Sveučilište u Zagrebu
FAKULTET KEMIJSKOG INŽENJERSTVA I TEHNOLOGIJE

Maja Karnaš

KUMARINSKI DERIVATI 1,2,4-TRIAZOLA – SINTEZA I BIOLOŠKO DJELOVANJE VEZANO ZA PRIMJENU U ZAŠTITI BILJA

DOKTORSKI RAD

Zagreb, 2024

University of Zagreb
FACULTY OF CHEMICAL ENGINEERING AND TECHNOLOGY

Maja Karnaš

COUMARIN DERIVATIVES OF 1,2,4-TRIAZOLE – SYNTHESIS AND BIOLOGICAL ACTIVITY RELATED TO PLANT PROTECTION APPLICATION

DOCTORAL THESIS

Zagreb, 2024

Sveučilište u Zagrebu
FAKULTET KEMIJSKOG INŽENJERSTVA I TEHNOLOGIJE

Maja Karnaš

KUMARINSKI DERIVATI 1,2,4-TRIAZOLA – SINTEZA I BIOLOŠKO DJELOVANJE VEZANO ZA PRIMJENU U ZAŠTITI BILJA

DOKTORSKI RAD

Mentori:
Prof. dr. sc. Vesna Rastija
Prof. dr. sc. Marijana Hranjec

Zagreb, 2024

University of Zagreb
FACULTY OF CHEMICAL ENGINEERING AND TECHNOLOGY

Maja Karnaš

COUMARIN DERIVATIVES OF 1,2,4-TRIAZOLE – SYNTHESIS AND BIOLOGICAL ACTIVITY RELATED TO PLANT PROTECTION APPLICATION

DOCTORAL THESIS

Supervisors:
PhD. Vesna Rastija, Full Professor
PhD. Marijana Hranjec, Full Professor

Zagreb, 2024

SAŽETAK

U okviru doktorskog rada sintetizirani su kumarinski 1,2,4-triazoli s mogućim višestrukim biološkim učincima vezanim za primjenu u zaštiti bilja. Sinteza je provedena kao *one-pot* reakcija kumarinskih hidrazida (**1** – **3**) i različito supstituiranih izotiocijanata u niskotemperaturnom eutektičkom otapalu kolin-klorid : urea (molni omjer 1 : 2), miješanjem na magnetskoj miješalici pri 80 °C. Rezultat su tri serije spojeva: prva, u kojoj je 1,2,4-triazolni prsten povezan -CH_2- poveznicom na položaju 4, a hidroksilna je skupina na položaju 7 kumarina (**1a** – **k**), te druga serija derivata s 1,2,4-triazolnim prstenom povezanim eterskom poveznicom na položaju 7 kumarina i metilnom skupinom na položaju 4 kumarina (**2a** – **o**). Treća, dodatna serija derivata od druge se razlikuje po nedostatku metilne skupine na položaju 4 kumarina (**3a** – **h**). Korištenje niskotemperaturnog eutektičkog otapala omogućilo je provođenje reakcije u jednom stupnju, bez primjene katalizatora i organskih otapala štetnih za okoliš. Zbog pojednostavljene izolacije i pročišćavanja produkata, skraćeno je vrijeme trajanja postupka u odnosu na konvencionalnu i druge objavljene metode. Derivati kumarinskih 1,2,4-triazola su dobiveni u prinosima od 25 do 91 %, a njihove su strukture potvrđene masenom spektrometrijom te ^{1}H i ^{13}C NMR spektroskopijom. Svim priređenim spojevima računalno je procijenjena sličnost s pesticidima te toksičnost za čovjeka i okoliš. Utvrđeno je kako većina spojeva ima povoljan toksikološki profil u odnosu na komercijalne triazolne pesticide. Antifungalno djelovanje *in vitro* ispitano je na četiri fitopatogene gljive, a antibakterijsko djelovanje na dvije fitopatogene i dvije zemljišno-korisne bakterije. Kumarinski 1,2,4-triazoli uspješno su inhibirali rast micelija gljiva, s izraženim antifungalnim djelovanjem druge serije derivata na *Sclerotiniu sclerotiorum* i *Fusarium oxysporum*. Sintetizirani spojevi nisu imali antibakterijski učinak ni na patogene ni na zemljišno-korisne bakterije. Na temelju rezultata antifungalnog djelovanja provedena je analiza kvantitativnog odnosa strukture i aktivnosti (QSAR) spojeva. Model za djelovanje na *S. sclerotiorum* razvijen je pomoću tri deskriptora (*nR=Ct*, *RDF095m* i *HATS1e*) i može objasniti 79 % inhibitornog djelovanja kumarinskih 1,2,4-triazola. Model za djelovanje na *F. oxysporum* razvijen je pomoću četiri deskriptora (*R4u+*, *nAROR*, *RDF080e* i *Mor11u*), a može objasniti 77 % inhibitornog djelovanja spojeva. Pouzdanost razvijenih modela potvrđena je metodama unutarnje i vanjske procjene valjanosti. Potencijalni mehanizam antifungalnog djelovanja istražen je metodom molekulskog uklapanja spojeva u enzime važne za rast micelija (sterol 14α-demetilazu, hitinaze A i B, *N*-miristoil transferazu) te enzime potrebne za razaranje stanične stijenke domaćina (proteinazu K, endoglukanazu I te endopoligalakturonaze I i II). Spojevi su se dobro uklopili u aktivna mjesta

