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Synthesis of novel 1,2,4-triazoles and their evaluation of 5-LOX inhibition and antimicrobial activity

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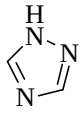
ABSTRACT

*A novel series of 1,2,4-triazoles were designed, synthesised and screened for their 5-LOX inhibition and antimicrobial activity. Among the tested compounds, compounds **6b** and **6e** showed potent 5-LOX inhibition with an IC_{50} of **6.98** and **8.0** µg/mL respectively, **6c** and **6e** were the promising compounds in antibacterial assay, where as compound **6c** was found to be lead compound in antifungal assay.*

Keywords: 5-((3-(4-substituted phenyl)-5-mercapto-4*H*-1,2,4-triazol-4-yl) diazenyl) naphthalene-2-ol, 5-LOX inhibition activity and antimicrobial activity.

INTRODUCTION

Triazole and its derivatives represent an important class of heterocyclic compounds. They possess a wide range of having biological applications and are used in the synthesis of drugs [1,2]. Triazole derivatives are also used in the synthesis of antibiotics, fungicides, herbicides and plant growth hormone insulators and are potentially good corrosion inhibitions [3-5]. The vicinal triazoles, also known as 1, 2, 3-triazoles are five-membered, unsaturated heterocyclic, the ring consists of three sequentially linked nitrogen atoms and two carbons. The parent compound has one unlocated hydrogen atom, as indeed do all derivatives with hydrogen joined to a ring. Triazoles refer to either one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$. The two isomers are 1*H*-1,2,4-triazole **(1)** and 1*H*-1,2,3-triazole **(2)**



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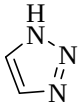

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Like the azoles, triazoles are used in many antifungal drugs and fungicides, but the triazole based drugs are more selective for fungi than the azole-based antifungal compounds [6]. The triazole antifungal drugs include fluconazole, isavuconazole, itraconazole, voriconazole, pramiconazole and posaconazole. The triazole plant protection fungicides include epoziconazole, triadimenol, propiconazole, metconazole, cyproconazole, tebuconazole, flusilazole and paclobutrazol [7]. Out of the two triazoles, 1, 2, 4-triazole possess a wide variety of activities. During the last few decades, a considerable attention has been devoted to synthesis of 1, 2, 4-triazole derivatives possessing comprehensive bioactivities such as antimicrobial [8-10], anti-inflammatory [11], analgesic [12], anti-tumor [13], antihypertensive [14] and antiviral [15].

MATERIALS AND METHODS

3.1 Materials and methods

All the chemicals of analytical grade were obtained from Sigma Aldrich and are used without further purification. Melting points (m.p) were recorded on Kumar capillary melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded from KBr discs on Thermo Nicolet (Model: 6700) spectrophotometer. The NMR (^{1}H & ^{13}C) spectra were recorded on Bruker 400 MHz Avance-II spectrophotometer using tetramethyl silane as an internal standard. The progress of the reaction was monitored by Merck TLC silica gel plates and purity of the target compounds was achieved by Merck silica gel (100-200 mesh).

*3.2. Synthesis of substituted benzylidene hydrazines **(2a-e)***

In a 50 ml R.B. flask, various *p*-substituted benzaldehydes **(1a-e)** (1 mmol) were dissolved in absolute EtOH (20ml) and hydrazine hydrate (2 mmol) in EtOH was added and the reaction mixture was stirred at 0 ^{o}C and refluxed for 1 h, in the presence of 6N HCl. The progress of the reaction was monitored by TLC. After completion of the reaction, EtOH was evaporated and on neutralization with a 10% aqueous solution of sodium bicarbonate yields substituted benzylidene hydrazines: **(2a-e).**

*3.3. Synthesis of Potassium -2-(substituted benzylidene) hydrazine carbodithioate **(3a-e)***

Substituted benzylidene hydrazines **(2a-e)** (1mmol) were dissolved in absolute EtOH (20ml) containing KOH (2 mmol) at ambient temperature and CS_2 (2 mmol) was added in parts. This mixture was agitated for 16 h and diluted with diethyl ether (50 ml). The product was filtered off and vacuum dried. The potassium dithiocarbozinate **(3a-e)** salt, were obtained in nearly quantitative yield.

