Organoiodine(III) Mediated Synthesis of Novel Symmetrical *Bis*([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazines as Antibacterial and Antifungal Agents

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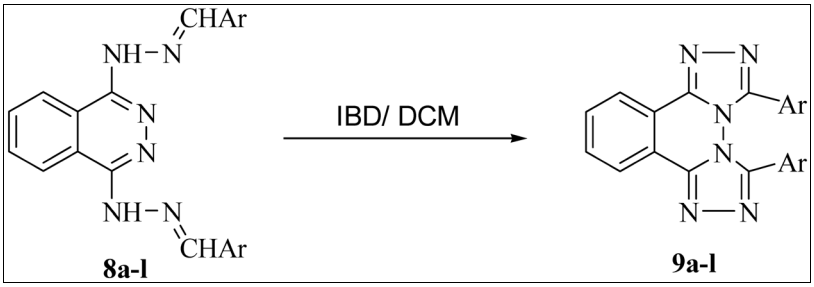

8a-l **9a-l**

A series of new symmetrical 3,6-*bis*(aryl)*bis*([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazines **9a-l** has been conveniently synthesized by oxidative cyclization of 1,4-*bis*(substituted benzalhydrazino)phthalazines **8a-l** promoted by iodobenzene diacetate under mild conditions (12 examples, up to 93% yield). All the 12 compounds were tested *in vitro* for their antibacterial activity against two Gram-positive bacteria, namely, *Staphylococcus aureus*, *Bacillus subtilis* and two Gram-negative bacteria, namely, *Escherichia coli* and *Pseudomonas aeruginosa*. All the synthesized compounds were also tested for their antifungal action against two fungi, *Aspergillus niger* and *Aspergillus flavus*.

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INTRODUCTION

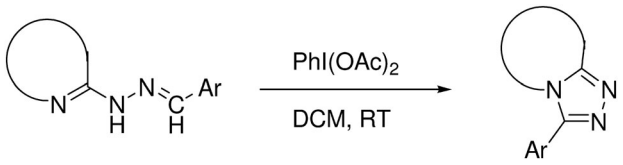
Triazoles constitute an important class of heterocyclic compounds. In particular, fused heterocyclic 1,2,4-triazoles have acquired much importance because of their diverse applications such as antifungal [1], bactericidal [1,2], anxiolytic [3, 4], anticonvulsant [5], herbicidal [6], antidepressants [7], and anti-inflammatory [8–10]. Among these, the commonly known systems are generally triazoles fused to pyridines, pyridazines, pyrimidines, pyrazines, and triazines. Although there are not many triazoles fused to phthalazines, a number of them are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities. In a report by Iszhii *et al*. [11], 1,2,4-triazolo[3,4-a]phthalazine, 3-methyl-1,2,4-triazolo[3,4-a]phthalazine, and 3-ethyl-1,2,4-triazolo[3,4-a] phthalazine have been reported as potent inhibitors of cyclic adenosine monophosphate phosphodiesterase, equal to theophlylline in potency and possess smooth muscle relaxant activity.

Most methods for the preparation of fused 1,2,4-triazole derivatives are based on heterocyclic hydrazones or hydrazides as precursors. However, these methods have some restrictions as regards their applicability and the use of toxic reagents such as phosphorus oxychloride [12], lead tetraacetate [12,13], or bromine [13, 14]. Organohypervalent iodine reagents have emerged as reagents of choice for various synthetically useful transformations due to their low toxicity, ready availability, and ease of handling [15–18]. In recent years, hypervalent iodine reagents have been successfully proven their usefulness as agents for oxidative cyclization of benzal hydrazone to synthesize 1,2,4-triazoles (Scheme 1) [19–23].

We have developed iodine(III) mediated methodology for the synthesis of heterocyclic systems bearing symmetrical triazole ring fused with different heterocyclic moieties such as pyridines **1**, pyrimidines **2**, **3**, **4**, and quinolines **5** as shown in Figure 1 [19–23].

Biological evaluations of the fused triazolo compounds synthesized during these studies have shown that they were generally associated with antibacterial activity. In particular, *bis*-1,2,4-triazolo[4,3-a][4,3-c]pyrimidines **4** have been found more active than simple 1,2,4-triazolo[4,3-a] [4,3-c]pyridine **1**, 1,2,4-triazolo[4,3-a][4,3-c]pyrimidines **2**

Scheme 1. General plan for synthesis of fused 1,2,4-triazole.



and **3**. Moreover, some of the *bis*-1,2,4-triazolo[4,3-a][4,3-c] pyrimidines **4** were associated with substantially higher antibacterial activity than some commercial antibiotics [21].

Keeping above points in view, we report herein the synthesis of symmetrical *bis*-1,2,4-triazolo-[3,4-a:4′,3′-c] phthalazines **9a-l** by the oxidative cyclization of 1,4-*bis*-2-(substituted benzylidenehydrazinyl)phthalazines **8a-l** using iodobenzene diacetate (IBD) in dichloromethane with an expectation to find new and more potent antibacterial and antifungal agents.

