Antimicrobial Evaluation and Synthesis of 3,10-Disubstituted-6,13-[bis(5-aryl-3-mercapto-1,2,4-triazolyl)dipiperazino]-bis[s-triazolo-(3,4-b)-1,3,4-thiadiazino-(5,6-b;5',6'-e)cyclohexa-1,4-dienes

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Summary: Synthesis of some 3,10-disubstituted-6,13-[bis(5-aryl-3-mercapto-1,2,4-triazolyl) dipiperazino]-bis[s-triazolo-(3,4-b)-1,3,4-thiadiazino-(5,6-b;5',6'-e)cyclohexa-1,4-dienes 3(a-i) have been reported. All the synthesized compounds have been characterized by elemental and spectral (IR, ^{1}H- NMR and Mass) analysis. Furthermore, above said compounds were evaluated for their antibacterial and antifungal respectively against selected microbial pathogens. Compound 3h displayed the most biological potency.

Keywords: Substituted cyclohexa-1, 4-dienes, antibacterial, antifungal activity.

Introduction

Heterocycle have been found very essential to living beings and explored pivotal role in their different metabolic activities. 1, 2, 4-triazoles exist in variety of therapeutic agents including antiviral (ribavarin), anti-migraine **(**rizatriptan), antifungal (flucanazole), antianxiety compounds (alprazolam). In medicinal chemistry, 1,2,4-triazoles [1-3] displayed diversity in biological activity as antibacterial [4-5], antifungal [5], anti-inflammatory [6], anticancer [7], antianxiety [8], antidepressant [8] etc. On the other hand the biological spectrum of substituted piperazines [9-11] have been claimed time to time by various scientific workers. In the present work, we have adopted the chemical strategy to derive some novel derivatives of piperazine and substituted 1, 2, 4-triazole by using simple conventional methodology in laboratory.

Results and Discussion

Reaction of 3-phenyl-4-amino-5-mercapto triazole [12] with chloranil in presence of anhydrous sodium acetate yielded compound **1**, which on further condensation reaction with morpholine furnished compound **2**. The desired moieties **3(a-i)** were obtained by the reaction of compound **2** and 3-aryl-4-amino-5-mercapto triazoles [12]. The entire synthesized compounds *i.e*. **1**, **2** and **3(a-i)** were evaluated for antibacterial and antifungal activity respectively against the selected panel of pathogens. Disk diffusion method was adopted for antibacterial activity while serial plate dilution method was for antifungal activity. The inhibitory activity (mm) was summarized in Table-**1**. On the basis of S A R, the prepared derivatives **1**, **2** and **3(a-i)** explored mild to moderate antibacterial and antifungal spectrum. The compound **1** was found active only *P. vulgaris* while compound **2** bearing morpholine was active against *E. coli*, *K. pneumoniae*, *P. vulgaris* and *A. fumigatus*. Target derivatives obtained by the incorporation of substituted 1,2,4-triazole with compound **2** which resulted into remarkable biological potency than the earlier tested derivative **1** & **2**. In vitro screening data revealed that incorporation of substituted 1, 2, 4-triazoles brought remarkable inhibitory properties. Among the screened compounds **3(a-i)**, phenyl substituted compound **3a** showed poor inhibition in comparison of the remaining derivatives while 2-chloro substituted compound **3h** was found the most potent candidate as showed better inhibition in comparison to reference drugs. Methoxy substituted phenyl rings bearing compounds **3b**, **3c** & **3d** showed almost similar antibacterial as well as antifungal inhibitory property. In compounds **3e** and **3f**; 4-hydroxy benzyl substitution bearing derivative **3h** displayed better inhibition than the derivative **3e** possessing 4-ethoxy phenyl substitution. Chloro substituted derivatives elicited better inhibition among the prepared series of compounds **3(a-i)**; 2-chloro phenyl substitution reasoned significant antimicrobial approach in compound **3h** while 4-chloro phenyl substitution in compound **3i** caused better inhibition but lesser than the **3h**.

