Tetrahedron 71 (2015) 139–146

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Useful four-carbon synthons en route to monastrol analogs

Amr M. Abdou [a,b], S. Botros [c], Rasha A. Hassan [b,c], Mona M. Kamel [c], Douglass F. Taber [b,*], Azza T. Taher [c]

[a] Department of Microbiology and Immunology, National Research Center, Dokki, Giza, Egypt
[b] Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716, USA
[c] Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 25 September 2014
Received in revised form 5 November 2014
Accepted 6 November 2014
Available online 15 November 2014

Keywords:
Monastrol analogs
Ester exchange
Biginelli
Anti-cancer activity

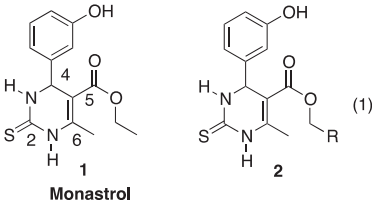
ABSTRACT

A simple protocol has been established for the preparation of a family of crystalline *N*-aryl γ-hydroxycrotonamides, useful four-carbon synthons. These were further elaborated to analogs of monastrol having variant ester sidechains, that were evaluated for their anticancer activity employing the NCI 60 cell line panel.

1. Introduction

Multicomponent reactions (MCRs),[1–5] are of increasing importance in organic and medicinal chemistry because of their simplicity, high atom economy, and bond forming efficiency, allowing the rapid establishment of molecular complexity. Further, MCRs can provide products with the diversity needed for the discovery of new lead compounds or for lead optimization.[6–10] One such MCR is the Biginelli reaction,[11] a procedure developed over a century ago. This is a one pot protocol for the assembly of dihydropyrimidin-2(1H)-ones via the acid-catalyzed cyclocondensation reaction of an aldehyde, a β-ketoester, and urea or thiourea.

Over the past decade, dihydropyrimidin-2(1H)-ones and their derivatives have attracted considerable attention in organic and medicinal chemistry as they display diverse pharmacological and therapeutic properties.[12–14] In particular, monastrol **1** (Eq. 1) is a cell-permeable inhibitor[15] of the mitotic kinesin Eg5, a motor protein responsible for the formation and maintenance of the bipolar spindle in mitotic cells.



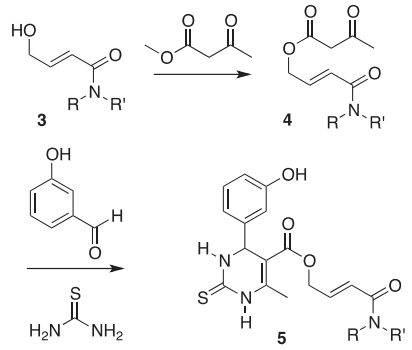
(1) (1)

Monastrol is considered as a lead molecule for the development of new anticancer drugs. Although a relatively weak inhibitor (IC_{50}=14 μM),[15] monastrol has been used as a model compound for the development of more potent analogs. Several publications have described the optimization of monastrol-based dihydropyrimidine analogs.[11c,16–24] To summarize these studies[17,19,22,23] the thiocarbonyl group at position 2, the 3-hydroxyphenyl group at position 4 as well as the methyl group at position 6 are all necessary for high inhibitory effect of the mitotic kinesin Eg5. On the contrary, structural modifications at position 5 did not abolish drug activity and many of those products showed encouraging results.[24]

Given these results, it seemed reasonable to prepare monastrol analogs **2** having variant ester sidechains. To date, no study of such variant esters of monastrol had appeared. We hypothesized that the additional esters should probably be of comparable polarity to monastrol itself. This led us to consider amides such as **3** (Scheme 1), that could be exchanged with acetoacetate to prepare the β-ketoesters **4**, that would in turn participate in the MCR. We adopted this approach when simple alkene metathesis[25] between allyl acetoacetate and an acrylamide derivative gave little of the desired cross product. The nearest literature analogy to this approach was the work of Kaushik,[24] who employed a variety of simple alkyl β-ketoesters in the Biginelli reaction, although not to prepare monastrol analogs.

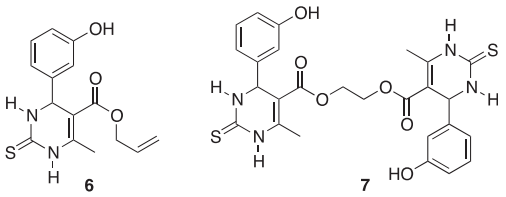
* Corresponding author. E-mail address: taberdf@udel.edu (D.F. Taber).

http://dx.doi.org/10.1016/j.tet.2014.11.022
0040-4020/



Scheme 1. Plan for the synthesis.

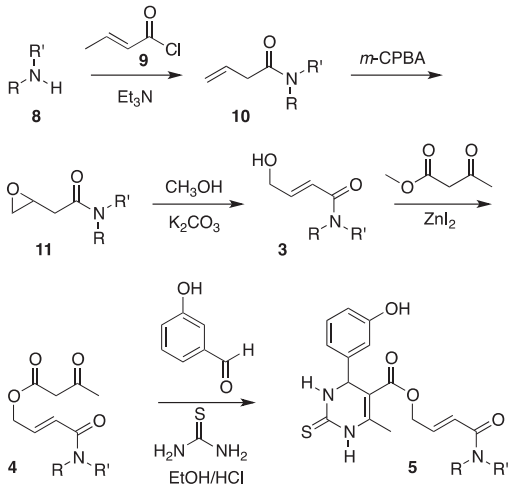
In the course of these studies, we also prepared the simple allyl ester **6** (Eq. 2) as well as the homodimer **7** and evaluated their anticancer properties.



