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ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY OF 4,5-DIHYDROPYRIMIDINE-5-CARBONITRILE DERIVATIVES: THEIR SYNTHESIS AND SPECTRAL ELUCIDATION

MOHAMMAD MUMTAZ ALAM[1], MYMOONA AKHTER[1], ASIF HUSAIN[1], AKRANTH MARELLA[1], OM PRAKASH TANWAR[1], RAHMAT ALI[1], SYED MISBAHUL HASAN[2], HARISH KUMAR[3], RASHIDUDDIN HAIDER[4] and MOHAMMAD SHAQUIQUZZAMAN*[1]

[1]Drug Designing and Medicinal Chemistry Lab, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi – 110062, India
[2]Pharmacy Department, College of Health Sciences, P. O. Box 2994, Sakaka, Kingdom of Saudia Arabia
[3]Ranbaxy Research Laboratories, Gurgaon, India
[4]Al-Hafeez Degree College, Veer Kunwar Singh University, Arrah-802301, India

Abstract: Thirteen new 6-(1-*H*-indole-2-yl)-4-oxo-2-[2-(substituted-benzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile derivatives were synthesized. The title compounds, hydrazones, were synthesized by reaction of hydrazine group of 2-hydrazinyl-4-(1-*H*-indole-2-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**2**) with different substituted aromatic aldehydes using a mixture (2:8, v/v) of glacial acetic acid and alcohol. The required intermediate compound **2** was synthesized from 2-mercapto-4-(1-*H*-indole-2-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile **1** upon nucleophilic attack by the hydrazine hydrate. Compound **1** was synthesized by modified Biginelli condensation method using indole-3-carbaldehyde, ethyl cyanoacetate and thiourea. The compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic and antimicrobial actions. Among the newer derivatives, one compound i.e., 6-(1-*H*-indole-2-yl)-4-oxo-2-[2-(2,6-dichlorobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**7**) emerged as lead compound having 71.14% inhibition of edema and 12.5 µg/mL MIC against both bacterial and fungal strains.

Keywords: cyanopyrimidine, Schiff's base, anti-inflammatory, analgesic, antimicrobial

Indole moiety was identified as one of the heterocyclic moieties for development of newer, better and safe anti-inflammatory agents after the discovery of indomethacin in 1963. Indole and its derivatives constitute the active class of compounds with wide range of biological activities, such as anti-inflammatory (1, 2), anti-microbial (3–5), anti-bacterial (6), anticonvulsant (7, 8), anti tuberculosis (9), antimalarial (10), anticancer (11) and cardiovascular (12).

The purines and pyrimidines are also valuable leads for drug design and discovery due to their key roles in various cellular processes. Since the discovery of 2-thiopyrimidine as antimetabolite more than half a century ago, hundreds of thiopyrimidine derivatives have been synthesized and evaluated for different kinds of biological activities. Pyrimidine and condensed pyrimidine derivatives possessing anti-inflammatory and analgesic activities are well documented in the literature (13–15). In addition to these activities, compounds containing pyrimidine moiety have been reported to possess numerous activities like hypoglycemic and hypolipidemic (16), antifungal (17), anticancer (18) and antiviral (19). Pyrimidine derivatives are also reported to possess dual activity e.g., anti-inflammatory and antimicrobial (20, 21). In continuation of our efforts (22, 23) in search of compounds with dual biological profile like anti-inflammatory and antimicrobial, we have synthesized a number of pyrimidine derivatives containing indole moiety as one of the substituents and evaluated them for biological activities.

EXPERIMENTAL

Chemistry

Chemicals were purchased from Merck and Sigma-Aldrich as 'synthesis grade' and used with-

* Corresponding author: e-mail: shaqiq@gmail.com, phone: + 91 11 26059688, ext. 5667, +91-9990663405; Fax: +91 11 26059663

out further purification. Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G plates (Merck No. 5544) using toluene : ethyl acetate : formic acid (5:4:1, v/v/v) as solvent system and the spots were located either under ultraviolet light or through exposure to iodine vapors.

The IR spectra were measured using a Bruker α-T spectrophotometer. ^{1}H-NMR spectra were recorded on Bruker Avance-400 MHz in $CDCl_3$ or DMSO-d_6 with tetramethylsilane (TMS) as an internal standard; chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS. Mass spectra were recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Spectral data are consistent with assigned structures. Elemental analyses were performed on a Perkin-Elmer-model-240 analyzer (C, H, N) and found within the range of ± 0.4% of theoretical values.

