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Research Article

Synthesis, Characterization and biological activity of indole-2-carboxylic acid derivatives

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Abstract

Synthesis of indole-2-carboxylic acid derivatives has attracted considerable attention in view of therapeutic applications. In the presented research work, a series of 3-[*N*,*N*-Dialkylamine(oxo)acetyl]-1-propyl-1*H*-indole-2-carboxylic acids was synthesized from 1-Propyl-1H-indole-2-carboxylic acid. The synthesis was carried out by treating 1-Propyl-1H-indole-2-carboxylic acid with oxalyl chloride in dry dichloromethane and condensation of different secondary amines with 1-propyl-1*H*-indole-2-carbohydrazide forms the titled compounds (OND 1 – OND 10). All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were screened for their antibacterial activities against *Staphylococcus aureus*, *Staphylococcus pyogenus*, *Escherichia coli* and *Pseudomonas aeruginosa* and for antifungal activity against *Candida albicans* and *Asperigillus niger*. The biological activities of the synthesized compounds have been compared with standard drugs like Ampicillin and Nystatin. The compounds exhibited significant antibacterial and moderate antifungal activities. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis and the antimicrobial screening of the new compounds are reported.

Keywords: Indole, Antibacterrial, Antifungal, 2^{0} Amine

1. Introduction

Indole is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. Indole alkaloids have been proved to be medicinally important natural compounds.

Indole compounds include the plant hormone Auxin, the anti-inflammatory drug indomethacin, the β-blocker pindolol, and the naturally occurring hallucinogen dimethyltryptamine.

The indole skeleton is one of the most attractive frameworks with a wide range of biological and pharmacological activities. This physiologically important nucleus is abundantly found in therapeutic agents as well as in natural products. Many researchers have described synthesis of indole and its derivatives along with its applications in literature.

A large number of heterocyclic compounds containing the indole ring are associated with diverse pharmacological properties such as Analgesic[1], Antiallergic[2], Antibacterial[3], Anticonvulsant[4,5], Antifungal[6], Antihistaminic[7], Anti-inflammatory[8-10], Anticancer[11,12], Antiviral[13], Anthelminthic[14], Anti hypertensive[16], Cardiovascular[17], Antioxidant[18].

Thus the efficient synthesis of novel substituted indole derivative compounds still represent highly pursued target. The substitution of oxalyl amines at the 3- position of Indole ring markedly influences the antimicrobial activity.

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2. Experimental

2.1 Material and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a Bruker Ac 400 MHz spectrometer. Purity of the synthesized compounds was checked by HPLC Agilent. The results are in agreements with the structures assigned.

2.2 Preparation of 1-Propyl-1*H*-indole-2-carboxylic acid

To a stirred suspension of K_2CO_3 (2.72 g, 0.02 mol) and 1*H*-indole-2-carboxylic acid (1.61 g, 0.01 mol) in dry DMF (10 ml), 1-bromopropane (1.18 ml, 0.013 mol) was added dropwise after 5 minute. The resultant solution was stirred for 5 hour at room temperature, and then poured onto crushed ice; the product was isolated and washed with water and hexane to give pure product. Yield: 93 %, mp 80-83^{0}C.

2.3 General procedure for the preparation of Indole-2-carboxylic acid derivatives (OND-1 TO OND-10)

To a stirred cooled (ice bath) solution of 1-propyl-1*H*-indole-2-carboxylic acid (2.03 g, 0.01 mol) in dry DCM (15 ml), oxalyl chloride (1.27 ml, 0.015 mol) was added dropwise in solution. The obtained solution was stirred at 0-5^{0}C for 10-15 minute then add TEA (1.68 ml, 0.012 mol) dropwise. The resulting solution was stirred at 0-5^{0}C for 30.0 minute and then at 25-30^{0}C for 1 hour. Dark yellow colored was formed. The solvent was removed *in vacuo*, the residue was dissolved in dry DCM (12 ml) and different secondary amine (0.012 mol) dropwise. The reaction mixture was stirred at 0-5^{0}C for 30.0 minute and then 25-30^{0}C for another 30.0 minute (monitored by TLC). The solvent was removed *in vacuo*. The product was dissolved in water and extracted with ethylacetate (25 ml × 3). The combined organic layers were washed with water followed by brine and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and the solid was triturated with hexane and resulting precipitate was filtered, washed with hexane and dried to give analytical pure product. The physical constants of the products are given in Table-1 and the scheme of titled compounds given in Scheme-1.

