Sveučilište u Zagrebu

FAKULTET KEMIJSKOG INŽENJERSTVA I TEHNOLOGIJE

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DOKTORSKI RAD

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Mentor:
prof. dr. sc. Tatjana Gazivoda Kraljević

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SAŽETAK

U ovom doktorskom radu je opisana sinteza, strukturna karakterizacija i biološka aktivnost novih derivata 2-arilbenzimidazola i 2-aril/hidrazonskih benzotiazola. Pored konvencionalnih sintetskih metoda korištene su i ekološki prihvatljive zelene metode poput sinteza potpomognutih mikrovalovima i ultrazvukom te mehanokemijske reakcije. Novi *O*-alkilni derivati (**12**–**27**) i 1,2,3-triazolni derivati (**28**–**33**) 2-arilbenzimidazola pripravljeni su reakcijama potpomognutim ultrazvukom. Derivati 2-arilbenzotiazola (**40**–**46**) s odgovarajućim aminoalkilnim supstituentima u *para*-položaju benzenskog prstena pripravljeni su konvencionalnom sintezom dok su derivati supstituirani u položaju 6 benzotiazolnog prstena atomom klora ili fluora (**56**–**67**) priređeni sintezom potpomognutom mikrovalovima. U svrhu odabira sinteze ciljanih derivata 2-arilbenzotiazola supstituiranog u različitim položajima benzenskog prstena 1,2,3-triazolom (**88**-**117**) provedena je optimizacija reakcijskih uvjeta na modelnoj reakciji uključujući konvencionalnu sintezu, sintezu potpomognutu ultrazvukom i mehanokemijsku sintezu. Budući da su mehanokemijskom sintezom postignuta najveća iskorištenja uz najkraće vrijeme trajanja reakcije 1,2,3-triazolni derivati 2-arilbenzotiazola (**118**–**156**) pripravljeni su mehanokemijskom Huisgenovom 1,3-dipolarnom cikloadicijom. Hibridi benzotiazola i *para*-supstituirane fenilne jezgre aminskim supstituentima premošteni hidrazonskom premosnicom (**169**–**202**) pripravljeni su mehanokemijskom sintezom bez otapala. 1,2,3-triazolni derivati benzimidazola s iminokumarinskom jezgrom priređeni su mikrovalovima potpomognutom sintezom odgovarajućih benzimidazolnih azida i terminalnih alkina.

Pripravljenim derivatima strukture su potvrđene spektroskopijom ^{1}H i ^{13}C NMR kao i dvodimenzijskim tehnikama NOESY, HSQC i HMBC. Antiproliferativno djelovanje *in vitro* pripravljenim spojevima je ispitano protiv niza staničnih tumorskih linija porijeklom iz čovjeka kao i na zdravim stanicama. Antibakterijska aktivnost *in vitro* je ispitana na Gram-pozitivnim i Gram-negativnim sojevima uključujući klinički rezistentne sojeve MRSA i VRE. Derivatima benzotiazola s 1,4-disupstituiranim 1,2,3-triazolnim prstenom (**118**–**156**) ispitana je i antivirusna aktivnost.

Najznačajnije antitumorsko djelovanje *in vitro* od svih ispitanih spojeva su pokazali spojevi **23** (K-562, Z-138, IC_{50} = 2.0 μM), **59** (CFPAC, IC_{50} = 1.03 μM) i **188** (CAPAN, IC_{50} = 0.6 μM, NCI-H460, IC_{50} = 0.9 μM). Najsnažnije selektivno antibakterijsko djelovanje su pokazali derivati benzimidazola **15**, **16** i **17** na *E. faecalis* (MIC = 0.25-1 mg/L), odnosno spoj **50** koji je

pokazao najizraženije djelovanje protiv soja MRSA 13276 (MIC = 2 μg/mL), dok je derivat **123** pokazao najsnažnije antivirusno djelovanje na HCoV-NL63 (EC_{50} = 34.1 μM).

Ključne riječi: benzimidazol, benzotiazol, hidrazon, 1,2,3-triazoli, klik kemija, mehanokemija, UZV, MW, antitumorsko djelovanje, antibakterijsko djelovanje, antivirusno djelovanje

ABSTRACT

This doctoral thesis describes the synthesis, structural characterization and biological activity of new 2-arylbenzimidazole and 2-aryl/hydrazone benzothiazole derivatives. In addition to conventional synthetic methods, ecologically acceptable green methods were also used, such as microwave- and ultrasound-assisted synthesis and mechanochemical reactions. Novel *O*-alkylated derivatives (**12**–**27**) and 1,2,3-triazole derivatives (**28**–**33**) of 2-arylbenzimidazoles were prepared by ultrasound-assisted reactions. Benzothiazole derivatives substituted with appropriate aminoalkyl substituent (**40**–**46**) were prepared by conventional synthesis, while derivatives substituted in position 6 of the benzothiazole ring with a chlorine or fluorine atom (**56**–**67**) were prepared by microwave-assisted synthesis. In order to select the synthetic method of target 2-arylbenzothiazole derivatives substituted in different positions of the benzene ring with 1,2,3-triazole (**88**-**117**), optimization of the reaction conditions was conducted on a model reaction including conventional synthesis, ultrasound-assisted and mechanochemical synthesis. Since mechanochemical synthesis achieved the highest yields with the shortest reaction time, 1,2,3-triazole derivatives of 2-arylbenzothiazole (**118**–**156**) were prepared by mechanochemical Huisgen 1,3-dipolar cycloaddition. Hybrids of benzothiazole and *para*-substituted phenyl ring with amine substituents bridged by a hydrazone moety (**169**–**202**) were prepared by *solvent-free* mechanochemical synthesis. 1,2,3-triazole benzimidazole derivatives with an iminocoumarin core were prepared by microwave-assisted synthesis of the corresponding benzimidazole azides and terminal alkynes.

The structures of the prepared derivatives were confirmed by ^{1}H and ^{13}C NMR spectroscopy as well as by two-dimension techniques NOESY, HSQC and HMBC. The antiproliferative activity *in vitro* of the prepared compounds was performed against a range human tumor cell lines as well as on healthy cells. Antibacterial activity *in vitro* was performed on Gram-positive and Gram-negative strains including clinically resistant strains of MRSA and VRE. Benzothiazole derivatives with a 1,4-disubstituted 1,2,3-triazole ring (**118**–**156**) were also tested for antiviral activity.

Among all the prepared derivatives the most significant antitumor activity *in vitro* showed compounds **23** (K-562, Z-138, IC_{50} = 2.0 μM), **59** (CFPAC, IC_{50} = 1.03 μM) and **188** (CAPAN, IC_{50} = 0.6 μM, NCI-H460, IC_{50} = 0.9 μM). The strongest selective antibacterial activity was shown by benzimidazole derivatives **15**, **16** and **17** on *E. faecalis* (MIC = 0.25-1 mg/L) and compound **50**, which showed the most pronounced activity against MRSA strain

13276 (MIC=2 μg/mL), while derivative **123** showed the strongest antiviral activity against HCoV-NL63 (EC50=34.1 μM).

Key words: benzimidazole, benzothiazole, hydrazone, 1,2,3-triazoles, click chemistry, mechanochemistry, US, MW, antitumor activity, antibacterial activity