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Synthesis, Characterization and Evaluation of Anti-Microbial Activity of Some Novel 1,2,4-Triazoles.

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ABSTRACT

Some novel 4-(aryliidineamino)-5-(1-(4-isobutylphenyl) ethyl)-4-yl-4H-1,2,4-triazole-3-thiols were prepared from the reaction of carbon-di-sulphide and hydrazine hydrate in water to produce thiocarbohydrazides. The thiocarbohydrazides is then refluxed with ibuprofen for 2 hour, followed by cooling to room temperature and washing with sodium bicarbonate solution to produce 5-(1-(4-isobutylphenyl) ethyl)-4H-1,2,4- triazole-3-thiole. It is then refluxed with different aldehydes in the presence of ethanol and HCl to produce the title compounds. The synthesized compounds are recrystallized from ethanol and are analyzed for their physical and spectral data. They are screened for their anti-microbial activity and it was found that the title compounds are found to have moderate to good antimicrobial activity.

Keywords: 1,2,4-triazoles, spectral analysis, anti-microbial activity.

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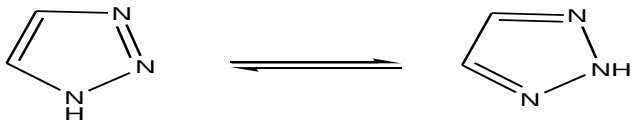
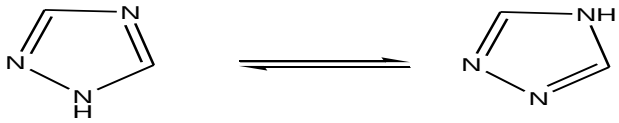
INTRODUCTION

Nowadays research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. The heterocycles are enjoying their importance as being the centre of activity. The nitrogen containing heterocycles are found in abundance in most of the medicinal compounds. The success of the imidazole as an important moiety of number of medicinal agents led to the introduction of the triazoles. The triazoles are said to be the isosters of imidazoles in which the carbon atom of imidazole is isosterically replaced by nitrogen. Triazoles are 5 membered rings, which contain two carbon and three nitrogen atoms. According to the position of nitrogen atoms, the triazoles exist in isomeric forms.

Two structural isomeric triazoles are known , the 1,2,3-(1,2,5) and the 1,2,4-(1,3,4), the former being known as osotriazole, and the latter as triazole. Each exists in two disimilar tautomeric forms. The different isomers are characterized by the position of the nascent hydrogen. Thus, 1,2,4-triazoles exist in two isomeric forms i.e. 1H and 4H.



osotraiazoles



triazoles

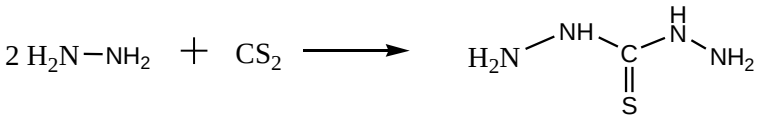
Compounds containing triazole nucleus finds a unique place in medicinal chemistry and play a significant role as they are associated with immense biological activity. Triazole derivatives have gained considerable attention owing to their effective biological activity and extensive use. A survey of literature reveals that 1,2,4-triazole derivatives are known for their biological activities like antibacterial[1], antifungal[2], anti-inflammatory[3], analgesic[4], anticonvulsant[5], diuretic[6], antitb[7], anti tumor[8] etc

In the present work, our aim was to incorporate 1, 2, 4-triazole moiety in the side chain of Ibuprofen, so that the synergistic anti-inflammatory and analgesic activity was achieved with less adverse effects.