(2)

2. Results and discussion

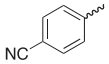
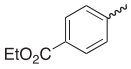
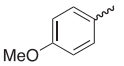
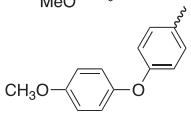
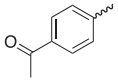
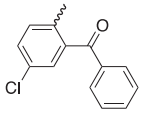
The key to our approach (Scheme 2) was the efficient assembly of the amides represented by structure **3**. We envisioned a three-step protocol beginning with the amine. Acylation could give the β,γ-unsaturated amide. Epoxidation followed by elimination would deliver the desired amides, that could be exchanged with methyl acetoacetate to give the Biginelli precursors **4**.



Scheme 2. Synthesis of monastrol analogs.

While the amides **10** might have been prepared from vinyl acetic acid,[26,27] or by other methods,[28–30] we were intrigued by the single report by Theodoru[31] of the condensation of the inexpensive crotonoyl chloride with tritylamine to prepare the corresponding β,γ-unsaturated amide. We applied this protocol to a series of amines (Table 1) and were pleased to observe that in each case a substantial amount of the β,γ-unsaturated amide was formed. The ratio between the isomers was calculated by integration of the ^{1}H NMR spectrum.[32–34]

Table 1
Reaction of amines with crotonoyl chloride

Entry	R	R′	Ratio[a]
1		H	73:27
2		H	64:36
3		H	72:28
4		H	83:17
5		H	88:12
6		H	68:32
7		H	60:40
8		C_2H_5	74:26
9		H	83:17
10		H	80:20
11		H	50:50

[a] Ratio of β, γ- to α, β- by integration of the ^{1}H NMR spectra of the crude product.

In the next step epoxidation was carried out on the crude product by exposure to *m*-chloroperbenzoic acid (*m*-CPBA) in dichloromethane for 48 h at room temperature.[35] Under these reaction conditions the *N*-substituted-3-butenamide underwent selective epoxidation to yield the *N*-substituted-β,γ-epoxyamide **11**, while the *N*-substituted-2-butenamide remained unreacted.

The mixture of *N*-substituted-β,γ-epoxybutanamide and the unreacted *N*-substituted-2-butenamide was not separated, but rather carried on to the target alcohols **3**.

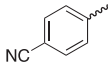
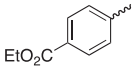
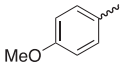
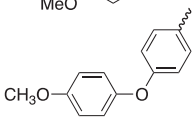
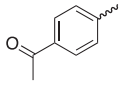
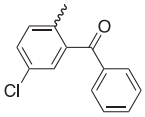
The lithium amide-mediated isomerization of epoxides into allylic alcohols is an attractive approach that has been thoroughly investigated due to its synthetic potential and interesting mechanistic features.[36] In the present investigation, the *N*-substituted-β,γ-epoxybutanamide **11** prepared from aniline was converted into the corresponding 4-hydroxy-2-butenamide **3a** by treatment with lithium diisopropylamide[36] in THF at 0 °C. The product formed

exclusively as the trans isomer as shown by the large coupling constants of the alkene hydrogens (15–17 Hz).

We then sought a more convenient protocol for the elimination. We investigated bases such as anhydrous K_2CO_3, triethylamine and DBU, and solvents including acetone, methanol, dichloromethane, and tetrahydrofuran. Two combinations proved particularly useful for the conversion of **11** into the corresponding alcohol, anhydrous K_2CO_3/methanol, and DBU/methanol, of which the former was more convenient (rt, 1 h). The product was the same as that obtained by the LDA procedure. A similar isomerization of one β,γ-epoxyamide into the corresponding trans allylic alcohol using methoxide in methanol has been reported.[37]

Each of the hydroxy amides so prepared (Table 2) was crystalline, except for **3h** and **3k**. Remarkably, only one such *N*-aryl γ-hydroxycrotonamide had previously been reported, prepared[38] by a less convenient procedure. The highly-functionalized amides of Table 2, readily purified on scale without chromatography, will be useful four-carbon synthons with many other applications.

Table 2
(E)-4-Hydroxy-*N*-substituted-2-butenamide **3a**–**3k**

Entry	R	R′	Compound	Yield[a]	Mp °C
1		H	**3a**	40	156–157
2		H	**3b**	43	165–167
3		H	**3c**	35	139–141
4		H	**3d**	38	178–180
5		H	**3e**	68	134–135
6		H	**3f**	36	157–160
7		H	**3g**	39	168–169
8		C_2H_5	**3h**	60	—
9		H	**3i**	48	134–136
10		H	**3j**	44	137–139
11		H	**3k**	38	—

[a] Isolated yields, three steps from starting amine.

