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Original article

Design, synthesis, antimicrobial, anti-inflammatory and analgesic activity of novel isoxazolyl pyrimido[4,5-*b*]quinolines and isoxazolyl chromeno[2,3-*d*] pyrimidin-4-ones

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ABSTRACT

Novel series of 2-methyl-3-{3-methyl-5-[(*E*)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydropyrimido [4,5-*b*]quinolin-4-ones **5** and 3-{3-methyl-5-[(*E*)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydro-2*H*-chromeno[2,3-*d*]pyrimidin-4-ones **7** have been synthesized from isoxazolyl cyanoacetamide synthon **2**. Compound **2** was obtained by reaction of 4-amino-3-methyl-5-styrylisoxazole **1** with ethyl cyanoacetate. Isoxazolyl pyrimido[4,5-*b*]quinolin-4-ones **5** were obtained from compounds **2** by condensation with *o*-nitro benzaldehyde followed by treatment with $SnCl_2$ and subsequent tandem *N*-acetylation and cyclodehydration with acetic anhydride. Compounds **2** were converted to isoxazolyl chromeno[2,3-*d*] pyrimidin-4-ones **7** by reaction with salicylaldehydes and subsequent cyclization with formaldehyde. Compounds **2**–**7** were characterized by IR, ^{1}H NMR, ^{13}C NMR, and Mass spectral data. The title compounds **5a**–**f** and **7a**–**g** were evaluated for their antimicrobial, anti-inflammatory and analgesic activity. Compounds **5d** and **7e** exhibited significant antimicrobial activity, potent anti-inflammatory and analgesic activities as that of standard drugs.

1. Introduction

The recent literature is enriched with progressive findings about the synthesis and pharmacological action of fused heterocycles. Since the discovery of 5-deaza flavin derivatives (**A**) as one of the naturally occurring essential redox coenzymes [1], extensive research in this field has been done to explore its functions in biological systems [2] and to develop a biomimetic model possessing the same basic skeleton [3,4]. 5-Deaza-10-oxaflavin (**B**) structure which is isosteric with 5-deaza flavin showed interesting biological activity [5] (Fig. 1). Similarly, the biological activity of substituted isoxazoles has made them a focus of medicinal chemistry over the years. Isoxazoles are potent analgesic, anti-inflammatory [6], antimicrobial [7], COX-2 inhibitory [8], antitubercular [9], and anticancer agents [10].

In view of this, we are of the opinion that, several pyrimidoquinolines (5-deaza flavin system) and chromenoquinolines (5-deaza-10-oxaflavin system) linked to isoxazole may possess remarkable pharmacological activity. Hence, we embarked on the synthesis of title compounds to improve specificity and efficacy of these scaffolds against micro organisms. In this context, we have utilized isoxazolyl cyanoacetamides as useful synthons, to build different heterocyclic systems, because the carbonyl and cyano functions of this synthon are suitably situated to enable reactions with common bidentate reagents. Moreover, the active hydrogen on C-2 of this compound can take part in a variety of condensation and substitution reactions. The main objective of the present study is to provide various organic heterocycles which can evolve as better chemotherapeutic agents. As a sequel to our studies on the synthesis and biological activity of heterocycles linked to isoxazole moiety [11–16], we undertook the synthesis of isoxazolyl pyrimido[4,5-*b*] quinolines and isoxazolyl chromeno[2,3-*d*]pyrimidin-4-ones in order to explore the pharmacological activity of these compounds. Herein, we report the discovery of pyrimidoquinolines and chromenoquinolines linked to isoxazole from isoxazolyl cyanoacetamide synthon and their antimicrobial, anti-inflammatory and analgesic activities.

2. Chemistry

The key intermediate, N_1-{3-methyl-5-[(*E*)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-cyanoacetamide **2** required for synthesis of target

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compounds was obtained by reacting4-amino-3-methyl-5-styrylisoxazole [17] **1** with ethyl cyanoacetate in refluxing ethanol. Compound **2** yielded the desired N_1-{3-methyl-5-[(*E*)-2-phenyl-1-ethenyl]-4-isoxazolyl}-(*E*)-2-cyano-3-(2-nitrophenyl)-2-propeanamides **3** on Knoevenagel condensation with *o*-nitro benzaldehyde in refluxing ethanol under the catalytic influence of piperidine. Further, compound **3** underwent reductive cyclization to afford the corresponding isoxazolyl quinoline derivatives *viz*., N_3-{3-methyl-5-[(*E*)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-amino-3-quinoline carboxamides **4a**–**f** on treatment with $SnCl_2$–EtOH. Finally, compounds **4** underwent tandem *N*-acetylation and cyclodehydration involving intramolecular cyclization to afford the title compounds *viz*., 2-methyl-3-{3-methyl-5-[(*E*)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydro pyrimido[4,5-*b*]quinolin-4-ones **5** on heating with acetic anhydride for 5 h (Scheme 1). The structures of the newly synthesized compounds **2**–**5** have been established based on their spectral (IR, ^{1}H NMR, ^{13}C NMR and MS) and analytical data.

Cyclocondensation of isoxazolyl cyanoacetamides **2** with salicylaldehydes in boiling absolute ethanol containing a catalytic amount of piperidine afforded the coumarin derivatives *viz*., N_3-[3-methyl-5-[(*E*)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-imino-2*H*-3-chromene carboxamides **6** in good yields (73–86%). The scope and generality of this reaction is illustrated by utilizing different substituted salicylaldehydes in the reaction to afford corresponding derivatives **6a**–**g** without any difficulty. Compounds **6** on heating with formalin in ethanol afforded the corresponding 3-{3-methyl-5-[(*E*)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydro-2*H*-chromeno[2,3-*d*]pyrimidin-4-ones (**7**) in good yields (Scheme 2). The structure of compounds **6** and **7** were confirmed by their spectroscopic (IR, ^{1}H NMR, ^{13}C NMR and MS) and analytical data.

3. Biological screening

3.1. Antimicrobial activity

The newly synthesized compounds **5a**–**f** and **7a**–**g** were evaluated for their *in vitro* antibacterial activity against Gram-positive bacteria *viz*. *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 511) and *Staphylococcus aureus* (MTCC 96) and Gram-negative bacteria *viz*. *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656) at 100 μg/mL concentration. The *in vitro* antibacterial activity of the tested compounds was assessed by minimum inhibitory concentration (MIC) using broth dilution method [18]. Ciprofloxacin was used as standard drug for comparison. The results of antibacterial screening (Table 1) reveal that the compounds **5a**–**f** and **7a**–**g** displayed a better activity and more active than the standard Ciprofloxacin. In series **5**, compounds **5d**, **5e**, and **5f** possessing chloro group as substituent on the benzene ring showed a better activity. Compound **5a** exhibited least activity because it has no substituent on benzene ring. In series **7**, compounds **7d**, **7e** and **7b**, **7c** carrying bromo and chloro groups as substituents on the benzene ring imparted a remarkable activity. However, the degree of inhibition varied both with the test compound and with the bacteria used in the present investigation. The compounds **7** having coumarin ring showed maximum activity by inhibiting the growth of all the bacteria under investigation to a greater extent in comparison with compounds **5**, and the standard drug Ciprofloxacin, and compounds **5d** and **7e** can be exploited for the formulation of bactericides after detailed study.

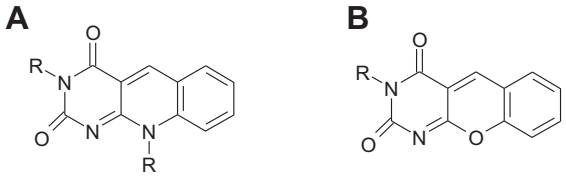


Fig. 1. Structures of biologically active 5-deaza flavine and 5-deaza-10-oxaflavin as design templates.

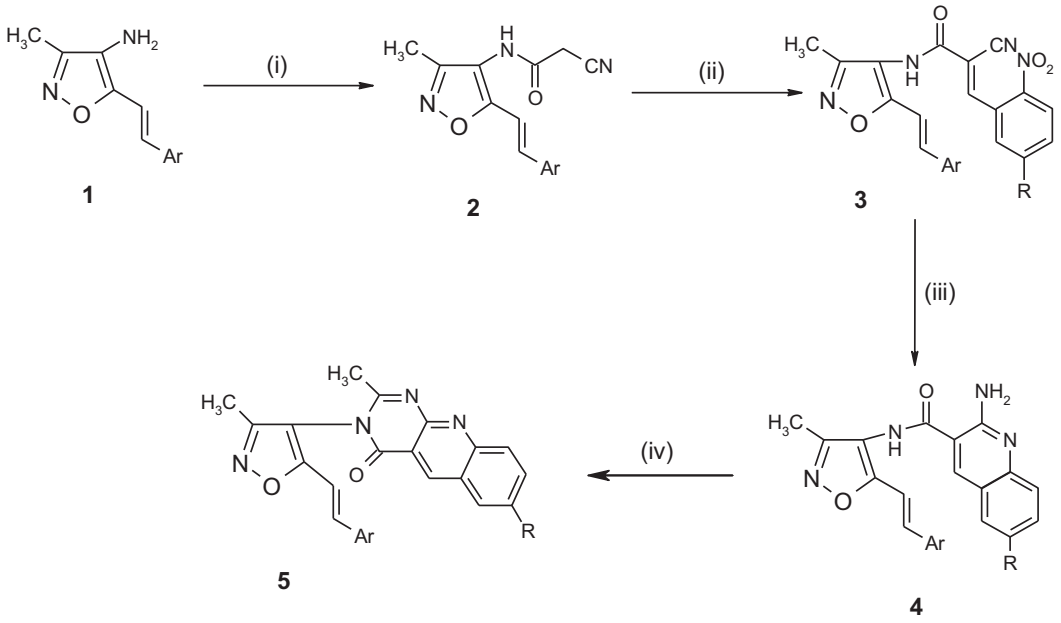
The title compounds **5a**–**f** and **7a**–**g** were also evaluated for their antifungal activity against *Fusarium oxysporum*, *Verticillium dahale*, *Alternaria solani*, *Rhizoctonia solani*, *Colletotrichum capsici* and *Pythium aphanidermatum* in acetone by agar cup bioassay method [19], using Clotrimazole as the standard drug. The antifungal activity results (Table 2) indicate that compounds **5a**–**f** and **7a**–**g** are significantly toxic towards all the fungi under investigation. In series **5**, compounds **5d**, **5e** and **5f**, exhibited high antifungal activity by inhibiting the growth of fungi to a remarkable extent, which may be due to the presence of chloro substituents on the benzene ring. In series **7**, compounds **7d**, **7e**, **7c** and **7b** possessing bromo, chloro and methyl substituents on benzene ring are highly toxic towards all the fungi. However, the degree of spore germination inhibition varied with the test compound as well as with the fungi under investigation. Compounds **5a**–**f** with pyrimidoquinoline ring is found to have high toxicity compared with that of compounds **7** bearing coumarin ring. The antifungal activity of the compounds **5a**–**f** and **7a**–**g** has shown better activity, when compared with the standard drug clotrimazole. It is note worthy that compounds **5d**, **5e**, **7e** and **7b** may be exploited for control of wilt diseases of different crops as fungicides after detailed study.