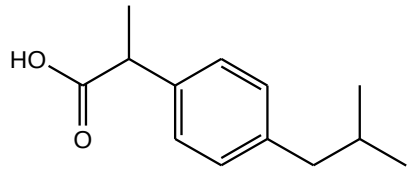
MATERIALS AND METHODS [9-14]

Synthetic scheme:

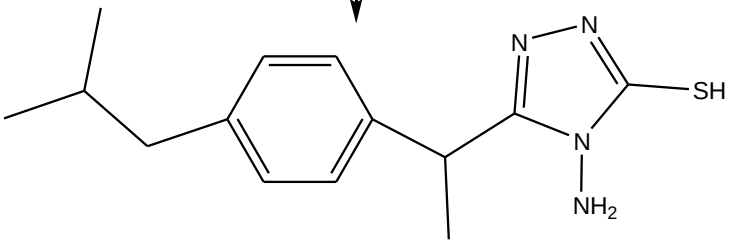
The Reaction Scheme is given in figure 1



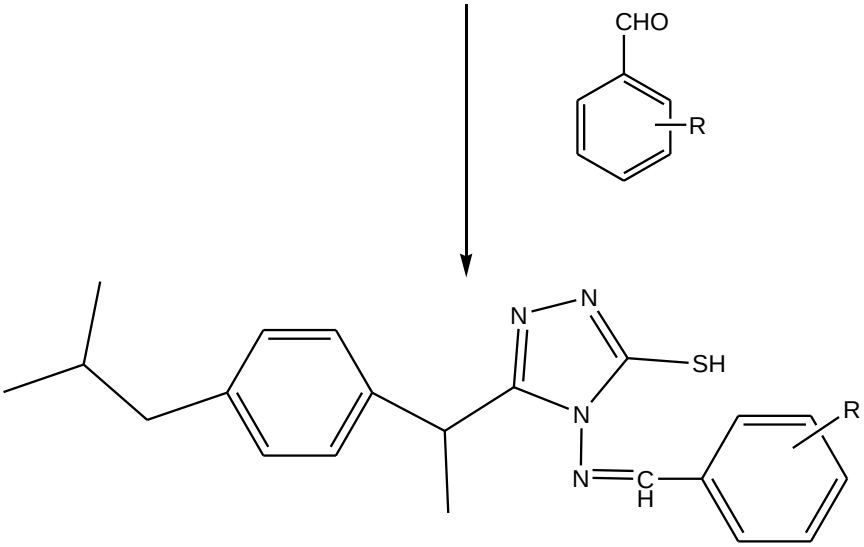
Thiocarbohydrazide
(1)



Ibuprofren



5-(1-(4-isobutylphenyl) ethyl)-4-amino-4H-1,2,4-triazole-3-thiol
(2)



4-(Arylidineamino)-5-(1-(4-isobutylphenyl) ethyl)-4-amino-4H-1,2,4-triazole-3-thiol
(3)
[T_1 to T_7]

Experimental Work

I.Synthesis of Thiocarbohydrazide

0.2 mole (12.6 ml) of carbon disulphide was added drop wise to vigorously stirred solution of hydrazine hydrate (95%) in water during 40-45 minutes. Then the temperature of the reaction was raised to 65˚ C. the reaction mixture was zapped inside a domestic microwave oven for 3minutes at 210 watts, then cooled to 0˚ C. the precipitated thiocarbohydrazide was filtered,

washed with ethanol followed by diethyl ether and then air dried. the product thus obtained was recrystallized from minimum amount of hot water containing a few drops of concentrated hydrochloric acid.

II. Synthesis of 5-(1-(4-isobutylphenyl) ethyl)-4H-1,2,4- triazole-3-thiole.

A well triturated mixture of ibuprofen (0.01 mol, 2.06 g) and Thiocarbohydrazide (0.01 mol, 1.06 g) was fused in a RB flask for 1 hour. Then it was colled to room temperature and washed with 5 % sodium bicarbonate solution to remove unreacted acid and again washed with water. The dried compound was recrystallized with ethanol. Yield: 76.55%

III. Synthesis of 4-(aryliidineamino)-5-(1-(4-isobutylphenyl) ethyl)-4-yl-4H-1,2,4-triazole-3-thiol (Schiff's Bases)

Equal mole of triazole and corresponding aldehydes in 25ml ethanol was treated with 0.5ml concentrated HCl and refluxed for 2 hour. After cooling the reaction mixture was filtered, air dried and recrystallized from ethanol.

ANTI-BACTERIAL SCREENING [15]

Agar Well Diffusion Method

The in-vitro antimicrobial activity of the target compounds was performed by agar well method (diffusion technique) against S. aureus and E. coli. The antibiotics Ertapenam, Netilmycin and Streptomycin were used as standard drugs for the study.

