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Synthesis, characterization and biological screening of novel indole derivatives for certain pharmacological activities

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Abstract
Indole derivatives are prepared by heating a mixture of phenyl hydrazine and substituted aromatic ketone for one hour in a water bath. Poured the heated mixture to a flask and added boiled acid (previously boiled). It was again heated and stirred for additional 10 minutes and added to ice water. All synthesized compounds were characterized by physicochemical properties, IR, NMR etc. The compounds are screened for different biological activities like antimicrobial, antzi inflammatory, analgesic, anticonvulsant, and antidepressant activities**.**

Keywords: indole, antimicrobial, anti-inflammatory, analgesic, antidepressant, anticonvulsant activities

Introduction
Medicinal chemistry [1]. And pharmaceutical chemistry are disciplines at the intersection of organic chemistry and pharmacology and various other biological specialities, where they are involved with the design, chemical synthesis and development of pharmaceutical agents or bio-active molecules (drugs). Discovery is the process of identifying novel active chemical compounds, often called hits, which are typically found by assay of compounds for a desired biological activity. Indole [2] is an aromatic heterocyclic organic compound with formula C_8H_7N. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. Indole is widely distributed in the natural environment and can be produced by a variety of bacteria [4]. As an intercellular signal molecule, indole regulates various aspects of bacterial physiology including spore formation, plasmid stability, and resistance to drugs, biofilm formation and virulence. The amino acid tryptophan is an indole derivative and the precursor of the neurotransmitter serotonin [5]. Indole is a solid at room temperature. It can be produced by bacteria as a degradation product of the amino acid tryptophan.

Materials and Methods
Indole derivatives [3]. were prepared by the reaction between phenylhydrazine and substituted aromatic ketone in the presence of sulphuric acid. All compounds were characteriszed by melting point determination, solubility, TLC, IR and NMR spectras. Then conducted pharmacological evaluation for antimicrobial, anti-inflammatory, analgesic, anticonvulsant and anti-depressant activities.

Experimental Part
Methodology for synthesis of phenylhydrazone
Dissolved 0.01 mmol of acetophenone and 1 mmol of phenyl hydrazine was heated in a reaction flask and stirred the reaction mixture for 10 minutes. Cool in ice bath until the phenylhydrazone is completely formed and then it was filtered. Washed the crystals with cold water. Allowed to air dry.Recrystallised from ethanol.

Synthesis of indole derivatives (a1-a5)
1.5 mmol of phenyl hydrazine was added to 10 ml of sulphuric acid(previously boiled)into a reaction flask and stirred additionally for 10 minutes. The mixture was poured into 25ml ice cold water. The precipitate formed should be filtered and recrystallized from ethanol.

Biological Evaluation
Experimental protocol was approved by Institutional Animal Ethical Committee, Pushpagiricollegeof pharmacy, Thiruvalla. All compounds were tested for oral acute toxicity study as per OECD guideline before evaluation of pharmacological activities.

Antimicrobial activity: Done by agar disc diffusion method [4]. against both gram positive and negative organisms. In disc diffusion method bacterial inoculum is prepared and inoculated into the entire surface of solid agar plate with a sterile cotton- tipped swab to form an even lawn. The paper disc 6mm in diameter impregnated with diluted test drug solution (500µg/ml in ethanol) was placed on the surface of each of agar plates using a sterile pair of forceps. The forceps were sterilized using flame. The plates were incubated for 2-3 days at 20 -25 ^{0}C and observed without opening them and the zone of inhibition was measured. The antibacterial screening was carried

out in a laminar air flow unit and all types of precautions were strictly maintained to avoid any type of contamination during the test.

Anti-inflammatory activity-in vitro protein denaturation method: A solution of 0.2 % w/v of Bovine Serum Albumin (BSA) was prepared in tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid [5]. Test drug of 100µg/ml concentration were prepared using ethanol as solvent. 50µl of each test drug was transferred to test tubes using micropipette. 5ml of 0.2% w/v BSA was added to the test tubes. The control consists of 5 ml of 0.2%w/v BSA solution and 50µl of alcohol. Diclofenac sodium 100µg/ml is used as standard. The test tubes were heated at 72^0C for 5 minutes and then cooled for 10 minutes. The absorbances of these solutions were determined using UV-VIS spectrophotometer at a wavelength of 660nm.

