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Department of Synthesis and Technology of Drugs, Department of General Chemistry
Department of Medical Microbiology, Medical University of Lublin

KRZYSZTOF SZTANKE, KAZIMIERZ PASTERNAK,
MAŁGORZATA SZTANKE, ANNA SIDOR-WÓJTOWICZ,
JANINA TRUCHLIŃSKA

Studies on the activity of some imidazo[2,1-c][1,2,4]triazine derivatives

Imidazo[2,1-c][1,2,4]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 d-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 a role in the receptor activation stage and was also observed in the first potent naturally occurring nonnitrogenous 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 for opioids side effects (2).

Previous studies concerning the synthesis and biological activity of imidazo[2,1-c][1,2,4]triazin-4(4*H*)-ones (7, 8, 9) carried out in the Department of Synthesis and Technology of Drugs disclosed some compounds with various aryl substituents at position 8, and with benzyl, substituted benzyl, methoxycarbonylmethyl substituents and 3-oxo group at position 3 to reveal a significant antinociceptive activity on the central nervous system in behavioral animal tests, and a low acute toxicity (LD_{50} ranging from over 1100 to over 2000 mg · kg^{-1} i.p.). On the other hand, the definite derivative of imidazo[2,1-c][1,2,4]triazine, viz. that with 4-chlorophenyl substituent at position 8 and with hydroxycarbonylmethyl substituent at position 3, showed a significant activity against all Gram-negative bacterial strains tested (10). Therefore, the newly obtained imidazo[2,1-c][1,2,4]triazine derivatives were tested to exclude or confirm their potential antimicrobial (antibacterial and antifungal) activity deduced from the literature data (6, 10).

The following compounds obtained due to the reaction of appropriate 1-arylimidazolidin-2-one hydrazones by condensation with diethyl oxalate (compounds I, II), diethyl isonitrosomalonate (compounds III, IV) and pyruvic acid (V) (9, 11, 12) were tested in relation to bacterial, fungal and moulds strains:

I. 8-(2-chlorophenyl)-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine;
II. 8-(2,3-dimethylphenyl)-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c][1,2,4]triazine;
III. ethyl 8-phenyl-6,7-dihydro-4-oxo-imidazo[2,1-c][1,2,4]triazine-3-formate;
IV. ethyl 8-(4-chlorophenyl)-6,7-dihydro-4-oxo-imidazo[2,1-c][1,2,4]triazine-3-formate;
V. 3-methyl-8-(2-methoxyphenyl)-7,8-dihydro-6H-imidazo[2,1-c][1,2,4]triazin-4-one.

The structure of the above mentioned compounds was confirmed by elemental analysis and spectral data: nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) and mass spectra (MS). The purity of all the compounds synthesized was checked by TLC. Thin-layer chromatography was carried out on commercial Merck SiO_2 60 F_{254} plates having fluorescence indicator; the spots were visualized with UV light λ = 254 nm. These compounds were characterized by solubility in dimethylformamide and dimethylsulfoxide.

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 1).

Table 1. Microorganism cultures used for 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 discs (ϕ 5mm) impregnated with dissolved in dimethylsulfoxide (DMSO) compound at concentrations of 100 mg/ml and 200 mg/ml 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, 5).

Prediction of potential biological activity. Potential biological properties of the investigated imidazo[2,1-c][1,2,4]triazines were evaluated using computer programme PASS (Prediction of Activity Spectra for Substances); http://www.ibmh.msk.su/service.htm. This programme predicts the biological activity spectrum for a compound on the basis of its structural formula. It estimates the probability of the molecule to be active (Pa) and inactive (Pi) for each type of activity from the biological activity spectrum. The most probable biological activities predicted by PASS programme are shown in Table 2.

Table 2. The most probable (Pa>70%) types of biological activity of tested imidazo[2,1-c][1,2,4]triazines predicted by PASS programme

Comp.	Pa	Pi	Activity	Pa	Pi	Comp.
I	95.1	0.1	GABA A receptor antagonist	94.4	0.1	II
	92.1	0.2	Antineurogenic pain	91.3	0.2	
	83.0	0.4	Cyclic GMP phosphodiesterase inhibitor	79.9	0.5	
	78.9	0.3	GABA receptor antagonist	76.8	0.3	
	73.0	1.2	Analgesic, non-opioid			
III	96.3	0.1	GABA A receptor antagonist	95.6	0.1	IV
	82.7	0.2	GABA receptor antagonist	81.6	0.2	
	80.9	0.5	Cyclic GMP phosphodiesterase inhibitor	79.6	0.5	
V	96.1	0.1	GABA A receptor antagonist			
	82.4	0.4	Cyclic GMP phosphodiesterase inhibitor			
	81.0	0.2	GABA receptor antagonist			

RESULTS AND DISCUSSION

In connection with a significant effect on the central nervous system of structural analogues of the above mentioned compounds, it seemed worthwhile to carry out microbiological screening (antibacterial and antifungal) to exclude or confirm their potential antimicrobial activity, deduced from the previous studies and from the literature data (6, 10).

