Sveučilište u Zagrebu

FAKULTET KEMIJSKOG INŽENJERSTVA I TEHNOLOGIJE

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SINTEZA I STRUKTURNA KARAKTERIZACIJA NOVIH DERIVATA BENZIMIDAZOLA I BENZOTIAZOLA KAO POTENCIJALNIH ANTIPROLIFERATIVNIH AGENSA S ANTIOKSIDATIVNIM DJELOVANJEM

DOKTORSKI RAD

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SAŽETAK

Sinteza i strukturna karakterizacija novih derivata benzimidazola i benzotiazola kao potencijalnih antiproliferativnih agensa s antioksidativnim djelovanjem

U ovom radu opisana je sinteza nekoliko klasa derivata benzimidazola i benzotiazola kojima je ispitana njihova antiproliferativna i antioksidativna aktivnost. U linearnoj višestupanjskoj sintezi novih konjugata benzazola primijenjeni su klasični sintetski pristupi kao i neke suvremene sintetske metode, uključujući sintezu u ekološki prihvatljivim otapalima ili sintezu potpomognutu mikrovalovima. Novi akrilonitrilni derivati *N*-supstituiranih derivata benzazola **32–71** i **77–107** priređeni su aldolnom kondenzacijom odgovarajućih cijanometilbenzazola s benzaldehidima koji imaju promjenjivi broj metoksi i hidroksi-skupina te 4-*N*,*N*-dimetilamino i 4-*N*,*N*-dietilamino skupine. Derivati Schiffovih baza supstituirani benzimidazolom **117–132** priređeni su kondenzacijom *N*-supstituiranih 2-aminobenzimidazola **108–115** s odgovarajućim 4-*N*,*N*-dimetilamino i 4-*N*,*N*-dietilamino supstituiranim benzaldehidima. Derivati iminokumarina i kumarina **134–149** te amidino-supstituirani derivati kumarina **164–175** priređeni su ciklokondenzacijom različito supstituiranih 2-hidroksibenzaldehida s odgovarajućim 2-cijanometilbenzimidazolima, te iz kumarinskih aldehida u kondenzaciji s odgovarajućim 4-amidino supstituiranim 1,2-fenilendiaminima uz korištenje *p*-benzokinona kao oksidansa. Amidino-supstituirani benzimidazoli **193–216** priređeni su kondenzacijom 5-supstituiranih salicilaldehida s odgovarajućim 4-amidino supstituiranim 1,2-fenilendiaminima. Amidino-supstituirani benzotiazoli **179–181** i **216–227** različito supstituiranih 2-hidroksibenzaldehida i odgovarajućih zwitter iona u ledenoj octenoj kiselini. Metoksi-supstituirani karboksamidi **235–262** priređeni su kondenzacijom benzoilnih klorida s *N*-supstituiranim derivatima 2-aminobenzimidazola. Hidroksi-supstituirani amidni derivati *N*-benzimidazola **263–268** i **280–286** priređeni su uklanjanjem zaštitnih metoksi skupina, korištenjem BBr_3 na niskim temperaturama, te benzilnih zaštitnih skupina katalitičkom hidrogenacijom uz Pd/C u metanolu. Amidino-supstituirani derivati benzamida **287–293** priređeni su kiselo-kataliziranom Pinnerovom reakcijom iz odgovarajućih cijano-supstituiranih polaznih spojeva. Strukture novosintetiziranih spojeva potvrđene su ^{1}H i ^{13}C NMR spektroskopijom, a nekim je spojevima struktura dodatno okarakterizirana i masenom spektrometrijom.

Svim priređenim spojevima ispitana je antiproliferativna aktivnost *in vitro* na niz staničnih linija humanih karcinoma i zdravih stanica, dok je ispitivanje antioksidativne aktivnosti *in vitro* provedeno primjenom spektroskopskih DPPH, FRAP i ABTS metoda. Amidino-supstituiranim derivatima **164–175** i **179–181** ispitana je antiviralna aktivnost *in vitro* na nekoliko sojeva virusa, te su neki od derivata pokazali jako dobru i selektivnu aktivnost prema pojedinim sojevima virusa. Derivatima Schiffovih baza **117–132** te amidino-supstituiranim benzazolima **193–216** i **217–228** ispitana i antibakterijska aktivnost prema Gram-pozitivnim i Gram-negativnim bakterijama. Iz dobivenih rezultata ispitivanja biološke aktivnosti i SAR studije, utvrđeno je da na antioksidativnu aktivnost značajno utječe broj metoksi i hidroksi skupina na fenilnom prstenu, te supstituent na dušikovom atomu benzimidazolne jezgre. Najizraženiji utjecaj na povećanje antiproliferativne aktivnosti pokazuje 4-*N*,*N*-dietilamino skupina smještena na položaju 7 kumarinskog prstena i fenilnom prstenu akrilonitrilnih derivata te izobutilni supstituent na *N* atomu benzimidazolne jezgre. Nekim od najaktivnijih derivata benzazola dodatno su ispitani mehanizmi biološkog djelovanja te je tako utvrđeno da neki derivati akrilonitrila i iminokumarina djeluju kao inhibitori polimerizacije tubulina, dok je amidnim derivatima ispitana i antioksidativna aktivnost u stanicama. Dokazano je i da najbolju antiviralnu aktivnost ima kumarinski derivat benzimidazola supstituiran nesupstituiranim amidinom koji inhibira ranu fazu replikacijskog ciklusa virusa, odnosno sintezu virusne RNA.

