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Anti-Inflammatory Screening of Some Novel Coumarin Derivatives

Jayendra Kanzariya[a], Prachi Trivedi[a], Sanjay Hadiyal[b], Ashish Dhamshaniya[b], Sangh Partap[c], Kaushik Neogi[d], Shradhaha Nakum[d], Jatin Upadhyay[a]*, Anamik Shah[a]*

[a]Department of Chemistry, Saurashtra University, Rajkot, India.
[b] Matushri Virbaima Mahila Science & Home Science College, Rajkot-36005
[c]Department of Pharmaceutical Chemistry, School of Pharmaceutical, Education and Research, Jamia Hamdard University, New Delhi, India
[d]Department of Pharmaceutics, Indian Institute of Technology, BHU, Varanasi, India
E-mail: jayu_bsc@yahoo.co.in
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Abstract: Multicomponent reactions are persuasive approach for developing a chemically diverse set of heterocyclic scaffolds with high yield. Using by conventional as well as microwave , synthesis of a series of new *9-nitro-4-phenyl-2-(pyrimidin-2-yl)-1,2-dihydro-5H-chromeno[4,3-d]pyrimidin-5-one* and assessed for their anti-inflammatory activity using the Carrageenan-induced hind paw edema method. Compounds EJ115-J, EJ115-E, & EJ115-A showed significant ($p < 0.001$) reduction of rat paw edema volume after 1 hr from the administration of the Carrageenan compared to the reference drug, indomethacin. Compounds EJ115-J and EJ115-E showed the highest anti-inflammatory activity, surpassing indomethacin after 4 hr with 65.61% and 60.99% inhibition, respectively.

Keywords: Biginelli Reaction, 4-hydroxy coumarin, pyrimidine-2-carboximidamide, Microwave irradiation, Anti inflammatory activity.

1.0 Introduction

Inflammation is a local reaction of the vascular and supporting elements of a tissue to injury resulting in the formation of a protein-rich exudates; it is a protective response of the nonspecific immune system that serves to localize, neutralize, or to destroy an injurious agent in preparation for the process of healing. The cardinal signs of inflammation are rubor (redness), calor (heat), dolor (pain), tumor (swelling), and functionlaesa (loss of function). Cause of inflammation includes physical agents, chemical agents, immunological reactions, and infection by pathogenic organism [1]. Inflammation is divided into acute and chronic patterns. The characteristics of acute inflammation are the exudation of fluid and

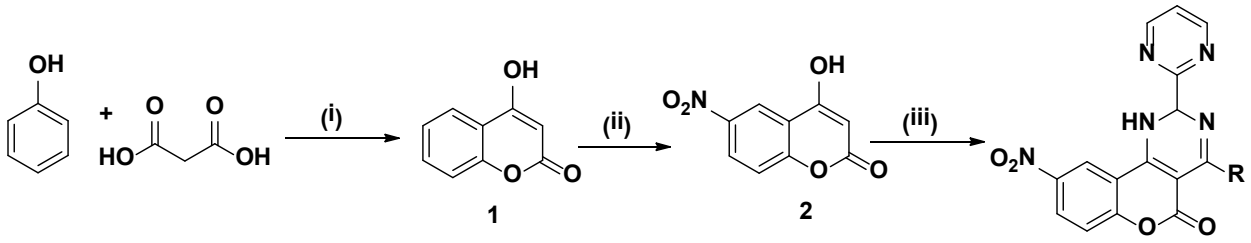
plasma proteins (oedema) and the emigration of leukocytes, predominantly neutrophils. Chronic inflammation is considered to be inflammation of prolonged duration (weeks or months) in which active inflammation, tissue destruction, and attempts at repair are proceeding simultaneously. Chronic inflammation includes some of the most common and disabling human diseases, such as rheumatoid arthritis, atherosclerosis, tuberculosis, and chronic lung diseases [2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the choice treatment in various inflammatory diseases such as arthritis, rheumatisms as well as to relieve the aches and pain of everyday life [3]. Classical NSAIDs exhibit their action by restricting the biosynthesis of prostaglandin, some of which are pro-inflammatory. This is essentially brought about by inhibiting the rate limiting cyclooxygenase (COX) enzyme involved in the inflammatory cascade [4]. Among different types of NSAIDs, imidazole and fused imidazole with six-membered rings which occupy central position are used as analgesic and anti-inflammatory agents [5].

