10.37021/ijper.v1i2.5

RESEARCH ARTICLE

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

Alex Martin*

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-sulfide and hydrazine hydrate in water to produce thiocarbohydrazides. The thiocarbohydrazides is then refluxed with ibuprofen for 2 hours, 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.

How to cite this article: Martin, A. (2019). Synthesis, Characterization, and Evaluation of Anti-Microbial Activity of Some Novel 1,2,4-Triazoles. Int. J. Pharm. Edu. Res., 1(2):47-51.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Nowadays, research is concentrated on the introduction of new and safe therapeutic agents of clinical importance. The heterocycles are enjoying their importance as being the center 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 a number of medicinal agents led to the introduction of the triazoles. The triazoles are said to be the isosteres of imidazoles in which the carbon atom of imidazole is isostatically replaced by nitrogen. Triazoles are five-membered rings, which contain two carbon and three nitrogen atoms. According to the position of nitrogen atoms, the triazoles exist in isomeric forms.

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

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 the 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, we aimed 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.

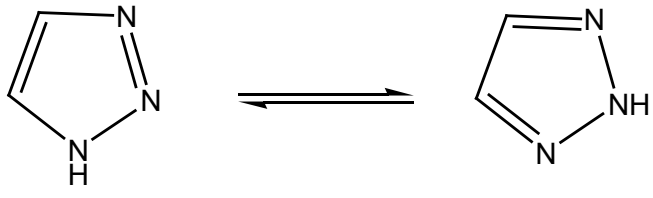
MATERIALS AND METHODS [9-14]

Synthetic scheme: The Reaction Scheme is given in Figure 1:

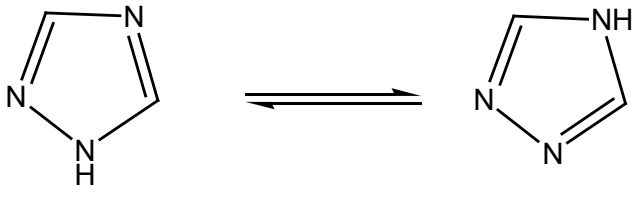
EXPERIMENTAL WORK

Synthesis of Thiocarbohydrazide

0.2 mole (12.6 ml) of carbon disulfide was added dropwise to vigorously stirred solution of hydrazine hydrate (95%) in water during 40-45 minutes. Then



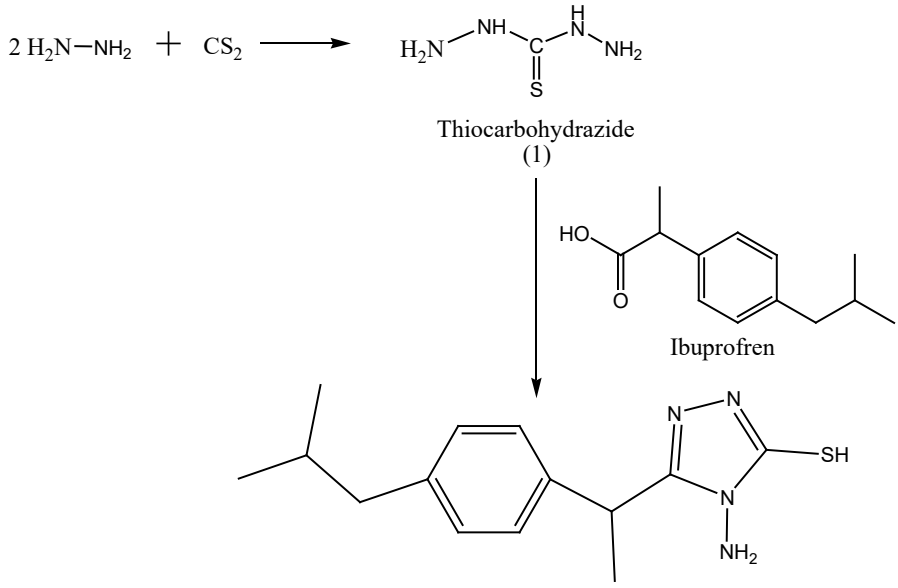
osotraiazoles



triazoles

Department of Pharmaceutical Chemistry, Ram-Eesh Institute of Vocational and Technical Education, Dr. A.P.J. Abdul Kalam Technical University, Greater Noida, India.

