SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME SCHIFF BASES OF [4-(AMINO) -5- PHENYL- 4H-1, 2, 4-TRIAZOLE- 3- THIOL]

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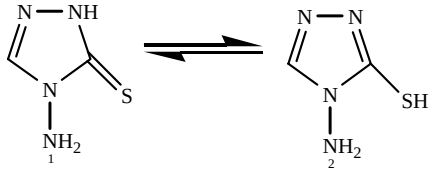
ABSTRACT

The basic nucleus 4-(amino)-5-phenyl-l-4H-1,2,4-triazole-3-thiol was prepared by cyclisation of potassium dithiocarbazinate with hydrazine hydrate using water as solvent under reflux condition for 3-4 h. The compound which has been synthesized successfully was subjected to addition reaction with different aldehydes to synthesize Schiff bases. The compounds were confirmed by physical parameters (solubility, melting point), chromatographic methods (TLC) and at last spectroscopic methods (IR, NMR, and Mass). In order to ascertain the pharmaceutical application, the selective pharmacological screening of the derivatives was carried out according to the standard procedures. The compounds were screened for their antianxietic activity by elevated plus maze method, antidepressant activity by forced swim test. Among the synthesized compounds, the Schiff bases of benzaldehyde (5e), furfuraldehyde (5d) and 2,4-dichloro benzaldehyde (5a) showed extremely significant activities. Results indicate that these compounds may be potential candidates for managing CNS disorders. However further studies are required to substantiate the same which are underway in our lab.

Keywords: Triazole, Schiff bases, antianxiety, antidepressant.

INTRODUCTION

In the last two decades, the chemistry of 1,2,4 - triazole and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. 1,2,4-triazole moieties have incorporated into variety of therapeutically interesting drug candidates including antiviral (ribavarin), anti migraine **(**rizatriptan), antifungal (flucanazole), antianxiety compounds (alprazolam). Moreover sulphur containing heterocyclic compounds represent an important group of compounds that are promising on practical application (Holla et al., 2003 ; Wu et al., 2007).The pharmacological importance of heterocycles derived from 1,2,4-triazole paved the way towards active research in a triazole chemistry. As a result, variety of new compounds was being added to this field every year. A number of attempts were made to improve the activity of these compounds by varying the substituents on the triazole nucleus (Dimova et al., 2001; Mathew et al., 2007; Banachiwicz et al., 2004). Among these the mercapto and amino group substituted 1,2,4-triazole ring (1&2) system have been reported for antimicrobial, anticancer, diuretic and hypoglycemic activities.



In view of these it has been planned to synthesize some newer 1,2,4-triazoles having mercapto and Schiff bases substitution. Since antianxiety& antideprassant activities had not been reported for our titled compounds, these activities were evaluated.

MATERIAL AND METHODS

All the synthetic work was done by procuring available laboratory grade reagents and analytical grade solvents. The solvents and reagents were purified and dried according to the procedure given in Vogel's text book of practical organic chemistry. TLC was preformed to monitor the reactions and to determine the purity of the products. Further the compounds were purified by recrystallisation using suitable solvents. The melting points of the synthesized compounds were determined in open capillaries using a Veego VMP-1 apparatus and expressed in ^{o}C and are uncorrected. The IR spectrum of compounds was recorded on a Shimadzu FT-IR spectrometer using the KBr pellet technique and is expressed in cm^{-1}. ^{1}H-NMR spectra were recorded on a Bruker DRX-300 (300 MHz FT-NMR) using $CDCl_3$ as solvent and TMS as Internal standard. Mass spectra were obtained using Shimadzu LC-MS 2010A mass spectrometer.

Synthesis of benzoic acid hydrazide from methyl benzoate (Compound-1)

Methyl benzoate (1.36 ml, 0.01M) in 25ml of ethanol is taken in a round bottom flask. To that hydrazine hydrate (0.70 ml, 0.15M) is added and reSfluxed for 4 hours. The total volume of the solution is reduced to half and it is cooled in ice water. The solid is precipitated out and recrystallised with ethanol.

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Synthesis of potassium dithiocarbazinate from benzoic acid hydrazide (Compound-2)

To a solution of potassium hydroxide (8.5 g, 0.15M) in absolute ethanol (125ml), benzoic acid hydrazide (1.36 g, 0.1M) and carbon disulphide (14.5 ml, 0.15M were added and the mixture was stirred for 16 hrs. To the resulting solution anhydrous ether (250ml) was added and precipitated potassium dithiocarbazinate was collected by filtration, washed with diethyl ether and dried. The potassium salt obtained in quantitative yield was directly used without purification.

