SYNTHESIS AND PHARMACOLOGICAL ACTIVITIES OF 7-AZAINDOLE DERIVATIVES

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ABSTRACT

As a part of our program to discover novel analogues of 7-azaindole (1H-Pyrrolo[2,3-b] pyridine) having useful biological activities, some derivatives have been synthesized and evaluated for their analgesic and hypotensive activity. Compounds evaluated by thermal stimuli (tail immersion method) at the dose of 50 mg/kg of body weight revealed significant analgesic activity. Pethidine was used as reference drug. Same compounds tested at the dose of 75mg/kg of body weight showed toxicity. Compounds tested for their effect on blood pressure in normotensive rat produced slight fall in blood pressure.

Keywords: 7-azaindole, analgesic, hypotensive activity, synthesis.

INTRODUCTION

The 7-azaindole nucleus has proven to be an interesting and important model for working on its own synthesis and synthesizing its different analogues for number of purposes (Hugon *et al*., 2007; Marminon *et al*., 2003; Kelly *et al*., 1997 and Anthony, 1972). In past Several decades indole derivatives have attracted the interest of scientists for their potential biological activities (Song *et al*., 2007; Lebouvier *et al*., 2007; Guillard *et al*., 2007 and Trejo *et al*., 2003). Different acylated azaindole compounds have been reported for analgesic and anti-inflammatory activities (Viaud Marie-Claude *et al*., 1977). Several other indole derivatives have been prepared as cyclooxygenase inhibitors and were found useful as anti-inflammatory agents (Shen, *et al*., 1963 and Matsuoka *et al*., 1996). One indole derivative designated as indomethacin has demonstrated a high degree of anti-inflammatory and analgesic activity. The azaindole derivatives were also found to possess blood pressure lowering activity and acting as effective coronary vasodilator, cardiovascular and potent hypotensive agent.

Synthesis and investigation of azaindole derivatives for different pharmacological and biological effects have been an important subject of our research (Saify 1982; Yasmin *et al*., 1984 and Saify *et al*., 1994).

EXPERIMENTAL

General

Reactions were monitored by TLC using pre-coated silica gel, GF-254 and were visualized under ultraviolet light at 254 nm and 366 nm on HP-UVIS Desaga (Heidelberg). Iodine vapors were also employed for the detection of spots. All melting points were recorded on Gallenkamp melting point apparatus and are uncorrected. Anhydrous calcium sulphate from E. Merck was used for drying reaction product. Ultraviolet (UV) spectra were recorded in methanol/DMSO 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 Varian Massen spectrometer MAT 311A spectrometer. Nuclear magnetic resonance (^{1}HNMR) spectra were recorded in DMSO-d_6 on Bruker AM-300 spectrometer operating at 300 MHz.

Analgesic Activity

Antinociceptive effect against thermal stimuli (tail flick method) was performed according to the method of di-Stasi *et al* (Luiz *et al*., 1988). Male Albino mice weighing between 20-30g, were used in the study. Animals were kept individually in plastic cages in the same environmental conditions with free access to water and standard rodent diet for about three days before the experiment. Test Compounds were dissolved in water for injection/DMSO (30%), injected intraperitoneally to the test groups at the doses of 50 mg/kg body weight. Pethidine HCl was also administered at the same dose to the standard group. Control groups were supplied with only vehicle. Readings were taken at 30, 60, 90, 120, 150 and 180 minutes after administration of compounds, mean of the three readings was considered as the post drug reaction time. Tail flick latency difference (TFLD) or mean increase in latency after the administration of the compound was used to measure the analgesia produced by test and standard drugs.

Effect on blood pressure

Male Wistar rats weighing 200-250g were anesthetized with an intraperitoneal injection of sodium pentothal (70-

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90mg/kg body weight). The compounds were administered intravenously.