General procedure for the synthesis of 2-mercapto-4-(1-*H*-indol-2-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (1)

Indole-3-carbaldehyde (1 mmol), ethyl cyanoacetate (1 mmol) and thiourea (1 mmol) were dissolved in absolute alcohol. Potassium carbonate (3 mmol) was added to this reaction mixture and refluxed for 2 h. The solvent was concentrated and poured into ice cold water with stirring. The solution was neutralized with glacial acetic acid, which causes the separation of compound **1**, which was filtered, washed with water and recrystallized from methanol. Yield: 75%; m.p. 227–228°C; R_f: 0.72; IR (cm^{-1}): 3272 (-NH of amide), 3254 (NH of indole), 3225 (-NH), 2222 (C=N), 1672 (C=O), 1182 (C=S); ^{1}H-NMR (δ, ppm): 6.78 (s, 1H, H-2, indole ring), 6.92 (d, 1H, *J* = 8.0 Hz, H-7, indole ring), 7.03 (t, 1H, *J* = 7.6 Hz, H-6, indole ring), 7.10 (t, 1H, *J* = 7.6 Hz, H-5, indole ring), 7.60 (d, 1H, *J* = 8.0 Hz, H-4, indole ring), 9.48 (s, 1H, NH), 10.46 (s, 1H, NH of indole ring), 12.04 (bs, 1H, NH-C=O).

General procedure for the synthesis of 2-hydrazinyl-4-(1-*H*-indol-2-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2)

Compound **1** (1 mmol) was dissolved in absolute ethanol and hydrazine hydrate (99%; 4 mmol) was added and refluxed for 1 h. The reaction mixture was allowed to cool, which causes the separation of solid. The precipitated product was filtered and washed with water. It was recrystallized from ethanol. Yield: 68%; m.p. 293–294°C; R_f: 0.76; IR (cm^{-1}): 3281 (-NH of amide), 3258 (NH of indole), 3246 (-NH), 3228, 3210 (-NH_2), 2218 (C=N), 1682 (C=O); ^{1}H-NMR (δ, ppm): 3.90 (bs, 3H, $NHNH_2$), 6.74 (s, 1H, H-2, indole ring), 6.90 (d, 1H, *J* = 8.0 Hz, H-7, indole ring), 7.01 (t, 1H, *J* = 7.6 Hz, H-6, indole ring), 7.06 (t, 1H, *J* = 7.6 Hz, H-5, indole ring), 7.56 (d, 1H, *J* = 8.0 Hz, H-4, indole ring), 10.40 (s, 1H, NH of indole ring), 11.92 (bs, 1H, NH-C=O).

General procedure for the synthesis of 6-(1-*H*-indol-2-yl)-4-oxo-2-[2-(substituted benzylidene) hydrazinyl]-4,5-dihydropyrimidine-5-carbonitriles (3-15)

Compound **2** (1 mmol) was dissolved in a mixture of glacial acetic acid and alcohol (2:8, v/v). To this solution, alcoholic solution of substituted aromatic aldehydes (1.1 mmol) were added and refluxed for 5–6 h. The solvent was concentrated to half of volume and the mixture was poured into ice water. The precipitate obtained was filtered, washed with water and recrystallized from methanol.

6-(1*H*-Indol-2-yl)-4-oxo-2-(2-benzylidene-hydrazinyl)-4,5-dihydropyrimidine-5-carbonitrile (3).

Yield: 60%, m.p. 239–240°C, R_f: 0.72; IR (cm^{-1}): 3288 (NH of amide), 3244 (NH of indole ring), 3226 (NH), 2207 (C=N), 1681 (C=O), 1609 (N=CH); ^{1}H-NMR (δ, ppm): 6.72 (s, 1H, H-2, indole ring), 6.84 (d, 1H, *J* = 8.0 Hz, H-7, indole ring), 6.98 (t, 1H, *J* = 7.6 Hz, H-6, indole ring), 7.08 (t, 1H, *J* = 7.6 Hz, H-5, indole ring), 7.14–7.29 (m, 5H, arylidene ring), 7.64 (d, 1H, *J* = 8.0 Hz, H-4, indole ring), 8.03 (s, 1H, N=CH), 10.69 (s, 1H, NH of indole ring), 11.74 (s, 1H, NH), 12.08 (s, 1H, NHCO). MS (m/z): 354 (M^+).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(2-chlorobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (4)

Yield: 58%; m.p. 255–256°C; R_f: 0.78; IR (cm^{-1}): 3265 (NH of amide), 3252 (NH of indole ring), 3218 (NH), 2211 (C=N), 1674 (C=O), 1604 (N=CH); ^{1}H-NMR (δ, ppm): 6.76 (s, 1H, H-2, indole ring), 6.90 (d, 1H, *J* = 8.4 Hz, H-7, indole ring), 7.06 (t, 1H, *J* = 8.0 Hz, H-6, indole ring), 7.14 (t, 1H, *J* = 8.0 Hz, H-5, indole ring), 7.43–7.56 (m, 4H, arylidene ring), 7.70 (d, 1H, *J* = 8.4 Hz, H-4, indole ring), 8.07 (s, 1H, N=CH), 10.63 (s, 1H, NH of indole ring), 11.79 (s, 1H, NH), 12.03 (s, 1H, NHCO). MS (m/z): 389 (M^+), 391 (M + 2).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(4-chlorobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (5)

