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Antimicrobial activity of some novel triazole-3-thione containing substituted piperazine moiety

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ABSTRACT

A novel series of triazole analogues possessing substituted piperazine were synthesized and their structures were elucidated using elemental analysis, IR, ^{1}H-NMR and mass spectral data. The synthesized compounds were tested for their in-vitro antimicrobial activity against the microbial strains i.e., Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Pseudomonas aeruginosa, Aspergillus niger and Candida albicans using disk diffusion method. Some of compounds displayed noticeable biological activity against all the pathogenic microorganisms tested including Pseudomonas aeruginosa and Candida albicans responsible for nosocomial infection. Structure activity relationship among the synthesized compounds was also studied.

Key words: benzimidazole, Piperazine, antimicrobial activity.

INTRODUCTION

Over the past few decades, gradually increasing drug resistance in the treatment of infectious disease indicate a crucial problem in antimicrobial therapy and necessitates continuing research into novel classes of antimicrobials [1]. Literature is flooded with antimicrobial properties of triazoles [2-4]. Keeping the above facts in view, we considered it of interest to synthesize some novel triazoles analogues for their antimicrobial activity.

MATERIALS AND METHODS

Chemistry

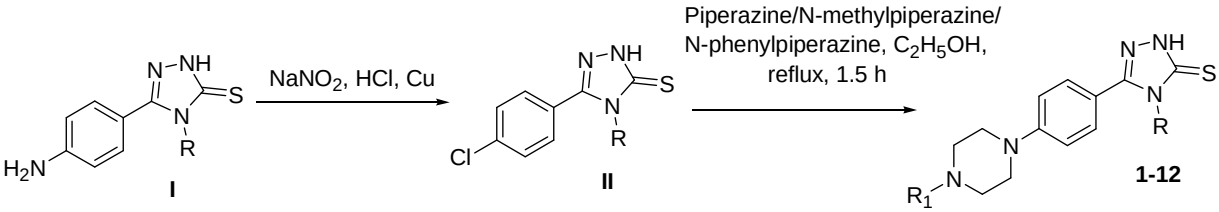
All the chemicals and solvents used in this study were purchased from Fisher-Scientific, Himedia and Sd-fine Chemicals Limited. Melting points were determined by open capillary method and are uncorrected. Elemental analysis was done using an elemental analyzer Heraeus Carlo Erba-1108, IR spectra were recorded on a Perkin Elmer IR spectrophotometer (KBr disc), NMR spectra on a Bruker DRX-300 NMR spectrometer (DMSO-d_6, TMS) and the electrospray mass spectra on a Micromass Quattro II triple-quadrupole mass spectrometer (Methanol).

The title compounds were prepared using the synthetic strategy depicted in Figure 1. 5-(4-Amino-phenyl)-4-substituted-2,4-dihydro-[1,2,4]triazole-3-thione **I** was prepared according the reported procedure [5-7]. 5-(4-chloro-phenyl)-4-methyl-2,4-dihydro-[1,2,4]triazole-3-thione **II** was obtained by diazotization of **I** in hydrochloric acid in the presence of copper powder. In the last step, compound **II** was refluxed with piperazine or substituted-piperazine in the presence of ethanol resulted in the formation of title compounds **01-12**.

General procedure for synthesis of 5-(4-chloro-phenyl)-4-methyl-2,4-dihydro-[1,2,4]triazole-3-thione **II:** The equimolar quantities of compound **II** and $NaNO_2$ were reacted in a round bottomed flask placed in ice-bath in the presence of hydrochloric acid and Cu powder with constant stirring. The crude product obtained was recrystallized with ethanol.

General procedure for synthesis of [5-(4-substituted-piperazin-1-yl)-phenyl]-4-substituted-2,4-dihydro-[1,2,4]triazole-3-thione **01-20**: The reaction mixture comprising of compound **II** and suitable substituted piperazine were refluxed for 2 h. After cooling to room temperature, water was added to reaction mixture resulted in precipitation of compound, which was washed with water filtered, recrytallized with ethanol.

Figure 1: Scheme for synthesis of Triazole-3-thiones.



