Sveučilište u Zagrebu

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DOKTORSKI RAD

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SAŽETAK

Novi konjugati imidazo[4,5-*b*]piridina: sinteza, strukturna karakterizacija i biološka aktivnost

U okviru doktorskog rada opisana je sinteza, strukturna karakterizacija i biološka aktivnost novih derivata imidazo[4,5-*b*]piridina. Za sintezu novih spojeva korištene su klasične metode organske sinteze, sinteza potpomognuta mikrovalovima, paralelna sinteza i organometalna kemija. Akrilonitrilni derivati **21**–**40** i **58**–**89** priređeni su aldolnom kondenzacijom 2-cijanometil imidazo[4,5-*b*]piridina s različitim benzaldehidima. Ispitan je utjecaj supstitucije u položaju *N*3 imidazo[4,5-*b*]piridinske jezgre, broj i položaj metoksi i hidroksi skupina, te prisutnost *N*,*N*-dialkilnog supstituenta u *p*-položaju fenilne jezgre na biološku aktivnost i spektroskopske karakteristike. Nadalje, priređeni su iminokumarinski derivati imidazo[4,5-*b*]piridina u reakciji ciklokondenzacije 2-cijanometilnih derivata i različitih salicilbenzaldehida. Novi derivati **97**–**119** supstituirani su u položajima 6 i 7 na iminokumarinskoj jezgri te na *N*3 dušikovom atomu imidazo[4,5-*b*]piridinske jezgre. Amino-supstituirani iminokumarini **102** i **111** priređeni su redukcijom nitro skupine. Derivati **103**, **112** i **119** priređeni su kao hidrokloridne soli radi bolje topljivosti. 2-fenilamidino-supstituirani derivati imidazo[4,5-*b*]piridina **126**–**133** priređeni su iz cijano-supstituiranih prekursora **122–125**. Pinnerovom reakcijom u dva stupnja, priređeni su derivati supstituirani cikličkim amidinima, a *one pot* reakcijom s reagensom LiHMDS priređeni su derivati s nesupstituiranim amidinom. Reakcijom metiliranja priređeni su derivati **134**–**136** unutar iste serije spojeva. Suzukijevom reakcijom u položaju 6 imidazo[4,5-*b*]piridinske jezgre, priređeni su derivati **142**–**146** i **149**–**154** koji su, ovisno o korištenoj bornoj kiselini, u *p*-položaju fenilnog prstena supstituirani različitim skupinama. Redukcijom nitro skupine priređeni su amino-supstituirani derivati **147** i **155** te su prevedeni u hidrokloridne soli **148** i **156**. Serija *N*3-metil-supstituiranih amidina **163**–**171** priređena je iz cijano-supstituiranih prekursora **152**, **161** i **162** Pinnerovom reakcijom u dva stupnja te *one pot* reakcijom s LiHMDS. Serija konjugata imidazo[4,5-*b*]piridina s amidino-supstituiranim benzazolima **182**–**190** priređena je kondenzacijom odgovarajućih amidino-supstituiranih prekursora **173**–**181** s imidazo[4,5-*b*]piridin-6-karbaldehidom **172**. Benzimidazolni derivati **182–193, 185–186** i **188–189** priređeni su iz 4-amidino-1,2-fenilendiamina uz *p*-benzokinon kao oksidans, dok su benzotiazolni derivati **184**, **187** i **190** priređeni kondenzacijom 5-amidinobenzentiolatima s karbaldehidom **172**.