RESULTS AND DISCUSSION

Chemistry. Based on the synthetic route given in Scheme 1, it was anticipated that oxidation of 1,4-*bis*-2-(substituted benzylidenehydrazinyl)phthalazines **8a-l** with two equivalents of IBD might afford 3,6-*bis*(aryl)-*bis*-1,2,4-triazolo-[3,4-a:4′,3′-c]phthalazine **9a-l**. Accordingly, a solution of 1,4-*bis*(-2-benzylidenehydrazinyl)phthalazine **8a** in dichloromethane was treated with IBD at room temperature. The reaction, indeed, afforded 3,6-diphenyl *bis*-1,2,4-triazolo-[3,4-a:4′,3′-c]phthalazine **9a**, which was obtained as a crystalline product (Scheme 2). Encouraged by the feasibility of this reaction, we studied oxidative cyclization of *bis*-hydrazones **8b-l** of various araldehydes with electron-donating and electron-withdrawing substituents. The method worked nicely for the synthesis of other derivatives **9b-l** (Scheme 2). Structures of all the new compounds **9a-l** were elaborated by their spectral data (IR, ^{1}H-NMR, ^{13}C-NMR, and mass) and elemental analyses.

1,4-*Bis*-2-(substituted benzylidenehydrazinyl)phthalazines **8a-l** required for this study were synthesized by condensation of 1,4-dihydrazinophthalazine with various araldehydes. Structures of all the new 1,4-*bis*-2-(substituted benzylidenehydrazinyl)phthalazines **8a-l** were confirmed by analyzing their spectral data (IR and ^{1}H-NMR). Structures of known 1,4-*bis*-2-(substituted benzylidenehydrazinyl)phthalazines **8a-l** were confirmed by comparing their melting points and spectral data with those reported in the literature [24]. Physical data of all the synthesized compounds are summarized in Table 1.

The probable mechanistic way for the intramolecular oxidative cyclization of 1,4-*bis*-2-(substituted benzylidenehydrazinyl)phthalazines **8a-l** to afford 3,6-*bis* (aryl)*bis*([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazines **9a-l** is analogous to our previous report [22] and is outlined in Scheme 3. The first step involves the electrophillic attack of iodobenzene diacetate on **8** to form organoiodine(III) intermediate **A**, which generates another intermediate bis-nitrile amide, **B**, along with expulsion of two molecules of iodobenzene and acetic acid each. The nitrile amide undergoes cyclization to give the product **9**.

Pharmacology. All the 3,6-*bis*(aryl)*bis*([1,2,4]triazolo) [3,4-a:4′,3′-c]phthalazines **9a-l** were screened for their antibacterial activity against two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121) and two Gram-negative bacteria (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741). The title compounds were also evaluated for their antifungal activity against two fungi, *A. niger* and *A. flavus*.

Compounds **9h** and **9j** showed most significant activity against *B. subtilis* with diameter of zone of inhibition 21 and 20 mm whereas these compounds were also found to be most effective against *S. aureus*, showing the zone of inhibition of 18 mm; however, rest of compounds showed fair activity against Gram-positive bacterial strains (Table 2). In the whole series, the MIC of various tested chemical compounds ranged between 64 and 256 μg mL^{-1} against Gram-positive bacteria. Compounds **9h** and **9j** were found to be most potent amongst all the synthesized compounds with the lowest MIC of 64 μg mL^{-1} against *S. aureus*. Five compounds, **9b**, **9f**, **9h**, **9i**, and **9j** exhibited activity against *B. subtilis* with MIC of 64 μg mL^{-1} (Table 3).

Additionally, compounds **9a-l** were also screened for their antifungal activity against *A. flavus* and *A. niger*. Compounds **9b**, **9d**, **9j**, and **9l** showed more than 50% of mycelial growth inhibition against *A. flavus* whereas in case

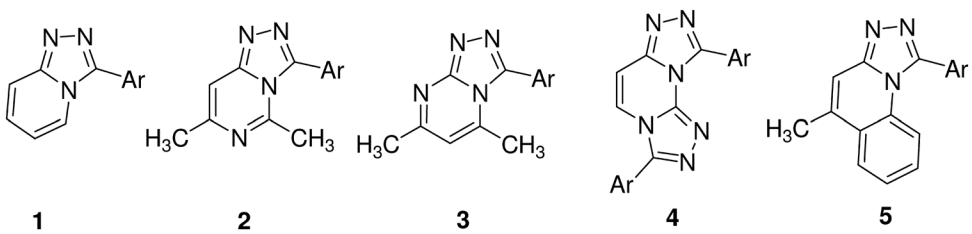


Figure 1. 1,2,4-Triazole fused with different heterocyclic moieties.

Scheme 2. Synthesis of 3,6-*bis*(aryl)*bis*([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazines **9a-l**.

