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**Syntezy chiralnych pochodnych selenoorganicznych
o potencjalnej aktywności antyoksydacyjnej
i antynowotworowej**

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Praca doktorska
wykonana w Katedrze Chemii Organicznej
Wydziału Chemii
Uniwersytetu Mikołaja Kopernika w Toruniu

Toruń, 2024

*Panu Profesorowi Jackowi Ścianowskiemu,
składam serdeczne podziękowania
za nieocenioną pomoc i wsparcie,
za cenne wskazówki, cierpliwość i życzliwość
okazane podczas przygotowania niniejszej pracy doktorskiej.*

*Rodzicom - za ich wsparcie, miłość i wiarę we mnie i moje możliwości
na każdym etapie mojej edukacji.*

*Mamie za nieskończoną cierpliwość w wysłuchiwaniu
moich dylematów życiowych i zawodowych.*

*Tacie za nieustanne trzymanie kciuków
i poczucie, że robię coś ważnego.*

*Przyjaciółom - za ich obecność, cierpliwość i wsparcie,
które dodawały mi siłę w trudnych momentach.*

*Agacie i Magdalenie – za współpracę, inspirację i serdeczność zarówno jako
współpracownicom, jak i wspaniałym koleżankom.*

*Wszystkim Pracownikom Katedry Chemii Organicznej - za możliwość rozwijania się
w wyjątkowej atmosferze, która czyniła codzienną pracę przyjemnością.*

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Wykaz skrótów

- ABTS** - kwas 2,2'-azyno-bis(3-etylobenzotiazolino-6-sulfonowy)
- Ebselen** - 2-fenylo-1,2-benzizoselenazol-3(2*H*)-on
- DPPH** - 2,2-difenylo-1-pikrylohydrazyl
- DTT^{ox}** – disulfid ditionu
- DTT^{red}** – ditionitrol
- Gln 80** - glutamina
- GPx** - peroksydaza glutationowa
- GR** - reduktaza glutationowa
- GSH** - glutation
- GSSG** - forma utleniona glutationu
- HL-60** – linia komórkowa raka białaczki promielocytowej
- HPLC** - wysokosprawna chromatografia cieczowa
- HUVEC** – linia normalnych komórek śródbłonna żyły pępowinowej
- IC₅₀** - stężenie hamujące badanej substancji potrzebne do zahamowania w 50% wzrostu komórek w hodowli
- MCF-7** – linia komórkowa raka piersi
- NADPH** - zredukowana forma dinukleotydu nikotynoamidoadeninowego
- NMR** - magnetyczny rezonans jądrowy
- PhSH** – tiofenol
- ROS** - reaktywne formy tlenu
- SARS-CoV-2** - wirus z rodziny koronawirusów, który powoduje chorobę COVID-19
- Sec 450** – selenocysteina
- TLC** - chromatografia cienkowarstwowa
- Trp 158** – tryptofan
- TrxR** – reduktaza tioredoksyny

1. Streszczenie w języku polskim

Biologiczna aktywność pochodnych selenoorganicznych jest w dużej mierze związana z ich zdolnością do naśladowania aktywności peroksydazy glutationowej (GPx). GPx jako antyoksydacyjny selenoenzym utrzymuje homeostazę redoks w organizmie poprzez katalityczny rozkład nadtlenu nieorganicznych i organicznych do wody lub alkoholu w obecności glutationu lub innych endogennych tioli jako współkatalizatorów. Nadmierna produkcja reaktywnych form tlenu (ROS) powoduje niekontrolowaną proliferację i apoptozę komórek. Stan ten nazywany również stresem oksydacyjnym prowadzi do wielu często występujących schorzeń, w tym chorób układu sercowo-naczyniowego, neurodegeneracji, starzenia się i uszkodzenia komórek prowadzących do procesów nowotworzenia.

Synteza nowych pochodnych selenoorganicznych o dużej aktywności antyoksydacyjnej i przeciwnowotworowej przy zachowaniu niskiej toksyczności jest obecnie jednym z głównych celów badań dotyczących farmakologicznego ich zastosowania oraz realizowanej przeze mnie rozprawy doktorskiej. Głównym celem mojej pracy było wprowadzenie chiralnych grup funkcyjnych w pobliżu atomu selenu, aby zbadać wpływ stereochemii centrów stereogenicznych na ich aktywność biologiczną. Synteza i przebadanie aktywności związków będących w stosunku do siebie enancjomerami, epimerami czy diastereoizomerami może wykazać różne oddziaływania między określonymi układami biologicznymi, a ściśle określoną strukturą przestrzenną. W trakcie badań otrzymałam 70 związków selenoorganicznych, wśród których znajduje się 7 achiralnych oraz 63 chiralnych pochodnych, które są w stosunku do siebie enancjomerami lub diastereoizomerami. Modyfikowane są na atomie azotu lub tlenu grupami alkilowymi acyklicznymi i cyklicznymi z dodatkowymi pierścieniami aromatycznymi i grupami hydroksylowymi a także na atomie selenu grupami fenyłowymi lub acetylowymi. Wszystkie pochodne przebadalam jako potencjalne antyoksydanty oraz środki cytotoksyczne.

Pierwszą zsyntetyzowaną przeze mnie grupą związków selenoorganicznych były *N*-podstawione benzizoselenazolony, spośród których wyróżniły się pochodne *N*-2-hydroksy-1-indanylowe jako prooksydanty. Pochodne z konfiguracją (*S*) atomu węgla C1 wykazywały najwyższą aktywność cytotoksyczną zarówno wobec linii komórkowej raka piersi MCF-7 oraz raka białaczki promielocytowej HL-60 ze wszystkich zsyntetyzowanych w tej pracy związków. Diselenidy odznaczały się wyjątkowymi

właściwościami redukcji nadtlenu wodoru w teście Iwaoki NMR, których aktywność znacząco wzrastała w wyniku obecności ugrupowania *N*-hydroksyindanylowego. Otrzymałam, również diselenid *N-trans*-2-hydroksy-1-indanylowy, wykazujący silne działanie przeciwnowotworowe wobec linii komórkowej HL-60, który jest nietoksyczny dla komórek normalnych śródbłonna żyły pępowinowej (HUVEC). Wprowadzenie dodatkowej grupy fenylowej przy atomie selenu pozwoliło na otrzymanie silnego inhibitora dla wzrostu komórek raka piersi MCF-7, którym okazał się *N-(1S,2R)-(-)-cis*-(2-hydroksy-1-indanylo)-2-(fenyloseleno)benzamid. Zoptymalizowanie metody otrzymywania chiralnych β -karbonylofenyloselenidów, pokazało iż kluczowym etapem syntezy tych związków jest wygenerowanie karboanionu z wyjściowego ketonu. Wprowadzenie dodatkowej grupy karbonylowej zamiast pierścienia fenylowego znacząco polepszyło aktywności antyoksydacyjne i cytotoksyczne.

Ostatni etap rozprawy doktorskiej obejmował syntezę nieznaną w literaturze nowej grupy związków β -karbonylofenyloselenidów, posiadających grupę estrową w pozycji *orto* do atomu selenu. Związki te okazały się bardzo dobrymi zmiataczami wolnych rodników, a terpenowa pochodna *O-(-)-mentylo*-2-((2-oksopropyl)seleno)benzoesan wykazywała aktywność przeciwutleniającą podobną do Troloxu.

2. Summary in English

The biological activity of organoselenium derivatives is related mainly to their ability to mimic the activity of glutathione peroxidase (GPx). GPx, as an antioxidant selenoenzyme, maintains redox homeostasis in the body by catalytically decomposing inorganic and organic peroxides to water or alcohol in the presence of glutathione or other endogenous thiols as co-catalysts. Excessive reactive oxygen species (ROS) production causes uncontrolled cell proliferation and apoptosis. This condition, also called oxidative stress, leads to many common diseases, including cardiovascular diseases, neurodegeneration, ageing and cell damage, leading to carcinogenesis.

The synthesis of new organoselenium derivatives with high antioxidant and anticancer activity while maintaining low toxicity is currently one of the leading research goals on their pharmacological application and my doctoral dissertation. The main goal of my work was to introduce chiral functional groups near the selenium atom to study the influence of the stereochemistry of stereogenic centers on their biological activity. Synthesis and testing of the activity of compounds that are enantiomers, epimers or diastereomers can show different interactions between specific biological systems and a strictly defined spatial structure. During the research, I obtained 70 organoselenium compounds, including 7 achiral and 63 chiral derivatives that are enantiomers or diastereomers in relation to each other. They are modified on the nitrogen or oxygen atom with acyclic and cyclic alkyl groups with additional aromatic rings and hydroxyl groups and on the selenium atom with phenyl or acetyl groups. I tested all derivatives as potential antioxidants and cytotoxic agents. The first group of organoselenium compounds I synthesized were *N*-substituted benzoselenazolones, among which *N*-2-hydroxy-1-indanyl derivatives stood out as pro-oxidants. Derivatives with the (*S*) configuration of the C1 carbon atom showed the highest cytotoxic activity against the MCF-7 breast cancer cell line and the HL-60 promyelocytic leukemia cell line out of all the compounds synthesized in this work. Diselenides were characterized by exceptional hydrogen peroxide reduction properties in the Iwaoka NMR test, the activity of which increased significantly due to the presence of the *N*-hydroxyindanyl group. I also obtained *N-trans*-2-hydroxy-1-indanyl diselenide, which showed strong anticancer activity against the HL-60 cell line, which is non-toxic to normal umbilical vein endothelial cells (HUVEC). The introduction of an additional phenyl group at the selenium atom allowed obtaining a strong inhibitor of MCF-7 breast cancer cell

growth, which turned out to be *N*-(1*S*,2*R*)-(-)-*cis*-(2-hydroxy-1-indanyl)-2-(phenylselenide)benzamide. Optimization of the method for obtaining chiral β -carbonyl phenyl selenides showed that the key step in the synthesis of these compounds is the generation of a carbanion from the starting ketone. Introducing an additional carbonyl group instead of the phenyl ring significantly improved antioxidant and cytotoxic activities. The doctoral dissertation's last stage included synthesizing a new group of β -carbonyl phenyl selenide compounds unknown in the literature, having an ester group in the ortho position to the selenium atom. These compounds proved to be excellent free radical scavengers, and the terpene derivative *O*-(-)-menthyl-2-((2-oxopropyl)selenyl)benzoate showed antioxidant activity similar to Trolox.

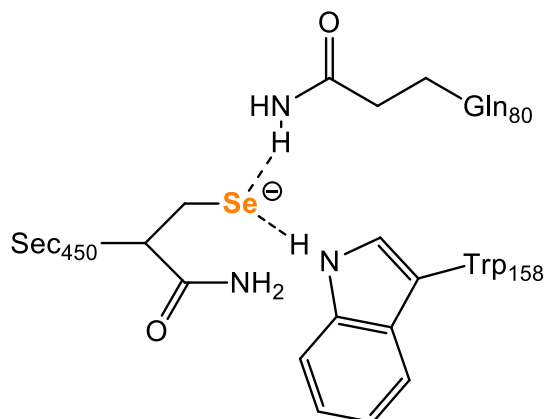
3. Wprowadzenie do rozprawy doktorskiej

Związki selenoorganiczne z uwagi na ich wyjątkowe właściwości znalazły szerokie zastosowanie w syntezie organicznej jako reagenty o charakterze elektrofilowym, nukleofilowym i wolnorodnikowym [1]. „Zielone” katalizatory selenoorganiczne stosowane są w wielu reakcjach, w tym np. w utlenianiu ketonów/aldehydów metodą Baeyera-Villigera, przekształceniu alkenów w epoksydy lub diole oraz utlenianiu alkoholi i związków zawierających azot [2,3]. Stosowane zamiast metali do aktywacji nadtlenu wodoru, zapewniają wysoką reaktywność i selektywność w licznych przemianach syntetycznych [4]. Ponadto prowadzone są również intensywne poszukiwania biologicznie aktywnych pochodnych stanowiących podstawę projektowania nowych selenoterapeutyków [5]. Połączenia selenoorganiczne posiadają właściwości dzięki którym mogą być zastosowane jako związki przeciwutleniające [6], przeciwnowotworowe [7], przeciwbakteryjne [8], przeciwcukrzycowe [9], przeciwwirusowe [10], przeciwzapalne [11] oraz jako terapeutyki w chorobie Alzheimera [12].

Biochemiczna rola selenu związana jest z jego obecnością w strukturze chiralnego aminokwasu L-selenocysteiny, który tworzy centrum aktywne unikalnego antyoksydacyjnego selenoenzymu - peroksydazy glutationowej (GPx) [13]. GPx katalizuje redukcję szkodliwych nadtlenków przez co obniża poziom reaktywnych form tlenu (ROS) i odgrywa kluczową rolę w homeostazie redoks organizmu [14]. Nadmierna produkcja ROS powoduje niekontrolowaną proliferację i apoptozę komórek [6]. Stan ten nazywany również stresem oksydacyjnym prowadzi do wielu często występujących schorzeń, w tym chorób układu sercowo-naczyniowego, neurodegeneracji, starzenia się i uszkodzeń komórek prowadzących do procesów nowotworowych [15,16].

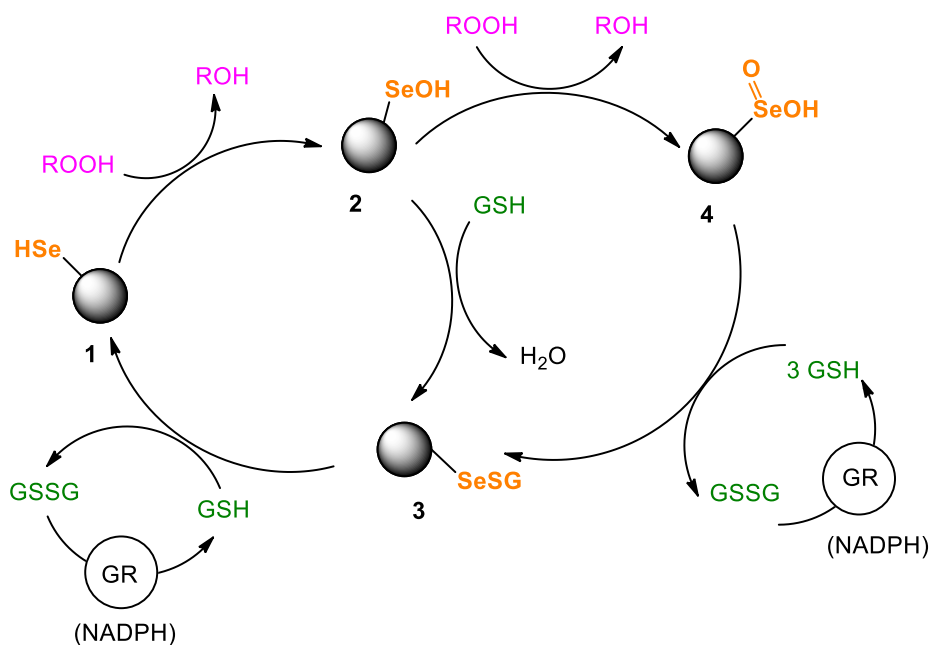
Peroksydaza glutationowa wykorzystuje glutation (GSH) lub inne endogenne tiole, jako kofaktora wykorzystywanego w redukcji wodoronadtlenków, takich jak H_2O_2 i wodoronadtlenków organicznych ROOH. GPx chroni błony lipidowe i inne składniki komórkowe przed utlenianiem [17]. Miejsce aktywne tego selenoenzymu (Rysunek 1) zawiera trzy aminokwasy, tj. selenocysteinę (Sec 450), glutaminę (Gln 80) i tryptofan (Trp 158) [18]. Atom Se utrzymywany jest w aktywnej, zjonizowanej formie dzięki specyficznemu środowisku aminokwasów, które znajdują się w odległości wiązania wodorowego od Sec tworząc tzw. triadę katalityczną [19]. Zwiększona nukleofilowość atomu selenu nasila aktywność katalityczną całego selenoenzymu. Dodatkowo, trwałość

wiązania C–Se, zapobiega zatruciu selenem oraz zmniejsza ryzyko jego nadmiernego utleniania [17].



Rysunek 1. Miejsce aktywne peroksydazy glutationowej [20]

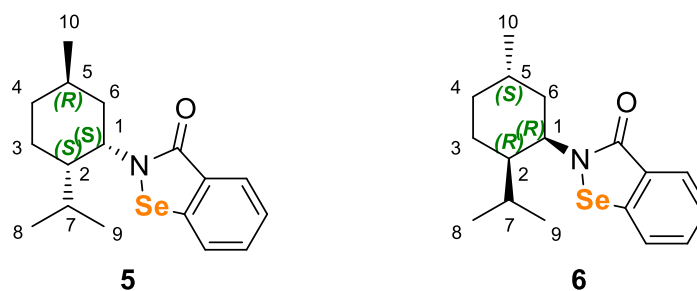
Mechanizm redukcji nadtlenków przez GPx przedstawiony jest na schemacie 1. W pierwszym etapie cyklu katalicznego selenol (E-SeH) **1** jest utleniany do kwasu selenenowego (E-SeOH) **2**. W przypadku zwiększonego stresu oksydacyjnego, następuje utlenienie związku pośredniego E-SeOH **2** do stabilnego kwasu seleninowego (E-SeO₂H) **4**. W obu przypadkach zarówno związek **2** jak i **4** redukowany jest poprzez wolny tiol GSH, tworząc pośredni selenylosulfid (E-SeSG) **3**. Związek **3** poddany działaniu GSH redukuje się za pośrednictwem tiolu do (E-SeH) **1**. W rezultacie regeneruje się miejsce kataliczne enzymu z jednoczesnym utlenieniem GSH do disulfidu GSSG. Reduktaza glutationowa (GR) przy pomocy NADPH przekształca GSSG w wolny GSH [21].



Schemat 1. Cykl eliminacji nadtlenków ROOH przez GPx [17]

Naturalna aktywność biologiczna GPx zainspirowała wiele grup badawczych do syntezy pochodnych selenoorganicznych, które mogą naśladować aktywność antyoksydacyjną tego enzymu [3]. Liczne testy *in vitro* i *in vivo* dowiodły, że związki te mogą być z powodzeniem stosowane jako środki farmakologiczne [22]. Jednak znalezienie biodostępnych cząsteczek selenoorganicznych o wysokim potencjale redoks i niskiej toksyczności jest nadal kluczowym problemem w tej dziedzinie badań. Właściwe zaprojektowanie leku może pomóc wyeliminować ten problem i umożliwić konstruowanie związków, które mogą selektywnie oddziaływać z określonymi receptorami w organizmie człowieka [23]. Z tego powodu, celem mojej pracy była synteza pochodnych selenoorganicznych wykazujących wysoki potencjał antyoksydacyjny i przeciwnowotworowy, które są bezpieczne dla komórek normalnych.

Różnica w aktywności biologicznej mimetyków GPx, które są w stosunku do siebie epimerami lub enancjomerami różniącymi się określoną trójwymiarową orientacją atomów, została zaprezentowana przez grupę badawczą Ścianowskiego. Wykorzystując enancjomerycznie czyste terpenyloawe aminy z układów *p*-mentanu, karanu i pinanu, zsyntetyzowano szereg *N*-terpenylobenzizoselenazol-3(2*H*)-onów obejmujący pary enancjomerów i diastereoizomerów [24]. Otrzymane pochodne zostały zbadane jako środki cytotoksyczne na linii komórkowej MCF-7, czyli raka piersi. Wyniki aktywności przeciwnowotworowej przedstawiono jako IC₅₀ (ang. inhibitory concentration) co oznacza stężenie hamujące badanej substancji potrzebne do zahamowania w 50% wzrostu komórek w hodowli, w stosunku do wzrostu komórek kontrolnych [25]. Niższe wartości IC₅₀ wskazują na większą skuteczność związku. Wyjątkowe wartości IC₅₀ zazwyczaj wynoszą poniżej 1 μM dla bardzo silnych inhibitorów, do 10 μM dla silnych inhibitorów, a do 50 μM dla środków cytotoksycznych o umiarkowanym działaniu. Znacząco odmienny wynik aktywności przeciwnowotworowej uzyskano np. dla enancjomerów **5** i **6** otrzymanych z (+) i (-)-neomentyloaminy (Rysunek 2). Zaobserwowano, że pochodna **5** ma prawie 7 razy wyższą aktywność cytotoksyczną niż pochodna **6**. Zmiany w stereochemii tych związków mogą wpływać na stabilność, zdolność wiązania oraz ich metabolizm, co skutkuje różnymi poziomami aktywności biologicznej.



IC₅₀ (MCF-7)

12,4 ± 0,4 μM

85,5 ± 4,0 μM

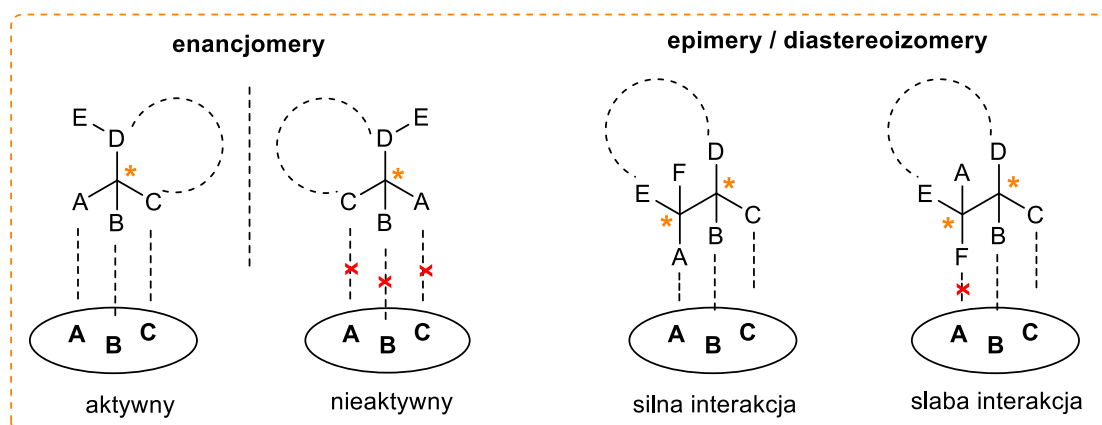
Rysunek 2. Elementy struktury wpływające na reaktywność związków 5, 6

Struktura przestrzenna cząsteczki bioaktywnej jest ważną cechą wpływającą na jej oddziaływanie z centrami aktywnymi białek i cały cykl biochemiczny. Różną aktywność biologiczną enancjomerów obserwowano wcześniej również w przypadku znanych leków, gdzie jeden enancjomer był potencjalnym środkiem terapeutycznym, a drugi wykazywał znaczną toksyczność, jak np. w dobrze znanym przypadku (*R*)- i (*S*)-talidomidu [26].

Przeciwne enancjomery/diastereoizomery mogą wykazywać silniejszą lub słabszą aktywność w wyniku zmiany interakcji lub indukcji powinowactwa do innych domen docelowych. Transformacja jednego związku do innego izomeru, poprzez zmianę układu wiązań (regioizomery) lub trójwymiarowej orientacji atomów (epimery/ enancjomery), może zmienić aktywność biologiczną cząsteczki [27] (Schemat 2).

Stereoselektywna interakcja lek-białko/enzym

R* - centrum stereogeniczne



Schemat 2. Oddziaływanie różnych izomerów z docelowymi miejscami wiązania

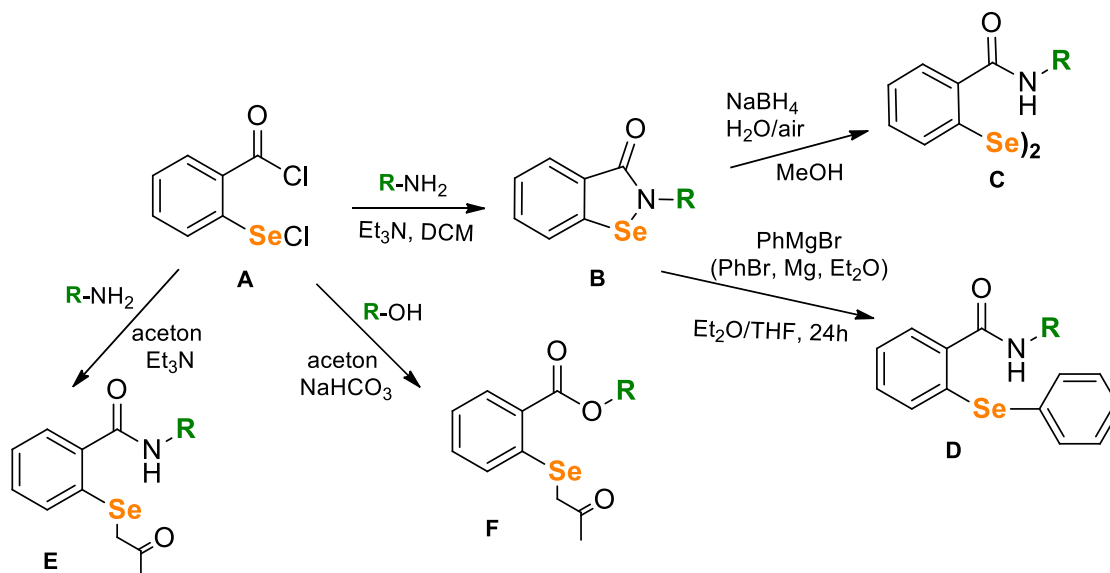
Chiralny związek, posiadający ściśle ustaloną orientację podstawników, które pasują do pewnych domen białkowych, może wykazywać lepszą aktywność biologiczną. Przeciwnie, jego enancjomer, ze względu na przeciwną konfigurację asymetrycznego centrum węgla, może być biologicznie nieaktywny lub wykazywać działania niepożądane [28]. Dodatkowo, jeśli więcej niż jedno centrum chiralne jest obecne w cząsteczce, możemy zaobserwować dwa epimery/ diastereoizomery z silniejszą lub słabszą interakcją lek-białko/enzym. Reaktywność można również modulować przez przekształcenie związku do jego regioizomeru, posiadającego inną organizację atomów, ale ten sam wzór cząsteczkowy. W ten sposób można zmienić siłę wiązania lub rozwinąć powinowactwo do innych domen białkowych.

Każdy izomer może wykazywać inne właściwości biochemiczne i farmakologiczne, w tym transport, biodostępność, selektywność, metabolizm i wydalanie [29]. Optymalizacja stereochemiczna leku może prowadzić do zwiększenia jego biodostępności i efektywności terapeutycznej.

Różnorodne właściwości związków selenoorganicznych, takie jak możliwość aplikacji jako katalizatorów w syntezie organicznej, aktywność przeciwutleniająca oraz ich potencjalne zastosowania terapeutyczne, nadal przyczyniają się do niemającego zainteresowania tą tematyką wielu grup badawczych. Zgłębienie zagadnienia stereochemii w chemii selenoorganicznej oraz zrozumienie wpływu konfiguracji przestrzennej na aktywność biologiczną i właściwości chemiczne tych związków jest jednym z najmniej zbadanych tematów w tej dziedzinie badań. Chociaż znane są przykłady chiralnych pochodnych selenoorganicznych syntetyzowanych jako związki bioaktywne, wpływ określonych centrów stereogenicznych, w szczególności różnica między aktywnością enancjomerów, na bioaktywność cząsteczek nie został jeszcze dogłębnie zbadany. Z tego powodu, głównym celem mojej pracy była synteza nowych chiralnych związków selenoorganicznych oraz zbadanie wpływu stereochemii na ich aktywność biologiczną.

Wśród mimetyków GPx najbardziej znaną grupę pochodnych tworzą *N*-podstawione benzisoselenazolony, w tym szeroko przebadany ebselen (2-fenyl-1,2-benzisoselenazol-3(2*H*)-on) o wszechstronnym potencjale terapeutycznym. Testowany jest klinicznie pod kątem różnych chorób, w tym jako lek na neurologiczne zaburzenia afektywne dwubiegunowe [30], jako środek na ochronę słuchu przed uszkodzeniami wywołanymi hałasem [31], a także jako potencjalny lek na choroby zakaźne, w tym infekcje wywołane przez SARS-CoV-2 (COVID-19) [32]. Z tego względu, pierwszym

celem mojej pracy była synteza serii chiralnych *N*-podstawionych benzisoselenazolonów (**B**), przebadanie ich aktywności antyoksydacyjnej i przeciwnowotworowej oraz wykazanie czy konfiguracja poszczególnych centrów asymetrycznych znacząco wpłynie na reaktywność Se-pochodnych. Wszystkie grupy związków selenoorganicznych, które stanowiły przedmiot moich badań przedstawione są na Schemacie 3.



Schemat 3. Droga syntezy grup związków zrealizowanych w ramach rozprawy doktorskiej

Zdolność do naśladowania aktywności GPx wykazują również odpowiednie pochodne benzisoselenazolonu t.j. selenidy, diselenidy, selenoestry i selenole [20]. Diselenidy (C), ze względu na niższą energię wiązania Se-Se (172 kJ/mol), niż wiązanie Se-N (193kJ/mol) w benzisoselenazolonach, stanowiły kolejny cel moich badań [33]. Niższa energia wiązania wskazuje na słabsze oddziaływanie atomów między sobą przez co wiązanie jest bardziej reaktywne.

Doniesienia odnośnie modyfikacji struktury benzisoselenazolonów poprzez wprowadzenie dodatkowego pierścienia aromatycznego, powodującego zwiększenie aktywności biologicznych otrzymanych związków [34,35], stanowiły inspirację do syntezy kolejnej grupy chiralnych pochodnych *N*-podstawionych fenyloselenidów (D).

Sugerując się budową GPx w miejscu aktywnym, nowe związki selenoorganiczne projektowano tak, aby atom selenu występował w otoczeniu heteroatomu np. azotu lub tlenu [18]. W kolejnej syntetyzowanej przeze mnie grupie związków różnicowałam strukturę chiralnych związków selenoorganicznych poprzez wprowadzenie dodatkowej grupy karbonylowej (2-oksopropylowej), dzięki czemu otrzymałam nowe chiralne *N*-

sfunkcjonalizowane β -karbonyloselenidy (**E**). Aktualności problemu badawczego dowodzi również fakt niedawnych doniesień literaturowych na temat modyfikacji niesymetrycznych alkilowo-arylowych selenidów, które posiadają silne właściwości przeciwnowotworowe [36,37].

Wykorzystanie estryfikacji w modyfikacji leków pozwala rozwiązać problem biodostępności wielu znanych środków bioaktywnych [38]. Zastosowanie tej modyfikacji w związkach selenoorganicznych, może poprawić rozpuszczalność oraz biodostępność tych pochodnych. Z tego powodu kolejnym i jednocześnie ostatnim etapem mojej pracy było zbadanie wpływu grupy estrowej na bioaktywność selenidów β -karbonylowych poprzez zastąpienie grupy *o*-amidowej podstawnikiem *o*-estrowym. W tym celu zsyntetyzowałam nieznane dotąd w literaturze selenidy β -karbonylowofenyłowe posiadające grupę *o*-estrową (**F**) i oceniłam, czy modyfikacja ta zmienia ich właściwości przeciwutleniające i przeciwnowotworowe.

4. Cel pracy

Ideą pracy doktorskiej było:

1. Zaprojektowanie i opracowanie wydajnej metody syntezy oraz otrzymanie nowych grup związków selenoorganicznych:
 - a) **chiralnych N-funkcjonalizowanych benzisoselenazol-3(2H)-onów**, podstawionych przy atomie azotu różnymi ugrupowaniami alifatycznymi i aromatycznymi,
 - b) **chiralnych N-funkcjonalizowanych diselenidów** zawierających grupę *o*-amidową, poprzez transformację wiązania Se-N w układ diselenidowy Se-Se,
 - c) **chiralnych N-funkcjonalizowanych fenyloselenidów** zawierających grupę *o*-amidową, poprzez wprowadzenie dodatkowego pierścienia aromatycznego,
 - d) **chiralnych N-funkcjonalizowanych β -karbonylofenyloselenidów** zawierających grupę *o*-amidową i dodatkową grupę karbonylową poprzez podstawienie atomu selenu grupą 2-oksopropylową,
 - e) **achiralnych i chiralnych O-funkcjonalizowanych β -karbonylofenyloselenidów** zawierających grupę *o*-estrową i dodatkową grupę karbonylową poprzez funkcjonalizację atomu selenu grupą 2-oksopropylową.
2. Zastosowanie otrzymanych związków jako katalizatorów antyoksydacyjnych oraz zbadanie aktywności cytotoksycznej uzyskanych pochodnych, a także określenie wpływu konfiguracji poszczególnych centrów stereogenicznych na bioaktywność wszystkich pochodnych reprezentujących pary enancjomerów lub diastereoizomerów.

5. Spis publikacji stanowiących podstawę rozprawy doktorskiej

P1. – praca przeglądowa

M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; J. Ścianowski,
„*Organoselenium compounds as antioxidants*”, *Arkrivoc.*, **2023**, part v, 211

P2.

A. Laskowska; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka;
A. Wojtczak; J. Ścianowski,
„*Attachment of Chiral Functional Groups to Modify the Activity of New GPx Mimetics*”,
Materials, **2022**, 15, 2068

Supporting information: <https://www.mdpi.com/article/10.3390/ma15062068/s1>

P3.

A. Laskowska; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska,
K. Gach-Janczak, A. Janecka; J. Ścianowski,
„*Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents*”,
RCS Advances, **2023**, 13, 14698

Supporting information:

<https://www.rsc.org/suppdata/d3/ra/d3ra02475j/d3ra02475j1.pdf>

P4.

A. Laskowska; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska,
A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski,
„*Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity
Evaluation—Part I*”, *Materials*, **2024**, 17, 899

Supporting information: <https://www.mdpi.com/article/10.3390/ma17040899/s1>

P5.

A. Laskowska; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska,
A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski,
„*Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity
Evaluation—Part II*”, *Molecules*, **2024**, 29, 2866

Supporting information: <https://www.mdpi.com/article/10.3390/molecules29122866/s1>

6. Omówienie publikacji wchodzących w skład rozprawy doktorskiej

6.1 Publikacja 1 [P1]

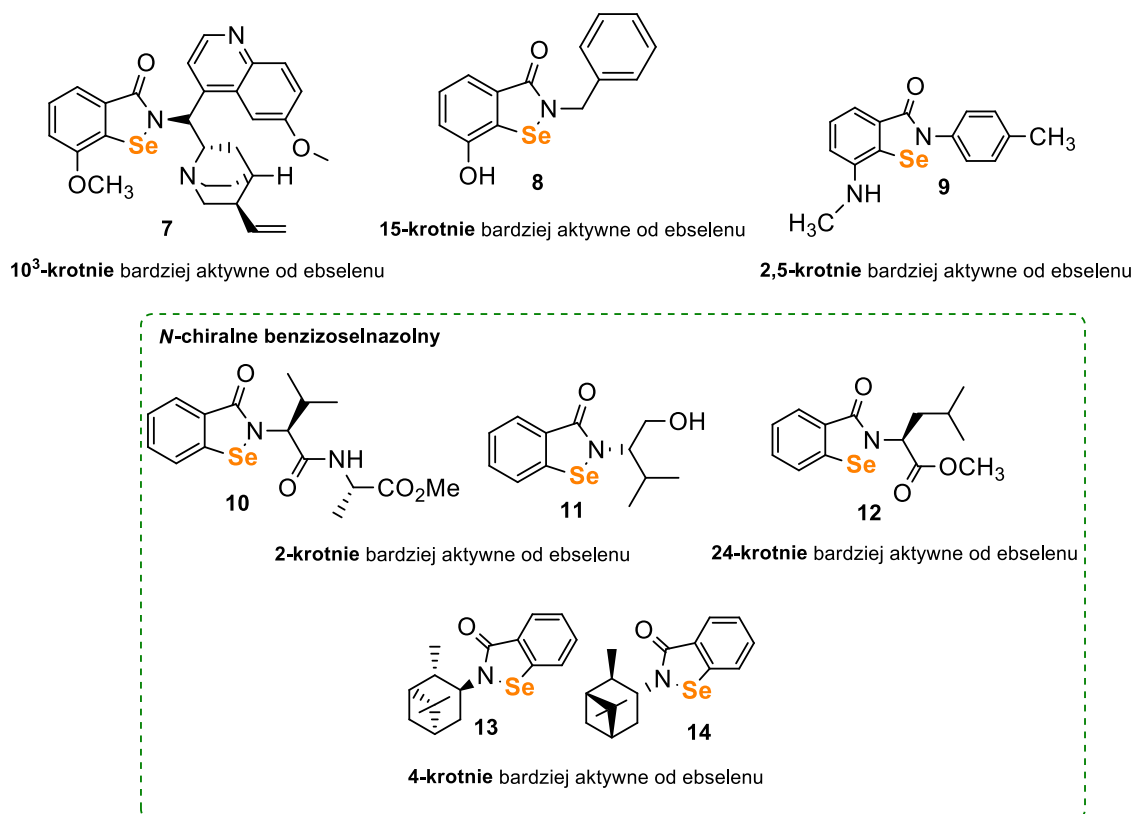
Związki selenoorganiczne jako antyoksydanty

Prezentowana praca przeglądowa jest podsumowaniem stanu wiedzy o otrzymanych w ciągu ostatnich dwunastu lat związkach selenoorganicznych o działaniu przeciwutleniającym. Opisane zostały związki, które mogą okazać się pomocne w medycynie jako suplementy zapobiegające chorobom wywołanym stresem oksydacyjnym. Ponadto przedstawiono cząsteczki selenoorganiczne, które mają możliwość zastosowania jako dodatki do żywności zapobiegające utlenianiu lub jako nanocząstki i polimery zawierające atom selenu w przemyśle materiałowym.

We wstępie opisano wpływ reaktywnych form tlenu (ROS) i stresu oksydacyjnego na organizmy żywe, a także rolę i podział przeciwutleniaczy. Wśród antyoksydantów możemy wyróżnić cząsteczki pochodzenia naturalnego i syntetycznego [39,40]. Do układów endogennych wytwarzanych przez organizm, zaliczamy pochodne enzymatyczne (np. peroksydaza glutationowa (GPx), reduktaza tioredoksynowa (TrxR) i nieenzymatyczne (np. glutation, ferrytyna, albumina). Natomiast, egzogenne przeciwutleniacze pochodzące ze źródeł naturalnych, takich jak rośliny (np. flawonoidy, karotenoidy, witaminy) lub minerały (selen, cynk, mangan) dostarczane są poprzez odpowiednią dietę. Druga grupa przeciwutleniaczy to związki pochodzenia syntetycznego, dostarczane organizmowi w postaci suplementów diety (np. kwas *L*-askorbinowy). Dalej omówiono znaczenie selenoenzymu – peroksydazy glutationowej (GPx) oraz jej cykl katalityczny. Szczegółowo przedstawione zostały również najczęstsze metody pomiaru aktywności antyoksydacyjnej, które również zastosowałam w swoich badaniach. Pierwszą opisaną pośrednią metodą oceny aktywności przeciwutleniającej był test GSH (zredukowanej formy glutationu) sprzężony z reduktazą glutationową (GR) z użyciem kofaktora NADPH [41]. Kolejną opisaną metodą jest test PhSH, w której wykorzystywany jest benzenotiol (PhSH) jako alternatywa dla glutationu [42]. Test ABTS polega na ocenie zdolności związków selenoorganicznych do odbarwiania roztworu rodnika ABTS• (kwasu 2,2'-azyno-bis(3-etylobenzotiazolino-6-sulfonowego) [43]. Opisywany jest również test utleniania ditiotreitolu (DTT^{red}) do disiarczku (DTT^{ox}), gdzie szybkość reakcji rejestrowana jest za pomocą spektroskopii magnetycznego rezonansu jądrowego (NMR) [44]. Metoda DPPH polega na mierzeniu zdolności

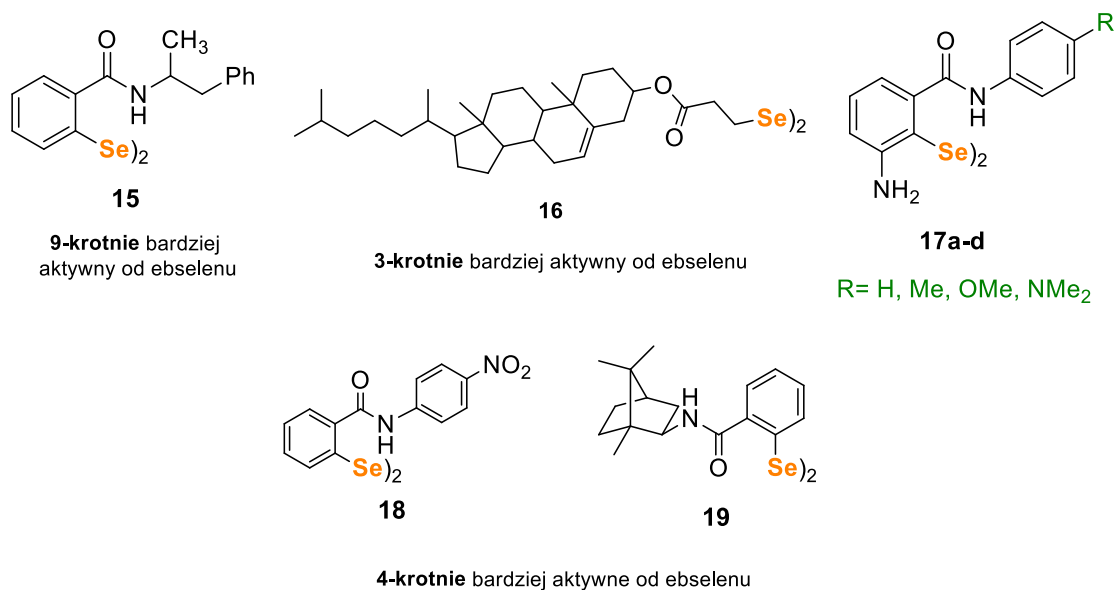
związków do zmiatania wolnych rodników 2,2-difenylo-1-pikrylohydrazylu (DPPH•) poprzez obserwację zmiany barwy roztworu z fioletowej na żółtą, co świadczy o antyoksydacyjnej aktywności badanego związku [45].

Główna część artykułu, czyli opis przeciwutleniaczy w medycynie została podzielona na sekcje: (a) ebselenu i jego pochodnych, (b) diselenidów i ich pochodnych oraz (c) innych związków selenoorganicznych jako przeciwutleniaczy. W sekcji odnośnie pochodnych ebselenu wykazano szerokie możliwości aplikacyjne ebselenu i scharakteryzowano jego modyfikacje, które wpływają na polepszenie zdolności naśladowania GPx. Związek **7**, wykazywał znacznie wyższą aktywność przeciwutleniającą w porównaniu do innych benzisoselenazonów prezentowanych w tym przeglądzie i jest 10^3 -krotnie bardziej aktywny niż ebselen [46]. Obecność grupy hydroksylowej w związku **8** czy $-NHCH_3$ w związku **9** zwiększała aktywność podobną do GPx odpowiednio 15-krotnie i 2,5-krotnie [47,48]. Interesującymi doniesieniami są przykłady chiralnych benzisoselenazonów **10-14**, które wykazują znacznie wyższą aktywność przeciwutleniającą w porównaniu do ebselenu (Rys. 3) [24,49–51].



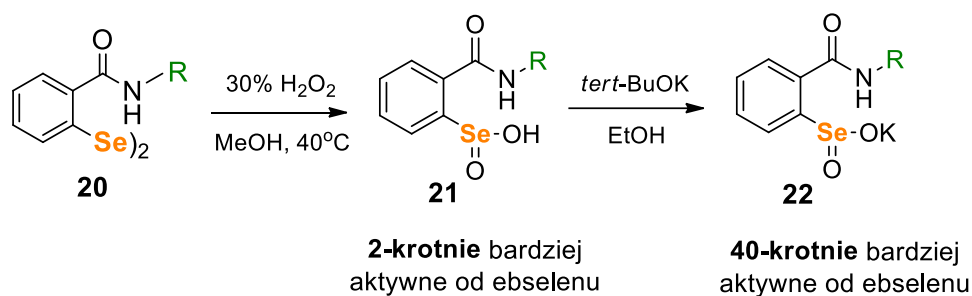
Rysunek 3. Benzisoselenazonolny **7-14** o lepszej aktywności antyoksydacyjnej od ebselenu

W kolejnym podrozdziale przedstawiono ciekawe doniesienia odnośnie syntetyzowanych przez kilka wiodących grup badawczych diselenidów i ich pochodnych. Związki te zostały najszerszej opisane ze względu na dominującą ilość doniesień literaturowych. Braga i współpracownicy otrzymali diselenid na bazie amidu **15**, który był 9-krotnie bardziej aktywny niż ebselen [52]. Ta sama grupa badawcza potwierdziła, że diselenid **16** zawierający w swojej strukturze cholesterol miał działanie przeciwutleniające 3-krotnie większe niż ebselen [53]. W 2021 r. Singh, Kumar i współpracownicy syntetyzowali szereg diselenidów **17a-d**. Zauważyli oni, że podstawniki oddające elektrony zwiększają działanie potencjalnych przeciwutleniaczy, a największą aktywność przeciwutleniającą zaobserwowano dla diselenidu **17d** (2-krotnie bardziej aktywnego niż ebselen) [54]. W grupie badawczej Ścianowskiego zsyntetyzowano diselenidy podstawnikami *N*-aryłowymi **18** [55] i *N*-chiralnymi **19** [49] (Rys. 4).



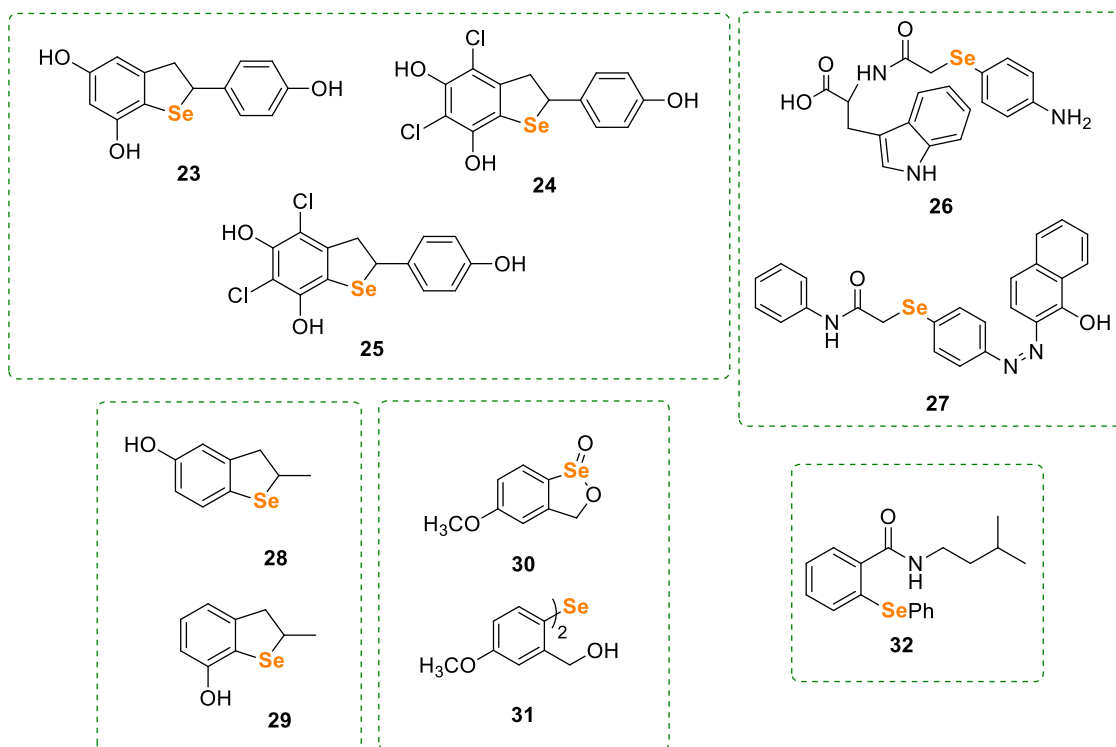
Rysunek 4. Diselenidy **15-19** o lepszej aktywności antyoksydacyjnej od ebselenu

Liczne modyfikacje i przekształcenia diselenidów do selenoli, selenylosulfidów, kwasów selenowych i seleninowych, selenotlenków czy do diselenidów z grupą aminową oraz amidową znacznie zwiększały aktywność antyoksydacyjną podobną do GPx [54,56,57]. W przypadku odpowiednich pochodnych diselenidowych: kwasów *N*-alkiloseleninowych **21** oraz rozpuszczalnych w wodzie soli potasowych tych kwasów **22** zaobserwowano aż 40-krotne polepszenie właściwości przeciwutleniających w porównaniu do ebselenu (Schemat 4). [58]



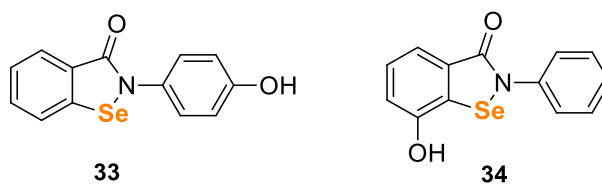
Schemat 4. Synteza kwasów seleninowych **22** i soli potasowych tych kwasów **22**

W dalszej części opisano wpływ grup hydroksylowej [59] i metoksy [60,61] na aktywność katalityczną związków selenoorganicznych oraz reaktywność selenidów i niesymetrycznych fenyloselenidów wobec nadtlenków [34]. Przedstawiono również benzoselenofeny **23-25** na bazie resweratrolu (naturalnego silnego przeciwutleniacza), które okazały się być 1,5 razy bardziej od niego aktywne [62]. Innymi przykładami związków wykazującymi podobną aktywność przeciwutleniającą do naturalnych antyoksydantów (tutaj witaminy C) są związki **26** i **27** [63]. W przypadku związków z dodatkową grupą hydroksylową, stwierdzono że, pozycja *orto* jest najbardziej korzystnym umiejscowieniem dla tej grupy w stosunku do atomu selenu. Związek **28** wykazywał 9-krotnie większą aktywność GPx w porównaniu do ebselenu. Z kolei cząsteczka **29** (z grupą OH w pozycji *para*) był tylko nieco bardziej aktywny niż związek referencyjny [59]. Badania efektu wprowadzenia grupy metoksy na aktywność antyoksydacyjną pokazały, że grupa metoksy w pozycji *para* (związki **30-31**) w stosunku do atomu selenu zwiększała aktywność katalityczną. W przeciwieństwie do tego, grupy *m*-metoksy mają niewielki wpływ, a podstawniki *o*-metoksy hamują zdolność redukcji nadtlenków [60,61]. Z kolei, niesymetryczny fenyloselenid **32**, wykazywał 2-krotnie wyższą aktywność przeciwutleniającą niż ebselen [34].



Rysunek 5. Inne związki selenoorganiczne **23-32** o potencjalnej aktywności podobnej do GPx

W następnym rozdziale opisano związki selenoorganiczne jako przeciwutleniacze stosowane w różnych gałęziach przemysłu, gdzie wykazano, że ebselenole hamowały peroksydację kwasu linolowego w tym samym czasie (związek **33**) lub znacznie lepiej (związek **33**) co α -tokoferol (Rys. 6) [47].



Rysunek 6. Struktury ebselenoli **33** i **34** o wysokiej aktywności antyoksydacyjnej

W ostatniej części przeglądu przedstawiono przykłady projektowania nanocząstek i nanokompozytów selenowych, które posiadają wyjątkową zdolność multioksydacyjną, naśladując wewnątrzkomórkowe przeciwutleniacze enzymatyczne i nieenzymatyczne, które stanowią system maszynierii przeciwutleniającej [64]. Dodatkowo opisano polimery zawierające atom selenu, które wykazują właściwości przeciwutleniające, co wskazuje na szerokie spektrum możliwości zastosowania ich w przemyśle materiałowym [65,66].

W niniejszej pracy przedstawiono różnorodne klasy związków, a znaczna ilość publikacji zgromadzonych w przeglądzie, wraz z ciągłym napływem nowych doniesień

literaturowych, stanowiła podstawę do podjęcia tematu przedstawionego w pracy doktorskiej. Fakt ten świadczy o ciągłym zainteresowaniu wielu grup badawczych poszukiwaniem związków selenoorganicznych o wysokim potencjale redoks. Prezentowana praca przeglądowa stanowi uzasadnienie otrzymania pochodnych benzisoselenazol-3(2*H*)-onów, diselenidów, fenyloselenidów (publikacje [P2], [P3], [P4]). Szeroka różnorodność pochodnych selenoorganicznych wskazuje na duże zróżnicowanie kierunków badań, stwarzając wiele możliwości badawczych.

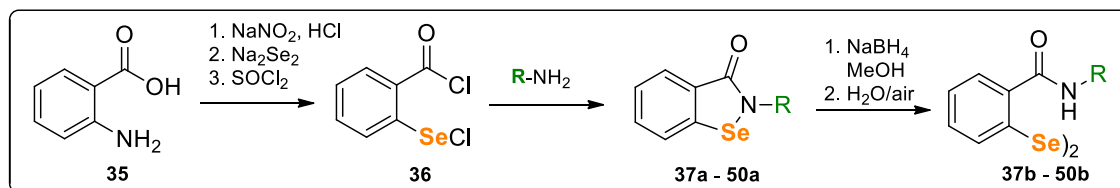
6.2 Publikacja 2 [P2]

Dołączenie chiralnych grup funkcyjnych w celu modyfikacji aktywności nowych mimetyków GPx

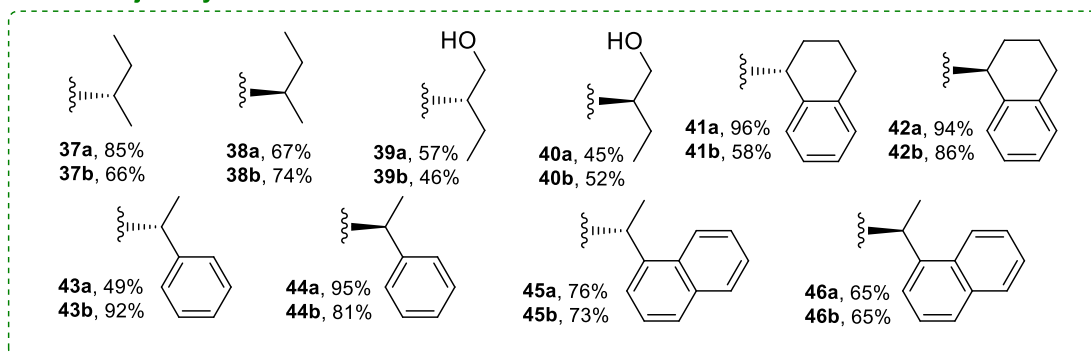
Przeciwne enancjomery/diastereoizomery mogą wykazywać silniejszą lub słabszą aktywność w stosunku do tych samych bądź innych domen docelowych. Bazując na tym założeniu, celem badań prezentowanych w niniejszej publikacji było zidentyfikowanie szczególnych cech strukturalnych, które wpływają na aktywność antyoksydacyjną i cytotoksyczną selenoorganicznych mimetyków GPx. Ocena aktywności biologicznej wykazała, że konfiguracja poszczególnych centrów węglowych może modulować reaktywność cząsteczek. Chiralne związki selenoorganiczne otrzymałam w dwóch postaciach *N*-podstawionych benzisoselenazol-3(2*H*)-onów, z reaktywnym wiązaniem Se-N (193kJ/mol) oraz odpowiednich difenylodiselenidów, posiadających połączenie Se-Se z niższą energią wiązania (172 kJ/mol).

