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Research Article

Synthesis, Characterization and Evaluation of Analgesic and Anti-inflammatory Activities of Some Novel Indoles

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Abstract

Purpose: *Long-term clinical usage of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with significant side effects - gastrointestinal lesions, bleeding and nephrotoxicity. Therefore, the discovery of new safer antiinflammatory drugs represents a challenging goal for this research area.*
Methods: *Various derivatives of 3-(2-aminopyrimidin-4-yl) indoles viz. 4-(4-substitutedphenyl)-6-(2-(4-substitutedphenyl)-1H-indol-3-yl) pyrimidin-2-amine (4a-4r) were synthesized by cyclization of (3-(4-substitutedphenyl)-1-(2-(4-substitutedphenyl)-1H-indol-3-yl) prop-2-en-1-one) of indole with guanidine hydrochloride in the presence of sodium isopropoxide. Their structures were confirmed by FTIR, ^{1}H NMR and elemental analysis. These compounds were investigated for their analgesic, inflammatory and ulcerogenic activities.*
Results: *All the compounds tested (4a-4r) showed analgesic and inflammatory activities. Seven compounds (4d, e, h, j, k, q, p) out of 18 compounds showed antiinflammatory activity comparable to that of the reference standard, indomethacin, but with much lower ulcerogenic action. Compounds 4j and 4k showed 87.4 and 88.2 % inhibition of paw edema, 78.5 and 76.6 % protection against acetic acid-induced writhings and 0.89 and 1.12 of severity index, respectively, compared to 92.7 %, 82.8 % and 2.2, respectively, for indomethacin.*
Conclusion: *The results show that incorporation of an appropriately substituted pyrimidine ring in indole nucleus can afford molecules with good analgesic and anti-inflammatory activities but with reduced gastric irritation.*

Keywords: *Synthesis, Indole, Pyrimidine, Anti-inflammatory activity, Analgesic activity, Ulcerogenic effect*

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INTRODUCTION

Non-steroidal anti-inflammatory agents (NSAIDS) continue to be one of the most widely used groups of therapeutic agents. They are used for the treatment of inflammation including pain, pyrexia and rheumatoid arthritis. With increasing life expectancy, it is not surprising that the development of newer NSAIDs continues at a rapid pace. Almost all the NSAIDs currently in clinical use are highly acidic in nature and suffer from the drawback of gastrointestinal toxicity [1].

Indole alkaloids have proved to be medicinally important natural compounds. Indole ring constitutes an important template for drug design such as the classical NSAIDs indomethacin and indoxole. Furthermore, indole derivatives have been reported to possess promising biological activities including analgesic, anti-inflammatory [2-5], antihypertensive [6], anticonvulsant [7], antimicrobial [8-9] and selective cyclooxygenase 2 COX 2 inhibitory activities [10-12]. Thus, efficient synthesis of novel substituted indole derivative compounds still represent highly pursued objective. The substitution of heterocyclic moiety at the 3-position of indole ring markedly influences anti-inflammatory activity [13].

Design and synthesis of novel NSAIDs continues to be explored in order to achieve agents with better efficacy and safety profile. The diarylheterocycle class of compounds has been investigated extensively as COX 2 inhibitors. In contrast, relatively few reports document structural modifications of the existing NSAIDs into better drugs [14]. Thus, a major objective of this study was to explore the indole nucleus of indomethacin, an NSAID, for structural modification in order to produce potential antiinflammatory agents. Furthermore, since pyrimidine derivatives have also been reported to possess potent anti-inflammatory activity [15-17], a second objective of this work, therefore, was to synthesize some indole derivatives possessing a pyrimidine moiety at 3- position of 2-phenyl indoles to obtain compounds (4a-4r) with superior biological activities.

EXPERIMENTAL

Materials

All the reagents and solvents used in the synthesis work were of laboratory grade and procured from either SD Fine Chemicals, Qualigens or Sigma, India. All the solvents were dried and distilled before use. The animals used were procured from our institute's animal house.

Synthesis of 2-(4-substitutedphenyl) indole (1a-1c)

Synthesis of 2-(4-substitutedphenyl) indole (1a-1c) was carried out by the procedure of Fischer Indole synthesis [18]. *p*-Substituted acetophenone phenylhydrazone was prepared by warming a mixture of 0.04175 mole of acetophenone / *p*-methoxyaceto-phenone / *p*-chloroacetophenone and 0.04175 mole of phenyl hydrazine with 60 mLl of ethanol and few drops of glacial acetic acid. Filtered The reaction mixture was filtered, and the solid washed first with dilute HCl and then with 12 mL of cold rectified spirit. It was recrystallized with ethanol and 4 g of the crude phenylhydrazone was placed in a beaker containing 25.71 g of polyphosphoric acid The mixture was heated over a boiling water bath and maintained at 100 ^{0}C for 10 min. Cold water (65 ml) was added, stirred well, filtered and washed well with water.

