Sveučilište u Zagrebu

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SINTEZA, STRUKTURNA KARAKTERIZACIJA I BIOLOŠKA ISPITIVANJA NOVIH HIBRIDA BENZAZOLA

DOKTORSKI RAD

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Mentor:
Prof. dr. sc. Tatjana Gazivoda Kraljević

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Faculty of chemical engineering and technology

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SYNTHESIS, STRUCTURAL CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL BENZAZOLE HYBRIDS

DOCTORAL DISSERTATION

Mentor:
Prof. dr. Tatjana Gazivoda Kraljević

Zagreb, 2025.

SAŽETAK

U ovom doktorskom radu opisana je sinteza i strukturna karakterizacija novih derivata benzoksazola i benzimidazola supstituiranih u položaju 2 arilnim supstituentima te njihova antiproliferativna, antibakterijska i antivirusna aktivnost *in vitro*. U sintezi novih derivata i njihovih prekursora su pored konvencionalnih sintetskih metoda primijenjeni i pristupi temeljeni na principima zelene kemije poput sinteza potpomognutih mikrovalovima i ultrazvukom, mehanokemijske sinteze te sinteze u niskotemperaturnim eutektičkim otapalima (*eng. Deep Eutectic Solvents,* DESs). Derivati 2-arilbenzoksazola **49**–**84** priređeni su trostupanjskom sintezom uključujući u prvom stupnju sintezu *O*-alkiliranih benzaldehida **1**–**12** reakcijom alkiliranja 4-hidroksibenzaldehida, potom reakciju kondenzacije s 2-aminofenolom uz nanočestice cinkova oksida kojom su sintetizirane Schiffove baze **13**–**48**, koje su u zadnjem stupnju reakcijom ciklokondenzacije prevedene u derivate 2-arilbenzoksazola **49**–**84**. Schiffove baze i derivati 2-arilbenzoksazola sintetizirani održivim metodama priređeni su u visokim iskorištenjima usporedivima s konvencionalnom sintezom, no uz značajno smanjenje reakcijskog vremena iz čega je razvidno da se primijenjene održive sinteze mogu učinkovito koristiti i u pripravi sličnih klasa spojeva različito supstituiranih na benzoksazolnom prstenu. 1,2,3-triazolni derivati benzoksazola **116**–**151** pripravljeni su četverostupanjskom sintezom. U prvom stupnju sintetizirane su Schiffove baze **85**–**93** kondenzacijom benzaldehida s odgovarajućim 2-aminofenolima, potom su njihovom ciklokondenzacijom s natrijevim cijanidom pripravljeni 2-arilbenzoksazoli **94**–**102**, koji su reakcijom propargiliranja prevedeni u *O*-propragilirane derivate **103**–**111**. Bakrom(I) kataliziranom klik-reakcijom u zadnjem stupnju s prethodno pripravljenim azidima **112**–**115** sintetizirani su 1,2,3-triazolni derivati benzoksazola **116**–**151**. Optimizacijom reakcijskih uvjeta za pripravu 1,2,3-triazolnih derivata benzoksazola i Schiffovih baza kao prekursora, uz reakcije potpomognute mikrovalovima i ultrazvukom te mehanokemijske reakcije, dodatno je ispitan utjecaj dvokomponentnog DES-a kolin-klorid/glicerol i trokomponentnog sustava D-sorbitol/urea/NH_4Cl, pri čemu su se reakcije u dvokomponentnom DES-u pokazale najučinkovitijima od svih primijenjenih metoda. Paladijem kataliziranom Sonogashirinom reakcijom prethodno sintetiziranih 3-bromarilnih derivata benzoksazola **157**–**161** s alifatskim ili aromatskim terminalnim alkinima pripravljeni su 3-alkinilarilni derivati benzoksazola **162**–**173**. Derivati 2,5-diarilbenzimidazola **183**–**191** sintetizirani su Suzuki-Miyaurinom reakcijom unakrsnog spajanja priređenih 5-klorbenzimidazolnih derivata **174**–**182** s fenilboronskom

