Synthesis of some new 5-aryl-4-[2-(4- substituted phenyl amine)-ethyl]-2,4-dihydro-[1,2,4]–triazole-3-thiones as possible antimicrobial agents.

B. B. L. Srivastava*
Department of Chemistry,
Mahatma Gandhi PG College,
Gorakhpur. UP. India.

*Correspondence.
* bbl_mgpg@yahoo.in

Abstract

5-aryl-4-[2-(4-subsituted phenyl amine)-ethyl]-2,4-dihydro-[1,2,4]- triazole-3-thiones (4a-i) was prepared by treatment of 4-(2-Hydroxy ethyl)-5-aryl -2,4-dihydro-3H-1,2,4-triazole-3-thiones (2) with different substituted aromatic amines (3). The compounds were screened for antibacterial activity against three bacteria viz *staphylococcus aurous* (Sa), *Entercocous faceless* (Ef) and *Escherichia Coli* (Ec) involving two strains of (Sa) and (Ef) and only one of (Ec) as recommended by NCCLS Moxifloxacin and Linezolid were taken as the reference standards.

Key words - Hydroxy ethyl triazole, Antibacterial activity.

Introduction

In the, course of the study of new antimicrobial agents, it was thought of interest to study the effect of triazole and their derivatives. A large number of triazole derivatives possessing diverse pharmacological properties like antimicrobial[1-4] antiviral[5], antiinflammatory[6], analgesic[7], antitumorial[8] and antihypertensive[9] activities. In addition to triazole, amines have also been shown to posses potent activity like antibacterial[10], antifungal[11] and insecticidal[12] etc. Keeping

these observation in view, we have synthesized nine new titled derivatives, incorporating these two moieties by the condensation 4-(2-hydroxy-ethyl)-5-aryl-2,4-dihydro-3H-1,2,4-triazole-3-thione with different substituted aromatic amines (Scheme route). The structures of compounds were deduced on the basis of their elemental analysis, Mass fragmentation and spectral data (IR, NMR). The characterization data of the compounds [4(a-i)] were given in table I.

Material and Methods

Melting points were taken in open capillary and are uncorrected compounds were checked for their homogeneity by TLC on silica gel G. Infra red spectra were determined on shimadazu 8201 PC (4000-350cm-1) in KBr. NMR spectra on a Bruker DRX300 instrument using TMS as internal standard (Chemical shift in δ ppm). All the compounds gave satisfactory result for C,H and N.

2-Aryl-5-mercapto-1,3,4-oxadiazole.(1)

A mixture of benzoic acid hydrazide (0.1 mole), KOH (0.1 mole), carbon disulphide (20 ml), was taken in 100 ml ethanol and then refluxed for 20 hours. After monitoring the reaction mixture, the excess ethanol was distilled off. The reaction mixture was cooled and then poured into ice cold water, where upon a solid appeared which was filtered. The filtrate on acidification with concentrated hydrochloric acid resulted in a precipitate which was filtered. this process was repeated two or three times till all the compound was precipitated out. The solid was dried under vacuum. The compounds were recrystallized from ethanol.

Molecular formal 1(a) $C_8H_6N_2OS$ yield 80% M.P. 220^0C.

The ^{1}H NMR spectrum exhibited characteristic bands at cm^{-1}.

^{1}HMR (δ in ppm) δ 7.56 (m, 5H, Ar, H), δ7.34 (S, 1H, H), 7.91 (d, 1H, ArC-NH)

4-(2-Hydroxyethyl)-5-aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones.(2)

A suspension (0.05 mole) of oxadiazole (1), (0.05 mole) of ethanol amine was refluxed for 8 hrs. It was observed that at the time of the reaction the color of the reaction mixture firstly changed to green and finally changed to reddish in color. The resultant reaction mixture was poured into ice

cold water (50 ml) containing concentrated hydrochloric acid (10ml) precipitation occurred in a matter of five minutes. The precipitate was filtered off and dried in vaccuo. Recrystallization from dilute ethanol afforded a crystalline mass. Molecular formula (29) is $C_{10}H_{11}N_3O_3$ yield 60% M.P. 115^0C. The NMR spectrum exhibited characteristic bands at cm^{-1}.

NMR ($CDcl_3$) (in δ ppm) 7.3-7.6 (m, 5H, Ar H), 2.79 (t, 2H, N-CH_2), 8.15 (S, 1H, -CH_2OH) Found C: 54.29, H:4.94, N:19.05, S:14.51 required C:54.30, H:4.98, N.19.00, S:14.48.

5-Aryl-4-[2-(4-Substituted phenylamine)-ethyl]-2,4-dihydro-[1,2,4]-triazole-3-thiones.4(a-i).

