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Regioselective addition reactions at C-2 of 3,4-dihydropyrimidinones. Synthesis and evaluation of multifunctionalized tetrahydropyrimidines

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ABSTRACT

Multifunctionalized tetrahydropyrimidines derivatives have been synthesized from Biginelli 3,4-dihydropyrimidin-2-(1*H*)-thiones (DHPMs) efficiently. The transformation includes desulfurization of DHPMs with Raney-Ni and subsequent regioselective C-2 functionalization using a variety of *C*-nucleophiles with simultaneous activation with ethyl chloroformate. Functionalized pyrimidine derivatives containing bulky substituents at C-2 of the pyrimidine ring are cytostatic.

1. Introduction

The elaboration of heterocycles represents one of the most vibrant research areas in organic chemistry and has a rich history within the realm of fragment-based drug design.[1] 3,4-Dihydropyrimidin-2-(1*H*)-ones (DHPMs) and their appropriately functionalized derivatives have interesting pharmacological profiles. These are potent antihypertensive agents,[2] mitotic kinesin inhibitors,[3–6] α_{1a}-adrenergic receptor antagonists,[7] or hepatitis B virus replication inhibitors[8] and depict a variety of other biological effects.[9,10] Consequently, synthetic investigations on DHPMs have received extensive attention by both synthetic as well as medicinal chemists. Although a large number of DHPM derivatives have been prepared in a single-pot Biginelli multi component reaction (MCR) and its variants, very useful and convincing structural variability of these interesting heterocycles have been achieved by chemical functionalization of the six positions around the DHPM core.[11] We have also recently reported highly regio- as well as chemoselective functionalization of Biginelli DHPMs at the C-6 methyl[12] position alone or in combination with N-1 (leading to bicyclic products), N-1 and N-3 positions with a wide variety of substituents.[13,14] Likewise, elaboration of the C-4 position of DHPMs through addition of *C*-nucleophiles on the precursor pyrimidinones has been achieved. These reactions proceeded with high regio- and chemoselectivity and without any chemical activation of pyrimidinone.[15–17]

Multifunctionalized pyrimidine derivatives form basic skeleton of a wide range of biologically active molecules and are often procured through multistep routes. Many important pyrimidine derivatives such as Bay 41–4109 **1**, Bay 39-5493 **2** (non-nucleosidic inhibitor of hepatitis B virus),[8] HAP-1 **3**,[18] *N*-cyano-iminopyrimidine **4** (potent antimycotic agent),[19] the hypocholesterolemic agent Rosuvastatin (Ca salt) **5** (HMG-CoA reductase inhibitor)[20] and the potent anticancer drug Gleevec **6** (a tyrosine kinase inhibitor)[21] contain a C-2 functionalized pyrimidine core (Fig. 1). Biginelli DHPMs could be visualized as ideal precursors for obtaining pyrimidine derivatives through simple transformations.

A survey of recent literature revealed different approaches to access pyrimidines from pyrimidinone derivatives.[22,23] For example, pyrimidines bearing a sulfone moiety at C-2, obtained through treatment of 2-thioxopyrimidines with oxone can react with nucleophiles[23] to obtain 2-substituted pyrimidines. Alternatively, a palladium(0)-catalyzed copper(I)-mediated coupling of boronic acids with cyclic thioamides has been used.[24] Kang et al. have reported a two-step procedure for efficient transformation of Biginelli DHPMs to pyrimidines via PyBroP-mediated coupling.[25] Similarly, pyrimidines have been reported via palladium catalyzed Suzuki Sonogashira cross-coupling and Eschenmoser sulfide contraction reactions.[26,27] However, these useful approaches are multistep as well as involve expensive reagents and lack generality in many instances. Regioselective addition reactions at C-2 position

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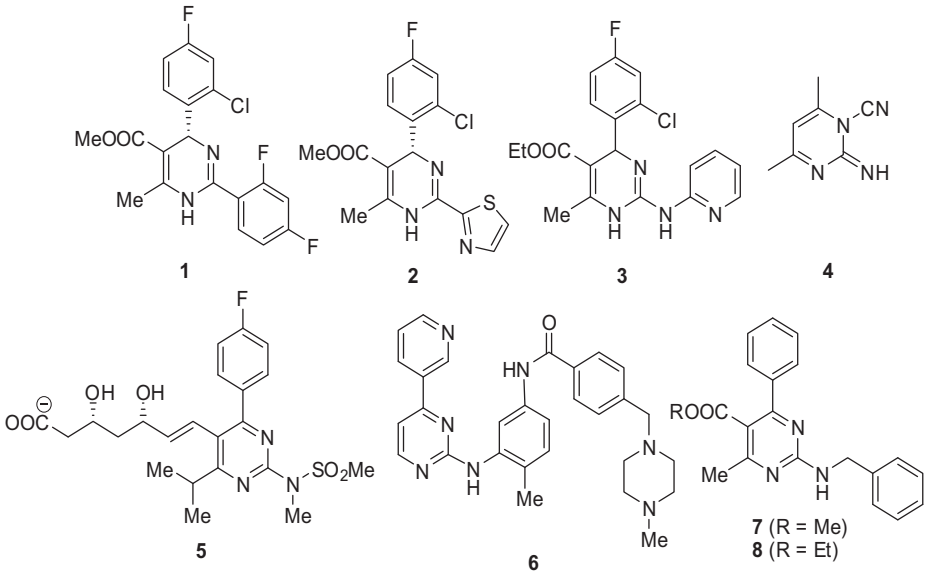
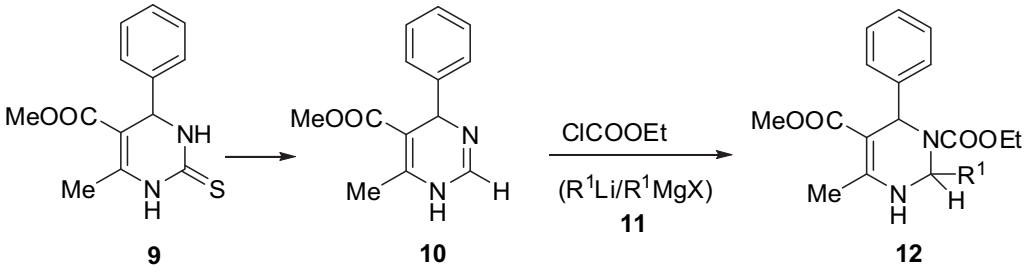


