Sveučilište u Zagrebu

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DOKTORSKI RAD

Mentor:
prof. dr. sc. Silvana Raić-Malić

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University of Zagreb

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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF NOVEL *N*-HETEROCYCLIC DERIVATIVES AND THEIR METAL COMPLEXES

DOCTORAL DISSERTATION

Mentor:
Prof. dr. Silvana Raić-Malić

Zagreb, 2025.

SAŽETAK

Istraživanja u okviru doktorskog rada, usmjerena na razvoj novih *N*-heterocikličkih spojeva s antiproliferativnom aktivnošću, obuhvaćaju sintezu derivata heterocikličkih farmakofora, poput purina, pirimidina, kinolina i kumarina, s izoksazolnom, 1,2,3-triazolnom ili aldoksim-eterskom poveznicom. Odabrani spojevi s piridinskim prstenom korišteni su kao bidentatni ligandi u sintezi monometalnih i heterobimetalnih kompleksa s renijem(I), manganom(I) i rutenijem(II). Sinteza prekursora te novih *N*-heterocikličkih derivata, liganada i metalnih kompleksa provedena je višestupnjevitim sintezama primjenom konvencionalnih metoda u kombinaciji s održivim sintetskim metodama zelene kemije. Za pripravu 1,2,3-triazolnih i izoksazolnih derivata korištene su regioselektivne reakcije 1,3-dipolarne cikloadicije. Enantiomerno čisti azidoalkoholi ((*R*),(*S*)-**16b**–(*R*),(*S*)-**18b** i (*R*),(*S*)-**20b**) dobiveni su enantioselektivnom biokatalitičkom reakcijom *para*-supstituiranih stiren-oksida uz natrijev azid i enzim halogenhidrin-dehalogenazu. Uvedeni su arilni, arilalkinilni i alkinilni supstituenti primjenom Suzuki–Miyaurine i Sonogashirine reakcije unakrsnog spajanja katalizirane paladijem. Antiproliferativna aktivnost novosintetiziranih derivata ispitana je *in vitro* na tumorskim i zdravim stanicama. Dobiveni rezultati omogućili su uvid u odnos strukture i antiproliferativne aktivnosti ispitivanih spojeva, a najaktivniji derivati dodatno su ispitani u svrhu razjašnjavanja mehanizma njihova djelovanja te su im određena fizikalno-kemijska i ADME svojstva. Aril-supstituirani triazolni derivat 6-klorpurina **40a** (HepG2, IC_{50} = 0.64 μM, SI = 28) i pirol[2,3-*d*]pirimidina **45a** (MCF-7, IC_{50} = 0.5 μM, SI = 40) pokazali su selektivnu submikromolarnu aktivnost na stanice HepG2 i MCF-7, pri čemu je aktivnost derivata **45a** na stanice MCF-7 bila bolja od referentnog lijeka 5-fluoruracila (IC_{50} = 1.0 μM, SI = 0.9). 6-arilalkinil-supstituirani purinski derivati **59b** (IC_{50} = 1.5 μM, SI = 7.3) i **60b** (IC_{50} = 1.9 μM, SI = 11.9) pokazali su značajnu inhibitornu aktivnost na stanice Capan-1 te dobra farmakokinetička svojstva. Kompleks $\mathbf{60b_{Re}}$ imao je snažnu, ali neselektivnu aktivnost na stanične linije Capan-1 (IC_{50} = 1.6 μM, SI = 1.8) i Z-138 (IC_{50} = 1.8 μM, SI = 1.6). Rezultati molekulskog uklapanja pokazali su da u odnosu na ostale ispitivane spojeve kompleks $\mathbf{60b_{Re}}$ ima najpovoljniju energiju vezanja (ΔG_vezanja = −63.19 kcal/mol) u domenu SH2 proteina STAT3, što ukazuje na stvaranje stabilnog kompleksa i potvrđuje njegov potencijal kao kandidata za razvoj selektivnih inhibitora signalnog puta STAT3. Koordinacijom pirimidinskih i furo[2,3-*d*]pirimidinskih izoksazolnih liganada s Re(I) postignuto je povećanje antiproliferativne aktivnosti, a posebno su se istaknuli trikarbonilni renijevi(I) kompleksi $\mathbf{68d'_{Re}}$ (CCRF-CEM, IC_{50} = 1.5 μM, SI = 27.2) i $\mathbf{70f'_{Re}}$ (THP-1, IC_{50} = 2.2 μM). Dodatna ispitivanja pokazala su da spojevi **68g'**, **69f'** i **70f'** u stanicama HuT-78 uzrokuju smanjenje reaktivnih