Coumarin (2H-1-benzopyran-2-one) and its analogs comprise a very large class of compounds which are naturally found in plants [6]. Coumarins have attracted considerable attention due to their wide spectrum of pharmacological and biological activities as anti-coagulant, antitumor, antifungal, antiviral, antibacterial and anti-inflammatory agents [7]. Coumarins represent the core structure for many pharmaceutical compounds which have beneficial effects on human health [8,9]. Furthermore, it has been already reported that coumarin is a potential nucleus for the development of anti-inflammatory drugs [10–15]. Its hydroxyaromatic derivatives (5- or 6- or 7-hydroxycoumarin) show even more potent anti-inflammatory activity [16]. Motivated by the attempt to discover a new coumarin series with improved potency and COX-2 selectivity, we designed and synthesized some new *9-nitro-4-substituted-2-(pyrimidin-2-yl)-1H-chromeno [4,3-d]pyrimidin-5(2H)-one derivatives* (EJ115A-EJ115U) (Scheme 1). The anti-inflammatory effect of the newly synthesized compounds and a reference drug (Indomethacin) was evaluated by the Carrageenan-induced paw edema method.

2.0 Result and discussion

2.1 Chemistry

In the first step, 4-Hydroxycoumarin (1) was prepared according to shah and co-workers [17]. The 4-Hydroxy-6-nitro-2H-chromen-2-one (2) was synthesized by a solution of $NaNO_3$ (0.52 g, 6.17 mmol) in an ice-cooled sulfuric acid (20 mL) was added 4 -hydroxycoumarin (1.00 g, 6.17 mmol). After stirred at 0 ^{0}C for 1 h, the mixture was poured into an ice-cooled water to precipitate the product. Upon filtration, the crude product was recrystallized from EtOAc/hexanes (7:3) to give a white solid [18]. The 9-nitro-4-substituted-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one were prepared by two methods i.e. conventional method and microwave method. In conventional method, a mixture of 6-nitro 4-hydroxy coumarine (1.0 mmol), pyrimidine-2-carboximidamide (1.0 mmol) and aldehyde (1.0 mmol) was charged in 5 ml methanol. Catalytic amount of HCl was added, and the reaction mass was hated at 65 ^{0}C, for 4 hr reaction. After completion of the reaction, it was filtered and filtrate was evaporated and purified by column chromatography using pet ether: ethyl acetate(6:4). In microwave synthesis, a mixture of 9-nitro-4-phenyl-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (1.0 mmol), pyrimidine-2-carboximidamide (1.0 mmol), aldehyde (1.0 mmol), was charged in 5 ml methanol. Catalytic amount of HCl was added, and the reaction mass was hated at 75 ^{0}C, for 10 min, in microwave



Reaction and Condition
(i) POCl3 & Zinc Chloride, heat at 70 °C for 8-10hr
(ii) NaNO3 and H2SO4 at 0°C for 1hr.
(iii) pyrimidine-2-carboximidamide, substituted benzaldehyde and Con.HCl, Reflux 4 hr

Scheme 1.

reaction. Consumption of starting material was confirmed by TLC using Hexane: Ethyl acetate. After completion of the reaction, it was filtered and filtrate was evaporated and purified by column chromatography using pet ether: ethyl acetate(80:20).

The completion of reactions and purity of synthesized compounds was confirmed by TLC and column chromatography using pet ether: ethyl acetate and Hexane: Ethyl acetate as solvent system and melting points were recorded. The structures were characterized by elemental and spectral data analyses. The EJ115A to EJ115U recorded IR spectra in the range of 1602 to 1741 and 3300 to 3325 cm^{-1} which shows the appearance of C=O and NO_2 absorption bands respectively. A broad singlet proton signal (-NHD_2O exchangeable) was shown by H^1-NMR spectra at δ 10.06 to 11.5 ppm. Compound EJ115-A to EJ115U with ^{13}C NMR spectra as a prototype revealed two upfield peaks at δ 156.2 ppm of C=O carbons and the C=N carbon appeared downfield at δ 164.4 ppm.

2.2 Pharmacology

In the present investigation, the in vivo anti-inflammatory activity was evaluated for all the newly synthesized compounds (EJ-115A-EJ-115U) using the Carrageenan-induced rat paw edema protocol. The paw edema volume was evaluated 1, 2 and 4 h after the induction of inflammation. The anti-inflammatory activity of the tested compounds and reference drug (Indomethacin) were determined as the increase in paw edema volume and the results are summarized in Table 1 and as percentage inhibition (% inhibition) and summarized in Table 2. Results were expressed as the mean ± SE difference between control and treated animals using one way analysis of variance (ANOVA), followed by a Tukey-Kramer test for multiple comparisons.

Table2. The anti-inflammatory activity of the tested compounds and reference drug (Indomethacin) in carrageenan-induced rat paw edema assay, Values are expressed as mean ± SEM, (n = 8).

