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Short communication

Organoiodine(III) mediated synthesis of 3,9-diaryl- and 3,9-difuryl-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidines as antibacterial agents

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Abstract

Nine 3,9-diaryl- and 3,9-difuryl-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidines (**3a–i**) have been synthesized by the oxidation of bis-2,4-pyrimidinylhydrazones of various araldehydes and furfural with 2 equiv of iodobenzene diacetate (IBD) in dichloromethane and tested in vitro for their antibacterial activity. Three compounds, namely 3,9-di-(4′-fluorophenyl)-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidine (**3f**), 3,9-di-(4′-nitrophenyl)-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidine (**3g**) and 3,9-di-(5′-nitro-2′-furyl)-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidine (**3i**), were associated with substantially higher antibacterial activity than some commercial antibiotics against Gram-positive bacteria namely *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*; two Gram-negative bacteria namely *Salmonella typhi* and *Escherichia coli* at MIC (minimum inhibitory concentration) 10 μg/ml.

Keywords: Iodobenzene diacetate; Bistriazolopyrimidines; Antibacterial activity

1. Introduction

In our previous papers [1,2], we reported the synthesis of fused 1,2,4-triazolopyridines and 1,2,4-triazolopyrimidines. Some of the compounds were found to possess strong antibacterial activity. Encouraged by these results, we got interested in extending the scope of this approach for the synthesis of bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidines (**3a–i**) by using iodobenzene diacetate (IBD) as antibacterial agents.

2. Chemistry

The synthetic pathway (Scheme 1) used for the synthesis of 3,9-diaryl- and 3,9-difuryl-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidines **3** is similar as reported in previous papers [1–3]. Thus, treatment of substituted pyrimidinyl bishydrazones (**2a–i**) with 2.2 equiv of IBD [4–7] in dichloromethane (DCM) for about 1 h at room temperature afforded desired products 3,9-diaryl- and 3,9-difuryl-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidines **3** in high yields. The bishydrazones (**2a–i**) were obtained by the condensation of 2,4-dihydrazinopyrimidine **1** with different aromatic/heteroaromatic aldehydes.

The structures of all bistriazolopyrimidines **3a–i** and hydrazones **2a–i** were elaborated by their spectral data (IR, ^{1}H NMR and MS) and elemental analysis.

The I (III) mediated oxidative cyclization of **2** to **3** is significant for the following reasons: (i) The method is eco-friendly, (ii) It involves mild conditions, (iii) There is possibility of using this approach for the synthesis of a wide variety of bis- and tris-triazolo heterocyclic compounds of potential biological interest.

Abbrevations: IBD, iodobenzene diacetate; DCM, dichloromethane; MIC, minimum inhibitory concentration; *B. subtilis*, *Bacillus subtilis*; *Sa. typhi*, *Salmonella typhi*; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; *E. coli*, *Escherichia coli*; MHA, Muller–Hinton agar; SCDA, soyabean casein digest agar; MTCC, microbial type culture collection and gene bank.

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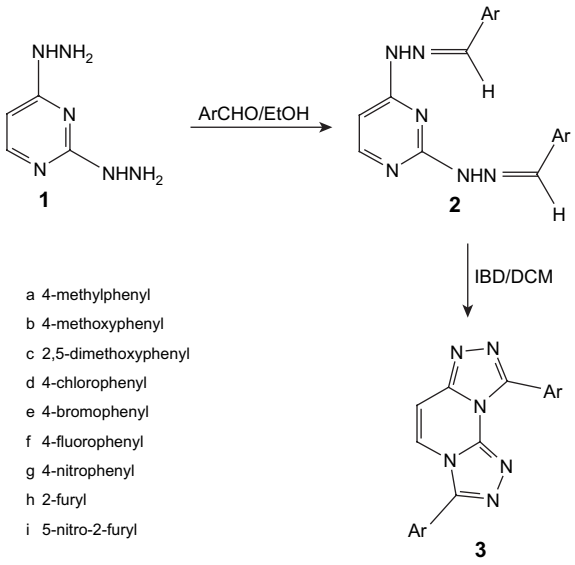

Scheme 1.

3. Biological investigation and results

Compounds **3a–i** were tested in vitro for their antibacterial activity against three Gram-positive bacteria namely *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*; two Gram-negative bacteria namely *Salmonella typhi* and *Escherichia coli*. 3,9-Di-(4′-fluorophenyl)-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidine (**3f**), 3,9-di-(4′-nitrophenyl)-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidine (**3g**) and 3,9-di-(5′-nitro-2′-furyl)-bis-1,2,4-triazolo[4,3-*a*][4,3-*c*]pyrimidine (**3i**), were associated with substantially higher antibacterial activity than some commercial antibiotics (Tables 1 and 2 and Fig. 1).