Pierwszym etapem badań była synteza *N*-podstawionych benzisoselenazol-3(2*H*)-onów. Procedura opierała się na reakcji chlorku 2-(chloroseleno)benzoilu **36** z dostępnymi handlowo enancjomerycznie czystymi aminami. Chlorek kwasowy **36** otrzymałam w wyniku reakcji chlorku tionylu z kwasem diselenodibenzoowym. Kwas ten natomiast otrzymałam z kwasu antranilowego **35** poprzez utworzenie soli diazoniowej tego kwasu, a następnie reakcji z diselenkiem sodu. Otrzymane związki **37a-50a** przekształciłam w diselenidy **37b-50b** za pomocą procedury redukcji i utleniania - redukcji borowodorkiem sodu i utleniania na powietrzu. Zsyntetyzowałam szereg nowych chiralnych benzisoselenazol-3(2*H*)-onów i odpowiednich diselenidów zawierających grupę *o*-amidową, podstawionych przy atomie azotu różnymi

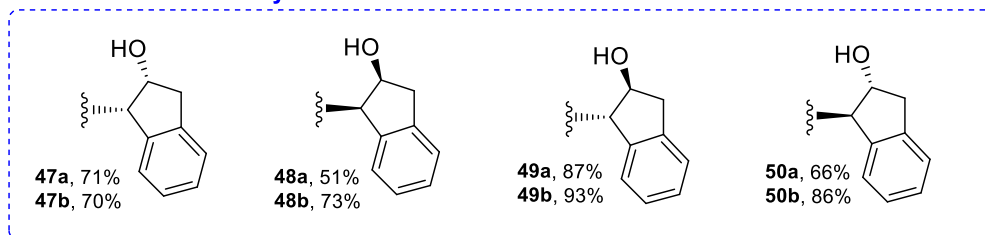
ugrupowaniami alifatycznymi i aromatycznymi. Zarówno benzisoselenazolony (45-96%), jak i diselenidy (46-93%) zsyntetyzowałam z dobrymi wydajnościami (Schemat 5)



enancjomery

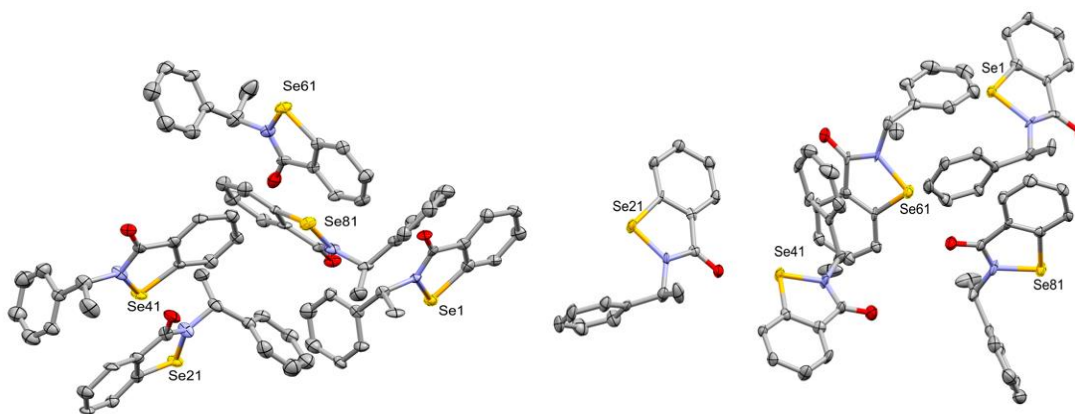


diastereoizomery



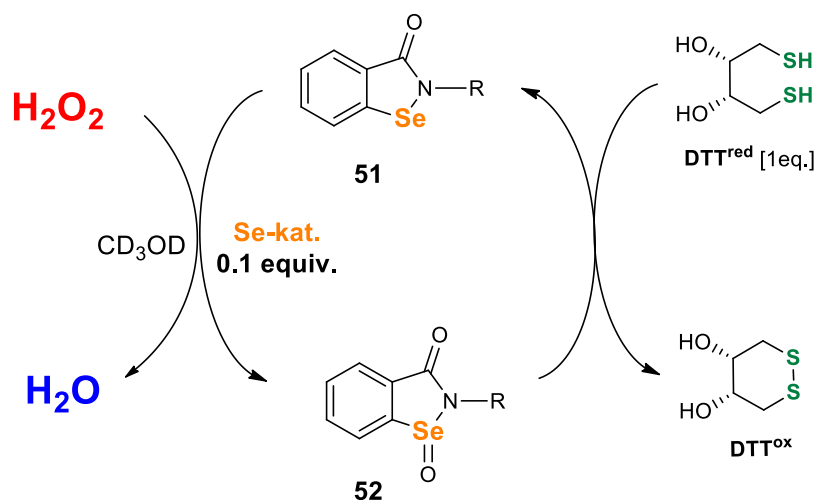
Schemat 5. Syntetyzowane chiralne benzisoselenazolony **37a-50a** i diselenidy **37b-50b**

Określono struktury krystaliczne enancjomerów **43a/44a** (Rysunek 7). Asymetryczna część struktury krystalicznej *N*-[*(R)*-(+)- α -metylobenzyl]-1,2-benzisoselenazol-3(2*H*)-onu **43a** składa się z 5 cząsteczek, we wszystkich wykryto konfigurację (*R*) centrum chiralnego. W przypadku związku **44a**, część asymetryczna składa się również z 5 cząsteczek benzisoselenazolonu, dla wszystkich określono konfigurację (*S*) centrum chiralnego.



Rysunek 7. Asymetryczna część konstrukcji **43a** i **44a**. ADP wykreślono przy poziomie prawdopodobieństwa 50%.

Zbadałam aktywność antyoksydacyjną wszystkich pochodnych metodą opartą na NMR zaprezentowaną przez Iwaokę i współpracowników [67]. Zdolność do redukcji nadtlenu wodoru mierzono szybkością utleniania ditiotreitolu do dwusiarczku (DTT^{red} do DTT^{ox}). Aktywny katalizator Se powstaje w wyniku utlenienia selenidu **51** do selenotlenku **52** za pomocą nadtlenu wodoru. Ditiotreitól (DTT^{red}) redukuje związek **52** do wyjściowego selenidu **51**. Szybkość zanikania substratu (DTT^{red}) oszacowano rejestrując zmiany w widmie ¹H NMR, w określonych odstępach czasu. Równanie reakcji (Schemat 6) i wyniki testu antyoksydacyjnego przedstawiono poniżej (Tabela 1).



Schemat 6. Równania reakcji utleniania i redukcji podczas zbadania aktywności antyoksydacyjnej

Tabela 1. Wyniki pomiaru aktywności przeciwutleniającej dla pochodnych **37a-50a** oraz **37b-50b**

Se-kat.[0.1 equiv.]	Pozostały DTT ^{red} [%] ± SEM			
	5 min	15 min	30 min	60 min
<i>Benzisoselenazolony</i>				
37a/38a	90,3 ± 1,9	88,4 ± 1,6	86,4 ± 1,7	80,5 ± 0,4
39a/40a	86,6 ± 4,6	83,3 ± 4,6	77,0 ± 3,3	60,6 ± 1,2
41a/42a	91,0 ± 0,1	88,3 ± 2,6	85,2 ± 3,7	77,3 ± 4,5
43a/44a	77,1 ± 4,7	58,5 ± 0,6	38,0 ± 6,7	16,1 ± 1,0
45a/46a	90,9 ± 1,9	89,3 ± 2,1	88,3 ± 3,01	84,7 ± 4,6
47a/48a	84,0 ± 4,6	79,6 ± 5,0	73,8 ± 2,2	62,4 ± 2,4
49a/50a	87,4 ± 3,5	86,0 ± 2,9	83,1 ± 1,5	78,0 ± 3,1
<i>Diselenidy</i>				
37b/38b	80,2 ± 4,4	65,0 ± 1,4	47,4 ± 1,4	29,5 ± 4,3
39b/40b	73,4 ± 0,9	50,5 ± 1,4	30,1 ± 4,5	19,5 ± 3,2
41b/42b	89,5 ± 2,6	83,2 ± 4,5	80,1 ± 2,4	75,3 ± 2,9
43b/44b	76,4 ± 2,6	54,3 ± 4,3	37,3 ± 0,6	26,1 ± 0,2
45b/46b	87,3 ± 3,3	84,6 ± 3,3	82,4 ± 3,6	76,4 ± 3,9
47b/48b	44,3 ± 1,3	13,5 ± 5,2	2,3 ± 2,3	0
49b/50b	14,2 ± 2,9	2,1 ± 1,3	0	0
Ebselen	75	64	58	52

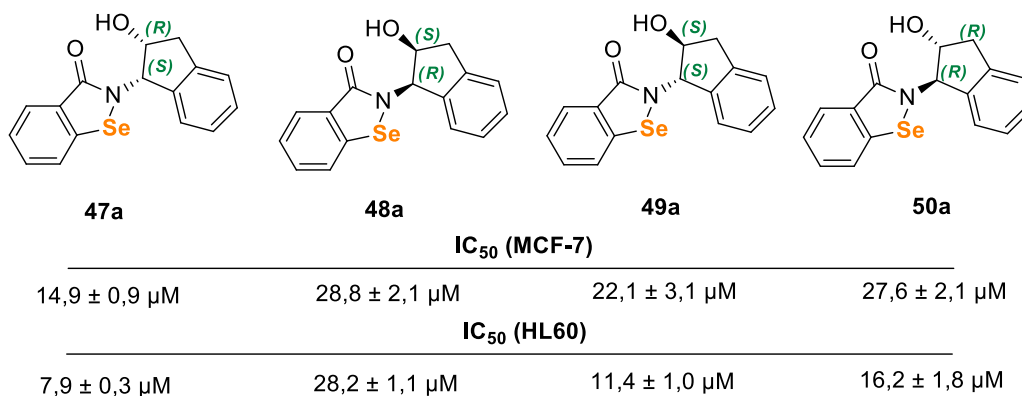
Aktywność przeciwutleniająca wśród poszczególnych par enancjomerów była taka sama. Zasadniczo lepsze wyniki uzyskałam dla diselenidów **37b-50b** niż dla odpowiednich benzisoselenazolonów **37a-50a**. Z pochodnych ebselenu najwyższą aktywność wykazywały związki z podstawnikiem α -metylobenzylowym **43a/44a**. Najlepsze właściwości do redukcji H₂O₂ zaobserwowałam dla pochodnych indanylowych posiadających grupy hydroksylowe *cis* (**47b/48b**) i *trans* (**49b/50b**), przy całkowitej konwersji ditiolu odpowiednio po 30 i 15 minutach. W porównaniu do pochodnych benzisoselenazolonów z tymi samymi podstawnikami (**49a/50a**) odpowiadające im diselenidy (**49b/50b**) około 18 razy szybciej redukowały H₂O₂.

Aktywność cytotoksyczną wszystkich związków mierzyłam za pomocą testu żywotności komórek (MTT) na liniach komórkowych ludzkiej białaczki promielocytowej HL-60 i raka piersi MCF-7 [68] w ramach współpracy z Uniwersytetem Medycznym w Łodzi. W przypadku benzisoselenazolonów największy potencjał antyproliferacyjny zaobserwowałam także dla pochodnej hydroksyindanylowej **47a** (Tabela 2).

Tabela 2. Działanie antyproliferacyjne związków 10a-23a

Związek	IC ₅₀ [μM] ± SEM	
	MCF-7	HL-60
37a	36,5 ± 4,4	48,5 ± 6,3
38a	19,2 ± 1,3	71,2 ± 0,1
39a	38,3 ± 1,3	26,0 ± 1,7
40a	35,1 ± 0,5	33,3 ± 0,5
41a	62,3 ± 3,8	18,5 ± 0,5
42a	46,0 ± 0,8	26,5 ± 3,5
43a	32,8 ± 2,8	16,1 ± 0,0
44a	38,8 ± 0,8	16,8 ± 0,4
45a	27,2 ± 0,1	47,5 ± 6,2
46a	41,1 ± 1,3	52,6 ± 0,2
47a	14,9 ± 0,9	7,9 ± 0,3
48a	28,8 ± 2,1	28,2 ± 1,1
49a	22,1 ± 3,1	11,4 ± 1,0
50a	27,6 ± 2,1	16,2 ± 1,8

Bioaktywność *N*-(2-hydrokso-1-indanylo)-1,2-benzizoselenazol-3(2*H*)-onów 47a-50a zależała od stereochemii atomu węgla C1 podstawnika przyłączonego do atomu azotu. Wartości IC₅₀ były niższe dla konfiguracji (*S*) atomu węgla C1. Biorąc pod uwagę stereochemię atomu węgla C2 z przyłączoną grupą hydroksylową, możemy zauważyć, że nie wpływa ona na reaktywność cząsteczek (Rysunek 8).



Rysunek 8. Aktywność enancjomerów i diastereoizomerów pochodnych 20a-23a

Większość diselenidów wykazywała cytotoksyczność tylko w stosunku do linii komórkowej HL-60, z wyjątkiem pochodnej 39b (IC₅₀ 37,00 ± 4,25 μM). Sugeruje to, że obecność wiązania Se-N jest potrzebna do wpływania na proliferację komórek MCF-7. Ponadto, aktywność cytotoksyczną zaobserwowałam wyłącznie dla diselenidów zawierających dodatkową grupę hydroksylową. Pochodne hydroksybutylowe 39b i 40b dały najniższe wartości IC₅₀ odpowiednio 8,67 ± 0,14 μM i 10,10 ± 0,49 μM. Najsilniej

działające związki oceniano także przy użyciu ludzkich komórek śródbłonna HUVEC. Diselenid *N-trans*-2-hydroksy-1-indanylu **50b** wykazał selektywne działanie antyproliferacyjne bez toksyczności wobec normalnych komórek (Tabela 3).

Tabela 3. Aktywność antyproliferacyjna związków **10b-23b**

Związek	IC50 [μM] \pm SEM		
	MCF-7	HL-60	HUVEC
37b	>100	>100	
38b	>100	>100	
39b	37,00 \pm 4,25	8,67 \pm 0,14	9,92 \pm 0,07
40b	>100	10,10 \pm 0,49	
41b	>100	>100	
42b	>100	>100	
43b	>100	>100	
44b	>100	>100	
45b	>100	>100	
46b	>100	>100	
47b	>100	20,00 \pm 0,16	
48b	>100	21,75 \pm 2,08	
49b	>100	12,40 \pm 0,33	18,95 \pm 0,20
50b	>100	13,00 \pm 0,41	>100

Uzyskane wyniki wykazały, że ugrupowanie hydroksybutylowe oraz hydroksyindanylowe przyłączone do atomu azotu benzisoselenazolonu lub odpowiedniego diselenidu umożliwiają poprawę bioaktywności związków selenoorganicznych i można je uznać za przydatne farmakofory w dalszych modyfikacjach z optymalną interakcją lek-białko/enzym.

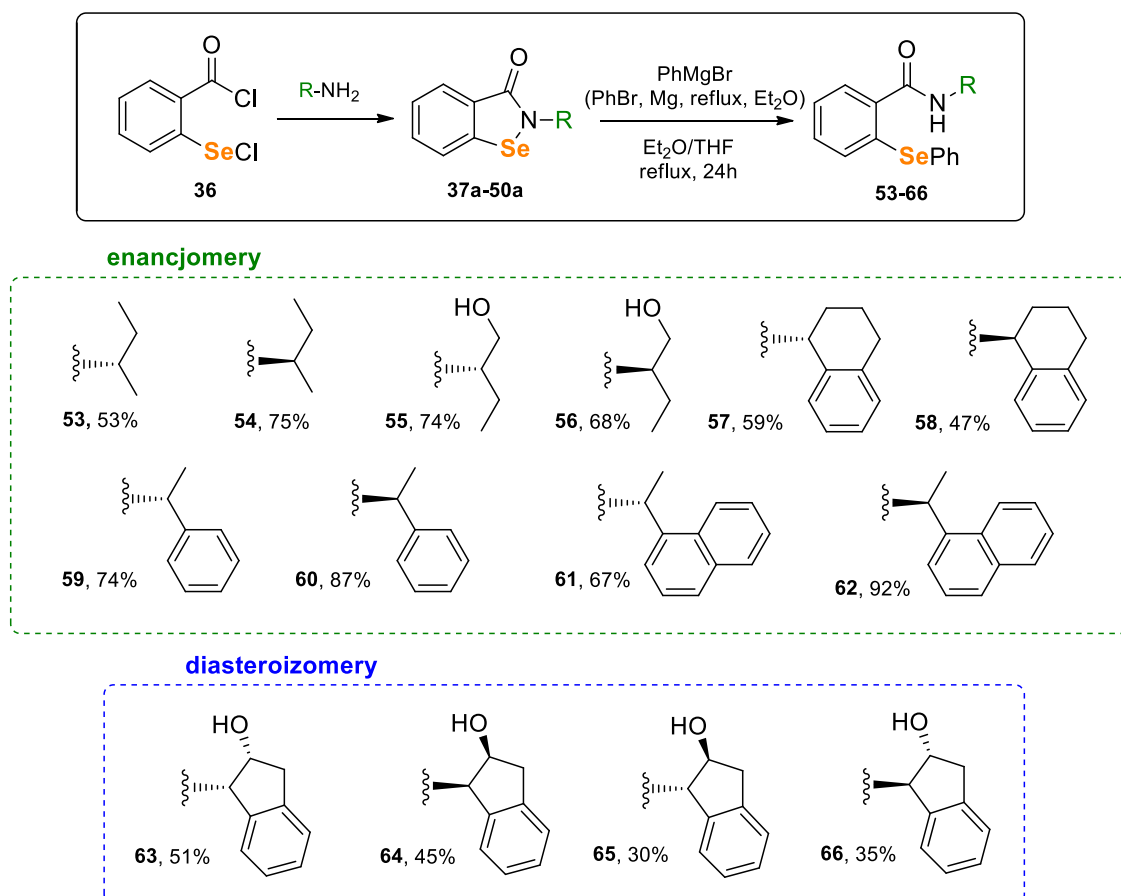
6.3 Publikacja 3 [P3]

Łatwa synteza chiralnych fenyloselenidów jako nowych przeciwutleniaczy i środków cytotoksycznych

Modyfikowanie pierwotnego rdzenia benzisoselenazolonu może znacząco wpłynąć na właściwości fizykochemiczne cząsteczki, zmieniając w ten sposób farmakokinetykę i działanie biochemiczne potencjalnego leku. W niektórych przypadkach wymiana Se-N na wiązanie Se-C_{ar} i wprowadzenie dodatkowego pierścienia aromatycznego zwiększyła aktywność cytotoksyczną chiralnych *N*-terpenowych benzisoselenazolonów [34].

Celem niniejszej publikacji było zbadanie, czy obecność grupy fenyloselenidowej może nasilać działanie antyoksydacyjne i antyproliferacyjne. Zsyntetyzowane pochodne są parami enancjomerów i diastereoizomerów, co dodatkowo pozwoliło określić, czy struktura przestrzenna cząsteczki może zwiększać potencjał terapeutyczny.

Pierwszym etapem badań była synteza benzisoselenazolnów **37a-50a**, a następnie fenyloselenidów **53-66**, poprzez reakcję z odczynnikami Grignarda. W tym celu nieznacznie zmodyfikowałam procedurę zaproponowaną przez Młochowskiego i współpracowników [69]. Do roztworu bromku fenylo-magnezowego wytworzonego przez ogrzewanie bromobenzenu i Mg^0 w eterze dietylowym pod chłodnicą zwrotną, dodałam odpowiedni benzisoselenazoln **37a-50a** rozpuszczony w Et_2O lub THF. Ze względu na niską rozpuszczalność substratów **37a-50a**, czas reakcji musiał zostać wydłużony do 24h. Wszystkie produkty **53-66** otrzymałam z dobrą wydajnością (Schemat 7).

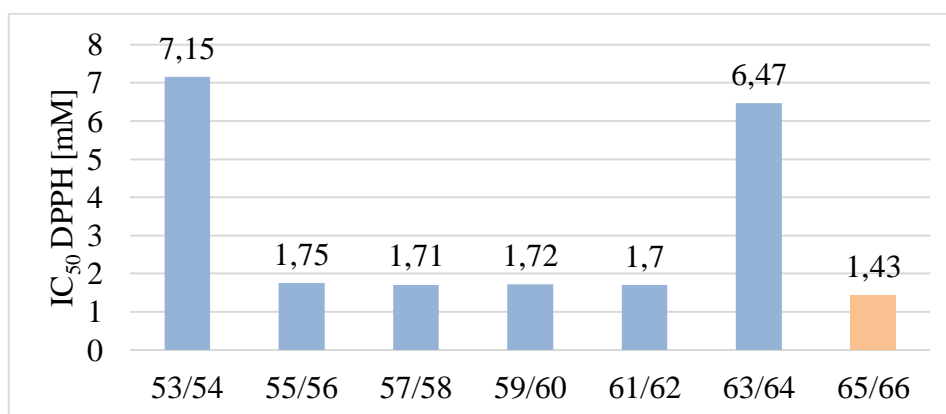


Schemat 7. Syntetyzowane chiralne fenyloselenidy **53-66**

Kolejnym etapem, było zbadanie potencjału antyoksydacyjnego wszystkich zsyntetyzowanych związków za pomocą dwóch testów. Pierwszy jest to test Iwaoki

i współpracowników przedstawiony w opisie publikacji 2 [P2]. We wszystkich przebadanych związkach konwersja ditiolu do disulfidu była niższa niż w obecności ebselenu.

Jako drugi test użyty do oceny aktywności przeciwutleniającej otrzymanych związków wykorzystałam test wychwytywania rodników 2,2'-difenyl-1-pikrylhydrazylu (DPPH). Test ten stosowany jest do ilościowego określania potencjału antyoksydacyjnego standardowych przeciwutleniaczy tj. kwas askorbinowy, α -tokoferol, kwas galusowy i Trolox [69]. Procent hamowania rodnika DPPH określa się jako wartość IC_{50} , czyli jako stężenie przeciwutleniacza wymagane do zmniejszenia o 50% początkowego stężenia DPPH [70]. Metoda ta opiera się na pomiarach spektrofotometrycznych, w których zakładamy, że mechanizm zależy od transferu pojedynczego elektronu (SET) oraz transferu atomu wodoru (HAT) [71]. Wyniki tego testu przedstawiono poniżej (Rysunek 9).



Rysunek 9. Wartości IC_{50} dla testu DPPH dla związków 53-66

Największy potencjał antyoksydacyjny zaobserwowałam dla fenylselenidu *N-trans*-2-hydroksy-1-indanylu **65/66**. Natomiast, jego konformery *cis* wykazywały znacznie niższą aktywność.

Podobnie jak w przypadku związków zsyntetyzowanych w publikacji 2 [P2], aktywność cytotoksyczną określiłam na liniach komórkowych białaczki HL-60 oraz raka piersi MCF-7. Największą aktywność cytotoksyczną zaobserwowano dla indanylofenylselenidów posiadających grupy 2-hydroksylowe *cis* (**64**) i *trans* (**65**, **66**) (Tabela 4).

Tabela 4. Działanie antyproliferacyjne związków **63-66**

Związek	IC ₅₀ [μM] ± SEM	
	MCF-7	HL-60
63	>150	>150
64	16,65 ± 1,36	>150
65	83,38 ± 1,94	69,50 ± 0,71
66	63,75 ± 0,35	55,45 ± 3,01

Fenyloselenidy z grupą *trans*-2-hydroksy-1-indanylu **65/66** wykazują podobnie niską aktywność w stosunku do obu linii komórkowych. Z kolei, w przypadku związku **64** z grupą (1*R*,2*S*)-(+)-*cis*-2-hydroksy-1-indanylu można zauważyć znacznie lepszą skuteczność hamowania linii komórkowej MCF-7. Wobec tego, wydaje się że bioselektywność może być silniej powiązana z rodzajem związków selenowych, niezależnie od chiralności podstawnika indanylowego.

Modyfikowanie pierwotnego rdzenia benzisoselenazolonu poprzez wprowadzenie grupy fenyloselenidowej, nie poprawia bioaktywności otrzymanych pochodnych w porównaniu do odpowiednich benzisoselenazolonów z ugrupowaniem Se-N i diselenidów zawierających wiązanie Se-Se. Uzyskane wyniki potwierdzają, że również dla tego typu związków selenoorganicznych obecność ugrupowania *N*-indanylowego wzmacnia właściwości przeciwutleniające i przeciwnowotworowe.

6.4 Publikacja 4 [P4]

Synteza nowych chiralnych selenidów β-karbonylowych o działaniu przeciwutleniającym i przeciwnowotworowym – część I

Duża reaktywność atomu selenu oraz łatwe jego wprowadzanie w strukturę substratu, poprzez reakcje selenenylacji i selenocyklizacji [72], pozwala na efektywne otrzymywanie nowych pochodnych selenoorganicznych, dostarczając szeroką gamę zróżnicowanych związków heteroorganicznych w łagodnych warunkach reakcji. Zróżnicowane modyfikacje strukturalne i badanie ich wpływu na bioaktywność, przyczynia się do znalezienia idealnej kombinacji cech strukturalnych, umożliwiających zmaksymalizowanie biodostępności i efektywności terapeutycznej syntetyzowanych pochodnych.

W prezentowanej publikacji zmodyfikowałam pierwotną strukturę ebselenu poprzez wprowadzenie ugrupowania 2-(2-oksopropyl)selenidowego. Synteza szeregu

nowych *N*-funkcjonalizowanych β -karbonyloselenidów w postaci *N*-chiralnych par enancjomerycznych i diastereoizomerycznych pozwoliła na określenie wpływu konfiguracji poszczególnych centrów stereogenicznych na aktywność katalityczną i antyproliferacyjną cząsteczek. Ponadto, oceniony został wpływ dodatkowej grupy karbonylowej na reaktywność otrzymanych Se-pochodnych.

Pierwszym etapem pracy była optymalizacja warunków reakcji syntezy *N*-podstawionych 2-((2-oksopropyl)seleno)benzamidów. Zastosowanie metody zaprezentowanej przez Z. Zhang i współpracowników [36][37] pozwoliło na otrzymanie planowanego produktu z wydajnością wynoszącą zaledwie 30% (próba 1). Sugerując się pierwszą metodą stosowaną do otrzymywania tego typu związków z 2010 r. przez Z. Zhang i współpracowników [73], w celu wytworzenia odpowiedniego karbanionu, zastosowałam wodorowęglan sodu, co nieznacznie zwiększyło wydajność reakcji do 41% (próba 2). Użycie 0,75 M roztworu NaHCO₃ (próby 3 i 4) również nie poprawiło skuteczności procedury. Kluczowe znaczenie miała kolejność dodawania odczynników. Zasadniczym etapem tej reakcji było zmieszanie ketonu z wodorowęglanem sodu w celu wytworzenia karboanionu do późniejszego acylowania atomu selenu. Najlepszy wynik uzyskano stosując nadmiar acetonu jako substratu i rozpuszczalnika, uzyskując produkt końcowy z wydajnością 87% (próba 6, Tabela 5).

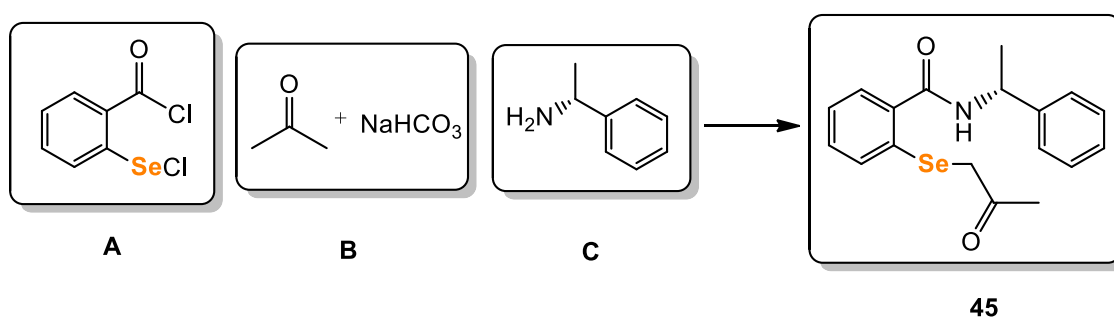
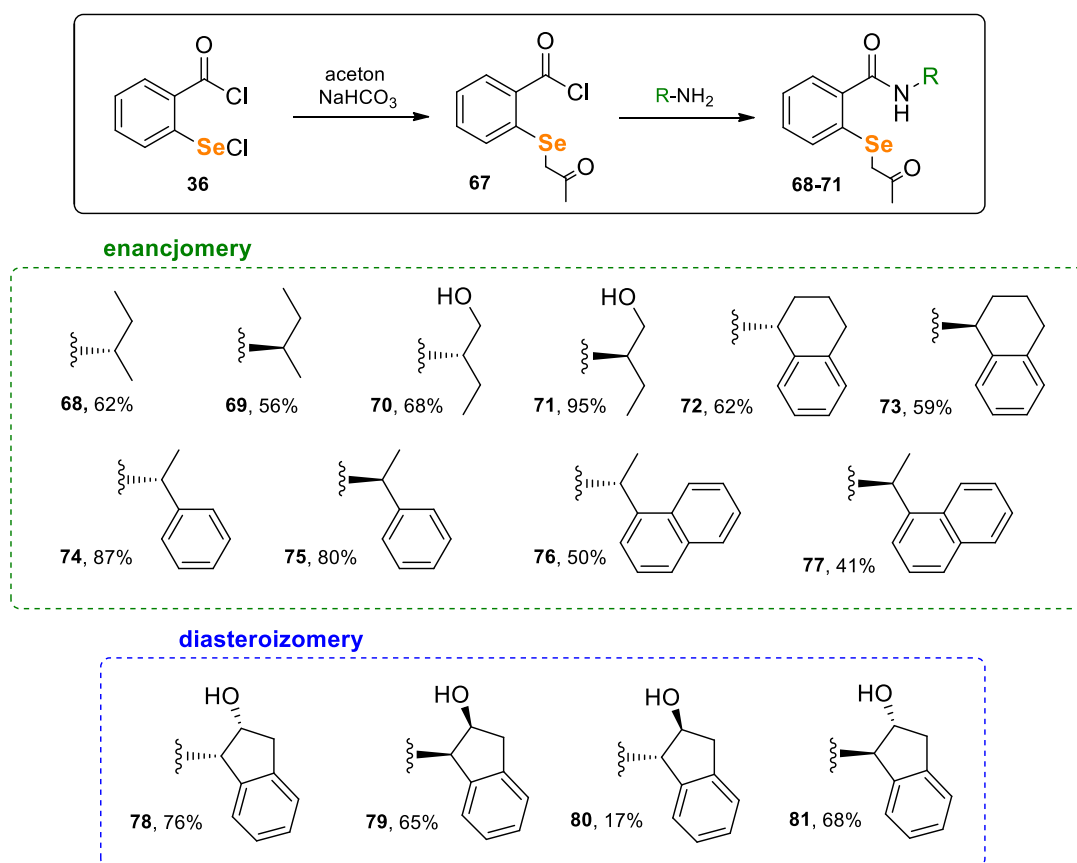


Tabela 5. Optymalizacja warunków reakcji

Próba	Rozpuszczalnik	NaHCO ₃	Warunki i kolejność dodawania odczynników	Wydajność [%]
1	DCM	-	1. A + aceton, rt, 1 h 2. C , rt, 20 min	30
2	DCM	1 eq.	1. A + B , rt, 1h 2. C , rt, 3 h	41

3	-	1 eq. (0,75 M NaHCO ₃ /H ₂ O)	1. A + C , rt, 20min 2. B , 0°C do rt, 2 h	37
4	Aceton	1 eq. (0,75 M NaHCO ₃ /H ₂ O)	1. B , rt, 25min + A , 0°C do rt, 1h 2. C , rt, 2 h	0
5	THF	1 eq.	1. B , rt, 30min + A , rt, 1 h 2. C , rt, 3 h	65
6	Aceton	1 eq.	1. B , rt, 30min + A , rt, 1 h 2. C , rt, 3 h	87

Zastosowałam zoptymalizowaną procedurę do syntezy serii β -karbonylowych selenidów jako par enancjomerycznych i diastereomerycznych z dobrymi wydajnościami (Schemat 8). W tym celu, chlorek 2-(chloroseleno)benzoilu **36** przekształciłam *in situ* w acetylowany selenid **67**, który następnie reagował z enancjomerycznie czystymi aminami.



Schemat 8. Syntetyzowane chiralne β -karbonyloselenidy **68-81**

Zdolności antyoksydacyjne otrzymanych związków oceniałam za pomocą testu NMR przedstawionego przez Iwaokę i współpracowników. W taki sam sposób jak w opisie publikacji 2 [P2]. Wyniki przeprowadzonej analizy przedstawiłam w tabeli 6.

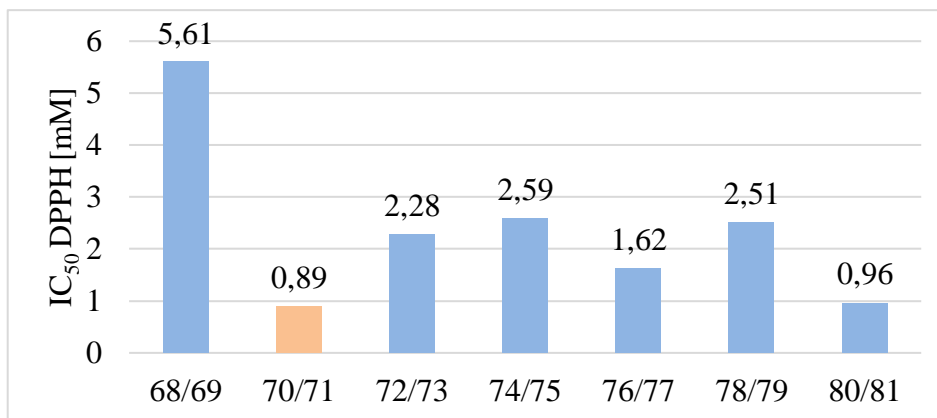
Tabela 6. Wyniki pomiaru aktywności przeciwutleniającej.

Se-kat.[0.1 equiv.]	Pozostały DTT ^{red} [%] ± SEM			
	5 minut	15 minut	30 minut	60 minut
<i>Acetoselenidy</i>				
68/69	78,7 ± 5,4	61,2 ± 4,8	44,2 ± 2,8	12,8 ± 3,7
70/71	72,0 ± 7,4	61,3 ± 6,1	48,8 ± 3,7	21,7 ± 1,7
72/73	85,6 ± 0,1	78,2 ± 0,2	69,3 ± 0,1	56,4 ± 0,5
74/75	90,6 ± 2,2	90,2 ± 0,7	82,8 ± 1,4	73,7 ± 0,5
76/77	93,1 ± 0,1	90,1 ± 0,6	83,9 ± 0,8	70,9 ± 0,2
78/79	72,5 ± 2,5	52,2 ± 0,2	27,2 ± 2,7	6,4 ± 4,3
80/81	79,1 ± 0,3	56,6 ± 5,3	30,2 ± 3,3	10,7 ± 2,4
Ebselen	75	64	58	52

Aktywność przeciwutleniająca wszystkich par enancjomerycznych była taka sama. Generalnie najwyższy potencjał antyoksydacyjny zaobserwowałam dla acetoselenidów z ugrupowaniem *N*-indanylowym, które posiadają grupę 2-hydroksylową w konfiguracji *cis* (**78/79**) i *trans* (**80/81**). Porównując wynik związków **78/79** z wartościami zmierzonymi dla odpowiednich pochodnych z ugrupowaniem *N*-(*cis*-2-hydroksy-1-indanylowym) z publikacji 2 [**P2**] i publikacji 3 [**P3**], można zaobserwować, że diselenidy posiadające reaktywne i podatne na rozszczepienie wiązanie Se-Se są najskuteczniejszymi reduktorami H₂O₂. Jednakże obecność grupy β-karbonyloselenidowej zamiast ugrupowania fenyloselenidowego lub wiązania Se-N w benzisoselenazolach znacząco zwiększała zdolność redukcji H₂O₂.

Warto również wspomnieć, iż wprowadzenie dodatkowych pierścieni aromatycznych do łańcuchów *N*-alkilowych, bez przyłączenia jakiegokolwiek polarnej grupy OH, wydaje się zmniejszać potencjał redukcji H₂O₂ (pochodne **72/73**, **74/75**, **76/77**, Tabela 8). Pochodne *N*-(2-butyłowe) **68/69** i te posiadające dodatkową grupę hydroksylową **70/71** były odpowiednio 4- i 2,5-krotnie bardziej aktywne niż dobrze znany mimetyk GPx ebselen.

Następnie, otrzymane związki zbadalam pod kątem ich zdolności do wychwytywania wolnych rodników za pomocą testu 2,2-difenylo-1-pikrylhydrazylu (DPPH) przedstawionym w publikacji 3 [**P3**]. Dodatkowo, obliczyłam równoważną zdolność antyoksydacyjną Troloxu (TEAC). Wyniki uzyskane dla testu DPPH przedstawiono na rysunku 10.



Związek	68/69	70/71	72/73	74/75	76/77	78/79	80/81
TEAC [mM TE·1g ⁻¹]	0,1	0,47	0,16	0,12	0,23	0,19	0,39

Rysunek 10. Wartości IC₅₀ oraz TEAC dla testu DPPH dla związków 68-81

Wszystkie badane pochodne wykazywały aktywność przeciwutleniającą wobec rodnika DPPH, jednakże była ona różna zależnie od dołączonych podstawników. Najwyższą zdolność neutralizacji rodnika DPPH, wykazywały enancjomery **70/71**, aczkolwiek niższą niż Trolox (IC₅₀=0,17 mM). W strukturze związków **68/69** atom azotu jest podstawiony chiralnym alifatycznym łańcuchem butylowym, natomiast związki **70/71** posiadają dodatkową grupę OH. Różnica w wychwytywaniu wolnych rodników jest około 5-krotna, może to wskazywać, że dodatkowe wprowadzenie grupy hydroksylowej przyczynia się do zwiększenia potencjału antyoksydacyjnego.

Działanie cytotoksyczne zbadalam na liniach komórkowych raka piersi MCF-7 i białaczki promielocytowej HL-60. Enancjomery *N*-(1-hydroksy-2-butanylo)selenidu **70/71** posiadają najwyższą aktywność antyproliferacyjną (Tabela 7).

Tabela 7. Potencjal cytotoksyczny związków 68-71 oraz 78-81

Związek	IC ₅₀ [μM] ± SEM	
	MCF-7	HL-60
68	46,1 ± 1,6	181 ± 11
69	55 ± 1,4	101 ± 14
70	39,2 ± 0,9	14,4 ± 0,5
71	35,7 ± 0,6	16,2 ± 1,1
78	39,2 ± 0,7	27,3 ± 0,5
79	296 ± 5	225 ± 2
80	37 ± 0,3	23,7 ± 1,1
81	38 ± 1,1	23,8 ± 1,1
Oksaliplatyna	35 [36,37]	0,8 [38]

Wyniki dla β -karbonyloselenidu z podstawnikiem *N*-(1-hydroksy-2-butanylowym), porównałam z wartościami IC₅₀ zmierzonymi dla odpowiednich fenyloselenidów, benzisoselenazonów i diselenidów (Tabela 8). Wyraźne różnice pomiędzy aktywnościami można zauważyć na linii komórkowej HL-60. Acetoselenidy są 2 razy bardziej aktywne niż odpowiadające im benzisoselenazony i nieco mniej cytotoksyczne niż diselenidy. Dodatkowo, wymiana grupy β -karbonylowej na pierścień fenylowy znacząco zmniejszyła aktywność antyproliferacyjną pochodnej selenoorganicznej.

Tabela 8. Aktywność cytotoksyczna dla pochodnych *N*-(1-hydroksy-2-butanylu)

<i>Se-pochodna</i>	IC ₅₀ [μ M]			
	MCF-7	HL-60	MCF-7	HL-60
β -karbonyloselenid	39,2 \pm 0,9	14,4 \pm 0,5	35,7 \pm 0,6	16,2 \pm 1,1
Fenyloselenid	>150	>150	>150	>150
Benzisoselenazon	38,3 \pm 1,3	26,0 \pm 1,7	35,1 \pm 0,5	33,3 \pm 0,5
Diselenid	37,0 \pm 4,3	8,7 \pm 0,1	>100	10,1 \pm 0,5

Jak przedstawiono w poprzednich pracach [P2] oraz [P3], obecność podstawnika indanylowego przyłączonego do atomu azotu benzisoselenazonów i odpowiadających im diselenidów, a także fenyloselenidów nasilała działanie cytotoksyczne. Dzieje się to również w przypadku β -karbonyloselenidów. Dodatkowo, biorąc pod uwagę zależność chiralności od aktywności w pochodnych *N-cis*-2-hydroksy-1-indanylowych **78** i **79**, zaobserwowano, że gdy stereochemia centrów węglowych C1 i C2 była 1*R*,2*S*, bioaktywność wobec obu linii komórkowych znacznie spadła w porównaniu z izomerem 1*S*,2*R*. *Trans* enancjomery **80** i **81** również wykazywały prawie 10-krotnie większą reaktywność niż selenid **79**. Badania potwierdzają, że podstawniki 1-hydroksy-2-butanylowy i 2-hydroksy-1-indanylowy umożliwiają poprawę bioaktywności związków selenoorganicznych i można je wybrać jako możliwe farmakofory w celu dalszego opracowywania potencjalnych Se-terapeutyków.

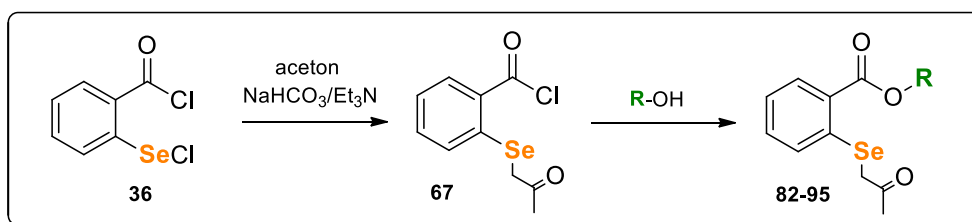
6.5 Publikacja 5 [P5]

Synteza nowych chiralnych selenidów β -karbonylowych o działaniu przeciwutleniającym i przeciwnowotworowym – część II

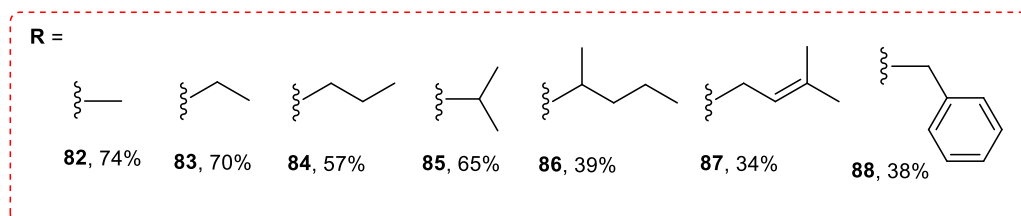
Wiązanie estrowe jest niezbędne w kilku szlakach biochemicznych ze względu na jego łatwą hydrolizę przez enzymy esterazy, które rozkładają ester na kwas karboksylowy i alkohol [74]. Wprowadzenie ugrupowania estrowego w kontekście opracowywania leków pozwala rozwiązać problem biodostępności i rozpuszczalności wielu znanych środków bioaktywnych [75][38]. Z tych powodów, postanowiłam zmodyfikować syntetyzowane związki selenoorganiczne dodając do struktury wiązanie estrowe.

W prezentowanej publikacji przedstawiłam metodę syntezy selenidów β -karbonylowofenylowych posiadających grupę *o*-estrową z podstawnikami alkilowymi oraz aryłowymi wśród, których znajdują się chiralne oraz monoterpeneowe ugrupowania. Zmodyfikowanie struktury selenidu β -karbonylowofenylowego poprzez zastąpienie grupy *o*-amidowej podstawnikiem *o*-estrowym, pomogło określić wpływ grupy estrowej na aktywność antyoksydacyjną i przeciwnowotworową związków selenoorganicznych.

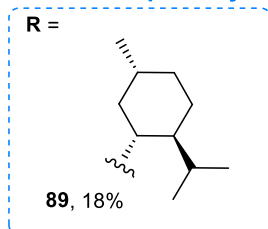
Pierwszy etap badań obejmował syntezę *O*-podstawionych pochodnych chlorku kwasowego kwasu 2-((2-oksopropyl)seleno)benzoowego. W tym celu, chlorek 2-(chloroseleno)benzoilu **36** przekształciłam *in situ* w acetylowany selenid **67**, który następnie reagował z dostępnymi handlowo alkoholami. Początkowo do powstania karboanionu, który wykorzystano do acylowania atomu selenu wykorzystałam wodorowęglan sodu. Jednak, w przypadku kilku użytych alkoholi wydajność reakcji była niska. W następnej próbie zastąpiłam NaHCO₃ trietyloaminą, co znacznie zwiększyło wydajność reakcji. Trietyloamina wydała się być kluczowa do utworzenia karboanionu i usunięcia chlorowodoru powstałego w reakcji. Dzięki, opracowanej metodzie otrzymałam serię selenidów β -karbonylowych: siedem achiralnych pochodnych alkilowych i arylowych **53-59** i siedem związków optycznie czynnych, w tym pochodną (-)-mentolu **60** oraz trzy pary enancjomerów **61-66** (Schemat 9).



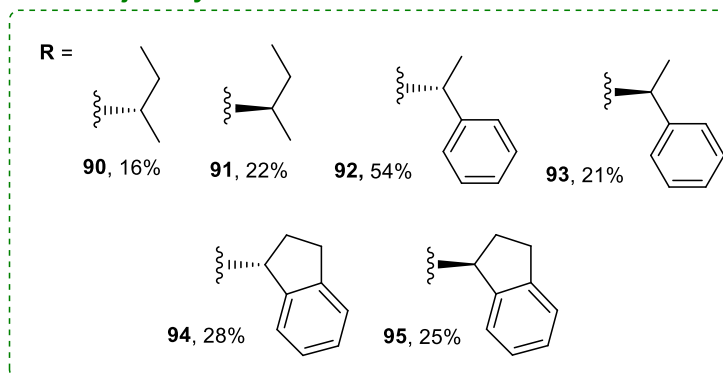
achiralne alkylove grupy



szkielet terpenowy



enancjomery

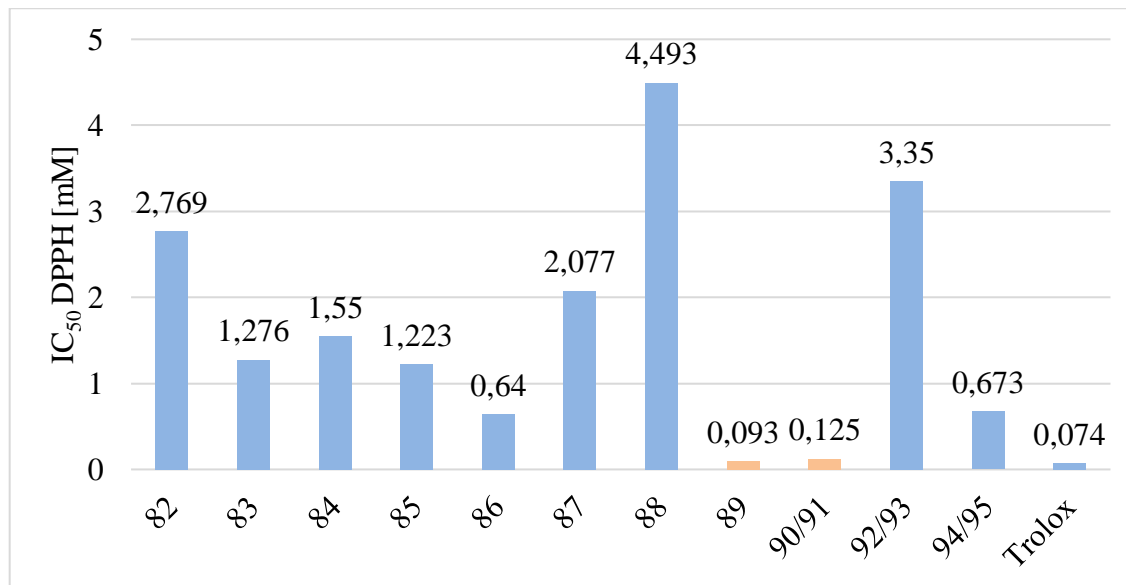


Schemat 9. Syntetyzowane acetoselenoestry **82-95**

W celu oceny potencjału antyoksydacyjnego otrzymanych związków wykorzystałam dwa testy: metodę opartą na redukcji ditiolu do disulfidu przedstawioną w opisie publikacji 2 [P2] oraz metodę wychwytywania wolnych rodników DPPH przedstawioną w opisie publikacji 3 [P3]. Wyniki testu przeciwutleniającego Iwaoki, wykazały, że najlepszym przeciwutleniaczem z tej grupy związków jest *O*-(propylo)-2-((2-oksopropylo)seleno)benzoesan **84**. Jednakże, konwersja uzyskana dla wszystkich pochodnych była mniej efektywna niż dla ebselenu i otrzymanych wcześniej pochodnych z grupą *o*-amidową **68-81** opisanych w publikacji 4 [P4]. Sugeruje to, że obecność podstawnika *o*-estrowego zamiast grupy *o*-amidowej w strukturze selenidów β -karbonylowych znacząco obniża zdolność do redukcji H₂O₂. W przypadku enancjomerów z grupą *O*-2-butylową **90/91** aktywność ta zmalała aż 9-krotnie, porównując z ich odpowiednikami z grupą *o*-amidową **68/69**.

Drugą metodą, którą wykorzystałam do zbadania aktywności przeciwutleniających otrzymanych związków był test pod kątem ich zdolności do

wychwytywania wolnych rodników. Wyniki uzyskane dla testu DPPH przedstawiono na Rysunku 11.



Związek	82	83	84	85	86	87
TEAC	0,027	0,058	0,048	0,061	0,116	0,036
Związek	88	89	90/91	92/93	94/95	
TEAC	0,017	0,793	0,590	0,022	0,110	

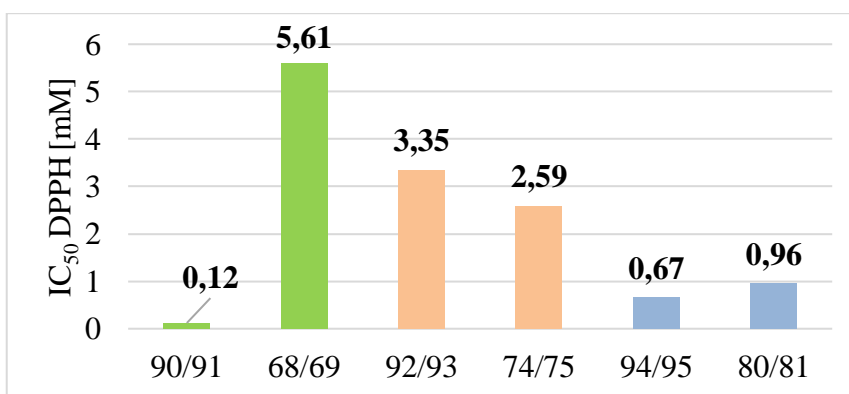
Rysunek 11. Wartości IC₅₀ oraz TEAC dla testu DPPH dla związków 82-95

Przebadano wszystkie otrzymane *O*-pochodne 2-((2-oksopropyl)seleno)benzoesanowe, w każdym przypadku wykryto utratę absorbancji rodnika DPPH. Związek **89** wykazywał najlepsze zdolności wychwytywania wolnych rodników. Po 15 minutach od rozpoczęcia reakcji wartość IC₅₀ wyniosła 0,093 mM. Porównując tę wartość z IC₅₀ uzyskanym dla Troloxu (0,074 mM) można stwierdzić, że *O*-((1*R*,2*S*,5*R*)-(-)-2-izopropyl-5-metylocykloheksyl)-2-((2-oksopropyl)seleno) benzoesan **89** wykazuje zauważalną aktywność wychwytywania rodników DPPH zbliżoną do Troloxu. Związek ten różnił się od pozostałych badanych pochodnych obecnością ugrupowania terpenowego w swojej strukturze. Monoterpeny bez sprzężonych wiązań π, takie jak mentol, nie wykazywały znaczących właściwości wymiatania wolnych rodników w teście DPPH [76]. Właściwości przeciwutleniających nie wykazywał również *N*-(-)-mentyl-1,2-benzizoselenazol-3(2*H*)-on **7** w teście NMR utleniania ditiolu do disulfidu, natomiast związek ten charakteryzował się najlepszą aktywnością przeciwnowotworową wobec komórek MCF-7 [24]. Moje badania wykazują, że obecność w strukturze ugrupowania terpenowego może pozwolić na

otrzymanie nie tylko związków o interesujących właściwościach przeciwnowotworowych ale i również przeciwutleniających.

W przypadku pozostałych badanych chiralnych związków **90-95** zdolność hamowania wolnych rodników była najwyższa dla estrów z grupą (*sec*)-butylową **90/91**, a najniższa w przypadku związków z podstawnikiem α -metylobenzylowym **92/93**.

Analizując wpływ wprowadzenia wiązania estrowego do struktury związków selenoorganicznych zamiast grupy *o*-amidowej nie można jednoznacznie, określić wpływu tej modyfikacji na aktywność antyoksydacyjną. Związki z grupą (*sec*)-butylową **90/91** były 43 razy bardziej aktywne niż te same pochodne z grupą *o*-amidową **68/69**. Natomiast, w przypadku enenjomerów z grupą α -metylobenzylową **92/93** właściwości wychwytywania wolnych rodników nie poprawiły się. Nieznaczny wzrost aktywności wykazały pochodne **94/95**, w porównaniu do podobnych związków zsyntetyzowanych z ugrupowaniem indanylowym **80/81**, ale bez grupy hydroksylowej.



