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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

New dihydropyridine derivatives: anti-inflammatory, analgesic and docking studies

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Abstract The present article describes synthesis of new diethyl 2,6-dimethyl-4-(4-(2-substituted amino-2-oxoethoxy) phenyl)-1,4-dihydropyridine-3,5-dicarboxylates (**6a–10b)** following multistep synthetic route. Structures of newly synthesized intermediates and title compounds were established by spectral and elemental analyses. The final compounds were screened for their in vivo anti-inflammatory and analgesic activities by carrageenan-induced paw oedema and tail immersion methods, respectively. Moreover, molecular docking studies were carried out for active compounds **6c**, **6d**, **7d**, **8** and **10b** to study their mode of action, meanwhile in vivo results indicated that these compounds displayed rapid onset of anti-inflammatory action and exhibited prominent activity when compared with the standard drug. Compounds **6d** and **7d** carrying amide functionality showed the highest anti-inflammatory as well as analgesic activities. The molecular docking results emphasised the in vivo data and all docked molecules were found to display very low binding constant values in nanomolar scale.

Keywords Dihydropyridines · Amide · Anti-inflammatory · Analgesic · Docking studies

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Introduction

White blood cells are the main soldiers in our body and they protect us from infection and foreign substances and unwanted materials. This primary response of the body to the pathogens and injury is referred as inflammation (Kawai and Akira, 2006). Sometimes, however, the white blood cells and their inflammatory chemicals cause damage to the body's tissues. In some diseases, the body's defence system inappropriately triggers an inflammatory response when there are no foreign substances to fight off. Generally, non-steroidal anti-inflammatory drugs (NSAIDs) are extensively used against such inflammatory responses. An ideal anti-inflammatory drug should obstruct any type of uncontrolled inflammatory stimulations and should not interfere with normal defence action. In fact, the NSAIDs inhibit the action of cyclooxygenase (COX-1 and COX-2) enzymes (Chowdhury *et al*., 2009 and Puig *et al*., 2000), which catalyses biosynthesis of prostaglandins (PGs). However, the use of these NSAIDs may lead to several side effects including gastric injury and that might later cause gastric ulceration and renal injury (Navidpour *et al*., 2006 and Dannhardt and Kiefer, 2001). Therefore, there is an immense need for new anti-inflammatory agents which do not show such side effects. Further, most of the reported NSAIDs act as anti-inflammatory as well as analgesic agents. These agents produce their therapeutic effects by inhibiting various prostaglandin substances involved in development of pain, inflammation as well as regulation of body temperature (Modi *et al*., 2012). Therefore, it is desirable that new anti-inflammatory agents rather possess analgesic property also.

Dihydropyridine (DHP) derivatives are an important class of compounds in medicinal chemistry owing to their wide range of biological applications. They are well-known

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calcium channel blockers. In addition, they display various activities (Coburn *et al*., 1988), viz. antihypertensive (Jiang *et al*., 1999 and Schleifer, 1999) hypnotic (Godfraid *et al*., 1986), anticoagulant (Kumar *et al*., 2011), antihypoxic, antiischemic (Khadilkar and Borkar, 1998), antitubercular (Kharkar *et al*., 2002) and antimicrobial (Sirisha *et al*., 2011) activities. In particular, several DHP derivatives were also reported as potent anti-inflammatory agents (Mishra and Mishra, 2007; Sadanandam *et al*., 1994 and Komoda *et al*., 2010). Interestingly, DHPs were found to bind adenosine receptors (A_1, A_2, A_3) effectively in the brain and bring about therapeutic effects successfully (Ismail *et al*., 1995). In fact, in most of the cases, A_3 adenosine receptor antagonists are being sought as potential anti-inflammatory agents (Beaven *et al*., 1994).

Amide is an important pharmacophoric linkage that is shown to possess wide spectrum of biological activity. Several amide derivatives were reported in the literature as prominent anti-inflammatory agents (Raghavendra *et al*., 2012; Galanakis *et al*., 2004; Takahashi and Miyazawa, 2012; Onkol *et al*., 2010 and Papadopoulou *et al*., 2005). Moreover, they were shown to be powerful antimicrobial (Padmavathi *et al*., 2011), antitubercular (Thomas *et al*., 2011) as well as anticancer (Lu *et al*., 2010) agents. More interestingly, amide group was found to increase selectivity of anti-inflammatory agents towards COX-2 enzyme (Kalgutkar *et al*., 2000). It was reported that conversion of indomethacin, meclofenamic acid and ketoprofen to their corresponding amides made them as selective COX-2 inhibitors.

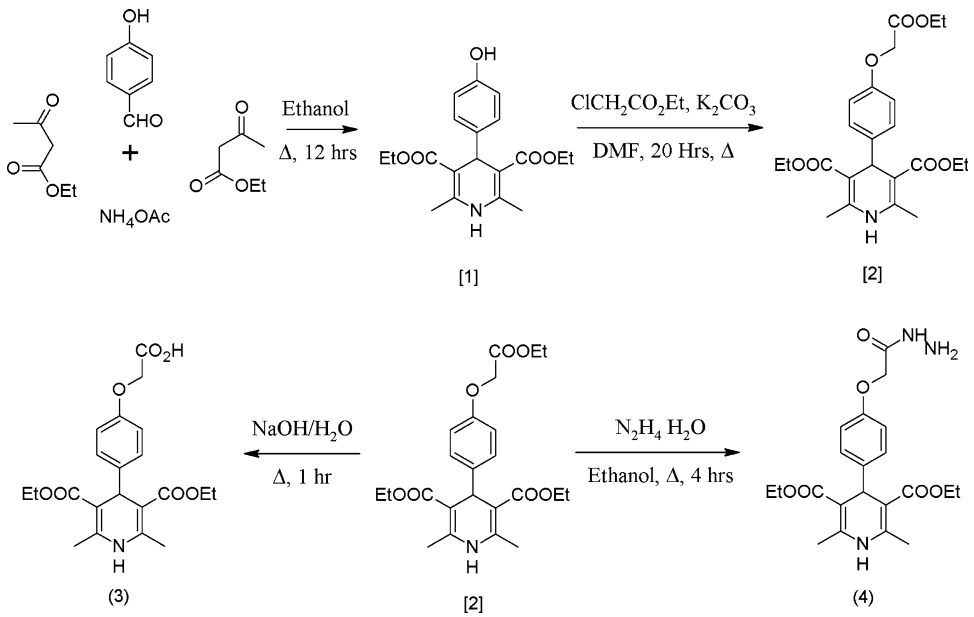
Keeping all the above points in view, it was contemplated to design and synthesize new series of dihydropyridine derivatives (**6a**–**10b**) carrying amide functionality to explore their in vivo anti-inflammatory and analgesic properties following standard methods, with the expectation that resulting molecules would display good pharmacological action. Accordingly, title compounds were synthesized and characterized by spectral data. Later on, they were subjected to in vivo anti-inflammatory and analgesic activities by carrageenan-induced paw oedema and tail immersion methods, respectively. Most of the tested compounds exhibited prominent activity. Finally, theoretical bioassay study was done using Molinspiration server and found that PPAR-γ is the most suitable receptor for docking studies compared to other possible targets. Hence, docking studies were undertaken for most active agents to study their mode of interaction with PPAR-γ and thereby to get supportive coordination between in silico and in vivo anti-inflammatory results. All compounds displayed good binding energy and low inhibition constants. Compound **6d** carrying substituted amino benzothiazole emerged as the most active anti-inflammatory as well as analgesic agent. The docking results are also compliment the in vivo data.

