ANTI-NOCICEPTIVE AND ANTIMICROBIAL EVALUATION OF MANNICH BASES OF 1*H*-INDOLES

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ABSTRACT: A series of several novel mannich bases of indoles (1A-L) have been synthesized from indole by reacting with respective aldehyde and the various compounds containing secondary amine. The synthesized compounds were characterized by IR and ^{1}HNMR spectral analysis. All the synthesized compounds were evaluated for their anti-nociceptive activity in mice by Eddy's hot plate method. The reaction time was compared with standard drug pentazocine. *In vitro* antimicrobial activity of the synthesized compounds were screened against the Gram positive microorganisms such as *Staphylococcus aureus*, *Streptococcus pyogenes,* Gram negative microorganisms such as *Escherchia coli*, *Klebsiella aerogenes* and fungus *Candida albicans* by cup plate method. The zone of inhibition of the synthesized compounds was compared with the standard drug amikacin and ketoconazole respectively. All the synthesized compounds exhibited significant anti-nociceptive activity and showed moderate antimicrobial activity.

Key words: Mannich base, Indole, anti-nociceptive, antibacterial, antifungal

1. INTRODUCTION

The field of medicinal chemistry has evolved from an emphasis on the synthesis, isolation and characterization of drugs to an increased awareness of the biochemistry of diseases states and the design of drugs for the prevention of diseases. An important aspects of medicinal chemistry has been a great deal of success in understanding relationship with chemical structure and its biological activity. The derivatives of heterocyclic compounds have their own importance due to the good biological activities. Among the wide variety of heterocycles, the compounds bearing nitrogen atom as hetero atom have played an important role in medicinal chemistry.

A benzopyrrole, Indole is one of the heterocyclic compounds containing pyrrole, a five member unsaturated ring composed of one nitrogen atom fused with benzene ring. Indole moiety is a versatile lead molecule in pharmaceutical development and has wide range of biological activities such as analgesic (Kamal, et al., 1988), anti-inflammatory (Srivastava, et al., 1993), anti-psychotic (Ashok, et al., 2003), anticonvulsant (Lai, et al., 2005), antileukemic (Pal, et al., 2001), antiproliferative (Michelle, et al., 2002), anti-microbial (Gadginamath, et al., 1999), antifungal (Nizamuddin, et al., 1999), anti-HIV (Romano, et al., 2005), cytotoxic (Calderon, et al., 2003) activities. Pyrrole undergoes aromatic electrophllic substitution at C-2, but the benzopyrrole forms mainly C-3 substituted products. It is due to the fact that the transition state of C-3 substituted indole is more stable due to benzenoid structure whereas in the transition state of C-2 substituted product in benzenoid system is disrupted. Mannich bases of Indole derivatives by the reaction with different aldehydes and amines were not reported in the literature. All these observations and essential role of the substituted indole derivatives prompted us to synthesize novel indole derivatives by mannich reaction at C-3 position and to evaluate their anti-nociceptive and antimicrobial activities.

2. EXPERIMENTAL

2.1. Materials and method

Melting points were determined by open-ended capillary tube on the electrical melting point apparatus and were uncorrected. The purity of the compounds were checked by TLC using Silica Gel as stationary phase and chloroform-methanol (9:1) as eluent and the spots were visually detected in an Iodine chamber. The structure of the synthesized compounds was elucidated by IR spectra in υ_{max} (cm^{-1}) on FT-IR (Shimadzu-8400 series) using KBr disc technique and ^1H NMR spectra in δ units (ppm) relative to an internal standard of tetramethylsilane on ^1H NMR (Brucker 400 MHz) in DMSO-d_6. Elemental analysis determinations for final compounds were performed on Carlo Erba 108 and the analyses.