Yield: 66%; m.p. 265–266°C; R_f: 0.76; IR (cm^{-1}): 3277 (NH of amide), 3250 (NH of indole ring), 3221 (NH), 2213 (C=N), 1678 (C=O), 1611 (N=CH); ^{1}H-NMR (δ, ppm): 6.79 (s, 1H, H-2, indole ring), 6.92 (d, 1H, *J* = 8.4 Hz, H-7, indole ring), 7.08 (t, 1H, *J* = 8.0 Hz, H-6, indole ring), 7.12 (t, 1H, *J* = 8.0 Hz, H-5, indole ring), 7.42 (d, 2H, *J* = 8.8 Hz, H-2',6', arylidene ring), 7.70 (d, 2H, *J* = 8.8 Hz, H-3',5', arylidene ring), 7.74 (d, 1H, *J* = 8.4 Hz, H-4, indole ring), 8.09 (s, 1H, N=CH), 10.58 (s, 1H, NH of indole ring), 11.64 (s, 1H, NH), 12.10 (s, 1H, NHCO). MS (m/z): 389 (M^+), 391 (M + 2).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(4-bromo-benzylidene)-hydrazinyl)-4,5-dihydropyrimidine-5-carbonitrile (**6**)

Yield: 62%; m.p. > 300°C; R_f: 0.74; IR (cm^{-1}): 3283 (NH of amide), 3261 (NH of indole ring), 3234 (NH), 2217 (C=N), 1685 (C=O), 1608 (N=CH); ^{1}H-NMR (δ, ppm): 6.68 (s, 1H, H-2, indole ring), 6.94 (d, 1H, *J* = 8.0 Hz, H-7, indole ring), 7.04 (t, 1H, *J* = 7.6 Hz, H-6, indole ring), 7.10 (t, 1H, *J* = 7.6 Hz, H-5, indole ring), 7.40 (d, 2H, *J* = 8.4 Hz, H-2',6', arylidene ring), 7.62 (d, 2H, *J* = 8.4 Hz, H-3',5', arylidene ring), 7.72 (d, 1H, *J* = 8.0 Hz, H-4, indole ring), 8.12 (s, 1H, N=CH), 10.61 (s, 1H, NH of indole ring), 11.69 (s, 1H, NH), 12.07 (s, 1H, NHCO). MS (m/z): 433 (M^+), 435 (M + 2).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(2,6-dichlorobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**7**)

Yield: 56%; m.p. 259–260°C; R_f: 0.71; IR (cm^{-1}): 3294 (NH of amide), 3270 (NH of indole ring), 3220 (NH), 2221 (C=N), 1680 (C=O), 1613 (N=CH); ^{1}H-NMR (δ, ppm): 6.78 (s, 1H, H-2, indole ring), 6.90 (d, 1H, *J* = 8.4 Hz, H-7, indole ring), 7.08 (t, 1H, *J* = 8.0 Hz, H-6, indole ring), 7.14 (t, 1H, *J* = 8.0 Hz, H-5, indole ring), 7.44 (d, 1H, *J* = 8.8 Hz, H-4', arylidene ring), 7.61 (d, 2H, *J* = 8.8 Hz, H-3',5', arylidene ring), 7.76 (d, 1H, *J* = 8.4 Hz, H-4, indole ring), 8.14 (s, 1H, N=CH), 10.71 (s, 1H, NH of indole ring), 11.84 (s, 1H, NH), 12.14 (s, 1H, NHCO). MS (m/z): 423 (M^+), 425 (M + 2).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(2-hydroxy-benzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**8**)

Yield: 60%; m.p. 289–290°C; R_f: 0.80; IR (cm^{-1}): 3490 (OH), 3285 (NH of amide), 3258 (NH of indole ring), 3224 (NH), 2215 (C=N), 1677 (C=O), 1615 (N=CH); ^{1}H-NMR (δ, ppm): 6.74 (s, 1H, H-2, indole ring), 6.88 (d, 1H, *J* = 8.0 Hz, H-7, indole ring), 7.06 (t, 1H, *J* = 7.6 Hz, H-6, indole ring), 7.11 (t, 1H, *J* = 7.6 Hz, H-5, indole ring), 7.34–7.58 (m, 4H, arylidene ring), 7.69 (d, 1H, *J* = 8.0 Hz, H-4, indole ring), 8.05 (s, 1H, N=CH), 10.64 (s, 1H, NH of indole ring), 11.38 (s, 1H, OH), 11.90 (s, 1H, NH), 12.16 (s, 1H, NHCO). MS (m/z): 370 (M^+).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(4-nitrobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**9**)