Results and discussion

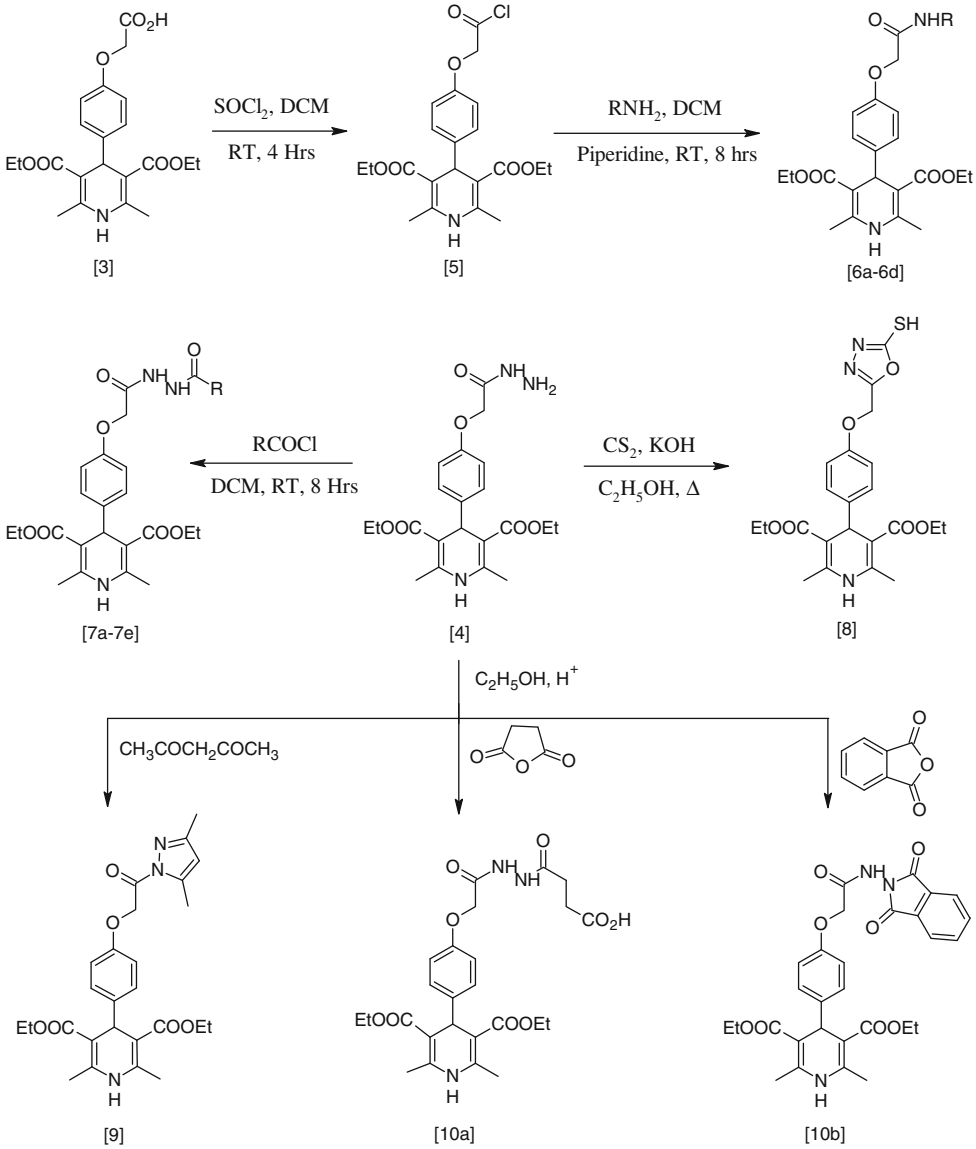
Chemistry

The required intermediates and final compounds **6a**–**10b** were synthesized according to Schemes 1 and 2, respectively. The core ring, dihydropyridine was constructed according to Hantzsch synthesis (Fassihi *et al*., 2009) by refluxing 4-hydroxybenzaldehyde with two equivalents of ethyl acetoacetate and ammonium acetate in ethanol. Then,

Scheme 1 Synthetic route of intermediates



Scheme 2 Synthesis of target compounds



the phenolic group was alkylated with ethyl chloroacetate in DMF under nitrogen atmosphere to obtain the product **2**. Under this condition, only phenolic OH group, but not NH group of DHP ring underwent alkylation. The alkaline hydrolysis of ester **2** afforded the corresponding acid **3** which was later conveniently converted to acid chloride **5** and was coupled with various amines to obtain amides **6a**–**d** in good yield. The ester **2** was transformed to its hydrazide **4** by refluxing it with hydrazine hydrate in ethanol for about 4 h. At this reaction condition, the two ester groups on DHP ring were found to be stable. Another set of amides **7a**–**e** were obtained by coupling hydrazide **4** with different acid chlorides in the presence of piperidine base. In another series, the intermediate **4** was cyclised to form 1,3,4-oxadiazole-2-thiol (**8**) by treating compound **4** with carbon disulphide and potassium hydroxide followed by subsequent neutralization. On the other hand, hydrazide **4** was condensed with acetyl acetone in alcoholic medium containing trace of glacial acetic acid to obtain pyrazole derivative **9**. Finally, intermediate **4** was treated with succinic anhydride and phthalic anhydride under acidic medium, to achieve **10a** and **10b**, respectively, in good yield. Interestingly, reaction of **4** with succinic anhydride led to ring opening reaction and resulted in an carboxylic acid derivative **10a**, while that with phthalic anhydride resulted in substituted pthalimide derivative **10b**.

Newly synthesized target compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectral techniques followed by elemental analysis. Formation of DHP ring was confirmed by FTIR spectrum of **1**, where it showed prominent peaks at 3337 and 1656 cm^{-1}, due to NH/OH and ester groups, respectively. This was also confirmed by its ^{1}H NMR spectrum, wherein it displayed singlets at δ 9.09 and δ 8.77 ppm, which are attributed to

phenolic OH and NH protons of DHP ring, respectively. A multiplet and a triplet at δ 4.04 and δ 1.15 ppm, respectively correspond to two OCH_2CH_3 attached to DHP ring were intern support the structure. Another characteristic singlet at δ 4.74 ppm was observed corresponding to C_4 CH proton also support the proposed structure of **1**. In ^{1}H NMR spectrum of compound **2**, disappearance of OH peak at δ 9.09 ppm clearly established that phenolic hydroxyl group was alkylated but not NH group of DHP ring. In FTIR spectrum of **3**, appearance of new broad peak at 3343 cm^{-1} and a shift in carbonyl stretching frequency from 1739 to 1723 cm^{-1} clearly confirmed hydrolysis of ester **2**. This was supported by ^{1}H NMR spectrum, wherein, a quartet and a triplet peaks that correspond to ester group was disappeared. The intermediate acid chloride **5** was directly used for next step without purification, as it is highly reactive. The formation of amides, viz. **6a** was confirmed by its FTIR spectrum, wherein a new peak at 1672 cm^{-1} corresponding to amide group appeared. This was also confirmed by its ^{1}H NMR spectrum, wherein a new peak at δ 9.31 ppm was observed.

