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Original article

Synthesis and pharmacological investigation of 3-(substituted 1-phenylethanone)-4-(substituted phenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates

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ABSTRACT

Fifteen new ethyl 6-methyl-2-methoxy-3-(substituted 1-phenylethanone)-4-(substituted phenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates (**6a**–**o**) have been synthesized in a two step reaction. In first step ethyl acetoacetate, *s*-methylisourea and appropriate benzaldehydes reacted in a single step reaction to obtain ethyl 6-methyl-2-methoxy-4-(substituted phenyl)-1, 4-dihydropyrimidine-5-carboxylates (**4a**–**e**). Second step involves synthesis of reaction between substituted phenacyl bromides and 1-4-dihydropyrimidine-5-carboxylates (**6a**–**o**). Their structures are confirmed by IR, ^{1}H NMR, mass and elemental analyses. The compounds were tested for antihypertensive activity by non-invasive tail-cuff, and evaluated by carotid artery cannulation method for determining the diastolic blood pressure. Hypertension was induced by DOCA-salt. Anti-inflammatory activity was carried out by carrageenan induced rat-paw oedema method. Test compounds **6b**, **6c**, **6e**, **6f**, **6j**, **6h**, **6k**, **6l**, **6m**, **6n** and **6o** exerted comparative antihypertensive activity at 10 mg/kg dose level compared to nifedipine. Compounds **6j**, **6m** and **6o** showed excellent results on evaluation by direct method. Test compounds **6a**–**6h**, **6l**, **6m**, **6n** and **6o** exerted moderate to comparative anti-inflammatory activity at the 100 mg/kg dose level compared to indomethacin. Their further investigation for analgesic activity and acute ulcerogenesis was carried out, compounds **6m**, **6f**, **6k**, **6o** showed excellent to good analgesic activity and low ulcerogenic activity.

1. Introduction

Similar groups/structures often exhibit similar biological activities. However, they usually exhibit different potency. The traditional structure–activity relationship (SAR) is a useful tool in the search for new drugs. However, SAR is usually determined by making minor changes to the structure of the existing compound and assessing the effect on its biological activity. Similarly, structural analogy has played vital role in designing compounds with higher potency. One of such structural analogy is seen between 4-aryl-1, 4-dihydropyridines (DHPs) of the nifedipine type and dihydropyrimidines (DHPMs). In 1893 Italian chemist Pietro Biginelli reported on the acid-catalyzed cyclocondensation reaction of ethyl acetoacetate, benzaldehyde and urea. The reaction was carried out simply by heating a mixture of three components dissolved in ethanol with a catalytic amount of hydrochloric acid at reflux temperature. The product of this novel one pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3, 4-dihydropyrimidine-2(1*H*)-one [1].

The synthetic potential of this new heterocyclic synthesis remained unexplored for quite some time. In the 1970s and 1980s interest slowly increased, and the scope of the original cyclocondensation reaction was gradually extended by variation of all three building blocks, allowing access to a large number of multifunctionalized dihydropyrimidines [2].

In the past decades, a broad range of biological effects, including antiviral [3], antitumor [4], antibacterial [5] and anti-inflammatory [6] activities have been ascribed to these partly reduced pyrimidine derivatives. More recently, DHPMs have emerged as, for e.g., orally active antihypertensive agents [7]. A very recent highlight in this context been the identification of the structurally rather simple DHPM monastrol as a mitotic kinesin motor protein inhibitor and potential new lead for the development of anticancer drugs [8]. Appropriately functionalized DHPM derivatives have emerged as potent calcium channel modulators [9]. Apart from synthetic DHPM derivatives several marine natural products with interesting biological activities containing the dihydropyrimidine-5-carboxylate core have recently been isolated. Most among these are the batzelladine alkaloids A and B which inhibit the binding of HIV

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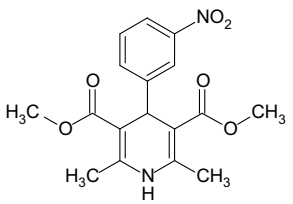
envelop protein gp-120 to human CD4 cells and therefore, are potential new leads for AIDS therapy [10].

2. Chemistry

The chemistry of pyrimidine-5-carboxylates has been of great interest. 4-Aryl-1, 4-dihydropyridines of the nifedipine type (I, II) are most studied class of organic calcium channel modulators, since their introduction. They have become drugs of immense importance for the treatment of hypertension, cardiac arrhythmias, etc. In recent years, interest has also been focused on aza-analogues such as dihydropyrimidines of types III and IV which show similar pharmacological profile to classical dihydropyridine calcium channel modulators, the reported lead compounds show superiority in potency and duration of activity.



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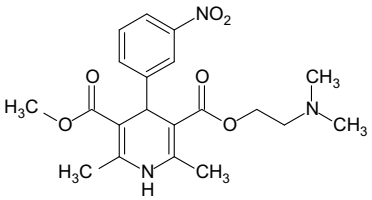

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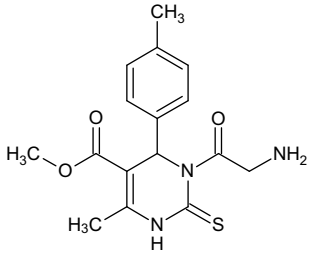

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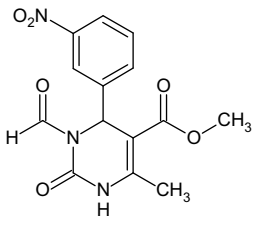


IV

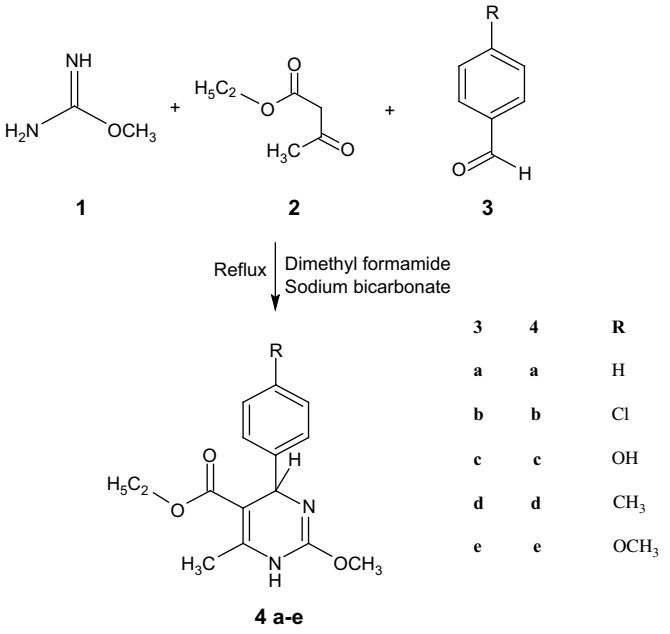
The pyrimidine-5-carboxylate substituted at third position, i.e., on nitrogen in the pyrimidine ring gives antihypertensive activity similar to nifedipine-type calcium channel modulator, substitution at the third nitrogen is possible and the resultants can be strong contenders of anti-inflammatory, antihypertensive activities [11–18]. It involves the application of Biginelli reaction and its modifications.

In the first step three-component reaction involving *o*-methylisourea hydrogensulfate, ethyl acetoacetate and substituted benzaldehydes (**3a**–**e**) reacted in the presence of sodium bicarbonate and dimethylformamide to form the substituted ring nucleus compounds (**4a**–**e**) (Scheme 1).