3.2. Anti-inflammatory activity

The anti-inflammatory activity of the newly synthesized compounds, **5a**–**f** and **7a**–**g**, were evaluated by applying carrageenan-induced paw edema test in rats [20], using Indomethacin as a reference drug for comparison. Results were expressed as mean ± S.E. Difference between vehicle control and treatment groups were tested using one way Analysis of variance (ANOVA) followed by the least significant difference (L.S.D.) Methods of statistical analysis were done according to Armitage et al. [21]. The anti-inflammatory activity data (Table 3) indicated that all the tested compounds in 60 min prior to carrageenan injection at dose of 10 mg/kg weight caused significant inhibition of paw edema response. A few among them have significant acute as well as residual anti-inflammatory activity. Compounds **7b**, **7d**, **7e** and **5e** caused significant decrease in paw edema after, 2, 3, 4 h drug administration, while **5b**, **5c** and **5d** gave their response after 2 h of administration and continued to the third hour. Compounds **7f** and **7c** showed the effect only after 2 h, but compounds **7g** and **5f** significantly decreased the paw edema after 4 h of post administration. On the other hand, compounds **5a** and **7a** were inactive towards carrageenan-induced edema in comparison to the reference drug Indomethacin, which markedly and significantly inhibited the paw edema after 2, 3, 4 h, of carrageenan injection. Thus compounds **7b**, **7d**, **7e**, **5e**, **5b**, **5c**, **5d**, **7f**, **7c** and **5f** have good anti-inflammatory activity. Among all the test compounds, it is interesting to note that compounds **5d** and **7e** showed best anti-inflammatory activity. The results are illustrated in Table 3.

3.3. Analgesic activity

The analgesic activity of the newly synthesized compounds **5a**–**f** and **7a**–**g** were also evaluated by applying the Hot plate test [22] (central analgesic activity), using Indomethacin as a reference drug (10 mg/kg-Po). The analgesic activity was measured at 0, 1 and



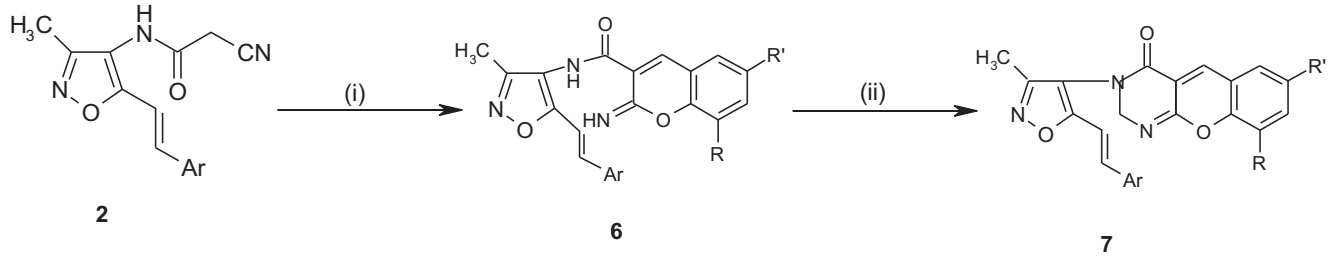
2: a, Ar = C_6H_5; b, Ar = 4-$CH_3C_6H_4$; c, Ar = 2-$CH_3C_6H_4$; d, Ar = 4-$OCH_3C_6H_4$;
e, Ar = 4-ClC_6H_4; f, Ar = 2-ClC_6H_4

3, 4 and **5**: a, Ar = C_6H_5, R = H; b, Ar = 4-$CH_3C_6H_4$, R = H; c, Ar = 4-$CH_3OC_6H_4$, R = H;
d, Ar = C_6H_5, R = Cl; e, Ar = 4-$CH_3C_6H_4$, R = Cl; f, Ar= 4-$CH_3OC_6H_4$, R = Cl;

Scheme 1. Synthesis of isoxazolyl pyrimido[4,5-*b*]quinolines (**5a**–**f**). Reagents and conditions. (i) Ethyl cyanoacetate, ethanol, Δ; (ii) *o*-nitro benzaldehyde, ethanol, piperidine, Δ; (iii) $SnCl_2$-ethanol; (iv) acetic anhydride, Δ.

2 h time intervals after pain induction. The results were recorded as the average value of six administrations and the percentage increase of the reaction time in comparison with the basal values. The results were presented in Table 4, and expressed as mean ± S.E. Difference between vehicle control and treatment groups were tested using one way Analysis of variance (ANOVA) followed by the least significant difference (L.S.D.) Methods of statistical analysis were done according to Armitage et al. [21]. A comparative study of the analgesic activity of the test compounds relative to the reference drug at different time intervals indicated that test compounds exhibited moderate analgesic activity at 60 min of reaction time (Table 4).

Compounds **7e**, **7c** and **5f** showed significant analgesic activity higher than that obtained by reference drug Indomethacin, after 1 h and 2 h post administration. While the compounds **5b**, **5c** and **5d** exhibited equipotent analgesic effects or slightly less than that of Indomethacin, after 1 and 2 h of their administration. Compounds **7b**, **7d** and **5e** exhibited significant analgesic activity higher than or slightly equipotent to Indomethacin only after 2 h of administration. Compounds **7g** and **7f** exhibited the analgesic effect after 1 h of administration only. Compounds **5a** and **7a** have no analgesic activity in comparison to the base line of same group 1 h and 2 h post administration. Therefore, compounds **7b**, **7c**, **5b**, **5c**, **7e**, **7d** and **5f** have significant analgesic activity. Among all the test



6 and **7**; a, Ar = C_6H_5, R =R'=H; b, Ar = 4-$CH_3C_6H_4$, R=H, R'= Cl; c, Ar = C_6H_5, R=R'= Cl;
d, Ar = 4-$OCH_3C_6H_4$, R=H, R'= Br; e, Ar = C_6H_5, R =R'= Br; f, Ar = C_6H_5, R=H,
R'= CH_3; g, Ar = C_6H_5, R=H, R'= 4-OCH_3

Scheme 2. Synthesis of isoxazolyl chromeno[2,3-*d*]pyrimidin-4-ones (**7a**–**g**). Reagents and conditions; (i) Salicylaldehyde, absolute ethanol, piperidine, Δ; (ii) Formalin, ethanol, Δ.

Table 1
Antibacterial activity of isoxazolyl pyrimido[4,5-*b*]quinolines (**5a**–**f**) and isoxazolyl chromeno[2,3-*d*]pyrimidin-4-ones (**7a**–**g**).

Compound	Minimum inhibitory concentration in μg/mL (MIC)					
	Gram +ve bacteria			Gram −ve bacteria		
	B. subtilis	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
5a	20	22	20	24	20	20
5b	19	23	25	23	18	15
5c	18	22	25	22	20	16
5d	8	16	9	10	7	5
5e	12	11	10	10	14	12
5f	12	10	13	14	11	14
7a	18	12	16	14	18	18
7b	14	16	15	11	18	18
7c	15	14	16	15	17	16
7d	12	10	8	15	13	10
7e	9	7	8	6	5	8
7f	15	18	16	12	15	18
7g	18	12	22	24	17	18
Ciprofloxacin	20	24	28	25	22	20

Negative control (acetone) – No activity.
Values are indicated in μg/mL.

compounds, it is interesting to note that, compound **7e** showed good analgesic activity. The results are illustrated in Table 4.

4. Conclusion

In conclusion, we report the synthesis of novel isoxazolyl pyrimido[4,5-*b*]quinolines and isoxazolylchromeno[2,3-*d*]pyrimidin-4-ones, using inexpensive and commercially available materials with potential medicinal properties. This synthesis benefits from a simple method of purification, which does not require chromatography. This ease of purification compliments this synthetic technology practical, easy to perform and facile. The newly synthesized novel isoxazolyl pyrimido[4,5-*b*]quinolines and isoxazolyl chromeno[2,3-*d*]pyrimidin-4-ones **5a**–**f** and **7a**–**g** were evaluated for their antimicrobial, anti-inflammatory and analgesic activity. It has been found that the derivatives **7b**, **7d**, **7e**, **5d** and **5f** exhibited good antimicrobial, anti-inflammatory and analgesic activity compared to the reference drugs. Among all the test compounds, it is interesting to note that compounds **5d** and **7e** showed best antimicrobial, anti-inflammatory and analgesic activity.

5. Experimental section

All the melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Analytical TLC was performed on Merck precoated 60 F_{254} silica gel plates. Visualization was done by exposure to iodine vapor. IR spectra (KBr pellet) were recorded on a Perkin–Elmer BX series FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. ^{13}C NMR spectra were recorded on a Bruker 75 MHz spectrometer. Chemical shift values are given in ppm (δ) with tetramethyl silane as an internal standard. Mass spectral measurements were carried out by EI method on a Jeol JMC-300 spectrometer at 70 eV. Elemental analyses were performed on a Carlo Erba 106 and Perkin–Elmer model 240 analyzers.

5.1. General procedure for the synthesis of N_1-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-cyanoacetamides (**2a**–**f**)

A mixture of compound **1** (1 mmol) and ethyl cyanoacetate (1 mmol) in ethanol (15 mL) were refluxed for 5 h. The reaction mixture was cooled and the solid obtained was filtered off and recrystallized from ethanol to give isoxazolyl cyanoacetamide **2**.

5.1.1. N_1-{3-Methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-cyanoacetamide (**2a**)

Yield 75%, mp 142–146 °C. IR (KBr) cm^{-1}: 3250 (NH), 2195 (C≡N), 1680 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.23 (s, 3H, CH_3), 3.72 (s, 2H, CH_2CN), 6.74 (d, 1H, CH=CH, J = 12 Hz), 6.91 (d, 1H, CH=CH, J = 12 Hz), 7.03–7.78 (m, 5H, ArH), 8.51 (s, 1H, CONH,

Table 2
Antifungal activity of isoxazolyl pyrimido[4,5-*b*]quinolines (**5a**–**f**) and isoxazolyl chromeno[2,3-*d*]pyrimidin-4-ones (**7a**–**g**) μg/mL.

Compound	Minimum inhibitory concentration in μg/mL (MIC)					
	F. oxysporum	*V. dahale*	*A. solani*	*R. solani*	*C. capsici*	*P. aphanidermatum*
5a	16	14	10	12	15	18
5b	12	12	10	14	18	15
5c	10	14	16	14	16	18
5d	8	9	10	11	11	15
5e	10	11	13	12	14	10
5f	11	13	12	14	16	17
7a	16	18	20	12	20	18
7b	9	10	8	11	12	9
7c	16	18	14	15	16	22
7d	18	14	12	14	12	20
7e	9	8	7	6	10	8
7f	20	22	20	24	18	22
7g	12	20	18	12	17	23
Clotrimazole	28	26	22	25	20	30

Negative control (acetone) – No activity.