Compd Code	R	R_1	Compd Code	R	R_1
1	CH_2CH_3	H	7	CH_2-CH=CH_2	C_6H_5
2	CH_2CH_3	CH_3	8	CH_2-CH=CH_2	$COCH_3$
3	CH_2CH_3	C_6H_5	9	C_6H_5	H
4	CH_2CH_3	$COCH_3$	10	C_6H_5	CH_3
5	CH_2-CH=CH_2	H	11	C_6H_5	C_6H_5
6	CH_2-CH=CH_2	CH_3	12	C_6H_5	$COCH_3$

Compound 3: MP (°C) 208-209°C; yield 60%; IR (cm^{-1}) (KBr) 3038.2 (Aromatic C-H str), 1603.3 & 1502.8 (Aromatic C-C str), 1618.3 (C=N group of triazine), 1239.6 (C=S group of triazine), 2926.8 (aliphatic C-H str), 1445.2 (aliphatic C-H def); ^{1}H NMR (300 MHz, DMSO-d_6,

TMS, δ ppm): 6.42-7.15 (m, 9H, ArH), 7.22 (s, 1H, NH), 3.48 (t, 4H, CH_2 of Piperazine towards benzene), 2.56 (t, 4H, CH_2 of Piperazine towards ethyl group), 1.14 (t, 3H, $C\underline{H}_3CH_2$), 2.37 (m, 2H, $CH_3C\underline{H}_2$); ESI-MS (Methanol) *m/z* 366.17 ($[M+H]^+$)

Compound 7: MP (°C) 226-228°C; yield 58%; IR (cm^{-1}) (KBr) 3040.6 (Aromatic C-H str), 1601.5 & 1502.7 (Aromatic C-C str), 1618.9 (C=N group of triazine), 1238.5 (C=S group of triazine), 3020.4 (alkene C-H str); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.41-7.18 (m, 9H, ArH), 7.25 (s, 1H, NH), 3.44 (t, 4H, CH_2 of Piperazine towards benzene), 2.58 (t, 4H, CH_2 of Piperazine towards ethyl group), 5.18 (d, 2H, $C\underline{H}_2$=CH-CH_2), 5.78 (t, 1H, CH_2=C$\underline{H}$-CH_2), 3.08 (d, 2H, CH_2=CH-$C\underline{H}_2$); ESI-MS (Methanol) *m/z* 378.17 ($[M+H]^+$)

Compound 11: MP (°C) 250-252°C; yield 65%; IR (cm^{-1}) (KBr) 3038.3 (Aromatic C-H str), 1602.5 & 1502.0 (Aromatic C-C str), 1618.3 (C=N group of triazine), 1238.8 (C=S group of triazine); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.45-7.13 (m, 14H, ArH), 7.26 (s, 1H, NH), 3.48 (t, 8H, CH_2 of Piperazine); ESI-MS (Methanol) *m/z* 414.17 ($[M+H]^+$)

Antimicrobial activity

The antimicrobial properties of the compounds were investigated against bacterial strains *i.e.*, *Proteus mirabilis* (MTCC-425), *Pseudomonas aeruginosa* (MTCC-424), *Bacillus subtilis* (MTCC-619), and *Staphylococcus aureus* (MTCC-96) and fungal strains *i.e.*, *Aspergillus niger* (MTCC-1344) and *Candida albicans* (MTCC-227) using disk diffusion method [8, 9]. (Table 1) Nutrient Agar [10] was employed as culture media for antibacterial studies. For antimycotic evaluation against *Aspergillus niger*, Czapek yeast extract agar [10] was employed. Malt yeast Agar [10] with pH 7.0 was employed as culture media in antimicrobial studies against *Candida albicans*. Norfloxacin and Clotrimazole were used as standard drug for antibacterial and antifungal studies respectively. The sterilization of the culture medias, petridishes and other glasswares was done by autoclaving at 15 1b/sq inch pressure for 30 min. For antibacterial studies, incubation was carried out at 37$\pm$1ºC for 48 h except for *Bacillus subtilis* where incubation was carried out at 26$\pm$1º C for similar time period. Incubation conditions for *Aspergillus niger* and *Candida albicans* was 25 $\pm$ 1ºC for 72 h.