On reaction with various substituted phenacyl bromides **5a**–**c**, they undergo nucleophilic substitution reaction in the presence of a base such as pyridine, to form their respective derivatives (**6a**–**o**) (Scheme 2).

3. Pharmacology

Antihypertensive activity of synthesized compounds was carried out by model or method of Deoxycorticosterone Acetate salt (DOCA-salt) induced hypertension in rats [19–21]. The non-invasive method to determine systolic blood pressure (SBP), invasive method to determine diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) for determining the changes in blood



3	4	R
a	a	H
b	b	Cl
c	c	OH
d	d	CH_3
e	e	OCH_3

1 2 3

4 a-e

Scheme 1.

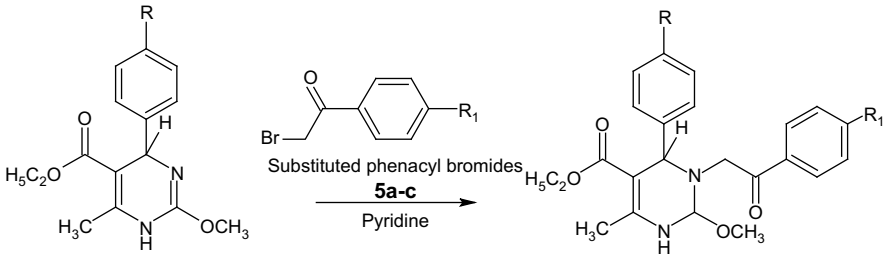
pressure were performed using Power Lab/4SP with ML135 Dual Bio Amp computerized BP monitor automatic cardiovascular system (AD instruments Pvt. Ltd., Australia). Anti-inflammatory activity of synthesized compounds was carried out by carrageenan induced rat-paw oedema [22] method using UGO BASTILE Plethysmometer 7140. Analgesic activity was carried out by acetic acid induced writhing method [23]. Acute ulcerogenesis test was done according to Cioli et al. [24].

4. Results and discussion

All the compounds synthesized are novel. These derivatives were obtained from the two step synthesis, their structures were confirmed by IR, NMR and elemental analyses. We have shown that 2-hetero-1, 4-dihydropyrimidines can be synthesized with selective substitution of the *para*-substituted electrophiles at N3 position. This selectivity is believed to be due to electron density at N3 and N1. The former being richer in electron density is more reactive and produces products of exclusive functionalization at N3. Fifteen derivatives were synthesized, antihypertensive activity was carried out initially for all the test compounds, those compounds which were found out to show significant activity by non-invasive (Tail-cuff method) technique were further evaluated for antihypertensive activity by direct cannulation method. Anti-inflammatory activity was carried out followed by analgesic and acute ulcerogenesis studies.

4.1. Antihypertensive activity

Antihypertensive activity carried out by the non-invasive method gave the systolic blood pressure (SBP), from which the observations are summarized in Table 1. For structure–activity studies we choose the aromatic substitutions that are commonly employed in dihydropyridines. 4-Methoxy derivative **6n** has remarkable antihypertensive activity. 3, 4-Disubstituted methoxy derivative **6o** has shown good antihypertensive activity at 10 mg/kg. Although 3-methoxy 4-hydroxy derivative (**6i**) is less potent than the corresponding **6n** and **6o**. 3, 4-Dichloro analogues are moderately potent than **6n** and **6o**. Data are presented as



5	R_1
a	H
b	Cl
c	OCH_3

Comp.	6a	6b	6c	6d	6e	6f	6g	6h	6i	6j	6k	6l	6m	6n	6o
R	H	H	H	Cl	Cl	OCH_3	OH	OH	OH	CH_3	CH_3	CH_3	OCH_3	OCH_3	OCH_3
R_1	H	Cl	OCH_3	H	Cl	Cl	H	Cl	OCH_3	H	Cl	OCH_3	H	Cl	OCH_3

Scheme 2.

means ± S.E.M., a repeated measures analysis of variance was used to obtain the statistical significance between and within groups. Differences were considered statistically significant at a *P* level lower than 0.05 and *F* value for all compounds are *F*: 22.33 ± 0.5. Their results for percentage inhibition are as shown in Table 2 and Fig. 1 respectively.

From the ANOVA (Analysis of Variance) and % inhibition studies, it was concluded that except compounds **6a**, **6d**, **6g** and **6i** all other compounds showed significant antihypertensive activity. Those drugs that showed significant and encouraging results were tested at lower dose levels of 5 and 2.5 mg/kg respectively.

Those compounds that showed significant activity by tail-cuff method were further evaluated for their antihypertensive activity by direct cannulation of the carotid artery. Antihypertensive activity carried out by cannulation method gave the diastolic blood pressure (DBP), from which the observations are given in Table 3 Data are presented as means ± S.E.M., a repeated measures analysis of variance was used to obtain the statistical significance between and within groups. Differences were considered statistically significant at a *P* level lower than 0.05 and *F* value for all compounds are *F*: ±0.5. Their results are as shown in Table 4 and Fig. 2 respectively.

4.2. Anti-inflammatory activity

Anti-inflammatory activity data is summarized in Table 5. The effect of structural modification on potency *in vitro*, and anti-inflammatory activity with a series of dihydropyrimidines is summarized in Table 6. The structural activity data suggest that potency *in vitro* for anti-inflammatory was optimized in **6m**. Aromatic substitution having methyl **6j** and hydroxyl **6g** analogues being considerably less potent then corresponding **6m**. Other compounds show moderate to low activity. Data are presented as means ± S.E.M., a repeated measures analysis of variance was used to obtain the statistical significance between and within groups. Differences were considered statistically significant at a *P* level lower than 0.05 and *F* value for all compounds are *F*: 34.61 ± 0.5, their results are as shown in Tables 5 and 6, and Fig. 3 respectively.

$P < 0.0001$ and the *F* value for all compounds are also statistically (99.99%) significant (*F*: 34.61 ± 0.5). The readings obtained from rat-paw oedema method are given in Table 5.

From the ANOVA and % inhibition studies, it was concluded that compounds **6i** and **6j** show low anti-inflammatory activity,

Table 1
Antihypertensive activity data obtained by tail-cuff method at 10 mg/kg dose.

