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STUDIES ON THE SYNTHESIS OF NEW DERIVATIVES OF 8-ARYL-4-IMINO-2,3,7,8-TETRAHYDRO-IMIDAZO [2,1-*c*][1,2,4] TRIAZIN-3 (6*H*)-ONE WITH AN EXPECTED BIOLOGICAL ACTIVITY

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Abstract: Reaction of 1-aryl-2-hydrazono-imidazolidines with ethyl oxamate furnished novel derivatives of imidazo [2,1-*c*][1,2,4] triazine

Keywords: 8-aryl-4-imino-2,3,7,8-tetrahydro-imidazo [2,1-*c*][1,2,4] triazin-3 (6*H*)-ones, synthesis, antimicrobial screening; prediction of biological activity; PASS programme

Previously, all known imidazo [2,1-*c*][1,2,4] triazines have been prepared and tested as cardiovascular agents (1, 2), antimicrobial agents (3), herbicides (4), central nervous system stimulants (5), and Maillard reaction inhibitors (6), 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, articular sclerosis, retinitis or diseases caused by aging, such as senile cataract, atherosclerosis, etc., by inhibiting the Maillard reaction.

Previous studies concerning the synthesis of imidazo [2,1-*c*][1,2,4] triazin-4 (4*H*)-ones (9-11, 13) carried out in the Department of Synthesis and Technology of Drugs have disclosed some compounds with various aryl substituents at position 8, and with benzyl, substituted benzyl, methoxycarbonylmethyl and hydroxyl substituents 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} in the range 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 (12).

Taking into account the results of the former studies concerning the synthesis and biological action of structurally similar derivatives of the same heterocyclic ring system, which antinociceptive or antimicrobial action were confirmed, it seemed worthwhile synthesizing some new derivatives of 8-aryl-4-imino-2,3,7,8-tetrahydro-imidazo [2,1-*c*] [1,2,4] triazin-3 (6*H*)-one and to carry out their antimicrobial screening to confirm or exclude their potential antimicrobial activity, deduced from the previous studies and from the literature data (3, 12). The title compounds [**II a-e**] were obtained in the reaction of appropriate 1-aryl-2-hydrazono-imidazolidines (1-arylimidazolidin-2-one hydrazones) with ethyl oxamate (Scheme 1). The structures of the obtained compounds were supported by elemental analyses and spectral data (^{1}H NMR, IR).
The starting substrates **I a-e** were prepared according to the procedure reported in a previous paper (8).

EXPERIMENTAL

Chemistry

Chemicals (carbon disulfide, methyl iodide, hydrazine hydrate, ethyl oxamate) were purchased from Merck as „synthesis grade" and used without further purification. The melting points (m. p.) were determined on a Boetius apparatus and given uncorrected. Compounds **II a-e** were recrystallized from DMF. 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 a fluorescence indicator; the spots were visualized with UV light λ = 254 nm. PMR spectra were recorded on a Tesla BS-567A 100 MHz spectrometer in DMSO-d_6, with TMS as an internal standard. IR spectra were recorded in KBr using a Specord IR-75 spectrophotometer.

8-ARYL-4-IMINO-2,3,7,8-TETRAHYDRO-IMIDAZO [2,1-*c*][1,2,4] TRIAZIN-3 (6*H*) ONES [II a-e].

Method A (general procedure)

Ethyl oxamate (0.05 mole) was added to the suspension of appropriate 1-aryl-2-hydrazono-imi-

dazolidine hydroiodide (0.05 mole) in 80 cm^3 of n--butanol. The reaction mixture was stirred, and triethylamine (5 mL) was added. Then, the reaction was carried out under reflux for 4-6 h. During that time, the precipitation of a solid was observed. The crude product obtained after cooling was collected by filtration, washed with cold methanol, and finally purified by recrystallization from DMF.

Method B (general procedure)

Free base of 1-aryl-2-hydrazono-imidazolidine (0.05 mole) was dissolved in 60 mL of ethanol. 0.05 mole of ethyl oxamate was added and the mixture was heated under reflux for 7-9 h. The mixture was kept overnight in a fridge, the precipitate yielded was collected by filtration and was purified by recrystallization from DMF.

Physicochemical data of the synthesized compounds are collected in Table 1.

Microbiology

Assay of antimicrobial activity *in vitro*

The synthesized compounds were tested for their antimicrobial (antibacterial and antifungal) activities by the disc-diffusion method by Kirby-Bauer, using a Mueller-Hinton medium for bacteria and the same medium with 4 % glucose for fungi. The tested microorganisms were isolated from clinical specimens analysed in 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 2).