Acid-catalyzed ester exchange had been known for some time.[39] Bader[40] was the first to report the more facile transesterification of β-ketoesters. Since that time, catalysis of this process has been reported using 4-dimethylaminopyridine,[41] DBU,[42] titanium tetraalkoxide,[43] distannoxanes,[44] *p*-toluenesulfonic acid,[45] and Zn metal with a catalytic amount of iodine.[46] The β-ketoesters **4** (Table 3) required for the Biginelli condensation were prepared in good yield by transesterification of methyl acetoacetate with the 4-hydroxy-2-butenamides **3** using the ZnI_2 protocol.[46]

Table 3
Preparation of transesterification products

Entry	Compound	Yield[a]
1	**4a**	61
2	**4b**	67
3	**4c**	62
4	**4d**	51
5	**4e**	56
6	**4f**	57
7	**4g**	75
8	**4h**	62
9	**4i**	61
10	**4j**	55
11	**4k**	55

[a] Isolated yield, based on the starting alcohol.

As we approached the preparation of the Biginelli products, we were concerned as to whether or not the β-keto esters that we had prepared would stand up to the reaction conditions, HCl in ethanol at reflux. In fact, we were able to achieve (Table 4) reasonable preparative yields of the Biginelli products. These polar products were most easily purified by concentration of the reaction mixture followed by direct chromatography of the crude product. To complete the set, esters **6** and **7** (Eq. 2) were prepared from, respectively, allyl acetoacetate and the acetoacetate of ethylene glycol. The Biginelli reaction failed with **4j** and **4k**.

Table 4
Preparation of the Biginelli products

Entry	Compound	Yield[a]
1	**4a**	33
2	**4b**	31
3	**4c**	31
4	**4d**	38
5	**4e**	47
6	**4f**	32
7	**4g**	33
8	**4h**	34
9	**4i**	33

[a] Isolated yields based on the starting β-ketoester.

3. In vitro antitumor screening

While the primary focus of this project was to develop a diversity-oriented approach to Biginelli esters, the products were evaluated against the NCI 60-cell tumor lines.[47–49] The screen was performed with a single dose of 10 μM of each of the synthesized compounds. The screening methodology has been described in detail elsewhere.[50] The data (Supplementary data) were reported as mean graph of the percent growth of the treated cells, and presented as percentage growth inhibition (GI%).

It is clear that the ester of the Biginelli products had a significant effect on activity. Esters **5a**–**5d** and **5h** with less polar phenyl substituents had no significant activity, nor did **6** or **7**. The Biginelli products with more polar substituents, **5e**, **5f**, **5g**, and **5i**, began to show some activity, with **5f** and **5g** being the more noteworthy.

This suggests that such esters have potential for further investigation.

4. Conclusion

We have developed a general strategy for the preparation of monastrol derivatives with variant esters. Although none of the derivatives prepared in the course of this study is active enough in itself to warrant further development, the variation of response from one to another suggests that the ester side chain can modulate the monastrol antineoplastic activity. The four-carbon hydroxy amides **3a**–**3k** prepared in the course of this study, many of which are crystalline, will be of general utility in organic synthesis.

5. Experimental section

5.1. General procedures

^{1}H NMR and ^{13}C NMR spectra were recorded as solutions in deuteriochloroform ($CDCl_3$), deuteriodimethylsulfoxide (DMSO-d_6) or deuterioacetone (CD_3COCD_3) at 400 or 600 and 100 or 150 MHz, respectively. Chemical shifts were given in ppm (δ) using TMS as an internal standard. Coupling constants (J) were given in Hz. ^{13}C multiplicities were determined with the aid of a JVERT pulse sequence, differentiating the signals for methylene and quaternary carbons as 'u' from methyl and methine carbons as 'd'. The infrared (IR) spectra were determined as neat samples. Accurate mass was done using a high resolution time-of-flight mass spectrometer with either electron impact (EI) direct insertion or liquid injection field desorption ionization (LIFDI) with a tolerance of 10.0 ppm. R_f values indicated refer to thin-layer chromatography (TLC) on 2.5×10 cm, 250 μ analytical commercial plates precoated with silica gel GF, developed in the solvent system indicated. The plates were visualized by UV light and staining with cerium ammonium molybdate (CAM) or $KMnO_4$, and the melting points were measured in open capillary tubes and are uncorrected. Toluene and dichloromethane were distilled from calcium hydride under dry nitrogen. MTBE is methyl *tert*-butyl ether and PE is 30–60 petroleum ether. All reactions were stirred magnetically.

The following known compounds were prepared according to the literature procedures:

Ethane-1,2-diyl bis(3-oxobutanoate)[51]
2-Propenyl 3-oxobutanoate[46]
1-Methoxy-4-(4-nitrophenoxy)benzene[52]
4-(4-Methoxyphenoxy)aniline[52]
N-Phenyl-2-propenamide[53]

5.1.1. Preparation of 4-hydroxy-N-substituted-2-butenamides **3a**–**3k**

5.1.1.1. Step 1. Synthesis of N-substituted-3-butenamides 1 (general procedure). A mixture of aromatic amine (1 mmol) in dry CH_2Cl_2 was cooled to 0 °C in an ice bath under nitrogen atmosphere. The mixture was stirred vigorously while triethylamine (4.3 mmol) was added portionwise. Then a solution of crotonoyl chloride (1.45 mmol) in dry CH_2Cl_2 was slowly added dropwise. The reaction mixture was stirred at 0 °C for 30 min. The reaction was quenched by adding 5% aqueous hydrochloric acid. The organic layer was isolated and washed with saturated aqueous $NaHCO_3$ solution and then with water until neutral. The organic extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a mixture of the *N*-substituted-3-butenamide and the *N*-substituted-2-butenamide.