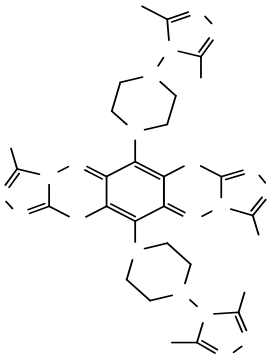

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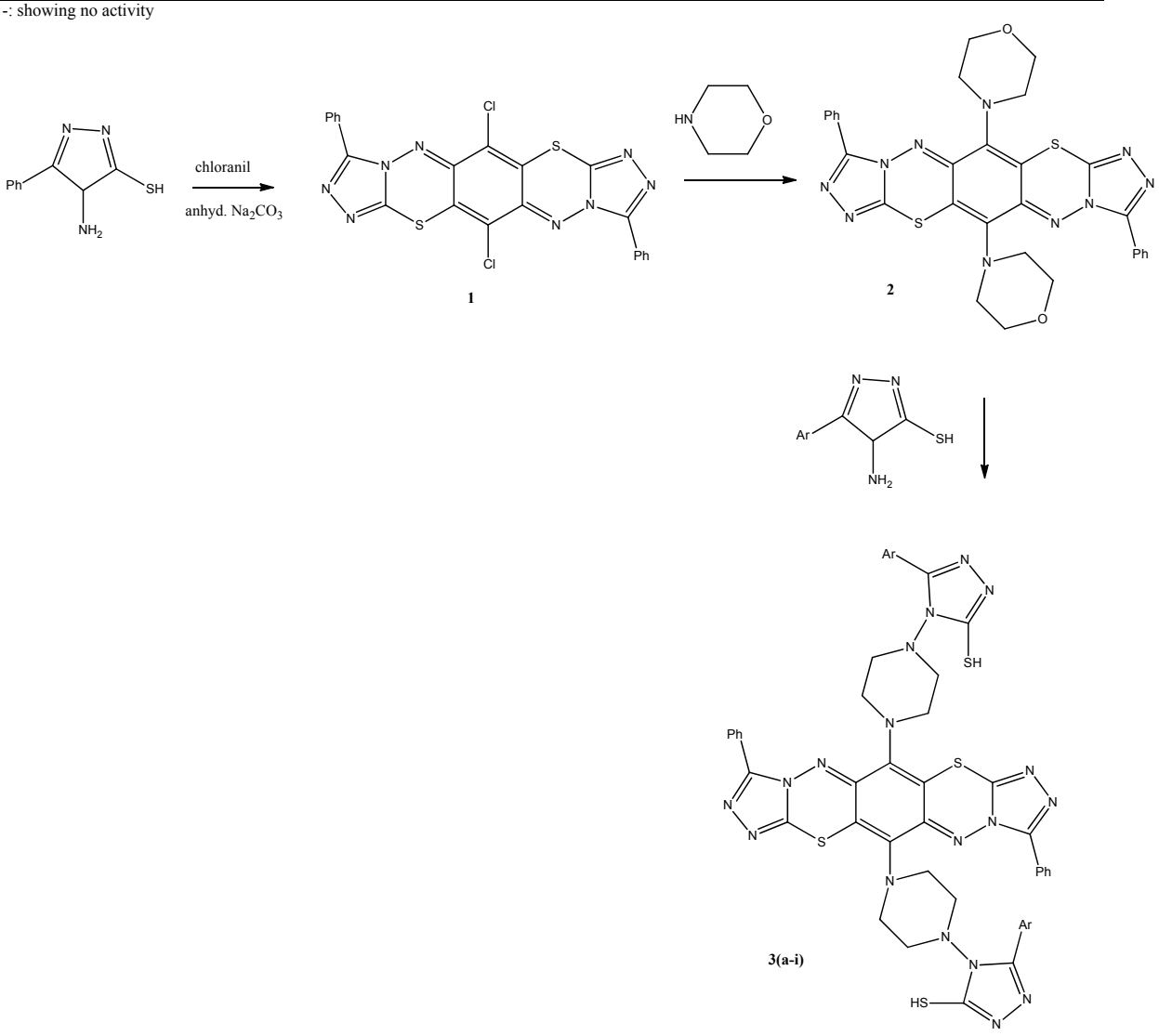
Table-1: Antibacterial and antifungal screening data for the synthesized compounds 1, 2 & 3(a-i).