Conversion of ester **2** to a key intermediate **4** was verified by FTIR spectrum of hydrazide **4**, wherein, shifting of carbonyl stretching frequency from 1739 cm^{-1} to lower frequency 1656 cm^{-1} was observed. Also, its ^{1}H NMR spectrum displayed two singlets at δ 9.24 and δ 4.28 ppm confirming the presence of hydrazidic NH and NH_2 groups. Formation of various amides **7a–e** from hydrazide **4** was proved by their ^{1}H NMR spectra. A shift in peak from δ 9.24 to δ 10.19 ppm in ^{1}H NMR spectrum of **7a** that corresponds to amide NH proton, confirmed its structure. Similar pattern of peaks were observed for remaining amide derivatives of the series also. Conversion of hydrazide **4** to 1,3,4-oxadiazole **8** was evidenced by its FTIR and ^{1}H NMR spectra, wherein carbonyl and amine stretching peaks disappeared and new characteristic peak at δ 14.65 ppm that corresponds to tautomeric CSNH proton of 1,3,4-oxadiazole ring appeared. Similarly, conversion of hydrazide to pyrazole derivative **9** was validated by its FTIR spectrum where peaks that correspond to hydrazide group disappeared. Moreover, its ^{1}H NMR spectrum showed two new peaks at δ 2.45 and 2.19 ppm which are attributed to two allylic methyl groups attached to pyrazole ring. In FTIR spectrum of **10a**, a new broad peak at 3390 cm^{-1} and shift of carbonyl stretching frequency to 1718 cm^{-1} were observed, confirming the presence of carboxylic group. A new peak at δ 12.08 ppm in ^{1}H NMR spectrum confirms the ring opening of succinic anhydride. Disappearance of NH_2 peak at δ 4.28 ppm in **10b** clearly established the conversion of hydrazide **4** to phthalimide derivative **10b**. Finally, structures of all the target compounds were confirmed by their ^{13}C NMR and mass spectral data. The spectral data of all the title compounds are summarized in the experimental section and their characterization data are tabulated in Table 1.

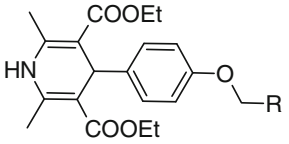
Biological results

The in vivo works have been performed in accordance with the ethical standards on animal experimentations. From the in vivo anti-inflammatory results, it is clear that newly synthesized target compounds are reasonably active. It was observed that the presence of electron donating groups on aryl ring brought about enhancement in the activity. Also, the presence of methyl group at para position of phenyl group in compound **6c** resulted in improved activity. Similar result was observed for compound **7a** which was more active than un-substituted derivative **7b**. Compound **6d** carrying 2-amino benzothiazole group displayed the highest activity when compared with standard drug, i.e. diclophenac. However, less activity was observed for compound **6b** bearing two methyl groups at 2nd and 6th positions of aryl ring. This may be owing to steric hindrance offered by two methyl groups adjacent to amide functionality of the molecule. As the literature reveals, thiophene carboxamide derivatives are good anti-inflammatory agents (Fakhr *et al*., 2009 and Hazra *et al*., 2007). Similarly, in our study compounds **7c**, **7d** and **7e** bearing thiophene pharmacophore exhibited moderate-to-good anti-inflammatory activity. In addition, un-substituted thiophene derivative, **7d** (43.95) displayed improved activity when compared with those possessing halogen substituents adjacent to carbonyl groups. Moreover, compound **8** containing 1,3,4-oxadiazole-2-thiol showed good activity (42.43) while compound **9** carrying substituted pyrazole displayed moderate anti-inflammatory action (13.65). Furthermore, compounds **6c** (43.94), **6d** (47.74) and **7d** (43.95) carrying substituted carboxamide groups, **8** (42.43), **10a** (34.85) and **10b** (34.10) possessing 1,3,4-oxadiazole-2-thiol, a carboxylic acid and phthalimide moieties, respectively showed prominent activity and displayed rapid inhibition just after injection of carrageenan irritant. Results of analgesic activity screening indicated that almost all tested compounds exhibited moderate activity. Particularly, samples **6d** (7.23 $\pm$ 0.02) and **7d** (7.02 $\pm$ 0.025) displayed the highest activity in 60–90 min duration and their results were comparable with standard drug diclophenac potassium. In conclusion, compounds **6c**, **6d**, **7d**, **8**, **10a** and **10b** showed enhanced activity both in anti-inflammatory and analgesic studies with rapid onset of action when compared with the standard. The anti-inflammatory results are tabulated in Table 2. Analgesic data are given in Table 3.

Molecular docking studies

The observed in vivo anti-inflammatory and analgesic potency for the new dihydropyridine derivatives prompted

Table 1 Characterisation data of final compounds **6a–d**, **7a–e**, **8**, **9**, **10a–b**



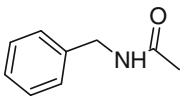
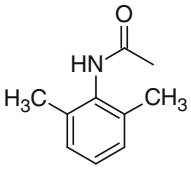
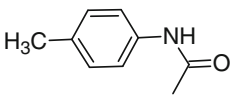
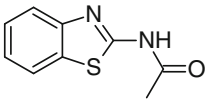
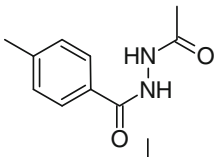
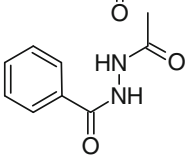
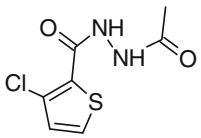
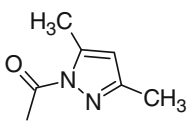
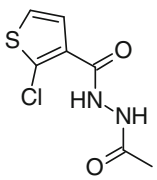
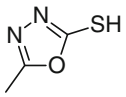
Compound	R	Mol. formula	Mol. weight (g)	M.P. (°C)	Yield (%)
6a		$C_{28}H_{32}N_2O_6$	492.5	165	82
6b		$C_{29}H_{34}N_2O_6$	506.6	176	78
6c		$C_{28}H_{32}N_2O_6$	492.5	116	84
6d		$C_{28}H_{29}N_3O_6S$	535.6	98	80
7a		$C_{29}H_{33}N_3O_7$	535.6	119	76
7b		$C_{28}H_{31}N_3O_7$	521.5	145	78
7c		$C_{26}H_{28}ClN_3O_7S$	562.0	132	80
7d		$C_{26}H_{29}N_3O_7S$	527.5	148	82
7e		$C_{26}H_{28}ClN_3O_7S$	562.0	138	79
8		$C_{22}H_{25}N_3O_6S$	459.5	173	72

Table 1 continued

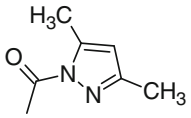
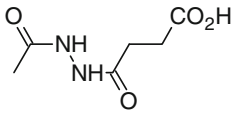
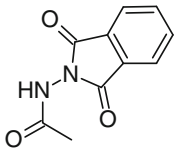
Compound	R	Mol. formula	Mol. weight (g)	M.P. (°C)	Yield (%)
9		$C_{26}H_{31}N_3O_6$	481.5	182	78
10a		$C_{25}H_{31}N_3O_9$	517.5	184	82
10b		$C_{29}H_{29}N_3O_8$	547.5	140	80

Table 2 Anti-inflammatory activity data of final compounds

Compounds	Percentage inhibition			
	½ hr	1 h	2 h	3 h
Standard	16.67	41.03	61.11	65.26
6a	08.33	16.05	08.90	31.14
6b	15.92	26.28	47.79	53.89
6c	43.94	55.13	62.22	61.07
6d	47.74	54.50	64.45	65.86
7a	16.67	35.92	46.67	47.31
7b	09.10	29.50	44.45	44.91
7c	10.61	39.73	57.24	56.28
7d	43.95	55.12	62.20	61.07
7e	12.89	42.30	52.78	53.30
8	42.43	54.50	61.70	61.08
9	13.65	32.70	46.11	48.50
10a	34.85	42.95	57.78	56.88
10b	34.10	42.30	57.22	59.28