Corresponding Author: Alex Martin, Department of Pharmaceutical Chemistry, Ram-Eesh Institute of Vocational and Technical Education, Dr. A.P.J. Abdul Kalam Technical University, Greater Noida, India. e-mail id: aalexmartin@rediffmail.com



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]

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 a minimum amount of hot water containing a few drops of concentrated hydrochloric acid.

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%

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

An equal mole of triazole and corresponding aldehydes in 25ml ethanol was treated with 0.5ml concentrated HCl and refluxed for 2 hours. 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 the 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 on another plate for two standard drugs and control. Dimethyl sulphoxide (DMSO) was used as control. After the 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 the 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 a 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.

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)-4h-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)-4h-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)-4h-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)-4h-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)-4h-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

Table 1

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-chlorophenyl	$C_{21}H_{23}ClN_4S$	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

Table 2: Data of The Antibacterial Activity of Compound T6

		Mean zone of inhibition (mm)	
Compound	*Dose (µg/ml)*	*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

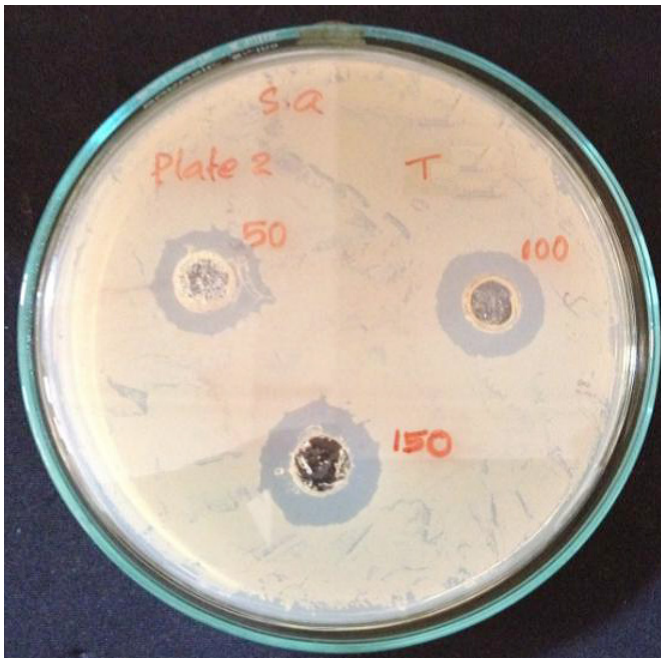


Figure 1: Antibacterial activity of T6 against E. coli

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

(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)-4h-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)-4h-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 were screened for antibacterial activity against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) using Agar well diffusion method.

The mean zone of inhibition of compound T6 was compared with different concentrations of standard drugs like Ertapenam (10 mcg/disc), Netilmicin (30cg/disc) and Streptomycin (100 mcg/ml) and DMSO as the control.

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.

ACKNOWLEDGMENT

The authors are thankful to Prof. Dr. Jainendra Jain, Principal, Dept. of Pharmaceutical Chemistry, Ram-Eesh Institute of Vocational and Technical Education, Greater Noida, for providing Laboratory facilities, guidance and financial support.

