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Design, synthesis and pharmacological evaluation of some substituted dihydropyrimidines with L-/T-type calcium channel blocking activities

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ABSTRACT

New dihydropyrimidines bearing various lipophilic pharmacophores and functionalities at position 3 were designed and synthesized. The basic framework of the new compounds was designed to maintain the main structural requirements for calcium channel blocking activity of the known dihydropyridines and dihydropyrimidines calcium channel blockers. The newly synthesized compounds were evaluated as antagonists for $Ca_V1.2$ and $Ca_V3.2$ using the whole-cell patch clamp technique. Seven compounds (**4b**, **4c**, **6c, 9, 13c, 13e** and **17b**) showed promising dual calcium channel blocking activity and three compounds (**13b, 14b** and **17a**) were selective against Cav3.2. Their drug-likeness has been assessed using *Molinspiration* and *Molsoft* softwares. Their physicochemical properties and pharmacokinetic profiles recommend that they can be considered as drug-like candidates.

1. Introduction

Voltage-activated calcium channels are vital regulators of calcium entry into many excitable tissues. Different calcium channel isoforms have been identified and shown to mediate specialized cellular functions. Because of their fundamental nature, they are considered as important targets for therapeutic intervention in disorders ranging from hypertension and stroke to pain and epilepsy [1,2]. Within this approach, selective blockers of the different isoforms could target calcium channels implicated in particular pathologies. Among the different calcium channel blockers (CCBs), T-type (Ca_V3) blockers are emerging as potential therapeutic avenues for various neuropsychiatric pathologies such as pain, epilepsy, Parkinson disease, autism, addiction and anxiety [2,3]. T-type calcium channel activity is also essential for the function of the cardiovascular system [4]. Angiotensin promotes T-type calcium channels upregulation, which then triggers an increase in aldosterone secretion [5]. As a result, T-type calcium channels are considered excellent potential targets for developing novel antihypertensive drugs [6,7]. Traditionally, current cardiovascular agents include drugs that target the L-type ($Ca_V1.2$) isoform, which are recommended by the European Society of Hypertension (ESH) together with the European Society of Cardiology (ESC) in their published 2018 ESC/ESH Guidelines for the management of arterial hypertension [8]. Moreover, clinical studies reported that combined L- and T-types CCBs hold promise for better hypertension management as a result of prevention of cardio-renal injury than only selective L-type blockers [9].

Along these lines, there are continuous efforts to develop efficient calcium channel blockers. Many molecules belonging to several chemical entities have been shown to target various calcium channel isoforms [10–14]. The drug discovery sector has focused on studying their respective structure activity relationships to gain more information about such activity and selectivity. Within this approach, dihydropyridines (DHPs) and their bioisosters are considered as the best known class of CCBs. Although DHPs mainly target the L-type calcium channels [15], recent electrophysiological studies showed that some DHPs can also block T-type isoforms [16–19]. Since the introduction of nifedipine, the basic structure activity relationships of the DHP scaffold have been subjected to extensive investigation. The introduction of large lipophilic alkyl and aryl chains at positions 3 or 5 as esters leads to new potent second, third and fourth generation DHPs with improved pharmacokinetic and safety profiles [20–22]. Interestingly, such modification was also shown to modulate tissue selectivity [23–25]. In

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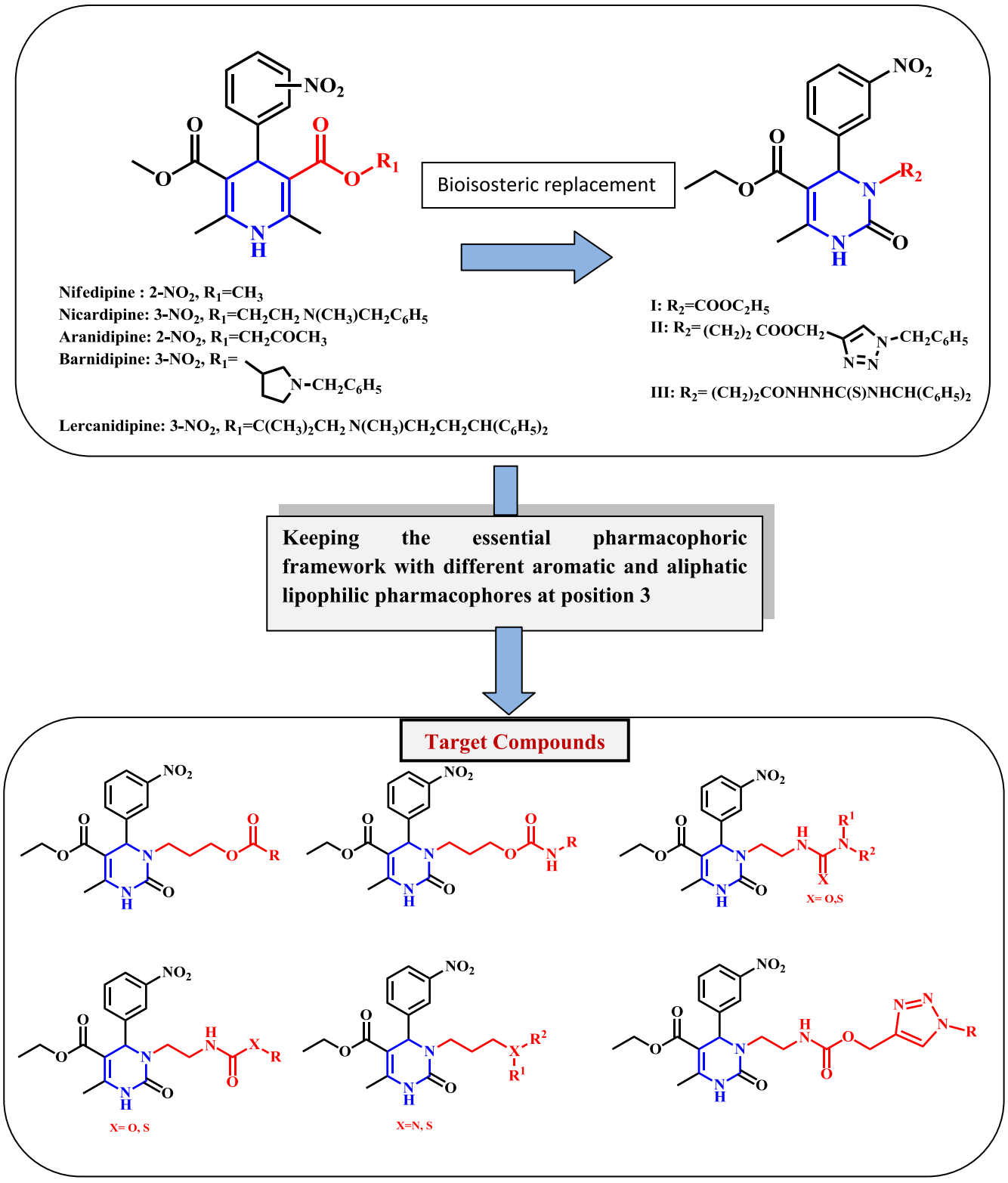
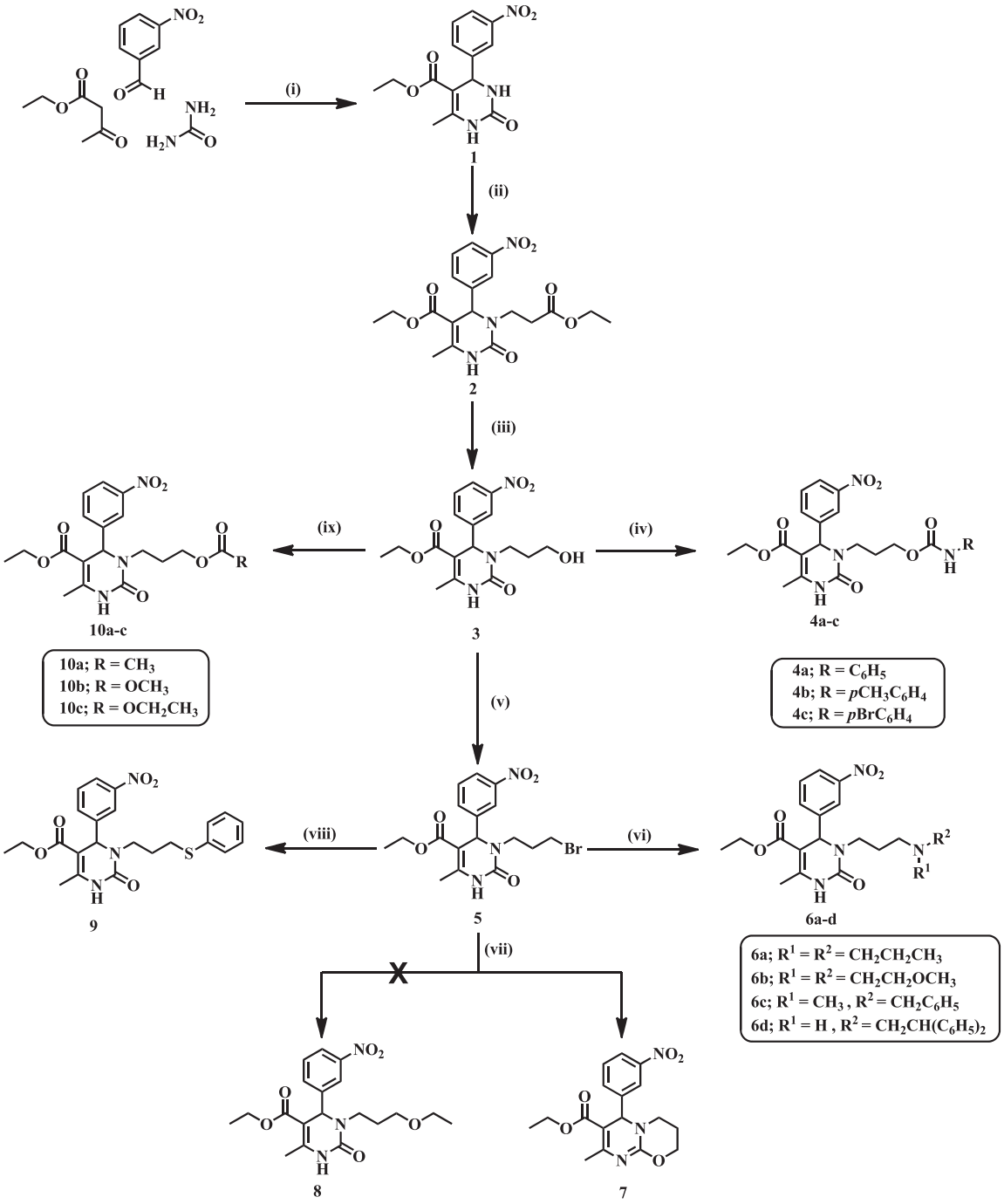


Fig. 1. Rational for the design of the target compounds.

continuation of the efficient systematic modification of DHPs, bioisosteric replacement of the core allowed the introduction of potent dihydropyrimidines (DHPMs) mimics. Literature survey revealed that multiple lead DHPMs (Fig. 1) have been developed and some were found to have superior potency and longer duration of action compared to the classical DHPs [14,26–31].