Compound (10 mg/kg)	Average systolic blood pressure (mmHg) at time (min)										
	0	15	30	60	120	180	240	300	360	400	460
6a	225 ± 4	222 ± 8	221 ± 9	195 ± 2	180 ± 4	175 ± 8	172 ± 6	165 ± 4	160 ± 6	158 ± 3	155 ± 3
6b	226 ± 8	224 ± 5	210 ± 5	193 ± 4	178 ± 5	161 ± 4	142 ± 4	138 ± 6	131 ± 7	125 ± 5	122 ± 8
6c	226 ± 8	224 ± 5	210 ± 5	193 ± 4	178 ± 5	161 ± 4	142 ± 4	138 ± 6	131 ± 7	125 ± 5	122 ± 8
6d	225 ± 4	222 ± 8	220 ± 9	190 ± 2	178 ± 4	172 ± 8	167 ± 6	160 ± 4	158 ± 6	152 ± 3	145 ± 3
6e	225 ± 6	223 ± 5	210 ± 6	195 ± 8	180 ± 5	160 ± 8	145 ± 3	140 ± 6	135 ± 7	125 ± 5	125 ± 7
6f	226 ± 3	224 ± 6	210 ± 3	193 ± 5	178 ± 7	161 ± 2	142 ± 5	134 ± 6	130 ± 7	125 ± 5	121 ± 8
6g	224 ± 4	222 ± 8	220 ± 9	190 ± 2	175 ± 4	172 ± 8	162 ± 6	160 ± 4	154 ± 6	150 ± 3	145 ± 3
6h	226 ± 2	224 ± 3	210 ± 8	190 ± 4	172 ± 5	160 ± 4	142 ± 4	135 ± 6	130 ± 7	125 ± 5	122 ± 8
6i	225 ± 4	222 ± 8	221 ± 9	195 ± 2	180 ± 4	175 ± 8	172 ± 6	165 ± 4	160 ± 6	158 ± 3	155 ± 3
6j	226 ± 4	222 ± 8	220 ± 9	193 ± 2	185 ± 4	175 ± 8	170 ± 6	165 ± 4	161 ± 6	155 ± 3	150 ± 3
6k	226 ± 8	220 ± 5	200 ± 5	193 ± 4	170 ± 5	161 ± 4	142 ± 4	138 ± 6	130 ± 7	125 ± 5	122 ± 8
6l	225 ± 8	220 ± 0	200 ± 5	191 ± 4	170 ± 0	161 ± 7	142 ± 2	135 ± 6	130 ± 7	125 ± 0	122 ± 2
6m	222 ± 3	220 ± 6	210 ± 3	193 ± 5	178 ± 7	161 ± 2	140 ± 5	131 ± 6	128 ± 7	123 ± 5	121 ± 8
6n	228 ± 3	220 ± 6	210 ± 3	183 ± 5	170 ± 7	161 ± 2	140 ± 5	131 ± 6	128 ± 7	113 ± 5	110 ± 5
6o	226 ± 3	220 ± 6	210 ± 3	190 ± 5	175 ± 7	160 ± 2	140 ± 5	128 ± 6	125 ± 7	120 ± 5	115 ± 8
Control	225 ± 2	224 ± 1	225 ± 1	224 ± 3	224 ± 4	224 ± 1	225 ± 1	224 ± 4	225 ± 2	224 ± 3	225 ± 2
Nifedipine	225 ± 1	221 ± 2	215 ± 1	195 ± 3	180 ± 2	168 ± 1	145 ± 2	125 ± 2	125 ± 1	122 ± 2	120 ± 3

Table 2
Comparative study of inhibition (%) for antihypertensive activity by tail-cuff method.

Compound	Inhibition (%)										
	0	15	30	60	120	180	240	300	360	420	480
6a	0.8	0.59	1.43	12.98	19.61	21.56	23.33	26.3	28.69	29.43	31.04
6b	0.71	0.7	6.49	13.78	20.46	27.98	36.74	38.24	41.52	45.0	46.48
6c	0.1	1.0	6.49	13.78	20.46	27.98	36.80	38.30	41.60	45.1	46.48
6d	0.8	0.91	1.87	15.21	20.5	22.9	25.62	28.35	29.7	32.1	35.57
6e	0.1	0.27	6.45	12.71	19.57	28.25	35.46	37.35	39.75	44.05	44.19
6f	0.1	0.1	6.58	13.74	20.37	28.07	36.76	40.02	42.0	44.05	46.06
6g	0.36	0.59	1.87	15.21	21.84	23.0	27.77	28.53	31.35	33.0	35.48
6h	0.9	1.0	6.36	15.12	23.13	28.43	36.73	39.58	41.97	44.05	45.48
6i	0.8	0.27	1.43	12.71	19.61	21.56	23.33	26.3	28.0	29.43	31.04
6j	0.5	0.59	1.87	13.74	17.38	27.98	36.80	38.30	41.97	44.05	45.48
6k	0.1	1.61	11.09	13.78	24.02	28.0	36.80	39.50	41.97	44.0	45.74
6l	0.1	1.82	11.09	14.67	24.0	28.1	36.90	39.58	41.97	44.05	46.30
6m	0.9	1.57	6.58	13.74	20.36	28.0	36.0	41.35	42.85	44.98	45.91
6n	0.9	1.57	6.58	18.19	23.82	28.0	36.0	41.35	42.85	49.39	51.90
6o	0.9	1.57	6.58	15.06	21.59	27.8	36.0	40.1	41.85	46.51	48.57
Control	–	–	–	–	–	–	–	–	–	–	–
Nifedipine	0.4	1.29	4.44	12.92	19.69	24.98	35.49	44.20	44.51	45.51	46.58

whereas, compounds **6a–6h**, **6l**, **6m**, **6n** and **6o** showed moderate to comparative activity of indomethacin, which was used as standard. Furthermore, those compounds which were found to be significant were screened at lower dose level of 50 and 25 mg/kg respectively.

4.3. Analgesic activity

The analgesic activity of the synthesized compounds **6a–6h** and **6k–6o** was evaluated by acetic acid induced writhing test. The activity showed that compound **6m** exhibited maximum analgesic activity (59.76%) and its activity was comparable with the standard drug ibuprofen (62.55%) at a dose of 20 mg/kg p.o. Compounds **6f**, **6k**, and **6o** showed good activity (51.33–53.17%). Results are presented in Table 7. Analysis of result showed that substitution of chlorine, methyl and methoxyl group in the ring structure resulted in increased activity.

4.4. Acute ulcerogenesis

The compounds, which showed significant anti-inflammatory activity, were screened for their ulcerogenic activity. The test was performed according to Cioli et al. The tested compounds showed ulcerogenic activity ranging from 0.16 to 0.76, whereas the standard drug ibuprofen showed high severity index of 1.97. The results indicate that compounds are devoid of ulcerogenic action. Results are presented in Table 7. Structural activity data for analgesic and ulcerogenic activity shows that both the activity were favored by 4-methoxy aromatic substitution **6o**, 4-hydroxy, 4-methyl substitutions being considerably less active compound with standard (ibuprofen). Other compounds show moderate to low activity.

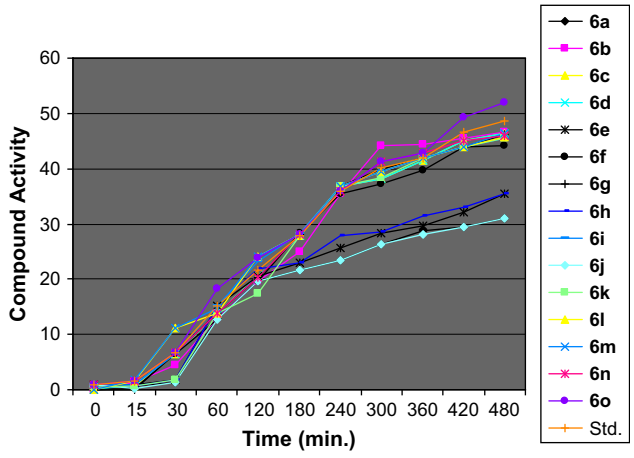


Fig. 1. Graph showing (%) decrease in systolic blood pressure (SBP).

5. Experimental section

5.1. Chemistry: general procedures

Chemicals were obtained from Fluka Chemical Co. (Germany). Melting points (m.p.) were detected with open capillaries using Thermonik Precision Melting point cum Boiling point apparatus (C-PMB-2, Mumbai, India) and are uncorrected. IR spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Japan). ^{1}H NMR was obtained using a Varian EM 390 Spectrophotometer (Shimadzu, Japan) using $CDCl_3$. All chemical shift values were recorded as δ (ppm). The purity of compounds was controlled by thin layer chromatography (Merck, silica gel, $HF_{254-361}$, type 60, 0.25 mm, Darmstadt, Germany). The elementary analysis was performed at RTM Nagpur University, India. Elementary analyses for C, H, N were within ±0.4% of theoretical values.

