Sveučilište u Zagrebu

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SINTEZA I BIOLOŠKA AKTIVNOST NOVIH 1,2,3-TRIAZOLNIH DERIVATA 2-SUPSTITUIRANIH BENZOKSAZOLA

DOKTORSKI RAD

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Mentor:

Prof. dr. sc. Tatjana Gazivoda Kraljević

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 1,2,3-TRIAZOLE DERIVATIVES OF 2-SUBSTITUTED BENZOXAZOLES

DOCTORAL DISSERTATION

Mentor:

Prof. dr. sc. Tatjana Gazivoda Kraljević

Zagreb, 2025.

SAŽETAK

U ovom doktorskom radu opisana je sinteza, strukturna karakterizacija i biološka aktivnost novih 1,2,3-triazolnih derivata 2-aminobenzoksazola **3**−**15** i 2-merkaptobenzokszaola **26**−**115**. 1,2,3-triazolni derivati **3**−**124** pripremljeni su konvencionalnim metodama, te sintezom u prirodnim niskotemperaturnim eutektičkim otapalima (eng. *Natural deep eutectic solvents*, NADES). 1,2,3-triazolni derivati 2-aminobenzoksazola **3**−**15** pripravljeni su trostupanjskom sintezom koja u prvom stupnju uključuje ciklizaciju benzoksazolnog prstena reakcijom *o*-aminofenola i di(imidazol-1-il)metanimina. Drugi korak uključuje propargiliranje pripremljenog 2-aminobenzoksazola **1** s propargil-bromidom u dimetilformamidu bez upotrebe baze, budući da upotrebom baze postoji mogućnost nastanka dipropargiliranog derivata. Sintetizirani propargilirani derivat 2-aminobenzoksazola **2** izoliran je kristalizacijom uz reduciranje volumena reakcijske smjese do početka kristalizacije. Pripremljeni propargiliriani derivat 2-aminobenzoksazola **2** u bakrom kataliziranoj Huisgenovoj 1,3-dipolarnoj cikloadiciji preveden je u 1,2,3-triazolne derivate 2-aminobenzoksazola **3**−**15** primjenom različitih azida pri povišenoj temperaturi u sustavu otapala *tert*-butanol/voda = 1/1. Katalizator u ovoj reakciji bio je Cu(I) koji je pripremljen *in situ* iz modre galice (izvor Cu(II)) i natrijevog askorbata koji djeluje kao reducens te reducira Cu(II) u Cu(I). Dodatkom natrijeva askorbata u suvišku omogućena je provedba reakcija bez dodatne inertizacije sustava. Dobiveni 1,2,3-triazolni derivati **3**−**15** izolirani su kristalizacijom iz reakcijske smjese ili ekstrakcijom. 1,2,3-triazolni derivati 2-merkaptobenzoskazola **26**−**115** pripemljeni su trostupanjskom sintezom koja u prvom stupnju uključuje ciklizaciju benzoksazolnog prstena reakcijom odgovarajućeg *o*-aminofenola i ugljik disulfida. Drugi korak uključuje propargiliranje pripremljenih 2-merkaptobenzoksazola **16**−**20** s propargil-bromidom u acetonu uz K_2CO_3 kao bazu. Sintetizirani propargilirani derivati 2-merkaptobenzoksazola **21**−**25** izolirani su kristalizacijom iz reakcijske smjese. Pripremljeni propargiliriani derivati 2-merkaptobenzoksazola **21**−**25** u bakrom kataliziranoj Huisgenovoj 1,3-dipolarnoj cikloadiciji prevedeni su u 1,2,3-triazolne derivate 2-merkaptobenzoksazola **26**−**115** primjenom različitih azida pri povišenoj temperaturi u sustavu otapala *tert*-butanol/voda = 1/1. Katalizator u ovoj reakciji bio je Cu(I) koji je pripremljen *in situ* iz modre galice (izvor Cu(II)) i natrijevog askorbata koji djeluje kao reducens te reducira Cu(II) u Cu(I). Dodatkom natrijeva askorbata u suvišku omogućena je provedba reakcija bez dodatne inertizacije sustava. Dobiveni 1,2,3-triazolni derivati **26**−**115** izolirani su kristalizacijom iz reakcijske smjese ili ekstrakcijom. Također, koristeći niskotemperaturno eutektičko otapalo kolin-klorid : etilen-glikol = 1:2 pripremljeno je devet