Figure 1. Medicinally potent C-2 functionalized pyrimidine derivatives.

of DHPMs appeared to be an attractive tool for introducing diversity at the C-2 position in order to probe the effect of substitution on the cytostatic activity. Herein, we report a general and practicable protocol for preparing a number of C-2 substituted pyrimidine derivatives and results of their cytostatic activity.

2. Results and discussion

We envisaged that electronegativity of the two nitrogen atoms could invoke considerable electrophilic character to the methine carbon (C-2) flanked by two nitrogen atoms in the 1,4-dihydropyrimidine derivative **10** (Scheme 1), and consequently, under appropriate reaction conditions, addition of nucleophiles **11** may be facilitated, exclusively at C-2 position, with or without any chemical activation of the nitrogen centers. This would allow synthesis of C-2 functionalized pyrimidine derivatives **12** with retention of chemical diversity at other positions. Such addition reactions at C-2 of **10** shall open a novel general route for accessing multifunctionalized pyrimidine derivatives, which are otherwise inaccessible through the conventional approaches. In view of facile desulfurisation[28] of **9**–**10**, using Raney-Ni, synthesis of **12** may be visualized as a single step approach from **10**. Indeed, it has been found that when **10** was treated with a number of carbon nucleophiles, with simultaneous activation of N-3 with ethyl chloroformate (Scheme 1), synthesis of a number of multifunctionalized pyrimidine derivatives **12**, bearing a range of substituents, such as alkyl, aryl, and heterocyclyl at its C-2 position was achieved with high regioselectivity and in a synthetically useful manner (Table 1). The overall reaction thus depicts a new carbon–carbon bond formation at the C-2 position. Representative compounds have also been screened for cytostatic activity. 3,4-Dihydropyrimidin-2-(1*H*)-thione **9** was obtained in quantitative yield through the reaction of methyl acetoacetate, benzaldehyde, and thiourea under acid catalysed conditions of Biginelli condensation reaction.[29] Desulfurisation[28] of **9** was achieved using Raney-Ni, under atmosphere of hydrogen gas and C-2 unsubstituted 5-methoxycarbonyl-6-methyl-4-phenyl-1,4-dihydropyrimidine **10** was obtained in 60% yield. Reaction of **10** with various nucleophilic species, such as carbanion of acetone, thiophene, Grignard reagents of alkyl groups (short, medium, and long



Scheme 1. Synthesis of C-2 elaborated tetrahydropyrimidines through regioselective C-2 addition reactions on 1,4-dihydropyrimidine derivative.

Table 1
Addition of carbon nucleophiles to 5-methoxycarbonyl-6-methyl-4-phenyl-1,4-dihydropyrimidine **10**. Formation of C-2 substituted DHPM derivatives **12** (Scheme 1)

Entry[a]	*C*-Nucleophile	Product	Yield[b] (%)	Mp (°C)
	11 R^1Li/MgX	**12** R^1		
1.	**11a** $LiCH_2COMe$	**12a** CH_2COMe	60	120–121
2.	**11b** 2-Thienyllithium	**12b** Thien-2yl	68	155–156
3.	**11c** MeMgI	**12c** Me	65	135–136
4.	**11d** EtMgBr	**12d** Et	64	128–129
5.	**11e** *n*-PrMgBr	**12e** *n*-Pr	67	120–121
6.	**11f** *n*-BuMgBr	**12f** *n*-Bu	70	96–97
7.	**11g** *n*-$C_5H_{11}MgBr$	**12g** *n*-C_5H_{11}	72	98–99
8.	**11h** *n*-$C_{11}H_{23}MgBr$	**12h** *n*-$C_{11}H_{23}$	62	Liquid
9.	**11i** AllylMgBr	**12i** allyl	70	95–96
10.	**11j** PhMgBr	**12j** Ph	68	140–141

[a] Compound **15** (Scheme 2) was also detected in 10–15% in each case.
[b] Isolated purified yields.

aliphatic chains), allyl and phenyl groups proceeded smoothly and exclusively at the C-2 position in the presence of ethyl chloroformate.

When a solution of carbanion of acetone[30] **11a** (LDA, THF, −78 °C) and ethyl chloroformate were slowly added, simultaneously, to a stirring solution of **10** in anhydrous THF maintained at −5 °C, **12a** was obtained in 60% isolated yield. The structure of **12a** was confirmed through the spectral and physical data (vide experimental). To provide an unambiguous characterization of **12a**, the structure was additionally confirmed from single crystal X-ray analysis (Fig. 2). Interestingly, in this reaction a side product **15** (Scheme 2) was also obtained.