Prompted by the above considerations, the current study describes the synthesis of series of substituted DHPMs bearing different lipophilic moieties at position 3 (Fig. 1), to mimic the essential structural requirements for calcium channel blocking activity in parent DHPs and lead DHPM CCBs [29,30]. The N3 substituents were selected to approximately mimic length and lipophilic nature of the corresponding side chain in the newer DHPs generations. All newly synthesized compounds were evaluated as blockers for $Ca_V1.2$ and $Ca_V3.2$ applying



Reagents : (i) HCl ; (ii) $CH{=}CHCOOC_2H_5$ / KF / Al_2O_3 ; (iii) $LiAlH_4$; (iv) RNCO / DCM ; (v) CBr_4 / PPh_3 / DCM ; (vi) NHR^1R^2 ; (vii) NaOEt / EtOH ; (viii) PhSH /K_2CO_3; (ix) RCOCl / Pyridine

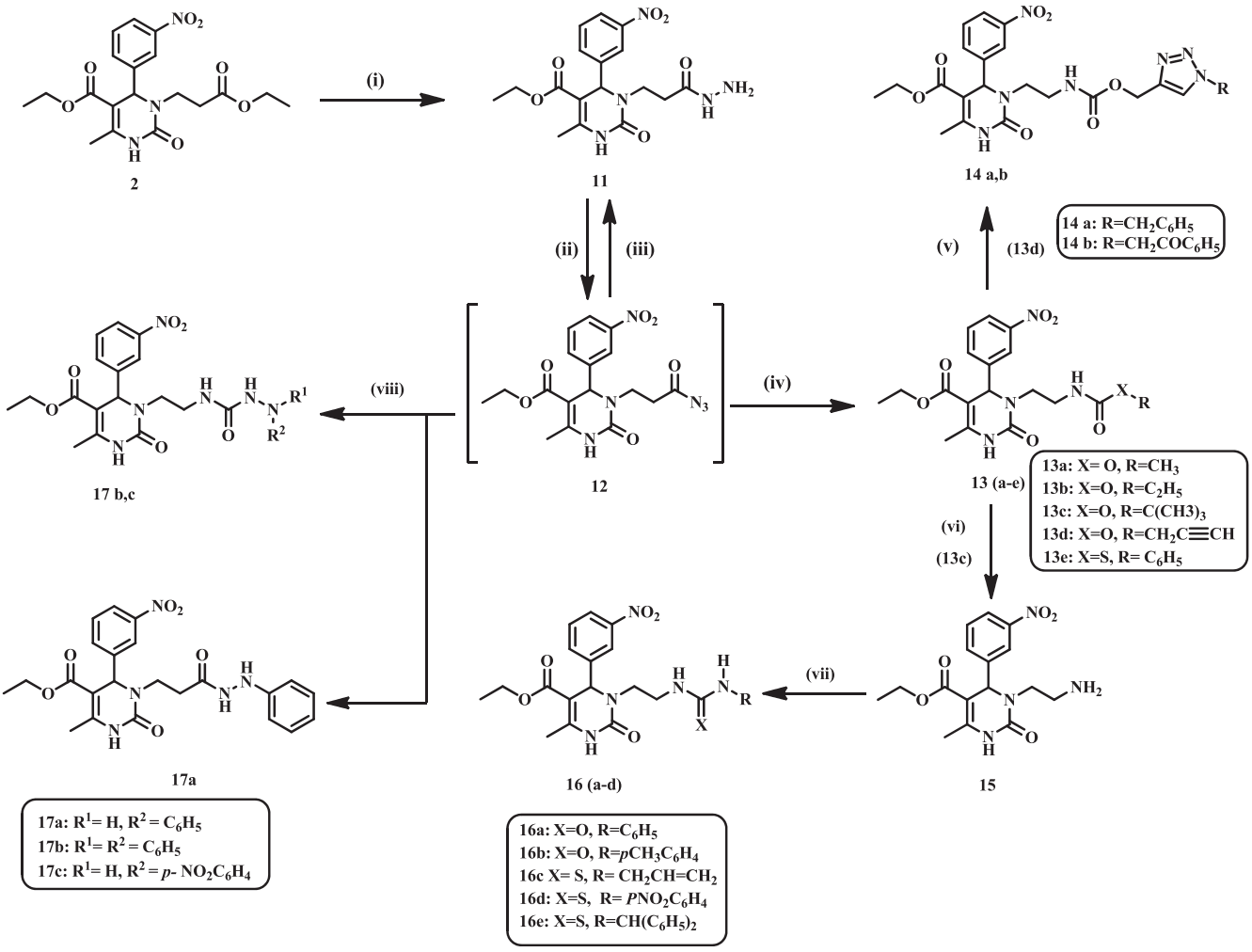
Scheme 1. Synthetic pathways for compounds **3–10.**

the whole-cell patch clamp technique in an attempt to identify the most potent and selective blockers.

2. Results and discussion

2.1. Chemistry

The synthetic strategies employed to obtain the intermediates and target compounds are illustrated in Schemes 1 and 2. In Scheme 1, the starting compound DHPM **(1)** was synthesized by one-pot three-component Biginelli reaction by heating at reflux temperature a mixture of urea, 3-nitrobenzaldehyde and ethyl acetoacetate in acidic medium [32]. The reaction mixture was then reacted with ethyl acrylate following green aza-Michael addition reaction catalysed by KF/Al_2O_3 [33] to obtain the key ethyl ester intermediate derivative **(2).** Regioselective reduction of the ethyl ester **(2)** with $LiAlH_4$ in THF afforded the corresponding alcohol **(3)** [34]. Generally the ring ester of Biginelli compounds is inert towards reduction. The unreactivity of the ester group may be attributed to the strong conjugation with the adjacent double bond [35]. The alcohol compound **(3)** was then utilized to synthesize several N^3-substituted 3,4-dihydropyrimidinones. The molecular structure of the alcohol **(3)** was confirmed on the basis of their spectral



Reagents : (i) NH_2NH_2 ; (ii) $NaNO_2$ /HCl / 0 °C ; (iii) NH_2NH_2 ; (iv) RXH ; (v) RN_3 /$CuSO_4$ /Sodium ascorbate ; (vi) CF_3COOH; (vii) RNCX / TEA ; (viii) $NH_2R^1R^2$

Scheme 2. Synthetic pathways for compounds **11–17.**

data. The infrared spectrum showed a characteristic band at 3439 cm^{-1} corresponding to −OH stretching of alcohol. The ^1H NMR spectrum showed D_2O exchangeable distorted triplet at δ 4.46–4.49 ppm corresponding to the −OH proton. Nucleophilic addition of alcohol **(3)** to substituted isocyanates in dry dichloromethane afforded the corresponding carbamates **(4a-c)** [36]. IR spectra of **(4a-c)** lacked the high frequency OH stretching absorption band and showed new NH and C=O stretching absorption bands attributed to side chain carbamate ester at 3314–3336 and 1682–1689 cm^{-1} respectively, while their ^1H NMR spectra showed a new D_2O-exchangeable singlet at δ 8.67–9.51 ppm owing to the carbamate-NH proton. The alcohol **(3)** was conveniently converted to the bromo derivative **(5)** by reaction with carbon tetrabromide in dry dichloromethane in presence of triphenylphosphine at room temperature [37]. Nucleophilic substitution of the bromo **(5)** with different substituted primary and secondary amines afforded the corresponding amino derivatives **(6a-d)** [38,39]. It was attempted to prepare the proposed ethoxypropyl derivative **(8)**, by refluxing compound **(5)** with sodium ethoxide in ethanol. Surprisingly, the tetrahydropyrimido [2,1-*b*][1,3]oxazine-7-carboxylate **(7)** was obtained instead. The structure of such unexpected compound was substantiated on basis of their IR, ^1H NMR and ^{13}C NMR spectral data. IR spectrum of **(7)** lacked the high frequency NH stretching absorption band and showed band at 1609 cm^{-1} corresponding to C=N group. Cyclization was unequivocally confirmed by HMBC for compound **(7)** as evidenced from the correlation between the side chain methylene protons OCH_2 at 4.36–4.40 ppm and C^{9a} at 155.17 ppm confirming the structure. Moreover, refluxing **(5)** with thiophenol in presence of anhydrous potassium carbonate yielded the targeted phenylthiopropyl derivative **(9)** following a reported procedure [40]. Meanwhile, nucleophilic addition reaction of alcohol **(3)** across the C=O bond of the acetyl chloride or alkyl chloroformate in anhydrous pyridine, afforded the corresponding acetyl **(10a)** [41,42] and carbonates (alkoxy carbonyloxy) derivatives **(10b)** and **(10c)** [43] respectively. Their IR spectra lacked the high frequency OH stretching absorption band present in their precursor alcohol **(3)** and showed a new C=O stretching absorption band attributed to side chain carbonyl of acetyl and carbonate compounds.

In Scheme 2, ethyl ester **(2)** was refluxed with hydrazine to yield the corresponding hydrazide **(11)** [44]. Nitrosation of hydrazide **(11)** in a mixture of acetic acid and diluted hydrochloric acid afforded the intermediate acyl azide **(12)** that was sufficiently pure for further rearrangement reactions [45,46]. The IR spectrum of acyl azide displayed strong absorption bands characteristic for azide stretching vibrations at

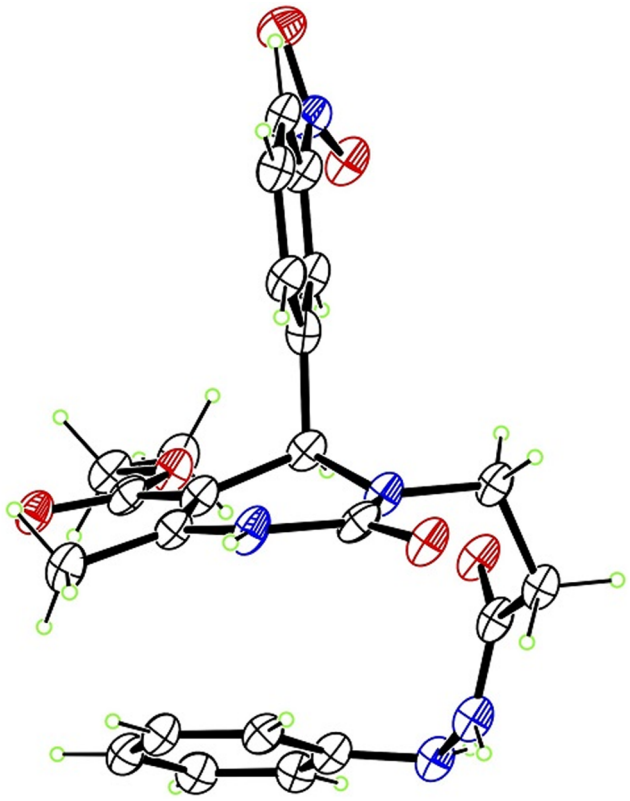


Fig. 2. ORTEP diagram of one of the two independent molecules of compound **17a**.

2126 and 2145 cm^{-1}. The azide intermediate was then utilized as a starting material for introducing the designed moieties to the DHPM ring core. Carbamates **(13a-d)** were readily prepared by refluxing azide intermediate in the appropriate alcohol [47,48]. Similarly, thiophenol was utilized to afford the corresponding thiocarbamate **(13e)** [49,50]. Cycloaddition of benzyl or phenacylazides and the propargyl carbamate **(13d)** through Cu catalyzed Huisgen reaction allowed the introduction of substitued 1,2,3-triazole ring to the DHPM core (compounds **14a & 14b**) [51]. Acid mediated *N*-Boc deprotection of the carbamate **(13c)** afforded the corresponding amine **(15)** [52]. Ureas and thioureas **(16a-e)** were synthesized by reacting the amine **(15)** and isocyanates [53] or isothiocyanate [54] in the presence of triethylamine. Treatment of azide intermediate with various hydrazines showed different reaction patterns. The reaction with 4-nitrophenyl hydrazine or 1,1-diphenyl hydrazine proceeded via Curtius rearrangement [55,56] affording the semicarbazides **(17b & 17c)** [47]. Unexpectedly, hydrazine or phenyl hydrazine afforded the corresponding hydrazides **(11 & 17a)** under similar conditions [57]. The phenyl hydrazide derivative **(17a)** was unequivocally confirmed by IR, ^{1}H NMR and ^{13}C NMR spectral data in addition to X-ray crystallography (Fig. 2**)**.

