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Antibacterial action of novel 8-aryl-4-imino-2,3,7,8-tetrahydroimidazo[2,1-c][1,2,4]triazin--3(6H)-one derivatives

The bicyclic imidazo[2,1-*c*][1,2,4]triazine scaffold is the structural element of many synthetic, biologically valuable compounds, possessing a broad activity spectrum. Some derivatives of this ring system have been reported in the patent literature as the Maillard reaction inhibitors, which are useful for the treatment and/or prevention of various diabetes complications such as coronary disease, periphery circulatory disorder, renal disease, cerebrovascular disorder, diabetic neurosis, retinities, articular sclerosis or the diseases caused by ageing such as senile cataract, atherosclerosis etc. (2). Beside this, various imidazo[2,1-*c*][1,2,4]triazine derivatives have been produced as bactericides (5), central nervous system stimulants, cardiovascular, analgesic and antitumour agents (1, 6, 8–10).

Also, our previous studies have identified the definite derivative of 2-[4-oxo-8-(4-chlorophenyl)-2H-3,4,6,7-tetrahydroimidazo[2,1-c][1,2,4]triazin-3-yl]acetic acid with a significant selective anti-Gram-negative antibacterial activity (7). Prompted by these results, it seemed worthwhile to evaluate the potential biological action of novel 8-aryl-4-imino-2,3,7,8-tetrahydroimidazo[2,1-c][1,2,4]triazin-3(6H)-one derivatives in order to broaden or limit their possible biological activity spectrum. Simple susceptibility screening test using the disc-diffusion method was used to confirm or exclude their potential antibacterial activities deduced from the literature search and from our previous studies (5, 7). The following compounds obtained in the reaction of appropriate 1-aryl-2-hydrazonoimidazolidines with ethyl oxamate were evaluated for their antibacterial activity:

I. 8-(3,4-dichlorophenyl)-4-imino-2,3,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3(6*H*)-one;
II. 8-(2,6-dichlorophenyl)-4-imino-2,3,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3(6*H*)-one.

Although these compounds are new, they were obtained according to the procedure reported in the previous paper (11). Their structures were confirmed by elemental analyses and spectral data: nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) and mass spectra (MS). The purity of the compounds synthesized was checked by thin-layer chromatography. TLC experiments were performed on commercial Merck SiO_2 60 F_{254} plates having fluorescence indicator. The spots were visualized with UV light $\lambda = 254$ nm. The examined compounds were characterized by solubility in dimethylformamide and dimethylsulfoxide.

MATERIALS AND METHODS

Assay of antibacterial activity *in vitro* 8-Aryl-4-imino-2,3,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3(6*H*)-ones of the type I–II were tested for their antibacterial activities using the disc-diffusion method by Kirby-Bauer. The majority of test microorganisms were obtained from clinical specimens of the Laboratory of Medical Microbiology Department, Medical University of Lublin. The assayed collection included 48 strains of the following Gram-positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and 16 strains of Gram-negative one – *Escherichia coli* (Table 1).

Table 1. Microorganism cultures used to antibacterial screening

Group	Strain	Number of strains
Gram-positive bacteria	*Staphylococcus aureus*	21
	Staphylococcus epidermidis	15
	Streptococcus pyogenes	12
Gram-negative bacteria	*Escherichia coli*	16

In the disc-diffusion method, sterile paper discs (ϕ 5mm) impregnated with dissolved in dimethyl sulfoxide compound at a concentration of 100 μg mL^{-1} were used. Discs containing DMSO were used as a solvent control. The microorganisms' cultures were spread over the Mueller-Hinton agar for the tested bacteria in Petri dishes. Then, the paper discs impregnated with the solutions of the compounds tested were placed on the surface of the media inoculated with the microorganisms. The plates were incubated at 35°C/24 h for the microorganisms' cultures. After incubation, the growth inhibition zones around the discs were observed indicating that the examined compound inhibited the growth of microorganisms (3, 4). Each assay in this experiment was repeated three times. Ampicillin at a concentration of 200 μg mL^{-1} was used as a standard drug. Results are presented in Table 2.