Rysunek 12. Wartości IC₅₀ dla testu DPPH dla **90/91**, **92/93**, **94/95** i odpowiadających im pochodnych Se z grupą *o*-amidową.

Potencjał cytotoksyczny otrzymanych pochodnych zbadalam na liniach komórkowych raka piersi MCF-7 i białaczki promielocytowej HL-60. Najniższą wartość IC₅₀ zaobserwowałam dla *O*-(metylo)-2-((2-oksopropylo)seleno)benzoesanu **82** dla linii komórkowej MCF-7 i *O*-(2-pentylo)-2-((2-oksopropylo)seleno)benzoesanu **86** dla linii komórkowej HL-60 (Tabela 9).

Tabela 9. Potencjał cytotoksyczny związków **82**, **86**

Związek	IC ₅₀ [μM] ± SEM	
	MCF-7	HL-60
82	51,1 ± 0,6	97,2 ± 0,5
86	121,0 ± 0,4	84,0 ± 2,0
Oksaliplatyna	35	0,8

Porównując wyniki badań cytotoksycznych dla wszystkich otrzymanych przeze mnie grup związków selenoorganicznych t.j benzisoselenazonów, diselenidów, fenyloselenidów, acetoselenidów z selenidami β-karbonyłowymi z grupą *o*-estrową widać duże zróżnicowanie pomiędzy poszczególnymi grupami związków oraz pomiędzy enancjomerami i diastereoizomerami. Jako przykład przedstawiam porównanie wyników uzyskanych dla enancjomerów **92/93** i odpowiadających im pochodnych selenoorganicznych (Tabela 10).

Tabela 10. Aktywność cytotoksyczna dla pochodnych z grupą α-metylobenzylową

Se-pochodna	IC ₅₀ [μM]			
	A		B	
	MCF-7	HL-60	MCF-7	HL-60
Selenid β-karbonylowy z grupą <i>o</i> -estrową	176,0 ± 3,0	126,0 ± 2,5	107,0 ± 0,8	94,2 ± 3,0
Selenid β-karbonylowy z grupą <i>o</i> -amidową	235,0 ± 1,0	303,0 ± 3,0	237,0 ± 11,0	23,5 ± 1,4
Fenyloselenid	>150	>150	>150	>150
Benzisoselenazon	32,8 ± 2,8	16,1 ± 0,0	38,8 ± 0,8	16,8 ± 0,4
Diselenid	>100	>100	>100	>100

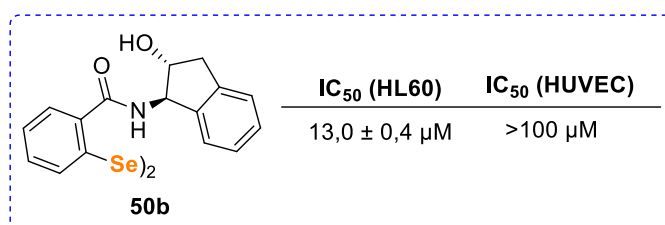
Porównując wartości IC₅₀ uzyskane dla pochodnych α-metylobenzylowych można zauważyć, że dla obu cząsteczek z grupą *o*-estrową i *o*-amidową, najlepszą aktywność uzyskano dla związków z konfiguracją *S* atomu węgla dla linii komórkowej HL-60. Dodatkowo, można zauważyć większą wrażliwość linii komórkowej HL-60 niż linii komórkowej MCF-7 na otrzymane pochodne z grupą α-metylobenzylową. Ogólnie

można zaobserwować, że zamiana ugrupowania *o*-amidowego na *o*-estrowe zwiększa potencjał cytotoksyczny otrzymanych β -karbonyloselenidów. Natomiast, spośród wszystkich otrzymanych pochodnych z grupą α -metylobenzylową to benzisoselnazolony wykazywały najsilniejszą aktywność cytotoksyczną.

Ważnym motywem w moich badaniach, szczególnie interesującym ze względu zmianę aktywności biologicznej poszczególnych enancjomerów i diastereoizomerów, była grupa hydroksyindanylowa. W tej pracy dla selenidu β -karbonylowego z grupą *o*-estrową otrzymałam pochodne z podstawnikiem indanylowym bez dodatkowej grupy hydroksylowej. Przyglądając się wynikom dla syntetyzowanych grup związków z podstawnikiem hydroksyindanylowym, można zauważyć mniejszą wrażliwość komórek nowotworowych na związki z wiązaniem estrowym **94/95**, jednak trudno określić, czy jest to efekt zastąpienia grupy amidowej grupą estrową w pozycji *orto*, czy też brak dodatkowej grupy hydroksylowej.

7. Podsumowanie

- 1) Opracowałam syntezę serii *N*-podstawionych chiralnych benzisoselenazol-3(2*H*)-onów i odpowiednich diselenidów z grupą *o*-amidową, tworząc szereg enancjomerów i diastereoizomerów. Otrzymanych 28 pochodnych posiada różne ugrupowania na atomie azotu, w tym alifatyczne acykliczne i cykliczne łańcuchy węglowe z dodatkowymi pierścieniami aromatycznymi i grupami hydroksylowymi. Wszystkie związki przetestowano jako przeciwutleniacze i środki przeciwnowotworowe. Uzyskane wyniki wykazały, że:
- Diselenidy są na ogół lepszymi przeciwutleniaczami, których aktywność znacząco wzrasta w wyniku obecności ugrupowania *N*-hydroksyindanylowego,
 - Działanie cytotoksyczne benzisoselenazolonów jest podobne zarówno w stosunku do linii komórkowych HL-60, jak i MCF-7, o największym potencjale antyproliferacyjnym dla pochodnych *N*-(2-hydroksy-1-indanylowych) mających konfigurację (*S*) atomu węgla C1 bezpośrednio połączonego z atomem azotu pierścienia selenazolonu,
 - Aktywność przeciwnowotworowa diselenidów ulega selektywnej ekspresji jedynie wobec linii komórkowych HL-60, a także przyłączenie grupy hydroksylowej wydaje się być niezbędne do uzyskania efektu cytotoksycznego wobec linii komórkowych ludzkiej białaczki promielocytowej.
 - Diselenid **50b** może wykazywać selektywne działanie cytotoksyczne przy minimalnym uszkodzeniu normalnych komórek.

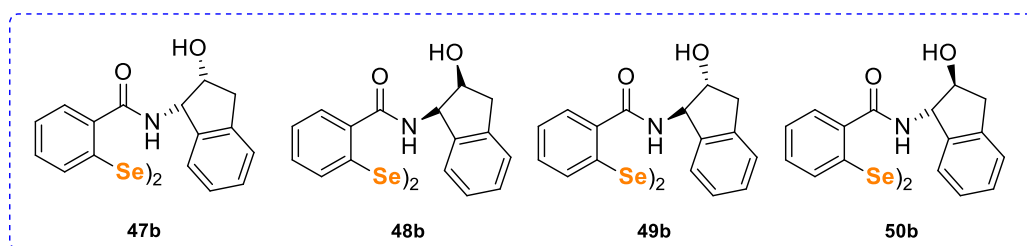


Rysunek 13. Aktywność cytotoksyczna związku **50b**

- 2) Opracowałam syntezę chiralnych niesymetrycznych difenyloselenidów posiadających dodatkową grupę *o*-amidową. Pochodne były podstawione na atomie azotu grupami chiralnymi, tworząc szereg par enancjomerów i diastereoizomerów. Zauważyłam, że wprowadzenie do struktury dodatkowego pierścienia aromatycznego nie poprawia bioaktywności pochodnych selenoorganicznych, w porównaniu do odpowiednich benzisoselenazonów i diselenidów.
- 3) Zoptymalizowałam metodę syntezy chiralnych β -karbonylofenyloowych selenidów i udowodniłam, że wygenerowanie karboanionu z wyjściowego ketonu jest kluczowe dla wysokiej wydajności reakcji. Procedurę tę wykorzystałam do syntezy *N*-podstawionych pochodnych w postaci par enancjomerycznych i diastereomerycznych, prezentując pierwsze przykłady niesymetrycznych (2-oksopropylo)seleno)benzamidów posiadających grupy chiralne przyłączone do atomu azotu. Po przetestowaniu otrzymanych związków jako przeciwutleniaczy i środków cytotoksycznych można stwierdzić, że:
 - a) W obu wykonanych testach DTT i DPPH najbardziej aktywne były pochodne posiadające grupy hydroksylowe – ugrupowania hydroksyindanylowe oraz hydroksybutylowe,
 - b) Obecność grupy β -karbonyloselenidowej zamiast ugrupowania fenyloselenidowego lub wiązania Se-N w benzisoselenazonach znacząco zwiększała zdolność redukcji H₂O₂,
 - c) Biorąc pod uwagę zależność chiralności od aktywności, zaobserwowałam, że konfiguracje atomu węgla C1 i C2 pochodnych *N*-2-hydroksy-1-indanylowych wpływają na ich potencjał antyproliferacyjny, przy czym najniższe wartości IC₅₀ zauważyłam dla związków z konfiguracją (*S*) atomu węgla C1.
- 4) Opracowałam metodologię umożliwiającą otrzymanie nowej grupy związków selenoorganicznych β -karbonylofenyloselenidów, posiadających grupę estrową w pozycji *orto*. Kluczowym etapem syntezy było zastosowanie trietyloaminy podczas acylowania selenidu. Otrzymałam pierwsze pochodne z rusztowaniami alkiloachiralnymi i chiralnymi, w tym również pary enancjomerów. Otrzymane

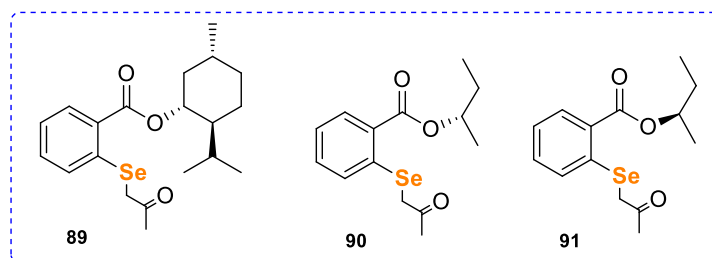
pochodne zbadalam pod kątem aktywności przeciwutleniającej i cytotoksycznej. Analiza wyników, wykazała że:

- a) Zamiana grupy amidowej na grupę estrową znacząco obniża właściwości redukcyjne H_2O_2 ,
 - b) Pochodne estrowe są lepszymi zmiataczami wolnych rodników. W teście DPPH wynik uzyskany dla związku **89** miał zbliżoną wartość IC_{50} do wartości dla Troloxu,
 - c) Aktywność cytotoksyczna pochodnych z grupą *o*-estrową nie uległa poprawie poprzez zamianę wiązania amidowego na estrowe.
- 5) Wszystkie otrzymane pochodne przebadalam *in vitro* pod kątem aktywności antyoksydacyjnej. Związki, które wyróżniały się największym potencjałem, to pochodne:
- a) W teście DTT **47b/48b** oraz **49b/50b**;



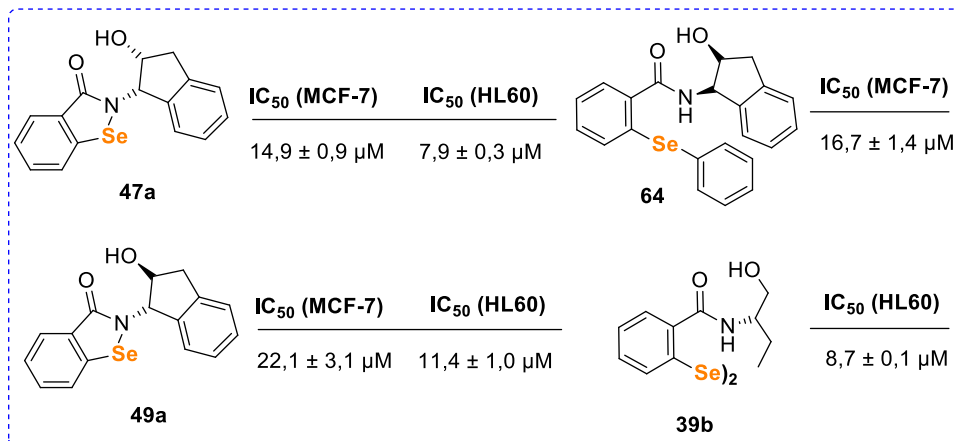
Rysunek 14. Struktury związków **47b/48b** oraz **49b/50b** o najwyższej aktywności antyoksydacyjnej w teście DTT

- b) W teście DPPH **89**, **90** oraz **91**;



Rysunek 15. Struktury związków **89**, **90** oraz **91** o najwyższej aktywności antyoksydacyjnej w teście DPPH

6) Wszystkie otrzymane pochodne zostały poddane testom pod kątem ich aktywności cytotoksycznej. Najwyższy potencjał antyproliferacyjny wykazały pochodne **47a**, **49a** oraz **64** dla linii komórkowej MCF-7 a dla linii komórkowej HL-60 związki **47a**, **49a** oraz **39b**.



Rysunek 16. Struktury związków **39b**, **47a**, **49a** oraz **64** o najwyższej aktywności cytotoksycznej

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9. Wykształcenie i osiągnięcia naukowe

I. Edukacja i zatrudnienie

2014-2018 - studia stacjonarne inżynierskie, kierunek: Chemia i technologia żywności
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Tytuł pracy inżynierskiej: „*Synteza i reakcje benzisosenazol-3(2H)-onów pochodnych p-mentanu i pinanu*”-promotor: prof. dr hab. Jacek Ścianowski

2018-2019 - studia stacjonarne magisterskie, kierunek: Chemia
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Tytuł pracy magisterskiej: „*Syntezy kwasów N-alkilo-2 (karboksyamido) benzenoseleninowych*”- promotor: prof. dr hab. Jacek Ścianowski

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Promotor - prof. dr hab. Jacek Ścianowski

Promotor pomocniczy- dr Agata Pacuła-Miszewska

2023-obecnie – Specjalistka ds. badań i rozwoju, Noctiluca S. A

II. Dorobek naukowy

a) Publikacje:

1) M. Obieziurska; A. J. Pacuła; **A. Laskowska**; A. Długosz-Pokorska;
M. Krzemiński; A. Janecka; J. Ścianowski, „ *N-Terpenyl Benzisosenazolones—
Evaluation of the Particular Structure-Bioactivity Relationship*”, *Proceedings*, **2019**, *41*,
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J. Ścianowski, „*Water-soluble seleninic salts – step towards enhanced bioavailability*”,
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- 3) A. J. Pacuła-Miszewska; **A. Laskowska**; A. Kmieciak; M. Zielińska-Błajet; M. P. Krzemiński; J. Ścianowski, „*Chiral Aziridine Sulfide N(sp³),S-Ligands for Metal-Catalyzed Asymmetric Reactions*”, *Symmetry*, **2021**, 13, 502
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- 5) A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; **A. Laskowska**; H. Kaczmarek; J. Ścianowski, „A new method for creating Se–N intramolecular bonds using UV radiation” *New J. Chem.*, **2022**, 46, 20053
- 6) M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; J. Ścianowski, "Organoselenium compounds as antioxidants" *Arkivoc*, **2023**, v, 212-235
- 7) **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; K. Gach-Janczak; A. Janecka; J. Ścianowski, „Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents”, *RCS Advances*, **2023**, 13, 14698
- 8) A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; **A. Laskowska**; H. Kaczmarek; J. Ścianowski, „New greener approach for the Se–N bond formation”, *Phosphorus, sulfur, and silicon*, **2023**, 199(1), 58
- 9) J. Ścianowski; A. J. Pacuła-Miszewska; **A. Laskowska**; M. Obieziurska-Fabisiak, „Synthesis and applications of new chiral terpenyl organoselenium compounds” *Phosphorus, sulfur, and silicon*, **2023**, 199(1), 2
- 10) **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; A. Długosz-Pokorska; K. Gach-Janczak; J. Ścianowski, „Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I”, *Materials*, **2024**, 17, 899
- 11) **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; A. Długosz-Pokorska; K. Gach-Janczak; J. Ścianowski, „Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part II”, *Molecules*, **2024**, 29, 2866

b) Zgłoszenie patentowe:

1) M. Obieziurska; A. J. Pacuła; **A. Laskowska**; A. Długosz-Pokorska; M. Krzemiński; A. Janecka; J. Ścianowski, *N-alkilowe sole kwasu 2-(karboksyamido)-benzenoselenenowego oraz sposób ich wytwarzania*. P.431055

c) Konferencje

• Wykłady:

1) J. Ścianowski; A. J. Pacuła; M. Obieziurska; **A. Laskowska**, *Selenoorganiczne pochodne terpenów – syntezy i aplikacje*, 62. Zjazd Naukowy Polskiego Towarzystwa Chemicznego, 2-6.09.2019, Warszawa (współautorka wykładu)

2) J. Ścianowski; A. J. Pacuła-Miszewska; **A. Laskowska**; M. Obieziurska-Fabisiak, *Divers Transformations Leading to New Potent GPx Mimetics*, 1st International Electronic Conference on Catalysis Science, 10-30.11.2020 (współautorka wykładu)

3) J. Ścianowski; A. J. Pacuła-Miszewska; **A. Laskowska**; M. Obieziurska-Fabisiak, *Synthesis and applications of new chiral organoselenium compounds*, 9th Workshop of the multidisciplinary network SeS Redox and Catalysis, 8-10.09.2022 r, Łódź (współautorka wykładu)

• Komunikaty:

1) M. Obieziurska; A. J. Pacuła; **A. Laskowska**; J. Ścianowski, *Syntezy chiralnych pochodnych benziselenazol-3(2H)-onu o właściwościach antyoksydacyjnych*, IX Seminarium „Postępy w syntezie związków nieracemicznych”, 17-20.10.2018, Karpacz (współautorka komunikatu)

2) M. Obieziurska, A.J. Pacuła; **A. Laskowska**; A. Długosz-Pokorska; M. Krzemiński; A. Janecka; J.Ścianowski, *N-Terpenyl benzisosenazolones - evaluation of the particular structure-bioactivity relationship*, The 23rd International Electronic Conference on Synthetic Organic Chemistry (współautorka komunikatu)

3) **A. Laskowska**; M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; J. Ścianowski, *Synteza chiralnych pochodnych diselenidów*, e-zjazd wiosenny SSPTChem 2021, 27-29.05.2021 (osobista prezentacja komunikatu)

4) **A. Laskowska**; M. Obiezurska-Fabisiak; A. J. Pacuła-Miszewska; J. Ścianowski, *Aplikacje chiralnych amin w syntezie nowych selenoorganicznych antyoksydantów i środków cytotoksycznych*, XIV Kopernikańskie Seminarium Doktoranckie, 20-22.09.2021 r., Toruń (osobista prezentacja komunikatu)

5) A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; **A. Laskowska**; M. Zboński; J. Ścianowski, *New greener approach for the Se-N bond formation*, 9th Workshop of the multidisciplinary network SeS Redox and Catalysis, 8-10.09.2022 r, Łódź (współautorka komunikatu)

6) **A. Laskowska**; M. Obiezurska-Fabisiak; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka; J. Ścianowski, *Porównanie właściwości antyoksydacyjnych i biologicznych nowych mimetyków GPx, modyfikowanych chiralnymi grupami funkcyjnymi*, X Seminarium "Postępy w syntezie związków niereacemicznych", 19-20.10.2022 r., Wrocław (osobista prezentacja komunikatu)

- **Postery:**

1) A. J. Pacuła; M. Obieziurska; **A. Laskowska**; J. Ścianowski; K. Kaczor; J. Antosiewicz; A. Janecka; T. Janecki, *Nowe pochodne benzisoselenazol-3(2H)-onu N-funkcjonalizowane monoterenami*, XI ogólnopolskie Sympozjum Chemii Organicznej XIOSCO, 8-11.04.2018, Warszawa (współautorka posteru)

2) M. Obieziurska; A. J. Pacuła; **A. Laskowska**; J. Ścianowski, *Incorporation of the Thiocarbonyl Group in the Structure of Redox-Active Organoselenium Molecules*, XXI International Symposium „Advances in the chemistry of heteroorganic compounds”, 23.11.2018, Łódź (współautorka posteru)

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5) A. J. Pacuła; M. Obieziurska; **A. Laskowska**; A. Długosz-Pokorska; M. Krzemiński; A. Janecka; J. Ścianowski, *Application of Terpens in the Synthesis of New*

Se-Antioxidants, XXII International Symposium „Advances in the chemistry of heteroorganic compounds”, 22.11.2019, Łódź (współautorka posteru)

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7) **A. Laskowska**; M. Obieziurska; A. J. Pacuła; A. Długosz-Pokorska; A. Janecka; J. Ścianowski, *Nowe mimetyki peroksydazy glutationowej- kwasy seleninowe o właściwościach antyoksydacyjnych i cytotoksycznych*, Kopernikańskie E-Seminarium Doktoranckie, 07.09.2020 (osobista prezentacja posteru)

8) A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; **A. Laskowska**; A. Długosz-Pokorska; M.Krzemiński; A. Janecka; J. Ścianowski, *N-Terpenyl benziososelenazolones -evaluation of the particular structure bioactivity relationship*, 2nd International Conference on CHEMISTRY FOR BEAUTY AND HEALTH, 12-14.05.2021, Poznań (współautorka posteru)

9) J. Ścianowski, M. Obieziurska-Fabisiak; **A. Laskowska**; A. Długosz-Pokorska; M.Krzemiński; A. Janecka; A. J. Pacuła-Miszewska, *Selenoorganic compouds with antioxidative and cytotoxic activity*, 2nd International Conference on CHEMISTRY FOR BEAUTY AND HEALTH, 12-14.05.2021, Poznań (współautorka posteru)

10) **Laskowska**; M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; J. Ścianowski, *Chiralne diselenidy o potencjalnych właściwościach antyoksydacyjnych i cytotoksycznych*, 63. Zjazd Naukowy Polskiego Towarzystwa Chemicznego, 13-16.09.2021, Łódź (osobista prezentacja posteru)

11) J. Ścianowski, M. Obieziurska; A. J. Pacuła, **A. Laskowska**; A. Długosz-Pokorska; A. Janecka, *Pochodne selenoorganiczne o potencjalnej aktywności przeciwutleniającej i cytotoksycznej*, 63. Zjazd Naukowy Polskiego Towarzystwa Chemicznego, 13-16.09.2021, Łódź (współautorka posteru)

12) M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; J. Ścianowski, *A new UV method for the synthesis of benziososelenazolones supported by UV irradiation*, 22nd Tetrahedron Symposium "Catalysis for a sustainable world", 28.06-1.07.2022, Lizbona, Portugalia

13) **A. Laskowska**; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka; J. Ścianowski, *Modification of new organoselenium compounds with chiral functional*

groups, 9th Workshop of the multidisciplinary network SeS Redox and Catalysis, 8-10.09.2022 r, Łódź (osobista prezentacja posteru)

14) M. Obieziurska-Fabisiak; **A. Laskowska**; A. J. Pacuła-Miszewska; M. Zboiński, J. Ścianowski, *Incorporation of the sulfur atom in the structure of organoselenium molecules*, 9th Workshop of the multidisciplinary network SeS Redox and Catalysis, 8-10.09.2022 r, Łódź (współautorka posteru)

15) A. J. Pacuła-Miszewska, M. Obieziurska-Fabisiak; **A. Laskowska**; M. Zboiński, J. Ścianowski, *New greener approach for the Se-N bond formation*, 9th Workshop of the multidisciplinary network SeS Redox and Catalysis, 8-10.09.2022 r, Łódź (współautorka posteru)

16) **A. Laskowska**; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka; J. Ścianowski, *Synthesis and application of chiral benzisoselenazol-3(2H)-ones*, XXIII International Symposium Advances in the Chemistry of Heteroorganic Compounds” Łódź (Poland), 28.10.2022 (osobista prezentacja posteru)

17) A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; **A. Laskowska**; M. Zboiński; J. Ścianowski, *New UV-initiated approach for the intramolecular Se-N bond formation*, XXIII International Symposium Advances in the Chemistry of Heteroorganic Compounds” Łódź (Poland), 28.10.2022 (współautorka posteru)

18) M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; M. Zboiński; J. Ścianowski, *The influence of O/S exchange on the antioxidant activity of ebselen derivatives*, XXIII International Symposium Advances in the Chemistry of Heteroorganic Compounds” Łódź (Poland), 28.10.2022 (współautorka posteru)

19) **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Długosz-Pokorska; A. Janecka; J. Ścianowski, *Chiral diselenides with antioxidant and cytotoxic activity*, 15th International Conference on the Chemistry of Se and Te, Florianopolis (Brazil), 28.11-02.12.2022 (osobista prezentacja posteru)

20) A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; **A. Laskowska**; J. Ścianowski, *New UV-light initiated intramolecular Se-N bond formation*, 15th International Conference on the Chemistry of Se and Te” Florianopolis (Brazil), 28.11-02.12.2022 (współautorka posteru)

21) **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Długosz-Pokorska; A. Jastrzębska; J. Ścianowski, *Synteza chiralnych fenylselenidów jako nowych przeciwutleniaczy i środków cytotoksycznych*, „65. Zjazd Polskiego Towarzystwa Chemicznego”, PTChem 2023, Toruń, 18-22 września 2023 (autorka posteru)

- 22) M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; J. Ścianowski, *Selenocyklizacja jako nowe podejście w syntezie dihydrobenzo[1,4]oksaselenin*, „65. Zjazd Polskiego Towarzystwa Chemicznego”, PTChem 2023, Toruń, 18-22 września 2023 (współautorka posteru)
- 23) J. Ścianowski; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; **A. Laskowska**, *Indukowana światłem UV nowa metoda tworzenia wewnątrzcząsteczkowego wiązania Se-N*, „65. Zjazd Polskiego Towarzystwa Chemicznego”, PTChem 2023, Toruń, 18-22 września 2023 (współautorka posteru)
- 24) M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; D. Łukaszewicz; A. Jastrzębska; A. Długosz-Pokorska; K. Gach-Janczak; J. Ścianowski, *Antioxidant and anticancer properties of N-substituted with long carbon chain benzoselenazones and diselenides*, XXIII International Symposium Advances in the Chemistry of Heteroorganic Compounds” Łódź (Poland), 24.11.2023 (współautorka posteru)
- 25) J. Ścianowski, M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; D. Łukaszewicz; *Synthesis of dihydrobenzo[1,4]oxaselenines as a new approach for the selenocyclization reaction*, XXIII International Symposium Advances in the Chemistry of Heteroorganic Compounds” Łódź (Poland), 24.11.2023 (współautorka posteru)
- 26) **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Długosz-Pokorska; A. Jastrzębska; J. Ścianowski, *New β -carbonylphenylselenides o-functionalized with ester groups as potential antioxidant and cytotoxic agents*, „XVII-Kopernikańskie Seminarium Doktoranckie”, Toruń, 06-07.06.2024 (autorka posteru)
- 27) **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; A. Długosz-Pokorska; K. Gach-Janczak; J. Ścianowski, *New β -carbonyl phenyl selenides with o-ester group as potential antioxidant and anticancer agents*, ”The 11-th Workshop of the Selenium and Sulfur Redox and Catalysis Network (WSeS-11)”, Toruń, 25-26.07.2024 (autorka posteru)
- 28) **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; A. Długosz-Pokorska; K. Gach-Janczak; J. Ścianowski, *Synthesis of new chiral β -carbonyl phenyl selenides with o-amide group as antioxidant and anticancer agents*, „The 11-th Workshop of the Selenium and Sulfur Redox and Catalysis Network (WSeS-11)”, Toruń, 25-26.07.2024 (współautorka posteru)

III. Staż zagraniczny

20.06-19.07.2021 - staż zagraniczny w ramach programu NAWA PROM w grupie prof. Claudio Santi, Università Degli Studi di Perugia, Włochy

IV. Działalność organizacyjna

Członek Komitetu Organizacyjnego konferencji:

- 1) „65. Zjazd Polskiego Towarzystwa Chemicznego”, Toruń, 18-22 września 2023
- 2) The 11-th Workshop of the Selenium and Sulfur Redox and Catalysis Network (WSeS-11)”, 25-26.07.2024, Toruń

V. Nagrody, wyróżnienia i granty

- 1) Grantów Młodych Naukowców WCH UMK (*4 granty*):
– granty nr 2092/2019, 492/2020, PDB/granty wydziałowe, WCH/2022.Zad13.05.89.
- 2) Grant „Grants4NCUStudents” nr 60/2022/Grants4NCUStudents
- 3) Nagroda za najlepszy poster naukowy przedstawiony na „The 11-th Workshop of the Selenium and Sulfur Redox and Catalysis Network”

10. Publikacje wchodzące w skład rozprawy doktorskiej

Publikacja 1 [P1]

Organoselenium compounds as antioxidants

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Dedicated to Prof. Józef Drabowicz on the occasion of his 76th anniversary

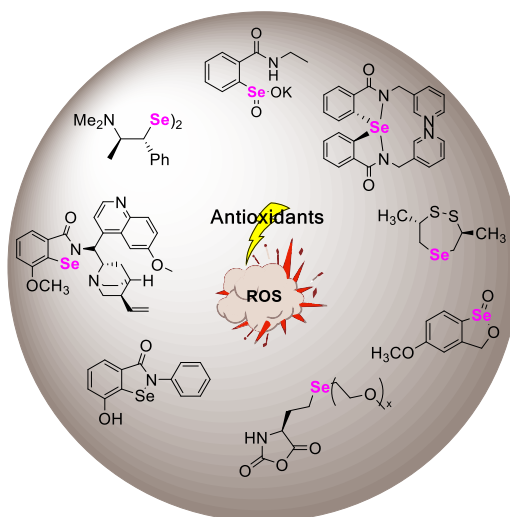
Received 10-14-2022

Accepted 11-17-2022

Published on line 12-01-2022

Abstract

Reactive oxygen species (ROS) are responsible for many of civilization's diseases, including cancer, diabetes, and Alzheimer's disease, decomposition of products in the food industry, and deterioration of physicochemical properties of polymers and nanomaterials. In recent years, several organoselenium compounds have been synthesized and used as peroxide scavengers, which are the source of many antioxidant substances. This review aims to collect and divide the organoselenium compounds obtained in the last twelve years with antioxidant activity, which can prove helpful in a) medicine as supplements preventing diseases caused by oxidative stress, b) as food additives preventing oxidation, or c) in the materials industry as Se-containing nanoparticles and polymers. In addition, the most common methods for determining GPx-like antioxidant activity are presented.



Keywords: GPx mimics, organoselenium compounds, antioxidants

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1. Introduction

The formation of reactive oxygen species (ROS) in living organisms is related to many basic biological processes, such as the respiratory chain, metabolism of purine nucleotides, microsomal hydroxylation cycle, and reactions involving oxidoreductases. The products of these reactions, which include the superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), should be reduced in subsequent biochemical reactions and safely removed from the body. However, may not be the case for various reasons, for example, stress, improper diet, and strenuous exercise.¹

The imbalance between the rate of ROS formation and the efficiency of the antioxidant system is known as oxidative stress. Excessive production of ROS with the simultaneous depletion of antioxidant reserves causes the oxidation of fatty acids, proteins, and DNA. Efforts to reduce oxidative stress can be manifested in the clinical improvement of patients, while excessive production of ROS is the primary cause or secondary complication of the disease. Toxic oxidation products are the initiators of several diseases, such as atherosclerosis, hypertension, diabetes, inflammation, Alzheimer's disease, and cancer.¹

Antioxidants are compounds that possess the ability to prevent or inhibit the oxidation of other chemicals, and among them, we find molecules of both natural and synthetic origin.^{2,3} The biological systems include endogenous compounds produced by the organism, which in turn can be divided into derivatives of enzymatic (e.g., glutathione peroxidase (GPx), thioredoxin reductase (TrxR), superoxide dismutase (SOD), and catalase) and non-enzymatic (e.g., lipoic acid, glutathione, ferritin, albumin) origin. When endogenous antioxidants are unable to protect the body against the effects of ROS, there is a need for exogenous antioxidants derived from natural sources, such as plants (e.g., flavonoids, phenolic acids, carotenoids, organosulfur compounds, vitamins) or minerals (selenium, zinc, manganese) provided via appropriate diet. The second group of antioxidants is compounds of synthetic origin, delivered to organisms in the form of dietary supplements and bioequivalent to their natural forms (e.g., vitamin C compared to chemically synthesized *L*-ascorbic acid). Synthetic antioxidants are also utilized as additives to prevent the oxidation of unstable ingredients in the food, pharmaceutical,

cosmetic, and materials industries. The classifications of antioxidants, along with the most representative examples, are shown in Figure 1.⁴

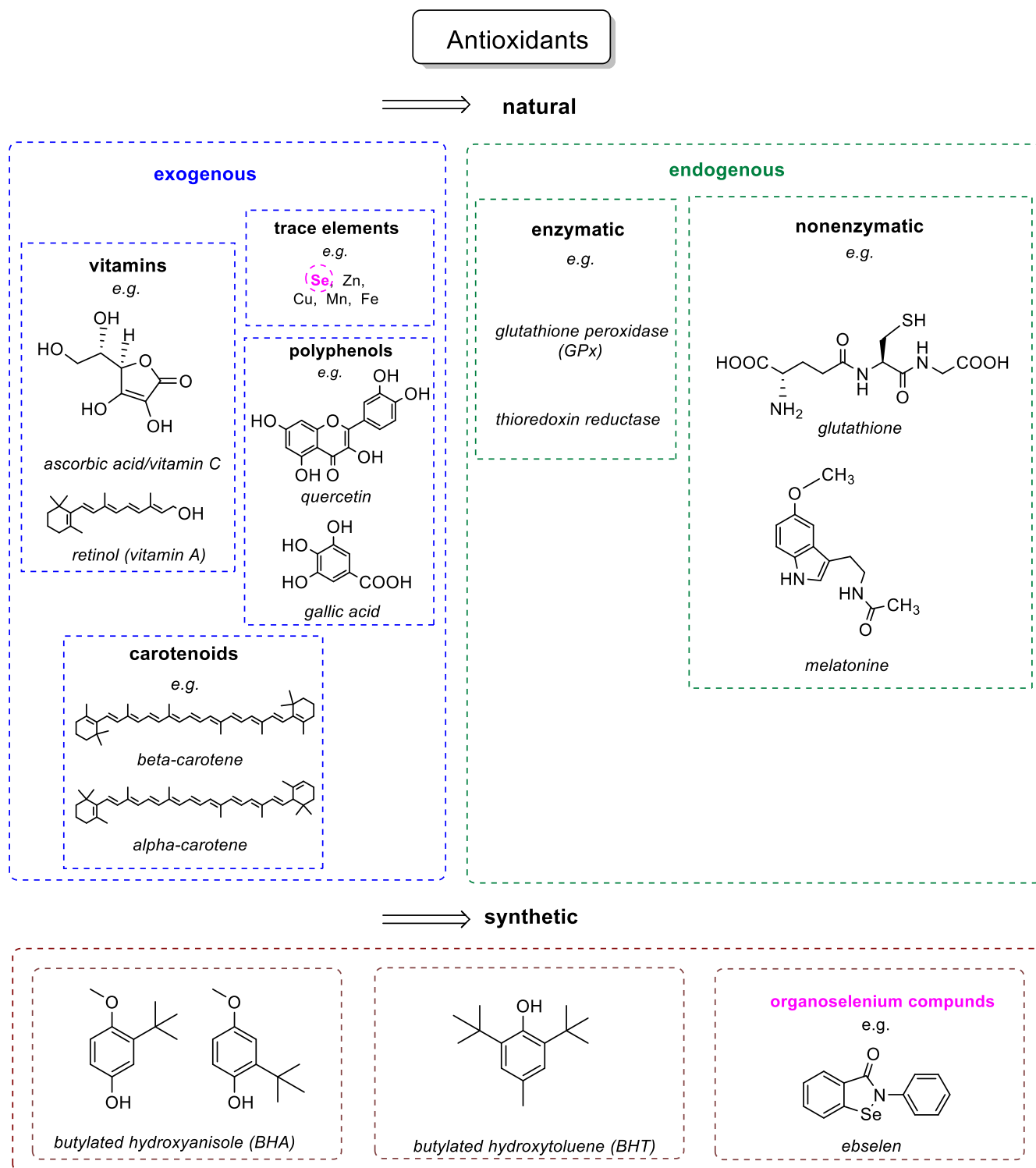


Figure 1. Antioxidants, classification with examples.

In the biological context, selenium was considered for many years only as a poison and carcinogen.^{5,6,7A} breakthrough moment that changed the perception of selenium and revealed its positive face came in the second half of the 20th century. At that time, two researchers, Schwarz and Foltz, confirmed that trace amounts

of selenium are necessary for the proper functioning of animals and human organisms.⁸ Selenium biology has developed rapidly in recent decades and is now known in various forms as an essential trace element in living organisms. Nutritional functions of selenium in the human body are provided by the action of 25 selenoproteins. A key role in effectively reducing harmful peroxides is played by one, so far the best known in mammals, selenoenzyme - glutathione peroxidase (GPx). The biological activity of this protein is related to the presence of selenocysteine (Sec) in its active site.⁹ Since these discoveries, the incorporation of a selenium atom in the structure of various small molecules enabled the design of many potential Se-therapeutics. Numerous publications in the field of medicinal chemistry present the significant biological potential of organoselenium compounds in diversified activity assays.^{10,11,12,13}

Glutathione peroxidase (GPx) catalyzes the reduction of H_2O_2 and other organic peroxides using glutathione (GSH) as a cofactor.¹⁴ Under physiological conditions, in the first step, active selenol (E-SeH) **1** is oxidized to selenenic acid (E-SeOH) **2**. Acid **2** reacts with glutathione (GSH) to form the selenenyl sulfide **3**. Regeneration of selenol **1** takes place as a result of the reaction of sulfide (E-Se-SG) **3** with another molecule of glutathione (GSH) and the release of its oxidized form - disulfide (GSSG). In oxidative stress conditions, when the level of hydrogen peroxide (H_2O_2) is high and glutathione (GSH) is low, over-oxidation of selenol takes place, and the formation of seleninic acid (E-SeOOH) **4** occurs (Figure 2).¹⁵

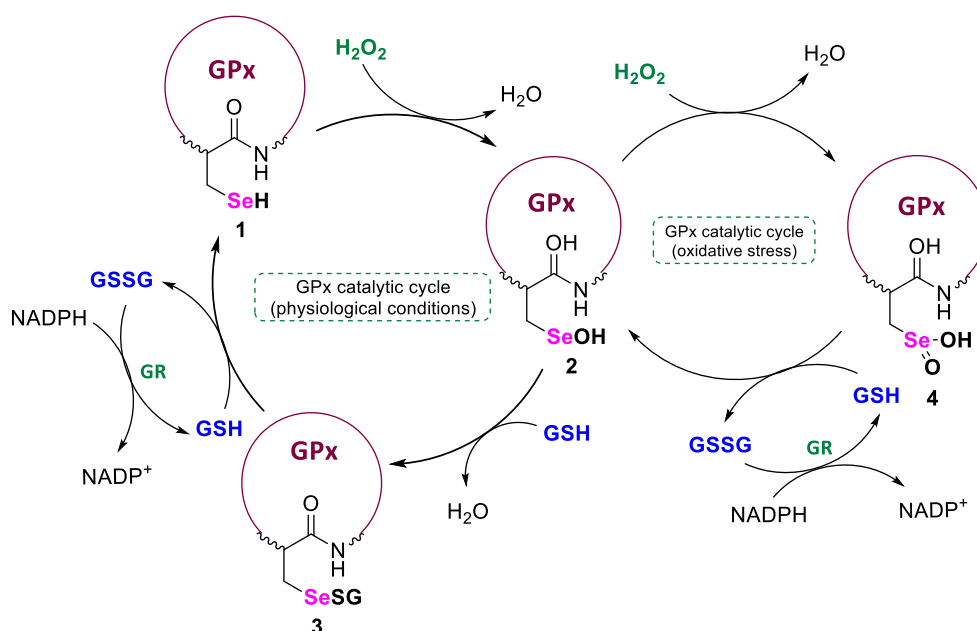


Figure 2. GPx catalytic cycle. GSH: reduced form of glutathione; GSSG: the oxidized dimeric form of glutathione; GR: glutathione reductase.

Looking at the GPx catalytic cycle, it should be noted that catalysis will be possible only if the chalcogen atom is easily reduced to the appropriate nucleophilic form and only if non-reversible overoxidation is prevented. The unique property of selenenic and seleninic species compared to sulfur analogs results from their easy oxidation-reduction reactions with the participation of thiols. The formation of sulfonic acid is irreversible¹⁶ and sulfinic acid can be reduced only in a few cases by sulfiredoxin.¹⁷ Therefore, an additional evolutionary benefit of using selenocysteine in the redox protein instead of cysteine is evident.¹⁸ An in-depth analysis of the structure and understanding of the mechanism of GPx activity placed the mentioned selenoenzyme in the center of chemists' attention. Scientists are trying to synthesize organoselenium compounds that are specific mimetics of GPx and

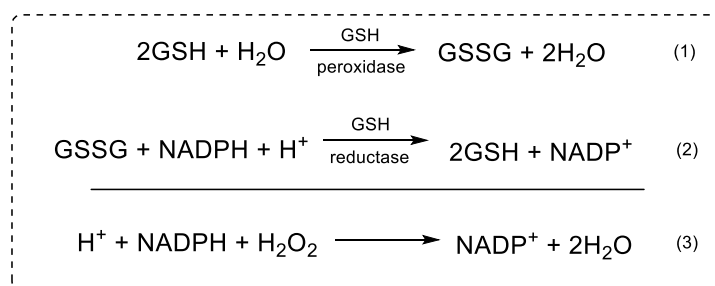
thus possess potent antioxidant activity. In this review, we have collected information from the last twelve years on topics covering the roles of organoselenium compounds as antioxidants in a) medicine and b) in materials chemistry and the food industry.

2. Methods of Measuring GPx-like Antioxidant Activity

This section presents the most common methods of measuring antioxidant activity that has been used in the studies listed below.

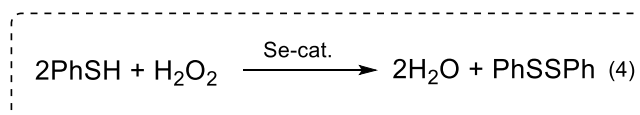
Method A: GSH/GR coupled assay¹⁹

The glutathione reductase (GR) coupled assay was the first indirect method to evaluate GPx-mimic activity, developed by Wilson *et al.* The GR enzyme, at the expense of the cofactor NADPH (β -nicotinamide adenine dinucleotide 2'-phosphate), catalyzes the reduction of the oxidized form of glutathione (GSSG), formed during the catalytic action, back to GSH. The initial reduction rates (v_0) of NADPH are recorded by using UV spectroscopy at 340 nm. The GPx-like catalytic activity is studied using various peroxides for this reaction, e.g., hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (*t*-BuOOH), or cumene hydroperoxide (Cum-OOH). The half and summary equations of the involved reaction are shown below (Eqn 1,2,3).



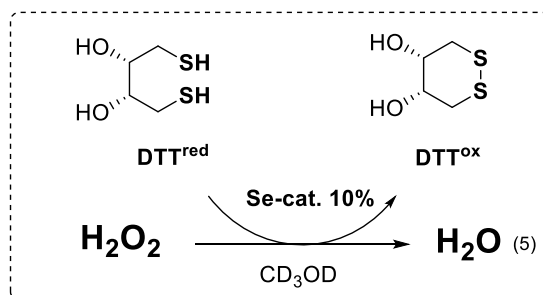
Method B: PhSH assay²⁰

In this direct method developed by Tomoda *et al.*, benzenethiol (PhSH) is used as an alternative to glutathione. The reduction of hydrogen peroxide in the presence of PhSH with simultaneous formation of diphenyl disulfide (PhSSPh) is assessed using different techniques: a) spectrophotometrically through the UV absorption increase at 305 nm due to PhSSPh formation; b) using the HPLC analysis because the amount of PhSSPh formed is determined by the time required for 50% conversion of PhSH to PhSSPh ($t_{1/2}$ values) and calculated as the peak areas at different time intervals. The equation of the described reaction is shown below (Eqn 4).



Method C: DTT^{red}/ DTT^{ox} NMR assay^{21,22}

The GPx-like antioxidant activity of compounds can be assessed using the test presented by Iwaoka *et al.* The organoselenium catalyst reduces hydrogen peroxide (H_2O_2) and is regenerated in the presence of dithiothreitol (DTT^{red}). The rate of the reaction is measured using ¹H NMR spectroscopy in CD_3OD ²¹ or D_2O .²² The appearance of signals representing the formed disulfide (DTT^{ox}) in specific time intervals is recorded. The equation of the reaction is shown below (Eqn 5).



Method D: DPPH assay²³

Shaaban *et al.* described another easy method to assess the radical scavenging activities of organoselenium compounds and nutritional products. The antioxidant activity of a compound is estimated by its ability to reduce stable DPPH[•] radical (purple color in methanol) to DPPHH (colorless) by the decrease in the absorbance at 517 nm.

Method E: ABTS assay²⁴

In this method presented by Shaaban *et al.*, the antioxidant activity of organoselenium compounds is assessed by their ability to decolorize the ABTS[•] (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals and the corresponding radical-scavenging activity is estimated by the decrease in the absorbance at 734 nm.

3. Antioxidants in Medicine

3.1. Ebselen and its derivatives

Ebselen (*N*-phenylbenzisoselenazol-3(2*H*)-one) **5** is one of the first synthetic glutathione peroxidase (GPx) mimetics to catalyze essential reactions involved in protecting cells from oxidative damage and free radicals.^{25,26,27} Currently, it is in the second and third phases of clinical trials as a therapeutic in treating diseases caused mainly by oxidative stress (Figure 3).²⁸ This section presents recent advances in synthesis of new ebselen analogs with enhanced GPx-like activity. Modifications of the ebselen **5** structure mainly include the replacement or substitution of the phenyl group on the nitrogen atom (ring B) or the connection of various substituents on the benzamide ring A.

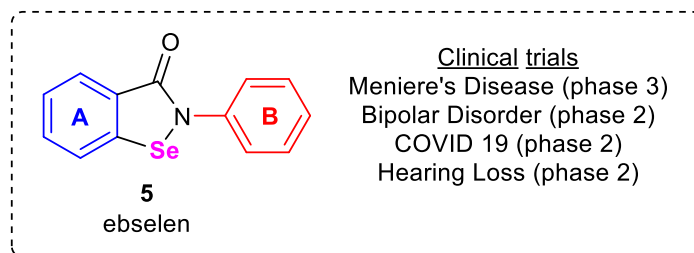


Figure 3. Structure of ebselen.

3.1.1. Modification of ebselen rings A and B. A series of benzisoselenazolones with additional substituents on the benzamide ring was examined in terms of antioxidant activity by Kumar *et al.* (2014) utilizing Method B.²⁰ The high GPx-like activity of benzisoselenazolone **6** (Figure 4), which forms selenol intermediate **8**, suggests the presence of the bulky *N*-quininamine substituent and the *ortho*-CH₃ benzamide substituent stabilizes compound

8, which regenerates benzoselenazolone **6** through its reaction with H_2O_2 (Figure 4). Compound **6** showed a much higher antioxidant activity than other benzoselenazolones presented in this article and is 10^3 -fold more active than ebselen **5**.²⁹

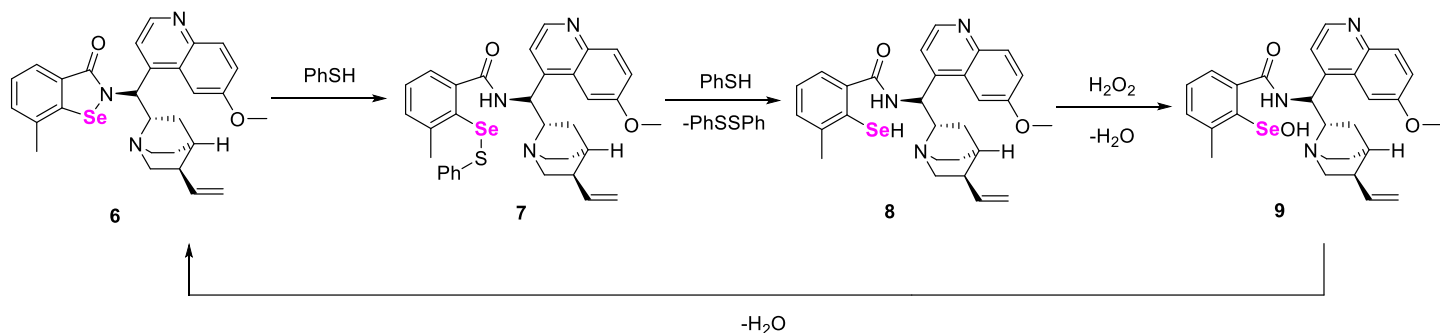


Figure 4. The high GPx-like activity of benzoselenazolone **6** and generation of selenol **8**.

The GPx-like activity of the ebselenol series was also described by Kumar *et al.* in 2016 and compared with ebselen **5** using GSH/ H_2O_2 and GSH/*t*-BuOOH assays (Method A).¹⁹ All obtained compounds possessed antioxidant activity higher than ebselen **5**. Moreover, all derivatives were more active when the oxidant was H_2O_2 . The compounds were also assessed as GPx-mimics using Method B.²⁰ Ebselenol **10** (Figure 5) was a 15-fold more active catalyst than ebselen **5**. The authors suggest that such high activity of compound **10** was most likely a result of the hydroxyl group and the selenium center proximity.³⁰ In 2021, Kumar *et al.* synthesized *N*-methyl ebselenamine compounds and then assessed their antioxidant activity (Method A),¹⁹ noting that the excellent GPx-like activity of the obtained derivatives may be related to the close proximity of the -NHMe group and the selenium atom. Of all the compounds, derivative **11** showed the highest antioxidant activity (2.5-fold more active than ebselen **5** and 5-fold more active than α -tocopherol).³¹

In 2014, a group of Chinese researchers obtained a new series of multi-target directed ligands derived from ebselen **5** and tested them for catalytic reduction of H_2O_2 using Method A.¹⁹ The results indicated that compound **12** (Figure 5) exhibited about 1.5-fold higher antioxidant activity than ebselen **5**.³²

3.1.2. Modification of ebselen ring B. In 2011, a series of di- and tripeptide-based ebselen analogs was synthesized by Satheeshkumar and Mugesh. The GPx-like antioxidant activity was studied using Method A.¹⁹ The antioxidant activity of these compounds depends significantly on the nature of the peptide moiety attached to the nitrogen atom of the selenazole ring. Compound **13** (Figure 5), which possesses a Val-Ala peptide, showed the highest antioxidant activity in all three peroxide assays (about 2-fold higher than ebselen **5**). The authors suggest that the (Val-Ala) dipeptide facilitates the formation of active selenol, which is directly involved in the scavenging of peroxides (See Figure 2).³³

In 2014, in Wirth's research group, a series of new *N*-chiral benzoselenazolones was synthesized. The GPx-like activity of all derivatives was determined by Method B²⁰ (using a high-performance liquid chromatography (HPLC) assay) and by Method A¹⁹ (by the UV-Vis assay), each with two different peroxides, H_2O_2 and CumOOH as the substrates. Most of the obtained derivatives showed GPx-like activity similar to that of ebselen **5**. In contrast, the highest antioxidant activity was observed for the derivative **14** (Figure 5) with the hydroxyl group (about two-fold higher than that of ebselen **5** in Method A¹⁹).³⁴

In the last seven years, several *N*-alkyl,³⁵ *N*-aryl,³⁶ and chiral benzoselenazolones^{37,38,39} were obtained by Ścianowski *et al.* The evaluation of the antioxidant activity was based on Method C.²¹ The compounds **15-23** that exhibited the highest antioxidant activity in each of the mentioned groups are presented in Figure 5. In the

same research group, the oxygen atom of the carbonyl group was replaced with a sulphur atom by synthesizing the benzoselenazolones, and the obtained derivatives were assessed in terms of their GPx-like antioxidant activity (Method C²¹). The best peroxide scavenger was *N*-propyl benzoselenazole-3(2*H*)-thione (2-fold more active than ebselen 5).⁴⁰

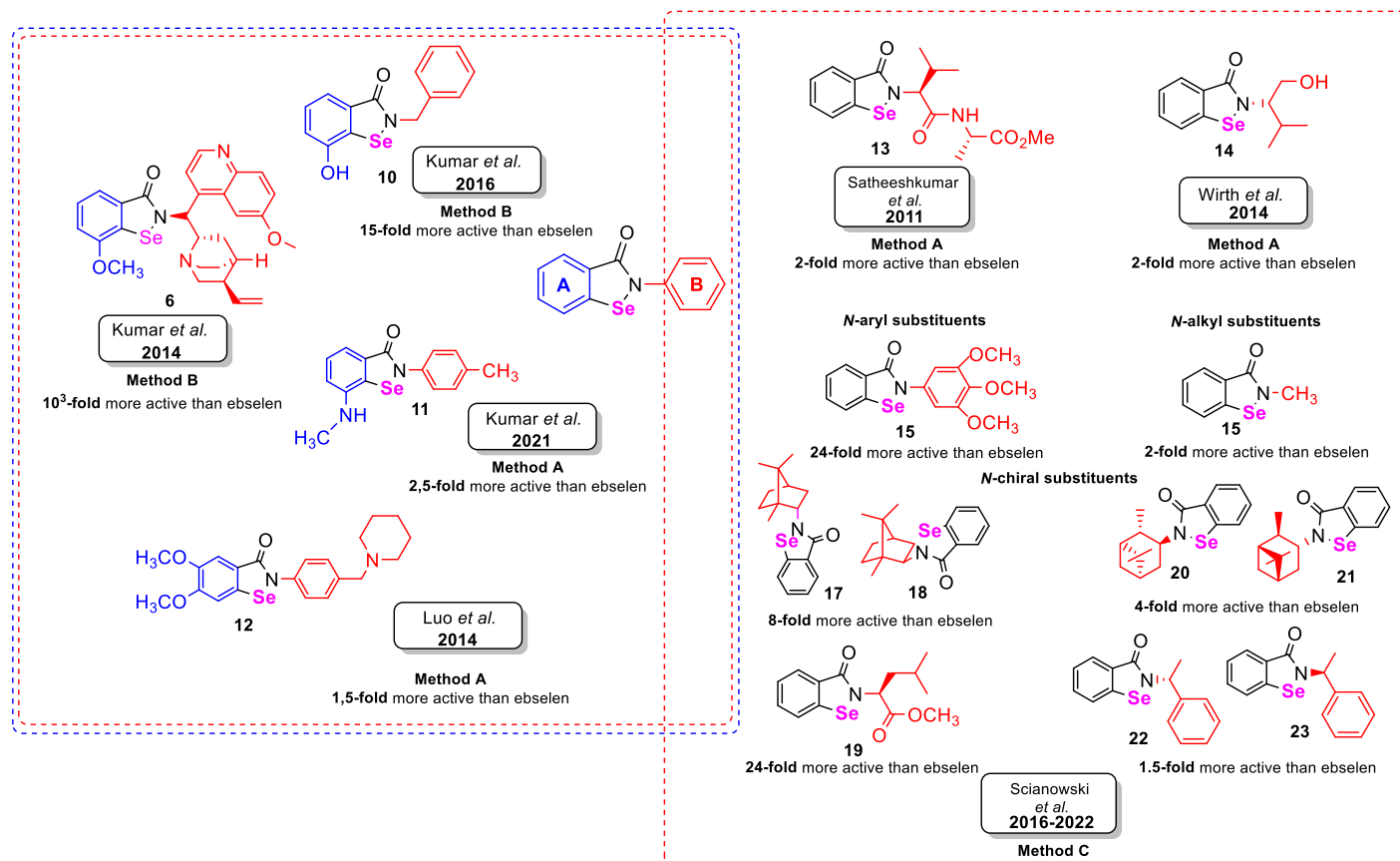


Figure 5. Ebselen analogs 6-23 with potential GPx-like activity.