2.2. Electrophysiology

Calcium channel blocking activities of the newly synthesized compounds were assessed by a whole-cell patch clamp recording assay using $Ca_V1.2$ and $Ca_V3.2$ channels (Fig. 3). Currents from tsA-201 cells transiently transfected with rat $Ca_V1.2$ (L-type) and ancillary calcium channel β1b and α2δ subunit cDNA were recorded, and % inhibition by each test compound (at a concentration of 10 μM) was determined to measure the resting state block. (Fig. 3**a)** shows robust inhibition of $Ca_V1.2$ by compounds (**4b, 6c** and **13e)** (greater than 60%) and moderate inhibition by compounds (**4c, 9, 13c** and **17b)** (around 50%). The remaining compounds mediated less than 50% block. Results recorded from similar experiments with cells transfected with the $Ca_V3.2$ (T-Type) α1 subunit are represented in Fig. 3b. Robust inhibition of $Ca_V3.2$ was observed with ten compounds (**4b, 4c, 6c, 9, 13b, 13c, 13e, 14b, 17a** and **17b)** (approximately equal to or greater than 80%) with compound **4c** showing almost complete T-type inhibition. The remainder of the test compounds were less active. Collectively, seven compounds (**4b**, **4c**, **6c, 9, 13c, 13e** and **17b**) were found to be effective blockers of both $Ca_V1.2$ and $Ca_V3.2$ channels with slightly higher T-type current inhibition suggesting preferential antagonism of $Ca_V3.2$ over $Ca_V1.2$ and three compounds (**13b, 14b** and **17a**) were selective against Cav3.2. For comparison, the selective L-type channel inhibitor nifedipine mediates block of Cav1.2 channels in tsA-201 cells with sub micromolar affinity [19] and there are potent non selective Cav3.2 channel inhibitors that fully block this channel at the screening concentration used here [58].

2.3. In silico prediction of physicochemical properties and drug-likeness data for the active compounds

Over the last few decades, research indicated that some bioactive molecules may fail to become good drug candidates due to low bioavailability [59]. Hence, the drug discovery sector now utilizes molecular property prediction as a useful tool to identify useful drug candidates. Herein we applied computational methods to assess whether our most active compounds possess the correct parameters to exhibit drug-likeness or not. *Molinspiration* [60] online software was used to predict the main molecular descriptors formulating Lipinski's rule of five [61] which states that cell permeability and oral bioavailability are likely to occur if at least three of the following rules are obeyed: molecular weight (M.W) ≤ 500 Da, *n*-octanol-water partition coefficient (log P) ≤ 5, number of hydrogen bond acceptors (HBA) ≤ 10, number of hydrogen bond donors (HBD) ≤ 5. *Molinspiration* is also used to predict Lipiniski' s violation. In addition, it calculates the number of rotatable bonds (NROTB) and topological polar surface area (TPSA) ($Å^2$) which have been reported as very good descriptors of oral bioavailability of drugs [62]. It was estimated that the TPSA values for most known drugs are below 140–150 $Å^2$ [63,64]. TPSA was used to calculate percentage of absorption applying the following equation: %ABS = 109–0.345 TPSA. *In silico* physicochemical properties data of the most active compounds are listed in Table 1**.**

Among the tested compounds, two compounds **(6c, 13e)** are in full accordance to Lipinski's rule of five. Four compounds showed a slight violation regarding number of hydrogen bond acceptors **(13b, 13c** and **17a)** or log P **(9).** Other compounds displayed additional violations. TPSA values of (**6c, 9** and **13e)** were below 140 $Å^2$. Other compounds showed slightly higher TPSA in the range of 142.80–148.83 $Å^2$ (below 150 $Å^2$) except for compound (**14b)** (190.59 $Å^2$). In addition, most of tested compounds displayed reasonable %ABS in the range of 57.65–71.84 % indicating a good predicted oral bioavailability except for compound (**14b)** with slightly lower absorption (43.25%).

Molsoft software [65] was applied to predict solubility as well as drug likeness model score **(**Table 2**)**. It is known that about 80% of drugs in market have an estimated solubility above 0.0001 mg/L. Interestingly, all tested compounds fulfill the solubility requirement. Drug-likeness model score has been defined as a combined effect of physico-chemical properties, pharmacokinetics and pharmacodynamics of a compound and it is represented by a numerical value [66]. Compounds having zero or negative value should not be considered as drug-like. As illustrated in (Table 2**)**, all tested compounds have positive drug likeness values except for (**13c)**, which indicates that most of the active compounds have good predicted drug-likeness potential. Taking all together, it is concluded that the most active compounds exhibited reasonable physicochemical properties with good predicted drug-likeness values, which might raise them to be drug candidates.

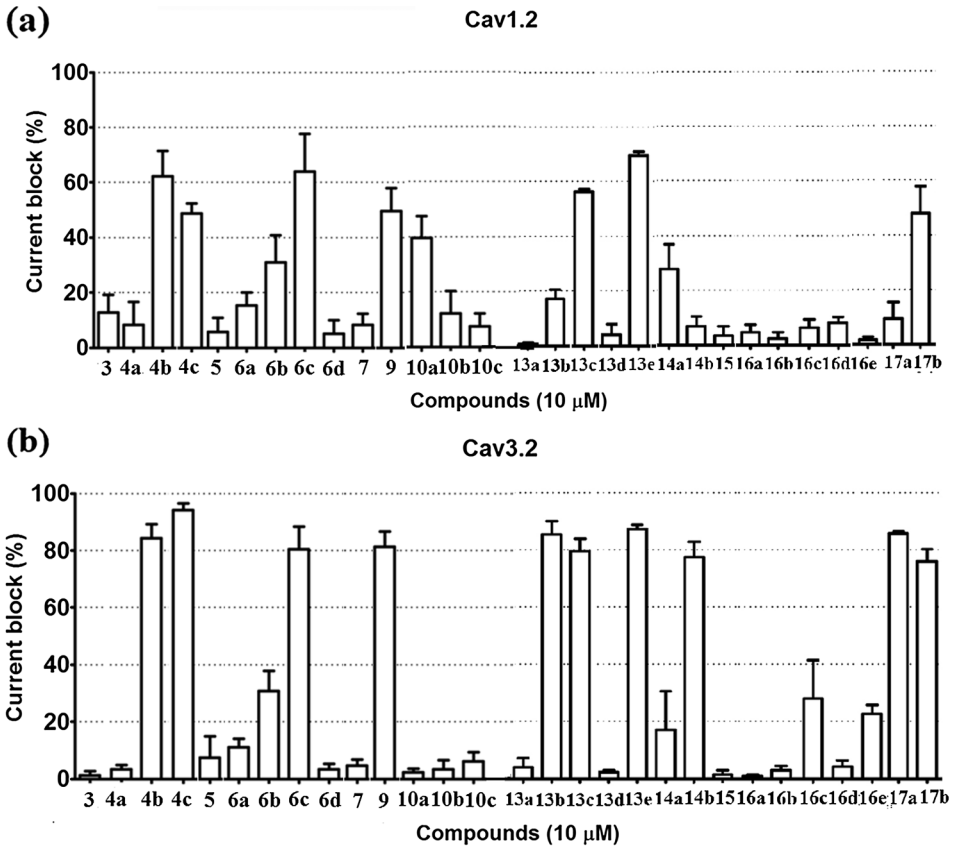


Fig. 3. (a) Tonic block of rat $Ca_V1.2$ (L-type) induced by a 10 mM application of test compounds (n = 4–5 per channel). (b) Tonic block of human $Ca_V3.2$ (T-type) with the same compounds (n = 4–5 per channel, at 10 μM). Error bars reflect standard errors.

2.4. Structure activity relationship

In the present study N3 substituents were selected to approximately mimic length and lipophilic nature of the corresponding side chain in the newer DHPs generations. In order to gain more information about the structure activity relationship, the scope was extended to modify the corresponding side chain ester in the parent DHPs to various N3-functionalities in the target compounds. A deep insight in the structures of the synthesized compounds revealed that among the carbamates (**4a-c)** only the p-substituted compounds (**4b** and **4c)** showed relatively better antagonism towards $Ca_V1.2$ and $Ca_V3.2$. Reduction of ester (**2)** affording the alcohol derivative (**3)** decrease the potency towards both calcium channels. Similarly, the bromo derivative (**5)** was found to be inactive. Introduction of single hetero atom attached to alkyl or aralkyl moieties in compounds (**6a-d** and **9)** conserved the calcium channel blocking activity. Surprisingly, (**6c** and **9)** showed the highest activity. The unexpected tetrahydropyrimido[2,1-*b*][1,3]oxazine-7-carboxylate (**7)** did not show any significant activity. The acetylated derivative (**10a)** revealed only slight L-current blockade. However, replacement the acetyl moiety with carbonate (**10b** and **10c)** was detrimental to both $Ca_V1.2$ and $Ca_V3.2$ blocking activity. Within the carbamates and thiocarbamates series (**13a-e),** only the the tertbutylcarbamate (**13c)**

Table 1
In silico physicochemical properties data of active compounds as predicted by *Molinspiration.*

Code	LogP[a]	M.Wt[b]	HBA[c]	HBD[d]	Lipinski's Violation	TPSA[e]	%ABS[f]	Volumes $(A)^3$	NROTB[g]
4b	5.38	496.52	11	2	2	142.80	59.73	441.59	11
4c	5.74	561.39	11	2	3	142.80	59.73	442.91	11
6c	4.80	466.54	9	1	0	107.70	71.84	430.80	11
9	5.43	455.54	8	1	1	104.47	68.43	402.79	10
13b	3.34	420.42	11	2	1	142.80	59.73	370.18	10
13c	3.92	448.48	11	2	1	142.80	59.73	403.00	10
13e	4.81	484.53	10	2	0	133.56	62.92	417.37	10
14b	3.63	591.58	15	2	2	190.59	43.25	509.27	14
17a	3.94	467.48	11	3	1	145.59	58.77	411.64	10
17b	5.94	558.60	12	3	3	148.83	57.65	495.83	11

[a] LogP: logarithm of compound partition coefficient between *n*-octanol and water.
[b] M.Wt: molecular weight.
[c] HBA: number of hydrogen bond acceptors.
[d] HBD:number of hydrogen bond donors.
[e] TPSA: polar surface area.
[f] %ABS: percentage of absorption.
[g] NROTB: number of rotatable bonds.

Table 2
In silico drug-likeness data of active compounds (*Molsoft*).

Code	S[a] (mg/L)	Drug-likeness model score
4b	0.37	0.24
4c	0.08	0.40
6c	4.07	1.27
9	1.24	0.26
13b	38.48	0.44
13c	5.16	-0.23
13e	1.37	0.82
14b	2.22	0.38
17a	1.97	0.15
17b	0.58	0.58
Nifedipine	44.88	-0.33
Nicardipine	4.97	1.67
Lercanidipine	0.01	1.39

S[a] aqueous solubility.

and the thiocarbamate (**13e)** showed reasonable antagonism towards $Ca_V1.2$ and $Ca_V3.2$ while the ethylcarbamate (**13b)** showed remarkable selective inhibition of $Ca_V3.2$. The propargyl and methyl carbamates (**13a,d)** did not reveal any significant activity. Replacement of the propargyl moiety with the substituted 1,2,3-triazole ring (**14b)** increased the blockade activity towards $Ca_V3.2$ over that against $Ca_V1.2$. Conversion of the tertbutylcarbamate (**13c)** to the corresponding ureas and thioureas **(16a-e)** dramatically decreased the activity. Finally the phenyl hydrazide (**17a)** displayed selective T-current blockade, while the semicarbazide derivative (**17b)** displayed dual L/T-current blockade activity.