General procedure for the synthesis of 2-(4-substitutedphenyl) indole-3-carbaldhyde (2a-2c)

Synthesis of 2-(4-substitutedphenyl) indole-3-carbaldehyde (2a-2c) was carried out by Vielsmayer Haack reaction. Anhydrous dimethylformamide DMF (25 mL) was stirred at 0 ^{o}C for 15 min and 0.088 mole of phosphorous oxychloride was added to it

dropwise and stirred at 0 °C for 1 h. Indole (0.044 mole) was dissolved in anhydrous DMF and added dropwise to the above mixture < 10 °C. The mixture was warmed to 35 - 40 °C and stirred for 1.5 h. Water (50 mL) was added to the mixture, boiled for 2h, cooled and kept overnight. A solid mass, obtained after addition of water (30 mL), was recrystallized from 90 % ethanol.

2a IR (cm^{-1} , KBr): 3421, 3165, 3059, 2332, 1710,1581,1445, 1247,854,748; ^{1}H NMR (DMSO-d_6): δ 7.21-7.32 (m, 3H, Ar), 7.50 (d, J=8Hz,1H, Ar), 7.66 (d, J=8Hz,2H, Ar), 7.80 (d, J=8Hz,2H, Ar),8.20 (d, J=8Hz, 1H, Ar), 9.96 (s, 1H, CHO), 12.48 (brs, 1H, NH). **2b** IR (cm^{-1} , KBr): 3421, 3146, 3059, 2330, 1720,1581,1445, 738; ^{1}H NMR (DMSO-d_6): δ 7.23-7.33 (m, 2H, Ar), 7.55 (d, J=8Hz,1H, Ar), 7.71 (d, J=8Hz,2H, Ar), 7.82 (d, J=8Hz,2H, Ar),8.20 (d, J=8Hz, 1H, Ar), 9.91 (s, 1H, CHO), 12.41 (brs, 1H, NH). **2c** IR (cm^{-1} , KBr): 3360, 3062, 2329, 1734,1550, 1249,741; ^{1}H NMR (DMSO-d_6): δ 3.49 (s, 3H, -OCH_3) δ 7.21-7.42 (m, 3H, Ar), 7.69 (d, J=8Hz,2H, Ar), 7.88 (d, J=8Hz,2H, Ar),8.24 (d, J=8Hz, 1H, Ar), 9.98 (s, 1H, CHO), 12.42 (brs, 1H, NH).

General procedure for the synthesis of (E)-3-(2-(4-sunstitutedphenyl)-1H-indol-3-yl)-1-(4-sunstitutedphenyl) prop-2-en-1-one (3a-3r)

Indole-3-carbaldehyde, 4-sustituted aceto-phenone and piperidine (0.015 mole each) were mixed with 20 mL ethylene glycol. The solution was refluxed at 160 – 180 °C for 3 - 4 h and cooled. The resulting solid was filtered and washed with 95 % ethanol. **3a** IR (cm^{-1}, KBr): 3235, 3146, 3010, 1650,1575,1445, 745; ^{1}H NMR (DMSO-d_6): δ 6.98 (d, J=7Hz, 1H,=CH-Ar), 7.10-8.23 (m, 14H, Ar), 8.08 (d, J=7Hz, 1H-COCH=),12.44 (brs, 1H, NH).

3b IR (cm^{-1} , KBr): 3233, 3120, 3010, 2289, 1632,1550,1445, 738; ^{1}H NMR (DMSO-d_6): δ 6.82 (d, J=8 Hz, 1H =CH-Ar), 7.10-8.20 (m, 13H, aromatic), 8.05 (d, J=12Hz, 1H-COCH=),12.41 (brs, 1H, NH). **3c** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 781; ^{1}H NMR (DMSO-d_6): δ 2.37 (s, 1H, -CH_3), 6.96 (d, J=12 Hz, 1H =CH-Ar), 7.19-8.23 (m, 13H, aromatic), 8.05 (d, J=13 Hz, 1H-COCH=),12.09 (brs, 1H, NH). **3d** IR (cm^{-1} , KBr): 3450, 3360, 3126, 3015, 2250, 1632,1552,1438, 781; ^{1}H NMR (DMSO-d_6): δ 7.06 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 7.89 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH). **3e** IR (cm^{-1} , KBr): 3410,3360, 3290, 3126, 3015, 2250, 1632,1552,1438, 781; ^{1}H NMR (DMSO-d_6): δ 5.68 (s, 1H, NH_2) 6.99 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.02 (d, J=13 Hz, 1H-COCH=), 9.05 (s, 1H, -OH),12.39 (brs, 1H, NH). **3f** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 1243; ^{1}H NMR (DMSO-d_6): δ 3.51 (s, 3H, -OCH_3), 5.68 (s, 1H, NH_2) 6.79 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.06 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH). **3g** IR (cm^{-1} , KBr): 3235, 3146, 3010, 2310, 1650,1575,1445, 745; ^{1}H NMR (DMSO-d_6): δ 6.88 (d, J=7Hz, 1H,=CH-Ar), 7.10-8.23 (m, 14H, Ar), 8.18 (d, J=7Hz, 1H-COCH=),12.44 (brs, 1H, NH). **3h** IR (cm^{-1} , KBr): 3233, 3120, 3010, 2289, 1632,1550,1445, 763; ^{1}H NMR (DMSO-d_6): δ 6.92 (d, J=8 Hz, 1H =CH-Ar), 7.10-8.20 (m, 13H, aromatic), 8.15 (d, J=12Hz, 1H-COCH=),12.41 (brs, 1H, NH). **3i** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 781; ^{1}H NMR (DMSO-d_6): δ 2.37 (s, 1H, -CH_3), 6.95 (d, J=12 Hz, 1H =CH-Ar), 7.19-8.23 (m, 13H, aromatic), 7.99 (d, J=13 Hz, 1H-COCH=),12.09 (brs, 1H, NH). **3j** IR (cm^{-1} , KBr): 3450, 3360, 3126, 3015, 2250, 1632,1552,1438, 781; ^{1}H NMR (DMSO-d_6): δ 6.85 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.03 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH). **3k** IR (cm^{-1} , KBr): 3416, 3360, 3290, 3126, 3015, 2250, 1632,1552,1438, 781; ^{1}H NMR (DMSO-d_6): δ 5.68 (s, 1H, NH_2) 6.72 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.12 (d,