5.1.1. General procedure for preparation of compounds ***4a–e***

A mixture of *o*-methylisourea hydrogensulfate (60 mmol), ethyl acetoacetate (55 mmol), and substituted benzaldehydes (50 mmol)

Table 3
Antihypertensive activity data obtained by direct cannulation of carotid artery method at 10 mg/kg dose.

Compound (10 mg/kg)	Average diastolic blood pressure (mmHg) at time (min)						
	0	15	30	60	120	180	240
6b	195 ± 8	190 ± 5	176 ± 5	150 ± 4	136 ± 5	121 ± 4	102 ± 4
6c	198 ± 8	191 ± 5	178 ± 5	149 ± 4	136 ± 5	121 ± 4	102 ± 4
6e	196 ± 6	191 ± 5	178 ± 6	149 ± 8	135 ± 5	120 ± 8	105 ± 3
6f	195 ± 3	189 ± 6	174 ± 3	150 ± 5	137 ± 7	121 ± 2	102 ± 5
6h	197 ± 2	192 ± 3	175 ± 8	151 ± 4	136 ± 5	120 ± 4	102 ± 4
6j	197 ± 4	190 ± 8	176 ± 9	150 ± 2	137 ± 4	125 ± 8	100 ± 6
6k	196 ± 8	190 ± 5	175 ± 5	151 ± 4	135 ± 5	121 ± 4	102 ± 4
6l	196 ± 8	189 ± 0	173 ± 5	149 ± 4	136 ± 0	121 ± 7	102 ± 2
6m	195 ± 3	190 ± 6	175 ± 3	151 ± 5	136 ± 7	121 ± 2	101 ± 5
6n	196 ± 3	191 ± 6	176 ± 3	148 ± 5	137 ± 7	121 ± 2	102 ± 5
6o	196 ± 3	188 ± 6	172 ± 3	148 ± 5	134 ± 7	118 ± 2	100 ± 5
Control	195 ± 2	195 ± 1	196 ± 1	195 ± 3	195 ± 4	195 ± 1	196 ± 1
Nifedipine	197 ± 1	190 ± 2	173 ± 1	149 ± 3	135 ± 2	118 ± 1	101 ± 2

Table 4
Comparative study of inhibition (%) for antihypertensive activity by carotid artery cannulation method.

Compound	Inhibition (%)						
	0	15	30	60	120	180	240
6b	0.3	2.35	9.99	22.99	30.14	37.77	47.78
6c	1.8	1.84	8.97	23.50	30.14	37.77	47.78
6e	0.7	1.84	8.92	23.29	30.65	38.08	46.30
6f	0.05	2.81	11.11	22.93	29.52	37.87	47.73
6h	1.02	1.43	10.35	22.47	30.14	38.28	47.78
6j	1.12	2.20	9.79	23.09	30.65	35.52	48.69
6k	0.8	2.35	10.50	22.47	30.39	37.77	47.78
6l	0.8	3.12	11.52	22.52	30.04	37.62	47.88
6m	0.05	2.30	10.62	22.89	30.87	37.87	48.24
6n	0.5	1.79	10.09	23.52	30.12	37.87	47.73
6o	0.5	3.33	12.13	23.52	31.06	39.41	48.75
Control	–	–	–	–	–	–	–
Nifedipine	0.9	2.51	11.72	23.55	30.80	39.41	48.39

was mixed together with sodium bicarbonate (200 mmol) and 100 mL of dimethylformamide, reaction mixture was heated at 70 °C for 12 h. After cooling to room temperature, the mixture was diluted with 150 mL of brine and extracted with ether (2 × 150 mL), dried over magnesium sulfate and excess of solvent was removed under reduced pressure. After complete drying the product was collected and recrystallized from ethanol. The structure of compounds was confirmed by IR, ^{1}H NMR, mass and element analyses.

*5.1.1.1. Ethyl 6-methyl-2-methoxy-4-(phenyl)-1, 4-dihydropyrimidine-5-carboxylate (**4a**).* Yield: 64.47%, m.p. 202–204 °C. R_f: 0.56 (chloroform–benzene 50:50). IR (KBr) ν = 1687.6 cm^{-1} (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H, N_1–H), 8.15 (d, J = 7.9 Hz, 1H, aromatic), 8.11 (s, 1H, aromatic), 7.64 (d, J = 7.9 Hz, 1H, aromatic), 7.51 (t, J = 7.9 Hz, 1H, aromatic), 7.32 (m, 5H, aromatic), 5.98, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.19 (m, 2H, ethyl ester), 2.39 (s, 3H, methyl) and 1.40–1.70 (d, J = 7.38 Hz, 2H, ethyl ester). ESMS: m/z (MH^+) 274. Anal. ($C_{15}H_{18}N_2O_3$) C(65.68/65.66), H(6.61/6.50), N(10.21/10.24).

*5.1.1.2. Ethyl 6-methyl-2-methoxy-4-(4-hydroxyphenyl)-1, 4-dihydropyrimidine-5-carboxylate (**4b**).* Yield: 64%, m.p. 216–218 °C. R_f: 0.71 (chloroform–benzene 20:80). IR (KBr) ν = 1687.6 cm^{-1} (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H, N_1–H), 8.15 (d, J = 7.9 Hz, 1H, aromatic), 8.13 (s, 1H, aromatic), 7.64 (d, J = 7.9 Hz, 1H, aromatic), 7.51 (t, J = 7.9 Hz, 1H, aromatic), 7.42 (m, 5H, aromatic), 5.90, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.11 (m, 2H, ethyl ester), 2.49 (s, 3H, methyl) and 1.18 (t, J = 7.38 Hz, 3H, ethyl ester). ESMS: m/z (MH^+) 289. Anal. ($C_{15}H_{18}N_2O_4$) C(62.06/62.09), H(6.25/6.22), N(9.65/9.69).

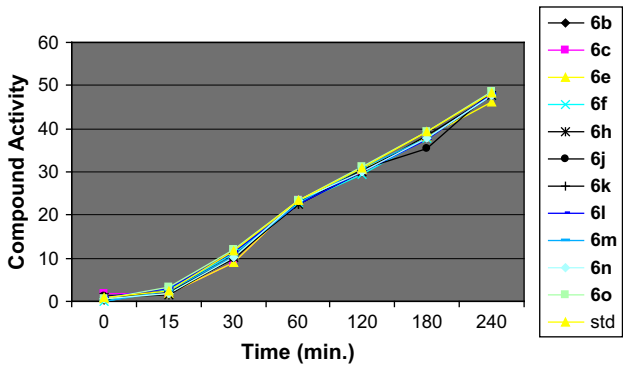


Fig. 2. Graph showing (%) decrease in diastolic blood pressure (DBP) by carotid artery cannulation method.

Table 5
Anti-inflammatory activity data at 10 mg/kg dose.

