7-Azaindole Derivatives as Potential Antibacterial agents

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ABSTRACT:

Bacteria, the most familiar of infectious agents, cause 90 percent of hospitalized infections in developing countries, although they compete with viruses for being the most diversified. Bacteria cause most of the serious, short-term infections and can treat with the use of antibiotics. Bacterial infections are caused by the presence and growth of microorganisms that damage host tissue. The extent of infection is generally determined by how many organisms are present and the toxins they release.

The common infections caused by bacteria are urinary tract and kidney infections, strep throat, boils, typhoid, cholera, tetanus, gangrene, diphtheria, whooping cough, anthrax, Lyme disease, and most of the serious cases of dysentery, meningitis and pneumonia. Among these infections children are at high risk with dysentery, meningitis and pneumonial type of infections.

An aging population, childhood immunization and an increased number of immunocompromised patients has changed the epidemiology of bacterial infections. This new focus will drive the attention of researchers for further development of new antibacterial agents in future.

During last decade a considerable attention has been focused on azaindole analogues as antimicrobial agent. They showed significant response against a number of Gram positive and Gram negative bacteria and fungi. These compounds proved to have high, moderate and weak inhibitory effect against the tested Gram positive and Gram negative bacteria.

During the course of present work, it is decided to synthesized novel derivatives of 7-Azaindol to screen various biological activity. In this paper antibacterial , antifungal and cytotoxic activity are reported.

INTRODUCTION:

Several references have been reorted in the literature regarding the antibacterial activity of indole. Wibberly et.al 1965 therefore have prepared 2-phenyl and 2-pyridyl indoles for comparison of their activity with the corresponding isatogens. These workers have found that invitro antibacterial screening test on 2-pyridyl, 2-yl isatogens showed interesting activity.

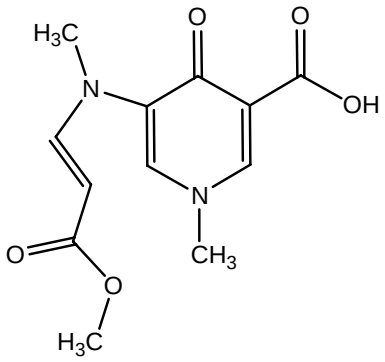
7 Azaindole derivatives were prepared and tested against various gram positive and gram negative organism.Splotter in 1995 showed that isatagens may be synthesized in certain instances from the corresponding styryl compounds, on exposure to sunlight of their solution in benzene. They demonstrated that the method was also successful for the synthesis of 2-dimethyl-amino phenyl) -3-oxo-3-H- pyrrolo-(2,3 C) pyridine 1 oxide; from 4-(4'-dimethyl amino styrryl) -3-nitropyridine and for the synthesis fo 2-(4'dimrthylamino phenyl)3-oxo[3H] – pyrrlol[3,2-b] pyridine 1 oxide.

Antibacterial screening of various compounds showed that isatogens and 3-oxo-[3H] – prrrlol-pyridine -1-oxide were all effective against gram positive organism, but only 2 phenyl isatogens and 2 –phenyl isatogens showed a broad spectrum of activity. The inactivity of 2-phenyl-2-H-indolone, and the hydrate of 2-pyridyl 2- 3H indolone, and the hydrate of 2-pyridyl-2- 3H indolone suggest that the 1-oxide group is essential for growth inhibition.

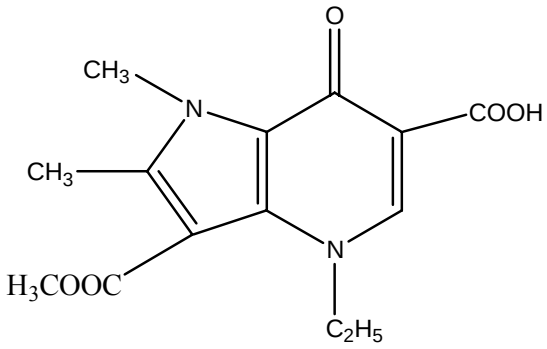
Much effort has been expended on the antimicrobial activity of azaindole derivatives[1]. They showed significant response against a number of Gram positive and Gram negative bacteria[2] and fungi [3]. The azaisatogens synthesized by Hooper et al., [4] were effective against Gram positive organisms. Also the 2-pyridyl-6-azaindoles showed a broad spectrum of antibacterial activity and were generally more effective than the analogous indoles.

