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POTENTIAL ANTIBACTERIAL AGENTS
Part I. Synthesis of Substituted 1-Arylaminomethyl Benzotriazoles

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Condensation of 1,2,3-benzotriazole with primary aromatic amines, viz, 2-amino, 5-chlorobenzophenone; 4-(N-morpholino)-aniline; 4-(N-piperidino)-aniline and 3-acetyl aniline, under the conditions of Mannich reaction, afforded the hitherto unreported substituted 1-arylaminomethyl benzotriazoles which were assigned structures (I to IV) on the basis of their H-nmr and mass spectral data. Anti-bacterial activity determination showed compounds (I to IV) to inhibit the growth of *Salmonella typhi, Pseudomonas spp.* and *Escherichia coli.* Compounds I and II were also found active against *Bacillus subtilis.*

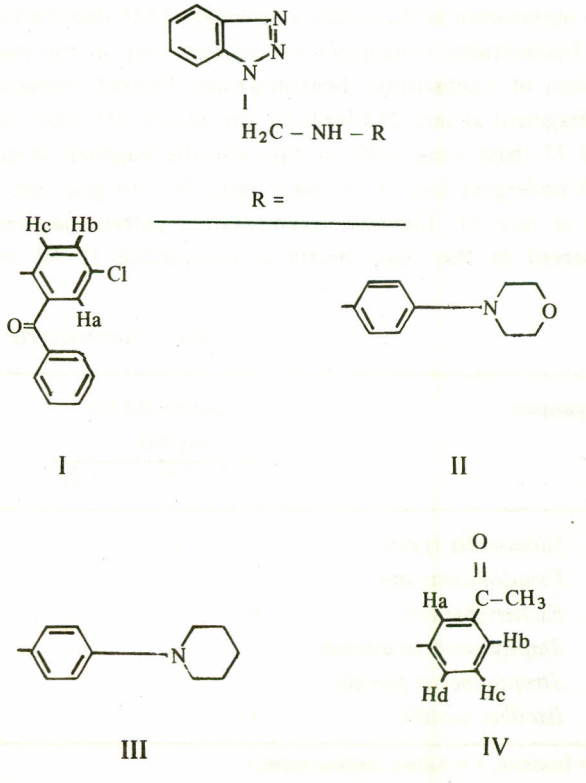
Key words: Mannich Reaction; 1-Arylaminomethylbenzotriazole; Antibacterial Activity.

INTRODUCTION

The usefulness of the Mannich reaction in the synthesis of potential biologically active compounds is well documented in the literature [1]. The N-Mannich bases, in particular, are reported to display anticonvulsant, sedative [2], anthelmintic [3], antiviral and antibacterial [4-6] activities. The tetracycline N-Mannich base has been shown to exhibit a high order of antibacterial activity [7,8] which is attributed to the structural modification of tetracycline caused by the Mannich reaction.

In earlier reports 1,2,3-benzotriazole has been reported to undergo a Mannich reaction with secondary amines only, to yield a 1-substituted product, while polymeric products were obtained with primary aromatic amines. Licari *et al.* [9] were the first to report the successful condensation of benzotriazole with formaldehyde and aniline to furnish a crystalline Mannich base which was assigned the structure of 1-anilinomethyl benzotriazole. No evidence, however, was provided by these authors to exclude the possibility of getting a 2-substituted product. Varma [10] reinvestigated this reaction and has conclusively established that the substitution of anilinomethyl group takes place at position 1 of benzotriazole.

The present studies describe the reaction of benzotriazole with formaldehyde and substituted primary aromatic amines, namely, (a) 2-amino-5-chloro benzophenone, (b) 4-(N-morpholino)-aniline, (c) 4-(N-piperidino)-aniline and (d) 3-acetyl aniline to yield the hitherto unreported substituted 1-aryl aminomethyl benzotriazoles which were assigned structures (I to IV) on the basis of their H-nmr and mass spectral data. The new N-Mannich bases thus synthesised were tested for antibacterical activity, following the method of Prescott and Dunn [11] using as test organisms *Salmonella typhi, Pseudomonas spp. Escherichia coli, Staphylococcus aureus, Streptococcus faecalis,* and *Bacillus subtilis.* The results shown in Table 1 indicate that compounds (I to IV) inhibited to growth of



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Salmonella typhi, Pseudomonas spp and *Escherichia coli* but showed no activity against *Staphylococcus aureus* and *S treptococcus faecalis.* Compound I and II were also found to inhibit the growth of *Bacillus subtilis.*

In the H-nmr spectrum of I, a sharp singlet resonating at δ 7.28 (1H, S, Ar-H) was attributed to the isolated aromatic proton Ha. Two doublets centred at δ 7.75 (1H, d, Ar-H ; J = 8.3 Hz) and δ 8.1 (1H, d, Ar-H : J = 8.3 Hz) were due to the *ortho* oriented aromatic protons Hc and Hb. A doublet centred at δ 6.25 (2H, d, -CH $_2$-NH-, J = 6Hz) was ascribed to the methylene group, while a triplet resonating at δ 9.4 (1H, t, -CH_2-NH-, J = 6Hz) was assigned to the proton of the imino (-NH-) group. The low field absorption of (-NH-) group at δ 9.4 may be due to the presence of electronegative oxygen of the neighbouring carbonyl group which is intramoleculary hydrogen bonded with the (-NH-) group.

