Sveučilište u Zagrebu
FAKULTET KEMIJSKOG INŽENJERSTVA I TEHNOLOGIJE

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DJELOVANJE KUMARINSKIH DERIVATA 1,2,4-TRIAZOLA NA FITOPATOGENE KUKCE I NECILJANE ORGANIZME

DOKTORSKI RAD

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Sažetak

U ovom radu ispitana je insekticidna djelotvornost 33 hibrida kumarina i 1,2,4-triazola prema fitopatogenim kukcima te njihov utjecaj na odabrane neciljane organizme u standardiziranim *in vivo* pokusima. *In vivo* biološko djelovanje spojeva provedeno je na tri vrste fitopatogenih kukaca; repičin sjajnik (*Brassicogethes aeneus* (Fabricius 1775)), veliki voskov moljac (*Galleria mellonella* L.), voćna mušica (*Drosophila melanogaster*) te pet vrsta korisnih organizama u zaštiti bilja; entomopatogene nematode (*Steinernema feltiae*, *Heterorhabditis bacteriophora*), božja ovčica (*Adalia bipunctata.*), medonosna pčela (*Apis mellifera*) i grabežljiva stjenica (*Orius laevigatus*). Na repičinom sjajniku (*Brassicogethes aeneus*) svi spojevi postigli su potpuni mortalitet do trećeg dana, od kojih je najdjelotvornii bio spoj koji sadrži benzilnu skupinu na položaju N-4 triazolnog prstena (**2c**, 100,00 % mortaliteta) te spoj bez supstituenta na istom položaju (**2o,** 100,00 % mortaliteta). Iako je veći broj spojeva osmi dan tretmana pokazalo značajno insekticidno djelovanje na *Drosophila melanogaster*, derivat 4-metilkumarina s 3-bromofenilnom grupom na N-4 položaju triazolnog prstena (**2f**) istaknuo se visokim mortalitetom već četvrti dan (86,36 %). Na *Galleria mellonella* većina spojeva pokaza la je odgođeno djelovanje (niski udjeli kukuljenja trećega dana), s kasnijim snažnim učinkom kod više derivata (**1j**, **2c**, **2n**, **2a**, **3a**, **2i** ≥ 90 % mortaliteta do osmoga dana). Najdjelotvorniji spojevi su derivati 4-metilkumarina s fenilnom (**2c**, 96,67 % kukuljica) i klorofenilnom (**2n**, 96,67 % kukuljica) skupinom na N-4 položaju triazolnog prstena. Među neciljanim organizmima, *Orius laevigatus* bio je vrlo osjetljiv (za većinu spojeva ≥ 90 % mortaliteta do drugoga dana), što upućuje na potrebu oprezne primjene u sustavima biološke zaštite. Entomopatogene nematode pokazale su izraženu međuvrsnu različitost, pri čemu je veći broj spojeva pokazao visok postotak mortaliteta protiv *Steinernema feltiae* (**3f**, **2g**, **1k**, **2l** od 83 do 100,00 % mortaliteta), u usporedbi s otpornijom *Heterorhabditis bacteriophora*. Mortalitet niti jednog ispitanog spoja na *Adalia bipunctata* se statistički značajno ne razlikuje od standardnog insekticida ($p < 0,05$). Kod *Apis mellifera* uočeno je sporo djelovanje s najvećim učincima tek do desetoga dana uz najviši mortalitet kod spojeva **2b** (84,62 %) i **2c** (84,62 %). Kombinirajući ciljani učinak i očuvanje neciljanih organizama, istaknula su se tri kandidata: **1b, 2i** i **2n**. Svi spojevi pokazali su statistički značajno slabiju inhibiciju kolinesteraza u odnosu na standardni inhibitor donepezil. Razvijeni su modeli kvantitativnog odnosa strukture i aktivnosti (QSAR) za insekticidno djelovanje protiv *Brassicogethes aeneus* te *Drosophila melanogaster*. Model za insekticidno djelovanje na *B. aeneus* izračunat je metodom višestruke linearne regresije, dok je model za insekticidno djelovanje na *D. melanogaster* izračunat

metodom umjetnih neuronskih mreža. Molekulsko uklapanje kumarinskih 1,2,4-triazola provedeno je na acetilkolinesterazi, butirilkolinesterazi, kristaliziranom kompleksu glutamatom aktiviranog kloridnog kanala i Fab-fragment protutijela (GluClcryst-Fab kompleks), dopaminskom transporteru *D. melanogaster* (dDAT) i kimernom acetilkolinskom vezivnom proteinu (Ac-AChBP). Rezultati molekulskog uklapanja ukazuju kako se najdjelotvorniji spoj najvjerojatnije veže na glutamatom aktivirani kloridni kanal, uzrokujući pojaćani protok kloridnih iona, hiperpolarizaciju staničnih membrana i smrt kukca.