Code	1hour	2 hour	4 hour
EJ115-A	6.30±1.85	7.11±1.75	8.48±1.44
EJ115-B	7.95±1.13	8.81±1.22	10.47±1.33
EJ115-C	10.34±1.85	11.66±1.66	13.31±1.88
EJ115-D	11.33±1.52	12.75±1.69	14.14±1.72
EJ115-E	4.70±1.08	5.53±1.17	7.17±1.30
EJ115-F	7.50±0.42	9.08±0.92	10.74±1.23
EJ115-H	8.22±0.822	9.66±0.839	10.78±0.937
EJ115-I	13.34±0.75	14.33±0.589	15.19±0.619
EJ115-J	4.28±1.07	5.32±1.12	6.32±0.974

EJ115-L	6.76±.723	7.73±0.87	8.79±0.67
EJ115M	11.068±1.44	12.24±1.45	13.67±1.57
EJ115-O	8.22±1.18	9.37±1.37	10.72±1.70
EJ115-R	12.27±0.815	12.82±1.02	13.98±0.973
EJ115-S	6.72±1.23	7.912±0.887	9.22±0.849
EJ115-T	11.48±1.07	12.166±.853	13.422±.916
EJ115-U	7.45±0.940	8.71±1.103	9.70±1.14
Control	14.19±0.613	15.33±0.561	18.38±0.607
Indomethacine	3.606±.602	4.60±0.846	5.58± 0.366

In general, the data listed in Table 1 indicate that all of the newly synthesized compounds significantly ($p < 0.001$) reduce the rat paw edema volume 4 h after administration of the carrageenan compared to the reference drug, indomethacin. Compounds EJ115-J, EJ115-E, & EJ115-A showed a remarkable reduction of rat paw edema volume 1 h after administration of the Carrageenan compared to the reference drug, Indomethacine. All the tested compounds showed significant ($p < 0.01–0.001$) reduction of rat paw edema volume 4 h after the administration of the Carrageenan compared to the reference drug, Indomethacine (figure. 1).

Table 3: % Inhibition of acute inflammation (Carrageenan-induced paw edema) (EJ115A-EJ115U) (n = 8).

Group	% Inhibition of acute inflammation		
	1 h	2 h	4 h
EJ115-A	55.60	53.62	53.86
EJ115-B	43.97	42.53	43.03
EJ115-C	27.13	23.93	27.58
EJ115-D	20.15	16.82	23.06
EJ115-E	66.87	63.92	60.99
EJ115-F	47.14	40.76	41.56
EJ115-H	42.07	36.98	41.34
EJ115-I	5.99	6.52	17.35
EJ115-J	69.83	65.29	65.61
EJ115-L	52.36	49.57	52.17
EJ115M	22.05	20.15	25.62
EJ115-O	42.07	38.87	41.16
EJ115-R	13.53	16.37	23.93
EJ115-S	52.64	48.4	49.83
EJ115-T	19.09	20.67	27.05
EJ115-U	47.49	43.18	47.22
Control	0	0	0
Indomethacine	74.63	69.99	69.64

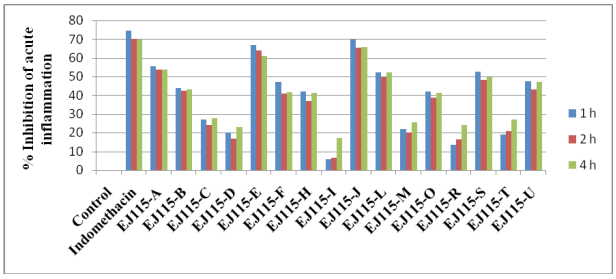


Figure 1. % Inhibition of acute inflammation (Carrageenan-induced paw edema)

3. Conclusion

In the present study, a series *9-nitro-4-substituted-2-(pyrimidin-2-yl)-1H-chromeno [4,3-d]pyrimidin-5(2H)-one derivatives* was synthesized and assessed for their anti-inflammatory activity using the Carrageenan-induced hind paw edema method. All the synthesized compounds exhibited significant anti-inflammatory activity, especially compounds EJ115-J and EJ115-E which showed maximum anti-inflammatory activity exceeding that of the reference Indomethacine itself. Furthermore, a molecular docking study of the entire synthesized compound was carried out to understand the binding interaction between the new compounds with the COX-1 and COX-2 enzymes. The results of this study suggest a good binding interaction which explains the significant biological activity. Further investigation of the binding mode and optimization of the structure of this promising series of compounds will be carried out in the future.

4. Experimental

4.1 General

All the chemicals used were of laboratory grade and procured from E. Merck (Darmstadt, Germany) and S.D. Fine Chemicals (Mumbai, India). Melting points were determined by open capillary tubes in a Hicon melting point apparatus

(Hicon, New Delhi, India) and are uncorrected. Purity of the compounds was checked by thin-layer chromatography (TLC) plates (silica gel G) by using ethyl acetate: Hexane (4:6), which were visualized by exposing to iodine vapours and UV light. The FT-IR spectra were recorded on (IR affinity SHIMADZU) FTIR spectrophotometer using KBr pellets; υ_{max} values are given in cm^{-1} and ^{1}H NMR spectra were recorded on Bruker model DRX-300 and 400MHz NMR spectrometer (^{1}H at 300 and 400 MHz, ^{13}C at 100 MHz) in DMSO-d_6. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as an internal standard and coupling constants (*J* values) are expressed in Hz. Mass spectra were recorded on LCMS/MS (PerkineElmer and LABINDIA, Applied Biosystem) model no. API 3000, presented as m/z. Elemental analysis (C, H and N) were undertaken with Perkin-Elmer model 240C analyzer. Analyses for C, H, and N were within ±0.4% of the theoretical values.