2.2. Procedure for the synthesis of novel mannich base of indoles

Equimolar quantities (0.01 mol) of indole and the respective primary amine or secondary amine were dissolved in methanol (30 mL) in a beaker under perfect ice-cold condition and stirred constantly. To this solution the respective aldehyde (0.01mol) was added slowly and stirred for 4-6 h under ice-cold condition. The content was kept overnight in the freezer (Rajamanickam, et al., 2006). The corresponding crystals of novel mannich base of indoles obtained were recrystallized from alcohol. The physical data and spectral data were recorded in Table 1 and Table 2 respectively.

Table1. Physical data of newly synthesized indole derivatives

Comp. code	Molecular formula	M.W.	m.p. (^{0}C)	R_f value	% yield	Elemental analysis % calculated (% found)			
						C	H	N	O
1A	$C_{17}H_{16}N_2O$	264	82	0.8213	69.40	77.25 (77.22)	6.10 (6.08)	10.60 (10.58)	6.05 (6.02)
1B	$C_{22}H_{20}N_2$	312	97	0.9012	62.66	84.58 (84.56)	6.45 (6.44)	8.97 (8.96)	-
1C	$C_{17}H_{16}N_2O_2$	280	77	0.7353	70.18	72.84 (72.82)	5.75 (5.74)	9.99 (9.98)	11.42 (11.40)
1D	$C_{25}H_{24}N_2O_2$	384	119	0.6944	64.20	78.10 (78.08)	6.29 (6.26)	7.29 (7.28)	8.32 (8.30)
1E	$C_{22}H_{18}N_2O$	326	110	0.7683	62.80	80.96 (80.94)	5.56 (5.55)	8.58 (8.56)	4.90 (4.88)
1F	$C_{27}H_{22}N_2$	374	137	0.9142	65.78	86.60 (86.58)	5.92 (5.90)	7.48 (7.46)	-
1G	$C_{22}H_{18}N_2O_2$	342	125	0.8498	61.44	77.17 (77.16)	5.30 (5.29)	8.18 (8.16)	9.35 (9.34)
1H	$C_{30}H_{26}N_2O_2$	446	131	0.8768	63.36	80.69 (80.68)	5.87 (5.86)	6.27 (6.26)	7.17 (7.16)
1I	$C_{24}H_{23}N_3O$	369	85	0.7021	72.30	78.02 (78.04)	6.27 (6.28)	11.37 (11.38)	4.33 (4.34)
IJ	$C_{29}H_{27}N_3$	417	122	0.8363	65.25	83.42 (83.28)	6.52 (6.50)	10.06 (10.02)	-
1K	$C_{24}H_{23}N_3O_2$	385	107	0.8612	71.25	74.78 (74.72)	6.01 (5.98)	10.90 (10.88)	8.30 (8.28)
1L	$C_{32}H_{31}N_3O_2$	489	141	0.7294	66.76	78.50 (78.46)	6.38 (6.35)	8.58 (8.56)	6.54 (6.52)