kisikovih vrsta (20 %), značajno reduciraju potencijal mitohondrijske membrane (više od 50 %) te induciraju zaustavljanje staničnog ciklusa u fazi G1/G0. Kinolinski derivat **94** pokazao je selektivnu submikromolarnu aktivnost na stanice Capan-1, DND-41, HL-60 i Z-138 (IC_{50} = 2.1–4.7 μM, SI = 21–48), uz povoljna farmakokinetička svojstva (Papp(AB) = 15×10^{-6} cm/s, LBF = 58 %). Istraživanjem molekulskog uklapanja potvrđeno je vezivanje spoja **94** za protein Bcl-2 (ΔG = –84.98 kcal/mol). Kumarinski trikarbonilni renijev(I) kompleks $\mathbf{123_{Re}}$ selektivno je inhibirao rast stanica HuT78 (IC_{50} = 2.4 μM, SI = 8.7), a analiza staničnog ciklusa pokazala je da $\mathbf{123_{Re}}$ dovodi do nakupljanja stanica u fazi G0/G1 (47.3 %) te smanjenje stanica u fazi G2/M (20.4 %) staničnog ciklusa. Ferocenski derivat **155f** s *N*-metilpirolnim supstituentom i esterskom poveznicom imao je selektivnu antiproliferativnu aktivnost na stanice HepG2 (IC_{50} = 5.0 μM, SI = 20), dok je 2,6-diklorpurinski derivat ferocena **137** pokazao snažno, ali neselektivno djelovanje na stanice MCF-7 (IC_{50} = 2.0 μM, SI = 1). Elektrokemijskom analizom potvrđena je redoks aktivnost ferocenskog i 1,2,3-triazolnog fragmenta u spojevima **137** i **155f**, što ukazuje na njihovu sposobnost generiranja reaktivnih kisikovih vrsta (ROS) u biološkim uvjetima.

Ključne riječi: *N*-heterociklički derivati, organometalni kompleksi, antiproliferativno djelovanje, molekulsko modeliranje, svojstva ADME