4.1.1. General procedure for the synthesis of the 9-nitro-4-substituted-2-(pyrimidin-2-yl)-1H-chromeno [4,3-d]pyrimidin-5(2H)-one derivatives

To a clean RBF, a mixture of (A) 6-nitro 4-hydroxy coumarine (1.0 mmol), (B) pyrimidine-2-carboximidamide (1.0 mmol), (C) aldehyde (1.0 mmol), was charged in 5 ml methanol. Catalytic amount of HCl was added, and the reaction mass was hated at 65 ^{0}C, for 4 hr reaction. Consumption of starting material was confirmed by TLC using Hexane: Ethyl acetate(6:4) . After completion of the reaction, it was filtered and filtrate was evaporated and purified by column chromatography using pet ether: ethyl acetate(80:20).

4.1.2. General procedure for the synthesis of the 9-nitro-4-phenyl-2-(pyrimidin-2-yl)-1H-chromeno [4,3-d]pyrimidin-5(2H)-one derivatives prepared in Microwave Irradiation.

To a clean 30ml microwave vial , a mixture of (A) 9-nitro-4-phenyl-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (1.0 mmol), pyrimidine-2-carboximidamide (1.0 mmol) and aldehyde (1.0 mmol) was charged in 5 ml methanol. Catalytic amount of HCL was added, and the reaction mass was hated at 75 ^{0}C, for 10 min in microwave reaction. Consumption of starting material was confirmed by TLC using Hexane: Ethyl acetate(6:4). After completion of the reaction, it was filtered and filtrate was evaporated and purified by column chromatography using pet ether: ethyl acetate.

4.1.3. Spectral Data of 9-nitro-4-phenyl-2-(pyrimidin-2-yl)-1H-chromeno [4,3-d] pyrimidin-5(2H)-one derivatives

4-(4-(tert-butyl) phenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115-a) Yield: 71%; mp, 196-198 °C; IR (KBr) (cm^{-1}): 3314, 3000, 2950, 1080, 1715, 1452, 1602, 1276; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 1.23 (s, 9H, CH_3), 6.3 (s, 1H), 7.08 (d, J = 5.3 Hz, 2H, ArH), 7.30 – 7.21 (m, 2H, ArH), 7.45 – 7.31 (m, 4H, ArH), 7.62 (s, 2H), 8.01 – 7.88 (m, 2H, ArH), 11.52(s, 1H, NH). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 174.2, 168.6, 162.2, 158.0, 154.7, 153.7, 146.4, 137.0, 129.6, 127.0, 125.9, 124.7, 121.3, 117.8, 117.3, 88.6, 34.3 and 31.4. MS (m/z) 455[M^+], 456 [M+1]; HRMS calcd for $C_{25}H_{21}N_5O_4$: 397.2002; Found: 397.2015; Anal. Calcd. for $C_{25}H_{21}N_5O_4$: C, 65.93; H, 4.65; N, 15.38; Found: C, 65.97; H, 4.67; N, 15.41%.

4-(2,5-dimethoxyphenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115b) Yield: 85%; mp, 190-192 °C; IR (KBr) (cm^{-1}): 3324, 2990, 2945, 1070, 1720, 1460, 1610, 1260. ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 3.7 (s, 6H, OCH3), 6.2 (s, 1H,), 6.8 (d, 2H, ArH, J=2.0 HZ), 7.1 (t, 1H,ArH, J=9.2 Hz),

7.5 (d, 2H,ArH, J=8.8Hz,), 8.5 (d, 2H, ArH, J=2.8 Hz), 8.6 (d, 2H, ArH, J=4.8Hz), 11.5 (s, 1H, NH). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 173.2, 164.2, 160.9, 158.0, 155.1, 153.7, 153.7, 150.9, 146.4, 131.9, 127.0, 124.7, 121.3, 118.8, 117.8, 114.3, 87.9, and 56.7. MS (m/z): 459[M^+], 460[M+1]; HRMS. Calcd. for $C_{23}H_{17}N_5O_6$: 397.2002; Found: 397.2015; Anal. Calcd. for $C_{23}H_{17}N_5O_6$: C, 60.13; H, 3.73; N, 15.24; Found: C, 60.16; H, 3.76; N, 15.27%.