Table 3
Anti-inflammatory activity of the isoxazolyl pyrimido[4,5-*b*]quinolines (**5a**–**f**) and isoxazolyl chromeno[2,3-*d*]pyrimidin-4-ones (**7a**–**g**).

Group[a]	Volume of edema (mL)[b]			
	1 h	2 h	3 h	4 h
5a	74.2 ± 5.4	86.0 ± 8.6	88.1 ± 7.5	90.4 ± 6.3
5b	53.6 ± 7.1	70.8 ± 5.7[c]	74.4 ± 4.6[c]	86.2 ± 5.1
5c	53.1 ± 5.4	58.2 ± 3.9[c]	64.6 ± 2.6[c]	79.5 ± 2.4
5d	50.7 ± 6.1	60.5 ± 6.7[c]	66.3 ± 6.4[c]	69.2 ± 4.2[c]
5e	60.2 ± 6.8	65.4 ± 5.9[c]	69.6 ± 8.1[c]	71.8 ± 8.3
5f	59.4 ± 7.2	72.6 ± 3.8	78.4 ± 2.4	74.3 ± 6.2[c]
7a	65.1 ± 7.2	82.1 ± 6.9	83.9 ± 2.3	79.4 ± 7.3
7b	62.4 ± 8.1	72.4 ± 6.3[c]	80.3 ± 7.2[c]	76.5 ± 6.7[c]
7c	64.2 ± 6.4	75.1 ± 3.5[c]	80.3 ± 3.1	85.2 ± 2.6
7d	59.6 ± 8.4	64.2 ± 7.8[c]	67.4 ± 6.5[c]	70.6 ± 6.1[c]
7e	51.5 ± 7.8	64.2 ± 8.4[c]	59.2 ± 7.2[c]	54.6 ± 6.8[c]
7f	86.2 ± 3.8	76.4 ± 8.1[c]	86.3 ± 7.1	88.5 ± 2.4
7g	51.8 ± 2.9	88.4 ± 5.9	82.3 ± 4.2	77.4 ± 2.3[c]
Control	59.2 ± 6.4	89.4 ± 7.3	96.2 ± 2.5	91.2 ± 2.3
Indomethacin	48.8 ± 5.0	43.6 ± 4.9[c]	46.4 ± 4.3[c]	47.8 ± 5.4[c]

[a] Dose levels, po: test compounds (10 mg/kg b.wt.), Indomethacin (10 mg/kg b.wt.).
[b] Values are expressed as means ± S.E. (number of animals $N = 6$ rats).
[c] Statistically significant compared to respective control values, $P < 0.05$ (Dunnett's test).

D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.25 (CH_3), 28.62 (CH_2), 100.55 (Isoxazole-C_4), 119.40 (=CH), 120.35 (=CH), 125.00 (Ar–C), 126.22 (Ar–C), 128.00 (Ar–C), 128.72 (Ar–C), 128.85 (Ar–C), 130.05 (Ar–C), 132.23 (CN), 156.55 (Isoxazole-C_3), 159.80 (Isoxazole-C_5), 182.20 (C=O). EI-MS (70 eV) *m/z*: 268 $[M + H]^+$. Anal. Calcd for $C_{15}H_{13}N_3O_2$: C, 67.41; H, 4.86; N, 15.73. Found: C, 67.45; H, 4.84; N, 15.76%.

5.1.2. N_1-{3-Methyl-5-[(E)-2-(4-methylphenyl)-1-ethenyl]-4-isoxazolyl}-2-cyanoacetamide (**2b**)

Yield 80%, mp 167–169 °C. IR (KBr) cm^{-1}: 3270 (NH), 2200 (C≡N), 1685 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.26 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 3.94 (s, 2H, CH_2CN), 6.63 (d, 1H, CH=CH, $J = 12$ Hz), 6.82 (d, 1H, CH=CH, $J = 12$ Hz), 6.94–7.53 (m, 4H, ArH), 8.31 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.01 (CH_3), 26.52 (Ar–CH_3), 28.69 (CH_2), 100.20 (Isoxazole-C_4), 120.05 (=CH), 120.45 (=CH), 125.20 (Ar–C), 127.01 (Ar–C), 128.15 (Ar–C), 128.55 (Ar–C), 128.90 (Ar–C), 129.98 (Ar–C), 132.15 (CN), 156.40 (Isoxazole-C_3), 159.50 (Isoxazole-C_5), 182.10 (C=O). EI-MS (70 eV) *m/z*: 282 $[M + H]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_2$: C, 68.32; H, 5.33; N, 14.94. Found: C, 68.37; H, 5.28; N, 14.90%.

Table 4
Analgesic activity of isoxazolyl pyrimido[4,5-*b*]quinolines (**5a**–**f**) and isoxazolyl chromeno[2,3-*d*]pyrimidin-4-ones (**7a**–**g**).

Group[a]	Reaction time (S)[b]		
	0 h	1 h	2 h
5a	13.4 ± 0.85	14.8 ± 0.58	16.5 ± 1.19
5b	13.8 ± 1.13	21.3 ± 1.54[c]	21.1 ± 1.27[c]
5c	13.7 ± 0.70	21.7 ± 1.35[c]	21.4 ± 1.13[c]
5d	13.4 ± 1.02	21.2 ± 1.13[c]	21.5 ± 1.15[c]
5e	17.5 ± 1.35	20.8 ± 1.20	22.3 ± 1.38[c]
5f	16.8 ± 1.15	25.1 ± 2.49[c]	22.6 ± 0.71[c]
7a	15.9 ± 0.84	17.7 ± 0.78	15.7 ± 0.87
7b	16.8 ± 0.97	19.1 ± 1.04	24.7 ± 1.21[c]
7c	17.2 ± 0.87	28.3 ± 1.67[c]	21.9 ± 2.64[c]
7d	14.5 ± 1.05[c]	18.8 ± 0.95[c]	22.6 ± 1.49[c]
7e	15.1 ± 0.93	23.3 ± 1.48[c]	23.4 ± 1.02[c]
7f	13.3 ± 1.02	21.2 ± 1.13[c]	21.5 ± 1.15
7g	13.8 ± 1.13	21.3 ± 1.57[c]	13.4 ± 0.91
Control	16.4 ± 0.65	16.2 ± 0.85	16.4 ± 1.20
Indomethacin	17.3 ± 0.80	21.8 ± 0.34[c]	22.4 ± 0.30[c]

[a] Dose levels, po: test compounds (10 mg/kg b.wt.), Indomethacin (10 mg/kg b.wt.).
[b] Values are expressed as means ± S.E. (number of animals $N = 6$ rats).
[c] Statistically significant compared to respective control values, $P < 0.05$ (Dunnett's test).

5.1.3. N_1-{3-Methyl-5-[(E)-2-(2-methylphenyl)-1-ethenyl]-4-isoxazolyl}-2-cyanoacetamide (**2c**)

Yield 84%, mp 155–157 °C. IR (KBr) cm^{-1}: 3300 (NH), 2180 (C≡N), 1670 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.34 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.75 (s, 2H, CH_2CN), 6.62 (d, 1H, CH=CH, $J = 12$ Hz), 6.81 (d, 1H, CH=CH, $J = 12$ Hz), 6.95–7.42 (m, 4H, ArH), 8.67 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.75 (CH_3), 26.45 (Ar–CH_3), 28.75 (CH_2), 100.75 (Isoxazole-C_4), 119.95 (=CH), 120.15 (=CH), 124.95 (Ar–C), 126.10 (Ar–C), 128.10 (Ar–C), 128.65 (Ar–C), 128.90 (Ar–C), 130.11 (Ar–C), 132.40 (CN), 156.40 (Isoxazole-C_3), 159.75 (Isoxazole-C_5), 182.55 (C=O). EI-MS (70 eV) *m/z*: 282 $[M + H]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_2$: C, 68.32; H, 5.33; N, 14.94. Found: C, 68.30; H, 5.35; N, 14.97%.

5.1.4. N_1-{5-[(E)-2-(4-Methoxyphenyl)-1-ethenyl]-3-methyl-4-isoxazolyl}-2-cyanoacetamide (**2d**)

Yield 74%, mp 133–136 °C. IR (KBr) cm^{-1}: 3275 (NH), 2190 (C≡N), 1680 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.23 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 3.96 (s, 2H, CH_2CN), 6.72 (d, 1H, CH=CH, $J = 12$ Hz), 6.93 (d, 1H, CH=CH, $J = 12$ Hz), 7.04–7.52 (m, 4H, ArH), 8.81 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.15 (CH_3), 28.10 (CH_2), 63.75 (OCH_3), 100.15 (Isoxazole-C_4), 119.35 (=CH), 120.40 (=CH), 125.11 (Ar–C), 126.10 (Ar–C), 128.09 (Ar–C), 128.58 (Ar–C), 128.76 (Ar–C), 130.15 (Ar–C), 132.16 (CN), 156.45 (Isoxazole-C_3), 159.75 (Isoxazole-C_5), 181.98 (C=O). EI-MS (70 eV) *m/z*: 298 $[M + H]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.05; N, 14.14. Found: C, 64.69; H, 5.01; N, 14.19%.

5.1.5. N_1-{5-[(E)-2-(4-Chlorophenyl)-1-ethenyl]-3-methyl-4-isoxazolyl}-2-cyanoacetamide (**2e**)

Yield 78%, mp 185–187 °C. IR (KBr) cm^{-1}: 3280 (NH), 2150 (C≡N), 1680 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.34 (s, 3H, CH_3), 3.73 (s, 2H, CH_2CN), 6.65 (d, 1H, CH=CH, $J = 12$ Hz), 6.84 (d, 1H, CH=CH, $J = 12$ Hz), 6.92–7.54 (m, 4H, ArH), 8.56 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.55 (CH_3), 28.75 (CH_2), 100.35 (Isoxazole-C_4), 119.15 (=CH), 120.33 (=CH), 125.45 (Ar–C), 127.23 (Ar–C), 128.11 (Ar–C), 128.65 (Ar–C), 128.90 (Ar–C), 130.17 (Ar–C), 132.23 (CN), 156.45 (Isoxazole-C_3), 159.75 (Isoxazole-C_5), 182.11 (C=O). EI-MS (70 eV) *m/z*: 302 $[M + H]^+$. Anal. Calcd for $C_{15}H_{12}N_3O_2Cl$: C, 59.80; H, 3.98; N, 13.95. Found: C, 59.83; H, 4.01; N, 13.92%.