4. Experimental

4.1. Chemical synthesis

Melting points were determined in open capillaries in electrical melting point apparatus and are uncorrected. The IR (KBr) and ^{1}H NMR spectra were recorded on Buck Scientific IR M-500 and Bruker (300 MHz) spectrophotometers, respectively. All the compounds gave satisfactory analytical results (with in ±0.4 of the theoretical values).

2,4-Dihydrazinopyrimidine **1** was synthesized according to the literature procedure commencing with uracil [8–10].

4.1.1. Hydrazones *2a–i*

4.1.1.1. General procedure. 2,4-Dihydrazinopyrimidine (**1**) was dissolved in ethanol and aryl/hetaryl aldehydes were added to it. The contents were refluxed on a water bath for 1 h and allowed to stand at room temperature. The crystalline solid, thus obtained, was filtered, washed with ethanol and dried to afford bishydrazones **2**.

*4.1.1.2. Characterization data of hydrazones **2a–i**.* Compound **2a**: m.p. 178–181 °C; yield 71%; IR 3312 cm^{-1} NHstr.; ^{1}H NMR δ 2.40 (s, 6H, CH_3), 8.09 (d, 1H, $J = 5.7$ Hz), 6.85 (d, 1H, $J = 5.7$ Hz), 7.80 (s, 2H, –N=CH), 7.55–7.61 (m, 4H), 7.17–7.22 (m, 4H).

Compound **2b**: m.p. 148–150 °C; yield 51%; IR 3387 cm^{-1} NHstr.; ^{1}H NMR δ 3.90 (s, 6H, OCH_3), 7.85 (d, 1H,

Table 2
Minimum inhibitory concentration (MIC) of **3a–i** against five test bacteria by using agar dilution assay

Compound	MIC (μg/ml)				
	Sa	St	Se	Ec	Bs
3a	>64	>64	>64	>64	8
3b	64	64	>64	>64	64
3c	8	8	8	>64	32
3d	32	8	>64	>64	8
3e	>64	32	>64	>64	>64
3f	4	64	4	4	2
3g	1	1	2	2	1
3h	0.5	64	64	64	8
3i	8	1	1	2	0.5
Cefaclor	4	2	4	4	8
Linezolid	4	2	8	8	2

Sa – *S. aureus* (MTCC 3160), St – *Sa. typhi* (MTCC 733), Se – *S. epidermidis* (MTCC 2639), Ec – *E. coli* (MTCC 51) and Bs – *B. subtilis* (MTCC 121).

Table 1
In vitro antibacterial spectrum of chemically synthesized compounds by using agar diffusion assay

Compound	Diameter of zone of growth inhibition (mm)[a]				
	Sa	St	Se	Ec	Bs
3a	–	–	–	–	20.83 ± .37
3b	10.83 ± .37	10 ± .81	–	–	10 ± .57
3c	20.16 ± .37	20 ± .57	20 ± .81	–	15.16 ± .37
3d	15 ± .57	20 ± .81	–	–	20 ± .81
3e	–	15 ± .57	–	–	–
3f	24.83 ± .37	10 ± .57	25 ± .81	–	25 ± .57
3g	30 ± .81	30 ± .57	25.16 ± .37	25 ± .57	30 ± .81
3h	40 ± .57	10 ± .81	10 ± .57	10 ± .81	20 ± .57
3i	20.83 ± .37	35.16 ± .37	35 ± .57	30 ± .81	40 ± .57
DMSO	8	7.83	7.16	7.16	7.83

– No activity.
Sa – *S. aureus* (MTCC 3160), St – *Sa. typhi* (MTCC 733), Se – *S. epidermidis* (MTCC 2639), Ec – *E. coli* (MTCC 51) and Bs – *B. subtilis* (MTCC 121).
[a] Mean of six replicates; ± standard deviation.