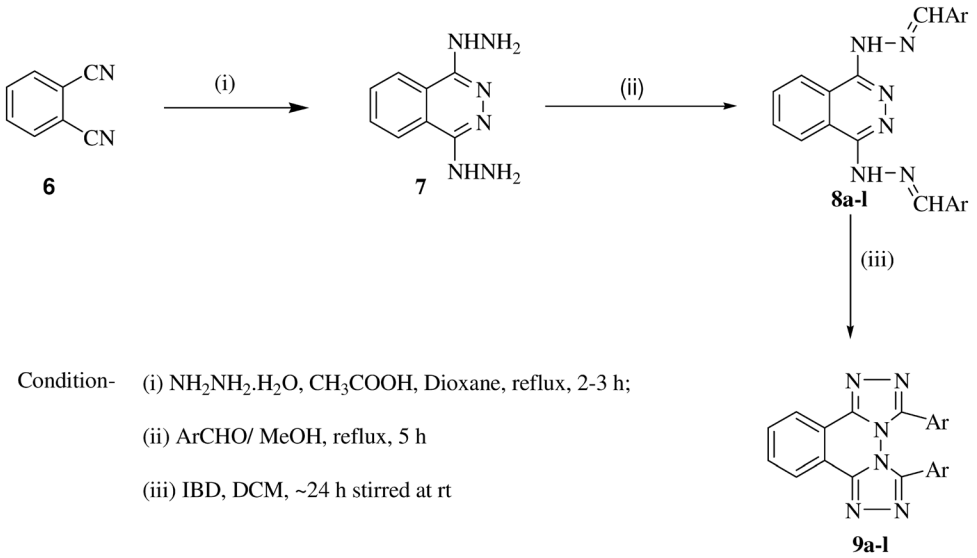


Table 1

Physical data of all the synthesized compounds **8a-l** and **9a-l**.

					Mp (°C)	
Entries	Ar	Product	Time (h)	Yield (%)	Obs	Lit
1	C_6H_5	**8a**	5	76	148–150	150 [25]
2	4-$CH_3C_6H_5$	**8b**	5	70	170–172	–
3	4-ClC_6H_5	**8c**	5	78	180–182	182 [25]
4	4-BrC_6H_5	**8d**	5	72	290–291	–
5	4-$OCH_3C_6H_5$	**8e**	5	77	159–160	160 [25]
6	4-FC_6H_5	**8f**	5	75	216–217	–
7	2-C_4H_3S (2-thienyl)	**8g**	5	77	170–172	–
8	4-$NO_2C_6H_5$	**8h**	5	80	>300	–
9	2-$OCH_3C_6H_5$	**8i**	5	76	110–112	112 [25]
10	2,4-$Cl_2C_6H_4$	**8j**	5	70	246–247	–
11	2-ClC_6H_5	**8k**	5	79	144–145	145 [25]
12	3-ClC_6H_5	**8l**	5	74	136–137	–
13	C_6H_5	**9a**	24	89	330[a]	–
14	4-$CH_3C_6H_5$	**9b**	24	81	332–333	–
15	4-ClC_6H_5	**9c**	24	90	353–354	–
16	4-BrC_6H_5	**9d**	24	88	360	–
17	4-$OCH_3C_6H_5$	**9e**	24	86	312–313	–
18	4-FC_6H_5	**9f**	24	85	>360	–
19	2-C_4H_3S (2-thienyl)	**9g**	24	91	315–316	–
20	4-$NO_2C_6H_5$	**9h**	24	93	264–265	–
21	2-$OCH_3C_6H_5$	**9i**	24	80	298–299	–
22	2,4-$Cl_2C_6H_4$	**9j**	24	82	270–271	–
23	2-ClC_6H_5	**9k**	24	80	266–267	–
24	3-ClC_6H_5	**9l**	24	86	286–287	–

of *A. niger*, five compounds, **9b**, **9d**, **9h**, **9j**, and **9l** showed more than 50% inhibition (Table 4). Compound **9j** possessed the maximum antifungal activity against both the tested fungi with maximum mycelial growth inhibition of 57.7 and 61.1% against *A. flavus* and *A. niger*, respectively.

A comparison of the antibacterial activity of these newly synthesized *bis*-1,2,4-triazolo-[3,4-a:4′,3′-c]phthalazines **9a-l** with our previously reported *bis*-1,2,4-triazolo[4,3-a][4,3-c]pyrimidines **4** reveals that latter compounds were more active than former. The reason for the loss in antibacterial activity is not clear.

Scheme 3. Probable mechanism for intramolecular cyclization of **8a-l** to **9a-l**.