	Antibacterial inhibition (mm)				Antifungal inhibition (mm)			
Comp. no.	**S. *aureus***	**E. *coli***	**K. *pneumoniae***	**P. *vulgaris***	**A. *fumigatus***	**C. *glabrata***	**C. *albacans***	**C. *krusei***
1	-	-	-	5	-	-	-	-
2	-	5	4	5	6	-	-	-
3a	-	5	4	5	8	-	-	-
3b	5	-	4	12	10	5	10	6
3c	-	10	12	10	8	6	5	10
3d	10	12	10	14	-	10	7	7
3e	12	-	15	17	-	-	-	10
3f	10	12	12	15	10	4	5	-
3g	12	10	14	8	-	9	7	9
3h	15	22	18	16	16	12	-	12
3i	12	15	15	16	12	-	-	-
Ampicillin trihydrate (std.)	16	20	20	20	-	-	-	-
Fluconazole (std.)	-	-	-	-	20	15	16	15
DMF (control)	-	-	-	-	-	-	-	-

-: showing no activity



Ar = C_6H_5, 2-OH.C_6H_4, 3-OH.C_6H_4, 4-OH.C_6H_4, 4-C_2H_5O.C_6H_4, 4-OH.$C_6H_4CH_2$, 4-C_2H_5O.$C_6H_4CH_2$, 2-Cl.C_6H_4, 4-Cl.C_6H_4

Scheme 1

Experimental

Material and Methods

All the chemicals used for the preparation of desired derivatives, were obtained from Sisco Research Laboratories (SRL), Mumbai, India; Qualigen Fine Chemicals, Mumbai, India; E. Merck Ltd., New Delhi, India. The reference drugs ampicillin and fluconazole were procured from Ind-Swift, Pharmaceutical, Panjab, India and Dr. Reddy Lab., Hyderabad, India. The melting points of the compounds were determined in open glass capillaries with the help of thermonic melting points apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. The homogeneity of all the newly synthesized compounds were routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. Elemental analysis was performed in Heraeus CHN rapid analyser. The results were found within the ±0.4% of theoretical values. Infrared spectra were recorded on KBr pellets on a Perkin Elmer system 2000 FTIR spectrometer and ^{1}H- NMR spectra on Bruker DPX 200 using TMS as internal standard.

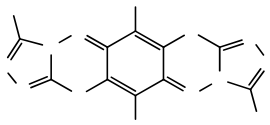
Biological Study

Antimicrobial Evaluation

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. All microbial strains, *i.e*. bacterial as well as fungal; were clinical isolates, identified with conventional morphological and biochemical methods. Microorganisms employed antibacterial studies were *Staphylococcus aureus*, *Escherichia coli*, *Klabsiella pneumoniae* and *Proteus vulgaris*. Disk diffusion method [13-14] was used for determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 ^{0}C for an hour. The test compounds were prepared with different concentrations using DMF. One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were for placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 ^{0}C for 24 h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. Bacterial inhibition of the tested compounds against the tested bacteria strains are recorded in Table-**1**. On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against *Aspergillus fumigatus* (plant isolate), *Candida glabrata*, *Candida albacans* and *Candida krusei* in DMSO by the serial plate dilution method [15-16]. Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of the corresponding species. Agar media (20 ml) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 ^{0}C for 1 h. Using an agar punch wells were made into each well labeled. A control was also prepared in triplicate and maintained at 37 ^{0}C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The inhibition values of the tested compounds against the tested fungal strains are recorded in Table-**1**.

Synthesis of 3,10-Disubstituted-6,13-dichloro-bis[s-triazolo-(3,4-b)-1,3,4-thiadiazino-(5,6-b;5',6'-e)cyclohexa-1,4-dienes **(1)**

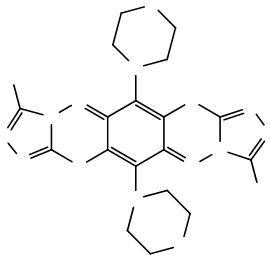
An ethanolic mixture of 3-phenyl-4-amino-5-mercapto triazole (0.15M), chloranil (0.07M) and anhydrous sodium acetate (0.001M) was heated to reflux for 3-4h. The resulting reaction mass was allowed to keep at room temperatre for 4 h., brown-red coloured solid settled down, filtered, washed properly with water and recrystallized from methanol. Yield: 70%; m.p.: 255 °C; R_f : 0.50. Anal. Calcd. for $C_{22}H_{10}Cl_2N_8S_2$: C, 50.68; H, 1.93; N, 21.49%. Found: C, 50.60; H, 1.95; N, 21.48%. IR (KBr, cm^{-1}): 1615 (C=N), 1520 (C-N), 1205 (N-N=C), 665 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm): 7.00-7.80(m, 10H, Ph). MS (*m*/*z*, %): 521.40.