5.1.6. N_1-{5-[(E)-2-(2-Chlorophenyl)-1-ethenyl]-3-methyl-4-isoxazolyl}-2-cyanoacetamide (**2f**)

Yield 80%, mp 190–192 °C. IR (KBr) cm^{-1}: 3250 (NH), 2150 (C≡N), 1675 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, CH_3), 3.71 (s, 2H, CH_2CN), 6.63 (d, 1H, CH=CH, $J = 12$ Hz), 6.82 (d, 1H, CH=CH, $J = 12$ Hz), 6.93–7.42 (m, 4H, ArH), 8.65 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.20 (CH_3), 28.61 (CH_2), 100.65 (Isoxazole-C_4), 119.38 (=CH), 120.33 (=CH), 125.11 (Ar–C), 127.45 (Ar–C), 128.15 (Ar–C), 128.45 (Ar–C), 128.93 (Ar–C), 130.17 (Ar–C), 132.01 (CN), 156.25 (Isoxazole-C_3), 159.78 (Isoxazole-C_5), 182.03 (C=O). EI-MS (70 eV) *m/z*: 302 $[M + H]^+$. Anal. Calcd for $C_{15}H_{12}N_3O_2Cl$: C, 59.80; H, 3.98; N, 13.95. Found: C, 59.76; H, 4.02; N, 13.91%.

*5.2. General procedure for the synthesis of N_1-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-(E)-2-cyano-3-(2-nitrophenyl)-2-propenamides (**3a–f**)*

A mixture of compound **2** (1 mmol) and *o*-nitro benzaldehyde (1 mmol) in ethanol (15 mL) containing a few drops of piperidine (3 drops) was refluxed for 4 h. The reaction mixture was cooled and the solid obtained was filtered off and recrystallized from ethanol to give **3**.

*5.2.1. N_1-{3-Methyl-5-[(E)-2-(2-phenyl)-1-ethenyl]-4-isoxazolyl}-(E)-2-cyano-3-(2-nitro phenyl)-2-propenamide (**3a**)*

Yield 80%, mp 161–163 °C. IR (KBr) cm^{-1}: 3200 (NH), 2175 (C≡N), 1680 (C=O), 1560, 1350 (NO_2). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 3H, CH_3), 6.01 (s, 1H, CH=C–CN), 6.62 (d, 1H, CH=CH, J = 12 Hz), 6.85 (d, 1H, CH=CH, J = 12 Hz), 6.93–7.62 (m, 9H, ArH), 8.51 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.26 (CH_3), 100.43 (isoxazole-C_4), 108.92 (=CH), 119.32 (=CH), 120.35 (=C–CN), 121.25 (=CH), 125.00 (Ar–C), 125.52 (Ar–C), 126.22 (Ar–C), 126.30 (Ar–C), 126.50 (Ar–C), 128.00 (Ar–C), 128.62 (Ar–C), 128.85 (Ar–C), 130.05 (Ar–C), 130.08 (Ar–C), 132.06 (Ar–C), 132.23 (Ar–C), 145.80 (=CH–Ar), 156.55 (isoxazole-C_3), 159.72 (isoxazole-C_5), 182.11 (C=O). EI-MS (70 eV) m/z: 401 $[M + H]^+$. Anal. Calcd for $C_{22}H_{16}N_4O_4$: C, 66.00; H, 4.00; N, 14.00. Found: C, 66.05; H, 4.04; N, 13.95%.

*5.2.2. N_1-{3-Methyl-5-[(E)-2-(4-methylphenyl)-1-ethenyl]-4-isoxazolyl}-(E)-2-cyano-3-(2-nitrophenyl)-2-propenamide (**3b**)*

Yield 82%, mp 182–185 °C. IR (KBr) cm^{-1}: 3215 (NH), 2167 (C≡N), 1670 (C=O), 1560, 1370 (NO_2). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.21 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 6.08 (s, 1H, CH=C–CN), 6.62 (d, 1H, CH=CH, J = 12 Hz), 6.84 (d, 1H, CH=CH, J = 12 Hz), 6.93–7.52 (m, 8H, ArH), 8.81 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.28 (CH_3), 28.75 (Ar–CH_3), 100.11 (isoxazole-C_4), 108.75 (=CH), 118.99 (=CH), 120.11 (=C–CN), 121.10 (=CH), 125.26 (Ar–C), 125.72 (Ar–C), 126.17 (Ar–C), 126.47 (Ar–C), 126.43 (Ar–C), 128.10 (Ar–C), 128.53 (Ar–C), 128.72 (Ar–C), 130.03 (Ar–C), 130.10 (Ar–C), 132.09 (Ar–C), 132.17 (Ar–C), 145.75 (=CH–Ar), 156.43 (isoxazole-C_3), 159.70 (isoxazole-C_5), 182.19 (C=O). EI-MS (70 eV) m/z: 415 $[M + H]^+$. Anal. Calcd for $C_{23}H_{18}N_4O_4$: C, 66.66; H, 4.34; N, 13.52. Found: C, 66.62; H, 4.30; N, 13.55%.

*5.2.3. N_1-{5-[(E)-2-(4-Methoxyphenyl)-1-ethenyl]-3-methyl-4-isoxazolyl}-(E)-2-cyano-3-(2-nitro phenyl)-2-propenamide (**3c**)*

Yield 76%, mp 170–172 °C. IR (KBr) cm^{-1}: 3210 (NH), 2180 (C≡N), 1670 (C=O), 1570, 1360 (NO_2). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.21 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.10 (s, 1H, CH=C–CN), 6.64 (d, 1H, CH=CH, J = 12 Hz), 6.81 (d, 1H, CH=CH, J = 12 Hz), 6.92–7.54 (m, 8H, ArH), 8.56 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.20 (CH_3), 63.82 (OCH_3), 100.40 (isoxazole-C_4), 109.01 (=CH), 119.11 (=CH), 120.31 (=C–CN), 121.29 (=CH), 125.07 (Ar–C), 125.50 (Ar–C), 126.28 (Ar–C), 126.42 (Ar–C), 126.63 (Ar–C), 128.11 (Ar–C), 128.63 (Ar–C), 128.70 (Ar–C), 130.01 (Ar–C), 130.09 (Ar–C), 132.08 (Ar–C), 132.15 (Ar–C), 145.75 (=CH–Ar), 156.40 (isoxazole-C_3), 159.68 (isoxazole-C_5), 182.01 (C=O). EI-MS (70 eV) m/z: 431 $[M + H]^+$. Anal. Calcd for $C_{23}H_{18}N_4O_5$: C, 64.18; H, 4.18; N, 13.02. Found: C, 64.24; H, 4.12; N, 13.08%.

*5.2.4. N_1-{3-Methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-(E)-3-(5-chloro-2-nitrophenyl)-2-cyano-2-propenamide (**3d**)*

Yield 70%, mp 156–158 °C. IR (KBr) cm^{-1}: 3210 (NH), 2176 (C≡N), 1685 (C=O), 1550, 1350 (NO_2). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, CH_3), 6.00 (s, 1H, CH=C–CN), 6.61 (d, 1H, CH=CH, J = 12 Hz), 6.82 (d, 1H, CH=CH, J = 12 Hz), 6.93–7.56 (m, 8H, ArH), 8.82 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.23 (CH_3), 100.39 (isoxazole-C_4), 108.79 (=CH), 119.11 (=CH), 120.28 (=C–CN), 121.15 (=CH), 125.11 (Ar–C), 125.48 (Ar–C), 126.21 (Ar–C), 126.28 (Ar–C), 127.53 (Ar–C), 128.03 (Ar–C), 128.51 (Ar–C), 128.72 (Ar–C), 129.98 (Ar–C), 130.07 (Ar–C), 132.09 (Ar–C), 132.26 (Ar–C), 145.73 (=CH–Ar), 156.43 (isoxazole-C_3), 159.65 (isoxazole-C_5), 182.20 (C=O). EI-MS (70 eV) m/z: 435 $[M + H]^+$. Anal. Calcd for: $C_{22}H_{15}N_4O_4Cl$: C, 60.82; H, 3.45; N, 12.90. Found: C, 60.89; H, 3.40; N, 12.97%.

*5.2.5. N_1-{3-Methyl-5-[(E)-2-(4-methylphenyl)-1-ethenyl]-4-isoxazolyl}-(E)-3-(5-chloro-2-nitrophenyl)-2-cyano-2-propenamide (**3e**)*

Yield 71%, mp 200–202 °C. IR (KBr) cm^{-1}: 3215 (NH), 2181 (C≡N), 1680 (C=O), 1560, 1365 (NO_2). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.32 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 6.20 (s, 1H, CH=C–CN), 6.61 (d, 1H, CH=CH, J = 12 Hz), 6.83 (d, 1H, CH=CH, J = 12 Hz), 6.95–7.62 (m, 7H, ArH), 8.84 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.21 (CH_3), 29.01 (Ar–CH_3), 100.21 (isoxazole-C_4), 108.65 (=CH), 119.23 (=CH), 121.01 (=C–CN), 121.25 (=CH), 125.09 (Ar–C), 125.52 (Ar–C), 126.28 (Ar–C), 126.30 (Ar–C), 127.42 (Ar–C), 128.06 (Ar–C), 128.43 (Ar–C), 128.52 (Ar–C), 129.99 (Ar–C), 130.08 (Ar–C), 132.03 (Ar–C), 132.17 (Ar–C), 145.76 (=CH–Ar), 156.41 (isoxazole-C_3), 159.45 (isoxazole-C_5), 182.26 (C=O). EI-MS (70 eV) m/z: 449 $[M + H]^+$. Anal. Calcd for $C_{23}H_{17}N_4O_4Cl$: C, 61.60; H, 3.79; N, 12.50. Found: C, 61.68; H, 3.71; N, 12.57%.

*5.2.6. N_1-{5-[(E)-2-(4-Methoxyphenyl)-1-ethenyl]-3-methyl-4-isoxazolyl}-(E)-3-(5-chloro-2-nitrophenyl)-2-cyano-2-propenamide (**3f**)*

Yield 84%, mp 188–190 °C. IR (KBr) cm^{-1}: 3200 (NH), 2188 (C≡N), 1675 (C=O), 1570, 1365 (NO_2). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.21 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.10 (s, 1H, CH=C–CN), 6.64 (d, 1H, CH=CH, J = 12 Hz), 6.82 (d, 1H, CH=CH, J = 12 Hz), 6.94–7.63 (m, 7H, ArH), 8.62 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.29 (CH_3), 64.23 (OCH_3), 100.21 (isoxazole-C_4), 108.63 (=CH), 119.18 (=CH), 120.15 (=C–CN), 121.32 (=CH), 125.01 (Ar–C), 125.48 (Ar–C), 126.18 (Ar–C), 126.36 (Ar–C), 127.41 (Ar–C), 128.03 (Ar–C), 128.53 (Ar–C), 128.76 (Ar–C), 130.11 (Ar–C), 130.20 (Ar–C), 132.09 (Ar–C), 132.31 (Ar–C), 145.71 (=CH–Ar), 156.45 (isoxazole-C_3), 159.61 (isoxazole-C_5), 182.16 (C=O). EI-MS: m/z 465 $[M + H]^+$. Anal. Calcd for $C_{23}H_{17}N_4O_5Cl$: C, 59.48; H, 3.66; N, 12.06. Found: C, 59.52; H, 3.71; N, 12.01%.