*3.4. Synthesis of substituted-5-phenyl-4H-1,2,4-triazol-3-thiol **(4a-e)***

Hydrazine hydrate (2mmol) was added slowly to the compounds **(3a-e)** in water (100 ml), with stirring and was refluxed on a water bath until the evolution of H_2S gas ceased. The progress of the reaction was monitored TLC. It was then cooled and carefully acidified with HCl. The solid thus separated, was filtered, washed with water and purified by column chromatography and recrystallized from EtOH to yield **(4a-e).**

*3.5. Synthesis of substituted-5-phenyl-4H-1,2,4-triazole-3 thiodinitrogen chloride salt **(5a-e)***

Compounds **(4a-e)** were dissolved in DCM (30ml) and 6N HCl (30ml) was added at 0 ^{o}C. To this biphasic system, a saturated aqueous solution of $NaNO_2$ (10ml) was added drop wise. After stirring for 30 min at 0 ^{o}C, NaN_3 (3 mmol) was added at 0 ^{o}C. Stirring was maintained for 30 min and the mixture was allowed to room temperature. The two phases were separated, and the aqueous phase was extracted with DCM. The combined organic layers were washed with aqueous solution of $NaHCO_3$, and brine solution and dried over Anhy. Na_2SO_4 and filtered on active charcoal bed. Evaporation of the solvent in vaccuo to yield crude azides **(5a-e).**

*3.6. Synthesis of 5-((3-(substituted phenyl)-5-mercapto-4H-1,2,4-triazol-4yl) diazenyl) naphthalene-2-ol **(6a-e)***

The compounds **5a-e** were suspended in a 1:1 mixture of DCM and water (10ml) and 2-napthol in DCM (10ml) was added. The resulting mixture was stirred at room temperature, till TLC analysis indicated completion of the reaction. The mixture was diluted with DCM (5ml) and water (5ml). The organic layer was separated and the water phase was extracted again with DCM (5ml). The combined organic layer was dried over Na_2SO_4. Removal of the solvent in vaccuo yields crude compounds which were purified by column chromatography using silica gel (100-200 mesh) and 6:4 (EtOAc : *n*-Hexane) as eluents and were further recrystallized from chloroform–hexane to afford the corresponding substituted 1,2,4-triazoles **(6a-e)**

*5-((3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)diazenyl) naphthalene-2-ol **(6a):***

m.p: 232 ^{o}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.5 (s, 1H, -OH), 7.0-7.3 (m, 6H, Ar-H), 7.5-8.4 (m, 4H, Ar-H), 12.9 (s, 1H, -SH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 109.3, 110.2, 117.5, 119.2, 120.4, 123.1, 125.6, 126.2, 126.9, 127.4, 128.4, 129.8, 131.3, 148.4, 150.1, 156.1. IR (KBr, cm^{-1}): 1398 (C-N), 1636 (N=N), 2852 (S-H), 3431 (O-H). ESI-MS: *m/z* 347.12 $[M+H]^+$, HPLC: Mobile phase-Methanol,Retention time – 3.416 min, Purity – 97.43%

*5-((3-(4-chlorophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl) diazenyl)naphthalene-2-ol **(6b):***

m.p: 221^{o}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.6 (s, 1H, -OH), 7.2-7.8, (m, 6H, Ar-H), 8.0-8.3 (m, 4H, Ar-H), 13.6 (s, 1H, -SH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 108.9, 110.3, 116.2, 118.9, 121.7, 123.2, 124.2, 126.1, 127.8, 128.7, 130.8, 131.1, 133.2, 146.1, 149.3, 155.0, IR (KBr, cm^{-1}): 761 (C-Cl), 1321.9 (C-N), 1572 (C=C), 1627.9 (N=N), 2551 (S-H), 3409 (O-H). ESI-MS: *m/z* 381.15 $[M+H]^+$, HPLC: Mobile phase-Methanol, Retention time - 2.790 min, Purity-99%

5-((3-(4-bromophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl) diazenyl)naphthalene-2-ol ***(6c):***
m.p: 211°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.35 (s, 1H, -OH), 6.95-7.5 (m, 6H, Ar-H), 7.4-8.2 (m, 4H, Ar-H), 13.0 (s, 1H, -SH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 109.1, 110.4, 116.7, 119.6, 120.7, 123.1, 124.5, 126.6, 127.2, 128.8, 130.3, 131.2, 133.3, 146.5, 149.8, 155.3. IR (KBr, cm^{-1}): 797 (-C-Br), 1324.9 (C-N), 1572 (C=C), 1629.9 (N=N), 2554 (S-H), 3412 (O-H). ESI-MS: *m/z* 425 $[M+H]^+$ 427 $[M+H+2]^+$, HPLC: Mobile phase-Methanol, Retention time-3.393 min, Purity-99.04%