2.3.1 3-[Morpholin-4-yl(oxo)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-1)

Purity by HPLC: 94 %; IR (KBr): 3446 (O-H, str), 3023 (Ar, C-H str), 2947 (C-H str), 2805 (C-H str), 1706 (acid, C=O str), 1633 (amide, C=O str), 1528 (Ar, C=C str), 1440 (Ar, C=C str), 1256 (C-H ban), 1152 (C-O str) cm^{-1}; 1H NMR (400 MHz, $CDCl_3$): δppm 0.92-0.96 (t, *J*=7.32 Hz, 3H, CH_3), 1.07-1.25 (m, 2H, CH_2), 1.63-1.75 (m, 4H, 2CH_2), 3.62-3.66 (t, *J*=7.08 Hz, 2H, CH2), 3.77-3.87 (m, 4H, 2CH_2), 7.30-7.45 (m, 3H, ArH), 8.30-8.31 (d, *J*=7.92 Hz, 1H, ArH), 9.77 (s, 1H, OH). 13C NMR (100 MHz, CDCl3): δ ppm 10.28, 21.81, 41.89, 46.36, 66.27, 112.36, 116.38, 123.07, 123.85, 126.41, 127.48, 129.99, 135.13, 159.00, 166.94, 187.66; MS: *m/z* = 344 $[M]^+$; Anal. Calcd for C18H20N2O5: C, 62.78; H, 5.85; N, 8.13. Found: C, 62.67; H, 5.47; N, 8.09%.

2.3.2 3-[(Diethylamino)(oxo)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-2)

Purity by HPLC: 91 %; IR (KBr): 3412, 3044, 2983, 2861, 1710, 1673, 1552, 1444, 1287, 1175 cm-1; 1H NMR (400 MHz, CDCl3): δ ppm 0.86-0.89 (t, *J*=7.4 Hz, 3H, CH3), 1.29-1.34 (m, 6H, 3CH3), 1.51-1.60 (m, 2H, CH2), 3.41-3.44 (t, *J*=7.08 Hz, 2H, CH2), 3.55-3.61 (m, 4H, 2CH2), 7.28-7.30 (m, 1H, ArH), 7.32-7.36 (m, 1H, ArH), 7.45- 7.47 (d, *J*=8.2 Hz, 1H, ArH), 8.31-8.33 (d, *J*=8.0 Hz, 1H, ArH), 10.11 (s, 1H, OH). 13C NMR (100 MHz, CDCl3): δ ppm 10.17, 12.50, 13.73, 21.66, 39.35, 42.72, 66.95, 112.71, 116.09, 122.99, 123.54, 125.99, 127.59, 130.06, 135.24, 158.88, 167.89, 188.32; MS: *m/z* = 330 $[M]^+$; Anal. Calcd for C18H22N2O4: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.36; H, 6.61; N, 8.37%.

2.3.3 3-[(4-Phenylpiperazin-1-yl)(oxo)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-3)

IR (KBr): 3502, 3101, 2961, 2852, 1708, 1678, 1533, 1456, 1248, 1156 cm^{-1}; MS: *m/z* = 419 $[M]^+$; Anal. Calcd for C24H25N3O4: C, 68.72; H, 6.01; N, 10.02. Found: C, 68.59; H, 5.89; N, 9.95%.

2.3.4 3-[Oxo(piperidin-1-yl)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-4)

IR (KBr): 3432, 3513, 3033, 2988, 2857, 1700, 1689, 1578, 1482, 1252, 1162 cm^{-}1; MS: *m/z* = 343 $[M+1]^+$; Anal. Calcd for C19H22N2O4: C, 66.65; H, 6.48; N, 8.18. Found: C, 66.53; H, 6.31; N, 8.06%.

2.3.5 3-[(4-Methylpiperazin-1-yl)(oxo)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-5)

IR (KBr): 3501, 3055, 2971, 2868, 1711, 1693, 1561, 1483, 1291, 1158 cm^{-1}; MS: *m/z* = 357 $[M]^+$; Anal. Calcd for $C_{19}H_{23}N_3O_4$: C, 63.85; H, 6.49; N, 11.76. Found: C, 63.77; H, 6.45; N, 11.70%

2.3.6 3-[(4-Ethylpiperazin-1-yl)(oxo)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-6)

IR (KBr): 3522, 3033, 2999, 2905, 1715, 1694, 1499, 1432, 1240, 1160 cm^{-1}; MS: *m/z* = 371 $[M]^+$; Anal. Calcd for $C_{20}H_{25}N_3O_4$: C, 64.67; H, 6.78; N, 11.31. Found: C, 64.63; H, 6.72; N, 11.28%.

2.3.7 3-[(*N*,*N*-Diisopropylamino)(oxo)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-7)

IR (KBr): 3488, 3097, 2986, 2971, 1718, 1701, 1497, 1422, 1285, 1178cm-1; MS: *m/z* = 358 $[M]^+$; Anal. Calcd for $C_{20}H_{26}N_2O_4$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.97; H, 7.28; N, 7.78%.