J=13 Hz, 1H-COCH=), 9.05 (s, 1H, -OH),12.39 (brs, 1H, NH). **3l** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 1243, 756; ^{1}H NMR (DMSO-d_6): δ 3.51 (s, 3H, -OCH_3), 5.68 (s, 1H, NH_2) 6.98 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.06 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH). **3m** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 1243, 756; ^{1}H NMR (DMSO-d_6): δ 3.51 (s, 3H, -OCH_3), 5.68 (s, 1H, NH_2) 6.97 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.96 (m, 13H, aromatic), 7.98 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH). **3n** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 1243, 756; ^{1}H NMR (DMSO-d_6): δ 3.51 (s, 3H, -OCH_3), 5.68 (s, 1H, NH_2) 6.84 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.02 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH). **3o** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 1243, 756; ^{1}H NMR (DMSO-d_6): δ 3.51 (s, 3H, -OCH_3), 5.68 (s, 1H, NH_2) 6.89 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.06 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH). **3p** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 1243, 756; ^{1}H NMR (DMSO-d_6): δ 3.51 (s, 3H, -OCH_3), 5.68 (s, 1H, NH_2) 6.79 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.15 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH).**3q** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 1243, 756; ^{1}H NMR (DMSO-d_6): δ 3.51 (s, 3H, -OCH_3), 5.68 (s, 1H, NH_2) 6.92 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.11 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH).**3r** IR (cm^{-1} , KBr): 3360, 3126, 3015, 2250, 1632,1552,1438, 1243, 756; ^{1}H NMR (DMSO-d_6): δ 3.51 (s, 3H, -OCH_3), 5.68 (s, 1H, NH_2) 6.96 (d, J=12 Hz, 1H =CH-Ar), 7.16-7.98 (m, 13H, aromatic), 8.09 (d, J=13 Hz, 1H-COCH=), 9.15 (s, 1H, -OH),12.39 (brs, 1H, NH).

General procedure for the synthesis of 4-(4-substitutedphenyl)-6-(2-(4-substitutedphenyl)-1H-indol-3-yl) pyrimidin-2-amine (4a-4r)

Chalcones were cyclized with guanidine hydrochloride in the presence of sodium isopropoxide (synthesized in situ by adding sodium metal in isopropanol to give pyrimidines as follows:

To a solution of 1.1 moles of guanidine hydrochloride in 50 mL of isopropanol, 1.1 moles of sodium metal was added. The reaction mixture was refluxed for 2 h, chalcone (3a-3r, 1.0 equivalent) were added to it and then refluxed for 10 h. Solvent was removed from the reaction mixture under reduced pressure. Water (50 mL) was added and the aqueous phase extracted with 20 mL of ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The resulting product was recrystallized from ethanol, and their melting point, % yield and molecular formula are given in Table 1 and Fig 1.

