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Preliminary communication

Organoiodine (III) mediated synthesis of 3-aryl/hetryl-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidines as antibacterial agents

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Abstract

Synthesis of some new 3-aryl/hetryl-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidines **(3a–k)** has been accomplished by the oxidation of 4,6-dimethyl-2-pyrimidinylhydrazones of various aldehydes with iodobenzene diacetate in dichloromethane. Nine new compounds (**3b–g** and **3i–k**) were tested in vitro for their antibacterial activity. Two compounds, namely 3-(4′-pyridyl)-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidine **(3k)** and 3-(3′,4′-dimethoxyphenyl)-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidine **(3f)**, were associated with substantially higher antibacterial activity than some commercial antibiotics against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Salmonella typhi* at MIC (minimum inhibitory concentration) i.e. 10 μg/ml.

Keywords: Iodobenzene diacetate; Triazolopyrimidines; Antibacterial activity

1. Introduction

Fused heterocyclic 1,2,4-triazoles have acquired much importance because of their CNS depressant [1], antiallergy [2], antimicrobial [3] and anti-inflammatory [4] properties. The use of organohypervalent iodine compounds as oxidizing reagents has received considerable attention in organic synthesis [5–13]. Since reactions involving hypervalent iodine reagents are mostly carried out under the mild reaction conditions, these developments have offered superior alternative to the reported traditional methods [14]. In a previous paper [15], we reported the synthesis of fused 1,2,4-triazolopyridines. 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 new 1,2,4-triazolo[4,3-a]pyrimidines **(3a–k)** by using iodobenzene diacetate as antibacterial agents.

2. Chemistry

The synthetic Scheme 1 used for the synthesis of 3-aryl-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidines **3** is same as reported earlier [15,16]. Thus, treatment of substituted pyrimidinyl hydrazones **(2a–k)** with 1.1 equivalent of IBD in dichloromethane (DCM) for about 1 h at room temperature afforded desired products 3-aryl-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidines **3** in high yields. The hydrazones **(2a–k)** were obtained by the condensation of 2-hydrazino-4,6-dimethylpyrimidine **1** with different aromatic/hetero-aromatic aldehydes.

The structures of all the new triazolopyrimidines **3a–k** and hydrazones **2a–k** were elaborated by their spectral data (IR, ^{1}HNMR and MS) and elemental analysis.

Thus, the present study provides an efficient way of synthesising new 11 fused 1,2,4-triazolo[4,3-a]pyrimidines the-

Abbreviations: IBD, iodobenzene diacetate; DCM, dichloromethane; NA, nutrient agar; MIC, minimum inhibitory concentration; *B. subtilis*, *Bacillus subtilis*; *S. typhi*, *Salmonella typhi*; *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*.

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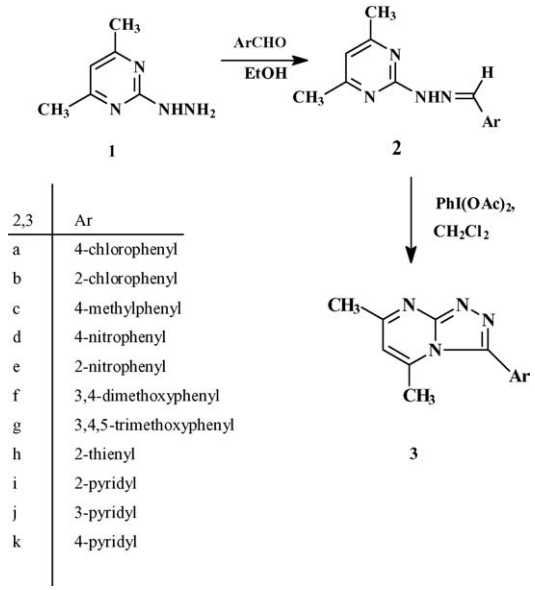

Scheme 1.

reby illustrating the generality of our previous I(III) mediated approach.

3. Biological investigation and results

Compounds **3b–g** and **3i–k** were tested in vitro for their antibacterial activity against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Salmonella typhi*. 3-(4′-Pyridyl)-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidine (**3k**) and 3-(3′,4′-dimethoxyphenyl)-5,7-dimethyl-1,2,4-triazolo-[4,3-a]pyrimidine (**3f**) were associated with substantially higher antibacterial activity than some commercial antibiotics (Table 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}HNMR spectra were recorded on Buck Scientific IR M-500 and Bruker (300 MHz) spectrometers, respectively. All the new compounds gave satisfactory analytical results (within ±0.4 of the theoretical values).

