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SYNTHESIS AND EVALUATION OF ANTI-MICROBIAL ACTIVITY OF CERTAIN NOVEL 3-SUBSTITUTED-4-AMINO-5-MERCAPTO-1,2,4-TRIAZOLES AND THEIR SCHIFF BASES

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ABSTRACT

A novel series of Schiff bases, based on new 3-substituted-4-amino-5-mercapto-1,2,4-triazole was prepared by reaction where initially. A mixture of di-substituted aromatic benzoic acid and thiocarbohydrazide was heated in the presence of alcohol to get the intermediate compound 3-substituted-4-amino-5-mercapto-1,2,4-triazole**(Int-i)**. Finally, **(Int-i)** on reaction with different substituted aromatic aldehydes in ethanol yielded the title compound**(SNa-c)**. The synthesized compounds were analyzed by TLC. Melting points were determined by precision melting point apparatus in open capillaries and are uncorrected. Characterization of compounds was done by IR, ^{1}H-NMR and Mass analysis. The synthesized compound was further evaluated for antibacterial and antifungal activity using cup-plate method.

KEYWORDS: 3-substituted triazole, Antimicrobial activity, Cup plate method, Schiff base.

INTRODUCTION

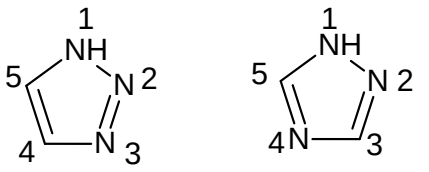
A microbial infection occurs when a microbe, such as a virus, bacteria, or fungi, enters a person's body and multiplies, producing disease. The microorganism enters the body through one of several entry points, including the respiratory tract, gastrointestinal tract, urogenital tract or skin breaks. The symptoms depend on the pathogen and the location of the illness.

Redness, heat, swelling, discomfort and swollen lymph nodes are all symptoms of a bacterial infection.

Infections are caused by the immune system's attempt to eliminate the invading pathogen. In many circumstances, the immune system can prevent these bacteria from replicating within the body. If not, major harm can occur. These microorganismsrelies on the host's body to survive, reproduce, and colonize. These contagious microscopic creatures, known as pathogens, replicate rapidly.

Triazole is an important heterocyclic moiety that occupies a unique position in heterocyclic chemistry, due to its large number of biological activities.

Triazole has two likely isomers based on where the nitrogen atom is located in the moiety.[1] One of the most significant families of heterocycles containing nitrogen that have demonstrated a range of biological activity are triazoles, which include 1,2,3-triazole and 1,2,4-triazole.[2] Many ring systems with this heterocyclic core have been combined into a wide range of therapeutically interesting medicinal molecules and triazole is a building block of tremendous importance in drug prospects.[3]



1,2,3-triazole 1,2,4-triazole

Isomers of triazole

Due to their numerous biological activities and extensive spectrum of medicinal qualities, substituted triazoles have drawn a lot of attention during the past years. Among the most important five-member heterocyclic compounds, the triazole moiety is a feature of both natural and artificial molecules.

MATERIAL AND METHODS

The chemicals and reagents used in the present project were of AR grade and LR grade, purchased from Merck, Sigma, Qualigens, NR Chem, Rolex, S.D.Fine Chem. Ltd.

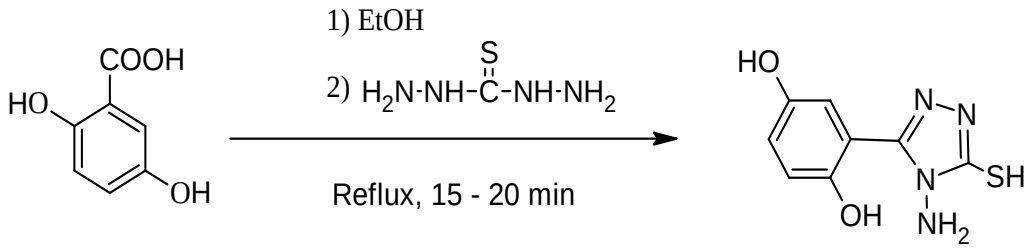
Melting points were determined by precision melting point apparatus in open capillaries and are uncorrected. All the compounds were recrystallized using suitable solvents. Purity of the

compounds was checked by thin layer chromatography using silica gel G, as stationary phase and various ratio of chloroform and methanol (4:2) was used as mobile phase. The spots resolved were visualized as brown colored spots by using iodine chamber.