Synthesis of 3,10-Disubstituted-6,13-dimorpholino-bis[s-triazolo-(3,4-b)-1,3,4-thiadiazino-(5,6-b;5',6'-e)cyclohexa-1,4-dienes **(2)**

To a solution of compound 1(0.01M) in DMF (100 ml), morpholine (0.021M) and powder potassium carbonate was added at 0-5 ^{0}C. The resulting slurry was stirred until it attained room temperature. After, the reaction mass refluxed for 1h.,cooled to room temperature, dumped on crushed

ice-water, filtered, washed, dried and recystallized from ethanol. Yield: 66%; m.p.: 278 °C; R_f : 0.64. Anal. Calcd. for $C_{30}H_{26}O_2N_{10}S_2$: C, 57.86; H, 4.21; N, 22.49%. Found: C, 57.82; H, 4.25; N, 22.50%. IR (KBr, cm^{-1}): 1611 (C=N), 1523 (C-N), 1210 (-N-N=C), 669 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm): 6.90-7.72(m, 10H, Ph). MS (*m*/*z*, %): 622.72.



Synthesis of 3,10-disubstituted-6,13-[bis(5-aryl-3-mercapto-1,2,4-triazolyl)dipiperazino]-bis[s-triazolo-(3,4-b)-1,3,4-thiadiazino-(5,6-b;5',6'-e)cyclohexa-1,4-dienes **3(a-i*)***

To a solution of compound 2 (0.01M) in dry pyridine (100 mL), different substituted triazoles (0.02M) was added. The reaction mixture refluxed for 1-3 h. After refluxing, excess of solvent was removed and the residue neutralized with dilute hydrochloric acid. The solid separated out was washed with water and recrystallized from appropriate solvents to furnish compounds **3(a-i)**.

3a: Yield: 60%; m.p.: 213 °C; R_f : 0.61. Anal. Calcd. for $C_{46}H_{38}N_{18}S_4$: C, 56.89; H, 3.94; N, 25.96%. Found: C, 56.86; H, 3.95; N, 26.00%. IR (KBr, cm^{-1}): 1608 (C=N), 1521 (C-N), 1206 (-N-N=C), 673 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm): 13.52(s, 2H, 2 X SH), 6.60-7.93(m, 20H, Ph & Ar), 1.10-1.00 (m, 16H, 2 X 8H, morpholine). MS (*m*/*z*, %): 971.17.

3b: Yield: 58%; m.p.: 252 °C; R_f : 0.68. Anal. Calcd. for $C_{46}H_{38}N_{18}O_2S_4$: C, 55.07; H, 3.82; N, 25.13%. Found: C, 55.06; H, 3.85; N, 25.14%. IR (KBr, cm^{-1}): 1612 (C=N), 1525 (C-N), 1213 (-N-N=C), 670 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm): 13.48(s, 2H, 2 X SH), 10.55(s, 2H, 2 X OH), 6.70-8.00(m, 18H, Ph & Ar), 1.15-1.02 (m, 16H, 2 X 8H, morpholine). MS (*m*/*z*, %): 1003.17.

3c: Yield: 52%; m.p.: 279 °C; R_f : 0.61. Anal. Calcd. for $C_{46}H_{38}N_{18}O_2S_4$: C, 55.07; H, 3.82; N, 25.13%. Found: C, 55.08; H, 3.82; N, 25.11%. IR (KBr, cm^{-1}): 1610 (C=N), 1518 (C-N), 1207 (-N-N=C), 672 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm): 13.53(s, 2H, 2 X SH), 10.46(s, 2H, 2 X OH), 6.71-7.93(m, 18H, Ph & Ar), 1.14-1.00 (m, 16H, 2 X 8H, morpholine). MS (*m*/*z*, %): 1003.17.

3d: Yield: 58%; m.p.: 250 °C; R_f: 0.66. Anal. Calcd. for $C_{46}H_{38}N_{18}O_2S_4$: C, 55.07; H, 3.82; N, 25.13%. Found: C, 55.00; H, 3.84; N, 25.16%. IR (KBr, cm^{-1}): 1618 (C=N), 1522 (C-N), 1216 (-N-N=C), 667 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm): 13.40(s, 2H, 2 X SH), 10.50(s, 2H, 2 X OH), 6.62-8.10(m, 18H, Ph & Ar), 1.18-1.03 (m, 16H, 2 X 8H, morpholine). MS (*m*/*z*, %): 1003.17.