IR (KBr cm^{-1}): 1662 (C=O str, amide), 3020 (Ar C-H str), 3300 (N-H str), 1487 (C-N str).

Synthesis of 4[amino]-5-phenyl-4H-1,2,4-triazole-3-thiol from potassium dithiocarbazinate (Compound-3)

A suspension of potassium salt dithiocarbazinate (4.44g, 0.02M), hydrazine hydrate (2ml, 0.04M) and water (80ml) was refluxed for 3 hrs. The color of the reaction mixture changed to green, hydrogen sulphide was evolved and a homogenous solution resulted. A white solid was precipitated by dilution with cold water (100ml) and acidification with concentrated hydrochloric acid. The product was filtered, washed with cold water (2×30 ml) and recrystallised from ethanol.

IR (KBr cm^{-1}): 943.3 (N-C-S str), 1278 (N-N-C str), 3365 (N-H str), 696.33 (C-S str), 3082 (Ar C-H str), 1446 (C-N str).

NMR (ppm): 7.7 (m, **5H**, Ar-H), 7.9 (s, **2H**, NH_2), 14.6 (s, **1H, S**-H).

Mass (m/z): M^+ calculated-192.24, found-194.90.

Synthesis of Schiff bases (Compound-4a-f)

A mixture of 4[amino]-5-phenyl-4H-1,2,4-triazole-3-thiol (1.98g, 0.001mol), respective aromatic aldehyde (0.001mol) and 4-5 drops of concentrated sulphuric acid in ethanol medium was refluxed for 3 hrs. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallised with hot ethanol.

Compound No.4a

IR (KBr cm^{-1}): 943.2 (N-C-S str), 3034 (Ar-H str), 696 (C-S str), 1446 (C-N str), 762.62 (C-Cl str)

NMR (ppm) : 7.5 (m, 5H, Ar-H), 7.8 (m, **5H**, Ar-H), 14.7 (s, 1H, SH), 10.3 (s, 1H, N=CH).

Mass (m/z) : M^+ calculated -349.24, found -362.35

Compound No. 4b

IR (KBr cm^{-1}): 943.2 (N-C-S str), 3134 (Ar-H str), 706 (C-S str), 1476 (C-N str).

NMR (ppm) : 7.5 (m, **5H**, Ar-H), 7.85 (m, **5H**, Ar-H), 14.1 (s, **1H**, SH), 10 .1 (s, 1H, N=CH).

Mass (m/z) : M^+ calculated -323.42, found -324.

Compound No. 4c

IR (KBr cm^{-1}): 943.2 (N-C-S str), 3144 (Ar-H str), 698.4 (C-S str), 1305.85 (C-N str).

NMR (ppm): 7.5 (m, **5H**, Ar-H), 7.85 (m, **5H**, Ar-H), 14.6 (s, **1H**, SH), 10.1 (s, 1H, N=CH).

Mass (m/z) : M^+ calculated -326.37, found -327.50.

Compound No. 4d

IR (KBr cm^{-1}): 943.2 (N-C-S str), 3144, 3099 (Ar-H str), 698.4 (C-S str), 1305.85 (C-N str).

NMR (ppm) : 7.5 (m, **5H**, Ar-H), 7.85 (m, **5H**, Ar-H), 14.6 (s, 1H, SH), 10.2 (s, 1H, N=CH).

Mass (m/z): M^+ calculated -270.31, found -272.65.

Compound No.4e

IR (KBr cm^{-1}): 943.2 (N-C-S str), 3144, 3099 (Ar-H str), 698.4 (C-S str), 1305.85 (C-N str).

NMR (ppm): 7.5 (m, **5H**, Ar-H), 7.85 (m, **5H**, Ar-H), 14.6 (s, **1H**, SH), 8.7 (s, 1H, N=CH).

Mass (m/z): M^+ calculated -280.35, found -276.00.

Compound No. 4f

IR (KBr cm^{-1}): 943.2 (N-C-S str), 3099 (Ar-H str), 678.4 (C-S str), 1405.85 (C-N str).

NMR (ppm): 7.1 (m, 5H, Ar-H), 7.85 (m, 5H, Ar-H), 14.6 (s, 1H, SH), 9.7 (s, 1H, N=CH).