9-nitro-2-(pyrimidin-2-yl)-4-(3,4,5-trimethoxyphenyl)-5H-chromeno[4,3-d] pyrimidin-5-one (EJ115c) Yield: 91%; mp 188-190 °C; IR (KBr) (cm^{-1}): 3300, 3000, 2972, 1710, 1612, 1492, 1006, 1228, 1344; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 3.64 (s, 9H,OCH_3), 6.2 (s,1H), 6.4(s, 2H, ArH), 7.5(d, 2H,J=9.2Hz, ArH), 8.3 (dd, 2H,J=7.6Hz, ArH), 8.6 (d, 2H, J=2.8Hz, ArH), 10.02 (s, 1H, NH). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 173.3, 171.1, 162.2, 158.0, 153.7, 151.9, 146.4, 143.9, 133.8, 127.07, 124.7, 121.3, 117.8, 110.2, 88.6, 60.6 and 56.7. MS (m/z): 487[M^+], 488 [M+1]; Anal. Calcd. for $C_{24}H_{17}N_5O_7$: C, 59.14; H, 3.52; N, 14.37; Found: C, 59.17; H, 3.54; N, 14.39%.

9-nitro-4-phenyl-2-(pyrimidin-2-yl)-5H-chromeno[4,3-d]pyrimidin-5-one (EJ115d) Yield: 87 %; mp, 176-178 °C; IR (KBr) (cm-1): 3310, 2972, 1710, 1614, 1529, 1220, 981; 1H NMR (400 MHz, DMSO-d6) δ(ppm): 8.58 (d, 2H,J=2.8Hz, ArH), 8.34-8.37 (dd, 2H, ArH), 7.5 (d, 2H,J=9.2Hz, ArH), 7.12-7.22 (m, 5H, ArH), 6.2 (s, 1H), 13.5 (s, 1H, NH). 13CNMR (100 MHz, DMSO-d6) δ (ppm): 166.4, 163.8, 156.9, 142.8, 140.6, 127, 126.5, 125.6, 125.3, 120.6, 119.9, 117.9, 104.2, 48.6, and 40.3. MS (m/z): 397[M+], 398 [M+1]; Anal. Calcd. for C21H11N5O7: C, 63.48; H, 2.79; N, 17.63; Found: C, 63.50; H, 2.81; N, 17.65;%.

4-(4-(benzyloxy)phenyl)-9-nitro-2-(pyrimidin-2-yl)-5H-chromeno[4,3-d]pyrimidin-5-one (EJ115e) Yield: 81%; mp, 170-172 °C; IR (KBr) (cm^{-1}): 3307, 3017, 2980, 1717, 1615, 1480, 1010, 1230, 1350; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 5.1 (s, 2H, CH_2), 6.3 (s, 1H), 6.9 (d, 2H, J=8.0Hz, ArH), 7.16-7.19 (t, 1H,ArH), 7.22-7.23 (m, 4H, ArH), 7.3 (d, 2H,J=7.6Hz, ArH), 7.42-7.56 (m, 3H, ArH), 7.5 (d, 1H, J = 8.0Hz, ArH), 8.5 (d, 2H, J=7.6Hz, ArH), 11.2 (s, 1H, NH). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 168.7, 167.6, 162.9, 160.4, 158.7, 153.7, 153.7, 146.4, 137.0, 130.9, 130.3, 130.4, 128.3, 128.1, 128.1, 127.0, 124.7, 121.3, 117.8, 117.3, 115.4, 88.6 and 70.8. MS (m/z): 503[M^+], 504[M+1]; Anal. Calcd. for $C_{28}H_{17}N_5O_5$: C, 66.80; H, 3.40; N, 13.91; Found: C, 66.83; H, 3.42; N, 13.94%.

4-(3-fluorophenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115f) Yield: 86%; mp, 176-178 °C; IR (KBr) (cm^{-1}): 3309, 3011, 1717, 1650, 1490, 1015, 1230, 1350; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.23 (s, 1H), 6.90-6.99 (m, 3H, ArH), 7.23-7.47 (m, 1H, ArH), 7.5 (d, 2H, J=8.8Hz,ArH), 8.37-8.38 (m, 3H, ArH), 8.6 (d, 2H,J=2.8Hz, ArH), 12.30 (s, 1H, NH). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 173.2, 171.8, 164.7, 162.6, 158.0, 153.7, 153.5, 140.5, 131.5, 129.8, 126.0, 125.6, 121.8, 121.3, 118.3, 118.2, 116.9, 115.6 and 88.5. MS (m/z): 417[M^+], 418[M+1]; Anal.Calcd.for $C_{21}H_{12}FN_5O_4$: C, 60.43; H, 2.90; N, 16.78; Found: C, 60.45; H, 2.93; N, 16.80%.