Yield: 54%; m.p. 253–254°C; R_f: 0.75; IR (cm^{-1}): 3279 (NH of amide), 3246 (NH of indole ring), 3233 (NH), 2216 (C=N), 1673 (C=O), 1609 (N=CH); ^{1}H-NMR (δ, ppm): 6.78 (s, 1H, H-2, indole ring), 6.94 (d, 1H, *J* = 8.4 Hz, H-7, indole ring), 7.08 (t, 1H, *J* = 8.0 Hz, H-6, indole ring), 7.16 (t, 1H, *J* = 8.0 Hz, H-5, indole ring), 7.70 (d, 1H, *J* = 8.4 Hz, H-4, indole ring), 8.04 (d, 2H, *J* = 8.4 Hz, H-2',6' arylidene ring), 8.16 (s, 1H, N=CH), 8.23 (d, 2H, J=8.4Hz, H-3',5', arylidene ring), 10.52 (s, 1H, NH of indole ring), 11.69 (s, 1H, NH), 12.09 (s, 1H, NHCO). MS (m/z): 399 (M^+).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(4-methoxybenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**10**).

Yield: 62%; m.p. 241–242°C; R_f: 0.74; IR (cm^{-1}): 3286 (NH of amide), 3254 (NH of indole ring), 3236 (NH), 2212 (C=N), 1675 (C=O), 1605 (N=CH); ^{1}H-NMR (δ, ppm): 3.84 (s, 3H, OCH_3), 6.72 (s, 1H, H-2, indole ring), 6.96 (d, 1H, *J* = 8.0 Hz, H-7, indole ring), 7.05 (m, 3H, H-3',5', arylidene ring merged with H-6 of indole ring), 7.13 (t, 1H, *J* = 7.6 Hz, H-5, indole ring), 7.68 (d, 1H, *J* = 8.0 Hz, H-4, indole ring), 7.84 (d, 2H, *J* = 8.4 Hz, H-2',6', arylidene ring), 8.04 (s, 1H, N=CH), 10.58 (s, 1H, NH of indole ring), 11.92 (s, 1H, NH), 12.04 (s, 1H, NHCO). MS (m/z): 384 (M^+).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(3,4-dimethoxybenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**11**)

Yield: 68%; m.p. 247–248°C; R_f: 0.76; IR (cm^{-1}): 3290 (NH of amide), 3256 (NH of indole ring), 3230 (NH), 2218 (C=N), 1688 (C=O), 1604 (N=CH); ^{1}H-NMR (δ, ppm): 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.66 (s, 1H, H-2, indole ring), 6.94 (d, 1H, *J* = 7.6 Hz, H-7, indole ring), 7.04 (t, 1H, *J* = 7.2 Hz, H-6, indole ring), 7.08 (d, 1H, *J* = 7.6 Hz, H-5', arylidene ring), 7.16 (t, 1H, *J* = 7.2 Hz, H-5, indole ring), 7.48 (d, 1H, *J* = 7.6Hz, H-6' arylidene ring), 7.60 (s, 1H, H-2', arylidene ring), 7.72 (d, 1H, *J* = 7.6 Hz, H-4, indole ring), 8.00 (s, 1H, N=CH), 10.73 (s, 1H, NH of indole ring), 11.88 (s, 1H, NH), 12.01 (s, 1H, NHCO). MS (m/z): 414 (M^+).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(3-methoxy-4-hydroxy-benzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (12)

Yield: 64%; m.p. 233–234°C; R_f: 0.72; IR (cm^{-1}): 3470 (OH), 3274 (NH of amide), 3248 (NH of indole ring), 3222 (NH), 2226 (C=N), 1670 (C=O), 1610 (N=CH); ^{1}H-NMR (δ, ppm): 3.86 (s, 3H, OCH_3), 6.68 (s, 1H, H-2, indole ring), 6.81 (d, 1H, *J* = 7.6 Hz, H-5', arylidene ring), 6.90 (d, 1H, *J* = 7.6 Hz, H-7, indole ring), 7.08 (t, 1H, *J* = 7.2 Hz, H-6, indole ring), 7.12 (t, 1H, *J* = 7.2 Hz, H-5, indole ring), 7.16 (d, 1H, *J* = 7.6 Hz, H-6' arylidene ring), 7.76 (d, 1H, *J* = 7.6 Hz, H-4, indole ring), 7.82 (s, 1H, H-2', arylidene ring), 8.06 (s, 1H, N=CH), 10.78 (s, 1H, NH of indole ring), 11.52 (s, 1H, OH), 11.96 (s, 1H, NH), 12.11 (s, 1H, NHCO). MS (m/z): 400 (M^+).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(4-dimethylamino-benzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (13)