Table 2. Spectral data of novel indole derivatives

Comp. code	IR (KBr disc) υ_{max} (cm^{-1})	^1H NMR (DMSO-d_6) δ (ppm)
1A	3384.46 (NH), 3310.22 (NH amide), 3076.44 (Ar.CH), 2810.12 (CH), 1646.22 (C=O), 1456 (CH_3), 1265 (C-N)	1.52 (s, CH_3), 4.74 (d, CH), 6.68-7.96 (m, Ar.CH), 8.2 (d, NH amide), 10.24 (brs, NH)
1B	3442.62 (NH), 2816.24 (CH), 1464.26 (CH_3), 1272.55 (C-N)	1.46 (s, CH_3), 4.16 (d, CH), 6.78-7.64 (m, Ar.CH), 10.16 (brs, NH)
1C	3490.66 (OH), 3346.27 (NH), 3083 (Ar.CH), 2828.42 (CH), 1705 (C=O), 1451.33 (CH_3), 1266.24 (C-N)	1.54 (s, CH_3), 4.28 (d, CH), 6.76-7.92 (m, Ar.CH), 10.08 (brs, NH), 11.4 (brs, OH)
1D	3521.98 (OH), 3328.26 (NH), 3092.42 (Ar.CH), 1690.44 (C=O), 1456.24 (CH_3), 1278.42 (C-N)	1.62 (s, CH_3), 4.02 (d, CH), 6.44-7.76 (m, Ar.CH), 10.22 (brs, NH), 11.36 (brs, OH)
1E	3368.45 (NH), 3308.66 (NH amide), 3056 (Ar.CH), 2809.13(CH) 1647.12 (C=O), 1267.52 (C-N)	5.92 (s, CH), 6.72-8.22 (m, Ar.CH), 8.48 (d, NH amide), 10.16 (brs, NH)
1F	3413.56 (NH), 2832.44 (CH), 1268.26 (C-N)	5.24 (s, CH), 6.66-7.88 (m, Ar.CH), 10.02 (brs, NH)
1G	3504.82 (OH), 3346.26 (NH), 3078.34 (Ar.CH), 2828.42 (CH), 1706.12 (C=O), 1265.56 (C-N)	4.06 (s, NH), 5.52 (s, CH), 6.74-7.96 (m, Ar.CH), 10.08 (brs, NH), 11.12 (brs, OH)
1H	3376.64 (NH), 3068 (Ar.CH), 2808.66 (CH), 1652.96 (C=O), 1452.98 (CH_3), 1262 (C-N)	2.32 (s, CH_3), 5.18 (s, CH), 6.28-8.12 (m, Ar.CH), 10.14 (brs, NH)
1I	3367.48 (NH), 3328.84 (NH amide), 3064.68(Ar.CH), 2820 (CH) , 1700.13(C=O), 1444.36(CH_3), 1264.24 (C-N)	2.68 (s, CH_3), 6.16 (s, CH), 6.76-8.28 (m, Ar.CH), 8.12 (d, NH amide), 10.22 (brs, NH)
1J	3399.30 (NH), 3056 (Ar.CH), 2822.63 (CH), 1456.34(CH_3), 1259.96 (C-N)	2.46 (s, CH_3), 5.24 (s, CH), 6.58-7.82 (m, Ar.CH), 10.62 (brs, NH)
1K	3508.32 (OH), 3364.59 (NH), 3050.21 (Ar.CH), 2980.92 (CH), 1700.13 (C=O), 1455.16 (CH_3), 1299.46 (C=N)	2.28 (s, CH_3), 4.12 (s, NH), 5.26 (s, CH), 6.58-8.12 (m, Ar.CH), 10.28 (brs, NH), 10.98 (brs, OH)
1L	3392.94 (NH), 3086.22 (Ar.CH), 2819.28 (CH), 1666.36 (C=O), 1456.82 (CH_3), 1261.16 (C-N)	2.56 (s, CH_3), 2.92 (s, CH_3), 5.18 (s, CH), 6.32-7.76 (m, Ar.CH), 10.46 (brs, NH), 11.2 (brs, OH)

2.3. Animals

Adult albino mice of either sex (20 – 30 g) were used for this study. All the animals were fed with standard pellets and water was provided *ad libitum* and maintained under standard laboratory conditions. The animal study was approved by institutional animal ethical committee of Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, India (AKCP/CPCSEA/509/F(1a)/2007).

2.4. Anti-nociceptive activity

All newly synthesized compounds were screened for anti-nociceptive activity by Eddy's Hot Plate Method (Rajamanickam, et al., 2008). The animals were divided into 12 groups of 6 mice each. Group 1 served as control and received vehicle (2% v/v Tween 80) where as group 2 was treated with standard drug pentazocine (5 mg/mL, i.p.). The rest of the groups were treated with newly synthesized indole derivatives (10 mg/mL, i.p.). The increase in the basal reaction time was noted on Eddy's hot plate before and after treatment of standard drug and synthesized compounds at 30, 60, 120 and 240 min. All the results (Mean ± SEM) were statistically analyzed by students " *t* " test and tabulated in Table 3.

