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NOVEL ARYL-SUBSTITUTED BICYCLIC HETEROCYCLES: SYNTHESIS, ANTIPROLIFERATIVE AND ANTIPARASITIC ACTIVITY

DOCTORAL THESIS

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NOVI ARIL-SUPSTITUIRANI BICIKLIČKI HETEROCIKLI: SINTEZA, ANTIPROLIFERATIVNA I ANTIPARAZITSKA AKTIVNOST

DOKTORSKI RAD

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SUMMARY

This thesis describes the synthesis and biological activity of novel benzothiazole, benzoxazole, and imidazo[1,2-*a*]pyridine Schiff base ligands, along with their ruthenium(II) metal complexes, as well as 6-unsubstituted, 6-halogen-, 6-amidino-, 6-arylmidamide-, and 6-amidoxime-substituted benzothiazoles, and 5(6)-amidino and 5(6)-arylimidamide benzimidazoles. In addition to conventional synthetic methods, environmentally friendly techniques were also employed, including syntheses assisted by microwave and ultrasound irradiation, along with mechanochemical reactions.

The prepared benzazole derivatives were evaluated *in vitro* for their antiproliferative activity against several tumour cell lines as well as normal cells. The evaluated Schiff base ligands exhibited moderate to low inhibitory activity, while most Ru(II) complexes demonstrated anticancer activity, with IC_{50} values lower than 10 μM. Notably, the imidazo[1,2-*a*]pyridine complex **17d$_{Ru}$** (IC_{50} = 2.0 μM) showed the highest activity against HuT78 cells. The majority of amidino-substituted benzothiazole derivatives displayed good antiproliferative activity, with derivatives **31b–31d** exhibiting the highest activity on SW620 (**31b**: IC_{50} = 0.25 μM; **31c**: IC_{50} = 0.35 μM; **31d**: IC_{50} = 0.36 μM) cell lines, while derivative **31d** was the most potent against HeLa cells (IC_{50} = 0.38 μM). However, amidino benzothiazoles were also toxic on normal cell lines. Among amidino benzimidazoles, the 1-benzyl-1,2,3-triazole analogue **88a** showed selective and marked antiproliferative activity on THP1 cells in the nanomolar range (IC_{50} = 0.8 μM, SI = 70). Amidino- , arylimidamide- and amidoxime-substituted benzothiazoles and benzimidazoles were also assessed for their antitrypanosomal and antibacterial activity. The results of the antibacterial activity evaluations showed that amidino benzimidazoles exhibit better antibacterial activity compared to amidino benzothiazoles, with compounds **85a–85c** (MICs = 1–4 μg/mL) demonstrating the most significant growth inhibition of *M. catarrhalis* and efflux-deficient *E. coli.* From the *in vitro* evaluation of antitrypanosomal activity, amidino-substituted benzothiazole **28c** (IC_{50} = 0.09 μM) had the most prominent but nonselective antitrypanosomal activity, whereas arylimidamide benzothiazole **115c** (IC_{50} = 1.17 μM, SI > 150) demonstrated excellent and selective antitrypanosomal activity against *T. brucei.* The 6-amidoxime benzothiazole analogue **138b** (IC_{50} = 0.92 μM, SI = 18), which exhibits selective antitrypanosomal activity and has a good ADME profile, was chosen for *in vivo* evaluation of its antitrypanosomal effects on *T. brucei* infected mice.

Compounds **28b**, **28c**, **29c**, **30c**, **31b–31d**, **32c**, **78a**, **78b**, **85a–85c**, **86a**, **86c**, **87a**, **112b**, **115c**, **118a**, **138a**, and **138b** that exhibited the best antiproliferative and antiparasitic activity were additionally evaluated for their binding affinity with DNA/RNA. Binding assays indicated that the amidoximes **138a** and **138b** bind to DNA/ RNA by intercalation, whereas the arylimidamides **112b**, **115c**, and **118a** displayed groove binding to DNA/ RNA. DNA binding assays of amidino benzothiazole derivatives **29c**, **32c**, and **78a** indicated minor groove binding, while amidino benzothiazole derivatives **28c**, **30c**, and **31b–31d** indicated intercalation modes of DNA interaction. In contrast, DNA binding assays of amidino benzimidazoles **85a–85c**, **86c**, and **87a** suggested an intercalative mode of binding.

Furthermore, the imidazo[1,2-*a*]pyridine Schiff base ligand **17b** and its metal complex **17b$_{Ru}$**, along with amidino benzothiazole **76c**, as well as amidino benzimidazoles **85c**, **88a–88c**, and **89c**, which exhibited strong antiproliferative activity, were tested in cell-cycle perturbation experiments on HuT78 cells. The results indicated that the cytotoxic effect of the metal complex **17b$_{Ru}$** was mediated by its impact on DNA synthesis and by disrupting the mitochondrial membrane potential. In contrast, the disruption of mitochondrial membrane potential caused by the tested amidine benzimidazoles **85c**, **88a–88c**, and **89c** can lead to cytotoxicity and cell death through apoptosis and/or necrosis.