*5.3. General procedure for the synthesis of N_3-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-amino-3-quinolinecarboxamides (**4a–f**)*

A mixture of compound **3** (1 mmol) and $SnCl_2 \cdot 2H_2O$ (2 mmol) in ethanol (15 mL) was refluxed for 6 h. After cooling down to room temperature, the reaction mixture was poured in to ice-cold water. The precipitate that formed was filtered off, washed with ethanol and recrystallized from ethanol to get product **4**.

*5.3.1. N_3-{3-Methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-amino-3-quinolinecarboxamide (**4a**)*

Yield 70%, mp 185–187 °C. IR (KBr) cm^{-1}: 3300 (CONH), 3290, 3210 (NH_2), 1680 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.23 (s, 3H, CH_3), 6.21 (s, 2H, NH_2, D_2O exchangeable), 6.74–7.63 (m, 10H, ArH & 2H, CH=CH), 9.20 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.21 (CH_3), 100.36 (isoxazole-C_4), 108.72

(=CH), 120.42 (=CH), 121.21 (Ar–C), 125.06 (Ar–C), 125.56 (Ar–C), 126.18 (Ar–C), 126.28 (Ar–C), 126.52 (Ar–C), 126.73 (Ar–C), 128.00 (Ar–C), 128.56 (Ar–C), 128.96 (Ar–C), 130.05 (Ar–C), 130.25 (Ar–C), 132.08 (quinoline ring-C_3), 132.33 (quinoline ring-C_4), 145.72 (quinoline ring-C_2), 153.25 (isoxazole-C_3), 159.80 (isoxazole-C_5), 181.90 (C=O). EI-MS (70 eV) *m/z*: 371 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}N_4O_2$: C, 71.35; H, 4.86; N, 15.13. Found: C, 71.41; H, 4.80; N, 15.18%.

*5.3.2. N_3-{3-Methyl-5-[(E)-2-(4-methylphenyl)-1-ethenyl]-4-isoxazolyl}-2-amino-3-quinolinecarboxamide (**4b**)*

Yield 78%, mp 210–212 °C. IR (KBr) cm^{-1}: 3325 (CONH), 3300, 3200 (NH_2), 1670 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 6.53 (s, 2H, NH_2, D_2O exchangeable), 6.82–7.86 (m, 9H, ArH & 2H, CH=CH), 9.02 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.30 (CH_3). 28.63 (Ar–CH_3), 100.23 (isoxazole-C_4), 108.53 (=CH), 120.38 (=CH), 121.20 (Ar–C), 125.11 (Ar–C), 125.73 (Ar–C), 126.26 (Ar–C), 126.32 (Ar–C), 126.67 (Ar–C), 126.79 (Ar–C), 128.04 (Ar–C), 128.43 (Ar–C), 128.69 (Ar–C), 130.17 (Ar–C), 130.33 (Ar–C), 132.11 (quinoline ring-C_3), 132.42 (quinoline ring-C_4), 145.65 (quinoline ring-C_2), 153.11 (isoxazole-C_3), 159.73 (isoxazole-C_5), 181.68 (C=O). EI-MS (70 eV) *m/z*: 385 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}N_4O_2$: C, 71.87; H, 5.20; N, 14.58. Found: C, 71.80; H, 5.14; N, 14.64%.

*5.3.3. N_3-{5-[(E)-2-(4-Methoxyphenyl)-1-ethenyl]-3-methyl-4-isoxazolyl}-2-amino-3-quinolinecarboxamide (**4c**)*

Yield 72%, mp 194–196 °C. IR (KBr) cm^{-1}: 3350 (CONH), 3325, 3200 (NH_2), 1670 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.32 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 5.94 (s, 2H, NH_2, D_2O exchangeable), 6.38–7.85 (m, 9H, ArH & 2H, CH=CH), 9.02 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.28 (CH_3), 65.66 (OCH_3), 100.31 (isoxazole-C_4), 108.58 (=CH), 120.38 (=CH), 121.17 (Ar–C), 125.03 (Ar–C), 125.48 (Ar–C), 126.11 (Ar–C), 126.30 (Ar–C), 126.49 (Ar–C), 126.70 (Ar–C), 128.11 (Ar–C), 128.43 (Ar–C), 128.82 (Ar–C), 130.09 (Ar–C), 130.33 (Ar–C), 132.17 (quinoline ring-C_3), 132.43 (quinoline ring-C_4), 145.76 (quinoline ring-C_2), 153.13 (isoxazole-C_3), 159.73 (isoxazole-C_5), 181.56 (C=O). EI-MS (70 eV) *m/z*: 401 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}N_4O_3$: C, 69.00; H, 5.00; N, 14.00. Found: C, 68.08; H, 5.06; N, 14.05%.

*5.3.4. N_3-{3-Methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-amino-6-chloro-3-quinolinecarboxamide (**4d**)*

Yield 65%, mp 200–202 °C. IR (KBr) cm^{-1}: 3315 (CONH), 3300, 3210 (NH_2), 1680 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, CH_3), 5.93 (s, 2H, NH_2, D_2O exchangeable), 6.82–7.54 (m, 9H, ArH & 2H, CH=CH), 8.80 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.23 (CH_3), 100.28 (isoxazole-C_4), 108.63 (=CH), 120.35 (=CH), 121.15 (Ar–C), 125.07 (Ar–C), 125.43 (Ar–C), 126.26 (Ar–C), 126.30 (Ar–C), 126.49 (Ar–C), 127.45 (Ar–C), 128.15 (Ar–C), 128.47 (Ar–C), 128.73 (Ar–C), 130.11 (Ar–C), 130.23 (Ar–C), 132.11 (quinoline ring-C_3), 132.42 (quinoline ring-C_4), 145.62 (quinoline ring-C_2), 153.15 (isoxazole-C_3), 159.76 (isoxazole-C_5), 181.70 (C=O). EI-MS (70 eV) *m/z*: 405 $[M + H]^+$. Anal. Calcd for: $C_{22}H_{17}N_4O_2Cl$: C, 65.34; H, 4.20; N, 13.86. Found: C, 65.30; H, 4.24; N, 13.82%.

*5.3.5. N_3-{3-Methyl-5-[(E)-2-(4-methylphenyl)-1-ethenyl]-4-isoxazolyl}-2-amino-6-chloro-3-quinolinecarboxamide (**4e**)*

Yield 85%, mp 178–180 °C. IR (KBr) cm^{-1}: 3315 (CONH), 3300, 3220 (NH_2), 1670 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 6.04 (s, 2H, NH_2, D_2O exchangeable), 7.05–7.83 (m, 8H, ArH & 2H, CH=CH), 8.92 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.28 (CH_3), 28.53 (Ar–CH_3), 100.17 (isoxazole-C_4), 108.41 (=CH), 120.33 (=CH), 121.19 (Ar–C), 125.16 (Ar–C), 125.63 (Ar–C), 126.21 (Ar–C), 126.28 (Ar–C), 126.81 (Ar–C), 127.47 (Ar–C), 128.06 (Ar–C), 128.41 (Ar–C), 128.58 (Ar–C), 130.19 (Ar–C), 130.41 (Ar–C), 132.16 (quinoline ring-C_3), 132.46 (quinoline ring-C_4), 145.58 (quinoline ring-C_2), 153.09 (isoxazole-C_3), 159.61 (isoxazole-C_5), 181.51 (C=O). EI-MS (70 eV) *m/z*: 419 $[M + H]^+$. Anal. Calcd for $C_{23}H_{19}N_4O_2Cl$: C, 66.02; H, 4.54; N, 13.39. Found: C, 66.09; H, 4.48; N, 13.31%.

*5.3.6. N_3-{5-[(E)-2-(4-Methoxyphenyl)-1-ethenyl]-3-methyl-4-isoxazolyl}-2-amino-6-chloro-3-quinolinecarboxamide (**4f**)*

Yield 81%, mp 218–220 °C; IR (KBr) cm^{-1}: 3350 (CONH), 3320, 3200 (NH_2), 1675 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.31 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 6.12 (s, 2H, NH_2, D_2O exchangeable), 7.04–7.85 (m, 8H, ArH & 2H, CH=CH), 9.21 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.21 (CH_3), 65.09 (OCH_3), 100.28 (isoxazole-C_4), 108.51 (=CH), 120.56 (=CH), 121.19 (Ar–C), 125.11 (Ar–C), 125.35 (Ar–C), 126.15 (Ar–C), 126.42 (Ar–C), 126.53 (Ar–C), 127.59 (Ar–C), 128.01 (Ar–C), 128.33 (Ar–C), 128.72 (Ar–C), 130.11 (Ar–C), 130.39 (Ar–C), 132.22 (quinoline ring-C_3), 132.53 (quinoline ring-C_4), 145.78 (quinoline ring-C_2), 153.11 (isoxazole-C_3), 159.61 (isoxazole-C_5), 181.43 (C=O). EI-MS (70 eV) *m/z*: 435 $[M + H]^+$. Anal. Calcd for $C_{23}H_{19}N_4O_3Cl$: C, 63.59; H, 4.37; N, 12.90. Found: C, 63.50; H, 4.30; N, 12.96%.

*5.4. General procedure for the synthesis of 2-methyl-3-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydropyrimido[4,5-b]quinolin-4-ones (**5a**–**f**)*

A solution of **4** (1 mmol) in acetic anhydride (15 mL) was refluxed for 5 h. The reaction mixture was allowed to cool down to room temperature The precipitate that formed was filtered off, washed with ethanol and recrystallized from ethanol to give compound **5**.

*5.4.1. 2-Methyl-3-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydropyrimido[4,5-b]quinolin-4-one (**5a**)*

Yield 85%, mp 220–222 °C. IR (KBr) cm^{-1}: 1670 (C=O), 1640 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.23 (s, 3H, isoxazole CH_3), 2.41 (s, 3H, pyrimidine-CH_3), 6.63–7.54 (m, 10H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.44 (isoxazole-CH_3), 22.45 (pyrimidine-CH_3), 108.03 (isoxazole-C_4), 110.15 (=CH), 111.12 (=CH), 123.05 (Ar–C), 125.00 (Ar–C), 126.42 (Ar–C), 126.95 (Ar–C), 127.93 (Ar–C), 128.83 (Ar–C), 128.91 (Ar–C), 129.34 (Ar–C), 131.15 (Ar–C), 133.70 (Ar–C), 134.65 (Ar–C), 135.11 (Ar–C), 135.88 (Ar–C), 140.44 (Ar–C), 147.08 (Ar–C), 153.55 (isoxazole-C_3), 156.57 (isoxazole-C_5), 162.80 (pyrimidine ring-C), 172.50 (C=O). EI-MS (70 eV) *m/z*: 395 $[M + H]^+$. Anal. Calcd for $C_{24}H_{18}N_4O_2$: C, 73.09; H, 4.56; N, 14.21. Found: C, 73.03; H, 4.61; N, 14.26%.