Table 3. Anti-nociceptive activity of newly synthesized indole derivatives

Treatment	Basel Reaction Time (sec) (Mean ± SEM) before Treatment	Reaction Time (sec) after administration (Mean ± SEM)			
		30 min	60 min	120 min	240 min
Control	3.33 ± 0.26	3.37 ± 0.22	3.24 ± 0.28	3.46 ± 0.32	3.42 ± 0.62
Standard	3.81 ± 0.28	10.25 ± 0.44**	12.85 ± 0.42**	13.20 ± 0.24**	14.92 ± 0.68**
1A	3.14 ± 0.48	4.92 ± 0.34	5.94 ± 0.38*	8.94 ± 0.32**	9.74 ± 0.58**
1B	3.36 ± 0.42	5.42 ± 0.28*	7.22 ± 0.64**	9.42 ± 0.56**	10.72 ± 0.86**
1C	3.68 ± 0.42	6.66 ± 0.20**	6.74 ± 0.20**	7.95 ± 0.38**	7.50 ± 0.42**
1D	3.52 ± 0.44	7.94 ± 0.32**	12.94 ± 0.52**	14.64 ± 0.22**	14.24 ± 0.84**
1E	2.03 ± 0.76	4.94 ± 0.28	5.96 ± 0.32*	7.95 ± 0.32**	8.76 ± 0.14**
1F	3.16 ± 0.14	4.98 ± 0.22	6.78 ± 0.46**	9.16 ± 0.64**	9.86 ± 0.64**
1G	3.22 ± 0.18	5.28 ± 0.84*	7.12 ± 0.28**	8.66 ± 0.42**	9.92 ± 0.78**
1H	3.14 ± 0.38	6.12 ± 0.42**	7.66 ± 0.72**	9.28 ± 0.46**	11.26 ± 0.94**
1I	3.49 ± 0.38	5.75 ± 0.26*	5.95 ± 0.72*	7.32 ± 0.82**	7.94 ± 0.20**
IJ	3.89 ± 0.42	4.96 ± 0.72	5.39 ± 0.42*	6.72 ± 0.26**	6. 98 ± 0.48**
1K	3.83 ± 0.34	4.84 ± 0.62	5.42 ± 0.56*	6.54 ± 0.38**	6.84 ± 0.32**
1L	3.34 ± 0.42	6.74 ± 0.26**	11.28 ± 0.36**	12.88 ± 0.76**	13.96 ± 0.66**

**$P < 0.01$ and *$P < 0.05$ statistically (Mean ± SEM) significant from control group (n=6)

2.5. Test microorganisms

Gram positive organisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, Gram negative organisms such as *Escherchia coli*, *Klebsiella aerogenes* and fungus *Candida albicans* were used for this study. All the bacterial cultures were obtained from microbiology lab, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamilnadu, India.

2.6. Antibacterial activity

The antibacterial activity was performed by cup-plate method (Anbu, et al., 2008). All the synthesized compounds were dissolved in 2 % v/v Tween 80 at a concentration of 10 mcg/mL. The respective bacterial culture was spread (swabbed) into the nutrient agar plates for uniform distribution of colonies. Using a sterile cork borer, 8 mm wide well was made on each agar plates. All the synthesized compounds (10 mcg/mL) were poured into each wells using a sterile micropipette and Amikacin (10 mcg/mL) were used as standard. The plates were incubated for 24 h at 37 ^{o}C. After incubation, the zone of inhibition was measured and the values were tabulated in Table 4. All the experiments were done in triplicate.