250 ml of the standard agar medium taken in a conical flask was allowed to soak for 5 minutes and then autoclaved for 15 minutes at 120˚ C and then poured in 20 ml quantity into previously washed and sterilized petri dishes. The fresh bacterial culture was obtained by inoculating bacteria into peptone water liquid and incubating at 37 ± 2˚ C for 18-24 hours. After culture media solidification, bacterial culture was introduced into the surface of sterile glass plates and a sterile glass spreader was used for even distribution of the inoculums. Then three wells are made at equal distances by using a sterile steel cork borer (8 mm diameter). Into these wells different concentrations of synthesized compounds were introduced. Similarly these wells were made in another plate for two standard drugs and a control. Dimethyl sulphoxide (DMSO) was used as control. After introduction of the synthesized compounds and standard antibiotics, the plates were placed in a refrigerator at 8-10˚ C for proper diffusion of drugs into the media. After two hours of cold incubation, the petri plates were transferred to incubator and maintained at 37 ± 2˚ C for 18-24 hours. After the incubation period, the petri plates were observed for growth inhibition zone by using the vernier scale. The results were evaluated by comparing the growth

inhibition zone shown by the synthesized compounds with standard drugs. The results are presented as the mean value of the growth inhibition zone measured in millimeters. The synthesized compounds were dissolved in minimum quantity of DMSO and adjusted to make up the volume with distilled water to get different concentrations.

RESULTS AND DISCUSSIONS:

Physical property data of oxadiazole derivatives.

Synthesized compounds were characterized by analytical and spectral analysis. The purity of the novel synthesized compounds was ascertained for consistency in melting point and R_f value TLC by Silica Gel G.

Code	Derivative s	Mol. Formula	Mol Wt.	Recrystallizing solvent	M.P. (˚C)	Yield.	R_f values.
T_1	benzyl	$C_{21}H_{24}N_4S$	364.518	ethanol	99-201	82%	0.41
T_2	4-methoxy phenyl	$C_{22}H_{26}N_4OS$	394.544	ethanol	192-195	85%	0.48
T_3	3,4,5-tri methoxy phenyl	$C_{24}H_{30}N_4O_3S$	454.596	ethanol	210-213	76%	0.54
T_4	4-chloro phenyl	$C_{21}H_{23}ClN_4S$	398.962	ethanol	180-182	69%	0.42
T_5	4-hydroxy phenyl	$C_{21}H_{24}N_4OS$	380.517	ethanol	196-198	66%	0.52
T_6	4-hydroxy-3-methoxy phenyl	$C_{22}H_{26}N_4O_2S$	410.543	ethanol	194-196	82%	0.51
T_7	4-dimethyl aminophenyl	$C_{23}H_{29}N_5S$	407.586	ethanol	230-232	74%	0.47

Characterization

Formation of 1,2,4-triazole derivatives (T_1 to T_7) was confirmed by IR, ^{1}HNMR and Mass spectral data. The characterization data of the synthesized compounds has been given below:

T_1: 4-(benzylideneamino)-5-(1-(4-isobutylphenyl)ethyl)-4*h*-1,2,4-triazole-3-thiol

MF: $C_{21}H_{24}N_4S$, MW: 364.518, M.P.: 199-201˚C, R_f : 0.41 (Benzene:Methanol - 8:2). IR(cm^{-1}): 3160.56 (C-H), 1538.58 (C-C, Ar-C), 2976.88 (C-H, Aliphatic), 1313.76 (C-N), 1612.52 (C=N), 1561.77 (N=N), 2578.52 (S-H), 615.06 (C-S).

^{1}HNMR (ppm):7.385-8.004 (m, Ar-H), 13.412 (s, SH, 1H), 9.835 (s, N=CH, 1H), 3.692-3.717 (m, CH, 1H), 2.485-2.505 (d, CH2, 2H), 2.401-2.085 (m, CH, 1H), 1.114-1.135 (d, CH3, 9H). Mass spectra: (M^+ peak) = 364.3590

T_2: 4-{(e)-[(4-methoxyphenyl)methylidene]amino}-5-(1-(4-isobutyl phenyl) ethyl)-4*h*-1,2,4-triazole-3-thiol.