Yield: 60%; m.p. 269–270°C; R_f: 0.70; IR (cm^{-1}): 3285 (NH of amide), 3268 (NH of indole ring), 3234 (NH), 2224 (C=N), 1676 (C=O), 1607 (N=CH); ^{1}H-NMR (δ, ppm): 2.34 (s, 6H, 2 × CH_3), 6.72 (s, 1H, H-2, indole ring), 6.88 (m, 3H, H-3',5', arylidene ring merged with H-7, indole ring), 7.06 (t, 1H, *J* = 7.6 Hz, H-6, indole ring), 7.12 (t, 1H, *J* = 7.6 Hz, H-5, indole ring), 7.78 (d, 1H, *J* = 8.0 Hz, H-4, indole ring), 7.86 (d, 2H, *J* = 8.0 Hz, H-2',6', arylidene ring), 8.08 (s, 1H, N=CH), 10.48 (s, 1H, NH of indole ring), 11.86 (s, 1H, NH), 12.08 (s, 1H, NHCO). MS (m/z): 397 (M^+).

6-(1*H*-Indol-2-yl)-4-oxo-2-[2-(4-methylbenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (14)

Yield: 52%; m.p. 263–264°C; R_f: 0.74; IR (cm^{-1}): 3280 (NH of amide), 3252 (NH of indole ring), 3238 (NH), 2219 (C=N), 1679 (C=O), 1606 (N=CH); ^{1}H-NMR (δ, ppm): 2.36 (s, 3H, CH_3), 6.70 (s, 1H, H-2, indole ring), 6.90 (d, 1H, H-7, indole ring), 7.05 (t, 1H, *J* = 7.2 Hz, H-6, indole ring), 7.14 (t, 1H, *J* = 7.2 Hz, H-5, indole ring), 7.34–7.56 (m, 4H, H-2',3',5',6' arylidene ring), 7.74 (d, 1H, *J* = 7.6 Hz, H-4, indole ring), 8.04 (s, 1H, N=CH), 10.50 (s, 1H, NH of indole ring), 11.76 (s, 1H, NH), 12.05 (s, 1H, NHCO). MS (m/z): 368 (M^+).

6-(1H-Indol-2-yl)-4-oxo-2-[2-(2,4,6-trimethylbenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (15)

Yield: 50%; m,p, 225–226°C; R_f: 0.78; IR (cm^{-1}): 3284 (NH of amide), 3260 (NH of indole ring), 3231 (NH), 2214 (C=N), 1673 (C=O), 1607 (N=CH); ^{1}H-NMR (δ, ppm): 2.32 (s, 3H, CH_3), 2.46 (s, 6H, 2 × CH_3), 6.74 (s, 1H, H-2, indole ring), 6.82 (s, 2H, H-3',5', arylidene ring), 6.88 (d, 1H, *J* = 7.6 Hz, H-7, indole ring), 7.08 (t, 1H, *J* = 7.2 Hz, H-6, indole ring), 7.10 (t, 1H, *J* = 7.2 Hz, H-5, indole ring), 7.60 (d, 1H, *J* = 7.6 Hz, H-4, indole ring), 8.01 (s, 1H, N=CH), 10.56 (s, 1H, NH of indole ring), 11.74 (s, 1H, NH), 12.06 (s, 1H, NHCO). MS (m/z): 396 (M^+).

Pharmacology

Protocol of animal experiments has been approved by the Institutional Animal Ethics Committee (IAEC). All the compounds synthesized were evaluated for their anti-inflammatory activity; most potent compounds were further evaluated for analgesic activity and ulcerogenic studies.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema volume method of Winter et al. (24). Wistar rats (150–200 g) were randomly divided into groups of six animals. Group I was kept as control and was administered vehicle only, groups II was kept as standard and received indomethacin (10 mg/kg *p.o.*), other groups received test drugs in dose molecularly equivalent to indomethacin. Drug solutions were prepared as a homogeneous suspensions in aqueous solution of sodium CMC (0.5% w/v) and were administered orally to the animals. Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 mL was injected subcutaneously into the sub-plantar region of the right hind paw of each rat, 30 min after the administration of the test compounds and standard drugs. The paw volume was measured by saline displacement shown on screen of digital plethysmometer (Ugo Basile) at 2 and 3 h after carrageenan injection. All the results are expressed as the mean ± SEM. The edema volume in control group (V_c) and edema volume in groups treated with test compounds (V_t) was measured and the percentage inhibition of edema was calculated using the formula:

$$\text{Anti-inflammatory activity (\% inhibition)} = [(V_c - V_t)/ V_c] \times 100$$

where, V_c = paw volume of control group, V_t = paw volume of test group.

