TALLINN UNIVERSITY OF TECHNOLOGY
DOCTORAL THESIS
73/2023

Design of Acetylcholinesterase Reactivators and Poly(ADP-ribose) Polymerase Inhibitors Based on Hydroxamic Acids and Oximes

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TAL TECH PRESS

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This dissertation was accepted for the defence of the degree of Doctor of Philosophy in Chemistry on 17/11/2023

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Denys Bondar

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signature

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ISSN 2585-6898 (publication)
ISBN 978-9916-80-089-8 (publication)
Printed by Koopia Niini & Rauam

TALLINNA TEHNIKAÜLIKOOL
DOKTORITÖÖ
73/2023

Hüdroksaamhapetel ja oksiimidel põhinevate atsetüülkoliinesteraasi reaktivaatorite ja polü(ADP-riboos)polümeraasi inhibiitorite disain

DENYS BONDAR

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Contents

List of Publications

This thesis was prepared based on the following publications by the author:

I Velihina, Ye.; Scattolin, Th.; **Bondar, D**.; Pil'o, S.; Obernikhina, N.; Kachkovskyi, O.; Semenyuta, I.; Caligiuri, I.; Rizzolio, F.; Brovarets, V.; Karpichev, Y.; Nolan S. P. Synthesis, *in silico* and *in vitro* Evaluation of Novel Oxazolopyrimidines as Promising Anticancer Agents. *Helv. Chim. Acta*, **2020**, 103 (12), #e2000169.

II **Bondar, D**.; Kapitanov, I. V.; Pulkrabkova, L.; Soukup, O.; Jun, D.; Botelho, F. D.; França, T. C. C.; Kuča, K.; Karpichev, Y. N-substituted arylhydroxamic acids as acetylcholinesterase reactivators. *Chem.-Biol. Interact.*, **2022**, 110078.

III **Bondar, D**.; Bragina, O.; Lee, J. Y.; Semenyuta, I.; Järving, I.; Brovarets, V.; Wipf, P.; Bahar, I.; Karpichev, Y. Hydroxamic Acids as PARP-1 Inhibitors: Molecular Design and Anticancer Activity of Novel Phenanthridinones, *Helv. Chim. Acta,* **2023**, #e202300133.

Author's Contribution to the Publications

Contribution to the papers in this thesis are:

I The author carried out synthetic experiments, prepared final compounds for characterization and biological studies. The author contributed to the final manuscript preparation and compilation of supporting material.

II The author planned synthesis and carried out the chemical experiments. The author participated in biological studies and complied the supporting information (characterization of intermediates and reaction products). The author played a major role in the final manuscript preparation.

III The author planned and carried out the synthesis. The author participated in biological studies and played a major role in the final manuscript preparation and complied the supporting information (characterization of intermediates and reaction products).

Author's Other Publications and Conference Presentations

Other Publications:

I Bolkvadze, V.; **Bondar, D**.; Vaher, M.; Halling, E.; Gorbatsova, J.; Mazina-Šinkar, J. The influence of organic solvents on phenylethylamines in capillary zone electrophoresis. *J. Chromatogr. A*, **2022**, 1675.

II Vasiliev, G.; Kubo, A.-L.; Vija, H.; Kahru, A.; **Bondar, D**.; Karpichev, Y.; Bondarenko, O. Synergistic antibacterial effect of copper and silver nanoparticles and their mechanism of action. *Sci. Rep.*, **2023**, 9202.

Oral Presentations:

I Designing N-Substituted Arylhydroxamic Acids as Catalytic Acetylcholinesterase Reactivators. *GSFMT conference 2020*, Tallinn, Estonia, 4-5.2.**2020**

II Oxime-Functionalized Nanodiamonds as a Platform for Treatment of Organophosphate Poisoning. *2020 Virtual MRS Spring Meeting & Exhibit*, Boston, 27.11 – 4.12.**2020**.

III Oxime-Functionalized Nanodiamonds as A Platform for Treatment of Organophosphate Poisoning. *European Advanced Materials Congress*, Stockholm, Sweden, 22-27.08.**2021**.

IV Nanodiamonds for targeted drug delivery, Invited speaker, Nanodiamonds for targeted drug delivery. Invited speaker, *V International Conference "Pharmacology and Pharmaceutical Technology"*, Kyiv, Ukraine, 26.11.**2021**.

V Drug coating design of nanodiamonds for targeted delivery. *5th International Symposium on Nanoparticles, Nanomaterials, and Applications 2022*, Caparica, Portugal, 24-27.01.**2022**

Poster Presentations:

I Designing N-substituted arylhydroxamic acids as catalytic acetylcholinesterase reactivators, *10th International Conference of Chemistry Toulouse-Kiev*, Toulouse, France, 2-4.06.**2019**.

II Scaffold hopping approach for designing new 7-piperazin- and 7-(1,4-diazepan)-substituted [1,3]oxazolo[5,4-d]pyrimidines as prospective anticancer agents, *10th International Conference of Chemistry Toulouse-Kiev*, Toulouse, France, 2-4.06.**2019**.

III Oxime-Functionalized Nanodiamonds as a Platform for Treatment of Organophosphate Poisoning, *2020 Virtual MRS Spring Meeting & Exhibit*, Boston, 27.11-4.12.**2020**.

IV Nanodiamonds as a Platform for Biomedical Application. *GSFMT Scientific Conference 2021*, Tartu, Estonia, 14–15.06.**2021**.

V Functional antimicrobial surfaces with Cu(II) polyoxotungstates, *5th International Symposium on Nanoparticles, Nanomaterials, and Applications 2022*, Caparica, Portugal, 24-27.01.**2022**

Abstract

Design of AChE Reactivators and PARP Inhibitors Based on Hydroxamic Acids and Oximes

Since the pioneering work by Edwards and Pearson on the α-effect in reactivity, the concept of supernucleophiles has been extensively studied and remains an important subject of modern physical organic chemistry.