5.1.1.2. Step 2. Epoxidation of N-substituted-3-butenamide (general procedure). The crude mixture of *N*-substituted butenamides (1.0 mmol) in CH_2Cl_2 was cooled to 0 °C in an ice bath. A solution of *m*-chloroperoxybenzoic acid (2.0 mmol) in dichloromethane was added dropwise over 10–15 min. The ice bath was removed and the reaction mixture was left stirring at room temperature for 48 h. The reaction mixture was washed with 5% aqueous NaOH and extracted 3 times with CH_2Cl_2. The organic extracts were combined, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a mixture of *N*-substituted-3,4-epoxybutanamide and unreacted *N*-substituted-2-butenamide.

5.1.1.3. Step 3. Opening and base induced isomerization of epoxide. (*E*)-4-Hydroxy-*N*-phenyl-2-butenamide (**3a**): The crude mixture containing *N*-phenyl-3,4-epoxybutanamide and (*E*)-*N*-phenyl-2-butenamide (1.98 g, 1 equiv) was combined with anhydrous K_2CO_3 (4.60 g, 3.0 equiv) in methanol (30 mL). The reaction was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed using MTBE/CH_2Cl_2 and acetone/CH_2Cl_2 to give a white solid (0.76 g, yield 40% (3 steps from starting amine)); mp 156–157 °C; TLC R_f=0.23 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3335, 3310, 3294, 3136, 1675, 1634, 1549, 1339, 1096; 763; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.18 (t, J=5.2 Hz, 2H), 5.12 (t, J=5.2 Hz, 1H), 6.35 (d, J=15.3 Hz, 1H), 6.88 (dt, J=15.3, 3.6 Hz, 1H), 7.04 (t, J=7.2 Hz, 1H), 7.31 (t, J=7.8 Hz, 2H), 7.67 (d, J=7.8 Hz, 2H), 10.05 (s, 1H); ^{13}C NMR (150 MHz, DMSO) δ u: 164.0, 139.8, 60.8, d: 145.4, 129.2, 123.6, 122.8, 119.6; HRMS calcd for $C_{10}H_{11}NO_2$ (M^+) 177.0790, found 177.0782.

*5.1.1.3.1. (E)-4-Hydroxy-N-(2,4,6-trimethylphenyl)-2-butenamide (**3b**).* White solid, yield 43% (3 steps from starting amine); mp 165–167 °C TLC R_f=0.25 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3251, 2921, 2851, 1635, 1521, 1093, 712; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.08 (s, 6H), 2.23 (s, 3H), 4.15–4.17 (m, 2H), 5.10 (t, J=5.4 Hz, 1H), 6.36 (d, J=15.4 Hz, 1H), 6.80 (dt, J=15.4, 3.6 Hz, 1H), 6.88 (s, 2H), 9.27 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ u: 163.8, 135.7, 135.2, 133.0, 60.7; d: 144.4, 128.7, 122.3, 40.3, 21.0, 18.6; HRMS calcd for $C_{13}H_{18}NO_2$ ($M+H)^+$ 220.1338, found 220.1341.

*5.1.1.3.2. (E)-N-(4-Fluorophenyl)-4-hydroxy-2-butenamide (**3c**).* White solid, yield 35% (3 steps from starting amine); TLC R_f=0.20 (MTBE/CH_2Cl_2, 6:4); mp: 139–141 °C; IR (neat, cm^{-1}) 3298, 3273, 3151, 2913, 1687, 1643, 1509, 1215, 1105, 836; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.18 (ddd, J=5.6, 3.6, 2 Hz, 2H), 5.15 (t, J=5.2 Hz, 1H), 6.32 (dt, J=15.2, 2.0 Hz, 1H), 6.88 (dt, J=15.2, 3.6 Hz, 1H), 7.13–7.17 (m, 2H), 7.66–7.70 (m, 2H), 10.13 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ u:163.8, 158.4 (d, J=240 Hz), 136.2, 136.2, 60.7; d: 145.6, 122.5, 121.25 (d. J=10 Hz), 115.75 (d, J=30 Hz); HRMS calcd for $C_{10}H_{11}FNO_2$ ($M+H)^+$ 196.0774, found 196.0771.

*5.1.1.3.3. (E)-N-(4-Cyanophenyl)-4-hydroxy-2-butenamide (**3d**).* Yellow solid, yield 38% (3 steps from starting amine); mp 178–180 °C; TLC R_f=0.16 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3298, 3273, 3151, 2913, 1687, 1643, 1509, 1215, 1105, 836; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.19–4.21 (m, 2H), 5.19 (t, J=5.2 Hz, 1H), 6.37 (dt, J=15.2, 2.2 Hz, 1H), 6.96 (dt, J=15.2, 3.6 Hz, 1H), 7.78 (d, J=9.2 Hz, 2H), 7.85 (d, J=9.2 Hz, 2H), 10.51 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ u: 164.6, 144.0, 119.6, 105.3, 60.7; d: 147.2, 133.7, 122.0, 119.6; HRMS calcd for $C_{11}H_{11}N_2O_2$ ($M+H)^+$ 203.0821, found 203.0822.