*5.4.2. 2-Methyl-3-{3-methyl-5-[(E)-2-(4-methylphenyl)-1-ethenyl]-4-isoxazolyl}-3,4-dihydropyrimido[4,5-b]quinolin-4-one (**5b**)*

Yield 82%, mp 208–210 °C. IR (KBr) cm^{-1}: 1680 (C=O), 1645 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, isoxazole CH_3), 2.42 (s, 3H, CH_3), 2.51 (s, 3H, pyrimidine-CH_3), 6.84–7.52 (m, 9H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.32 (isoxazole-CH_3), 22.28 (pyrimidine-CH_3), 23.35 (Ar–CH_3), 107.04 (isoxazole-C_4), 109.10 (=CH), 111.02 (=CH), 122.08 (Ar–C), 125.10 (Ar–C), 125.40 (Ar–C), 126.49 (Ar–C), 126.90 (Ar–C), 128.43 (Ar–C), 128.31 (Ar–C), 129.14 (Ar–C), 130.25 (Ar–C), 133.20 (Ar–C), 133.25 (Ar–C), 134.21 (Ar–C), 135.48 (Ar–C), 141.24 (Ar–C), 146.08 (Ar–C), 152.55 (isoxazole-C_3), 156.07 (isoxazole-C_5), 161.70 (pyrimidine ring-C), 171.40 (C=O). EI-MS (70 eV) *m/z*: 409 $[M + H]^+$. Anal. Calcd for $C_{25}H_{20}N_4O_2$: C, 73.52; H, 4.90; N, 13.72. Found: C, 73.50; H, 4.82; N, 13.80%.

*5.4.3. 3-{5-[(E)-2-(4-Methoxyphenyl)-1-ethenyl]3-methyl–4-isoxazolyl}-2-methyl-3,4-dihydropyrimido[4,5-b]quinolin-4-one (**5c**)*

Yield 74%, mp 215–217 °C. IR (KBr) cm^{-1}: 1670 (C=O), 1640 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.21 (s, 3H, isoxazole CH_3), 2.45 (s, 3H, CH_3), 2.52 (s, 3H, pyrimidine-CH_3), 3.86 (s, 3H, OCH_3), 6.82–7.83 (m, 9H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.34 (isoxazole-CH_3), 22.23 (pyrimidine-CH_3), 64.33 (OCH_3), 108.11 (isoxazole-C_4), 110.23 (=CH), 111.23 (=CH), 123.10 (Ar–C), 125.04 (Ar–C), 126.56 (Ar–C), 126.82 (Ar–C), 127.81 (Ar–C), 128.72 (Ar–C), 128.83 (Ar–C), 129.17 (Ar–C), 131.03 (Ar–C), 133.54 (Ar–C), 134.51 (Ar–C), 135.06 (Ar–C), 135.67 (Ar–C), 140.36 (Ar–C), 147.17 (Ar–C), 153.61 (isoxazole-C_3), 156.43 (isoxazole-C_5), 162.69 (pyrimidine ring-C), 172.99 (C=O). EI-MS (70 eV) *m/z*: 425 $[M + H]^+$. Anal. Calcd for $C_{25}H_{20}N_4O_3$: C, 70.75; H, 4.71; N, 13.20. Found: C, 70.70; H, 4.76; N, 13.25%.

*5.4.4. 7-Chloro-2-methyl-3-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydropyrimido[4,5-b]quinolin-4-one (**5d**)*

Yield 79%, mp 206–208 °C. IR (KBr) cm^{-1}: 1675 (C=O), 1640 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, isoxazole CH_3), 2.51 (s, 3H, pyrimidine-CH_3), 6.92–7.84 (m, 9H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.23 (isoxazole-CH_3), 24.66 (pyrimidine-CH_3), 108.18 (isoxazole-C_4), 110.26 (=CH), 111.08 (=CH), 123.17 (Ar–C), 125.08 (Ar–C), 126.51 (Ar–C), 126.85 (Ar–C), 127.87 (Ar–C), 128.91 (Ar–C), 129.01 (Ar–C), 129.26 (Ar–C), 131.09 (Ar–C), 133.65 (Ar–C), 134.57 (Ar–C), 135.19 (Ar–C), 135.78 (Ar–C), 140.31 (Ar–C), 147.10 (Ar–C), 153.35 (isoxazole-C_3), 156.50 (isoxazole-C_5), 162.76 (pyrimidine ring-C), 172.86 (C=O). EI-MS (70 eV) *m/z*: 429 $[M + H]^+$. Anal. Calcd for $C_{24}H_{17}N_4O_2Cl$: C, 67.28; H, 3.97; N, 13.08. Found: C, 67.37; H, 3.90; N, 13.02%.

*5.4.5. 7-Chloro-2-methyl-3-{3-methyl-5-[(E)-2-(4-methylphenyl)-1-ethenyl]-4-isoxazolyl}-3,4-dihydropyrimido[4,5-b]quinolin-4-one (**5e**)*

Yield 74%, mp 188–190 °C. IR (KBr) cm^{-1}: 1680 (C=O), 1640 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.14 (s, 3H, isoxazole CH_3), 2.42 (s, 3H, CH_3), 2.53 (s, 3H, pyrimidine CH_3), 7.04–7.85 (m, 8H, ArH & 2H, CH=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.36 (isoxazole-CH_3), 25.22 (pyrimidine-CH_3), 28.55 (Ar–CH_3), 107.11 (isoxazole-C_4), 109.19 (=CH), 111.11 (=CH), 122.17 (Ar–C), 125.20 (Ar–C), 125.35 (Ar–C), 126.51 (Ar–C), 126.85 (Ar–C), 128.55 (Ar–C), 128.51 (Ar–C), 129.18 (Ar–C), 130.17 (Ar–C), 133.17 (Ar–C), 133.14 (Ar–C), 134.27 (Ar–C), 135.33 (Ar–C), 141.21 (Ar–C), 146.16 (Ar–C), 152.43 (isoxazole-C_3), 156.15 (isoxazole-C_5), 161.64 (pyrimidine ring-C), 171.83 (C=O). EI-MS (70 eV) *m/z*: 409 $[M + H]^+$. EI-MS (70 eV) *m/z*: 443 $[M + H]^+$. Anal. Calcd for $C_{25}H_{19}N_4O_2Cl$: C, 67.87; H, 4.29; N, 12.66. Found: C, 67.80; H, 4.22; N, 12.71%.

*5.4.6. 7-Chloro-3-{5-[(E)-2-(4-methoxyphenyl)-1-ethenyl]-3-methyl-4-isoxazolyl}-2-methyl-3,4-dihydropyrimido[4,5-b] quinolin-4-one (**5f**)*

Yield 86%, mp 200–202 °C. IR (KBr) cm^{-1}: 1670 (C=O), 1640 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, isoxazole CH_3), 2.52 (s, 3H, pyrimidine CH_3), 3.81 (s, 3H, OCH_3), 6.84–7.52 (m, 8H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.29 (isoxazole-CH_3), 22.32 (pyrimidine-CH_3), 64.28 (OCH_3), 108.17 (isoxazole-C_4), 110.29 (=CH), 111.21 (=CH), 123.17 (Ar–C), 125.13 (Ar–C), 126.43 (Ar–C), 126.71 (Ar–C), 127.88 (Ar–C), 128.65 (Ar–C), 128.78 (Ar–C), 129.13 (Ar–C), 131.11 (Ar–C), 133.49 (Ar–C), 134.59 (Ar–C), 135.03 (Ar–C), 135.58 (Ar–C), 140.29 (Ar–C), 147.21 (Ar–C), 153.56 (isoxazole-C_3), 156.50 (isoxazole-C_5), 162.73 (pyrimidine ring-C), 173.01 (C=O). EI-MS (70 eV) *m/z*: 459 $[M + H]^+$. Anal. Calcd for $C_{25}H_{19}N_4O_3Cl$: C, 65.50; H, 4.14; N, 9.17. Found: C, 65.58; H, 4.07; N, 9.10%.

*5.5. General procedure for the synthesis of N_3-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-imino-2H-3-chromenecarboxamides (**6a–g**)*

To a solution of compound **2** (1 mmol) in absolute ethanol (20 mL) containing piperidine (0.5 mL), salicylaldehyde (1 mmol) was added. The reaction mixture was heated under reflux for 3 h and then allowed to cool. The precipitate obtained on pouring the reaction mixture in to crushed ice was filtered off, washed with ethanol, dried and recrystallized from ethanol to afford **6**.

*5.5.1. N_3-{3-Methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-2-imino-2H-3-chromenecarboxamide (**6a**)*

Yield 73%, mp 235–237 °C. IR (KBr) cm^{-1}: 3300 (CONH), 3215 (C=NH), 1670 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.42 (s, 3H, CH_3), 6.24 (s, 1H, =CH), 6.91–7.63 (m, 9H, ArH & 2H, CH=CH), 9.24 (s, 1H, =NH), 10.20 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.25 (CH_3), 108.35 (isoxazole-C_4), 110.96 (=CH), 115.12 (=CH), 121.26 (Ar–C), 123.43 (Ar–C), 126.01 (Ar–C), 126.15 (Ar–C), 126.41 (Ar–C), 127.56 (Ar–C), 128.80 (Ar–C), 129.06 (Ar–C), 130.08 (Ar–C), 130.72 (Ar–C), 131.19 (Ar–C), 132.73 (Ar–C), 134.00 (Ar–C), 136.56 (Ar–C), 155.15 (isoxazole-C_3), 159.01 (isoxazole-C_5), 160.01 (C=NH), 181.01 (C=O). EI-MS (70 eV) *m/z*: 372 $[M + H]^+$. Anal. Calcd for $C_{22}H_{17}N_3O_3$: C, 71.15; H, 4.58; N, 11.32. Found: C, 71.20; H, 4.53; N, 11.37%.