4a IR (cm^{-1}, KBr): 3410, 3355, 3175, 3126, 3015, 1648,1590,1418, 1243, 756; ^{1}H NMR (DMSO-d_6): δ 5.58 (s, 2H, NH_2), 7.10-8.16 (m, 15H, aromatic), 11.92 (brs, 1H, NH) ; Elemental (CHN) Analysis: Anal. Calcd for $C_{24}H_{18}N_4$ C, 79.54; H, 5.01; N, 15.46 Found: C, 79.46; H, 5.02; N, 15.48. **4b** IR (cm^{-1} , KBr): 3405, 3360,3218, 1640,1587,1425, 1241, 746; ^{1}H NMR (DMSO-d_6): δ 5.61 (s, 2H, NH_2), 7.20-8.22 (m, 14H, aromatic), 11.87 (brs, 1H, NH); Elemental Analysis: Anal. Calcd for $C_{24}H_{17}ClN_4$ C, , 72.63; H, 4.32; Cl, 8.93; N, 14.12 Found: C, 72.62; H, 4.36; N, 14.07. **4c** IR (cm^{-1} , KBr): 3412, 3361,3233, 3116, 1635,1620,1436, 1215, 756; ^{1}H NMR (DMSO-d_6): δ 2.39 (s, 1H, -CH_3), 5.42 (s, 2H, NH_2), 7.16-8.21 (m, 14H, aromatic), 12.07 (brs, 1H, NH); Elemental Analysis: Anal. Calcd for $C_{25}H_{20}N_4$ C, 79.76; H, 5.35; N, 14.88 Found: C, 79.70; H, 5.39; N, 14.92. **4d** IR (cm^{-1} , KBr): 3456, 3350, 3120, 3015, 1629,1591,1438, 1241, 766; ^{1}H

Table 1: Physical data for synthesized pyrimidinyl indole derivatives (4a-4r)

Compound	R	R'	Molecular formula	MP (°C)	Yield (%)	Rf values
4a	H	H	$C_{24}H_{18}N_4$	150-153	62.33	0.72
4b	H	4-Cl	$C_{24}H_{17}ClN_4$	167-171	55.23	0.66
4c	H	4-CH_3	$C_{25}H_{20}N_4$	164-169	72.56	0.56
4d	H	4-OH	$C_{24}H_{18}N_4O$	150-153	66.73	0.65
4e	H	4-NH_2	$C_{24}H_{19}N_5$	152-156	58.19	0.73
4f	H	4-OCH_3	$C_{25}H_{20}N_4O$	189-192	61.84	0.65
4g	Cl	H	$C_{24}H_{17}ClN_4$	201-203	79.34	0.77
4h	Cl	4-Cl	$C_{24}H_{16}C_{l2}N_4$	179-181	58.12	0.69
4i	Cl	4-CH_3	$C_{25}H_{19}ClN_4$	205-208	62.18	0.70
4j	Cl	4-OH	$C_{24}H_{17}ClN_4O$	216-218	67.28	0.66
4k	Cl	4-NH_2	$C_{24}H_{18}ClN_5$	224-226	65.61	0.75
4l	Cl	4-OCH_3	$C_{25}H_{19}ClN_4O$	216-218	63.14	0.68
4m	OCH_3	H	$C_{25}H_{20}N_4O$	197-199	59.42	0.67
4n	OCH_3	4-Cl	$C_{25}H_{19}ClN_4O$	189-192	49.55	0.64
4o	OCH_3	4-CH_3	$C_{26}H_{22}N_4O$	167-180	68.28	0.71
4p	OCH_3	4-OH	$C_{25}H_{20}N_4O_2$	147-150	65.58	0.73
4q	OCH_3	4-NH_2	$C_{25}H_{21}N_5O$	171-173	68.13	0.74
4r	OCH_3	4-OCH_3	$C_{26}H_{22}N_4O_2$	213-216	63.66	0.65

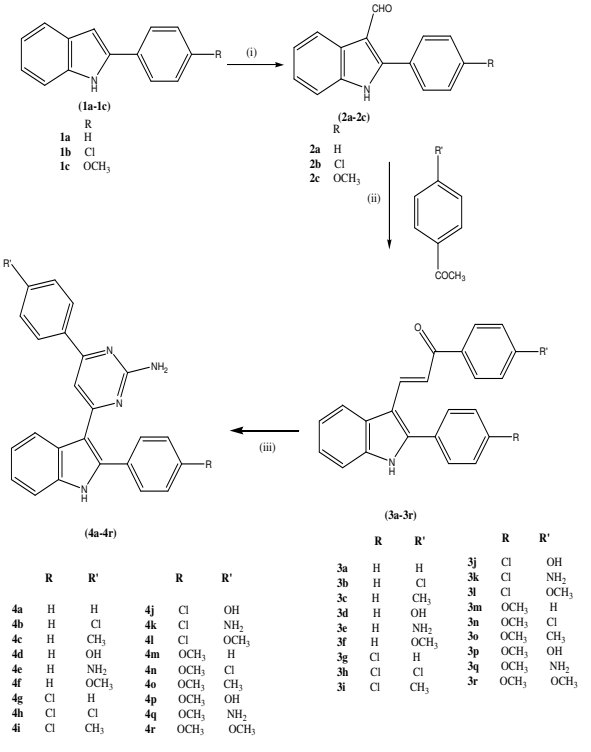

Fig 1: Scheme for synthesis of the pyrimidines
Reagent and conditions: (i) anhydrous DMF, POCl3; (ii) piperidine, ethylene glycol, reflux; (iii) guanidine HCl, sodium isopropoxide, reflux.

