SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF 2-(5-MERCAPTO-3-SUBSITUTED-1,5-DIHYDRO-[1,2,4]TRIAZOLE-4-YL)-ISOINDOLE-1, 3-DIONE

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ABSTRACT

Nitrogen containing heterocycles have got special attention in Pharmaceutical Chemistry due to their excellent Medicinal potential. The 1,2,4-triazole nucleus is associated with diverse pharmacological activities such as antibacterial, antifungal, hypoglycemic, antihypertensive, analgesic, anti-inflammatory, anti-tumor, anti-viral, urease inhibition and many other properties. The increasing importance of 1,2,4-Triazoles as potent biologically active agents prompted authors to synthesize new series of 4-Amino-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiols.

Firstly some 4-Amino-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol were synthesized by hydrazide of benzoic acid and its different derivatives and then these 1,2,4-triazole derivatives reacted with pthalic anhydride to obtain different 2-(5-Mercapto-3-subsituted-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione. The physicochemical properties such as physical appearance, melting point, solubility and spectral analysis such as ^{1}H NMR, Mass, IR of synthesized compound done for structural confirmation. Antimicrobial activity of these synthesized compounds was carried out against several strains of bacteria and fungi by Zone diffusion method. These compounds possess good antimicrobial activity. Amikacin and fluconazole were used as standard drug for antimicrobial activity.

Key words 1,2,4-triazole, synthesis, Antimicrobial, substitution, triazole-3-thiols

INTRODUCTION

Now a days research for new drug discovery concentrated towards the development of safe therapeutic agents with clinical importance. In recent year heterocyclic compounds analogues and derivatives have attracted strong interest due to their useful biological and pharmacological properties. Azoles are important five membered heterocyclic rings containing at least one nitrogen atom like Isoxazole, Thiazole, Pyrazole and Triazole. 1,2,4 triazoles are one of the important moiety of medicinal agents which fulfill the requirements of new drug discovery. The important class of compounds formed by 1,2,4-triazole and its derivatives exhibit a broad spectrum of biological activities depending on the substitution pattern around the ring [1–5]. 1,2,4-Triazoles and their derivatives constitute an important class of organic compounds with diverse agricultural, industrial and biological activities including anti-microbial, sedative, anti-convulsant, anti-inflammatory and other properties, and consequently the synthesis of these heterocycles has received considerable attention in recent years. Some of the present day drugs such as Ribavirin (antiviral agent), Rizatriptan (antimigraine agent), Alprazolam (anxiolytic agent), Fluconazole and Itraconazole (antifungal agents) are the best examples for potent molecules possessing triazole nucleus [6-14].

Thus, in continuation to our lasting interest towards chemistry and pharmacological properties of 1,2,4-triazole in present study, we have designed and synthesized a series of 1,2,4-triazole derivatives having different functionality and determined their antimicrobial activity against various Gram +ve, Gram-ve bacteria and fugal strains.

MATERIAL AND METHODS

All the chemicals used are LR grade of Loba chemicals and Merk. Melting point of synthesized compounds were determined using Melting point

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apparatus and were found uncorrected. Purity of compound checked by thin layer chromatography using silica gel G as stationary phase and various combinations of Benzene: acetone, as mobile phase. The spot resolved were visualized as brown colored spots by using iodine chamber. The techniques employed for the characterization of the synthesized compounds were IR spectra, 1H-NMR spectra, MASS spectra. The IR spectra of synthesized compounds were recorded using KBr pellets in range of 4000-400 cm^{-1} on a Fourier Transform IR Spectrometer and the frequency were recorded in wave number. ^{1}H-NMR and ^{13}C NMR Spectra of synthesized compounds were recorded on DRX-300 MHz Bruke. Mass Spectra of synthesized compounds were recorded in range of 10-1000 Dalton on a DARTMS and provided in [M+H] +ion.

Synthesis

Synthesis of methyl benzoate (2a-2h)

Benzoic acid (0.01 mole) in 20 ml of methanol and 0.5 ml conc. Sulfuric acid was refluxed for 12 hrs. and poured into ice. The product was isolated and treated with standard sodium bicarbonate solution to give desired compounds.

Synthesis of benzoic acid hydrazide (3a-3h)

A mixture methyl benzoate (0.01 mole) and hydrazine hydrate (0.5 g, 0.01 mole) was heated for 9 hrs. and poured into ice. The product was isolated and crystallized from ethanol.