In the H-nmr spectrum of II, a doublet resonating at δ 6.06 (2H, d, -CH2-NH- ; J = 6Hz) was assigned to the methylene group while a triplet centred at δ 4.56 (1H, t, -CH_2-NH-, J = 6Hz) was attributed to the imino group (-NH-). In the H-nmr spectra of compounds III and IV, the chemical shifts for the imino and methylene groups (-CH_2-NH-) were very close to that recorded for compound II.

The mass spectrum of I shows molecular ion at m/z 362 which undergoes McLafferty rearrangement leading to fragments at m/z 243 and 119. This is in agreement with the observation of Lawrence and Waight [12] that the loss of 1-substituent constitutes the primary step in the mass spectra of 1-substituted benzotriazoles. Further break-up of fragment at m/z 243 leads to ions at m/z 215, 180, 105 and 77 (base peak, 100 %). Likewise the fragment at m/z 119 undergoes loss of 28 mass units (N_2) to give rise to ion at m/z 91. Identical fragmentation pattern has been observed in the mass spectra of compounds II, III and IV.

EXPERIMENTAL

Melting points were taken on the Buchi-510 melting point apparatus and are uncorrected. The i.r. spectra were measured in KBr on a Jasco A-302 spectrophotometer. The H-nmr spectra were recorded on Brucker AM-100 MHz spectrometer using TMS as internal reference. Mass spectra were taken on Finningen Mat 112-S instrument at 70 eV. The petroleum ether used referred to fraction with boiling point 60-80^o.

2-Amino, 5-chloro-benzophenone [13], 4-(N-morpholino)- aniline [14] and 4-(N-piperidino)-aniline [15] used in this work were prepared according to the published procedures.

Preparation of 1-(2-benzoyl-4-Chlorophenylaminomethyl)-Benzotriazole-I. Benzotriazole (2.38 g; 0.02 mole) was dissolved in 20 ml. boiling methanol. Formalin (2 ml, 29 %), 2-amino-5-chloropbenzophenone (4.62 g, 0.02 mole) and glacial acetic acid (4 drops) were added to it with good stirring. The contents were refluxed (½ hr.) on steam bath till all solid dissolved. Refluxing continued for another 4 hr. and left overnight at room temperature. The separated solid was filtered off and crystallized from benzene to give 1-(2-benzoyl-4-chlorophenylamino-methyl)-benzotriazole-I as yellow needles, mp. 159-60^o (6.69g, 91.8 %). I.R., 3350 (-NH-), 1640 (C=O), 1600 and 1590 cm^{-1} (Aryl) ; H-nmr, δ 6.25 (2H, d, CH_2-NH-, J = 6Hz), δ 7.28 (1H, S, Ar-Ha), δ 7.35-7.6 (9H, aromaric protons), δ 7.75 (1H, d, Ar-Hc, J = 8.3 Hz), δ 8.1 (1H, d, Ar-Hb, J=8.3 Hz), δ 9.4 (1H, t, -CH_2-NH-, J = 6Hz). Mass, m/z 362 (20 %, M^+), 333 (2 %), 243 (74 %), 215 (36 %), 180 (16 %), 166 (16 %), 132 (28 %), 119 (38 %), 105 (26 %), 91 (44 %) and 77 (100 % base peak).

Preparation of 1-(4-morpholino)-phenylaminomethyl)-benzo triazole-(II). Benzotriazole (2.38g, 0.02 mole) was dissolved in 20 ml boiling methanol. Formalin (2 ml, 29 %),

Table 1. Antibacterial activity of compounds (1 to IV)

Organism	Compound (I) mg/ml			Compound (II) mg/ml			Compound (III) mg/ml			Compound (IV) mg/ml		
	5	2.5	1.25	5	2.5	1.25	5	2.5	1.25	5	2.5	1.25
1. *Salmonella typhi*	+	+	–	+	+	+	+	+	–	+	+	+
2. *Pseudomonas spp.*	+	–	–	+	+	+	+	+	–	+	+	+
3. *Escherichia coli*	+	–	–	+	+	–	+	–	–	+	+	+
4. *Staphylococcus aureus*	–	–	–	–	–	–	–	–	–	–	–	–
5. *Streptococcus faecalis*	–	–	–	–	–	–	–	–	–	–	–	–
6. *Bacillus subtilis*	+	–	–	+	–	–	–	–	–	–	–	–

– = Inactive, + = Active (antibacterial.)

