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SYNTHESIS OF 4-AMINO-5-ARYL-1, 2, 4-TRIAZOLES AND SCREENING FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

4-Amino-5-aryl-1, 2, 4-triazoles were synthesized and tested for antibacterial activities against different species of gram positive and gram negative bacteria. 4-Amino-5-aryl-1, 2, 4-triazoles were obtained by cyclization of the potassium salts of appropriately substituted dithiocarbazinic acid with hydrazine hydrate. The new synthesized compounds were characterized using IR spectra, ^{1}H NMR and elemental analysis.

Key words: 1, 2, 4-Triazoles, Antibacterial, IR, ^{1}H NMR.

INTRODUCTION

1, 2, 4- triazoles and its derivatives represent one of the most biological active classes of compounds possessing a wide spectrum of activities. 1, 2, 4 – Triazole nucleus is associated with diverse pharmacological activities such as antibacterial[1], antifungal[2], hypoglycemic[3], analgesic[4], antihypertensive[5] and anti–inflammatory[6] activities.

The scientific literature also states that the antibacterial[7] activities of thiourea derivatives are due to the presences of the –NH – C (S) – NH – function in the molecule and the changes in this activity depend on the nature of its substituents. These observations prompted us to synthesize some new triazoles and to investigate their antibacterial activities.

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EXPERIMENTAL

General: The melting points of synthesized compounds were determined in open glass capillaries containing liquid paraffin and are uncorrected. The IR spectra of compound were recorded in KBr on FTIR spectrophotometer. The ^{1}H NMR was recorded on Brucker 300 MHz instrument in DMSO/$CDCl_3$ using TMS as an internal standard.

Synthesis of derivatives

(i) **Synthesis of methyl esters of acids (1a-f)**

These were synthesized by esterification of isonicotinic acid, benzoic acid, 1-naphthyl acetic acid, trichloroacetic acid, phenyl acetic acid and salicylic acid respectively using excess methanol in the presence of sulphuric acid[8].

(ii) **Synthesis of hydrazides of acids (2 a-f)**

These were prepared by the reaction of the corresponding methyl esters **(1a-f)** with hydrazine hydrate[9-11].

(iii) **Synthesis of potassium salts of substituted dithiocarbazinic acids (3a-f)**

A mixture of **(2a-f)** (0.01 mol), CS_2 (0.15 mol) and KOH (0.15 mol) in absolute ethanol (350 mL) was heated under the reflux for 10 hrs, cooled to room temperature and diluted with dry ether (200 mL). The precipitate, that appeared, was filtered, washed with 2 x 50 mL of ether and vacuum dried.

(iv) **Synthesis of 4-amino-5-aryl-1, 2, 4-triazoles (4a-f)**

To a suspension of **(3a-f)** (0.002 mol), hydrazine hydrate (0.04 mol) and water (4 mL) were added and the mixture was refluxed with stirring for several hours, until the evolution of H_2S had ceased. After dilution with water (100 mL) and the acidification with HCl, the precipitates were filtered, washed with 2 x 30 mL of water and recrystallized from ethanol-water.

The reaction scheme is given in Fig.1. The melting points, yields and elemental analysis of these compounds are given in Table 1.

(4a) IR (cm^{-1}): 1240(C=S), 1558(C=N), 1160 (C-N), 730 (C-H).

^{1}H NMR (δ): 7.2-7.28 (4H,s, aromatic), 8.9-8.92 (1H, s, NH), 2.9-2.92(2H, s, NH_2).

(4b) **IR (cm^{-1}):** 1244 (C=S), 1521 (C=N), 1140 (C-N), 2925 (NH_2 str).

^{1}H NMR (δ): 7.8-7.9 (5H,s, aromatic), 7.7-7.72 (1H, s, NH), 4.32-4.34 (2H, s, NH_2).

(4c) **IR (cm^{-1}):** 1240 (C=S), 1519(C=N), 1130 (C-N), 3053 (NH_2), 2920 (CH_2).

^{1}H NMR (δ): 8.0-8.02 (7H,s, aromatic), 7.7-7.72 (H, s, NH), 4.32-4.34(2H, s, NH_2) 2.40-2.42 (2H, m, CH_2).

(4d) **IR (cm^{-1}):** 778.81 (C-Cl), 1268 (C=S), 1598 (C=N), 1138 (C-N), 3057 (NH_2), 2916 (NH).