3.2. Diselenides and their derivatives

In addition to benzoselenazolones, diselenides constitute the second significant group of compounds with potential antioxidant activity similar to GPx. Diphenyl diselenide (PhSe)₂ is the simplest compound among diselenides exhibiting antioxidant potential higher than ebselen, probably because of intramolecular interactions that stabilize its selenylsulfide intermediate, preventing the reduction of H₂O₂. In the recent years, a number of diselenides have been synthesized and evaluated *in vitro* for GPx-like antioxidant activity by several leading research groups.

3.2.1. Diselenides obtained by Braga *et al.* and Rocha *et al.* Chiral diselenides and their derivatives from (-)-ephedrine were prepared and monitored as GPx mimetics by Braga *et al.* (2012). Diselenide **24** and derivative **25** showed catalytic performance 11.5- and 4-fold higher, respectively, compared to the standard (PhSe)₂.⁴¹ By combining the structures of ebselen **5** and derivative **24** (the highest antioxidant activity in the previous work), Braga and co-workers obtained amido-based diselenide **26**, which was 9- and 3-times more active than the standard benzisoselenazolone **5** and (PhSe)₂.⁴² The same research group confirmed that alkyl diselenide **27** containing a cholesterol unit in its structure possessed an antioxidant effect 3-fold higher than ebselen **5**.⁴³ Aniline-based diselenide **28** substituted with the *p*-CF₃ group was 5- and 2-times more active than ebselen **5** and (PhSe)₂, respectively. The obtained results showed that the catalytic efficiency increased with the electron-withdrawing capacity of the substituent in the *para* position. The amino group participates in stabilizing the selenolate intermediate through a hydrogen bond with the selenium atom, creating a zwitterionic form.⁴⁴

In 2012, Braga, in cooperation Hassan and Rocha, obtained β-amino-based diselenides and disulfides. Diselenide **29** showed a very significant antioxidant potential (5-times higher than (PhSe)₂) and non-toxic effect.⁴⁵ The same scientists continued the idea of synthesizing organoselenium compounds with a heteroatom close to the selenium atom, noting the interesting GPx-like activity of this type of derivative. In 2015, they presented the synthesis of aliphatic and aromatic 2-picolyamide-based diselenides with proximal non-bonding Se--O interactions. The aromatic compound **30** possessed about 5-times higher antioxidant potential than (PhSe)₂.⁴⁶ Three years later (2018), Braga and Rocha conducted the synthesis of a new class of chiral diselenoamino acid derivatives from phenylalanine and valine. Diselenide **31** showed antioxidant activity similar to (PhSe)₂. The obtained results suggested that the catalytic activity of the GPx mimetics presented in this paper depends on the steric effects that can be influenced by the number of carbon atoms between the selenium atom and the amino acid residue and/or by the amino acid lateral residue.⁴⁷

In 2014, Ibrahim, Rocha *et al.* revealed that an amino group in amino diselenides drastically enhances their GPx-like catalytic activities by synthesizing 1-(2-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine **32** and comparing the results obtained with activity observed for (PhSe)₂ (two times higher activity than (PhSe)₂).⁴⁸ In 2019, the same compound **32** was tested *in vitro* and *in vivo* in mice, showing no acute toxicity.⁴⁹

The GPx-like activity for all the diselenides and their derivatives **24-32** was evaluated according to Method B.²⁰ The relative activity observed for the individual derivatives **24-32** and their structures are summarized in Figure 6.

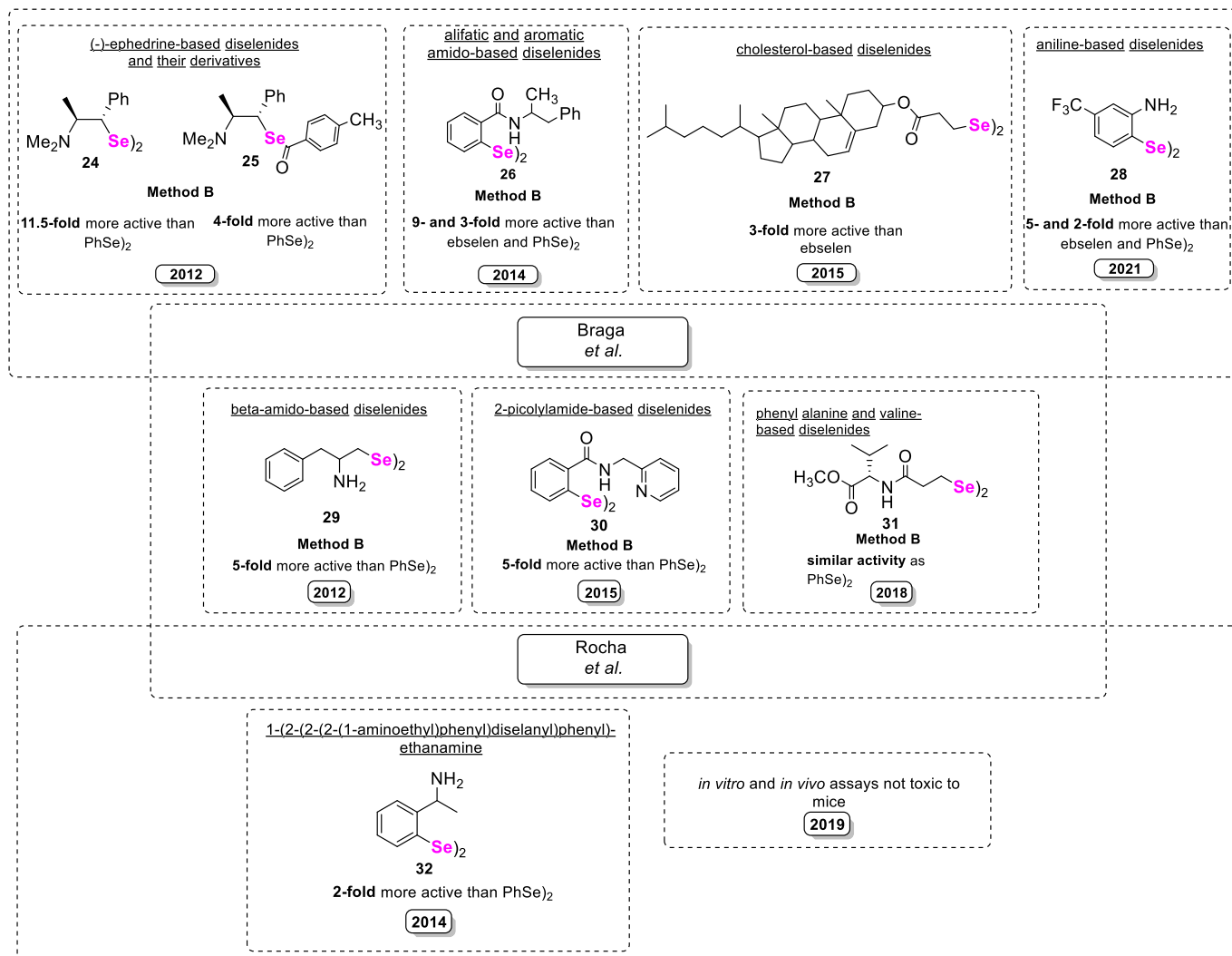


Figure 6. Diselenides and their derivatives **24-32** with potential GPx-like activity assessed by Braga, Rocha *et al.*

3.2.2. Diselenides designed by Singh *et al.* Another research group specialized in synthesizing organoselenium compounds with potential antioxidant activity is that of Singh *et al.* In 2014, they assessed the antioxidant potential of nicotinoyl-based organoselenium compound **33** (2,2'-diselenobis[3-amidopyridine]), and it was two-times more active than ebselen **5**.⁵⁰ The results obtained in this work inspired the authors to synthesize new pyridine-based GPx mimics by substituting suitable functional groups. In the same year, an article appeared about the synthesis of pyridoxine-derived diselenides and other organoselenium derivatives (selenides, selones, seleninic acids, selenosulfides) was performed. Among all derivatives, the lower potential was observed for selenide **34**, selone **35**, selenosulfide **38** and the highest for diselenide **36** (1.5-fold more active than ebselen **5**) and seleninic acid **37** (2-fold active than ebselen **5**).⁵¹ In the next paper (2015), Singh *et al.* proposed to modify diselenide **36** by introducing a bromine atom in the 6-position of the pyridine ring. This substitution increased the antioxidant activity of derivative **39** (2-fold more active than ebselen **5**).⁵² In 2021, Singh, Kumar *et al.* received a series of diselenides **40a-d**, selenazolonamines **41a-d**, selenoxides **42a-d**. They noticed that electron-donating substituents dramatically increased the antioxidant potential. Moreover, the selenoxides **42a-d** showed a GPx-like activity higher than the corresponding selenazolonamines **41a-d**. The highest antioxidant activity was observed for diselenide **40d** (2-fold more active than ebselen **5**).⁵³ The GPx-like activities of all

compounds **33-42** were assessed by utilizing Method A.¹⁹ The relative activities for the individual derivatives **33-42** and their structures are summarized in Figure 7.

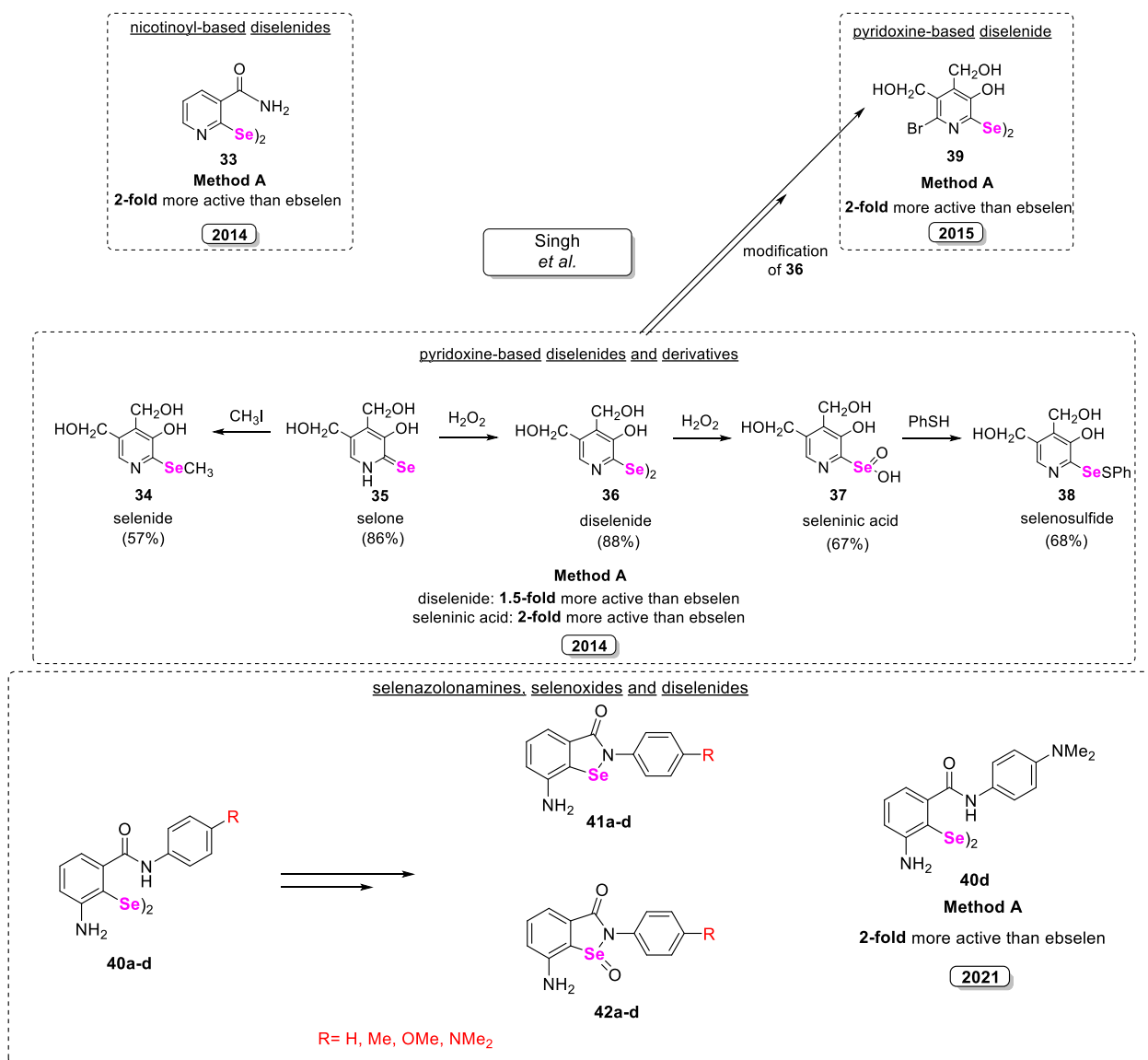


Figure 7. Diselenides and their derivatives **33-42** with potential GPx-like activity assessed by Singh *et al.*

3.2.3. Diselenides synthesized by Mugesh *et al.* As mentioned above, the high activity of amine-based diselenides is related to the presence of basic amino groups which can deprotonate the selenol to generate a more reactive selenolate in the catalytic cycle of GPx (See Figure 2). Mugesh *et al.*, in various studies, assessed the antioxidant activity of *tert*- and *sec*-amine-based diselenides. Modification of the aromatic *tert*-amino diselenide **43** ring⁵⁴ by introducing a 4- or 6-methoxy group increased the antioxidant activity of the new derivatives **44** and **45**.⁵⁵ The *sec*-amino diselenides **46a-d** containing alkyl substituents turned out to be unstable and rapidly cyclized to the corresponding isoselenazolones **47a-d**. These, in turn, showed GPx-mimetic activity 2- and 3-fold higher than ebselen **5**. Stable diselenides **48a-d** presented lower antioxidant activity than isoselenazolones **47a-d** and marginally better than ebselen **5**.⁵⁶ The antioxidant activity was also assessed for diselenide **49** and compared with the derivative **44**.⁵⁷ The GPx-mimic activity for derivatives **43-48** was evaluated

using Method A¹⁹ and for compound **49** using Method B.²⁰ The relative activities for the individual derivatives **43-49** and their structures are summarized in Figure 8.

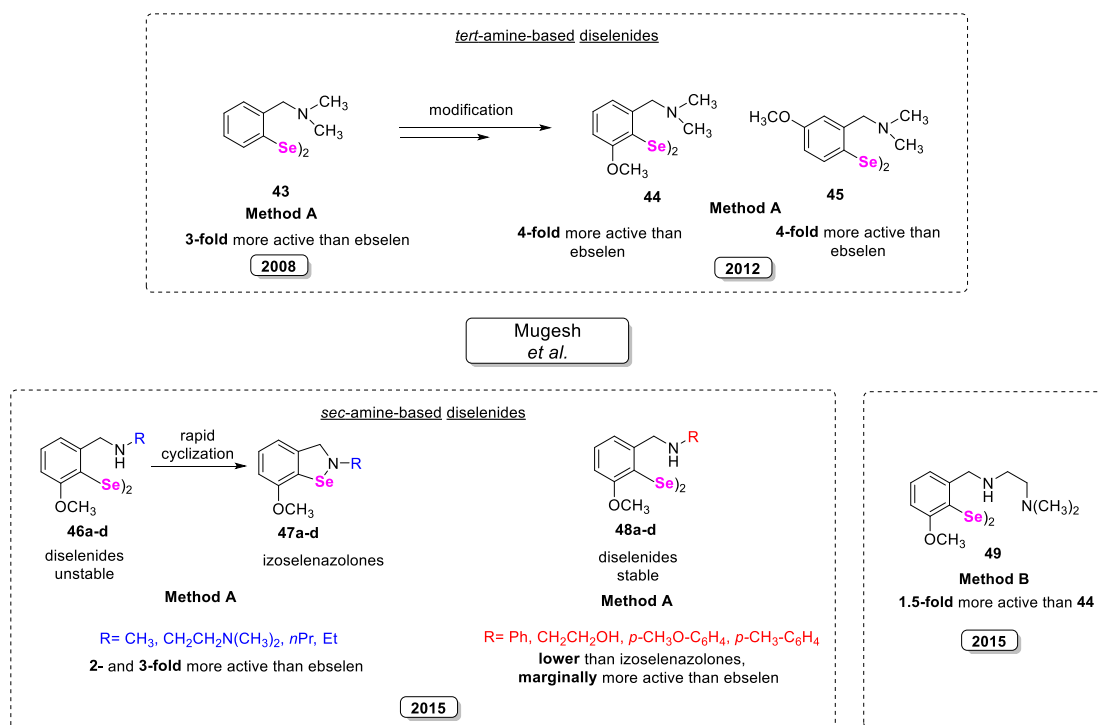


Figure 8. Diselenides and their derivatives **43-49** with potential GPx-like activity assessed by Mugesh *et al.*

3.2.4. Diselenides designed by Ścianowski *et al.* *N*-alkyl,³⁵ *N*-aryl,³⁶ and *N*-chiral^{37,39} amido-based diselenides were synthesized in the Ścianowski research group. A significant increase in the antioxidant potential was observed for appropriate diselenide derivatives: *N*-alkyl selenenic acids **52a-f** and water-soluble potassium salts of these acids **53a-f**.⁵⁸ The salts **53a-f** showed the highest GPx-mimetic activity among all compounds presented by the Ścianowski group. Additionally, the solubility of these salts **53a-f** in water gives them great potential in pharmacology. The GPx-mimic activity for all derivatives **50-57** was assessed using Method C.^{21,22} The relative activities for the individual derivatives **50-57** and their structures are summarized in Figure 9.

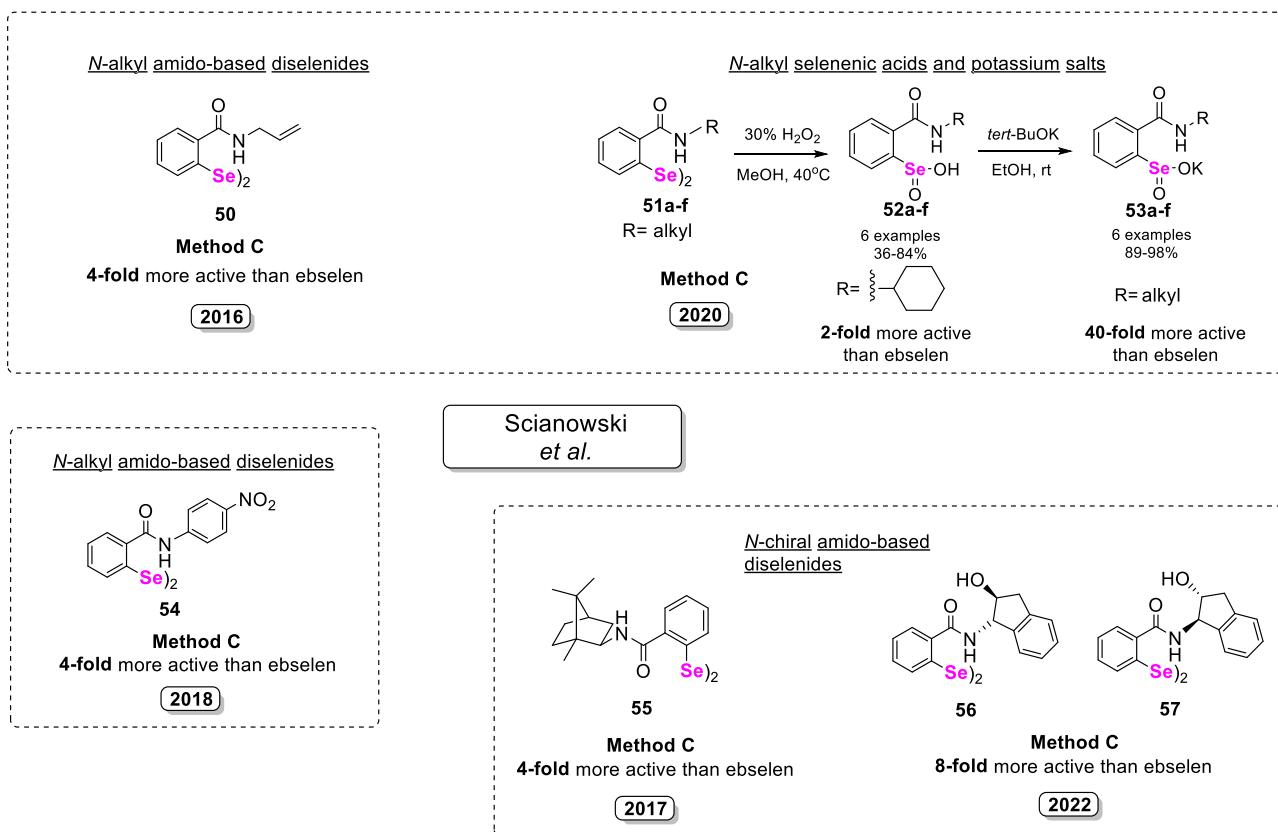


Figure 9. Diselenides and their derivatives **50-57** with potential GPx-like activity assessed by Ścianowski *et al.*

3.3. Other organoselenium compounds as antioxidants

Braga *et al.* (2012) assessed the GPx-like catalytic activity of selenides and selenoxides using Method B.²⁰ The selenoxide **58** with an amino chelating group showed 3-fold higher activity than ebselen **5**. Selenide **59** was a poorer catalyst than selenoxide **58**, but showed catalytic activity comparable to ebselen **5**.⁵⁹ In 2016, Manichetti and Braga synthesized different benzo[*b*][1,4]selenazines. The best antioxidant potential tested using Method C²¹ was observed for benzoselenazine **60** which was lower than that of diphenyldiselenide.⁶⁰

The antioxidant activity of dithiaselenepanes was assessed using Method C²¹ by Capperucci *et al.* (2016). According to this assay 3,7-dimethyl-1,2,5-dithiaselenepane **61** was more active than (PhSe)₂. Intriguingly, the more hindered dithiaselenepane **62** showed lower catalytic efficiency than **61**.⁶¹ In 2019, a series of cyclic and open-chain selenides was obtained within the same research group. They suggested that the nature of the functional groups close to the selenium atom strongly influenced the catalytic antioxidant properties of organoselenides. β-Seleno nitriles **63** and **64** and 2-oxo-1,4-oxaselenane **65** were the best catalysts according to Methods A¹⁹ and C.²¹ As in the previous report,⁶¹ also, in this case, a higher GPx-like activity of cyclic selenides compared to acyclic analogs was observed.⁶² In the same research group (2015), resveratrol-based benzoselenophenes **66-68** were obtained, for which the antioxidant activity was assessed using Method D.²³ All the selenophene derivatives **66-68** were more efficient than resveratrol (about 1.5-fold more active) when tested under the same conditions.⁶³

Singh *et al.* (2017) prepared phenolic 2,3-dihydrobenzo[*b*]selenophene antioxidants bearing an HO-group in *ortho*, *meta* and *para* positions with respect to the Se atom. Compound **70**, carrying the phenolic group in *ortho* position, was found to be the best catalyst, three-fold more active than (PhSe)₂. Compound **69** (HO-group

in *para* position) was slightly more active than the reference compound.⁶⁴ The antioxidant activity was determined using Method B.²⁰

Quinoline derivatives containing selenium were synthesized by Alves *et al.* in 2021. For these compounds, the antioxidant activity was assessed using Method D²³ and Method E.²⁴ The obtained results indicated that compounds **71** and **72** were effective DPPH and ABTS radical scavengers, respectively.⁶⁵

Shaaban *et al.*, in their recent papers (2018, 2022),^{66,67} obtained a series of selenides and determined their GPx-like antioxidant activity using Methods D²³ and E.²⁴ In the first article, the quinoid-based *N*-substituted maleanilic acid **73** and its corresponding methyl ester **74** were more active (approximately 1.5-fold) than ebselen **5**.⁶⁶ Compounds **75** and **76** showed GPx-like activity similar using Method D²³ like vitamin C.⁶⁷

Iwaoka *et al.*, in their reports (2010, 2015, 2017),^{21,68,69} assessed the antioxidant activity of water-soluble cyclic **77**, **81**, **83-86** and linear selenides **78-80**, **82** using Methods A¹⁹ and C²¹ in water and methanol. As a result, they managed to formulate some general characteristics of the compounds that contribute to the increased GPx-mimic activity: 1) the most preferred cyclic ring size is five, 2) generally, the reactivity of the substituents increases in the series NH₂ < HO < CO₂H in aqueous medium and *vice versa* in methanol, 3) in most cases a greater number of substituents increases the activity of the compound, 4) the stereo configuration of the substituents does not affect the activity of the compounds in water, unlike in methanol.^{21,68,69}

Mugesh *et al.* (2012, 2015) tested GPx-like antioxidant activity for diaryl selenides bearing amide moieties and spirodiazaselenuranes using Method A.¹⁹ The reactivity of the selenides **87**, **89** and the spirodiazaselenuranes **88**, **90** indicated that the substituents attached to the nitrogen atom have a significant effect on the antioxidant activity. It has been observed that the introduction of electron-withdrawing groups generally decreased, while the introduction of electron-donating groups significantly increased the GPx activity of both diaryl selenides and spirodiazaselenuranes.^{70,71}

The effect of introducing the methoxy group (in proximity to the selenium atom) on GPx-like antioxidant activity was investigated in cyclic selenium esters (2014)⁷² and a series of *o*-(hydroxymethyl)phenyl selenides (2016)⁷³ by Press and Back. In both cases, it was observed that a single electron-donating methoxy group in *para* position to selenium increased the catalytic activity. In contrast, *m*-methoxy groups have little effect, and *o*-methoxy substituents inhibit the activity. Moreover, the effects of multiple methoxy groups were not cumulative. The best peroxide scavengers (Method B²⁰) were cyclic selenium ester **91** and selenide **92**.

McNeil and Back (2016) found that the dimeric form **94** acted roughly twice as fast in Method A¹⁹ than the monomer **93** due to two redox centers in **94** instead of one in **93**. Therefore, dimeric form **94** better mimicked the multivalent selenoenzyme GPx, which possessed four redox-active selenocysteine moieties.⁷⁴

In 2020, Ścianowski *et al.* presented a new method for the synthesis of *N*-substituted unsymmetrical phenylselenides with an *o*-amido function. The highest H₂O₂-scavenging potential (Method C²¹) was observed for the derivative **95** with *N*-(3-methylbutyl) substituent. The phenylselenide **95** showed 2-fold higher activity than ebselen **5**.⁷⁵

The structures of all the derivatives mentioned above **58-95**, with high antioxidant activity, are summarized in Figure 10.

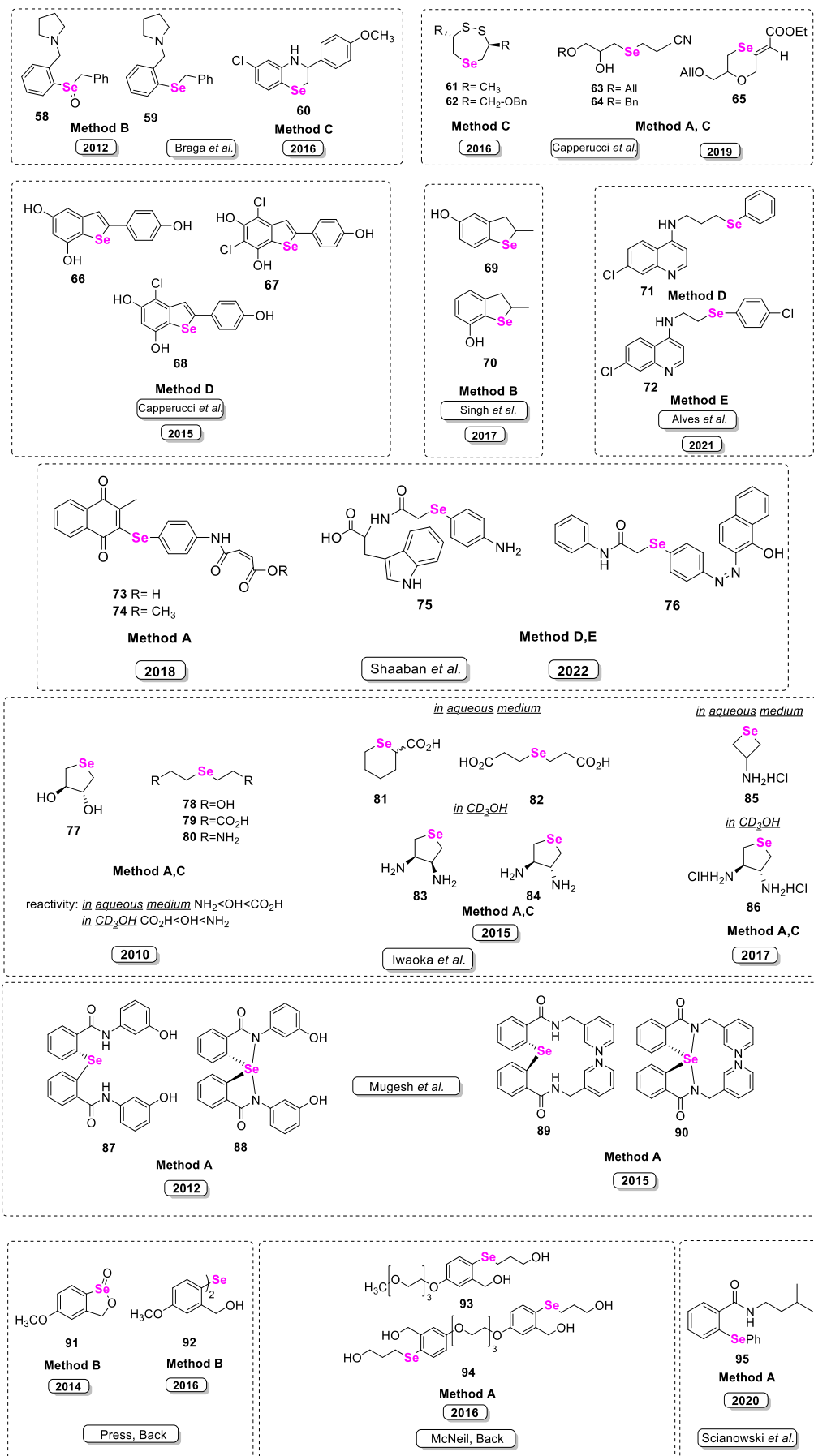


Figure 10. Other organoselenium compounds 58-95 with potential GPx-like activity.

4. Antioxidants in Other Areas

In the food industry, antioxidants are considered food additives. They protect food against fatty acid oxidation (rancidity), extend the time of shelf life consumption, and prevent the loss of food value. They react with primary oxidation products (mainly fats), creating less reactive radicals. For the general population, the main source of Se in the diet are organic forms (generally greater than 80%).⁷⁶

Ebselenols substituted with a hydroxyl group in the benzeniselenazolone ring were evaluated for their capacity to inhibit peroxidation of linoleic acid in a two-phase chlorobenzene/water system open to the atmosphere using HPLC with UV detection assay.^{77,78} While ebselenols **97** inhibited peroxidation at the same time as the standard α -tocopherol, ebselenols **96** stopped this process for much longer. In the absence of a reducing agent (ascorbic acid), ebselenol **96** scavenged peroxide radicals with a stoichiometric number as high as $n = 3$ (for comparison, α -tocopherol is known to trap two peroxy radicals per molecule, so the stoichiometric number is $n = 2$). The presented properties of ebselenols **96**, **97** (Figure 11) indicate that these compounds can be used in food technology (as antioxidants protecting food against autooxidation of fatty acids and thus prolonging the shelf time), in polymer producers, or the oil industry (as antioxidants protecting against autooxidation, which corresponds to the oxidative deterioration of organic materials).⁷⁹

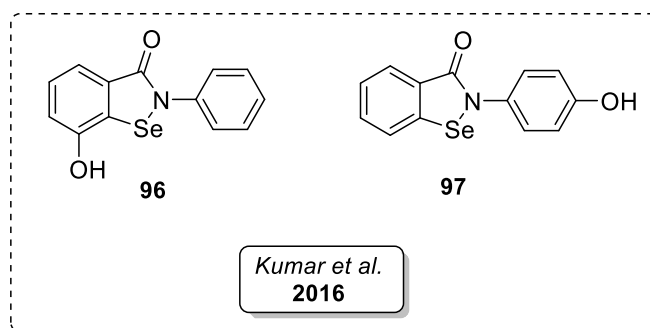


Figure 11. The structures of ebselenols **96** and **97** with high antioxidant activity.

Unique physical, chemical, and antioxidant properties have made selenium nanoparticles (SeNPs), particularly popular.⁸⁰ Additionally, SeNPs, due to their higher bioavailability and lower toxicity compared to other chemical forms of selenium, may be a promising source of selenium in the diet.⁸¹

In 2018, Huang *et al.* designed the first Se@pDA self-assembly nanocomposite that exhibited remarkable ROS scavenging property due to excellent GPx-mimic ability of selenium and polydopamine (pDA) reducibility (Figure 12). Compared to a single composite, the Se@pDA nanocomposite possessed exceptional multi-antioxidative capacity mimicking intracellular enzymatic and non-enzymatic antioxidants that constitute the antioxidant machinery system. Experimental data have shown that Se@pDA nanozymes can be effective in ameliorating the oxidative damage caused by ROS in lipids as well as in DNA, compared to individual Se or pDA nanoparticles.⁸²

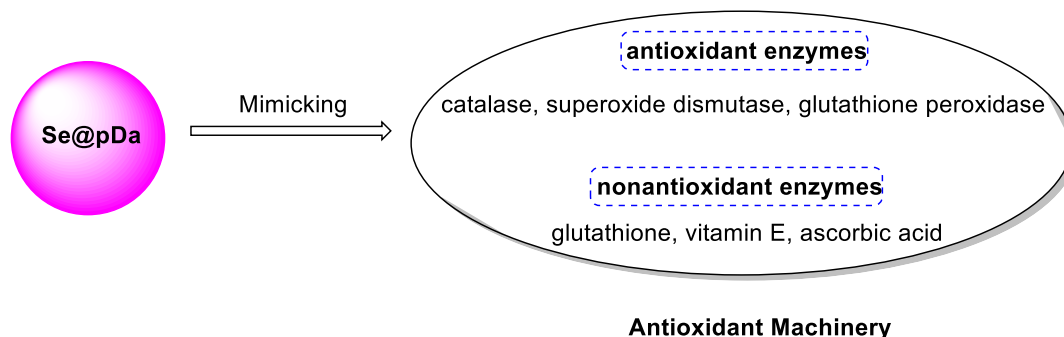


Figure 12. Illustration of Se@pDA nanozyme as a mimic of intracellular antioxidant machinery.

Selenium-containing polymers, due to the unique properties of selenium, are susceptible to various stimuli, such as light, radiation, or redox reaction. The most interesting factor, in the context of this review, is the redox (oxidative- and reductive-reactivity) stimulus.

P(EG_x-SeHC) **99**, water-soluble selenium-containing polymers, were prepared by ring-opening polymerization (ROP) of EG_x-SeHC **98** (derived from selenohomocysteine (SeHC)) and oligoethylene glycol by Lu *et al.* in 2019 (Figure 13).⁸³ The obtained polymer, is a promising protein mimic because of its peptide skeletons and helical conformations on the one hand, and on the other hand, due to the presence of selenocysteine, it shows redox responsivity. Compared to the oxidation of homocysteine-derived polypeptides,⁸⁴ which normally requires six hour treatment with 1% acetic acid and 300 mM of 1% H₂O₂ at 38 °C, P(EG_x-SeHC), **99** could be oxidized with 20 mM H₂O₂ in one hour in water at room temperature to obtain **100**. The presented features indicate that selenopolypeptides could find potential applications as chiral materials, stimuli-responsive carriers, autoxidation, and anti-inflammatory agents.

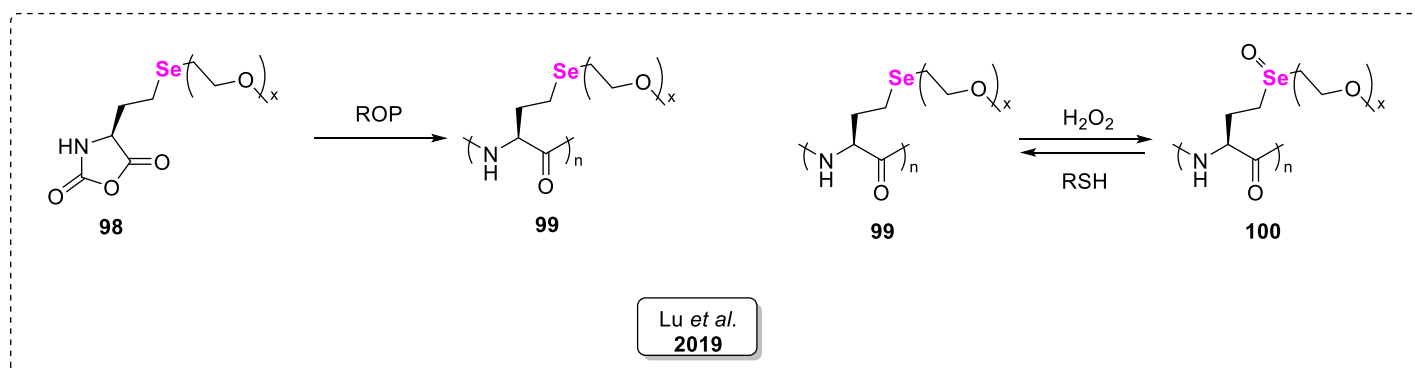


Figure 12. Preparation and oxidation of P(EG_x-SeHC) **99**.

Redox reactions play a significant role in drug delivery and the release of selenium-containing polymers. This is because selenoxide and selenone possess better hydrophilicity than selenium. Xu *et al.*, in their papers (2010, 2013),^{85,86} presented amphiphilic block polymers with selenide moieties in the main chain or in the side chain. The polymers self-assembled to form micelles, and then they were structurally disrupted under the mild conditions of 0.1% H₂O₂, releasing the desired compound. X-ray photoelectron spectroscopy (XPS) and ⁷⁷Se NMR measurements in both cases showed that transformation from selenide to selenone is associated with the change of hydrophobic to hydrophilic character.^{85,86}

5. Conclusions

Antioxidants play a significant role in protecting the body against the negative effects of ROS, excess of which is the initiator of many diseases. The development of antioxidants containing selenium in their structure is strongly related to the progress in synthesizing compounds capable of mimicking the catalytic cycle of the essential antioxidant selenoenzyme - glutathione peroxidase. Benzeneselenazoles, diselenides, selenides, selenoxides, or selenoesters are compounds that, in connection with their GPx-like activity, can be considered as potential antioxidant drugs in medicine. Ebselenols can additionally be used as diet additives in the food industry. On the other hand, the antioxidant properties of Se-containing nanoparticles and polymers provide a wide spectrum of possibilities for their use in the materials industry. This article reviews the literature from the past 12 years to 2022.

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Authors' Biographies



Magdalena Obieziurska-Fabisiak was born in Inowroclaw, Poland in 1992. At the Nicolaus Copernicus University in Toruń, she obtained her Master's degree in 2016, and in 2021 she received her Ph.D. degree in Organic Chemistry. Currently, she is working there as a research assistant. During her Ph.D. studies, she completed practice at the group of Prof. Claudio Santi (University of Perugia). Her research interest is focused on the synthesis and biological activity of new organoselenium catalysts.



Agata Pacuła-Miszewska obtained her Master's degree in Pharmacy at the Medical University of Gdańsk in 2012. In 2018, she received her PhD degree in Organic Chemistry at the Nicolaus Copernicus University in Toruń and is currently working there as an assistant professor. During her PhD studies she has completed a practice at the group of Prof. Claudio Santi (University of Perugia), Prof. Thomas Wirth (Cardiff University) and a post-doctoral internship at Prof. Stefano Menichetti Group (University of Florence). Her research interest is focused on the synthesis and activity evaluation of biologically potent organoselenium compounds.



Anna Laskowska received her Engineering degree in Chemistry and Food Technology in 2018 and her Master's degree in Chemistry in 2019 at the Nicolaus Copernicus University in Torun. Currently, she is pursuing her Ph.D. in the AST Doctoral School from the same university under the supervision of Professor Jacek Ścianowski in the Faculty of Chemistry and Department of Organic Chemistry.



Jacek Scianowski graduated from the University of Lodz in 1989. He prepared his Ph.D. thesis at the Nicolaus Copernicus University in Toruń in 1998. Currently, he works at NCU as a Full Professor and Head of the Department of Organic Chemistry. His research interests have encompassed natural product chemistry, the application of terpenes in asymmetric synthesis, chalcogen chemistry, particularly the use of selenium and tellurium derivatives in organic transformations, and medicinal chemistry.

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Publikacja 2 [P2]

Article

Attachment of Chiral Functional Groups to Modify the Activity of New GPx Mimetics

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Abstract: A series of new chiral benzenoselenazol-3(2*H*)-ones and their corresponding diselenides bearing an *o*-amido function substituted on the nitrogen atom with various aliphatic and aromatic moieties were synthesized. All derivatives representing pairs of enantiomers or diastereoisomers were obtained to thoroughly evaluate the three-dimensional structure–activity correlation. First, benzenoselenazol-3(2*H*)-ones were synthesized by reacting 2-(chloroseleno)benzoyl chloride with an appropriate enantiomerically pure amine. Then, the Se–N bond was cleaved by a reduction–oxidation procedure using sodium borohydride and then air oxidation to obtain the corresponding diselenides. All derivatives were tested as antioxidants and anticancer agents. In general, the diselenides were more reactive peroxide scavengers, with the highest activity observed for 2,2′-diselenobis[*N*-(1*S*,2*S*)-(-)-*trans*-2-hydroksy-1-indanyl]bezamide]. The most cytotoxic derivative towards human promyelocytic leukemia HL-60 and breast cancer MCF-7 cell lines was *N*-[(1*S*,2*R*)-(-)-*cis*-2-hydroksy-1-indanyl]-1,2-benzoselenazol-3(2*H*)-one. The structure–activity relationship of the obtained organoselenium derivatives was discussed.

Keywords: benzenoselenazol-3(2*H*)-ones; diselenides; pharmacophore; antioxidant activity; antiproliferative activity



Citation: Laskowska, A.; Pacuła-Miszewska, A.J.; Długosz-Pokorska, A.; Janecka, A.; Wojtczak, A.; Ścianowski, J. Attachment of Chiral Functional Groups to Modify the Activity of New GPx Mimetics. *Materials* **2022**, *15*, 2068. <https://doi.org/10.3390/ma15062068>

Academic Editor: Daniela Iannazzo

Received: 21 February 2022

Accepted: 10 March 2022

Published: 11 March 2022

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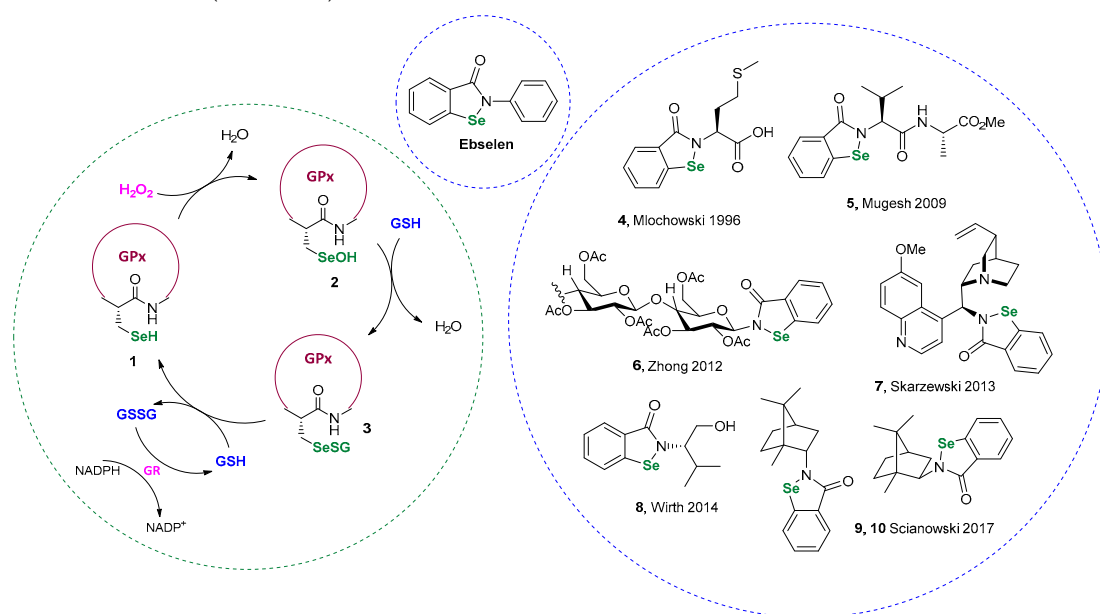
1. Introduction

As the human body is a combination of permanent changes in concentration, tension and movement, the overall biochemical processes that occur are far from maintaining equilibrium. All the reactions and transformation taking place, in most cases carried out by asymmetrical molecules—irregular yet perfectly fitted to one another—create an excellently designed system in this seemingly chaotic phenomenon. The key element which is crucial for proper enzyme function, structure and cell metabolism is the homochirality of L-amino acids and D-sugars. These small molecules impose the chirality of more complex structures such as proteins and nucleic acids, which subsequently force the chirality of the whole cell [1–3]. As a result, potential drugs generally possess fixed stereocenters to efficiently interact with specific receptors that have a characteristic spatial structure at their binding side [4–6].

Additionally, the primary biochemical role of selenium is associated with its activity as the chiral amino acid L-selenocysteine that forms the active center of the antioxidant enzyme glutathione peroxidase. This enables the elimination of excessive reactive oxygen species production and redox homeostasis preservation [7,8]. The chiral amino acid environment of GPx, which surrounds and interacts with L-Sec, is essential for stabilizing L-selenocysteine in its active-selenol form **1** [9]. As can thus be deduced, the spatial structure of the bioactive

molecule is an important feature that influences the activity of the enzyme and the entire biochemical cycle. The same assumption can be superimposed on drugs, where one enantiomer can be a potential therapeutic with the other exhibiting significant toxicity, as in the well-known case of (*R*)- and (*S*)-thalidomide [10].

To date, a multitude of GPx mimetics possessing an organoselenium moiety that imitates the characteristic activity of *L*-Sec was presented and proved efficient in both in vitro and in vivo assays [11,12]. Among them, *N*-substituted benzoselenazolones, represented by the versatile potential selenotherapeutic Ebselen, create the most explored group of derivatives with several chiral examples, including functionalized amino acids 4 and 5 [13,14], sugars 6 [15], alkaloids 7 [16], alcohols 8 [17] and terpenes 9 and 10 [18] (Scheme 1).



Scheme 1. GPx activity cycle and the structure of known chiral benzoselenazolones 4–10.

However, the difference in the biological activity of GPx mimetics, possessing distinct 3-dimensional orientation of atoms that serve to compare their epimeric/enantiomeric forms, has hardly been evaluated but was originally presented by our research group. Using enantiomerically pure terpene amines from *p*-menthane, carane and pinane systems, a series of *N*-terpenylbenzoselenazol-3(*2H*)-ones including pairs of enantiomers and diastereoisomers, were synthesized [19]. In our previous studies, we observed that antiproliferative activity towards the breast cancer cell line MCF-7 was increased by the presence of a 2-methylbutyl carbon chain (structures 11 and 12) [18]. The most potent terpene derivatives 13 and 15 confirmed this assumption. However, a significantly different result was obtained for the enantiomer of compounds 13–14 which envisioned the chirality of C1 and C4 carbon centers as the key element influencing reactivity, and that the difference in activity between the two enantiomers may be significant (Figure 1).

Herein, we planned to address this issue and identify the particular structural features that affect the biological activity of organoselenium GPx mimetics. The opposite enantiomers/diastereoisomers can exhibit stronger or weaker activity due to the change of interaction or affinity induction to other target domains. The biological activity evaluation will show whether the configuration of particular carbon centers can modulate the reactivity of the molecules. The chiral organoselenium compounds will also be obtained in two forms—the aforementioned *N*-substituted benzoselenazol-3(*2H*)-ones, with a reactive Se–N bond (193 kJ/mol), and the corresponding diselenides, possessing a Se–Se functionality with lower bond energy (172 kJ/mol) (Figure 2) [20,21].

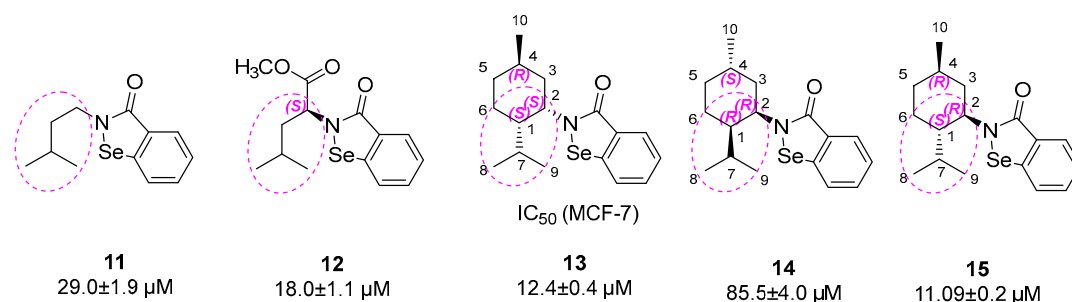


Figure 1. Structural elements influencing the reactivity of compounds 11–15.

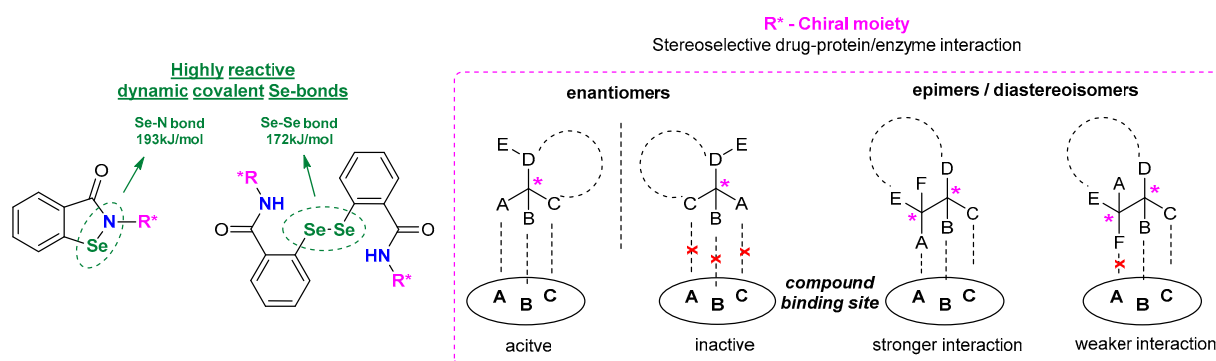


Figure 2. Structural features of the designed compounds—interaction with the target binding sites.

As presented in Scheme 1, the reactive selenol of GPx has to covalently bind with inter- or intracellular thiols to regenerate the primary reactive form. The ease of breaking the Se-bond determines the hydrogen peroxide reduction and enzyme regeneration rate. This way, the synthesis of the presented compounds will enable recognizing the influence of the chiral moiety and the reactivity of two different types of organoselenium groups on the bioactivity of the GPx mimetics.

2. Materials and Methods

2.1. General

NMR spectra were recorded on Bruker Avance III/400 or Bruker Avance III/700 (Karlsruhe, Germany) for ¹H and 176.1 MHz or 100.6 MHz for ¹³C (see Supplementary Materials). Chemical shifts were recorded relative to SiMe₄ (δ0.00) or solvent resonance (CDCl₃ δ7.26, CD₃OD δ3.31). Multiplicities were given as: s (singlet); d (doublet); dd (double doublet); ddd (double doublet); t (triplet); dt (double triplet); and m (multiplet). The ⁷⁷Se NMR spectra were recorded on Bruker Avance III/400 or Bruker Avance III/700 with diphenyl diselenide as an external standard. NMR spectra were carried out using the ACD/NMR Processor Academic Edition. Melting points were measured with a Büchi Tottoli SPM-20 heating unit (Büchi Labortechnik AG, Flawil, Switzerland) and were uncorrected. Elemental analyses were performed on a Vario MACRO CHN analyzer (Elementar Analysensysteme GmbH, Langensfeld, Germany). Optical rotations were measured in 10 mm cells with a polAAR 3000 polarimeter (Optical Activity Limited, Ramsey, United Kingdom). Column chromatography was performed using Merck 40-63D 60 Å silica gel (Merck, Darmstadt, Germany).

2.2. Procedures and Analysis Data

Compounds were synthesized according to the previously presented procedure [18].
N-[(S)-(+)-*sec*-butyl]-1,2-benzisoselenazol-3(2H)-one **18a**

Yield: 85%; mp 51–52 °C; [α]_D²⁰ = +40 (c = 1.12, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 0.84 (t, J = 7.0 Hz, 3H), 1.26 (d, J = 7.0 Hz, 3H), 1.58–1.69 (m, 2H), 4.39–4.44 (m, 1H), 7.41–7.44 (m, 1H_{ar}), 7.58–7.62 (m, 1H_{ar}), 7.79–7.82 (m, 1H_{ar}),

8.03–8.06 (m, 1H_{ar}) ¹³C NMR (700 MHz, DMSO) δ = 11.40 (CH₃), 21.74 (CH₃), 30.57 (CH₂), 51.31 (CH), 126.24 (2xCH_{ar}), 127.78 (CH_{ar}), 129.26 (C_{ar}), 131.74 (CH_{ar}), 139.22 (C_{ar}), 166.67 (C=O) ⁷⁷Se NMR 700 MHz, DMSO) δ = 804.33 ppm; IR = 2964, 2873, 1738, 1589, 1562, 1443, 1323, 1247, 1217, 1147, 1046, 1020, 788, 737, 697, 591, 509, 416 cm⁻¹. Elemental Anal. Calcd for C₁₁H₁₃NOSe (255.02): C, 51.98; H, 5.15; N, 5.51; Found C, 51.93; H, 5.15; N, 5.49.