3e: Yield: 48%; m.p.: 292 °C; R_f: 0.70. Anal. Calcd. for $C_{50}H_{46}N_{18}O_2S_4$: C, 56.69; H, 4.38; N, 23.80%. Found: C, 56.71; H, 4.33; N, 24.00%. IR (KBr, cm^{-1}): 1609 (C=N), 1520 (C-N), 1211 (-N-N=C), 671 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm): 13.56(s, 2H, 2 X SH), 6.58-7.88(m, 18H, Ph & Ar), 1.95(q, 4H, 2X 2H, -CH_2-), 1.37(t, 6H, 2X 3H, –CH_3), 1.18-1.08 (m, 16H, 2 X 8H, morpholine). MS (*m*/*z*, %): 1059.28.

3f: Yield: 55%; m.p.: 262 °C; R_f: 0.56. Anal. Calcd for $C_{48}H_{42}N_{18}O_2S_4$: C, 55.91; H, 4.11; N, 24.45%. Found: C, 55.79; H, 4.10; N, 24.47%. IR (KBr, cm^{-1}): 1616 (C=N), 1518 (C-N), 1214 (-N-N=C), 668 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm): 13.61(s, 2H, 2 X SH), 10.30 (s, 2H, 2 X OH), 6.58-7.96(m, 18H, Ph & Ar), 3.96(s, 4H, 2 X -CH_2-Ph), 1.11-1.00 (m, 16H, 2 X 8H, morpholine). MS (*m*/*z*, %): 1003.17.

3g: Yield: 50%; m.p.: 196 °C; R_f: 0.63. Anal. Calcd. for $C_{52}H_{50}N_{18}O_2S_4$: C, 57.44; H, 4.63; N, 23.19%. Found: C, 57.49; H, 4.61; N, 23.23%. IR (KBr, cm^{-1}): 1621 (C=N), 1512 (C-N), 1208 (-N-N=C), 666 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ/ ppm): 13.49 (s, 2H, 2 X SH), 6.60-7.99 (m, 18H, Ph & Ar), 3.90 (s, 4H, 2 X -CH_2-Ph), 1.85 (q, 4H, 2X 2H, -CH_2-), 1.32 (t, 6H, 2X 3H, –CH_3), 1.14-1.04 (m, 16H, 2 X 8H, morpholine). MS (*m*/*z*, %): 1087.33.

3h: Yield: 53%; m.p.: 282 °C; R_f : 0.70. Anal. Calcd. for $C_{46}H_{36}Cl_2N_{18}S_4$: C, 53.12; H, 3.49; N, 24.24%. Found: C, 53.19; H, 3.46; N, 24.35%. IR (KBr, cm^{-1}): 1617 (C=N), 1516 (C-N), 1214 (-N-N=C), 672 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm):13.40(s, 2H, 2 X SH), 6.71-8.02(m, 18H, Ph & Ar), 1.16-1.00 (m, 16H, 2 X 8H, morpholine). MS (*m*/*z*, %): 1040.06.

3i: Yield: 48%; m.p.: 264 °C; R_f : 0.63. Anal. Calcd. for $C_{46}H_{36}Cl_2N_{18}S_4$: C, 53.12; H, 3.49; N, 24.24%. Found: C, 53.11; H, 3.48; N, 24.30%. IR (KBr, cm^{-1}): 1616 (C=N), 1522 (C-N), 1218 (-N-N=C), 670 (C-S-C). ^{1}H-NMR ($CDCl_3$, δ / ppm):

13.49 (s, 2H, 2 X SH), 6.56-8.00(m, 18H, Ph & Ar), 1.19-0.99 (m, 16H, 2 X 8H, morpholine). MS (*m*/*z*, %): 1040.06.

Conclusion

On the basis of study of biological activity data, it may be concluded that compounds **3(a-i)** displayed mild to moderate antibacterial and antifungal activities. Incorporation of 1, 2, 4-triazoles and presence of chloro group as substituent at **2nd** position brought remarkable increase in biological potency. Compound **3h** explored the most potent antimicrobial spectrum and deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed [17-19].

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