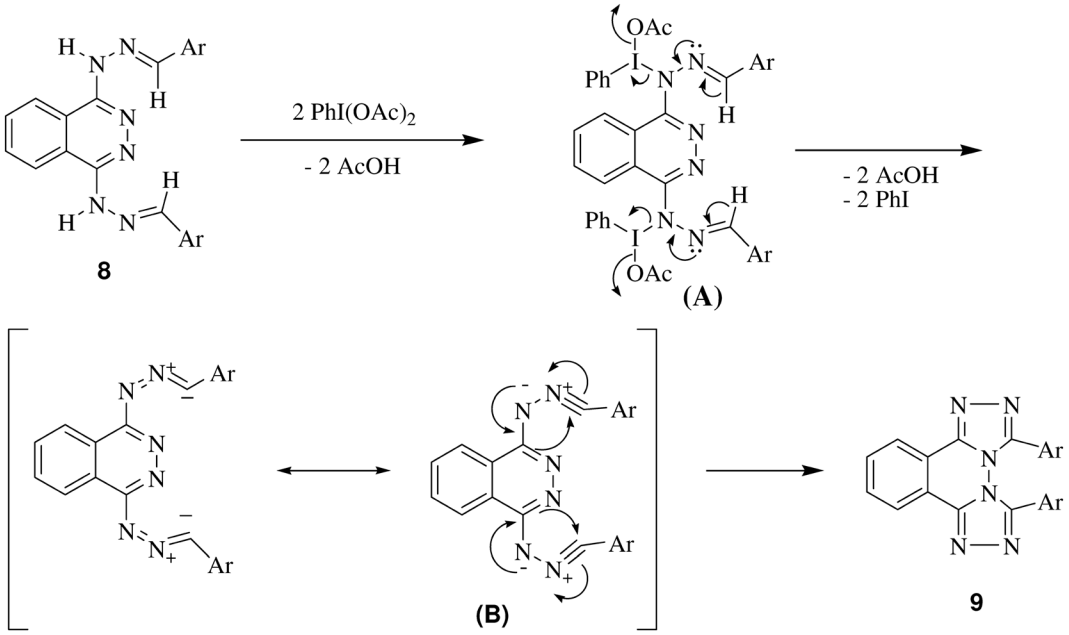


Table 2

In vitro antibacterial activity of chemical compounds through agar well diffusion method.

	Diameter of growth of inhibition zone (mm)[a]			
Compounds	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
9a	15.6[a] ± 0.57[b]	16 ± 0	–	–
9b	16.3 ± 0.57	18.3 ± 0.57	–	–
9c	15.3 ± 0.57	16.6 ± 0.57	–	–
9d	15 ± 0	14.3 ± 0.57	–	–
9e	15.6 ± 0.57	16.3 ± 0.57	–	–
9f	16.3 ± 0.57	18.6 ± 0.57	–	–
9g	15 ± 0	16.6 ± 0.57	–	–
9h	18.6 ± 0.57	20.6 ± 0.57	–	–
9i	16.3 ± 0.57	18 ± 0	–	–
9j	18.3 ± 0.57	19.6 ± 0.57	–	–
9k	15.6 ± 0.57	14.6 ± 0.57	–	–
9l	14.3 ± 0.57	16.3 ± 0.57	–	–
Ciprofloxacin	26 ± 0	24 ± 0	25 ± 0	25.3 ± 0.57

–, No activity.
[a]Values, including diameter of the well (8 mm), are means of three replicates.
[b]± Standard deviation.

CONCLUSIONS

We have described herein an efficient and convenient synthesis of symmetrical 3,6-*bis*(aryl)*bis*([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazines *via* the oxidative cyclization of 1,4-*bis*-2-(substituted benzylidenehydrazinyl)phthalazines, thereby emphasizing the increasing utility of hypervalent iodine(III) mediated methods, and this method stands as a feasible alternative to this class of heterocycles. All the compounds synthesized were tested *in vitro* for their antibacterial and antifungal activity. Compounds **9h** and **9j** were found to be most potent among all the synthesized compounds with the lowest MIC of 64 μg mL^{-1} against *S. aureus*. Five compounds, **9b**, **9f**, **9h**, **9i**, and **9j** exhibited activity against *B. subtilis* with MIC of 64 μg mL^{-1}. On the other hand, compound **9j** also possessed the maximum antifungal activity against both the tested fungi.

Table 3

Minimum inhibitory concentration (MIC) (μg mL^{-1}) of compounds by using macro dilution method.

	Minimum inhibitory concentrations (μg mL^{-1})	
Compounds	*Staphylococcus aureus*	*Bacillus subtilis*
9a	128	128
9b	128	64
9c	128	128
9d	128	256
9e	128	128
9f	128	64
9g	128	128
9h	64	64
9i	128	64
9j	64	64
9k	128	256
9l	256	128
Ciprofloxacin	5	5

Table 4

In vitro antifungal activity of synthetic chemical compounds through poisoned food method.

	Mycelial growth inhibition (%)	
Compounds	*Aspergillus flavus*	*Aspergillus niger*
9a	45.5	48.8
9b	51.1	55.5
9c	48.8	50
9d	55.5	58.8
9e	44.4	47.7
9f	50	44.4
9g	48.8	45.5
9h	48.8	52.5
9i	44.4	50
9j	57.7	61.1
9k	50	47.7
9l	55.5	56.6
Fluconazole	77.7	81.1

EXPERIMENTAL

Chemical synthesis. Melting points (mps) were taken on slides in an electrical apparatus Labindia visual melting range apparatus and are uncorrected. Calibration of melting point apparatus was done using benzoic acid as reference. IR spectra were recorded on a PerkinElmer 1800 FTIR spectrophotometer.^{1}H NMR spectra were recorded on a Bruker 300 MHz and 400 MHz instrument using tetramethylsilane as an internal standard.

Synthesis of 1,4-bis-2-(substituted benzylidenehydrazinyl) phthalazines (8a-l). *General procedure.* A mixture of 1,4-dihydrazinophthalazine (0.01 mol), appropriate araldehyde (0.02 mol) dissolved in methanol with few drops of acetic acid was refluxed for 5 h on water bath. The mixture was then poured in crushed ice, and the resulting solid was washed with sodium bisulfite, dried, and crystallized from methanol to afford pure 1,4-*bis*-2-(substituted benzylidenehydrazinyl)phthalazines **8a-l.**

Characterization data of unknown 1,4-bis-2-(substituted benzylidenehydrazinyl) phthalazines (8). *1,4-Bis(-2-(4-methylbenzylidene) hydrazinyl)phthalazine (8b).* Mp 170–172°C; yield 70%; IR (ν_{max}, cm^{-1}, KBr): 3495, 1605, 1574, 1528, 1466, 1381, 1327, 1203, 1126; ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.35 (s, 6H, 2 CH_3), 7.11–7.79 (m, 12H), 8.65 (s, 2H, —N═CH), 10.0 (bs, 2H, NH).