Bayomi et al., [5] described the synthesis of various pyrrolo (3, 2-b) pyridine-6-carboxylic acid derivatives as potential antimicrobial agents against several Gram positive and Gram negative organisms. The results of microbial evaluation in vitro of most of the compounds exhibited moderate activity. Remarkable compounds active against *S.sonnei*

are 1, 4-dimethyl-3-carbomethoxy, 7-oxo-pyrrolo (3, 2-b) pyridine-6-carboxylic acid and 1-methyl, 4-ethyl, 3-carbomethoxy 7-oxo-pyrrolo (3, 2-b) pyridine-6-carboxy-lic acid.

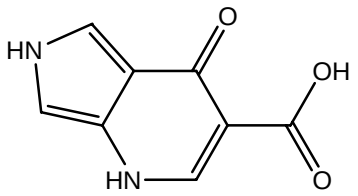


1,4-dimethyl-3-carbomethoxy,7-oxo-pyrrolo[3,2-b]pyridine-6-carboxylic acid



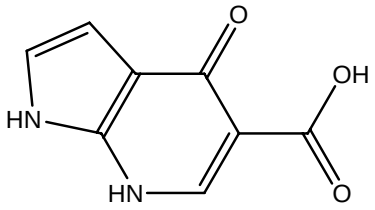
1-methyl, 4-ethyl, 3-carbomethoxy 7-oxo-pyrrolo [3, 2-b] pyridine-6-carboxylic acid

In a very next communication Bayomi et al,[6] reported the synthesis of a series of 1, 4-dihydro-4-oxo-pyrrolo (3, 4-b) pyridine-3-carboxylic acid as an extension of the interest in fused pyrrolopyridines as potential antimicrobial agent. Few compounds of this series were found to exhibit a relatively broad spectrum of activity.



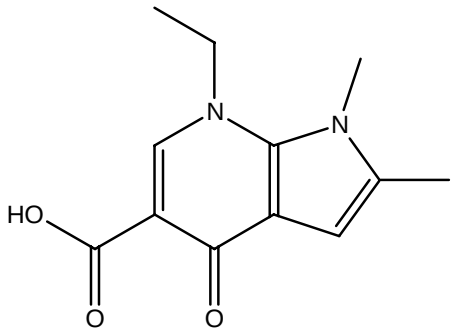
1, 4-dihydro-4-oxo-pyrrolo (3, 4-b) pyridine-3-carboxylic acid

During last decade a considerable attention has been focused on azaindole analogues as antimicrobial agent[7]. A series of four, 7-dihydro-4-oxo-7-azaindole-5-carboxylic acid has been synthesized by Toja and his co-workers [8].



7-dihydro-4-oxo-7-azaindole-5-carboxylic acid

One compound in the series of Toja et al., is 4, 7-dihydro-4-oxo-1, 2-dimethyl-7-ethyl-7azaindole-5-carboxylic acid was found most potent antibacterial agent of the compound evaluated.



4, 7-dihydro-4-oxo-1, 2-dimethyl-7-ethyl-7azaindole-5-carboxylic acid

Mohamed et al., [9] introduced some new azaindole derivatives as antimicrobial agents. These compounds proved to have high moderate and weak inhibitory effect against the tested Gram positive and Gram negative bacteria. In a similar attempt[10] some more azaindole derivatives were prepared showing satisfactory antibacterial activity.

On the basis of related biological activity which have been already reported.[11-18]

MATERIALS AND METHODS:

GENERAL METHOD OF SYNTHESIS:

Equimolar quantities of 7-azaindole and substituted phenacyl halide and/or 2-(2-Bromoethyl)-2, 5, 5-trimethyl-1, 3-dioxane were dissolved in approximately 25 ml of acetone in separate conical flasks and then mixed together in another conical flask. The reaction mixture was stirred at room temperature for 2 to 3 hrs. After completion of the reaction solid precipitates were obtained. The reaction was monitored by TLC, (solvent system of $CHCl_3$-MeOH in different proportions). The resultant compound was filtered and washed with acetone and/or mixture of acetone and ether to remove the unreacted starting materials. The crude precipitates were recrystallized several times from ethanol and/or the mixture of solvents to give the pure crystals of compound.

