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Antagonism of L-type Ca^{2+} channels $Ca_V1.3$ and $Ca_V1.2$ by 1,4-dihydropyrimidines and 4*H*-pyrans as dihydropyridine mimics ☆

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ABSTRACT

The L-type calcium channel (LTCC) $Ca_V1.3$ is regarded as a new potential therapeutic target for Parkinson's disease. Calcium influx through $Ca_V1.3$ LTCC during autonomous pacemaking in adult dopaminergic neurons of the *substantia nigra pars compacta* is related to the generation of mitochondrial oxidative stress in animal models. Development of a $Ca_V1.3$ antagonist selective over $Ca_V1.2$ is essential because $Ca_V1.2$ pore-forming subunits are the predominant form of LTCCs and are abundant in the central nervous and cardiovascular systems. We have explored 1,4-dihydropyrimidines and 4*H*-pyrans to identify potent and selective antagonists of $Ca_V1.3$ relative to $Ca_V1.2$ LTCCs. A library of 36 dihydropyridine (DHP)-mimic 1,4-dihydropyrimidines and 4*H*-pyrans was synthesized, and promising chiral compounds were resolved. The antagonism studies of $Ca_V1.3$ and $Ca_V1.2$ LTCCs using DHP mimic compounds showed that dihydropyrimidines and 4*H*-pyrans are effective antagonists of DHPs for $Ca_V1.3$ LTCCs. Some 1,4-dihydropyrimidines are more selective than isradipine for $Ca_V1.3$ over $Ca_V1.2$, shown here by both calcium flux and patch-clamp electrophysiology experiments, where the ratio of antagonism is around 2–3. These results support the hypothesis that the modified hydrogen bonding donor/acceptors in DHP-mimic dihydropyrimidines and 4*H*-pyrans can interact differently with DHP binding sites, but, in addition, the data suggest that the binding sites of DHP in $Ca_V1.3$ and $Ca_V1.2$ LTCCs are very similar.

1. Introduction

L-type voltage-gated calcium channels (LTCCs) are membranous multimeric proteins that underlie important physiological properties,[1] such as cardiac action potential generation, calcium homeostasis, E–C coupling, and hormone secretion. Several isoforms[2] are localized in neuronal, neurosecretory, and skeletal muscle tissue with different pharmacological and biophysical properties. Recently, we reported[3] that an unusual engagement of $Ca_V1.3$ LTCCs adult dopaminergic neurons of the *substantia nigra pars compacta* (SNc) during pacemaking renders SNc neurons vulnerable in animal models of Parkinson's disease (PD). This engagement is decreased by the application of isradipine, a 1,4-dihydropyridine (DHP) antagonist of LTCCs in adult neurons. In the presence of the DHP, SNc dopamine (DA) neurons switch to a calcium-independent form of pacemaking, lowering mitochondrial stress with no obvious loss of function at the cellular or behavioral level. This strategy is neuroprotective in PD animal models[4] and clinical trials conducted in Denmark[5] and United Kingdom[6] showed that by treating hypertension with DHP calcium channel blockers the onset of Parkinson's disease is reduced. However, treatment of presently available LTCC blockers, such as isradipine, diltiazem, and verapamil, for the protection of SNc neurons from Parkinson's disease progression, is limited because $Ca_V1.2$ LTCCs also interact with these molecules.[7] $Ca_V1.3$ and $Ca_V1.2$ are the predominant neuronal LTCC isoforms; $Ca_V1.2$ LTCCs are the major isoform (~90%) and are also found in abundance in cardiac tissue of the sinoatrial node, participating heavily in cardiac pacemaking.[8] Although DHPs hold the potential to ameliorate the PD effect, they also block $Ca_V1.2$ LTCCs and might provide undesirable effects to the cardiovascular system.

The antagonism of $Ca_V1.3$ LTCCs has only recently attracted attention with studies recently reported by us[9,10] and other research groups.[11,7a] As described in our earlier paper,[10] our initial effort focused on DHP analogues to verify the difference between

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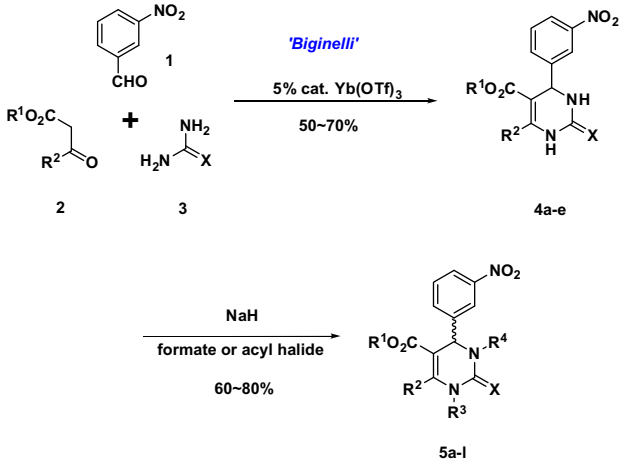
$Ca_V1.3$ and $Ca_V1.2$; many DHPs were synthesized and found to be potent, but all were poorly selective or not selective for $Ca_V1.3$ channels. From this study we confirmed that *ortho*- or *meta*-nitro phenyl substituted DHPs are more potent and slightly selective toward $Ca_V1.3$. It was also found that hydrogen bonding with the nitrogen of 1,4-dihydropyridine is essential to maintain the bioactivity and selectivity toward $Ca_V1.3$ LTCCs. Since the modification of the dihydropyridine ring was limited, our efforts to explore DHPs were shifted to diverse alternative scaffolds, such as 1,4-dihydropyrimidines[12,13] and 4*H*-pyrans (Fig. 1).[14] These scaffolds are known to display similar pharmacological properties to DHP calcium channel blockers, but have potential diverse structural variations. Asymmetric modification of the DHP scaffold to 1,4-dihydropyrimidines or 4*H*-pyrans can provide a chiral center as well as different hydrogen-bonding donor/acceptors at or near the pyrimidine/pyran ring. Therefore, these modifications allow the exploration of the steric and electronic demand on the pyrimidine/pyran ring region for the development of small-molecule inhibitors that can selectively antagonize $Ca_V1.3$ over $Ca_V1.2$ LTCCs. In this study, various 1,4-dihydropyrimidines and 4*H*-pyrans were synthesized and their antagonism toward voltage dependent $Ca_V1.3$ and $Ca_V1.2$ LTCCs were studied.

2. Results

2.1. Chemistry

On the basis of previous DHPs studies and lead compounds isradipine and nitrendipine, we chose 3-nitrophenyl as the aromatic ring. Changes were made to the dihydropyridine scaffold (pyrimidinone, pyrimidinethione, and hydropyran), to each of the carboxylates (R^1, R^2), and to the alkyl side chain (R^3) (Fig. 1); methyl, ethyl, and isopropyl were selected for derivatization of the carboxylates (R^1, R^2) or the alkyl side chain (R^3). With these substitutions, we are able to exam hydrogen-bonding acceptor/donor effects and the influence on its neighboring group, especially related to the steric bulkiness.

The synthesis of pyrimidinones and pyrimidinethiones were similar; they were readily prepared by a Biginelli three-component condensation with an aldehyde, a β-ketoester, and a urea or thiourea (Scheme 1).[15] Because traditional Biginelli reactions, which are catalyzed by HCl or H_2SO_4 in an alcohol solvent, give low yields with possible transesterification at high temperature, we used Lewis acid catalyzed procedures[16] in a non-alcohol solvent. Condensation of 3-nitrobenzaldehyde, alkyl acetoacetate, and urea or thiourea in the presence of Yb(OTf)3 afforded 6-alkyl-5-(alkoxycarbonyl)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one (**4a–c**) or 6-alkyl-5-(alkoxycarbonyl)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-thione (**4d,e**) in good yields. To provide structural similarity with lead molecules isradipine or nitrendipine, N-carbonylation or N-carbamation of Biginelli products **4a–e** was performed with isobutyryl chloride or alkyl chloroformates (e.g., $ClCO_2Et$, $ClCO_2iPr$), in the presence of NaH or TEA, to obtain **5a–l**.



Scheme 1. Synthesis of dihydropyrimidines via Biginelli reaction.

4*H*-Pyrans were also prepared by a three-component condensation with a cyanoacetate, an aldehyde, and a β-ketoester (Scheme 2). Condensation of 3-nitrobenzaldehyde, cyanoacetate, and alkyl acetoacetate in a protic solvent afforded 3,5-diakyl 2-amino-6-methyl-4-(3-nitrophenyl)-4*H*-pyran-3,5-dicarboxylates (**8a–c**) in good yields. N-Acylation of **8a–c** with acetyl chloride or isobutyric anhydride in the presence of TEA provided **9a–c** in excellent yields.