1,4-Bis(-2-(4-bromobenzylidene)hydrazinyl)phthalazine (8d). Mp 290–291°C; yield 72%; IR (ν_{max}, cm^{-1}, KBr): 3402, 1612, 1574, 1520, 1481, 1389, 1327, 1211, 1126, 1065, 1003; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.58–8.45 (m, 12H), 10.65 (s, 2H, —N═CH), 11.80(bs, 2H, NH).

1,4-Bis(-2-(4-fluorobenzylidene)hydrazinyl)phthalazine (8f). Mp 216–217°C; yield 75%; IR (ν_{max}, cm^{-1}, KBr): 3340, 1597, 1504, 1389, 1304, 1234, 1149, 1111, 1049; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.20–8.69 (m, 12H), 11.50 (s, 2H, —N═CH); 11.90 (s, 2H, NH).

1,4-Bis(-2-thiophen-2-ylmethylene)hydrazinyl)phthalazine (8g). Mp 170–172°C; yield 77%; IR (ν_{max}, cm^{-1}, KBr): 3380, 1582, 1474, 1373, 1219, 1111, 1041; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.10–8.80 (m, 10H), 10.54 (s, 2H, —N═CH); 11.60 (s, 2H, NH).

1,4-Bis(-2-(4-nitrophenylidene)hydrazinyl)phthalazine (8h). Mp > 300°C; yield 80%; IR (ν_{max}, cm^{-1}, KBr): 3402, 1589, 1512, 1381, 1335, 1203, 1134, 1103, 1003; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.78–8.60 (m, 12H), 11.15 (s, 2H, —N═CH); 12.20 (s, 2H, NH).

1,4-Bis(-2-(2,4-dichlorophenylidene)hydrazinyl)phthalazine (8j). Mp 246–247°C; yield 70%; IR (ν_{max}, cm^{-1}, KBr): 3387, 1589, 1528, 1466, 1381, 1335, 1219, 1142, 1103, 1049, 1003; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.30–8.77 (m, 10H), 11.00 (s, 2H, —N═CH); 12.10 (s,2H, NH).

1,4-Bis(-2-(3-chlorophenylidene)hydrazinyl)phthalazine (8l). Mp 136–137°C; yield 74%; IR (ν_{max}, cm^{-1}, KBr): 3387, 1566, 1528, 1474, 1373, 1335, 1265, 1219, 1119, 1003; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.28–8.71 (m, 12H), 9.80 (s, 2H, —N═CH); 11.90 (s, 2H, NH).

Synthesis of 3,6-bis(aryl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazines (9a-l). *General procedure.* To a stirred suspension/solution of 1,4-*bis*-2-(substituted benzylidenehydrazinyl)phthalazine **8** (0.01 mol) in dichloromethane (25 mL), IBD (0.02 mol) was added in portionwise. After some time, a clear dark brown solution was observed, but in some cases, it remained as suspension. Reaction was monitored by thin layer chromatography. After completion of reaction, stirring was stopped and reaction mixture was concentrated on water bath to evaporate the DCM. To the resulting residue, ethanol (5–10 mL) was added, and the mixture was heated on a water bath and filtered. On cooling at room temperature, solid separated out was filtered and washed with cold alcohol to give pure triazolophthalazine **9**.

Characterization data of 3,6-bis(aryl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazines (9a-l). *3,6-Diphenylbis-1,2,4-triazolo-[3,4-a:4′,3′-c]phthalazine (9a).* IR (ν_{max}, cm^{-1}, KBr): 1620, 1566, 1466, 1366; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.09–7.21 (m, 6H), 7.34–7.36 (d, 4H, J = 8.4 Hz), 7.92–7.95 (dd, 2H, J = 3.3 Hz, J' = 5.7 Hz), 8.52–8.55 (dd, 2H, J = 3.3 Hz, J' = 5.7 Hz); ^{13}C-NMR (DMSO-d_6, δ, ppm): 120.95, 124.03, 124.24, 131.05, 132.75, 133.06, 145.93, 147.25, 148.34; MS (ESI) m/z = 363.17 ($M + H^+$).

Anal. Calcd. for $C_{22}H_{14}N_6$ (362.13): C, 72.92; H, 3.89; N, 23.19. Found: C, 72.80; H, 3.78; N, 23.10.

3,6-Di-p-tolylbis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9b). IR (ν_{max}, cm^{-1}, KBr): 1612, 1566, 1466, 1366; ^{1}H-NMR (DMSO-d_6,

δ, ppm): 2.22 (s, 6H, $2CH_3$), 6.89–6.91 (d, 4H, J = 7.5 Hz), 7.90–7.93 (m, 2H), 8.50–8.53 (m, 2H); ^{13}C-NMR (DMSO-d_6, δ, ppm): 21.31, 121.29, 123.95, 124.41, 129.05, 129.10, 132.07, 140.05, 146.60, 148.02; MS (ESI) m/z = 391.30 ($M + H^+$).