4-(4-methoxyphenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115h) Yield: 92%; mp, 182-184 °C; IR (KBr) (cm^{-1}): 3313, 3020, 2980, 1711, 1615, 1480, 1009, 1221, 1352; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 3.70 (s,3H, OCH_3), 6.19(s, 1H), 6.78 (d, 2H, J=8.8Hz, ArH), 7.04 (d, 2H, J=8.8Hz, ArH), 7.54 (d, 2H,J=8.8Hz, ArH), 8.35-8.80 (dd, 2H,ArH), 8.57(d, 2H, J=2.8Hz, ArH), 11.5 (s, 1H, NH). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 174.2, 173.5, 165.2, 164.3, 159.1, 154.9, 153.5, 148.7, 130.4, 127.3, 126.8,

123.7, 121.3, 118.8, 117.8, 115.1, 90.6 and 58.4. MS (m/z): 429[M^+], 430[M+1]; Anal. Calcd. for $C_{22}H_{15}N_5O_5$: C, 61.54; H, 3.52; N, 16.31; Found: C, 61.57; H, 3.55; N, 16.34%.

9-nitro-2-(pyrimidin-2-yl)-4-(thiophen-3-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115i) Yield: 76%; mp, 160-162 °C; IR (KBr) (cm^{-1}): 1089, 1712, 1606, 1492,1217 , 1002, 3010, 3307; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.0 (s, 1H), 6.55 (s, 1H,ArH), 7.14-7.18 (t, 1H,ArH), 7.3 (d, 1H,J=9.2Hz, ArH), 7.4 (d, 2H,J=3.2Hz, ArH), 7.5 (d, 1H, J=7.6Hz, ArH), 7.7 (d, 1H, J=7.6Hz,ArH), 8.6 (d, 2H, J=8.4Hz, ArH), 11.2 (s, 1H,NH).^{13}CNMR(100 MHz, DMSO-d_6) δ (ppm): 173.2, 168.3, 159.1, 157.7, 153.7, 146.4, 144.1, 134.4, 127.0, 126.8, 124.7, 121.3, 117.8, 117.3 and 92.1. MS (m/z): 405[M^+], 406[M+1]; Anal. Calcd. for $C_{19}H_{11}N_5O_4S$: C, 56.29; H, 2.74; N, 17.28; Found: C, 56.32; H, 2.77; N, 17.30%.

4-(2-chlorophenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115j) Yield: 84%; mp, 182-184 °C; IR (KBr) (cm^{-1}): 3324, 3015, 1708, 1610, 1495, 1025, 1220, 1341, 740; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.19 (s, 1H), 7.14-7.17 (m, 3H, ArH), 7.3 (d, 2H, J=), 7.4 (d, 1H,J=7.6Hz, ArH), 7.50-7.53 (t, 1H, ArH), 7.90 (d, 1H, J=2.8Hz, ARH), 8.62 (d,2H, J=3.2Hz, ArH), 11.3 (s, 1H, NH). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 169.2, 167.4, 160.9, 158.0, 153.9, 153.7, 146.4, 138.5, 135.3, 133.9, 131.6, 130.0, 127.3, 127.7, 124.7, 121.3, 117.8, 117.3 and 87.9. MS (m/z): 433[M^+], 434[M+1], 435[M+2]; Anal. Calcd. for $C_{21}H_{12}ClN_5O_6$: C, 58.14; H, 2.79; N, 16.14; Found: C, 58.17; H, 2.82; N, 16.17%.

4-(3,4-dimethoxyphenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115k) Yield: 87%; mp, 190-192 °C; IR (KBr) (cm^{-1}): 3300, 3024, 2972, 1710, 1612, 1492, 1006, 1228, 1344; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 3.74 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.19 (s,1H), 6.66-6.69 (t, 2H, ArH), 6.71 (d, 1H, J=4.4Hz, ArH), 7.52 (d,2H, J = 4.4Hz, ArH), 8.34-8.37 (dd, 2H), 8.60 (d,2H,J=2.8Hz, ArH), 11.30 (s, 1H, NH). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 173.2, 171.8, 162.2, 158.0, 153.7, 153.7, 153.1, 150.0, 146.4, 130.6, 127.0, 124.7, 123.1, 121.3, 117.8, 117.3, 114.1, 114.0, 88.6, 57.7 and 56.7. MS (m/z): 459[M^+], 460[M+1]; Anal. Calcd. for $C_{23}H_{17}N_5O_6$: C, 60.13; H, 3.73; N, 15.24; Found: C, 60.15; H, 3.76; N, 15.27%.