General method of preparation of compounds

A solution of reactant (0.5mmole) was added to a stirred solution of 1H-Pyrrolo (2,3-b) pyridine (I) (0.5mmole) in acetone(45-55ml). Reaction mixture stirred for 4-8 hours followed by refluxing. Process of reaction was monitored through thin layer chromatography. The crude solid product was filtered and washed with acetone. The product thus obtained was purified through recrystallization by using methanol and ether. The pure compound was dried in desiccator over anhydrous calcium sulphate. Melting point was recorded and spectral data obtained to confirm the structure of compound.

7-(2-(2,5,5-trimethyl-1,3-dioxan-2-yl)ethyl)-1H-pyrrolo-(2,3-b)-pyridinium bromide (II): White needle shaped crystals (74%) mp 170 $\pm$ 2^0C. ^{1}H-NMR (d_6 -DMSO, 300 MHz): δ 1.89 (s, 3H, C*H*$_3$-7'), 2.48-2.57 (m, 8H, *H*-9, C*H*$_3$-8', C*H*$_3$-9'), 3.31 (s, 4H, *H*-4'a, *H*-4'b, *H*-6'a, *H*-6'b), 4.76-4.95 (m, 2H, *H*-8), 6.99 (dd, 2H, $J_{2,3}$ = 2.6 Hz, *H*-2, *H*-3), 7.63-7.68 (m, 1H, *H*-5), 8.15 (d, 1H, *J* = 3.3 Hz, *H*-4), 8.66-8.72 (m, 1H, *H*-6). EIMS m/z: 275, (M^+-Br, $C_{16}H_{23}N_2O_2$) 188, 131, 118, 90, 71, 55. IR ν_{max} (KBr) cm^{-1}: 3200, 1510, 1340, 1130, 799.UV λ_{max} (MeOH) nm: 293, 226, 200.

7-(3-Nitrophenacyl)-1H-pyrrolo-(2,3-b)-pyridinium bromide (III) :White needle shaped crystals (77%) mp 224 $\pm$ 2^0C. ^{1}H-NMR (d_6-DMSO, 300 MHz): δ 3.32 (s, 2H, *H*-8), 7.02 (d, 1H, $J_{1,2}$ = 2.6 Hz, *H*-2), 7.03 (d, 1H, $J_{2,1}$ = 2.6 Hz, *H*-3), 7.74-7.79 (m, 1H, *H*-4), 7.99 (dd, 2H, $J_{4,5}$ = 5.5, $J_{5,6}$ = 5.6 Hz, *H*-5, *H*-6), 8.61-8.67 (m, 2H, *H*-5',*H*-6'), 8.78-8.79 (m, 1H, *H*-4'), 8.88-8.89 (m, 1H, *H*-2'), 13.3 (s, 1H, N*H*). EIMS m/z: 282 (M^+-Br, $C_{15}H_{12}N_3O_3$) 280, 252, 206, 194, 150, 104 64. IR ν_{max} (KBr) cm^{-1}: 3100, 2790, 1690, 1515, 1350, 730.UV λ_{max} (MeOH) nm: 297, 227, 198.

RESULTS AND DISCUSSION

Results of analgesic activity are represented in table 1. The parent compound (I) investigated for analgesic activity showed insignificant activity (Haider, 1995). Compound II showed highly significant ($p<0.01$) effect at 30 minutes which lasted for 3 hours, producing the peak effect at 150 minutes. This compound exhibited greater analgesic activity in comparison with pethidine with higher TFLD values at 30, 90, 120, 150 and 180 minutes except at 60 minutes.

The same derivative (II) when tested at the dose of 75 mg/kg produced toxicity by showing 30% mortality.

Nitro-phenacyl (meta-nitro) derivative (III) tested at the dose of 50mg/kg and showed remarkable effect ($p<0.01$) after 60 minutes of administration, The compound showed the peak effect from 120 to 150 minutes. Analgesia produced by this compound is comparable with pethidine at 90 and 120 minutes and showed greater TFLD value at 150 minutes. This compound produced tremors and convulsions and showed 50% mortality when

Table 1: Analgesia produced by 7-(2-(2,5,5-trimethyl-1,3-dioxan-2-yl)ethyl)-1H-pyrrolo-(2,3-b)-pyridinium bromide (II) and 7-(3-Nitrophenacyl)-1H-pyrrolo-(2,3-b)-pyridinium bromide (III) against thermal stimuli (tail immersion method)