Synthesis of potassium-benzoic acid hydrazide dithiocarbamate (4a-4h)

A mixture of benzoic acid hydrazide (0.01 mole), KOH (0.84 g, 0.015 mole) and 1.5 ml CS2 in absolute alcohol was stirred for 21 hrs. and product was isolated from diethyl ether.

Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole- 3-thiol (5a-5h)

Potassium salt (0.01 mole) was taken in hydrazine hydrate and heated up to the evolution of H2S gas cussed nearly 5 hrs. in water bath. The reaction mixture was poured into crushed ice and treated withnglacial Acetic acid. The product was filtered and purified by KOH treatment and crystallized from ethanol.

Synthesis of 4-Amino-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiols (6a-6h)

A mixture of triazole (0.01 mol) and phthalic anhydride (0.01 mol), in butanol (20 mL) was heated under reflux for 4h. Then the solution was concentrated . A solid product was obtained by filtration which was crystallized from ethanol.

Anti microbial activity: Antimicrobial activity of compounds were done by Agar Diffusion Method with Standard cultures of Gram positive bacteria viz *Staphylococcus aureus, Bacillus subtilis* and *Bacillus cerus* and Gram negative bacteria viz *Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa* species and 2 fungal strains of *A. fumigatus, C. albicans* were obtained from Department of microbiology, SHIATS, Allahabad.

