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Synthesis and antibacterial activity of substituted 1,2,3,4-tetrahydropyrazino [1,2-*a*] indoles

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Abstract—A series of substituted 1,2,3,4-tetrahydropyrazino [1,2-*a*] indole derivatives have been synthesized and tested against the Gram positive and Gram negative strains of bacteria namely *Staphylococcus aureus* (MTCCB 737), *Salmonella typhi* (MTCCB 733), *Pseudomonas aeruginosa* (MTCCB 741), *Streptomyces thermonitrificans* (MTCCB 1824) and *Escherichia coli* (MTCCB 1652). All synthesized compounds showed mild to moderate activity. However, compounds **4d**–**f** were found to have potent activity against pathogenic bacteria used in the study. Their MIC ranged from 3.75 to 60 μg/disc. In vitro toxicity tests demonstrated that toxicity of **4d**–**f** was not significantly different than that of gentamycin. However, at higher concentration (1000–4000 μg/ml) difference was highly significant.

Since the introduction of penicillin in the 1940s, antibiotics have a history of success in controlling morbidity due to infectious diseases. But, as a consequence of frequent use, bacterial resistance to known classes of antibiotics has become a severe global problem in recent years and presents a continuous clinical challenge.[1–3] Resistance can result from modification of an antibacterial target or from functional bypassing of that target, or it can be contingent on impermeability, efflux or enzymatic inactivation of the drug.[4] There are serious concerns that untreatable pathogens may develop at an alarming rate in the near future. Strategies to address this challenge include the design of improved versions of antibacterial classes already in clinical use and the use of drug combinations. The application of these strategies can be quite successful, but a high risk of rapid resistance development remains. Thus, an urgent need for new potent classes of antibiotics with novel modes of action persists.

Pyrazino [1,2-*a*] indoles have attracted a great deal of attention due to their therapeutic uses as serotonin antagonist,[5] thrombolytic,[6] in cardiovascular diseases,[7] antidepressant, anxiolitics,[8] central nervous system depressants,[9] anticonvulsants,[10] antihistaminic,[11] protein kinase C inhibitors,[12] 5-HT_{2A},[13] 5-HT_{2C}[13,14] and selective imidazoline I_2 receptor ligands.[15] The antibacterial activity of the indole derivatives has not been much studied. One report shows the activity of pyrazinoate towards resistant *Mycobacterium tuberculosis*.[16] Some triazino [5,6-*b*] indoles have been reported to have antifungal properties.[17]

2-(3-Methyl-1*H*-indol-1-yl) ethylamine **2** was obtained by the reaction of 3-methylindole **1** with 2-chloroethylamine hydrochloride in CH_3CN in the presence of NaOH and Bu_4NHSO_4 by reported procedures.[18] Key benzotriazolyl intermediate 2-(1*H*-1,2,3,4-benzotriazol-1-ylmethyl)-10-methyl-1,2,3,4-tetrahydropyrazino [1,2-*a*] indole **5** and nucleophilic substituted derivative **6a**,**b** and compounds **7**–**9** were obtained according to our