2-Substituted 1,4-dihydropyrimidines[17] were prepared as shown in Scheme 3. The strategy for the synthesis of 2-substituted 1,4-dihydropyrimidines seemed straightforward; instead of urea or thiourea, as used in the previous Biginelli condensation, 2-methyl-2-thiopseudourea was chosen to provide the desired products. However, the acid catalyzed Biginelli three-component condensation was slow and led to several side products. Alternatively, condensation of 3-nitrobenzaldehyde with the β-ketoester first to give α-benzylidene-β-keto ester **10**, followed by tandem Michael

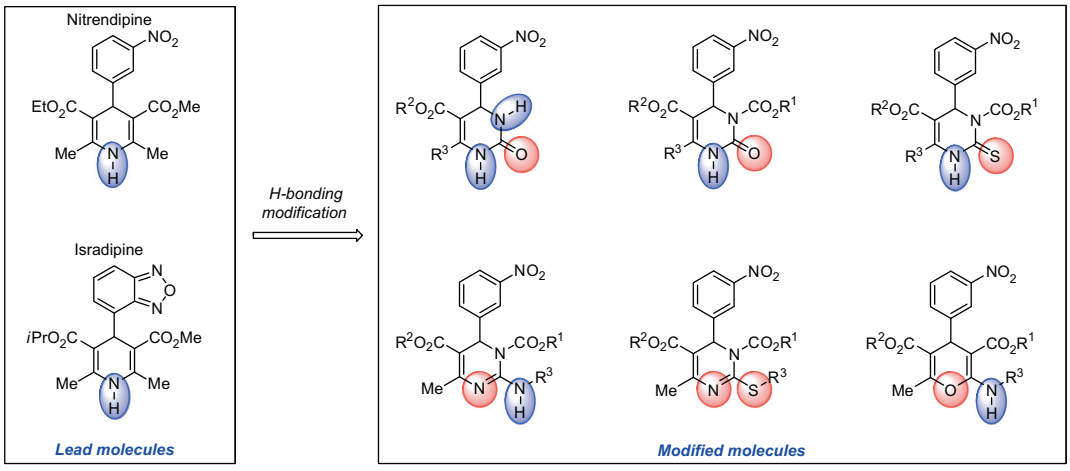
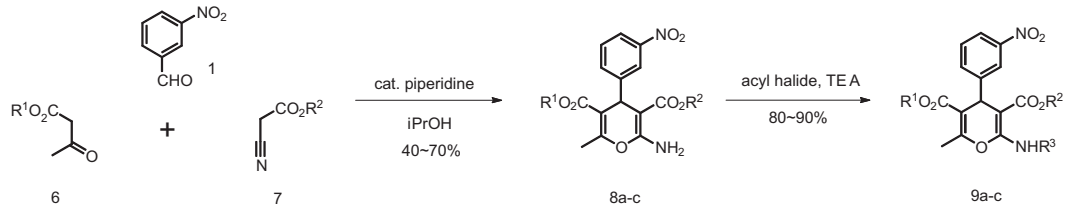
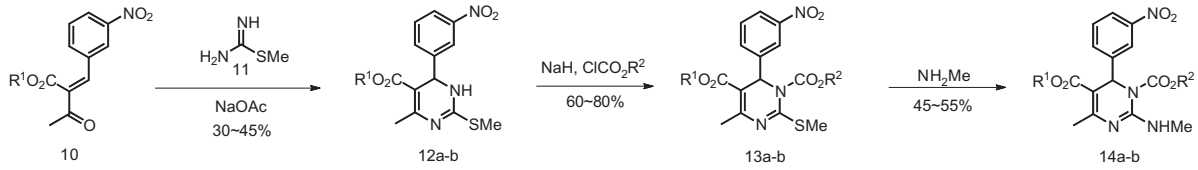


Figure 1. 1,4-Dihydropyrimidines and 4*H*-pyrans that are mimics of DHPs and are synthesized in this study.



Scheme 2. Synthesis of substituted 2-aminopyranes via three component in situ condensation and cyclization.



Scheme 3. Synthesis of substituted dihydropyrimidines via Atwal modified Biginelli reaction.

addition and condensation with 2-methyl-2-thioseudourea sulfate (**11**) in the presence of NaOAc, afforded alkyl 4-methyl-2-(methylthio)-6-(3-nitrophenyl)-1,6-dihydropyrimidine-5-carboxylates (**12a–b**) in moderate yields. Compounds **12a–b** were allowed to react with alkyl chloroformate in the presence of NaH to give **13a–b**. Substitution of the methylthio group with methylamine proceeded well and provided 2-methylamine substituted dihydropyrimidines **14a–b** in moderate yields.

Since each enantiomer of DHPs and dihydropyrimidines is known to have different pharmacological effects on the target calcium channels, three analogues with the best combination of relative potency and selectivity (**5a**, **5b**, **5i**) were chosen, and their racemates were resolved to greater than 97% ee using chiral HPLC,[18] with a Chiralcel OD-H column, to explore chirality-dependent bioactivity.

2.2. Biological results

The biological assay process started by preparing cultures of the two different LTCC-containing HEK293 cells. The $Ca_V1.3$/$Ca_V1.2$ Ca^{2+} channel assays involved subjecting the DHP mimic molecules to a high-throughput screen using a FLIPR™ Tetra (fluorometric imaging plate reader, Molecular Devices) system and a calcium assay kit (Fluo 8). The IC_{50} values for each compound were determined by dose–response curves with 12 concentration points (1 nM–50 μM) in triplicate, to obtain an IC_{50} value and an associated standard deviation (Table 1 through Table 4). The standard deviations associated with the calcium channel FLIPR assay were usually <10%, so we were confident in drawing conclusions from the data. Of the 36 molecules put through the selectivity/potency determination, 28 were found to have selectivity greater than our standard compound isradipine (selectivity is 0.5 in this study), and six molecules (**5b,e,g,i**; (*R*)-**5b**, (*R*)-**5i**) were shown to inhibit $Ca_V1.3$ Ca^{2+} channel with high potency (<1 μM).

To get further biological confirmation of the antagonism of dihydropyrimidines, we performed whole-cell patch-clamp electrophysiology experiments in the voltage-clamp mode on HEK293 cells stably expressing $Ca_V1.2$ and $Ca_V1.3$ LTCCs and applied compound (*R*)-**5i** (Fig. 2a). Barium currents were evoked from use of a voltage step from −80 mV to 0 mV for 100 ms. During this relatively short voltage step, application of (*R*)-**5i** at 1 μM and 10 μM not only significantly inhibited the peak barium current, in keeping with results from our FLIPR calcium assay, but also potently inhibited the end barium current, which is characteristic of DHPs preference for the inactivated state of L-type calcium channels (Fig. 2b,c).

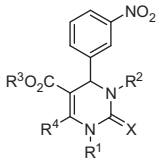
3. Discussion

Various dihydropyrimidine alkyl esters displayed IC_{50} values in the range of 0.2–100 μM at both $Ca_V1.3$ and $Ca_V1.2$ calcium channels (Table 1). Carbamates (R^2) at the N3 positions of the dihydropyrimidine ring appear to increase potency. Comparing the IC_{50} values of **4a–e** (>15 μM) with other carbamates, it is apparent that modification to a carbamate at the N3 nitrogen near the aromatic ring leads to a gain in potency toward both $Ca_V1.3$ and $Ca_V1.2$ (Table 1). It seems that the alkyl carbamates (R^2) and esters (R^3) at C5 are essential for good interactions with the calcium channels; this result is in agreement with the previous SAR of DHPs.[10] When the alkyl ester at C5 was changed from methyl to ethyl and isopropyl, there was little, if any, difference in potency. However, replacement of the methyl group (R^4) to isopropyl on C6, such as in **4c**, **5c**, **5d**, **5h**, and **5l**, made the molecules less potent relative to the methyl analogue. The more bulky steric requirement at this position appears to be important for potency toward both $Ca_V1.3$ and $Ca_V1.2$ LTCCs. However, these various alkyl substitutions seem not to be strongly correlated with $Ca_V1.3$ selectivity. As observed with the N1 acyl derivatives (**5d**, **5e**) in Table 1, acyl substitution of the other nitrogen on the pyrimidinone ring (**5k,l**) did not improve the potency of either calcium channel. Replacement of the oxygen at the C2 position of the dihydropyrimidine ring with a sulfur (X = S) gave an ~5-fold more potent antagonist toward $Ca_V1.3$ for the dihydropyrimidinethiones (compare **5a** to **5b** and **5f** to **5g**). However, these molecules were 1.5- to 3-fold less selective than the dihydropyrimidinones.