The cell density of each inoculum was adjusted with hemocytometer in order to procure a final concentration of approximately 10^5 CFU ml^{-1}. During antimicrobial evalution the medium after sterilization was poured into sterile petridishes under aseptic conditions in a laminar flow chamber. When the medium in the plate solidified, 0.5 ml of (10^5 CFU ml^{-1}) culture of test organism was inoculated and uniformly spread over the agar surface using a sterile L-shaped glass rod. Solutions of the test compound (100 µg/ml) were prepared by dissolving the test compound in dimethyl formamide (DMF). The sterile filter paper disc (8mm diameter) were moistened with the test compounds solution in DMF of specific concentration (100µg/disc) placed on the agar culture plates that had been previously inoculated with specific microorganisms. Controls were maintained with DMF. Inhibition zones were measured and the diameter was calculated in millimeters. All the tests were performed in triplicate for determination of MIC's.

Table 1. Antimicrobial activity[#] of the synthesized compounds using disc-diffusion method (100 µg/ 8mm disc)

Compound	Zone of Inhibition (mm)					
	Antibacterial activity				Antifungal activity	
	SA	BA	PM	PA	AN	CA
1.	12	11	12	-	12	-
2.	12	11	13	11	12	11
3.	16	15	16	15	14	16
4.	12	13	-	13	-	13
5.	14	13	-	15	-	12
6.	14	13	11	-	13	-
7.	15	14	14	14	11	12
8.	15	14	15	14	13	13
9.	14	15	13	12	13	13
10.	15	14	15	14	14	14
11.	17	14	16	17	15	17
12.	14	13	14	14	13	15
Norfloxacin	24	17	22	20	NT	NT
Clotrimazole	NT	NT	NT	NT	21	23

NT= Not tested.

- (dash) = No activity

[#]Microbial strains were procured from Institute of Microbial Technology (IMTECH) Chandigarh, INDIA.

Abbreviations: SA = Staphylococcus aureus; BA = Bacillus subtilis; PM = Proteus mirabilis; PA= Pseudomonas aeruginosa; AN = Aspergillus niger; CA = Candida albicans.

RESULTS AND DISCUSSION

The structures of the compounds were elucidated on the basis of elemental analysis, IR, ^{1}H-NMR and mass spectroscopy. The results of elemental analysis (C, H, N estimation) were found to be within ± 0.4% of the theoretical values. All the final compounds have strong absorption around 3045 cm^{-1} which is evidence for the presence of aromatic C—H bonds. Presence of aromatic C—C bonds was confirmed by presence of absorption band around 1602 and 1502 cm^{-1}. IR data also confirms the presence of specific functional groups present in the final synthesized compounds. The chemical shift of all other carbons of final compounds was seen as expected. The mass spectra of title compounds were in conformity with the assigned structure.

Out of all the compounds evaluated for antimicrobial studies, compound no. **11** showed appreciable antibacterial activity against all six microbial strains used (zone of inhibition in disk diffusion method- 17 mm against *Staphylococcus aureus*, 14 mm against *Bacillus subtilis*, 16 mm against *Proteus mirabilis*, 17 mm against *Pseudomonas aeroginosa*, 15 mm against *Aspergillus niger* and 17 mm against *Candida albican*). On comparison of results it has been found that antimicrobial activity of test compounds changes on varying R group attached to triazole as follows: C_6H_5 > CH_2CH_3 > CH_2-CH=CH_2. On the other hand, in terms of R_1 substitutions of piperazine antimicrobial activity of synthesized compounds can be arranged in the following way: C_6H_5 > CH_3 > $COCH_3$. Compounds with phenyl ring were found to possess considerable activity in comparison to methyl and other groups. The considerable antimicrobial activity of active compounds may be attributed to the high lipophilicity due to presence of phenyl substitutions that might have facilitated better penetration of the compounds inside the microbial strains.

CONCLUSION

A novel series of triazole-3-thiones possessing substituted piperazine were synthesized for their antimicrobial activity. In the present studies, *[5-(4-phenyl-piperazin-1-yl)-phenyl]-4-phenyl-2,4-dihydro-[1,2,4]triazole-3-thione* came out as the most active compound, exhibiting antimicrobial activity all the microbial strains tested. The results obtained showed that the majority of the compounds exhibited antimicrobial activity. These new data might be beneficial in the future development of triazole-3-thiones as novel antimicrobial agent.

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