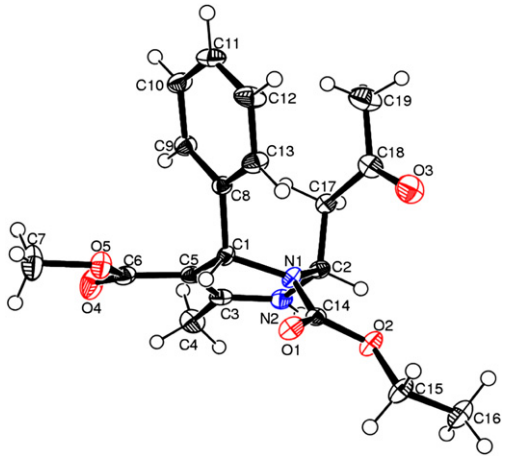
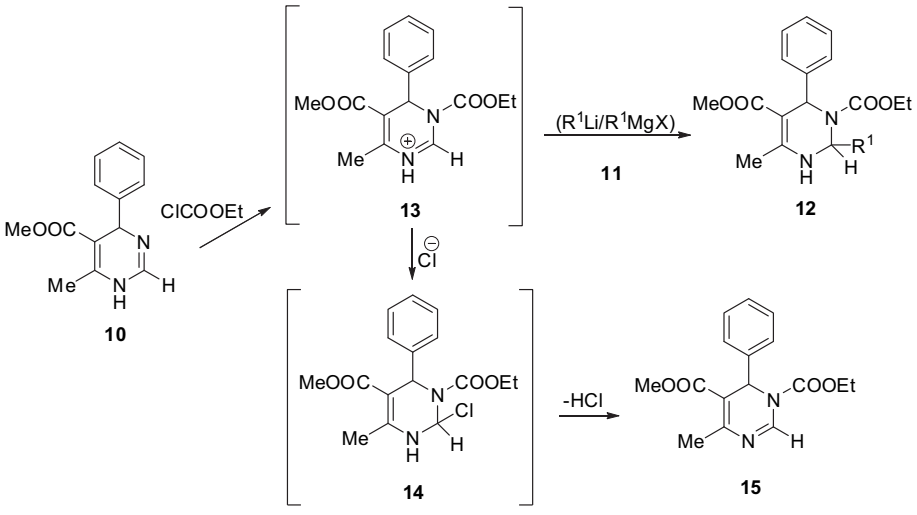


Figure 2. Ortep diagram of **12a** (CCDC-735335) showing stereo view of the molecule and the numbering scheme used in the structure analysis. (N2, N1, C1, C3 positions may be read as N1, N3, C4, and C6, respectively, following IUPAC nomenclature scheme).

C–C bonds. Thus, treatment of **10** with freshly prepared methyl magnesium iodide at −5 °C, under an atmosphere of dry nitrogen gas furnished 3-ethoxycarbonyl-5-methoxycarbonyl-2,6-dimethyl-4-phenyl-1,2,3,4-tetrahydropyrimidine **12c** in 65% yield (Table 1). Other Grignard reagents[32] bearing aliphatic carbon chains of length varying from C_{1-5} and C_{11} reacted with **10**, analogously at −5 °C under an atmosphere of nitrogen to furnish the corresponding pyrimidine derivatives **12d**–**h** (Table 1, entries 4–8) in good yields. Similar reaction with allylmagnesium bromide furnished C-2 addition product **12i** in 70% yield after column chromatography. The reaction of phenylmagnesium bromide with **10** was also very facile and furnished the pyrimidine derivative **12j** in 68% yield. While the deprotection of carbamate from N-3 positions of products **12a**–**j** using $LiAlH_4$ in methanol[17] (stirring at 0 °C to room temperature and then reflux), NaOMe in refluxing methanol[33] or tetrabutyl ammonium fluoride (TBAF) in THF[34] could not be achieved, the current methodology served the purpose of appending a variety of substituents at the C-2 position, regioselectively and in a synthetically useful manner.

The formation of the products **12** and **15** can be visualized to be proceeding through the mechanism shown in Scheme 2. Thus, during the simultaneous addition of ethyl chloroformate as well as pre-generated anion (R^1Li/MgX) to a solution of **10**, the in situ generated intermediate species **13** can undergo reaction with **11** to furnish **12**, as well as with chloride ion, formed as a consequence of the N-3 activation, leading to **14**, which upon dehydrohalogenation may give **15**, which may also arise from **13** through deprotonation. In support of this mode of reaction, when under similar reaction conditions, a solution of **10** was stirred with ethyl chloroformate, **15** was formed in 45% yield.

Representative members[22] of the newly synthesized compounds **12** were evaluated for their inhibitory effect against the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), human T-lymphocyte (CEM), and human cervix carcinoma (HeLa) cells (Table 2). Whereas, pyrimidine derivatives



Scheme 2. Proposed mode of addition reactions of R^1Li/R^1MgX to **10**.

Likewise, red colored anion **11b** formed as a result of lithiation of thiophene[31] using *n*-BuLi at −40 °C upon quenching with **10**, in the presence of ethyl chloroformate resulted in the formation of the corresponding product **12b** (Table 1).

The nucleophilic addition reaction of Grignard reagents to the activated carbon–carbon and carbon–heteroatom double bonds constitutes one of the most versatile methods for the formation of

bearing acetylmethyl, methyl, ethyl and *n*-propyl substituents at the C-2 position (i.e., **12a**, **12c**, **12d**, and **12e**, Table 2) depicted a marginal cytostatic activity of the test compounds (IC_{50}: 56–434 μM), more bulky alkyl groups such as butyl (**12f**) or pentyl (**12g**) rendered a 5- to 10-fold higher antiproliferative activity (IC_{50}: 23–45 μM). The C2-phenyl substituted pyrimidine derivative **12j** showed a higher cytostatic activity against HeLa cells.

Table 2
Inhibitory effect against the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), human T-lymphocyte (CEM) and human cervix carcinoma (HeLa) cells

Compound	IC_{50}[a](μM)			
	L1210	FM3A	CEM	HeLa
12a	330±52	434±93	412±124	210±23
12c	146±130	385±163	169±13	114±30
12d	206±16	168±15	134±59	65±3
12e	128±9	142±11	56±12	98±72
12f	37±4	34±7	45±8	28±2
12 g	37±3	35±3	42±10	23±2
12j	75±7	77±8	76±20	10±18

[a] Inhibitory concentration (50%).