*5.1.1.3.4. (E)-4-Hydroxy-N-(4-ethoxycarbonylphenyl)-2-butenamide (**3e**).* For the preparation of this alcohol, ethanol was used instead of methanol with anhydrous potassium carbonate for the epoxide isomerization to prevent transesterfication. White solid, yield 68% (3 steps from starting amine); mp 134–135 °C; TLC R_f=0.2 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3311, 3116, 2982, 1683, 1597, 1539, 1278, 770; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (t, J=7.2 Hz, 3H), 4.20 (t, J=5.2 Hz, 2H), 4.28 (q, J=7.2 Hz, 2H), 5.18 (t, J=5.2 Hz, 1H), 6.38 (d, J=15.2 Hz, 1H), 6.94 (dt, J=15.2, 3.6 Hz, 1H), 7.81 (d, J=8.8 Hz, 2H), 7.92 (d, J=8.8 Hz, 2H), 10.42 (s, 1H); ^{13}C NMR

(100, MHz, DMSO) δ u: 165.8, 164.4, 144.2, 124.5, 60.9, 60.7; d: 146.6, 130.7, 122.3, 119.0, 14.7; HRMS calcd for $C_{13}H_{15}NO_4$ (M^+) 249.1001, found 249.1001.

*5.1.1.3.5. (E)-4-Hydroxy-N-(4-methoxyphenyl)-2-butenamide (**3f**).* Buff solid, yield 36% (3 steps from starting amine); mp 157–160 °C; TLC R_f=0.25 (acetone/CH_2Cl_2, 1:1); IR (neat, cm^{-1}) 3296, 3132, 2959, 2930, 2837, 1635, 1511, 1500, 1244, 831; ^1H NMR (400 MHz, DMSO-d_6) δ 3.72 (s, 3H), 4.17 (t, *J*=5.2 Hz, 2H), 5.12 (t, *J*=5.2 Hz, 1H), 6.30 (d, *J*=15.2 Hz, 1H), 6.81–6.86 (m, 1H), 6.89 (d, *J*=8.8 Hz, 2H), 7.58 (d, *J*=8.8 Hz, 2H), 9.94 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ u: 163.5, 155.6, 133.0, 60.7; d: 144.8, 122.8, 121.0, 114.3, 55.6; HRMS calcd for $C_{11}H_{13}NO_3$ (M^+) 207.0895, found 207.0897.

*5.1.1.3.6. (E)-4-Hydroxy-N-[4-(4-methoxyphenoxy)phenyl]-2-butenamide (**3g**).* Yellow solid, yield 39% (3 steps from starting amine); mp 168–169 °C; TLC R_f=0.22 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3278, 2837, 1541, 1604, 1501, 1241, 834; ^1H NMR (400 MHz, DMSO-d_6) δ 3.74 (s, 3H), 4.18 (t, *J*=5.2 Hz, 2H), 5.13 (t, *J*=5.2 Hz, 1H), 6.32 (d, *J*=15.2 Hz, 1H), 6.83–6.88 (m, 1H), 6.89–6.95 (m, 6H), 7.64 (d, *J*=8.8 Hz, 2H), 10.06 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ u: 163.7, 155.7, 153.6, 150.6, 135.0, 60.7; d: 145.2, 122.7, 121.2, 120.4, 118.5, 115.4, 55.8; HRMS calcd for $C_{17}H_{17}NO_4$ (M^+) 299.1158, found 299.1173.

*5.1.1.3.7. (E)-4-Hydroxy-N-ethyl-N-phenyl-2-butenamide (**3h**).* Orange oil, yield 60% (3 steps); TLC R_f=0.28 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3396, 3061, 2975, 2933, 2873, 1633, 1615, 1592, 1495, 769, 701; ^1H NMR (400 MHz, $CDCl_3$) δ 1.14 (t, *J*=7.1 Hz, 3H), 2.32 (s, 1H), 3.82 (q, *J*=7.1 Hz, 2H), 4.19 (s, 2H), 5.91 (d, *J*=15.2 Hz, 1H), 6.95 (dt, *J*=15.2, 4.2 Hz, 1H), 7.14 (d, *J*=7.6 Hz, 2H), 7.34 (t, *J*=7.6 Hz, 1H), 7.41 (t, *J*=7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 165.3, 141.7, 62.1, 44.5; d: 144.1, 129.6, 128.4, 127.8, 120.5, 13.0; HRMS calcd for $C_{12}H_{15}NO_2$ (M^+) 205.1103, found 205.1093.

*5.1.1.3.8. (E)-N-(4-Acetylphenyl)-4-hydroxy-2-butenamide (**3i**).* Yellow solid, yield 46% (3 steps from starting amine); mp 134–136 °C; TLC R_f=0.20 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3307, 3109, 2924, 1671, 1594, 1532, 1275, 1179, 837; ^1H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H), 4.19–4.22 (m, 2H), 5.18 (t, *J*=5.4 Hz, 1H), 6.38 (dt, *J*=15.2, 2.0 Hz, 1H), 6.95 (dt, *J*=15.2, 3.4 Hz, 1H), 7.80 (d, *J*=8.6 Hz, 2H), 7.94 (d, *J*=8.6 Hz, 2H), 10.42 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ u: 197.0, 164.4, 144.2, 132.1, 60.8; d: 146.7, 130.0, 122.3, 118.9, 26.9; HRMS calcd for $C_{12}H_{14}NO_3$ $(M+H)^+$ 220.0974, found 220.0965.