Despite their widespread application in organic synthesis due to their low cost, versatility, and well-studied chemistry, hydroxamic acids and oximes, typical supernucleophiles, face challenges in medicinal chemistry due to their presumably fragile N-O bond, detrimental to compound chemical stability, drug efficiency, and metabolite safety. This study aims to demonstrate the utility of hydroxamic acids and oximes in drug design through adressing two medical problems: organophosphate (OP) poisoning and the development of anticancer agents. For OP poisoning treatment, nanodiamond-grafted oximes were prepared and studied to overcome BBB limitations, based on visualization of tight junctions and actin cytoskeleton with confocal microscopy to follow internalization and localization of the nanoparticles and their aggregates inside the MDCK and HUVEC cells. Optimization of antidote structure and surface modification techniques can further enhance the efficacy of the nanodiamond-based reactivators of human acelylcholinesterase (AChE) inhibited by OPs. A rational design of *N*-substituted aryl hydroxamic acids was lead to optimization of their reactivation capacity for AChE inhibited by OPs; the *N*-butyl derivatives were shown to have a better-balanced combination of properties.

For anticancer activity, the cyclic aryl hydroxamic acids, *N*-(benzyloxy)- and *N*-(hydroxy)phenanthridinones, were prepared and investigated for the first time as PARP inhibitors aside from their well-known application as HDAC inhibitors. The oxazolopyrimidine scaffold was explored due to its versatility in drug design. Isomeric oxazolo[4,5-*d*]pyrimidines and oxazolo[5,4-*d*]pyrimidines were designed to inhibit VEGFR2 and modulation of their antitumor activity was possible by structural modification, offering prospects for therapeutic use.

In conclusion, this study expands the horizons of α-nucleophiles in drug design and involved preparation of various substituted oximes and hydroxamic acids, oxazolopyrimidines with valuable theraupetic properties thereby enriching drug libraries with valuable candidates.

Lühikokkuvõte
Hüdroksaamhapetel ja oksiimidel põhinevate AChE reaktivaatorite ja PARP inhibiitorite disain

Alates Edwardsi ja Pearsoni teedrajavast tööst reaktiivsuse α-efekti valdkonnas, on supernukleofiilide kontseptsiooni põhjalikult uuritud ning see on tänapäevase füüsikalise orgaanilise keemia üheks tähtsaimaks uurimisobjektiks.

Tänu supernukleofiilide madalale hinnale, mitmekülgsusele ja põhjalikult uuritud keemiale on need orgaanilises sünteesis laialdaselt kasutusel. Vaatamata sellele, on tüüpiliste supernukleofiilide – hüdroksaamhapete ja oksiimide kasutamine meditsiinilises keemias tõsine väljakutse, nende eeldatavalt nõrga N-O sideme tõttu, mis vähendab ühendite keemilist stabiilsust, potentsiaalsete ravimite efektiivsust ja kaasnevate metaboliitide ohutust. Selle uuringu eesmärgiks on demonstreerida hüdroksaamhapete ja oksiimide kasulikkust ravimite väljatöötamisel, käsitledes kahte meditsiinilist probleemi: orgaanilise fosfaadi (OP) mürgistust ja vähivastaste ainete väljatöötamist. OP-mürgistuse raviks valmistati ette ja uuriti nanoteemandiga poogitud oksiime, et ületada BBB piirangud, mis põhinevad tugevate ühenduste ja aktiini tsütoskeleti visualiseerimisel konfokaalmikroskoopiaga, et jälgida nanoosakeste ja nende agregaatide sisestamist ja lokalisatsiooni MDCK ja HUVEC rakkudes. Antidoodi struktuuri ja pinna modifitseerimise tehnikate optimeerimine võib veelgi suurendada OP-de poolt inhibeeritud inimese atselüülkoliinesteraasi (AChE) nanoteemandipõhiste reaktivaatorite efektiivsust. *N*-asendatud arüülhüdroksaamhapete ratsionaalne disain viis nende reaktiveerimisvõime optimeerimiseni OP-de poolt inhibeeritud AChE suhtes. Näidati, et *N*-butüülderivaatidel on paremini tasakaalustatud omaduste kombinatsioon.

Vähivastase aktiivsuse uurimise eesmärgil valmistati tsüklilised arüülhüdroksaamhapped, *N*-(bensüüloksü)- ja *N*-(hüdroksü)fenantridinoonid ning neid katsetati esmakordselt PARP inhibiitoritena, lisaks nende hästituntud kasutusele HDAC inhibiitoritena. Oksasolopürimidiini struktuuri uuriti selle mitmekülgsuse tõttu ravimite väljatöötamisel. Isomeersed oksasolo[4,5-*d*]pürimidiinid ja oksasolo[5,4-*d*]pürimidiinid disainiti inhibeerimaks VEGFR2 ning nende kasvajavastase toime moduleerimine oli läbi struktuuri muutuste võimalik, pakkudes väljavaateid terapeutiliseks kasutamiseks.

Kokkuvõtteks, antud uuring laiendab α-nukleofiilide kasutusvõimalusi ravimite väljatöötamisel ja erinevate asendatud oksiimide ja hüdroksaamhapete sünteesi võimalusi, rikastades seeläbi väärtuslike terapeutiliste omadustega oksasolopürimidiinidel põhinevate ravimite raamatukogusid väärtuslike ravimikandidaatidega.

Curriculum vitae

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Teaching expertise and supervision

2022	Green Chemistry Metrics, exercise tutorial (graduate course)
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