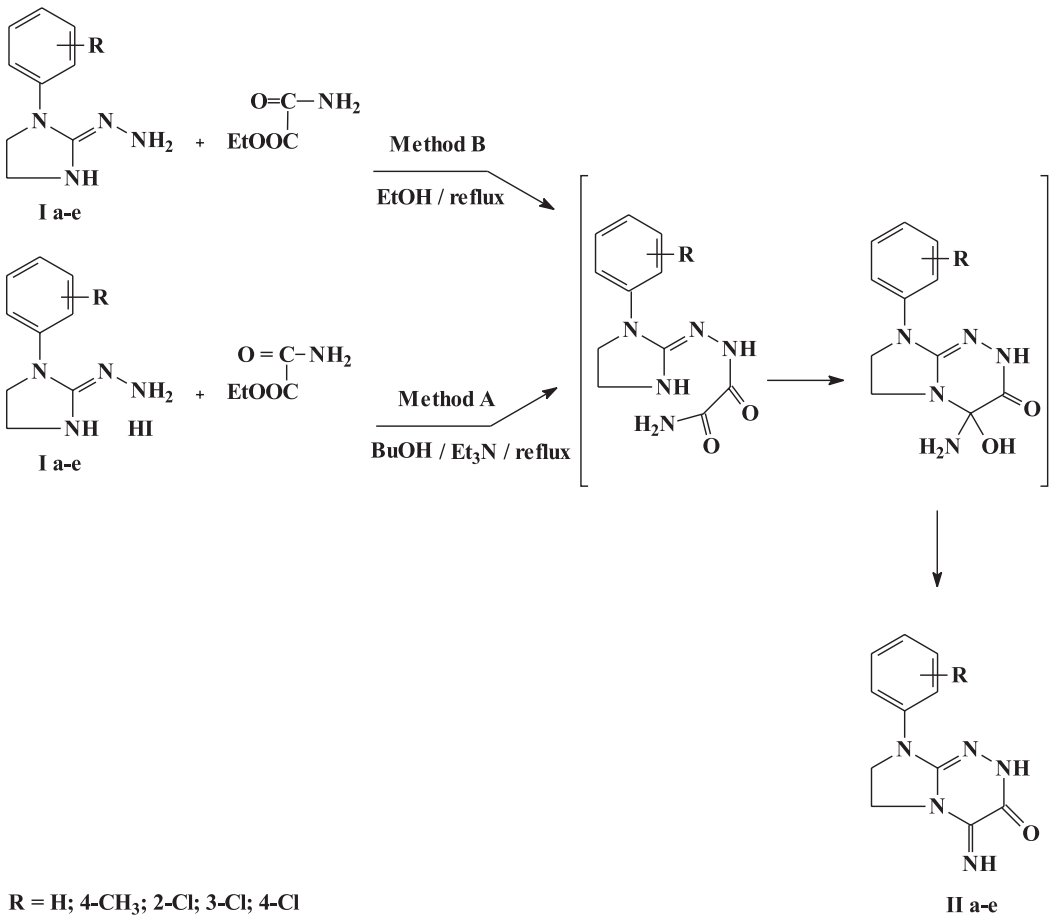
In the disc-diffusion method, sterile paper disc (ϕ 5mm) impregnated with a dissolved in dimethylsulfoxide (DMSO) compound at concentrations of 100 μg×mL^{-1} and 200 μg×mL^{-1} were used. Discs containing DMSO were used as control. The microorganism cultures were spread over the following appropriate media: Mueller-Hinton agar for *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus agalactiae, Escherichia coli, Pseudomonas aeruginosa, Proteus spp., Klebsiella pneumoniae, Enterobacter aerogenes*, and Sabouraud 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

Table 1. Physicochemical properties of 8-aryl-4-imino-2,3,7,8-tetrahydro-imidazo[2,1-*c*][1,2,4]triazin-3(6*H*)-ones