A mixture of compound (2) (0.01 mol) and substituted aromatic amine (0.01 mol) in ethanol (10 ml) was refluxed on a water bath for 5 hrs under anhydrous condition. After monitoring the reaction mixture, it was cooled and poured into ice cold water. A precipitate was obtained, which was filtered, dried and recrystallised from ethanol. All the compounds were prepared by the same procedure.

The titled compound 4 (a-i) were characterized on the basis of their characterization data as listed in table I.

Pharmacological Studies

Determination of Antibacterial activity: (in vitro)

Three compounds belonging to the category 5-Aryl-4-[2-(4-Substituted phenyl amine)-ethyl]-2,4-dihydro-[1,2,4]-triazole-3-thiones. (4-(a-i) were evaluated only against three bacteria viz *staphylococcus aurous* (Sa), *Entercocous faceless* (Ef) and *Escherichia Coli* (Ec) involving two strains of (Sa) and (Ef) and only one of (Ec) as recommended by NCCLS Moxifloxacin and Linezolid were taken as the reference standards.

Methods and Materials

The test bacteria were maintained on nutrient agar slants (composition g/l, peptone 5, Sodium chloride 5, beef extracts 15, Yeast extract 15, ph 7.2, and agar (20). Testing was done in nutrient broth. After incubation with a loopful of culture from the slant, the broth was incubated at

37 ± 1^0C for 24 hours. Fresh broth (20 ml) was seeded with 0.25 ml of 24 hours broth culture and two fold dilution methods were followed. The test sample was dissolve in dimethyl sulphoxide (DMSO) to obtain a 10 ml solution and 0.2ml solution to the test material was to 1.8ml of the seeded broth and this formed the first dilution. One ml of this was diluted with a further 1 ml of the seeded broth, to prepare the second dilution and so on till six such dilutions were obtained. A set of tubes containing only seeded broth was kept as control and suitable solvent controls were also maintained. After incubation for 24 hrs the last tube with no visible growth of the micro organism was taken to represent the minimum inhibitory concentration (MIC) of the test sample expressed in μg/ml. The antibacterial data of the tested compounds are recorded in table II.

Result & Discussion

Three compounds of the category; 5-Aryl-4-[2-(4-substituted phenyl amine)-ethyl]-2,4-dihydro-[1,2,4] triazole-3-thiones (table II) were evaluated against three bacteria *viz*. *staphylococcus aurous* (two strains viz; MSSA and MRSA), *Entercoccous faceless* (two strains viz. VSE and VRE) and *Escherichia coli* (one strain). Less significant antibacterial activity has been observed by these compounds against all the bacterial strains; however certain generalization can be drawn on the basis of available data incorporated in table II for these compounds. thus compound No 1 bearing R=Cl and R' = NO_2 was found to have MIC value 256 and 128 against vancomycin susceptible enterococcal (VSE) infection, and *vancomycin resistant entercoccal* (VRE) infection, respectively while the same compound was found with more than 256 MIC value against both the strains of *Staphylococcus aurous* (MSSA and MRSA). It is seen that when both the substituent are nitro (R-R'=NO_2) the antimicrobial activity decreased considerably as is evident activity data present in table II. A hydroxy and nitro substituent did not improve the activity.

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Table I

Characterization data of

5-Aryl-4-[2-(4-substituted phenylamine)-ethyl]-2,4-dihydro-[1,2,4] triazole-3-thiones

Compd.	R	R^1	Mol. Formula	Mol wieght	M.p. (^{0}c)	Yield (%)	Elemental Analysis			
							C	H	N	S
Va	p-Cl	p-NO_2	$C_{16}H_{14}ClNO_2S$	375.83	240	80	51.10 (51.13)	**3.78 (3.75)**	**18.64 (18.63)**	**8.50 (8.53)**
Vb	p-NO_2	p-NO_2	$C_{16}H_{14}N_6O_4S$	386.39	148	85	49.71 (49.74)	3.63 **(3.65)**	21.78 **(21.75)**	8.35 **(8.30)**
Vc	p-OH	p-NO_2	$C_{16}H_{15}N_5O_3S$	357.39	140	65	53.74 (53.77)	4.25 **(4.23)**	19.64 **(19.60)**	8.94 **(8.97)**
Vd	p-MeO	p-NO_2	$C_{17}H_{17}N_5O_3S$	371.41	158	60	54.94 (54.97)	4.62 **(4.61)**	18.84 **(18.86)**	8.65 **(8.63)**
Ve	3,5-Dinitro	p-NO_2	$C_{16}H_{13}N_7O_6S$	431.39	110	75	44.52 (44.55)	3.02 **(3.04)**	22.76 **(22.73)**	7.46 **(7.43)**
*Vf	H	p-NO_2	$C_{16}H_{15}N_5O_2S$	341.39	95	70	56.25 (56.29)	4.45 **(4.43)**	20.54 **(20.51)**	9.35 **(9.39)**
Vg	p-Cl	p-Cl	$C_{16}H_{14}N_2O_4S$	365.28	180	65	52.65 (52.61)	3.81 **(3.86)**	15.32 **(15.34)**	8.82 **(8.78)**
Vh	p-OH	p-Cl	$C_{16}H_{15}ClN_4OS$	346.84	175	72	55.42 (55.41)	4.35 **(4.36)**	16.20 **(16.15)**	9.28 **(9.25)**
Vi	p-OH	P-OCH_3	$C_{17}H_{18}N_4O_2S$	342.42	165	80	59.64 (59.63)	5.33 **(5.30)**	16.32 **(16.36)**	9.40 **(9.36)**