Table 3 Analgesic activity data of final compounds

Compound	Tail flick latency in secs			
	0 min	30 min	60 min	90 min
Control	3.35 ± 0.028	3.23 ± 0.005	3.34 ± 0.005	3.25 ± 0.005
Standard	3.23 ± 0.017	6.25 ± 0.02	6.95 ± 0.02	7.45 ± 0.02
6a	3.17 ± 0.017	4.56 ± 0.008	5.73 ± 0.020	6.8 ± 0.017
6b	3.25 ± 0.028	4.92 ± 0.005	6.1 ± 0.057	6.9 ± 0.028
6c	3.24 ± 0.003	5.15 ± 0.028	5.91 ± 0.023	7.12 ± 0.012
6d	3.23 ± 0.033	5.28 ± 0.005	6.03 ± 0.018	7.23 ± 0.020
7a	3.25 ± 0.005	4.99 ± 0.101	6.11 ± 0.063	6.92 ± 0.014
7b	3.20 ± 0.020	4.62 ± 0.005	5.87 ± 0.014	6.37 ± 0.004
7c	3.16 ± 0.013	4.34 ± 0.034	5.67 ± 0.002	6.45 ± 0.034
7d	3.23 ± 0.003	4.92 ± 0.014	6.23 ± 0.024	7.02 ± 0.025
7e	3.27 ± 0.026	4.85 ± 0.004	5.74 ± 0.014	6.46 ± 0.004
8	3.21 ± 0.003	4.97 ± 0.031	6.07 ± 0.004	6.97 ± 0.017
9	3.27 ± 0.027	4.76 ± 0.004	5.93 ± 0.032	6.78 ± 0.012
10a	3.18 ± 0.015	4.96 ± 0.027	6.07 ± 0.018	6.87 ± 0.023
10b	3.29 ± 0.032	5.03 ± 0.026	6.16 ± 0.003	7.21 ± 0.002

us to carry out molecular docking studies of selected active molecules. Currently, computer-aided docking studies are being widely used to predict binding orientations of new drug candidates with protein-receptor sites (Lengauer and Rarey, 1996). The in silico studies were done using AutoDock 4.0 (Morris *et al*., 2009) software and results were found to be in good correlation with in vivo data, which established the anti-inflammatory and analgesic efficacy of new target molecules.

In recent days, the computer-aided bioassay method is widely used to identify suitable receptors and interaction of drugs with those targets (Sousa *et al*., 2006; Suresh *et al*., 2011). Also, it has been established that computer-aided results were found to compliment that of in vitro biochemical assay (Lewis *et al*., 2011). Based on these observations, the predictive bio-assay study was carried out for the final compounds using MOLINSPIRATION server, and it was found that new ligands are most likely to be Nuclear Receptor Modulators rather than being classical enzyme inhibitors (COX) or GPCR ligands. Among nuclear receptors, peroxisome proliferator-activated receptor gamma (PPAR-γ or PPARG) protein is most suitable receptor for nuclear-receptor activators (Gronemeyer *et al*., 2004; Barros *et al*., 2010) and is having diverse ligand binding capacity for anti-inflammatory agents as they were shown to act by inhibiting cyclooxygenase (COX) activity (Desvergne and Wahli, 1995). Hence, in the present study, PPAR-γ protein was taken as

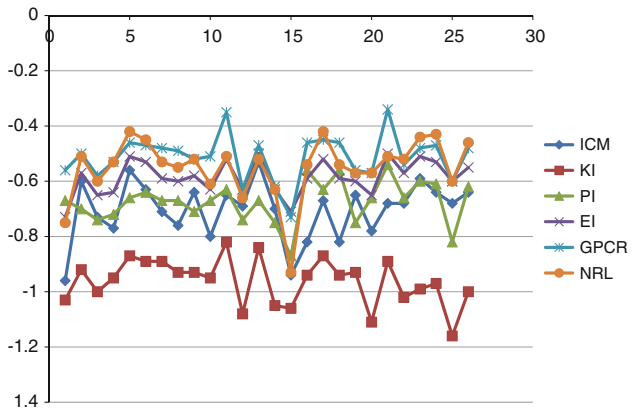


Fig. 1 Graphical representation of computer-aided theoretical bio-assay results. *ICM* ion channel modulators, *KI* kinase inhibitors, *PI* protease inhibitors, *EI* enzyme inhibitors, *GPCR* G-protein coupled receptor, *NRL* nuclear receptor ligand

receptor rather than COX-2 enzyme. The results of theoretical bio-assay study were pictorially represented in Fig. 1. The computational work was carried out on windows7 platform with Intel Corei3 processor and 4 GB DD3 memory system. The required PPAR-γ structure was recovered from protein data bank (PDB ID: 3ADS). The LIGPLOT and CASTp (Dundas *et al*., 2006) analyses were undertaken to recognize any residues present in the active site.

The active site comprises of about fourteen amino acid residues, viz. LEU330, LEU333, MET348, MET364, LEU353, ILE341, GLU343, SER342, ILE281, PHE282, CYS285, SER289, ILE326 and ARG288. Compounds **6c**, **6d**, **7d**, **8** and **10b** with prominent anti-inflammatory activity were selected for docking study and they were found to involve in strong hydrogen bonding interactions with any of these amino acid residues. All the five ligands displayed physical contacts through hydrogen bonding with the active pocket residues. Specifically, at least one of the two ester groups present on DHP ring was engaged in hydrogen bonding with protein-receptor site. Beside the ester group, phenoxy ether and carboxamide groups were also found to participate in interactions with various amino acids present in the active site. The cumulative interactions of these groups of new target molecules with protein-receptor are perhaps responsible for their good bio-activity.

The ligands exhibited very good binding energies ranging from −9.63 to −10.70 kcal/mol. In addition, they showed low inhibition constants in nanomolar scale. These values are significant when compared to that of standard drug (diclofenac sodium), which exhibited binding energy of -6.6 kcal/mol and very high binding constant value (14,320 nM). Diclofenac sodium exhibited two hydrogen bond interactions with ARG288 and SER289 with a bond length of 1.835 and 1.883 Å, respectively. Compound **6c** displayed two hydrogen bond interactions with MET364 and ARG288 residues with bond distance of 2.181 and 2.081 Å, respectively. Also, it exhibited good binding energy of −10.17 kcal/mol and binding constant of 34.92 nM. Ligand **6d** exhibited one hydrogen bonding interaction with GLU343 (2.145 Å) in its most suitable orientation and showed the highest binding energy of −10.70 kcal/mol and the lowest binding constant value (14.37 nM), which clearly indicated its strong affinity towards receptor. Compound **7d** involved in three hydrogen bonding interactions with SER342 (2.196 Å), SER289 (1.902 Å) and ARG 288 (2.412 Å) and displayed very good binding energy (−10.18 kcal/mol) as well as low inhibition constant (34.36 nM). Similar kinds of results were obtained for ligands **8** and **10b** also. In general, compounds with CONH groups exhibited good binding interactions with the protein-receptor than substituted cyclic systems. Particularly, compound **6d** bearing substituted 2-amino benzothiazole ring displayed the highest binding affinity towards receptor which is in good correlation with in vivo results. The results of docking studies are summarised in Table 4 and the ligand-receptor interaction of active compounds are shown pictorially in Figs. 2 and 3.