I.R Spectra was recorded on KBr beam splitter in DTGS KBr detector on Bruker Spectrophotometer with resolution of 4000-500 cm^{-1}. The ^{1}H-NMR spectra was reported on instrument SA/AD/INS/001 NMR Spectrophotometer using DMSO as Solvent and at varian 400MHz and frequencies were recorded in wave numbers. Mass spectra was done by LC-MS technique on instrument SA/AD/INS/051.

Synthesis

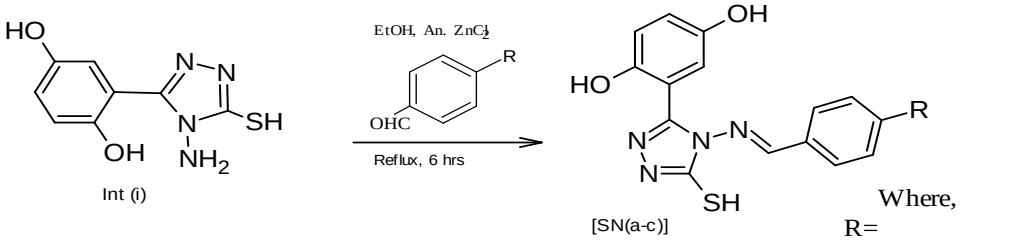
Step A: Synthesis of (2,5-dihydroxyphenyl)-4-amino-5-mercapto-1,2,4-triazole (Int-i)



2,5-dihydroxy benzoic acid int (i)

A mixture of 2,5-disubstituted benzoic acid (0.01 mol), thiocarbohydrazide (0.01 mol) and Ethanol contained in a round bottom flask was heated in oil bath until contents melted. The mixture was maintained at this temp for 20 minutes. The product obtained on cooling was washed with sodium carbonate solution to remove un-reacted acid if any. The product was then washed with water and collected by filtration. The solid product was then recrystallized from a suitable solvent.

Step B: 2-(5-mercapto-4-(4-substituted benzylidene)amino)-4H-1,2,4-triazol-3-yl) benzene-1,4-diol.(SNa-c)



Where,

R=

SN-a) $-NO_2$

SN-b) -Cl

SN-c) $-OCH_3$

The mixture of **int (i)** (0.01 mol) and substituted benzaldehyde (0.01 mol) is taken in round bottom flask containing 30 ml of absolute ethanol. To this a pea size anhydrous $ZnCl_2$ is added and the mixture is refluxed for 6 hrs. The yellow colour solid separates out. The product is then filtered and washed with water to remove unreacted $ZnCl_2$. The solid product was then recrystallized from a suitable solvent.

Table 1: Physical Characterization Data of Synthesized Compounds [SN-(a-c)].

Comp. Code	R	Molecular Formula	Molecular Weight	m.p (^{0}C)	Yield (%)	Rf value	S	Elemental Analysis Calculated(found)%		
								C	H	N
SN-a	$-NO_2$	$C_{15}H_{11}N_5O_4S$	356	217	82	0.59	E.D	50.42 (50.39)	3.10 (3.12)	19.60 (19.57)
SN-b	-Cl	$C_{15}H_{11}ClN_4O_2S$	346	187	53	0.61	E.D	51.95 (51.94)	3.10 (3.12)	16.16 (16.13)
SN-c	$-OCH_3$	$C_{16}H_{14}N_4O_3S$	342	180	75	0.69	E.D	56.13 (56.15)	4.12 (4.09)	16.36 (16.32)

S: Solvent for recrystalization

E.D: Mixture of Ethanol & Dimethylformamide in the ratio of 1:2

Spectral characterization of synthesized compounds. [SN-(a-c)]

2-(5-mercapto-4-(4-nitrobenzylidene)amino)-4H-1,2,4-triazol-3-yl)benzene-1,4-diol (SN-a):IR(KBr)cm^{-1}: 3040(Ar, C-H str), 2500(S-H str), 1605(Ar, C=N str), 1512(Ar, C=C str), 1512 (N-O str), 1335(O-H bend), 1252(C-N str), 1096(C-O str), 845(1,4-disubstituted Ar. ring) **MS (m/z):**The molecular ion peak M^+ agrees with the molecular weight 356 of the compound under investigation and it is confirmed. The fragment peaks are obtained at m/e 303, 225). **^{1}H-NMR (DMSO/400MHz)-**It contains 11H protons: 7.2 (s, 2H, OH), 7.8-8.4 (m, 7H, Ar-H), 8.6-8.8 (m, 1H, N=CH), 12.00 (bs, 1H, SH).