kiselinom. 1,2,3-triazolni derivati hibrida benzimidazola i benzoksazola **199**–**208** sintetizirani su četverostupanjskom sintezom. U prvom stupnju su pripravljeni 2-merkaptobenzoksazoli **192** i **193** koji su s 2-klormetilbenzimidazolom prevedeni u hibride **194** i **195** te propargilirani na dušikovom atomu. 1,2,3-triazolni hibridi **199**–**208** pripravljeni su u zadnjem stupnju bakrom(I)-kataliziranom klik-reakcijom *N*-propargiliranih hibrida benzoksazola i benzimidazola **196** i **197** s odgovarajućim azidima **112–115** i **198**. Strukture novopripravljenih derivata potvrđene su ^{1}H- i ^{13}C-NMR spektroskopijom, a nekim spojevima dvodimenzijskim metodama HMBC, HSQC i NOESY te masenom spektrometrijom. Svim sintetiziranim derivatima 2-arilbenzazola ispitano je antiproliferativno djelovanje *in vitro* na niz tumorskih staničnih linija porijeklom iz čovjeka te antibakterijska aktivnost *in vitro* na Gram-pozitivne i Gram-negativne bakterije. Odabranim klasama benzoksazola i benzimidazola ispitana je i antivirusna aktivnost *in vitro*. Od svih ispitanih spojeva, najizraženiju antiproliferativnu aktivnost protiv stanica karcinoma pluća (NCI-H460, IC_{50} = 0.4 μM) pokazali su 5-brom-2-arilbenzoksazol supstituiran morfolinskim prstenom u položaju 4 fenilnog supstituenta **60** i 1,2,3-triazolni derivat 5-klorbenzoksazola **150**, te protiv adenokarcinoma gušterače 3-alkinilfenilni derivat benzoksazola **170** (Capan-1, IC_{50} = 0.8 μM) i 2,5-diarilni derivat benzimidazola **188** (Capan-1, IC_{50} = 0.6 μM) supstituirani morfolinom u položaju 4 fenilnog supstituenta. Najsnažniju antibakterijsku aktivnost pokazali su 5-klorbenzoksazolni derivat supstituiran piperidinskim prstenom **68** (*P. aeruginosa*, MIC = 0.25 μg/mL; *E. faecalis*, MIC = 0.5 μg/mL) te 1,2,3-triazolni derivat 5-brombenzoksazola supstituiran pirolidinom **145** (*E. faecalis*, MIC = 1 μg/mL). Najizraženiju antivirusnu aktivnost pokazali su prema koronavirusu 3-heptinilfenilni derivat 5-klorbenzoksazola **162** (HCoV-229E, EC_{50} = 3.8 μM), derivat 5-klorbenzimidazola supstituiran pirolidinskim prstenom **186** (HCoV-OC43, EC_{50} = 3.2 μM) i 1,2,3-triazolni hibrid 5-klorbenzimidazola i benzoksazola **204** (HCoV-OC43, EC_{50} = 3.2 μM; Zika virus, EC_{50} = 4.7 μM). Iz analize odnosa strukture i biološke aktivnosti razvidno je da na biološku aktivnost utječe prisustvo halogena na položaju 5 benzazolnog prstena te vrsta aminoalkilnog supstituenta u položaju 4 ili 3 fenila supstituiranog na položaj 2 benzazola.

Ključne riječi: benzoksazol, benzimidazol, hibridi benzoksazola i benzimidazola, 1,2,3-triazol, klik-kemija, mehanokemija, UZV, MW, niskotemperaturna eutektička otapala, antitumorska aktivnost, antibakterijska aktivnost, antivirusna aktivnost