Conclusions

In the present work, new DHP derivatives were successively synthesised and characterised by spectral techniques. All the target molecules were subjected to in vivo anti-inflammatory and analgesic screening. Their results indicated that compound **6c**, **6d**, **7d**, **8**, **10b** displayed rapid onset of anti-inflammatory action and their results were excellent when compared with standard drug diclophenac. Also, molecular docking studies were carried out for the highly active molecules and their results were in agreement with in vivo data. The predictive bioactivity study revealed that new target molecules were most likely to be nuclear receptor modulators. They showed very good binding energies and low binding constants in the nanomolar scale. Particularly, compounds **6d** and **7d** comprising amide

Table 4 Binding energy and binding constants of the target compounds

Ligands	Binding energy (kcal/mol)	Intermolecular energy (kcal/mol)	Total internal energy (kcal/mol)	No of hydrogen bonds	Inhibition constant (nM)
6c	−10.17	−13.16	−0.71	2	34.92
6d	−10.7	−13.68	−0.97	1	14.37
7d	−10.18	−13.76	−2.15	3	34.36
8	−9.79	−10.38	−0.22	2	67.21
10b	−9.63	−12.32	−1.99	3	86.61

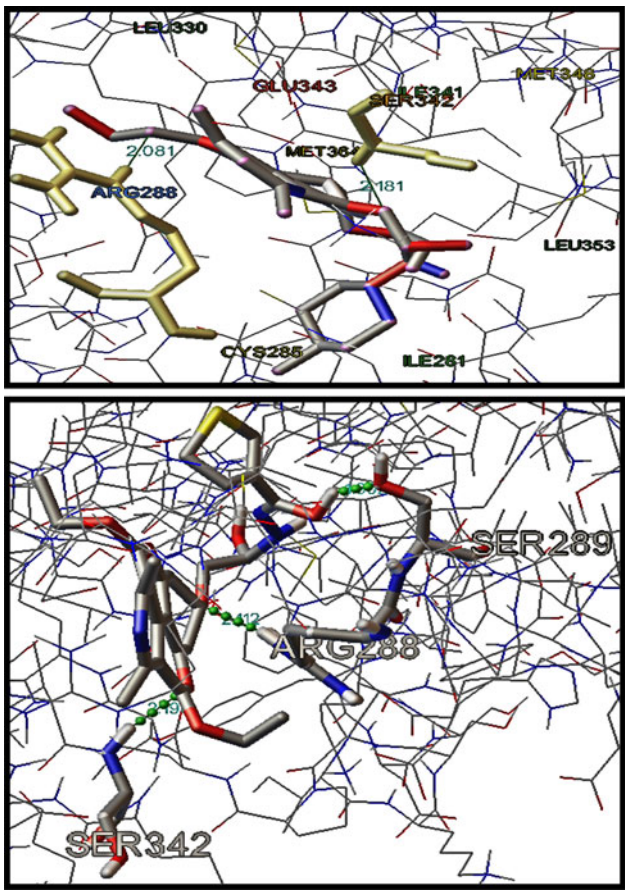


Fig. 2 Pictorial representation of ligand-receptor interactions of **6c** and **7d**

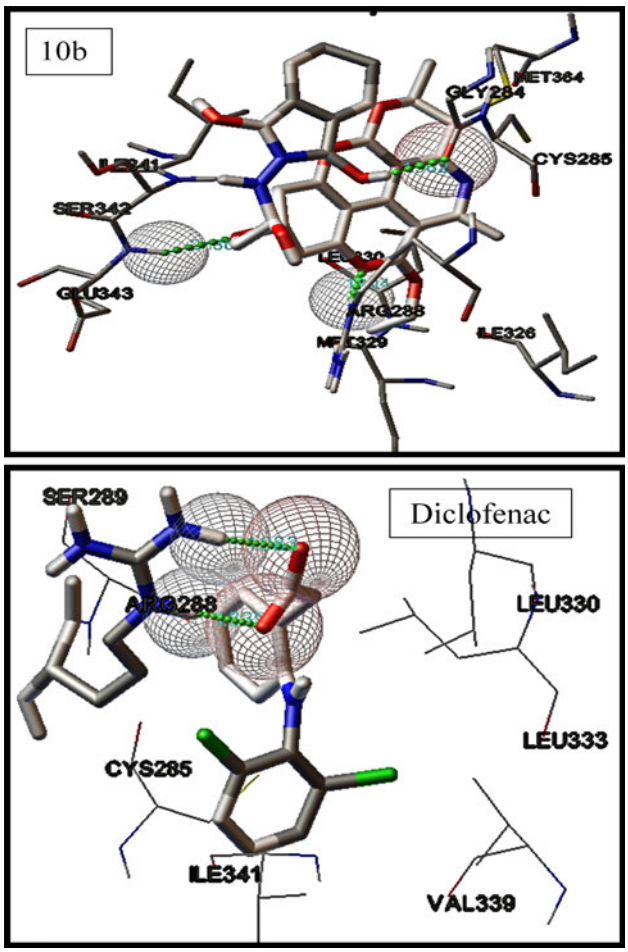


Fig. 3 Representation of molecular interactions of **10b** and diclofenac with receptor

functional group displayed the highest anti-inflammatory activity and low binding constant values. Also, they exhibited very good analgesic property and hence emerged as lead compounds. All docked molecules displayed high binding energy and about thousand time low binding constants when compared with standard diclophenac sodium, which clearly proves the potency of the designed target molecules. Thus, suitable modification of this DHP structure may further enhance its activity and can be a good scaffold for the development of newer bio-active materials.

Experimental

Chemistry

All the chemicals used in the present work procured from Sigma Aldrich and Lanchaster (UK). All the solvents are of analytical grade. They were purchased and used as such without any further purification. The progress of the reaction was monitored by thin layer chromatography, performed on a Silica gel 60 F254 coated aluminium sheet. Melting points were determined on open capillaries using a Stuart SMP3 (BIBBY STERLIN Ltd. UK) apparatus and were uncorrected. Infrared spectra were recorded on a Nicolet Avatar 5700 FTIR (Thermo Electron Corporation). ^{1}H NMR and ^{13}C NMR spectra were obtained with Bruker-400 MHz and 100 MHz FT-NMR spectrometer using TMS as internal reference and DMSO-d_6 as solvent. Elemental analyses were performed on a Flash EA1112 CHNS analyzer (Thermo Electron Corporation). Mass spectra were recorded on LC-MSD-Trap-XCT_Plus Mass Spectrometer.

*Procedure for synthesis of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**1**)*

A mixture of 4-hydroxybenzaldehyde (2 g, 16.3 mmol) ethyl acetoacetate (2.1 mL, 32.7 mmol) and ammonium acetate (1.9 g, 24.5 mmol) in 20 mL of ethanol was refluxed for 12 h. The reaction mixture was cooled on ice bath and solid separated was filtered, washed with ethanol and dried under vacuum. The product was recrystallized using ethanol/DMF mixture. Yield 60 %, m.p. 240 °C. FTIR (ATR, cm^{-1}): 3337, 2980, 1656, 1221. ^{1}H NMR

(DMSO-d_6, 400 MHz, δ ppm): 9.09 (s, 1H), 8.77 (s, 1H), 6.94–6.91 (d, 2H, J = 12 Hz), 6.59–6.56 (d, 2H, J = 12 Hz), 4.74 (s, 1H), 4.04–3.92 (m, 4H), 2.23 (s, 6H), 1.15–1.11 (t, 6H, 8.0 Hz). MS (m/z, %): 346.4. Anal. Calcd. for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 66.01; H, 6.69; N, 4.05.

*Procedure for synthesis of diethyl 4-(4-(2-ethoxy-2-oxoethoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**2**)*

To a clear solution of **1** (3 g, 8.7 mmol) in 30 mL of DMF, ethyl chloroacetate (1.1 mL, 9.01 mmol) and K_2CO_3 (2.4 g, 17.3 mmol) were added with stirring. The reaction mixture was heated at 80 °C for 20 h under nitrogen and cooled to room temperature. It was then quenched to ice cold water with stirring. Resulting solid was filtered, washed with water and dried. This was recrystallized with hot ethanol. Yield 80 %, m.p. 106 °C. FTIR (ATR, cm^{-1}): 3351, 2979, 2905, 1739, 1686, 1221. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.73 (s, 1H), 7.04–7.02 (d, 2H, J = 6.8 Hz), 6.74–6.72 (d, 2H, J = 6.8 Hz), 4.78 (s, 1H), 4.66 (s, 2H), 4.16–4.11 (m, 2H), 4.00–3.93 (m, 4H), 2.23 (s, 6H), 1.20–1.16 (t, 3H, J = 7.2 Hz), 1.13–1.10 (t, 3H, J = 6.8 Hz). MS (m/z, %): 432.2. Anal. Calcd. for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.77; N, 3.25; O, 25.96. Found: C, 63.96; H, 6.74; N, 3.23; O, 25.91.