1,2,3-triazolnih derivata 2-merkaptobenzoksazola **116**−**124**. Iako su iskorištenja bila niža nego kod konvencionalnih otapala, upotrebom DES-a spoj **123** dobiven je u skoro 3 puta većem iskorištenju. Pripremljenim derivatima struktura je potvrđena ^{1}H-, ^{13}C-NMR-om i masenom spektrometrijom. Antiproliferativna akitvnost *in vitro* sintetiziranih 1,2,3-triazolnih derivata 2-aminobenzoksazola **3**−**15** i 2-merkaptobenzokszaola **26**−**115** ispitana je na osam tumorskih staničnih linija porijeklom iz čovjeka: glioblastom (LN-229), adenokarcinom gušterače (CAPAN-1), rak debelog crijeva (HCT-116), ne-male stanice raka pluća (NCI-H460), akutna limfoblastična leukemija (DND-41), akutna mijeloična leukemija (HL-60), kronična mijeloična leukemija (K-562) i non-Hodgkinov limfom (Z-138). Od svih benzoksazolnih derivata, najizraženije djelovanje prema svim ispitanim tumorskim staničnim linijama (IC_{50} =1.0−2.1 μM), a osobito prema DND-41 (IC_{50} =1.0 μM) pokazao je 1,2,3-triazolni derivat 2-merkaptobenzoksazola u kojem je triazolni prsten supstituiran kumarinom **40**. Odabranim derivatima 2-merkaptobenzoksazola **41**−**115** ispitana je antibakterijska aktivnost prema Gram-pozitivnim bakterijama: *Enterococcus faecalis* (*E. faecalis*) i *Staphylococcus aureus* (*S. aureus*) i Gram-negativnim bakterijama: *Escherichia coli (E.coli), Klebsiella pneumoniae* (*K. pneumoniae*) i *Pseudomonas aeruginosa* (*P. aeruginosa*) standardnom metodom mikrorazrjeđivanja bujona prema preporučenim smjernicama Instituta za kliničke i laboratorijske standarde. Usporedbom antibakterijske aktivnosti svih ispitanih 1,2,3-triazolnih derivata 2-merkaptobenzoksazola **41**−**115**, uočeno je da je na aktivnost značajno utjecala supstitucija u položaju 5 benzoksazolnog prstena. Vidljivo je da je antibakterijska aktivnost obrnuto proporcionalna elektronegativnosti supstituenata u položaju 5. Najaktivniji derivati **45** i **47** bili su iz skupine 2-merkapto-5-metilbenzoksazolnih derivata, pri čemu metilna skupina ima najmanju elektronegativnost u odnosu na supstituente ostalih derivata (5-metoksi, 5-klor i 5-fluor2-merkaptobenzoksazolni derivati). Spoj **45**, u kojemu je triazolni prsten supstituiran metil(trimetilsilil)nom skupinom, pokazao je najizraženije djelovanje prema Gram-pozitivnoj bakteriji *S. aureus* (MIC = 1 mg L^{-1}), te Gram-negativnim bakterijama *K. pneumoniae* (MIC = 2 mg L^{-1}) i *P. aeruginosa* (MIC = 1 mg L^{-1}). Osim toga, spoj **45** pokazao je umjereno djelovanje prema Gram-pozitivnoj bakteriji *E. faecalis* (MIC = 8 mg L^{-1}) i Gram-negativnoj bakteriji *E.coli* (MIC = 16 mg L^{-1}).

Ključne riječi: benzoksazol, 1,2,3-triazol, klik-kemija, zelena kemija, prirodna niskotemperaturna eutektička otapala, antitumorsko djelovanje, antibakterijsko djelovanje