MF: $C_{22}H_{26}N_4OS$, MW: 394.544, M.P.: 192-195˚C, R_f: 0.48 (Benzene:Methanol = 8:2). IR (cm^{-1}): 3155.68 (C-H, Ar-H), 1538.93 (C-C, Ar-C), 2993.32 (C-H, Aliphatic), 1309.77 (C-N), 1605.80 (C=N), 1571.44 (N=N), 2566.31 (S-H), 609.61 (C-S), 1243.99 (C-O-C).

T_3: 4-{(e)-[(3,4,5-trimethoxyphenyl)methylidene]amino}-5-(1-(4-isobutyl phenyl) ethyl)-4*h*-1,2,4-triazole-3-thiol

MF: $C_{24}H_{30}N_4O_3S$, MW: 454.596, M.P.: 210-213˚C, R_f: 0.54 (Benzene: Methanol = 8:2). IR(cm^{-1}): 3158.19 (C-H, Ar-H), 1538.70 (C-C, Ar-C), 2996.56 (C-H, Aliphatic), 1307.34 (C-N), 1573.99 (C=N), 1563.99 (N=N), 2573.15 (S-H), 595.57 (C-S), 1236.00 (C-O-C)

T_4: 4-{(e)-[(4-chlorophenyl)methylidene]amino}-5-(1-(4-isobutyl phenyl) ethyl)-4*h*-1,2,4-triazole-3-thiol

MF: $C_{21}H_{23}ClN_4S$, MW: 398.962, M.P.: 180-182˚C, R_f: 0.42 (Benzene : Methanol = 8:2) IR(cm^{-1}): 3192.49 (C-H, Ar-H), 1508.17 (C-C, Ar-C), 2957.56 (C-H, Aliphatic), 1327.30 (C-N), 1595.36 (C=N), 1560.17 (N=N), 2559.55 (S-H), 596.22 (C-S), 1083.73 (Chlorobenzene)

T_5: 4-{(e)-[(4-hydroxyphenyl)methylidene]amino}-5-(1-(4-isobutyl phenyl) ethyl)-4*h*-1,2,4-triazole-3-thiol

MF: $C_{21}H_{24}N_4OS$, MW: 380.517, M.P.: 196-198˚ C, R_f: 0.52 (Benzene : Methanol = 8:2) IR(cm^{-1}): 3148 (C-H, Ar-H), 1551.86 (C-C, Ar-C), 3001.98 (C-H, Aliphatic), 1330.38 (C-N), 1605.62 (C=N), 1551.86 (N=N), 2572.14 (S-H), 618.11 (C-S), 3351.72 (O-H), 1219.46 (C-O)

T_6: 4-{(e)-[(4-hydroxy-3-methoxyphenyl)methylidene]amino}-5-(1-(4-isobutyl phenyl) ethyl)-4*h*-1,2,4-triazole-3-thiol

MF: $C_{22}H_{26}N_4O_2S$, MW:410.543, M.P.: 194-196˚ C, R_f: 0.51 (Benzene : Methanol = 8:2) IR(cm^{-1}): 3109.62 (C-H, Ar-H), 1544.75 (C-C, Ar-C), 2835.98 (C-H, Aliphatic), 1315.23 (C-N), 1602.97 (C=N), 1585.73 (N=N), 2561.84 (S-H), 618.58 (C-S), 1260.88 (C-O-C), 3373.05 (O-H),1221.85(C-O).

^{1}HNMR (ppm): 6.777-8.540 (m, Ar-H, 7H), 13.499 (s, SH, 1H), 10.359 (s, N=CH, 1H), 11.618 (s, OH, 1H), 5.531 (s, OCH_3, 3H), 3.831-3.871 (m, CH, 1H), 2.442-2.462 (d, CH_2, 2H), 1.816-1.897 (m, CH, 1H), 1.091-1.112 (d, CH_3, 9H). Mass Spectra (M^+ peak): 410.3684

T_7: 4-{(e)-[(4-(dimethylamino)phenyl)methylidene]amino}-5-(1-(4-isobutyl phenyl) ethyl)-4*h*-1,2,4-triazole-3-thiol

MF: $C_{23}H_{29}N_5S$, MW: 407.586, M.P.: 230-232˚ C, R_f: 0.47 (Benzene : Methanol = 8:2)

IR(cm^{-1}): 3292.49 (C-H, Ar-H), 1515.28 (C-C, Ar-C), 2969.06 (C-H, Aliphatic), 1300.65 (C-N), 1593.85 (C=N), 1553.66 (N=N), 2568.07 (S-H), 594.56 (C-S).

