SYNTHESIS OF SOME N^1-ARYL/HETEROARYLAMINOMETHYL/ETHYL - 1,2,4 – TRIAZOLES AND THEIR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

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ABSTRACT

A group of 18 N^1-aryl/heteroarylamino/methyl/ethyl-1,2,4-triazoles was synthesized (Scheme 1) by condensation of hydroxymethyl derivative of 1,2,4-triazole and appropriate aromatic/heteroaromatic amines (Route I) and by reaction of 1,2,4-triazole, acetaldehyde and few aromatic/heteroaromatic amines (Route II). UV, IR and ^{1}H NMR spectroscopy determined their structure. All the synthesized compounds were screened for their antibacterial and antifungal activities against *Escherichia coli, Bacillus subtilis, Salmonella enteritidis, Staphylococcus aureus, Aspergillus niger* and *Candida albicans.*

INTRODUCTION

The recent findings that 1,2,4-triazole nucleus is associated with diverse biological activities such as: analgesic, antiasthmatic, diuretic, antihypertensive, antibacterial, antifungal and antiinflammatory properties [1-4], prompt us to synthesise some new 1,2,4-triazole derivatives and to investigate their antibacterial and antifungal activities. Mannich reaction (aminomethylation) of some heterocycles (benzotriazoles, benzimidazoles), formaldehyde and aliphatic and aromatic amines is a well known process [5-12]. N-hydroxymethyl derivatives of heterocycles under the influence of amines, can also give corresponding Mannich bases [12,13]. It is also known that some aminomethyl heterocycles possess biological [6-11] and corrosion-inhibition activity [5,12,13], they can be used as additives in greasy oils [14-17] or in photopolymerizing paints for improving adhesion [18].

This study is focused on the synthesis of a set of N^1-aryl/heteroarylaminomethyl/ethyl-1,2,4-triazoles (using 1H-1,2,4-triazole, aryl/heteroaryl amines and paraformaldehyde/acetaldehyde) and on the study of antibacterial and antifungal activities of these compounds.

EXPERIMENTAL

The melting points of compounds synthesized were determined on a Büchi 510 melting point apparatus and the values reported here are given uncorrected. The IR spectra were recorded in the range of 4000-400 cm^{-1} using the KBr disks on a Perkin-Elmer 297 spectrophotometer. The ^1H (250 MH_z) NMR spectra were recorded with a Bruker AC 250E spectrometer in DMSO-d_6 with TMS as an internal standard. UV spectra were recorded on a Varian Cary 219 spectrophotometer.

Synthesis of 1H-1-hydroxymethyl-1,2,4-triazole (2)

A mixture of 28g (0.04 mol) 1H-1,2,4-triazole (**1**), 12g (0.04mol) paraformaldehyde and 6 drops triethylamine is refluxed for 2.5 hours on the water bath. The crude precipitate is left over night at room temperature and after that is recrystallized from benzene. Yield 97-99%; Mp 65-7^{0}C; **IR:** 3500-3100 cm^{-1} (OH) st.; 1080 cm^{-1} (C-O, CH_2OH); **^{1}H NMR:** 8.00 and 8.60 ppm (s, 2H. triazole ring); 5.46 ppm (d, CH_2); 7.03 ppm (s, OH);

General procedure of N¹-aryl/heteroarylaminomethyl-1,2,4-triazoles (3a-n)

A mixture of 1H-1-hydroxymethyl-1,2,4-triazole (0,01 mol) (**2**) and corresponding aromatic/heteroaromatic amines (0.01 mol) in 96% ethanol is refluxed for some time (30 minutes to 4 hours), first it is kept at room temperature and then at –5^{0}C overnight. The precipitate is filtered off, washed with cold ethanol and recrystallized from appropriate solvent to give analytically pure product.

General procedure of N¹-aryl/heteroarylaminoethyl-1,2,4-triazoles (4a-d)

A mixture of 0,7g (0.01 mol) 1H-1,2,4-triazole (**1**), corresponding aromatic/heteroaromatic amines (0.01 mol) and 0.6 ml (0.01 mol) acetaldehyde in 96% ethanol is refluxed for some time (30 minutes to 1 hour), first it is kept at room temperature and then at –5^{0}C overnight. The precipitate is filtered off, washed with cold ethanol and recrystallized from appropriate solvent to give analytically pure product.

Microbiology

The filter paper disc method [19] was performed in Sabouraud dextrose broth and Mueller Hinton broth. These agar media were inoculated with 0.5ml of the 24h liquid cultures containing 10^7 microorganisms/ml. Filter paper discs (5mm diameter) saturated with each compound solution (1mg/ml; 5mg/ml and 10mg/ml DMSO) were placed on the indicated agar mediums. The incubation time was 24h at 37^{0}C for bacterial and 48h at 30^{0}C for *Candida sp.* Discs with DMSO were used as control. The diameter of zone inhibition (mm) was measured. The tests were repeated 3 times to confirm the findings.