2-Methylamino-1*H*-pyran compounds (**8a–9c**) containing esters (R^2, R^3) at both the C3 and C5 positions of the pyran ring exhibited moderate IC_{50} values (0.4–14 μM) for both $Ca_V1.3$ and $Ca_V1.2$ LTCCs, but did not display $Ca_V1.3$ selectivity (Table 2).

Acyl substitution of the 2-amino group of the pyran ring appears to be detrimental to the potency at $Ca_V1.3$ and $Ca_V1.2$. The acylation was more detrimental to the antagonism of $Ca_V1.2$ than to that of $Ca_V1.3$, resulting in a potency loss of ~10-fold for $Ca_V1.2$ and only ~5-fold loss for $Ca_V1.3$ (**9a–c**). These results suggest that the hydrogen bond donor near the pyrimidine ring is critical for strong binding with the target proteins in these series of molecules. This hydrogen bonding dependence is again effective, even after modification of the pyrimidinetriones to substituted thioethers (Table 3. **12a–13b**). These thioether molecules, which do not have hydrogen bond donors in their scaffold, are not potent antagonists. Methylamine substitution (**14a–b**) at the same position of the pyrimidine ring provides a hydrogen bond donor

Table 1
IC_{50} values and the selectivity of antagonism of $Ca_V1.3$ and $Ca_V1.2$ channels by compounds **4a–5l**



No.	R^1	X	R^2	R^3	R^4	IC_{50} (μM) $Ca_V1.3$	IC_{50} (μM) $Ca_V1.2$	Selectivity
Isradipine						0.221	0.107	0.5
4a	H	O	H	Me	Me	18.89 (±0.03)	11.67 (±0.04)	0.6
4b	H	O	H	*i*Pr	Me	46.62 (±0.72)	58.1 (±0.76)	1.3
4c	H	O	H	Me	*i*Pr	>100	>100	
4d	H	S	H	Me	Me	>100	54.97 (±0.73)	
4e	H	S	H	*i*Pr	Me	15.84 (±0.63)	10.7 (±0.59)	0.7
5a	H	O	CO_2Me	*i*Pr	Me	2.96 (±0.14)	2.48 (±0.14)	0.9
5b	H	S	CO_2Me	*i*Pr	Me	0.55 (±0.01)	0.35 (±0.01)	0.6
5c	H	O	CO_2Et	Me	*i*Pr	24.47 (±1.38)	14.84 (±0.64)	0.6
5d	H	O	CO*i*Pr	Me	*i*Pr	>100	56.58 (±5.5)	
5e	H	O	CO*i*Pr	*i*Pr	Me	0.99 (±0.04)	0.50 (±0.05)	0.5
5f	H	O	CO_2*i*Pr	Me	Me	4.60 (±0.5)	2.48 (±0.17)	0.6
5g	H	S	CO_2*i*Pr	Me	Me	0.88 (±0.01)	0.17 (±0.02)	0.2
5h	H	O	CO_2*i*Pr	Me	*i*Pr	10.07 (±0.39)	10.4 (±0.73)	1.0
5i	H	O	CO_2*i*Pr	*i*Pr	Me	0.66 (±0.02)	0.64 (±0.06)	1.0
5j	H	O	CO_2*i*Pr	*i*Pr	*i*Pr	4.31 (±0.43)	1.93 (±0.22)	0.5
5k	CO_2Me	O	CO_2Me	*i*Pr	Me	5.88 (±0.48)	6.13 (±0.37)	1.1
5l	CO_2Et	O	CO_2Et	Me	*i*Pr	13.93 (±0.02)	13.92 (±0.87)	1.0

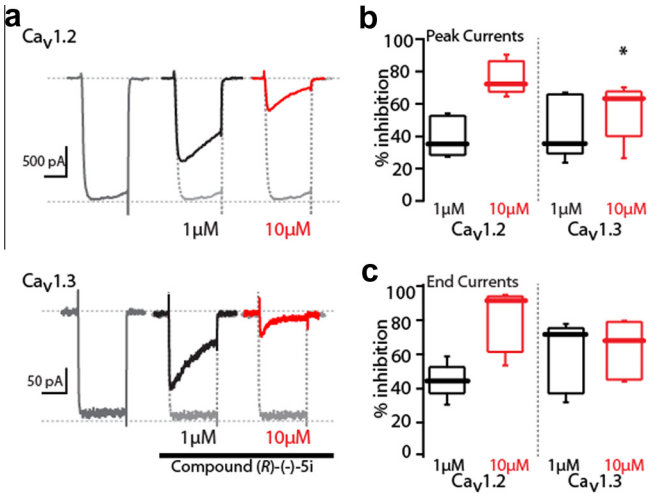
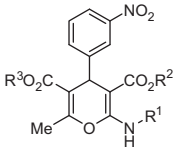


Figure 2. (a) Current traces from the activation of either $Ca_V1.2$ (top) or $Ca_V1.3$ (bottom) stably transfected HEK293 cells with the application of compound (*R*)-**5i** at 1 μM (black) or 10 μM (red). The horizontal bar represents 50 ms. (b) Population data representing the percentage of inhibition of the peak currents (first 50 ms of current trace) resulting from application of compound (*R*)-**5i** at 1 μM (black) or 10 μM (red) on HEK293 cells containing either $Ca_V1.2$ (left: black, $n = 5$, median = 35.3%; red, $n = 5$, median = 72.6%) or $Ca_V1.3$ (right: black, $n = 5$, median = 35%; red, $n = 5$, median = 63.3%) L-type calcium channels, in which the difference of inhibition produced by 10 μM (red) treatment of compound (*R*)-**5i** on $Ca_V1.2$ and $Ca_V1.3$ L-type calcium channels was deemed significant by the Mann–Whitney Rank Sum Test ($^*p < 0.05$). (c) Population data representing the percentage of inhibition of the end currents (last 50 ms of current trace) resulting from application of compound (*R*)-**5i** at 1 μM (black) or 10 μM (red) on HEK293 cells containing either $Ca_V1.2$ (left: black, $n = 5$, median = 44.7%; red, $n = 5$, median = 91.5%) or $Ca_V1.3$ (right: black, $n = 5$, median = 71.8%; red, $n = 5$, median = 68.0%). L-type calcium channels, in which neither treatment group was deemed significant by the Mann–Whitney Rank Sum Test.

and a slight gain in potency toward both $Ca_V1.3$ and $Ca_V1.2$. When methylthio was substituted by a methyl thiocarbonate (**15**), the potency was increased about 5-fold.

Table 2
IC_{50} values and the selectivity of antagonism of $Ca_V1.3$ and $Ca_V1.2$ channels by compounds **8a–9c**



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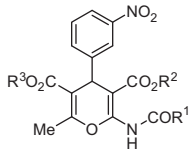

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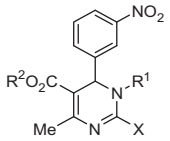
No.	R^1	R^2	R^3	IC_{50} (μM) $Ca_V1.3$	IC_{50} (μM) $Ca_V1.2$	Selectivity
8a	H	Et	Me	2.72 (±0.05)	0.58 (±0.02)	0.2
8b	H	*i*Pr	Me	1.82 (±0.14)	0.41 (±0.06)	0.2
8c	H	*i*Pr	*i*Pr	1.39 (±0.15)	0.86 (±0.03)	0.6
9a	*i*Pr	Et	Me	9.95 (±0.04)	4.23 (±0.16)	0.4
9b	Me	Et	Me	10.03 (±0.52)	4.75 (±0.23)	0.5
9c	Me	*i*Pr	Me	13.62 (±0.17)	6.11 (±0.16)	0.5

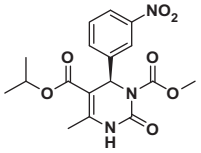
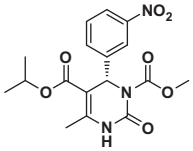
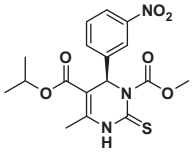
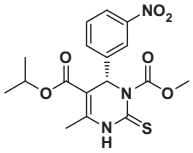
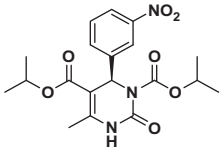
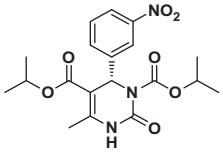
From the optically resolved compounds, it is apparent that the (*R*)-isomers are more potent (>4-fold) and slightly more selective for $Ca_V1.3$ than are the (*S*)-isomers. Compound (*R*)-5i was the most potent analogue in the study (IC_{50} of $Ca_V1.3$ is 0.51 μM) and had some selectivity for $Ca_V1.3$ channels (IC_{50} of $Ca_V1.2$/IC_{50} of $Ca_V1.3 = 2.1$) in our FLIPR calcium assay; the IC_{50} of isradipine was 0.22 μM for $Ca_V1.3$ and 0.107 μM for $Ca_V1.2$. Even though (*R*)-5i is ~2-fold weaker of an antagonist, its $Ca_V1.3$ selectivity is 4-fold greater than that of isradipine in the FLIPR calcium assay. Although the ratio of % inhibition of peak current ($Ca_V1.3$/$Ca_V1.2$) with compound (*R*)-5i in our patch-clamp experiment is 0.99 for 1 μM and 0.87 for 10 μM, this reflects a much improved selectivity than that for DHPs like isradipine (ratio = 0.69 at 0.3 μM).[9] Interestingly, compound (*R*)-**5i** displayed more potent inhibition during the later part of the voltage step. The resulting end currents for both $Ca_V1.2$ and $Ca_V1.3$ LTCCs were inhibited more potently than the peak currents, an action most attributed to the state-dependent block by DHPs.[19]