Anal. Calcd. for $C_{22}H_{14}N_6$ (390.16): C, 73.83; H, 4.65; N, 21.52. Found: C, 73.74; H, 4.63; N, 21.40.

3,6-Bis(4-chlorophenyl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9c). IR (ν_{max}, cm^{-1}, KBr): 1605, 1566, 1466, 1404; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.21–7.24 (d, 4H, J = 8.1 Hz), 7.40–7.42 (d, 4H, J = 8.1 Hz), 7.93–7.96 (m, 2H), 8.53–8.56 (m, 2H); ^{13}C-NMR (DMSO-d_6, δ, ppm): 121.25, 124.04, 126.07, 128.88, 131.15, 132.29, 135.67, 146.78, 146.89; MS (ESI) m/z = 431.12 ($M + H^+$).

Anal. Calcd. for $C_{22}H_{12}Cl_2N_6$ (430.05): C, 61.27; H, 2.80; N, 19.49. Found: C, 61.15; H, 2.73; N, 19.39.

3,6-Bis(4-bromophenyl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9d). IR (ν_{max}, cm^{-1}, KBr): 1628, 1566, 1466, 1396, 1366; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.34 (s, 4H), 7.94 (s, 2H), 8.53 (s, 2H); ^{13}C-NMR (DMSO-d_6, δ, ppm): 121.24, 124.04, 124.65, 126.40, 131.30, 131.83, 132.30, 146.87; MS (ESI) m/z = 521 ($M + H^+ + 2$), 338.42 (base peak).

Anal. Calcd. for $C_{22}H_{12}Br_2N_6$ (517.95): C, 50.80; H, 2.33; N, 16.16. Found: C, 50.75; H, 2.25; N, 16.08.

3,6-Bis(4-methoxyphenyl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9e). IR (ν_{max}, cm^{-1}, KBr): 1612, 1535, 1466, 1368; ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.98 (s, 6H, $2OCH_3$), 6.64 (s, 4H), 7.26 (s, 4H), 7.89 (s, 2H), 8.49 (s, 2H); ^{13}C-NMR (DMSO-d_6, δ, ppm): 55.66, 114.23, 119.63, 121.31, 123.89, 130.88, 131.98, 146.49, 147.90, 160.64; MS (ESI) m/z = 423.25 ($M + H^+$).

Anal. Calcd. for $C_{24}H_{18}N_6O_2$ (422.15): C, 68.24; H, 4.29; N, 19.89. Found: C, 68.17; H, 4.25; N, 19.79.

3,6-Bis(4-fluorophenyl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9f). IR (ν_{max}, cm^{-1}, KBr): 1612, 1535, 1466, 1366; ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.98–7.04 (m, 4H), 7.43–7.47 (m, 4H), 7.94 (s, 2H), 8.53 (s, 2H); ^{13}C-NMR (DMSO-d_6, δ, ppm): 115.78, 116.08, 121.15, 123.71, 124.04, 131.79, 131.91, 132.32, 146.79, 146.94, 161.59, 164.88; MS (ESI) m/z = 399.20 ($M + H^+$).

Anal. Calcd. for $C_{22}H_{12}F_2N_6$ (398.11): C, 66.33; H, 3.04; N, 21.10. Found: C, 66.32; H, 3.0; N, 21.05.

3,6-Di(thiophen-2-yl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9g). IR (ν_{max}, cm^{-1}, KBr): 1620, 1558, 1466, 1412; ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.796 (s, 1H), 7.13 (s, 2H), 7.57 (s, 2H), 7.92 (s, 3H), 8.85 (s, 3H); ^{13}C-NMR (DMSO-d_6, δ, ppm): 121.21, 124.01, 126.88, 128.48, 130.13, 131.51, 132.33, 143.35, 146.79, 172.51; MS (ESI) m/z = 374.44 ($M + H^+$).

Anal. Calcd. for $C_{18}H_{10}N_6S_2$ (374.04): C, 57.74; H, 2.69; N, 22.44. Found: C, 57.72; H, 2.66; N, 22.41.

3,6-Bis(4-nitrophenyl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9h). IR (ν_{max}, cm^{-1}, KBr): 1605, 1520, 1466, 1342; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.71 (d, 4H, J = 8.4 Hz), 7.74 (m, 4H), 7.94–7.97 (d, 4H, J = 8.4 Hz); ^{13}C-NMR (DMSO-d_6, δ, ppm): 121.36, 124.02, 127.24, 128.78, 128.91, 130.30, 132.16, 146.77, 147.93; MS (ESI) m/z = 453.1 ($M + H^+$), 338.41 (base peak).

Anal. Calcd. for $C_{22}H_{12}N_8O_4$ (452.1): C, 58.41; H, 2.67; N, 24.77. Found: C, 58.34; H, 2.55; N, 24.65.

3,6-Bis(2-methoxyphenyl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9i). IR (ν_{max}, cm^{-1}, KBr): 1612, 1520, 1474, 1366; ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.72 (s, 6H, 2 OCH_3), 6.56–6.88 (m, 4H), 7.01–7.24 (m, 2H), 7.42–7.64 (m, 2H), 8.00 (s, 2H), 8.50 (s, 2H); ^{13}C-NMR (DMSO-d_6, δ, ppm): 55.39, 55.58, 111.27, 115.94, 120.38, 120.87, 124.24, 130.32, 132.63, 133.17, 145.38, 146.81, 156.75; MS (ESI) m/z = 423.25 ($M + H^+$).

