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SYNTHESIS OF SOME 7-AZAINDOLE DERIVATIVES: THEIR CYTOTOXICITY AND ANTIBACTERIAL ACTIVITY

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In the course of pharmaco-chemical studies, a number of new 7-azaindole derivatives have been prepared. The spectroscopic techniques such as ^{1}H NMR, EIMS, UV and IR were utilized for their structure elucidation. These compounds were tested against a variety of Gram + ve and Gram-ve bacteria. These compounds were also screened for their cytotoxicity against *Artemia salina*. Compound IV displayed significant antibacterial activity amongst all the tested compounds. Whereas compound VI was found to be the most significant cytotoxic when screened through brine shrimp bioassay.

Key words: 7-Azaindole, Cytotoxicity, Antibacterial activity.

Introduction

^{1}H-pyrrolo (2,3-b) pyridine (7-azaindole) derivatives have been drawing much attention in biochemical and physico-chemical studies [1] because of their characteristic condensed ring-systems. Pyrrolopyridine (azaindole) has similar skeleton to that of indole except that it possesses one more nitrogen in the form of a basic function which can actually change the physical, chemical and thus biological properties of the compound.

The chemistry of 4,5,6 and 7-monoazaindoles has earlier been surveyed in a comprehensive review [2]. Hooper *et al.* [3] synthesized isatogens and related compounds for antibacterial screening. The isatogens and 3-oxo-3H-pyrrolopyridine-1-oxides were all effective against Gram-positive organisms, but only 2-phenylisatogen and 2-pyride-2'-ylisatogen showed a broad spectrum of activity. The pyrrolo (2,3-c) pyridines were generally more effective than the analogous indoles although the 3-diazo group conferred a broad spectrum of activity in the 2-substituted indoles. S.M. Boyomi *et al.* [4,5] reported the antibacterial activity of two series of fused pyrrolo pyridine and 1, 4-dihydro, 4-oxopyrrolo (3,4-b) pyridine-3-carboxylic acid. In the previous communications [6,7] from our laboratory we have reported the preparation and biological activities of some 7-azaindole derivatives. Our continued efforts extend the synthesis and extensive evaluation of biological activities of some more 7-azaindole derivatives.

Experimental

Melting points were determined on a Gallenkamp apparatus and are un-corrected. Proton nuclear magnetic resonance spectra were recorded on a Bruker AM-300 spectrometer operating at 300 MHz. The chemical shifts are reported in δ values in parts per million (ppm) and coupling constant in Hz. Infra red spectra were taken with a Pye-Unicam SP-800 spectrometer and ultraviolet spectra were measured on JASCO IRA-I. Purity of the products was checked on T.L.C. plates coated with silica gel PF_{245}.

General procedure. Equimolar quantities of 7-azaindole and substituted phenacyl halide (purchased from Aldrich Chemical Co. Ltd.) were dissolved separately in acetone, then mixed together in round bottom flask with constant stirring for 30 min. Then the reaction mixture was kept at room temperature until precipitate was formed; after crystalization, the solution was filtered. The crystals were washed and dried. The spectral studies were carried out by spectroscopic techniques.

7-(4'-Methylphenacyl)-1H-pyrrolo (2,3-b) pyridinium bromide (I). ^{1}H-NMR DMSO (300 MHz) δ : 8.78 (1H, dd, J = 7.85, 1.09 Hz, H-6), 8.47 (1H, dd, J = 6.73, 1.09 Hz, H-4), 8.12 (2H, d, J = 7.93 Hz, H- 2',6'), 7.73 (1H, d, J = 3.57 Hz, H-2), 7.66 (1H, dd, J = 7.85, 6.73 Hz, H-5), 7.45 (2H, d, J = 7.93 Hz, H-3',5'), 6.99 (1H, d, J = 3.57 Hz, H-3), 6.49 (2H, s, N-CH_2), 2.48 (3H, s, Ar-CH_3).

EIMS *m/z* (%): M^+ ($C_{16}H_{15}N_2O$), 251 (16), 160 (27), 133 (46), 132 (2), 118 (71) and 76 (18).

IR ν_{max} (KBr) cm^{-1}: 3300, 2900, 1680, 1600 and 1120.