Table 3
IC_{50} values and the selectivity of antagonism of $Ca_V1.3$ and $Ca_V1.2$ channels by compounds **12a–15**



No.	X	R^1	R^2	IC_{50} (μM) $Ca_V1.3$	IC_{50} (μM) $Ca_V1.2$	Selectivity
12a	SMe	H	Me	10.62 (±0.21)	3.29 (±0.38)	0.3
12b	SMe	H	*i*Pr	1.86 (±0.11)	0.42 (±0.08)	0.2
13a	SMe	CO_2*i*Pr	Me	5.58 (±0.81)	16.13 (±0.09)	3.0
13b	SMe	CO_2Me	*i*Pr	10.67 (±5.95)	5.54 (±3.5)	0.4
14a	NHMe	CO_2*i*Pr	Me	4.22 (±0.3)	1.81 (±0.29)	0.4
14b	NHMe	CO_2Me	*i*Pr	3.25 (±0.01)	2.45 (±0.37)	0.8
15	SCO_2Me	CO_2Me	*i*Pr	1.11 (±0.1)	1.02 (±0.08)	0.9

Table 4
IC_{50} values and the selectivity of antagonism of $Ca_V1.3$ and $Ca_V1.2$ channels by chiral compounds **5a–b** and **5i**

Name	Structure	ee %	IC_{50} (μM) $Ca_V1.3$	IC_{50} (μM) $Ca_V1.2$	Selectivity
(*R*)-(−)-**5a**		>99	1.91 (±0.26)	2.36 (±0.44)	1.2
(*S*)-(+)**5a**		>99	35.10 (±1.81)	21.74 (±0.17)	0.6
(*R*)-(−)-**5b**		98	0.56 (±0.04)	0.50 (±0.05)	0.9
(*S*)-(+)-**5b**		>99	12.8 (±0.43)	9.87 (±0.04)	0.8
(*R*)-(-)-**5i**		97	0.51 (±0.05)	1.02 (±0.08)	2.1
(*S*)-(+)-**5i**		98	1.96 (±0.04)	3.07 (±0.11)	1.6

This activity with compound (*R*)-5bi suggests a generalizable property for DHPs.

4. Conclusions

Recently, we identified the $Ca_V1.3$ LTCC as a new potential therapeutic target for Parkinson's disease. We have explored DHP-mimic analogues in an attempt to identify potent and selective antagonists for the $Ca_V1.3$ channel relative to the $Ca_V1.2$ channel on the basis of the known LTCC antagonists isradipine and nitrendipine. A library of 36 DHP mimic dihydropyrimidines and 4*H*-pyrans was synthesized, and related chiral compounds were resolved. Antagonism studies with $Ca_V1.3$ and $Ca_V1.2$ LTCC using these DHP mimic compounds showed that dihydropyrimidines and 4*H*-pyrans are alternative antagonists to DHPs for $Ca_V1.3$ LTCC; however, these DHP mimics bind almost equally to $Ca_V1.3$ and $Ca_V1.2$. The

ratio percent antagonism ranged from 0.2 to 3. In general, the modified hydrogen bond donor/acceptors in the DHP mimic dihydropyrimidines and 4*H*-pyrans interact similarly with their binding sites. This also suggests that the binding sites for DHPs in $Ca_V1.3$ and $Ca_V1.2$ are very similar.

5. Experimental section

5.1. Bioactivity assay

IC_{50} values were determined using a FLIPR assay we developed and Fluo-8 calcium dye and HEK293 cells stably expressing $Ca_V1.3$ and $Ca_V1.2$ LTCCs. Rat $Ca_V1.3\alpha1D$ (GenBank accession number: AF370010), rat $Ca_{V\beta}3$ (GenBank accession number: M88751), rat $Ca_V\alpha2\delta$-1 (GenBank accession number: AF286488), and rabbit $Ca_V1.2\alpha1C$ (GenBank accession number: P15381) complementary DNAs were used for the construction. The constructs were provided by Dr. Diana Lipscombe (Brown University) and Dr. Johannes Hell (University of Iowa). More specific protocols for transfection of HEK293 cells with $Ca_V1.3$ and $Ca_V1.2$ constructs as well as the FLIPR assay are as previously described in the literature.[9,10]

Whole-cell electrophysiology was conducted as in our previous study.[9] Specifically, after 24–48 h of incubation at 37 °C on poly-D-lysine treated coverslips, stably transfected HEK-293 cells underwent whole-cell patch-clamp electrophysiology. The external solution contained the following (in mM): 140 NaCl, 1 $MgCl_2$, 10 $BaCl_2$, 10 HEPES, 10 dextrose, 10 sucrose, and 20 CsCl at pH 7.4 and an osmolarity at ~320 $mOSml^{-1}$. The test compound stock solutions in DMSO (100 mM or just DMSO) were diluted with the external solution to the desired concentration (10^{-9} to 10^{-3} M), which was perfused (2 mL/min) into the recording chamber while measuring the evoked barium currents. In experiments where concentration–response curves were obtained, local perfusion of the desired concentration was employed. Barium currents were measured from whole-cell voltage patch-clamp recordings using the Pulse 8.4 software data acquisition system (HEKA, Germany). Signals were low-pass filtered at 1 kHz, digitized (sampled) at 10 kHz, and were amplified with an Axopatch 200B patch-clamp amplifier (Axon Instruments). Barium currents were evoked by a depolarizing voltage step from a holding potential of −80 mV to 0 mV for 100 ms at a frequency of 0.05 Hz at room temperature (22–25 °C). Patch pipettes were pulled from thin-wall borosilicate glass coated with dental wax and maintained a resistance of approximately 3–5 mΩ. Internal pipette solutions contained the following (in mM): 180 NMG (*N*-methyl-D-glucosamine), 40 HEPES, 4 $MgCl_2$, 12 phosphocreatine, 0.1 leupeptin, 2 Na_2ATP, 0.5 Na_3GTP, 5 BAPTA, pH 7.2–7.3 and an osmolarity at ~290 $mOSml^{-1}$. Electrophysiological signals were analyzed using Clampfit® 9.2 (Axon Instruments) and IgorPro®6 software.

5.2. General chemistry methods

All starting reagents were purchased from Sigma–Aldrich (Milwaukee, WI). ^{1}H NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer with a direct cryoprobe. Chemical shifts are reported as δ values in parts per million downfield from TMS (δ 0.0) as the internal standard in $CDCl_3$. Electrospray mass spectra were obtained on a Micromass Quattro II spectrometer. Thin-layer chromatography was carried out on E. Merck precoated silica gel 60 F254 plates. An Agilent 971-FP flash purification system with various Superflash 50 silica gel cartridges was used for flash column chromatography. The direct chiral resolutions of racemic **5a**, **5b**, and **5i** were performed using a Chiralcel OD-H HPLC column (Daicel, 250 × 4.6 mm i.d., 5 mm). Hexanes and isopropanol were used as the mobile phases. The operation temperature was 25 °C, and the flow rate was 1 ml/min with 254 nm UV detection.

5.2.1. General procedure for the synthesis of 6-alkyl-5-(alkyloxycarbonyl)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one/thione (4a–e)

A mixture of the 3-nitrobenzaldehyde (1 mmol), β-ketoester (1 mmol), urea or thiourea (1.5 mmol), and $Yb(OTf)_3$ (5 mol %) in toluene (30 mL) was heated at 100 °C overnight. After cooling to room temperature, brine (50 mL) and ethyl acetate (50 mL) were added, and the organic layer was partitioned, dried with $MgSO_4$, and concentrated by vacuum evaporation. The crude product was purified by recrystallization using ethyl acetate and hexanes to give **4a–e** in 50–70% yields.