Mass (m/z) : M^+ calculated -296.35, found -297.00.

The physical characterization data are given in table 1.

Pharmacological screening

Animals

Inbred male albino mice (Swiss strain) weighing between 20-30 g were used in the study. They were housed under standard laboratory conditions for a week before the experiments. The housing conditions were maintained at controlled temperature (23°C) and humidity (50%). They received a standard diet and water ad libitum. The animals were transferred to the laboratory 1 hr before the start of the experiment. The institutional ethical committee approved the study. Experiments were conducted between 9 to 14 h. Each mouse was used only once.

Table 1: Physical parameters for the synthesized compounds

Compound No.	Molecular weight	Melting point °C	% Yield	Rf values	Solvent system
3	192.24	198-200	48	0.14	Pet ether: Chloroform 9:1
4a	349.24	213-215	46	0.16	Benzene: Acetone 9:1
4b	323.42	180-184	44	0.22	Benzene: Acetone 9:1
4c	326.37	210-212	50	0.16	Benzene: Acetone 9:1
4d	270.31	200-205	47	0.19	Benzene: Acetone 9:1
4e	280.35	190-192	52	0.20	Benzene: Acetone 9:1
4f	296.35	197-198	51	0.18	Benzene: Acetone 9:1

Anti anxiety activity

Elevated Plus Maze Test

Anxiolytic compounds, by decreasing anxiety, increases open arm exploration time on an elevated plus maze apparatus. Plus maze for mice consisted of two open (16×5 cm) and two closed arms (16×5×12 cm) facing each other with an open roof. The entire maze is elevated at a height of 25 cm. The animals (18 in total) were divided into three groups; control, standard and test, containing six animals each. All the test drugs and standard drug were given intraperitonially. Dose 25mg/kg body weight was selected for the test compound and 2 mg/kg weight for the standard drug. The control group received the vehicle alone (0.5% carboxymethyl cellulose) and 2 mg/kg body weight for the standard drug Diazepam. Each mouse was placed in the central platform facing to open arm. The numbers of entries into open and closed arms and the time spent in the respective arms were recorded for a 5 min period. The percentage preference to open arms [(open/open + closed) × 100] was calculated for each mouse and is presented in table 2 (Zangrossi et al., 2004).

Anti depressant activity

The activity was screened by Forced swim test, as explained earlier (Sharma R et al., 2008). Forced swimming test is a behavioral test used to predict the efficacy of the antidepressant. The synthesized compounds (30 mg/kg) and standard drug Diazepam were suspended in a 0.5% aqueous solution of carboxymethylcellulose (CMC) and were injected intraperitoneally (i.p), 30 min prior to the test. Control animals received the vehicle only. The mice were forced to swim individually in a glass jar (tank) (25×12×25 cm^3) containing fresh water of 15 cm height and maintained at 25±3 ^oC. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remains floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 minute of a total 6 minute test. The changes in immobility duration were studied after administering drugs in separate groups of 6 animals. The percentage change from control [(Test ×100)-100 was calculated for each mouse, the results are presented in table 3.

RESULTS

All the synthesized final compounds were first purified by successive recrystallisation using appropriate solvents. The purity of the synthesized compounds was checked by performing thin layer chromatography and by determining melting points. Then the synthesized compounds were subjected to spectral analysis such as IR, NMR and mass spectra to confirm the structures. All the spectras were consistent with the structures. The 2,4-dichloro benzaldehyde (5a), benzaldehyde (5e), furfuraldehyde (5d) substituted mercapto triazoles showed extremely

Table 2: Antianxiety activity of synthesized compounds: Elevated plus Maze Model

Group No.	Compounds	Average time spent in arm (seconds)		% preference to open arm
		Open	Closed	
1	Control	23.33 ± 1.801	39.96 ± 2.838	14.09 ± 1.739
2	Diazepam	4.90 ± 1.029***	89.18 ± 3.348***	3.69 ± 0.7843***
3	4a	14.91 ± 1.685*	63.53 ± 3.259**	9.61 ± 0.5856*
4	4b	13.66 ± 1.694*	61.19 ± 3.209**	7.91 ± 0.4218*
5	4c	13.27 ± 1.770**	6816 ± 6.360***	8.77 ± 3.3458*
6	4d	13.13 ± 1.870**	67.16 ± 6.260***	8.57 ± 3.3578*
7	4e	14.66 ± 1.994*	63.19 ± 3.409**	8.11 ± 0.4028*
8	4f	14.56 ± 1.984*	62.19 ± 3.429**	8.11 ± 0.4528*

Results are expressed as mean ± SEM obtained from 6 animals.
*** - Extremely significant P < 0.001, ** -moderately significant P < 0.01, * - significant P < 0.05, when compared with normal control.