UV λ_{max} (MeOH) nm: 296, 258 and 203.

7-(3'-Methoxyphenacyl)-1H-pyrrolo (2,3-b) pyridinium bromide (II). ^{1}H-NMR DMSO (300 MHz)δ : 8.77 (1H, dd, J = 7.83, 1.45 Hz, H-6), 8.34 (1H, dd, J= 5.67, 1.45 Hz, H-5), 7.80 (2H,dd, J=8.06, 2.01 Hz, H-6'), 7.70 (1H, d, J=3.65 Hz, H-2), 7.65 (1H, dd, J=7.83, 5.67 Hz, H-5), 7.52 (1H, d, J=2.01 Hz, H-2'), 7.48 (1H, dd, J=8.06 Hz, H-5'), 7.42 (1H, dd, J=7.45, 2.01 Hz, H-4'), 7.12 (1H,d, J=3.65 Hz, H-3), 6.54 (2H, s, N-CH_2), 3.93 (3H, s, Ar-OCH_3).

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EIMS *m/z*(%): M^+ ($C_{16}H_{15}N_2O_2$), 267 (9), 237 (100), 223 (25), 195 (3) and 132 (16).

IR ν_{max} (KBr) cm^{-1}: 3400, 2910, 1690, 1580, 1470 and 1340.

UV λ_{max} (MeOH) nm: 301, 248, 221 and 200.

7-(4'-Methoxyphenacyl)-1H-pyrrolo (2,3-b) pyridinium bromide (III). ^{1}H-NMR D_2O (300 MHz) δ: 8.81 (1H, dd, J=7.79, 1.06 Hz, H-6), 8.60 (1H, d, J=6.06 Hz, H-4), 7.98 (1H, d, J=8.17 Hz, H-2', 6'), 7.90 (1H, d, J=3.32 Hz, H-2), 7.69, (1H, dd, J=7.79, 6.06 Hz, H-5), 7.48 (1H, d, J=8.17 Hz, H-3', 5) 6.91 Hz, 1H, d, J=3.46 Hz, H-3), 6.54 (2H, s, N-CH_2), 4.02 (3H, s, Ar-OCH_3).

EIMS *m/z* (%): M^+ ($C_{16}H_{15}N_2O_2$), 267 (16) 208 (23), 160 (32), 135 (80), 118 (20), 107 (40) and 76 (11).

IR ν_{max} (KBr) cm^{-1} 2900, 1680, 1590 and 1450.

UV λ_{max} (MeOH) nm: 295, 258 and 203.

7-(3',4'-Dihydroxyphenacyl)- 1H-pyrrolo (2, 3-b) pyridinium chloride (IV). ^{1}H-NMR D_2O (300 MHz) δ: 8.72 (1H, d, J=7.85 Hz, H-6), 8.24 (1H, d, J=6.80 Hz, H-4), 7.70 (1H, dd, J=8.46, 2.21 Hz, H-6'), 7.67 (1H, d, J=3.65Hz, H-2), 7.62(1H, d, J=7.85, 6.80 Hz, H-5), 7.55 (1H, d, J=2.19 Hz, H-2'), 7.06 (1H, d, J=8.46 Hz, H-5'), 6.97 (1H, d, J=3.65 Hz, H-3), 6.48 (2H, s, N-CH_2), 4.83 (1H, br-s, OH), 4.65 (1H, br-s, OH).

EIMS *m/z* (%): M^{-1} ($C_{15}H_{12}N_2O_3$), 269 (2), 239 (14), 193 (3), 165 (12), 137 (15), 118 (100) and 109 (25).

IR ν_{max} (KBr) cm^{-1}: 3100, 1670, 1595 and 1290.

UV λ_{max} (MeOH) nm: 289, 227 and 208.

7-(4'-Bromophenacyl)-1H-pyrrolo (2,3-b) pyridinium bromide (V). ^{1}H-NMR D_2O (300 MHz) δ: 8.64 (1H, dd, J=7.81, 1.06 Hz, H-6), 8.35 (1H, dd, J=5.27, 1.06 Hz, H-4), 8.04 (2H, d, J=8.82 Hz, H-2',6), 7.88 (2H, d, J=8.82 Hz, H-3',5'), 7.70 (1H, d, J=3.62,H-2), 7.67 (1H, dd, J=7.81, 5.27Hz, H-5), 7.00 (1H, d, J=3.62 Hz,H-3), 5.84 (2H, s, N-CH_2).