CONFIRMATIONAL TECHNIQUES:

Melting point:

All melting points were recorded on Gallenkamp melting point apparatus and are uncorrected.

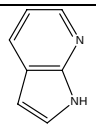
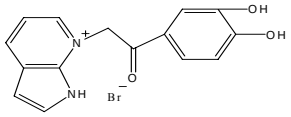
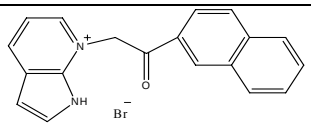
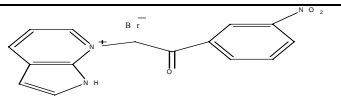
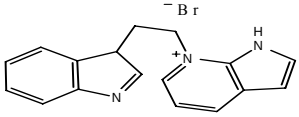
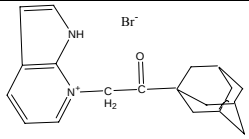
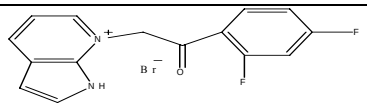
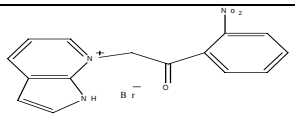
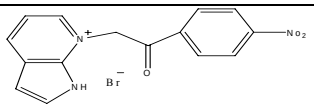
Chromatography:

Silica gel type 60 P254 of E. Merck was used for preparing TLC plates. Spots on plates were detected by Iodine using Iodine tank.

Spectroscopy:

Ultraviolet (UV) spectra were recorded in methanol on a Hitachi U-3200 spectrophotometer. Infra Red (IR) spectra were measured on a Shimadzu IR 460 spectrophotometer using KBR disc. Mass spectra (MS) were determined on Massen spectrometer MAT 311A Varian Bermen spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded in d6-DMSO on Bruker AM-300 spectrometer at 300 MHz.

Table: Structures of compound with their IUPAC name

Compound	IUPAC Name	Structures
1	1 H-pyrrolo[2,3-b]pyridine (7-Azaindol)	
2	1-[3-(3,4-dihydroxypehyl) 3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide	
3	1-[3-(2-naphthyl) 3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide	
4	1-[3-(3-nitrophenyl) 3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide	
5	1-[2-(1H-INDOL-3-yl) ethyl] 7H-pyrrolo[2,3-b]pyridine-1-ium;bromide	
6	1-[3-(1-adamantyl) 3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide	
8	1-3-[(2,4-diflorophenyl) 3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide	
9	1-[3-(2-nitrophenyl) 3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide	
10	1-[(4-nitrophenyl) 3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide	

Compound-2:
Molecular Formula: C_{15} H_{12} N_2 O_3 Br
Yield = 79%

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

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

EIMS *m/z* (relative int., %): 268 (M^+-HBr, C_{15} H_{12} N_2 O_3 , 4), 239(14), 193(3), 165(12), 137(15), 118(100) and 109(25).

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=3.65 Hz, H-2), 7.62 91H, dd, 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) and 6.48 (2H, s, H-2").

Compound-3:
Molecular Formula C_{19} H_{15} N_2 O Br = 367.23 amu.
Yield = 86.2%

UV = 340, 293, 250, 224, 203.

IR = 3755, 2923, 1687, 1361, 987, 823.5, 783, 545, 474.5

EIMS = *m/z* (relative int, %): 286 ($M^{+,}$ C_{19} H_{15} N_2 O, 7.4) 259(8.2), 258(62), 257(52), 155(15), 81(18), 77(22).

Compound-4:
Molecular Formula C_{15} H_{12} N_3 O_3 Br =362.178 amu
Yield=80%

UV = 299.6, 228, 200nm

IR = 3232, 2916, 1965, 1797, 1697, 1529, 1475, 1356, 1224.9, 1172.8, 1091.5, 930, 883, 799, 598, 519, 481.2

EIMS = *m/z* (relative int., %): 282(M^+, C_{15} H_{12} N_3 O_3, 9.8), 252(100), 236(41), 206(57), 193(6.1), 150(15.6), 131(53.5), 118(13.3), 90(6.2), 77(17.1), 63(6.6).