4,6-Dimethyl-2-hydrazinopyrimidine **1** was synthesized according to the literature procedure commencing with urea and acetylacetone [17].

*4.1.1. Hydrazones **2a–k***

The hydrazones **2** required for the oxidative cyclization were prepared by the condensation of 2-hydrazino-4,6-dimethylpyrimidine **1** with different aromatic and heteroaromatic aldehydes in ethanol with a trace of glacial acetic acid [18,19].

4.1.1.1. Characterization data of new hydrazones are given. **2a**: m.p. 84–86 °C, yield 95.93%. IR cm^{-1} 3239.8 N-Hstr.; ^{1}HNMR.δ 2.24 (s, 6H, C_4–CH_3, C_6–CH_3), 6.51 (s, 1H, –N=C–H), 7.31–7.41 (d, 2H, C_2'–H, C_3'–H), 7.62–7.71 (d, 2H, C_5'–H, C_6'–H), 8.00 (s, 1H, C_5–H).

2b:m.p. 182–184 °C, yield 51.93%. IR cm^{-1} 3215.8 N-Hstr.; ^{1}HNMR. δ 2.33 (s, 6H, C_4–CH_3, C_6–CH_3), 6.50 (s, 1H, –N=C–H), 7.17–7.21 (1H, m, C_5'–H), 7.25–7.29 (2H, m, C_4'–H, C_6'–H), 8.13–8.16 (1H, dd, J = 1.8, 7.8 Hz, C_3'–H).

2c:m.p. 170–172 °C, yield 46.66%. IR cm^{-1} 3257.9 N-Hstr.; ^{1}HNMR. δ 2.24 (s, 6H, C_4–CH_3, C_6–CH_3), 2.10 (s, 3H, C_4'–CH_3), 6.46 (s, 1H, –N=C–H), 7.10–7.13 (d, 2H, C_2'–H, C_3'–H), 7.56–7.59 (d, 2H, C_5'–H, C_6'–H), 8.00 (s, 1H, C_5–H).

2d: m.p. Ref. [18]; m.p. 166–167/170–172 °C, yield 78.51%.

2e:m.p. 199–201 °C, yield 87.66%. IR cm^{-1} 3185.6 N-Hstr.; ^{1}HNMR δ 2.37 (s, 6H, C_4–CH_3, C_6–CH_3), 6.54 (s, 1H, –N=C–H), 8.45 (s, 1H, C_5–H), 7.42–7.43 (1H, m, C_5'–H), 7.55–7.57 (m, 1H, C_4'–H), 7.94–7.96 (1H, m, C_6'–H), 8.28–8.31 (m, 1H, C_3'–H).

2f:m.p. 180–181 °C, yield 31.46%. IR cm^{-1} 3213.8 N-Hstr.; ^{1}HNMR. δ 2.35 (s, 6H, C_4–CH_3, C_6–CH_3), 3.86 (s, 3H, C_4–OCH_3), 6.46 (s, 1H, –N=C–H), 3.91(s, 3H, C_3'–OCH_3), 7.43–7.44 (d, 1H, C_5'–H), 6.77–6.80 (d, 1H, C_2'–H), 7.17–7.20 (dd, 1H, C_6'–H), 7.6 (s, 1H, C_5–H).

2g:m.p. 170–172 °C, yield 56.78%. IR cm^{-1} 3565.6 N-Hstr.; ^{1}HNMR δ 2.10 (s, 6H, C_4–CH_3, C_6–CH_3), 6.47 (s, 1H, –N=C–H), 8.5 (s, 1H, C_5–H), 3.85 (s, 3H, C_4'–OCH_3), 3.86 (s, 6H, C_3'–OCH_3, C_5'–OCH_3), 7.02 (s, 2H, C_2'–H, C_6'–H).

2h:m.p. 123–125 °C, yield 54.29%. IR cm^{-1} 3203.7 N-Hstr.; ^{1}HNMR δ 2.10 (s, 6H, C_4–CH_3, C_6–CH_3), 6.47 (s, 1H, –N=C–H), 8.71(s, 1H, C_5–H), 7.04–7.07 (m, 1H, C_4'–H), 7.27–7.29 (d, 1H, C_5'–H, J = 5.1 Hz), 7.35–7.36 (d, 1H, C_3'–H, J = 3.6 Hz).