*5.5.2. N_3-{3-Methyl-5-[(E)-2-(4-methylphenyl)-1-ethenyl]-4-isoxazolyl}-6-chloro-2-imino-2H-3-chromenecarboxamide (**6b**)*

Yield 86%, mp 228–230 °C. IR (KBr) cm^{-1}: 3300 (CONH), 3200 (C=NH), 1680 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.34 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 6.20 (s, 1H, =CH), 6.92–7.80 (m, 7H, ArH & 2H, CH=CH), 9.02 (s, 1H, =NH), 10.01 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.28 (CH_3), 26.43 (Ar–CH_3), 109.01 (isoxazole-C_4), 110.93 (=CH), 115.11 (=CH), 121.15 (Ar–C), 123.46 (Ar–C), 126.00 (Ar–C), 126.09 (Ar–C), 126.36 (Ar–C), 127.43 (Ar–C), 128.72 (Ar–C), 129.00 (Ar–C), 130.01 (Ar–C), 130.56 (Ar–C), 131.20 (Ar–C), 132.56 (Ar–C), 134.11 (Ar–C), 136.43 (Ar–C), 155.11 (isoxazole-C_3), 159.00 (isoxazole-C_5), 160.11 (C=NH), 181.23 (C=O). EI-MS (70 eV) *m/z*: 420 $[M + H]^+$. Anal. Calcd for $C_{23}H_{18}N_3O_3Cl$: C, 65.87; H, 4.29; N, 10.02. Found: C, 65.80; H, 4.22; N, 10.08%.

*5.5.3. N_3-{3-Methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-6,8-dichloro-2-imino-2H-3-chromenecarboxamide (**6c**)*

Yield 85%, mp 194–196 °C. IR (KBr) cm^{-1}: 3251 (CONH), 3220 (C=NH), 1670 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.41 (s, 3H, CH_3), 6.30 (s, 1H, =CH), 6.90–7.84 (m, 7H, ArH & 2H, CH=CH), 9.31 (s, 1H, =NH), 10.20 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.21 (CH_3), 108.45 (isoxazole-C_4), 111.01 (=CH), 115.20 (=CH), 121.25 (Ar–C), 123.41 (Ar–C), 126.06 (Ar–C), 127.08 (Ar–C), 127.48 (Ar–C), 127.56 (Ar–C), 128.73 (Ar–C), 129.10 (Ar–C), 130.01 (Ar–C), 130.55 (Ar–C), 131.23 (Ar–C), 132.65 (Ar–C), 134.08 (Ar–C), 136.63 (Ar–C), 155.09 (isoxazole-C_3), 159.06 (isoxazole-C_5), 160.03 (C=NH), 181.20 (C=O). EI-MS (70 eV) *m/z*: 440 $[M + H]^+$. Anal. Calcd for $C_{22}H_{15}N_3O_3Cl_2$: C, 60.13; H, 3.41; N, 9.56. Found: C, 60.17; H, 3.45; N, 9.52%.

*5.5.4. N_3-{5-[(E)-2-(4-Methoxyphenyl)-1-ethenyl]-3-methyl-4-isoxazolyl}-6-bromo-2-imino-2H-3-chromenecarboxamide (**6d**)*

Yield 76%, mp 209–211 °C. IR (KBr) cm^{-1}: 3250 (CONH), 3155 (C=NH), 1675 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 6.13 (s, 1H, =CH), 6.80–7.64 (m, 7H, ArH & 2H, CH=CH), 9.42 (s, 1H, =NH), 10.24 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.30 (CH_3), 64.73 (OCH_3), 108.31 (isoxazole-C_4), 110.88 (=CH), 115.11 (=CH), 121.15

(Ar–C), 123.41 (Ar–C),126.03 (Ar–C), 126.18 (Ar–C), 127.43 (Ar–C), 127.51 (Ar–C), 128.61 (Ar–C), 129.11 (Ar–C), 130.01 (Ar–C), 131.21 (Ar–C), 132.71 (Ar–C), 133.62 (Ar–C), 134.01 (Ar–C), 136.43 (Ar–C), 155.16 (isoxazole-C_3), 159.08 (isoxazole-C_5), 160.03 (C=NH), 181.11 (C=O). EI-MS (70 eV) *m/z*: 480 $[M + H]^+$. Anal. Calcd for $C_{23}H_{18}N_3O_4Br$: C, 57.62; H, 3.75; N, 8.76. Found: C, 57.68; H, 3.70; N, 8.70%.

*5.5.5. N_3-{3-Methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-6,8-dibromo-2-imino-2H-3-chromenecarboxamide (**6e**)*

Yield 80%, mp 216–218 °C. IR (KBr) cm^{-1}: 3250 (CONH), 3200 (C=NH), 1680 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.42 (s, 3H, CH_3), 6.20 (s, 1H, =CH), 6.81–7.74 (m, 7H, ArH & 2H, CH=CH), 9.02 (s, 1H, =NH), 10.34 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.28 (CH_3), 108.16 (isoxazole-C_4), 110.86 (=CH), 115.16 (=CH), 121.32 (Ar–C), 123.41 (Ar–C), 126.06 (Ar–C), 126.43 (Ar–C), 127.51 (Ar–C), 127.56 (Ar–C), 128.73 (Ar–C), 129.01 (Ar–C), 130.11 (Ar–C), 130.61 (Ar–C), 131.10 (Ar–C), 132.53 (Ar–C), 134.03 (Ar–C), 136.51 (Ar–C), 155.10 (isoxazole-C_3), 159.00 (isoxazole-C_5), 160.07 (C=NH), 181.21 (C=O). EI-MS (70 eV) *m/z*: 528 $[M + H]^+$. Anal. Calcd for $C_{22}H_{15}N_3O_3Br_2$: C, 50.09; H, 2.84; N, 7.96. Found: C, 50.01; H, 2.90; N, 7.90%.

*5.5.6. 2-Imino-6-methyl-N-(3-methyl-5-styrylisoxazol-4-yl)-2H-chromene-3-carboxamide (**6f**)*

Yield 75%, mp 204–208 °C. IR (KBr) cm^{-1}: 3280 (CONH), 3215 (C=NH), 1670 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.40 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 6.24 (s, 1H, =CH), 6.90–7.64 (m, 8H, ArH & 2H, CH=CH), 9.24 (s, 1H, =NH), 10.20 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.22 (CH_3), 26.53 (Ar–CH_3), 109.03 (isoxazole-C_4), 110.86 (=CH), 115.23 (=CH), 121.11 (Ar–C), 123.23 (Ar–C), 126.11 (Ar–C), 126.18 (Ar–C), 126.31 (Ar–C), 127.40 (Ar–C), 128.73 (Ar–C), 129.03 (Ar–C), 130.11 (Ar–C), 130.55 (Ar–C), 131.21 (Ar–C), 132.51 (Ar–C), 134.08 (Ar–C), 136.44 (Ar–C), 155.10 (isoxazole-C_3), 159.06 (isoxazole-C_5), 160.13 (C=NH), 181.30 (C=O). EI-MS (70 eV) *m/z*: 386 $[M + H]^+$. Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 71.68; H, 4.93; N, 10.90. Found: C, 71.72; H, 4.89; N, 10.85%.

*5.5.7. 2-Imino-6-methoxy-N-(3-methyl-5-styrylisoxazol-4-yl)-2H-chromene-3-carboxamide (**6g**)*

Yield 74%, mp 217–219 °C. IR (KBr) cm^{-1}: 3275 (CONH), 3200 (C=NH), 1675 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.40 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 6.20 (s, 1H, =CH), 6.94–7.62 (m, 8H, ArH & 2H, CH=CH), 9.21 (s, 1H, =NH), 10.24 (s, 1H, CONH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 11.26 (CH_3), 66.03 (OCH_3), 108.28 (isoxazole-C_4), 110.73 (=CH), 115.23 (=CH), 121.10 (Ar–C), 123.39 (Ar–C), 126.02 (Ar–C), 126.17 (Ar–C), 126.40 (Ar–C), 127.41 (Ar–C), 128.51 (Ar–C), 129.01 (Ar–C), 130.10 (Ar–C), 131.26 (Ar–C), 132.69 (Ar–C), 134.08 (Ar–C), 136.40 (Ar–C), 155.15 (isoxazole-C_3), 159.06 (isoxazole-C_5), 160.08 (C=NH), 181.20 (C=O). EI-MS (70 eV) *m/z*: 402 $[M + H]^+$. Anal. Calcd for $C_{23}H_{19}N_3O_4$: C, 68.82; H, 4.73; N, 10.47. Found: C, 68.75; H, 4.78; N, 10.40%.

*5.6. General procedure for the synthesis of 3-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydro-2H-chromeno[2,3-d] pyrimidin-4-ones (**7a**–**g**)*

A mixture of compound **6** (1 mmol) and formalin (37%, 1 mmol) was refluxed in ethanol (15 mL) for 4 h. The reaction mixture after cooling to room temperature was poured in to ice-cold water. The separated solid was filtered and recrystallized from ethanol to get pure compound **7**.

*5.6.1. 3-{3-Methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydro-2H-chromeno [2,3-d]pyrimidin-4-one (**7a**)*

Yield 70%, mp 226–228 °C. IR (KBr) cm^{-1}: 1660 (C=O), 1640 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 3H, isoxazole CH_3), 4.52 (s, 2H, CH_2), 6.51 (s, 1H, =CH), 6.84–7.63 (m, 9H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.02 (CH_3), 65.30 (N–CH_2–N), 109.87 (isoxazole-C_4), 111.05 (=CH), 115.20 (=CH), 121.35 (Ar–C), 123.55 (Ar–C), 126.07 (Ar–C), 126.10 (Ar–C), 126.33 (Ar–C), 127.55 (Ar–C), 128.88 (Ar–C), 129.07 (Ar–C), 130.05 (Ar–C), 130.88 (Ar–C), 131.19 (Ar–C), 132.80 (Ar–C), 134.02 (Ar–C), 136.52 (Ar–C), 155.28 (isoxazole-C_3), 159.00 (isoxazole-C_5), 160.05 (pyrimidine ring-O–C=N), 175.05 (C=O). EI-MS (70 eV) *m/z*: 384 $[M + H]^+$. Anal. Calcd for $C_{23}H_{17}N_3O_3$: C, 72.06; H, 4.43; N, 10.96. Found: C, 72.10; H, 4.47; N, 10.92%.

*5.6.2. 7-Chloro-3-{3-methyl-5-[(E)-2-(4-methylphenyl)-1-ethenyl]-4-isoxazolyl}-3,4-dihydro-2H-chromeno[2,3-d]pyrimidin-4-one (**7b**)*

Yield 78%, mp 203–204 °C. IR (KBr) cm^{-1}: 1680 (C=O), 1660 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 3H, isoxazole CH_3), 2.53 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 6.40 (s, 1H, =CH), 6.80–7.64 (m, 7H, ArH & 2H, CH=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.72 (CH_3), 22.04 (Ar–CH_3), 64.20 (N–CH_2–N), 109.37 (isoxazole-C_4), 110.09 (=CH), 116.40 (=CH), 121.05 (Ar–C), 122.25 (Ar–C), 126.47 (Ar–C), 126.50 (Ar–C), 126.13 (Ar–C), 127.05 (Ar–C), 128.48 (Ar–C), 129.67 (Ar–C), 130.00 (Ar–C), 130.28 (Ar–C), 131.59 (Ar–C), 132.20 (Ar–C), 134.42 (Ar–C), 137.32 (Ar–C), 154.28 (isoxazole-C_3), 158.00 (isoxazole-C_5), 161.05 (pyrimidine ring-O–C=N), 176.25 (C=O). EI-MS (70 eV) *m/z*: 432 $[M + H]^+$. Anal. Calcd for $C_{24}H_{18}N_3O_3Cl$: C, 66.82; H, 4.17; N, 9.74. Found: C, 66.78; H, 4.21; N, 9.70%.