^{1}H NMR (δ): 7.9-7.92 (1H,s, NH), 4.02-4.1 (2H, s, NH_2)

(4e) **IR (cm^{-1}):** 1250 (C=S), 1499 (C=N), 1120 (C-N), 3250 (NH_2), 2900 (CH_2)

^{1}H NMR (δ): 7.9-7.92 (5H,s), 4.30-4.32 (2H, s, NH_2), 9.0-9.02 (1H, s, NH), 2.22-2.24 (2H, M CH_2).

(4f) **IR (cm^{-1}):** 1252 (C=S), 1500 (C=N), 1125 (C-N), 3250 (NH_2), 2910 (OH, NH str.).

^{1}H NMR (δ): 8.02-8.04 (4H,s), 9.0-9.02 (1H, s, OH), 9.4-9.42 (s, NH), 4.02-4.08 (2H,s, NH_2)

Biological evaluation

The cup-plate method was performed using nutrient agar broth. These agar media was inoculated with 0.5 mL of the 24 hrs liquid culture containing 10^7 micro organism/mL. Plates discs saturated with solution of each compound (conc. 10 mg/mL in DMSO) were placed on the indicated agar medium. The incubation time was 24 hrs at 36°C to 37°C for *Staphylococcus aureus*, *Bacillus subtilus*, *Echerichia coli* and *Salmonella enteritidis*. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones. The tests were repeated to confirm the findings and the average of the reading was taken into consideration.

Antibacterial activity

The cup-plate method[12] was employed for the *in vitro* study of antibacterial effects against *Staph. aureus*, *B. subtilus*, *E. coli* and *S. entertidis*. The method was based on diffusion of antibacterial compound from reservoir nutrient agar medium such that the growth of the microorganism is inhibited as circular zone around the bore. The inhibitory effects of compounds **(4a-f)** against these organisms are given in Table 2. The screening results indicate that not all compounds exhibited antibacterial activities. It can be noted that **(4b)** and **(4e)** showed the greatest inhibitory effect against one or more types of bacteria as compared to other aryl derivatives. **(4b)** showed the greatest effect on *S. entertidis* compared to other derivatives, and also showed average effect on *Staph. aureus* along with **(4e)** and **(4f)**. But **(4b)** has shown less effect against *E. coli* and *B. subtilus* then **(4d)**; **(4e)** and **(4f)** showed poor effect against *E. coli*. **(4a)** exhibited similar actions against *E. coli*, *S. entertidis* and *Staph. aureus* but not showed any effect against *B. subtilus*. **(4b)** showed poor inhibitory zone against *E. coli* and *B. subtilus*, **(4c)** showed poor inhibitory zone against *B. subtilus* and *Staph. aureus* but not shown any inhibitory zone against *E. coli* and *S. entertidis*. **(4d)** exhibits the average effect against *E. coli* and good effect against other organism but exhibits no inhibitory zone against *S. entertidis* and *Staph. aureus*. **(4e)** was found to effective against all bacteria taken for study. This may be due to the phenyl ring and NH_2 group at 4-position. **(4e)** showed the similar effect with *E. coli*, *B. subtilus* and *Staph. aureus*. **(4f)** showed the similar effect with poor inhibition zone against *E. coli* and *S. entertidis* and no inhibition against *B. subtilus* and *Staph. aureus*.

RESULTS AND DISSUSION

The aim of this work was to synthesize 4-amino-5-aryl-1, 2, 4-triazoles (as per reaction scheme given in Fig.1). In order to achieve this aim, it was necessary to first synthesize esters **(1a-f)** of some acids like isonicotinic acid, benzoic acid, 1-naphthyl acetic acid, trichloroacetic acid, phenyl acetic acid and salicylic acid, respectively **(a-f)**. Esters were prepared by the rection of methyl alcohol in presence of sulphuric acid. After esterification, hydrazides **(2a-f)** were prepared. The next step was the conversion of the derivatives **(2a-f)** into the corresponding 4-amino-5-aryl-1, 2, 4-triazoles. The purity of the isolated compound ws checked by TLC in different solvents at different stages.

When **(2a-f)** was refluxed in ethanol with CS_2 and KOH the corresponding potassium salts of the substituted dithiocarbazinic acid **(3a-f)** were obtained. The structures of compounds **(3a-f)** were established by their IR and ^1H NMR spectra. The IR absorption due to the C=O and C=S functions appeared at 1660-1600 cm^{-1} and 1280-1240 cm^{-1},

respectively. The absorption bands associated with other functional groups appeared to be at the expected region. The ^{1}H NMR spectra of compounds **(3a-f)** (in DMSO –d_6) exhibited a multiplet in the aromatic region at 6.83-7.91 ppm.