NMR (DMSO-d_6): δ 5.65 (s, 2H, NH_2), 7.12-8.10 (m, 14H, aromatic), 9.14 (s, 1H, -OH),12.13 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{24}H_{18}N_4O$ C, 76.17; H, 4.79; N, 14.81; O, 4.23 Found: C, 76.12; H, 4.69; N, 14.82. **4e** IR (cm^{-1} , KBr): 3416, 3356, 3126, 3011, 1632,1599,1448, 1241; ^1H NMR (DMSO-d_6): δ 5.40 (s, 2H, NH_2), 5.72 (s, 1H, NH_2), 7.13-8.22 (m, 14H, aromatic), 12.18 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{24}H_{19}N_5$ C, 76.37; H, 5.07; N, 18.55Found: C, 76.36; H, 5.06; N, 18.49. **4f** IR (cm^{-1} , KBr): 3402, 3362, 3128, 1638,1591,1438, 1242, 756; ^1H NMR (DMSO-d_6): δ 3.58 (s, 3H, -OCH_3), 5.64 (s, 2H, NH_2), 7.12-8.24 (m, 14H, aromatic), 11.89 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{25}H_{20}N_4O$ C, 76.51; H, 5.14; N, 14.28; O, 4.08 Found: C, 76.48; H, 5.18; N, 14.26. **4g** IR (cm^{-1} , KBr): 3450,3368, 3122, 3010, 1632,1598,1438, 1243, 763; ^1H NMR (DMSO-d_6): δ 5.51(s, 2H, NH_2), 7.10-8.26 (m, 14H, aromatic), 12.26 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{24}H_{17}ClN_4C$, 72.63; H, 4.32; Cl, 8.93; N, 14.12 Found: C, 72.67; H, 4.34; N, 14.09. **4h** IR (cm^{-1} , KBr): 3433, 3362, 3126, 3016, 1652,1589,1436, 1243, 748; ^1H NMR (DMSO-d_6): δ 5.59 (s, 2H, NH_2), 7.08 - 8.31 (m, 13H, aromatic), 12.19 (brs, 1H, NH); Elemental Analysis: Anal. Calcd for $C_{24}H_{16}C_{l2}N_4$ C, 66.83; H, 3.74; Cl, 16.44; N, 12.99Found: C, 66.84; H, 3.69; N, 19.92. **4i** IR (cm^{-1} , KBr): 3456, 3350, 3120, 3015, 1630,1591,1438, 1241, 766; ^1H NMR (DMSO-d_6): δ 2.40 (s, 3H, CH_3), 5.64 (s, 2H, NH_2), 7.16-8.09 (m, 13H, aromatic), 12.17 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{25}H_{19}ClN_4$ C73.08; H, 4.66; Cl, 8.63; N, 13.64 Found: C, 73.04; H, 4.69; N, 13.61. **4j** IR (cm^{-1} , KBr): 3460, 3365, 3123, 1636,1592,1438, 1241, 756; ^1H NMR (DMSO-d_6): δ 5.46 (s, 2H, NH_2), 7.19-8.03 (m, 13H, aromatic), 9.18 (s, 1H, -OH),12.27 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{24}H_{17}ClN_4O$ C, 69.82; H, 4.15; Cl, 8.59; N, 13.57; O, 3.88 Found: C, 69.80; H, 4.12; N, 13.54. **4k** IR (cm^{-1} , KBr): 3418, 3362, 3126, 3016, 1632,1588,1436, 1243, 748; ^1H NMR (DMSO-d_6): δ 5.62 (s, 2H, NH_2) 5.71 (s, 2H, NH_2), 7.14-8.01 (m, 13H, aromatic), 12.21 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{24}H_{18}ClN_5$ C, 69.98; H, 4.40; Cl, 8.61; N, 17.00 Found : C, 69.93; H, 4.43; N, 17.03. **4l** IR (cm^{-1} , KBr): 3422, 3362, 3123, 1638,1590,1438, 1243, 756; ^1H NMR (DMSO-d_6): δ, 3.46 (s, 3H, -OCH_3), 5.44 (s, 2H, NH_2), 7.14-8.02 (m, 13H, aromatic), 12.17 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{25}H_{19}ClN_4O$ C, 70.34; H, 4.49; Cl, 8.30; N, 13.12; O, 3.75Found C, 70.32; H, 4.43; N, 13.10. **4m** IR (cm^{-1} , KBr): 3512, 3356, 3126, 3012, 1630,1589,1448, 1241, 746; ^1H NMR (DMSO-d_6): δ 3.47 (s, 3H, -OCH_3), 5.54 (s, 2H, NH_2), 7.10-8.01 (m, 14H, aromatic),12.31 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{25}H_{20}N_4O$ C, 76.51; H, 5.14; N, 14.28; O, 4.08 Found C, 76.49; H, 5.12; N, 14.21. **4n** IR (cm^{-1} , KBr): 3456, 3350, 3120, 3015, 1630,1591,1438, 1241, 766; ^1H NMR (DMSO-d_6): δ 3.48 (s, 3H, -OCH_3), , 5.64 (s, 2H, NH_2), 7.29-8.11 (m, 13H, aromatic), 12.20 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{25}H_{19}ClN_4O$ C, 70.34; H, 4.49; Cl, 8.30; N, 13.12; O, 3.75 Found: C, 70.31; H, 4.46; N, 13.10. **4o** IR (cm^{-1} , KBr): 3436, 3360, 3126, 3015, 1632,1599,1432, 1243, 756; ^1H NMR (DMSO-d_6): δ 2.37 (s, 3H, CH_3), 3.49 (s, 3H, -OCH_3), 5.61 (s, 2H, NH_2), 7.20-8.04 (m, 13H, aromatic), 12.18 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{26}H_{22}N_4O$ C, 76.83; H, 5.46; N, 13.78; O, 3.94 Found: C, 76.79; H, 5.47; N, 13.74. **4p** IR (cm^{-1} , KBr): 3458,3432, 3351, 3123, 1632,1596,1438, 1247, 756; ^1H NMR (DMSO-d_6): δ 3.46 (s, 3H, -OCH_3), 5.55 (s, 2H, NH_2), 7.21-8.12 (m, 13H, aromatic), 9.16 (s, 1H, -OH),12.28 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{25}H_{20}N_4O_2$ C, 73.51; H, 4.94; N, 13.72; O, 7.83 Found: C, 73.48; H, 4.88; N, 13.74. **4q** IR (cm^{-1} , KBr): 3410, 3349, 3116, 3019, 1630,1579,1438, 1243, 747; ^1H NMR (DMSO-d_6): δ 3.42 (s, 3H, -OCH_3), 5.58 (s, 2H, NH_2), 5.70 (s, 2H, NH_2), 7.01-7.98 (m, 13H, aromatic), 12.24 (brs, 1H, NH) ; Elemental Analysis: Anal.