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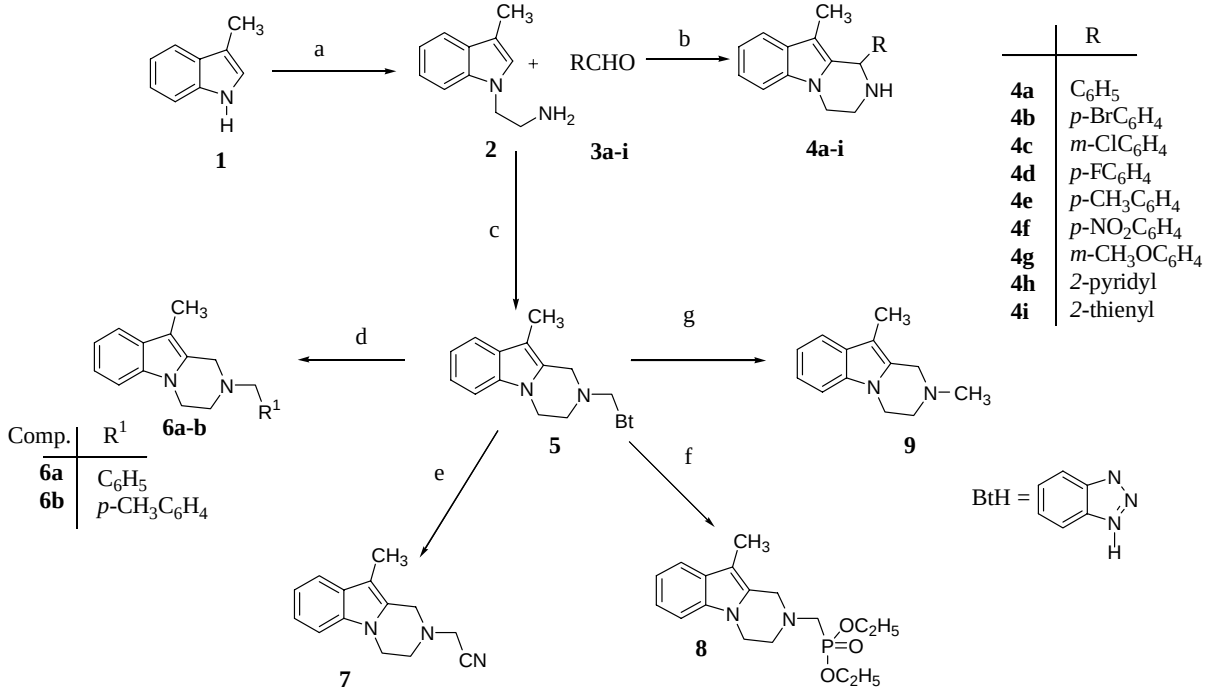
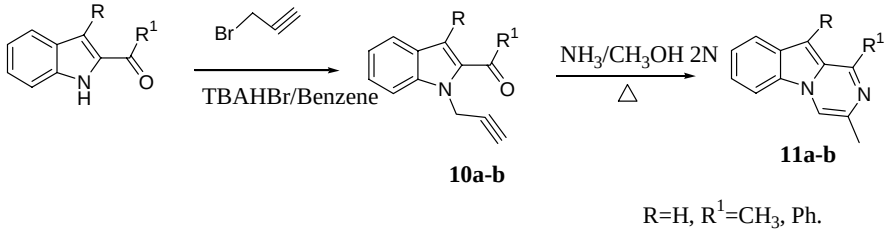
published procedure.[18] 1-Substituted 1,2,3,4-tetrahydropyrazino [1,2-*a*] indoles **4a**–**i** were obtained as a racemic mixture in high yields by the reaction of 2-(3-methyl-1*H*-indol-1-yl) ethylamine **2b** with benzotriazole and aldehydes **3a**–**i** in the presence of catalytic amount of $AlCl_3$ in CH_2Cl_2 (Scheme 1).[19] The structures of new compounds **4c** and **4g** are clearly supported by their ^1H, ^{13}C NMR spectra and microanalysis.[20] The ^1H NMR spectra showed NC*H*-pyrazino singlet signal for **4a**–**i** at ~5.3 ppm. Presence of exchangeable N*H*-pyrazino was confirmed by deuterium exchange. 1,3-Dimethylpyrazino [1,2-*a*] indole **11a** and 3-methyl-1-phenyl-pyrazino [1,2-*a*] indole **11b** were prepared by the reaction of 1-propargyl-2-acetylindole **10a** and 1-propargyl-2-benzoylindole **10b** in dry ammonia and methanol (Scheme 2).[21] 1-Propargyl-2-acetylindole **10a** and 1-propargyl-2-benzoylindole **10b** were synthesized by standard procedure[22] starting from 2-acetyl-1*H*-indole[23] and 2-benzoyl-1*H*-indole.[24]

The in vitro antibacterial activity was tested by disc diffusion method[25] using pathogenic strains of *Staphylococcus aureus*, *Salmonella typhi*, *Streptomyces thermonitrificans*, *Pseudomonas aeruginosa* and *Escherichia coli*. The experimental[27] result of antibacterial activity indicated a variable degree of efficacy of the compounds against different strains of bacteria (Table 1). Compound **4a** showed strong activity against *P. aeruginosa* (MIC 3.75 μg/disc); however, it did not show any effect on other strains of bacteria even up to a concentration of 60.00 μg/disc. Similarly the **4c** was effective against *P. aeruginosa* and *S. thermonitrificans* only, the MIC being 3.75 and 15.00 μg/disc, respectively. Significant activity was observed with **4d**–**4f** against all the bacterial strains used in the study and their MIC ranged from 7.50 to 60.00 μg/disc. Other compounds appeared to be broad spectrum, as they showed mild to moderate effect on most of the strains, although the compound with pyrazinoate moiety was shown earlier to inhibit the growth of *M. tuberculosis* at a concentration of 0.5–32 μg/ml. However, pyrazino [1,2-*a*] indoles have not been investigated. We, for the first time, synthesized substituted pyrazino [1,2-*a*] indoles and tested for the antimicrobial properties. The activity of the compound