*5.1.1.3.9. (E)-N-[4-Chloro-2-(phenylcarbonyl)phenyl]-4-hydroxy-2-butenamide (**3j**).* Canary yellow solid, yield 44% (3 steps from starting amine); mp 137–139 °C; TLC R_f=0.45 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3309, 3101, 2922, 2852, 1683, 1637, 1577, 1508, 1245, 948, 700; ^1H NMR (400 MHz, $CDCl_3$) δ 2.10 (s, 1H), 4.43 (s, 2H), 6.30 (dt, *J*=15.2, 2.0 Hz, 1H), 7.07 (dt, *J*=15.2, 3.6 Hz, 1H), 7.48–7.52 (m, 4H), 7.62 (d, *J*=7.2 Hz, 1H), 7.68 (d, *J*=7.2 Hz, 2H), 8.64 (d, *J*=9.2 Hz 1H), 10.89 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 198.6, 164.2, 139.1, 137.8, 124.5, 123.2, 62.0; d: 144.9, 134.1, 133.0, 132.8, 129.9, 128.6, 127.4, 123.1; HRMS calcd for $C_{17}H_{14}ClNO_3$ (M^+) 315.0662, found 315.0652.

*5.1.1.3.10. (E)-4-Hydroxy-N-(1-phenylethyl)-2-butenamide (**3k**).* Yellow oil, yield 38% (3 steps); TLC R_f=0.15 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3280, 3266, 3063, 2974, 1671, 1629, 1544, 1494, 1094, 699; ^1H NMR (400 MHz, $CDCl_3$) δ 1.45 (d, *J*=6.8 Hz, 3H), 4.12 (s, 2H), 4.29 (s, 1H), 5.11 (p, *J*=6.8 Hz, 1H), 6.09 (d, *J*=15.4 Hz, 1H), 6.80 (dt, *J*=15.4, 3.6 Hz, 1H), 7.05 (d, *J*=7.9 Hz, 1H), 7.19–7.24 (m, 1H), 7.27–7.28 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 165.4, 143.3, 61.4; d: 143.4, 128.6, 127.2, 126.2, 122.3, 48.9, 21.9; HRMS calcd for $C_{12}H_{15}NO_2$ (M^+) 205.1103, found 205.1112.

*5.1.1.4. Transesterfication of methyl acetoacetate. 5.1.1.4.1. (E)-4-(Phenylamino)-4-oxo-2-buten-1-yl 3-oxobutanoate (**4a**).* A mixture of methyl acetoacetate (1.06 g, 9.12 mmol), (E)-4-hydroxy-*N*-phenyl-2-butenamide (0.81 g, 4.56 mmol) and zinc metal (0.60 g, 9.12 mmol) were combined in toluene (15.0 mL). A catalytic amount of iodine (0.10 g) was added and the reaction was stirred and heated at 115–120 °C for 3 h while the flask was open to allow methanol evaporation. The reaction was quenched with saturated aqueous NH_4Cl (25.0 mL) and filtered through Celite. The filtered reaction mixture was extracted with diethyl ether (3×100 mL). The organic extracts were combined and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed to give compound **4a** as a yellow oil (0.73 g, 61% yield); TLC R_f=0.41 (MTBE/CH_2Cl_2, 8:2); IR (neat, cm^{-1}) 3306, 3137, 3062, 2932, 1716, 1683, 1651, 1151; ^1H NMR (400 MHz, $CDCl_3$) δ 2.21 (s, 3H), 3.47 (s, 2H), 4.73 (dd, *J*=4.9, 1.4 Hz, 2H), 6.29 (d, *J*=15.4 Hz, 1H), 6.87 (dt, *J*=15.4, 4.9 Hz, 1H), 7.07 (t, *J*=7.3 Hz, 1H), 7.26 (d, *J*=7.2 Hz, 2H), 7.58 (d, *J*=8.0 Hz, 2H), 8.80 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 200.7, 166.5, 162.8, 137.8, 63.5, 49.8; d: 137.5, 129.0, 125.3, 124.5, 120.0, 30.4; HRMS calcd for $C_{14}H_{15}NO_4$ (M^+) 261.1001, found 261.1005.

*5.1.1.4.2. (E)-4-[(2,4,6-Trimethylphenyl)amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4b**).* White solid, yield 67%; mp 122–124 °C; TLC R_f=0.532 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3229, 3027, 2922, 2853, 1743, 1718, 1635, 1529, 1150, 857; ^1H NMR (400 MHz, $CDCl_3$) δ 2.11 (s, 6H), 2.25 (s, 3H), 2.29 (s, 3H), 3.54 (s, 2H), 4.77 (s, 2H), 6.29 (d, *J*=15.6 Hz, 1H), 6.88 (s, 3H), 7.53 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 200.6, 166.5, 163.6, 135.1, 131.0, 63.7, 49.8; d: 136.9, 129.3, 128.8, 125.1, 30.4, 20.9, 18.3; HRMS calcd for $C_{17}H_{21}NO_4$ (M^+) 303.1471, found 303.1481.

*5.1.1.4.3. (E)-4-[(4-Fluorophenyl)amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4c**).* Yellowish brown oil, 62% yield; TLC R_f=0.49 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3292, 3077, 1747, 1716, 1682, 1646, 1544, 1508, 1212, 836; ^1H NMR (400 MHz, $CDCl_3$) δ 2.26 (s, 3H), 3.53 (s, 2H), 4.77 (d, *J*=4.8 Hz, 2H), 6.23 (d, *J*=15.6 Hz, 1H), 6.88 (dt, *J*=15.6, 4.8 Hz, 1H), 6.96 (t, *J*=8.4 Hz, 2H), 7.52 (dd, *J*=8.4, 4.4 Hz, 2H), 8.48 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.0, 166.5, 163.3, 159.4 (d, *J*=240 Hz), 133.9, 63.6, 49.7; d: 137.5, 125.5, 122.05 (d, *J*=10 Hz), 115.6 (d, *J*=23 Hz), 30.4; HRMS calcd for $C_{14}H_{14}FNO_4$ (M^+) 279.0907, found 279.0924.