*5.6.3. 7,9-Dichloro-3-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydro-2H-chromeno[2,3-d]pyrimidin-4-one (**7c**)*

Yield 80%, mp 220–223 °C. IR (KBr) cm^{-1}: 1675 (C=O), 1660 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, isoxazole CH_3), 4.20 (s, 2H, CH_2), 6.51 (s, 1H, =CH), 6.92–7.64 (m, 7H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.25 (CH_3), 65.44 (N–CH_2–N), 109.79 (isoxazole-C_4), 111.16 (=CH), 115.26 (=CH), 121.41 (Ar–C), 123.61 (Ar–C), 126.23 (Ar–C), 126.18 (Ar–C), 126.43 (Ar–C), 127.65 (Ar–C), 128.81 (Ar–C), 129.02 (Ar–C), 130.13 (Ar–C), 130.75 (Ar–C), 131.23 (Ar–C), 132.61 (Ar–C), 134.00 (Ar–C), 136.48 (Ar–C), 155.20 (isoxazole-C_3), 159.03 (isoxazole-C_5), 160.09 (pyrimidine ring-O–C=N), 175.45 (C=O). EI-MS (70 eV) *m/z*: 452 $[M + H]^+$. Anal. Calcd for $C_{23}H_{15}N_3O_3Cl_2$: C, 61.19; H, 3.32; N, 9.31. Found: C, 61.23; H, 3.36; N, 9.27%.

*5.6.4. 7-Bromo-3-{3-methyl-5-[(E)-2(4-methoxyphenyl)-1-ethenyl]-4-isoxazolyl}-3,4-dihydro-2H-chromeno[2,3-d] pyrimidin-4-one (**7d**)*

Yield 82%, mp 200–204 °C. IR (KBr) cm^{-1}: 1687 (C=O), 1655 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.40 (s, 3H, isoxazole CH_3), 3.62 (s, 3H, OCH_3), 4.64 (s, 2H, CH_2), 6.40 (s, 1H, =CH), 6.80–7.62 (m, 7H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.24 (CH_3), 64.23 (OCH_3), 65.45 (N–CH_2–N), 109.72 (isoxazole-C_4), 111.10 (=CH), 115.23 (=CH), 121.37 (Ar–C), 123.41 (Ar–C), 126.01 (Ar–C), 126.13 (Ar–C), 126.39 (Ar–C), 127.52 (Ar–C), 128.83 (Ar–C), 129.09 (Ar–C), 130.10 (Ar–C), 130.81 (Ar–C), 131.10 (Ar–C), 132.79 (Ar–C), 134.00 (Ar–C), 136.39 (Ar–C), 155.17 (isoxazole-C_3), 159.06 (isoxazole-C_5), 160.15 (pyrimidine ring-O–C=N), 175.55 (C=O). EI-MS (70 eV) *m/z*: 492 $[M + H]^+$. Anal. Calcd for $C_{24}H_{18}N_3O_4Br$: C, 58.65; H, 3.66; N, 8.55. Found: C, 58.69; H, 3.62; N, 8.51%.

*5.6.5. 7,9-Dibromo-3-{3-methyl-5-[(E)-2-phenyl-1-ethenyl]-4-isoxazolyl}-3,4-dihydro-2H-chromeno[2,3-d]pyrimidin-4-one (**7e**)*

Yield 86%, mp 199–201 °C. IR (KBr) cm^{-1}: 1670 (C=O), 1655 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.30 (s, 3H, isoxazole CH_3), 4.42 (s, 2H, CH_2), 6.61 (s, 1H, =CH), 6.92–7.80 (m, 7H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.22 (CH_3), 65.40 (N–CH_2–N), 109.71 (isoxazole-C_4), 111.10 (=CH), 115.21 (=CH), 121.38 (Ar–C), 123.60 (Ar–C), 126.27 (Ar–C), 126.10 (Ar–C), 127.41 (Ar–C), 127.69 (Ar–C), 128.78 (Ar–C), 129.08 (Ar–C), 130.21 (Ar–C), 130.64 (Ar–C), 131.42 (Ar–C), 132.58 (Ar–C), 134.12 (Ar–C), 136.35 (Ar–C), 155.18 (isoxazole-C_3), 159.15 (isoxazole-C_5), 160.21 (pyrimidine ring-O–C=N), 175.65 (C=O). EI-MS (70 eV) *m/z*: 540 $[M + H]^+$. Anal. Calcd for $C_{23}H_{15}N_3O_3Br_2$: C, 51.20; H, 2.78; N, 7.79. Found: C, 51.24; H, 2.74; N, 7.75%.

*5.6.6. 2,3-Dihydro-7-methyl-3-(3-methyl-5-styrylisoxazol-4-yl) chromeno[2,3-d]pyrimidin-4-one (**7f**)*

Yield 78%, mp 221–223 °C. IR (KBr) cm^{-1}: 1660 (C=O), 1640 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, isoxazole CH_3), 2.52 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 6.51 (s, 1H, =CH), 6.84–7.63 (m, 8H, ArH & 2H, CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.34 (CH_3), 23.04 (Ar–CH_3), 64.26 (N–CH_2–N), 109.30 (isoxazole-C_4), 110.13 (=CH), 116.46 (=CH), 121.02 (Ar–C), 122.23 (Ar–C), 126.47 (Ar–C), 126.59 (Ar–C), 126.10 (Ar–C), 127.15 (Ar–C), 128.40 (Ar–C), 129.63 (Ar–C), 130.02 (Ar–C), 130.21 (Ar–C), 131.66 (Ar–C), 132.27 (Ar–C), 134.45 (Ar–C), 137.39 (Ar–C), 154.32 (isoxazole-C_3), 158.10 (isoxazole-C_5), 161.15 (pyrimidine ring-O–C=N), 176.68 (C=O). EI-MS (70 eV) *m/z*: 398 $[M + H]^+$. Anal. Calcd for $C_{24}H_{19}N_3O_3$: C, 72.54; H, 4.78; N, 10.57. Found: C, 72.59; H, 4.73; N, 10.50%.

*5.6.7. 2,3-Dihydro-7-methoxy-3-(3-methyl-5-styrylisoxazol-4-yl) chromeno[2,3-d]pyrimidin-4-one (**7g**)*

Yield 74%, mp 211–213 °C. IR (KBr) cm^{-1}: 1660 (C=O), 1640 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 3H, isoxazole CH_3), 3.60 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 6.54 (s, 1H, =CH), 6.80–7.62 (m, 8H, ArH & 2H, CH=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.31 (CH_3), 64.13 (OCH_3), 65.34 (N–CH_2–N), 109.61 (isoxazole-C_4), 111.17 (=CH), 115.31 (=CH), 121.42 (Ar–C), 123.55 (Ar–C), 126.09 (Ar–C), 126.21 (Ar–C), 126.43 (Ar–C), 127.61 (Ar–C), 128.75 (Ar–C), 129.21 (Ar–C), 130.20 (Ar–C), 130.79 (Ar–C), 131.21 (Ar–C), 132.65 (Ar–C), 134.21 (Ar–C), 136.44 (Ar–C), 155.23 (isoxazole-C_3), 159.18 (isoxazole-C_5), 160.21 (pyrimidine ring-O–C=N), 175.66 (C=O). EI-MS (70 eV) *m/z*: 414 $[M + H]^+$. Anal. Calcd for $C_{24}H_{19}N_3O_4$: C, 69.73; H, 4.60; N, 10.16. Found: C, 69.77; H, 4.56; N, 10.12%.

5.7. Pharmacological screening

5.7.1. Antibacterial assay

The ready-made nutrient broth medium (Himedia, 24 g) was suspended in distilled water (100 mL) and heated until it dissolved completely. The medium and test tubes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound was dissolved in acetone and a concentration of 100 μg/mL of the test compound was added in the first test tube, which was serially diluted. A fixed volume of 0.5 mL of overnight culture was added in all the test tubes which were incubated at 37 °C for 24 h. After 24 h these tubes were measured for turbidity.

5.7.2. Antifungal assay

For the antifungal assay, the ready-made potato dextrose agar medium (Himedia, 39 g) was suspended in distilled water (100 mL) and heated until it dissolved completely. The medium and glass petri-dishes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. The medium was poured in to sterile petri-dishes under aseptic conditions in a Laminar flow chamber. When the medium in the plates solidified, 0.5 mL of the culture (one-week-old) of fungal spore suspension was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving test compound in acetone. Agar inoculation, cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup, different concentrations of test solution were added. Controls were maintained with acetone and clotrimazole. The treated and the controls were kept at room temperature for 72–96 h. The Minimum inhibitory Concentration (MIC) was recorded in μg/mL. Three to four replicates were maintained for each treatment.

5.7.3. Anti-inflammatory analysis

The albino rats weighing 150–180 g were used throughout the assay. They were kept in the animal house under standard condition of light and temperature with free access to food and water. The rats were randomly divided into groups, each of six rats. One group of six rats was kept as a control and another group received the standard drug Indomethacin (at a dose of 10 mg/kg body weight po). All the test compounds were dissolved in 0.05 mL solution of DMSO and given 60 min before the commencement of the study. After that 0.1 mL of 1% W/V carrageenan solution in normal saline was injected into the subplanter region of the left hind paw under light ether anesthesia 1 h after oral administration (po) of the test compound. The paw volume of each rat was measured immediately by mercury plethysmo meter and re-measured again 1, 2, 3, and 4 h after administration of DMSO. The edema was expressed as an increase in the volume of paw, and the % of edema inhibition for each rat and each group was calculated as follows:

$$\%\text{Inhibition} = \frac{(V_t - V_o)\text{control} - (V_t - V_o)\text{tested compound}}{(V_t - V_o)\text{control}} \times 100$$

Where

V_t = Volume of edema at specific time interval and V_o = Volume of edema at zero time interval.

5.7.4. Analgesic analysis

Albino rats weighing 150–180 g were used throughout this assay. They were kept in the animal house under standard condition of light and temperature with free access to food and water. The animals were randomly divided into groups each of six rats. One group of six rats was kept as a control and another group received the standard drug Indomethacin (at a dose of 10 mg/kg body weight po). The tested compounds were administrated orally at a dose of 10 mg/kg and Indomethacin was used as a reference drug (10 mg/kg). The recorded values were the average of six administrations ± S.E. and the percentage increase of the comparison with the basal values.

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