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Synthesis and Antifungal Activity of 4-amino-5-aryl-1, 2, 4-triazoles

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A few 4-amino-5-aryl-1, 2, 4-triazoles were synthesized and tested for antifungal activity against *Aspergillus niger* and *Candida albicans*. 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.

Keywords: 1, 2, 4- Triazoles, Antifungal activity, IR, ^{1}H-NMR.

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

The scientific literature also states that antiviral[6] and 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 antifungal activities.

EXPERIMENTAL

The melting points of synthesized compounds were determined in open glass capillaries using Kjeldahl flask containing liquid paraffin and are uncorrected. The IR Spectra of compound were recorded in KBr on RED FTIR Spectrophotometer. The ^{1}H NMR was recorded on Brucker 300 Mhz instrument in DMSO/$CDCl_3$ using TMS as internal standard.

Synthesis of derivatives

Synthesis of methyl esters of acids (1A-F)

These were synthesized by esterification of Isonicotinic acid, Benzoic Acid, 1-Naphthyl Acetic Acid, Trichloro Acetic Acid, Phenyl Acetic Acid and Salicylic Acid respectively, using excess methanol in the presence of Sulphuric Acid[12].

Synthesis of Hydrazides of Acids (2 A-F)

These were prepared by the reaction of the corresponding methyl esters (1A-F) with Hydrazine hydrate[13, 14, 15].

Synthesis of Potassium salts of substituted Dithiocarbazinic Acids (3A-F)

A mixture of 2A-F (0.01mol), 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 × 50 ml of ether and vacuum dried.

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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 (4ml) were added and the mixture was refluxed with stirring with 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 re-crystalised from ethanol water.

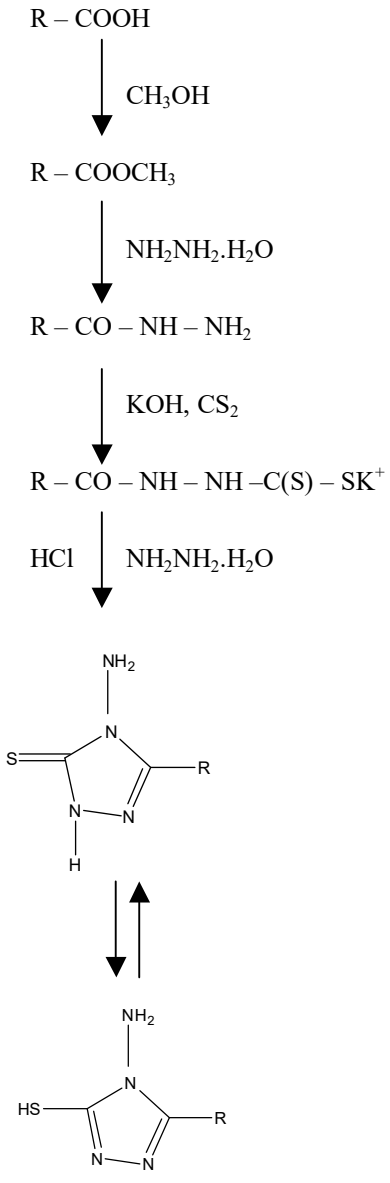

Fig. 1. Reaction Scheme

The reaction scheme is given in Fig. 1.

4A: IR (cm^{-1}): 1240(C=S), 1558 (C=N), 1160 (C-N), 730 (C-H).
^{1}H NMR: 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 ster).
^{1}H NMR: 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: 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: 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: 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 stre.).
^{1}H NMR: 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 microorganism/ml. Plates discs saturated with solution of each compound (conc. 10 mg/ml in DMSO) were place on the indicated agar medium. The incubation time was 48 hrs at 30º C for *Candida albicans* and *Aspergillus niger* species. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones. The tests were repeated to confirm the finding and the average of the reading was taken into consideration.

Anti fungal Activity

The cup-plate method[11] was employed for the in-vitro study of anti fungal effects against *A. niger* and *C. albicans.* The method was based on diffusion of antifungal 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 1.

Table 1. Physical and Analytical Data of Compounds Synthesized

Comp.	R Mol. Wt.	Mol. Formula (ºC)	M.P.	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

The screening results indicate that not all compound exhibited anti fungal activities. It can be noted that 4B was more effective with average inhibition zone area compared to other compounds against *A. niger*. 4E was the more effective with average inhibition zone area compare to other compounds against *C. albicans*. 4E also effective against *A.niger* but 4B was not having any inhibition zone. 4C showed antifungal activity against *C. albicans* with similar inhibition zone of 4F, which was also effective against *C. albicans.* 4D was effective against *A. niger* with poor inhibition zone. 4A was not shown antifungal activity with no inhibition zone. 4B and 4D was not effective against *C. albicans.*

RESULTS AND DISSCUSION

The aim of this work was the synthesis of 4-amino-5-aryl-1, 2, 4-triazoles (scheme). In order to achieve this aim it was necessary to first synthesizes esters (1A-F) of some acids Isonicotinic acid, Benzoic Acid, 1-Naphthyl Acetic Acid, Trichloro Acetic Acid, Phenyl Acetic Acid and Salicylic Acid respectively, (A-F). Ester was prepared by the reaction of methyl alcohol in presence of Sulphuric acid. After esterification, hydrazides (2A-F) were prepared. The next step was the conversion of derivatives (2A-F) into the corresponding 4-amino-5-aryl-1, 2, 4-triazoles. The purity of the isolated compound was 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 found. The structure of compound 3A-F was established by their IR and ^{1}H NMR Spectra. The IR absorption due to the C=O and C=S functions appeared at 1660-1600 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) of the 4A-F series; all the compounds prepare were novel. The melting points, yields and elemental analysis of these compounds are given in Table 2. 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-

Table 2.

Compound	*A. niger*	*C. albicans*
4A	-	-
4B	++	-
4C	-	+
4D	+	-
4E	+	++
4F	-	-

Concentration = 10mg/ml
Greatest inhibition zone - ++++
Good inhibition zone - +++
Average inhibition zone - ++
Poor inhibition zone - +
No inhibition zone - -

[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 characteristic bands around 3306-3152cm^{-1}(OH and NH stretch), 3103-2955cm^{-1}(C-H from Ar-H stretch), 2972-2788cm^{-1}(C-H from CH_2 stretch), 1626-1599cm^{-1} (C=C), 1583-1514cm^{-1}(C=N), 1534-1480cm^{-1} (N-H).

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