*5.1.1.4.4. (E)-4-[(4-Cyanophenyl)amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4d**).* Yellow solid, 51% yield; mp 102–105 °C; TLC R_f=0.40 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3326, 3106, 2927, 2225, 1746, 1715, 1696, 1595, 1528, 1176; ^1H NMR (400 MHz, $CDCl_3$) δ 2.31 (s, 3H), 3.85 (s, 2H), 4.85 (dd, *J*=4.5, 1.7 Hz, 2H), 6.28 (d, *J*=15.4 Hz, 1H), 6.99 (dt, *J*=15.4 Hz, 4.4 Hz, 1H), 7.61 (d, *J*=8.6 Hz, 2H), 7.77 (d, *J*=8.6 Hz, 2H), 8.34 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 200.9, 166.5, 163.4, 142.2, 118.9, 106.9, 63.5, 49.7; d: 139.0, 133.3, 124.7, 119.9, 30.5; HRMS calcd for $C_{15}H_{14}N_2O_4$ (M^+) 286.0954, found 286.0949.

*5.1.1.4.5. (E)-4-[(4-Carboxyphenyl)amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4e**).* Orange oil, 56% yield; TLC R_f=0.45 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3481, 3338, 3118, 2983, 1746, 1713, 1599, 1538, 1278, 1174, 1107, 771; ^1H NMR (400 MHz, $CDCl_3$) δ 1.40 (t, *J*=7.2 Hz, 3H), 2.32 (s, 3H), 3.58 (s, 2H), 4.37 (q, *J*=7.2 Hz, 2H), 4.87 (dd, *J*=6.4, 2.0 Hz, 2H), 6.28 (dt, *J*=15.2, 1.8 Hz, 1H), 7.00 (dt, *J*=15.2, 4.4 Hz, 1H), 7.70 (d, *J*=8.4 Hz, 2H), 7.90 (s, 1H), 8.03 (d, *J*=8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 200.8, 166.3, 163.1, 141.9, 130.8, 126.1, 63.5, 61.0, 49.8; d; 138.6, 124.8, 119.0, 77.4, 30.5, 14.4; HRMS calcd for $C_{17}H_{19}NO_6$ (M^+) 333.1212, found 333.1223.

*5.1.1.4.6. (E)-4-[(4-Methoxyphenyl)amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4f**).* Yellow solid, 57% yield; mp 136–138 °C; TLC R_f=0.44 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3303, 2934, 2360, 1748, 1716, 1683, 1540, 1511, 1244, 1172, 831; ^1H NMR (600 MHz, $CDCl_3$) δ 2.29 (s, 3H), 3.54 (s, 2H), 3.79 (s, 3H), 4.80–4.82 (m, 2H), 6.21 (d, *J*=15.0 Hz, 1H), 6.85 (d, *J*=8.4 Hz, 2H), 6.91 (dt, *J*=15.0, 4.8 Hz, 1H), 7.48 (d, *J*=8.4 Hz, 2H), 7.64 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ d: 200.8, 166.5, 163.0, 156.5, 130.9, 63.7, 49.8; d: 137.2, 125.5, 121.9,

114.1, 55.5, 30.4; HRMS calcd for $C_{15}H_{17}NO_5$ (M^+) 291.1107, found 291.1101.

*5.1.1.4.7. (E)-4-[[4-(4-Methoxyphenoxy)phenyl]amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4g**).* Yellow solid, 75% yield; mp 146–148 °C; TLC R_f=0.43 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3282, 2937, 2838, 1739, 1715, 1635, 1507, 1241, 1226, 834; ^1H NMR (400 MHz, $CDCl_3$) δ 2.33 (s, 3H), 3.58 (s, 2H), 3.82 (s, 3H), 4.87 (dd, *J*=4.0, 1.2 Hz, 2H), 6.24 (d, *J*=15.2 Hz, 1H), 6.87–7.00 (m, 7H), 7.49–7.53 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 200.6, 166.4, 162.7, 155.8, 155.1, 150.3, 132.4, 63.6, 49.9; d: 137.5, 125.1, 121.7, 120.5, 118.2, 114.8, 55.7, 30.4; HRMS calcd for $C_{21}H_{21}NO_6$ (M^+) 383.1369, found 383.1374.

*5.1.1.4.8. (E)-4-[Ethyl(phenyl)amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4h**).* Yellow oil, 62% yield; TLC R_f=0.28 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3480, 3061, 2975, 2934, 2874, 1747, 1717, 1668, 1403, 770, 702; ^1H NMR (400 MHz, $CDCl_3$) δ 1.08–1.12 (m, 3H), 2.13–2.15 (m, 3H), 3.33 (d, *J*=3.2 Hz, 2H), 3.80 (p, *J*=7.2 Hz, 2H), 4.62 (s, 2H), 5.83 (d, *J*=14.8 Hz, 1H), 6.79–6.85 (m, 1H), 7.13 (d, *J*=6.4 Hz, 2H), 7.33–7.43 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 200.0, 166.3, 164.2, 141.5, 63.8, 49.6, 44.4; d:136.9, 129.6, 129.5, 128.3, 127.9, 123.6, 30.2, 12.9; HRMS calcd for $C_{16}H_{19}NO_4$ (M^+) 289.1314, found 289.1333.