ABSTRACT

This research describes the synthesis, structural characterization and biological activity of novel 1,2,3-triazole derivatives of 2-aminobenzoxazole **3**−**15** and 2-mercaptobenzoxazole **26**−**115**. 1,2,3-triazole derivatives **3**−**124** were prepared utilizing conventional methods and green chemistry methods (i.e. *Natural deep eutectic solvents*, NADES). Preparation of 1,2,3-triazole derivatives of 2-aminobenzoxazole **3**−**15** began with the reaction between *o*-aminophenol and di(1H-imidazol-1-yl)methanimine followed by *N*-alkylation of the prepared 2-aminobenzoxazole **1** using propargyl-bromide without a base, since utilization of a base yielded the dipropargylated product. Utilizing corresponding azides in the copper catalysed Huisgen 1,3-dipolar cycloaddition at elevated temperatures, the prepared propargylated 2-aminobenzoxazole **2** was transformed to 1,2,3-triazole derivatives of 2-aminobenzoxazole **3**−**15**. The catalyst in this reaction was Cu(I) which was prepared *in situ* from $CuSO_4$ x 5 H_2O (source of Cu(II)) and sodium ascorbate (reducing agent) which reduced Cu(II) to Cu(I). Excess of sodium ascorbate enabled the conduction of this reaction without inert conditions. The prepared 1,2,3-triazole derivatives **3**−**15** were isolated with crystallization from the reaction mixture or extraction. The first step in the preparation of 1,2,3-triazole derivatives of 2-mercaptobenzoxazole was the reaction between the corresponding *o*-aminophenol and carbon disulfide (CS_2). Comparable to 2-aminobenzoxazole derivatives, the second step was the alkylation of the prepared benzoxazole-2-thiols **16**−**20** with propargyl-bromide and K_2CO_3 as base. The prepared propargylated derivatives of 2-mercaptobenzoxzole **21**−**25** were isolated by crystallization from acetone/water solvent system. The propargylated 2-mercaptobenzoxazole derivatives **21**−**25** were transformed to 1,2,3-triazole derivatives *via* the copper catalysed Huisgen 1,3-dipolar cycloaddition using the corresponding azides. Furthermore, using the deep eutectic solvent cholin-chloride : ethylene-glycol = 1:2, nine 1,2,3-triazole derivatives of 2-mercaptobenzoxazole were prepared **116**−**124**. Although the yields were lower as opposed to conventional solvents, compound **123** was prepared with almost 3 times higher yield. Structure of the prepared compounds was confirmed with ^{1}H-, ^{13}C-NMR and mass spectrometry. Antiproliferative activity *in vitro* of the prepared 1,2,3-triazole derivatives of 2-aminobenzoxazole **3**−**15** and 2-mercaptobenzoxazole **26**−**115** was determined against eight human tumour cell lines: glioblastoma (LN-229), pancreatic adenocarcinoma (Capan-1) colorectal carcinoma (HCT-116), non-small lung carcinoma (NCI-H460), acute lymphoblastic leukaemia (DND-41), acute myeloid leukaemia (HL-60), chronic myeloid leukaemia (K-562) and non-Hodgkin lymphoma (Z-138). Among all tested derivatives, compound **40**, where the

triazole ring was substituted with coumarin moiety exhibited the best antiproliferative activity against all tested cell lines (IC_{50} =1.0−2.1 μM) and especially against DND-41 cells (IC_{50} =1.0 μM). Antibacterial activity of chosen 1,2,3-triazole derivatives of 2-mercaptobenzoxazole **41**−**115** towards Gram-positive bacteria: *Enterococcus faecalis* (*E. faecalis*) and *Staphylococcus aureus* (*S. aureus*) and Gram-negative bacteria: *Escherichia coli (E.coli), Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) was determined utilizing the standard microdilution method following the recommendations form the Institute for clinical and laboratory standards. The results indicated that the substitution at position 5 of the benzoxazole ring had a crucial impact on the antibacterial activity. The antibacterial activity was inversely proportional to the electronegativity of the substituent at position 5. The most active compounds **45** and **47** were from the 5-methyl-2-mercaptobenzoxazole series, where the methyl group is the least electronegative (compared to 5-methoxy, 5-chloro and 5-fluoro derivatives). The most potent antibacterial activity towards Gram-positive bacteria *S. aureus* (MIC = 1 mg L^{-1}) and Gram-negative bacteria *K. pneumoniae* (MIC = 2 mg L^{-1}) and *P. aeruginosa* (MIC = 1 mg L^{-1}) exhibited compound **45**, where the triazole ring was substituted with methyl(trimethylsilyl) group. Furthermore, compound **45** exhibited moderate antibacterial activity towards Gram-positive bacteria *E. faecalis* (MIC = 8 mg L^{-1}) and Gram-negative bacteria *E.coli* (MIC = 16 mg L^{-1}).

Key words: benzoxazole, 1,2,3-triazole, click-chemistry, green chemistry, natural deep eutectic solvents, antitumor activity, antibacterial activity