demetilaze, hitinaza A i B, proteinaze K te endopoligalakturonaza I i II. Unatoč dobrim energijama uklapanja, spojevi nisu uspjeli ostvariti interakcije u aktivnim mjestima *N*-miristoiltransferaze i endoglukanaze. Iz dobivenih rezultata može se zaključiti kako su derivati druge serije kumarinskih triazola potencijalni inhibitori demetilaze, obje hitinaze ili proteinaze K, dok su derivati prve serije spojeva inhibitori endopoligalakturonaza. Spoj **2j** ističe se svojim antifungalnim djelovanjem i procjenjenom niskom toksičnosti, što ga čini dobrim kandidatom za razvoj novog aktivnog sastojka sredstava za zaštitu bilja.

Ključne riječi: kumarinski 1,2,4-triazoli; antibakterijsko djelovanje; antifungalno djelovanje; kvantitativni odnos strukture i aktivnosti; molekulsko uklapanje; procjena toksičnosti; zaštita bilja

SUMMARY

In this doctoral thesis, coumarin 1,2,4-triazoles with potential multiple biological effects related to application in plant protection were synthesized. The synthesis was carried out as a one-pot reaction of coumarin hydrazides (**1** – **3**) and differently substituted isothiocyanates in a deep eutectic solvent, choline chloride : urea (molar ratio 1 : 2), by stirring with a magnetic stirrer at 80 °C. The result is three series of compounds: the first in which the 1,2,4-triazole ring is connected by a -CH_2- linker at position 4 and the hydroxyl group is at position 7 of the coumarin (**1a** – **k**), and the second series of derivatives with a 1,2,4-triazole ring connected by an ether linker at position 7 of the coumarin and a methyl group at position 4 of the coumarin (**2a** – **o**). The third, additional series of derivatives, differs from the second by the absence of a methyl group at position 4 of coumarin (**3a** – **h**). The use of a deep eutectic solvent enabled to carry out the reaction in one step without the need to use catalysts and environmentally harmful organic solvents. The simplified isolation and purification of the products shortened the duration of the process compared to conventional and other published methods. The derivatives of coumarin 1,2,4-triazoles were obtained in yields of 25 to 91 %, and their structures were confirmed by mass spectrometry and ^{1}H and ^{13}C NMR spectroscopy. All prepared compounds were evaluated *in silico* for their similarity to pesticides and their toxicity to humans and the environment. Most of the compounds were found to have a favorable toxicological profile compared to commercial triazole pesticides. The antifungal activity was tested *in vitro* on four phytopathogenic fungi, and the antibacterial activity on two phytopathogenic and two soil-beneficial bacteria. Coumarin 1,2,4-triazoles successfully inhibited the growth of fungal mycelia, with the second series of derivatives showing pronounced antifungal activity on *Sclerotinia sclerotiorum* and *Fusarium oxysporum*. The synthesized compounds did not show antibacterial activity, neither on the pathogens nor on the soil-beneficial bacteria. Based on the results of antifungal activity, a quantitative structure-activity relationship (QSAR) analysis of the compounds was performed. The model for the effect on *S. sclerotiorum* was developed using three descriptors (*nR=Ct*, *RDF095m* and *HATS1e*) and can explain 79 % of the inhibitory effect of coumarin 1,2,4-triazoles. The model for the effect on *F. oxysporum* was developed using four descriptors (*R4u+*, *nAROR*, *RDF080e* and *Mor11u*) and can explain 77 % of the inhibitory effect of the compounds. The reliability of the developed models was confirmed by internal and external validation methods. The potential mechanism of antifungal activity was investigated by means of molecular docking of the compounds to the enzymes important for mycelial growth (sterol 14α-demethylase, chitinases A and B, *N*-myristoyltransferase) and

enzymes necessary for host cell wall destruction (proteinase K, endoglucanase I and endopolygalacturonase I and II). The compounds fit well into the active sites of demethylase, chitinases A and B, proteinase K, and endopolygalacturonase I and II. Despite good fitting energies, the compounds failed to achieve interactions in the active sites of *N*-myristoyltransferase and endoglucanase. From the obtained results, it can be concluded that the derivatives of the second series of coumarin triazoles are potential inhibitors of demethylase, chitinases or proteinase K, while the derivatives of the first series of compounds are inhibitors of endopolygalacturonases. Compound **2j** stands out for its antifungal activity and its estimated low toxicity, which emphasized it as a good candidate for the development of a new active ingredient for plant protection products.

Keywords: coumarin 1,2,4-triazoles; antibacterial activity; antifungal activity; quantitative structure-activity relationship; molecular docking; toxicity evaluation; plant protection