Anal. Calcd. for $C_{24}H_{18}N_6O_2$ (422.15): C, 68.24; H, 4.29; N, 19.89. Found: C, 68.19; H, 4.21; N, 19.82.

3,6-Bis(2,4-dichlorophenyl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c] phthalazine (9j). IR (ν_{max}, cm^{-1}, KBr): 1597, 1558, 1450, 1366; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.41 (s, 1H), 7.33–7.36 (d, 1H, J = 8.4 Hz), 7.46–7.49 (d, 2H), 7.55 (s, 1H), 7.62–7.65 (d, 1H, J = 8.4 Hz), 7.98–8.02 (dd, 2H, J = 8.4 Hz, J' = 5.1 Hz), 8.57–8.61 (dd, 2H, J = 8.4 Hz, J' = 5.1 Hz); ^{13}C-NMR (DMSO-d_6, δ, ppm): 120.50, 120.62, 124.21, 124.34, 124.58, 125.17, 128.37, 128.44, 129.85, 132.76, 132.94, 133.29, 133.81, 134.29, 137.10, 137.20, 143.58, 143.91, 146.18, 146.33; MS (ESI) m/z = 501.02 ($M + 2H^+$).

Anal. Calcd. for $C_{22}H_{10}Cl_4N_6$ (497.97): C, 52.83; H, 2.02; N, 16.80. Found: C, 52.80; H, 2.0; N, 16.74.

3,6-Bis(2-chlorophenyl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9k). IR (ν_{max}, cm^{-1}, KBr): 1597, 1566, 1435, 1366; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.18 (s, 2H), 7.31–7.38 (m, 4H), 7.57–7.59 (d, 2H, J = 5.7 Hz), 7.80 (s, 2H), 8.58 (s, 2H); ^{13}C-NMR (DMSO-d_6, δ, ppm): 120.62, 124.22, 124.30, 125.61, 126.16, 127.99, 128.20, 130.42, 132.01, 132.69, 132.80, 133.03, 144.83, 145.01, 145.99, 146.08; MS (ESI) m/z = 431.12 ($M + H^+$).

Anal. Calcd. for $C_{22}H_{12}Cl_2N_6$ (430.05): C, 61.27; H, 2.80; N, 19.49. Found: C, 61.22; H, 2.74; N, 19.30.

3,6-Bis(3-chlorophenyl)bis([1,2,4]triazolo)[3,4-a:4′,3′-c]phthalazine (9l). IR (ν_{max}, cm^{-1}, KBr): 1612, 1566, 1450, 1366; ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.24–7.42 (m, 8H), 7.96 (s, 2H), 8.54 (s, 2H); ^{13}C-NMR (DMSO-d_6, δ, ppm): 121.12, 124.10, 127.50, 128.67, 129.01, 130.44, 130.90, 132.45, 133.71, 146.34, 146.93; MS (ESI) m/z = 431.12 ($M + H^+$).

Anal. Calcd. for $C_{22}H_{12}Cl_2N_6$ (430.05): C, 61.27; H, 2.80; N, 19.49. Found: C, 61.20; H, 2.73; N, 19.35.

Biological assay. ***Test microorganisms and medium.*** Total six microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121); two Gram-negative bacteria (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741) and two fungi, *A. niger* and *A. flavus*, the ear pathogens isolated from the patients of Kurukshetra [25], were used in the present study for evaluation of antimicrobial activity of the chemical compounds. All the cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were subcultured on Nutrient agar whereas fungi on Sabouraud dextrose agar and incubated aerobically at 37°C.

In vitro antibacterial activity. The antibacterial activity of synthesized chemical compounds was evaluated by the agar-well diffusion method. All the cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of $\sim 1.5 \times 10^8$ cfu mL^{-1}. Mueller Hinton agar medium (20 mL) was poured into each Petri plate, and the agar plates were swabbed with 100-μL inocula of each test bacterium and kept for 15 min for adsorption. Using sterile cork borer of 8-mm diameter, wells were bored into the seeded agar plates, and these were loaded with a 100 μL volume with concentration of 2.0 mg mL^{-1} of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 h. Antibacterial activity of each synthetic compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi antibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as a positive control. The experiments were performed in triplicates,

and the mean values of the diameter of inhibition zones with ± standard deviation were calculated [26,27].

Determination of Minimum Inhibitory Concentration. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the various compounds against bacterial strains was tested through a macrodilution tube method as recommended by NCCLS [28]. In this method, various test concentrations of chemically synthesized compounds were made from 256 to 0.5μg mL^{-1} in sterile tubes No. 1–10. Sterile Mueller Hinton Broth (100 μL) was poured in each sterile tube followed by addition of 200-μL test compound in tube 1. Two-fold serial dilutions were carried out from the tube 1 to the tube 10, and excess broth (100 μL) was discarded from the last tube No.10. To each tube, 100 μL of standard inoculum (1.5×10^8 cfu mL^{-1}) was added. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37°C for 24 h.