*Procedure for the synthesis of 2-(4-(3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy)acetic acid (**3**)*

To a solution of ester **2** (1 g, 2.32 mmol), in 10 mL of ethanol, sodium hydroxide (0.1 g in 10 mL water) solution was added. The resulting mixture was refluxed for one hr. It was then cooled to room temperature and neutralized with conc. HCl. The crude compound **3** was recrystallized from ethanol-chloroform mixture. Yield 76 %, m.p. 177 °C. FTIR (ATR, cm^{-1}): 3342(br), 2982, 2912, 1723, 1671, 1203. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.74 (s, 1H), 7.04–7.02 (d, 2H, J = 8 Hz), 6.73–6.71 (d, 2H, J = 8 Hz), 4.78 (s, 1H), 4.58 (s, 2H), 4.00–3.97 (m, 4H), 2.24 (s, 6H), 1.15–1.11 (t, 3H, J = 7.2 Hz). MS (m/z, %): 404.4. Anal. Calcd. for $C_{21}H_{27}NO_7$: C, 62.52; H, 6.25; N, 3.47; O, 27.76. Found: C, 62.46; H, 6.24; N, 3.42; O, 27.72.

*Procedure for synthesis of 2-(4-(3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy) acetic acid hydrazide (**4**)*

To a clear solution of **2** (2.5 g, 5.8 mmol) in ethanol (25 mL), hydrazine hydrate (0.5 mL, 10 mmol) was added, and the mixture was refluxed for 4 h. It was then cooled to room temperature to get crude hydrazide **4**. The resulting solid was filtered, washed with ethanol and recrystallized from ethanol/DMF mixture. Yield 90 %, m.p. 192 °C. FTIR (ATR, cm^{-1}): 3348, 3228, 3224, 3099, 2978, 1672, 1656, 1203. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.24 (s, 1H), 8.73 (s, 1H), 7.04–7.02 (d, 2H, J = 8.8 Hz), 6.77–6.75 (d, 2H, J = 8.8 Hz), 4.78 (s, 1H), 4.39 (s, 2H), 4.28 (s, 2H), 4.01–3.94 (m, 4H), 2.23 (s, 6H), 1.14–1.10 (t, 6H, J = 7.0 Hz). MS (m/z, %): 418.1. Anal. Calcd. for $C_{21}H_{27}N_3O_6$. C, 60.42; H, 6.52; N, 10.07; O, 23.0. Found: C, 60.34; H, 6.57; N, 10.02; O, 22.97.

*Procedure for synthesis of diethyl 4-(4-(2-chloro-2-oxoethoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5**)*

To a solution of compound **3** (1 g, 2.32 mmol) in methylene dichloride (10 mL), thionyl chloride (0.35 mL, 4.64 mmol) was added followed by one drop of DMF. The resulting solution was stirred at 50 °C for about 4 h. The solvent was evaporated under reduced pressure, and the resulting crude product was taken as such for next step without any purification.

*General procedure for synthesis of amides (**6a–d**)*

The intermediate acid chloride **5** (0.1 g, 0.22 mmol) was dissolved in 5 mL of methylene dichloride and reacted with different amines (0.22 mmol) in the presence of piperidine (0.02 mL, 0.22 mmol). This reaction mixture was stirred at room temperature for 8 h. The precipitated product was filtered, washed well with methylene dichloride and dried. The crude product was recrystalized from ethanol-DMF mixture. Same procedure was followed for the synthesis of **7a–e**, wherein intermediate **4** was made use instead of **5**. Their characterization and spectral data are as follows.

*Diethyl 4-(4-(2-(benzylamino)-2-oxoethoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6a**)*

Yield 82 %, m.p. 165 °C. FTIR (ATR, cm^{-1}): 3392 (br), 2960, 2872, 1672, 1663, 1203. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.31 (s, 1H), 8.75 (s, 1H), 7.76–6.77 (m, 9H), 4.87 (s, 1H), 4.62 (s, 2H), 4.24 (s, 2H), 4.01–3.93 (m, 4H), 2.23 (s, 6H), 1.14–1.10 (t, 6H, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.2, 167.5, 157.3, 141.8, 142.1, 134.4, 126.8, 125,4, 116.0, 112.2, 68.7, 62.5, 43.3, 16.4, 14.3. MS (m/z, %): 493.6. Anal. Calcd. for $C_{28}H_{32}N_2O_6$. C, 68.28; H, 6.55; N, 5.69; O, 19.49. Found: C, 68.24; H, 6.53; N, 5.62; O, 19.46.

*Diethyl 4-(4-(2-(2,6-dimethylphenylamino)-2-oxoethoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6b**)*

Yield 78 %, m.p. 176 °C. FTIR (ATR, cm^{-1}): 3350, 3091, 2981, 2912, 1672, 1666, 1195. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.04 (s, 1H), 8.74 (s, 1H), 7.82–6.80 (m, 7H), 4.86 (s, 1H), 4.62 (s, 2H), 4.01–3.96 (m, 4H), 2.32 (s, 6H), 2.23 (s, 6H), 1.14–1.10 (t, 6H, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.2, 167.6, 157.4, 141.2, 137.3, 134.2, 129.8, 126.7, 124.2, 114.5, 113.1, 66.8, 61.4, 43.2, 16.3, 14.7, 14.2. MS (m/z, %): 507.4. Anal. Calcd. for $C_{29}H_{34}N_2O_6$. C, 68.76; H, 6.76; N, 5.53; O, 18.95. Found: C, 68.64; H, 6.73; N, 5.52; O, 18.89.

*Diethyl 4-(4-(2-(p-toluidino)-2-oxoethoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6c**)*

Yield 84 %, m.p. 116 °C. FTIR (ATR, cm^{-1}): 3315, 3091, 2981, 2923, 1686, 1667, 1235. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.83 (s, 1H), 8.74 (s, 1H), 7.78–6.76 (m, 8H), 4.88 (s, 1H), 4.63 (s, 2H), 4.01–3.95 (m, 4H), 2.32 (s, 3H), 2.23 (s, 6H), 1.14–1.11 (t, 6H, J = 6.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 169.3, 167.4, 158.1, 143.4, 138.3, 135.7, 129.7, 121.5, 114.3, 112.5, 66.8, 61.7, 43.2, 24.3, 16.4, 14.2. MS (m/z, %): 493.4. Anal. Calcd. for $C_{28}H_{32}N_2O_6$. C, 68.28; H, 6.55; N, 5.69; O, 19.49. Found: C, 68.14; H, 6.53; N, 5.62; O, 19.49.

*Diethyl 4-(4-(2-(benzothiazol-2-ylamino)-2-oxoethoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6d**)*

Yield 80 %, m.p. 98 °C. FTIR (ATR, cm^{-1}): 3315, 3150, 3066, 2981, 1672, 1215. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 11.12 (s, 1H), 8.75 (s, 1H), 7.78–6.68 (m, 8H), 4.84 (s, 1H), 4.62 (s, 2H), 4.00–3.96 (m, 4H), 2.23 (s, 6H), 1.14–1.10 (t, 6H, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 176.2, 169.3, 167.2, 155.6, 150.2, 148.7, 134.6, 130.2, 125.6, 124.3, 122.3, 114.5, 112.4, 66.7, 61.7, 42.9, 16.3, 14.2. MS (m/z, %): 536.4. Anal. Calcd. for $C_{28}H_{29}N_3O_6S$. C, 62.79; H, 5.46; N, 7.85; O, 17.92. Found: C, 62.64; H, 5.43; N, 7.82; O, 17.90.