N-[(*R*)-(-)-*sec*-butyl]-1,2-benzisoselenazol-3(2*H*)-one **19a**

Yield: 67%; mp 50–52 °C; [α]_D²⁰ = -38 (c = 1.24, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 0.84 (t, J = 7.0 Hz, 3H), 1.26 (d, J = 6.3 Hz, 3H), 1.57–1.69 (m, 2H), 4.39–4.44 (m, 1H), 7.40–7.44 (m, 1H_{ar}), 7.58–7.62 (m, 1H_{ar}), 7.79–7.82 (m, 1H_{ar}), 8.03–8.06 (m, 1H_{ar}) ¹³C NMR (400 MHz, DMSO) δ = 11.42 (CH₃), 21.77 (CH₃), 30.57 (CH₂), 51.26 (CH), 126.22 (CH_{ar}), 126.27 (CH_{ar}), 127.76 (CH_{ar}), 129.28 (C_{ar}), 131.71 (CH_{ar}), 139.25 (C_{ar}), 166.64 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 802.56 ppm; IR = 2965, 2873, 1737, 1589, 1563, 1443, 1323, 1246, 1216, 1148, 1047, 1020, 788, 737, 697, 592, 508, 414 cm⁻¹. Elemental Anal. Calcd for C₁₁H₁₃NOSe (255.02): C, 51.98; H, 5.15; N, 5.51; Found C, 51.95; H, 5.16; N, 5.47.

N-[(*S*)-(+)-1-hydroxy-2-butanyl]-1,2-benzisoselenazol-3(2*H*)-one **20a**

Yield: 45%; mp 108–109 °C; [α]_D²⁰ = +47 (c = 0.47, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 0.81 (t, J = 7.7 Hz, 3H), 1.53–1.60 (m, 1H), 1.71–1.77 (m, 1H), 3.52–3.56 (m, 1H), 3.64–3.68 (m, 1H), 4.40–4.44 (m, 1H), 5.14 (t, J = 5.6 Hz, 1H), 7.39–7.42 (m, 1H_{ar}), 7.53–7.61 (m, 1H_{ar}), 7.81 (dd, J₁ = 1.4 Hz, J₂ = 7.0 Hz, 1H_{ar}), 8.01–8.04 (m, 1H_{ar}) ¹³C NMR (700 MHz, DMSO) δ = 11.09 (CH₃), 24.98 (CH₂), 56.94 (CH), 63.77 (CH₂), 125.96 (2xCH_{ar}), 127.72 (CH_{ar}), 128.58 (C_{ar}), 131.63 (CH_{ar}), 140.55 (C_{ar}), 167.32 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 839.29 ppm; IR = 3224, 2959, 2871, 1590, 1562, 1444, 1338, 1310, 1252, 1082, 1020, 791, 739, 676, 601, 549, 483, 422 cm⁻¹. Elemental Anal. Calcd for C₁₁H₁₃NO₂Se (271.01): C, 48.90; H, 4.85; N, 5.18; Found C, 48.92; H, 4.85; N, 5.11.

N-[(*R*)-(-)-1-hydroxy-2-butanyl]-1,2-benzisoselenazol-3(2*H*)-one **21a**

Yield: 57%; mp 109–110 °C; [α]_D²⁰ = -41 (c = 0.83, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 0.81 (t, J = 7.7 Hz, 3H), 1.53–1.60 (m, 1H), 1.71–1.77 (m, 1H), 3.51–3.57 (m, 1H), 3.64–3.68 (m, 1H), 4.38–4.44 (m, 1H), 5.12 (t, J = 4.9 Hz, 1H), 7.39–7.42 (m, 1H_{ar}), 7.57–7.61 (m, 1H_{ar}), 7.80–7.83 (m, 1H_{ar}), 8.02 (d, J = 7.7 Hz, 1H_{ar}) ¹³C NMR (400 MHz, DMSO) δ = 11.10 (CH₃), 24.96 (CH₂), 56.87 (CH), 63.73 (CH₂), 125.96 (CH_{ar}), 125.98 (CH_{ar}), 127.70 (CH_{ar}), 128.57 (C_{ar}), 131.63 (CH_{ar}), 140.56 (C_{ar}), 167.30 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 837.92 ppm; IR = 3233, 2959, 2871, 1591, 1562, 1444, 1338, 1310, 1252, 1081, 1020, 791, 741, 676, 600, 549, 484, 421 cm⁻¹. Elemental Anal. Calcd for C₁₁H₁₃NO₂Se (271.01): C, 48.90; H, 4.85; N, 5.18; Found C, 48.93; H, 4.84; N, 5.18.

N-[(*R*)-(-)-1,2,3,4-tetrahydro-1-naphthyl]-1,2-benzisoselenazol-3(2*H*)-one **22a**

Yield: 96%; mp 217–220 °C; [α]_D²⁰ = -50 (c = 0.58, CHCl₃) (lit. mp 212–214 °C; [α]_D²⁰ = -0.53 (c = 0.075, CHCl₃) [17])

¹H NMR (700 MHz, DMSO) δ = 1.81–1.87 (m, 1H), 1.95–2.05 (m, 2H), 2.09–2.15 (m, 1H), 2.76–2.81 (m, 1H), 2.87–2.92 (m, 1H), 5.60 (t, J = 7 Hz, 1H), 7.02–7.06 (m, 1H_{ar}), 7.13–7.25 (m, 3H_{ar}), 7.43–7.47 (m, 1H_{ar}), 7.59–7.62 (m, 1H_{ar}), 7.84–7.89 (m, 1H_{ar}), 7.96 (d, J = 7.7 Hz, 1H) ¹³C NMR (400 MHz, DMSO) δ = 20.57 (CH₂), 29.16 (CH₂), 30.77 (CH₂), 51.93 (CH), 126.05 (CH_{ar}), 126.32 (CH_{ar}), 126.48 (CH_{ar}), 127.86 (CH_{ar}), 128.13 (CH_{ar}), 128.75 (CH_{ar}), 129.40 (CH_{ar}), 129.50 (CH_{ar}), 131.95 (C_{ar}), 136.21 (C_{ar}), 138.12 (C_{ar}), 139.77 (C_{ar}), 166.88 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 830.37 ppm; IR = 2919, 2857, 1588, 1561, 1491, 1441, 1308, 1268, 1245, 1154, 1082, 769, 733, 677, 572, 534, 511, 481, 418 cm⁻¹. Elemental Anal. Calcd for C₁₇H₁₅NOSe (329.03): C, 62.20; H, 4.61; N, 4.27; Found C, 62.25; H, 4.62; N, 4.29.

N-[(*S*)-(+)-1,2,3,4-tetrahydro-1-naphthyl]-1,2-benzisoselenazol-3(2*H*)-one **23a**

Yield: 94%; mp 218–220 °C; [α]_D²⁰ = +54 (c = 0.42, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 1.77–1.84 (m, 1H), 1.92–2.02 (m, 2H), 2.06–2.12 (m, 1H), 2.72–2.79 (m, 1H), 2.83–2.89 (m, 1H), 5.57 (t, J = 7 Hz, 1H), 7.00–7.03 (m, 1H_{ar}), 7.10–7.22 (m, 3H_{ar}), 7.40–7.44 (m, 1H_{ar}), 7.55–7.59 (m, 1H_{ar}), 7.84–7.86 (m, 1H_{ar}), 7.93 (d, J = 7.7 Hz, 1H) ¹³C NMR (400 MHz, DMSO) δ = 20.58 (CH₂), 29.16 (CH₂), 30.77 (CH₂), 51.92 (CH), 126.06 (CH_{ar}), 126.31 (CH_{ar}), 126.47 (CH_{ar}), 127.85 (CH_{ar}), 128.11 (CH_{ar}), 128.76 (CH_{ar}), 129.40 (CH_{ar}),

129.50 (CH_{ar}), 131.93 (C_{ar}), 136.22 (C_{ar}), 138.12 (C_{ar}), 139.77 (C_{ar}), 166.86 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 830.01 ppm; IR = 2921, 2853, 1589, 1561, 1492, 1441, 1308, 1269, 1245, 1154, 1081, 769, 734, 676, 572, 534, 512, 481, 419 cm⁻¹. Elemental Anal. Calcd for C₁₇H₁₅NOSe (329.03): C, 62.20; H, 4.61; N, 4.27; Found C, 62.29; H, 4.62; N, 4.21.

N-[(*R*)-(+)-*α*-methylbenzyl]-1,2-benzisoselenazol-3(2*H*)-one **24a**

Yield: 49%; mp 108–110 °C; [α]_D²⁰ = +128 (c = 1.04, CHCl₃) (lit. mp 116–117.5 °C; [α]_D²⁰ = +120 (c = 1.00, C₂H₅OH) [13])

¹H NMR (700 MHz, DMSO) δ = 1.68 (d, J = 7 Hz, 3H), 5.67 (q, J = 7 Hz, 1H), 7.30–7.33 (m, 1H_{ar}), 7.36–7.42 (m, 4H_{ar}), 7.43–7.45 (m, 1H_{ar}), 7.58–7.61 (m, 1H_{ar}), 7.83 (dd, J₁ = 0.7 Hz, J₂ = 7.7 Hz, 1H_{ar}), 7.99 (d, J = 7.7 Hz, 1H) ¹³C NMR (700 MHz, DMSO) δ = 20.86 (CH₃), 52.37 (CH), 126.18 (CH_{ar}), 126.32 (CH_{ar}), 127.40 (2xCH_{ar}), 127.83 (CH_{ar}), 128.11 (CH_{ar}), 128.99 (2xCH_{ar}), 129.03 (C_{ar}), 131.88 (CH_{ar}), 139.58 (C_{ar}), 142.61 (C_{ar}), 166.34 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 819.68 ppm; IR = 2921, 1590, 1560, 1492, 1441, 1308, 1249, 1154, 1115, 1059, 759, 738, 695, 610, 562, 538, 504, 480, 420 cm⁻¹. Elemental Anal. Calcd for C₁₅H₁₃NOSe (303.02): C, 59.61; H, 4.34; N, 4.63; Found C, 59.68; H, 4.35; N, 4.60.

N-[(*S*)-(–)-*α*-methylbenzyl]-1,2-benzisoselenazol-3(2*H*)-one **25a**

Yield: 95%; mp 109–111 °C; [α]_D²⁰ = –123 (c = 1.02, CHCl₃) (lit. mp 116–117.5 °C; [α]_D²⁰ = –120 (c = 1.00, C₂H₅OH) [13])

¹H NMR (700 MHz, DMSO) δ = 1.68 (d, J = 7 Hz, 3H), 5.67 (q, J = 7 Hz, 1H), 7.30–7.33 (m, 1H_{ar}), 7.37–7.42 (m, 4H_{ar}), 7.42–7.45 (m, 1H_{ar}), 7.58–7.61 (m, 1H_{ar}), 7.83 (dd, J₁ = 1.4 Hz, J₂ = 7.7 Hz, 1H_{ar}), 8.00 (d, J = 7.7 Hz, 1H) ¹³C NMR (700 MHz, DMSO) δ = 20.86 (CH₃), 52.41 (CH), 126.18 (CH_{ar}), 126.32 (CH_{ar}), 127.40 (2xCH_{ar}), 127.83 (CH_{ar}), 128.11 (CH_{ar}), 128.99 (2xCH_{ar}), 129.02 (C_{ar}), 131.89 (CH_{ar}), 139.59 (C_{ar}), 142.60 (C_{ar}), 166.36 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 820.47 ppm; IR = 2924, 1590, 1561, 1492, 1441, 1307, 1245, 1158, 1059, 759, 738, 695, 610, 560, 538, 504, 448, 416 cm⁻¹. Elemental Anal. Calcd for C₁₅H₁₃NOSe (303.02): C, 59.61; H, 4.34; N, 4.63; Found C, 59.63; H, 4.34; N, 4.58.

N-[(*S*)-(–)-1-(1-naphthyl)ethyl]-1,2-benzisoselenazol-3(2*H*)-one **26a**

Yield: 76%; mp 136–138 °C; [α]_D²⁰ = –241 (c = 0.72, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 1.78 (d, J = 7.0 Hz, 3H), 6.37 (q, J = 6.3 Hz, 1H), 7.40–7.44 (m, 1H_{ar}), 7.50–7.56 (m, 3H_{ar}), 7.60–7.63 (m, 1H_{ar}), 7.82 (d, J = 7 Hz, 1H_{ar}), 7.84–7.89 (m, 2H_{ar}), 7.94–8.02 (m, 3H_{ar}) ¹³C NMR (700 MHz, DMSO) δ = 19.72 (CH₃), 48.32 (CH), 123.37 (CH_{ar}), 123.97 (CH_{ar}), 125.67 (CH_{ar}), 126.13 (CH_{ar}), 126.30 (CH_{ar}), 126.44 (CH_{ar}), 127.29 (CH_{ar}), 127.90 (CH_{ar}), 129.00 (C_{ar}), 129.21 (CH_{ar}), 129.48 (CH_{ar}), 131.63 (C_{ar}), 131.82 (CH_{ar}), 133.99 (C_{ar}), 137.91 (C_{ar}), 139.73 (C_{ar}), 165.81 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 816.43 ppm; IR = 2924, 2853, 1590, 1562, 1442, 1341, 1309, 1245, 1079, 1020, 798, 736, 674, 605, 523, 505, 453 cm⁻¹. Elemental Anal. Calcd for C₁₉H₁₅NOSe (353.03): C, 64.78; H, 4.29; N, 3.98; Found C, 64.82; H, 4.29; N, 3.90.

N-[(*R*)-(+)-1-(1-naphthyl)ethyl]-1,2-benzisoselenazol-3(2*H*)-one **27a**

Yield: 65%; mp 135–137 °C; [α]_D²⁰ = +245 (c = 0.62, CHCl₃) (lit. mp 130–132 °C; [α]_D²⁰ = +2.62 (c = 0.28, CHCl₃) [17])

¹H NMR (700 MHz, DMSO) δ = 1.78 (d, J = 7.7 Hz, 3H), 6.37 (q, J = 6.3 Hz, 1H), 7.40–7.44 (m, 1H_{ar}), 7.50–7.56 (m, 3H_{ar}), 7.60–7.63 (m, 1H_{ar}), 7.82 (d, J = 7 Hz, 1H_{ar}), 7.84–7.89 (m, 2H_{ar}), 7.94–8.02 (m, 3H_{ar}) ¹³C NMR (700 MHz, DMSO) δ = 19.74 (CH₃), 48.29 (CH), 123.38 (CH_{ar}), 123.96 (CH_{ar}), 125.68 (CH_{ar}), 126.14 (CH_{ar}), 126.30 (CH_{ar}), 126.44 (CH_{ar}), 127.28 (CH_{ar}), 127.88 (CH_{ar}), 129.02 (C_{ar}), 129.21 (CH_{ar}), 129.46 (CH_{ar}), 131.63 (C_{ar}), 131.80 (CH_{ar}), 133.99 (C_{ar}), 137.94 (C_{ar}), 139.73 (C_{ar}), 165.77 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 815.51 ppm; IR = 2923, 2853, 1590, 1561, 1456, 1440, 1343, 1309, 1268, 1079, 1020, 797, 739, 675, 606, 522, 504, 453 cm⁻¹. Elemental Anal. Calcd for C₁₉H₁₅NOSe (353.03): C, 64.78; H, 4.29; N, 3.98; Found C, 64.77; H, 4.30; N, 3.89.

N-[(1*S*,2*R*)-(–)-*cis*-2-hydroxy-1-indanyl]-1,2-benzisoselenazol-3(2*H*)-one **28a**

Yield: 71%; mp 187–189 °C; [α]_D²⁰ = –97 (c = 0.61, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 2.86–2.89 (m, 1H), 3.14–3.18 (m, 1H), 4.57–4.61 (m, 1H), 5.56 (d, J = 4.9 Hz, 1H), 5.95 (d, J = 4.9 Hz, 1H), 6.90 (d, J = 7.7 Hz, 1H_{ar}), 7.14 (t, J = 7.0 Hz, 1H_{ar}), 7.23 (t, J = 7.7 Hz, 1H_{ar}), 7.28–7.31 (m, 1H_{ar}), 7.38–7.41 (m, 1H_{ar}), 7.55–7.59 (m, 1H_{ar}),

7.87–7.90 (m, 1H_{ar}), 7.95 (d, J = 7.7 Hz, 1H) ¹³C NMR (400 MHz, DMSO) δ = 39.52 (CH₂), 61.36 (CH), 73.06 (CH), 124.73 (CH_{ar}), 125.68 (CH_{ar}), 125.71 (CH_{ar}), 125.78 (CH_{ar}), 126.91 (CH_{ar}), 127.76 (CH_{ar}), 127.86 (CH_{ar}), 128.37 (CH_{ar}), 131.83 (CH_{ar}), 141.06 (C_{ar}), 141.46 (C_{ar}), 142.28 (C_{ar}), 168.25 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 877.05 ppm; IR = 3259, 2922, 2853, 1621, 1533, 1458, 1446, 1349, 1311, 1259, 1047, 1026, 733, 680, 517, 475, 415 cm⁻¹. Elemental Anal. Calcd for C₁₆H₁₃NO₂Se (331.01): C, 58.19; H, 3.97; N, 4.24; Found C, 58.11; H, 3.96; N, 4.29.

N-[(1*R*,2*S*)-(+)-*cis*-2-hydroxy-1-indanyl]-1,2-benzisoselenazol-3(2*H*)-one **29a**

Yield: 51%; mp 187–189 °C; [α]_D²⁰ = +100 (c = 0.59, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 2.89–2.92 (m, 1H), 3.17–3.21 (m, 1H), 4.60–4.64 (m, 1H), 5.59 (d, J = 4.2 Hz, 1H), 5.98 (d, J = 4.9 Hz, 1H), 6.93 (d, J = 7.7 Hz, 1H_{ar}), 7.16 (t, J = 4.9 Hz, 1H_{ar}), 7.26 (t, J = 7.7 Hz, 1H_{ar}), 7.31–7.34 (m, 1H_{ar}), 7.41–7.44 (m, 1H_{ar}), 7.58–7.62 (m, 1H_{ar}), 7.90–7.93 (m, 1H_{ar}), 7.98 (d, J = 7.7 Hz, 1H) ¹³C NMR (400 MHz, DMSO) δ = 39.56 (CH₂), 61.37 (CH), 74.07 (CH), 124.74 (CH_{ar}), 125.68 (CH_{ar}), 125.71 (CH_{ar}), 125.78 (CH_{ar}), 126.90 (CH_{ar}), 127.76 (CH_{ar}), 127.86 (CH_{ar}), 128.37 (CH_{ar}), 131.82 (CH_{ar}), 141.06 (C_{ar}), 141.47 (C_{ar}), 142.26 (C_{ar}), 168.24 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 877.00 ppm; IR = 3234, 2950, 2853, 1602, 1566, 1457, 1445, 1337, 1312, 1271, 1061, 1019, 756, 738, 677, 517, 476, 417 cm⁻¹. Elemental Anal. Calcd for C₁₆H₁₃NO₂Se (331.01): C, 58.19; H, 3.97; N, 4.24; Found C, 58.13; H, 3.98; N, 4.25.

N-[(1*S*,2*S*)-(+)-*trans*-2-hydroxy-1-indanyl]-1,2-benzisoselenazol-3(2*H*)-one **30a**

Yield: 87%; mp 204–206 °C; [α]_D²⁰ = +212 (c = 0.55, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 2.80–2.84 (m, 1H), 3.24–3.28 (m, 1H), 4.43 (kw, J = 5.6 Hz, 1H), 5.57 (d, J = 4.9 Hz, 1H), 5.70 (d, J = 5.6 Hz, 1H), 7.10 (d, J = 7.7 Hz, 1H_{ar}), 7.20–7.24 (m, 1H_{ar}), 7.31 (d, J = 4.1 Hz, 2H_{ar}), 7.44–7.48 (m, 1H_{ar}), 7.60–7.63 (m, 1H_{ar}), 7.87–7.90 (m, 1H_{ar}), 7.98 (d, J = 7.7 Hz, 1H) ¹³C NMR (300 MHz, DMSO) δ = 39.01 (CH₂), 65.63 (CH), 78.88 (CH), 125.18 (CH_{ar}), 125.46 (CH_{ar}), 126.14 (CH_{ar}), 126.31 (CH_{ar}), 127.32 (CH_{ar}), 127.80 (CH_{ar}), 128.88 (CH_{ar}), 129.01 (C_{ar}), 131.95 (CH_{ar}), 139.56 (C_{ar}), 140.85 (C_{ar}), 140.95 (C_{ar}), 167.38 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 824.06 ppm; IR = 3091, 2923, 2850, 1590, 1562, 1460, 1445, 1329, 1312, 1268, 1072, 1021, 793, 743, 733, 676, 516, 458 cm⁻¹. Elemental Anal. Calcd for C₁₆H₁₃NO₂Se (331.01): C, 58.09; H, 3.98; N, 4.26; Found C, 58.15; H, 3.97; N, 4.22.

N-[(1*R*,2*R*)-(–)-*trans*-2-hydroxy-1-indanyl]-1,2-benzisoselenazol-3(2*H*)-one **31a**

Yield: 66%; mp 205–207 °C; [α]_D²⁰ = –214 (c = 0.57, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 2.80–2.85 (m, 1H), 3.24–3.28 (m, 1H), 4.43 (kw, J = 5.6 Hz, 1H), 5.56 (d, J = 4.9 Hz, 1H), 5.69 (d, J = 5.6 Hz, 1H), 7.10 (d, J = 9.1 Hz, 1H_{ar}), 7.20–7.24 (m, 1H_{ar}), 7.31 (d, J = 7.7 Hz, 2H_{ar}), 7.44–7.47 (m, 1H_{ar}), 7.60–7.63 (m, 1H_{ar}), 7.88–7.90 (m, 1H_{ar}), 7.98 (d, J = 7.7 Hz, 1H) ¹³C NMR (400 MHz, DMSO) δ = 39.33 (CH₂), 65.71 (CH), 78.93 (CH), 125.24 (CH_{ar}), 125.51 (CH_{ar}), 126.20 (CH_{ar}), 126.35 (CH_{ar}), 127.36 (CH_{ar}), 127.85 (CH_{ar}), 128.95 (CH_{ar}), 129.04 (C_{ar}), 131.98 (CH_{ar}), 139.62 (C_{ar}), 140.94 (C_{ar}), 141.03 (C_{ar}), 167.42 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 823.38 ppm; IR = 3090, 2923, 2852, 1590, 1562, 1460, 1445, 1329, 1312, 1269, 1071, 1021, 793, 744, 733, 676, 516, 457 cm⁻¹. Elemental Anal. Calcd for C₁₆H₁₃NO₂Se (331.01): C, 58.19; H, 3.97; N, 4.24; Found C, 58.15; H, 3.97; N, 4.22.

2,2'-diselenobis[*N*-(*S*)-(+)-*sec*-butylbezamide] **18b**

Yield: 66%; mp 233–235 °C; [α]_D²⁰ = +43 (c = 0.24, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 0.92 (t, J = 7.7 Hz, 3H), 1.18 (d, J = 7.0 Hz, 3H), 1.50–1.60 (m, 2H), 3.92–3.97 (m, 1H), 7.31–7.34 (m, 1H_{ar}), 7.36–7.39 (m, 1H_{ar}), 7.69 (d, J = 7.7 Hz, 1H_{ar}), 7.79 (d, J = 7.7 Hz, 1H_{ar}), 8.44 (d, J = 7.7 Hz, 1H_{ar}) ¹³C NMR (700 MHz, DMSO) δ = 11.15 (CH₃), 20.59 (CH₃), 29.26 (CH₂), 47.33 (CH), 126.55 (CH_{ar}), 128.30 (CH_{ar}), 130.41 (CH_{ar}), 131.79 (CH_{ar}), 132.31 (C_{ar}), 134.35 (C_{ar}), 167.36 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 443.25' ppm; IR: 3312, 2968, 2927, 2870, 1738, 1612, 1583, 1535, 1448, 1435, 1366, 1353, 1302, 1285, 1229, 1216, 1163, 1146, 1027, 871, 743, 677, 645, 541, 472, 446 cm⁻¹. Elemental Anal. Calcd for C₂₂H₂₈N₂O₂Se₂ (512.08): C, 51.77; H, 5.53; N, 5.49; Found C, 51.72; H, 5.53; N, 5.52.

2,2'-diselenobis[*N*-(*R*)-(–)-*sec*-butylbezamide] **19b**

Yield: 74%; mp 234–235 °C; $[\alpha]_D^{20} = -41$ (c = 0.30, CHCl₃);

¹H NMR (700 MHz, DMSO) $\delta = 0.91$ (t, J = 7.7 Hz, 3H), 1.18 (d, J = 7.0 Hz, 3H), 1.50–1.60 (m, 2H), 3.92–3.97 (m, 1H), 7.31–7.34 (m, 1H_{ar}), 7.36–7.39 (m, 1H_{ar}), 7.69 (d, J = 8.4 Hz, 1H_{ar}), 7.79 (d, J = 7.7 Hz, 1H_{ar}), 8.44 (d, J = 7.7 Hz, 1H_{ar}) ¹³C NMR (700 MHz, DMSO) $\delta = 11.15$ (CH₃), 20.60 (CH₃), 29.27 (CH₂), 47.32 (CH), 126.54 (CH_{ar}), 128.30 (CH_{ar}), 130.41 (CH_{ar}), 131.78 (CH_{ar}), 132.32 (C_{ar}), 134.36 (C_{ar}), 167.35 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) $\delta = 443.17$ ppm; IR: 3311, 2968, 2927, 2870, 1738, 1613, 1583, 1535, 1448, 1435, 1366, 1353, 1302, 1284, 1229, 1216, 1164, 1145, 1027, 872, 743, 678, 645, 528, 472, 446 cm⁻¹. Elemental Anal. Calcd for C₂₂H₂₈N₂O₂Se₂ (512.08): C, 51.77; H, 5.53; N, 5.49; Found C, 51.70; H, 5.53; N, 5.45.

2,2'-diselenobis[N-(S)-(+)-1-hydroxy-2-butanylbezamide] **20b**

Yield: 52%; mp 200–202 °C; $[\alpha]_D^{20} = +65$ (c = 0.31, CHCl₃);

¹H NMR (700 MHz, DMSO) $\delta = 0.92$ (t, J = 7.7 Hz, 3H), 1.44–1.52 (m, 1H), 1.66–1.73 (m, 1H), 3.41–3.46 (m, 1H), 3.49–3.53 (m, 1H), 3.86–3.93 (m, 1H), 4.73 (t, J = 6.3 Hz, 1H), 7.31–7.35 (m, 1H_{ar}), 7.36–7.40 (m, 1H_{ar}), 7.70 (dd, J₁ = 1.4 Hz, J₂ = 8.4 Hz, 1H_{ar}), 7.84 (dd, J₁ = 1.4 Hz, J₂ = 8.4 Hz, 1H_{ar}), 8.31 (d, J = 8.4 Hz, 1H_{ar}) ¹³C NMR (700 MHz, DMSO) $\delta = 11.05$ (CH₃), 24.12 (CH₂), 53.94 (CH), 63.51 (CH₂), 126.50 (CH_{ar}), 128.43 (CH_{ar}), 130.40 (CH_{ar}), 131.82 (CH_{ar}), 132.38 (C_{ar}), 134.27 (C_{ar}), 167.91 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) $\delta = 444.00$ ppm; IR: 3278, 3045, 2969, 2870, 1738, 1622, 1583, 1530, 1453, 1366, 1306, 1283, 1216, 1179, 1165, 1076, 1060, 1048, 1025, 873, 727, 692, 644, 557, 447 cm⁻¹. Elemental Anal. Calcd for C₂₂H₂₈N₂O₄Se₂ (544.04): C, 48.72; H, 5.20; N, 5.16; Found C, 48.65; H, 5.21; N, 5.11.

2,2'-diselenobis[N-(R)-(-)-1-hydroxy-2-butanylbezamide] **21b**

Yield: 46%; mp 199–201 °C; $[\alpha]_D^{20} = -67$ (c = 0.41, CHCl₃);

¹H NMR (700 MHz, DMSO) $\delta = 0.89$ (t, J = 7.0 Hz, 3H), 1.41–1.49 (m, 1H), 1.63–1.70 (m, 1H), 3.38–3.43 (m, 1H), 3.46–3.50 (m, 1H), 3.83–3.89 (m, 1H), 4.69 (t, J = 5.6 Hz, 1H), 7.27–7.31 (m, 1H_{ar}), 7.33–7.37 (m, 1H_{ar}), 7.67 (dd, J₁ = 0.7 Hz, J₂ = 8.4 Hz, 1H_{ar}), 7.81 (dd, J₁ = 1.4 Hz, J₂ = 7.7 Hz, 1H_{ar}), 8.27 (d, J = 8.4 Hz, 1H_{ar}) ¹³C NMR (700 MHz, DMSO) $\delta = 11.05$ (CH₃), 24.12 (CH₂), 53.95 (CH), 63.51 (CH₂), 126.50 (CH_{ar}), 128.43 (CH_{ar}), 130.42 (CH_{ar}), 131.82 (CH_{ar}), 132.36 (C_{ar}), 134.27 (C_{ar}), 167.92 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) $\delta = 443.96$ ppm; IR: 3276, 3047, 2969, 2870, 1738, 1622, 1584, 1530, 1453, 1365, 1306, 1283, 1216, 1179, 1165, 1076, 1060, 1047, 1025, 873, 727, 691, 644, 557, 448 cm⁻¹. Elemental Anal. Calcd for C₂₂H₂₈N₂O₄Se₂ (544.04): C, 48.72; H, 5.20; N, 5.16; Found C, 48.69; H, 5.20; N, 5.09.

2,2'-diselenobis[N-(R)-(-)-1,2,3,4-tetrahydro-1-naphthylbezamide] **22b**

Yield: 58%; mp 266–268 °C; $[\alpha]_D^{20} = -167$ (c = 0.21, CHCl₃);

¹H NMR (700 MHz, DMSO) $\delta = 1.69$ –1.81 (m, 1H), 1.81–1.88 (m, 1H), 1.94–2.05 (m, 2H), 2.72–2.82 (m, 2H), 5.25 (q, J = 7 Hz, 1H), 7.09–7.19 (m, 3H_{ar}), 7.23–7.31 (m, 2H_{ar}), 7.34–7.39 (m, 1H_{ar}), 7.74 (dd, J₁ = 1.4 Hz, J₂ = 7.7 Hz, 1H_{ar}), 7.81 (dd, J₁ = 1.4 Hz, J₂ = 7.7 Hz, 1H_{ar}), 9.05 (d, J = 9.1 Hz, 1H) ¹³C NMR (700 MHz, DMSO) $\delta = 20.84$ (CH₂), 29.37 (CH₂), 30.30 (CH₂), 48.02 (CH), 126.35 (CH_{ar}), 126.60 (CH_{ar}), 127.24 (CH_{ar}), 128.35 (CH_{ar}), 128.56 (CH_{ar}), 129.27 (CH_{ar}), 130.52 (CH_{ar}), 131.99 (C_{ar}), 132.62 (CH_{ar}), 134.02 (C_{ar}), 137.73 (C_{ar}), 137.76 (C_{ar}), 167.61 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) $\delta = 445.03$ ppm; IR: 3279, 2940, 2923, 1627, 1583, 1530, 1486, 1430, 1335, 1256, 1080, 1024, 878, 865, 758, 682, 593, 447 cm⁻¹. Elemental Anal. Calcd for C₃₄H₃₂N₂O₂Se₂ (660.08): C, 62.01; H, 4.90; N, 4.25; Found C, 61.95; H, 4.91; N, 4.17.

2,2'-diselenobis[N-(S)-(+)-1,2,3,4-tetrahydro-1-naphthylbezamide] **23b**

Yield: 86%; mp 266–268 °C; $[\alpha]_D^{20} = +166$ (c = 0.25, CHCl₃);

¹H NMR (700 MHz, DMSO) $\delta = 1.72$ –1.84 (m, 1H), 1.85–1.91 (m, 1H), 1.97–2.08 (m, 2H), 2.74–2.85 (m, 2H), 5.28 (q, J = 8.4 Hz, 1H), 7.12–7.21 (m, 3H_{ar}), 7.26–7.34 (m, 2H_{ar}), 7.38–7.42 (m, 1H_{ar}), 7.77 (dd, J₁ = 1.4 Hz, J₂ = 7.7 Hz, 1H_{ar}), 7.85 (dd, J₁ = 1.4 Hz, J₂ = 7.7 Hz, 1H_{ar}), 9.08 (d, J = 9.1 Hz, 1H) ¹³C NMR (700 MHz, DMSO) $\delta = 20.84$ (CH₂), 29.37 (CH₂), 30.30 (CH₂), 48.02 (CH), 126.36 (CH_{ar}), 126.60 (CH_{ar}), 127.24 (CH_{ar}), 128.35 (CH_{ar}), 128.56 (CH_{ar}), 129.28 (CH_{ar}), 130.52 (CH_{ar}), 131.99 (C_{ar}), 132.62 (CH_{ar}), 134.01 (C_{ar}), 137.72 (C_{ar}), 137.76 (C_{ar}), 167.62 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) $\delta = 445.22$ ppm; IR: 3275, 2939, 2922, 1627, 1583, 1530, 1486, 1430, 1334, 1255, 1079, 1024, 878, 864, 758,

681, 593, 427 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{34}\text{H}_{32}\text{N}_2\text{O}_2\text{Se}_2$ (660.08): C, 62.01; H, 4.90; N, 4.25; Found C, 62.00; H, 4.89; N, 4.21.

2,2'-diselenobis[N-(R)-(+)- α -methylbenzylbezamide] 24b

Yield: 92%; mp 200–202 $^{\circ}\text{C}$; $[\alpha]_D^{20} = +101$ (c = 0.47, CHCl_3) (lit. mp 219–220 $^{\circ}\text{C}$; $[\alpha]_D^{20} = +172$ (c = 1.00, dioxane) [13])

^1H NMR (700 MHz, DMSO) $\delta = 1.52$ (d, J = 7 Hz, 3H), 5.17–5.22 (m, 1H), 7.23–7.27 (m, 1H_{ar}), 7.33–7.39 (m, 4H_{ar}), 7.43–7.45 (m, 2H_{ar}), 7.67 (dd, J₁ = 1.4 Hz, J₂ = 7.7 Hz, 1H_{ar}), 7.92 (dd, J₁ = 1.4 Hz, J₂ = 7.7 Hz, 1H_{ar}), 9.09 (d, J = 7.7 Hz, 1H) ^{13}C NMR (700 MHz, DMSO) $\delta = 22.61$ (CH_3), 49.30 (CH), 126.57 (2 \times CH_{ar}), 126.58 (CH_{ar}), 127.19 (CH_{ar}), 128.60 (CH_{ar}), 128.75 (2 \times CH_{ar}), 130.46 (CH_{ar}), 132.03 (CH_{ar}), 132.53 (C_{ar}), 133.78 (C_{ar}), 144.95 (C_{ar}), 167.15 (C=O) ^{77}Se NMR (700 MHz, DMSO) $\delta = 444.99$ ppm; IR: 3312, 3061, 3033, 2976, 2930, 1625, 1583, 1524, 1451, 1431, 1376, 1325, 1207, 1133, 1091, 873, 709, 696, 674, 644, 472, 443 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{30}\text{H}_{28}\text{N}_2\text{O}_2\text{Se}_2$ (608.05): C, 59.41; H, 4.65; N, 4.62; Found C, 59.31; H, 4.64; N, 4.55.

2,2'-diselenobis[N-(S)-(–)- α -methylbenzylbezamide] 25b

Yield: 81%; mp 200–202 $^{\circ}\text{C}$; $[\alpha]_D^{20} = -104$ (c = 0.63, CHCl_3) (lit. mp 219–220 $^{\circ}\text{C}$; $[\alpha]_D^{20} = -172$ (c = 1.00, dioxane) [13])

^1H NMR (700 MHz, DMSO) $\delta = 1.52$ (d, J = 7 Hz, 3H), 5.17–5.23 (m, 1H), 7.23–7.27 (m, 1H_{ar}), 7.32–7.40 (m, 4H_{ar}), 7.43–7.46 (m, 2H_{ar}), 7.66–7.70 (m, 1H_{ar}), 7.92 (dd, J₁ = 0.7 Hz, J₂ = 7.7 Hz, 1H_{ar}), 9.09 (d, J = 8.4 Hz, 1H) ^{13}C NMR (700 MHz, DMSO) $\delta = 22.62$ (CH_3), 49.30 (CH), 126.57 (2 \times CH_{ar}), 126.58 (CH_{ar}), 127.19 (CH_{ar}), 128.61 (CH_{ar}), 128.75 (2 \times CH_{ar}), 130.45 (CH_{ar}), 132.03 (CH_{ar}), 132.52 (C_{ar}), 133.76 (C_{ar}), 144.96 (C_{ar}), 167.15 (C=O) ^{77}Se NMR (700 MHz, DMSO) $\delta = 445.01$ ppm; IR: 3310, 3059, 3032, 2975, 1623, 1583, 1522, 1448, 1431, 1376, 1324, 1207, 1132, 1090, 872, 696, 671, 644, 471, 443 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{30}\text{H}_{28}\text{N}_2\text{O}_2\text{Se}_2$ (608.05): C, 59.41; H, 4.65; N, 4.62; Found C, 59.32; H, 4.65; N, 4.59.

2,2'-diselenobis[N-(S)-(–)-1-(1-naphthyl)ethylbezamide] 26b

Yield: 70%; mp 262–64 $^{\circ}\text{C}$; $[\alpha]_D^{20} = -96$ (c = 0.35, CHCl_3);

^1H NMR (700 MHz, DMSO) $\delta = 1.66$ (d, J = 7.7 Hz, 3H), 5.95–6.01 (m, 1H), 7.31–7.36 (m, 2H_{ar}), 7.49–7.56 (m, 2H_{ar}), 7.58–7.62 (m, 1H_{ar}), 7.65–7.69 (m, 2H_{ar}), 7.86 (d, J = 8.4 Hz, 1H_{ar}), 7.90–7.94 (m, 1H_{ar}), 7.95–7.99 (m, 1H_{ar}), 8.26 (d, J = 8.4 Hz, 1H_{ar}), 9.26 (d, J = 7.7 Hz, 1H) ^{13}C NMR (700 MHz, DMSO) $\delta = 21.87$ (CH_3), 45.64 (CH), 123.11 (CH_{ar}), 123.63 (CH_{ar}), 125.95 (CH_{ar}), 126.09 (CH_{ar}), 126.60 (CH_{ar}), 126.73 (CH_{ar}), 127.85 (CH_{ar}), 128.67 (CH_{ar}), 129.17 (CH_{ar}), 130.40 (CH_{ar}), 130.90 (CH_{ar}), 132.03 (C_{ar}), 132.45 (C_{ar}), 133.76 (C_{ar}), 133.90 (C_{ar}), 140.44 (C_{ar}), 167.10 (C=O) ^{77}Se NMR (700 MHz, DMSO) $\delta = 442.72$ ppm; IR: 3290, 2922, 1625, 1583, 1530, 1452, 1429, 1338, 1308, 1284, 1258, 1180, 1132, 1025, 871, 792, 773, 735, 693, 641, 448 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{38}\text{H}_{32}\text{N}_2\text{O}_2\text{Se}_2$ (708.08): C, 64.59; H, 4.56; N, 3.96; Found C, 64.46; H, 4.55; N, 3.89.

2,2'-diselenobis[N-(R)-(+)-1-(1-naphthyl)ethylbezamide] 27b

Yield: 73%; mp 262–264 $^{\circ}\text{C}$; $[\alpha]_D^{20} = +98$ (c = 0.32, CHCl_3);

^1H NMR (700 MHz, DMSO) $\delta = 1.66$ (d, J = 7.7 Hz, 3H), 5.55–6.01 (m, 1H), 7.31–7.36 (m, 2H_{ar}), 7.49–7.56 (m, 2H_{ar}), 7.58–7.61 (m, 1H_{ar}), 7.66–7.69 (m, 2H_{ar}), 7.86 (d, J = 8.4 Hz, 1H_{ar}), 7.90–7.93 (m, 1H_{ar}), 7.96–7.98 (m, 1H_{ar}), 8.26 (d, J = 8.4 Hz, 1H_{ar}), 9.26 (d, J = 7.7 Hz, 1H) ^{13}C NMR (700 MHz, DMSO) $\delta = 21.87$ (CH_3), 45.64 (CH), 123.12 (CH_{ar}), 123.64 (CH_{ar}), 125.96 (CH_{ar}), 126.09 (CH_{ar}), 126.59 (CH_{ar}), 126.71 (CH_{ar}), 127.85 (CH_{ar}), 128.67 (CH_{ar}), 129.17 (CH_{ar}), 130.41 (CH_{ar}), 130.91 (CH_{ar}), 132.03 (C_{ar}), 132.64 (C_{ar}), 133.79 (C_{ar}), 133.91 (C_{ar}), 140.45 (C_{ar}), 167.10 (C=O) ^{77}Se NMR (700 MHz, DMSO) $\delta = 443.08$ ppm; IR: 3292, 3063, 2976, 2927, 1624, 1583, 1531, 1450, 1432, 1337, 1306, 1283, 1258, 1180, 1131, 1083, 1026, 997, 878, 793, 773, 736, 725, 692, 641, 447 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{38}\text{H}_{32}\text{N}_2\text{O}_2\text{Se}_2$ (708.08): C, 64.59; H, 4.56; N, 3.96; Found C, 64.52; H, 4.57; N, 3.90.

2,2'-diselenobis[N-(1S,2R)-(–)-cis-2-hydroxy-1-indanylbezamide] 28b

Yield: 70%; mp 241–243 $^{\circ}\text{C}$; $[\alpha]_D^{20} = -149$ (c = 0.39, CHCl_3);

^1H NMR (700 MHz, DMSO) $\delta = 2.90$ –2.95 (m, 1H), 3.14 (dd, J₁ = 4.9 Hz, J₂ = 9.1 Hz, 1H), 4.56–4.61 (m, 1H), 5.17–5.21 (m, 1H), 5.49 (dd, J₁ = 5.6 Hz, J₂ = 8.4 Hz, 1H), 7.21–7.27 (m, 2H_{ar}), 7.28–7.35 (m, 3H_{ar}), 7.40–7.44 (m, 1H_{ar}), 7.78 (d, J = 8.4 Hz, 1H_{ar}), 8.01 (d, J = 7.7 Hz, 1H_{ar}), 8.59 (d, J = 8.4 Hz, 1H_{ar}) ^{13}C NMR (700 MHz, DMSO) $\delta = 39.92$ (CH_2), 58.10 (CH), 72.54 (CH), 124.94 (CH_{ar}), 125.35 (CH_{ar}), 126.63 (CH_{ar}), 126.86 (CH_{ar}), 128.00 (CH_{ar}),

129.00 (CH_{ar}), 130.42 (CH_{ar}), 132.16 (CH_{ar}), 132.66 (C_{ar}), 133.62 (C_{ar}), 141.44 (C_{ar}), 142.02 (C_{ar}), 168.21 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 445.85 ppm; IR: 3288, 3046, 2976, 2926, 1622, 1582, 1529, 1428, 1337, 1305, 1281, 1216, 1080, 1047, 997, 873, 792, 772, 735, 691, 558, 447 cm⁻¹. Elemental Anal. Calcd for C₃₂H₂₈N₂O₄Se₂ (664.04): C, 58.01; H, 4.26; N, 4.23; Found C, 58.05; H, 4.27; N, 4.17.

2,2'-diselenobis[N-(1R,2S)-(+)-cis-2-hydroxy-1-indanylbezamide] 29b

Yield: 73%; mp 241–243 °C; [α]_D²⁰ = +152 (c = 0.41, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 2.91–2.95 (m, 1H), 3.14 (dd, J₁ = 4.9 Hz, J₂ = 16.1 Hz, 1H), 4.46 (qd, J₁ = 2.1 Hz, J₂ = 4.9 Hz, 1H), 5.18 (d, J = 4.9 Hz, 1H), 5.49 (dd, J₁ = 5.6 Hz, J₂ = 8.4 Hz, 1H), 7.21–7.27 (m, 2H_{ar}), 7.28–7.35 (m, 3H_{ar}), 7.40–7.44 (m, 1H_{ar}), 7.78 (d, J = 8.4 Hz, 1H_{ar}), 8.01 (d, J = 7.7 Hz, 1H_{ar}), 8.59 (d, J = 8.4 Hz, 1H_{ar}) ¹³C NMR (700 MHz, DMSO) δ = 39.70 (CH₂), 58.10 (CH), 72.53 (CH), 124.94 (CH_{ar}), 125.34 (CH_{ar}), 126.63 (CH_{ar}), 126.86 (CH_{ar}), 128.00 (CH_{ar}), 129.00 (CH_{ar}), 130.42 (CH_{ar}), 132.15 (CH_{ar}), 132.65 (C_{ar}), 133.62 (C_{ar}), 141.43 (C_{ar}), 142.02 (C_{ar}), 168.21 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 445.85 ppm; IR: 3289, 3047, 2974, 2927, 1622, 1583, 1530, 1431, 1337, 1306, 1282, 1210, 1082, 1046, 997, 877, 793, 772, 735, 692, 558, 447 cm⁻¹. Elemental Anal. Calcd for C₃₂H₂₈N₂O₄Se₂ (664.04): C, 58.01; H, 4.26; N, 4.23; Found C, 58.09; H, 4.27; N, 4.17.

2,2'-diselenobis[N-(1S,2S)-(-)-trans-2-hydroxy-1-indanylbezamide] 30b

Yield: 93%; mp 234–236 °C; [α]_D²⁰ = +153 (c = 0.33, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 2.76 (dd, J₁ = 7.7 Hz, J₂ = 15.4 Hz, 1H), 3.18 (dd, J₁ = 7.7 Hz, J₂ = 15.4 Hz, 1H), 4.46 (kwintet, J = 7 Hz, 1H), 5.29 (t, J = 7.7 Hz, 1H), 5.41 (d, J = 5.6 Hz, 1H), 7.17–7.23 (m, 4H_{ar}), 7.30–7.34 (m, 1H_{ar}), 7.37–7.41 (m, 1H_{ar}), 7.76 (dd, J₁ = 0.7 Hz, J₂ = 7.7 Hz, 1H_{ar}), 7.88 (dd, J₁ = 0.7 Hz, J₂ = 7.7 Hz, 1H_{ar}), 9.03 (d, J = 8.4 Hz, 1H) ¹³C NMR (700 MHz, DMSO) δ = 39.34 (CH₂), 62.39 (CH), 77.89 (CH), 124.41 (CH_{ar}), 125.16 (CH_{ar}), 126.59 (CH_{ar}), 127.19 (CH_{ar}), 128.17 (CH_{ar}), 128.64 (CH_{ar}), 130.54 (CH_{ar}), 132.09 (CH_{ar}), 132.67 (C_{ar}), 133.88 (C_{ar}), 140.46 (C_{ar}), 142.18 (C_{ar}), 168.32 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 445.85 ppm; IR: 3254, 2919, 1630, 1584, 1530, 1456, 1456, 1428, 1345, 1272, 1214, 1122, 1065, 919, 871, 685, 646, 579, 517, 447 cm⁻¹. Elemental Anal. Calcd for C₃₂H₂₈N₂O₄Se₂ (664.04): C, 58.01; H, 4.26; N, 4.23; Found C, 58.05; H, 4.27; N, 4.19.

2,2'-diselenobis[N-(1R,2R)-(-)-trans-2-hydroxy-1-indanylbezamide] 31b

Yield: 86%; mp 233–235 °C; [α]_D²⁰ = -150 (c = 0.33, CHCl₃);

¹H NMR (700 MHz, DMSO) δ = 2.76 (dd, J₁ = 7.7 Hz, J₂ = 15.4 Hz, 1H), 3.18 (dd, J₁ = 7.7 Hz, J₂ = 15.4 Hz, 1H), 4.46 (kwintet, J = 7 Hz, 1H), 5.30 (t, J = 7.7 Hz, 1H), 5.40 (d, J = 5.6 Hz, 1H), 7.15–7.24 (m, 4H_{ar}), 7.30–7.34 (m, 1H_{ar}), 7.36–7.42 (m, 1H_{ar}), 7.76 (dd, J₁ = 0.7 Hz, J₂ = 7.7 Hz, 1H_{ar}), 7.88 (dd, J₁ = 0.7 Hz, J₂ = 7.7 Hz, 1H_{ar}), 9.01 (d, J = 8.4 Hz, 1H) ¹³C NMR (700 MHz, DMSO) δ = 39.31 (CH₂), 62.36 (CH), 77.88 (CH), 124.41 (CH_{ar}), 125.16 (CH_{ar}), 126.59 (CH_{ar}), 127.20 (CH_{ar}), 128.17 (CH_{ar}), 128.64 (CH_{ar}), 130.52 (CH_{ar}), 132.09 (CH_{ar}), 132.67 (C_{ar}), 133.84 (C_{ar}), 140.44 (C_{ar}), 142.17 (C_{ar}), 168.32 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 445.76 ppm; IR: 3257, 2920, 1630, 1584, 1531, 1458, 1429, 1345, 1272, 1213, 1123, 1066, 920, 871, 658, 649, 580, 518, 448 cm⁻¹. Elemental Anal. Calcd for C₃₂H₂₈N₂O₄Se₂ (664.04): C, 58.01; H, 4.26; N, 4.23; Found C, 57.92; H, 4.27; N, 4.18.

2.3. Antioxidant Activity Assay

The antioxidant activity assay was prepared via the method presented by Iwaoka [22].

2.4. MTT Viability Assay

The MTT assay was based on the method of Mosmann [23].

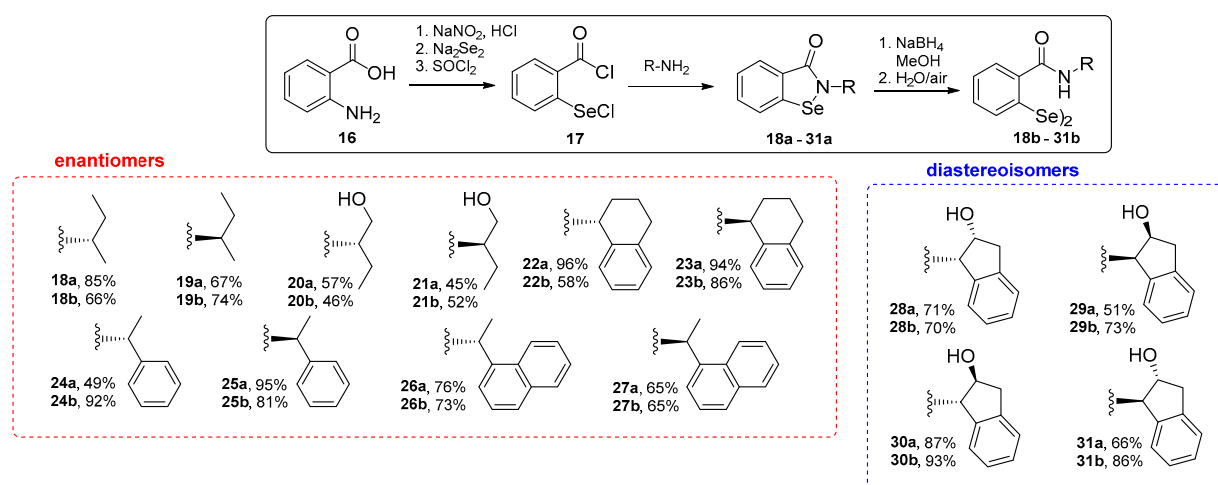
2.5. Crystal Structure Determination

The crystal structures of **24a** and **25a** were determined. The X-ray diffraction data were collected at a temperature of 100(1) K with a Rigaku XtaLAB Synergy CCD diffractometer using CuKα radiation λ = 1.54184 Å. Both structures were solved by direct methods and refined with the full-matrix least-squares method on F² with the use of the SHELX2017 [24,25] program package. The absorption corrections were used with CrysAlisPro 1.171.41.120a

(Rigaku OD, 2021), [26]. The hydrogen atoms were located from the electron density maps, and their positions were constrained in the refinement. Details of the diffraction experiments and crystal structures are presented in the Supplemental Materials. The crystallographic data were deposited with the Cambridge Crystallographic Data Centre, the CCDC numbers: 2014858 and 2014859 for **24a** and **25a**, respectively.

3. Results and Discussion

The first step of the research involved the synthesis of *N*-substituted benzoselenazolone-3(2*H*)-ones. The procedure was based on the reaction of 2-(chloroseleno)-benzoyl chloride **17**, obtained according to our previously reported method [18], with commercially available chiral amines. Then, the obtained compounds **18a–31a** were transformed into diselenides **18b–31b** by a sodium borohydride reduction and air oxidation protocol. Both benzoselenazolones (45–96%) and diselenides (46–93%) were synthesized in good yields (Scheme 2).



Scheme 2. Synthesized chiral benzoselenazolones **18a–31a** and diselenides **18b–31b**.

Furthermore, the crystal structures of enantiomers **24a/24b** were determined. The asymmetric part of the crystal structure of *N*-[(*R*)-(+)- α -methylbenzyl]-1,2-benzisoselenazolone-3(2*H*)-one **24a** consists of five molecules (Supplemental Materials). The single molecule with the atom numbering scheme is shown in Figure 3. In all of them, the (*R*) configuration of the chiral center was detected. The molecular conformation differs slightly between the molecules, with the dihedral angles between the phenyl ring and the benzoselenazolone moiety being 84.8(8)°, 82.3(9)°, 85.1(9)°, 76.3(9)° and 83.2(9)° for molecules 1–5, respectively. Significant differences in the conformation of molecules 1–5 were detected, affecting the positions of the methyl groups relative to the benzoselenazolone moieties. The representative Se1–N2–C11–C12 torsion angle of its equivalents in molecules 2–5 are 102.2(15)°, 82.7(19)°, 93.9(19)°, 85(2)° and 64(2)°. The network of short intermolecular Se...O contacts involving four molecules is found in the structure. The respective distances are Se1...O4[3/2 - x, 1 - y, 1/2 + z] 2.592(13), Se21...O44[x, y, 1 + z] 2.640(13), Se41...O24 2.627(18), Se61...O84[x, y, -1 + z] 2.761(14) and Se81...O64 2.677(16) Å.