Compound (10 mg/kg)	Average paw volume			
	0 h	1 h	3 h	5 h
6a	1.15 ± 0.01	1.12 ± 0.02	1.10 ± 0.02	1.10 ± 0.01
6b	1.20 ± 0.02	1.18 ± 0.02	1.15 ± 0.02	1.10 ± 0.02
6c	1.20 ± 0.01	1.17 ± 0.01	1.15 ± 0.01	1.10 ± 0.01
6d	1.15 ± 0.02	1.12 ± 0.02	1.10 ± 0.02	1.10 ± 0.01
6e	1.14 ± 0.01	1.12 ± 0.02	1.10 ± 0.02	1.10 ± 0.01
6f	1.21 ± 0.02	1.17 ± 0.02	1.14 ± 0.02	1.0 ± 0.02
6g	1.14 ± 0.01	1.12 ± 0.02	1.10 ± 0.02	1.10 ± 0.01
6h	1.20 ± 0.01	1.18 ± 0.02	1.14 ± 0.02	1.10 ± 0.01
6i	1.18 ± 0.01	1.16 ± 0.02	1.15 ± 0.02	1.14 ± 0.01
6j	1.17 ± 0.01	1.16 ± 0.01	1.16 ± 0.01	1.16 ± 0.01
6k	1.21 ± 0.02	1.17 ± 0.02	1.14 ± 0.02	1.0 ± 0.02
6l	1.16 ± 0.02	1.14 ± 0.02	1.12 ± 0.02	1.10 ± 0.01
6m	1.18 ± 0.01	1.14 ± 0.02	1.11 ± 0.01	0.9
6n	1.21 ± 0.02	1.16 ± 0.02	1.13 ± 0.02	1.10 ± 0.02
6o	1.21 ± 0.01	1.16 ± 0.01	1.12 ± 0.01	1.0 ± 0.01
Control	1.86 ± 0.02	1.86 ± 0.01	1.86 ± 0.02	1.86 ± 0.01
Indomethacin	1.11 ± 0.01	1.04 ± 0.01	0.91 ± 0.02	0.93 ± 0.01

*5.1.1.3. Ethyl 6-methyl-2-methoxy-4-(4-methylphenyl)-1, 4-dihydropyrimidine-5-carboxylate (**4c**).* Yield: 76%, m.p. 144–147 °C. R_f: 0.78 (chloroform–benzene 20:80). IR (KBr) ν = 1687.6 cm^{-1} (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1H, N_1–H), 8.15 (d, J = 7.9 Hz, 1H, aromatic), 8.17 (s, 1H, aromatic), 7.74 (d, J = 7.9 Hz, 1H, aromatic), 7.51 (t, J = 7.9 Hz, 1H, aromatic), 7.32 (m, 5H, aromatic), 5.98, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.10 (m, 2H, ethyl ester), 2.59 (s, 3H, methyl) and 1.18 (t, J = 7.38 Hz, 3H, ethyl ester). ESMS: m/z (MH^+) 288. Anal. ($C_{16}H_{20}N_2O_3$) C(66.65/66.68), H(6.99/6.96), N(9.72/9.70).

*5.1.1.4. Ethyl 6-methyl-2-methoxy-4-(4-methoxyphenyl)-1, 4-dihydropyrimidine-5-carboxylate (**4d**).* Yield: 75%, m.p. 157–159 °C. R_f: 0.64 (chloroform–benzene 20:80). IR (KBr) ν = 1687.6 cm^{-1} (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H, N_1–H), 8.14 (d, J = 7.9 Hz, 1H, aromatic), 8.16 (s, 1H, aromatic), 7.66 (d, J = 7.9 Hz, 1H, aromatic), 7.54 (t, J = 7.9 Hz, 1H, aromatic), 7.33 (m, 5H, aromatic), 5.96, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.11 (m, 2H, ethyl ester), 2.342 (s, 3H, methyl) and 1.14 (t, J = 7.38 Hz, 3H, ethyl ester). ESMS: m/z (MH^+) 305. Anal. ($C_{16}H_{20}N_2O_4$) C(63.14/63.16), H(6.62/6.63), N(9.20/9.21)

Table 6
Comparative study results for inhibition (%) at 10 mg/kg dose for anti-inflammatory activity with indomethacin as standard.

Compound	% Inhibition [1 − (D_t/D_c) × 100]			
	0 h	1 h	3 h	5 h
6a	38.09	39.83	41.03	42.05
6b	35.57	36.62	38.36	42.0
6c	35.62	37.09	38.41	42.65
6d	38.25	39.83	41.03	42.05
6e	38.82	39.83	41.03	42.05
6f	35.83	37.15	38.76	47.26
6g	38.84	39.83	40.91	42.05
6h	35.62	36.62	38.76	42.05
6i	36.62	37.63	38.25	39.94
6j	39.09	37.78	37.85	38.89
6k	35.83	37.15	38.76	47.26
6l	37.15	38.82	39.83	42.05
6m	36.62	38.82	40.55	52.63
6n	35.83	37.78	39.48	42.0
6o	35.80	37.70	39.83	47.2
Control	–	–	–	–
Indomethacin	41.0	46.38	51.84	52.63

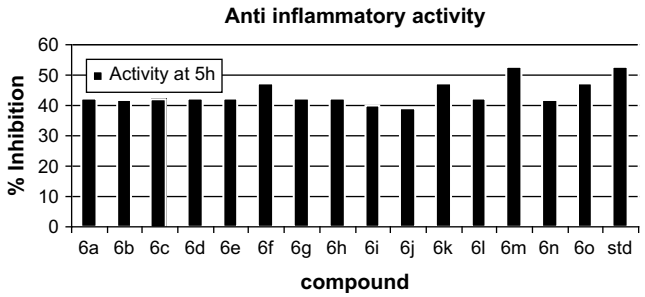


Fig. 3. Graph showing decrease in inflammation compared with Std. (indomethacin).

*5.1.1.5. Ethyl 4-(4-chlorophenyl)-6-methyl-2-methoxy-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**4e**).* Yield: 64%, m.p. 240–245 °C. R_f: 0.74 (chloroform–benzene 20:80). IR (KBr) ν = 1687.6 cm^{-1} (C═O), 1392.5, 3176 (N–H), 1292.22 (C–N). ^1H NMR ($CDCl_3$) δ 8.31 (s, 1H, N_1–H), 8.17 (d, J = 7.9 Hz, 1H, aromatic), 8.14 (s, 1H, aromatic), 7.66 (d, J = 7.9 Hz, 1H, aromatic), 7.54 (t, J = 7.9 Hz, 1H, aromatic), 7.30 (m, 5H, aromatic), 5.96, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.11 (m, 2H, ethyl ester), 2.49 (s, 3H, methyl) and 1.18 (t, J = 7.38 Hz, 3H, ethyl ester). ESMS: m/z (MH^+) 307. Anal. ($C_{15}H_{17}ClN_2O_3$) C(58.35/58.40), H(5.55/5.51), N(9.07/9.09).

*5.1.2. General procedure for preparation of compounds **6a–o***

A mixture of ethyl 6-methyl-2-methoxy-4-(substituted phenyl)-1, 4-dihydropyrimidine-5-carboxylates **4a–e** (0.01 mmol) and substituted phenacyl bromides **5a–c** (0.01 mmol) were taken in dichloromethane (20 mL) as solvent and pyridine (1.5 mL) as a catalyst. The resultant mixture was refluxed for 7 h. Then it was cooled to room temperature and poured on to crushed ice. It was kept overnight and filtered to obtain solid, which was dried and recrystallized from ethanol.

*5.1.2.1. Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone)-4-(phenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6a**).* Yield: 80.88%, m.p. 96–99 °C. R_f: 0.67 (ethanol–benzene 40:60). IR (KBr) ν = 1687.6 cm^{-1} (C═O), 1392.5, 3176 (N–H), 1292.22 (C–N). ^1H NMR ($CDCl_3$) δ 7.78 (d, J = 7 Hz, 1H), 7.64 (t, J = 7.0 Hz, 1H), 7.55 (t, J = 7 Hz, 1 H), 7.37 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.11 (s, 1H), 4.8–4.6 (m, 1H), 3.42 (q, J = 6 Hz, 2H), 3.7 (s, 3H), 2.46 (s, 3H), 0.96 (t, J = 6 Hz, 3H). ESIMS: m/z (MH^+) 394. Anal. ($C_{23}H_{26}N_2O_4$) C(70.03/70.06), H(6.64/6.66), N(7.10/7.09).