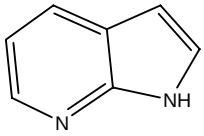
Treatment (IM)	Dose mg/kg	Mean increase in latency + SEM (s)					
		30 min	60 min	90 min	120 min	150 min	180 min
Control (II)	—	0.55 ± 0.10	0.66 ± 0.10	0.42 ± 0.14	0.36 ± 0.15	0.38 ± 0.14	0.37 ± 0.08
Compound II	50	2.27**±0.19	2.67**± 0.28	2.85**± 0.34	3.17**± 0.48	3.37**± 0.43	1.64**± 0.28
Control (III)	—	0.31 ± 0.04	0.32 ± 0.03	0.39 ± 0.08	0.36 ± 0.09	0.31 ± 0.05	0.36 ± 0.07
Compound (III)	50	1.02 ± 0.34	1.25**± 0.17	1.94** ± 0.12	2.15** ± 0.10	2.15**± 0.05	0.56 ± 0.18
Pethidine	50	2.26*±0.63	3.52** ± 0.46	2.82** ± 0.13	2.57** ± 0.23	1.92** ± 0.07	1.57** ± 0.20

Significant differences by student "t" test *$p<0.05$, **$p<0.01$as compared to control n=5

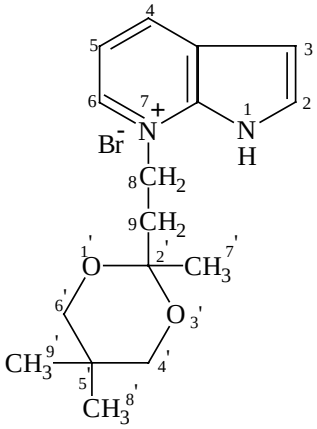
Table 2: Effect of 7-(2-(2,5,5-trimethyl-1,3-dioxan-2-yl)ethyl)-1H-pyrrolo-(2,3-b)-pyridinium bromide (II) and 7-(3-Nitrophenacyl)-1H-pyrrolo-(2,3-b)-pyridinium bromide (III) on blood pressure in normotensive rat

Compound No.	Dose mg/kg	Response	% decrease in systolic/diastolic pressure
II	10	Hypotensive	13/14
III	10	Hypotensive	9/10

tested at the dose of 75mg/kg.



(I)



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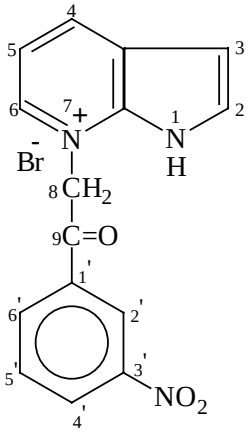

(III)

Considering the promising results of analgesic activity at the dose of 50 mg/kg and toxicity at the dose of 75mg/kg body weight further investigations are needed to evaluate the therapeutic index of these compounds.

The parent compound tested for its effect on blood pressure was found hypotensive (Haider, 1995). In present study both the compounds were evaluated for blood pressure lowering activity in normotensive anesthetized rat. Administration of 10mg/kg elicited a slight decrease in blood pressure. The hypotensive response was reversible and completely returned to pretreatment level after a minute.

Encouraging analgesic and hypotensive results showed the potential of the compounds to be further evaluated for the same as well other activities at different doses and using different experimental models.

REFERENCES

Anthony J (1972). Verbiscar Synthesis of 1-p-chlorobenzyl-7-azaindole-3-.alpha.-piperidylmethanol as a potential antimalarial agent. *J. Med. Chem.*, **15**(2): 149-152.

Guillard J, Decrop M, Gallay N, Espanel C, Boissier E, Herault O and Viaud-Massuard MC (2007). Synthesis and biological evaluation of 7-azaindole derivatives, synthetic cytokinin analogues. *Bioorg Med Chem Lett.*, **17**(7): 1934-1937.