Ključne riječi: benzimidazol, benzotiazol, heterocikli, antiproliferativna aktivnost, antioksidativna aktivnost, antiviralna aktivnost, antibakterijska aktivnost

ABSTRACT

Synthesis and structural characterization of novel benzimidazole and benzothiazole derivatives as potential antiproliferative agents with antioxidative activity

This thesis describes the synthesis of several classes of benzimidazoles and benzothiazoles in order to investigate their antiproliferative and antioxidant activity. By using multi-step linear synthesis of new benzazole conjugates, classical synthetic approaches as well as some modern synthetic methods, including synthesis in environmentally friendly solvents or microwave-assisted synthesis, were applied. New acrylonitrile derivatives of *N*-substituted benzazoles **32–71** and **77–107** were prepared by aldol condensation of the corresponding cyanomethylbenzazoles with benzaldehydes with a variable number of methoxy and hydroxy groups and 4-*N*,*N*-dimethylamino and 4-*N*,*N*-diethylamino groups. Benzimidazole derived Schiff bases **117–132** were prepared by condensation of *N*-substituted 2-aminobenzimidazoles **108–115** with corresponding 4-*N*,*N*-dimethylamino and 4-*N*,*N*-diethylamino-substituted benzaldehydes. Iminocoumarin and coumarine derivatives **134–149** and amidino-substituted coumarine derivatives **164–175** obtained by cyclocondensation of substituted 2-hydroxybenzaldehydes with corresponding 2-cyanomethylbenzimidazoles as well as from coumarine aldehydes with corresponding 4-amidino substituted 1,2-phenylenediamines using *p*-benzoquinone as an oxidant. Amidino-substituted benzimidazoles x-y were synthesized within the condensation of 5-substituted salicylaldehydes with corresponding 4-amidino substituted 1,2-phenylenediamines. Amidino-substituted with benzothiazoles **179–181** i **216–227** were prepared by condensation of differently substituted 2-hydroxybenzaldehydes and corresponding zwitter ions in glacial acetic acid. Methoxy-substituted carboxamides **235–262** were prepared by condensation of benzoyl chlorides with *N*-substituted 2-aminobenzimidazole derivatives. Hydroxy-substituted amide derivatives of *N*-benzimidazole **263–268** and **280–286** obtained by removing protective methoxy groups, using BBr3 at low temperatures, and benzyl protective groups by catalytic hydrogenation with Pd/C in methanol. Amidino-substituted benzamide derivatives **287–293** were synthesized via acid-catalyzed Pinner reaction from the corresponding cyano-substituted starting precursors. Structures of all newly prepared compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy while some of them were additionally characterized by mass spectrometry.

All prepared compounds were tested for their antiproliferative activity *in vitro* on a number of human cancer cell lines as well as normal fibroblasts, while antioxidant activity *in vitro* was performed using spectroscopic DPPH, FRAP and ABTS methods. Amidino-substituted derivatives **164–175** and **179–181** were tested for antiviral activity *in vitro* on several virus strains, and some compounds have shown pronounced and selective activity against some viruses. Schiff base derived benzazoles **117–132** and amidino-substituted benzazoles **193–216** and **217–228** were tested for antibacterial activity against Gram-positive and Gram-negative bacteria. Results obtained from evaluation of biological activity and SAR studies, revealed that the antioxidant activity is affected by the number of methoxy and hydroxy groups on the phenyl ring as well as the substituent on the nitrogen atom of the benzimidazole nucleus. The strongest impact on the enhancement of antiproliferative activity was observed by 4-*N*,*N*-diethylamino group placed at the position 7 on coumarin ring and phenyl ring of acrylonitrile derivatives. The isobutyl substituent on the N atom of the benzimidazole core has the greatest influence on increasing the activity of the synthesized compounds. Some of the most active benzazole derivatives were additionally evaluated to study their mechanisms of biological action and it was confirmed that some of the acrylonitrile and iminocoumarin derivatives have proven to be tubuline polymerization inhibitors, while for amide derivatives the antioxidative activity was tested also in the cells. It has been proven that the most promising antiviral activity has been possessed by coumarine derived benzimidazole substituted with amidine group being inhibitor of an early step in the replication cycle of virus, respectively the synthesis of viral RNA.

Key words: benzimidazole, benzothiazole, heterocycles, antiproliferative activity, antioxidant activity, antiviral activity, antibacterial activity