*5.1.2.2. Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(phenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6b**).* Yield: 45%, m.p. 158–161 °C. R_f: 0.63 (ethanol–benzene 40:60). IR (KBr) ν = 1687.6 cm^{-1} (C═O), 1392.51, 3203 (N–H), 1325.01 (C–Cl) 1292.22 (C–N). ^1H NMR ($CDCl_3$) δ 7.76 (d, J = 7 Hz, 1H), 7.72 (t, J = 7.0 Hz, 1H), 7.56 (t, J = 7 Hz, 1H), 7.39 (d, J = 7.0 Hz, 1H), 7.05 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.89 (s, 1H), 4.9–4.7 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.8 (s, 3H), 2.45 (s, 3H), 0.98 (t, J = 6 Hz, 3H). ESIMS: m/z (MH^+) 429. Anal. ($C_{23}H_{25}$ ClN_2O_4) C(64.41/64.44), H(5.88/5.90), N(6.5/6.2).

Table 7
Analgesic and ulcerogenic activity of compounds **6a–6h** and **6k–6o**.

Compound (50 mg/kg)	Analgesic activity (% protection)	Ulcerogenic activity (severity index)
6a	40.14	0.16
6b	41.66	0.45
6c	45.80	0.74
6d	37.87	0.45
6e	37.33	0.20
6f	51.33	0.20
6g	40.33	0.18
6h	45.66	0.45
6k	53.17	0.76
6l	43.33	0.56
6m	59.76	0.75
6n	49.56	0.45
6o	51.33	0.20
Control	–	0.00
Ibuprofen	62.55	1.97

*5.1.2.3. Ethyl 6-methyl-2-methoxy-3-[1-(4-methoxyphenyl) ethanone]-4-(phenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6c**).* Yield: 64%, m.p. 108–110 °C. R_f: 0.67 (ethanol–benzene 40:60). IR (KBr) ν = 1691.6 cm^{-1} (C═O), 1390.58, 3245 (N–H), 1355.86 (O–CH_3), 1292.22 (C–N). ^1H NMR ($CDCl_3$) δ 7.76 (d, J = 7 Hz, 1H), 7.64 (t, J = 7.0 Hz, 1H), 7.55 (t, J = 7 Hz, 1H), 7.39 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.94 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.9 (s, 3H), 2.45 (s, 3H), 0.96 (t, J = 6 Hz, 3H). ESIMS: m/z (MH^+) 424. Anal. ($C_{24}H_{28}N_2O_5$) C(67.91/67.93), H(6.65/6.68), N(6.60/6.63).

*5.1.2.4. Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone)-4-(4-chlorophenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6d**).* Yield: 60%, m.p. 115–120 °C. R_f: 0.67 (ethanol–benzene 40:60). IR (KBr) ν = 1690.6 cm^{-1} (C═O), 1390.58, 3245 (N–H), 1355.86 (O–CH_3), 1325.01 (C–Cl), 1292.22 (C–N). ^1H NMR ($CDCl_3$) δ 7.77 (d, J = 7 Hz, 1H), 7.62 (t, J = 7.0 Hz, 1H), 7.58 (t, J = 7 Hz, 1H), 7.35 (d, J = 7.0 Hz, 1H), 7.03 (d, J = 10 Hz, 2H), 6.52 (d, J = 10 Hz, 2H), 5.91 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.3 (s, 3H), 2.47 (s, 3H), 0.95 (t, J = 6 Hz, 3H). ESIMS: m/z (MH^+) 429. Anal. ($C_{23}H_{25}$ ClN_2O_4) C(64.41/64.38), H(5.88/5.89), N(6.53/6.50).

*5.1.2.5. Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-chlorophenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6e**).* Yield: 55%, m.p. 110–115 °C. R_f: 0.67 (ethanol–benzene 40:60). IR (KBr) ν = 1690.6 cm^{-1} (C═O), 1390.58, 3245 (N–H), 1355.86 (O–CH_3), 1330, 1325.01 (C–Cl), 1292.22 (C–N). ^1H NMR ($CDCl_3$) δ 7.75 (d, J = 7 Hz, 1H), 7.64 (t, J = 7.0 Hz, 1H), 7.45 (t, J = 7 Hz, 1H), 7.38 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.67 (d, J = 10 Hz, 2H), 5.95 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.9 (s, 3 H), 2.44 (s, 3H), 0.99 (t, J = 6 Hz, 3H). ESIMS: m/z (MH^+) 464. Anal. ($C_{23}H_{24}Cl_2N_2O_4$) C(59.62/59.60), H(5.22/5.27), N(6.05/6.08).

*5.1.2.6. Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-methoxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6f**).* Yield: 65%, m.p. 115–120 °C. R_f: 0.67 (ethanol–benzene 40:60). IR (KBr) ν = 1690.6 cm^{-1} (C═O), 1390.58, 3245 (N–H), 1355.86 (O–CH_3), 1325.01 (C–Cl), 1292.22 (C–N). ^1H NMR ($CDCl_3$) δ 7.76 (d, J = 7 Hz, 1H), 7.62 (t, J = 7.0 Hz, 1H), 7.55 (t, J = 7 Hz, 1H), 7.34 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.91 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.7 (s, 3H), 2.45 (s, 3H), 0.96 (t, J = 6 Hz, 3H). ESIMS: m/z (MH^+) 459. Anal. ($C_{24}H_{27}ClN_2O_5$) C(62.81/62.85), H(5.93/5.90), N(6.10/6.15).

*5.1.2.7. Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone)-4-(4-hydroxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6g**).* Yield: 78%, m.p. 131–134 °C. R_f: 0.57 (ethanol–benzene 40:60). IR (KBr) ν = 1691.6 cm^{-1} (C═O), 1390.51, 3176 (N–H), 1296.22 (C–N). ^1H NMR ($CDCl_3$) δ 7.74 (d, J = 7 Hz, 1H), 7.62 (t, J = 7.0 Hz, 1H), 7.55 (t, J = 7 Hz, 1 H), 7.44 (d, J = 7.0 Hz, 1H), 7.06 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.91 (s, 1H), 4.8–4.4 (m, 1H), 3.97 (q, J = 6 Hz, 2H), 3.7 (s, 3H), 2.47 (s, 3H), 0.95 (t, J = 6 Hz, 3H). ESIMS: m/z (MH^+) 411. Anal. ($C_{23}H_{26}N_2O_5$) C(67.30/67.35), H(6.38/6.35), N(6.82/6.85).

*5.1.2.8. Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-hydroxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6h**).* Yield: 57%, m.p. 136–138 °C. R_f: 0.57 (ethanol–benzene

40:60). IR (KBr) ν = 1687.6 cm^{-1} (C═O), 1390.51, 3203 (N–H), 1342.01 (C–Cl), 1296.22 (C–N). ^1H NMR ($CDCl_3$) δ 7.76 (d, J = 7 Hz, 1H), 7.68 (t, J = 7.0 Hz, 1H), 7.56 (t, J = 7 Hz, 1H), 7.35 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.91 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.7 (s, 3H), 2.45 (s, 3H), 0.99 (t, J = 6 Hz, 3H). ESIMS: m/z (MH^+) 444. Anal. ($C_{23}H_{25}ClN_2O_5$) C(62.09/62.11), H(5.66/5.65), N(6.30/6.34).