It is also worth noticing that the cytostatic activity of the individual test compounds was independent of the nature of the tumor cell line (either murine vs human, or carcinoma vs leukemia (lymphoma)). It would now be imperative to further explore the C-2 position on the pyrimidine to potentiate the cytostatic activity of the test compounds.

3. Conclusion

In summary, a simple and efficient method for the synthesis of novel multifunctionalized tetrahydropyrimidines from Biginelli dihydropyrimidin-2-(1*H*)-thiones has been devised. The methodology holds potential for the introduction of a number of tailor-made nucleophilic fragments at the C-2 position of DHPMs in a synthetically useful manner. The nature of the C-2 substituent modulates the cytostatic activity in cell culture.

4. Experimental

4.1. General

The solvents: acetone (K_2CO_3), diethyl ether, hexane, and tetrahydrofuran (THF) (Na–benzophenone ketyl), alkyl halides ($CaCl_2$), thiophene (refluxing with Na–metal and then dried over KOH pellets), diisopropylamine (KOH pellets) were adequately dried and drawn under N_2 atmosphere using hypodermic glass syringes. Low boiling reagents were distilled and stored over 4 Å molecular sieves. Freshly prepared *n*-BuLi (2.0–2.3 N in hexanes) standardized using diphenylacetic acid was used. Lithium diisopropyl amide (LDA) was generated from equimolar quantities of diisopropylamine and *n*-BuLi at 0 °C. Grignard reagents were prepared using activated magnesium metal and appropriate alkyl halides in anhydrous diethyl ether. Reactions were run under a blanket of dry nitrogen gas in a sealed (rubber septum, Aldrich) round-bottomed flasks. Organometallic reagents were added using cannula. The low temperature was achieved by using slush of liquid nitrogen with appropriate solvent.

Melting points were determined in open capillaries and are uncorrected. ^{1}H NMR (300 and 400 MHz) spectra were recorded on Jeol FT-AL-300 and Bruker-400 MHz multinuclear spectrometers, using deuterated solvents. All chemical shifts are reported in part per million (δ) relative to tetramethylsilane (TMS, δ 0.0) used as internal reference standard. ^{13}C NMR spectra were recorded at 75 MHz on Jeol FT-AL-300 and 100 MHz on Bruker-400 instruments. EI mass spectra (MS) were recorded on Bruker Daltonics esquire 3000 spectrometer. Elemental analyses were performed on FLASH EA 112 (Thermo Electron Corporation) analyzer and the results are quoted in %. For monitoring the progress of a reaction and for comparison purpose, thin layer chromatography (TLC) was performed on pre-coated aluminium sheets Merck (60 F_{254}, 0.2 mm) using an appropriate solvent system. The chromatograms were visualized under UV light. The compounds were purified using flash chromatography using silica gel (60–120 mesh) and mixtures of ethyl acetate/hexane as eluent.

4.2. Synthesis of 5-methoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2-(1*H*)-thione 9

A solution of methyl acetoacetate (0.35 mol), benzaldehyde (0.35 mol), and thiourea (0.52 mol) in methanol (30–40 mL) containing dilute hydrochloric acid (0.5 mL) was refluxed till the reaction was completed (TLC). The product got directly precipitated in the reaction flask as off-white solid, which was filtered and washed with water (2×50 mL). Recrystallization from ethanol gave the pure 5-methoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2-(1*H*)-thione **9** in 90% yield as an off-white solid; [found: C, 59.51; H, 5.11; N, 10.45; S, 12.11. $C_{13}H_{14}N_2O_2S$ requires C, 59.54; H, 5.34; N, 10.68; S, 12.21%]; R_f (45% EtOAc/hexane) 0.5; mp: 205–206 °C (methanol); δ_H (300 MHz, $CDCl_3$+DMSO-d_6) 9.46 (1H, s, D_2O exchangeable, NH), 8.78 (1H, s, D_2O exchangeable, NH), 7.24–7.41 (5H, m, ArH), 5.35 (1H, d, *J* 2.7 Hz, C4–H), 3.62 (3H, s, ester–CH_3), 2.36 (3H, s, CH_3); δ_C (75 MHz, $CDCl_3$) 174.2, 165.6, 144.1, 142.7, 128.0, 127.2, 126.2, 101.1, 54.6, 50.5, 17.3; *m*/*z* 285 (M+23).

4.3. Desulfurisation of 5-methoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2-(1*H*)-thione 9. Synthesis of C-2 unsubstituted DHPM derivative 10

A solution of compound **9** (5 mmol) in methanol (50 mL) was treated with excess of Raney-Ni (2 g) and refluxed for approximately 6 h under hydrogen atmosphere. After complete desulfurisation (TLC), reaction was filtered through a Celite bed and the solvent was removed under reduced pressure. Crystallization of the crude product from methanol gave the pure C-2 unsubstituted product, i.e., 5-methoxycarbonyl-6-methyl-4-phenyl-1,4-dihydropyrimidine **10** in 60% yield as an off-white solid; [found: C, 67.78; H, 6.01; N, 12.05. $C_{13}H_{14}N_2O_2$ requires C, 67.82; H, 6.08; N, 12.17]; R_f (20% EtOAc/hexane) 0.5; mp: 175–176 °C (DCM); ν_{max} (KBr): 2877, 1687, 1626 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.12–7.33 (5H, m, ArH), 6.91 (1H, s, C2–H), 5.53 (1H, s, C4–H), 3.60 (3H, s, ester–CH_3), 2.25 (3H, s, CH_3); δ_C (75 MHz, $CDCl_3$) 167.2, 144.8, 142.5, 128.4, 127.3, 126.9, 100.0, 57.4, 50.9, 18.9; *m*/*z* 231 (M+1).