3. Conclusion

The aim of the present investigation was to synthesize some new dihydropyrimidines bearing various lipophilic pharmacophores and functionalities at position 3 and evaluation for their calcium channel blockade activities. The newly synthesized compounds were evaluated for their ability to block $Ca_V1.2$ and $Ca_V3.2$ channels utilising whole-cell patch clamp. Seven compounds (**4b**, **4c**, **6c, 9, 13c, 13e** and **17b**) showed promising dual calcium channel blocking activity and three compounds (**13b, 14b** and **17a**) were selective against Cav3.2. Computational prediction of drug likeness showed reasonable physicochemical properties and satisfactory predicted pharmacokinetic parameters. Such type of compounds would represent a fruitful matrix for further development of more potent and selective CCBs that deserve further investigation and derivatization in order to explore the scope and limitation of its biological activities.

4. Experimental

4.1. Chemistry

All chemicals were purchased from commercial sources. Flash column chromatography separation was performed using Acros organics silica gel 40–60 μm, 60 Å using combination of ethyl acetate and hexanes. Preparative thin layer chromatography was performed using UNIPLATE ™1500μm silica gel plates with UV 254 preparative layer. Whatman and sigma TLC plates were utilized for thin layer chromatography and visualization was done using UV fluorescence at 254 nm. Melting points were recorded on a Mel-Temp, Laboratory devices, Inc and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 MHz & 600 MHz instrument using DMSO-d_6 as solvent unless otherwise stated. ^{1}H NMR Spectra are reported in order; multiplicity, number of protons and signals were characterized as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet), br s (broad signal), q (quartet). Chemical shifts are relative to TMS as an internal standard. Mass spectra were recorded on ThermoFinnigan MAT95XL high resolution magnetic sector mass spectrometer, using electrospray ionization method. The IR spectra were recorded on ZnSe crystal at 8 cm^{-1} resolution in Nicolet 380 ATR-FTIR spectrophotometer (Thermo electron Corporation, Madison, WI). DHPM **(1)** was prepared according to previously published reaction conditions [32].

4.1.1. Ethyl 3-[2-(ethoxycarbonyl)ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate *(2)*

To a solution of DHPM **(1)** (4.5 g, 15 mmol) and the appropriate ethyl acrylate (15 mmol) in anhydrous DMF (30 mL), KF/neutral alumina (10 mol %) was added in one portion. The mixture was stirred at room temperature for 1–5 days. The completion of the reaction was monitored by TLC using 1:1 toluene: acetone. The catalyst was removed by filtration. The filtrate was then poured into ice-cold water (500 mL). The obtained product was filtered, washed with water, dried and purified by crystallization from ethanol*; Yield:* 4.6 g (76.3%); MP: 121 °C [14].

4.1.2. Ethyl 3-(3-hydroxypropyl)-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate *(3)*

To a solution of the ester compound **(2)** (2 g, 5 mmol) in dry THF (100 mL), $LiAlH_4$ (0.6 g, 16 mmol) was added portion-wise while stirring at 0 °C. The mixture was left to slowly warm up to room temperature under stirring for 2 days. After completion, the reaction was quenched by ice-cold water (200 mL), filtered and the residue was washed with hot ethyl acetate (100 mL × 3). The filtrate was then extracted with ethyl acetate. The combined organic layer was washed with water and brine solution then dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography using gradient ethyl acetate in hexanes. *Yield:* 0.7 g (39%); MP: 145 °C; IR (KBr, cm^{-1}): 3439 (OH), 3216 (NH), 1701, 1678 (C═O), 1635 (C═N), 1523, 1342 (NO_2), 1234, 1075 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.17 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 1.48–1.56 (m, 1H, CH), 1.63–1.71 (m, 1H, CH), 2.26 (s, 3H, C^6—CH_3), 2.81–2.88 (m, 1H, CH), 3.36–3.44 (m, 2H under DMSO, CH_2), 3.56–3.65 (m, 1H, CH), 3.96–4.09 (m, 2H, $CH_3\underline{CH_2}$), 4.46–4.49 (*dist.* t, 1H, OH, D_2O-exchangeable), 5.36, 5.46 (2 s, 1H, C^4—H), 7.39–8.20 (m, 4H, ArH), 9.48, 9.54, 9.57 (3 s, 1H, NH, D_2O-exchangeable); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ ppm: 14.20, 18.47, 21.06, 30.01, 42.32, 58.42, 60.48, 100.82, 122.34, 123.24, 129.92, 133.23, 144.49, 147.29, 148.25, 154.02, 165.14; HRMS (ESI) calcd. for $C_{17}H_{22}N_3O_6[M + 1]^+$: 364.1503; Found: 364.1509.

4.1.3. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-3-[3-({arylcarbamoyl}oxy) propyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate *(4a-c)*

To a solution of alcohol **(3)** (0.36 g, 1 mmol) in anhydrous DCM (5 mL), the appropriate substituted isocyanate (1 mmol) was added. The reaction mixture was stirred over night at room temperature and then concentrated under reduced pressure. The residue was purified by flash chromatography using gradient ethylacetate in hexanes to afford the pure carbamates.

*4.1.3.1. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-3-[3-({phenylcarbamoyl} oxy)propyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(4a)**. Yield:* 0.4 g (83%); MP: 160 °C; IR (KBr, cm^{-1}): 3336, 3215 (NH), 1728, 1689, 1637 (C═O), 1598 (C═N), 1526, 1347 (NO_2), 1241, 1216, 1091, 1030 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.18 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 1.84–2.06 (m, 3H, CH_2 and CH), 2.37 (s, 3H, C^6—CH_3), 3.00–3.07 (m, 1H, CH), 3.82–3.86 (m, 1H, CH), 4.00–4.09 (m, 2H, $CH_3\underline{CH_2}$), 4.16 (t, J = 6 Hz, 1H, CH), 5.62 (s, 1H, C^4—H), 7.00 (t, J = 8 Hz, 1H, ArH), 7.28 (t, J = 8 Hz, 2H, ArH), 7.56 (d, J = 8 Hz, 2H, ArH), 7.65 (t, J = 8 Hz, 1H, ArH), 7.86 (d, J = 8 Hz, 1H, ArH), 8.17 (d, J = 8 Hz, 1H, ArH), 8.30 (s, 1H, ArH), 8.67, 8.99 (2 s, 2H, 2NH, D_2O-exchangeable); HRMS (ESI) calcd. for $C_{24}H_{27}N_4O_7[M + 1]^+$:483.1874; Found: 483.1872.

4.1.3.2. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-3-[3-({p-tolylcarbamoyl} oxy)propyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(4b)***. *Yield:* 0.36 g (73.5%); MP: 165 °C; IR (KBr, cm^{-1}): 3332, 3222 (NH), 1728, 1682, 1640 (C═O), 1595 (C═N), 1527, 1350 (NO_2), 1241, 1220, 1095, 1035 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.14 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 1.73–1.80 (m, 1H, CH), 1.86–1.93 (m, 1H, CH), 2.24 (s, 3H, CH_3), 2.27 (s, 3H, C^6−CH_3), 2.89–2.96 (m, 1H, CH), 3.67–3.73 (m, 1H, CH), 3.92–4.02 (m, 2H, CH_2), 4.06 (q, J = 6 Hz, 2H, $CH_3\underline{CH_2}$), 5.49 (s, 1H, C^4−H), 7.08 (d, J = 8 Hz, 2H, ArH), 7.36 (d, J = 8 Hz, 2H, ArH), 7.68 (t, J = 8 Hz, 1H, ArH), 7.78 (d, J = 8 Hz, 1H, ArH), 8.17 (d, J = 8 Hz, 1H, ArH), 8.20 (s, 1H, ArH), 9.51, 9.64 (2 s, 2H, 2NH, D_2O-exchangeable); ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.68, 17.58, 20.25, 26.88, 42.27, 59.13, 59.56, 61.80, 99.23, 118.21, 121.46, 122.78, 129.04, 130.33, 131.19, 133.26, 136.41, 145.05, 147.73, 148.54, 151.59, 153.48, 164.74; HRMS (ESI) calcd. for $C_{25}H_{29}N_4O_7$ [M + 1] $^+$: 497.2031; Found: 497.2038.

4.1.3.3. Ethyl 3-[3-({(4-bromophenyl)carbamoyl}oxy)propyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(4c)***. *Yield:* 0.33 g (59%); MP: 140 °C; IR (KBr, cm^{-1}): 3314, 3224 (NH), 1729, 1683, 1640 (C═O), 1592 (C═N), 1527, 1350 (NO_2), 1240, 1216, 1095, 1033 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.19 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 1.86–1.96 (m, 1H, CH), 1.98–2.08 (m, 1H, CH), 2.37 (s, 3H, C^6−CH_3), 3.01–3.11 (m, 1H, CH), 3.82–3.89 (m, 1H, CH), 4.01–4.19 (m, 4H, $CH_3\underline{CH_2}$ and CH_2), 5.62 (s, 1H, C^4−H), 7.43 (d, 12 Hz, 2H, ArH), 7.53 (d, J = 8 Hz, 2H, ArH), 7.65 (t, J = 8 Hz, 1H, ArH), 7.86 (d, J = 8 Hz, 1H, ArH), 8.17 (d, J = 8 Hz, 1H, ArH), 8.30 (s, 1H, ArH), 8.82, 9.02 (2 s, 2H, 2NH, D_2O-exchangeable); HRMS (ESI) calcd. for $C_{24}H_{26}BrN_4O_7$ [M + 1]$^+$: 561.0979; Found: 561.0961 and calcd. For $C_{24}H_{26}$ [81]BrN_4O_7 [M + 1]: 563.0959; Found: 563.0987.

4.1.4. Ethyl 3-(3-bromopropyl)-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate ***(5)***

To a solution of alcohol **(3)** (0.36 g, 1 mmol) and carbon tetrabromide (3 mmol) in anhydrous DCM (5 mL) at 0 °C, triphenylphosphine (0.78 g, 3 mmol) was added in portions while stirring over 10 min. The mixture was left to attain room temperature under stirring for 20 min. After completion, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography using gradient ethylacetate in hexanes to afford the pure product. *Yield:* 0.33 g (78%); MP: 167 °C; IR (KBr, cm^{-1}): 3208 (NH), 1699, 1679 (C═O), 1636 (C═N), 1528, 1348 (NO_2), 1226, 1084 (C−O−C); ^{1}H NMR ($CHCl_3$, 400 MHz) δ ppm: 1.26 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 1.98–2.06 (m, 1H, CH), 2.17–2.24 (m, 1H, CH), 2.38 (s, 3H, C^6−CH_3), 2.99–3.06 (m, 1H, CH), 3.38–3.48 (m, 2H, CH_2), 3.78–3.85 (m, 1H, CH), 4.08–4.21 (m, 2H, $CH_3\underline{CH_2}$), 5.49 (s, 1H, C^4−H), 7.54 (t, J = 8 Hz, 1H, ArH), 7.74 (d, J = 8 Hz, 1H, ArH), 8.17 (d, J = 8 Hz, 1H, ArH), 8.24 (s, 1H, ArH), 9.15 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ ppm: 14.24, 18.51, 30.55, 44.34, 60.39, 60.53, 100.46, 122.30, 123.20, 129.89, 133.23, 144.29, 147.84, 148.35, 153.36, 165.20; HRMS (ESI) calcd. for $C_{17}H_{20}BrN_3O_5Na$ [M + Na] $^+$:448.0479; Found: 448.0487 & found for $C_{17}H_{20}$[81]BrN_3O_5Na [M + Na]:450.0488.