RESULT AND DISCUSSION

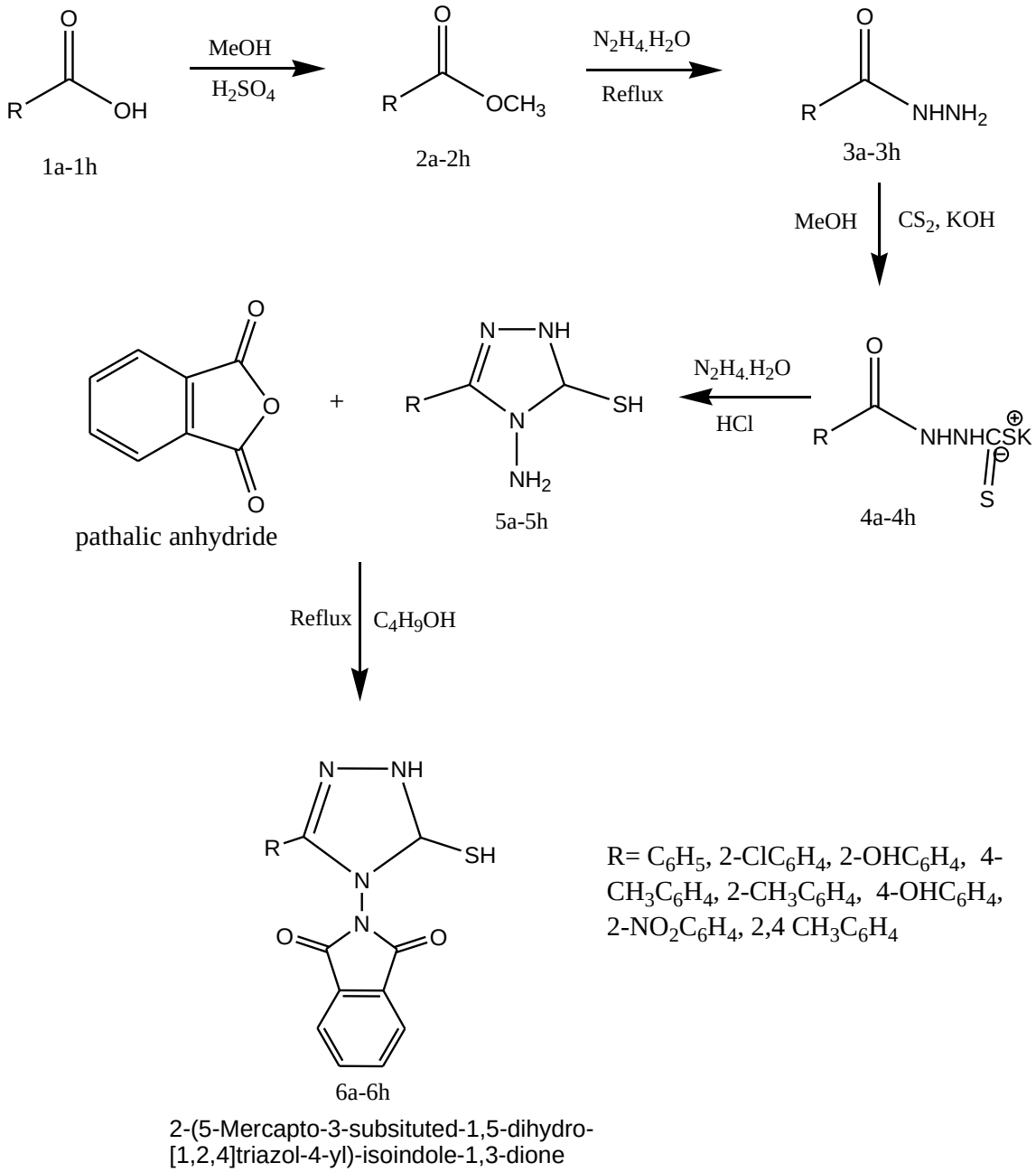
In present work firstly some 4-Amino-5-

Table 1. properties of synthesized compounds

COMPOUND	MOL. FORM.	MELTING POINT (°C)	MOL. WT.	% YIELD
6a	$C_{16}H_{12}N_4O_2S$	262	324	87
6b	$C_{16}H_{11}ClN_4O_2S$	210	358.8	58
6c	$C_{16}H_{12}N_4O_3S$	192	340	59
6d	$C_{17}H_{14}N_4O_2S$	268	338	57
6e	$C_{16}H_{11}N_5O_4S$	260	369	59
6f	$C_{17}H_{14}N_4O_2S$	252	338	88
6g	$C_{16}H_{12}N_4O_3S$	276	340	59
6h	$C_{18}H_{16}N_4O_2S$	260	516.9	82

subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol(5a-5h) were synthesized by hydrazide of benzoic acid and its different derivatives and then these 1,2,4-triazole derivatives reacted with pthalic anhydride to obtain different 2-(5-Mercapto-3-subsituted-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione(6a-6h). Eight different derivatives of 2-(5-Mercapto-3-subsituted-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione was synthesized, the properties of these comounds givent in table.1 and characterized by different spectral analysis and results for these derivatives are as follows :

Fig.1. synthetic scheme for compounds 6a-6h



2-(5-Mercapto-3- phenyl-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione (6a)

mp 262°C; yield: 87%; IR(KBr, cm-1) : 3363.97s, 3483.56s (N-H), 2362.88s (C-H), 1633.76s, 1681.98s (C=O), 1506.46s, 1471.74sc (CH2), 1114.89s,

1182.40s (C-C), 840.99s (C-C), 752.26s (C-S); 1H-NMR(CDCl3) δ: 8.25(d, Ar-CH), 8.05(d), 7.85(d), 7.5(m, Ar-CH), 4.8(s, NH), 3.3(N-CH2), 2.2(CH2); m/e: 324.07 (100.0%), 325.07 (20.3%), 326.06 (4.5%), 326.07 (2.3%)

2-(5-Mercapto-3-(2-chloro phenyl)-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione (6b)

mp 210°C; yield: 58%; IR(KBr, cm-1) : 3173.01s, 3066.92s, 2968.56s, 2928.04s(C-H), 2854.74s(N-H), 2386.74s(C-N), 1650s(C=O), 987.59b(C-H), 962.72w(C-N), 711.76s(C-S); 1H-NMR (CDCl3) δ: 7.2-7); .5 (m), 7.9-7-8(d), 5,3.3,2.2(s), 7.2(s, 2H, Ar-H), 5(s, N-H), 2.2(s, CH_2), 3.3(N-CH_2), m/e: 358.03 (100.0%), 360.03 (32.8%), 359.03 (20.2%), 361.03 (6.9%), 360.02 (4.4%), 360.04 (1.5%), 362.02 (1.4%)

2-(5-Mercapto-3-(2-hydroxy phenyl)-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione (6c)

mp 192°C; yield: 59%; IR(KBr, cm-1): 3171.08s, 3066.92s, 2989.76s (C-H), 2929.77s (CH2), 2789.16s, 2852.81s (N-H), 2368.86s (C-N), 1448.59s (CH2), 1647.26sc (C=O), 1072.46s (C-N), 1022.31s (C-C), 987.00w (N-H), 848.00s (C-N), 711.76s (C-S); 1H-NMR(CDCl3) δ: 4.8(s, N-H), 2.2(CH)2, 3.3(N-CH2), 7.8(m, C-N of triazole), 7.4(d, CH of benzene); m/e: 340.06 (100.0%), 341.07 (18.1%), 342.06 (4.7%), 342.07 (2.3%), 341.06 (2.3%)