4.4. Synthesis of 3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-(2-oxopropyl)-4-phenyl-1,2,3,4-tetrahydropyrimidine 12a and 3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidine 15

To a solution of diisopropylamine (17.39 mmol) in THF (2 mL), *n*-BuLi (17.39 mmol) was added dropwise at −78 °C, under nitrogen atmosphere. The solution was allowed to warm to 0 °C and stirred for an additional 10 min. The solution was cooled to −78 °C and pre-cooled THF (25 mL at −78 °C) was added with stirring. Acetone (17.39 mmol) dissolved in THF (10 mL) was then introduced via hypodermic syringe and the solution stirred for additional 10 min to ensure complete deprotonation to monoanion **11a**. The monoanion **11a** was then introduced with the help of a cannula, to a round-bottomed flask containing a solution of **10** (4.34 mmol) in anhydrous THF (20 mL) with simultaneous addition of ethyl chloroformate (4.34 mmol) at −5 °C. Reaction was warmed to room temperature and stirring was continued to complete the reaction (TLC), after which a saturated aqueous solution of NH_4Cl was introduced. The reaction was extracted with ethyl acetate (3×25 mL) and the extract washed once with brine (100 mL). The extract was dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. Column chromatography of the residue over silica gel (60–120 mesh), using mixtures of hexane and ethyl acetate furnished two products. The product **12a** was isolated in

60% yield as a white solid; [found: C, 63.07; H, 6.83; N, 7.70. $C_{19}H_{24}N_2O_5$ requires C, 63.33; H, 6.66; N, 7.77]; R_f (15% EtOAc/hexane) 0.65; mp: 120–121 °C (DCM); ν_{max} (KBr): 3280, 2971, 1720, 1621 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.23–7.36 (5H m, ArH), 6.42 (1H, br, NH), 5.78 (1H, br, C2–H), 5.29 (1H, br, C4–H), 4.30 (2H, br, ester–CH_2), 3.56 (3H, s, ester–CH_3), 2.40 (3H, s, CH_3), 2.01–2.31 (2H, m, –CH_2), 1.85 (3H, s, –$COCH_3$), 1.29 (3H, br, CH_3); δ_C (100 MHz, $CDCl_3$) 207.67, 167.4, 128.4, 127.5, 127.3, 62.3, 59.4, 50.6, 30.0, 14.7; *m*/*z* 383 (M+23). The product 3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidine **15** was obtained in 15% yield also as white solid; [found C, 63.55; H, 6.35; N, 8.88. $C_{16}H_{18}N_2O_4$ requires C, 63.57; H, 5.96; N, 9.27]; R_f (15% EtOAc/hexane) 0.65; mp: 70–71 °C (DCM); ν_{max} (KBr): 3310, 2956, 1702, 1621 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.03 (1H, s, C2–H), 7.26–7.35 (5H, m, ArH), 6.05 (1H, s, C4–H), 4.25 (2H, m, ester–CH_2), 3.67 (3H, s, ester–CH_3), 2.44 (3H, s, CH_3), 1.30 (3H, t, *J* 7.2 Hz, CH_3); δ_C (100 MHz, $CDCl_3$) 166.2, 151.4, 144.6, 140.5, 128.6, 128.5, 127.6, 111.6, 63.7, 53.3, 51.6, 22.1, 14.2; *m*/*z* 303 (M+1).

*4.4.1. Alternate procedure for **15**.* To a solution of **10** (4.33 mmol) in THF, ethyl chloroformate (4.76 mmol) was added slowly at −5 °C under nitrogen atmosphere. Reaction was warmed to room temperature and stirring was continued to complete the reaction (TLC), after which a saturated aqueous solution of NH_4Cl was introduced. The reaction was extracted with ethyl acetate (3×25 mL) treated in sequence with brine. The extract was dried over anhydrous Na_2SO_4 and the mixture was concentrated under reduced pressure. The corresponding product **15** was isolated after column chromatography using silica gel (60–120 mesh) and mixtures of ethyl acetate and hexane as eluent.

4.5. Synthesis of 3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-(thiophen-2-yl)-4-phenyl-1,2,3,4-tetrahydropyrimidine 12b

To a solution of thiophene (17.39 mmol) in THF, *n*-BuLi (17.39 mmol) was added slowly at −40 °C under nitrogen atmosphere. The temperature of the mixture was held between −30 °C and −20 °C for an hour, and then lowered to −78 °C. Dark red colored anion of thiophene **11b** was added to solution of the electrophile **10** (4.34 mmol) in anhydrous THF (25 mL) with simultaneous addition of ethyl chloroformate (4.34 mmol) at −5 °C. Reaction was warmed to room temperature and stirring was continued to complete the reaction (TLC), after which a saturated aqueous solution of NH_4Cl was introduced. The reaction was extracted with ethyl acetate (3×25 mL) and the extract washed once with brine (100 mL). The extract was dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The corresponding products **12b** (68%) and **15** (14%) were isolated after column chromatography using mixtures of ethyl acetate and hexane as eluent. Compound **12b** was obtained as a white solid; [found: C, 62.07; H, 5.83; N, 7.17. $C_{20}H_{22}N_2O_4S$ requires C, 62.17; H, 5.69; N, 7.25]; R_f (15% EtOAc/hexane) 0.60; mp: 155–156 °C (DCM); ν_{max} (KBr): 3388, 1666, 1607 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.89–7.00 (5H, m, ArH), 6.82 (1H, br, NH), 6.43–6.53 (3H, m, ArH), 6.37 (1H, br, C2–H), 4.99 (1H, br, C4–H), 4.34 (2H, q, *J* 6.9 Hz, ester–CH_2), 3.46 (3H, s, ester–CH_3), 2.51 (3H, s, CH_3), 1.29 (3H, t, *J* 7.2 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 155.6, 144.1, 128.1, 127.7, 127.6, 127.4, 127.2, 126.4, 126.1, 125.3, 124.6, 96.3, 68.9, 63.3, 62.5, 62.2, 50.6, 14.7, 14.3; *m*/*z* 409 (M+23).