4-(N-morpholino)-aniline (3.56 g, 0.02 mole) and glacial acetic acid (4 drops) were added to it with constant stirring. The reaction mixture was refluxed (4½ hr.) on steam bath and then left overnight at room temperature. The separated product was filtered off and crystallized from methanol to yield dirty white needles of 1-(4-(N-morpholino)-phenylaminomethyl)-benzotriazole-II, mp. 179-80° (4.78 g, 63.5 %). I.R., 3250 (-NH), 1520 cm^{-1} (aryl). H-nmr, δ 3.02 (4H, t, -CH_2-N-CH_2-, J = 6Hz), δ 3.81 (4H, t, -CH_2-O-CH_2-, J = 6Hz), δ 4.56 (1H, t, CH_2-NH-, J = 6.6 Hz), δ 6.09 (2H, d, -CH_2-NH-, J = 6.6 Hz), δ 6.78-7.58 (8H, Ar-H). Mass m/z 309 (M^+, 2 %), 190 (77 %), 177 (12 %), 132 (100 %), 119 (78 %), 94 (68 %), 91 (62 %), 84 (14 %), 77 (28 %), 64 (54 %) and 56 (27 %).

Preparation of 1-(4-(N-piperidino)-phenylaminomethyl)-benzotriazole-(III). To benzotriazole (2.38 g, 0.02 mole) dissolved in methanol (20 ml) was added with stirring formalin (2 ml., 29 %), 4-(N-piperidino)-aniline (3.52 g, 0.02 mole) and glacial acetic acid (4 drops). The reaction mixture was refluxed (4 hr) on steam bath and then left overnight at room temperature. The seperated solid was filtered off and crystallized from ethylacetate-petroleum ether to afford 1-(4-(piperidino)-phenylaminomethyl)-benzotriazole(III) as white needles; mp, 190-1° (4.48 g, 73 %). I.R., 3350 (-NH-), 1520 Cm^{-1} (aryl). H-nmr, δ 1.6 (6H, m, $(CH_2)_3$), δ 2.95 (4H, t, $(N(CH_2)_2)$, J = 6Hz), δ 4.53 (1H, t, -CH_2-NH-, J = 6.6Hz), δ 6.0 (2H, d, -CH_2-NH-, J = 6.6Hz), δ 6.03-7.5, (8H, Ar-H). Mass m/z 307 (1 %, M^+), 188 (100 % base peak), 187 (90 %), 173 (3 %), 159 (10 %), 147 (14 %), 132 (32 %), 119 (36 %), 83 (13 %), 77 (28 %) and 64 (31 %).

Preparation of 1-(3-acetophenyl-aminomethyl)-benzotriazole (IV). To benzotriazole (2.38 g, 0.02 mole) dissolved in boiling methanol (20 ml) was added accompanied by stirring formalin (2 ml, 28 %), 3-acetophenone (2.7 g, 0.02 mole) and glacial acetic acid (4 drops). The reaction mixture was refluxed (4.5 hr.) on a steam bath and left overnight at room temperature. The seperated product on filtration and crystallization from ethylacetate-petroleum ether afforded 1-(3-acetophenylaminomethyl)-benzotriazole(IV) as light pale coloured rods, m.p. 180-1° (4.45 g, 83 %). I.R., 3200 (-NH-), 1698 (C=O), 1600 and 1590 cm^{-1} (aryl). H-nmr, δ 2.06 (3H, S, Ar-CO-CH_3), δ 5.15 (1H, t, -CH_2-NH-, J = 6 Hz), δ 6.16 (2H, d, -CH_2-NH-, J = 6Hz), δ 7.4 (1H, S, Ar-Ha), δ 7.49 (4H, m, Ar-H), δ 7.6 (1H, dd, Ar-Hb, J = 8.3, 1.0 Hz), δ 7.75 (1H, dd, Ar-Hd, J = 8.3, 1.0 Hz), δ 8.1 (1H, dd, Ar-Hc, J = 8.3, 8.3 Hz). Mass m/z 266 (2 %, M^+). 147 (80 %), 132 (100 %) base peak), 119 (48 %), 104 (64 %), 91 (46 %).

Antibacterial activity

Following the procedure of Prescott and Dunn [11], the antibacterial activity of compounds (I to IV) was determined using concentrations of 5.0 to 1.25 mg/ml. The test organisms employed were *Salmonella typhi, Pseudomonas spp. Escherichia coli; Staphylococcus aureus, Streptococcus faecalis* and *Bacillus subtilis.* The results recorded in Table I indicated that, whereas compounds (I to IV) showed no activity against *Staphylococcus aureus* and *Streptococcus faecalis,* they inhibited the growth of *Salmonella typhi, Pseudomonas spp.* and *Escherichia coli.* In this study compound (IV) was shown to be the most active, followed by compound (II) in order of activity. The results in Table I also indicate compounds (I) and (II) to be active against *Bacillus subtilis* though at higher concentration of 5.0 mg/ml.

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