5.2.1.1. 6-Methyl-5-(methyoxycarbonyl)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one (4a). White powder, mp: 283–285 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.37 (s, 1H), 8.10 (dt, J = 7.8, 1.7 Hz, 1H), 8.05 (t, J = 2.0 Hz, 1H), 7.90 (d, J = 3.5 Hz, 1H), 7.64 (m, 2H), 5.27 (d, J = 3.5 Hz, 1H), 3.51 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.0, 152.3, 150.1, 148.3, 147.1, 133.4, 130.7, 122.8, 121.3, 98.5, 53.8, 51.4, 18.4. MS (ESI): calcd for $C_{13}H_{13}N_3O_5$ $[M+H]^+$, 292.09; found: 292.25.

5.2.1.2. 6-Methyl-5-(isopropyloxycarbonyl)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one (4b). White powder, mp: 206–208 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.19 (t, J = 2.0 Hz, 1H), 8.15 (ddd, J = 8.1, 2.4, 1.1 Hz, 1H), 7.70 (dt, J = 7.8, 1.3 Hz, 1H), 7.65 (s, 1H), 7.52 (t, J = 7.9 Hz, 1H), 5.82 (t, J = 2.6 Hz, 1H), 5.53 (d, J = 3.1 Hz, 1H), 4.97 (hept, J = 6.3 Hz, 1H), 2.39 (s, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.06 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.6, 152.5, 148.3, 146.9, 145.8, 132.9, 129.9, 123.1, 122.0, 100.9, 68.0, 55.4, 22.1, 21.7, 18.9. MS (ESI): calcd for $C_{15}H_{17}N_3O_5$ $[M+H]^+$, 320.12; found: 320.27.

5.2.1.3. 6-Isopropyl-5-(methyoxycarbonyl)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one (4c). White powder, mp: 162–165 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.08–9.02 (m, 1H), 8.10 (dt, J = 5.6, 2.7 Hz, 1H), 8.06 (d, J = 2.1 Hz, 1H), 7.94–7.88 (m, 1H), 7.68–7.58 (m, 2H), 5.25 (d, J = 3.5 Hz, 1H), 4.13 (hept, J = 7.0 Hz, 1H), 3.50 (s, 3H), 1.12 (dd, J = 10.7, 7.0 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.0, 158.4, 152.8, 148.3, 146.9, 133.4, 130.8, 122.9, 121.2, 97.4, 53.5, 51.6, 27.5, 19.6, 19.3. MS (ESI): calcd for $C_{15}H_{17}N_3O_5$ $[M+H]^+$, 320.12; found: 320.34.

5.2.1.4. 6-Methyl-5-(methyoxycarbonyl)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-thione (4d). White powder, mp: 244–247 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.55 (s, 1H), 9.82 (d, J = 3.7 Hz, 1H), 8.17 (dt, J = 7.4, 2.1 Hz, 1H), 8.08 (t, J = 1.9 Hz, 1H), 7.76–7.62 (m, 2H), 5.34 (d, J = 3.2 Hz, 1H), 3.57 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.4, 165.4, 147.9, 146.3, 145.2, 133.0, 130.5, 122.8, 121.0, 99.5, 53.3, 51.3, 17.3. MS (ESI): calcd for $C_{13}H_{13}N_3O_4S$ $[M+H]^+$, 308.07; found: 308.14.

5.2.1.5. 6-Methyl-5-(isopropyloxyarbonyl)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-thione (4e). White powder, mp: 203–205 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.14 (t, J = 2.0 Hz, 1H), 8.01 (dd, J = 8.1, 2.1 Hz, 1H), 7.67 (dt, J = 7.6, 1.3 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 6.77 (s, 1H), 5.14 (m, 1H), 5.08 (s.1H), 4.11 (m, 2H), 2.35 (d, J = 6.0 Hz, 6H), 1.91–1.67 (m, 4H), 1.67–1.46 (m, 4H), 1.25 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.4, 167.1, 150.2, 148.0, 145.4, 145.3, 134.5, 128.7, 123.0, 121.3, 103.1, 102.9, 60.0, 40.0, 32.8, 32.6, 23.7, 23.6, 19.3, 19.2, 14.2. MS (ESI): calcd for $C_{22}H_{26}N_2O_6$ $[M+H]^+$, 415.19; found: 415.42.

5.2.2. General procedure for the synthesis of 1,5-dialkyl 4-alkyl-6-(3-nitrophenyl)-2-oxo/thioxo-2,3-dihydropyrimidine-1,5-(6*H*)-dicarboxylate (5a–l)

A mixture of **4a–e** (1 mmol) and NaH (60% in oil, 1.1 mmol) in THF (30 mL) was treated with alkyl chloroformate (1.1 mmol) or

acyl halide (1.1 mmol) at 0 °C under argon. After stirring 5 h at room temperature, the mixture was diluted with brine (50 mL) and ethyl acetate (50 mL). The organic layer was partitioned, dried with $MgSO_4$, and concentrated by vacuum evaporation. The crude product was purified by flash chromatography using ethyl acetate and hexanes to afford **5a–l** in 60∼80% yields.

5.2.2.1. 1-Methyl 5-isopropyl 4-methyl-6-(3-nitrophenyl)-2-oxo-2,3-dihydropyrimidine-1,5(6*H*)-dicarboxylate (5a).

White powder, Mp: 132–135 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.52 (s, 1H), 8.24 (s, 1H), 8.15 (m, 1H), 7.74 (dt, J = 7.9, 1.3 Hz, 1H), 7.52 (t, J = 8.0 Hz, 1H), 6.38 (s, 1H), 5.08 (hept, J = 6.2 Hz, 1H), 3.91 (s, 3H), 2.42 (s, 3H), 1.31 (d, J = 6.2 Hz, 3H), 1.21 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.2, 153.6, 150.2, 148.3, 146.3, 142.1, 132.9, 129.7, 123.2, 122.0, 104.5, 68.6, 56.2, 54.7, 22.0, 21.8, 18.1. MS (ESI): calcd for $C_{17}H_{19}N_3O_7$ $[M+H]^+$, 378.13; found: 378.27. (*R*)-(−)-**5a** t_r = 11.9 min, (*S*)-(+)-**5a** t_r= 23.0 min [Chiralcel OD-H column, hexanes/IPA 85:15, 1.0 mL/min].

5.2.2.2. 1-Methyl 5-isopropyl 4-methyl-6-(3-nitrophenyl)-2-thioxo-2,3-dihydropyrimidine-1,5(6*H*)-dicarboxylate (5b).

Pale yellow powder, mp: 244–247 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.91 (s, 1H), 8.23 (t, J = 2.1 Hz, 1H), 8.16 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H), 7.74 (m, 1H), 7.53 (t, J = 8.0 Hz, 1H), 6.39 (s, 1H), 5.14 (hept, J = 6.2 Hz, 1H), 3.94 (s, 3H), 2.41 (s, 3H), 1.33 (d, J = 6.3 Hz, 3H), 1.28 (d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 176.3, 164.3, 154.4, 148.4, 143.6, 141.4, 132.5, 129.8, 123.2, 121.7, 107.0, 69.1, 56.2, 54.9, 22.0, 21.9, 17.7. MS (ESI): calcd for $C_{17}H_{19}N_3O_6S$ $[M+H]^+$, 394.11; found: 394.22. (*R*)-(−)-**5b** t_r = 11.4 min, (*S*)-(+)-**5b** t_r = 15.6 min [Chiralcel OD-H column, hexanes/IPA 90:10, 1.0 mL/min].

5.2.2.3. 1-Ethyl 5-methyl 4-isopropyl-6-(3-nitrophenyl)-2-oxo-2,3-dihydropyrimidine-1,5(6*H*)-dicarboxylate (5c).

White powder, mp: 192–194 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.31 (s, 1H), 8.21 (t, J = 2.1 Hz, 1H), 8.16 (ddd, J = 8.2, 2.1, 0.9 Hz, 1H), 7.75 (dt, J = 7.7, 1.2 Hz, 1H), 7.51 (t, J = 7.9 Hz, 1H), 6.43 (s, 1H), 4.37 (q, J = 7.2 Hz, 2H), 4.24 (hept, J = 7.0 Hz, 1H), 3.77 (m, 3H), 1.37 (t, J = 7.1 Hz, 3H), 1.24 (dd, J = 28.6, 7.0 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.0, 155.7, 153.3, 150.1, 148.4, 141.6, 133.2, 129.8, 123.3, 122.0, 102.2, 64.2, 55.3, 51.9, 27.4, 19.6, 19.5, 14.3. MS (ESI): calcd for $C_{18}H_{21}N_3O_7$ $[M+H]^+$, 392.15; found: 392.34.

5.2.2.4. 6-Isopropyl-5-(methyoxycarbonyl)-4-(3-nitrophenyl)-3-isobut -yl-3,4-dihydropyrimidin-2(1*H*)-one (5d).