2-(5-mercapto-4-(4-chlorobenzylidene)amino)-4H-1,2,4-triazol-3-yl)benzene-1,4- diol (SN-b): 3032 (Ar,C-H str), 1630 (Ar, C=N str), 1545 (Ar, C=C str), 1332 (O-H bend),1077 (C=O str), 818 (1,4-disubstituted Ar. ring), 663 (C-Cl). **MS (m/z):**The molecular ion peak M^+ agrees with the molecular weight 346 of the compound and it is confirmed. **^{1}H-NMR (DMSO/400MHz)-**7.0 (s, 2H, OH), 7.7-8.6 (m, 7H, Ar-H), 8.7 (m, 1H, N=CH), 11.86 (bs, 1H, SH).

2-(5-mercapto-4-(4-methoxybenzylidene)amino)-4H-1,2,4-triazol-3-yl)benzene-1,4-diol (SN-c): 3414 (O-H str), 3010 (Ar, C-H str), 2820 (C-H str of CH_2), 2520 (S-H str), 1652,1590 (Ar, C=C str), 1590 (Ar, C=N str), 1352 (O-H str), 1255 (C-N str), 1185 (C-O str),

823 (1,4-disubstituted Ar. ring) **MS (m/z):**The molecular ion peak M^+ agrees with the molecular weight 342. **^{1}H-NMR (DMSO/400MHz)-**3.53(s,3H, OCH_3),7.4 (s, 2H, OH), 7.6-8.5 (m, 7H, Ar-H), 8.75 (m, 1H, N=CH), 11.95 (bs, 1H, SH).

Biological evaluation

Antibacterial and Antifungal evaluation

The Antimicrobial activity was estimated by comparing the inhibition of growth of sensitive micro- organisms produced by known concentration of the synthetic compound to be examined against a reference substance.

Method of analysis

Cup-plate method (Agar well diffusion method)- The agar cup plate method depends upon diffusion of the antibiotic from a vertical agar (well) cylinder through a solidified agar layer on a petri dish. Sterile Agar was inoculated by suspension of the microbial inoculum. Then a disc with diameter of 6 to 8 mm was punched aseptically, and then the antimicrobial solution at desired concentration was introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain entirely in a zone around the cylinder containing a solution of the substance to be tested.[24-26]

Preparation of medium

Antibiotic Assay Medium no. 19 was taken in 600 ml of purified water, heated to boiling to dissolve the medium completely the pH of media was checked. If required sufficient quantity of 1 M sodium hydroxide or 1 M hydrochloric acid was added and distributed in 200 ml flasks as required, so that after sterilization as quantity as per required for analysis, sterilized by autoclaving it at15 lbs pressure (121^0C) for 15 min.

Preparation of the sample solution

5 mg/ml and 10 mg/ml samples were prepared to inoculate into well.

Preparation of the standard solution

1 mg of each sample was weighed and dissolved/diluted with 1 ml DMSO in volumetric flask. Vortex for 1-2 min to effect the dissolution, 100 µl was used directly to inoculate.

Preparation of test Organism and Suspension

Stock culture: *S.aureus* ATCC no.6538, *B.subtilis* ATCC no.19659, *E. coli* ATCC no. 8739**,** *P.aeruginosa* ATCC no. 15442**,** *A.niger* ATCC no. 11414, *C.albicans* ATCC no. 10231

A loopful of suspension ATCC. 6538 was streak on two slants of preincubated Nutrient agar. The slants were Incubated at 30-35^{0}C for 24 hours in an incubator After incubation the growth from incubated slant was picked and inoculated in 3 ml of saline solution and vortex to prepare the uniform suspension. The O.D. of culture was adjusted to approx. 60-70 % OD at 530 nm using sterile saline and calorimeter. After adjusting O.D. the test organisms were stored in refrigeration at 2-8^{0}C.

Note: Approximately viable count is 10^0 to 10 cfu/ml against 60-70 % OD at 530 nm

Plate preparation for analysis

Each 2 ml of culture suspension of *S. aureus, B. subtilis, E. coli, P. aeruginosa, A. niger and C. albicans* was used to inoculate separately in 200 ml of sterile molten and cooled medium at 40^{0}C - 45^{0}C Antibiotic Assay Medium no. 19. 15-20 ml of sterilized agar medium was poured into a sterile petri plate with the help of sterile measuring cylinder and a depth of 3 to 4 mm was given. This was allowed to cool at room temperature by placing the dishes or plates on a level surface. The plates were kept in refrigerator for 15 to 20 minutes for hardening ensuring that the layers of medium are uniform in thickness. On each plate 4-5 agar cups were made using 8-10 mm SS borer. The plates were than labelled for sample, standard and negative control samples and analysed.