4.6. General procedure for the synthesis of pyrimidine derivatives 12c–j

Appropriate Grignard reagent **11c–j** (17.39 mmol) prepared using activated magnesium metal and alkyl/aryl/allyl halide in anhydrous ether, was transferred with the help of cannula to a solution of **10** (4.34 mmol) in anhydrous THF maintained at −5 °C, under nitrogen atmosphere with simultaneous addition of ethyl chloroformate (4.34 mmol). Reaction was warmed to room temperature and stirring was continued to complete the reaction (TLC), after which a saturated aqueous solution of NH_4Cl was introduced. The reaction was extracted with ethyl acetate (3×25 mL) treated with brine. The extract was dried over anhydrous Na_2SO_4 and the mixture was concentrated under reduced pressure. The following products have been isolated after column chromatography.

*4.6.1. 3-Ethoxycarbonyl-5-methoxycarbonyl-2,6-dimethyl-4-phenyl-1,2,3,4-tetrahydropyrimidine (**12c**).* White solid; [found: C, 64.07; H, 6.83; N, 8.70. $C_{17}H_{22}N_2O_4$ requires C, 64.15; H, 6.91; N, 8.80]; R_f (15% EtOAC/hexane) 0.6; mp: 135–136 °C (DCM); ν_{max} (KBr): 3320, 2975, 1705, 1620 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.17–7.60 (5H, m, ArH), 6.40 (1H, s, NH), 5.60 (1H, br, C2–H), 4.54 (1H, br, C4–H), 4.24 (2H, m, ester–CH_2), 3.51 (3H, s, ester–CH_3), 2.41 (3H, s, CH_3), 1.36 (3H, t, *J* 7.2, CH_3), 0.83 (3H, d, *J* 6.6 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 171.4, 160.1, 154.9, 138.3, 128.0, 127.8, 127.4, 126.8, 73.6, 67.2, 60.4, 51.4, 22.7, 14.7,14.3d; *m*/*z* 341 (M+23).

*4.6.2. 3-Ethoxycarbonyl-5-methoxycarbonyl-2-ethyl-6-methyl-4-phenyl-1,2,3,4-tetrahydropyrimidine (**12d**).* White solid; [found: C, 65.07; H, 7.13; N, 8.39. $C_{18}H_{24}N_2O_4$ requires C, 65.06; H, 7.22; N, 8.43]; R_f (15% EtOAC/hexane) 0.6; mp: 128–129 °C (DCM); ν_{max} (KBr): 3318, 2976, 1702, 1621 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.16–7.37 (5H, m, ArH), 6.39 (1H, s, NH), 5.35 (1H, s, C2–H), 4.69 (1H, s, C4–H), 4.26 (2H, q, *J* 7.2 Hz, ester–CH_2), 3.54 (3H, s, ester–CH_3), 2.40 (3H, s, CH_3), 1.36 (3H, br, CH_3), 1.14 (2H, m, CH_2), 0.52 (3H, t, *J* 7.5 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 167.6, 155.6, 127.9, 127.5, 126.8, 103.3, 92.9, 62.2, 51.5, 50.5, 32.3, 14.7, 9.66, 6.79; *m*/*z* 355 (M+23).

*4.6.3. 3-Ethoxycarbonyl-5-methoxycarbonyl-6-methyl-4-phenyl-2-propyl-1,2,3,4-tetrahydropyrimidine (**12e**).* Off-white solid; [found: C, 65.81; H, 7.49; N, 8.01. $C_{19}H_{26}N_2O_4$ requires C, 65.89; H, 7.51; N, 8.09]; R_f (15% EtOAC/hexane) 0.6; mp: 120–121 °C (DCM); ν_{max} (KBr): 3315, 2970, 1702, 1628 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.17–7.35 (5H, m, ArH), 6.37 (1H, br, NH), 5.37 (1H, br, C2–H), 4.70 (1H, br, C4–H), 4.26 (2H, m, ester–CH_2), 3.58 (3H, s, ester–CH_3), 2.40 (3H, s, CH_3), 0.78–1.41 (4H, m, 2×CH_2), 0.49 (3H, t, *J* 6.4 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 167.7, 155.7, 128.0, 127.6, 126.9, 63.4, 62.3, 51.7, 50.6, 38.4, 29.7, 21.5, 18.4, 14.8, 13.3; *m*/*z* 369 (M+23).

*4.6.4. 3-Ethoxycarbonyl-5-methoxycarbonyl-2-butyl-6-methyl-4-phenyl-1,2,3,4-tetrahydropyrimidine (**12f**).* White solid; [found: C, 66.47; H, 7.77; N, 7.69. $C_{20}H_{28}N_2O_4$ requires C, 66.66; H, 7.77; N, 7.77]; R_f (15% EtOAC/hexane) 0.6; mp: 96–97 °C (DCM); ν_{max} (KBr): 3320, 2966, 1700, 1620 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.16–7.37 (5H, m, ArH), 6.37 (1H, s, NH), 5.43 (1H, s, C2–H), 4.72 (1H, s, C4–H), 4.30 (2H, q, *J* 7.5 Hz, ester–CH_2), 3.53 (3H, s, ester–CH_3), 2.40 (3H, s, CH_3), 0.74–1.42 (9H, m, CH_3, and 3×CH_2), 0.59 (3H, t, *J* 6.9 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 167.7, 155.6, 127.9, 127.5, 126.8, 62.2, 50.5, 36.0, 29.6, 27.1, 21.9, 14.7, 13.6; *m*/*z* 383 (M+23).