Schiffove baze imidazo[4,5-*b*]piridina **194–197** priređene su iz 6-supstituiranog karbaldehida imidazo[4,5-*b*]piridina s različitim anilinima, dok su Schiffove baze **202–214** priređene iz 2-aminofenil-supstituiranih imidazo[4,5-*b*]piridina s različitim benzaldehidima. Kako bi se ispitao utjecaj supstituenta u *N*3 položaju imidazo[4,5-*b*]piridinske jezgre, priređeni su nesupstituirani derivati, kao i derivati s metilnim i fenilnim supstituentom. Proučavan je utjecaj prisutnosti *N*,*N*-dialkilnih supstituenata kao i OH skupine na antiproliferativnu i antibakterijsku aktivnost. Priređene su i dvije klase amidnih derivata imidazo[4,5-*b*]piridina koje se međusobno razlikuju samo prema položaju amidne veze. Derivati **217–218**, **230**, **231 i 234** priređeni su reakcijama između benzoilnog klorida i amino-supstituiranih derivata uz prisutnost trietilamina, dok je derivat **222** priređen katalitički. Amidino-supstituirani derivati **219**–**220** te **232–233** priređeni su Pinnerovom reakcijom u dva stupnja. Amino-supstituirani derivati **233** i **235** priređeni su reakcijom redukcije te su prevedeni u hidrokloridne soli **224** i **236**. Strukture svih priređenih ciljanih spojeva i prekursora detaljno su analizirane korištenjem ^{1}H i ^{13}C NMR spektroskopije, te su za pojedine spojeve dodatno potvrđene masenom spektrometrijom. Detaljna spektroskopska karakterizacija u svrhu pronalaženja novih potencijalnih optičkih pH-senzora, provedena je za odabrane spojeve iz klasa akrilonitrila, iminokumarina i Schiffovih baza, te su pK_a vrijednosti određene eksperimentalno i računalnim metodama. Svim ciljanim novopriređenim spojevima ispitana je antiproliferativna aktivnost *in vitro* na nekoliko staničnih linija humanih karcinoma, dok je za najaktivnije derivate istražen mehanizam biološkog djelovanja koji je dodatno potvrđen, za neke derivate, i računalnom analizom. Antioksidativna aktivnost derivata iz klasa akrilonitrila i iminokumarina *in vitro* ispitana je spektroskopskim metodama DPPH, FRAP i ABTS te elektrokemijski, dok je mehanizam antioksidativnog djelovanja potvrđen računalnom analizom. Najaktivnijim amidinskim derivatima dodatno je potvrđen mehanizam biološkog djelovanja ispitivanjem interakcije s c*t*-DNK. Odabranim derivatima iz klasa iminokumarina, amidina, Schiffovih baza i amida ispitana je antibakterijska aktivnost *in vitro* na nekoliko sojeva Gram pozitivnih i Gram negativnih bakterija.

Ključne riječi: imidazo[4,5-*b*]piridin, antiproliferativna aktivnost, antioksidativna aktivnost, antibakterijska aktivnost, pH-senzori, računalna analiza

SUMMARY

Novel imidazo[4,5-*b*]pyridine conjugates: synthesis, structural characterization and biological activity