II	R	Formula Molec. mass	Yield (%) in method A	Yield (%) in method B	M.p. °C	Analyses calc. / found %C	%H	%Cl	%N	IR (KBr) cm^{-1}	PMR (ppm)
a	H	$C_{11}H_{11}N_5O$ 229.24	68	62	307-10	57.63 57.8	4.84 4.8	– –	30.55 30.4	1688 (C=O) 1577 (C=N)	4.2 (s,4H, $2CH_2$); 7.02-7.65 (m, 5H, $CH_{arom.}$); 7.89 (s, 1H, NH); 8.3 (s, 1H, NH)
b	4-CH_3	$C_{12}H_{13}N_5O$ 243.27	73	67	297-99	59.25 59.1	5.39 5.4	– –	28.79 28.7	1691 (C=O) 1582 (C=N)	2.27 (s, 3H, CH_3); 4.38 / 4.54 (*J=9.1 Hz, J' = 7.6 Hz*)(dd, 4H, $2CH_2$); 7.21 (d,2H, $CH_{arom.}$); 7.47 (d, 2H, $CH_{arom.}$); 7.68 (s, 1H, NH); 8.08 (s, 1H, NH)
c	2-Cl	$C_{11}H_{10}ClN_5O$ 263.68	65	64	215-18	50.11 49.9	3.82 3.8	13.45 13.5	26.56 26.6	1691 (C=O) 1581 (C=N)	3.96 / 4.17 (*J= 9 Hz, J' =7.6 Hz*)(dd, 4H, $2CH_2$); 7.3-7.61 (m, 4H, $CH_{arom.}$); 7.73 (s, 1H, NH); 8.13 (s, 1H, NH)
d	3-Cl	$C_{11}H_{10}ClN_5O$ 263.68	60	56	253-56	50.11 50.2	3.82 3.7	13.45 13.3	26.56 26.7	1690 (C=O) 1578 (C=N)	4.2 (s, 4H, $2CH_2$);7.08-7.80 (m, 4H, $CH_{arom.}$); 7.95 (s, 1H, NH); 8.36 (s, 1H, NH)
e	4-Cl	$C_{11}H_{10}ClN_5O$ 263.68	63	67	282-84	50.11 50.2	3.82 3.9	13.45 13.3	26.56 26.5	1694 (C=O) 1582 (C=N)	4.18 (s, 4H, $2CH_2$); 7.27 (d, 2H, $CH_{arom.}$); 7.53 (d, 2H, $CH_{arom.}$); 7.91 (s, 1H, NH); 8.31 (s, 1H, NH)

Table 2. 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



Scheme 1.

were placed on the surface of the media inoculated with the microorganism. The plates were incubated at 35°/24 h for the microorganism cultures. After incubation, the zones of growth inhibition around the discs were observed indicating that the examined compound inhibits the growth of microorganism (14).

RESULTS AND DISCUSSION

Chemistry

New derivatives of 8-aryl-4-imino-2,3,7,8-tetrahydro-imidazo [2,1-*c*][1,2,4] triazin-3 (6*H*)-one were obtained in the reaction of appropriate 1-aryl-

Table 3. The most probable types of biological activity of 8-aryl-4-imino-2,3,7,8-tetrahydro-imidazo[2,1-*c*][1,2,4]triazin-3(6*H*)-ones predicted by PASS program

Compound	Pa (%)	Pi (%)	Activity
II a	97.1	0.1	GABA A receptor antagonist
	94.4	0.1	Antineurogenic pain
	84.9	0.7	Analgesic, non-opioid
	83.9	0.2	GABA receptor antagonist
II b	96.0	0.1	GABA receptor antagonist
	92.3	0.2	Antineurogenic pain
	80.8	0.2	GABA receptor antagonist
	81.0	0.8	Analgesic, non-opioid
II c	96.0	0.1	GABA A receptor antagonist
	91.8	0.2	Antineurogenic pain
	81.2	0.2	GABA receptor antagonist
	74.8	1.0	Analgesic, non-opioid
II d	96.0	0.1	GABA A receptor antagonist
	91.1	0.2	Antineurogenic pain
	83.2	0.7	Analgesic, non-opioid
	81.6	0.2	GABA receptor antagonist
II e	96.4	0.1	GABA A receptor antagonis
	92.2	0.2	Antineurogenic pain
	82.5	0.2	GABA receptor antagonist
	82.9	0.7	algesic, non-opioid

-2-hydrazono-imidazolidines with ethyl oxamate. The above mentioned reaction can be carried out either by starting from 1-aryl-2-hydrazono-imidazolidine hydroiodide in the presence of triethylamine (method A), or from free base (method B) with comparable yields. The reactions were carried out by heating in alcoholic medium for 4-9 h. The reaction conditions were established experimentally. The course of reaction includes the formation of intermediate products (with concomitant liberation of a molecule of ethanol) and finally the reaction leads to formation of 8-aryl-2,3,7,8-tetrahydroimidazo [2,1-c][1,2,4] triazin-3 (6*H*)-one derivatives (with the liberation of a hydrogen oxide molecule) as is illustrated in Scheme 1. The scrutiny of ^{1}H NMR, IR spectra confirms that under the reaction conditions, the formation of bicyclic ring system (imidazo [2,1-*c*][1,2,4] triazine) is accompanied with the liberation of ethanol and a hydrogen oxide molecule. NMR spectral characteristics of the imidazo [2,1-*c*][1,2,4] triazines revealed in their ^{1}H NMR spectra the signals of H6 and H7 as two double doublet at ca. 4.2 ppm and 4.4 ppm with the coupling constans of $J \sim$ 9Hz and $J' \sim 7.6$ Hz, or as a broad singlet at ca. 4.2 ppm. A small distance between these two signals can be due to the restriction in conformation of the imidazolidine ring as an effect of the fusion with triazine, and resulting from almost perfect planarity and equalization of both methylene group character. The multiplet at about 7.02–7.80 ppm was characteristic of aromatic protons. In addition, two singlet signals of both NH groups in the range 7.68-7.95 ppm and 8.08-8.36 ppm (exchangeable with D_2O), integrating of one proton and one proton, respectively were found in the ^{1}H NMR spectra of compounds **II a–e**. IR spectral characteristic of imidazo [2,1-*c*][1,2,4] triazines revealed in their IR spectra the absorption bands at about 1691 (C=O) and about 1580 (C=N) cm^{-1}, which confirmed as well the formation of cyclic products. From the IR spectral data (Table 1), there is a clear relationship between the frequency of ring carbonyl group C=O and the frequency of the C=N bond at the ring junction and the difference in their frequencies is of the order of 109-112 cm^{-1}. These data are in full agreement with the literature data (15).