Scheme 1. Synthesis of substituted 1,2,3,4-tetrahydropyrazino [1,2-*a*] indoles. Reagents and conditions: (a) $ClCH_2CH_2NH_2HCl$, NaOH, TBAHS, CH_3CN, reflux, 36 h; (b) benzotriazole, dichloromethane, catalytic $AlCl_3$, 25 °C, 85–96%; (c) benzotriazole, HCHO (2 equiv), 25 °C stirring; (d) R^1MgX, THF, reflux; (e) NaCN, DMSO, 25 °C, 36 h; (f) $P(OEt)_3$, $ZnBr_2$, DCM, 25 °C, 24 h; (g) $NaBH_4$, THF, reflux,12 h.



Scheme 2. Reaction conditions: molar ratio indole **10a–b**, 1/2 M NH_3 in MeOH = 1:20, sealed tube.

Table 1. Minimum inhibitory concentrations of substituted-1,2,3,4-tetrahydropyrazino [1,2-*a*] indoles by disc diffusion assay

Compound	MIC (μg/disc)				
	Staphyloccus aureus	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Streptomyces thermonitrificans*	*Escherichia coli*
4a	—	—	03.75	—	—
4b	15.0	60.0	60.0	15.0	—
4c	—	—	15.0	03.75	—
4d	30.0	30.0	30.0	07.5	15.0
4e	30.0	15.0	60.0	30.0	15.0
4f	30.0	30.0	30.0	15.0	30.0
4g	60.0	30.0	—	15.0	30.0
4h	03.75	—	—	—	—
4i	60.0	30.0	—	—	—
5	30.0	60.0	—	—	—
6a	15.0	60.0	60.0	60.0	—
6b	15.0	30.0	60.0	60.0	—
7	15.0	30.0	60.0	30.0	—
8	—	—	60.0	60.0	—
9	30.0	30.0	30.0	30.0	—
11a	60.0	60.0	60.0	60.0	60.0
11b	60.0	60.0	60.0	60.0	60.0
Gentamycin	1.0	1.0	0.5	1.0	1.0
Doxycycline HCl	1.0	1.0	1.0	1.0	2.0

(—) means no activity.

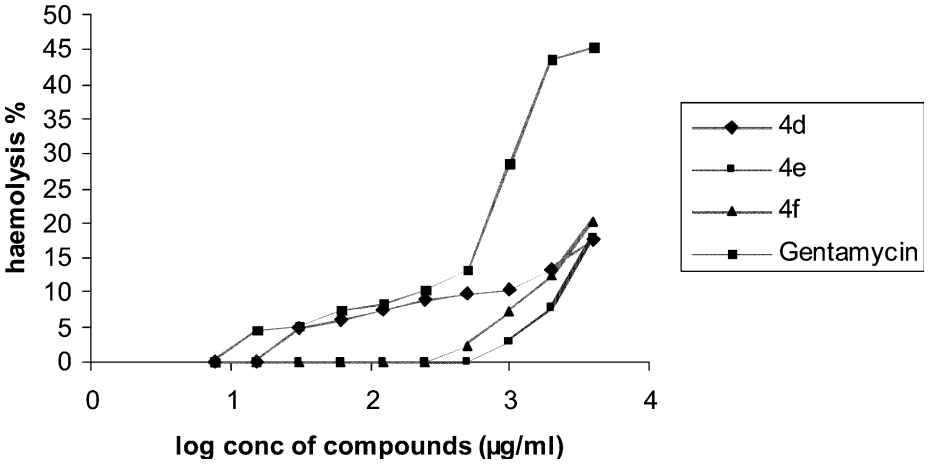


Figure 1. Cytotoxicity of substituted pyrazino [1,2-*a*] indoles **4d–f** analysed by haemolytic assay.

appeared to be associated with the 1 or 2 substitution on the 1,2,3,4-tetrahydropyrazino [1,2-*a*] indoles. The presence of NH group with substitution on 1-position of compound **4a–i** showed better activity than compounds **5–9** with substituents present on 2-position. Reactivity data of all the compounds revealed that the presence of free NH group might be responsible for the requisite activity. It was observed that the presence of 4-bromophenyl and 3-chlorophenyl substituents at 1-position in compounds **4b–c** showed significant activity against *S. aureus* and *S. thermonitrificans*, respectively. However, compounds **4d–f** with the presence of 4-fluorophenyl, 4-methylphenyl and 4-nitrophenyl at 1-positon showed significant activity against all the bacteria used in the study (Table 1). Enhanced activity of 1-substituted 1,2,3,4-tetrahydropyrazino [1,2-*a*] indoles (**4a–i)** as compared to that of 2-substituted 1,2,3,4-tetrahydropyrazino [1,2-*a*] indoles (**5–9)** might be due to the availability of lone pair on the pyrazino nitrogen atom as well as structure of the pyrazino ring.[18]