4.1.5. Ethyl 3-[3-(substitutedamino)propyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(6a-d)***

A mixture of the bromo compound **(5)** (0.42 g, 1 mmol) and the appropriate amine (20 mmol) in absolute ethanol (20 mL) was heated under reflux for 6–9 h. After reaction completion, the mixture was concentrated under reduced pressure, diluted with water (100 mL) and extracted with ethyl acetate (100 mL × 3). The combined organic layer was washed thoroughly with water and brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was chromatographed by preparative thin layer chromatography using ethyl acetate as mobile phase to afford the pure product.

4.1.5.1. Ethyl 3-[3-(dipropylamino)propyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate ***(6a)***. *Yield:* 0.31 g (71%); Oil ; IR (KBr, cm^{-1}): 3215 (NH), 1675 (C═O), 1637 (C═N), 1529, 1347 (NO_2), 1230, 1080 (C−O−C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 0.84 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 1.22 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 1.40 (q, J = 6 Hz, 2H, $CH_3\underline{CH_2}$), 1.52–1.81 (m, 3H, CH_2 and CH), 2.27–2.33 (m, 3H, $\underline{CH_3}CH_2$), 2.39 (s, 3H, C^6−CH_3), 2.93–3.10 (m, 1H, CH), 3.27–3.52 (m, 1H, CH), 3.63–3.85 (m, 2H, $CH_3\underline{CH_2}$), 4.00–4.16 (m, 3H, $CH_3\underline{CH_2}$ and CH), 5.46, 5.59 (2 s, 1H, C^4−H), 7.68 (t, J = 8 Hz, 1H, ArH), 7.84–7.88 (m, 1H, ArH), 8.18 (d, J = 8 Hz, 1H, ArH), 8.28 (s, 1H, ArH), 8.75 (s, 1H, NH, D_2O-exchangeable); HRMS (ESI) calcd. for $C_{23}H_{35}N_4O_5$ [M + 1] $^+$: 447.2602; Found: 447.2613.

4.1.5.2. Ethyl 3-[3-(bis{2-methoxyethyl}amino)propyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(6b)***. *Yield:* 0.2 g (42.5%); Oil; IR (KBr, cm^{-1}): 3211 (NH), 1673 (C═O), 1639 (C═N), 1528, 1347 (NO_2), 1232, 1082 (C−O−C); ^{1}H NMR ($CHCl_3$, 400 MHz) δ ppm: 1.08–1.18 (m, 6H, $\underline{CH_3}CH_2$ and OCH_3), 1.56–1.74 (m, 1H, CH), 1.93 (s, 3H, OCH_3), 2.27 (s, 3H, C^6−CH_3), 2.46–2.49 (m, 1H, CH), 2.59 (t, J = 6 Hz, 2H, CH_2), 2.73–2.82 (m, 1H, CH), 3.14–3.27 (m, 5H, 2 CH_2 and CH), 3.55 (t, J = 6 Hz, 2H, CH_2), 3.91–4.08 (m, 4H, $CH_3\underline{CH_2}$ and CH_2), 5.29, 5.41 (2 s, 1H, C^4−H), 7.35–7.48 (m, 1H, ArH), 7.67–7.73 (m, 1H, ArH), 8.03–8.06 (m, 1H, ArH), 8.11, 8.15 (2 s, 1H, ArH), 9.31 (s, 1H, NH, D_2O-xchangeable); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ ppm: 14.01, 14.10, 18.21, 20.80, 22.27, 23.04, 25.09, 43.64, 45.02, 48.89, 52.18, 53.57, 58.56, 59.55, 59.77, 60.00, 60.17, 63.46, 65.53, 70.91, 71.48, 100.05, 100.71, 121.98, 123.11, 128.46, 129.65, 129.96, 131.83, 131.93, 133.17, 133.30, 144.02, 144.72, 148.03, 152.92, 155.22, 159.04, 165.15, 165.71, 170.88; HRMS (ESI) calcd. for $C_{23}H_{35}N_4O_7$ [M + 1] $^+$:479.2500; Found:479.2507.

4.1.5.3. Ethyl 3-[3-(benzyl{methyl}amino)propyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(6c)***. *Yield:* 0.3 g (65%); MP: 126 °C; IR (KBr, cm^{-1}): 3210 (NH), 1703, 1683 (C═O), 1635 (C═N), 1524, 1349 (NO_2), 1227, 1082 (C−O−C); ^{1}H NMR ($CHCl_3$, 400 MHz) δ ppm: 1.21 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 1.66–1.76 (m, 1H, CH), 1.81–1.88 (m, 1H, CH), 2.15 (s, 3H, NCH_3), 2.34 (s, 3H, C^6−CH_3), 2.36–2.38 (m, 1H, CH), 2.46–2.50 (m, 1H, CH), 2.87–2.96 (m, 1H, CH), 3.41–3.51 (m, 2H, CH_2), 3.72–3.79 (m, 1H, CH), 4.03–4.18 (m, 2H, $CH_3\underline{CH_2}$), 5.52 (s, 1H, C^4−H), 7.22–7.32 (m, 5H, ArH), 7.51 (t, J = 8 Hz, 1H, ArH), 7.72 (d, J = 8 Hz, 1H, ArH), 8.15 (d, J = 8 Hz, 1H, ArH), 8.24 (s, 1H, ArH), 9.06 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ ppm: 14.23, 18.53, 25.40, 42.07, 43.89, 54.33, 60.16, 60.25, 61.97, 100.32, 122.36, 123.02, 127.01, 128.25, 128.87, 129.81, 133.18, 138.97, 144.74, 147.96, 148.26, 153.17, 165.29; HRMS (ESI) calcd. for $C_{25}H_{31}N_4O_5$ [M + 1] $^+$:467.2289; Found:467.2296.

4.1.5.4. Ethyl 3-[3-({2,2-diphenylethyl}amino)propyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(6d)***. *Yield:* 0.1 g (18.5%); Oil; IR (KBr, cm^{-1}): 3220, 3085 (NH), 1674 (C═O), 1639 (C═N), 1529, 1348 (NO_2), 1234, 1079 (C−O−C); ^{1}H NMR ($CHCl_3$, 400 MHz) δ ppm: 1.22, 1.26 (2 t, J^1 = 8 Hz, J^2 = 8 Hz, 3H, $\underline{CH_3}CH_2$), 1.58–1.78 (m, 3H, CH_2 & $CH_2\underline{NH}$, D_2O-exchangeable), 2.04, 2.32, 2.35 (3 s, 3H, C^6−CH_3), 2.65 (t, J = 6 Hz, 1H, CH), 2.78–2.85 (m, 1H,CH), 3.20 (d, J = 8 Hz, 1H, CH), 3.31 (d, J = 8 Hz, 1H, CH), 3.41–3.59, 3.65–3.77 (m, 1H, CH), 3.97–4.18 (m, 3H, CH & $CH_3\underline{CH_2}$), 4.26 (d, J = 8 Hz, 0.6H, C*H*), 4.46 (t, J = 8 Hz, 0.3H, CH), 5.40, 5.44 (2 s, 1H, C^4−H), 7.15–7.38 (m, 10H, ArH), 7.47, 7.52 (2 t, J^1 = 8 Hz, J^2 = 8 Hz, 1H, NH, D_2O-exchangeable), 7.59–7.79 (m, 2H, ArH), 8.07–8.23 (m, 2H, ArH); HRMS (ESI) calcd. for $C_{31}H_{35}N_4O_5$ [M + 1] $^+$: 543.2602; Found: 543.2602.

*4.1.6. Ethyl 8-methyl-6-(3-nitrophenyl)-2,3,4,6-tetrahydropyrimido[2,1-b][1,3]oxazine-7-carboxylate **(7)***

A mixture of the bromo derivative **(5)** (0.42 g, 1 mmol) and NaOEt (1.2 mmol) in absolute ethanol (20 mL) was stirred under reflux for 8 h. After reaction completion, the mixture was concentrated under reduced pressure and the residue was purified by flash chromatography utilizing gradient ethyl acetate in hexanes. *Yield:* 0.3 g (88%); MP: 111 °C; IR (KBr, cm^{-1}): 1694 (C═O), 1610 (C═N), 1530, 1345 (NO_2), 1247, 1216, 1086, 1058 (C−O−C); ^{1}H NMR ($CHCl_3$, 400 MHz) δ ppm: 1.20 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 2.05–2.10 (m, 1H, CH), 2.26–2.32 (m, 1H, CH), 2.37 (s, 3H, C^6−CH_3), 3.22–3.35 (m, 2H, CH_2), 4.02–4.13 (m, 3H, $CH_3\underline{CH_2}$, CH), 4.36–4.40 (m, 1H, CH), 5.36 (s, 1H, C^4−H), 7.55 (t, J = 8 Hz, 1H, ArH), 7.80 (d, J = 8 Hz, 1H, ArH), 8.16 (d, J = 8 Hz, 1H, ArH), 8.20 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ ppm: 14.23, 22.38, 23.27, 45.11, 59.69, 63.64, 65.53, 100.84, 122.05, 123.26, 130.06, 133.38, 144.05, 148.11, 155.17, 159.23, 165.85; HRMS (ESI) calcd. for $C_{17}H_{20}N_3O_5$ [M + 1] $^+$:346.1397; Found: 346.1397.

*4.1.7. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-3-[3-(phenylthio)propyl]-1,2,3,4-tetrahydro pyrimidine-5-carboxylate **(9)***

A mixture of thiophenol (0.1 mL, 1 mmol) and anhydrous K_2CO_3 (0.14 g, 1 mmol) in anhydrous acetone (20 mL) was stirred at room temperature for 30 min. The bromo compound **(5)** (1 mmol) was then added and the reaction mixture was refluxed for 8 h. The reaction mixture was concentrated under reduced pressure and the obtained residue was purified by chromatography or preparative TLC with gradient eluent of 0–90% ethyl acetate in hexanes *Yield:*0.24 g (53%); MP: 111 °C; IR (KBr, cm^{-1}): 3210 (NH), 1705, 1680 (C═O), 1635 (C═N), 1524, 1344 (NO_2), 1270, 1232, 1083, 1021 (C−O−C); ^{1}H NMR ($CHCl_3$, 400 MHz) δ ppm: 1.24 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 1.77–1.86 (m, 1H, CH), 1.90–1.98 (m, 1H, CH), 2.36 (s, 3H, C^6−CH_3), 2.87–3.03 (m, 3H, CH_2 and CH), 3.78–3.85 (m, 1H, CH), 4.04–4.19 (m, 2H, $CH_3\underline{CH_2}$), 5.42 (s, 1H, C^4−H), 7.16–7.20 (m, 1H, ArH), 7.24–7.32 (m, 4H, ArH), 7.49 (t, J = 8 Hz, 1H, ArH), 7.71 (d, J = 8 Hz, 1H, ArH), 8.13 (d, J = 8 Hz, 1H, ArH), 8.20 (s, 1H, ArH), 9.34 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ ppm: 14.23, 18.50, 26.79, 30.90, 44.56, 60.19, 60.32, 100.36, 122.31, 123.11, 126.25, 128.98, 129.40, 129.85, 133.16, 135.71, 144.42, 147.93, 148.24, 153.34, 165.20; HRMS (ESI) calcd. for $C_{23}H_{26}N_3O_5S$ [M + 1]$^+$: 456.1588; Found: 456.1575.

*4.1.8. Ethyl 3-(3-substitued propyl)-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate **(10a-c)***

A mixture of alcohol **(3)** (0.36 g, 1 mmol) and acetyl chloride (1.7 mmol) (for **4a**) or the proper alkyl chloroformate (1.2 mmol) (for **3b** &**3c**) in anhydrous pyridine (3 mL) was stirred overnight at room temperature under nitrogen. The reaction was diluted with water (100 mL), extracted with ethyl acetate (100 mL × 3). The combined organic layer was washed with water and brine then dried over anhydrous sodium sulfate and evaporated under reduced pressure. The pure compounds were isolated by flash chromatography utilizing gradient ethyl acetate in hexanes.