2i:m.p. 108–110 °C, yield 45.27%. IR cm^{-1} 3172.8 N-Hstr.; ^{1}HNMR δ 2.10 (s, 6H, C_4–CH_3, C_6–CH_3), 6.52 (s, 1H, –N=C–H), 7.93 (s, 1H, C_5–H), 7.25–8.10 (m, 4H, C_3'–H, C_4'–H, C_5'–H C_6'–H).

2j:m.p. 152–153 °C, yield 65.60%. IR cm^{-1} 3179.5 N-Hstr.; ^{1}HNMR δ 2.36 (s, 6H, C_4–CH_3, C_6–CH_3), 6.51(s, 1H, –N=C–H), 7.83 (s, 1H, C_5–H), 7.23–7.27 (dd, 1H, C_5'–H, J = 4.8, 8.1 Hz), 8.14–8.18 (dd, 1H, C_4'–H, J = 2.1, 6.0 Hz), 8.48–8.50 (distorted doublet, 2H, C_2'–H, C_6'–H).

2k:m.p. 171–172°C, yield 67.40%. IR cm^{-1} 3179.5 N-Hstr.; ^{1}HNMR δ 2.37 (s, 6H, C_4–CH_3, C_6–CH_3), 6.54 (s, 1H, –N=C–H), 7.76 (s, 1H, C_5–H), 7.54–7.56 (dd, 2H, C_3'–H, C_5'–H, J = 2.1, 5.1 Hz), 8.54–8.56 (dd, 2H, C_2'–H, C_6'–H, J = 1.5, 4.5 Hz).

*4.1.2. 3-Aryl-5,7-dimethyl-1,2,4-triazolo [4,3-a] pyrimidines **3a–k***

4.1.2.1. General procedure. To a stirred solution of pyrimidinylhydrazones **2** (0.01 mol) in DCM (25 ml) at room temperature, IBD (0.01 mol) was added in four to five por-

Table 1
In vitro antibacterial spectrum of chemically synthesized compounds

Compound	Concentration (μg ml^{-1})	Inhibition (%)			
		B. subtilis	*E. coli*	*S. aureus*	*S. typhi*
3b	500	Nil	Nil	Nil	Nil
	100	Nil	Nil	Nil	Nil
	50	Nil	Nil	Nil	Nil
3c	500	Nil	Nil	Nil	Nil
	100	Nil	Nil	Nil	Nil
	50	Nil	Nil	Nil	Nil
3d	500	Nil	16.29	43.67	100
	100	Nil	Nil	42.11	100
	50	Nil	Nil	33	92.5
3e	500	Nil	Nil	Nil	100
	100	Nil	Nil	Nil	100
	50	Nil	Nil	Nil	100
3f	500	100	100	100	100
	100	100	100	100	100
	50	100	100	100	100
	10	97.5	98.24	100	100
3g	500	Nil	20	Nil	100
	100	Nil	16.67	Nil	100
	50	Nil	10	Nil	100
3i	500	Nil	100	100	100
	100	Nil	100	100	100
	50	Nil	100	100	100
	10	Nil	93.64	83.34	100
3j	500	90	45	100	100
	100	86.67	35	100	100
	50	83.34	15.79	100	100
	10	57	Nil	100	98.25
3k	500	100	100	100	100
	100	100	100	100	100
	50	100	100	100	100
	10	100	100	100	100
Ciprofloxacin	500	86.96	89.29	93.85	100
	10	51.67	48.58	45.72	Nil
Chloramphenicol	500	94.08	88.47	92.43	85.22
	10	46.67	40	38.58	Nil
Streptomycin	500	91.9	94.62	84.35	89.7
	10	36.25	36	31	Nil
Ampicillin	500	88.22	84.17	89.53	91.18
	10	36	Nil	20	Nil

tions during 5 min. The solvent was evaporated on a steam bath and the residual mass containing product and iodobenzene triturated with petroleum ether to give solid product, which was recrystallized from methanol to yield pure triazolopyrimidines **3**.

*4.1.2.2. Characterization data of new triazolopyrimidines **3a–k** are given.* **3a**:m.p. 198–199 °C (decomp.), yield 40.94%. ^{1}HNMR δ 2.23 (s, 3H, C_5–CH_3), 2.63 (s, 3H, C_7–CH_3), 6.53 (s, 1H, C_6–H); 7.51 (s, 4H, C_2'–H, C_3'–H, C_5'–H, C_6'–H). *m/z* M^+ 258.