Antibacterial Activity Screening

The novel derivative of 1, 2, 4-triazole i.e. compound T6 was screened for anti-bacterial activity against *E. coli* (Gram negative) and *S. aureus* (Gram positive) using Agar well diffusion method. Mean zone of inhibition of compound T6 was compared with different concentration of standard drugs like Ertapenam (10 mcg/disc), Netilmicin (30cg/disc) and Streptomycin (100 mcg/ml) and DMSO as the control.

Table 2: Data of the Antibacterial Activity of Compound T6

Compound	Dose (µg/ml)	Mean zone of inhibition (mm)	
		S. aureus	E. coli
T_6	50	14	ND
	100	21	ND
	150	25	29
	300	ND	33
	500	ND	39
Ertapenam (10mcg/disc)		43	36
Netilmicin (30mcg/disc)		29	21
Streptomycin (100mcg/ml)		24	23
DMSO		-	-

ND- Not Determined

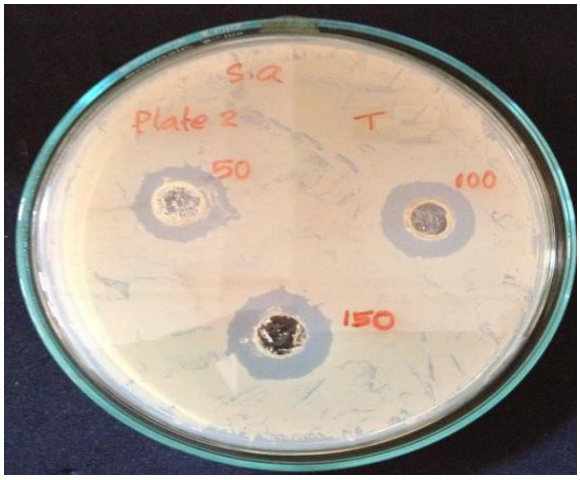


Figure:1: Antibacterial activity of T6 against E. coli.

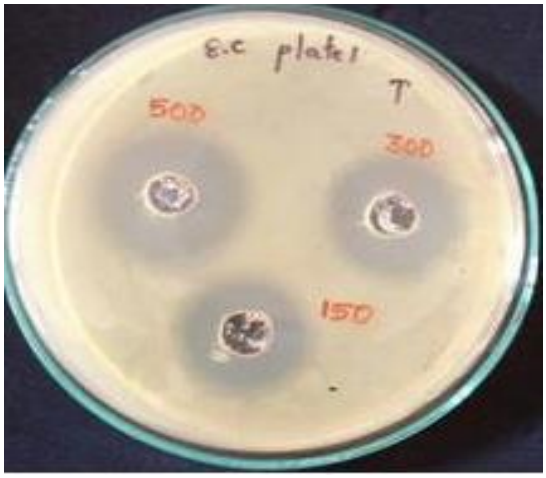


Figure: 2: Antibacterial activity of T6 against E. coli.

CONCLUSION

Seven different novel derivatives of 1, 2, 4-triazole were synthesized by reaction with seven different aromatic aldehydes. The yield of all the synthesized compounds was found to be in the range of 69-82 %. The titled compounds were characterized by pysico-chemical parameters like melting point and R_f value. The structure of all the synthesized compounds was characterized by IR, NMR and Mass spectra. The spectral data also supported the assigned structure by showing the characteristic absorption peaks.

The test compound i.e. T_6 was found to be significantly active towards Gram positive bacteria at a concentration of 150 mcg/ml as compared to Streptomycin which is active at a concentration of 100 mcg/ml. Also T_6 showed significant activity towards Gram negative bacteria at a concentration of 150 mcg/ml as compared to Streptomycin which showed activity at a concentration of 100 mcg/ml. Antibacterial activity of T_6 was found to be less as compared to standard drugs Ertapenam and Netilmycin.

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