* Ir (KBr) (in r cm^{-1}): 1561 (Ar-NO_2), 1616 (C=N), 1253 (C=S), 3476 and 3384 (sec. NH)

* ^{1}H NMR ($CDCl_3$) (in δ ppm): 6.3-7.9 (m, 9H, ArH), 8.2 (s, 1H, CSNH), 4.00 (s, 1H, NH), 3.6 (t, 2H, $ArNHCH_2$), 3.9 (t, H, –N-CH2)

* Mass fragmentation peaks m/z (rat) 341, base pead=137, and other Important peaks are 295, 311, 197, 264, 98, 137, 204, 238 etc.

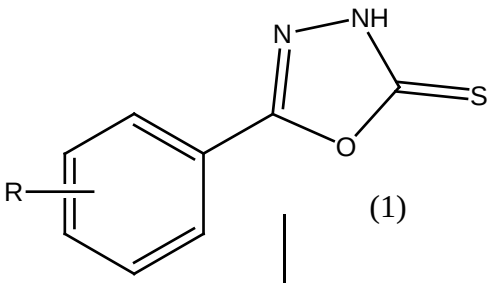
Table II

Antibacterial activity data of 5 – Artyl -4- [2-(Substituted phenyl amine)-ethyl] – 2, 4-dihydro – [1,2,4] triazole -3- thiones

Compound	R	R^1	Minimum Inhibitory Concentration (MIC) in hg/ml.				
			S		Ef		Ec
			DRCC 035 MSSA	DRCC 018 MRSA	DRCC 034 VSE	DRCC 153 VRE	DRcc 018
1	Cl	No_2	>256	>256	256	128	>256
2	No_2	No_2	>256	>256	>256	>256	>256
3	OH	No_2	>256	>256	256	256	>256
Moxifloxacin			0.6	0.6	0.25	0.25	0.03
Linezolid			2.0 (2.4)	1.0 (1.2)	2.0 (2.4)	1.0 (2.4)	>32

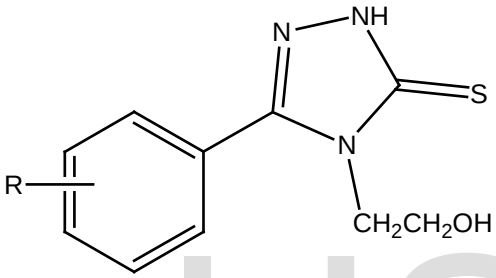
Sa = Staphylococcus aureus, Ef = Enterococcus faccalis; Ec = escheriehia coli.

Scheme route



(1)

$NH_2CH_2CH_2OH$



(2)

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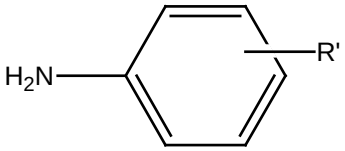

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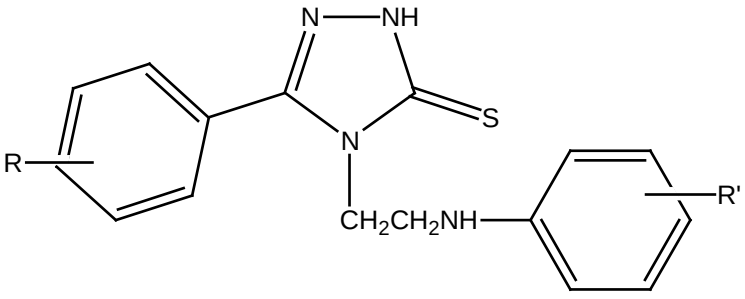

4(a-i)