4-(2-hydroxyphenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115l) Yield: 55%; mp 178-180 °C; IR (KBr) (cm^{-1}): 1000, 1091, 1384, 1219,1427,1529,1612, 1712, 2972, 3021, 3350, 3412; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.19 (s,1H), 6.81-6.89 (t,1H,ArH), 7.07 (s,1H, ArH), 7.16-7.18 (t,1H, ArH), 7.31 (d,2H,J=4.4Hz, ArH), 7.40 (d,1H, J=4.8Hz ArH), 7.46 (d,2H, J=4.4Hz, ArH), 8.53 (d,2H, J=4.8Hz, ArH), 12.20 (s, 1H, NH).^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 171.2, 164.3, 161.9, 160.9, 158.0, 153.7, 153.7, 146.4, 135.0, 131.7, 127.0, 124.7, 122.0, 121.3, 120.5, 118.1, 117.8, 117.3 and 87.9. MS (m/z): 415[M^+], 416[M+1]; Anal. Calcd. for $C_{21}H_{13}N_5O_5$: C, 60.72; H, 3.15; N, 16.86; Found: C, 60.75; H, 3.18; N, 16.89%.

(E)-9-nitro-2-(pyrimidin-2-yl)-4-styryl-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115m) Yield: 61%; mp 182-184 °C; IR (KBr) (cm^{-1}): 916, 1335, 1620, 1658, 1440, 2950, 1741, 1244, 1014; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 5.70 (s,1H), 6.36 (d,1H,,J=2.0Hz, ArH), 6.7 (s, 1H), 7.19-7.52 (m, 7H, ArH), 8.3 (d, 4H, J=12.4Hz, ArH), 11.5 (s, 1H, NH); ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 165.7, 162.2, 142.7, 137.3, 130.0, 128.4, 128.0, 126.8, 125.9, 119.9, 117.8, 117.2, 104.3 and 91.2. MS (m/z): 425[M^+], 426[M+1]; Anal. Calcd. for $C_{23}H_{15}N_5O_6$: C, 64.94; H, 3.55; N, 16.46; Found: C, 64.97; H, 3.58; N, 16.49%.

4-(4-fluorophenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115p) Yield: 89%; mp 178-180 °C; IR (KBr) (cm^{-1}): 3310, 3011, 1717, 1650, 1491, 1015, 1233, 1352; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.24(s, 1H), 6.91-7.00(m, 3H, ArH), 7.22-7.25(t, 1H,ArH), 7.53(d, 2H, J=9.2Hz, ArH), 8.35-8.38(m, 2H, ArH), 8.58(d, 2H,J=2.8Hz, ArH), 10.80 (s, 1H, NH); ^{13}CNMR(100 MHz, DMSO-d_6) δ (ppm): 166.3, 163.5, 163.3, 161.1, 156.3, 144.1, 144.0, 142.8, 129.7, 129.6, 126.0, 122.6, 120.8, 120.2, 117.3, 113.4, 113.2, 112.2, 112.0 and 103.8. MS (m/z): 417[M^+], 418[M+1]; Anal. Calcd. for $C_{21}H_{12}FN_5O_4$: C, 60.43; H, 2.90; N, 16.78; Found: C, 60.46; H, 2.92; N, 16.81%.

4-(4-isopropylphenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115r) Yield: 87%; mp 192-194 °C; IR (KBr) (cm^{-1}): 3310, 3015, 2975, 2950, 1717, 1650, 1490, 1010, 1230, 1357; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 1.16 (s, 6H, CH3), 2.99-3.05 (m, 1H, CH), 6.20(s, 1H, CH*) 7.15-7.18 (t, 1H, ArH), 7.42(d, 2H, J=2.8Hz, ArH), 7.64(d, 2H, J=8.4Hz, ArH), 7.68(d, 2H, J=7.2Hz, ArH), 8.30(d, 1H, J=7.6Hz, ArH), 8.89(d, 2H, J=7.2Hz, ArH), 10.95(s, 1H, NH).^{13}CNMR(100 MHz, DMSO-d_6) δ (ppm): 177.2, 172.6, 162.3, 158.0, 153.7, 155.7, 152.1, 146.4, 137.5, 132.2, 127.0, 126.1, 125.1, 121.3, 117.8, 117.3, 88.6, 36.2 and 25.1. MS (m/z): 441[M^+], 442[M+1]; Anal. Calcd. for $C_{24}H_{19}N_5O_4$: C, 65.30; H, 4.34; N, 15.86; Found: C, 65.33; H, 4.37; N, 15.89%.