In the crystal structure of *N*-[(*S*)-(+)- α -methylbenzyl]-1,2-benzisoselenazolone-3(2*H*)-one **25a**, the asymmetric part also consists of five benzoselenazolone molecules. For all molecules, the *S* configuration of the benzyl C11 chiral centers was found. The dihedral angles between the phenyl and benzoselenazolone moieties in molecules 1–5 are 84.6(3)°, 85.8(4)°, 82.5(4)°, 82.7(4)° and 76.4(4)°. Due to the opposite enantiomer, the Se1–N2–C11–C12 torsion angles have negative signs: -102.5(6)°, -94.5(8)°, -81.8(9)°, -65.6(9)° and -84.4(10)° for molecules 1–5, respectively. In **25a**, the similar network of Se...O intermolecular interactions is found, with the respective distances Se1...O4[1 - x, -1/2 + y, 1/2 - z] 2.592(5), Se21...O44[x, -1 + y, z] 2.633(6), Se41...O24 2.621(5), Se61...O84 2.685(6) and Se81...O64[x, 1 + y, z] 2.779(6) Å.

The antioxidant activity of all the obtained compounds was evaluated using the broadly used test presented by Iwaoka [22]. The Se-catalyst reduces hydrogen peroxide and regenerates in the presence of dithiol (DTT^{red}). The rate of the reaction is measured using ¹H NMR spectroscopy. The appearance of signals representing the formed disulfide (DTT^{ox}) in specific time intervals is recorded (Table 1).

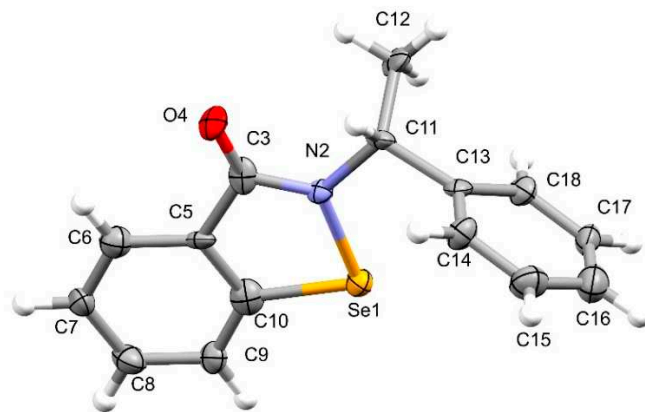


Figure 3. Molecule 1 of **24a** with atomic displacement parameters plotted at a 50% probability level. The atom-numbering scheme is analogous for all molecules in both enantiomers **24a** and **25a**.

Table 1. Results of the antioxidant activity measurement.

Catalyst (0.1 equiv.)	Remaining DTT ^{red} (%)			
	5 min	15 min	30 min	60 min
Benzisoselenazolones				
18a/19a	90	88	86	80
20a/21a	86	83	77	60
22a/23a	91	88	85	77
24a/25a	77	59	38	16
26a/27a	90	89	88	84
28a/29a	84	80	74	62
30a/31a	87	86	83	78
Diselenides				
18b/19b	80	65	47	29
20b/21b	73	50	30	19
22b/23b	89	83	80	75
24b/25b	76	54	37	26
26b/27b	87	84	82	76
28b/29b	44	13	2	0
30b/31b	14	2	0	0
Ebselen	75	64	58	52

The antioxidant activity of all enantiomeric pairs was the same. In general, better results were obtained for diselenides **18b–31b** than for the corresponding benzoselenazolones **18a–31a**. The best H₂O₂-scavenging properties were observed for the indanyl derivatives possessing a *cis* (**28b/29b**) and *trans* (**30b/31b**) 2-hydroxy group, with the total dithiol conversion after 30 and 15 min, respectively.

The cytotoxic activity of Se-compounds was measured using a cell viability assay (MTT) on human promyelocytic leukemia HL-60 and breast cancer MCF-7 cell lines [23]. In

the case of benzisoselenazolones, the highest antiproliferative potential was also observed for the hydroxyindanyl derivative **28a** (Table 2).

Table 2. The antiproliferative activity of compounds **18a–31a**.

Compound	IC ₅₀ (μM) ± SEM	
	HL-60	MCF-7
18a	48.5 ± 6.3	36.5 ± 4.4
19a	71.2 ± 0.1	19.2 ± 1.3
20a	26.0 ± 1.7	38.3 ± 1.3
21a	33.3 ± 0.5	35.1 ± 0.5
22a	18.5 ± 0.5	62.3 ± 3.8
23a	26.5 ± 3.5	46.0 ± 0.8
24a	16.1 ± 0.0	32.8 ± 2.8
25a	16.8 ± 0.4	38.8 ± 0.8
26a	47.5 ± 6.2	27.2 ± 0.1
27a	52.6 ± 0.2	41.1 ± 1.3
28a	7.9 ± 0.3	14.9 ± 0.9
29a	28.2 ± 1.1	28.8 ± 2.1
30a	11.4 ± 1.0	22.1 ± 3.1
31a	16.2 ± 1.8	27.6 ± 2.1

The bio-activity of the *N*-(2-hydroxy-1-indanyl)-1,2-benzisoselenazol-3(2*H*)-ones **28a–31a** depended on the stereochemistry of the C1 carbon of the *N*-substituent. The IC₅₀ values were lower for the (*S*)-configuration of C1, directly connected to the nitrogen atom. The stereochemistry of C2, with the attached hydroxy group, seemed to not influence the reactivity (Figure 4).

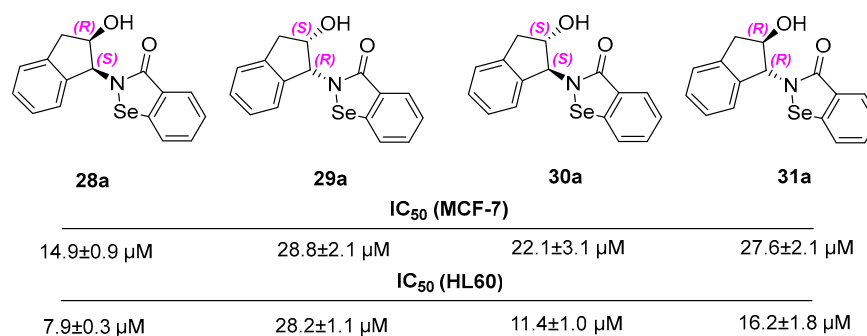


Figure 4. Different activity of diastereomeric hydroxyindanyl derivatives **28a–31a**.

The corresponding diselenides only expressed cytotoxicity towards the HL-60 cell line, except for derivative **20b** (IC₅₀ 37.00 ± 4.25 μM). This suggests that the Se–N bond is needed to influence the proliferation of MCF-7 cells. Additionally, the cytotoxic activity was exclusively observed for diselenides bearing an additional hydroxy group. The hydroxybutyl derivatives **20b** and **21b** gave the lowest IC₅₀ values 8.67 ± 0.14 μM and 10.10 ± 0.49 μM, respectively. Most potent compounds were also evaluated using human endothelial cells HUVEC. The *N-trans*-2-hydroxy-1-indanyl diselenide **31b** revealed a selective antiproliferative activity with no toxicity towards normal cells (Table 3).

Table 3. The antiproliferative activity of compounds **18b–31b**.

Compound	IC ₅₀ (μM) ± SEM		
	HL-60	MCF-7	HUVEC
18b	>100	>100	
19b	>100	>100	
20b	8.67 ± 0.14	37.00 ± 4.25	9.92 ± 0.07
21b	10.10 ± 0.49	>100	
22b	>100	>100	
23b	>100	>100	
24b	>100	>100	
25b	>100	>100	
26b	>100	>100	
27b	>100	>100	
28b	20.00 ± 0.16	>100	
29b	21.75 ± 2.08	>100	
30b	12.40 ± 0.33	>100	18.95 ± 0.20
31b	13.00 ± 0.41	>100	>100

4. Conclusions

Herein, we reported the synthesis of chiral *N*-substituted benzoselenazol-3(2*H*)-ones and the corresponding diselenides with *o*-amido function, creating a series of enantiomers and diastereoisomers. The 28 obtained derivatives possess various moieties on the nitrogen atom, including aliphatic acyclic and cyclic carbon chains with additional aromatic rings and hydroxy groups. All compounds were tested as antioxidants and anticancer agents. The obtained results revealed that: diselenides are generally better antioxidants with a significant activity enhancement by the presence of *N*-hydroxyindanyl moiety; the cytotoxic activity of benzoselenazolones is similar towards both HL-60 and MCF-7 cell lines with the highest antiproliferative potential for *N*-(2-hydroxy-1-indanyl) derivatives having a (*S*)-configuration of the C₁_{indanyl} carbon directly connected to the nitrogen atom of the selenazolone ring; the anticancer activity of diselenides is only selectively expressed towards HL-60 cell lines, revealing that the potential to influence the proliferation of breast cancer cells MCF-7 is connected to the presence of the benzoselenazolone core; the attachment of a hydroxy group seems to be essential to obtain a cytotoxic effect on human promyelocytic leukemia cell lines; the IC₅₀ values obtained for selected diselenides using human endothelial cells HUVEC showed that these compounds can express a selective cytotoxic effect with a minimal disruption of the normal cells. It can be concluded that the hydroxyindanyl moiety attached to the nitrogen atom of the benzoselenazolone or the *o*-amidodiselenide core enables the improvement of the bio-activity of the Se-compounds and can be considered a useful motif in further modification.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ma15062068/s1>, 1. ¹H, ¹³C and ⁷⁷Se NMR spectra of benzoselenazol-3(2*H*)-ones **18a–31a**; 2. ¹H, ¹³C and ⁷⁷Se NMR spectra of diselenides **18b–31b**; 3. Crystallographic data of benzoselenazol-3(2*H*)-ones **24a** and **25a**.

Author Contributions: Conceptualization, J.Š.; methodology, J.Š. and A.J.P.-M.; formal analysis, A.L., A.D.-P., A.J., A.J.P.-M. and A.W.; investigation, A.L., A.D.-P. and A.J.P.-M.; writing—original draft preparation, A.L. and A.J.P.-M.; writing—review and editing, J.Š., A.J. and A.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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Publikacja 3 [P3]


 Cite this: *RSC Adv.*, 2023, **13**, 14698

Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents†

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Organoselenium compounds are well-known for their unique biological properties, including antioxidant, anticancer and anti-inflammatory. They result from the presence of a particular Se-moiety enclosed in a structure that provides physicochemical features necessary for effective drug–target interactions. Looking for a proper drug design that considers the influence of each structural element has to be conducted. In this paper, we have synthesized a series of chiral phenylselenides, possessing an additional *N*-substituted amide moiety, and evaluated their antioxidant and anticancer potential. The presented derivatives, as a group of enantiomeric and diastereomeric pairs, enabled a thorough investigation of the 3D structure–activity dependence in correlation with the presence of the phenylselenyl group as the potential pharmacophore. The *N*-indanyl derivatives possessing a *cis*- and *trans*-2-hydroxy group were selected as the most promising antioxidants and anticancer agents.

Received 13th April 2023

Accepted 8th May 2023

DOI: 10.1039/d3ra02475j

rsc.li/rsc-advances

Introduction

Over the years, organoselenium chemistry has expanded through a multitude of research fields including medicine, catalysis and materials science. Examples of its diverse applicability include antibacterial¹ and redox-responsive materials;² utility as catalysts in oxidation, reduction and rearrangement reactions, as well as stereoselective reactions;³ and in dietary supplementation as an essential micro-nutrient.⁴ Taking into consideration the nutritional and biochemical properties of naturally abundant organoselenium compounds like selenocysteine, selenomethionine and selenium as an element itself, one of the leading directions of development in the field is medicinal chemistry. As one of the most evaluated small antioxidant molecules, organoselenium compounds can be considered leading players in the race for market-approved drugs. The ability to mimic the antioxidant selenoenzyme glutathione peroxidase (GPx) determines its high redox-modulating properties.⁵ Over the years, they have also been proven to exhibit various

biological activities including anticancer, anti-inflammatory, and antibacterial.^{6–11} However, issues associated with bioavailability and side effects remain major obstacles in the design of safe Se-therapeutics.

Since the discovery of the well-known peroxide scavenger ebselen (*N*-phenylbenzisoselenazol-3(2*H*)-one) a lot has been accomplished in the search for the ideal structural modifications that would maximize the therapeutic potential of the Se-moiety and reduce its toxicity.^{12–16} Modifying the primary benzisoselenazolone core can significantly affect the physicochemical properties of the molecule, thus changing the pharmacokinetics and biochemical outcome of the potential drug. We have recently observed that in some cases, the exchange of the Se–N for a Se–C_{ar} bond and incorporation of an additional aromatic ring enhances the cytotoxic activity of chiral *N*-terpene benzisoselenazolones **1** and **3**.¹⁷ The IC₅₀ values decreased approximately six and twelve times for *N*-menthyl **2** and *N*-caranyl phenylselenide **4**, respectively (Fig. 1).

These results prompted us to investigate if the presence of the phenylselenyl group can enhance the antiproliferative effect. There are few methods that enable to obtain unsymmetrical phenylselenides with a secondary amide group: starting from corresponding benzisoselenazolones through the reaction with Grignard reagent (method A);¹⁸ based on the Ru-catalyzed selenation of benzamides (method B);¹⁹ and Cu-catalyzed nucleophilic substitution of *o*-iodobenzamides (method C), previously reported by our research group¹⁷ (Scheme 1). Herein, we have utilized one of the known

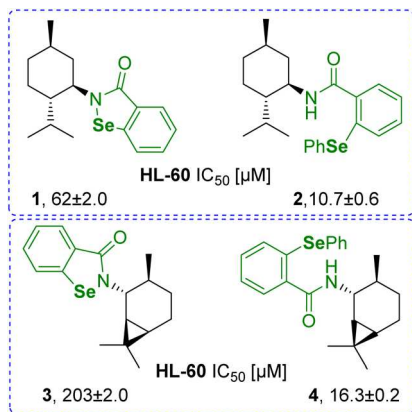
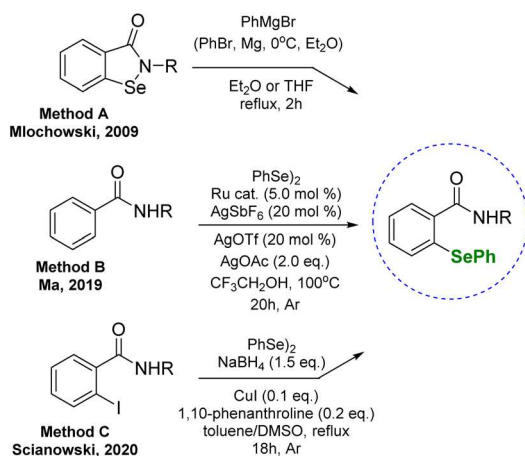
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† Electronic supplementary information (ESI) available: ¹H, ¹³C and ⁷⁷Se NMR spectra of phenylselenides **6–19** are available. See DOI: <https://doi.org/10.1039/d3ra02475j>

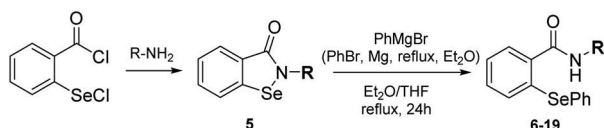


Fig. 1 *N*-terpene phenylselenenides with enhanced bio-activity.Scheme 1 Methods for the preparation of diphenylselenenides mono-substituted with *o*-amido function.

protocols to synthesize a series of chiral unsymmetrical diphenyl selenenides and evaluated their antioxidant and anti-cancer activity. The presented derivatives are pairs of enantiomers and diastereoisomers, which additionally allows determining whether the molecule's spatial structure can, in this case, influence the drug–target interaction and increase the therapeutic potential.

Results and discussion

The first stage of the research involved the synthesis of phenylselenenides 6–19. For this purpose, we have slightly modified the procedure proposed by Młochowski and co-workers (Method A, Scheme 1). To a solution of phenyl



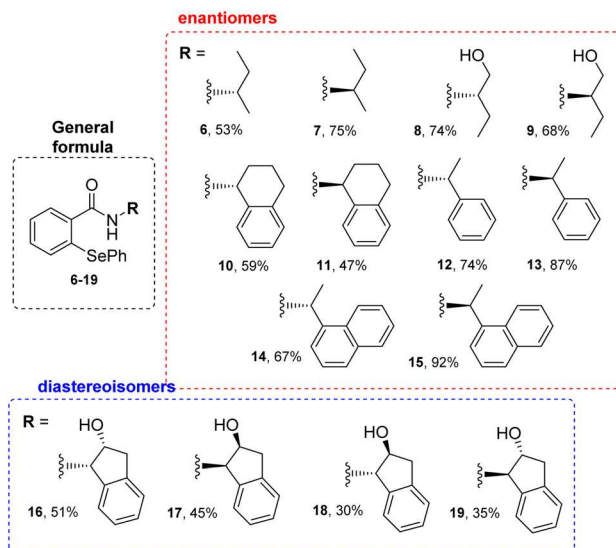
Scheme 2 Synthesis of phenylselenenides 6–19.

magnesium bromide, formed by refluxing PhBr and Mg⁰ in diethyl ether, appropriate *N*-substituted benzisoselenazolone 5 (obtained previously through the reaction of 2-(chloroseleno)benzoyl chloride with an appropriate enantiomerically pure amine²⁰) dissolved in Et₂O or THF was added (Scheme 2).

Due to low solubility of the starting materials 5, the reaction time had to be elongated to 24 h. All products 6–19 were obtained in good yields (Scheme 3). Surprisingly, our recently reported protocol (Method C, Scheme 1) was not efficient for these type of bulky substrates, *e.g.* compound 15 was obtained in only 38% yield, contrary to Method A which furnished the final phenylselenide 15 in 92%.

The antioxidant activity of all synthesized compounds was evaluated by two assays. First, by the procedure presented by Iwaoka and co-workers.²¹ Herein, the particular selenide 6–19, serving as the Se-catalyst, is first oxidized by hydrogen peroxide to the corresponding selenoxide 20. Then, the activated catalyst 20 transforms the dithiol DTT^{red} to the disulfide DTT^{ox}. The progress of the reaction, disappearance of signals correlated with DTT^{red} and appearance of those associated with DTT^{ox}, is monitored by the changes in ¹H NMR spectra in specific time intervals. Unfortunately, in all cases, the conversion was less efficient than in the presence of ebselen (Table 1).

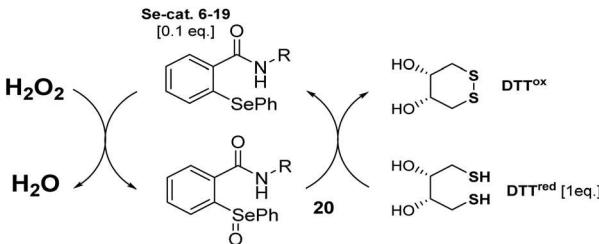
In the second study, the popular 2,2'-diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging assay (RSA) was used to measure the total antioxidant capacity for all obtained compounds. It has been applied to quantify antioxidant activity in many samples using antioxidant standards such as ascorbic acid, butylated hydroxyl toluene, α -tocopherol, butylated hydroxyl anisole, gallic acid, and trolox.²² The results of this assay were expressed as IC₅₀ values (Table 2). The IC₅₀ value is defined as the antioxidant concentration required to reduce 50% of the initial DPPH concentration.²³ The percentage of inhibition against the DPPH radical was



Scheme 3 Synthesized phenylselenenides 6–19.



Table 1 Results of the antioxidant activity measurement



Se-catalyst [0.1 eq.]	Remaining DTT ^{red} [%]			
	5 min	15 min	30 min	60 min
Phenylselenides				
6/7	87	86	86	85
8/9	91	85	81	76
10/11	88	84	79	75
12/13	88	85	83	83
14/15	89	86	84	82
16/17	87	85	83	81
18/19	88	85	83	80
Ebselen	75	64	58	52

Table 2 Results collected from the DPPH radical scavenging assay

Compound	IC ₅₀ _{DPPH} [μmol L ⁻¹]
6/7	7149.2
8/9	1752.6
10/11	1714.3
12/13	1715.1
14/15	1704.7
16/17	6470.4
18/19	1429.3

calculated with the following equation: inhibition (%) = $\frac{(A - B)/A}{A} \times 100$, where A and B are the absorptions of the blank samples and working standards. The DPPH[•] test is based on

Table 3 The antiproliferative activity of compounds 6–19

Compound	IC ₅₀ [μM] ± SEM	
	HL-60	MCF-7
6	>150	>150
7	>150	>150
8	>150	>150
9	>150	>150
10	>150	>150
11	>150	>150
12	>150	>150
13	>150	>150
14	>150	>150
15	>150	>150
16	>150	>150
17	>150	16.65 ± 1.36
18	69.50 ± 0.71	83.38 ± 1.94
19	55.45 ± 3.01	63.75 ± 0.35

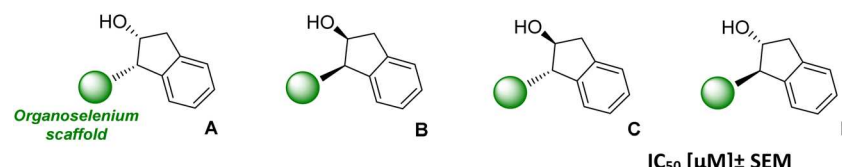
spectrophotometric mixed mode assays based on single electron transfer (SET), hydrogen atom transfer (HAT), and proton-coupled electron transfer (PCET) mechanisms. The DPPH radical can only be dissolved in organic media thus, this assay has a higher affinity toward lipophilic than hydrophilic antioxidants. The highest antioxidant capacity was observed for the *trans*-indanyl diastereoisomers **18/19**. Surprisingly, the *cis*-indanyl conformers **16/17** were approximately 4 times less active.

Next, the antiproliferative activity of derivatives **6–19** was evaluated towards leukemia HL-60 and breast cancer MCF-7 cell lines.²⁴ The highest cytotoxic potential was observed for the indanyl phenylselenides possessing the *cis* (**17**) and *trans* (**18**, **19**) 2-hydroxy group (Table 3).

As presented in our previous papers,²⁰ the presence of the indanyl substituent attached to the nitrogen atom of benzisoseleazolones and corresponding diselenides also enhances the cytotoxic effect. A comparison of all 3 types of indanyl Se-derivatives is presented in Table 4. Benzisoseleazolones (A–D) show a moderate cytotoxic activity and it is similar towards both tested cell lines with IC₅₀ values ranging from 7.9 ± 0.3 to 28.8 ± 2.1 μM. In the case of all diselenides (A–D), the cytotoxicity against MCF-7 cells is low (IC₅₀ > 100 μM), whereas HL-60 cell line is far more sensitive (IC₅₀ < 20 μM).

Phenylselenides with a *trans*-hydroxy group (C and D) on the indanyl scaffold present similar low activity to both cell lines, however, in the case of *cis*-hydroxy-indanyl derivative B the MCF-7 cell line is inhibited more effectively (IC₅₀ = 16.7 ± 1.4 μM). Thus, bio-selectivity seems to be strongly related to the type of the Se-moiety, regardless of the chirality of the indanyl substituent.



Table 4 The antiproliferative activity of *N*-indanyl Se-derivatives


	A		B	
Se-derivative	MCF-7	HL-60	MCF-7	HL-60
Phenylselenide	>150	>150	16.6 ± 1.3	>150
Benzisoselenazolone	14.9 ± 0.9	7.9 ± 0.3	28.8 ± 2.1	28.2 ± 1.1
Diselenide	>100	20.0 ± 0.1	>100	21.7 ± 2.1
	C		D	
Se-derivative	MCF-7	HL-60	MCF-7	HL-60
Phenylselenide	83.3 ± 1.9	69.5 ± 0.7	63.7 ± 0.3	55.4 ± 3.0
Benzisoselenazolone	22.1 ± 3.1	11.4 ± 1.0	27.6 ± 2.1	16.2 ± 1.8
Diselenide	>100	12.4 ± 0.3	>100	13.0 ± 0

Conclusions

In conclusion, we have developed a facile synthesis of chiral unsymmetrical diphenylselenides possessing an additional *o*-amide group on one of the aromatic rings. The derivatives were *N*-substituted with chiral groups forming a series of enantiomeric and diastereomeric pairs. Although the phenylselenanyl group seems not to be improving the bio-activity of the organoselenium derivatives, in contrary to corresponding benzisoselenazolones with Se–N moiety and diselenides equipped with a Se–Se bond, the obtained results confirm that also for these type of Se-derivatives, the presence of the *N*-indanyl scaffold enhance the antioxidant and anticancer properties. Thus, the *cis*- and *trans*-indanyl moiety can be considered a pharmacophore necessary for the optimal drug–target interaction.

Experimental

General

NMR spectra were recorded on Bruker Avance III/400 or Bruker Avance III/700 (Karlsruhe, Germany) for ^1H and 176.1 MHz or 100.6 MHz for ^{13}C (see ESI†). Chemical shifts were recorded relative to SiMe_4 (δ 0.00) or solvent resonance (CDCl_3 δ 7.26, CD_3OD δ 3.31). Multiplicities were given as: s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet), and m (multiplet). The ^{77}Se NMR spectra were recorded on Bruker Avance III/400 or Bruker Avance III/700 with diphenyl diselenide as an external standard. NMR spectra were carried out using ACD/NMR Processor Academic Edition. Melting points were measured with a Büchi Tottoli SPM-20 heating unit (Büchi Labortechnik AG, Flawil, Switzerland) and were uncorrected. Elemental analyses were performed on a Vario MACRO CHN analyzer. Optical rotations were measured in 10 mm cells with a polAAR 3000 polarimeter. Column chromatography was performed using Sigma Aldrich 60 Å (52–73 Å) 63–200 μm silica gel (Merck, Darmstadt, Germany).

General procedure for the synthesis of phenylselenides 6–19

To a suspension of elemental magnesium in diethyl ether, phenyl bromide was slowly added (during this time, the mixture was continuously heated to 40 °C). The reaction mixture was stirred and refluxed until all magnesium was dissolved (≈ 2 h) at 40 °C under anaerobic conditions. Appropriate *N*-substituted benzisoselenazol-3(2*H*)-one, dissolved in diethyl ether or THF THF (**Et**₂O: **6**, **7**, **8**, **9**, **12**, **13**, **14**, **15**; THF: **10**, **11**, **16**, **17**, **18**, **19**) was slowly added. The reaction was stirred at 40 °C for 24 h, poured on water, and extracted with DCM. The combined organic layers were dried over anhydrous magnesium sulfate and evaporated. The crude product was purified by column chromatography (silica gel, DCM). Characterization of all obtained products is presented in ESI file.†

Antioxidant activity assays

DTT-assay. To a solution of compounds **6–19** (0.015 mmol) and dithiothreitol DTT^{red} (0.15 mmol) in 1.1 mL of CD_3OD , 30% H_2O_2 (0.15 mmol) was added. ^1H NMR spectra were measured right after the addition of hydrogen peroxide and then in specific time intervals. The concentration of the substrate was determined according to the changes in the integration on the ^1H NMR spectra.²¹

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH[•]) method. The antioxidant capacity of each compound was determined by spectrophotometric DPPH[•] method. The discussed compounds **6–19** were dissolved in methanol (5 mL), next different values of the obtained solutions were added to 0.5 mL DPPH[•] methanolic solution (0.30 mmol L⁻¹) and made up to 2.5 mL with methanol. The mixture was shaken vigorously and left in darkness for 15 min. The absorbance was measured at 517 nm against a reagent blank (2 mL of methanol with 0.5 mL of DPPH[•] methanolic solution) using a 1 cm quartz cell in a Shimadzu UV-1601 spectrophotometer.



The 50% DPPH' inhibition ($IC_{50_{DPPH}}$) was calculated based on linear regression analyses (the relationship curve of %DPPH scavenging *versus* concentrations of the respective sample).

The calibration curve was prepared using working solutions of 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) in methanol between 0.02–0.10 mmol mL⁻¹ and resulted in the equation: %DPPH' = 0.5155C_{TE} + 1.8291.

MTT viability assay. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay, which measures the activity of cellular dehydrogenases, was based on the method of Mosmann.²⁴ Briefly, cells were seeded into 24-well plates (about 8 × 10⁴ cells per mL) and then left to adhere and grow for 24 h. Subsequently, the tested compounds in the medium were added to a final concentration of 0–150 μM for 48 h, followed by the addition of 100 μL MTT, 5 mg mL⁻¹ in PBS, for the next 2 h. Then, the plates were centrifuged, the supernatant was discarded and remaining insoluble formazan crystals were dissolved in 1 mL DMSO. The absorbance of the blue formazan product was measured at 560 nm using FlexStation 3 Multi-Mode Microplate Reader (Molecular Devices, LLC, CA, USA) and compared with control (untreated cells). All experiments were performed three times in triplicate. The concentration of the tested compounds required to inhibit cell viability by 50% (IC_{50}) was calculated from concentration–response curves.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Financial support from the NCU research grant “Excellence Initiative – Debuts – III edition” number 03.01.00002702, Zad.6. IDUB/Debiuty_3_ and program for financing the costs of publication of doctoral students under the program “Initiative of excellence - Research University”.

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Publikacja 4 [P4]

Article

Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I

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Abstract: A series of unsymmetrical phenyl β -carbonyl selenides with *o*-amido function substituted on the nitrogen atom with chiral alkyl groups was obtained. The compounds form a series of enantiomeric and diastereomeric pairs and present the first examples of this type of chiral Se derivatives. All obtained selenides were further evaluated as antioxidants and anticancer agents to define the influence of the particular stereochemistry of the attached functional groups on the bioactivity of the molecules. The highest H₂O₂ reduction potential was observed for *N*-(*cis*-2-hydroxy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide, and the best radical scavenging properties for *N*-((-1-hydroxy-2-butanyl)-2-((2-oxopropyl)selanyl)benzamide. Also, both enantiomers of the *N*-(1-hydroxy-2-butanyl) selenide expressed the highest cytotoxic potential towards human promyelocytic leukemia HL-60 cell line with similar IC₅₀ values 14.4 ± 0.5 and 16.2 ± 1.1 μM, respectively. On the other hand, breast cancer cell line MCF-7 was most sensitive to *N*-((*R*)-(-)-1-hydroxy-2-butanyl)-2-((2-oxopropyl)selanyl)benzamide (IC₅₀ of 35.7 ± 0.6 μM). The structure–activity dependence of the obtained Se derivatives was discussed, and the most potent compounds were selected.

Keywords: 2-((2-oxopropyl)selanyl)benzamides; pharmacophore; antioxidant activity; antiproliferative activity



Citation: Laskowska, A.; Pacuła-Miszewska, A.J.; Obieziurska-Fabisiak, M.; Jastrzębska, A.; Długosz-Pokorska, A.; Gach-Janczak, K.; Ścianowski, J. Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I. *Materials* **2024**, *17*, 899. <https://doi.org/10.3390/ma17040899>

Academic Editor: Pavel Kopel

Received: 9 January 2024

Revised: 12 February 2024

Accepted: 13 February 2024

Published: 15 February 2024



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1. Introduction

Finding relationships between the chemical structure and biological activity is an approach that enables the proper design and further selection of potential drug candidates. Various structural features of the evaluated compounds have to be considered, including the installation of functional groups that influence the physicochemical properties of the molecule, the bonding arrangement of the compound's basic skeleton, and its chirality. The selection and incorporation of pharmacophores that can interact and fit into specific domains of the receptor and improve the molecule's bioavailability allow the potential drug's target bioproperty to be achieved [1–3]. In this context, organoselenium compounds, creating a diversified group of derivatives with remarkable redox properties, are constantly being modified and evaluated to find the perfect combination of structural features to maximize their biocapacity and toxicity reduction [4–6].

The importance and applicability of Se compounds have been highlighted in various research fields, including catalysis [7], material science [8], and medicinal chemistry [9,10]. Due to the high reactivity of the selenium atom and facile introduction into the structure of the substrate, through selenenylation and selenocyclization reactions [11], new Se derivatives can be efficiently obtained, providing a broad range of diversified heteroorganic compounds under mild reaction conditions. Among this group of derivatives,

N-substituted benzisoselenazolones **1** occupy an important place because of their unique ability to act as an artificial L-selenocysteine-like catalyst and mimic the activity of the antioxidant selenoenzyme glutathione peroxidase (GPx) [12]. It should be highlighted that the family of glutathione peroxidase includes eight isozymes and five of them are selenocysteine-containing proteins. Some of them are being studied with cancerous diseases. But, on the other hand, different GPx subtypes play different roles in tumors, and the mechanisms of action need to be studied further [13–16]. In the presented catalytic cycle of H₂O₂ reduction by GPx, the active selenol of L-Sec **2**, maintained in its ionized form through a hydrogen bond network with the neighboring amino acid environment (glutamine and tryptophan residues [17]) is first oxidized to the selenenic acid **3**, and further regenerated in the presence of two GSH molecules. The spatial structure of the potential GPx mimetic, which needs to interact with the homochiral L-amino acid residues of the enzyme, influences the efficiency of the entire biochemical cycle. In our research, we synthesized and evaluated the antioxidant and anticancer properties of various *N*-substituted benzisoselenazolones, including chiral *N*-terpenyl and aminoacid derivatives [18]. We have additionally explored the possibility of improving their catalytic properties by converting the Se-N bond into different functional groups that could also imitate the active selenol of L-Sec, like corresponding diselenides **5** [19], phenylselenides **6** [20], seleninic acids, and their potassium salts **7** [21]. Herein, we have further differentiated the primary structure by synthesizing a series of new *N*-functionalized β -carbonyl selenides **8** (Figure 1).

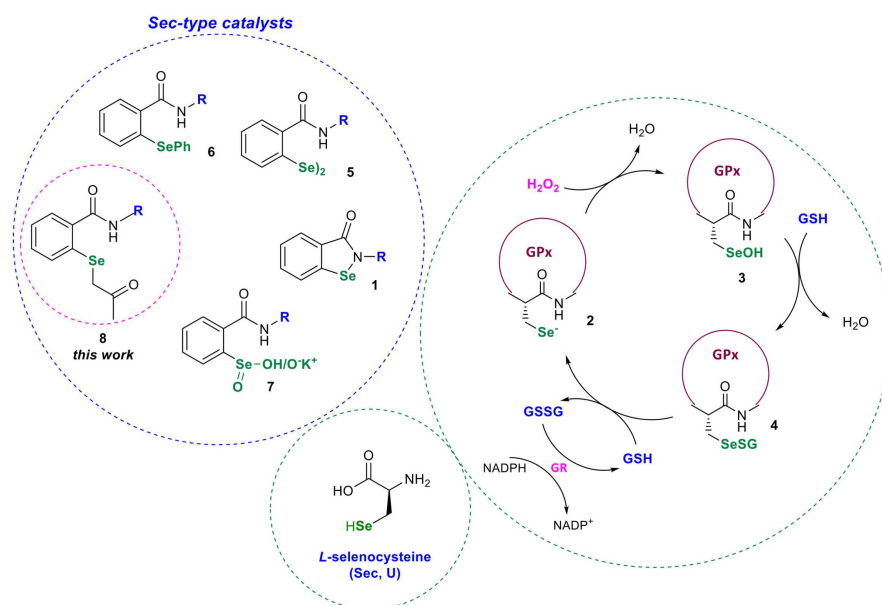


Figure 1. GPx activity cycle and examples of Sec-type catalysts **1**, **5**–**8**.

The synthesis of this type of selenide **8** in the form of *N*-chiral enantiomeric and diastereomeric pairs will enable us to conclude the influence of the configuration of particular carbon centers on the catalytic and antiproliferative activity of the molecules. Additionally, the ability of the 2-(2-oxopropyl)selanyl moiety to mimic the selenocysteine selenolate anion and the influence of an additional carbonyl moiety will be evaluated.

2. Results and Discussion

The first step of this research involved synthesizing *N*-substituted 2-((2-oxopropyl)selanyl)-benzamide derivatives. The first example of this type of compound, derived from D-glucosamine, was obtained by Z. Zhang and co-workers in 2010 [22]. The reaction of 2-(chloroseleno)benzoyl chloride with 1,3,4,6-tetra-O-acetyl-2-deoxy- β -D-glucopyranosylamine, performed in acetone and catalyzed by sodium bicarbonate, gave the final product with 60% yield. Then, Sh. Zhang et al. obtained a series of β -carbonyl selenides through a modified procedure

using dichloromethane as the solvent and eliminating NaHCO_3 [23,24]. Herein, we have optimized the reaction conditions based on the synthesis of selenide **9**. The method presented recently by Sh. Zhang and co-workers furnished the final product with only a 30% yield (entry 1). The addition of sodium bicarbonate slightly increased the efficiency of the procedure (entry 2). Using a 0.75 M solution of NaHCO_3 (entries 3 and 4) also did not improve the reaction yield. The order in which the reagents were added significantly influenced the overall process. The reaction's key step was mixing the ketone with sodium bicarbonate to generate the carbanion for the subsequent acylation of the selenium atom. The best result was obtained using an excess of acetone as both the substrate and the solvent, furnishing the final product with 87% yield (entry 6, Table 1).

Table 1. Optimization of reaction conditions.

Entry	Solvent (Solubilization of A)	NaHCO_3	Conditions and Order of Addition	Yield (%)
1	DCM	-	1. A + acetone, rt, 1 h 2. C, rt, 20 min	30
2	DCM	1 eq.	1. A + B, rt, 1 h 2. C, rt, 3 h	41
3	-	1 eq. (0.75 M $\text{NaHCO}_3/\text{H}_2\text{O}$)	1. A + C, rt, 20 min 2. B, 0 °C to rt, 2 h	37
4	Acetone	1 eq. (0.75 M $\text{NaHCO}_3/\text{H}_2\text{O}$)	1. B (acetone + H_2O solution of NaHCO_3 , rt, 25 min) + A, 0 °C to rt, 1 h 2. C, rt, 2 h	0
5	THF	1 eq.	1. B (acetone + NaHCO_3 , rt, 30 min) + A, rt, 1 h 2. C, rt, 3 h	65
6	Acetone	1 eq.	1. B (acetone + NaHCO_3 , rt, 30 min) + A, rt, 1 h 2. C, rt, 3 h	87

The selected conditions proved efficient for a variety of chiral amines. In one-pot synthesis, 2-(chloroseleno)-benzoyl chloride was first transformed in situ to the acetylated selenide **10**. Subsequent reactions with commercially available enantiomerically pure amines furnished a series of β -carbonyl selenides as enantiomeric and diastereomeric pairs in good yields (Scheme 1).

The ability to reduce hydrogen peroxide was evaluated by a conventionally used assay presented by Iwaoka and co-workers [25]. The method is based on the oxidation of the Se catalyst to its active species **25** by H_2O_2 , which is later reduced to its initial form by dithiothreitol (DTT^{red}). The dithiol DTT is simultaneously transformed into a disulfide (DTT^{ox}). ^1H NMR spectra, recorded in specific time intervals, present the rate of the dithiol formation, which equals the rate of H_2O_2 reduction. The results of the performed analysis are shown in Table 2.

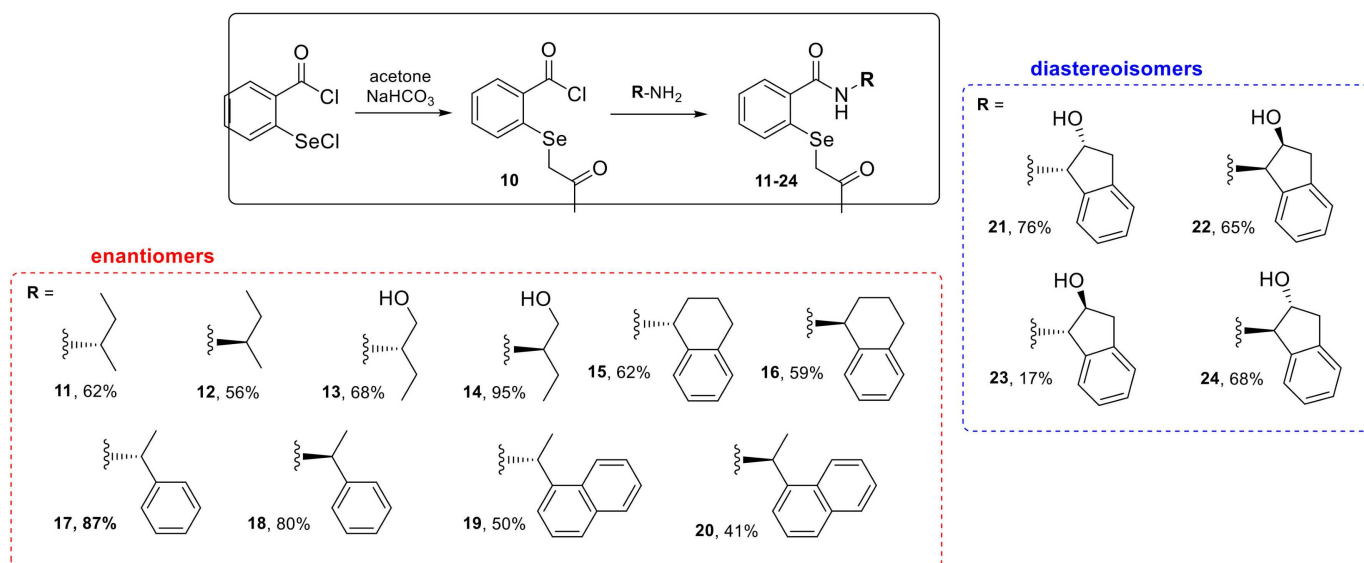
Scheme 1. Synthesized chiral β -carbonyl selenides 11–24.

Table 2. Results of the antioxidant activity measurement.

Catalyst (0.1 equiv.)	Remaining DTT ^{red} (%)			
	5 min	15 min	30 min	60 min
11/12	79 ± 5.4	61 ± 4.8	44 ± 2.8	13 ± 3.7
13/14	72 ± 7.4	61 ± 6.1	48 ± 3.7	21 ± 1.7
15/16	86 ± 0.1	78 ± 0.2	69 ± 0.1	56 ± 0.5
17/18	91 ± 2.2	90 ± 0.7	82 ± 1.4	73 ± 0.5
19/20	93 ± 0.1	90 ± 0.6	83 ± 0.8	70 ± 0.2
21/22	72 ± 2.5	52 ± 0.2	27 ± 2.7	6 ± 4.3
23/24	79 ± 0.3	56 ± 5.3	30 ± 3.3	10 ± 2.4
Ebselen	75	64	58	52

All enantiomeric and diastereomeric pairs presented the same H_2O_2 -scavenging potential. In general, the highest reactivity was observed for both *N*-indanyl selenides possessing the 2-hydroxy group in *cis* (21/22) and *trans* (23/24) configuration, with the DTT^{red} reduced to 6 and 10%, respectively. The best result obtained for compounds 21/22 was compared to the values measured for corresponding *N*-(*cis*-2-hydroxy-1-indanyl) Se derivatives. As presented in Figure 2, it can be observed that diselenides possessing a reactive and susceptible to facile cleavage Se-Se bond are the most efficient H_2O_2 scavengers. However, the presence of a β -carbonyl selenanyl group, instead of a phenylselenanyl moiety or a Se-N bond of the benzisoselenazolone core, significantly enhanced the H_2O_2 reduction potential.

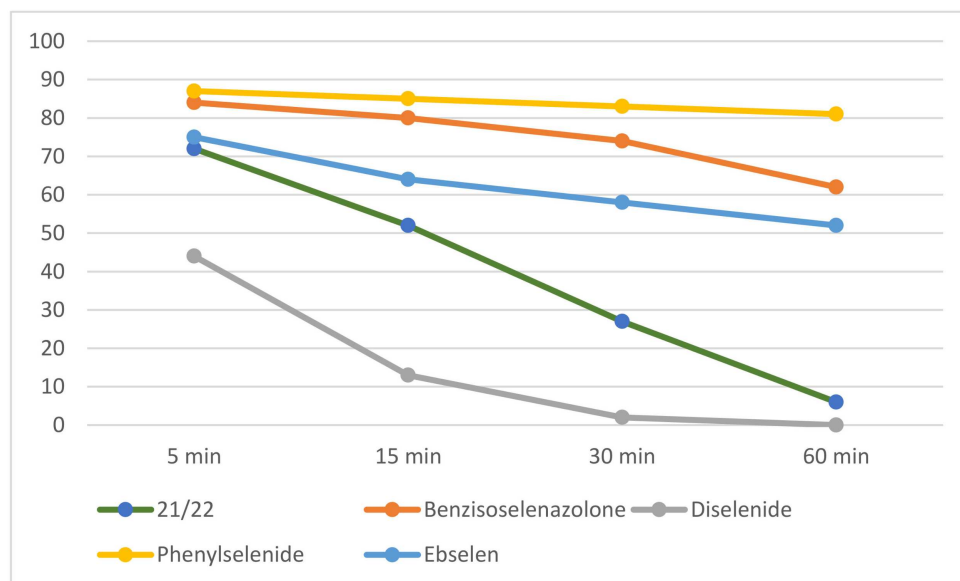


Figure 2. Results of the antioxidant activity measurements of corresponding Se derivatives.

It also has to be noted that the sterically undeveloped *N*-(2-butyl) derivatives **11/12** and those possessing an additional hydroxy group **13/14** were 4 and 2.5 fold, respectively, more active than the well-known GPx-mimetic ebselen. The introduction of additional aromatic rings to the *N*-alkyl chains, without the attachment of any polar OH group, seems to reduce the H₂O₂ scavenging capacity (derivatives **15/16**, **17/18**, **19/20**, Table 2).

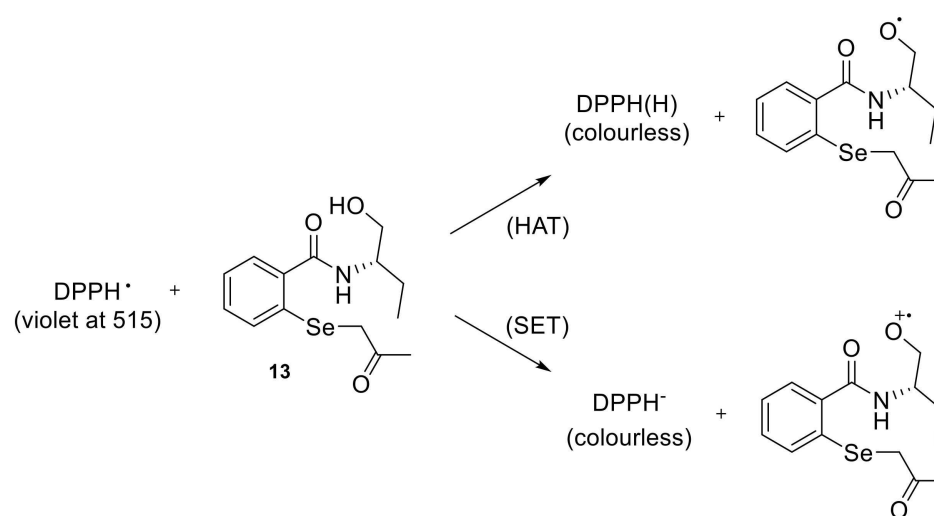
Next, the free radical scavenging activity of the tested selenides was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test (Table 3). This straightforward procedure relies on donating electrons from the tested compounds to neutralize the radical, changing the color of DPPH from purple to yellow [26,27]. The discussed assay depends on a mixed mode involving mechanisms such as hydrogen atom transfer, single electron transfer, and proton-coupled electron transfer [26]. The advantages of the DPPH test encompass low cost, effectiveness, experimental simplicity, reproducibility, applicability at ambient temperature, and the capability for result comparison with alternative radical scavenging methodologies [27–29]. In contrast to other free radicals, such as peroxy radicals, hydroxyl radicals, or superoxide radical anions, the DPPH radical exhibits stability [27]. On the other hand, DPPH is capable of reacting under certain conditions, even in the absence of antioxidants, for example, in the presence of oxygen or sunlight [30]. Nevertheless, due to the usually short time of reaction between tested compounds and DPPH radical (less than 60 min), these reactions do not affect the final result of the analysis.

Table 3. Results of DPPH test.

Compound	IC ₅₀ (mM)	Antioxidant Capacity (mM TE·1 g ⁻¹)
11/12	5.61	0.10
13/14	0.89	0.47
15/16	2.28	0.16
17/18	2.59	0.12
19/20	1.62	0.23
21/22	2.51	0.19
23/24	0.96	0.39

All tested selenides demonstrated the neutralization of the DPPH radical, nevertheless, all calculated values were higher than the IC₅₀ for Trolox (0.17 mM). The presence of different substituents affects their antioxidant activity. The scavenging capacity on DPPH

radicals was in the order of **13/14** > **23/24** > **19/20** > **21/22** > **15/16** > **17/18** > **11/12**. Additionally, the Trolox equivalent antioxidant capacity (TEAC) was calculated [31,32]. The obtained results were as follows: 0.19 (for **13/14**), 0.18 (for **23/24**), 0.10 (for **19/20**), 0.07 (for **21/22**, **15/16**, and **17/18**), and 0.03 for **11/12**. The tested compounds share a heteroaromatic ring of high electronegativity, and they only differ in their *N*-substituted amido group, which comes from chiral amines or amino alcohols. Obtained results indicated that enantiomers **13/14** were the most potent free radical inhibitors, which react with free radicals, particularly the hydroperoxide radical. In the structure of **13/14**, the nitrogen atom is substituted with a chiral aliphatic butyl chain possessing an OH group. The latter has been recognized as a hydrogen donor to quench electron mobility while interrupting the free radical chain reaction. Based on the mechanisms of the DPPH assay described in the literature [26,27,33,34], we assume that the reaction can occur by a combination of hydrogen atoms and/or a single electron transfer mechanism, presented for compound **13** in Scheme 2.



Scheme 2. A plausible mechanism for the DPPH reduction, where HAT—hydrogen atom transfer; SET—single electron transfer.

Studies of the antioxidant role of *N*-substituted benziselenazolones and their derivatives are scarce compared to those in biological substrates. Moreover, the IC₅₀ parameter is a characteristic property of a given antioxidant only under certain conditions, so comparing the obtained results with those described in the literature is often difficult. We have observed different effects of substituents on the DPPH radical scavenging properties for the tested compounds. Regarding the antioxidant activity, additional insertion of the hydroxyl group contributes to the scavenging activities. However, from the obtained results, establishing correlations between the structure of β -carbonyl selenides and their DPPH neutralization proved challenging. It would be interesting to know the possible mechanisms of the antioxidant activity of aromatic and aliphatic substituents of the tested structures, typical to different potentially interesting molecules with antioxidant activity. Therefore, our future research will focus on this issue.

The antiproliferative activity of all Se compounds was measured using a cell viability assay (MTT) on breast cancer MCF-7 and human promyelocytic leukemia HL-60 cell lines [35]. In this case, the highest cytotoxic potential was observed for both enantiomers of *N*-(1-hydroxy-2-butanyl) selenide **13** and **14** with similar IC₅₀ values— 14.4 ± 0.5 and 16.2 ± 1.1 μ M (HL-60 cell line), respectively (Table 4).

Table 4. The antiproliferative activity of compounds 11–24.

Compound	IC ₅₀ [μM] ± SEM	
	MCF-7	HL-60
11	46.1 ± 1.6	181 ± 11
12	55 ± 1.4	101 ± 14
13	39.2 ± 0.9	14.4 ± 0.5
14	35.7 ± 0.6	16.2 ± 1.1
15	115 ± 21	111 ± 3.5
16	188.3 ± 0.3	142 ± 1.7
17	235 ± 1	303 ± 3
18	237 ± 11	23.5 ± 1.4
19	87.4 ± 0.5	101 ± 0.6
20	72.4 ± 0.8	84.5 ± 0.6
21	39.2 ± 0.7	27.3 ± 0.5
22	296 ± 5	225 ± 2
23	37 ± 0.3	23.7 ± 1.1
24	38 ± 1.1	23.8 ± 1.1
Oxaliplatin	35 [36,37]	0.8 [38]

Comparing the results obtained for selenides **13** and **14** to the IC₅₀ values measured for corresponding phenylselenides, benzeneselenazoles, and diselenides, substituted on the nitrogen atom with the same *N*-(1-hydroxy-2-butanyl) scaffolds, we can observe a structure–activity dependence relative to the type of attached Se moiety. In general, the difference in cytotoxic potential is more visible towards HL-60 cell lines. Selenides **13** and **14** are 2-fold more active than corresponding benzeneselenazoles and slightly less cytotoxic than the *N*-(1-hydroxy-2-butanyl) diselenides. Additionally, the exchange of the β-carbonyl group to a phenyl ring significantly decreases the antiproliferative activity of the Se-derivative (Table 5).

Table 5. The antiproliferative activity of *N*-(1-hydroxy-2-butanyl) Se derivatives.

Se-derivative	IC ₅₀ [μM] ± SEM			
	MCF-7	HL-60	MCF-7	HL-60
β-Carbonylselenide	39.2 ± 0.9	14.4 ± 0.5	35.7 ± 0.6	16.2 ± 1.1
Phenylselenide	>150	>150	>150	>150
Benzeneselenazole	38.3 ± 1.3	26.0 ± 1.7	35.1 ± 0.5	33.3 ± 0.5
Diselenide	37.00 ± 4.25	8.67 ± 0.14	>100	10.10 ± 0.49

In the context of interaction with chiral biological targets, the most interesting phenomenon is to observe a difference in the activity between two enantio- or diastereoisomers. It was observed in the case of *N*-*cis*-2-hydroxy-1-indanyl derivatives **21** and **22**. When the stereochemistry of the C1 and C2 carbon centers was 1*R*,2*S*, the bio-activity towards both cell lines significantly decreased compared to the 1*S*,2*R* isomer. The *trans* epimers **23** and **24** also presented almost 10-fold more reactive than selenide **22** (Figure 3).