ABSTRACT

This doctoral dissertation describes the synthesis and structural characterization of novel benzoxazole and benzimidazole derivatives substituted at position 2 with aryl substituents, as well as their antiproliferative, antibacterial, and antiviral activities *in vitro*. In the synthesis of new derivatives and their precursors, in addition to conventional synthetic methods, approaches based on green chemistry principles were also employed, including microwave- and ultrasound-assisted syntheses, mechanochemical synthesis, and synthesis in deep eutectic solvents (DESs). The 2-arylbenzoxazole derivatives **49**–**84** were synthesized through a three-step process. The first step involved the synthesis of *O*-alkylated benzaldehydes **1**–**12** by alkylation of 4-hydroxybenzaldehyde. These were then condensed with 2-aminophenol in the presence of zinc oxide nanoparticles to form Schiff bases **13**–**48**, which were cyclocondensed in the final step to yield the 2-arylbenzoxazole derivatives **49**–**84**. The Schiff bases and 2-arylbenzoxazole derivatives synthesized by sustainable methods were obtained in high yields comparable to those of conventional synthesis, but with significantly reduced reaction times, demonstrating that the applied sustainable methods can be effectively used for the synthesis of similar classes of compounds with different substitutions on the benzoxazole ring. The 1,2,3-triazole derivatives of benzoxazole **116**–**151** were prepared through a four-step synthesis. In the first step, Schiff bases **85**–**93** were obtained by condensing benzaldehydes with appropriate 2-aminophenols. Their cyclocondensation with sodium cyanide yielded 2-arylbenzoxazoles **94**–**102**, which were then propargylated to form *O*-propargyl derivatives **103**–**111**. In the final step, copper(I)-catalyzed click reactions with previously prepared azides **112**–**115** afforded the 1,2,3-triazole derivatives of benzoxazole **116**–**151**. Reaction condition optimization for the preparation of 1,2,3-triazole benzoxazole derivatives and their Schiff base precursors also involved microwave- and ultrasound-assisted reactions, as well as mechanochemical methods. The influence of a binary DES (choline chloride/glycerol) and a ternary system (D-sorbitol/urea/NH_4Cl) was evaluated, with reactions in the binary DES proving to be the most effective among all tested methods. 3-Alkynylaryl benzoxazole derivatives **162**–**173** were synthesized via palladium-catalyzed Sonogashira coupling of previously prepared 3-bromoaryl benzoxazole derivatives **157**–**161** with aliphatic or aromatic terminal alkynes. 2,5-Diarylbenzimidazole derivatives **183**–**191** were synthesized through a Suzuki–Miyaura cross-coupling reaction of 5-chlorobenzimidazole derivatives **174**–**182** with phenylboronic acid. The 1,2,3-triazole derivatives of benzimidazole–benzoxazole hybrids **199**–

208 were synthesized via a four-step process. In the first step, 2-mercaptobenzoxazoles **192** and **193** were prepared and reacted with 2-chloromethylbenzimidazole to yield hybrids **194** and **195**, which were then propargylated at the nitrogen atom. The 1,2,3-triazole hybrids **199**–**208** were obtained in the final step through copper(I)-catalyzed click reactions of *N*-propargylated benzoxazole–benzimidazole hybrids **196** and **197** with appropriate azides **112**–**115** and **198**. The structures of the newly synthesized derivatives were confirmed by ^{1}H and ^{13}C NMR spectroscopy, and for some compounds, by 2D techniques such as HMBC, HSQC, and NOESY, as well as mass spectrometry. All synthesized 2-arylbenzazoles were evaluated *in vitro* for antiproliferative activity against a panel of human cancer cell lines and for antibacterial activity against Gram-positive and Gram-negative bacteria. Selected classes of benzoxazoles and benzimidazoles were also evaluated for *in vitro* antiviral activity. Among all tested compounds, the most pronounced antiproliferative activity against lung cancer cells (NCI-H460, IC_{50} = 0.4 μM) was observed for the 5-bromo-2-arylbenzoxazole derivative **60** substituted with a morpholine ring at position 4 of the phenyl substituent, and for the 1,2,3-triazole derivative of 5-chlorobenzoxazole **150**. Against pancreatic adenocarcinoma (Capan-1), strong activity was observed for the 3-alkynylphenyl derivative of benzoxazole **170** (IC_{50} = 0.8 μM) and the 2,5-diaryl benzimidazole derivative **188** (IC_{50} = 0.6 μM) substituted with morpholine at position 4 of the phenyl ring. The strongest antibacterial activity was shown by the 5-chlorobenzoxazole derivative substituted with a piperidine ring **68** (*P. aeruginosa*, MIC = 0.25 μg/mL; *E. faecalis*, MIC = 0.5 μg/mL) and the 1,2,3-triazole derivative of 5-bromobenzoxazole substituted with pyrrolidine **145** (*E. faecalis*, MIC = 1 μg/mL). The most potent antiviral activity was observed for the 3-heptynylphenyl derivative of 5-chlorobenzoxazole **162** (HCoV-229E, EC_{50} = 3.8 μM), the 5-chlorobenzimidazole derivative substituted with a pyrrolidine ring 186 (HCoV-OC43, EC_{50} = 3.2 μM), and the 1,2,3-triazole hybrid of 5-chlorobenzimidazole and benzoxazole 204 (HCoV-OC43, EC_{50} = 3.2 μM; Zika virus, EC_{50} = 4.7 μM). Structure–activity relationship analysis revealed that the biological activity is influenced by the presence of halogens at position 5 of the benzazole ring and the type of aminoalkyl substituent at position 4 or 3 of the phenyl ring attached at position 2 of the benzazole.

Keywords: benzoxazole, benzimidazole, benzoxazole–benzimidazole hybrids, 1,2,3-triazole, click chemistry, mechanochemistry, ultrasound, microwave, deep eutectic solvents, antitumor activity, antibacterial activity, antiviral activity