*4-(4-(dimethylamino)phenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (*EJ115s) Yield: 85%; mp 168-170 °C; IR (KBr) (cm^{-1}): 3320, 3017, 2975, 2951, 1717, 1650, 1490, 1015, 1230, 1350; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 1.00 (s, 6H, CH_3), 6.27 (s, 1H), 7.36 (d, 2H, J=7.6Hz, ArH), 7.44 (s, 2H,ArH), 7.5 (d, 2H, J=8.8Hz,ArH), 8.35-8.38 (dd, 2H, ArH), 8.5 (d, 2H, J=8.4Hz, ArH), 10.80 (s, 1H, NH); ^{13}CNMR(100 MHz, DMSO-d_6) δ (ppm): 166.4, 163.3, 156.3, 142.8, 128.4, 126.1, 122.0, 120.2, 120.2, 117.3, 103.7, 52.4, 40.0, 38.8 and 36.0. MS (m/z): 442[M^+], 443[M+1]; Anal. Calcd. for $C_{23}H_{18}N_6O_4$: C, 62.44; H, 4.10; N, 19.00; Found: C, 62.47; H, 4.13; N, 19.04%.

4-(3,4-difluorophenyl)-9-nitro-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one (EJ115t) Yield: 85%; mp 168-170°C; IR (KBr) (cm^{-1}): 3320, 1344, 1712, 1620, 1402, 1529, 2972, 952, 1280, 1099; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.19 (s, 1H), 6.95-7.00 (m, 1H,ArH), 7.16-7.20 (t, 2H,ArH), 7.32-7.36 (m, 1H), 7.42 (d, 2H,J=7.6Hz,ArH), 8.20 (s, 1H, ArH), 8.36 (d, 1H, J=8.8Hz,ArH), 8.6 (d, 2H,j=8.8Hz,ArH), 11.1 (s, 1H, NH). ^{13}CNMR(100 MHz, DMSO-d_6) δ (ppm): 169.2, 167.8, 162.2, 158.0, 153.7, 153.3, 150.9, 148.1, 146.4, 136.1, 127.0, 125.2, 124.7, 121.3, 117.8, 117.7, 117.3, 115.6 and 88.6. MS (m/z): 435[M^+], 436[M+1]; Anal. Calcd. for $C_{20}H_{11}F_2N_5O_4$: C, 57.94; H, 2.55; N, 16.09; Found: C, 57.94; H, 2.55; N, 16.09%.

9-nitro-4-(pyridin-4-yl)-2-(pyrimidin-2-yl)-1H-chromeno[4,3-d]pyrimidin-5(2H)-one(EJ115u) Yield: 76%; mp 172-174 °C; IR (KBr) (cm^{-1}): 3325, 3007, 1720, 1651, 1495, 1027, 1230, 1354; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.37 (s, 1H), 7.59 (d,2H, J=4.0Hz,ArH),7.83 (d, 2H, j=5.6Hz, ArH), 8.36-8.39 (dd,2H, ArH), 8.55 (d,2H,J=2.8Hz,ArH), 8.59 (d, 2H,J=6.4Hz,ArH), 11.70 (s, 1H, NH); ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 174.1, 172.6, 161.2, 158.0, 155.6, 153.7, 152.4, 146.4, 141.4, 129.1, 124.7, 121.3, 125.8, 117.2 and 90.5. MS (m/z): 400[M^+], 401[M+1]; Anal. Calcd. for $C_{20}H_{12}N_6O_4$: C, 60.00; H, 3.02; N, 20.99; Found: C, 60.00; H, 3.02; N, 20.99%.

4.2 Pharmacology

All the pharmacological experiments were performed according to ethical principles after

Institutional Animal Ethics Committee (IAEC) approval. The investigations were conducted on albino mice of either sex (25-30 g) obtained from central animal house facility, Hamdard University, New Delhi-62 Registration no.173/ Go/Re/S/2000CPCSEA. The animals were housed under optimal conditions and allowed free access to standard pellet diet and water (*ad libitum*).

4.3 Preparation of test samples for bioassay

All test samples (50 mg/kg) were suspended in a mixture of distilled water and 0.5% sodium carboxyl methylcellulose (CMC) and were given intraperitoneally (i.p.) to the test animals. The animals of the control group received the same experimental handling except that the test drug treatment was replaced with appropriate volumes of the vehicle. Indomethacine (10 mg/kg) for anti-inflammatory were used as reference drugs.

4.3.1. Anti-inflammatory activity

4.3.2. Carrageenan-induced hind paw oedema test

For the determination of anti-inflammatory effect, the carrageenan-induced paw oedema model [19] was employed. Sixty minutes after the i.p administration of control, test samples and reference drug, each rat was injected with a freshly prepared 0.1 mL of 1% carrageenan suspension in physiological saline (0.9% NaCl) into subplantar tissue of the right hind paw. For the control, 10 mL/kg saline solution was administered. Paw oedema was measured every 90 min for 6h after induction of inflammation. Mean values of the treated groups were compared with those of the control group and analyzed using statistical methods.

Statistical analysis

Results were expressed as means ± s.e.m. Statistical significance was analyzed by using the one-way analysis of variance followed by Tukey's Multiple Comparison Test where $p < 0.05$ was accepted to be a significant difference.

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