*4.6.5. 3-Ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-pentyl-4-phenyl-1,2,3,4-tetrahydropyrimidine (**12g**).* White solid; [found: C, 67.21; H, 7.97; N, 7.39. $C_{21}H_{30}N_2O_4$ requires C, 67.37; H, 8.02; N, 7.48]; R_f (15% EtOAC/hexane) 0.6; mp: 98–99 °C (DCM); ν_{max} (KBr): 3316, 2976, 1709, 1621 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.16–7.60 (5H, m, ArH), 6.38 (1H, s, NH), 5.44 (1H, s, C2–H), 4.65 (1H, s, C4–H), 4.27 (2H, q, *J* 6.9 Hz, ester–CH_2), 3.53 (1H, s, CH_3), 2.40 (3H, s, CH_3), 0.78–1.37 (11H, m, CH_3, and 4×CH_2), 0.72 (3H, t, *J* 7.2 Hz, CH_3); δ_C

(75 MHz, $CDCl_3$) 167.7, 155.6, 127.9, 127.5, 126.8, 63.6, 62.1, 50.5, 36.3, 31.0, 24.6, 22.2, 21.3, 14.6, 13.8; *m*/*z* 397 (M+23).

*4.6.6. 3-Ethoxycarbonyl-5-methoxycarbonyl-6-methyl-4-phenyl-2-undecyl-1,2,3,4-tetrahydropyrimidine (**12h**).* Yellow liquid; [found: C, 70.58; H, 9.16; N, 6.06. $C_{27}H_{42}N_2O_4$ requires C, 70.74; H, 9.17; N, 6.11]; R_f (15% EtOAC/hexane) 0.6; ν_{max} (KBr): 3320, 2986, 1710, 1621 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.15–7.37 (5H, m, ArH), 6.36 (1H, s, NH), 5.44 (1H, s, C2–H), 4.71 (1H, s, C4–H), 4.30 (2H, m, ester–CH_2), 3.53 (3H, s, ester–CH_3), 2.40 (3H, s, CH_3), 0.69–1.42 (26H, t, *J* 7.2 Hz, 10×CH_2, and 2×CH_3); δ_C (75 MHz, $CDCl_3$) 167.7, 155.6, 127.9, 127.5, 126.8, 63.5, 62.1, 50.5, 31.8, 29.5, 29.4, 29.3, 29.2, 28.8, 25.0, 22.6, 14.6, 14.0; *m*/*z* 481 (M+23).

*4.6.7. 3-Ethoxycarbonyl-5-methoxycarbonyl-2-allyl-6-methyl-4-phenyl-1,2,3,4-tetrahydropyrimidine (**12i**).* Yellow solid; [found: C, 66.23; H, 6.98; N, 8.06. $C_{19}H_{24}N_2O_4$ requires C, 66.27; H, 6.97; N, 8.13]; R_f (15% EtOAC/hexane) 0.6; mp 95–96 °C (DCM); ν_{max} (KBr): 3310, 2988, 1711, 1640 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.18–7.35 (5H, m, ArH), 5.93 (1H, s, C4–H), 5.68 (1H, m, CH), 5.10 (2H, m, CH_2), 4.68 (2H, m, C4–H, and NH), 4.08 (2H, m, ester–CH_2), 3.61 (3H, s, ester–CH_3), 2.71 (2H, m, CH_2), 2.29 (3H, s, CH_3), 1.14 (3H, t, *J* 7.2 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 167.1, 155.0, 153.1, 144.3, 133.6, 127.8, 127.5, 126.6, 119.0, 99.4, 63.6, 61.4, 56.0, 50.5, 37.9, 21.1, 14.3; *m*/*z* 367 (M+23).

*4.6.8. 3-Ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2,4-diphenyl-1,2,3,4-tetrahydropyrimidine (**12j**).* White solid; [found: C, 69.23; H, 6.28; N, 7.21. $C_{22}H_{24}N_2O_4$ requires C, 69.47; H, 6.31; N, 7.36]; R_f (15% EtOAC/hexane) 0.6; mp 140–141 °C (DCM); ν_{max} (KBr): 3341, 1680 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.18–7.47 (10H, m, ArH), 6.90 (1H, s, NH), 5.83 (1H, m, C2–H), 4.64 (1H, m, C4–H), 3.86–3.99 (2H, m, ester–CH_2), 3.65 (3H, s, ester–CH_3), 2.55 (3H, s, CH_3), 0.93 (3H, t, *J* 7.2 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 152.2, 141.8, 128.6, 128.1, 128.0, 127.6, 127.3, 126.8, 125.3, 66.8, 61.4, 55.7, 50.7, 21.3, 14.0; *m*/*z* 403 (M+23).

4.7. Cytostatic activity assays

Murine leukemia L1210, murine mammary carcinoma FM3A, human T-lymphocyte CEM and human cervix carcinoma HeLa cells were suspended at 300,000–500,000 cells/mL in RPMI-1640 culture medium supplemented with 10% foetal bovine serum and 2 mM L-glutamine, and 100 μl of the cell suspensions were added to 100 μl of an appropriate dilution of the test compounds in 96-well-microtiter plates. After incubation at 37 °C for two (L1210 and FM3A) or three (CEM and HeLa) days, the cell number was determined using a Particle Counter ZI (Coulter, Analis, Ghent, Belgium). The number of the suspension cells could be counted directly; the number of the monolayer HeLa cells was counted after detachment of the cells upon trypsinization. The IC_{50} was defined as the compound concentration required for inhibiting cell proliferation by 50%.