White powder, mp: 163–165 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.72 (s, 1H), 8.16–8.11 (m, 2H), 7.71 (dd, J = 7.9, 1.5 Hz, 1H), 7.55–7.47 (m, 1H), 6.70 (s, 1H), 4.31 (hept, J = 7.0 Hz, 1H), 3.74 (s, 3H), 3.65 (hept, J = 6.7 Hz, 1H), 1.24 (ddd, J = 25.6, 11.7, 6.9 Hz, 12H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 179.3, 165.0, 155.5, 152.4, 148.4, 141.8, 133.5, 129.8, 123.1, 121.6, 102.8, 52.4, 51.9, 35.3, 27.2, 19.8, 19.7, 19.4, 19.4. MS (ESI): calcd for $C_{19}H_{23}N_3O_6$ $[M+H]^+$, 390.17; found: 390.26.

5.2.2.5. 6-Methyl-5-(isopropyloxycarbonyl)-4-(3-nitrophenyl)-3-isobu -tyl-3,4-dihydropyrimidin-2(1*H*)-one (5e).

White powder, mp: 181–183 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.98 (s, 1H), 8.21 (t, J = 2.0 Hz, 1H), 8.13 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H), 7.72 (dt, J = 7.7, 1.3 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 6.59 (s, 1H), 5.08 (hept, J = 6.3 Hz, 1H), 3.69 (hept, J = 6.7 Hz, 1H), 2.45 (s, 3H), 1.27 (dd, J = 39.1, 6.5 Hz, 6H), 1.21 (dd, J = 6.5, 2.4 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 179.4, 164.2, 152.2, 148.3, 146.1, 142.5, 133.2, 129.6, 123.0, 122.0, 104.9, 68.6, 53.7, 35.6, 22.0, 21.8, 19.8, 19.5, 17.7. MS (ESI): calcd for $C_{19}H_{23}N_3O_6$ $[M+H]^+$, 390.17; found: 390.32.

5.2.2.6. 1-Isopropyl 5-methyl 4-methyl-6-(3-nitrophenyl)-2-oxo-2,3-dihydropyrimidine-1,5(6*H*)-dicarboxylate (5f).

White powder, mp: 135–137 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.11 (s, 1H), 8.24 (t, J = 2.1 Hz, 1H), 8.16 (dd, J = 8.2, 2.1 Hz, 1H), 7.78–7.71 (m, 1H), 7.52 (t, J = 8.0 Hz, 1H), 6.37 (s, 1H), 5.11 (hept, J = 6.2 Hz, 1H), 3.75 (s, 3H), 2.44 (s, 3H), 1.35 (dd, J = 18.7, 6.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.3, 152.3, 150.6, 148.4, 147.2, 142.0, 133.0, 129.8, 123.2, 122.2, 103.6, 72.6, 55.7, 51.9, 21.8, 21.8, 17.9. MS (ESI): calcd for $C_{17}H_{19}N_3O_7$ $[M+H]^+$, 400.11; found: 400.18.

5.2.2.7. 1-Isopropyl 5-methyl 4-methyl-6-(3-nitrophenyl)-2-thioxo-2,3-dihydropyrimidine-1,5(6*H*)-dicarboxylate (5g).

White powder, mp: 205–206 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.02 (s, 1H), 8.21 (t, J = 2.1 Hz, 1H), 8.15 (ddd, J = 8.3, 2.3, 1.1 Hz, 1H), 7.73–7.69 (m, 1H), 7.54 (t, J = 4.0 Hz, 1H), 6.36 (s, 1H), 5.12 (h, J = 6.3 Hz, 1H), 3.79 (s, 3H), 2.43 (s, 3H), 1.37 (dd, J = 22.2, 6.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 176.3, 165.3, 153.4, 148.4, 144.3, 141.3, 132.9, 129.8, 123.3, 121.9, 106.0, 73.7, 55.9, 52.1, 21.9, 21.8, 17.6. MS (ESI): calcd for $C_{17}H_{19}N_3O_6S$ $[M+H]^+$, 394.11; found: 394.06.

5.2.2.8. 1-Isopropyl 5-methyl 4-isopropyl-6-(3-nitrophenyl)-2-oxo-2,3-dihydropyrimidine-1,5(6*H*)-dicarboxylate (5h).

White powder, mp: 186–187 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.78 (s, 1H), 8.23 (t, J = 2.1 Hz, 1H), 8.15 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H), 7.75 (dt, J = 7.9, 1.3 Hz, 1H), 7.52 (t, J = 8.0 Hz, 1H), 6.40 (s, 1H), 5.14 (hept, J = 6.3 Hz, 1H), 4.26 (hept, J = 7.0 Hz, 1H), 3.73 (s, 3H), 1.36 (dd, J = 9.0, 6.3 Hz, 6H), 1.28 (d, J = 7.1 Hz, 3H), 1.22 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.1, 155.9, 152.8, 150.4, 148.4, 141.9, 133.2, 129.8, 123.2, 122.1, 102.1, 72.5, 55.2, 51.9, 27.4, 21.8, 19.5, 19.5. MS (ESI): calcd for $C_{19}H_{23}N_3O_7$ $[M+H]^+$, 428.14; found: 428.26.

5.2.2.9. 1,5-Diisopropyl 4-methyl-6-(3-nitrophenyl)-2-oxo-2,3-dihydro-pyrimidine-1,5(6*H*)-dicarboxylate (5i).

Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.87 (s, 1H), 8.25 (t, J = 2.0 Hz, 1H), 8.15 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H), 7.73 (dt, J = 7.8, 1.3 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 6.33 (s, 1H), 5.08 (dhept, J = 10.1, 6.2 Hz, 2H), 2.42 (s, 3H), 1.34 (dd, J = 18.4, 6.2 Hz, 6H), 1.25 (dd, J = 50.5, 6.2 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.3, 152.3, 150.5, 148.3, 146.4, 142.4, 132.9, 129.7, 123.1, 122.3, 104.3, 72.5, 68.5, 56.0, 22.0, 21.9, 21.8, 21.7, 17.9. MS (ESI): calcd for $C_{19}H_{23}N_3O_7$ $[M+H]^+$, 428.14; found: 428.32. (*R*)-(−)-**5i** t_r = 19.9 min, (*S*)-(+)-**5i** t_r = 22.9 min [Chiralcel OD-H column, hexanes/IPA 95:5, 1.0 mL/min].

5.2.2.10. 1,5-Diisopropyl 4-isopropyl-6-(3-nitrophenyl)-2-oxo-2,3-dihy-dropyrimidine-1,5(6*H*)-dicarboxylate (5j).

White powder, mp: 196–198 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.24 (t, J = 2.0 Hz, 1H), 8.15 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H), 7.98 (s, 1H), 7.73 (dt, J = 7.8, 1.3 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 6.34 (s, 1H), 5.08 (m, 2H), 4.22 (hept, J = 7.0 Hz, 1H), 1.37 (d, J = 6.3 Hz, 3H), 1.34 (d, J = 6.2 Hz, 3H), 1.29 (d, J = 6.2 Hz, 3H), 1.23 (d, J = 7.1 Hz, 3H), 1.21 (d, J = 6.9 Hz, 3H), 1.17 (d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.0, 154.6, 152.6, 150.0, 148.3, 142.3, 133.0, 129.7, 123.1, 122.2, 103.0, 72.5, 68.5, 55.7, 27.3, 22.0, 21.9, 21.8, 21.8, 19.7, 19.6. MS (ESI): calcd for $C_{21}H_{27}N_3O_7$ $[M+H]^+$, 456.17; found: 456.33.

5.2.2.11. 1,3-Dimethoxycarbornyl-5-(isopropyloxycarbonyl)-6-methyl-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one (5k).

Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.28 (t, J = 2.0 Hz, 1H), 8.23–8.14 (m, 1H), 7.75 (dt, J = 7.8, 1.2 Hz, 1H), 7.55 (t, J = 8.0 Hz, 1H), 6.41 (s, 1H), 5.11 (hept, J = 6.3 Hz, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 2.50 (s, 3H), 1.32 (d, J = 6.3 Hz, 3H), 1.23

(d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.5, 153.7, 151.5, 148.5, 147.1, 145.7, 140.4, 132.9, 130.0, 123.5, 122.1, 111.9, 69.4, 55.8, 55.3, 54.9, 21.9, 21.8, 17.4. MS (ESI): calcd for $C_{19}H_{21}N_3O_9$ $[M+H]^+$, 436.14; found: 436.28.