Analgesic activity

Compounds **4**, **5**, **6**, **7** and **11** were tested for analgesic activity. Analgesic activity was carried out by using acetic acid induced writhing method (25) in

Swiss albino mice (25–30 g) of either sex. Mice were divided into group of six in each. Group I was taken as control and received CMC suspension only, group II received reference drug indomethacin and rest of the groups were treated orally with test drugs (10 mg/kg) suspended in 1.0% CMC. A 1% aqueous acetic acid solution (0.1 mL) was used as writhing inducing agent. Acetic acid solution was injected intraperitonealy 3 h after the treatment with reference and test drugs to the various groups, respectively, and writhings were noted for 10–15 min after the acetic acid administration. All the results are expressed as the mean ± SEM. The percentage protection was measured using the formula:

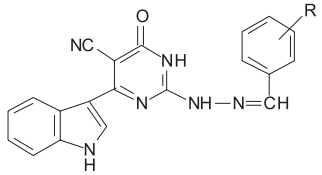
$$\text{Analgesic activity (\% protection)} = [(n - n')/n] \times 100$$

where, n = mean number of writhes of control group, n' = mean number of writhes of test group.

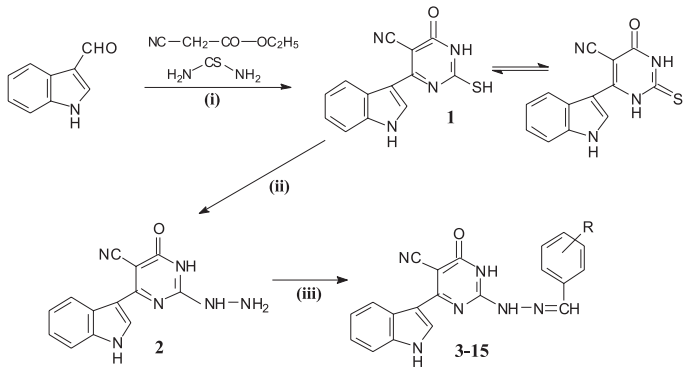
Acute ulcerogenesis

Acute ulcerogenesis test was done according to the reported method (26) for compounds selected for analgesic activity (compounds **4**, **5**, **6**, **7** and **11**). The studies were carried out on healthy Wistar rats (150–200 g) at a dose three times the anti-inflammatory dose *viz*. 30 mg/kg. The animals were divided into different groups of six animals each, group I served as control and received vehicle only and groups II received pure indomethacin 30 mg/kg. Other groups were administered with test compounds in doses molecularly equivalent to 30 mg/kg of indomethacin. Food but not water was removed

Table 1: Elemental analyses of compounds **3-15**.



Comp. No.	R	Molecular formula	Found (calcd.) (%)		
			C	H	N
3	H	$C_{20}H_{14}N_6O$ (354.37)	67.79 (67.54)	3.98 (3.97)	23.72 (23.74)
4	2-Cl	$C_{20}H_{13}ClN_6O$ (388.81)	61.78 (61.28)	3.37 (3.38)	21.61 (21.59)
5	4-Cl	$C_{20}H_{13}ClN_6O$ (388.81)	61.78 (61.52)	3.37 (3.37)	21.61 (21.60)
6	4-Br	$C_{20}H_{13}BrN_6O$ (433.27)	55.44 (55.28)	3.02 (3.01)	19.40 (19.39)
7	2,6-Cl_2	$C_{20}H_{12}Cl_2N_6O$ (423.26)	56.75 (56.84)	2.86 (2.85)	19.86 (19.85)
8	2-OH	$C_{20}H_{14}N_6O_2$ (370.37)	64.86 (64.69)	3.81 (3.81)	22.69 (22.70)
9	4-NO_2	$C_{20}H_{13}N_7O_3$ (399.37)	60.15 (60.52)	3.28 (3.27)	24.55 24.56)
10	4-OCH_3	$C_{21}H_{16}N_6O_2$ (384.40)	65.62 (65.27)	4.20 (4.19)	21.86 (21.88)
11	3,4-$(OCH_3)_2$	$C_{22}H_{18}N_6O_3$ (414.42)	63.76 (63.74)	4.38 (4.39)	20.28 (20.27)
12	3-OCH_3-4-OH	$C_{21}H_{16}N_6O_3$ (400.39)	63.00 (63.18)	4.03 (4.04)	20.99 (20.97)
13	4-$N(CH_3)_2$	$C_{22}H_{19}N_7O$ (397.44)	66.49 (66.17)	4.82 (4.81)	24.67 (24.68)
14	4-CH_3	$C_{21}H_{16}N_6O$ (368.40)	68.47 (68.12)	4.38 (4.37)	22.81 (22.82)
15	2,4,6-$(CH_3)_3$	$C_{23}H_{20}N_6O$ (396.45)	69.68 (69.53)	5.08 (5.07)	21.20 (21.19)



R = 3: H, 4: 2-Cl, 5: 4-Cl, 6: 4-Br, 7: 2,6-Cl_2, 8: 2-OH, 9: 4-NO_2, 10: 4-OCH_3, 11: 3,4-$(OCH_3)_2$, 12: 3-OCH_3, 4-OH, 13: 4-$N(CH_3)_2$, 14: 4-CH_3, 15: 2,4,6-$(CH_3)_2$

Reagents and conditions: (i): K_2CO_3, abs. ethanol, reflux; (ii): hydrazine hydrate, abs. ethanol, reflux; (iii): aromatic aldehyde, glacial acetic acid + ethanol (2:8, v/v), reflux.