Three or four field's singlets were observed at the 8.11-8.96 ppm region representing the protons of the OH group and the NH (thiasemicarbazide moiety), due to strong deshielding effect of the aromatic ring system and the thio carbonyl group. The ^{1}H NMR spectra of **(3a-f)** also exhibited the CH_2 – and CH – signals of the allyl group of multiplets and doublets between 4.09 and 5.83 ppm.

Further, the potassium salts upon reaction with hydrazine hydrate yielded the corresponding 4-Amino-5-aryl-1,2,4-triazoles **(4a-f),** All the compounds prepared were novel. The melting points, yields and elemental analysis of these compounds are given in Table 1. The structures of **(4a-f)** were established by their IR and ^{1}H NMR spectra. IR spectra also showed a band in the 1266-1249 cm^{-1} region due to C=S function, further supporting the predominance of the thion form in the solid state and the polar solvents [9,10].

The fact that the compound exists in thion-thiol tautomeric equilibrium is supported by the absence of characteristics (SH) absorption bands in the IR spectra. The IR spectra of the compound **(4a-f)** showed characteristics bands around 3306-3152 cm^{-1} (OH and NH stretch), 3103-2955 cm^{-1} (C-H from Ar-H stretch), 2972-2788 cm^{-1}(C-H from CH_2 stretch), 1626-1599 cm^{-1} (C=C), 1583-1514 cm^{-1} (C=N), 1534-1480 cm^{-1} (N-H).

Table 1. Physical and analytical data of compounds synthesized

Comp.	R	Mol. formula (Mol. wt.)	M. P. (^{o}C)	Yield (%)	Elemental analysis Calc./Found		
					C	H	N
(4a)	C_5H_4N-	$C_7H_7N_5S$ (193.2)	198-9	56.17	43.47	3.62	36.23
(4b)	C_6H_5-	$C_8H_8N_4S$ (192.2)	202-3	69.60	49.94	4.16	29.13
(4c)	$C_{11}H_9^-$	$C_{13}H_{12}N_4S$ (256.3)	209	72.02	60.86	4.68	21.84
(4d)	CCl_3-	$C_3H_3N_4Cl_3S$ (233.5)	223	30.3	15.41	1.28	23.98
(4e)	C_7H_7-	$C_9H_{10}N_4S$ (206.12)	205-6	52.4	52.39	4.85	27.16
(4f)	C_6H_5O-	$C_8H_8N_4OS$ (208.17)	218	35.42	46.11	3.84	26.90

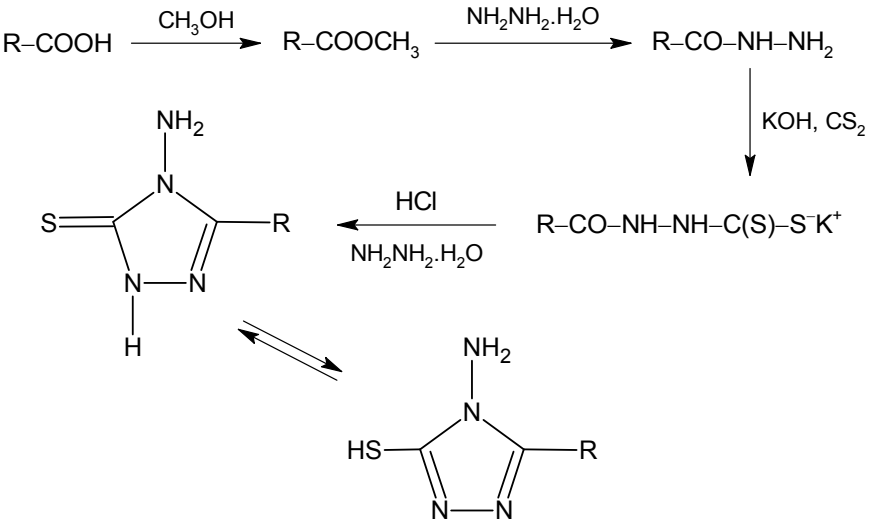

Fig. 1: Reaction scheme

Table 2.

Compound	*E. coli*	*B. subtilus*	*S. entertidis*	*Staph. aureus*
(4a)	+	-	+	+
(4b)	+	+	+++	++
(4c)	-	+	-	+
(4d)	++	+	-	-
(4e)	++	++	+	++
(4f)	+	-	+	+

Concentration = 10 mg/mL

Greatest inhibition zone	-	++++
Good inhibition zone	-	+++
Average inhibition zone	-	++
Poor inhibition	-	+
No inhibition zone	-	-

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