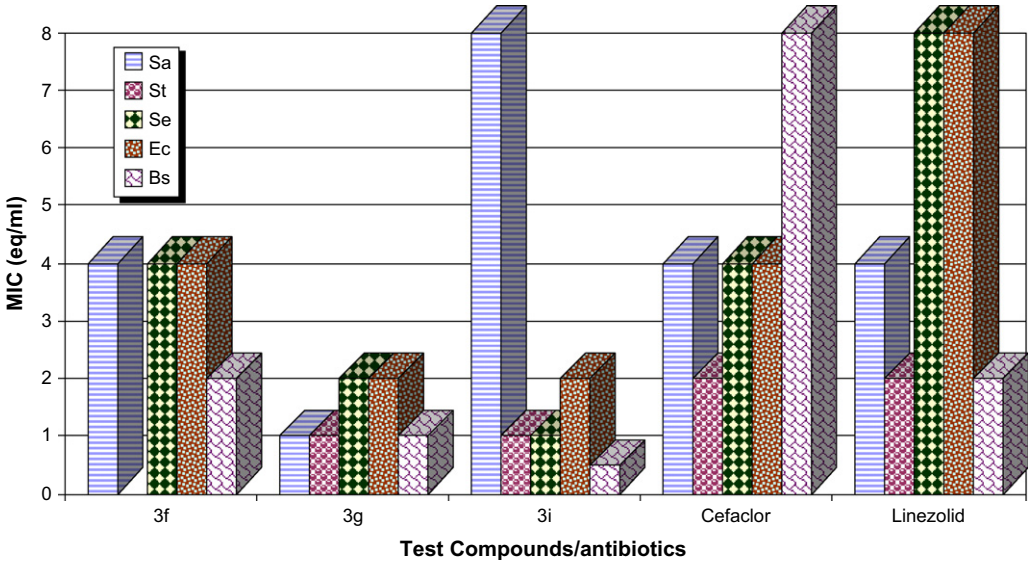

Fig. 1. Minimum inhibitory concentration (MIC) of **3a–i** against five test bacteria by using agar dilution assay.

$J = 5.7$ Hz), 6.99 (d, 1H, $J = 5.7$ Hz), 7.77 (s, 2H, −N═CH), 6.92–6.98 (m, 4H), 7.69–7.73 (m, 4H).

Compound **2c**: m.p. 160–162 °C; yield 54%; IR 3406 cm^{-1} NHstr.; ^1H NMR δ 3.89 (s, 12H, OCH_3), 7.78 (d, 1H, $J = 5.7$ Hz), 6.93 (d, 1H, $J = 5.7$ Hz), 7.73 (s, 2H, −N═CH), 7.04–7.07 (m, 6H).

Compound **2d**: m.p. 171–173 °C; yield 72%; IR 3388 cm^{-1} NHstr.; ^1H NMR δ 7.92 (d, 1H, $J = 5.7$ Hz), 7.12 (d, 1H, $J = 5.7$ Hz), 7.78 (s, 2H, −N═CH), 7.69–7.74 (m, 4H), 7.34–7.38 (m, 4H).

Compound **2e**: m.p. 180–183 °C; yield 65%; IR 3400 cm^{-1} NHstr.; ^1H NMR δ 8.10 (d, 1H, $J = 5.7$ Hz), 7.03 (d, 1H, $J = 5.7$ Hz), 7.75 (s, 2H, −N═CH), 7.54–7.58 (m, 4H), 7.77–7.81 (m, 4H).

Compound **2f**: m.p. 208–210 °C; yield 40%; IR 3199 cm^{-1} NHstr.; ^1H NMR δ 8.14 (d, 1H, $J = 5.7$ Hz), 6.91 (d, 1H, $J = 5.7$ Hz), 7.77 (s, 2H, −N═CH), 7.09–7.14 (m, 4H), 7.67–7.72 (m, 4H).

Compound **2g**: m.p. 262–265 °C; yield 81%; IR 3302 cm^{-1} NHstr.; ^1H NMR δ 8.12 (d, 1H, $J = 5.7$ Hz), 6.93 (d, 1H, $J = 5.7$ Hz), 7.73 (s, 2H, −N═CH), 8.17–8.25 (m, 4H), 7.62–7.67 (m, 4H).

Compound **2h**: m.p. 128–130 °C; yield 54%; IR 3225 cm^{-1} NHstr.; ^1H NMR δ 8.03 (d, 1H, $J = 5.7$ Hz), 6.93 (d, 1H, $J = 5.7$ Hz), 7.73 (s, 2H, −N═CH), 6.39–6.42 (m, 2H), 6.60–6.63 (m, 2H), 7.43 (d, 2H, $J = 3.6$ Hz).

Compound **2i**: m.p. 209–211 °C; yield 78%; IR 3153 cm^{-1} NHstr.; ^1H NMR δ 8.26 (d, 1H, $J = 5.7$ Hz), 7.02 (d, 1H, $J = 5.7$ Hz), 7.73 (s, 2H, −N═CH), 7.34 (d, 2H, $J = 3.9$ Hz), 7.53 (d, 2H, $J = 3.9$ Hz).