Compound-5:
Molecular Formula C_{17} H_{15} N_3 Br = 340.01 amu.
Yield = 75%

UV = 200, 221, 283, 289.

IR = 3437, 3375, 3217, 2917, 2074, 1837, 1616, 1462, 1360, 1297, 1102, 1005, 800.5, 726, 604, 543, 428.3

EIMS = *m/z* (relative intensity, %): 261(M^+, C_{17} H_{15} N_3, 2) 260(1), 225(1), 143(100), 142(20) 115(37), 103(5), 91(12), 82(10), 63(7).

Compound-6:
Molecular Formula C_{19} H_{23} N_2 O Br = 375.30 amu.
Yield =81.5%

UV = 299.6, 226.0

IR =3409, 2918, 2850, 2773, 1712, 1620, 1458, 1357, 1164, 1097, 887, 779, 727, 661, 536, 476.

EIMS = *m/z* (relative intensity; %): 295 (M^+, C_{19} H_{23} N_2 O, 7.3), 294(35), 237(2.6), 176(3), 159(15.5), 131(100), 93(18.4), 79(27).

Compound-7:

Molecular Formula: C_{15} H_{10} F_2 N_2 O Cl

Yield = 32.

UV λ_{max} (MeOH) nm: 301, 276 and 198.

IR υ_{max} (KBr) cm^{-1}: 3400, 2800, 1610, and 1560.

EIMS *m/z* (relative int., %): 272 (M^+-HBr, C_{15} H_{10} F_2 N_2 O , 53), 235 (6), 141(100), 132 (70), 118 (12) 113 (48) and 77(45).

H-NMR (D_2O, 300 MHz) δ: 8.79 (1H, dd, J=7.85, 1.96 Hz, H-6), 8.42 (1H, d, J=8.85 Hz, H-4), 8.21 (1H, dd, J=7.94, 5.42 Hz, H-6'), 7.96 (1H, d, J=3.40 Hz, H-2), 7.72 (1H, dd, J=8.85, 7.85 Hz, H-5), 7.41 (1H, m, H-3'), 7.32 (1H, d, J=3.40 (Hz, H-3), 7.21 (1H, dd, J=11.96, 7.94 Hz, H-5') and 6.18 (2H, s, H-2").

Compound- 8:

Molecular Formula: C_{15} H_{12} N_3 O_3 Br

Yield = 48%

UV λ_{max} (MeOH) nm: 290, 252 and 202.

IR υ_{max} (KBr) cm^{-1}: 3385, 2930, 1695, 1610 and 1340.

EIMS *m/z* (relative int., %): 282 (M^+-Br, C_{15} H_{12} N_3 O_3 , 10), 224(30), 164(10), 144 (4), 132(10) and 118(80).

H-NMR (CD_3OD, 300 MHz) δ: 9.22 (1h, DD, j=8.32, 1.21 Hz, H-6), 9.01 (1H, dd, J=6.18, 1.21 Hz, H-4) 8.96 (1H, dd, J=8.32, 6.18 Hz, H-5), 8.32 (1H, d, J=3.26 Hz, H-2), 8.32 (1H, dd, J=7.90, 1.68 Hz, H-6'), 8.31 (1H, ddd, J=8.30, 7.48, 1.68 Hz, H-4'), 7.93 91H, ddd, J=8.30, 7.90, 1.64 Hz, H-5'), 7.85 (1H, dd, J=8.30, 1.64 Hz, H-3'), 7.64 (1H, d, J=3.26 Hz, H-3) and 6.25 (2H, s, H-2").

ANTIBACTERIAL ACTIVITY

Method

Antibacterial and Antifugnal activities of all compounds were studied using disc diffusion method Disc diffusion assays were carried out using the method of Bauer et al.[19] as modified by Wilkins et al.[20] Stock solution of test compound (20,000µg/ml) was prepared by dissolving 20mg of test compound in 1ml of DMSO. Filter paper disc of about 6mm were sterilized by autoclaving at 15lb/in^2 pressure for about 30 minutes. Each disc was soaked in 10µl of the stock solution of compound extract in order to achieve a final concentration of 200µg/disc.