Table 3: Anti depressant activity of synthesized compounds: despair swim test model

Group No.	Treatment	No of animals	Dose (mg/kg)	Immobility time(In seconds)
1	Control	6	0.5	38.166 ± 1.778
2	Diazepam	6	0.5	79.166 ± 3.600**
3	4a	6	2.5	25.833 ± 1.956**
4	4b	6	2.5	22.833 ± 1.456**
5	4c	6	2.5	24.166 ± 1.579**
6	4d	6	2.5	24.166 ± 1.579**
7	4e	6	2.5	27.833 ± 1.887*
8	4f	6	2.5	24.434± 1.779**

Results are expressed as mean±SEM obtained from 6 animals. (*)-moderate, P < 0.05, (**)-significant, P< 0.001, when compared with normal control.

significant anti anxiety activities compared to the control. The dichloro benzaldehyde (5a), dimethylamino benzaldehyde (5b), vanillin (5c) and furfuraldehyde substituted mercapto triazoles showed significant antidepressant activity compared to the control.

DISCUSSION

We have synthesized benzoic acid hydrazide (compound 2) from methyl benzoate and hydrazine hydrate using absolute alcohol. The peak at 3300 of N-H str peak and peak at 1662 of amide C=O in IR spectra confirm the formation of compound 2. From the compound benzoic acid hydrazide, potassium dithiocarbazinate (compound-3) was synthesized. The compound-3 is confirmed by the presence of C=S str at 617 cm^{-1} in IR spectra. The potassium dithiocarbazinate was used to synthesize 4-[amino]-5-phenyl-l,4H-1,2,4-triazole-3-thiol by cyclisation process. The presence of N-C-S str at 943.3 cm^{-1}, N-N-C at 1278 cm^{-1}in IR spectra and the presence of SH and NH peak at 14.6 and 7.9 ppm in HNMR confirm the formation of the same. The nucleus 4-[amino]-5-phenyl-1,2,4-triazole-3-thiol was used to synthesise Schiff bases [5a-f]. The Schiff bases are confirmed by the absence of NH peak in IR spectra and the presence of N=CH at 10.3 ppm at NMR spectra. The compounds are bearing two important biological scaffolds 1,2,4-triazole and Schiff bases. The presence of -N-N-C- moiety along with mercapto group imparts activities. Also the

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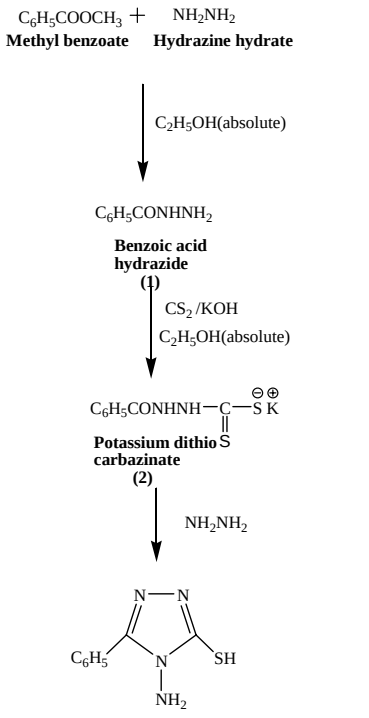
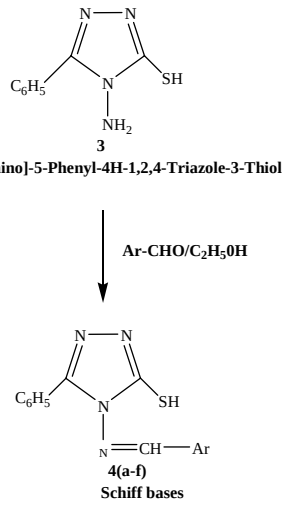


4[amino]-5-Phenyl-4H-1,2,4-Triazole-3-Thiol
(3)



aromatic/heterocyclic ring improves the CNS penetration. The Schiff bases are important class of compounds due to their flexibility, structural similarities with natural biological substances and also due to their presence of imine (-N=CH-) which imparts in elucidating the mechanism of transformation in biological system.

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