5.2.2.12. 1,3-Diethoxycarbornyl-5-(methoxycarbonyl)-6-methyl-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1H)-one (5l). Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.25 (d, J = 2.0 Hz, 1H), 8.17 (m, 1H), 7.75 (m, 1H), 7.54 (t, J = 8.0 Hz, 1H), 6.45 (s, 1H), 4.42 (q, J = 7.1 Hz, 2H), 4.20 (m, 2H), 3.83 (s, 3H), 3.72 (h, J = 7.1 Hz, 1H), 1.41 (m, 6H), 1.26 (d, J = 7.1 Hz, 3H), 1.20 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.3, 157.6, 153.3, 150.9, 148.4, 147.3, 139.8, 133.1, 129.9, 123.5, 121.7, 116.4, 64.8, 64.7, 55.1, 52.6, 31.5, 21.1, 18.4, 14.2, 13.8. MS (ESI): calcd for $C_{21}H_{25}N_3O_9$ $[M + H]^+$, 464.17; found: 464.33.

5.2.3. General procedure for the synthesis of 3,5-dialkyl 2-amino-6-methyl-4-(3-nitrophenyl)-4*H*-pyran-3,5-dicarboxylate (8a–c)

A mixture of the 3-nitrobenzaldehyde (1 mmol), cyanoacetate (1 mmol), acetoacetate (1 mmol), and catalytic piperidine in isopropyl alcohol (20 mL) was refluxed overnight. The reaction mixture was concentrated by vacuum evaporation, diluted with brine (50 mL) and ethyl acetate (50 mL). The organic layer was partitioned, dried with $MgSO_4$, and concentrated by vacuum evaporation. The crude product was purified by flash chromatography using ethyl acetate and hexanes to afford **8a–c** in 40–70% yields.

5.2.3.1. 3-Ethyl 5-methyl 2-amino-6-methyl-4-(3-nitrophenyl)-4*H*-pyran-3,5-dicarboxylate (8a). White powder, mp: 130–134 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.10 (t, J = 2.0 Hz, 1H), 8.02 (ddd, J = 8.2, 2.3, 1.1 Hz, 1H), 7.56 (dt, J = 7.6, 1.4 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 6.20 (s, 2H), 4.80 (d, J = 1.0 Hz, 1H), 4.05 (qd, J = 7.1, 1.0 Hz, 2H), 3.65 (s, 3H), 2.41 (d, J = 0.9 Hz, 3H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.5, 166.6, 158.4, 158.0, 148.6, 148.0, 134.5, 128.7, 123.4, 121.4, 109.3, 78.9, 59.8, 51.7, 37.6, 18.7, 14.3. MS (ESI): calcd for $C_{17}H_{18}N_2O_7$ $[M+H]^+$, 363.12; found: 363.22

5.2.3.2. 3-Isopropyl 5-methyl 2-amino-6-methyl-4-(3-nitrophenyl)-4*H*-pyran-3,5-dicarboxylate (8b). Yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.11 (t, J = 2.0 Hz, 1H), 8.01 (ddd, J = 8.2, 2.3, 1.1 Hz, 1H), 7.57 (dt, J = 7.7, 1.4 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 6.25 (s, 2H), 4.91 (hept, J = 6.3 Hz, 1H), 4.78 (d, J = 1.1 Hz, 1H), 3.65 (s, 3H), 2.40 (d, J = 1.0 Hz, 3H), 1.27 (d, J = 6.2 Hz, 3H), 0.98 (d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.0, 166.6, 158.3, 158.0, 148.8, 147.8, 134.6, 128.6, 123.5, 121.4, 109.2, 79.0, 67.1, 51.7, 37.7, 22.2, 21.7, 18.7. MS (ESI): calcd for $C_{18}H_{20}N_2O_7$ $[M + H]^+$, 399.12; found: 410.25.

5.2.3.3. 3,5-Diisopropyl 2-amino-6-methyl-4-(3-nitrophenyl)-4*H*-pyra-*n*-3,5-dicarboxylate (8c). Yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.11 (t, J = 2.0 Hz, 1H), 8.01 (ddd, J = 8.1, 2.3, 1.1 Hz, 1H), 7.56 (dt, J = 7.7, 1.4 Hz, 1H), 7.39 (t, J = 7.9 Hz, 1H), 6.25 (s, 2H), 4.92 (dhept, J = 15.7, 6.2 Hz, 2H), 4.75 (d, J = 1.1 Hz, 1H), 2.39 (s, 3H), 1.26 (dd, J = 15.8, 6.2 Hz, 6H), 1.01 (dd, J = 43.9, 6.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.1, 165.6, 158.3, 157.4, 148.9, 147.6, 134.8, 128.5, 123.9, 121.2, 109.4, 78.9, 68.3, 67.0, 37.8, 22.2, 21.9, 21.7, 21.6, 18.6. MS (ESI): calcd for $C_{20}H_{24}N_2O_7$ $[M + H]^+$, 405.17; found: 405.23.

5.2.4. General procedure for the synthesis of 3,5-dialkyl 2-acylamido-6-methyl-4-(3-nitrophenyl)-4*H*-pyran-3,5-dicarboxylate (9a–c)

The acyl halide (1.05 mmol) was added to the solution of **8a–c** (1 mmol) and triethylamine (1.2 mmol) in dichloromethane (30 mL) and stirred for 2 h at room temperature. After the reaction mixture was diluted with brine (50 mL) and dichloromethane (20 mL), the organic layer was partitioned, dried with $MgSO_4$, and concentrated by vacuum evaporation. The crude product was purified by flash chromatography using ethyl acetate and hexanes to afford **9a–c** in 80–90% yields.

5.2.4.1. 3-Ethyl 5-methyl 2-isobutyramido-6-methyl-4-(3-nitrophenyl)-4*H*-pyran-3,5-dicarboxylate (9a). White powder, mp: 142–144 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.95 (s, 1H), 8.14 (t, J = 2.0 Hz, 1H), 8.06 (ddd, J = 8.1, 2.3, 1.1 Hz, 1H), 7.59 (dt, J = 7.7, 1.4 Hz, 1H), 7.44 (t, J = 7.9 Hz, 1H), 4.92–4.88 (m, 1H), 4.13 (qd, J = 7.1, 1.1 Hz, 2H), 3.67 (s, 3H), 2.58 (hept, J = 6.9 Hz, 1H), 2.51 (d, J = 0.8 Hz, 3H), 1.26 (d, J = 6.9 Hz, 6H), 1.23 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.4, 168.1, 166.1, 158.9, 153.7, 148.0, 147.2, 134.5, 129.2, 123.5, 121.9, 108.7, 87.6, 61.1, 51.9, 37.5, 37.5, 19.2, 19.1, 18.7, 14.1. MS (ESI): calcd for $C_{21}H_{24}N_2O_8$ $[M+H]^+$, 433.16; found: 433.2.

5.2.4.2. 3-Isopropyl 5-methyl 2-acetacamido-6-methyl-4-(3-nitrophenyl)-4*H*-pyran-3,5-dicarboxylate (9b). White powder, mp: 105–110 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.77 (s, 1H), 8.12 (t, J = 2.0 Hz, 1H), 8.06 (ddd, J = 8.2, 2.3, 1.1 Hz, 1H), 7.58 (dt, J = 7.7, 1.3 Hz, 1H), 7.44 (t, J = 7.9 Hz, 1H), 4.89 (d, J = 1.0 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.67 (s, 3H), 2.49 (d, J = 0.9 Hz, 3H), 2.24 (s, 3H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.8, 167.5, 166.1, 158.7, 153.0, 148.0, 147.1, 134.4, 129.2, 123.5, 122.0, 108.8, 87.6, 61.2, 51.9, 37.5, 25.6, 18.7, 14.1. MS (ESI): calcd for $C_{19}H_{20}N_2O_8$ $[M + H]^+$, 405.13; found: 405.18.

5.2.4.3. 3,5-Diisopropyl 2-acetamido-6-methyl-4-(3-nitrophenyl)-4*H*-pyran-3,5-dicarboxylate (9c). White powder, mp: 131–134 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.84 (s, 1H), 8.13 (t, J = 2.0 Hz, 1H), 8.06 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H), 7.65–7.53 (m, 1H), 7.44 (t, J = 7.9 Hz, 1H), 5.01–4.89 (m, J = 6.3, 5.8 Hz, 1H), 4.86 (s, 1H), 3.67 (s, 3H), 2.49 (s, 3H), 2.23 (s, 3H), 1.31 (d, J = 6.3 Hz, 3H), 1.02 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.5, 167.3, 166.1, 158.6, 152.9, 147.9, 147.2, 134.5, 129.1, 123.6, 121.9, 108.7, 87.8, 69.0, 51.9, 37.6, 25.6, 22.0, 21.5, 18.7. MS (ESI): calcd for $C_{20}H_{22}N_2O_8$ $[M+H]^+$, 419.15; found: 419.21.