*4.1.2. 3,9-Diaryl- and 3,9-difuryl-bis-1,2,4-triazolo [4,3-a][4,3-c]pyrimidines **3a–i***

4.1.2.1. General procedure. To a stirred solution of bispyrimidinylhydrazones **2** (0.01 mol) in dichloromethane (25 ml) at room temperature, IBD (0.02 mol) was added in four to five portions for 5 min. The solvent was evaporated on a steam bath and the residual mass containing product and iodobenzene was triturated with petroleum ether to give solid product, which was then recrystallised from methanol to yield pure bis-triazolopyrimidines **3**.

*4.1.2.2. Characteriazation data of triazolopyrimidines **3a–i***. Compound **3a**: m.p. 98–102 °C; yield 62%; ^1H NMR δ 2.50 (s, 6H, CH_3), 7.86 (d, 1H, $J = 8.1$ Hz), 7.25 (d, 1H, $J = 8.1$ Hz), 8.18 (d, 2H, $J = 8.4$ Hz), 7.68 (d, 2H, $J = 8.4$ Hz), 7.40–7.45 (m, 4H); MS: *m/z*, M^+ 340.

Compound **3b**: m.p. 121–123 °C; yield 57%; ^1H NMR δ 3.83 (s, 6H, OCH_3), 7.9 (d, 1H, $J = 8.1$ Hz), 7.62 (d, 1H, $J = 8.1$ Hz), 8.17 (d, 2H, $J = 8.7$ Hz), 7.61 (d, 2H, $J = 8.7$ Hz), 7.02–7.05 (m, 2H), 6.90–6.94 (m, 2H); MS: *m/z*, M^+ 372.

Compound **3c**: m.p. 92–96 °C; yield 67%; ^1H NMR δ 3.85 (s, 12H, OCH_3), 7.62 (d, 1H, $J = 8.1$ Hz), 7.28 (d, 1H, $J = 8.1$ Hz), 7.12–7.23 (m, 6H); MS: *m/z*, M^+ 432.

Compound **3d**: m.p. 202–206 °C; yield 67%; ^1H NMR δ 7.85 (d, 1H, $J = 8.1$ Hz), 7.62 (d, 1H, $J = 8.1$ Hz), 8.35 (d, 2H, $J = 8.7$ Hz), 8.20 (d, 2H, $J = 8.7$ Hz), 7.51–7.56 (m, 4H); MS: *m/z*, M^+ 381.

Compound **3e**: m.p. 150–153 °C; yield 62%; ^1H NMR δ 7.94 (d, 1H, $J = 8.1$ Hz), 7.64 (d, 1H, $J = 8.1$ Hz), 8.30 (d, 2H, $J = 8.7$ Hz), 8.14 (d, 2H, $J = 8.7$ Hz), 7.68–7.75 (m, 4H); MS: *m/z*, M^+ 468.

Compound **3f**: m.p. 192–195 °C; yield 59%; ^1H NMR δ 7.90 (d, 1H, $J = 8.1$ Hz), 7.36 (d, 1H, $J = 8.1$ Hz), 8.38–8.45 (m, 2H), 8.24–8.29 (m, 2H), 7.18–7.23 (m, 4H); MS: *m/z*, M^+ 348.

Compound **3g**: m.p. 183–185 °C; yield 68%; ^1H NMR δ 8.16 (d, 1H, $J = 8.1$ Hz), 8.02 (d, 1H, $J = 8.1$ Hz), 8.4–8.5 (m, 4H), 8.11–8.25 (m, 4H); MS: *m/z*, M^+ 402.

Compound **3h**: m.p. 142–145 °C; yield 60%; ^1H NMR δ 8.48 (d, 1H, $J = 8.1$ Hz), 7.31 (d, 1H, $J = 8.1$ Hz), 8.5 (d,

1H, $J = 3.6$ Hz), 7.50–7.70 (dd, 2H, $J = 1.5$, 6.3 Hz), 7.40 (d, 1H, $J = 3.6$ Hz), 6.71–6.74 (m, 2H); MS: *m/z*, M^+ 292.

Compound **3i**: m.p. 162–165 °C; yield 48%; ^{1}H NMR δ 8.26 (d, 1H, $J = 8.1$ Hz), 7.61 (d, 1H, $J = 8.1$ Hz), 7.66 (d, 2H, $J = 3.9$ Hz), 7.45 (d, 2H, $J = 3.9$ Hz); MS: *m/z*, M^+ 382.