Sterile Petri plates were poured with about 18-20 ml of autoclaved Muller hinton agar[21]and were pre-incubated at 37 C for 18-24 hours. The test cultures were inoculated in about 4-5ml of Mueller hinton broth, incubated overnight at 37 C. Next day inoculated cultures were vortexed and a uniform lawn of culture was made on Mueller hinton agar plate after streaking sterile cotton swab in overnight broth culture. Plates were than air dried for 10-15 minutes, filter paper disc soaked in test compound solution were placed at different position of the plate. Plates were than incubated at 37 ^{0}C for 18-24 hours. Next day, the zone of inhibition around each disc was measured in millimeter.

Table 1: Results of compounds 1-6 agaist grams positive:

S.No.	Compound no.	1	2	3	4	5	6	7	8	9	10
1.	Staphlococcous . aureus AB188	28	10	7	8	-	7	-	7	-	-
2.	Staphlococcous epidermidis	-	-	9	7	-	7	-	-	-	-
3.	Methicillin Resistant Staphlococcous aureus 3	-	13	9	7	7	7	7	-	-	-
4.	Micrococcous luteus	7	20	15	20	7	11	-	11	-	-
5.	Micrococcous luteus ATCC 9341	-	21	-	-	-	13	7	7	-	-
6.	Bacillus subtilis ATCC	9	12	11	12	15	14	7	10	-	-
7.	Bacillus cereus ATCC	8	12	11	7	8	11	-	9	-	-
8.	Corynebacterium diphtheriae	8	15	9	8	15	14	-	10	-	-
9.	Corynebacterium Hofmanii	-	-	-	-	9	14	-	10	-	-
10.	Corynebacterium Xerosis	8	15	8	7	7	15	-	-	-	-
11.	Listeria monocytogene	7	-	9	7	-	7	-	-	-	-
12.	Streptococcous fecalis	7	-	8	7	7	10	-	-	-	-
13.	Microbacterium seregmotis	-	-	-	-	7	15	-	-	-	-

- = no activity

Table .2: Results of compounds 1-6 agaist grams negative

S.No.	**Compound no.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1.	Salmonella typhi ATCC	-	10	9	9	10	7	-	-	-	-
2.	Salmoinella paratyphi A	7	7	9	12	-	7	-	-	-	-
3.	Salmonella paratyphi B	-	17	10	11	-	-	-	-	-	-
4.	Shigella dysenteriae	20	11	8	8	-	7	-	-	-	-
5.	Proteus micrabilis	7	7	7	-	-	7	-	-	-	-
6.	Enterobecter sp.	7	17	7	8	7	8	-	10	-	-
7.	Escherichia coli ATCC	-	18	10	12	7	-	-	-	-	-
8.	Escherichia coli MDR	-	9	11	12	7	-	-	-	-	-
9.	Klebsiella pneumoniae	17	15	10	12	7	8	7	11	-	-
10.	Pseudomonas aerugonosa	8-	7	7	7-	-	9		-	-	-

- = no activity

Cytotoxicity Evaluation Using 3T3 Cell

Method

Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay.[22]For this purpose, 3T3(Mouse fibroblast) were cultured in Dulbecco's Modified Eagle's Medium, supplemented with 5% of foetal bovine serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 25 cm^3 flask, and kept in 5% CO_2 incubator at 37 ^oC. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of $1x10^5$ cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of compounds (1-100 µM). After 72 h, 50 µL MTT (2 mg/mL) was added to each well and incubated further for 4 hrs.Subsequently, 100µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a microplate ELISA reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition for 3T3 cells.

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Result and Discussion

7 Azaindole 1 and its substituted phenacyl derivatives 2- 9 were evaluated for their invitro antibacterial activity against 13 gram positive and 10 gram negative bacterial culture. The results are depicted in table1and 2. These compounds showed varied degree of activity. The parent compound showed negligible antibacterial activity against gram positive bacteria comparable to its derivatives except staphlococcous Aureus AB 188 . While compound 1 showed significant activity againt gram negative bacteria (shigella dysenteriae and Klebssiella pneumoniae).

Results indicated that compound 2-6 showed significant activity against different gram positive strains.while 7-9 showed weak activity. Compounds 2-4 exhibited significant activity against gram negative strains however; compounds 5-9 demonstrate no activity against the same strain.