EIMS *m/z* (%): M^{-1} ($C_{15}H_{11}BrN_2O$), 316 (8), 286 (100), 205 (15), 182 (25) and 131 (50).

IR ν_{max} (KBr) cm^{-1}: 3400, 2900, 1690, 1600, 1580 and 1450.

UV λ_{max} (MeOH) nm: 298, 261 and 200.

7-(4'-Chlorophenacyl)-1H-pyrrolo (2, 3-b) pyridinium bromide (VI). ^{1}H-NMR D_2O (300 MHz) δ: 8.76 (1H, d, J=7.85 Hz, H-6), 8.34 (1H, d, J=6.22 Hz, H-4), 8.12 (2H, d, J=8.62 Hz, H-2', 6'), 7.70 (2H, d, J=8.62 Hz, H-3',5'), 7.60 (1H, d, J=3.21 Hz, H-2), 7.54 (1H, dd, J=7.85, 6.22 Hz, H-5), 7.12 (1H, d, J=3.21 Hz, H-3), 6.02 (2H, s, N-CH_2).

EIMS *m/z* (%): M^{-1} ($C_{15}H_{11}ClN_2O$), 271 (10), 241 (100), 206 (8) and 139 (85).

IR ν_{max} (KBr) cm^{-1}: 3420, 2910, 1710 and 1680.

UV λ_{max} (MeOH) nm: 300, 252 and 202.

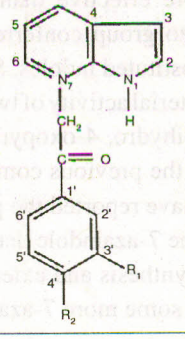
Antibacterial testing. All compounds were tested for their antibacterial activity by agar diffusion technique [8]. The overnight broth culture of bacteria in trypticase soya broth containing 10^7 cfu/ml was uniformly inoculated on the sensitest agar plates to obtain a confluent lawn. Stock solution of each compound was prepared in DMSO and 20 µl of each was applied to the sterile 6 mm filter paper disc. These were placed on the medium aseptically. Plates were incubated at 37°C for 24 hr and zones of inhibition were measured in mm.

Brine shrimp bioassay. Brine shrimp (*Artemia salina* Leach) eggs were hatched in a shallow plastic dish filled with brine solution (3.8% w/v). Unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into large compartment which was darkened while the smaller compartment was opened to ordinary light. After two days, nauplii were collected by pipette from the lighted side. Sample of the test compound was prepared by dissolving 20 mg of each compound in 2 ml of methanol. From this stock solution 500, 50 and 5 µl quantities were transfered to 9 vials, 3 for each dilution and one vial as control having 2 ml of methanol solvent, were allowed to evaporate overnight. After two days, when the shrimp larvae were ready, 1 ml of sea water was added to each vial, 10 shrimps were counted and added to each vial (30 shrimp/dilution) and volume was adjusted with each water to 5 ml per vial. After 24 hr, number of survivors was counted. Data were analyzed with Finney computer programme to determine LC_{50} [9].

Results and Discussion

All compounds were screened for their antibacterial activity against 23 Gram-ve and 12 Gram+ve bacterial cultures. Results are listed in Tables 2 and 3. Compound IV displayed

TABLE 1.



Comp.	R_1	R_2	M.P.(°C)	M.F.	M.W.	Yield(%)
I	H	CH_3	259-260	$C_{16}H_{15}BrN_2O$	331	68
II	OCH_3	H	252-253	$C_{16}H_{15}BrN_2O_2$	347	69
III	H	OCH_3	259-260	$C_{16}H_{15}BrN_2O_2$	347	72
IV	OH	OH	272-273	$C_{15}H_{13}ClN_2O_3$	304	79
V	H	Br	259-260	$C_{15}H_{12}Br_2N_2O$	396	83
VI	H	Cl	253-254	$C_{15}H_{12}BrClN_2O$	351	75