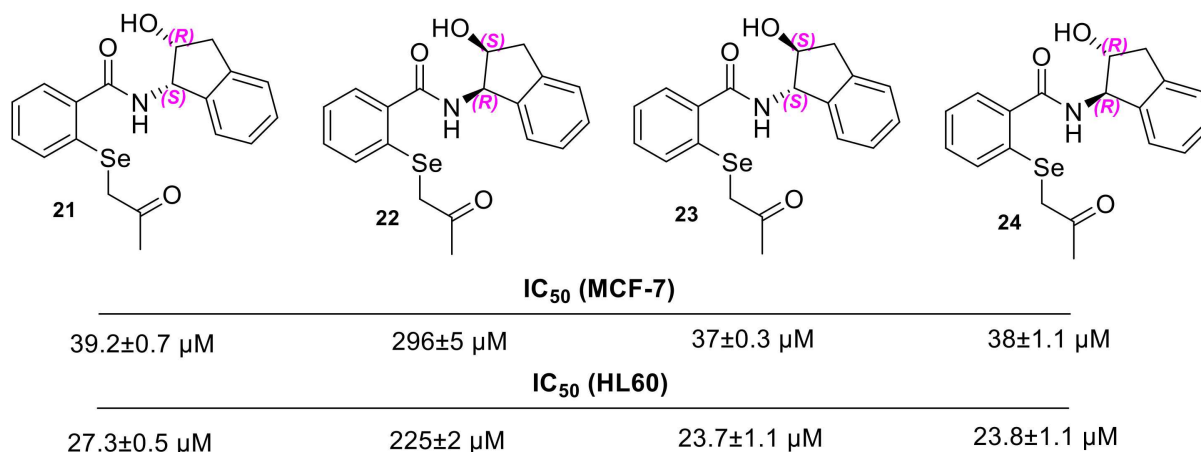


Figure 3. Different bio-activity of diastereomeric hydroxyindanyl selenides **21–24**.

3. Materials and Methods

3.1. General

NMR spectra were recorded on Bruker Avance III/400 or Bruker Avance III/700 (Karlsruhe, Germany) for ¹H and 176.1 MHz or 100.6 MHz for ¹³C (Supplementary Materials). Chemical shifts were recorded relative to SiMe₄ (δ0.00) or solvent resonance (CDCl₃ δ7.26, CD₃OD δ3.31). Multiplicities were given as s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet), and m (multiplet). The ⁷⁷Se NMR spectra were recorded on a Bruker Avance III/400 or Bruker Avance III/700 with diphenyl diselenide as an external standard. NMR spectra were carried out using ACD/NMR Processor Academic Edition. Melting points were measured with a Büchi Tottoli SPM-20 heating unit (Büchi Labortechnik AG, Flawil, Switzerland) and were uncorrected. Elemental analyses were performed on a Vario MACRO CHN analyzer. Optical rotations were measured in 10 mm cells with a polAAR 3000 polarimeter. Column chromatography was performed using Merck 40–63D 60Å silica gel (Merck, Darmstadt, Germany). HPLC analyses were recorded on Shimadzu LC-2060C with phenyl-hexyl column in liquid phase 5–95 or 50–99 (MeOH/H₂O); for chromatographs, see the Supplementary Materials (Figures S15–S28). All amines were commercially available and purchased from Merck (Merck, Darmstadt, Germany). Their chemical and optical purities were above 97%. The MCF-7 human breast adenocarcinoma cell line was acquired from the European Collection of Cell Cultures (ECACC, Nice, France). Cultivation involved the use of Minimum Essential Medium Eagle (MEME) supplemented with non-essential amino acids, antibiotics (100 mg/mL streptomycin and 100 U/mL penicillin, 2 mM glutamine, all sourced from Sigma-Aldrich, St. Louis, MO, USA), and 10% fetal bovine serum from Biological Industries (Beit-HaEmek, Israel). For the human leukemia cell line HL-60, obtained from the European Collection of Authenticated Cell Cultures (ECACC Nice, France), the culture medium was RPMI 1640 plus GlutaMax I (Invitrogen, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 μg/mL streptomycin and 100 U/mL penicillin). Cells were maintained at 37 °C in a 5% CO₂ atmosphere, allowing them to grow until reaching 80% confluence.

3.2. General Procedure and Analysis Data

NaHCO₃ (0.063 g, 0.75 mmol, 1 eq.) was stirred with acetone (2 mL) for 30 min, and then the solution of 2-chloroselenobenzoyl chloride (0.190 g, 0.75 mmol, 1 eq.) in acetone (1 mL) was added. After 1 h, amine (1 mmol, 1.3 eq.) was added, and the reaction was continued for 3 h (selenides **15–18**, **23**, **24**) or 12 h (selenides **11–14**, **19–22**) at room temperature. After the reaction was finished, the solvent was treated with water (10 mL) and then extracted with DCM. The combined organic layers were dried over magnesium sulfate, and the solvent was evaporated on a rotary evaporator. The crude product was

purified by column chromatography (silica gel, DCM) and then recrystallized from a mixture of DCM/heptane.

N-((*S*)-(+)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzamide **11**.

Yield: 62%; mp 73–74 °C; $[\alpha]_D^{20} = 33$ (c = 0.24, CHCl₃).

¹H NMR (400 MHz, DMSO) $\delta = 0.87$ (t, J = 7.6 Hz, 3H), 1.11 (d, J = 6.8 Hz, 3H), 1.44–1.52 (m, 2H), 2.20 (s, 3H), 3.73 (s, 2H), 3.80–3.85 (m, 1H), 7.24 (t, J = 7.6 Hz, 1H_{ar}), 7.38 (t, J = 8 Hz, 1H_{ar}), 7.50 (d, J = 8 Hz, 1H_{ar}), 7.55 (d, J = 7.6 Hz, 1H_{ar}), 8.21 (d, J = 8.8 Hz, 1H_{ar}) ¹³C NMR (400 MHz, DMSO) $\delta = 11.15$ (CH₃), 20.64 (CH₃), 28.67 (CH₃), 29.26 (CH₂), 35.66 (CH₂), 46.97 (CH), 125.80 (CH_{ar}), 128.28 (CH_{ar}), 129.74 (CH_{ar}), 131.00 (CH_{ar}), 132.40 (C_{ar}), 136.19 (C_{ar}), 167.58 (C=O), 205.03 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) $\delta = 319.53$ ppm; IR: 3291, 2960, 2932, 2872, 1694, 1636, 1585, 1532, 1457, 1427, 1356, 1264, 1230, 1141, 1039, 882, 743, 743, 533 cm⁻¹. Elemental Anal. Calcd for C₁₄H₁₉O₂Se (312.03): C, 53.85; H, 6.13; N, 4.49; Found C, 53.82; H, 6.12; N, 4.48; HPLC purity > 98%.

N-((*R*)-(-)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzamide **12**.

Yield: 56%; mp 72–73 °C; $[\alpha]_D^{20} = -32$ (c = 0.31, CHCl₃).

¹H NMR (400 MHz, DMSO) $\delta = 0.87$ (t, J = 7.6 Hz, 3H), 1.11 (d, J = 7.2 Hz, 3H), 1.42–1.50 (m, 2H), 2.20 (s, 3H), 3.73 (s, 2H), 3.80–3.89 (m, 1H), 7.24 (t, J = 7.2 Hz, 1H_{ar}), 7.36 (t, J = 8 Hz, 1H_{ar}), 7.50 (d, J = 8 Hz, 1H_{ar}), 7.55 (d, J = 7.6 Hz, 1H_{ar}), 8.21 (d, J = 8.4 Hz, 1H_{ar}) ¹³C NMR (400 MHz, DMSO) $\delta = 11.16$ (CH₃), 20.64 (CH₃), 28.67 (CH₃), 29.26 (CH₂), 39.67 (CH₂), 46.97 (CH), 125.80 (CH_{ar}), 128.28 (CH_{ar}), 129.75 (CH_{ar}), 131.00 (CH_{ar}), 132.40 (C_{ar}), 136.20 (C_{ar}), 167.58 (C=O), 205.03 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) $\delta = 319.58$ ppm; IR: 3299, 2963, 2928, 2873, 1695, 1626, 1583, 1535, 1455, 1431, 1357, 1283, 1230, 1147, 1031, 872, 746, 714, 546 cm⁻¹. Elemental Anal. Calcd for C₁₄H₁₉NO₂Se (312.03): C, 53.85; H, 6.13; N, 4.49; Found C, 53.86; H, 6.15; N, 4.49; HPLC purity > 97%.

N-((*S*)-(+)-1-hydroxy-2-butanyl)-2-((2-oxopropyl)selanyl)benzamide **13**.

Yield: 68%; mp 65–66 °C; $[\alpha]_D^{20} = 32$ (c = 0.39, CHCl₃).

¹H NMR (400 MHz, DMSO) $\delta = 0.89$ (t, J = 7.2 Hz, 3H), 1.33–1.45 (m, 1H), 1.58–1.67 (m, 1H), 2.20 (s, 3H), 3.40–3.49 (m, 2H), 3.73 (s, 2H), 3.78–3.83 (m, 1H), 4.65 (t, J = 5.6 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H_{ar}), 7.37 (t, J = 8 Hz, 1H_{ar}), 7.50 (d, J = 8 Hz, 1H_{ar}), 7.61 (d, J = 6.8 Hz, 1H_{ar}), 8.08 (d, J = 7.6 Hz, 1H_{ar}) ¹³C NMR (400 MHz, DMSO) $\delta = 11.05$ (CH₃), 24.08 (CH₂), 28.67 (CH₃), 39.64 (CH₂), 53.57 (CH), 63.52 (CH₂), 125.72 (CH_{ar}), 128.45 (CH_{ar}), 129.64 (CH_{ar}), 131.07 (CH_{ar}), 132.61 (C_{ar}), 135.95 (C_{ar}), 168.11 (C=O), 205.04 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) $\delta = 321.67$ ppm; IR: 3292, 2961, 2874, 1694, 1635, 1585, 1530, 1456, 1355, 1231, 1039, 1017, 880, 743, 685, 577 cm⁻¹. Elemental Anal. Calcd for C₁₄H₁₉NO₃Se (329.05): C, 51.22; H, 5.83; N, 4.27; Found C, 51.19; H, 5.81; N, 4.27; HPLC purity > 97%.

N-((*R*)-(-)-1-hydroxy-2-butanyl)-2-((2-oxopropyl)selanyl)benzamide **14**.

Yield: 95%; mp 66–67 °C; $[\alpha]_D^{20} = -35$ (c = 0.49, CHCl₃).

¹H NMR (400 MHz, DMSO) $\delta = 0.90$ (t, J = 7.6 Hz, 3H), 1.36–1.46 (m, 1H), 1.61–1.71 (m, 1H), 2.23 (s, 3H), 3.45–3.50 (m, 2H), 3.76 (s, 2H), 3.78–3.85 (m, 1H), 4.68 (t, J = 5.6 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H_{ar}), 7.38 (t, J = 8 Hz, 1H_{ar}), 7.52 (d, J = 8 Hz, 1H_{ar}), 7.63 (d, J = 8 Hz, 1H_{ar}), 8.10 (d, J = 8.4 Hz, 1H_{ar}) ¹³C NMR (400 MHz, DMSO) $\delta = 11.05$ (CH₃), 24.08 (CH₂), 28.67 (CH₃), 39.64 (CH₂), 53.57 (CH), 63.52 (CH₂), 125.72 (CH_{ar}), 128.45 (CH_{ar}), 129.64 (CH_{ar}), 131.06 (CH_{ar}), 132.61 (C_{ar}), 135.96 (C_{ar}), 168.11 (C=O), 205.04 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) $\delta = 321.67$ ppm; IR: 3291, 2961, 2872, 1694, 1636, 1585, 1532, 1457, 1355, 1230, 1039, 1018, 881, 743, 685, 576 cm⁻¹. Elemental Anal. Calcd for C, 51.22; H, 5.83; N, 4.27; Found C, 51.23; H, 5.84; N, 4.29; HPLC purity > 97%.

N-((*R*)-(-)-1,2,3,4-tetrahydro-1-naphthyl) 2-((2-oxopropyl)selanyl)benzamide **15**.

Yield: 62%; mp 115–116 °C; $[\alpha]_D^{20} = -35$ (c = 0.76, CHCl₃).

¹H NMR (400 MHz, DMSO) $\delta = 1.73$ –1.81 (m, 2H), 1.92–2.00 (m, 2H), 2.23 (s, 3H), 2.73–2.78 (m, 2H), 3.77 (s, 2H), 5.52–5.59 (m, 1H), 7.11–7.19 (m, 3H_{ar}), 7.21–7.28 (m, 2H_{ar}), 7.37 (t, J = 8 Hz, 1H_{ar}), 7.53 (d, J = 8 Hz, 1H_{ar}), 7.60 (d, J = 8 Hz, 1H_{ar}), 8.85 (d, J = 8.8 Hz, 1H) ¹³C NMR (700 MHz, DMSO) $\delta = 20.83$ (CH₂), 28.70 (CH₃), 29.32 (CH₂), 30.28 (CH₂), 35.77 (CH₂), 47.64 (CH), 125.84 (CH_{ar}), 126.29 (CH_{ar}), 127.16 (CH_{ar}), 128.32 (CH_{ar}), 128.48 (CH_{ar}), 129.22 (CH_{ar}), 129.84 (CH_{ar}), 131.17 (CH_{ar}), 132.62 (C_{ar}), 135.81 (C_{ar}), 137.65 (C_{ar}),

137.84 (C_{ar}), 167.84 (C=O), 205.03 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 321.23 ppm; IR: 3354, 2999, 2941, 1738, 1680, 1627, 1584, 1530, 1488, 1430, 1356, 1261, 1077, 738, 561 cm⁻¹. Elemental Anal. Calcd for C₂₀H₂₁NO₂Se (387.07): C, 62.18; H, 5.48; N, 3.63; Found C, 62.15; H, 5.51; N, 3.65; HPLC purity > 98%.

N-((*S*)-(+)-1,2,3,4-tetrahydro-1-naphthyl)-2-((2-oxopropyl)selanyl)benzamide **16**.

Yield: 59%; mp 114–115 °C; $[\alpha]_D^{20}$ = 34 (c = 0.74, CHCl₃).

¹H NMR (400 MHz, DMSO) δ = 1.74–1.81 (m, 2H), 1.90–1.99 (m, 2H), 2.22 (s, 3H), 2.63–2.68 (m, 2H), 3.77 (s, 2H), 5.51–5.60 (m, 1H), 7.14–7.18 (m, 3H_{ar}), 7.20–7.28 (m, 2H_{ar}), 7.37 (t, J = 7.2 Hz, 1H_{ar}), 7.53 (d, J = 7.2 Hz, 1H_{ar}), 7.59 (d, J = 7.6 Hz, 1H_{ar}), 8.83 (d, J = 8.8 Hz, 1H) ¹³C NMR (400 MHz, DMSO) δ = 20.82 (CH₂), 28.69 (CH₃), 29.30 (CH₂), 30.27 (CH₂), 35.78 (CH₂), 47.64 (CH), 125.85 (CH_{ar}), 126.28 (CH_{ar}), 127.16 (CH_{ar}), 128.31 (CH_{ar}), 128.46 (CH_{ar}), 129.22 (CH_{ar}), 129.87 (CH_{ar}), 131.16 (CH_{ar}), 132.56 (C_{ar}), 135.84 (C_{ar}), 137.65 (C_{ar}), 137.82 (C_{ar}), 167.85 (C=O), 205.03 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 319.81 ppm; IR: 3353, 2999, 2941, 1738, 1681, 1627, 1585, 1532, 1488, 1432, 1357, 1262, 1078, 739, 562 cm⁻¹. Elemental Anal. Calcd for C₂₀H₂₁NO₂Se (387.07): C, 62.18; H, 5.48; N, 3.63; Found C, 62.19; H, 5.47; N, 3.63; HPLC purity > 98%.

N-((*R*)-(+)- α -methylbenzyl)-2-((2-oxopropyl)selanyl)benzamide **17**.

Yield: 87%; mp 133–134 °C; $[\alpha]_D^{20}$ = 64 (c = 0.78, CHCl₃).

¹H NMR 400 MHz, DMSO) δ = 1.44 (d, J = 7.2 Hz, 3H), 2.19 (s, 3H), 3.72 (s, 2H), 5.07–5.13 (m, 1H), 7.17–7.24 (m, 1H_{ar}), 7.24–7.34 (m, 3H_{ar}), 7.35–7.42 (m, 3H_{ar}), 7.51 (d, J = 8 Hz, 1H_{ar}), 7.68 (d, J = 6 Hz, 1H_{ar}), 8.88 (d, J = 8 Hz, 1H) ¹³C NMR (300 MHz, DMSO) δ = 22.74 (CH₃), 28.67 (CH₃), 35.65 (CH₂), 48.97 (CH), 125.78 (CH_{ar}), 126.49 (2xCH_{ar}), 127.10 (CH_{ar}), 128.59 (CH_{ar}), 128.69 (2xCH_{ar}), 129.73 (CH_{ar}), 131.25 (CH_{ar}), 132.84 (C_{ar}), 135.38 (C_{ar}), 145.07 (C_{ar}), 167.31 (C=O), 204.99 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 322.26 ppm; IR: 3337, 2989, 2970, 1738, 1678, 1631, 1581, 1530, 1454, 1415, 1353, 1330, 1230, 1156, 755, 746, 686, 554, 541 cm⁻¹. Elemental Anal. Calcd for C₁₈H₁₉NO₂Se (361.06): C, 60.00; H, 5.32; N, 3.89; Found C, 59.95; H, 5.31; N, 3.89; HPLC purity > 98%.

N-((*S*)-(-)- α -methylbenzyl)-2-((2-oxopropyl)selanyl)benzamide **18**.

Yield: 80%; mp 132–133 °C; $[\alpha]_D^{20}$ = -61 (c = 0.60, CHCl₃).

¹H NMR 400 MHz, DMSO) δ = 1.46 (d, J = 7.2 Hz, 3H), 2.21 (s, 3H), 3.75 (s, 2H), 5.07–5.13 (m, 1H), 7.17–7.24 (m, 1H_{ar}), 7.24–7.34 (m, 3H_{ar}), 7.35–7.42 (m, 3H_{ar}), 7.53 (d, J = 8 Hz, 1H_{ar}), 7.69 (d, J = 6 Hz, 1H_{ar}), 8.91 (d, J = 8 Hz, 1H) ¹³C NMR (300 MHz, DMSO) δ = 22.72 (CH₃), 28.67 (CH₃), 35.67 (CH₂), 48.99 (CH), 125.81 (CH_{ar}), 126.50 (2xCH_{ar}), 127.10 (CH_{ar}), 128.59 (CH_{ar}), 128.70 (2xCH_{ar}), 129.73 (CH_{ar}), 131.25 (CH_{ar}), 132.78 (C_{ar}), 135.47 (C_{ar}), 145.06 (C_{ar}), 167.34 (C=O), 204.98 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 322.03 ppm; IR: 3336, 2989, 2970, 1738, 1678, 1630, 1582, 1529, 1455, 1414, 1354, 1331, 1232, 1157, 755, 747, 686, 555, 541 cm⁻¹. Elemental Anal. Calcd for C₁₈H₁₉NO₂Se (361.06): C, 60.00; H, 5.3; N, 3.89; Found C, 59.99; H, 5.30; N, 3.87; HPLC purity > 99%.

N-((*S*)-(-)-1-(1-naphthyl)ethyl)-2-((2-oxopropyl)selanyl)benzamide **19**.

Yield: 50%; mp 161–162 °C; $[\alpha]_D^{20}$ = -36 (c = 0.55, CHCl₃).

¹H NMR (400 MHz, DMSO) δ = 1.61 (d, J = 6.8 Hz, 3H), 2.21 (s, 3H), 3.75 (s, 2H), 5.90 (quin, J = 5.6 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H_{ar}), 7.40 (t, J = 7.6 Hz, 1H_{ar}), 7.44–7.57 (m, 5H_{ar}), 7.69 (d, J = 7.6 Hz, 1H_{ar}), 7.85 (d, J = 8 Hz, 1H_{ar}), 7.96 (d, J = 7.6 Hz, 1H_{ar}), 8.23 (d, J = 8 Hz, 1H_{ar}), 9.09 (d, J = 7.6 Hz, 1H) ¹³C NMR (400 MHz, DMSO) δ = 21.89 (CH₃), 28.65 (CH₃), 39.64 (CH₂), 45.31 (CH), 123.05 (CH_{ar}), 123.68 (CH_{ar}), 125.79 (CH_{ar}), 125.93 (CH_{ar}), 126.06 (CH_{ar}), 126.67 (CH_{ar}), 127.77 (CH_{ar}), 128.64 (CH_{ar}), 129.13 (CH_{ar}), 129.77 (CH_{ar}), 130.88 (C_{ar}), 131.25 (CH_{ar}), 132.76 (C_{ar}), 133.85 (C_{ar}), 135.47 (C_{ar}), 140.52 (C_{ar}), 167.26 (C=O), 204.93 (C=O) ⁷⁷Se NMR (400 MHz, DMSO) δ = 322.03 ppm; IR: 3378, 2971, 1738, 1693, 1633, 1537, 1455, 1356, 1257, 1133, 1031, 801, 781, 737, 643, 542 cm⁻¹. Elemental Anal. Calcd for C₂₂H₂₁NO₂Se (411.07): C, 64.39; H, 5.16; N, 3.41; Found C, 64.46; H, 5.15; N, 3.42; HPLC purity > 98%.

N-((*R*)-(+)-1-(1-naphthyl)ethyl)-2-((2-oxopropyl)selanyl)benzamide **20**.

Yield: 41%; mp 161–162 °C; $[\alpha]_D^{20}$ = 37 (c = 0.49, CHCl₃).

^1H NMR (400 MHz, DMSO) δ = 1.58 (d, J = 7.2 Hz, 3H), 2.18 (s, 3H), 3.73 (s, 2H), 5.87 (quin, J = 7.6 Hz, 1H), 7.25 (t, J = 7.2 Hz, 1H_{ar}), 7.37 (t, J = 7.2 Hz, 1H_{ar}), 7.45–7.64 (m, 5H_{ar}), 7.67 (d, J = 7.6 Hz, 1H_{ar}), 7.82 (d, J = 8 Hz, 1H_{ar}), 7.93 (d, J = 8 Hz, 1H_{ar}), 8.21 (d, J = 8 Hz, 1H_{ar}), 9.07 (d, J = 7.6 Hz, 1H) ^{13}C NMR (400 MHz, DMSO) δ = 21.89 (CH₃), 28.65 (CH₃), 39.61 (CH₂), 45.30 (CH), 123.05 (CH_{ar}), 123.68 (CH_{ar}), 125.78 (CH_{ar}), 125.93 (CH_{ar}), 126.06 (CH_{ar}), 126.66 (CH_{ar}), 127.77 (CH_{ar}), 128.63 (CH_{ar}), 129.12 (CH_{ar}), 129.77 (CH_{ar}), 130.88 (C_{ar}), 131.25 (CH_{ar}), 132.76 (C_{ar}), 133.85 (C_{ar}), 135.47 (C_{ar}), 140.51 (C_{ar}), 167.25 (C=O), 204.92 (C=O) ^{77}Se NMR (400 MHz, DMSO) δ = 322.07 ppm; IR: 3377, 2970, 1738, 1693, 1633, 1537, 1454, 1365, 1257, 1132, 1032, 801, 781, 737, 642, 542 cm⁻¹. Elemental Anal. Calcd for C₂₂H₂₁NO₂Se (411.07): C, 64.39; H, 5.16; N, 3.41; Found C, 64.44; H, 5.18; N, 3.43; HPLC purity > 98%.

N-((1*S*,2*R*)-(-)-*cis*-2-hydroxy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide **21**.

Yield: 76%; mp 111–112 °C; $[\alpha]_D^{20}$ = -45 (c = 0.38, CHCl₃).

^1H NMR (700 MHz, DMSO) δ = 2.26 (s, 3H), 2.86–2.90 (m, 1H), 3.08–3.13 (m, 1H), 3.80 (d, J = 5.6 Hz, 2H), 4.50–4.53 (m, 1H), 5.12 (d, J = 4.2 Hz, 1H), 5.38–5.42 (m, 1H), 7.19–7.25 (m, 2H_{ar}), 7.26–7.29 (m, 3H_{ar}), 7.41 (t, J = 7.7 Hz, 1H_{ar}), 7.56 (d, J = 7.7 Hz, 1H_{ar}), 7.79 (d, J = 7.7 Hz, 1H_{ar}), 8.32 (d, J = 9.1 Hz, 1H) ^{13}C NMR (700 MHz, DMSO) δ = 28.78 (CH₃), 35.75 (CH₂), 40.18 (CH₂), 57.85 (CH), 72.55 (CH), 124.81 (CH_{ar}), 125.31 (CH_{ar}), 125.76 (CH_{ar}), 126.77 (CH_{ar}), 127.88 (CH_{ar}), 128.98 (CH_{ar}), 129.60 (CH_{ar}), 131.37 (CH_{ar}), 133.13 (C_{ar}), 135.09 (C_{ar}), 141.36 (C_{ar}), 142.20 (C_{ar}), 168.33 (C=O), 205.10 (C=O) ^{77}Se NMR (700 MHz, DMSO) δ = 324.41 ppm; IR: 3283, 2947, 2926, 1724, 1634, 1583, 1562, 1477, 1423, 1353, 1227, 1080, 793, 737, 584 cm⁻¹. Elemental Anal. Calcd for C₁₉H₁₉NO₃Se (389.05): C, 58.77; H, 4.93; N, 3.61; Found C, 58.81; H, 4.90; N, 3.59; HPLC purity > 99%.

N-((1*R*,2*S*)-(+)-*cis*-2-hydroxy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide **22**.

Yield: 65%; mp 110–111 °C; $[\alpha]_D^{20}$ = 48 (c = 0.42, CHCl₃).

^1H NMR (700 MHz, DMSO) δ = 2.26 (s, 3H), 2.87–2.91 (m, 1H), 3.08–3.14 (m, 1H), 3.80 (d, J = 5.6 Hz, 2H), 4.51–4.55 (m, 1H), 5.12 (d, J = 4.2 Hz, 1H), 5.39–5.42 (m, 1H), 7.18–7.25 (m, 2H_{ar}), 7.26–7.29 (m, 3H_{ar}), 7.41 (t, J = 7.7 Hz, 1H_{ar}), 7.56 (d, J = 7 Hz, 1H_{ar}), 7.80 (d, J = 7.7 Hz, 1H_{ar}), 8.32 (d, J = 8.4 Hz, 1H) ^{13}C NMR (400 MHz, DMSO) δ = 28.78 (CH₃), 35.75 (CH₂), 40.18 (CH₂), 57.85 (CH), 72.55 (CH), 124.81 (CH_{ar}), 125.32 (CH_{ar}), 125.77 (CH_{ar}), 126.78 (CH_{ar}), 127.88 (CH_{ar}), 128.97 (CH_{ar}), 129.61 (CH_{ar}), 131.37 (CH_{ar}), 133.10 (C_{ar}), 135.12 (C_{ar}), 141.36 (C_{ar}), 142.19 (C_{ar}), 168.34 (C=O), 205.10 (C=O) ^{77}Se NMR (700 MHz, DMSO) δ = 325.43 ppm; IR: 3349, 2923, 2853, 1724, 1626, 1584, 1560, 1509, 1459, 1354, 1228, 1094, 795, 738, 580 cm⁻¹. Elemental Anal. Calcd for C₁₉H₁₉NO₃Se (389.05): C, 58.77; H, 4.93; N, 3.61; Found C, 58.76; H, 4.94; N, 3.60; HPLC purity > 98%.

N-((1*S*,2*S*)-(-)-*trans*-2-hydroxy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide **23**.

Yield: 17%; mp 112–114 °C; $[\alpha]_D^{20}$ = 35 (c = 0.38, CHCl₃).

^1H NMR (700 MHz, DMSO) δ = 2.26 (s, 3H), 2.71–2.79 (m, 1H), 3.13–3.19 (m, 1H), 3.80 (d, J = 7 Hz, 2H), 4.42 (quin, J = 7 Hz, 1H), 5.22 (t, J = 7.7 Hz, 1H), 5.39 (d, J = 5.6 Hz, 1H), 7.26–7.34 (m, 4H_{ar}), 7.28 (t, J = 6.3 Hz, 1H_{ar}), 7.41 (t, J = 7 Hz, 1H_{ar}), 7.57 (d, J = 5.6 Hz, 1H_{ar}), 7.70 (d, J = 7.7 Hz, 1H_{ar}), 8.84 (d, J = 5.6 Hz, 1H) ^{13}C NMR (300 MHz, DMSO) δ = 28.71 (CH₃), 35.67 (CH₂), 39.11 (CH₂), 61.97 (CH), 77.74 (CH), 124.34 (CH_{ar}), 125.11 (CH_{ar}), 125.76 (CH_{ar}), 127.11 (CH_{ar}), 128.09 (CH_{ar}), 128.62 (CH_{ar}), 129.66 (CH_{ar}), 131.31 (CH_{ar}), 132.95 (C_{ar}), 135.38 (C_{ar}), 140.26 (C_{ar}), 142.26 (C_{ar}), 168.55 (C=O), 205.08 (C=O) ^{77}Se NMR (700 MHz, DMSO) δ = 321.18 ppm; IR: 3303, 2997, 2940, 1738, 1677, 1642, 1541, 1364, 1232, 748, 647, 542, 517 cm⁻¹. Elemental Anal. Calcd for C₁₉H₁₉NO₃Se (389.05): C, 58.77; H, 4.93; N, 3.61; Found C, 58.67; H, 4.91; N, 3.62; HPLC purity > 98%.

N-((1*R*,2*R*)-(-)-*trans*-2-hydroxy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide **24**.

Yield: 68%; mp 112–114 °C; $[\alpha]_D^{20}$ = -31 (c = 0.58, CHCl₃).

^1H NMR (700 MHz, DMSO) δ = 2.26 (s, 3H), 2.74–2.79 (m, 1H), 3.13–3.19 (m, 1H), 3.80 (d, J = 7 Hz, 2H), 4.42 (quin, J = 7 Hz, 1H), 5.22 (t, J = 7.7 Hz, 1H), 5.39 (d, J = 5.6 Hz, 1H), 7.17–7.24 (m, 4H_{ar}), 7.29 (t, J = 7 Hz, 1H_{ar}), 7.41 (t, J = 6.3 Hz, 1H_{ar}), 7.57 (d, J = 6.3 Hz, 1H_{ar}), 7.70 (d, J = 6.3 Hz, 1H_{ar}), 8.84 (d, J = 8.4 Hz, 1H) ^{13}C NMR (300 MHz, DMSO) δ = 28.71 (CH₃), 35.68 (CH₂), 39.11 (CH₂), 61.97 (CH), 77.74 (CH), 124.32 (CH_{ar}), 125.11

(CH_{ar}), 125.75 (CH_{ar}), 127.12 (CH_{ar}), 128.10 (CH_{ar}), 128.63 (CH_{ar}), 129.68 (CH_{ar}), 131.31 (CH_{ar}), 132.97 (C_{ar}), 135.36 (C_{ar}), 140.27 (C_{ar}), 142.27 (C_{ar}), 168.54 (C=O), 205.08 (C=O) ⁷⁷Se NMR (700 MHz, DMSO) δ = 324.38 ppm; IR: 3305, 2997, 2942, 1738, 1677, 1642, 1541, 1365, 1229, 748, 647, 528, 517 cm⁻¹. Elemental Anal. Calcd for C₁₉H₁₉NO₃Se (389.05): C, 58.77; H, 4.93; N, 3.61; Found C, 58.71; H, 4.95; N, 3.63; HPLC purity > 97%.

3.3. Antioxidant Activity Evaluation

3.3.1. DTT Activity Assay

To a solution of compounds **11–24** (0.015 mmol) and dithiothreitol DTT^{red} (0.15 mmol) in 1.1 mL of CD₃OD, 30% H₂O₂ (0.15 mmol) was added. ¹H NMR spectra were measured right after the addition of hydrogen peroxide and then in specific time intervals. The concentration of the substrate was determined according to the changes in the integration on the ¹H NMR spectra [25], Supplementary Materials (Figures S29–S31).

3.3.2. DPPH Radical Scavenging Assay

The 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl test is based on the capacity of tested compounds to scavenge the stable free radical DPPH, and the obtained results are expressed as inhibitory concentration (IC₅₀).

The radical neutralization test was conducted according to the previously described method [39]. In brief, calibration curves were created for each tested compound by increasing volumes of selenides. Solutions in methanol to a 0.5 mL methanolic DPPH radical (0.3 mM) made up with methanol to the 2.0 mL. All solutions were measured in triplicate against a reagent blank (2 mL of methanol + 0.5 mL of DPPH methanolic solution) after 30 min at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan).

The inhibition ratio (%) was obtained from the following equation:

$$\text{inhibition ratio (\%)} = \frac{A1 - A0}{A0} \cdot 100\%, \text{ where :}$$

A1—absorbance of sample

A0—absorbance of the reagent blank.

Next, the 50% DPPH inhibition (IC₅₀) was calculated by the linear regression analysis between the radical scavenging percentage against the tested compound concentration. All obtained curves are presented in Supplementary Materials (Figures S32–S39).

Additionally, the antioxidant capacity (AC) of tested compounds was determined and expressed as millimoles of Trolox (TE) equivalent per 1 g of compounds. For this purpose, the calibration curve of inhibition ratio (%DPPH) vs. TE concentration was prepared and presented in the Supporting Information (Figure S22). Finally, the results of DPPH radical scavenging activity by β-carbonyl selenides were presented as Trolox equivalent antioxidant capacity (TEAC) and calculated as follows [31,32]:

$$\text{Trolox equivalent antioxidant capacity} = \frac{\text{TroloxIC}_{50}[\text{mM}]}{\text{Tested } \beta\text{-carbonylselenidesIC}_{50}[\text{mM}]}$$

3.4. MTT Viability Assay

The MTT (3-(4,5-diazol-2-yl)-2,5 diphenyl tetrazolium bromide) assay, which measures the activity of methylcellular dehydrogenases, was based on Mosmann's method [35]. Briefly, cells were seeded into 96-well plates (about 1.5 × 10⁴ cells per well, in 100 μL) and then left to adhere and grow for 24 h. Subsequently, 100 μL of the tested compounds in the medium was added to a final concentration of 0–250 μM for 24 h, followed by the addition of 100 μL MTT, 3 mg/mL in PBS, for the next 3 h. After the incubation, the medium was removed. The remaining insoluble formazan crystals were dissolved in 100 μL DMSO. The absorbance of the blue formazan product was measured at 570 nm in the plate reader spectrophotometer Infinite M200 (Tecan, Austria) and compared with the control (untreated cells). All experiments were performed three times in triplicate. The concentration of the

tested compounds required to inhibit cell viability by 50% (IC₅₀) was calculated using Microsoft Excel software for semi-log curve fitting with linear regression analysis.

4. Conclusions

Herein, we optimized the synthesis of chiral β -carbonyl selenides and proved that the generation of the carbanion from the starting ketone is crucial for the high efficiency of the reaction. This procedure was further used for the synthesis of *N*-substituted derivatives in the form of enantiomeric and diastereomeric pairs, presenting the first examples of unsymmetrical (2-oxopropyl)selanyl benzamides possessing chiral groups attached to the nitrogen atom. All compounds were tested as antioxidants by two assays: the DTT method, presenting the ability to reduce H₂O₂, and the DPPH procedure for evaluating radical scavenging capacity. In both cases, derivatives possessing hydroxy groups were the most active ones—selenides with 2-hydroxy group in *cis* (DTT assay) and *trans* configuration (DTT and DPPH method) and 1-hydroxy-2-butanyl substituent (DPPH assay). Additionally, the antiproliferative capacity towards breast cancer MCF-7 and human promyelocytic leukemia HL-60 cell lines was measured. Also, in this case, both enantiomers of *N*-(1-hydroxy-2-butanyl) selenides were the most potent cytotoxic agents with similar IC₅₀ values. Considering the chirality–activity dependence, we have observed that the configurations of C1 and C2 carbon of the *N*-2-hydroxy-1-indanyl derivatives influence their antiproliferative potential. The results indicate that the *N*-1-hydroxy-2-butanyl and *N*-2-hydroxy-1-indanyl substituents can be selected as possible pharmacophores to develop potential Se therapeutics with optimal drug–target interaction further.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ma17040899/s1>, Figure S1: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*S*)-(+)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzamide 11; Figure S2: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*R*)-(-)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzamide 12; Figure S3: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*S*)-(+)-1-hydroksy-2-butanyl)-2-((2-oxopropyl)selanyl)benzamide 13; Figure S4: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*R*)-(-)-1-hydroksy-2-butanyl)-2-((2-oxopropyl)selanyl)benzamide 14; Figure S5: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*R*)-(-)-1,2,3,4-tetrahydro-1-naphtyl)-2-((2-oxopropyl)selanyl)benzamide 15; Figure S6: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*S*)-(+)-1,2,3,4-tetrahydro-1-naphtyl)-2-((2-oxopropyl)selanyl)benzamide 16; Figure S7: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*R*)-(+)- α -metylbenzyl)-2-((2-oxopropyl)selanyl)benzamide 17; Figure S8: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*S*)-(-)- α -metylbenzyl)-2-((2-oxopropyl)selanyl)benzamide 18; Figure S9: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*S*)-(-)-1-(1-naphtyl)etyl)-2-((2-oxopropyl)selanyl)benzamide 19; Figure S10: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((*R*)-(+)-1-(1-naphtyl)etyl)-2-((2-oxopropyl)selanyl)benzamide 20; Figure S11: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((1*S*,2*R*)-(-)-*cis*-2-hydroksy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide 21; Figure S12: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((1*R*,2*S*)-(+)-*cis*-2-hydroksy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide 22; Figure S13: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((1*S*,2*S*)-(+)-*trans*-2-hydroksy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide 23; Figure S14: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *N*-((1*R*,2*R*)-(-)-*trans*-2-hydroksy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide 24; Figure S15: Chromatograph of *N*-((*S*)-(+)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzamide 11; Figure S16: Chromatograph of *N*-((*R*)-(-)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzamide 12; Figure S17: Chromatograph of *N*-((*S*)-(+)-1-hydroksy-2-butanyl)-2-((2-oxopropyl)selanyl)benzamide 13; Figure S18: Chromatograph of *N*-((*R*)-(-)-1-hydroksy-2-butanyl)-2-((2-oxopropyl)selanyl)benzamide 14; Figure S19: Chromatograph of *N*-((*R*)-(-)-1,2,3,4-tetrahydro-1-naphtyl)-2-((2-oxopropyl)selanyl)benzamide 15; Figure S20: Chromatograph of *N*-((*S*)-(+)-1,2,3,4-tetrahydro-1-naphtyl)-2-((2-oxopropyl)selanyl)benzamide 16; Figure S21: Chromatograph of *N*-((*R*)-(+)- α -metylbenzyl)-2-((2-oxopropyl)selanyl)benzamide 17; Figure S22: Chromatograph of *N*-((*S*)-(-)- α -metylbenzyl)-2-((2-oxopropyl)selanyl)benzamide 18; Figure S23: Chromatograph of *N*-((*S*)-(-)-1-(1-naphtyl)etyl)-2-((2-oxopropyl)selanyl)benzamide 19; Figure S24: Chromatograph of *N*-((*S*)-(-)-1-(1-naphtyl)etyl)-2-((2-oxopropyl)selanyl)benzamide 20; Figure S25: Chromatograph of *N*-((1*S*,2*R*)-(-)-*cis*-2-hydroksy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide 21; Figure S26: Chromatograph of *N*-((1*S*,2*R*)-(-)-

cis-2-hydroksy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide 22; Figure S27: Chromatograph of *N*-((1*S*,2*S*)-(+)-trans-2-hydroksy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide 23; Figure S28: Chromatograph of *N*-((1*R*,2*R*)-(-)-trans-2-hydroksy-1-indanyl)-2-((2-oxopropyl)selanyl)benzamide 24; Figure S29: Example of ¹H NMR spectra for antioxidant Iwaoka test after reaction time 5 min, 15 min, 30 min, 60 min for compound 23; Figure S30: Results of antioxidant activity measurement of integration from ¹H NMR spectra after reaction time 5 min and 15 min for all samples and compound 11–24; Figure S31: Results of antioxidant activity measurement of integration from ¹H NMR spectra after reaction time 30 min and 60 min for all samples and compound 11–24; Figure S32: Compound 11/12—calibration curve; Figure S33: Compound 13/14—calibration curve; Figure S34: Compound 15/16—calibration curve; Figure S35: Compound 17/18—calibration curve; Figure S36: Compound 19/20—calibration curve; Figure S37: Compound 21/22—calibration curve; Figure S38: Compound 23/24—calibration curve; Figure S39: Standard curves for the relationship: absorbance = function (concentration TE) (A), % DPPH = function (concentration of TE) (B).

Author Contributions: Conceptualization, A.J.P.-M. and J.Ś.; methodology, A.L., A.J.P.-M.; formal analysis, A.L., M.O.-F. and A.D.-P.; investigation, A.L. and A.D.-P.; resources, A.J.P.-M.; data curation, A.L. and A.D.-P.; writing—original draft preparation, A.J.P.-M., A.L. and A.J.; writing—review and editing, A.L., J.Ś. and A.D.-P.; visualization, A.L. and A.J.; supervision, J.Ś. and K.G.-J.; project administration, J.Ś.; funding acquisition, J.Ś., K.G.-J. and A.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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Publikacja 5 [P5]

Article

Synthesis of a New Class of β -Carbonyl Selenides Functionalized with Ester Groups with Antioxidant and Anticancer Properties—Part II

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Abstract: A series of phenyl β -carbonyl selenides with *o*-ester functionality substituted on the oxygen atom with chiral and achiral alkyl groups was synthesized. All compounds are the first examples of this type of organoselenium derivatives with an ester substituent in the ortho position. The obtained derivatives were tested as antioxidants and anticancer agents to see the influence of an ester functionality on the bioactivity of β -carbonyl selenides by replacing the *o*-amide group with an *o*-ester group. The best results as an antioxidant agent were observed for *O*-((1*R*,2*S*,5*R*)-(–)-2-isopropyl-5-methylcyclohexyl)-2-((2-oxopropyl)selanyl)benzoate. The most cytotoxic derivative against breast cancer MCF-7 cell lines was *O*-(methyl)-2-((2-oxopropyl)selanyl)benzoate and against human promyelocytic leukemia HL-60 was *O*-(2-pentyl)-2-((2-oxopropyl)selanyl)benzoate.

Keywords: 2-((2-oxopropyl)selanyl)benzoates; pharmacophore; antioxidant activity; antiproliferative activity



Citation: Laskowska, A.; Pacuła-Miszewska, A.J.; Obieziurska-Fabisiak, M.; Jastrzębska, A.; Długosz-Pokorska, A.; Gach-Janczak, K.; Ścianowski, J. Synthesis of a New Class of β -Carbonyl Selenides Functionalized with Ester Groups with Antioxidant and Anticancer Properties—Part II. *Molecules* **2024**, *29*, 2866. <https://doi.org/10.3390/molecules29122866>

Academic Editor: Vladimir A. Potapov

Received: 5 May 2024

Revised: 2 June 2024

Accepted: 13 June 2024

Published: 16 June 2024



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1. Introduction

Organic molecules, both of a synthetic and natural origin, are utilized in almost every field of industry, depending on their applicability, determined mainly by the particular functional groups incorporated into their structure. Among those, esters are one of the most exploited compounds, commonly known for their characteristic fragrance properties and widely used in the perfume industry [1]. In addition to the natural presence in essential oils and pheromones, the ester bond is also a dominating functionality in many primary (e.g., lipids and carbohydrates [2]) and secondary (e.g., lactones, terpenoids and steroids [3]) metabolites [4]. The ester linkage is essential for several biochemical pathways due to its ability and facile cleavage by esterases, hydrolase-type enzymes that split the ester into a carboxylic acid and alcohol [5–7]. In the context of drug development, the introduction of an ester moiety bears several advantages. The transformation of bioactive compounds like polar carboxyl or hydroxyl groups into an ester prodrug improves its solubility and bioavailability [8]. Increased membrane permeability facilitates the delivery and installation inside the cells, where the molecule can be hydrolyzed to its water-soluble active form [9,10]. Using esterification in drug modification solves the bioavailability problem of many known bioactive agents. Examples of drugs applied in their ester form are presented in Figure 1 [11–13].

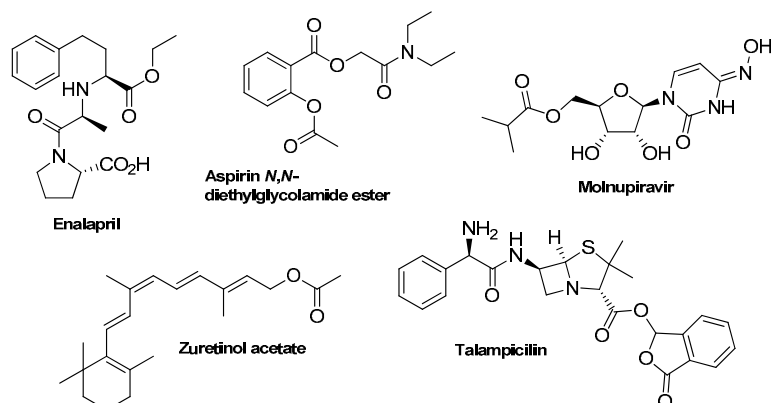
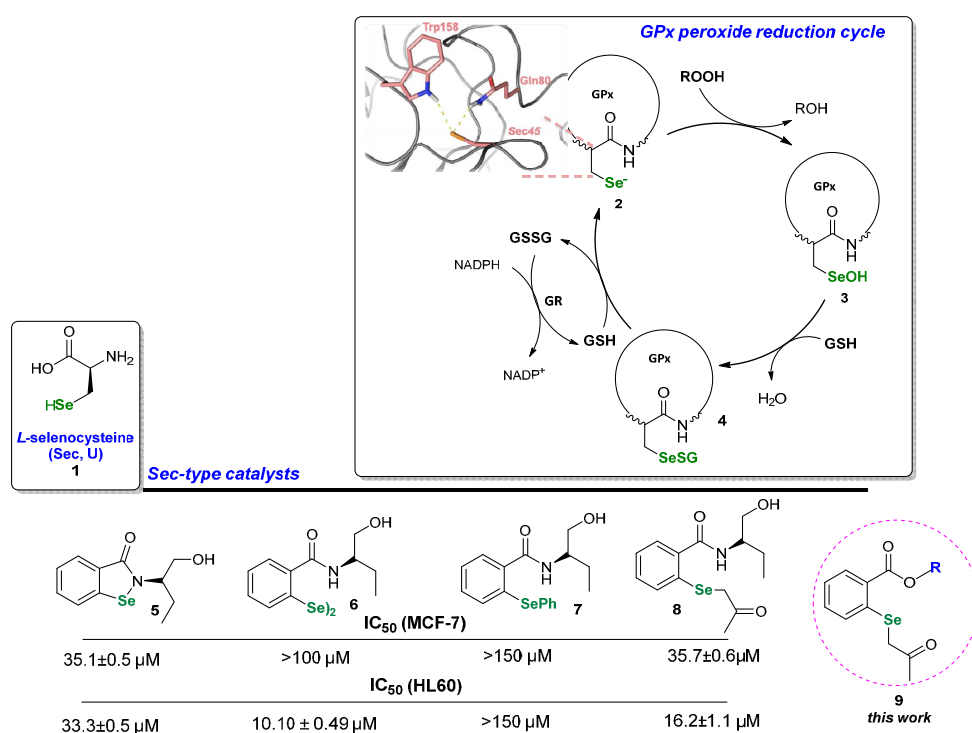


Figure 1. Examples of ester-type drugs.

Organoselenium compounds like selenides, diselenides and selenols are well known to possess a multitude of possible biological activities due to the ability to mimic the activity of several selenoenzymes, including the antioxidant enzyme glutathione peroxidase (GPx), and they are involved in thyroid physiology, thioredoxin reductase (TRx) and iodothyronine deiodinase (ID) [14–16]. A catalytic reduction in peroxides by GPx is presented in Scheme 1 [17]. *L*-selenocysteine **1** builds up the active side of the enzyme and catalyzes the ROOH reduction to ROH [18]. The Se atom of **2** is maintained in its active ionized form due to the specific amino acid environment—glutamine and tryptophan—creating the catalytic triad with *L*-Sec [19]. The formed seleninic acid **3** is reduced to the starting RSe[−] in a two-step process by two glutathione (GHS) molecules. Se-derivatives acting as artificial *L*-Sec can exhibit various bioactivities [20]. Until now, we have synthesized a diversified series of GPx mimetics, including benzoselenazolones **5**, corresponding diselenides **6** [21] and phenyl selenides **7** [22]. An evaluation of their antiproliferative activity revealed that installing a -SePh functionality significantly decreases the reactivity. To address this issue, we created a new type of selenide-type catalyst possessing a 2-(2-oxopropyl)selenyl group **8** and showed that this modification significantly enhanced the reactivity of the tested compounds [23].



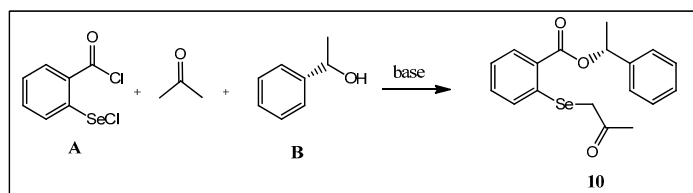
Scheme 1. GPx activity cycle and examples of Sec-type catalysts 5–9.

Herein, we wanted to examine the influence of an ester functionality on the bioactivity of β -carbonyl selenides by replacing the *o*-amide group with an *o*-ester substituent. For this purpose, we synthesized the first β -carbonyl phenyl selenides possessing an *o*-ester group and evaluated if this modification changes their antioxidant and anticancer properties.

2. Results and Discussion

The first step in the research involved synthesizing a new class of compounds—*O*-substituted 2-((2-oxopropyl)selenanyl)-benzoate derivatives. The procedure was based on the reaction of acetylated selenide **11**, formed in situ from 2-(chloroseleno)-benzoyl chloride **A** and acetone, with commercially available alcohols. Initially, the reaction of acetone with sodium bicarbonate yielded a carbanion which was used to acylate the selenium atom. A subsequent reaction with alcohol **B** furnished the final product **10** with only a 16% yield (entry 1, Table 1). In the case of a reaction with several other alcohols, the product was not formed. In the next attempt, NaHCO₃ was replaced with triethylamine. In this way, the reaction efficiency increased to 54% (entry 2, Table 1). The reaction's key step was mixing the ketone with triethylamine to form the carbanion and remove the hydrogen chloride produced in the reaction.

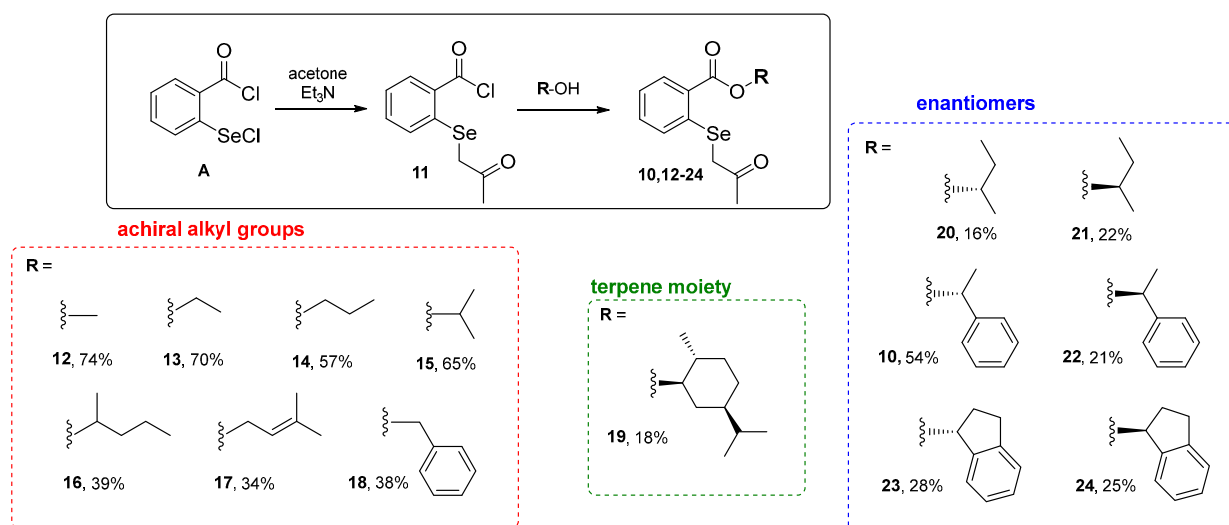
Table 1. Optimization of reaction conditions.



Entry	Solvent (Solubilization of A)	Base	Conditions and Order of Addition	Yield (%)
1	Acetone	NaHCO ₃	1. (acetone + NaHCO ₃ , rt, 30 min) + A , rt, 1 h 2. B , rt, 15 h	16
2	Acetone	Et ₃ N	1. (acetone + Et ₃ N, rt, 30 min) + A , rt, 1 h 2. B , rt, 15 h	54

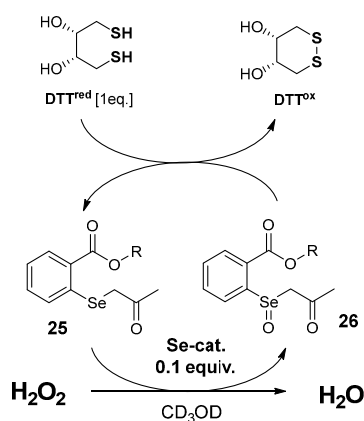
The selected conditions (entry 2, Table 1) were applied in further reactions with commercially available alkyl achiral and chiral alcohols, including monocyclic terpene alcohol. A series of β -carbonyl selenides, seven alkyl derivatives **12–18** and seven optically active compounds, including *O*-terpenyl selenide **19** and three pairs of enantiomers **10** and **20–24**, were obtained with good yields (Scheme 2).

All derivatives were evaluated as antioxidants by two assays. Firstly, the NMR-based method was used, as presented by Iwaoka and co-workers [24]. The ability to reduce hydrogen peroxide was measured by the rate of dithiothreitol oxidation to disulphide (DTTred to DTTox). The active Se-catalyst is formed through the oxidation of selenide **25** to selenoxide **26** by hydrogen peroxide. Dithiothreitol (DTTred) reduces compound **26** to the starting selenide **25**. The substrate (DTTred) disappearance rate was estimated by recording changes in the ¹H NMR spectrum in specific time intervals. The reaction equation and antioxidant test results (mean value \pm standard deviation) are shown below (Table 2), and all obtained results are presented in the Supplementary Materials (Tables S1 and S2).



Scheme 2. Synthesized β -carbonyl selenides with *o*-ester groups 10 and 12–24.

Table 2. Results of the antioxidant activity measurement.