Haider SM (1995). Synthesis of some 7-azaindole and nicotinamide derivatives having potential therapeutic activity", Ph.D. thesis, University of Karachi, p.50.

Hugon B, Anizon F, Bailly C, Golsteyn RM, Pierré A, Léonce S, Hickman J, Pfeiffer B, Prudhomme M (2007). Synthesis and biological activities of isogranulatimide analogues. *Bioorg Med Chem.*, **15**(17): 5965-5980.

Kelly TA, McNeil DW, Rose JM, David E, Shih CK and Grob PM (1997). Novel Non-Nucleoside Inhibitors of Human Immunodeficiency Virus Type 1 Reverse Transcriptase. 6. 2-Indol-3-yl- and 2-Azaindol-3-yl-dipyridodiazepinones. *J. Med. Chem.*, **40**(15): 2430-2433.

Lebouvier N, Pagniez F, Duflos M, LePape P, Na YM, Le Baut G and Le Borgne M (2007). Synthesis and antifungal activities of new fluconazole analogues with azaheterocycle moiety. *Bioorg Med Chem Lett.*, **17**(13): 3686-3689.

Luiz C Distasi, Mirtes Costa, Sigrid LJ, Medacolli, Mizue Kirizawa, Cecillia G Gomes and Gustaf Trolin (1988). Screening in mice of some medicinal plants used for analgesic purposes in the state of Sao Paulo. *J. Ethnopharmacol.*, **24**: 205.

Marminon C, Pierre A, Pfeiffer B, Perez V, Leonce S, Joubert A, Bailly C, Renard P, Hickman J and Prudhomme M (2003). Syntheses and Antiproliferative Activities of 7-Azarebeccamycin Analogues Bearing One 7-Azaindole Moiety. *J. Med. Chem.*, **46**(4): 609-622.

Matsuoka, Hiroharu, Maruyama, Noriaki, PCT (1996). *Int. Appl.* W097 30,030 (Cl. C07 D209/08), p.79.

Saify ZS (1982). Synthesis of new carbonic anhydrase inhibitor. *Pak. J. Pharm. Univ. Kar.*, **1**(1): 83-88.

Saify ZS, Haider SM, Ahmed M, Saeed M, Khan A and Siddiqui BS (1994). Synthesis of some 7-azaindole derivatives; their cytotoxicity and antibacterial activity. *Pak. J. Sci. & Indust. Res.*, **37**(10): 439-441.

Shen TY, Ellis RL, Windholz TB, Matzuk AR, Rosegay A, Lucas S, Holly FW and Wilson AN (1963). Non steroid anti-inflammatory agents. *J. Am. Chem. Soc.*, **85**: 488.

Song JJ, Reeves JT, Gallou F, Tan Z, Yee NK and Senanayake CH (2007). Organometallic methods for the synthesis and functionalization of azaindoles. *Chem. Soc. Rev.*, **36**(7): 1120-1132.

Trejo A, Arzeno H, Browner M, Chanda S, Cheng S, Comer DD, Dalrymple SA, Dunten P, Lafargue J, Lovejoy B, Freire-Moar J, Lim J, Mcintosh J, Miller J, Papp E, Reuter D, Roberts R, Sanpablo F, Saunders J, Song K, Villasenor A, Warren SD, Welch M, Weller P, Whiteley PE, Zeng L and Goldstein DM (2003). Design and Synthesis of 4-Azaindoles as Inhibitors of p38 MAP. *Kinase. J. Med. Chem*., **46**(22): 4702-4713.

Viaud Marie-Claude, Jamoneau Patricia and Baudin Marie-Laure (1997). Acylation of oxazolo[4,5-b]pyridine-2(3H)-ones, 2-phenyloxazolo[4,5-b]pyridines and pyrrolo-[2,3-b]pyridine-2(2H)-ones. *Tetrahedron*, **53**: 5159**.**

Yasmine Q and Saify ZS (1984). Synthesis of some 7-azaindole derivatives of potential therapeutic interest. *J. Pharm. Univ. Kar*., **3**(1): 27-29.