the most significant activity amongst all the tested compounds, against two Gram +ve micro organisms i.e *C. xerosis* and *B. bronchoseptica* and six Gram -ve cultures which are *S.typhi*, *S. typhi* para A, *Sh. boydii, Ent. cloacae, Prot. vulgaris* and *S. gallinarium*. The rest of the compounds did not exhibit any promising antibacterial activity. These compounds were also tested for their cytotoxicity towards *Artemia salina*. From the data recorded in Table 4, it is evident that only onederivative (VI) proved to be a significant cytotoxic agent while three others i.e. II, IV and V showed some cytotoxicity. Compounds I and III showed LC_{50}>1000 in brine shrimp bioassay.

A number of pyridinium salt derivatives have been investigated for carcinostatic activity [10]. Quaternary pyridinium salts have also been found to have bactericidal, fungicidal and herbicidal activity [11, 12]. Phenols constitute one of the oldest and most widely used classes of antimicorbial agents [13]. Although the compounds under study did not show encouraging results yet the greater antibacterial activity of compound IV than others may possibly be due to the presence of quaternary pyridinium as well as phenolic features in the molecule.

TABLE 2. PRIMARY SCREENING OF 7-AZAINDOLE DERIVATIVES AGAINST GRAM POSITIVE MICRO-ORGANISMS.

S. No.	Micro-organism	Zone of inhibition in mm for compound					
		I	II	III	IV	V	VI
1.	*C. diptheriae*	12	8	6	5	8	8
2.	*C. hoffmanii*	5	6	14	15	8	12
3.	*C. xerosis*	11	6	10	19	9	9
4.	*St. pyrogenes*	8	6	6	14	13	11
5.	*St. fecalis*	5	5	8	8	16	8
6.	*S. aureus*	5	6	7	11	11	9
7.	*S. epidermidis*	11	5	5	6	9	10
8.	*B. subtilis*	11	6	12	14	8	11
9.	*B. anthracis*	7	6	9	14	10	17
10.	*B. bronchoseptica*	12	8	10	16	17	8
11.	*List. monocytogenes*	8	5	6	9	6	8
12.	*List. ivanovii*	9	5	11	12	5	11

TABLE 3. PRIMARY SCREENING OF 7-AZAINDOLE DERIVATIVES AGAINST GRAM NEGATIVE MICRO-ORGANISMS.

S. No.	Micro-organism	Zone of inhibition in mm for compound					
		I	II	III	IV	V	VI
1.	*S. typhi*	11	9	11	20	7	8
2.	*S. typhi* para A	17	6	15	21	15	17
3.	*S. typhi* para B	12	6	12	14	8	15
4.	*S. typhimurium*	7	6	11	15	6	10
5.	*S. gallinarium*	13	6	9	16	14	9
6.	*S. pullorum*	11	8	9	13	9	9
7.	*Sh. dysenteriae*	9	5	5	9	10	9
8.	*Sh. flexneri*	10	7	9	10	9	10
9.	*Sh. sonnei*	14	6	9	10	7	11
10.	*Sh. boydii*	10	7	7	20	11	18
11.	*E. coli*	10	9	11	11	6	17
12.	*Ent. aerogenes*	6	6	7	7	7	10
13.	*Ent. cloacae*	12	10	7	22	12	8
14.	*Kl. pneumoniae*	6	15	10	12	9	10
15.	*K. ozaenae*	6	8	7	10	7	8
16.	*Ps. aeruginosa*	9	9	7	12	10	8
17.	*Vib. cholerae*	10	7	10	14	9	9
18.	*Prot. vulgaris*	8	8	8	20	10	8
19.	*Prot. mirabilis*	6	6	7	8	6	8
20.	*Ser. marcescens*	10	6	9	10	10	8
21.	*Aero-hydrophila*	10	6	8	12	10	12
22.	*Acineto. calcoaceticus*	7	5	7	12	5	11
23.	*Citro. freundii*	8	7	5	13	7	8

TABLE 4. BRINE SHRIMP BIOASSAY RESULT FOR AZAINDOLE DERIVATIVES.

Compound	LC_{50} in µg/ml
I	> 1000
II	468
III	> 1000
IV	643
V	113
VI	28

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