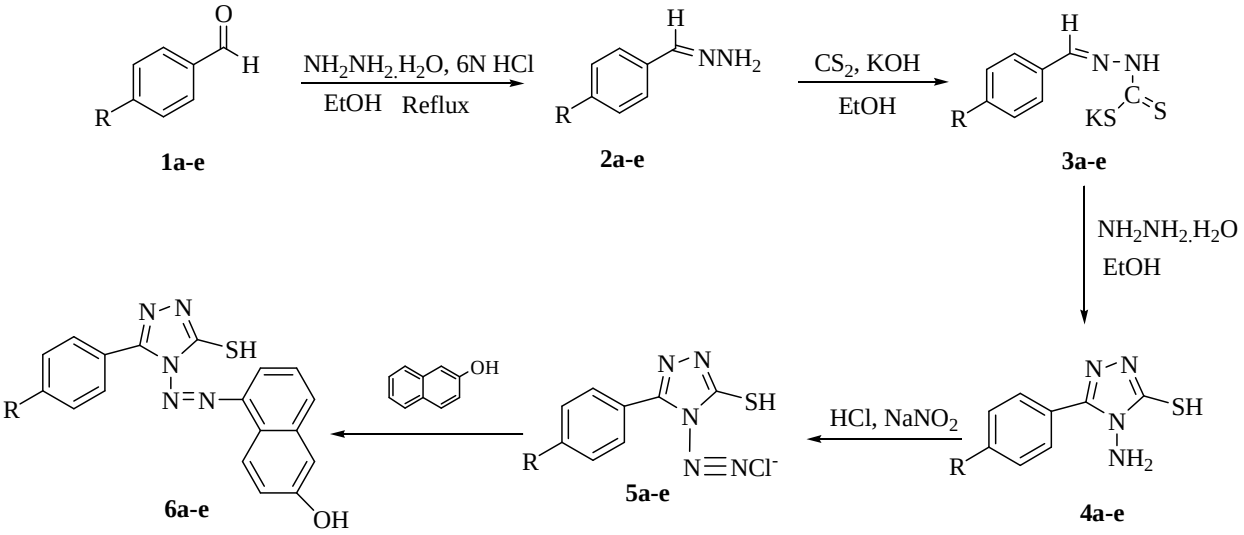
5-((3-(4-amino phenyl)-5-mercapto-4H-1,2,4-triazol-4-yl) diazenyl) naphthalene-2-ol ***(6d):*** mp: 296 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.6 (s, 1H, -OH); 5.8 (s, 2H, -NH2); 7.0-7.5 (m, 4H, Ar-H), 7.6-7.9 (m, 6H, Ar-H), 13.0 (s, 1H, -SH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 109.4, 110.6, 116.2, 119.7, 120.2, 123.6, 124.8, 126.2, 127.7, 128.9, 130.1, 131.7, 142.5, 146.3, 149.2, 155.7. IR (KBr, cm^{-1}): 1572 (C=C) 1591.8 (C=N), 1684.5 (N=N), 2549.4 (S-H), 3092.4 (O-H), 3430.3 (N-H). ESI-MS *m/z:* 362.17 $[M+H]^+$, HPLC: Mobile phase-Acetonitrile, Retention time-3.174 min, Purity-98.85%

5-((3-(4-nitro phenyl)-5-mercapto-4H-1,2,4-triazol-4-yl) diazenyl)naphthalene-2-ol ***(6e):***
m.p: 326 °C. ^{1}H NMR (CDCl3, 400 MHz): δ 4.6 (s, 1H, -OH), 7.0-7.8 (m, 6H, Ar-H), 8.3-8.4 (m, 4H, Ar-H), 13.0 (s, 1H, -SH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 109.2, 110.9, 116.8, 120.1, 121.6, 122.2, 123.9, 126.1, 126.8, 128.5, 130.3, 131.3, 142.3, 147.1, 149.4, 155.5, IR (KBr, cm−1): 927 (N-O), 1320.2 (C-NO2), 1572 (C=C), 1591.8 (C=N), 1684 (N=N), 2549 (S-H), 3092 (O-H). ESI-MS *m/z:* 392.09 $[M+H]^+$, HPLC: Mobile phase-Acetonitrile, Retention time-3.162 min, Purity-99%