Analysis

100 µl 1mg/ml solution A was added to agar cup labeled as STD.100 µl 1mg/ml Solution B was added to agar cup labeled for each compound ID (SN-a, SN-b & SN-c) labeled on plate. 100 µl DMSO was added to agar cup labeled as N (Negative). This dishes or plates were left standing for 15-20 min. at 2-8^{0}C or as appropriate, as a period of pre- incubation diffusion to minimize the effects of variation in time between the applications of the different solutions. These plates were than incubated for about 24-48 hours at the temperature 30-35^{0}C for bacteria and 20-25^{0}C for yeast and mould. After completion of incubation the diameters or areas of the circular inhibition zones were measured and the results were recorded. The activity was performed at different concentrations of 10mg/ml and 5mg/ml.

Table 2: Antibacterial activity of synthesized compounds[SN(a-c)] at 5mg/ml.

Compound code	Mean zone of inhibition in mm			
	Gram-positive organisms		Gram-negative organisms	
	S.aureus	*B. subtilis*	*E. coli*	*P.aeruginosa*
SN-a	01	03	08	10
SN-b	15	18	16	19
SN-c	02	03	01	04
Gentamycin (Standard)	36	36	36	36
DMSO (Control)	-	-	-	-

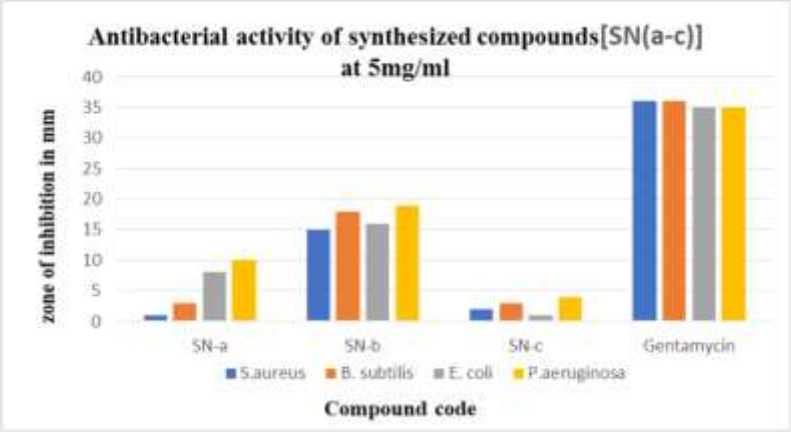


Fig. 1: Graphical representation of Antibacterial activity of compounds [SN(a-c)].

Table 3: Antibacterial activity of synthesized compounds[SN(a-c)] at 10mg/ml.

Compound code	Mean zone of inhibition in mm			
	Gram-positive organisms		Gram-negative organisms	
	S.aureus	*B. subtilis*	*E. coli*	*P.aeruginosa*
SN-a	02	01	14	18
SN-b	22	21	24	28
SN-c	04	02	02	04
Gentamycin (Standard)	36	36	36	36
DMSO Control	-	-	-	-

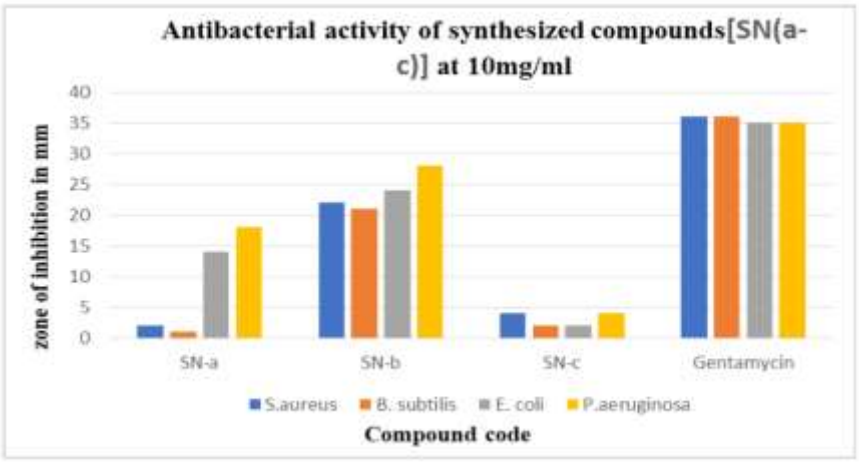


Fig. 2: Graphical representation of Antibacterial activity of compounds[SN(a-c)].

Table 4: Antifungal activityof synthesized compounds[SN(a-c)] at 5mg/ml.