2-(5-Mercapto-3-(2-methyl phenyl)-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione (6d)

mp 268°C; yield: 57%; IR(KBr, cm-1) : 3171.08s (C-H), 3096.92s (C-H), 2928.04s (CH2), 2852.81s (N-H), 2764.09s (CH2), 2364.81s, 1871.01co (C-N), 1500.00s (C=O), 1448.59sc (CH2), 1303.92s (N-H), 1074.00s (C-N), 1022.00s (C-C), 967.00w (N-H), 711.00s (C-S); 1H NMR(CDCl3) δ: 7.2-7.5(d), 7.9-7.8(d), 5,3.3,2.2(s), 7.2(s), 7.9(m), 5(s, N-H), 2.2(s, CH_2), 3.3(N-CH_2); m/e: 338.08 (100.0%), 339.09 (19.2%), 340.08 (4.7%), 340.09 (2.3%), 339.08 (2.3%)

2-(5-Mercapto-3-(2-nitro phenyl)-1,5-dihydro-[1,2,4] triazol-4-yl)-isoindole-1,3-dione (6e)

mp 260°C; yield: 59%; IR(KBr, cm-1): 3171.08s, 3066.92s, 2989.76s (C-H), 2929.77s (CH2), 2789.16s, 2852.81s (N-H), 2368.86s (C-N), 1647.26sc (C=O), 1448.59s (CH2), 1072.46s (C-N), 1022.31s (C-C), 987.00w (N-H), 848.00s (C-N), 711.76s (C-S); 1H-NMR(CDCl3) δ: 4.8(s, N-H), 2.2(CH)2, 3.3(N-CH_2), 7.8(m, C-N of triazole), 7.4(d, CH of benzene); m/e: 369.05 (100.0%), 370.06 (18.1%), 371.05 (4.8%), 370.05 (2.6%), 371.06 (2.5%)

2-(5-Mercapto-3-(4-methyl phenyl)-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione (6f)

mp 252°C; yield: 88%; IR(KBr, cm-1) : 3304.66s (N-H), 3068.85s, 1608.00s (C-H), 1664.62s (C=O), 1491.02sc (CH2), 1315.50s, 1371.03s (C-N), 1124.42s, 1170.83s (C-C), 1070.83s (C-N), 707.90s (C-S); 1H-NMR(CDCl3) δ: 7.5(m, Ar-CH), 7.3, 4.8(s, NH), 5(NH), 3.3(2H, N-CH2), 2.2(CH2); m/e: 338.08

Table 2. antibacterial activity of compounds and standard against different bacteria fungi

S.NO.	Compound	Zone of inhibition in mm							
		Gram(+) bacteria			Gram(-) bacteria			Fungi	
		S.a	B.s	B.c	E.c	P.v	P.a	A.f	C.a
1.	4a	5	6	5	4	5	7	9	12
2.	4b	7	5	8	6	4	6	9	11
3.	4c	Nil	7	Nil	5	6	6	1o	14
4.	4d	6	5	5	7	5	5	9	13
5.	4e	7	6	7	6	Nil	4	8	11
6.	4f	5	5	6	5	4	6	10	15
7.	4g	8	7	5	6	6	5	9	9
8.	8a	6	5	Nil	4	5	6	7	10
Control	DMF	1	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Standard	Standard	9	11	8	9	7	9	14	18

S.a: Staphylococcus aureus *B.s: Bacillus subtilis* *B.c: Bacillus cerus*
E.c: Escherichia coli *P.v: Proteus vulgaris* *P.a: Pseudomonas aeruginosa*
A.f: Aspergillus fumigatus *C.a: Candida albicans*

(100.0%), 339.09 (19.2%), 340.08 (4.7%), 340.09 (2.3%), 339.08 (2.3%)

2-(5-Mercapto-3-(4-hydroxy phenyl)-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione (6g)

mp 276°C; yield: 59%; IR(KBr, cm-1): 3171.08s, 3066.92s, 2989.76s (C-H), 2929.77s (CH2), 2789.16s, 2852.81s (N-H), 2368.86s (C-N), 1448.59s (CH2), 1647.26sc (C=O), 1072.46s (C-N), 1022.31s (C-C), 987.00w (N-H), 848.00s (C-N), 711.76s (C-S); 1H-NMR(CDCl3) δ: 4.8(s, N-H), 2.2(CH)2, 3.3(N-CH2), 7.8(m, C-N of triazole), 7.4(d, CH of benzene); m/e: 340.06 (100.0%), 341.07 (18.1%), 342.06 (4.7%), 342.07 (2.3%), 341.06 (2.3%)