3b:m.p. 208–209 °C, yield 77.41%. ^{1}HNMR δ 2.22 (s, 3H, C_5–CH_3), 2.64 (s, 3H, C_7–CH_3), 6.53 (s, 1H, C_6–H), 7.40–7.46 (1H, m, C_5'–H), 7.53–7.55 (2H, m, C_4'–H, C_6'–H), 7.60–7.63 (1H, dd, J = 1.3, 7.8 Hz, C_3'–H). *m/z* M^+ 258.

3c:m.p. 205–207 °C(decomp.), yield 82.82%. ^{1}HNMR δ 2.21 (s, 3H, C_5–CH_3), 2.45 (s, 3H, C_4–CH_3), 2.61 (s, 3H, C_7–CH_3), 6.49 (s, 3H, C_6–CH_3), 7.28–7.30 (d, 2H, C_3'–H, C_5'–H, J = 7.8 Hz), 7.41–7.44 (d, 2H, C_2'–H, C_6'–H, J = 7.9 Hz). *m/z* M^+ 238.

3d:m.p. 247–248 °C, yield 82.70%. ^{1}HNMR. δ 2.27 (s, 1H, C_5–CH_3), 2.66 (s, 1H, C_7–CH_3), 6.54 (s, 1H, C_6–H), 7.79–7.82 (d, 2H, C_3'–H, C_5'–H, J = 8.6 Hz). *m/z* M^+ 269.

3e:m.p. 226–227 °C, yield 92.74%. ^{1}HNMR δ 2.15 (s, 3H, C_5–CH_3), 2.65 (s, 3H, C_7–CH_3), 6.56 (s, 1H, C_6–H), 7.69–7.72 (1H, m, C_5'–H), 7.80–7.83 (m, 2H, C_4'–H,C_6'–H), 8.26–8.31 (m, 1H, C_3'–H). *m/z* M^+ 269.

3f:m.p. 151–153 °C, yield 52.41%. ^{1}HNMR δ 2.25 (s, 3H, C_5–CH_3), 2.62 (s, 3H, C_7–CH_3), 3.90 (s, 3H, C_4'–OCH_3), 3.96 (s, 3H, C_3'–OCH_3), 6.50 (s, 1H, C_6–H), 6.77–6.80 (d,

1H, C_2'–H), 7.39–7.41 (d,1H, C_5'–H), 7.15–7.16 (dd, 1H, C_6'–H). *m/z* M^+ 284.

3g:m.p. 160–162 °C, yield 72.78%. ^{1}HNMR δ 2.30 (s, 3H, C_5–CH_3), 2.66 (s, 3H, C_7–CH_3), 3.87 (s, 3H, C_4'–OCH_3), 3.93 (s, 3H, C_3'–OCH_3, C_5'–OCH_3), 6.52 (s, 1H, C_6–H), 7.09 (s, 2H, C_2'–H, C_6'–H). *m/z* M^+ 314.

3h:m.p. 90–92 °C, yield 40.48%. ^{1}HNMR δ 2.65 (s, 3H, C_5–CH_3), 2.80 (s, 3H, C_7–CH_3), 6.57 (s, 1H, C_6–H), 7.14–7.17 (m, 1H, C_4'–H), 7.45–7.46 (d, 1H, C_5'–H, J = 5.25 Hz), 7.95–7.96 (d, 1H, C_3'–H, J = 3.55 Hz). *m/z* M^+ 230.

3i:m.p. 148–150 °C, yield 80.80%. ^{1}HNMR δ 2.53 (s, 3H, C_5–CH_3), 2.64 (s, 3H, C_7–CH_3), 6.61 (s, 1H, C_6–H), 7.41–7.45 (dd, 1H, C_5'–H, J = 3.9, 5.4 Hz), 7.86–7.92 (ddd, 1H, C_4'–H, J = 1.5, 7.5, 7.8 Hz), 8.04–8.06 (d, 1H, C_3'–H, J = 7.2 Hz), 8.72–8.74 (d, 1H, C_6'–H, J = 5.1 Hz). *m/z* M^+ 225.

3j:m.p. 210–212 °C, yield 75.30%. ^{1}HNMR δ 2.25 (s, 3H, C_5–CH_3), 2.65 (s, 3H, C_7–CH_3), 6.57 (s, 1H, C_6–H), 7.46–7.49 (dd, 1H, C_5'–H, J = 4.2, 7.2 Hz), 7.95–7.96 (dd, 1H, C_4'–H, J = 1.8, 5.7 Hz), 8.62–8.63 (distorted doublet, 2H, C_2'–H). *m/z* M^+ 225.