Analgesic activity: Eddy's hot plate method: The mice were divided into 3 groups each containing six animals. Control group is treated with alcohol - water mixture (1omg/kg). Standard group is treated with diclofenac (10mg/kg) i.p and test (60mg/kg) is administered to third group. Maintained the hot plate temperature at 55ºC and noted the reaction time at 0,15,30,60 minutes [6].

Anticonvulsant activity: Maximal electro shock induced convulsion method: Rats were divided into 3 groups each containing six animals. Control group is treated with alcohol- water mixture (10ml/kg) orally. Standard group is treated with phenytoin (25mg/kg) i.p and test (60mg/kg) is administered to third group. Applied a current of 150 mA for 0.2 seconds and noted the abolition of extensor phase [7].

Antidepressant activity: Forced swim test: Rats of either sex were individually forced to swim in an open cylindrical container containing 30 cm of water. Treatment was given 60min prior to study as described by study design. All animals were forced to swim for 10 min and the duration of immobility was observed and measured during the final 2 min interval of the test. Each rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water [8]. A decrease in the duration of immobility is indicative of an antidepressant like effect. Control group is treated with vehicle, standard group is with clomipramine (15mg/kg), and test is with test compounds (60mg/kg).

Result and Discussion

Table 1

Sample Code	State	Colour	Molecular Formula	Molecular Weight	M.P (^{0}C)	Yield %w/w	R_F value
a1	Solid powder	Off white	C_9H_9N	131.17	76	78.8	0.42
a 2	Solid powder	Off white	C_8H_7NO	133.15	85	65.4	0.33
a 3	Solid powder	Dark brown	C_8H_6NBr	196.04	62	57.2	0.50
a4	Solid powder	Yellow	$C_8H_8N_2$	132.16	60	61.3	0.49
a 5	Solid powder	Yellowish green	C_8H_6NCl	151.59	80	64	0.62

Spectral Analysis

2-methyl-3-(4-methylphenyl)-1H-indole = a1

IR-(cm^{-1})3484.56- NH bond stretch, 1669.46-NH bond bend, 1263-methyl group, 2864-phenyl group, 829.43-ortho para substitution, NMR(δ in ppm) 10.3 –CH_3, 7.8-ArH, 1.9-CH_2

4-(2-methyl-1H-indole-3-yl) phenol = a2

IR-(cm^{-1}) 3477.80-NH bond stretch, 1652.10-NH bond bend, 1275.91-methylgroup, 1109.12-aromatic hydroxyl group, 883.28-para substitution. NMR (δ in ppm)- 5.817-OH, 8.193-NH, 7.852-ArH, 10.4-CH_3

3-(4-chlorophenyl)-2-methyl-1H-indole = a3

IR-(cm^{-1})3418.97-NH bond stretch, 1661.75-NH bond bend, 2821.67-chloro group, 1231.60-aromatic methyl group, 825.57-para substitution. NMR (δ in ppm)- 6.694-Cl, 9.689-CH_3, 3.029-NCH_3, 7.699-ArH

4-(2-methyl-1H-indole-3-yl) aniline = a4

IR(cm^{-1})-3449.84 - Presence of NH2 group, 1598.09-Presence of NH2 group, 1175.66 -Presence of CH_3, 1101.40 -Presence of ArH bond, 816.89-Para substitution,804.35 -Ortho para substitution. NMR (δ in ppm)- 6.965-OH, 8.310-NH_2, 7.890-ArH, 1.615-CH_2

3-(4-bromophenyl)-2-methyl-1H-indole = a5
IR-(cm^{-1})-3418.97-NH bond stretch, 1661.75-NH bond bend, 2821.67-chloro group, 1231.60-aromatic methyl group, 825.57-para substitution. NMR(δ in ppm)-6.694-Cl, 9.689-CH_3, 3.029-NCH_3, 7.699-ArH