RESULTS AND DISCUSSION

1H-1,2,4-triazole in reaction with formaldehyde/acetaldehyde and corresponding aromatic/heteroaromatic amines produces N¹-aryl/heteroarylaminomethyl/ethyl-1,2,4-triazoles (Scheme 1), that were not previously investigated. These compounds are of considerable synthetic interest as substances with potential biological activity.

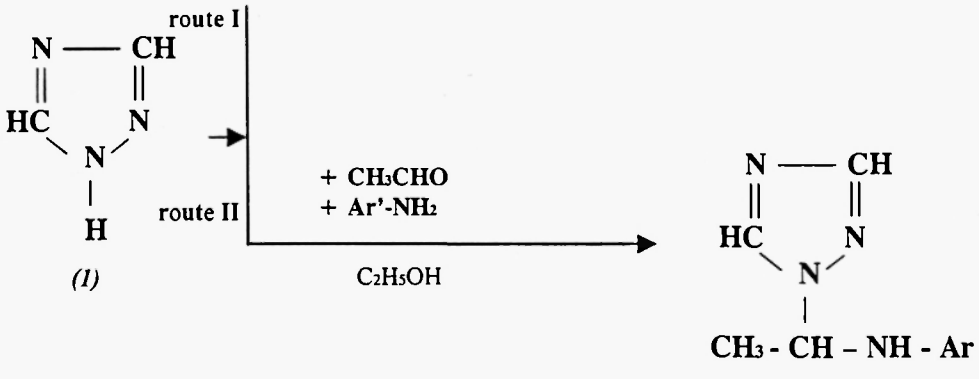
For that purpose substituted aromatic and heteroaromatic amines have been chosen and special attention has been paid to the electronic deficient heterocyclic amines.

Reactions of 1H-1-hydroxymethyl-1,2,4-triazole (**2**) with a number of aromatic/hetroaromatic amines in ethanol gave N^1-aryl/heteroarylaminomethyl-1,2,4-triazoles (**3a**-n) in a pure state according to the route I presented in scheme I. In order to achieve this purpose, it was necessary at first to convert the starting material (1H-1,2,4-triazole) in corresponding 1H-1-hydroxymethyl-1,2,4-triazole (**2**).

The 1H-1-hydroxymetyl-1,2,4-triazole (**2**) is produced by reaction of paraformaldehyde (*"in situ"* source of formaldehyde), 1H-1,2,4-triazole (**1**) and catalytical amount of triethylamine (Mp=65-67^{0}C). The structure of (**2**) was established by IR and ^{1}H NMR spectra. The IR absorption due to ν(OH)st and ν(C-O)st from CH_2OH appeared at 3500-3100 cm^{-1} and 1080 cm^{-1} respectively. The ^{1}H NMR spectra of this compound in DMSO-d_6 exhibited the following signals: 8.00 and 8.60 ppm singlets from triazole H protons; 5.46 ppm (H from CH_2) and 7.03 ppm (H from OH).

When (**2**) reacts with aromatic amines such as benzocaine; *o*- and *p*-aminobenzoic acids; *p*-chloro- and *p*-bromoaniline; *p*-toluidine; *p*-aminobiphenyl and *p*-phenylenediamine, the N^1-arylaminomethyl-1,2,4-triazoles are given (**3a**-h). Melting points, time of reaction, yield and elemental analysis of those compounds are given in Table1.

Scheme 1



Once more 1H-1-hydroxymethyl-1,2,4-triazole was used as starting material with heteroaromatic amines: 2-amino-, 4-methyl-2-amino, 6-methyl-2-amino-, 6-chloro-aminopyridine and 4-amino-1,2,4-triazole and N^1-heteroarylamino-methyl-1,2,4-triazoles (**3i-n**). Physical and analytical data of those compounds are given in Table1. IR, ^{1}H NMR and ^{13}C NMR spectral data (Table 2) established the structure of (**3a-n**).

The IR spectra of compounds (**3a**-n) showed characteristic absorption bands at 3440-3260 and 3140-3000 cm^{-1} which are due to NH and aromatic C-H functions, respectively; C-H aliphatic and C=N/C=C vibration appeared at 2980-2820 and 1620-1600 cm^{-1}, respectively. The absorption bands associated with other functional groups appeared in the expected regions. The ^{1}H NMR spectra of these compounds (in DMSO-d_6) exhibited 2H protons from triazole ring as singlets at 7.88-7.98 and 8.11-8.69 ppm. The ^{1}H NMR spectra exhibited also the 1H from NH and 2H from CH_2-signals between 5.22-8.77 and 5.81-5.42 ppm. The UV spectra of (**3a-n**) have been recorded in two solvents: water and ethanol, and they showed three absorption maxima or shoulders at 212-240, 263-267 and 282-295 nm (in water) and 221-247, 263-270 and 283-297 nm (in ethanol).

