

Synthesis and Antimicrobial Activity of Some 5-Phenyl-4-Substituted Amino-3-mercapto-(4H)-1,2,4-Triazoles

GOPALAKRISHNA RAO*, S. RAJASEKARAN AND M. ATTIMARAD

Al-Ameen College of Pharmacy
Hosur Road, Bangalore - 560 027

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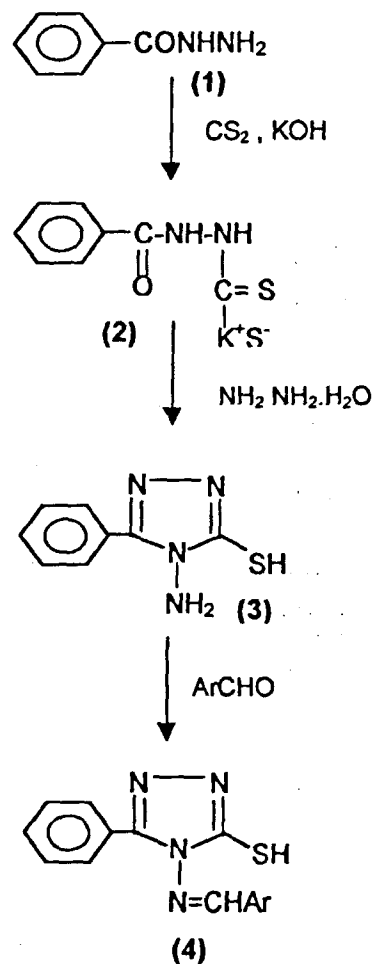
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Certain 5-phenyl-4-substituted amino-3-mercapto-(4H)-1,2,4-triazoles having different substitutions on the aromatic ring possessing the azomethine linkage were prepared and evaluated for their antimicrobial activity. The compounds with a chloro, bromo, hydroxyl and a nitro group on the aromatic ring showed good antimicrobial activity.

A number of substituted triazole derivatives¹⁻⁵ have been reported to possess antimicrobial activity. Compounds with azomethine linkage⁶ were also shown to possess good antimicrobial activity. Antimicrobial activity of 1,2,4-triazoles having a mercapto⁷⁻⁹ or mercapto acetic acid¹⁰ at different position have been reported. The antifungal activity of some amides related to 3-acylamino-2-aryl-1-triazolyl-2-butanol has also been reported¹¹. The title compounds possessing both triazole and azomethine linkage were synthesized and evaluated for antimicrobial activity.

Melting points were determined in open capillaries using a Stairwell melting point apparatus and are uncorrected. The structures of the compounds were supported by elemental analysis and spectra data. IR spectra were taken on a FTIR 8700 Shimadzu spectrometer using the KBr discs. PMR (D_3CCOCD_3) spectra were obtained using a EM390 CW-NMR 90MHz using TMS as internal standard. Mass spectra were recorded on a Finnigan Mat 8230 MS-GC. Elemental analysis (C, H, and N) was carried out on Carlo Ebra Strum DP 200.

The intermediate 5-phenyl-4-amino-3-mercapto-4H-triazole (1) was synthesized via aryl hydrazide using a reported method¹². The compound 4d was synthesized by refluxing equimolar quantities of 5-phenyl-4-amino-3-mercapto-4H-1,2,4-triazole (1 g, 0.0052 mol) and 4-chlorobenzaldehyde in absolute alcohol (20 ml) for 1 h. The reaction mixture was cooled and poured into ice cold



SCHEME 1

*For Correspondence

TABLE 1 : PHYSICAL DATA AND ANTIMICROBIAL ACTIVITY OF 4a-k

Compd. No.	Ar	m.p.(°)	% yield	B.s	S.a	E.c	P.a	A.n
4a	C ₆ H ₅	182	84	22	17	20	15	15
b	2-CH ₃ C ₆ H ₄	189	85	24	16	17	13	10
c	4-CH ₃ C ₆ H ₄	213-14	76	19	16	16	14	09
d	4-Cl C ₆ H ₄	212	92	20	20	20	13	10
e	3,4,5(OCH ₃) ₃ C ₆ H ₂	196	62	21	15	16	11	11
f	3(OCH ₃) 4OH C ₆ H ₃	196-97	81	22	16	19	09	15
g	2-OH 5Br C ₆ H ₄	215	62	22	15	20	12	12
h	2-NO ₂ C ₆ H ₄	207-09	84	19	15	19	10	08
i	3-NO ₂ C ₆ H ₄	198	80	20	20	17	Nil	16
j	2-OH C ₆ H ₄	208	84	22	18	25	Nil	09
k	4-N(CH ₃) ₂ C ₆ H ₄	215	74	21	18	18	10	17
	streptomycin			25	20	23	22	
	griseofulvin							23