Calcd for $C_{25}H_{21}N_5O$ C, 73.69; H, 5.19; N, 17.19; O, 3.93 Found: C, 73.63; H, 5.18; N, 17.16. **4r** IR (cm^{-1} , KBr): 3435, 3368, 3128, 3017, 1632,1595,1436, 1243, 746; ^1H NMR (DMSO-d_6): δ 3.45 (s, 3H, -OCH_3), 5.56 (s, 2H, NH_2), 7.13-8.11 (m, 13H, aromatic), 12.21 (brs, 1H, NH) ; Elemental Analysis: Anal. Calcd for $C_{26}H_{22}N_4O_2$ C, 73.92; H, 5.25; N, 13.26; O, 7.57 Found: C, 73.90; H, 5.22; N, 13.25.

Characterization of synthesized compounds

Melting point was determined in an open capillary on Veego electronic apparatus (model:-VMP-D) and are uncorrected. Infrared IR spectra of the synthesized compounds were recorded on Shimadzu 8400-S FT-IR spectrophotometer using potassium bromide while ^1H NMR spectra were recorded in DMSO-d_6 using NMR Varian-Mercury 300 MHz spectrometer and chemical shifts are reported as parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Carbon, hydrogen and nitrogen (C, H and N, respective) analyses were carried out on a CE Instruments EA 1110 analyzer. To monitor the reactions and establish the identity and purity of reactants and products, thin layer chromatography (TLC) was performed on microscopic glass slides (2 x 7.5 cm) coated with silica gel-G (Merck), using toluene-methanol and chloroform-methanol as the solvent systems; the spots were visualized by exposure to iodine vapors, dilute sulfuric acid spray or ultraviolet (UV).

Animals

Wistar rats (150 - 200 g) and albino mice (25 - 30 g) of either sex were selected for the experiments. The animals were acclimatized for a period of two weeks in our laboratory environment prior to the study. They were housed in polypropylene cages, maintained under standard laboratory conditions, andfed with standard diet and water *ad libitum*. The principles of National Institutes of Health NIH Guide for Care and Use of Laboratory Animals (pub. No. 85-23, revised 2002) and the instructions given by the institutional Animal Ethical Committee (which had approved the animal studies vide letter no. COPS/174/2009-10) were followed throughout the experiment.

Assessment of anti-inflammatory activity

Anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema model [19]. Wistar rats (150 - 200 g) were divided into groups of six animals each. Group 1 served as control group and received saline, group 2 received indomethacin (10 mg/kg) and other groups received test drugs in a dose of 20 mg/kg. The drugs were prepared as homogenous suspensions in saline (0.9 % NaCl) and administered orally to animals. One hour after drug administration, each rat received a sun planter injection of 0.1 ml of 1 % carrageenan solution in its left paw. Measurement of the hind paw volume was carried out using a plethysmometer prior to treatment (Vo), and 2 and 3 h (Vt) after drug administration. Inhibition of swelling by the drugs was calculated using Eq 1.

Inhibition (%) = {[(Vt- Vo) control-(Vt-Vo) treated]/ (Vt- Vo) control} x100 …… (1)

where Vt and Vo relates to the mean volume of the hind paw of the rats (n=6) before and after anti-inflammatory agent treatment, respectively. The results are shown in Table 2.