In vitro antifungal activity. The antifungal activity of the synthesized chemical compounds was evaluated by poisoned food technique. The molds were grown on Sabouraud dextrose agar (SDA) at 25°C for 7 days and used as inocula. About 15 mL of molten SDA (45°C) was poisoned by the addition of 100 μL volume of each compound having concentration of 4.0 mg mL^{-1}, reconstituted in the DMSO, poured into a sterile Petri plate and allowed to solidify at room temperature. The solidified poisoned agar plates were inoculated at the center with fungal plugs (8 mm diameter), obtained from the actively growing colony and incubated at 25°C for 7 days. DMSO was used as the negative control whereas fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of the fungal colonies was measured and expressed as percent mycelial inhibition determined by applying the formula given by Al-Burtamani *et al.* [29].

$$\text{Inhibition of mycelial growth } \% = (dc - dt)/dc \times 100,$$

Where, dc = average diameter of fungal colony in negative control plates, dt = average diameter of fungal colony in experimental plates.

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REFERENCES AND NOTES

[1] El-Hawash, S. A.; Habib, N. S.; Fanaki, N. H. Pharmazie 1999, 54, 808.
[2] Brown, D. J.; Iwai, Y. Aust J Chem 1979, 32, 2727.
[3] Tarzia, G.; Ocelli, E.; Toja, E.; Barone, D.; Corsico, N.; Gallico, L.; Luzzani, F. J Med Chem 1988, 31, 1115.
[4] Trust, R. I.; Albright, J. D. U.S. Patent 4242515, 1980; Trust, R. I.; Albright, J. D. Chem Abstr 1981, 94, 139815.
[5] Tarzia, G.; Ocelli, E.; Barone, D. Il Farmaco 1989, 44, 3.
[6] (a) Peignier, R.; Chne, A.; Cantregril, R., Mortier, J. Eur Patent 441718; (a) Peignier, R.; Chne, A.; Cantregril, R., Mortier, J. Chem Abstr 1991, 115, 208000; (b) Cantegril, R.; Chne, A.; Mortier, J.; Peignier, R. Eur Patent 483027; (b) Cantegril, R.; Chne, A.; Mortier, J.; Peignier, R. Chem Abstr 1992, 117, 131214.
[7] Sarges, S.; Howard, H. R.; Browne, R. G.; Lebel, L. A.; Seymour, P. A.; Koe, B. K. Med Chem 1990, 33, 2240.
[8] Holla, B. S.; Poorjary, N. K.; Rao, S. B.; Shivananda, M. K. Eur J Med Chem 2002, 37, 511.
[9] Holla, B. S.; Akberali, P. M.; Shivananda, M. K. Il Farmaco 2001, 56, 919.
[10] Bektaş, H.; Karaali, N.; Şahin, D.; Demirbaş, A.; Karaoglu, S. A.; Demirbaş, N. Molecules 2010, 15, 2427.
[11] Iszhii, A.; Kubo, K.; Deguchi, T.; Tanaka, M. Yakugaku Zasshi 1979, 99, 533; Chem Abstr 1979, 91, 133826z.
[12] Bower, J. D.; Doyle, F. P. J Chem Soc 1957, 727.
[13] Pollak, A.; Tišler, M. Tetrahedron 1966, 22, 2073.
[14] Gibson, M. S. Tetrahedron 1963, 19, 1587.
[15] Prakash, O.; Singh, S. P. Aldrichimica Acta 1994, 27, 15.
[16] Varvoglis, A. Tetrahedron 1994, 53, 1179.
[17] Wirth, T. Topics in Current Chemistry; Springer-Verlag: Berlin 2003, 224.
[18] Zhdankin, V. V. Arkivoc 2009, 1, 1–62.
[19] Sadana, A. K.; Mirza, Y.; Aneja, K. R.; Prakash, O. Eur J Med Chem 2003, 38, 533.
[20] Prakash, O.; Bhardwaj, V.; Kumar, R.; Tyagi, P.; Aneja, K. R. Eur J Med Chem 2004, 39, 1073.
[21] Prakash, O.; Kumar, R.; Kumar, R.; Tyagi, P.; Kuhad, R. C. Eur J Med Chem 2007, 42, 868.
[22] Prakash, O.; Kumar, R.; Sharma, D.; Naithani, R.; Kumar, R. Heteroatom Chem 2006, 17, 653.
[23] Kumar, R.; Nair, R. R.; Dhiman, S. S.; Sharma, J.; Prakash, O. Eur J Med Chem 2009, 44, 2260.
[24] Joshi, H.; Upadhyay, P.; Baxi, A. J. J Ind Chem Soc 1990, 67, 779.
[25] Aneja, K. R.; Sharma, C.; Joshi, R. Inter J Otorhinolaryngol 2010, 74, 604.
[26] Ahmad, I.; Beg, A. J. J Ethnopharmacol 2001, 74, 113.
[27] Andrews, J. M. J Antimicrob Chemother 2001, 48, 5.
[28] NCCLS, Method for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically Approved Standards, 5th ed., National Committee for Clinical Standards, PA. Villanova, 2000.
[29] Al-Burtamani, S. K. S.; Fatope, M. O.; Marwah, R. G.; Onifade, A. K.; Al-Saidi, S. H. J Ethnopharmocol 2005, 96, 107.