Rezultati izdvajaju nekoliko strukturnih motiva s potencijalom za razvoj selektivnih insekticida koji osiguravaju snažno suzbijanje ciljnih štetnika uz smanjeni rizik za ključne korisne organizme.

Ključne riječi: kumarinski 1,2,4-triazoli; zaštita bilja; insekticidno djelovanje; fitopatogeni kukci; neciljani organizmi; inhibicija kolinesteraza; kvantitativni odnos strukture i aktivnosti; procjena toksičnosti; molekulsko uklapanje

Summary

In this study, the insecticidal efficacy of 33 coumarin-1,2,4-triazole hybrids was evaluated against phytopathogenic insects, as well as their impact on selected non-target organisms in standardized *in vivo* assays. The *in vivo* activity of the compounds was assessed on three phytopathogenic insect species: pollen beetle (*Brassicogethes aeneus* (Fabricius 1775)), greater wax moth (*Galleria mellonella* L.), and fruit fly (*Drosophila melanogaster*), along with five beneficial organisms relevant for crop protection: entomopathogenic nematodes (*Steinernema feltiae*, *Heterorhabditis bacteriophora*), ladybird (*Adalia bipunctata*), honey bee (*Apis mellifera*), and predatory bug (Orius sp.). In *B. aeneus*, all compounds achieved complete mortality by the third day of the treatment, with the most effective being the derivative carrying a benzyl substituent at the N-4 position of the triazole ring (**2c**, 100,00 % mortality) and the unsubstituted analogue (**2o**, 100,00 % mortality). Although a greater number of compounds showed significant insecticidal effects on *D. melanogaster* by the eighth day of the treatment, the 4-methylcoumarin derivative with a 3-bromophenyl substituent at the N-4 position of the triazole ring (**2f**) stood out with high mortality already on the fourth day of the treatment (86,36 %). In *G. mellonella*, most compounds exhibited delayed effects (low pupation rates on third day of the treatment), followed by pronounced activity of several derivatives (**1j**, **2c**, **2n**, **2a**, **3a**, **2i**; ≥ 90,00 % mortality by eight day of the treatment). The most effective compounds were 4-methylcoumarin derivatives with phenyl (**2c**, 96,67 % pupation) and chlorophenyl (**2n**, 96,67 % pupation) groups at the N-4 triazole position. Among non-target organisms, *Orius laevigatus* proved to be highly sensitive (≥ 90,00 % mortality for most compounds by second day of the treatment), highlighting the need for caution in biological control systems. Entomopathogenic nematodes displayed marked interspecific variability, with higher susceptibility of *S. feltiae* (**3f**, **2g**, **1k**, **2l**; 83 - 100,00 % mortality) compared to the more resistant *H. bacteriophora*. In *Adalia bipunctata*, the mortality of none of the tested compounds differed significantly from the standard insecticide ($p < 0,05$). In *A. mellifera*, slow activity was observed, with the strongest effects only by tenth day of the treatment, notably for compounds **2b** (84,62 %) and **2c** (84,62 %). Balancing targeted efficacy with preservation of beneficial species, three promising candidates were identified: **1b**, **2i**, and **2n**. All compounds showed significantly weaker cholinesterase inhibition compared to the standard inhibitor donepezil. Quantitative structure-activity relationship (QSAR) models were developed for insecticidal activity against *B. aeneus* (multiple linear regression) and *D. melanogaster* (artificial neural networks). Molecular docking

of coumarin-1,2,4-triazoles was performed on acetylcholinesterase, butyrylcholinesterase, the crystallized glutamate-gated chloride channel complexed with a Fab fragment (GluClcryst-Fab), the *D. melanogaster* dopamine transporter (dDAT), and the chimeric acetylcholine-binding protein (Ac-AChBP). A molecular docking study indicated that the most active compound probably binds to glutamate-gated chloride channels, causing an increased flow of chloride ions, hyperpolarisation of cell membranes, and death of the insect.

The results highlight several structural motifs with potential for the development of selective insecticides that ensure strong suppression of target pests while reducing the risk to key beneficial organisms.

Keywords: coumarin 1,2,4-triazoles; crop protection; insecticidal activity; phytopathogenic insects; non-target organisms; cholinesterase inhibition; quantitative structure-activity relationship; toxicity assessment; molecular docking