5.2.5. General procedure for the synthesis of 4-(3-nitrophenyl)-1,4-dihydro-6-methyl-2-(methylthio)-5-pyrimidinecarboxylate (12a–b)

A mixture of the 2-[(3-nitrophenyl)methylene]-3-oxobutanoic acid alkyl ester (1 mmol), *S*-methylisothiourea hemisulfate salt (1 mmol), and sodium acetate (2 mmol) in dimethylformamide (10 mL) was stirred at 80 °C overnight. After cooling to room temperature, the mixture was diluted with brine (50 mL) and ethyl acetate (50 mL). The organic layer was partitioned, washed with brine (50 mL), dried with $MgSO_4$, and concentrated by vacuum evaporation. The crude product was purified by flash chromatography using ethyl acetate and hexanes to afford **12a–b** in 30–45% yields.

5.2.5.1. 5-Methyl 4-(3-nitrophenyl)-1,4-dihydro-6-methyl-2-(methylthio)-5-pyrimidinecarboxylate (12a). Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.17 (t, J = 2.0 Hz, 1H), 8.11 (ddd, J = 8.1, 2.3, 1.1 Hz, 1H), 7.70 (dt, J = 7.7, 1.4 Hz, 1H), 7.49 (t, J = 7.9 Hz, 1H), 6.38 (s, 1H), 5.83 (s, 1H), 3.70 (s, 3H), 2.44 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.8, 151.2, 148.4, 146.8, 145.3, 133.4, 129.2, 122.1, 122.0, 99.8, 59.2, 51.3, 19.0, 13.6. MS (ESI): calcd for $C_{14}H_{15}N_3O_4S$ $[M+H]^+$, 322.09; found: 322.15.

5.2.5.2. 5-Isopropyl 4-(3-nitrophenyl)-1,4-dihydro-6-methyl-2-(methylthio)-5-pyrimidinecarboxylate (12b). Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.20 (s, 1H), 8.14–8.08 (m, 1H),

7.69 (dt, J = 7.7, 1.3 Hz, 1H), 7.48 (t, J = 7.9 Hz, 1H), 6.45 (s, 1H), 5.84 (s, 1H), 5.03 (hept, J = 6.3 Hz, 1H), 2.44 (s, 3H), 2.36 (s, 3H), 1.26 (d, J = 6.2 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.8, 151.4, 148.2, 147.1, 144.9, 133.4, 129.1, 122.2, 122.1, 100.4, 67.5, 59.2, 22.1, 21.8, 18.8, 13.5. MS (ESI): calcd for $C_{16}H_{19}N_3O_4S$ $[M+H]^+$, 350.12; found: 350.21.

5.2.6. General procedure for the synthesis of 4-(3-nitrophenyl)-1,4-dihydro-6-methyl-2-(methylthio)-5-pyrimidinedicarboxylate (13a–b)

These molecules were prepared using the procedure for the synthesis of **5a–l**.

5.2.6.1. 3-Isopropyl, 5-methyl 4-(3-nitrophenyl)-1,4-dihydro-6-methyl-2-(methylthio)-5-pyrimidinedicarboxylate (13a).

Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.15 (dtd, J = 4.7, 2.3, 1.0 Hz, 2H), 7.60 (dt, J = 7.7, 1.4 Hz, 1H), 7.55–7.45 (m, 1H), 6.33 (s, 1H), 5.11 (hept, J = 6.3 Hz, 1H), 3.74 (s, 3H), 2.47 (s, 3H), 2.45 (s, 3H), 1.39 (dd, J = 21.4, 6.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.3, 158.7, 153.5, 152.2, 148.4, 141.8, 133.2, 129.7, 123.3, 122.4, 109.0, 72.9, 54.0, 51.8, 21.9, 21.8, 21.8, 15.6. MS (ESI): calcd for $C_{18}H_{21}N_3O_6S$ $[M+H]^+$, 408.12; found: 408.16.

5.2.6.2. 3-Methyl, 5-isopropyl 4-(3-nitrophenyl)-1,4-dihydro-6-methyl-2-(methylthio)-5-pyrimidinedicarboxylate (13b).

Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.15 (m, 2H), 7.61 (dt, J = 7.8, 1.4 Hz, 1H), 7.50 (t, J = 7.8 Hz, 1H), 6.33 (s, 1H), 5.09 (hept, J = 6.2 Hz, 1H), 3.91 (s, 3H), 2.47 (s, 3H), 2.46 (s, 3H), 1.31 (d, J = 6.2 Hz, 3H), 1.21 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.3, 158.0, 153.3, 152.8, 148.3, 141.9, 133.0, 129.7, 123.2, 122.1, 110.0, 68.4, 54.3, 54.3, 22.0, 21.9, 21.7, 15.5. MS (ESI): calcd for $C_{18}H_{21}N_3O_6S$ $[M+H]^+$, 408.12; found: 408.18.

5.2.7. General procedure for the synthesis of dialkyl 4-methyl-2-(methylamino)-6-(3-nitrophenyl)pyrimidine-1,5(6*H*)-dicarboxylate (14a–b)

A mixture of **13a,b** (1 mmol), methylamine HCl salt (10 mmol), and MeOH (5 mL) in a sealed tube was heated at 65 °C overnight. After cooling to room temperature, the mixture was diluted with brine (50 mL) and ethyl acetate (50 mL). The organic layer was partitioned, washed with brine (50 ml), dried with $MgSO_4$, and concentrated by vacuum evaporation. The crude product was purified by flash chromatography using ethyl acetate and hexanes to afford **14a–b** in 45–55% yields.

5.2.7.1. 1-Isopropyl 5-methyl 4-methyl-2-(methylamino)-6-(3-nitrophenyl)pyrimidine-1,5(6*H*)-dicarboxylate (14a).

White powder, mp: 91–93 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.20 (t, J = 2.0 Hz, 1H), 8.14 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H), 7.99 (s, 1H), 7.64 (dt, J = 7.8, 1.3 Hz, 1H), 7.48 (t, J = 8.0 Hz, 1H), 6.30 (s, 1H), 5.06 (hept, J = 6.3 Hz, 1H), 3.70 (s, 3H), 3.02 (d, J = 4.7 Hz, 3H), 2.45 (s, 3H), 1.42 (d, J = 6.2 Hz, 3H), 1.35 (d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.7, 158.2, 153.8, 149.4, 148.2, 143.5, 133.2, 129.7, 123.0, 122.5, 102.9, 72.5, 54.6, 51.2, 28.6, 23.3, 22.0, 21.7. MS (ESI): calcd for $C_{18}H_{22}N_4O_6$ $[M + H]^+$, 391.16; found: 391.11.

5.2.7.2. 5-Isopropyl 1-methyl 4-methyl-2-(methylamino)-6-(3-nitrophenyl)pyrimidine-1,5(6*H*)-dicarboxylate (14b).

Pale yellow powder, mp: 103–105 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.15 (s, 1H), 8.12 (ddd, J = 8.2, 2.3, 1.1 Hz, 1H), 7.80 (bs, 1H), 7.63 (dt, J = 7.7, 1.4 Hz, 1H), 7.47 (t, J = 7.9 Hz, 1H), 6.31 (s, 1H), 5.04 (hept, J = 6.2 Hz, 1H), 3.88 (s, 3H), 3.02 (d, J = 4.3 Hz, 3H), 2.42 (d, J = 0.8 Hz, 3H), 1.29 (d, J = 6.2 Hz, 3H), 1.18 (d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.8, 157.5, 154.6, 148.9, 148.2, 143.5, 132.9, 129.7, 122.9, 122.1, 103.9, 67.4, 54.6, 54.3, 28.7, 23.2, 22.2, 22.0. MS (ESI): calcd for $C_{18}H_{22}N_4O_6$ $[M+H]^+$, 391.16; found: 391.18.

5.2.7.3. 5-isopropyl 1-methyl 2-(acetylthio)-4-methyl-6-(3-nitrophenyl)pyrimidine-1,5(6*H*)-dicarboxylate (15).

Yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.15 (s, 1H), 8.04 (ddd, J = 8.2, 2.2, 1.0 Hz, 1H), 7.64 (ddt, J = 7.8, 1.7, 0.8 Hz, 1H), 7.41 (t, J = 8.0 Hz, 1H), 6.19 (s, 1H), 4.94 (m, 1H), 3.86 (s, 3H), 3.76 (s, 3H), 2.35 (s, 3H), 1.17 (d, J = 6.3 Hz, 3H), 1.06 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.4, 163.7, 154.6, 151.8, 148.4, 142.9, 140.3, 133.2, 129.9, 123.5, 122.3, 110.7, 69.5, 56.4, 55.8, 54.8, 21.9, 21.7, 15.9. MS (ESI): calcd for $C_{19}H_{21}N_3O_8S$ $[M+H]^+$, 452.11; found: 452.21.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.04.054.

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