RESULTS AND DISCUSSION

2.1 Chemistry

The target molecules **(6a−e)** were synthesized in 5 steps. Substituted benzaldehyde **(1a−e),** were treated with hydrazine hydrate and Hydrochloricacid to yield **2a-e**, which in presence of potassium hydroxide and carbon disulphide results in the formation of compounds **3a-e** which on further treatment with hydrazine hydrate in ethanol undergo cyclisation resulting in the formation of substituted-5-phenyl-4*H*-1,2,4-triazol-3-thiol **(4a-e).** Compounds **(4a-e)** were diazotized to form **5a-e**, which on further treatment with β-napthol results in the formation of target molecules **(6a-e).** All the target molecules were characterized by advanced spectroscopic data. The synthetic route for the target molecules was depicted in **Scheme-1**



1a,2a,3a,4a,5a,6a R=H, 1b,2b,3b,4b,5b,6b R= Cl, 1c,2c,3c,4c,5c,6c R= Br, 1d,2d,3d,4d,5d,6d R=NH_2, 1e,2e,3e,4e,5e,6e R=NO_2

Scheme-1: Synthesis of 5-((3-(4-substituted phenyl)-5-mercapto-4*H*-1,2,4-triazol-4-yl) diazenyl) naphthalene-2-ol (6a-e)

2.2 Bio evaluation

Prior to bio evaluation, the purity of all the target compounds **(6a-e)** was experimented by HPLC which was observed to be ≥ 97%. The 5-LOX inhibition and antimicrobial activity of the titled compounds **(6a-e)** were evaluated according to the reported procedures and the results are tabulated.

2.2.1 5-LOX inhibition activity

The *in vitro* anti-inflammatory activity of the target compounds **(6a-e)** were carried out according to the method described by Lip Yong Chung [16] employing "Curcumin" as standard. The 5-LOX inhibition assay was carried out at Laila Impex, Vijayawada, India in association with Dr. Trimoorthulu, president (R&D). The lipoxygenase inhibitory activity was expressed in terms of IC_{50} (µg/mL) which was presented in **table 1.**

Table: 1 IC_{50} values (µg/mL) of the target molecules (6a-e)

Entry	Compound	IC_{50} (µg/ml)
1	**6a**	>100
2	**6b**	**6.98**
3	**6c**	>100
4	**6d**	20.84
5	**6e**	8.0
6	**Standard***	12.79

** Curumin was used as standard.*

From the results in **table-1** It can be inferred that compound **6b** and **6e** exhibited potent activity with IC_{50} of 6.98 and 8.0 µg/mL respectively than the standard curcumin (12.79 µg/mL). The next best compound was **6d** with an IC_{50} of 20.84 µg/mL and the remaining compounds 6a and 6c has an IC_{50} of >100 µg/mL. The promising compound in the series **(6b)** was twice more active than curcumin (standard).

2.2.2 Antimicrobial activity

2.2.2.1 Antibacterial activity

The final compounds **(6a-e)** were screened for their antimicrobial activity by using agar well diffusion method [17]. *In vitro* antibacterial activity of the target compounds **(6a-e)** were screened at a single concentration (25µg/mL) against Gram positive bacteria *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441) and *Bacillus cereus* (MTCC 430), Gram negative bacteria *Pseudomonas aeruginosa* (MTCC 424) *Escherichia coli* (MTCC 443) and *Proteus vulgaris*. The inoculated sterilized nutrient agar media was poured into petri dishes and allowed to solidify 6mm wells were made on the agar surface, into each of these wells, 30µl of the test compound with different concentrations /reference standard/control was added by using a micropipette. Streptomycin was used as standard reference and DMSO was used as a control (solvent) which did not possess any inhibition zone. The plates were incubated at 37 ºC for 24 h for bacterial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated in **Table-2.**

Table :2. Zones of inhibition (mm) of the target compounds (6a-e) against tested bacterial strains

Entry	Compounds	Zone of inhibition in mm*					
		Gram negative bacteria			Gram positive bacteria		
		E. coli	*P. aeruginosa*	*P. vulgaris*	*S. aureus*	*B. cereus*	*B. subtilis*
1	**6a**	NA	NA	NA	8	NA	NA
2	**6b**	NA	NA	8	8	NA	NA
3	**6c**	8	9	9	10	8	9
4	**6d**	NA	NA	NA	8	NA	NA
5	**6e**	9	8	10	9	8	9
6	**Streptomycin**	10	9	12	11	10	11

** Zones of inhibition at 25µg/mL.*
NA = Not Active.

The compound **6a** and **6d** were found to be active against *S. aureus* with the zone of inhibition 8mm, **6b** was active against *P. vulgaris* and *S. aureus* and compounds **6c** and **6e** were found to be the active against all the tested bacterial strains. All the compounds were found to be active against *S. aureus.* Compounds **6c** and **6e** showed zone of inhibition 10mm against *S. aureus* and *P. vulgaris* respectively.