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References and notes

1. Hajduk, P. J.; Greer, J. *Nat. Rev. Drug Discovery* **2007**, *6*, 211–219.
2. Atwal, K. S.; Swanson, B. N.; Unger, S. E.; Floyd, D. M.; Moreland, S.; Hedberg, A.; O'Reilly, B. C. *J. Med. Chem.* **1991**, *34*, 806–811.
3. Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J. *Science* **1999**, *286*, 971–974.
4. Haggarty, S. J.; Mayer, T. U.; Miyamoto, D. T.; Fathi, R.; King, R. W.; Mitchison, T. J.; Schreiber, S. L. *Chem. Biol.* **2000**, *7*, 275–286.
5. Maliga, Z.; Kapoor, T. M.; Mitchison, T. J. *Chem. Biol.* **2002**, *9*, 989–996.
6. DeBonis, S.; Simorre, J. P.; Crevel, I.; Lebeau, L.; Skoufias, D. A.; Blangy, A.; Ebel, C.; Gans, P.; Cross, R.; Hackney, D. D.; Wade, R. H.; Kozielski, F. *Biochemistry* **2003**, *42*, 338–349.
7. Barrow, J. C.; Nantermet, P. G.; Selnick, H. G.; Glass, K. L.; Rittle, K. E.; Gilbert, K. F.; Steele, T. G.; Homnick, C. F.; Freidinger, R. M.; Ransom, R. W.; Kling, P.; Reiss, D.; Broten, T. P.; Schorn, T. W.; Chang, R. S. L.; O'Malley, S.; Olah, T. V.; Ellis, J. D.; Barrish, A.; Kassahun, K.; Leppert, P.; Nagarathnam, D.; Forray, C. *J. Med. Chem.* **2000**, *43*, 2703–2718.
8. Deres, K.; Schroeder, C. H.; Paessens, A.; Goldmann, S.; Hacker, H. J.; Weber, O.; Kramer, T.; Niewoehner, U.; Pleiss, U.; Stoltefuss, J.; Graef, E.; Koletzki, D.; Masantschek, R. N. A.; Reimann, A.; Jaeger, R.; Grob, R.; Beckermann, B.; Schlemmer, K.-H.; Haebich, D.; Ruebsamen-Waigmann, H. *Science* **2003**, *299*, 893–896.
9. Kappe, C. O. *Eur. J. Med. Chem.* **2000**, *35*, 1043–1052.
10. Singh, K.; Arora, D.; Singh, K.; Singh, S. *Mini-Rev. Med. Chem.* **2009**, *99*, 5–106.
11. Dallinger, D.; Kappe, C. O. *Pure Appl. Chem.* **2005**, *77*, 155–161.
12. Singh, K.; Singh, S.; Mahajan, A. *J. Org. Chem.* **2005**, *70*, 6114–6117.
13. Singh, K.; Singh, S. *Tetrahedron Lett.* **2006**, *47*, 8143–8146.
14. Singh, K.; Singh, K. *Tetrahedron* **2009**, *65*, 10395–10399.
15. Singh, K.; Arora, D.; Falkowaski, D.; Liu, Q.; Moreland, R. S. *Eur. J. Org. Chem.* **2009**, 3258–3264.
16. Singh, K.; Arora, D.; Singh, S. *Tetrahedron Lett.* **2007**, *48*, 1349–1352.
17. Singh, K.; Singh, S. *Tetrahedron* **2009**, *65*, 4106–4112.
18. Stray, S. J.; Bourne, C. R.; Punna, S.; Lewis, W. G.; Finn, M. G.; Zlotnick, A. Z. *PNAS* **2005**, *10*, 8138–8143.
19. Kreutzberger, A.; Sellheim, M. *J. Heterocycl. Chem.* **1985**, *22*, 721–723.
20. Watanabe, M.; Koike, H.; Ishiba, T.; Okada, T.; Seo, S.; Hirai, K. *Bioorg. Med. Chem.* **1997**, *5*, 437–444.
21. Capdeville, R.; Buchdunger, E.; Zimmermann, J.; Matter, A. *Nat. Rev. Drug Discovery* **2002**, *1*, 493–502.
22. While screening pyrimidine derivatives for their activity against a broad variety of DNA and RNA viruses (including HIV) in the appropriate cell culture models, we observed that while none of the dihydropyrimidinone derivatives showed appreciable activity against any of the investigated viruses at subtoxic concentrations, C-2 substituted pyrimidine derivatives **7** and **8** proved markedly cytostatic against MDCK cell cultures (IC_{50}: 0.9 μg/mL and 1.2 μg/mL, respectively). Compound **7** was more cytotoxic (MCC: 4–10 μg/mL) to confluent cell cultures (i.e., human embryonic lung cells, feline Crandell kidney cells) than **8** (MCC: ≥100 μg/mL) in these cell cultures. Singh, K.; Arora, D.; Balzarini, J. unpublished results.
23. Matloobi, M.; Kappe, C. O. *J. Comb. Chem.* **2007**, *9*, 275–284.
24. Lenger, A.; Kappe, C. O. *Org. Lett.* **2004**, *6*, 771–774.
25. Kang, F.-A.; Kodah, J.; Guan, Q.; Li, X.; Murray, W. V. *J. Org. Chem.* **2005**, *70*, 1957–1960.
26. Gholap, A. R.; Toti, K. S.; Shirazi, F.; Deshpande, M. V.; Srinivasan, K. V. *Tetrahedron* **2008**, *64*, 10214–10223.
27. Singh, S.; Schober, A.; Gebinoga, M.; Gross, A. G. *Tetrahedron Lett.* **2009**, *50*, 1838–1843.
28. Kashima, C.; Shimizu, M.; Katoh, A.; Omote, Y. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1799–1802.
29. Biginelli, P. *Gazz. Chim. Ital.* **1893**, *23*, 360–416.
30. Singh, K.; Singh, J.; Singh, H. *Tetrahedron* **1998**, *54*, 3567–3574.
31. Jones, E.; Moodie, I. M. *Org. Synth.* **1988**, *Coll. Vol. 6*, 979–980.
32. Hiers, G. S. *Org. Synth.* **1941**, *Coll. Vol. 1*, 550–552.
33. Comins, D. L.; Joseph, S. P.; Goehring, R. R. *J. Am. Chem. Soc.* **1994**, *116*, 4719–4728.
34. Jacquemard, U.; Beneteau, V.; Lefoix, M.; Routeir, S.; Merour, J.-Y.; Coudert, G. *Tetrahedron* **2004**, *60*, 10039–10047.