*4.1.8.1. Ethyl 3-(3-acetoxypropyl)-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate **(10a)**. Yield:* 0.3 g (75.5%); MP: 106 °C; IR (KBr, cm^{-1}): 3199 (NH), 1735, 1709, 1677 (C═O), 1638 (C═N), 1535, 1352 (NO_2), 1267, 1237, 1084, 1045 (C−O−C); ^{1}H NMR ($CHCl_3$, 400 MHz) δ ppm: 1.26 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 1.83–1.97 (m, 2H, CH_2), 2.06 (s, 3H, $COCH_3$), 2.37 (s, 3H, C^6−CH_3), 2.86–2.93 (m, 1H, CH), 3.79–3.86 (m, 1H, CH), 4.09–4.20 (m, 4H, $CH_3\underline{CH_2}$ and CH_2), 5.44 (s, 1H, C^4−H), 7.54 (t, J = 8 Hz, 1H, ArH), 7.73 (d, J = 8 Hz, 1H, ArH), 8.17 (d, J = 8 Hz, 1H, ArH), 8.22 (s, 1H, ArH), 9.06 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ ppm: 14.24, 18.56, 20.88, 26.63, 42.73, 60.11, 60.39, 61.84, 100.46, 122.29, 123.16, 129.94, 133.09, 144.31, 147.70, 148.27, 153.12, 165.23, 170.94; HRMS (ESI) calcd. for C_{19} $H_{24}N_3O_7$ [M + 1] $^+$: 406.1609; Found: 406.1613.

*4.1.8.2. Ethyl 3-[3-({methoxycarbony})oxy)propyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(10b)**. Yield:* 0.42 g (85.7%); MP: 116 °C; IR (KBr, cm^{-1}): 3198 (NH), 1749, 1708, 1675 (C═O), 1635 (C═N), 1535, 1354 (NO_2), 1267, 1235, 1083, 1030 (C−O−C); ^{1}H NMR ($CHCl_3$, 400 MHz) δ ppm: 1.27 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 1.86–2.06 (m, 2H, CH_2), 2.38 (s, 3H, C^6−CH_3), 2.92–2.99 (m, 1H, CH), 3.78 (s, 1H, OCH_3), 3.81–3.84 (m, 1H, CH), 4.09–4.21 (m, 4H, $CH_3\underline{CH_2}$ and CH_2), 5.45 (s, 1H, C^4−H), 7.54 (t, J = 8 Hz, 1H, ArH), 7.74 (d, J = 8 Hz, 1H, ArH), 8.16 (d, J = 8 Hz, 1H, ArH), 8.23 (s, 1H, ArH), 9.28 (s, 1H, NH, D_2O-xchangeable); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ ppm: 14.21, 18.44, 26.82, 42.68, 54.81, 60.23, 60.33, 65.37, 100.41, 122.31, 123.13, 129.89, 133.12, 144.34, 147.87, 148.28, 153.30, 155.62, 165.20; HRMS (ESI) calcd. for C_{19} $H_{24}N_3O_8$ [M + 1]$^+$: 422.1558; Found: 422.1559.

*4.1.8.3. Ethyl 3-[3-({ethoxycarbonyl}oxy)propyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(10c)**. Yield:* 0.4 g (92%); MP: 108 °C; IR (KBr, cm^{-1}): 3215 (NH), 1738, 1691, 1668 (C═O), 1641 (C═N), 1522, 1345 (NO_2), 1257, 1233, 1071, 1028 (C−O−C); ^{1}H NMR ($CHCl_3$, 400 MHz) δ ppm: 1.27 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 1.31 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 1.86–2.03 (m, 2H, CH_2), 2.38 (s, 3H, C^6−CH_3), 2.92–2.99 (m, 1H, CH), 3.77–3.84 (m, 1H, CH), 4.07–4.24 (m, 6H, 2 $CH_3\underline{CH_2}$ and CH_2), 5.45 (s, 1H, C^4−H), 7.54 (t, J = 8 Hz, 1H, ArH), 7.74 (d, J = 8 Hz, 1H, ArH), 8.17 (d, J = 8 Hz, 1H, ArH), 8.23 (s, 1H, ArH), 9.28 (s, 1H, NH, D_2O-xchangeable); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ ppm: 14.25, 18.50, 21.05, 26.87, 42.74, 60.23, 60.34, 64.07, 65.12, 100.47, 122.32, 123.13, 129.89, 133.11, 144.34, 148.28, 153.20, 155.03, 165.19, 171.18; HRMS (ESI) calcd. for $C_{20}H_{26}N_3O_8$ [M + 1]$^+$: 436.1714; Found: 436.1719.

*4.1.9. Ethyl 3-[2-(hydrazocarbonyl)ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetra hydropyrimidine-5-carboxylate **(11)***

To a solution of **2** in ethanol (50 mL, 1 mmol), hydrazine-hydrate (99%) (3 mL) was added and refluxed for 7 h. The reaction was then cooled, concentrated, diluted with water. The precipitate was washed with water, dried and recrystallized from ethanol; *Yield:* 3 g (80%); MP: 184 °C [14].

*4.1.10. Ethyl 3-(3-azido-3-oxopropyl)-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(12)***

A solution of $NaNO_2$ (5 mmol) in cold water (10 mL) was added dropwise while stirring to a cold solution (0–5 °C) of hydrazide **2** (1 mmol) in acetic acid (5 mL), 1 N HCl (2 mL), and water (15 mL). The reaction mixture was stirred at this temperature for 3–5 h. The precipitate was collected by filtration, washed thoroughly with cold water and dried.

Yield: 0.3 g (75%); IR (KBr, cm^{-1}): 3330 (NH), 2126,2145 (N_3), 1692, 1678 (C═O), 1630 (C═N), 1529, 1345 (NO_2), 1228, 1089 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.16 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 2.25 (s, 3H, C^6−CH_3), 2.82–2.89 (m, 1H, CH), 3.06–3.12 (m, 1H, CH), 3.22–3.27 (m, 1H, CH), 3.60–3.66 (m, 1H, CH), 3.95–4.09 (m, 2H, $CH_3\underline{CH_2}$), 5.44 (s, 1H, C^4−H), 7.66–8.18 (m, 4H, ArH), 9.60 (s, 1H, NH, D_2O-exchangeable).

*4.1.11. Ethyl 3-[2-({substitutedcarbonyl}amino)ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(13a-e)***

The crude azide intermediate **12** (1 mmol) was stirred in the appropriate alcohol or thiophenol (20 mL) under reflux for 6–12 h. The reaction mixture was concentrated under reduced pressure. The product separated was purified by flash chromatography using gradient ethyl acetate in hexanes.

*4.1.11.1. Ethyl 3-[2-({methoxycarbonyl}amino)ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(13a)**. Yield:* 0.28 g (70%); MP: 139 °C; IR (KBr, cm^{-1}): 3366, 3207 (NH), 1698, 1683 (C═O), 1631 (C═N), 1529, 1347 (NO_2), 1234, 1127,

1085, 1025 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.22 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 2.39 (s, 3H, C^6—CH_3), 3.04–3.09 (m, 1H, CH), 3.21–3.33 (m, 2H, CH_2), 3.55 (s, 3H, OCH_3), 3.72–3.79 (m, 1H, CH), 4.04–4.15 (m, 2H, $CH_3\underline{CH_2}$), 5.60 (s, 1H, C^4—H), 6.35 (s, 1H, NH, D_2O-exchangeable), 7.68 (t, J = 8 Hz, 1H, ArH), 7.85 (d, J = 8 Hz, 1H, ArH), 8.18 (d, J = 8 Hz, 1H, ArH), 8.27 (s, 1H, ArH), 8.67 (s, 1H, NH, D_2O-exchangeable).

^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.94, 17.63, 44.46, 51.24, 59.46, 59.67, 99.18, 121.56, 122.75, 130.38, 133.30, 144.97, 147.68, 148.45, 151.00, 151.57, 156.73, 164.71; HRMS (ESI) calcd. for $C_{18}H_{23}N_4O_7$ $[M + 1]^+$: 407.1561; Found: 407.1570.

4.1.11.2. Ethyl 3-[2-({ethoxycarbonyl}amino)ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(13b)***. *Yield:* 0.4 g (95%); MP: 165 °C; IR (KBr, cm^{-1}): 3367, 3213 (NH), 1700, 1685 (C═O), 1634 (C═N), 1527, 1353 (NO_2), 1233, 1129, 1087, 1037 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.12–1.17 (m, 6H, $2\underline{CH_3}CH_2$), 2.25 (s, 3H, C^6—CH_3), 2.75–2.85 (m, 1H, CH), 2.98–3.02 (m, 1H, CH), 3.12–3.21 (m, 1H, CH), 3.55–3.60 (m, 1H, CH), 3.91–4.08 (m, 4H, 2 $CH_3\underline{CH_2}$), 5.44 (s, 1H, C^4—H), 7.13 (s, 1H, NH, D_2O-exchangeable), 7.67–7.79 (m, 2H, ArH), 8.14–8.21 (m, 2H, ArH), 9.58 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.94, 14.53, 17.64, 44.47, 59.43, 59.57, 99.15, 121.59, 122.75, 130.38, 133.32, 145.00, 147.67, 148.43, 151.00, 151.53, 156.31, 164.70; HRMS (ESI) calcd. for $C_{19}H_{24}N_4O_7Na$ $[M + Na]^+$: 443.1537; Found: 443.1543.

4.1.11.3. Ethyl 3-[2-({tert-butoxycarbonyl}amino)ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(13c)***. *Yield:* 0.29 g (65%); MP: 180 °C; ; IR (KBr, cm^{-1}): 3387, 3209 (NH), 1701, 1683 (C═O), 1635 (C═N), 1528, 1346 (NO_2), 1234, 1171, 1130, 1084 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.14 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 1.36 (s, 9H, $C(CH_3)_3$), 2.23 (s, 3H, C^6—CH_3), 2.71–2.78 (m, 1H, CH), 2.92–2.99 (m, 1H, CH), 3.09–3.15 (m, 1H, CH), 3.51–3.59 (m, 1H, CH), 3.92–4.07 (m, 2H, $CH_3\underline{CH_2}$), 5.44 (s, 1H, C^4—H), 6.88 (s, 1H, NH, D_2O-exchangeable), 7.68–7.73 (m, 2H, ArH), 8.13–8.17 (m, 2H, ArH), 9.54 (s, 1H, NH, D_2O-exchangeable); HRMS (ESI) calcd. for $C_{21}H_{28}O_7N_4Na$ $[M + Na]^+$: 471.1850; Found: 471.1852.

4.1.11.4. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-3-[2-({(prop-2-yn-1-yloxy) carbonyl} amino)ethyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(13d)***. *Yield:* 0.27 g (63%); MP: 131 °C; IR (KBr, cm^{-1}): 3359, 3225 (NH), 2357 (C≡C), 1729, 1701, 1685 (C═O), 1634 (C═N), 1525, 1347 (NO_2), 1234, 1131, 1088, 1026 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.17 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 2.27 (s, 3H, C^6—CH_3), 2.81–2.87 (m, 1H, CH), 3.00–3.08 (m, 1H, CH), 3.16–3.25 (m, 1H, CH), 3.47 (s, 1H, C≡CH), 3.58–3.64 (m, 1H, CH), 3.98–4.10 (m, 2H, $CH_3\underline{CH_2}$), 4.54–4.65 (m, 2H, OCH_2), 5.46 (s, 1H, C^4—H), 7.42 (s, 1H, NH, D_2O-exchangeable), 7.67–7.76 (m, 2H, ArH), 8.16–8.18 (m, 2H, ArH), 9.59 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.95, 17.63, 44.41, 51.52, 59.46, 59.68, 76.96, 79.16, 99.21, 121.57, 122.75, 130.34, 133.33, 144.97, 147.70, 148.41, 151.58, 155.34, 164.70; HRMS (ESI) calcd. for $C_{20}H_{23}O_7N_4$ $[M + 1]^+$: 431.1561; Found: 431.1581.