Antimicrobial activity of all the synthesized compounds were tested against *Bacillus subtilis* (B.s), *Staphylococcus aureus* (S.a), *E.coli* (E.c), *P. auroginosa* (P. a), *Aspergillus niger* (A. n), at a concentration of 200 µg/0.1 ml for antibacterial and 100 µg/0.1 ml for antifungal activity.

water. The solid obtained was filtered, washed with cold water, dried and crystallized from aqueous ethanol yield: 1.5 g (92%); m.p: 212°. Found: C, 57.37; H, 3.36; N, 17.42; C₁₅H₁₁N₄SCl requires C, 57.23; H, 3.51; N, 17.79%. IR (KBr) cm⁻¹: 3100 (s, ArH); 2774 (w, SH); 1599 (s, C=N); 1501 (m), 1440 (m) (ArC=C); 1088 (m), 1012 (m), (Ar-H), 730 (m, Ar-Cl); 685 (s, Ar-H). PMR (CD₃COCD₃) δ : 10.11 (s, 1H, CH); 7.82-8.1 (d, 4H, ArH); 7.39-7.72 (m, 5H, ArH). Mass Spectra, m/e (%) : 314 (M+, 1), 177 (78), 137 (4), 118 (78), 91 (47), 77 (78), 63 (55) and 50 (100).

The compound 4a-k were prepared using a similar procedure as mentioned above.

All the compounds 4a-k have been screened for both antibacterial and antifungal activity using the cup-plate method¹³, using streptomycin as a positive control for antibacterial activity and griseofulvin as a positive control for antifungal activity. The bacteria employed were *B. subtilis*, *S. aureus*, *E. coli* and *P. aureginosa*, and the fungi employed was *A niger*. Dimethylsulfoxide was used as solvent for all the compounds and as a control. The zone of inhibition of bacterial growth as measured after

24 h, after incubating at 37°. The zone of inhibition of fungal growth was measured after 48 h after incubating at 28°. The result are tabulated in Table 1.

The compounds with a chloro, bromo or a hydroxy group (4d, 4g, 4j) on the aromatic ring possessing azomethine linkage showed potent antibacterial activity and all other compounds showed mild to moderate activity. The compound with a nitrogroup (4i) at the 3rd position on the aromatic ring possessing an azomethine linkage showed moderate antifungal activity and all other compounds showed weak activity.

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Antioxidant Activity of *Cuscuta reflexa* Stems

S.B. YADAV, VYASJI TRIPATHI*, R.K. SINGH¹ AND H.P. PANDEY¹

Department of Chemistry, Banaras Hindu University, Varanasi-221 005

¹Department of Biochemistry, Banaras Hindu University, Varanasi-221 005

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The *in vitro* antioxidant activity of *Cuscuta reflexa* stem extract has been investigated by estimating degree of non-enzymatic hemoglobin glycosylation measured colorimetrically at 440 nm. The ethyl acetate fraction of ethanol extract showed higher activity than the other fractions. The antioxidant activity of extracts are very close and identical in magnitude and comparable to that of standard antioxidant compounds used.

Cuscuta reflexa Roxb. known as Amarvel or Akashbel in Hindi, is a common golden yellow dodder like parasite belonging to the family Convolvulaceae. It is common throughout India growing on thorny or other shrubs as parasitic annuals. The plant useful in diseases of bile and blackbile as purge, and in affections of the brain such as fits, melancholy and insanity¹. It is also useful externally against itch and internally in protracted fevers, retention of wind and in duration of the liver². Recently a great deal of interest has been directed towards the bioactivity of flavonoids as dietary sources of antioxidant³. Since *Cuscuta* is one of the richest sources of flavonoids^{4,5}, the present investigation attempts to explore the antioxidant properties of this plant.

Shade dried stems were exhaustively extracted with petroleum ether and ethanol using a soxhlet extractor. The extracts were concentrated to dryness *in vacuo*. The ethanolic extract was subjected to silica gel column chromatography and eluted with benzene (EB), chloroform (EC), and ethylacetate (EEA). Further fractionation of the ethyl acetate fraction using silica gel column and elution with benzene-ethylacetate afforded C₆H₆:EtOAc (1:2) EBE-1 and C₆H₆:EtOAc (1:8) EBE-2.

Since non-enzymatic glycosylation of hemoglobin is an oxidation reaction⁶, an antioxidant is expected to inhibit this reaction. The degree of glycosylation of hemoglobin *in vitro* can be measured colorimetrically^{7,8}. Hemoglobin, 5 g/100 ml in 0.01 M phosphate buffer (pH 7.4) was incubated in presence of 2 g/100 ml concentration of glucose for 72 h in order to find out the best

*For Correspondence