Regarding the SAR all compounds have phenyl substitution having different group at phenyl ring. Compound 4, 8 and 9 are nitro derivative only the difference is in the position of methoxy group at phenyl ring. Compound 8 and 9 are devoid of any activity while compound 4 showed inhibitory activity against Micrococus leuteus and Bacillus subtilis ATCC in gram positive bacteria while it exhibited activity against Salmonella paratyphi A and B, E.coli ATCC, E.coli MDR, klebeiella pneumonia in gram negative bateria.

The antibacterial activity of 7 Azaindole derivatives may be attributed to mode of action having bacteristatic nature as due to its structural form; it can therefore with the synthesis of folic acid.

Compound were also screened for their cytotoxic activity on a fibroblast cell line with significant antibacterial and antifungal activity it is encouraging to observe that these compounds did not exhibited toxicity against fibroblast cells. The IC50 values are presented in table 3

References:

1. Takuo, K. and Hiroshi, Z., **(1975).**Jpn. Kokai **75**, 129, 751 (Cl. A61K), 14 Oct. 3 pp.

2. Uwe, P., Andreas, K., Thomas, S., Klaus, G., Dieter, B.K., Rainer, E., Georg, M.K. and Joachim Z.H., **(1992).** Eur. Pat. Appl**.** EP 520, 277 (CL.C07D471/04), 30 Dec. 28 pp.

3. Satoshi, M., Shinobu, I., Mitsuo, K. and Yoshiki, O., **(1992).**Bull. Chem. Soc. Jpn. **65**, 2992

4. Hooper, M., Patterson, D.A. and Wibberley, D.G., **(1965)** J. Pharm. Pharmacol**. 17**, 734

5. Bayomi, S.M., Price, K.E. and Sowell, J. W**., (1985) J**. Heterocyclic Chem**. 22**, 83
6. Bayomi, S.M., Price, K.E. and Sowell, J. W., **(1985)** J. Heterocyclic Chem**. 22**, 729

7. Mohammed, A.G., Diss**. (1987).** Abstr. Int*. B.* **47**, 4477

8. Toja, E., Tarzia, G., Ferrari, P. and Tuan, G., **(1986).** J. Heterocyclic Chem**.** 23, 1555

9. Mohamed, T.A., (1992) Bull. Fac. Sci. Assiut Univ**. 21**, 69
10. Mohamed, T.A., **(1992)** J. Chem. Technol. Biotechnol**. 55,** 239
11. Nausheen Mushtaq, Fozia Noor, Shamoona Takween, Shamim Akhter, Muhammad Arif, and Khalid. M. Khan, **(2008)** Pak. J.Pharm. Sci., **21(**1), pp.36-39.

12. S.A. Saecd, A. Khan, Z. S. Saify & S. M. Haider. . **(1997).** Proceedings of second International Conference on Pharmaceutical Sciences, Karachi,
13. S. M. Haider, M. Saeed, mansoor Ahrnad, Abdullah Khan, A. Yasmeen, T. Parveen & D. J. Haleem, Proceedings of 1" ISBBP symposium on Biochemistry and Biophysics, **1994.**
14. S. M. Haider, Mansoor Ahmed, M. Saeed, A. Khan and B. S. Siddiqui, **(1994).**Pakistan Journal of scientific and Industrial research, **37**(10),
15. Z.S Saify, *et al,* **(1984).** J. of Pharmacy, **3** (1),
16. Z.S.Saify et. al. (1986).Pak. Journal of Pharmacology, 2, 43-46,

17. Z.S Saify, 141**, (1973).**Ann. Se. Conf.,

18. Synthesis of some pyrrolopyridine and related compounds of potential biological interest. Ph.D. Thesis. Z.S.Saify **(1971)** King's College University of London.

19. Bauer AW, Kirby WMM & Sherries T. **(1996)** *American Journal of clinical pathology* **45**,493

20. Wilkins P. J., Grey P. C., Dreosti I. E**. (1972)** British Journal of Nutrition **27**,113-120

21. Mueller JH & Hinton J. **(1941).** *Procedings of society of exp Biology and Medicine* **48**, 330-333.
22. Mosmann, T.(**1983)** *J. Immunol. Methods*, **65,** 55-63.