5. Biological assay

5.1. Medium

Media used for the study were Muller–Hinton agar (MHA) and soyabean casein digest agar (SCDA) of the following composition; beef infusion 300 g/l, casein acid hydrolysate 17.5 g/l, starch 1.5 g/l, agar–agar 17 g/l and sterile distilled water 1000 ml, adjusted to pH 7.4 and casein enzymatic hydrolysate 17.0 g/l, papain digest of soyabean 3.0 g/l, NaCl 5.0 g/l, dipotassium phosphate 2.5 g/l, dextrose 2.5 g/l and sterile distilled water 1000 ml, adjusted to pH 7.3, respectively.

5.2. Primary screening

Primary screening of nine chemically synthesized compounds **3a–i** was done against three Gram-positive bacteria namely *S. aureus* (MTCC 3160), *S. epidermidis* (MTCC 2639) and *B. subtilis* (MTCC 121); two Gram-negative bacteria namely *Sa. typhi* (MTCC 733) and *E. coli* (MTCC 51) by well diffusion assay technique. The overnight cultures of all the bacteria were used for the assay and adjusted to 0.5 McFarland Standard, i.e. 1.5×10^8 CFU/ml [11]. The test bacterial cultures were set at 0.5 McFarland Standard using Wickerham paper. The stock solution (1 mg/l) of all the test chemicals was prepared by dissolving 1 mg of the test chemical in 1 ml of dimethylsulfoxide (DMSO). DMSO was used as control for all the test compounds.

Twenty milliliter of MHA and 500 μl of each test bacterial culture of overnight incubation adjusted at 0.5 McFarland were mixed and poured into sterilized and labelled Petri plates. The wells of 6 mm diameter were punched in the solidified agar plates. Test chemicals of 100 μl were added to individual wells. The loaded plates were incubated at 35 °C for 24 h. The diameter of zone of growth inhibition around each well was measured after incubation using a Vernier Caliper.

5.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation [12]. MIC of compounds against Gram-positive and Gram-negative test bacteria was determined by literature method [13]. MHA was used for MIC determination. All the test cultures were streaked on the SCDA and incubated overnight at 37 °C. Turbidity of all the bacterial cultures were adjusted to 0.5 McFarland Standard by preparing bacterial suspension of 4–6 isolated colonies. The cultures were further diluted 10-fold to get inoculum size of 1.2×10^7 CFU/ml. Stock solution of 4 mg/ml was prepared in DMSO and was appropriately diluted to get final concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.12 μg/ml. Standard antibiotics (linezolid, manufacturer - Alembic, batch no. 6893002; cefaclor, manufacturer - Glaxo, batch no. 1305911) were also diluted in same manner to make comparison. Each dilution (320 μl) was added to 20 ml of cooled 45 °C molten MHA (separate flask was taken for each dilution). After through mixing, the medium was poured in sterilized Petri plates. The test bacterial cultures were spotted in a predefined pattern by ascetically transferring 10 μl of each culture on the surface of presolidified agar plates. The spotted plates were incubated at 35 °C for 24 h.

6. Results and discussions

Nine chemically synthesized compounds were tested in vitro for their antibacterial activity against five test bacteria namely *S. aureus* (MTCC 3160), *S. epidermidis* (MTCC 2639), *B. subtilis* (MTCC 121) (Gram-positive), *Sa. typhi* (MTCC 733) and *E. coli* (MTCC 51) (Gram-negative) (Tables 1 and 2). Out of these nine compounds tested, three compounds, namely **3f**, **3g** and **3i** possessed excellent antibacterial activity against both Gram-positive and Gram-negative bacteria. The compound **3f** showed MIC 2–4 μg/ml against *S. aureus*, *S. epidermidis*, *E. coli* and *B. subtilis*, where as MIC 1–2 μg/ml was observed against *S. aureus*, *S. epidermidis*, *B. subtilis*, *Sa. typhi* and *E. coli* by compound **3g**. Another compound **3h** showed MIC of 0.5 μg/ml against *S. aureus*, followed by 0.6–2 μg/ml MIC against *B. subtilis*, *S. epidermidis*, *Sa. typhi* and *E. coli* (Table 2).

The antibacterial activity of compounds **3a–i** was also compared with two commercial antibiotics namely cefaclor and linezolid, as cefaclor is inhibitory against Gram-positive and Gram-negative bacteria and linezolid is active against drug resistant Staphylococci. Many of these compounds showed comparable activity as displayed in Table 2 and Fig. 1.

From the results obtained thus far, it is evident that bistriazolopyrimidines are more active than simple triazolopyrimidines and triazolopyridines. Apart from this, substituents in the aryl ring also play significant role.

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