4.1.11.5. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-3-[2-({(phenylthio) carbonyl}amino) ethyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(13e)***. *Yield:* 0.4 g (82.5%); MP: 148 °C; IR (KBr, cm^{-1}): 3333, 3209 (NH), 1685 (C═O), 1629 (C═N), 1525, 1344 (NO_2), 1235, 1204, 1127, 1087 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.14 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 2.26 (s, 3H, C^6—CH_3), 2.80–2.87 (m, 1H, CH), 3.12–3.17 (m, 1H, CH), 3.31–3.40 (m, 1H, CH), 3.62–3.66 (m, 1H, CH), 3.98–4.07 (m, 2H, $CH_3\underline{CH_2}$), 5.49 (s, 1H, C^4—H), 7.30–7.50 (m, 5H, ArH), 7.65–7.75 (m, 2H, ArH), 7.99–8.16 (m, 2H, ArH), 8.37 (s, 1H, NH, D_2O-exchangeable), 9.61 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.96, 17.68, 44.22, 59.47, 59.81, 99.27, 121.65, 122.79, 128.67, 128.74, 128.83, 130.42, 133.32, 134.83, 144.97, 147.67, 148.44, 151.51, 164.25, 164.73; HRMS (ESI) calcd. for $C_{23}H_{25}O_6N_4S$ $[M + 1]^+$: 485.1489; Found: 485.14804.

4.1.12. Ethyl 3-[2-({({1-substituterd-1H-1,2,3-triazol-4-yl}methoxy) carbonyl}amino) ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(14a,b)***

An equi-molar amounts of the propagyl derivative **(13d)** (1 mmol) and the appropriate azide (1 mmol) were dissolved in tetrahydrofuran (20 mL). To the reaction mixture, 5 mL of water, 5 mL of *tert*-butanol or methanol, and sodium ascorbate (300 mg) and copper sulphate (75 mg) were added and stirred overnight at room temperature. The mixture was evaporated under reduced pressure, diluted with water and extracted with ethyl acetate (100 mL × 3). The combined organic layer was washed with water (50 mL × 3) and brine then dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography with gradient eluent of 0–90% ethyl acetate in hexanes to offer the desired products.

4.1.12.1. Ethyl 3-[2-({({1-benzyl-1H-1,2,3-triazol-4-yl}methoxy)carbonyl}amino)ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(14a)***. *Yield:* 0.14 g (26%); MP: 150 °C; IR (KBr, cm^{-1}): 3349, 3211 (NH), 1709, 1693 (C═O), 1633 (C═N), 1525, 1347 (NO_2), 1235, 1131, 1089, 1029 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.05 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 2.17 (s, 3H, C^6—CH_3), 2.70–2.76 (m, 1H, CH), 2.89–2.95 (m, 2H, CH_2), 3.04–3.12 (m, 1H, CH), 3.86–3.98 (m, 2H, $CH_3\underline{CH_2}$), 4.94 (s, 2H, OCH_2), 5.36 (s, 1H, C^4—H), 5.52 (s, 2H, $\underline{CH_2}C_6H_5$), 7.20–7.32 (m, 6H, 5ArH & NH), 7.57–7.66 (m, 2H, ArH), 8.05 (s, 1H, triazolo C^5—H), 8.07–8.09 (m, 2H, ArH), 9.50 (s, 1H, NH, D_2O-exchangeable); HRMS (ESI) calcd. for $C_{27}H_{30}O_7N_7$ $[M + 1]^+$: 564.2201; Found: 564.2219.

4.1.12.2. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-3-[2-({({1-(2-oxo-2-phenylethyl)-1H-1,2,3-triazol-4-yl}methoxy)carbonyl}amino)ethyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate ***(14b)***. *Yield:* 0.15 g (25%); IR (KBr, cm^{-1}): 3256, 3130 (NH), 1695 (C═O), 1638 (C═N), 1527, 1348 (NO_2), 1227, 1122, 1086, 1050 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.19 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 2.38 (s, 3H, C^6—CH_3), 3.02–3.08 (m, 1H, CH), 3.23–3.35 (m, 2H, CH_2), 3.74–3.80 (m, 1H, CH), 4.00–4.14 (m, 2H, $CH_3\underline{CH_2}$), 5.15 (s, 2H, OCH_2), 5.61 (s, 1H, C^4—H), 6.17 (s, 2H, CH_2CO), 6.50 (s, 1H, NH, D_2O-exchangeable), 7.59–7.86 (m, 5H, ArH), 8.01 (s, 1H, triazolo C^5—H), 8.12–8.18 (m, 3H, ArH), 8.27 (s, 1H, ArH), 8.60 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 14.51, 18.32, 45.92, 56.51, 58.48, 60.55, 61.33, 101.21, 122.97, 123.57, 126.81, 129.00, 129.83, 130.97, 134.35, 134.96, 135.41, 144.18, 146.16, 148.88, 149.18, 153.09, 157.20, 165.78, 192.30, 206.26, 206.52; HRMS (ESI) calcd. for $C_{28}H_{30}O_8N_7$ $[M + 1]^+$: 592.2150; Found: 592.2158.

4.1.13. Ethyl 3-aminoethyl-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate ***(15)***

N-Boc-amine **13c** (1 mmol) was dissolved in anhydrous dichloromethane (20 mL). Trifluoroacetic acid (50–100 eq) was added slowly at RT. Stirring was continued overnight till consumption of the starting material. The mixture was concentrated in vacuum. The product was purified by preparative TLC using gradient ethyl acetate in hexanes. *Yield:* 0.16 g (45.5%); MP: 124 °C; IR (KBr, cm^{-1}): 3230, 3150, 3068 (NH_2 & NH), 1671 (C═O), 1646 (C═N), 1521, 1351 (NO_2), 1244, 1080 (C—O—C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.16 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 2.29 (s, 3H, C^6—CH_3), 2.95–3.01 (m, 3H, CH_2 & CH), 3.69–3.77 (m, 1H, CH), 3.94–4.09 (m, 2H, $CH_3\underline{CH_2}$), 5.53 (s, 1H, C^4—H), 7.71 (t, J = 8 Hz, 1H, ArH), 7.80 (d, J = 8 Hz, 1H, ArH), 8.08 (s, 2H, NH_2, D_2O-exchangeable), 8.19 (d, J = 8 Hz, 1H, ArH), 8.22 (s, 1H, ArH), 9.73 (s, 1H, NH, D_2O-exchangeable; HRMS (ESI) calcd. for $C_{16}H_{21}O_5N_4$ $[M + 1]^+$: 349.1506; Found: 349.1500.

*4.1.14. Ethyl 6-methyl-4-(3-nitrophenyl)-3-[2-(substituted ureido or thioureido)ethyl]-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(16a-d)***

A mixture of amine **(15)** (1 mmol) and triethanolamine (4 mL) in dichloromethane (10 mL) was stirred at room temperature for 10 min. The appropriate isocyanate or isothiocyanate (1.5 mmol) was added and stirring was continued overnight at room temperature. The mixture was evaporated to dryness under reduced pressure then crystallized from the proper solvent.

*4.1.14.1. Ethyl 6-methyl-4-(3-nitrophenyl)-3-[2-(3-phenylureido)ethyl]-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(16a)**. Yield:* 0.21 g (49%); cryst.solvent: acetone; MP: 216 °C; IR (KBr, cm^{-1}): 3374, 3213, 3110 (NH), 1712, 1679 (C═O), 1643 (C═N), 1536, 1357 (NO_2), 1245, 1088 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.03 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 2.21 (s, 3H, C^6−CH_3), 2.83–2.90 (m, 1H, CH), 3.13–3.28 (m, 2H, CH_2), 3.60–3.71 (m, 1H, CH), 3.82–3.92 (m, 2H, $CH_3\underline{CH_2}$), 5.48 (s, 1H, C^4−H), 6.21 (s, 1H, NH, D_2O-exchangeable), 6.87 (t, J = 8 Hz, 1H, ArH), 7.18 (t, J = 8 Hz, 2H, ArH), 7.34 (d, J = 4 Hz, 2H, ArH), 7.62 (t, J = 8 Hz, 1H, ArH), 7.72 (d, J = 8 Hz, 1H, ArH), 8.10 (d, J = 8 Hz, 1H, ArH), 8.12 (s, 1H, ArH), 8.53, 9.51 (2 s, 2H, 2NH, D_2O-exchangeable); ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.73, 17.54, 44.68, 59.48, 59.56, 99.41, 117.84, 121.26, 121.54, 122.75, 128.55, 130.31, 133.30, 140.11, 144.83, 147.65, 148.33, 151.80, 155.43, 164.79; HRMS (ESI) calcd. for $C_{23}H_{26}O_6N_5$ $[M + 1]^+$: 468.1878; Found: 468.1865.

*4.1.14.2. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-3-[2-(3-{p-tolyl}ureido) ethyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(16b)**. Yield:* 0.28 g (58%); cryst.solvent: acetone; MP: 214 °C; IR (KBr, cm^{-1}): 3367, 3214, 3091 (NH), 1704, 1689 (C═O), 1635 (C═N), 1531, 1350 (NO_2), 1244, 1073 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.08 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 2.21 (s, 3H, C^6−CH_3), 2.24 (s, 3H, p-tolyl-CH_3), 2.81–2.88 (m, 1H, CH), 3.12–3.26 (m, 2H, CH_2), 3.62–3.68 (m, 1H, CH), 3.88–4.00 (m, 2H, $CH_3\underline{CH_2}$), 5.49 (s, 1H, C^4−H), 6.13 (s, 1H, NH, D_2O-exchangeable), 7.01 (d, J = 8 Hz, 2H, ArH), 7.26 (d, J = 8 Hz, 2H, ArH), 7.66 (t, J = 8 Hz, 1H, ArH), 7.74 (d, J = 8 Hz, 1H, ArH), 8.14–8.15 (m, 2H, ArH), 8.40, 9.59 (2 s, 2H, 2NH, D_2O-exchangeable); HRMS (ESI) calcd. for $C_{24}H_{28}O_6N_5$ $[M + 1]^+$: 482.2034; Found: 482.2043.

*4.1.14.3. Ethyl 3-[2-(3-allylthioureido)ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(16c)**. Yield:* 0.18 g (40.5%); cryst.solvent: acetone (9:1); MP: 177 °C; IR (KBr, cm^{-1}): 3358, 3213, 3078 (NH), 1707, 1675 (C═O), 1640 (C═N), 1523, 1350 (NO_2), 1233, 1089 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.13 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 2.25 (s, 3H, C^6−CH_3), 2.97–3.02 (m, 1H, CH), 3.41–3.54 (m, 2H, CH_2), 3.58–3.65 (m, 1H, CH), 3.93–4.05 (m, 4H, $CH_3\underline{CH_2}$ & $NH\underline{CH_2}CH = CH_2$), 5.04–5.15 (m, 2H, $NHCH_2CH = \underline{CH_2}$), 5.50 (s, 1H, C^4−H), 5.77–5.87 (m, 1H, $NHCH_2\underline{CH} = CH_2$), 7.53 (s, 1H, NH, D_2O-exchangeable), 7.65–7.69 (m, 2H, ArH & NH D_2O-exchangeable), 7.75 (d, J = 8 Hz, 1H, ArH), 8.14–8.16 (m, 2H, ArH), 9.61 (s, 1H, 1NH, D_2O-exchangeable); ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.93, 17.62, 30.65, 41.72, 43.63, 59.45, 59.50, 99.22, 115.32, 121.74, 122.78, 130.31, 133.45, 145.00, 147.65, 148.37, 151.59, 164.67; HRMS (ESI) calcd. for $C_{20}H_{26}O_5N_5S$ $[M + 1]^+$: 448.1649; Found: 448.1640.