*5.1.2.9. Ethyl 6-methyl-2-methoxy-3-[1-(4-methoxyphenyl) ethanone]-4-(4-hydroxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6i**).* Yield: 60%, m.p. 106–108 °C. R_f: 0.57 (ethanol–benzene 40:60). IR (KBr) ν = 1691.6 cm^{-1} (C═O), 1390.51, 3245 (N–H), 2914.24 (O–H), 1296.22 (C–N). ^1H NMR ($CDCl_3$) δ 8.76 (d, J = 7 Hz, 1H), 7.62 (t, J = 7.0 Hz, 1H), 7.55 (t, J = 7 Hz, 1H), 7.34 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 6.0 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.9 (s, 3H), 2.45 (s, 3H), 0.99 (t, J = 6 Hz, 3H). ESIMS: m/z (MH^+) 441. Anal. ($C_{24}H_{28}N_2O_6$) C(65.44/65.47), H(6.41/6.45), N(6.36/6.32).

*5.1.2.10. Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone)-4-(4-methylphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6j**).* Yield: 62.5%, m.p. 116–118 °C. R_f: 0.76 (ethanol–benzene 40:60). IR (KBr) ν = 1691.6 cm^{-1} (C═O), 1390.51, 3176 (N–H), 1296.22 (C–N). ^1H NMR ($CDCl_3$) δ 9.75 (s, 1H), 8.5 (br s, 1H), 8.2 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.48 (t, J = 7.91 Hz, 1H), 6.7 (s, 1H), 6.4 (br, s, 1H), 5.05 (t, J = 6.3 Hz, 1H), 2.4 (s, 3H), 1.29 (d, J = 5.8 Hz, 3H), 1.16 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH^+) 409. Anal. ($C_{24}H_{28}N_2O_4$) C(70.57/70.55), H(6.91/6.96), N(6.86/6.85).

*5.1.2.11. Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-methylphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6k**).* Yield: 45.5%, m.p. 104–107 °C. R_f: 0.68 (ethanol–benzene 40:60). IR (KBr) ν = 1687.6 cm^{-1} (C═O), 1390.51, 3203 (N–H), 1342.01 (C–Cl) 1296.22 (C–N). ^1H NMR ($CDCl_3$) δ 9.75 (s, 1H), 8.5 (br s, 1H), 8.2 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.48 (t, J = 7.91 Hz, 1H), 6.7 (s, 1H), 6.4 (br s, 1H), 5.05 (t, J = 6.3 Hz, 1H), 2.4 (s, 3H), 1.29 (d, J = 5.8 Hz, 3H), 1.16 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH^+) 443. Anal. ($C_{24}H_{27}ClN_2O_5$) C(65.08/65.06), H(6.14/6.11), N(6.32/6.29).

*5.1.2.12. Ethyl 6-methyl-2-methoxy-3-[1-(4-methoxyphenyl) ethanone]-4-(4-methylphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6l**).* Yield: 66%, m.p. 157–159 °C. R_f: 0.68 (ethanol–benzene 40:60). IR (KBr) ν = 1693.6 cm^{-1} (C═O), 1390.51, 3245 (N–H), 1402 (CH_3–O–C), 1296.22 (C–N). ^1H NMR ($CDCl_3$) δ 9.71 (s, 1H), 8.4 (br s, 1H), 8.2 (s, 1H), 8.1 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.48 (t, J = 7.91 Hz, 1H), 6.8 (s, 1H), 6.4 (br s, 1H), 5.05 (t, J = 6.3 Hz, 1H), 2.6 (s, 3H), 1.29 (d, J = 5.8 Hz, 3H), 1.18 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH^+) 439. Anal. ($C_{25}H_{30}N_2O_5$) C(68.47/68.50), H(6.90/6.92), N(6.35/6.38).

*5.1.2.13. Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone)-4-(4-methoxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6m**).* Yield: 81%, m.p. 134–137 °C. R_f: 0.58 (ethanol–benzene 40:60). IR (KBr) ν = 1687.9 cm^{-1} (C═O), 1390.51, 3176 (N–H), 1402 (CH_3–O–C), 1296.22 (C–N). ^1H NMR ($CDCl_3$) δ 9.77 (s, 1H), 8.5 (br s, 1H), 8.4 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.6 (d, J = 7.4 Hz, 1H), 7.48 (t, J = 7.91 Hz, 1H), 6.9 (s, 1H), 6.4 (br s, 1H), 5.15 (t, J = 6.3 Hz, 1H), 2.4 (s, 3H), 1.29 (d, J = 5.8 Hz, 3H), 1.17 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH^+) 425. Anal. ($C_{24}H_{28}N_2O_5$) C(67.91/67.94), H(6.65/6.64), N(6.60/64).

*5.1.2.14. Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-methoxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6n**).* Yield: 74%, m.p. 155–158 °C. R_f: 0.78 (ethanol–benzene 40:60). IR (KBr) ν = 1689.8 cm^{-1} (C═O), 1402.15, 3204 (N–H), 1346.01 (C–Cl), 1402 (CH_3–O–C), 1296.22 (C–N). ^1H NMR ($CDCl_3$) δ 9.55 (s, 1H), 8.4 (br s, 1H), 8.3 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.40 (t, J = 7.91 Hz, 1H), 6.5 (s, 1H), 6.3 (br s, 1H), 5.10 (t, J = 6.3 Hz, 1H), 2.4 (s, 3H), 1.20 (d, J = 5.8 Hz, 3H), 1.18 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH^+) 459. Anal. ($C_{24}H_{27}ClN_2O_5$) C(62.81/62.85), H(5.93/5.95), N(6.10/6.11).

*5.1.2.15. Ethyl 6-methyl-2-methoxy-3-[1-(4-methoxyphenyl) ethanone]-4-(4-methoxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (**6o**).* Yield: 74%, m.p. 108–111 °C. R_f: 0.64 (ethanol–benzene 40:60). IR (KBr) ν = 1690.8 cm^{-1} (C═O), 1402.15, 3240 (N–H), 1402 (CH_3–O–C), 1402 (CH_3–O–C), 1296.22 (C–N). ^1H NMR ($CDCl_3$) δ 9.78 (s, 1H), 8.7 (br s, 1H), 8.2 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.52 (t, J = 7.91 Hz, 1H), 6.7 (s, 1H), 6.4 (br, s, 1H), 5.00 (t, J = 6.3 Hz, 1H), 2.2 (s, 3H), 1.25 (d, J = 5.8 Hz, 3H), 1.26 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH^+) 455. Anal. ($C_{25}H_{30}N_2O_6$) C(66.06/66.06), H(6.65/6.69), N(6.16/6.18).

5.2. Pharmacological study

5.2.1. Antihypertensive activity

5.2.1.1. Non-invasive tail-cuff method. The newly synthesized compounds **6a**–**o** were used for antihypertensive activity studies. Norwegian strain of inbred albino rats (male) weighing 200–250 g were used in experiment. Nifedipine was used as standard drug. All rats were housed in a temperature and humidity controlled room with 12-hour light/dark cycle. All rats were allowed free access to regular food and tap water. The drinking water was replaced by 1% w/v sodium chloride aqueous solution for rats used in DOCA experiments. All experimental work was carried out in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments in Animal (CPCSEA), India.