The in vitro cell cytotoxicity of **4d–f** was investigated using haemolytic assay.[26] Results demonstrated that concentrations up to 500 μg/ml of these compounds lysed 5–10% of erythrocytes. The percent lysis induced by concentration up to 500 μg/ml of gentamycin was not significantly different than that of compounds. However, at higher doses (1000–4000 μg/ml), the difference in toxicity was significant (Fig. 1).

Series of substituted pyrazino [1,2-*a*] indole have been synthesized and were found to have antibacterial activity against pathogenic strains of *S. aureus* (MTCCB 737), *S. typhi* (MTCCB 733), *P. aeruginosa* (MTCCB 741), *S. thermonitrificans* (MTCCB 1824) and *E. coli* (MTCCB 1652) (Table 1). The compounds **4d–f** were considered to be potent antibacterial agents due to significant activity against all the bacteria used in the study. The compounds **4d–f** being less toxic than gentamycin could be considered as safer drug candidates and can be taken up for the development of suitable antibacterial drug.

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20. Analytical data for 1-(3-methoxyphenyl)-10-methyl-12,3,4-tetrahydropyrazino [1,2-*a*] indole **4g**: white needles from EtOAc/hexanes; mp 124–126 °C; ^{1}H NMR δ 1.60 (s, 1H, NH), 1.84 (s, 3H, CH_3), 3.12–3.30 (m, 2H, H-3), 3.76 (s, 3H, OMe), 3.94–4.12 (m, 2H, H-4), 5.30 (s, 1H, H-1), 6.80–7.50 (m, 8H, Ar); ^{13}C NMR δ 8.2 (CH_3), 38.5 (C3), 41.5(C1), 54.2 (C4), 57.2 (C3′-OMe), 105.6 (C6), 107.8 (C4′), 108.3 (C10), 109.2 (C2′), 118.7 (C7), 119.1 (C6′), 119.5 (C9), 121.7 (C8), 122.4 (C5′), 131.1 (C9a), 139.5 (C1′), 142.1 (C5a), 143.2 (C10a), 154.4 (C3′); LCMS *m/z*, 293 (5%, M+1), 292 (30%, M), 291 (100%, M−1). Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.00; H, 9.58; N, 9.58. Found: C, 77.72; H, 9.51; N, 9.54. 1-(3-Chlorophenyl)-10-methyl-1,2,3,4-tetrahydropyrazino [1,2-*a*] indole **4c**: white needles from EtOAc/hexanes; mp 101–102 °C; ^{1}H NMR δ 1.84 (s, 4H, NH, CH_3), 3.12–3.24 (m, 2H, H-3), 3.84–4.12 (m, 2H, H-4), 5.31 (s, 1H, H-1), 7.04–7.42 (m, 8H, aromatic); ^{13}C NMR δ 8.2 (CH_3), 38.2 (C3), 44.6 (C1), 56.7 (C4), 108.5 (C6), 118.3 (C10), 118.9 (C7), 121.0 (C9), 125.1 (C8), 128.6 (C6′), 129.2 (C4′), 131.2 (C2′), 132.4 (C5′), 133.1 (C9a), 135.5 (C3′), 138.4 (C1′), 140.6 (C5a), 142.3 (C10a); LCMS *m/z* 297.1 (50%, M+1), 295 (100%, M−1), 280.1 (7%, M−15), 186.2 (12%, M−110). Anal. Calcd for $C_{18}H_{17}N_2Cl$: C, 72.80; H, 5.70; N, 9.40. Found: C, 72.52; H, 5.40; N, 9.21.
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27. Experimental detail: the different concentrations of compounds in the range from 3.75 to 60.00 μg/disc were loaded on the discs, which were placed on the agar surface of the culture plates. The plates were incubated at 37 °C and examined at 48 h for zone of inhibition, if any, around the discs. Gentamycin and doxycycline HCl were used in an assay as a standard control drug. An additional negative control disc without any sample but impregnated with the equivalent amount of solvent (DMSO) was also used in the assay. Lowest concentration of the compounds, which developed a zone of inhibition of minimum 6.0 mm diameter, was considered to be minimum inhibitory concentrations.