Within this work, the synthesis, structural characterization and biological activity of novel imidazo[4,5-*b*]pyridine derivatives was described. For the synthesis of targeted compounds, classical methods of organic chemistry, parallel synthesis, and microwave assisted synthesis as well as organometal chemistry were used. Acrylonitrile derivatives **21**–**40** and **58**–**89** were prepared in the aldol condensation reaction from 2-cyanomethylimidazo[4,5-*b*]pyridines and various benzaldehydes. Influence of *N*3 substitution on imidazo[4,5-*b*]pyridine core, the number and the position of hydroxy and methoxy groups as well as presence of *N*,*N*-dialkyl substituent placed at *p*-position of the phenyl ring on biological activity and spectroscopic characteristics was investigated. Iminocoumarin derivatives of imidazo[4,5-*b*]pyridine were prepared in the cyclocondensation reaction from 2-cyanomethyl derivatives and various salicyl benzaldehydes. Novel derivatives **97**–**119** were substituted at positions 6 and 7 of iminocoumarin core as well as at the position *N*3 of imidazo[4,5-*b*]pyridine nucleus. Amino-substituted iminocoumarins **102** and **111** were prepared by reduction of nitro group to amino. Derivatives **103**, **112** and **119** were prepared as hydrochloride salts to achieve better solubility. 2-phenylamidino-substituted imidazo[4,5-*b*]pyridine derivatives **126**–**133** were prepared from cyano-substituted precursors **122**–**125**. Two step Pinner reaction was used to prepare compounds substituted with cyclic amidines, while *one pot* reaction with LiHMDS was used to prepare derivatives with unsubstituted amidines. Derivatives **134**–**136** were prepared in the reaction of methylation. Suzuki coupling in the position 6 of imidazo[4,5-*b*]pyridine core was used to prepare derivatives **142–146** and **149–154** which were, depending on used boronic acid, substituted at the *p*–position of the phenyl ring with different substituents. Amino-substituted derivatives **147** and **155** were prepared by reduction of nitro group to amino and were further converted to corresponding hydrochloride salts **148** and **156**. *N*3-methyl-substituted amidine derivatives **163**–**171** were prepared from cyano-substituted precursors **152**, **161** and **162** in Pinner reaction and *one pot* recation with LiHMDS. Benzazole conjugates of imidazo[4,5-*b*]pyridine with amidino-substituted benzazoles **182**–**190** were synthesized by condensation of corresponding amidino-substituted precursors **173**–**181** with imidazo[4,5-*b*]pyridine-6-carbaldehyde **172**.

Benzimidazole derivatives **182**–**193**, **185**–**186** and **188–189** were prepared from 4-amidino-1,2-phenylenediamines with *p*-benzoquinone as an oxidant, while benzothiazole derivatives **184**, **187** and **190** obtained by condensation of 5-amidino-substituted benzenthiolates with carbaldehyde **172**. Schiff base imidazo[4,5-*b*]pyridine derivatives **194**–**197** was prepared from imidazo[4,5-*b*]pyridine-6-carbaldehyde **172** with various anilines, while the other **202**–**214** obtained from 2-aminophenyl-substituted imidazo[4,5-*b*]pyridines with benzaldehydes. In order to investigate the influence *N*3-substituents on imidazo[4,5-*b*]pyridine nucleus, methyl, phenyl as well as unsubstituted derivatives were prepared. The influence of *N*,*N*-dialkyl substituents and hydroxy groups placed at the phenyl ring on antiproliferative and antibacterial activity was studied. Two series of amide derivatives were prepared which differ only from position of amide bond. Derivatives **217**–**218**, **230**, **231** and **234** were prepared by reactions between benzoil chlorides and amino-substituted derivatives in the presence of triethylamine, while derivative **222** was prepared catalytically. Amidino-substituted derivatives **219–220** and **232–233** were prepared by Pinner reaction. Amino-substituted derivatives **233** i **235** were synthesized by reduction of nitro group and converted to hydrochloride salts **224** and **236**. Structures of targeted compounds and precursors were analysed by means of ^{1}H and ^{13}C NMR spectroscopy, and were further confirmed by mass spectrometry for selected compounds. Thorough spectroscopic characterization was conducted for selected acrylonitrile, iminocumarin and Schiff bases derivatives, while pK_a values were determined experimentally and computationally. All targeted compounds were evaluated for their antiproliferative activity *in vitro* on several human cancer cell lines. Mechanism of action was further investigated for the most active compounds and for some chosen derivatives confirmed by computational analysis. Acrylonitrile and iminocoumarin derivatives were tested for their antioxidative activity *in vitro* by using spectroscopic methods DPPH, FRAP and ABTS as well as electrochemically, while their mechanism of antioxidative action was explained by computational analysis. The most active amidine compounds were chosen studying the interaction with *ct*-DNA to further investigate their mechanism of action. Selected compounds from iminocoumarin, amidine and amide classes were tested for their antibacterial activity *in vitro* on some Gram positive and Gram negative bacterial strains.

Key words: imidazo[4,5-*b*]pyridine, antiproliferative activity, antioxidative activity, antibacterial activity, pH sensors, computational analysis