Table 1: ***Physical and analytical data of compounds 3a - n***

Comp	Ar	Mol. for. /(mol. wt.)	Mp (°C)	time	Yield (%)	Elem. analysis cal./found [%] C	H	N
3 a	-C_6H_4-$COOC_2H_5$ (p)	$C_{12}H_{14}N_4O_2$/246,27	129-130	30 min.	84.67	58.52 58.02	5.73 5.13	22.76 22.20
3 b	-C_6H_4-COOH (p)	$C_{10}H_{10}N_4O_2$/218,21	185-186	1 hour	83.56	55.04 54.18	4.62 5.39	25.67 24.80
3 c	-C_6H_4-COOH (o)	$C_{10}H_{10}N_4O_2$/218,21	170-171	45 min.	78.33	55.04 54.57	4.62 5.19	25.67 25.10
3 d	-C_6H_4-Cl (p)	$C_9H_9N_4Cl$/208,65	89-91	4 hours	96.69	51.81 51.22	4.35 4.41	26.85 25.60
3 e	-C_6H_4-Br (p)	$C_9H_9N_4Br$/253,11	66-68	4 hours	97.00	42.70 41.71	3.58 4.41	22.14 22.48
3 f	-C_6H_4-CH_3 (p)	$C_{10}H_{12}N_4$/88,23	64-66	3 hours	95.20	63.81 62.96	6.43 6.30	29.77 29.35
3 g	-C_6H_4-C_6H_5 (p)	$C_{15}H_{14}N_4$/250,30	124-125	2.5hours	71.29	71.97 71.45	5.64 6.10	22.38 22.69
3 h	-C_6N_4-NH-CH_2- 	$C_{12}H_{14}N_8$/270.30	150-151	2 hours	49.10	53.32 52.98	5.22 5.24	41.46 40.79
3 i	2-Pyridyl-	$C_8H_9N_5$/175.19	100-102	3 hours	96.88	54.85 53.90	5.18 5.11	39.98 39.38
3 j	4-Methyl-2- pyridyl -	$C_9H_{11}N_5$/185.21	108-109	3 hours	62.63	57.13 56.93	5.86 6.37	37.01 38.03
3 k	6- Methyl-2- pyridyl -	$C_9H_{11}N_5$/185.21	94-95	3 hours	76.32	57.14 56.93	5.86 6.25	37.01 36.76
3 l	5-Chloro-2-pyridyl-	$C_8H_8N_5Cl$/206.61	153-155	3 hours	95.87	46.59 46.05	3.91 3.98	33.96 33.15
3 m	2-Pyrimidyl-	$C_7H_8N_6$/176.18	149-150	3 hours	64.50	47.72 47.47	4.58 4.93	47.70 47.98
3 n	.1,2,4-Triazole-4-yl	$C_5H_7N_7$/165.16	123	4 hours	36.04	36.36 36.40	4.27 4.63	59.36 58.67

Acetaldehyde can also be used successfully as starting material for the condensation with 1,2,4-triazole and aromatic/heteroaromatic amines (Scheme 1, route II). N^1-aryl/heteroarylaminoethyl-1,2,4-triazoles (**4a**-d) have been obtained in good yield (Table 2) by mild heating of equimolar quantity of 1H-1,2,4-triazole, acetaldehyde and amines: benzocaine, *p*-nitroaniline; 2-amino-6-methylpyridine and 2-aminothiazole. The structure of new aminoethyl-1,2,4-triazole derivatives has been confirmed by their IR, ^{1}H NMR and UV spectra.

Table 2: ***Physical and analytical data of compounds 4a-d***

Comp.	Ar'	Mol. for. /(mol. wt.)	Mp (°C)	Time	Yield (%)	Elem. analysis cal./found [%] C	H	N
4 a	-C_6H_4-$COOC_2H_5$ (p)	$C_{13}H_{16}N_4O_2$/260,295	134-136	30 min.	41.67	59.98 59.45	6.19 6.41	21.53 22.07
4 b	-C_6H_4-NO_2 (p)	$C_{10}H_{11}N_5O_2$/233,237	149-150	20 min.	65.46	51.50 51.80	4.75 5.15	30.03 29.81
4 c	6-Methyl-2-pyridyl-	$C_{10}H_{13}N_5$/203.25	136	1 hour	72.00	59.10 59.77	6.45 5.95	34.56 33.87
4 d	2-Thiazolyl-	$C_7H_9N_5S$/195.25	171-172	1 hour	74.68	43.06 42.22	4.65 4.67	35.87 35.78