Table 2. Antibacterial activities of the tested 8-aryl-4-imino-2,3,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3(6*H*)-ones against the tested bacterial isolates using the disc-diffusion method

Comp.	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Streptococcus pyogenes*	*Escherichia coli*
I	++	+++	+++	+++
II	-	-	+++	+++
Standard	++	+	++	++

Results were interpreted in terms of the diameter of inhibition zone: (-): < 9 mm; (+): 10–15 mm; (++): 16–20 mm; (+++): > 20 mm

Standard: ampicillin at concentration of 200 μg mL^{-1}

RESULTS AND DISCUSSION

Susceptibility of Gram-positive and Gram-negative bacterial strains to newly obtained compounds were determined. The results are presented in Table 2. According to the data presented, the derivative I, bearing two lipophilic weak electron-withdrawing chlorine atoms at 3,4-positions of the phenyl ring, revealed a broad activity spectrum and simultaneously the highest potency. This compound was found to be effective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Escherichia coli*. Structurally similar compound II revealed lower activity spectrum than I. Thus, it has been proved that the replacement of 3,4-dichlorophenyl substituent in the 8-position of the heterobicycle for either 2,6-dichlorophenyl one (II) led to a complete loss of activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Simultaneously, this structural change did not influence the activity against *Streptococcus pyogenes* and *Escherichia coli*.

In conclusion, compound I at the examined concentration (100 μg mL^{-1}) inhibited the growth of all Gram-positive and Gram-negative bacterial strains tested. Moreover, this compound expressed antibacterial potency superior to that of ampicillin. Taking into account its significant antibacterial potency, the research in this field will be continued. It is likely to happen that its structural analogues should also be active.

CONCLUSIONS

1. The tested compounds (I–II) at the concentration of 100 $\mu g \cdot mL^{-1}$ had influence on the growth of the bacterial strains tested.
2. Compound I was the most effective of the series. It revealed antibacterial activity against all the tested strains of Gram-positive and Gram-negative bacteria. Moreover, its activity was superior to that of ampicillin.

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SUMMARY

The bicyclic imidazo-triazine scaffold is the structural element of many synthetic, biologically active compounds that display a wide spectrum of biological activity. Besides, it follows from the literature survey and previous studies that depending on the type of substituent certain derivatives of imidazo-triazine may also show antibacterial properties. The purpose of this study was to confirm or exclude the possible antibacterial activity of 8-aryl-4-imino-2,3,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3(6*H*)-ones. Microbiological tests were conducted on 48 Gram-positive and 16 Gram-negative bacterial strains. The studied compounds (I and II) at a concentration of 100 μg ·mL^{-1} were proved to be effective against microorganisms tested. Compound I revealed a broad activity spectrum and simultaneously the highest antibacterial potency. This compound in the tested concentration of 100 μg ·mL^{-1} was found to be effective against all the examined strains of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes* and *Escherichia coli.*

Aktywność przeciwbakteryjna nowych pochodnych 8-arylo-4-imino-2,3,7,8-tetrahydroimidazo [2,1-c][1,2,4]triazyno-3(6H)-onu

Bicykliczny układ imidazo-triazyny jest obecny w strukturze syntetycznych, biologicznie aktywnych związków wykazujących różnorodne działanie farmakologiczne. Ponadto z danych literatury wynika, że w zależności od podstawnika niektóre pochodne tego układu mogą wykazywać aktywność przeciwbakteryjną. Celem pracy było potwierdzenie lub wykluczenie potencjalnej aktywności przeciwbakteryjnej nowych pochodnych 8-arylo-4-imino-2,3,7,8-tetrahydroimidazo[2,1-*c*][1,2,4]triazyno-3(6*H*)-onu. Testy aktywności przeciwdrobnoustrojowej przeprowadzono na 48 szczepach bakterii Gram-dodatnich i 16 szczepach bakterii Gram-ujemnych. Przebadane związki w stężeniu 100 μg mL^{-1} wykazały aktywność przeciw badanym bakteriom. Związek I wykazał szerokie spektrum aktywności przeciwbakteryjnej. W badanym stężeniu hamował wzrost wszystkich testowanych szczepów *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes* oraz *Escherichia coli.*