2.2.2.2 Antifungal activity

All the synthesized compounds were evaluated *in vitro* antifungal activity against *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170). They were grown on potato dextrose agar medium. The plates were incubated at 28 °C for 24 h and the zone of inhibition was measured in mm. Fluconazole was used as a standard reference and DMSO was used as a solvent (control), which did not possess any inhibition zone. The results of the antifungal activities are tabulated in **Table-3.**

Table:3. Zones of inhibition (mm) of the target compounds (6a-e) against tested fungal strains

Entry	Compound	Concentration (µg/mL)	Zone of inhibition in mm	
			C. albicans	*S. cerevisiae*
1	**6a**	25	NA	NA
2	**6b**	25	NA	NA
3	**6c**	25	NA	10
4	**6d**	25	NA	NA
5	**6e**	25	NA	NA
6	Fluconazole	25	18	20

NA = Not Active

Among the compounds, the compound which bears electron with drawing $–NO_2$ **(6c)** was found to be active against *S. cerevisiae* with the zone of inhibition 10mm. The remaining compounds **(6a,b,d,e)** were found to be inactive against all the tested strains.

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REFERENCES

[1] Y. Unver, E. Dugdu, K. Sancak, M. Er, S.A. Karaoglu, *Turk J Chem*., **2009**, 33, 135.
[2] K. Sancak, Y. Unver, C. Kazak, E. Dugdu, B. Arslan, *Turk J Chem.*, **2010,** 34, 771.
[3] W. Li, Q. Wu, Y. Ye, M. Luo, L. Hu, Y. Gu, F. Niu, J. Hu, *Spectrochim. Acta A.*, **2004**, 60, 2343.
[4] M. Kritsanida, A. Mouroutsou, P. Marakos, N. Pouli, S. Papakonstantinou-Garoufalias C. Pannecouque, M.Witvrouw, E.D. Clercq, *II Farmaco*, **2002**, 57, 53.
[5] B.S Holla, K.N. Poorjary, B.S. Rao, M.K. Shivananda, *Eur. J. Med. Chem.*, **2002**, 37, 511.
[6] G.R. Kokil, P.V. Rewatkar, S. Gosain, S. Aggarwal, A. Verma, A. Kalra, S. Thareja, *Letters in drug Design & Discovery*., **2010**, 7, 46.
[7] U. Gisi, H. Sierotzki, A. Cook, A. McCaffery, *Pest Management Science.*, **2002**, 58, 859.
[8] B.S. Holla, R. Gonsalves, S. Shenoy, *II Farmaco.*, **1998**, 53, 574.
[9] S. Ersan, S. Nacak, R. Bercem, *II Farmaco*., **1998**, 53, 773.
[10] N.N. Gulerman, H.N. Dogan, S. Rollas, C. Johansson, C. Celik, *II Farmaco*., **2001**, 56, 953.
[11] J.R. Maxwell, D.A. Wasdahl, A.C. Wolfson, *J Med Chem.*, **1984**, 27, 1565.
[12] G. Turan-Zitouni, Z.A. Kapalancikli, K. Erol, F.S. Kilic, *II Farmaco*., **1999**, 54, 218.
[13] N. Demirbas, R. Ugurluoglu, A. Demirbas, *Bioorg Med Chem*., **2002**, 10, 3717.
[14] K. Paulvannan, T. Chen, R. Hale, *Tetrahedron*., **2000**, 56, 8071.
[15] M. Kritsanida, A. Mouroutsou, P. Marakos, N. Pouli, S. Papakonstantinou-Garoufalias, C. Pannecouque, M. Witvrouw, E.D. Clercq, *II. Farmaco.*, **2002**, 57, 253.
[16] L.Y. Chung, W.K. Soo, K.Y. Chan, M.R. Mustafa, S.H. Goh, Z. Imiyabir, *Pharmaceutical Biology*., **2009**, 47, 1142.
[17] Carrod. L.P and Grady F.D. *Antibiotics and Chemotherapy*, 3rd. ed, Churchill Livingstone: Edinburgh, **1972** p. 477.