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 all tested compounds in the examined concentrations (100 μg/ml and 200 μg/ml) 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 the tested compounds seemed to be profitable, particularly in the case of compounds possessing effect on the central nervous system, e.i. showing antinociceptive activity. This suggests that when taken orally they would have no negative effect on the human digestive tract microflora.

Based on PASS programme for all compounds (I-V) the most probable seemed to be GABA A receptor antagonistic activity (Pa about 95.5%, Pi 0.1%). Also, the inhibition of cyclic guanosine monophosphate (cGMP) was predicted as highly possible (Pa ranging 79.6–83.0%) for them. From the literature data it follows that substituted purinones as analogues of cGMP are potent phosphodiesterase (PDE) inhibitors *in vitro*. Besides, imidazo[5,1-f][1,2,4]triazin-4(3H)-ones are highly cGMP- PDE-5

selective and show IC_{50} values for PDE-3 and PDE-4 greater than 50 nM. In comparison to well known pyrazolo[4,3-d]pyrimidin-7-one phosphodiesterase inhibitors (for instance Sildenafil) the imidazotriazines reported in the literature indicate improved selectivity over PDE-1 and substantially improved PDE-5 inhibition (4). For compound I analgesic activity was found as highhly possible (Pa > 70%). It is noteworthy in this case that the estimated probability for the compound I i.e. 8-(2-chlorophenyl)-3,4-dioxo-2H,8H-6,7-dihydro-imidazo[2,1-c][1,2,4]triazine to be analgesic (73.0%) is nearing for a molecule of similar structure, having in position 8, 3-chlorophenyl substituent, instead of 2-chlorophenyl, i.e. 8-(3-chlorophenyl)-3,4-dioxo-2H,8H-6,7-dihydro-imidazo[2,1-c][1,2,4]triazine (82.2%), whose strong antinociception in doses 12.5-200 mg (0.00625–0.1 LD_{50}) and very weak acute toxicity (over 2000 mg kg^{-1}) in behavioral animal tests was confirmed (8). Both compounds I and II showed Pa > 50% in the categories of acute neurologic disorders treatment and neuroprotector. Additionally, compound I indicated Pa >50% in the categories of anticonvulsant, gout treatment, cannabinoid receptor antagonist, antiepileptic, excitatory amino acid antagonist and cyclic AMP phosphodiesterase inhibitor, while compound II in the categories of analgesic, antimigraine, cyclic AMP phosphodiesterase inhibitor and mediator release inhibitor. Both compounds II and III demonstrated Pa>50% in categories of anticonvulsant and lipoprotein lipase inhibitor. Additionally, compound III demonstrated Pa >50% in the category of antihypoxic and compound IV in the categories of benzodiazepine 1 receptor agonist and membrane permeability inhibitor. Compound V indicated Pa >50% in the categories of alpha 1d adrenoreceptor antagonist, tumour necrosis factor alpha release inhibitor, growth factor antagonist, 5-hydroxytryptamine 6 antagonist, calmodulin antagonist and renal vasodilator.

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SUMMARY

The purpose of this study was to exclude the potential antimicrobial activity of certain imidazo[2,1-c][1,2,4]triazines with an expected analgesic activity. 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 are the structural analogues of analgesic active imidazo [2,1c][1,2,4]triazines, possessing relatively low acute toxicity. 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 100mg/ml and 200mg/ml had no influence on the growth of microorganisms tested. Lack of this influence can be profitable in the case of analgesic active compounds. Also the most probable biological properties of investigated imidazo[2,1-c][1,2,4]triazines were evaluated using computer PASS programme.

Badanie aktywności pochodnych imidazo[2,1-c][1,2,4]triazyny

Celem pracy jest wykluczenie potencjalnej aktywności mikrobiologicznej pochodnych imidazo[2,1-c][1,2.4]triazyny o spodziewanej aktywności przeciwbólowej. 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 są strukturalnymi analogami aktywnych przeciwbólowo i nisko toksycznych imidazo[2,1-c][1,2,4]triazyn. Testy aktywności przeciwdrobnoustrojowej przeprowadzono na 106 szczepach bakteryjnych, 6 szczepach drożdżaków i 3 szczepach pleśniaków. Przebadane imidazo[2,1-c]triazyny w stężeniach 100 mg/ml i 200 mg/ml nie wykazały wpływu na wzrost testowanych drobnoustrojów. Brak tej aktywności wydaje się korzystny w przypadku związków odznaczających się aktywnością przeciwbólową. Ponadto określono najbardziej prawdopodobne typy aktywności badanych imidazo[2,1-c][1,2,4]triazyn przy zastosowaniu programu komputerowego PASS.