Analgesic activity

All the compounds were tested for analgesic activity, using the acetic acid induced writhing method [20] in albino mice (25 - 30 g) of either sex. A 0.6 % v/v solution of acetic acid was used as writhing-inducing agent. The test compounds and indomethacin were administered orally 1 h prior to acetic acid injection. The mice were divided into groups of six animals each. Group 1 served as control group and received saline, group 2 received indomethacin (10 mg/kg) while the

other groups received test drugs in a dose of 20 mg/kg. The drugs were prepared as homogenous suspensions in saline (0.9 % NaCl) and administered orally to the animals. The writhing-inducing agent was administered intraperitonially. The number of writhings within a period of 20 min after drug administration was counted for each of the animal groups. Protection was calculated using Eq 2.

Table 2: Anti-inflammatory effects of the synthesized pyrimidinyl indoles derivatives in carrageenan-induced rat paw edema

Compound	Inhibition (%, mean± SEM)	
	2 h	3 h
4a	55.9 ± 2.7	67.7 ± 3.3**
4b	44.0 ± 2.0	54.7 ± 3.2
4c	60.3 ± 2.6	71.9 ± 2.8**
4d	61.3 ± 1.7	72.5 ± 1.2**
4e	62.4± 2.1	69.7 ± 3.2**
4f	59.4 ± 2.6	68.9 ± 1.1
4g	49.3 ± 1.6	55.8 ± 1.4
4h	67.2 ± 2.4	77.7 ± 2.9**
4i	50.6 ± 1.6	62.1 ± 2.4
4j	79.3 ± 0.6	87.3 ± 2.1**
4k	76.7 ± 1.7	88.2 ± 1.5**
4l	57.8 ± 2.2	65.3 ± 1.3**
4m	49.4 ± 3.3	54.8 ± 4.1
4n	59.6 ± 1.6	71.5 ± 2.2**
4o	49.9 ± 2.3	61.4 ±1.9
4p	63.8 ± 1.0	75.0 ± 3.1**
4q	69.2 ± 1.6	78.9 ± 2.1**
4r	54.2 ± 2.2	68.3 ± 1.8**
Control	--	--
Indomethacin	84.1 ± 1.04	92.7 ± 1.79

*Dose of compound = 20 mg/kg; indomethacin dose = 10 mg/kg; ** values are significant at p < 0.01.*

$$\text{Protection (\%)} = 100(100 - W_1)./ W_1 \quad \ldots\ldots\ldots\ldots (2)$$

where W_1 is the number of writhins of test group and W_2 is the number of writhings in the control group. The results are expressed as % decrease in writhing with respect to control and presented in Table 3.

Evaluation of acute ulcerogenesis

The studies were carried out using healthy Wistar rats (150 - 200 g) at thrice the dose (60 mg/kg) given in the anti-inflammatory studies. [21]. The animals were divided into different groups of six animals each. Group 1 served as control and received vehicle (1% aqueous methylcellulose dispersion) only, group 2 received the standard drug (indomethacin, 60 mg/kg) while the other groups were administered test compounds in doses molecularly equivalent to 60 mg/kg of indomethacin. The animals were fasted 6 h prior to receiving a single dose of either vehicle, standard or test compound. Following drug treatment, the rats were fed normal diet and then sacrificed after chloroform anaesthesia 14 h later.. The gastric mucosa of the rats was examined with the aid of a magnifier. For each stomach, the severity of mucosal damage was assessed according to the following scoring system: 0 = ≤ 5 punctiform lesions; 1 = > 5 punctiform lesions; 2 = one to five small ulcers; 3 = more than five small ulcers of one large ulcer; 4 =

Table 3: Analgesic effect of synthesized pyrimidinyl indole derivatives in acetic acid-induced writhing in rats

Compound	No. of writhings (mean±SEM)	Decrease in writhing (%)
4a	15.3 ± 0.60	55.9
4b	17.8± 0.47	48.8
4c	13.0 ± 0.76	62.6**
4d	10.0 ± 0.85	71.3**
4e	11.5± 0.61	66.8**
4f	29.3 ± 0.57	44.4
4g	14.7 ± 0.66	57.8**
4h	6.2 ± 0.47	82.2**
4i	14.7 ± 0.84	57.7**
4j	7.5 ± 0.30	78.4**
4k	8.2 ± 0.47	76.4**
4l	13.8± 0.49	60.3**
4m	12.0 ± 0.57	65.4**
4n	15.5 ± 0.42	55.4
4o	12.7 ± 0.74	63.5**
4p	11.8 ± 0.87	66.0**
4q	7.2 ± 0.85	79.3**
4r	19.3 ± 0.61	44.5
Control	34.8 ± 0.87	-
Indomethacin	6.0 ± 0.42	82.7

*Dose of compound = 20 mg/kg; indomethacin dose = 10 mg/kg; ** values are significant at p < 0.01*

more than one large ulcer. The mean score of each treated group minus the mean score of the control group was taken as the 'severity index' of gastric damage ($p < 0.001$). The data are shown in Table 4.