Compound code	Mean zone of inhibition in mm	
	A.niger	***C.albicans***
SN-a	01	01
SN-b	04	03
SN-c	01	01
Griseofulvin (Standard)	16	16
DMSO Control	-	-

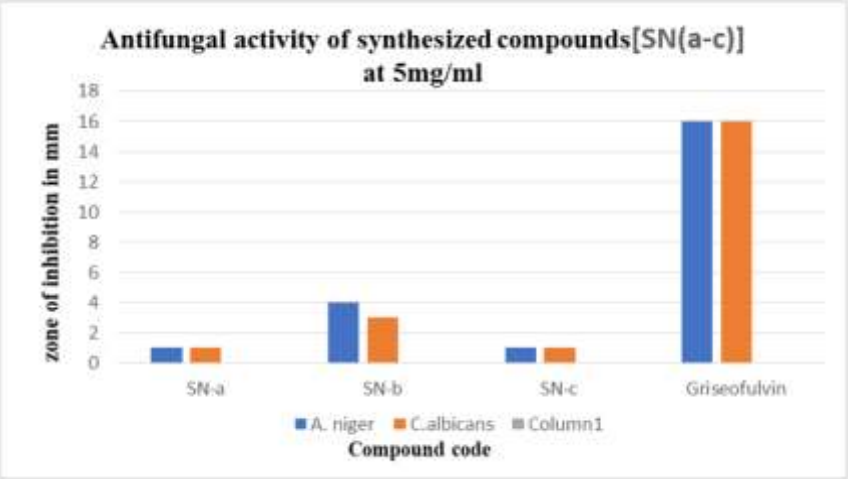


Fig. 3: Graphical representation of Antifungal activity of compounds[SN(a-c)].

Table 5: Antifungal activityof synthesized compounds[SN(a-c)] at10mg/ml.

Compound.code	Mean zone of inhibition in mm	
	A. niger	***C.albicans***
SN-a	02	01
SN-b	15	13
SN-c	14	13
Griseofulvin (Standard)	16	16
DMSO (Control)	-	-

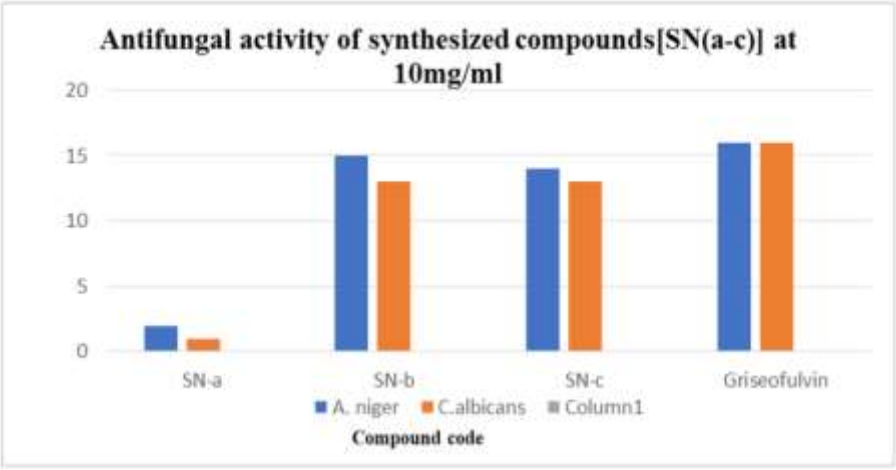


Fig. 4: Graphical representation of Antifungal activity of compounds.

RESULTS AND DISCUSSION

The synthesized substituted triazoles compounds **[SN-(a-c)]** was evaluated for antimicrobial activity. During the study it has been found that compound **SN-a** and **SN-b** possess good antibacterial activity against ***S. aureus*** and ***E.coli.*** Other representative compound of the series **SN–c** possesses moderate to weak antibacterial activity against selected bacterial strain when compared to reference standard Gentamycin.

Antifungal activity of the synthesized compounds **[SN(a-c)]** showed that, compound **SN-b** and **SN–c** possess good and compound **SN-a** possess poor inhibiting the growth of micro-organisms against fungal strain ***A.niger*** and ***C.albicans*** as compared to reference standard Griseofulvin.

CONCLUSION

Synthesized compounds were confirmed by physical characterization, spectral techniques like, IR, ^{1}H-NMR and mass spectroscopy, and the spectral data of compounds were in agreement with their structure.

Our study investigated that certain new substituted triazole derivatives displayed good to moderate anti-microbial activity in comparison with the reference standard. Thus, the present work provides a new outline of the study of anti-microbial activity of substituted triazole.

From the above results one can establish that the synthesized substituted 1,2,4-triazole can be rich source for the exploitation. Therefore, in search of new generation of the active compounds, it may be worthwhile to explore the possibility in this area by introducing different functional groups or by cyclization as substituents which may result into better pharmacological agents.

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