Scheme 1.

24 h before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass and compared with that done by indomethacin. For each stomach the mucosal damage was assessed according to the following scoring system: 0.5: redness, 1.0: spot ulcers, 1.5: hemorrhagic streaks, 2.0: ulcers > 3 but < 5, 3.0: ulcers > 5.

The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Antimicrobial activity

The newly prepared compounds (**4**, **5**, **6**, **7** and **11**) were also screened for their antibacterial activity against *Escherichia coli* (ATCC-8739) and *Staphylococcus aureus* (ATCC-29737) bacterial strains at a concentration of 100 mg/mL by turbidity method (27) using norfloxacin as standard. Antifungal activity of the compounds was determined against *Penicillium citrum* and *Rhizopus oryza* using fluconazole as standard. Compounds inhibiting growth of one or more of the above microorganisms were further tested for minimum inhibitory concentration (MIC).

Statistical analysis

Data were expressed as the mean ± standard error (SE) of the mean. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with *post hoc* analysis. The Dunnett's test was applied to identify significance among groups.

RESULTS AND DISCUSSION

Chemistry

Thirteen new compounds, 6-(1*H*-indol-2-yl)-4-oxo-2-[2-(substituted benzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile derivatives (**3**–**15**) were synthesized and the reaction sequence for the synthesis is outlined in Scheme 1.

The title compounds, hydrazones, were synthesized by reaction of hydrazine group of 2-hydrazinyl-4-(1*H*-indol-2-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**2**) with different substituted aromatic aldehydes using a mixture (2:8, v/v) of glacial acetic acid and alcohol. The required intermediate compound **2** was synthesized from 2-mercapto-4-(1*H*-indol-2-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile **1** upon nucleophilic attack by the hydrazine hydrate. The compound **1** was synthesized by modified Biginelli condensation method using indole-3-carbaldehyde, ethyl cyanoacetate and thiourea. Spectral data of all the newly synthesized

compounds were in full agreement with the proposed structures.

In general, infra-red spectra (IR) revealed peak around 1610 cm^{-1} that indicates formation of hydrazones, the title compounds. Similarly, -NH of amide group, cyano group (C=N) and C=O showed peaks in the range of 3265–3294, 2207–2226 and 1673–1688 cm^{-1}, respectively, which indicates the formation of cyanopyrimidine.

In the ^{1}H-NMR spectra the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet in the range of δ 8.00–8.16 ppm corresponding to arylidene linkage and a broad singlet in range of 12.01–12.16 ppm corresponding to protons of CO-NH of cyanopyrimidine ring, respectively.

All the newly synthesized compounds were screened for their anti-inflammatory activity using Winter et al. method and showed inhibition of edema ranging from 32.72–71.14%. Among the compounds tested for anti-inflammatory activity 6-(1*H*-indol-2-yl)-4-oxo-2-[2-(4-chlorobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile

Table 2: Anti-inflammatory and analgesic activity along with ulcerogenic effect of the synthesized compounds **3**–**15**.

Compound %	Inhibition ± SEM[a]		Analgesic activity (writhing test)[a]		Severity index[b]
	After 2 h	After 3 h	No. of writhes/30 min	% Protection	
Control	–	–	–	–	0.000.00
Indomethacin	67.85 ± 1.42	76.01 ± 0.81	16.34 ± 0.6667	60.302 ± 2.780	0.84 ± 0.17**
3	27.14 ± 1.11**	42.28 ± 0.98**	–	–	–
4	50.48 ± 1.09**	60.97 ± 1.51**	23.84 ± 1.078**	42.65 ± 2.227**	0.34 ± 0.17
5	54.52 ± 1.45**	65.45 ± 1.07**	23 ± 0.3651**	44.35 ± 2.412**	0.17 ± 0.11
6	56.43 ± 2.44**	67.07 ± 1.26**	22 ± 0.5774**	47.01 ± 1.129**	0.17 ± 0.11
7	59.28 ± 1.26**	71.14 ± 1.07	20.5 ± 0.4282**	50.36 ± 2.328*	0.17 ± 0.11
8	26.43 ± 1.64**	40.85 ± 1.74**	–	–	–
9	39.05 ± 1.83**	52.64 ± 1.55**	–	–	–
10	41.67 ± 1.71**	54.67 ± 1.35**	–	–	–
11	46.67 ± 1.26**	58.53 ± 1.61**	24.84 ± 0.8333**	40.03 ± 2.583**	0.34 ± 0.17
12	24.52 ± 2.54**	38.62 ± 1.77**	–	–	–
13	18.57 ± 1.52**	32.72 ± 1.42**	–	–	–
14	20.24 ± 2.23**	34.35 ± 1.76**	–	–	–
15	15.00 ± 1.09**	29.47 ± 0.73**	–	–	–

*p < 0.05; **p < 0.01. [a]Relative to their respective standard (indomethacin) and data were analyzed by one-way ANOVA followed by Dunnett's test for n = 6. [b]Relative to the control and data were analyzed by one-way ANOVA followed by Dunnett's test for n = 6.