Table 4: Ulcerogenic activity (severity index) of synthesized compounds on the gastric mucosa of rats

Compound	Severity index
4a	1.49 ± 0.22
4b	1.52 ± 0.33
4c	1.83 ± 0.30**
4d	0.94 ± 0.21**
4e	1.13 ± 0.24**
4f	1.44 ± 0.27**
4g	0.88 ± 0.33**
4h	0.89 ± 0.19**
4i	1.65 ± 0.34**
4j	0.89 ± 0.30**
4k	1.12 ± 0.47**
4l	1.93± 0.40
4m	1.21 ± 0.27**
4n	1.52 ± 0.42**
4o	1.96 ± 0.34
4p	0.98 ± 0.47**
4q	1.01 ± 0.25**
4r	1.56 ± 0.49**
Control	0.00
Indomethacin	2.24 ± 0.30**

*Compound/indomethacin dose =60 mg/kg; ** values are significant at p < 0.01.*

Statistical analysis

The data were analysed by one-way ANOVA followed by Dunnet's t-test using computerized GraphPad Prism software, version 5. All the results are expressed as mean ± SEM. *P* values < 0.01 were considered significant.

Anti-inflammatory activity

The inhibition of swelling in carrageenan-induced edema in rat paw brought about by oral administration of the drug is shown in Table 2.

All the synthesized compounds tested for anti-inflammatory activity showed inhibition of edema ranging from 54.8 to 88.2 %, especially compounds 4d, e, j, k, q and p which demonstrated very good anti-inflammatory activity. In particular, 4-(2-amino-6-(2-(4-chlorophenyl)-1H-indol-3-yl)pyrimidin-4-yl)phenol (4j, 87.4 %) and 4-(4-aminophenyl)-6-(2-(4-chlorophenyl)-1H-indol-3-yl)pyrimidin-2-amine (4k, 88.2 %) were equipotent with indomethacin (92.7%) in inhibiting paw edema.

RESULTS

Analgesic activity

Compounds (4a - 4r) were tested for analgesic activity at the same dose as for antiinflammatory activity. Analgesic activity, measured by % protection against writhing in mice afforded by the drugs, is shown in Table 3. The compounds tested showed analgesic activity in the range of 44.5 to 82.3 %. Compounds 4h and 4q were especially potent showing 82.3 and 79.4 % protection, respectively, against acetic acid-induced writhing which compares well with 82.8 % for indomethacin. The analgesic effects of the compounds were in a similar way to their anti-inflammatory effects.

Acute ulcerogenesis

Ulcerogenic effect was studied at 60 mg/kg in rats (thrice the dose used in the earlier tests) and the data are shown in Table 4. The ulcerogenic effect, measured by severity index, of compounds 4a - 4r (0.88 to 1.96) was appreciably less than that of indomethacin (2.16). Ulcerogenic effect was especially low for compounds 4d, 4g, 4h 4j and 4p with severity index of 0.94, 0.88, 0.89, 0.89 and 0.98, respectively.

DISCUSSION

The purpose of the present study was to examine whether molecular modification of

indole and pyrimidine would result in molecules with good analgesic/anti-inflammatory actions and less ulcerogenic effects. A series of compounds was synthesized and evaluated for biological activities. The data obtained indicate that compounds having indole nucleus with substituted phenyl ring at 2-position and substituted pyrimidine ring at 3-position are not only good analgesic and anti-inflammatory agents but exhibited less ulcerogenic effect. Pyrimidines having substituted phenyl ring at 6^{th} position were in general more active than unsubstituted ones, indicating that the presence of functional group may be helpful in orienting the molecule in active site. Pyrimidines having hydroxyl and amino group substituted at para position of phenyl ring at position-6 were found to be more active than others, indicating that the presence of groups forming hydrogen bonds may aid more efficient binding with the receptors. The presence of an electronegative group at para position of 2-phenyl ring on indole nucleus seems to be important for good antiinflammatory action.

These findings suggest that the newly synthesized compounds will produce less gastric irritation and may be considered as safer drugs for treating inflammatory conditions. Compounds with 4-chlorophenyl and 4-aminophenyl rings at 6^{th} position of the pyrimidine ring showed higher analgesic activity than other substitutions.

CONCLUSION

Various derivatives of 3-(2-aminopyrimidin-4-yl) indoles were successfully synthesized and screened for their analgesic, anti-inflammatory and ulcerogenic activities. The tested compounds showed comparable analgesic and anti-inflammatory activities with indomethacin but were less irritant to the gastric mucosa. The results obtained support our hypothesis that chemical hybridization of indole nucleus and pyrimidine ring at the appropriate position affords compounds with good analgesic and antiinflammatory activities, and less gastric irritation. Further studies are planned to include *in vitro* COX 2 assay as well as elucidation of quantitative structure-activity relationship, leading to the derivation of a pertinent equation.

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