Catalyst (0.1 Equiv.)	Remaining DTT ^{red} (%)			
	5 min	15 min	30 min	60 min
12	95.3 ± 0.2	95.2 ± 0.3	94.8 ± 0.4	94.4 ± 0.4
13	96.7 ± 0.1	96.5 ± 0.2	95.0 ± 0.1	94.9 ± 0.4
14	92.8 ± 0.1	88.5 ± 0.3	82.0 ± 1.9	75.0 ± 1.1
15	93.6 ± 0.2	93.0 ± 0.1	92.9 ± 0.2	92.6 ± 0.2
16	95.1 ± 0.1	94.9 ± 0.1	94.7 ± 0.1	94.4 ± 0.2
17	90.8 ± 0.2	90.6 ± 0.3	90.3 ± 0.3	89.2 ± 0.2
18	93.5 ± 0.1	91.5 ± 0.2	89.2 ± 0.3	85.8 ± 0.7
19	95.1 ± 0.1	94.9 ± 0.1	94.6 ± 0.1	94.3 ± 0.2
20/21	95.8 ± 0.1	94.4 ± 0.2	94.0 ± 1.5	92.1 ± 2.7
10/22	96.1 ± 0.1	95.7 ± 0.1	95.7 ± 0.3	94.9 ± 0.4
23/24	94.4 ± 0.9	93.9 ± 0.8	93.6 ± 0.7	92.8 ± 0.4
Ebselen	75	64	58	52

The best result was observed for *O*-(propyl)-2-((2-oxopropyl)selenyl)benzoate **14**. However, the conversion obtained for all derivatives was less efficient than for ebselen and the previously obtained derivatives with the *o*-amide group. As presented in Figure 2, it can be observed that the presence of an *o*-ester substituent instead of an *o*-amide group in the structure of β -carbonyl selenides significantly lowers the ability to reduce H₂O₂. In the

case of enantiomers 20/21 and their counterparts with the *o*-amide group, these properties are reduced by as much as 9 times.

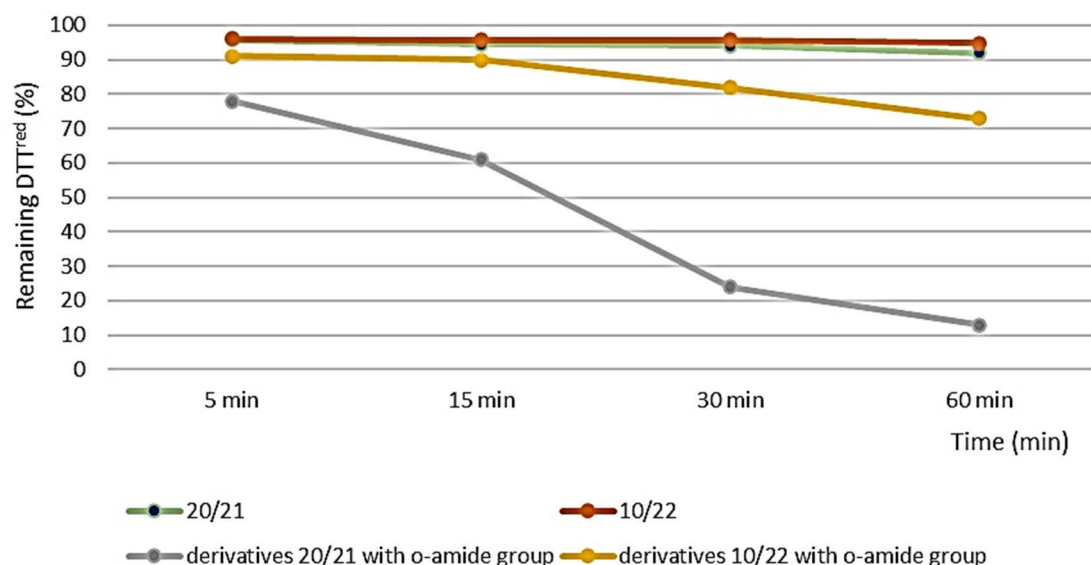


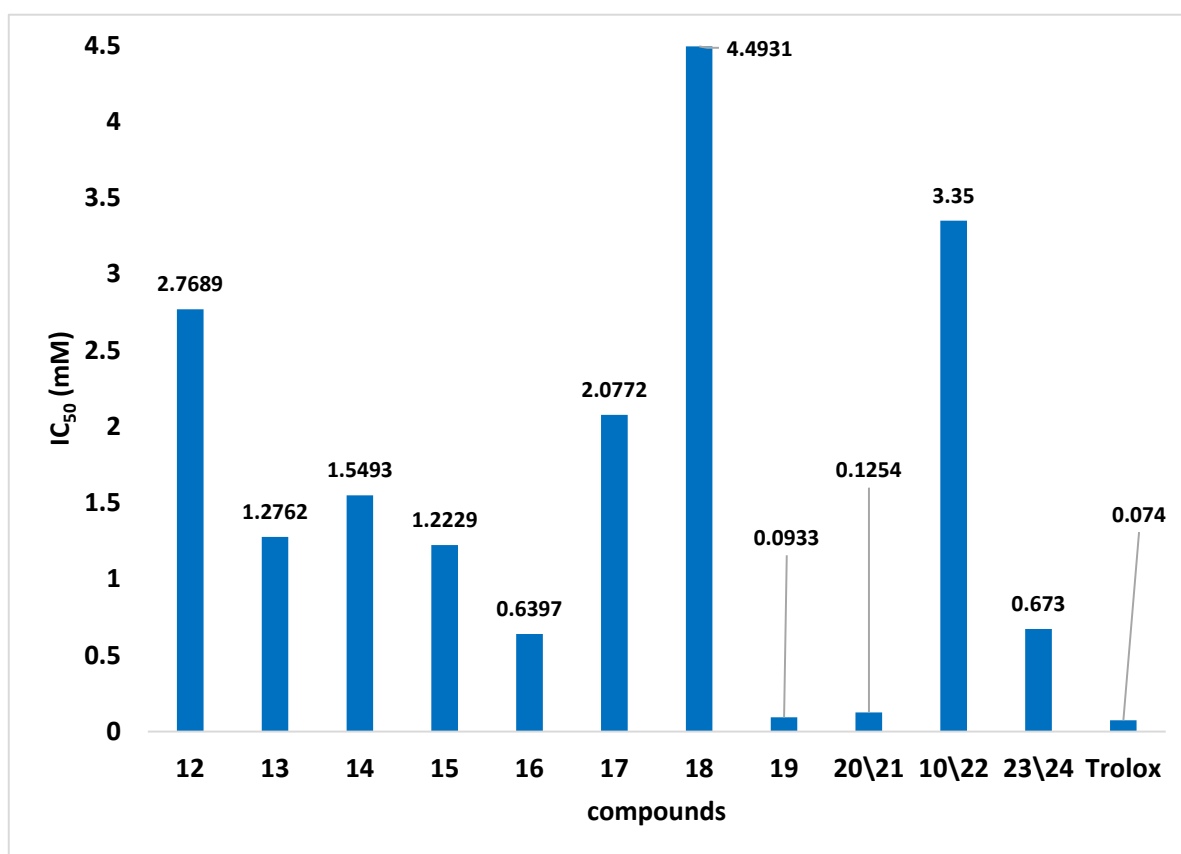
Figure 2. Results of the antioxidant activity measurements of 20/21, 10/22 and corresponding Se-derivatives with *o*-amide group.

During our previous research, we also obtained a series of benzoselenazolones and diselenides *N*-functionalized with long carbon chains terminated with ester groups. The obtained results indicate high reactivity of compounds possessing a Se–N bond along with the ester functionality. This suggests that the isoselenazolone core is essential for the elevated antioxidant potential of the ester-type Se-derivatives [25].

Because organoselenium compounds are well known for their ability to act as antioxidants and/or pro-oxidants, the compounds obtained were tested for their capacities to scavenge free radicals. The results obtained for the DPPH assay are presented in Figure 3 and listed in Table S1 (Supplementary Materials).

In this study, the selected β -carbonyl selenides with the *o*-ester group were tested, and the loss of DPPH radical absorbance was detected in the presence of all. As observed, compound 19 exhibited the best free-radical scavenging capabilities. The calculated IC_{50} value was 0.0933 mM at 15 min post-reaction initiation. Given the IC_{50} value obtained for Trolox (0.0744 mM) and the remaining tested compounds, the free-radical scavenging capacity of compound 19 can be considered noticeable.

All tested organoselenium derivatives contained the same 2-((2-oxopropyl)selenyl)benzoate core but differed in the attached esterification functional substituents. *O*-((1*R*,2*S*,5*R*)-(–)-2-isopropyl-5-methylcyclohexyl)-2-((2-oxopropyl)selenyl)benzoate 19 differed from the other studied derivatives by the presence of a terpene moiety in its structure. It surprised us because monoterpenes without conjugated π bonds, such as menthol, did not have satisfactory free-radical scavenging properties in the DPPH assay [26] and antioxidant activity in the ABTS test [27]. In our previous research, we obtained a new group of chiral benzoselenazol-3(2*H*)-ones substituted on the nitrogen atom with monoterpene moieties, among others, *p*-menthane [28]. The antioxidant activities of synthesized benzoselenazolones (such as *N*-menthyl-1,2-benzisoselenazol-3(2*H*)-one) were evaluated based on the Iwaoka test [24]. The pinene derivatives were the most effective, whereas the substrate conversion observed for the menthane derivative after 60 min was only 67%. However, this compound was characterized by the best anticancer activity against MCF-7 cells [28]. The latter, the literature data and the values of IC_{50} obtained in this study suggest that the presence of terpene moiety [26,27] in the structure may allow compounds with interesting antioxidant and anticancer properties to be obtained.



No	12	13	14	15	16	17
TEAC	0.0267	0.0580	0.0478	0.0605	0.1157	0.0356
No	18	19	20/21	10/22	23/24	
TEAC	0.0165	0.7931	0.5901	0.0221	0.1099	

Figure 3. The results of DPPH test for tested compounds where TEAC–Trolox equivalent antioxidant capacity ($IC_{50}^{Trolox}/IC_{50}^{compounds}$) was calculated.

In the case of the remaining tested compounds, the organoselenium derivatives of chiral alcohols (20/21, 10/22 and 23/24) exhibited better free-radical scavenging properties compared to those derived from aliphatic alcohols (12–17) or benzyl alcohol (18). Moreover, the capacity for free-radical inhibition among this group of compounds was the highest for butyl esters and the lowest for products incorporating methylbenzyl in their structure.

The inferior properties of inhibiting free radicals for organoselenium derivatives of aliphatic alcohols may stem from the absence of π bonds in the functional moiety. However, compound 17, containing a double bond, was not characterized by better free-radical quenching properties. The lowest TEAC value (0.0166) was determined for compound 18, which may be due to the appearance of an aromatic ring in the structure. A similar situation was observed by Wojtunik et al. [26] for *p*-cymene. It indicates that further research on these compounds should concentrate on elucidating their mechanisms of action as free-radical inhibitors.

The literature extensively discusses the relationship between the structures of well-known antioxidants (such as polyphenols) and their antioxidant activity. However, for the discussed compounds, the literature data are very scarce. For this reason, the IC_{50} values for the selected and obtained derivatives were compared with the results for analogous organoselenium compounds described previously [23] (Figure 4).

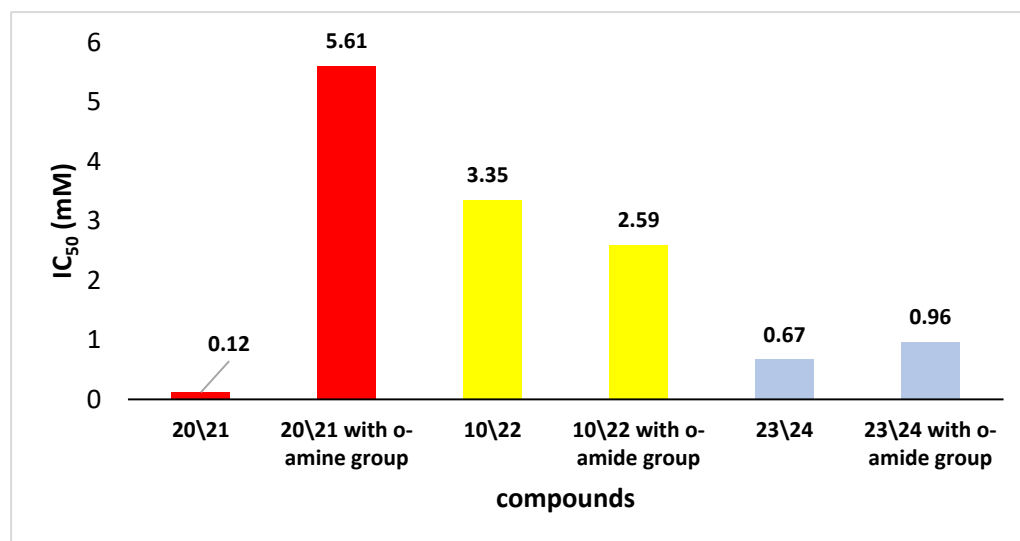


Figure 4. The IC₅₀ for the DPPH test of **20/21**, **10/22** and **23/24** and corresponding Se-derivatives with the *o*-amide group.

Given the obtained results, it is difficult to estimate how the replacement of functional groups allowed for organoselenium derivatives with better properties to inhibit the action of free radicals. For example, in the case of compounds **23/24**, these properties increased modestly, while for compounds with a *sec*-butyl group (**20/21**), they increased significantly. On the other hand, we did not notice a decrease in the IC₅₀ value for derivatives with an α -methyl benzyl group **10/22**. Generally, one of the compounds with the highest reactivity for derivatives from the *o*-amide group was observed for *N*-(*trans*)-indanyl selenide, for which the IC₅₀ value was 0.96 mM. Similar compounds synthesized in this work with an indanyl moiety **23/24** but without a hydroxyl group showed an even higher ability to scavenge free radicals.

Finally, the cytotoxic activity of the obtained compounds **10** and **12–24** against breast cancer MCF-7 and human promyelocytic leukemia HL-60 cell lines were assessed using the cell viability assay MTT [29]. The highest cytotoxic potential was observed for the methyl β -carbonyl selenoesters **12** for the MCF-7 cell line and *sec*-amyl β -carbonyl selenoesters **16** for the HL-60 cell line. All results are shown in Table 3.

Table 3. The antiproliferative activity of compounds **10** and **12–24**.

Compound	IC ₅₀ (μ M) \pm SEM	
	MCF-7	HL-60
12	51.1 \pm 0.6	97.2 \pm 0.5
13	129.5 \pm 1.2	147.0 \pm 5.0
14	166.0 \pm 3.0	97.2 \pm 0.1
15	273.0 \pm 3.0	105.0 \pm 1.0
16	121.0 \pm 0.4	84.0 \pm 2.0
17	194.0 \pm 5.0	103.0 \pm 2.0
18	133.0 \pm 2.0	110.0 \pm 5.0
19	121.0 \pm 10.0	243.0 \pm 8.0
20	313.0 \pm 10.0	147.5 \pm 2.0
21	243.0 \pm 10.0	89.4 \pm 0.9
10	176.0 \pm 3.0	126.0 \pm 2.5
22	107.0 \pm 0.8	94.2 \pm 3.0
23	123.0 \pm 0.4	94.4 \pm 3.0
24	136.0 \pm 3.0	94.2 \pm 0.7
Oxaliplatin	35 [30,31]	0.8 [32]

Previously, we determined the IC_{50} values for selenium derivatives like benzisoselenazolones, diselenides, phenyl selenides and β -carbonyl selenides with the *o*-amide group substituted with different achiral and chiral moieties, also in the form of enantiomeric and diastereomeric pairs. Through the analysis of these results, we can see the differentiation of values between individual groups of compounds and between enantiomers and diastereoisomers. As a representative example, a comparison of the results obtained for enantiomers **10/22** and corresponding Se-derivatives is presented (Table 4).

Table 4. The antiproliferative activity of α -methylbenzyl Se-derivatives.

Se-derivative	IC_{50} (μM) \pm SEM			
	A		B	
	MCF-7	HL-60	MCF-7	HL-60
β -carbonyl selenide with <i>o</i> -ester group	176.0 \pm 3.0	126.0 \pm 2.5	107.0 \pm 0.8	94.2 \pm 3.0
β -carbonyl selenide with <i>o</i> -amide group	235.0 \pm 1.0	303.0 \pm 3.0	237.0 \pm 11.0	23.5 \pm 1.4
Phenyl selenide	>150	>150	>150	>150
Benzisoselenazolone	32.8 \pm 2.8	16.1 \pm 0.0	38.8 \pm 0.8	16.8 \pm 0.4
Diselenide	>100	>100	>100	>100

Comparing the IC_{50} values obtained for the same α -methylbenzyl derivatives, we can notice that for both molecules with an *o*-ester and an *o*-amide group, the best activity was achieved for the *S* configuration for the HL-60 cell line. Additionally, a greater sensitivity of the HL-60 cell line compared to the MCF-7 cell line to the obtained derivatives with an α -methylbenzyl group can be noticed. We can also see an increase in the anticancer properties of *o*-ester selenides compared to phenyl selenides. In general, we can observe that the exchange of the *o*-amide to *o*-ester moiety enhances the cytotoxic potential of the obtained β -carbonyl selenides.

An important scaffold in our research, which is particularly interesting due to the chirality of compounds and the change in biological activity for individual enantiomers and diastereoisomers, was the hydroxyindanyl moiety. In this work, for β -carbonyl selenide with the *o*-ester group, we obtained derivatives with an indanyl substituent without an additional hydroxyl group; therefore, the values can not be compared in a direct way. Looking at the results for the other groups of compounds, we can notice lower sensitivity of cancer cells to compounds **23** and **24**, but it is difficult to determine whether this is the effect of replacing the amide group with an ester group in the *ortho* position or the lack of an additional hydroxyl group.

Looking ahead, our primary goals will involve advancing our understanding of apoptosis and DNA damage pathways, which are fundamental to the cytotoxicity mechanisms. By unravelling the molecular mechanisms governing these pathways, we will aim to elucidate the underlying basis for the anticancer effects of our compounds. Understanding apoptosis and DNA damage pathways is crucial because they regulate cell death and survival, especially in cancer.

3. Materials and Methods

3.1. General

The ^1H , ^{13}C NMR and ^{77}Se NMR spectra were recorded on Bruker Avance III/400 or Bruker Avance III/700 (Karlsruhe, Germany) and 176.1 MHz or 100.6 MHz (Supplementary Materials). The spectra were made in d_6 -DMSO solvent (CD_3OD δ 3.31), and chemical shifts were recorded relative to SiMe_4 or diphenyl diselenide as an external standard. NMR spectra were edited using ACD/NMR Processor Academic Edition. Multiplicities were given as s (singlet), d (doublet), dd (double doublet), t (triplet), dt (double triplet), q (quartet), sextet, septet and m (multiplet). Melting points were measured on the Büchi Tottoli SPM-20 heating unit (Büchi Labortechnik AG, Flawil, Switzerland) and were uncorrected. Vario MACRO CHN analyzer was used for elemental analyses. Optical rotations were measured with a polAAr 3000 polarimeter (Bury Road Industrial Estate, Ramsey, UK).

For column chromatography, Merck 40-63D 60Å silica gel (Merck, Darmstadt, Germany) was used. Alcohols were commercially available from Merck (Merck, Darmstadt, Germany). Their chemical purities were above 98%, and optical purities were above 97%. The MCF-7 (human breast adenocarcinoma) cell line was obtained from the European Collection of Cell Cultures (ECACC, Nice, France), and the HL-60 cell line (human leukemia) was obtained from the European Collection of Authenticated Cell Cultures (ECACC Nice, France).

3.2. General Procedure and Analysis Data

To acetone (2 mL) was added NaHCO_3 (0.063 g, 0.75 mmol, 1 eq.) (**12–15**) or Et_3N (0.095 g, 0.75 mmol, 1 eq) (**10**, **16–24**) with stirring for 30 min; then, the solution of 2-(chloroseleno)-benzoyl chloride (0.190 g, 0.75 mmol, 1 eq.) in acetone (1 mL) was added. After 1 h, alcohol (1 mmol, 1.3 eq.) was added, and the reaction was continued for 15 h at room temperature. The reaction was completed with water (10 mL) and was extracted with DCM. The combined organic layers were dried under magnesium sulfate, and the solvent was evaporated. The product was purified by column chromatography (silica gel, DCM).

O-(methyl)-2-((2-oxopropyl)selanyl)benzoate **12**

Yield: 74%; mp 74–75 °C;

^1H NMR (400 MHz, DMSO) δ = 2.27 (s, 3H), 3.87 (s, 2H), 3.90 (s, 1H), 7.32 (t, J = 7.7 Hz, 1H_{ar}), 7.52–7.59 (m, 2H_{ar}), 7.98 (d, J = 7.7 Hz, 1H_{ar}) ^{13}C NMR (400 MHz, DMSO) δ = 28.92 (CH₃), 35.44 (CH₂), 52.88 (CH₂), 125.75 (CH_{ar}), 127.98 (C_{ar}), 128.98 (CH_{ar}), 131.69 (CH_{ar}), 133.65 (CH_{ar}), 136.83 (C_{ar}), 167.04 (C=O), 204.90 (C=O) ^{77}Se NMR (400 MHz, DMSO) δ = 339.57 ppm; IR: 2951, 2913, 1693, 1583, 1460, 1433, 1397, 1357, 1288, 1274, 1252, 1193, 1145, 1101, 1056, 1031, 958, 747, 688 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{11}\text{H}_{12}\text{O}_3\text{Se}$ (271.17): C, 48.72; H, 4.46; Found C, 48.63; H, 4.57.

O-(ethyl)-2-((2-oxopropyl)selanyl)benzoate **13**

Yield: 70%; mp 60–61 °C;

^1H NMR (400 MHz, DMSO) δ = 1.32 (t, J = 7.2 Hz, 3H), 2.25 (s, 3H), 3.87 (s, 2H), 4.99 (q, J = 7.2 Hz, 1H), 7.30 (t, J = 7.2 Hz, 1H_{ar}), 7.47–7.56 (m, 2H_{ar}), 7.96 (d, J = 7.2 Hz, 1H_{ar}) ^{13}C NMR (400 MHz, DMSO) δ = 14.59 (CH₃), 28.93 (CH₃), 35.46 (CH₂), 61.69 (CH₂), 125.74 (CH_{ar}), 128.31 (C_{ar}), 128.97 (CH_{ar}), 131.64 (CH_{ar}), 133.57 (CH_{ar}), 136.70 (C_{ar}), 166.59 (C=O), 204.92 (C=O) ^{77}Se NMR (400 MHz, DMSO) δ = 338.99 ppm; IR: 2990, 2976, 2910, 2852, 1688, 1582, 1561, 1457, 1398, 1358, 1304, 1286, 1265, 1248, 1145, 1097, 1053, 1031, 1020, 961, 745, 687 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{12}\text{H}_{14}\text{O}_3\text{Se}$ (285.20): C, 53.98; H, 4.65; Found C, 53.81; H, 4.71.

O-(propyl)-2-((2-oxopropyl)selanyl)benzoate **14**

Yield: 57%;

^1H NMR (700 MHz, DMSO) δ = 0.98 (t, J = 7.0 Hz, 3H), 1.74 (sextet, J = 6.3 Hz, 2H), 2.27 (s, 3H), 3.90 (s, 2H), 4.25 (t, J = 7.0 Hz, 2H), 7.33 (t, J = 7.0 Hz, 1H_{ar}), 7.50–7.58 (m, 2H_{ar}), 7.99 (d, J = 7.7 Hz, 1H_{ar}) ^{13}C NMR (400 MHz, DMSO) δ = 10.86 (CH₃), 22.04 (CH₂), 28.93 (CH₃), 35.45 (CH₂), 67.01 (CH₂), 125.77 (CH_{ar}), 128.26 (C_{ar}), 128.98 (CH_{ar}), 131.62 (CH_{ar}), 133.60 (CH_{ar}), 136.74 (C_{ar}), 166.64 (C=O), 204.93 (C=O) ^{77}Se NMR (700 MHz, DMSO) δ = 338.76 ppm; IR: 2969, 2878, 1696, 1584, 1535, 1459, 1354, 1305, 1272, 1227, 1143, 1100,

1056, 1032, 739 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{13}\text{H}_{16}\text{O}_3\text{Se}$ (299.22): C, 52.18; H, 5.39; Found C, 52.31; H, 5.55.

O-(2-propyl)-2-((2-oxopropyl)selanyl)benzoate 15

Yield: 65%;

^1H NMR (400 MHz, DMSO) δ = 1.31 (d, J = 6.4 Hz, 6H), 2.25 (s, 3H), 3.86 (s, 2H), 5.13 (septet, J = 6.0 Hz, 1H), 7.30 (t, J = 6.4 Hz, 1H_{ar}), 7.46–7.55 (m, 2H_{ar}), 7.93 (d, J = 6.8 Hz, 1H_{ar}). ^{13}C NMR (400 MHz, DMSO) δ = 22.11 ($2 \times \text{CH}_3$), 28.94 (CH_3), 35.49 (CH_2), 69.36 (CH), 125.72 (CH_{ar}), 128.73 (C_{ar}), 128.97 (CH_{ar}), 131.57 (CH_{ar}), 133.49 (CH_{ar}), 136.54 (C_{ar}), 166.13 (C=O), 204.92 (C=O). ^{77}Se NMR (400 MHz, DMSO) δ = 337.89 ppm; IR: 2980, 2937, 1693, 1584, 1455, 1354, 1273, 1254, 1226, 1146, 1098, 1054, 1032, 738 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{13}\text{H}_{16}\text{O}_3\text{Se}$ (299.22): C, 52.18; H, 5.39; Found C, 52.42; H, 5.48.

O-(2-pentyl)-2-((2-oxopropyl)selanyl)benzoate 16

Yield: 39%;

^1H NMR (700 MHz, DMSO) δ = 0.91 (t, J = 7.0 Hz, 3H), 1.30 (d, J = 7.0 Hz, 3H), 1.33–1.45 (m, 2H), 1.56–1.64 (m, 1H), 1.65–1.73 (m, 1H), 2.27 (s, 3H), 3.89 (s, 2H), 5.08 (sextet, J = 7.0 Hz, 1H), 7.32 (t, J = 7.0 Hz, 1H_{ar}), 7.50–7.58 (m, 2H_{ar}), 7.96 (d, J = 7.7 Hz, 1H_{ar}). ^{13}C NMR (700 MHz, DMSO) δ = 14.22 (CH_3), 18.62 (CH_2), 20.28 (CH_3), 28.94 (CH_3), 35.50 (CH_2), 37.92 (CH_2), 72.26 (CH), 125.76 (CH_{ar}), 128.66 (C_{ar}), 128.99 (CH_{ar}), 131.51 (CH_{ar}), 133.51 (CH_{ar}), 136.61 (C_{ar}), 166.23 (C=O), 204.94 (C=O). ^{77}Se NMR (700 MHz, DMSO) δ = 339.07 ppm; IR: 2958, 2931, 2872, 1693, 1583, 1458, 1356, 1303, 1275, 1254, 1226, 1183, 1145, 1099, 1054, 1031, 738, 688 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{15}\text{H}_{20}\text{O}_3\text{Se}$ (327.28): C, 55.05; H, 6.16; Found C, 55.23; H, 6.24.

O-(3-methylbut-2-en-1-yl)-2-((2-oxopropyl)selanyl)benzoate 17

Yield: 34%;

^1H NMR (700 MHz, DMSO) δ = 1.75 (d, J = 9.1 Hz, 6H), 2.27 (s, 3H), 3.89 (s, 2H), 4.80 (d, J = 7.0 Hz, 2H), 5.44 (t, J = 7.0 Hz, 1H), 7.32 (t, J = 7.0 Hz, 1H_{ar}), 7.49–7.58 (m, 2H_{ar}), 7.96 (d, J = 7.7 Hz, 1H_{ar}). ^{13}C NMR (400 MHz, DMSO) δ = 18.42 (CH_3), 25.91 (CH_3), 28.93 (CH_3), 35.46 (CH_2), 62.27 (CH_2), 118.95 (CH), 125.74 (CH_{ar}), 128.23 (C_{ar}), 128.95 (CH_{ar}), 131.67 (CH_{ar}), 133.59 (CH_{ar}), 136.77 (C_{ar}), 139.59 (C_{ar}), 166.52 (C=O), 204.92 (C=O). ^{77}Se NMR (400 MHz, DMSO) δ = 339.21 ppm; IR: 2971, 2938, 1690, 1585, 1439, 1358, 1270, 1228, 1146, 1095, 1053, 1031, 942, 738, 687 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{15}\text{H}_{18}\text{O}_3\text{Se}$ (325.26): C, 55.39; H, 5.56; Found C, 55.61; H, 5.72.

O-(benzyl)-2-((2-oxopropyl)selanyl)benzoate 18

Yield: 38%;

^1H NMR (700 MHz, DMSO) δ = 2.27 (s, 3H), 3.91 (s, 2H), 5.37 (s, 2H), 7.30–7.35 (m, 2H_{ar}), 7.35–7.39 (m, 1H_{ar}), 7.42 (t, J = 7.7 Hz, 2H_{ar}), 7.47–7.50 (m, 2H_{ar}), 7.52–7.58 (m, 2H_{ar}), 8.02 (d, J = 7.7 Hz, 1H_{ar}). ^{13}C NMR (400 MHz, DMSO) δ = 28.94 (CH_3), 35.46 (CH_2), 67.07 (CH), 125.83 (CH_{ar}), 127.88 (C_{ar}), 128.58 ($2 \times \text{CH}_{\text{ar}}$), 128.71 (CH_{ar}), 129.03 ($2 \times \text{CH}_{\text{ar}}$), 129.45 (CH_{ar}), 131.79 (CH_{ar}), 133.79 (CH_{ar}), 136.32 (C_{ar}), 137.01 (C_{ar}), 166.38 (C=O), 204.96 (C=O). ^{77}Se NMR (400 MHz, DMSO) δ = 339.53 ppm; IR: 3030, 2924, 2853, 1696, 1584, 1631, 1455, 1355, 1303, 1270, 1251, 1227, 1141, 1096, 1053, 1031, 967, 738, 696 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{17}\text{H}_{16}\text{O}_3\text{Se}$ (347.27): C, 58.80; H, 4.64; Found C, 59.04; H, 4.71.

O-((1R,2S,5R)-(-)-2-isopropyl-5-methylcyclohexyl)-2-((2-oxopropyl)selanyl)benzoate 19

Yield: 18%; $[\alpha]_{\text{D}}^{20} = -29$ ($c = 0.20$, CHCl_3);

^1H NMR (700 MHz, DMSO) δ = 0.76 (d, J = 7 Hz, 3H), 0.89 (d, J = 7 Hz, 3H), 0.91 (d, J = 7 Hz, 3H), 1.08–1.18 (m, 2H), 1.22–1.29 (m, 1H), 1.48–1.60 (m, 2H), 1.84–1.94 (m, 2H), 1.97–2.05 (m, 1H), 2.22–2.28 (m, 1H), 2.27 (s, 3H), 3.89 (s, 2H), 4.86 (td, $J_1 = 4.9$ Hz, $J_2 = 11.2$ Hz, 1H), 7.33 (t, J = 7.0 Hz, 1H_{ar}), 7.47–7.56 (m, 2H_{ar}), 7.95 (d, J = 7.7 Hz, 1H_{ar}). ^{13}C NMR (400 MHz, DMSO) δ = 16.86 (CH_3), 20.94 (CH_3), 22.33 (CH_3), 23.62 (CH_2), 26.64 (CH), 28.95 (CH_3), 31.37 (CH), 34.15 (CH_2), 35.54 (CH_2), 40.98 (CH_2), 47.05 (CH), 75.30 (CH), 125.81 (CH_{ar}), 128.49 (C_{ar}), 129.04 (CH_{ar}), 131.46 (CH_{ar}), 133.56 (CH_{ar}), 136.76 (C_{ar}), 166.08 (C=O), 204.93 (C=O). ^{77}Se NMR (400 MHz, DMSO) δ = 338.30 ppm; IR: 2953, 2923, 2868, 1692, 1585, 1460, 1358, 1272, 1254, 1228, 1142, 1098, 1052, 1031, 958, 738 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_3\text{Se}$ (395.39): C, 60.75; H, 7.14; Found C, 60.89; H, 7.22.

O-((S)-(+)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzoate 20Yield: 16%; $[\alpha]_D^{20} = 50$ (c = 0.40, CHCl₃);

¹H NMR (700 MHz, DMSO) $\delta = 0.90$ (t, $J = 7.0$ Hz, 3H), 1.28 (d, $J = 11.2$ Hz, 3H), 1.58–1.72 (m, 2H), 2.25 (s, 3H), 3.85 (s, 2H), 4.99 (sextet, $J = 7.0$ Hz, 1H), 7.26–7.32 (m, 1H_{ar}), 7.44–7.57 (m, 2H_{ar}), 7.91–7.99 (dd, $J_1 = 2.1$ Hz, $J_2 = 9.8$ Hz, 1H_{ar}). ¹³C NMR (700 MHz, DMSO) $\delta = 10.00$ (CH₃), 19.75 (CH₃), 28.73 (CH₂), 28.95 (CH₃), 35.48 (CH₂), 73.71 (CH), 125.77 (CH_{ar}), 128.66 (C_{ar}), 128.98 (CH_{ar}), 131.52 (CH_{ar}), 133.52 (CH_{ar}), 136.59 (C_{ar}), 167.27 (C=O), 204.99 (C=O). ⁷⁷Se NMR (700 MHz, DMSO) $\delta = 338.89$ ppm; IR: 2969, 2924, 1693, 1584, 1457, 1434, 1355, 1304, 1273, 1254, 1226, 1144, 1127, 1099, 1053, 1029, 965, 739, 688 cm⁻¹. Elemental Anal. Calcd for C₁₄H₁₈O₃Se (313.25): C, 53.68; H, 5.79; Found C, 53.97; H, 5.86.

O-((R)-(–)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzoate 21Yield: 22%; $[\alpha]_D^{20} = -46$ (c = 0.35, CHCl₃);

¹H NMR (700 MHz, DMSO) $\delta = 0.92$ (t, $J = 7.0$ Hz, 3H), 1.30 (d, $J = 7.0$ Hz, 3H), 1.59–1.72 (m, 2H), 2.27 (s, 3H), 3.88 (s, 2H), 5.01 (sextet, $J = 7.0$ Hz, 1H), 7.31–7.36 (m, 1H_{ar}), 7.47–7.56 (m, 2H_{ar}), 7.91–7.99 (dd, $J_1 = 1.4$ Hz, $J_2 = 7$ Hz, 1H_{ar}). ¹³C NMR (700 MHz, DMSO) $\delta = 10.00$ (CH₃), 19.75 (CH₃), 28.74 (CH₂), 28.95 (CH₃), 35.49 (CH₂), 73.71 (CH), 125.77 (CH_{ar}), 128.67 (C_{ar}), 128.99 (CH_{ar}), 131.52 (CH_{ar}), 133.52 (CH_{ar}), 136.59 (C_{ar}), 167.27 (C=O), 204.98 (C=O). ⁷⁷Se NMR (700 MHz, DMSO) $\delta = 338.91$ ppm; IR: 2969, 2924, 1693, 1584, 1457, 1355, 1304, 1273, 1254, 1226, 1144, 1127, 1099, 1052, 1028, 966, 739, 688 cm⁻¹. Elemental Anal. Calcd for C₁₄H₁₈O₃Se (313.25): C, 53.68; H, 5.79; Found C, 53.54; H, 5.71.

O-((R)-(+)- α -methylbenzyl)-2-((2-oxopropyl)selanyl)benzoate 10Yield: 54%; $[\alpha]_D^{20} = 58$ (c = 0.24, CHCl₃);

¹H NMR (400 MHz, DMSO) $\delta = 1.60$ (d, $J = 6.4$ Hz, 3H), 2.23 (s, 3H), 3.86 (s, 2H), 6.04 (q, $J = 6.8$ Hz, 1H), 7.27–7.35 (m, 2H_{ar}), 7.38 (t, $J = 6.8$ Hz, 2H_{ar}), 7.42–7.49 (m, 2H_{ar}), 7.51–7.56 (m, 2H_{ar}), 8.07 (d, $J = 7.2$ Hz, 1H_{ar}). ¹³C NMR (400 MHz, DMSO) $\delta = 22.78$ (CH₃), 28.94 (CH₃), 35.47 (CH₂), 73.71 (CH), 125.82 (CH_{ar}), 126.39 (2 \times CH_{ar}), 128.11 (C_{ar}), 128.38 (CH_{ar}), 128.98 (CH_{ar}), 129.02 (2 \times CH_{ar}), 131.80 (CH_{ar}), 133.75 (CH_{ar}), 136.99 (C_{ar}), 141.99 (C_{ar}), 165.77 (C=O), 204.91 (C=O). ⁷⁷Se NMR (400 MHz, DMSO) $\delta = 339.53$ ppm; IR: 2980, 2929, 1695, 1584, 1456, 1355, 1302, 1270, 1252, 1144, 1099, 1053, 1029, 993, 760, 698 cm⁻¹. Elemental Anal. Calcd for C₁₄H₁₈O₃Se (361.29): C, 59.84; H, 5.02; Found C, 59.98; H, 4.95.

O-((S)-(–)- α -methylbenzyl)-2-((2-oxopropyl)selanyl)benzoate 22Yield: 21%; $[\alpha]_D^{20} = -59$ (c = 0.32, CHCl₃);

¹H NMR (700 MHz, DMSO) $\delta = 1.62$ (d, $J = 5.6$ Hz, 3H), 2.25 (s, 3H), 3.88 (s, 2H), 6.05 (q, $J = 6.3$ Hz, 1H), 7.29–7.36 (m, 2H_{ar}), 7.40 (t, $J = 6.3$ Hz, 2H_{ar}), 7.46–7.52 (m, 2H_{ar}), 7.53–7.58 (m, 2H_{ar}), 8.09 (d, $J = 6.3$ Hz, 1H_{ar}). ¹³C NMR (400 MHz, DMSO) $\delta = 22.77$ (CH₃), 28.94 (CH₃), 35.47 (CH₂), 73.71 (CH), 125.82 (CH_{ar}), 126.38 (2 \times CH_{ar}), 128.13 (C_{ar}), 128.32 (CH_{ar}), 129.00 (CH_{ar}), 129.01 (2 \times CH_{ar}), 131.79 (CH_{ar}), 133.74 (CH_{ar}), 136.97 (C_{ar}), 141.98 (C_{ar}), 165.77 (C=O), 204.89 (C=O). ⁷⁷Se NMR (400 MHz, DMSO) $\delta = 339.57$ ppm; IR: 2979, 2929, 1694, 1583, 1456, 1355, 1301, 1270, 1252, 1143, 1099, 1052, 1028, 993, 760, 698 cm⁻¹. Elemental Anal. Calcd for C₁₄H₁₈O₃Se (361.29): C, 59.84; H, 5.02; Found C, 60.02; H, 5.12.

O-((R)-(–)-2,3-dihydro-(1*H*)-inden-1-yl)-2-((2-oxopropyl)selanyl)benzoate 23Yield: 28%; $[\alpha]_D^{20} = -27$ (c = 0.31, CHCl₃);

¹H NMR (700 MHz, DMSO) $\delta = 2.14$ –2.23 (m, 1H), 2.27 (s, 3H), 2.55–2.61 (m, 1H), 2.88–2.97 (m, 1H), 3.09–3.17 (m, 1H), 3.91 (s, 2H), 6.36 (q, $J = 2.1$ Hz, 1H), 7.22–7.30 (m, 2H_{ar}), 7.33–7.40 (m, 2H_{ar}), 7.48 (d, $J = 1.4$ Hz, 1H_{ar}), 7.51–7.57 (m, 2H_{ar}), 7.91 (d, $J = 7.7$ Hz, 1H_{ar}). ¹³C NMR (700 MHz, DMSO) $\delta = 28.97$ (CH₃), 30.24 (CH₂), 32.37 (CH₂), 35.55 (CH₂), 79.71 (CH), 125.37 (CH_{ar}), 125.77 (CH_{ar}), 125.92 (CH_{ar}), 127.17 (CH_{ar}), 128.37 (C_{ar}), 129.03 (CH_{ar}), 129.58 (CH_{ar}), 131.76 (CH_{ar}), 133.64 (CH_{ar}), 136.73 (C_{ar}), 141.04 (C_{ar}), 144.86 (C_{ar}), 166.56 (C=O), 204.91 (C=O). ⁷⁷Se NMR (400 MHz, DMSO) $\delta = 337.85$ ppm; IR: 2970, 2927, 2852, 1692, 1584, 1460, 1355, 1271, 1251, 1227, 1141, 1099, 1053, 1031, 738 cm⁻¹. Elemental Anal. Calcd for C₁₉H₁₈O₃Se (373.30): C, 61.13; H, 4.86; Found C, 61.01; H, 4.97.

O-((S)-(+)-2,3-dihydro-(1*H*)-inden-1-yl)-2-((2-oxopropyl)selanyl)benzoate 24Yield: 25%; $[\alpha]_D^{20} = 24$ (c = 0.37, CHCl₃);

^1H NMR (700 MHz, DMSO) δ = 2.14–2.22 (m, 1H), 2.27 (s, 3H), 2.53–2.60 (m, 1H), 2.90–2.97 (m, 1H), 3.08–3.18 (m, 1H), 3.91 (s, 2H), 6.38 (q, J = 3.5 Hz, 1H), 7.21–7.30 (m, 2H_{ar}), 7.30–7.40 (m, 2H_{ar}), 7.48 (d, J = 7.7 Hz, 1H_{ar}), 7.52–7.58 (m, 2H_{ar}), 7.91 (d, J = 7.7 Hz, 1H_{ar})
 ^{13}C NMR (400 MHz, DMSO) δ = 28.97 (CH₃), 30.24 (CH₂), 32.36 (CH₂), 35.54 (CH₂), 79.70 (CH), 125.36 (CH_{ar}), 125.77 (CH_{ar}), 125.92 (CH_{ar}), 127.17 (CH_{ar}), 128.36 (C_{ar}), 129.02 (CH_{ar}), 129.58 (CH_{ar}), 131.76 (CH_{ar}), 133.64 (CH_{ar}), 136.74 (C_{ar}), 141.03 (C_{ar}), 144.86 (C_{ar}), 166.55 (C=O), 204.90 (C=O) ^{77}Se NMR (400 MHz, DMSO) δ = 337.85 ppm; IR: 2970, 2925, 2851, 1694, 1584, 1460, 1354, 1271, 1251, 1227, 1142, 1099, 1053, 1032, 740 cm⁻¹. Elemental Anal. Calcd for C₁₉H₁₈O₃Se (373.30): C, 61.13; H, 4.86; Found C, 61.26; H, 4.93.

3.3. Antioxidant Activity Evaluation

3.3.1. DTT Activity Assay

DDT activity assay for compounds **10** and **11–24** was performed according to the Iwaoka procedure [24].

3.3.2. The 2,2-di(4-tert-Octyl phenyl)-1-picrylhydrazyl (DPPH) Test

The assay for the neutralization of radicals was executed following the methodology delineated in our previous work [23]. The 50% decline in absorbance of the DPPH solution was derived from the curve with the tested compound concentration (mM) plotted against the absorbance. The obtained results are presented as the inhibitory concentration (IC₅₀) of the tested compounds. The absorbance value was measured after 15 min post-initiation. In addition to the compounds that were obtained, the antioxidant activity of Trolox was assessed for comparison purposes. The efficacy of the tested compounds in scavenging DPPH radicals was quantified and expressed as Trolox equivalent antioxidant capacity (TEAC). All details regarding the procedure are included in the Supplementary Materials.

3.4. MTT Viability Assay

The MTT (3-(4,5-diazol-2-yl)-2,5 diphenyl tetrazolium bromide) assay, which measures the activity of methylcellular dehydrogenases, was based on the method of Mosmann [29]. The MTT assay was executed following the methodology delineated in our previous work [23].

4. Conclusions

We developed an efficient method for synthesizing a new group of organoselenium compounds, β -carbonyl phenyl selenides, possessing an ester group in the ortho position. The key step in the synthesis was using triethylamine during the acylation of selenide. The first derivatives with alkyl achiral and chiral scaffolds were obtained. The obtained derivatives were tested for antioxidant and cytotoxic activity. The antioxidant activity was tested in two ways: the reduction in peroxide to water (Iwaoka test) and the quenching of free radicals (DPPH test). As a result of these studies, we observed that the exchange of the amide group to an ester group significantly lowers the H₂O₂ reduction properties. However, it was observed that the obtained ester derivatives are better free-radical scavengers. The best results were obtained for the compound O-((1*R*,2*S*,5*R*)-(-)-2-isopropyl-5-methylcyclohexyl)-2-((2-oxopropyl)selenyl)benzoate, for which the IC₅₀ value was close to the value for Trolox. Very good results of the DPPH test were also obtained for the *sec*-butyl and indanyl derivatives. In the case of cytotoxic activity, which was tested on two cell lines, MCF-7 (breast cancer) and HL-60 (promyelocytic leukemia), we did not notice a relevant improvement in these properties.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules29122866/s1>, Figure S1: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-(methyl)-2-((2-oxopropyl)selanyl)benzoate **12**; Figure S2: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-(ethyl)-2-((2-oxopropyl)selanyl)benzoate **13**; Figure S3: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-(propyl)-2-((2-oxopropyl)selanyl)benzoate **14**; Figure S4: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-(2-propyl)-2-((2-oxopropyl)selanyl)benzoate **15**; Figure S5: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-(2-pentyl)-2-((2-oxopropyl)selanyl)benzoate **16**; Figure S6: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-(3-methylbut-2-en-1-yl)-2-((2-oxopropyl)selanyl)benzoate **17**; Figure S7: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-(benzyl)-2-((2-oxopropyl)selanyl)benzoate **18**; Figure S8: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-((1*R*,2*S*,5*R*)-(-)-2-isopropyl-5-methylcyclohexyl)-2-((2-oxopropyl)selanyl)benzoate **19**; Figure S9: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-((*S*)-(+)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzoate **20**; Figure S10: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-((*R*)-(-)-*sec*-butyl)-2-((2-oxopropyl)selanyl)benzoate **21**; Figure S11: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-((*R*)-(+)- α -methylbenzyl)-2-((2-oxopropyl)selanyl)benzoate **10**; Figure S12: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-((*S*)-(-)- α -methylbenzyl)-2-((2-oxopropyl)selanyl)benzoate **22**; Figure S13: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-((*R*)-(-)-2,3-dihydro-(1*H*)-inden-1-yl)-2-((2-oxopropyl)selanyl)benzoate **23**; Figure S14: (a) ¹H NMR, (b) ¹³C NMR, and (c) ⁷⁷Se NMR spectra of *O*-((*S*)-(+)-2,3-dihydro-(1*H*)-inden-1-yl)-2-((2-oxopropyl)selanyl)benzoate **24**; Table S1: Results of antioxidant activity measurement of integration from ¹H NMR spectra after reaction time 5 min and 15 min for all compounds; Table S2: Results of antioxidant activity measurement of integration from ¹H NMR spectra after reaction time 30 min and 60 min for all compounds; Table S3: The results of DPPH Radical Scavenging Assay.

Author Contributions: Conceptualization, J.Ś.; Methodology, A.J.P.-M., K.G.-J. and J.Ś.; Formal analysis, A.L., M.O.-F., A.J. and A.D.-P.; Investigation, A.L., A.J.P.-M., A.J. and A.D.-P.; Data curation, A.L., M.O.-F. and A.D.-P.; Writing—original draft, A.L., A.J.P.-M. and A.J.; Writing—review & editing, A.D.-P., K.G.-J. and J.Ś.; Supervision, K.G.-J. and J.Ś.. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Medical University of Lodz grant number 503/1-156-02/503-11-001.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Conflicts of Interest: The authors declare no conflict of interest.

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11. Oświadczenia współautorów publikacji

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Oświadczam, że mój udział w niżej wymienionych publikacjach obejmował syntezy oraz oczyszczanie pochodnych selenoorganicznych, ich analizę (analiza widm NMR i IR, wyznaczenie temperatury topnienia, wyznaczenie skręcalności, wykonanie testów aktywności antyoksydacyjnej), zbieranie literatury, przygotowanie wstępnej wersji manuskryptów i Supplementary Materials.

1. M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; J. Ścianowski, *Organoselenium compounds as antioxidants*, *Arkivoc.*, **2023**, part v, 211
2. **A. Laskowska**; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka; A. Wojtczak; J. Ścianowski, *Attachment of Chiral Functional Groups to Modify the Activity of New GPx Mimetics*, *Materials*, **2022**, 15, 2068
3. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; K. Gach-Janczak, A. Janecka; J. Ścianowski, *Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents*, *RCS Advances*, **2023**, 13, 14698
4. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I*, *Materials*, **2024**, 17, 899
5. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part II*, *Molecules*, **2024**, 29, 2866

Toruń, 06.08.2024 r.


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Oświadczam, że mój udział jako promotora w niżej wymienionych publikacjach polegał na opracowaniu koncepcji pracy, koordynowaniu badań, nawiązywaniu współpracy z ośrodkami wykonującymi pomiary cytotoksyczności komórek nowotworowych, korekcie końcowych manuskryptów i korespondencji z redakcjami czasopism.

1. M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; J. Ścianowski, *Organoselenium compounds as antioxidants, Arkivoc.*, **2023**, part v, 211
2. **A. Laskowska**; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka; A. Wojtczak; J. Ścianowski, *Attachment of Chiral Functional Groups to Modify the Activity of New GPx Mimetics, Materials*, **2022**, 15, 2068
3. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; K. Gach-Janczak, A. Janecka; J. Ścianowski, *Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents, RCS Advances*, **2023**, 13, 14698
4. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I, Materials*, **2024**, 17, 899
5. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part II*, *Molecules*, **2024**, 29, 2866

Toruń, 06.08.2024 r.


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Oświadczam, że mój udział jako promotora pomocniczego w niżej wymienionych publikacjach polegał na pomocy w koordynowaniu badań, dyskusji nad uzyskanymi wynikami i pisaniu wstępnych wersji manuskryptów.

1. M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; A. Laskowska; J. Ścianowski, *Organoselenium compounds as antioxidants*, *Arkrivoc.*, **2023**, part v, 211
2. A. Laskowska; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka; A. Wojtczak; J. Ścianowski, *Attachment of Chiral Functional Groups to Modify the Activity of New GPx Mimetics*, *Materials*, **2022**, 15, 2068
3. A. Laskowska; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; K. Gach-Janczak, A. Janecka; J. Ścianowski, *Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents*, *RCS Advances*, **2023**, 13, 14698
4. A. Laskowska; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I*, *Materials*, **2024**, 17, 899
5. A. Laskowska; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part II*, *Molecules*, **2024**, 29, 2866

Toruń, 06.08.2024 r.



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Oświadczam, że mój udział w niżej wymienionych publikacjach polegał na pomocy w wykonywaniu pomiarów aktywności antyoksydacyjnej wybranych pochodnych selenoorganicznych i pisaniu wstępnej wersji manuskryptu do *Arkivoc*.

1. M. Obieziurska-Fabisiak; A. J. Pacuła-Miszewska; **A. Laskowska**; J. Ścianowski, *Organoselenium compounds as antioxidants, Arkivoc.*, **2023**, part v, 211
2. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; K. Gach-Janczak, A. Janecka; J. Ścianowski, *Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents, RCS Advances*, **2023**, 13, 14698
3. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I, Materials*, **2024**, 17, 899
4. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part II*, *Molecules*, **2024**, 29, 2866

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Oświadczam, że mój udział w niżej wymienionych publikacjach obejmował koordynowanie pomiarów potencjału antyoksydacyjnego pochodnych selenoorganicznych testem DPPH i przygotowaniu opisów do publikacji.

1. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; K. Gach-Janczak, A. Janecka; J. Ścianowski, *Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents*, *RCS Advances*, **2023**, *13*, 14698
2. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I*, *Materials*, **2024**, *17*, 899
3. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part II*, *Molecules*, **2024**, *29*, 2866

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Oświadczam, że mój udział w niżej wymienionych publikacjach polegał na wykonywaniu badań dotyczących cytotoksyczności pochodnych selenoorganicznych i korekcie manuskryptów.

1. A. Laskowska; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka; A. Wojtczak; J. Ścianowski, *Attachment of Chiral Functional Groups to Modify the Activity of New GPx Mimetics*, *Materials*, **2022**, *15*, 2068
2. A. Laskowska; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I*, *Materials*, **2024**, *17*, 899
3. A. Laskowska; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part II*, *Molecules*, **2024**, *29*, 2866

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Oświadczam, że mój udział w niżej wymienionych publikacjach polegał na koordynowaniu i wykonywaniu badań dotyczących cytotoksyczności pochodnych selenoorganicznych.

- 1. A. Laskowska;** A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; K. Gach-Janczak, A. Janecka; J. Ścianowski, *Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents*, *RCS Advances*, **2023**, *13*, 14698
- 2. A. Laskowska;** A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part I*, *Materials*, **2024**, *17*, 899
- 3. A. Laskowska;** A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska, A. Długosz-Pokorska; K. Gach-Janczak, J. Ścianowski, *Synthesis of New Chiral β -Carbonyl Selenides with Antioxidant and Anticancer Activity Evaluation—Part II*, *Molecules*, **2024**, *29*, 2866

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Oświadczam, że mój udział w niżej wymienionych publikacjach polegał na koordynowaniu badań dotyczących cytotoksyczności pochodnych selenoorganicznych oraz korekcie manuskryptów.

1. **A. Laskowska**; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka; A. Wojtczak; J. Ścianowski, *Attachment of Chiral Functional Groups to Modify the Activity of New GPx Mimetics, Materials*, **2022**, *15*, 2068
2. **A. Laskowska**; A. J. Pacuła-Miszewska; M. Obieziurska-Fabisiak; A. Jastrzębska; K. Gach-Janczak, A. Janecka; J. Ścianowski, *Facile synthesis of chiral phenylselenides as novel antioxidants and cytotoxic agents, RCS Advances*, **2023**, *13*, 14698



Łódź, 12.08.2024 r.

prof. dr hab. Anna Janecka

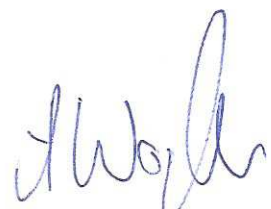
Prof. dr hab. Andrzej Wojtczak

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Oświadczam, że mój udział w poniższej publikacji polegał na wykonaniu i opracowaniu analizy krystalograficznej dwóch enancjomerów *N*-[α -metylobenzylo]-1,2-benzizoselenazonu.

1. **A. Laskowska**; A. J. Pacuła-Miszewska; A. Długosz-Pokorska; A. Janecka; A. Wojtczak; J. Ścianowski, *Attachment of Chiral Functional Groups to Modify the Activity of New GPx Mimetics*, *Materials*, **2022**, *15*, 2068

Toruń, 06.08.2024 r.


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