*Diethyl 4-(N′-(4-methyl benzoyl)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**7a**)*

Yield 76 %, m.p. 119 °C. FTIR (ATR, cm^{-1}): 3346, 3226, 3056, 2982, 1674, 1648, 1212. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.19 (s, 2H), 8.74 (s, 1H), 7.78–7.76 (d, 2H, J = 8 Hz), 7.29–7.27 (d, 2H, J = 8 Hz), 7.06–7.04 (d, 2H, J = 8.4 Hz), 6.84–6.82 (d, 2H, J = 8.4 Hz), 4.80 (s, 1H), 4.56 (s, 2H), 4.01–3.95 (m, 4H), 2.35 (s, 3H), 2.24 (s, 6H), 1.15–1.11 (t, 6H, J = 7.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.2, 166.9, 165.3, 155.9, 145.0, 141.7, 129.6, 128.9, 127.4, 114.0, 112.0, 66.1, 58.9, 39.7, 20.9, 18.1, 14.1. MS (m/z, %): 536.6. Anal. Calcd. for $C_{29}H_{33}N_3O_7$. C, 65.03; H, 6.21; N, 7.85; O, 20.91. Found: C, 64.98; H, 6.19; N, 7.83; O, 20.88.

*Diethyl 4-(N′-(benzoyl)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**7b**)*

Yield 78 %, m.p. 145 °C. FTIR (ATR, cm^{-1}): 3345, 3234, 3056, 2979, 1689, 1645, 1210. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.36 (s, 1H), 10.16 (s, 1H), 8.74 (s, 1H), 7.95–6.83 (m, 9H), 4.80 (s, 1H), 4.57 (s, 2H), 4.01–3.96 (m, 4H), 2.24 (s, 6H), 1.15–1.11 (t, 6H, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.2, 166.9, 165.4, 155.9, 145.1, 141.5, 131.2, 129.6, 128.7, 127.3, 114.1, 112.2, 66.1, 58.7, 39.7, 18.1, 14.1. MS (m/z, %): 522.3. Anal. Calcd. for $C_{28}H_{31}N_3O_7$. C, 64.48; H, 5.99; N, 8.06; O, 21.47. Found: C, 64.39; H, 5.92; N, 8.02; O, 21.48.

*Diethyl 4-(N′-(3-chloro thiophene-2-carbonyl)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**7c**)*

Yield 80 %, m.p. 132 °C. FTIR (ATR, cm^{-1}): 3342, 3238, 3017, 2965, 1664, 1645, 1212. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.24 (s, 1H), 10.18 (s, 1H), 8.74 (s, 1H), 7.95–6.85 (m, 6H), 4.79 (s, 1H), 4.58 (s, 2H), 4.01–3.95 (m, 4H), 2.24 (s, 6H), 1.15–1.11 (t, 6H, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.2, 166.6, 161.2, 155.8, 140.9, 138.1, 135.0, 129.8, 129.2, 114.1, 112.2, 67.1, 58.8, 40.0, 16.3, 14.2. MS (m/z, %): 562.9. Anal. Calcd. for $C_{26}H_{28}ClN_3O_7S$. C, 55.56; H, 5.02; N, 7.48; O, 19.93. Found: C, 55.49; H, 5.02; N, 7.42; O, 19.89.

*Diethyl 4-(N′-(thiophene-2-carbonyl)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**7d**)*

Yield 82 %, m.p. 148 °C. FTIR (ATR, cm^{-1}): 3348, 3242, 3009, 2965, 1669, 1647, 1209. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.19 (s, 1H), 10.11 (s, 1H), 8.74 (s, 1H), 7.82–6.81 (m, 7H), 4.81 (s, 1H), 4.54 (s, 2H), 4.01–3.96 (m, 4H), 2.24 (s, 6H), 1.15–1.11 (t, 6H, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.2, 166.6, 161.2, 155.8, 142.8, 138.1, 137.4, 129.8, 129.2, 114.2, 112.3, 67.1, 58.8, 39.8, 16.3, 14.2. MS (m/z, %): 528.9. Anal. Calcd. for $C_{26}H_{29}N_3O_7S$. C, 59.19; H, 5.54; N, 7.96; O, 21.23. Found: C, 59.08; H, 5.52; N, 7.91; O, 21.19.

*Diethyl 4-(N′-(2-chloro thiophene-3-carbonyl)-2-phenoxyacetohydrazide)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**7e**)*

Yield 79 %, m.p. 138 °C. FTIR (ATR, cm^{-1}): 3332, 3213, 2997, 2946, 1665, 1643, 1202. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.23 (s, 1H), 10.14 (s, 1H), 8.74 (s, 1H), 7.80–6.84 (m, 6H), 4.82 (s, 1H), 4.54 (s, 2H), 4.00–3.95 (m, 4H), 2.24 (s, 6H), 1.15–1.11 (t, 6H, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.2, 166.4, 164.8, 156.2, 141.3, 139.4, 137.6, 134.5, 129.8, 128.7, 118.9, 114.3, 112.3, 67.3, 58.8, 40.2, 16.3, 14.2. MS (m/z, %): 563.2. Anal. Calcd. for $C_{26}H_{28}ClN_3O_7S$. C, 55.56; H, 5.02; N, 7.48; O, 19.93. Found: C, 55.48; H, 4.98; N, 7.45; O, 19.91.

*Procedure for synthesis of diethyl 4-(4-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**8**)*

An ethanolic KOH (0.1 g in 10 mL C_2H_5OH) solution was prepared and hydrazide **4** (0.5 g, 1.21 mmol) was added to it. The resulting solution was cooled to 5 °C and to this carbon disulphide (0.2 mL, 2.42 mmol) was added while stirring. This mixture was stirred at room temperature for about one hour and then heated under reflux condition for 6 h. The resulting potassium salt was filtered and washed with ethanol. The salt was dissolved in water and acidified with dil. HCl under stirring. The resulting solid was filtered and recrystallised from ethanol–chloroform mixture. Yield 72 %, m.p. 173 °C. FTIR (ATR, cm^{-1}): 3334, 3082, 2933, 1662, 1492, 1213. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 14.65 (br, 1H), 8.74 (s, 1H), 7.07–7.05 (d, 2H, J = 8.8 Hz), 6.87–6.85 (d, 2H, J = 8.8 Hz), 5.15 (s, 1H), 4.79 (s, 2H), 4.00–3.94 (m, 4H), 2.23 (s, 6H), 1.13–1.09 (t, 6H, J = 7.6 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 178.0, 166.9, 159.6, 155.3, 145.1, 141.9, 128.4, 114.1, 101.9, 59.5, 58.1, 38.9, 18.1, 14.1. MS (m/z, %): 460.3. Anal. Calcd. for $C_{22}H_{25}N_3O_6S$. C, 57.50; H, 5.48; N, 9.14; O, 20.89. Found: C, 57.48; H, 5.47; N, 9.14; O, 20.85.