3k:m.p. 198–200 °C, yield 84.84%. ^{1}HNMR δ 2.30 (s, 3H, C_5–CH_3), 2.65 (s, 3H, C_7–CH_3), 6.60 (s, 1H, C_6–H), 7.54–7.56 (dd, 2H, C_3'–H, C_5'–H, J = 1.2, 4.8 Hz), 8.0–8.2 (dd, 2H, C_2/–H, C_6/–H, J = 1.2, 4.8 Hz). *m/z* M^+ 225.

5. In vitro antibacterial assays

Antibacterial testing of nine synthesized compounds was done in vitro by the following method. The stock solution (1 mg ml^{-1}) of the test chemical was prepared by dissolving 10 mg of the test chemical in 10 ml of DCM. The stock solution was suitably diluted with sterilized distilled water to get dilutions of 500, 100 and 50 μg ml^{-1}. Control for each dilution was prepared by diluting 10 ml of DCM instead of stock solution with sterilized distilled water.

Four bacteria, namely *B. subtilis, S. aureus* (Gram +ve, non-motile), *E. coli* (Gram –ve, non-motile) and *S. typhi* (Gram –ve, motile), were used for the antibacterial assay. Spread-plate method was used for *B. subtilis, E. coli* and *S. aureus* as they were non-motile. Pour-plate method was used for *S. typhi* (as it is motile). For this, 24-h-old broth cultures were diluted up to 10^{-3}. Fifty microliters of the test chemical of required dilution and 50 μl of 24-h-old culture broth of 10^{-3} dilutions were mixed to make a total volume of 0.1 ml. The mixture was then spread on the surface of nutrient agar plates with the help of a sterilized spreader. For control, 50 μl of respective dilution of DCM was added in to the broth medium. All the plates were incubated at 35 °C for 24 h and the colonies observed in the test and control plates were counted.

Twenty-four hour old broth culture of *S. typhi* was diluted to 10^{-3}. To the already labelled Petri plates, was added 500 μl of the test chemical of the required dilution and 500 μl of culture of 10^{-3} dilutions to make a total volume of 1 ml. Sufficiently, cooled NA medium was poured to the Petri plates and rotated them to mix the contents. For control, 500 μl of respective dilution of DCM were added in place of the dilution of test chemical. After solidification, plates were incubated at 35 °C for 24 h and the colonies observed in the test and control plates were counted to get percent inhibition by the test chemical.

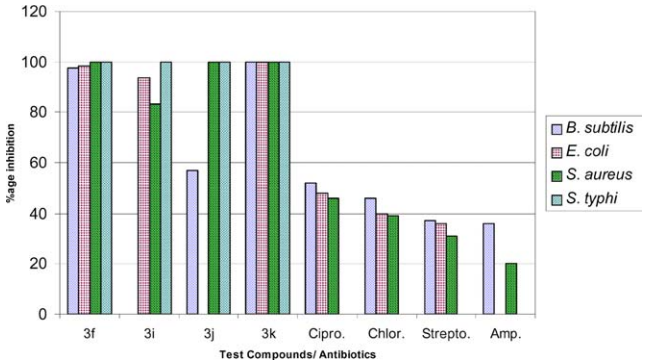

Fig. 1. In vitro antibacterial assay of test compounds/antibiotics at concentration 10 μg/ml.

6. Results and discussion

Nine synthesized compounds **3b–g** and **3i–k** were tested in vitro for their antibacterial activity against *B. subtilis, E. coli, S. aureus* and *S. typhi* by the method used by us [15]. Out of the nine compounds tested, **3f** and **3k** were most active against all the four test bacteria viz. *B. subtilis, E. coli, S. aureus* and *S. typhi* at all the four concentrations (500, 100, 50 and 10 μg ml^{-1}) showing 100% inhibition. The compound **3i** was also found to be inhibitory at all the four concentrations (500, 100, 50 and 10 μg ml^{-1}) for *E. coli, S. aureus* and *S. typhi* but it was completely inactive against *B. subtilis*. **3j** was also found to be 100% inhibitory against *S. aureus* and *S. typhi* at all the four concentrations, while the activity of the compound was low against *E. coli* and *B. subtilis* at all the concentrations. The activities of the compounds were also compared with the four commercial antibiotics (Ciprofloxacin, Chloramphenicol, Streptomycin, Ampicillin) and were found to be more potent than commercial antibiotics (Table 1 and Fig. 1). Thus, these compounds could be used as lead structure in pharmaceutical industry if they are nontoxic to the human system.

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