2.3.8 3-[Oxo(pyrrolidin-1-yl)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-8)

IR (KBr): 3489, 3098, 2978, 2891, 1706, 1681, 1499, 1488, 1280, 1171 cm^{-1}: MS: *m/z* = 328 $[M]^+$; Anal. Calcd for $C_{18}H_{20}N_2O_4$: C, 65.84; H, 6.14; N, 8.53. Found: C, 65.79; H, 6.09; N, 8.47%.

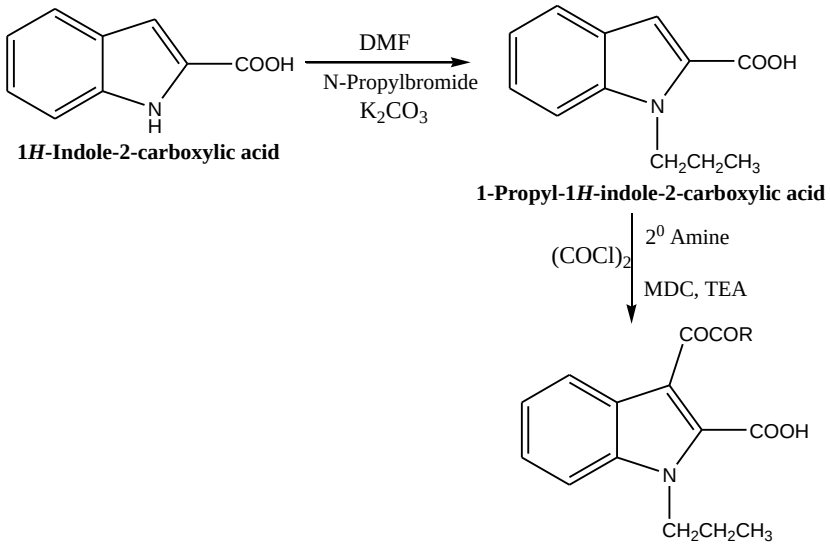
2.3.9 3-[(2-Methylpiperidin-1-yl)(oxo)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-9)

IR (KBr): 3569, 3099, 2966, 2898, 1720, 1696, 1583, 1467, 1281, 1184 cm^{-1}; MS: *m/z* = 356 $[M]^+$; Anal. Calcd for $C_{20}H_{24}N_2O_4$: C, 67.40; H, 6.79; N, 7.86. Found: C, 67.35; H, 6.70; N, 7.83%.

2.3.10 3-[(4-Methylpiperidin-1-yl)(oxo)acetyl]-1-propyl-1*H*-indole-2-carboxylic acid (IND-10)

IR (KBr): 3546, 3084, 2997, 2951, 1717, 1687, 1576, 1455, 1279, 1187cm^{-1}; MS: *m/z* = 356 $[M]^+$; Anal. Calcd for $C_{20}H_{24}N_2O_4$: C, 67.40; H, 6.79; N, 7.86. Found: C, 67.27; H, 6.73; N, 7.81%.

Scheme-1: Synthesis of Indole-2-carboxylic acid derivatives (OND-1 TO OND-10)



1*H*-Indole-2-carboxylic acid

1-Propyl-1*H*-indole-2-carboxylic acid

Indole -2-carboxylic acid Derivatives (OND-1 to OND-10)

Table-1: Physical constant of Indole-2-carboxylic acid derivatives (OND-1 TO OND-10)

Compd	Substitution (Ar)	M.F	M.W	Yield (%)
IND-1	Morpholine	$C_{18}H_{20}N_2O_5$	344.36	83
IND-2	Diethylamine	$C_{18}H_{22}N_2O_4$	330.37	80
IND-3	1-Phenylpiperazine	$C_{24}H_{25}N_3O_4$	419.47	79
IND-4	Piperidine	$C_{19}H_{22}N_2O_4$	342.38	89
IND-5	1-Methylpiperazine	$C_{19}H_{23}N_3O_4$	357.40	85
IND-6	1-Ethylpiperazine	$C_{20}H_{25}N_3O_4$	371.43	76
IND-7	Diisopropylamine	$C_{20}H_{26}N_2O_4$	358.43	80
IND-8	Pyrrolidine	$C_{18}H_{20}N_2O_4$	328.36	82
IND-9	2-Methylpiperidine	$C_{20}H_{24}N_2O_4$	356.41	84
IND-10	4-Methylpiperidine	$C_{20}H_{24}N_2O_4$	356.41	78

2.4 Antimicrobial Activity

All of the synthesized compounds **(IND-1 TO IND-10)** were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96 and *Streptococcus pyogenes* MTCC 442, two Gram-negative bacteria *Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 1688 and three fungal strains *Candida albicans* MTCC 227 and *Aspergillus Niger*

MTCC 282 taking Ampicillin and Nystatin as standard drugs. The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards.