Table 4. Anti-bacterial and Antifungal activities of synthesized indole derivatives

Compound code	Diameter of Zone of inhibition (mm)				
	S. aureus	*S. pyogenes*	*E. coli*	*K. aerogenes*	*C. albicans*
1A	18	10	22	11	16
1B	16	12	20	20	11
1C	19	9	18	10	12
1D	9	14	16	18	15
1E	26	9	18	19	14
1F	10	10	9	10	8
1G	18	12	15	18	16
1H	20	18	16	17	15
1I	9	12	9	9	12
IJ	16	20	21	18	18
1K	10	17	9	11	9
1L	21	18	13	15	14
Standard	25 (a)	22 (a)	24 (a)	20 (a)	17 (b)

n = 3, (a) - Amikacin, (b) - Ketoconazole

2.7. Antifungal activity

The antifungal activity was tested against *Candida albicans* by cup plate method (Rajasekaran et al., 2006). All the synthesized compounds were dissolved in 2 % v/v Tween 80 at a concentration of 10 mcg/mL. The fungal culture was spread (swabbed) into the sabouraud dextrose agar plates for uniform distribution of colonies. Using a sterile cork borer, 8 mm wide well was made on each agar plates.

All the synthesized compounds (10 mcg/mL) were poured into each wells using a sterile micropipette and Ketoconazole (10 mcg/mL) were used as standard. The plates were incubated for 48 h at 27 ºC. After incubation, the zone of inhibition was measured and the values were tabulated in Table 4. All the experiments were done in triplicate.

3. RESULTS AND DISCUSSION

Totally a series of 12 novel mannich bases of indole derivatives were synthesized. The melting points and Rf value of the synthesized compounds indicated the formation of new chemical analogues. The structure of the synthesized compounds was established by spectral (IR and ^{1}H NMR) as well as elemental analyses data. The IR and ^{1}H NMR spectrum of all the synthesized compounds showed the presence of NH band (3367.48 - 3442.62 cm^{-1}) and NH proton signal (10.08 – 10.62 ppm) of 1*H*-indole respectively which confirmed the reaction was not taken at this position. The IR and ^{1}H NMR spectrum of all the compounds showed the presence of CH stretching (2808.66 – 2880.92 cm^{-1}) and CH proton signal (4.02 – 6.16 ppm) respectively together with the absence of CH proton at C-3 position of indole confirmed the formation of mannich bases of indole derivatives.

The anti-nociceptive activitiy of the synthesized compounds were evaluated in albino mice. All the compounds exhibited highly significant anti-nociceptive activity at the dose of 10 mg/mL, i.p. when compared to standard drug pentazocine 10 mg/mL, i.p. All the compounds showed onset of action for anti-nociceptive activity at 30 min. and the maximum activity attained at 120 min which extended upto 240 min. The antimicrobial activities of the synthesized compounds were screened against Gram positive organisms such as *S. aureus*, *S. pyogenes*, Gram negative organisms such as *E. coli*, *K. aerogenes* and fungus *C. albicans* by cup-plate method. All the synthesized compounds exhibited mild to moderate antibacterial and antifungal activities at the concentration of 10 mcg/mL when compared with amikacin (10 mcg/mL) and ketoconazole (10 mcg/mL) respectively.

CONCLUSION

Twelve novel indole derivatives **(1A-L)** were synthesized by mannich reaction using indole with compounds containing active hydrogen and various aldehyde and characterized. All the spectral data accused the structure of the synthesized compounds. Anti-nociceptive and antimicrobial activities were evaluated for all the synthesized indole derivatives. The results showed that all the compounds showed highly significant anti-nociceptive activity and mild to moderate antibacterial and antifungal activities. All these results concluded that the newly synthesized indole derivatives led to intensive research and worthwhile contribution in medicinal chemistry.

ACKNOWLEDGEMENTS

The authors are thankful to the management, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamilnadu, India and for providing facilities and technical assistance. Also the authors are grateful to Dato' Prof. Dr. Nik Rahimah Nik Yacob, Vice-Chancellor and Dato' Edmund Santhara, CEO, Masterskill University College of Health Sciences, Malaysia, for their continuous encouragement and support.

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