The IR and NMR data of compounds **(4a-d)** support the proposed structure; their IR spectra in KBr disk showed absorption bands at about 3315-3220, 3120-3000 and 2980-2840cm^{-1} which are indicative of NH, C-H aromatic and C-H aliphatic vibrations, respectively. ^{1}H NMR spectra of **(4a-d)** in DMSO-d_6 exhibited the following signals: NH from triazole ring as singlet between 7.89-7.97 and 8.21-8.56 ppm; H proton from NH group (CH-NH-Ar function) at 5.08-8.72ppm. The signals associated with other functional groups appeared in the expected regions, such as CH_3- and –CH- signals between 1.67-1.85 and 5.95 and 6.40ppm. The UV spectra of **(4a-d)** have been also recorded in water and ethanol. They showed three absorption maxima or shoulders at 219-230, 254-264 and 282-284nm (in water) and 221-235, 256-2664 and 282-295 nm (in ethanol).

Table 3. ***Zone of inhibition (mm) of the compounds tested against various bacterial and fungal strains***

	Escherichia coli			*Bacillus subtilis*			[redacted]			*Staphylococcus aureus*			*Aspergillus niger*			*Candida albicans*		
Conc. [mg/ml]	*1*	*5*	*10*	*1*	*5*	*10*	*1*	*5*	*10*	*1*	*5*	*10*	*1*	*5*	*10*	*1*	*5*	*10*
3a	6	6	6	6	6	6	6	7.5	9.5	6.5	8	8.5	5.5	6	6	-	6	6
3b	-	-	6	-	6	6	6	6	6.5	-	-	-	-	-	-	-	-	-
3c	-	-	-	-	-	-	-	-	-	5.5	5.5	5.5	-	-	-	-	-	
3d	5.5	6	6	-	-	6	6	6	6.5	-	-	-	-	-	-	-	-	7
3e	6	6	6	-	5.5	6	5.5	5.5	6	-	-	-	-	-	6	6.5	7	7.5
3f	-	-	-	-	6	6.5	-	6	7.5	-	-	-	-	5.5	7	-	-	-
3g	5.5	5.5	5.5	5.5	5.5	5.5	-	6	6	-	-	-	-	5.5	6	-	-	-
3h	-	5.5	6.5	-	-	-	-	-	6	-	-	-	-	-	6	-	-	-
3i	5.5	5.5	6	-	-	7.5	6	6	6.5	-	-	-	-	-	-	-	-	-
3j	5.5	5.5	6	-	-	-	5.5	6	6	-	-	9	-	-	6	-	-	-
3k	5.5	5.5	6	-	-	5.5	-	-	7.5	-	-	9	-	-	-	-	-	-
3l	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3m	-	-	-	-	-	-	-	-	-	5.5	6	7	-	-	-	-	-	-
3n	-	6	6.5	-	-	8	6	6	7	-	-	6.5	-	-	-	-	-	-
4a	6.5	6.5	6.5	-	-	6	-	6	6.5	-	7	7	-	-	5.5	-	6.5	6.5
4b	7	-	5.5	-	-	6.5	-	-	-	-	-	-	-	-	-	-	-	-
4c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-
4d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- : No inhibition zone

Using 3 concentrations (1mg/ml, 5mg/ml and 10mg/ml DMSO), the newly synthesised compounds **(3a-n)** and **(4a-d)** were tested for "*in vitro*" antibacterial and antifungal activities against: *Escherichia coli, Bacillus subtilis, Salmonella enteritidis, Staphylococcus aureus, Aspergillus niger* and *Candida albicans* (Table 3). The tested compounds in the arylaminomethyl series **(3a-h)** were more active in the antibacterial and antifungal screening than the heteroarylaminomethyl derivatives **(3i-n)**. Among N^1-arylaminomethyl-1,2,4-triazoles **(3a-h)**, compound **(3a)** was found to be the most active of the compounds studied, with 9.5mm zone of inhibition against *S. enteritidis*.

In the N^1-heteroarylaminomethyl-1,2,4-triazole series **(3i-n)**, the highest activities (7.5mm) against *B. subtilis* and *S. enteritidis* were observed for compounds **(3i)** and **(3k)**, respectively. The results also showed that activity depends upon type and position of the substituents. For example 4-bromosubstituted arylaminomethyltriazole **(3e)** was more active than the 4-chloro analogue **(3d)** and 4- **(3j)** or 6-methyl heteroarylaminomethyltriazole **(3k)** were more active than the 5-chloro analogue **(3l)**. It must also be noted that most of the investigated compounds do not inhibit the growth of the fungus. In most cases zone of inhibition growth with increasing the concentration of tested compounds. Increasing the number of carbon atoms in aminomethyl/ethyl unit, in compounds **(4a-d)** coursed a decrease in antibacterial and antifungal activities (compared **3a** with **4a** and **3k** with **4c**).

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