2.4.1 Minimal Inhibition Concentration [MIC]

The main advantage of the Broth Dilution Method for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37^0C overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

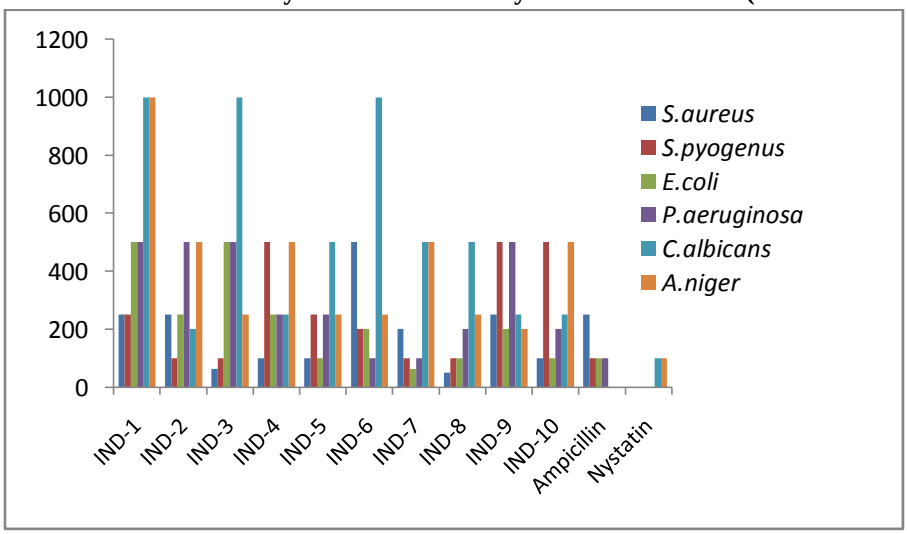
2.4.2. Methods used for screening

Each synthesized compounds was diluted obtaining 2000 µg mL^{-1} concentration, as a stock solution. Inoculum size for test strain was adjusted to 108 cfu (colony forming unit) per milliliter by comparing the turbidity. The results obtained from antimicrobial susceptibility testing are depicted in Table 2.

Table-2: Antimicrobial activity of indole-2-carboxylic acid derivatives (IND-1 TO IND-10)

Compound	Minimal Inhibitory Concentration (µg/ml)					
	Antibacterial Activity				Antifungal activity	
	S.aureus	***S.pyogenus***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
IND-1	250	250	500	500	1000	1000
IND-2	250	100	250	500	200	500
IND-3	62.5	100	500	500	1000	250
IND-4	100	500	250	250	250	500
IND-5	100	250	100	250	500	250
IND-6	500	200	200	100	1000	250
IND-7	200	100	62.5	100	500	500
IND-8	50	100	100	200	500	250
IND-9	250	500	200	500	250	200
IND-10	100	500	100	200	250	500
Ampicillin	250	100	100	100	NT	NT
Nystatin	NT	NT	NT	NT	100	100

Figure 1: Antimicrobial activity of Indole-2-carboxylic acid derivatives (IND-1 TO IND-10)



3. Results & Discussion

1*H*-indole-2-carboxylic acid with bromopropane in dry DMF forms the 1-Propyl-1*H*-indole-2-carboxylic acid. The mixture of 1-Propyl-1*H*-indole-2-carboxylic acid with oxalyl chloride and triethylamine in dry DCM at 0-5^{0}C and then at 25-30^{0}C for 1 hour stirring a yellow coloured product is formed. The residue was dissolved in dry DCM and added different secondary amines at same reaction conditions. All the synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique.

The data recorded in Table 2 indicated that compounds OND-3, OND-4, OND-5, OND-8 and OND-10 are more potent towards the *Staphylococcus aureus.* Remaining compounds are moderately potent towards the *Staphylococcus aureus.* Compounds OND-2, OND-3, OND-7 & OND-8 are moderately potent towards the *Streptococcus pyogenes.* Compound OND-7 is more potent towards the *Escherichia coli* and compounds OND-5, OND-8 and OND-10 were moderately potent towards the *Escherichia coli.* Compounds OND-6 and OND-7 are moderately potent towards the *Pseudomonas aeruginosa.* All these compounds are compared with the standard reference (Ampicillin) for their antibacterial activities. *Candida albicans* are moderately sensitive towards the OND-2 and OND-7. *Aspergillus Niger* is moderately sensitive towards the OND-9. All these compounds are compared with the standard reference (Nystatin) for their antifungal activities.

4. Conclusion

In this study, certain some indole-2-carboxylic acid derivatives were synthesized and evaluated for their antimicrobial activities. Results revealed that the compounds exhibited significant *in-vitro* activity. Compound OND-3, OND-7 and OND-8 are more potent. Remaining compounds also showed moderate to weak antimicrobial activities. The study would be a fruitful matrix for the development of indole-2-carboxylic acid derivatives for further bio-evaluation.

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