5.2.1.2. DOCA-salt hypertension. Rats were anesthetized by injecting pentobarbital injection administered intraperitoneally in a dose of 50 mg/kg body wt. It was placed on a heated surgical surface maintained at 37 °C. A flank incision was made to expose the left kidney, which was ligated and removed. This procedure of removing either of the kidneys is called as uninephrectomy. The incision was sutured. One week after uninephrectomy, rats were administered subcutaneously with injection of DOCA (30–50 mg/kg/week) and drinking water was replaced by 1% w/v sodium chloride aqueous solution. Control group of rats were uninephrectomized, injection of DOCA-salt was not administered to them and received vehicle injections and tap water [25].

5.2.1.2.1. Non-invasive blood pressure (NIBP) measurements. Indirect blood pressure (BP) was determined with a Power Lab/4SP with ML135 Dual Bio Amp and computerized BP monitor (AD instruments Pvt. Ltd., Australia). This system measures systolic blood pressure (SBP) by recording the cuff pressure at which the interrupted blood flow returns to the tail. Training the rats for tail-cuff blood pressure measurements was necessary to reduce the stress associated with the BP measurements and hence reduces the variability of BP with successive measurements. Training consisted of six sessions over 3 days. On day one, rats were introduced into plastic restrainer for 5 min per session. The tail-cuff was inflated five times in quick succession. By day three, the training was extended to 10 min per session. The effect of training was to reduce the standard deviation around mean BP. At the end of session rats were ready for BP recording. They were restrained by being placed into cylindrical restrainer. For better detection of tail pulse, the tail artery was dilated by placement of restrained mouse into thermostatically controlled Lucite box, heated at 33–34 °C, for 2–5 min before BP measurement was started [26].

Tail pulse was detected by passage of tail through a narrow tail-cuff sensor attached to the amplifier. BP measurements were started by automatic inflation of tail-cuff to greater than 200 mmHg and release of pressure. The results were recorded in form of graph. The computer provides two tracings that start and stop at the same time. The lower trace channel plots cuff pressure, which is calibrated at 500 mmHg at full scale. The tracing sharply rises when applied to the tail-cuff and falls off gradually during the 15–20 s of the test. The upper trace channel monitors pulse, with fluctuations about the centre line suddenly appearing at the onset of pulsations (Fig. 3). The first onset of pulse is taken as the systolic blood pressure. Initiation of pulse pressure was determined when the baseline amplitude increased in accordance to the set maximal inflated cuff pressures, maximal inflation was set at 200 mmHg.

Blood pressure recording was considered to be successful if the mouse did not move and a clear initial pulse could be seen. Ten tail-cuff measurements were made in a session. The BP for the session was accepted as the average of four BP readings that were within 5 mmHg or the average of 10 readings that were within 8 mmHg. BP measurements were done thrice per week for two weeks.

Twelve groups of six rats weighing between 200 and 250 g were made. Animals were operated and hypertension was induced as detailed above. Rats were trained for the experiment. On the first day of experiment the test compounds were administered by oral feeding using an oral feeding needle. Compounds **6a–o** as test compounds were prepared in 0.5% carboxymethyl cellulose and dosed 10 mg/kg orally. One group of animal was treated with standard drug nifedipine. One of the twelve groups was treated with vehicle.

Prior to dosing the animals, initial graph reading was taken to record the BP before administration of drug. After 1 h of dosing recordings were taken as mentioned above. Each compound was evaluated three times in one week, and average of the recordings was recorded accordingly.

The experiment was repeated at two different dose levels (5 and 2.5 mg/kg) after animal washing at interval of two weeks respectively, for those compounds that showed significant statistical differences between the test and control group. Average readings were calculated by employing ANOVA method.

5.2.1.3. Determination of blood pressure by cannulation. Direct blood pressure measurements were performed to study the diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) by cannulation technique employing Power Lab/4SP with ML135 Dual Bio Amp and computerized BP monitor (AD instruments Pvt. Ltd., Australia). Compounds **6b**, **6c**, **6e**, **6f**, **6h**, **6j**, **6k**, **6l**, **6m**, **6n** and **6o** were tested by direct cannulation method.

Animals were anesthetized with sodium pentobarbital (35 mg/kg i.p.), and the left carotid artery was exposed, cannulated and exteriorized between the scapulas. Blood pressure was measured directly from cannula using transducer attachments of the above instrument. After animals recovered from surgery and a baseline blood pressure was established, they were dosed orally with test compounds via feeding needle. Acute effects were determined by monitoring blood pressure at 15, 30, 60, 120 and 180 min after oral dosing. Average readings were calculated by employing ANOVA method.

5.2.2. Anti-inflammatory activity: carrageenan induced rat-paw oedema method

The method of Winter et al. [22] was employed with some modifications. All test samples were administered to animals at a 100 mg/kg dosage, as suspension in 0.5% carboxymethyl cellulose and administered orally. After 60 min of drug dose, the injection of 0.1 mL of solution of carrageenan (0.5 mg/25 mL) was injected into the sub-plantar tissue of the left hind paw of each rat. Out of this, one group was treated with indomethacin as standard (100 mg/kg). The initial volume of paw was measured within 30 s after carrageenan injection. Later on paw volume was measured after 1–5 h respectively. The relative increase in the paw volume was calculated in the individual animal of the control, test, and standard groups respectively. The % inhibition of oedema was calculated as follows:

$$\text{Anti} - \text{inflammatory activity } (\%\text{inhibition}) = [1-(D_t/D_c)\times 100]$$

where D_t is mean relative change in a paw volume in test group and D_c is mean relative change in paw volume in control group.

The experiment was repeated at two different dose levels (25 and 50 mg/kg) for compounds which showed significant statistical differences between the test and the control group. ANOVA was employed as the statistical method.

5.2.3. Analgesic activity: writhing test method

Analgesic activity was carried out by acetic acid induced writhing method [23] in Swiss albino mice (25–30 g). A 0.6% aqueous acetic acid solution was injected intraperitoneally (i.p.) to a volume of 0.1 mL used as writhing inducing agent. In each group six mice were kept. Mice were kept individually in test cage, before acetic acid injection and habituated for 30 min. Screening of analgesic activity was performed after p.o. administration of test compounds at a dose of 20 mg/kg. The compounds, which exhibited good anti-inflammatory activity comparable to that of indomethacin, were screened for analgesic activity. All compounds were dissolved in 1% CMC. Ibuprofen was used as reference drug. After 1 h of drug administration 0.10 mL of 1% acetic acid solution was given to mice intraperitoneally. Stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted for 5–15 min of acetic acid injection. The analgesic activity was expressed in terms of % inhibition. % Analgesic activity was calculated as follows:

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

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

Statistical analysis was done using Student's *t*-test. The percent protection in mice brought about by administration of the drug is shown in table.

5.2.4. Acute ulcerogenesis

Acute ulcerogenesis test was done according to Cioli et al. [24]. Albino rats (150–200 g) were divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after p.o. administration of test compounds or ibuprofen at the dose of 50 mg/kg. Control rats received p.o. administration of vehicle (suspension of 1% methyl cellulose). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The gastric mucosa of the rats was examined by means of a 4× binocular magnifier. The lesions were counted and divided into large (greater than 2 mm in diameter), small (1–2 mm) and punctiform (less than 1 mm). For each stomach the severity of mucosal damage was assessed according to the following scoring system: 0—no lesions or up to five punctiform lesions; 1—more than five punctiform lesions; 2—one to five small ulcers; 3—more than five small ulcers or one large ulcer; 4—more than one large ulcer.

The mean score of each treated group minus the mean score of the control group was considered the 'severity index' of gastric damage.

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