Keywords: amidines, arylimidamides, amidoximes, benzothiazoles, benzimidazoles, imidazo[1,2-*a*]pyridines, metal complexes, green synthesis, antiproliferative activity, antitrypanosomal activity, antibacterial activity

SAŽETAK

U ovom radu opisana je sinteza i biološka aktivnost novih liganda Schiffovih baza benzotiazola, benzoksazola i imidazo[1,2-*a*]piridina, zajedno s njihovim rutenij(II) metalnim kompleksima, kao i 6-nesupstituiranih, 6-halogen-, 6-amidino-, 6-arilimidamid- i 6-amidoksim-supstituiranih benzotiazola i 5(6)-amidino i 5(6)-arilimidamidnih benzimidazola. Uz konvencionalne sintetske metode, korištene su i ekološki prihvatljive sintetske metode, uključujući sintezu potpomognutu mikrovalnim i ultrazvučnim zračenjem, kao i mehanokemijsku sintezu.

Pripravljenim benzazolnim derivatima ispitana je antiproliferativna aktivnost *in vitro* na niz staničnih linija humanih karcinoma i zdrave stanice. Ispitani ligandi Schiffovih baza pokazali su umjerenu do nisku inhibitornu aktivnost, dok je većina Ru(II) kompleksa pokazala antikancerogeno djelovanje s IC_{50} vrijednostima nižim od 10 μM, pri čemu je kompleks imidazo[1,2-*a*]piridina **17d$_{Ru}$** (IC_{50} = 2.0 μM) pokazao najveću aktivnost na stanice HuT78. Većina amidino-supstituiranih derivata benzotiazola **31b–31d** imala je dobru antiproliferativnu aktivnost, s najizraženijom aktivnosti na stanice SW620 (**31b**: IC_{50} = 0.25 μM; **31c**: IC_{50} = 0.35 μM; **31d**: IC_{50} = 0.36 μM), dok je derivat **31d** bio najaktivniji na stanice HeLa (IC_{50} = 0.38 μM). Međutim, amidinobenzotiazoli također su bili toksični za normalne stanične linije. Među amidinobenzimidazolima, 1-benzil-1,2,3-triazolni analog **88a** pokazao je selektivnu i izraženu antiproliferativnu aktivnost na stanice THP1 u nanomolarnom području (IC_{50} = 0.8 μM, SI = 70). Amidino-, arilimidamid- i amidoksim-supstituiranim benzotiazolima i benzimidazolima također je ispitana antitripanosomska i antibakterijska aktivnost. Rezultati ispitivanja antibakterijskog djelovanja pokazali su da amidinobenzimidazoli imaju bolje antibakterijsko djelovanje u usporedbi s amidinobenzotiazolima, pri čemu su spojevi **85a–85c** (MIC = 1–4 μg/mL) pokazali najizraženiju inhibiciju rasta bakterijskih sojeva *M. catarrhalis* i *E. coli* s inaktiviranom efluksnom pumpom. Rezultati *in vitro* antitripanosomske aktivnosti pokazali su da je amidino-supstituirani benzotiazol **28c** (IC_{50} = 0.09 μM) imao najbolju, ali neselektivnu antitripanosomsku aktivnost, dok je arilimidamidni benzotiazol **115c** (IC_{50} = 1.17 μM, SI > 150) pokazao izvrsno i selektivno antitripanosomsko djelovanje protiv *T. brucei*. 6-amidoksimski benzotiazolni analog **138b** (IC_{50} = 0.92 μM, SI = 18) sa selektivnom antitripanosomskom aktivnosti i odgovarajućim ADME svojstvima odabran je za daljnja *in vivo* ispitivanja antitripanosomske aktivnosti na miševima zaraženim s *T. brucei*.

Spojevima **28b**, **28c**, **29c**, **30c**, **31b–31d**, **32c**, **78a**, **78b**, **85a–85c**, **86a**, **86c**, **87a**, **112b**, **115c**, **118a**, **138a** i **138b**, koji su pokazali nabolje antiproliferativno i antitripanosomsko

djelovanje, dodatno je ispitan afinitet vezivanja na DNK i RNK. Amidoksimski benzotiazoli **138a** i **138b** vezivali su se na DNK/RNK interkalacijom, dok su se arilimidamidni benzotiazoli **112b**, **115c** i **118a** vezivali u utor DNK/RNK. Testovi vezivanja amidinobenzotiazolnih derivata **29c**, **32c** i **78a** na DNK ukazali su na vezanje tih spojeva u mali utor DNK, dok su se amidinobenzotiazolni derivati **28c**, **30c** i **31b–31d** vezivali na DNK interkalacijom. Testovi vezivanja DNK i amidinobenzimidazola **85a–85c**, **86c** i **87a** također su ukazali na vezivanje spojeva na DNK interkalacijom.

Nadalje, imidazo[1,2-*a*]piridinska Schiffova baza **17b** i metalni kompleks **17b$_{Ru}$**, amidinobenzotiazol **76c** i amidinobenzimidazoli **85c**, **88a**–**88c** i **89c** pokazali su dobru antiproliferativnu aktivnost na stanice HuT78. Citotoksični učinak metalnog kompleksa **17b$_{Ru}$** posredovan je njegovim učinkom na sintezu DNK i narušavanjem potencijala mitohondrijske membrane. Nasuprot tome, narušavanje potencijala mitohondrijske membrane djelovanjem amidinobenzimidazola **85c**, **88a–88c** i **89c** dovelo je do njihovog citotoksičnog efekta i stanične smrti, odnosno apoptoze i/ili nekroze.

Ključne riječi: amidini, arilimidamidi, amidoksimi, benzotiazoli, benzimidazoli, imidazo[1,2-*a*]piridini, metalni kompleksi, zelena sinteza, antiproliferativno djelovanje, antitripanosomsko djelovanje, antibakterijsko djelovanje