Table 3: Antibacterial and antifungal study; MIC results.

Compounds	*Staphylococcus aureus*	*Escherichia coli*	*Rhizopus oryza*	*Penicillium citrum*
Norfloxacin	6.25	6.25	–	–
Fluconazole	–	–	6.25	6.25
4	50	> 100	50	> 100
5	50	50	50	> 100
6	50	50	25	25
7	12.5	12.5	12.5	12.5
11	25	25	50	50

MIC (μg/mL) = minimum inhibitory concentration, i.e., the lowest concentration to completely inhibit bacterial growth.

(**5**), 6-(1*H*-indol-2-yl)-4-oxo-2-[2-(4-bromobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**6**) and 6-(1*H*-indol-2-yl)-4-oxo-2-[2-(2,6-dichlorobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**7**) showed 65.45%, 67.07% and 71.14%, respectively, inhibition of edema. From the above pharmacological results following remarks can be made:

- Compounds show an increase in percentage inhibition of edema with an increase in electronegativity in substitution. Example: compound **7**, which is 2,6-dichloro substituted, showed 71.14% inhibition and compound **5,** which is 4-chloro substituted, showed 65.45% inhibition.
- Replacement of electronegative group by electropositive group shows a decline in inhibition. Example: compound **5** which is chloro substituted showed 65.45% inhibition and compound **14,** which is methyl substituted, showed 34.35% inhibition.
- With an increase in methoxy groups activity also increases. Example: compound **11**, which is 3,4-dimethoxy substituted, showed 58.53% inhibition and compound 10, which is 4-methoxy substituted, showed 54.67% inhibition.
- *p*-Substituted compounds were found to be more active than *o*-substituted ones. Example: compound **5** which is *p*-chloro substituted showed 65.45% inhibition and compound 4, which is *o*-chloro substituted, showed 60.97% inhibition.

The one way ANOVA test was applied and test compounds were found to be significantly active compared to the control.

Analgesic activity was carried out on albino mice by Seigmund et al. method. Compounds (**4**, **5**, **6**, **7** and **11**) showing more than 75% of inhibition of indomethacin in swelling induced by carrageenan were further tested for analgesic activity.

The compounds tested showed analgesic activity in the range of 40.03–50.36% with compound **7** the most active (50.36%) (Table 2). According to structure activity relationship, it is clear that the 6-(1*H*-indol-2-yl)-4-oxo-2-[2-(2,6-dichlorobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**7**) was found to be a good anti-inflammatory and analgesic agent.

The most active compounds were also tested for their gastric irritation and it was found that these agents were less irritant to gastric mucosa than the standard, as indicated by the severity index (SI). The tested compounds (**4**, **5**, **6**, **7** and **11**) showed severity index ranging from 0.17 to 0.34 in comparison to 0.84 ± 0.17 of the standard.

As our aim was to synthesize compounds with dual effect i.e., anti-inflammatory and antimicrobial activity, the compounds which showed promising activity were also tested for antibacterial and antifungal activity using cup plate method. Compound **7** was found to be active against both bacterial and fungal strain with MIC of 12.5 µg/mL. Compound **4**, **5** and **11** were found to be more active against bacterial strain as compared to fungal strain. Compound **6** was found to be more active against fungal strain.

CONCLUSION

Thirteen new 6-(1*H*-indol-2-yl)-4-oxo-2-[2-(substituted benzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile derivatives were synthesized and screened for dual inhibition i.e., anti-inflammatory and antimicrobial activity. It was interesting to note that three derivatives i.e., compounds **5**, **6** and **7** were found to have anti-inflammatory and analgesic properties. These compounds were also tested for ulcerogenic activity and showed superior GI safety profile which is indicated by severity index.

Among the newer derivatives, one compound i.e., 6-(1*H*-indol-2-yl)-4-oxo-2-[2-(2,6-dichlorobenzylidene)-hydrazinyl]-4,5-dihydropyrimidine-5-carbonitrile (**7**) emerged as a lead compound having 71.14% inhibition of edema and 12.5 µg/mL MIC values against both bacterial and fungal strains. It is conceivable that these derivatives could be further modified to develop more potent and safer anti-inflammatory and antimicrobial agents. Further studies to acquire more information about quantitative structure-activity relationship (QSAR) are in progress in our laboratory.

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