*Procedure for the synthesis of diethyl 4-(4-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**9**)*

To the ethanolic solution of **4** (0.5 g, 1.21 mmol), acetyl acetone (0.43 mL, 1.21 mmol) was added followed by addition of catalytic amount (0.2 mL) of glacial acetic acid and the resulting solution was refluxed for 5 h. It was cooled, filtered and recrystallised from ethanol–chloroform mixture. Yield 78 %, m.p. 182 °C. FTIR (ATR, cm^{-1}): 3326, 2996, 2934, 1665, 1214. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.73 (s, 1H), 7.04–7.02 (d, 2H, J = 8.8 Hz), 6.78–6.76 (d, 2H, J = 8.8 Hz), 6.22 (s, 1H), 5.39 (s, 1H), 4.79 (s, 2H), 4.02–3.96 (m, 4H), 2.45 (s, 3H), 2.23 (s, 6H), 2.19 (s, 3H), 1.14–1.11 (t, 6H, J = 7.0 Hz).). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 168.1, 166.9, 155.9, 152.3, 145.0, 143.5, 141.0, 128.2, 113.8, 101.9, 65.8, 58.9, 39.5, 18.2, 14.1, 13.5. MS (m/z, %): 482.5. Anal. Calcd. for $C_{26}H_{31}N_3O_6$. C, 64.85; H, 6.49; N, 8.73; O, 19.94. Found: C, 64.80; H, 6.47; N, 8.74; O, 19.91.

*Procedure for synthesis of 4-(2-(2-(4-(3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridin-4-yl)phenoxy)acetyl)hydrazinyl)-4-oxobutanoic acid (**10a**)*

A mixture of **4** (0.5 g, 1.21 mmol), succinic anhydride (0.11 g, 1.21 mmol) in 10 mL of ethanol was refluxed in the presence of 0.1 mL of glacial acetic acid for 6 h. After complete reaction, pH of the reaction mixture was made alkaline by adding sodium bicarbonate solution. The product was extracted with ethyl acetate and aqueous layer was neutralized with dil. HCl. Resulting solid was filtered and recrystalised from ethanol. Yield 82 %, m.p. 184 °C. FTIR (ATR, cm^{-1}): 3390 (br), 2978, 1718, 1658, 1221. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 12.08 (s, 1H), 9.99 (s, 1H), 9.81 (s, 1H), 8.73 (s, 1H), 7.04–7.02 (d, 2H, J = 8.4 Hz), 6.79–6.77 (d, 2H, J = 8.4 Hz), 4.78 (s, 1H), 4.48 (s, 2H), 4.01–3.95 (m, 4H), 2.43–2.42 (t, 2H, J = 3.2 Hz), 2.38–2.37 (t, 2H, J = 3.2 Hz), 2.23 (s, 6H), 1.14–1.11 (t, 6H, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.8, 166.8, 164.7, 156.1, 145.2, 141.1, 135.8, 113.9, 102.3, 65.9, 63.9, 58.9, 38.9, 18.1, 14.1. MS (m/z, %): 518.2. Anal. Calcd. for $C_{25}H_{31}N_3O_9$. C, 58.02; H, 6.04; N, 8.12; O, 27.82. Found: C, 57.92; H, 6.02; N, 8.09; O, 27.79.

*Procedure for the synthesis of diethyl 4-(4-(2-(1,3-dioxoisoindolin-2-ylamino)-2-oxoethoxy)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**10b**)*

A solution of **4** (0.5 g, 1.21 mmol) and phthaleic anhydride (0.2 g, 1.35 mmol) in 10 mL of ethanol containing 0.2 mL of glacial acetic acid was refluxed for 6 h with stirring. Upon completion of reaction, the reaction mixture was cooled, filtered and washed with ethanol. The solid product was recrystallised from ethanol–chloroform mixture. Yield 80 %, m.p. 140 °C. FTIR (ATR, cm^{-1}): 3224, 2981, 1668, 1222. ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.24 (s, 1H), 8.73 (s, 1H), 7.78–6.80 (s, 8H), 4.79 (s, 1H), 4.55 (s, 2H), 4.00–3.96 (m, 4H), 2.23 (s, 6H), 1.15–1.11 (t, 6H, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.8, 166.9, 164.9, 156.0, 145.0, 141.1, 135.6, 129.8, 123.8, 113.9, 102.3, 65.9, 63.9, 58.9, 38.9, 18.1, 14.1. MS (m/z, %): 548.5. Anal. Calcd. for $C_{29}H_{29}N_3O_8$. C, 63.61; H,

5.34; N, 7.67; O, 23.38. Found: C, 63.57; H, 5.32; N, 7.63; O, 23.39.

Anti-inflammatory study

Newly synthesized title compounds were screened for their in vivo anti-inflammatory properties following Carrageenan-induced Paw Oedema method (Yoshimasa *et al*., 1985) by taking Diclofenac sodium as standard drug. Male or female Wistar strain rats with a body weight between 100 and 150 g were used. The animals were weighed and divided into different groups (control, standard and the test groups) of five rats each. The animals were starved overnight. To ensure uniform hydration, the rats received 5 ml of water by stomach tube (controls) or the test drug (20 mg/kg) dissolved or suspended in the same volume. Thirty minutes later, the rats were given a subcutaneous injection of 0.05 ml of 1 % solution of Carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection, after 30, 60, 120 and 180 min. Results were expressed as mean, by one way ANOVA analysis followed by Dunnet's-*t* test and are summarised in Table 2. The in vivo work was performed in accordance with the ethical standards on animal experimentations.

Analgesic study

The analgesic study of active target compounds was done by Tail immersion method (Stasi *et al*., 1988) by taking Diclofenac potassium as standard drug. The animals were weighed and divided into different groups (control, standard and the test groups) of five rats each. Young female Wistar rats (170–210 g body weight) were used for the study. They were placed into individual restraining cages leaving the tail hanging out freely. The animals were allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds, the rat reacts by withdrawing the tail. The reaction time was recorded in 0.5 s units by a stopwatch. After each determination, the tail was carefully dried. The reaction time was determined before and periodically after either oral or subcutaneous administration of the test substance, e.g. after 0, 30, 60 and 90 min. The cut off time of the immersion was 15 s. The withdrawal time of untreated animals was between 1 and 5.5 s. A withdrawal time of more than 6 s therefore was regarded as a positive response. The analgesic results are tabulated in Table 3.

Molecular docking studies

The molecular docking studies were performed by using AutoDock version 4.0 software (Huey *et al*., 2007). The ligands were analysed for predictive bioactivity study by using MOLINSPIRATION server and they were found to be nuclear receptor modulator.

Peroxisome Proliferator- Activated Receptor (PPAR-γ) was used as receptor for our docking studies. Ligand bound crystallographic structure of protein receptors are available in Protein Data Bank (Berman *et al*., 2000). Since indomethacin is a well-known agonist of PPAR-γ receptor, in our study, the crystal structure of indomethacin coupled PPAR was taken, and structure of indomethacin was replaced by synthesized ligands for docking studies. LIGPLOT analysis was done to identify the residues present in the ligand-binding site. Additional residues were identified by CASTp analysis.

The ligands were drawn in Chem Draw Ultra 6.0 assigned with proper 2D orientation. The correct atom types and bond types were defined and hydrogen atoms were added. The lowest energy conformer of the molecule was selected by using PRODRG software (Schuttelkopf and van Aalten, 2004). This structure was selected and taken as input ligand structure in AutoDock, to carry out simulation studies.

The receptor was cleaned from any possible ligands before the docking studies. Receptor was modified for the docking studies by incorporating united atom charges, solvation parameters and polar hydrogens. Q site finder server was used to predict ligand-binding site. In addition, CASTp server was used to identify active site and to measure its cavity size. The auto grid program available in AutoDock server was used to generate grid maps. For each ligand, a best conformer having higher binding energy, minimum binding constant and maximum number of hydrogen bonding interactions was chosen. Finally, docking runs were performed and schematic diagram of protein–ligand interactions was generated in LIGPLOT server.

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Conflict of interest The authors declare that they have no conflict of interest.

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