2-(5-Mercapto-3-(2,4-dimethyl phenyl)-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione (6h)

mp 260°C; yield: 82%; IR(KBr, cm-1) : 3176.37s (C-H), 3343.54s, 3479.70s (C-H), 2390.96s (C-N), 1629.90s (C=O), 1503.38s, 1431.23sc (CH2), 1346.46s (C-N), 1101.39s , 1172.76s (C-C), 871.35s (C-N), 742.62s, 779.27s (C-S), 661.61op (C-H); 1H-NMR(CDCl3) δ: 8.1(d, Ar-CH), 7.4-(t, Ar substituted), 7(d), 6.7(t,), 3.3(HC-NH); m/e: 352.10 (100.0%), 353.10 (22.4%), 354.10 (5.3%), 354.11 (2.0%)

Antibacterial activity of all the compounds was carried out against all eight microorganisms. The Mueller-Hinton Agar media was used for sub culturing and for estimating antibacterial activity, while potato dextrose agar media was used for antifuncal activity. The various results are summarized in the table 2. Some compound showed good to moderate activity.

The research study reports the successful synthesis and antimicrobial activity of novel 2-(5-Mercapto-3-subsituted-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione carrying biologically active groups fig.1. Their antimicrobial activity study revealed that all the compounds tested showed moderate to good antibacterial activity against pathogenic strains.

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REFERENCES

1. Iraj Rahavi Ezabadi, Charalabos Camoutsis, Panagiotis Zoumpoulakis, Athina Geronikaki, Marina Sokovic, Jasmina Glamocˇilija and Ana Ciric, Bioorganic & Medicinal Chemistry 16 (2008) 1150–1161

2. Jianming Xu, Yongbing Cao, Jun Zhang, Shichong Yu, Yan Zou, Xiaoyun Chai, Qiuye Wu a, Dazhi Zhang, Yuanying Jiang, Qingyan Sun, European Journal of Medicinal Chemistry 46 (2011) 3142e3148

3. Nuray Ulusoy Guzeldemirci, Omer Kucukbasmac, European Journal of Medicinal Chemistry 45 (2010) 63–68

4. Xiaoyun Chai, Jun Zhang, Shichong Yu, Honggang Hu, Yan Zou, Qingjie Zhao, Zhigang Dan, Dazhi Zhang, Qiuye Wu, Bioorganic & Medicinal Chemistry Letters 19 (2009) 1811–1814

5. Nisha Aggarwal, Rajesh Kumar, Prem Dureja, J.M. Khurana, European Journal of Medicinal Chemistry (2011), doi: 10.1016/j.ejmech.2011.06.009

6. Sumesh Eswaran, Airody Vasudeva Adhikari b,N. Suchetha Shetty, European Journal of Medicinal Chemistry 44 (2009) 4637–4647

7. Seref Demirayaka, Kadriye Benklia, Kıymet Gu¨venb, Eur. J. Med. Chem. 35 (2000) 1037–1040

8. Yatin J. Mange , Arun M. Isloor , Shridhar Malladi , Shrikrishna Isloor , Arabian Journal of Chemistry (2011), doi:10.1016/j.arabjc.2011.01.033

9. Nasser S. A. M. Khalil, Carbohydrate Research 341 (2006) 2187–2199

10. Krzysztof Sztanke, Kazimierz Pasternak, Anna Sidor-Wo jtowicz, Janina Truchlin´ska and Krzysztof Jozwiak, Bioorganic & Medicinal Chemistry 14 (2006) 3635–3642

11. Qiu Qin He, Chao Mei Liu , Ke Li, Yong Bing Cao, Chinese Chemical Letters 18 (2007) 421–423

12. Xiaoyun Chai, Jun Zhang, Yongbing Cao,Yan Zou, Qiuye W, Dazhi Zhang, Yuanying Jiang, Qingyan Sun,European Journal of Medicinal Chemistry 46 (2011) 3167e3176

13. Hacer Bayrak, Ahmet Demirbas, Neslihan Demirbas, Sengul Alpay Karaoglu, European Journal of Medicinal Chemistry 44 (2009) 4362–4366

14. Haythem A. Saadeh, Ibrahim M. Mosleh, Amal G. Al-Bakri, Mohammad S. Mubarak, Monatsh Chem (2010) 141:471–478