Anti microbial activity - agar disc diffusion method

Table 2

Sl. No	Sample	Zone of inhibition in mm			
		Bacillus subtilis	***Pseudomonas aeruginosa***	***E.coli***	***Staphylococcus Aureus***
1	Standard (ciprofloxacin 10mcg)	4±.014**	3.5±.023**	3±.003**	4±.004**
2	a 1	11±.023	13±.043	10±.022	-
3	a 2	12±.001	-	-	-
4	a 3	32±.033**	26±.032**	30±.042**	36±.012**
5	a 4	20±.033**	18±.013**	16±.023**	20±.033**
6	a 5	26±.014**	20±.014**	22±.042**	26±.004**

Anti inflammatory activity- in vitro protein denaturation method

Table 3

Sl. No.	Group	Absorbance at 660nm	Percentage of inhibition
1	a1	0.696±0.022	45%
2	a2	0.459±0.042	63%
3	a3	0.201±0.011**	85%
4	a4	0.337±0.033**	74%
5	a5	0.195±0.043**	89%
6	Control	1.747±0.044	-
7	standard	0.1399±0.012**	92%

Analgesic Activity - Eddy's hot plate method

Table 4

Sl. no	Sample	Basal reaction before. drug admn. (sec)	Pain perception After drug administration (sec) Mean ± SEM		
			After 15minute	After 30 minute	After After 60 minute
1	a 1	4±0.30	4±0.30	4±0.36	3±0.30
2	a 2	3±0.33	5±0.35	6±0.40	5±0.47
3	a 3	4±0.22**	5±0.33**	9±0.33**	7±0.36**
4	a 4	3±0.42**	5±0.22**	5±0.36**	7±0.36**
5	a 5	3±0.22**	7±0.36**	9±0.30**	8±0.30**
6	Control	2±0.33	2±0.33	3±0.30	2±0.30
7	Standard Diclofenac sodium(10mg/kg)	5±0.42**	7±0.30**	10±0.25**	9±0.42**

Each value represent Mean ± SEM, n = 6, $p < 0.01$

Anti depressant activity- Forced swim test

Table 5

Sl. No	Group	Duration of immobility (sec)	Percentage change (%)
1	a 1	54±0.90	28
2	a 2	40±0.87	46
3	a 3	25±1.0**	66**
4	a 4	34±0.99	54
5	a 5	30±0.87**	60**
6	Standard	12±1.22**	84**
7	control	75±1.22	-

Anti convulsant activity - Maximal electro shock induced convulsion method

Table 6

Sl.No	Group	Time (in sec)				
		Tonic flexion	Tonic extensor	Clonic convulsion	Stupor	Death/ recovery
1.	a1	7±0.30	7±0.40	6±0.36	-	Death
2.	a2	6±0.36	4±0.42	4±0.49	110±0.42	Recovery

3.	a3	5±0.36	4±0.36	3±0.36	50±0.33	Recovery
4.	a4	5±0.36**	3±0.22**	2±0.30**	40±0.42**	Recovery
5.	a5	6±0.47	5±0.36	3±0.30	60±0.33	Recovery
6.	Standard	3±0.30**	-	2±0.33**	10±0.47**	Recovery
7.	control	5±0.36	10±0.30	3±0.30	-	Death

Each value represent Mean ± SEM, n = 6, $p < 0.01$

Conclusion

In the case of *antibacterial activity*, a3 a4 and a5 shows more activity. a3. has chloro group and a5 has bromo group.For antiinflammatory activity more active compound is a3. Compounds like a5 and a4 also have antiinflammatory activity. In case of analgesic activity a5 shows more activity. Compound a4 shows significant anticonvulsant effect.Compound a3 showed significant antidepressant activity. In this case indole substituted with halogens increases the activity. From our study it is concluded that presence of phenyl ring with EWG at 2 and 4 position in indole increases the activity.

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