SUMMARY

In the research focused on the development of new *N*-heterocyclic compounds with antiproliferative activity, compounds containing heterocyclic pharmacophores, such as purines, pyrimidines, quinolines and coumarins with isoxazole, 1,2,3-triazole or aldoxime-ether linkers were synthesized. Selected compounds with pyridine ring were used as bidentate ligands in the synthesis of monometallic and heterobimetallic complexes of rhenium(I), manganese(I) and ruthenium(II). The syntheses of the new *N*-heterocyclic derivatives, ligands and metal complexes were carried out by multistep syntheses using conventional and sustainable green synthetic methods. Regioselective 1,3-dipolar cycloadditions were used for the preparation of 1,2,3-triazole and isoxazole derivatives. Enantiomerically pure azido alcohols ((*R*),(*S*)-**16b**–(*R*),(*S*)-**18b** i (*R*),(*S*)-**20b**) were obtained by enantioselective biocatalytic azidolysis of *para*-substituted styrene oxides with sodium azide and the enzyme halohydrin dehalogenase. Aryl, arylalkynyl and alkynyl substituents were introduced using palladium-catalysed Suzuki-Miyaura and Sonogashira cross-coupling reactions. The antiproliferative activity was evaluated *in vitro* on tumour and healthy cell lines. The obtained results gave an insight into the relationship between the structure and the antiproliferative activity of the tested compounds. The most active derivatives were additionally evaluated to clarify their mechanism of action. Additionally, their physicochemical and ADME properties were determined. The aryl-substituted triazole derivative of 6-chloropurine **40a** (HepG2, IC_{50} = 0.64 μM, SI = 28) and pyrrole[2,3-*d*]pyrimidine **45a** (MCF-7, IC_{50} = 0.5 μM, SI = 40) showed selective submicromolar inhibitory activity against HepG2 and MCF-7 cells, with the activity of **45a** in MCF-7 cells exceeding that of the reference drug 5-fluorouracil (IC_{50} = 1.0 μM, SI = 0.9). The 6-arylalkynyl-substituted purine derivatives **59b** (IC_{50} = 1.5 μM, SI = 7.3) and **60b** (IC_{50} = 1.9 μM, SI = 11.9) demonstrated significant activity against the Capan-1 cell line. Complex **60b$_{Re}$** had strong but non-selective activity on the Capan-1 (IC_{50} = 1.6 μM, SI = 1.8) and Z-138 (IC_{50} = 1.8 μM, SI = 1.6) cell lines. The results of molecular modelling showed that the **60b$_{Re}$** complex achieved the most favourable binding energy (ΔG_binding = −63.19 kcal/mol) at the SH2 domain of the STAT3 protein compared to the other compounds tested, indicating the formation of a stable rhenium(I) complex and confirming its potential as a candidate for the development of new selective inhibitors of the STAT3 pathway. Coordination of pyrimidine and furo[2,3-*d*]pyrimidine isoxazole ligands with Re(I) led to enhanced antiproliferative activity with the rhenium(I) tricarbonyl complexes **68d'$_{Re}$** (CCRF-CEM, IC_{50} = 1.5 μM, SI = 27.2) and **70f'$_{Re}$** (THP-1, IC_{50} = 2.2 μM) demonstrating superior antiproliferative activity.

Further biological assays showed that compounds **68g'**, **69f'** and **70f'** caused a slight decrease of generation of reactive oxygen species in HuT-78 cells (20%), significant decrease of the mitochondrial membrane potential (more than 50%) and induced cell cycle arrest in the G1/G0 phase. Quinoline derivative **94** exhibited selective submicromolar activity against Capan-1, DND-41, HL-60, and Z-138 cells (IC_{50} = 2.1–4.7 μM, SI = 21–48), with a favourable pharmacokinetic profile (Papp (AB) = 15 × 10^{-6} cm/s, LBF = 58%). Molecular modelling study confirmed binding of **94** to the Bcl-2 protein (ΔG = –84.98 kcal/mol). The coumarin Re(I)-tricarbonyl complex $\mathbf{123_{Re}}$ showed selective antiproliferative activity against HuT78 cells (IC_{50} = 2.4 μM, SI = 8.7). Cell cycle analysis displayed that $\mathbf{123_{Re}}$ caused cell accumulation (47.3 %) in the G0/G1 phase and cell reduction (20.4 %) in the G2/M cell cycle phase. The ferrocene derivative **155f** with *N*-methylpyrrole substituent and ester bond showed selective antiproliferative activity on HepG2 cells (IC_{50} = 5.0 μM, SI = 20), while the 2,6-dichloropurine ferrocene derivative **137** had a strong but non-selective effect on MCF-7 cells (IC_{50} = 2.0 μM, SI = 1). Voltammetric analysis confirmed the redox activity of the ferrocene and 1,2,3-triazole fragments in **137** and **155f**, indicating their potentional of reactive oxygen species (ROS) generation.

Keywords: *N*-heterocyclic derivatives, organometallic complexes, antiproliferative activity, molecular modelling, ADME properties