REFERENCES

1. Rao G, Rajasekran S, Attimarad M. Synthesis and Antimicrobial activity of Some 5-phenyl-4- substituted amino-3-mercapto (4H) 1,2,4-triazoles. *Ind J Pharm Sci*. 2000; 6:475-479.
2. Jalilian AR, Sattari S, Bineshmarvasti M, Shafiee A, Daneshtalab M. Synthesis and in vitro antifungal and cytotoxicity evaluation of thiazolo-4H-1,2,4-triazoles and 1,2,3-thiadiazolo-4H-1,2,4-triazoles. *Arch Pharm (Weinheim).* 2000; 333:347-54.
3. Winter CA, Risley EA, Nuss GW. Carageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med*. 1962; 111:544–547
4. Hunashal RD, Ronad PM, Maddi VS, Satyanarayana D, Kamadod MA. Synthesis, anti inflammatory and analgesic activity of 2-[4-(substituted benzylideneamino)-5-(substituted phenoxy methyl)-4H-1,2,4-triazol-3-yl-thio] acetic acid derivatives. *Arab J Chem.* 2011; 1-9.
5. Chimirri A, Bevacqua F, Gitto R, Quartarone S, Zappala MD, Sarro A. Synthesis and anticonvulsant activity of new 1-H-triazolo[4,5-c]-2,3-benzodiazepines. *Med Chem Res* 1999; 9:203-12.
6. Zhou, S. N.; Zhang, L. X.; Jin, J. Y.; Zhang, A. J.; Lei, X. X.; Lin, J. S.; He, J.W.; Zhang, H. L. Synthesis and Biological Activities of Some Novel Triazolothiadiazines and Schiff Bases Derived from 1, 2, 4-Triazole. *Phosphorus, Sulfur Silicon Relat. Elem.* 2007; 182:419-432.
7. Pathak US, Rathod IS, Jain KS, Laddha NS, Kolhe KS. Synthesis of novel 1,2-disubstituted -6, 7-dimethyl-1*H*, 5*H*-thieno[2, 3-*d*]-1, 2, 4-triazolo[1,5-*a*]pyrimidin-5-ones. *Indian J. Chem*. 1997; 36B:566-571.
8. Holla, B. S.; Rao, B.S.; Sarojini, B. K.; Akberali P. M.; Kumari, N. S.Synthesis and studies on some new fluorine containing triazolothiadiazines as possible antibacterial, antifungal and anticancer agents. *Eur. J. Med. Chem.,* 2006; *41*:657-663.
9. Audrieth LF, Scott ES, Kippur PS. Hydrazine derivatives of the carbonic and thiocarbonic acids: The preparation and properties of Thiocarbohydrazide. *J Org Chem*. 1954; 19: 733-741.
10. Rao GK, Sen S, Rajendra B. Synthesis of 3-(4-chlorophenoxymethyl) -6-substituted phenyl-5,6-dihydro-s-triazolo-(3,4-b)-1,3,4-thiadiazoles as antimicrobial agents. *Indian Drugs*. 2004; 41(9):524-527.
11. Radha Rani B, Bahalerao UT, Rahman MF. Synthesis and biological activity of benzothiozolomethyl-oxadiazoles, -thiadiazoles, -triazoles. *Indian Journal of Chemistry*. 1990; 29B:995-998.
12. Ibrahim A, Rahman A, Abdu E. Synthesis of Certain 3-Pyridyl-1,2,4-triazolo-[3,4-B] -1,3,4-Thiadiazepines and Evaluation of their Possible Biological Activities. *Chem. Commun*. 1991; 56:1749-1756.
13. Holla BS, Veerendra B, Shivananda MK , Poojary B. Synthesis and characterization and anticancer activity studies on some Mannich bases derived from1,2,4 - triazoles. *Eur. J. Med. Chem.*. 2003; 38:759-767.
14. Wu J, Liu X, Cheng X, Cao Y, Wang D and Li Z. Synthesis of novel derivatives of 4-amino-3-(2-furyl)-5-mercapto-1,2,4-triazole as potential HIV-1NNRTIs. *Molecules*. 2007; 12:2003-2016.
15. Gaud RS, Gupta GD. *Practical Microbiology*, 3rd edition, Nirali Prakashan, Pune.2004; 111.