*4.1.14.4. Ethyl 6-methyl-4-(3-nitrophenyl)-3-[2-(3-{4-nitrophenyl} thioureido)ethyl]-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(16d)**. Yield:* 0.2 g (38.5%); the product was used as such without further purification; MP: 234 °C; IR (KBr, cm^{-1}): 3343, 3201, 3122 (NH), 1703, 1671 (C═O), 1646 (C═N), 1535, 1328 (NO_2), 1240, 1089 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.10 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 2.26 (s, 3H, C^6−CH_3), 2.81–2.88 (m, 1H, CH), 3.05–3.08 (m, 1H, CH), 3.59–3.74 (m, 3H, CH & CH_2), 3.91–3.98 (m, 2H, $CH_3\underline{CH_2}$), 5.54 (s, 1H, C^4−H), 7.66–7.77 (m, *4*H, ArH), 8.17–8.18 (m, 4H, ArH), 8.37, 9.66, 10.29 (3 s, 3H, 3NH, D_2O-exchangeable); HRMS (ESI) calcd. for $C_{23}H_{25}O_7N_6S$ $[M + 1]^+$: 529.1500; Found: 529.1491.

*4.1.14.5. Ethyl 3-[2-(3-benzhydrylthioureido)ethyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(16e)**. Yield:* 0.2 g (35%); %); cryst.solvent: acetone/DMF (9:1); MP: 206 °C; IR (KBr, cm^{-1}): 3328, 3204, 3090 (NH), 1680 (C═O), 1647 (C═N), 1529, 1345 (NO_2), 1248, 1093 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.10 (t, J = 6 Hz, 3H, $\underline{CH_3}CH_2$), 2.26 (s, 3H, C^6−CH_3), 2.94–3.00 (m, 1H, CH), 3.50–3.57 (m, 2H, CH_2), 3.64–3.68 (m, 1H, CH), 3.90–4.04 (m, 2H, $CH_3\underline{CH_2}$), 5.55 (s, 1H, C^4−H), 6.70 (s, 1H, $\underline{CH}(C_6H_5)_2$), 7.23–7.40 (m, 10*H*, ArH), 7.57 (s, 1H, NH, D_2O-exchangeable), 7.65 (t, J = 8 Hz, 1H, ArH), 7.75 (d, J = 8 Hz, 1H, ArH), 8.15 (d, J = 8 Hz, 1H, ArH), 8.17 (s, 1H, ArH), 8.48, 9.64 (2 s, 2H, 2NH, D_2O-exchangeable); ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.93, 17.65, 43.77, 59.46, 60.54, 64.90, 99.28, 121.75, 122.77, 126.93, 127.21, 127.30, 128.35, 130.27, 133.44, 142.40, 144.97, 147.66, 148.36, 151.66, 164.67; HRMS (ESI) calcd. for $C_{30}H_{32}N_5O_5S$ $[M + 1]^+$: 574.2119; Found: 574.2124.

*4.1.15. General procedure for preparation of compounds **(17a-c)***

The crude intermediate **12** was dissolved in anhydrous dichloromethane (10 mL) and stirred with the appropriate hydrazine (2 eq.) overnight at room temperature. The obtained product was filtered, washed with dichloromethane, dried and used as such without further purification.

*4.1.15.1. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-3-[2-(2-phenylhydrazocarbonyl)ethyl] -1,2,3,4-tetrahydropyrimidine-5-carboxylate **(17a)**. Yield:* 0.23 g (48%); MP: 161 °C; IR (KBr, cm^{-1}): 3279, 3248, 3126 (NH), 1683 (C═O), 1627 (C═N), 1534, 1352 (NO_2), 1239, 1085 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.09 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 2.23–2.35 (m, 4H, C^6−CH_3 and CH), 2.58–2.65 (m, 1H, CH), 2.99–3.05 (m, 1H, CH), 3.73–3.76 (m, 1H, CH), 3.88–4.02 (m, 2H, $CH_3\underline{CH_2}$), 5.53 (s, 1H, C^4−H), 6.63–7.14 (m, 5H, ArH), 7.66–8.16 (m, 4H, ArH), 9.64, 9.73 (2 s, 2H, 2NH, D_2O-exchangeable); ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.86, 17.64, 41.37, 59.38, 59.46, 99.27, 112.00, 118.37, 121.64, 122.79, 128.56, 130.40, 133.40, 144.97, 147.69, 149.05, 151.50, 164.70, 170.10; HRMS (ESI) calcd. for $C_{23}H_{26}O_6N_5$ $[M + 1]^+$: 468.1878; Found: 468.1883.

*4.1.15.2. Ethyl 3-[2-(2,2-diphenylhydrazinecarboxamido)ethyl]-6-methyl-4-(3-nitro phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(17b)**. Yield:* 0.28 g (50%); MP:233 °C; IR (KBr, cm^{-1}): 3418, 3199, 3079 (NH), 1711, 1680 (C═O), 1643 (C═N), 1530, 1347 (NO_2), 1239, 1087 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.12 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 2.25 (s, 3H, C^6−CH_3), 2.86–2.91 (m, 1H, CH), 3.11–3.12 (m, 2H, CH_2), 3.57–3.63 (m, 1H, CH), 3.91–4.06 (m, 2H, $CH_3\underline{CH_2}$), 5.47 (s, 1H, C^4−H), 7.02–7.29 (m, 10*H*, ArH), 7.61–8.21 (m, 4H, ArH), 8.93, 9.61 (2 s, 2H, 2NH, D_2O-exchangeable); ^{13}C NMR (DMSO, 100.63 MHz) δ ppm: 13.93, 17.64, 44.32, 59.46, 99.12, 119.28, 121.67, 122.75, 128.88, 130.32, 133.33, 145.05, 147.03, 147.61, 148.32, 151.71, 164.69; HRMS (ESI) calcd. for $C_{29}H_{31}N_6O_6$ $[M + 1]^+$: 559.2299; Found: 559.2308.

*4.1.15.3. Ethyl 6-methyl-4-(3-nitrophenyl)-3-[2-(2-{4-nitrophenyl} hydrazinecarbox-amido)ethyl]-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(17c)**. Yield:* 0.27 g (50.5%); MP: 146 °C; IR (KBr, cm^{-1}): 3354, 3238, 3087 (NH), 1709, 1681 (C═O), 1639 (C═N), 1528, 1330 (NO_2), 1236, 1085 (C−O−C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.16 (t, J = 8 Hz, 3H, $\underline{CH_3}CH_2$), 2.25 (s, 3H, C^6−CH_3), 2.76–2.91 (m, 1H, CH), 3.13–3.16 (m, 2H, CH_2), 3.60–3.64 (m, 1H, CH), 3.98–4.04 (m, 2H, $CH_3\underline{CH_2}$), 5.47 (s, 1H, C^4−H), 6.77–6.82 (m, 2H, ArH), 7.69–8.15 (m,6H, ArH), 8.33, 8.89, 9.61 (3 s, 3H, 3NH, D_2O-exchangeable); HRMS (ESI) calcd. for $C_{23}H_{26}O_8N_7$ $[M + 1]^+$: 528.1837; Found: 528.1847.

4.2. Calcium channel antagonism

4.2.1. Chemicals

Stock solutions of 10 or 30 mM concentrations of the test compounds were prepared in DMSO. The stock solutions were diluted before the experiments so that the concentration of DMSO was 0.1% or less in the final dilution. Calcium channel currents are not affected by a concentration of 0.1% DMSO or less.

4.2.2. Cell culture and transient transfection

Human embryonic kidney cells (HEK) tsA-201 cells were grown to 80–90% confluence at 37 °C (5% CO2) in Dulbecco's modiEed Eagle's medium (Life Technologies, Grand Island, NY). They were supplemented with 10% (vol/vol) fetal bovine serum (HyClone, Thermo Scientific, Pittsburgh, PA), 200 U/ml penicillin, and 0.2 mg/mL streptomycin (Life Technologies, Grand Island, NY). Cells were then suspended with 0.25% trypsin/ethylenediaminetetraacetic acid and plated onto glass coverslips in 10-cm culture dishes (Corning, Corning, NY) at 10% confluence 6 h before transfection. Calcium channel (5 μg) and green fluorescent protein marker (0.5 μg) DNAs were transfected into the cells with calcium phosphate. For $Ca_V1.2$, the additional $Ca_V\beta1b$ (5 μg) and $Ca_V\alpha2\delta1$ (5 μg) subunits were co-expressed. Cells were then transferred to 30 °C 16–18 h later following transfection and stored for two days before recording.

4.2.3. Electrophysiology

Cells on a glass coverslip were transferred into an external bath solution of 20 mM $BaCl_2$, 1 mM $MgCl_2$, 40 mM TEACl, 65 mM CsCl, 10 mM HEPES, 10 mM glucose, pH 7.4. Borosilicate glass pipettes (Sutter Instrument Co., Novato, CA, 3e5 MU) were filled with internal solution containing 140 mM CsCl, 2.5 mM $CaCl_2$, 1mM $MgCl_2$, 5 mM EGTA, 10 mM HEPES, 2 mM Na-ATP and 0.3 mM Na-GTP, pH 7.3. Whole-cell patch clamp recordings were carried out using an EPC 10 amplifier (HEKA Elektronik, Bellmore, NY) linked to a personal computer equipped with Pulse (V8.65) software (HEKA Elektronik, Bellmore, NY). After seal formation, the membrane beneath the pipette was ruptured and the pipette solution was allowed to dialyze into the cell for 2–5 min before recording. Voltage-dependent currents were leak corrected with an online P/4 subtraction paradigm. Data were recorded at 10 kHz and filtered at 2.9 kHz. T-type calcium currents were produced by depolarization from a holding potential of -110 mV to a test potential of -20 mV. L-type calcium currents were produced by depolarization from a holding potential of -90 mV to a test potential of +20 mV, with an inter-pulse interval of 20 s. The duration of the test pulse typically was 100 ms. For Cav1.2, the average series resistance was 5.69 ± 0.08 MΩ, the mean current density 64.03 ± 3.78 pA/pF, and the mean cell capacitance was 13.14 ± 0.21 pF (n = 114). For Cav3.2, the average series resistance was 5.62 ± 0.08 MΩ, the mean current density 148.60 ± 5.89 pA/pF, and the mean cell capacitance was 13.62 ± 0.20 pF (n = 114).

4.2.4. Data analysis and statistics

Data analysis was carried out using online analysis built-in Pulse software (HEKA Elektronik, Bellmore, NY). All graphs were prepared using GraphPad Prism 5 (GraphPad Software, La Jolla, CA). All data are given as mean values ± standard errors.

4.3. X-ray crystallography

The crystal structure of the hemi-acetone solvate of **17a** was determined using X-ray data collected at 90 K with CuKα radiation (1.54184 Å), on a Bruker Kappa Apex-II DUO diffractometer. $C_{23}H_{25}N_5O_6{\cdot}½\ C_3H_6O$, orthorhombic space group *Pbca*, a = 15.6466(19), b = 38.535(6), c = 16.009(2) Å, Z = 16, D_{calcd} = 1.367 g cm^{-3}. A total of 45,332 data was collected to θ = 59.1°, R = 0.092 for 3609 data with I > 2σ(I) of 6948 unique data and 671 refined parameters. The CIF has been deposited at the Cambridge Crystallographic Data Centre, CCDC 1863224.

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