Microbiology

Antibacterial and antifungal activities of obtained compounds were tested by the disc-diffusion method of Kirby-Bauer in relation to 54 strains of Gram-positive and 52 strains of Gram-negative bacteria, 6 strains of yeast-like fungi and 3 strains of moulds. It can be concluded from microbiological screening tests that compounds **II a-e** in examined concentrations (100 mg mL^{-1} and 200 mg mL^{-1}) had no influence on the growth of microorganisms tested.

These results allowed to limit the possible biological spectrum of activity of synthesized imidazo [2,1-*c*][1,2,4] triazine derivatives and exclude their potential antimicrobial activity. Lack of antimicrobial activity of tested compounds seemed to be pro-

fitable in the case of compounds possessing effect on the central nervous system i.e., showing antinociceptive activity and, therefore, in the future these compounds will be tested for their potential analgesic activity.

Prediction of biological activity

Imidazotriazines 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 ligands of δ-opioid receptors (SNC--80). These similar features according to a 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 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*][1,2,4] 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 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 (16).

Potential biological properties of the investigated imidazo [2,1-*c*][1,2,4] triazines were evaluated using a computer program PASS (Prediction of Activity Spectra for Substances); http://www.ibmh.msk.su/service.ntm. This program 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 program are shown in Table 3. For all compounds (**II a-e**) the most probable it seemed to be GABA A receptor antagonistic activity (*Pa* about 96.3 %, *Pi* 0.1 %). Additionally for all compounds (**II a**-**e**) analgesic activity was found as highly possible (*Pa* > 70 %). According to PASS program the probability for the obtained compounds to be analgesic is in the order: **II a** > **II d** > **II e** > **II b** > **II c**. It is noteworthy in this case that the estimated probability for compound **II d** i. e. 8-(3-chlorophenyl)-4-imino-2,3,7,8-tetrahydro-imidazo [2,1-*c*][1,2,4] triazin-3 (6*H*)-one to be analgesic (83.2 %) is imperceptibly greater than for a molecule of similar structure, having in 4 position the oxo-group, instead, the imino-group i.e., 8-(3-chlorophenyl)-2,3,7,8-tetrahydro-imidazo [2,1-*c*][1,2,4] triazin-3,4 (6*H*)-dione (82.2 %), whose a strong antinociception at doses 12.5-200 mg (0.00625–0.1 LD_{50}) and a very weak acute toxicity (over 2000 mg kg^{-1}) in behavioral animal tests were confirmed (13). The replacement of phenyl, 3-chlorophenyl, 4-chlorophenyl, 4-methylphenyl substituent at position 8 in the case of compounds **II a** (*Pa* = 84.9 %, *Pi* = 0.7 %), **II d** (*Pa* = 83.2 %, *Pi* = 0.7 %), **II e** (*Pa* = 82.9 %, *Pi* = 0.7 %), **II b** (*Pa* = 81.0, *Pa* = 0.8) by 2-chlorophenyl substituent decreased the probability to be analgesic in the case of compound **II c** to *Pa* = 74.8 %, *Pi* = 1.0 %. All compounds (**II a-e**) showed *Pa* > 50 % in the categories of septic shock treatment, inflammatory Bowel disease treatment, antiviral (Herpesviridae). Compounds **II a-b** and **II d-e** indicated *Pa* > 50 % in the category of antimigraine, compounds **II a** and **II c-e** in the category of antiischemic (cerebral), compounds **II c-e** in the categories of acute neurological disorders treatment, neuroprotector and compounds **II c-d** in the category of anticonvulsant. Only compound **II c** demonstrated *Pa* > 50 % in the categories of gout treatment, cannabinoid receptor antagonist, antineurotic and antiepileptic, compound **II a** in the category of tyrosine phosphatase inhibitor and compound **II b** in the category of calcium channel agonist.

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