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Exclusion of antimicrobial activity of some analgesic active imidazo[2,1-c][1,2,4]triazines

Imidazo[2,1-c]triazines reported herein contain in their chemical structure similar features (potential pharmacophore formations: the phenyl ring, the additional carbonyl group as the potential acceptor centre of hydrogen bond) to many morphine-like analgesics such as benzitramide, fentanyl, petidine and selective ligand of δ-opioid receptors (SNC-80). These similar features according to pharmacophore model introduced by Beckett with its further modifications can play an important role in expressing pharmacological activity, especially the analgesic action. The presence of carbonyl group in the structure of the obtained compounds can probably play a supporting role in binding with receptor due to the high negative potential present on the oxygen atom. On the other hand, the obtained imidazo[2,1-c]triazines have no basic nitrogen atom. The lack of this atom could play the role in the receptor activation stage and was also observed in the first potent naturally occurring non-nitrogenous KOR selective agonist – salvinorin A, the main active ingredient of *Salvia divinorum*, which has no action at the 5-HT_2 receptors, the principle molecular target responsible for the actions of classical hallucinogens (2).

The potential pharmacological activity of reported imidazotriazoles was confirmed based on experimental behavioral tests conducted on male Albino-Swiss mice (17–30 g) in the Department of Pharmacodynamics, Medical University of Lublin. The synthesized compounds possessing significant antinociceptive activity and an opioid-like mechanism of their analgesic activity as the result of the writhing and hot plate tests indicated on the central nervous system (CNS) of the laboratory animals correlated with relatively low acute toxicity (LD_{50} values in range from 1100 to over 2000 $mg \cdot kg^{-1}$ b.w. via i.p.) (6) were tested to exclude their potential antimicrobial (antibacterial and antifungal) activity resulted from the literature data (5).

The following compounds obtained due to the reaction of 1-arylimidazolidin-2-one hydrazones by cyclization reaction with ethyl oxalate were tested in relation to bacterial, fungal and moulds strains:

1. 8-phenyl-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine;
2. 8-(2-methylphenyl)-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine.
3. 8-(4-methylphenyl)-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine.
4. 8-(2-methoxyphenyl)-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine.
5. 8-(4-methoxyphenyl)-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine.
6. 8-(3-chlorophenyl)-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine.
7. 8-(4-chlorophenyl)-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine.
8. 8-(3,4-dichlorophenyl)-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine.

The structure of the above compounds was confirmed by elemental analysis and spectral data: nuclear magnetic resonance (^1H NMR, ^{13}C NMR) and mass spectra. Their purity was tested by means

of chromatography. These compounds were characterized by solubility in dimethylformamide and dimethylsulfoxide. These compounds showed antinociceptive activity in animal behavioral tests and relatively low acute toxicity (LD_{50} value in range from 1100 to over 2000 mg kg^{-1} b.w. via i.p.) (6).

MATERIAL AND METHODS

Assay of antimicrobial activity *in vitro*. The synthesized compounds were tested for their antimicrobial (antibacterial and antifungal) activities by disc-diffusion method by Kirby-Bauer, using Mueller-Hinton medium for bacteria and the same medium with 4% glucose for fungi. The tested microorganisms were isolated from clinical specimens of the Laboratory of Medical Microbiology Department, Medical University of Lublin. The assayed collection included 54 strains of Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus agalactiae*), 52 strains of Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus spp., Klebsiella pneumoniae, Enterobacter aerogenes*), 6 strains of yeast-like fungi (*Candida albicans*), 3 strains of moulds (*Aspergillus spp.*).

Table. Microorganism cultures used to microbiological screening

Group	Strain	Number of strains
Gram-positive bacteria	*Staphylococcus aureus*	21
	Staphylococcus epidermidis	15
	Streptococcus pyogenes	12
	Streptococcus agalactiae	6
Gram-negative bacteria	*Escherichia coli*	16
	Pseudomonas aeruginosa	12
	Proteus spp.	10
	Klebsiella pneumoniae	8
	Enterobacter aerogenes	6
Yeast-like fungi	*Candida albicans*	6
Moulds	*Aspergillus spp.*	3

In the disc-diffusion method, sterile paper disc (ϕ 5 mm) impregnated with dissolved in dimethylsulfoxide (DMSO) compound at concentrations of 100 mg ml^{-1} and 200 mg ml^{-1} were used. Discs containing DMSO were used as control. The microorganisms cultures were spread over the following appropriate media: Mueller-Hinton agar for *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus agalactiae, Escherichia coli, Pseudomonas aeruginosa, Proteus spp., Klebsiella pneumoniae, Enterobacter aerogenes*, and Saburoud agar for the yeast-like fungi (*Candida albicans*) and for the moulds (*Aspergillus spp.*) in Petri dishes. Then, the paper discs impregnated with the solutions of the compound tested were placed on the surface of the media inoculated with the microorganism. The plates were incubated at 35°/24 h for the microorganisms cultures. After incubation, the zones of growth inhibition around the discs were observed indicating that the examined compound inhibits the growth of microorganism (1, 3, 4).

RESULTS AND DISCUSSION

In connection with significant activity on the central nervous system of the above mentioned compounds, it seemed worthwhile to carry out microbiological screening (antibacterial and antifungal) to exclude their potential antimicrobial activity.

Antibacterial and antifungal activities were tested in relation to 106 strains of bacteria, 6 strains of yeast-like fungi and 3 strains of moulds. It can be concluded from microbiological screening tests that eight compounds in the examined concentrations (100 mg ml^{-1} and 200 mg ml^{-1}) had no influence on the growth of microorganisms tested. The microbiological screening tests afforded to limit the possible biological spectrum of activity of tested compounds.

Lack of antimicrobial activity of tested compounds seemed to be profitable particularly in the case of compounds possessing effect on the central nervous system, i.e. showing significant antinociceptive activity.

REFERENCES

1. Dzierżanowska D.: Antybiotykoterapia praktyczna. α-medica press, Bielsko Biała 1994.
2. Kaczor A., Matosiuk D.: Non-peptide opioid receptor ligands – recent advances. Part 1. Agonists. Curr. Med. Chem., 9, 17, 1567, 2002.
3. Kędzia W. B.: Diagnostyka mikrobiologiczna w medycynie. PZWL, Warszawa 1990.
4. National Committee for Clinical Laboratory Standards, Approved Standards, NCCLS Document M7 – A3, Villanova, Italy, 20, 2, 2002.
5. Novinson T. et al.: Synthesis and antimicrobial activity of some novel heterocycles. Azolo--as-triazines. J. Med. Chem., 19, 4, 517, 1976.
6. Sztanke K. et al.: Antinociceptive activity of new imidazolidine carbonyl derivatives. Part 4. Synthesis and pharmacological activity of 8-aryl-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazines. Eur. J. Med. Chem., 2004 (in press).

SUMMARY

The purpose of this study was to exclude the potential antimicrobial activity of certain analgesic active imidazo[2,1-c][1,2,4]triazines. These compounds contain in their chemical structure potential pharmacophore formations and features similar to those present in morphine-like analgesics and opioid receptor agonists containing no basic nitrogen atom. These compounds showed significant antinociceptive activity on central nervous system of tested animals, correlated with relatively low acute toxicity values (LD_{50} in range from 1100 to above 2000 mg kg^{-1} b.w. via i. p.). Microbiological tests were conducted on 106 strains of bacteria, 6 strains of yeast-like fungi and 3 strains of moulds. The examined imidazo[2,1-c][1,2,4]triazines in concentrations of 100 μg ml^{-1} and 200 μg ml^{-1} had no influence on the growth of microorganisms tested. Lack of this influence can be profitable in the case of analgesic active compounds.

Wykluczenie aktywności przeciwdrobnoustrojowej aktywnych przeciwbólowo imidazo[2,1-c][1,2,4]triazyn

Celem pracy jest wykluczenie potencjalnej aktywności mikrobiologicznej aktywnych przeciwbólowo imidazo[2,1-c][1,2.4]triazyn. W strukturze otrzymanych związków występują potencjalne ugrupowania farmakoforowe analogiczne do występujących w narkotycznych lekach

przeciwbólowych, a także agonistach receptora opioidowego pozbawionych zasadowego atomu azotu. Związki te wykazały znaczącą aktywność antynocyceptywną w ośrodkowym układzie nerwowym i niską toksyczność. Testy aktywności przeciwdrobnoustrojowej przeprowadzono na 106 szczepach bakteryjnych, 6 szczepach drożdżaków i 3 szczepach pleśniaków. Przebadane imidazo[2,1-c]triazyny w stężeniach 100 $\mu g\ ml^{-1}$ i 200 $\mu g\ ml^{-1}$ nie wykazały wpływu na wzrost testowanych drobnoustrojów. Brak tej aktywności wydaje się korzystny w przypadku związków odznaczających się aktywnością przeciwbólową.