*5.1.1.4.9. (E)-4-[(4-Acetylphenyl)amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4i**).* Orange solid, 61% yield; mp 128–130 °C TLC R_f=0.24 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3324, 3106, 2925, 1745, 1677, 1595, 1530, 1272, 1177, 840; ^1H NMR (400 MHz, $CDCl_3$) δ 2.27 (s, 3H), 2.57 (s, 3H), 3.53 (s, 2H), 4.80 (d, *J*=3.6 Hz, 2H), 6.30 (d, *J*=15.4 Hz, 1H), 6.96 (dt, *J*=15.4, 4.4 Hz, 1H), 7.72 (d, *J*=8.4 Hz, 2H), 7.91 (d, *J*=8.4 Hz, 2H), 8.77 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.9, 197.5, 166.5, 163.5, 142.7, 132.8, 63.6, 49.7; d: 138.4, 129.7, 125.2, 119.3, 30.4, 26.5; HRMS calcd for $C_{16}H_{17}NO_5$ (M^+) 303.1107, found 303.1121.

*5.1.1.4.10. (E)-4-[[4-Chloro-2-(phenylcarbonyl)phenyl]amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4j**).* Yellow oil, 55% yield; TLC R_f=0.6 (MTBE/PE, 7:3); IR (neat, cm^{-1}) 3734, 3282, 2937, 2838, 1739, 1715, 1635, 1507, 1241, 1226, 1033, 834; ^1H NMR (400 MHz, $CDCl_3$) δ 2.32 (s, 3H), 3.59 (s, 2H), 4.88 (dd, *J*=4.4, 1.6 Hz, 2H), 6.27 (dt, *J*=15.2, 1.6 Hz, 1H), 7.00 (dt, *J*=15.2, 4.8 Hz, 1H), 7.56 (m, 4H), 7.66 (t, *J*=7.3 Hz, 1H), 7.70–7.75 (m, 2H), 8.73 (d, *J*=9.2 Hz, 1H), 10.92 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 200.1, 198.7, 166.5, 163.4, 138.9, 137.8, 128.6, 124.5, 63.5, 49.8; d: 138.3, 134.1, 133.1, 132.9, 129.9, 127.6, 125.7, 123.2, 30.4; HRMS calcd for $C_{21}H_{18}ClNO_5$ (M^+) 399.0874, found 399.0865.

*5.1.1.4.11. (E)-4-[(1-Phenylethyl)amino]-4-oxo-2-buten-1-yl 3-oxobutanoate (**4k**).* Yellow oil, 55% yield; TLC R_f=0.5 (MTBE/CH_2Cl_2, 6:4); IR (neat, cm^{-1}) 3288, 3063, 3031, 2973, 2924, 2852, 1748, 1716, 1635, 1540, 1149, 700; ^1H NMR (400 MHz, $CDCl_3$) δ 1.48 (d, *J*=8.4 Hz, 3H), 2.25 (s, 3H), 3.49 (s, 2H), 4.74 (d, *J*=3.6 Hz, 2H), 5.12–5.19 (m, 1H), 6.07 (d, *J*=15.2 Hz, 1H), 6.51 (s, 1H), 6.78 (dt, *J*=15.2, 4.8 Hz, 1H), 7.24–7.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 200.6, 166.5, 164.0, 143.0, 63.7, 49.8; d: 136.3, 128.6, 127.4, 126.2, 125.3, 48.9, 30.3, 21.7; HRMS calcd for $C_{16}H_{19}NO_4$ (M^+) 289.1314, found 289.1306.

*5.1.1.5. Synthesis of dihydropyrimidines **5a**–**5i**. 5.1.1.5.1. (E)-4-Phenylamino-4-oxo-2-buten-1-yl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5a**).* A mixture of (*E*)-4-(phenylamino)-4-oxo-2-buten-1-yl 3-oxobutanoate (**4a**) (0.32 g, 1.23 mmol), 3-hydroxybenzaldhyde (0.43 g, 1.50 mmol), thiourea (0.11 g, 1.50 mmol), and conc aqueous HCl (0.15 mL) in ethanol (5.0 mL) were combined. The mixture was heated under reflux for 4 h. The reaction mixture was chromatographed directly to give the product as white solid (0.17 g, 33% yield); mp 130–132 °C; TLC R_f=0.25 (MTBE/CH_2Cl_2 2:8); IR (neat, cm^{-1}) 3262 (br), 2985, 1683, 1652, 1558, 1540, 1457, 1109, 754, 667; ^1H NMR (400 MHz,CD_3COCD_3) δ 2.49 (s, 3H), 4.84 (td, *J*=4.6, 2.0 Hz, 2H), 5.43 (d, *J*=3.6 Hz, 1H), 6.21 (dt, *J*=15.4, 2.0 Hz, 1H), 6.77–6.80 (m, 1H), 6.88 (d, *J*=7.6 Hz, 2H), 6.94 (dt, *J*=15.4, 4.6 Hz, 1H), 7.08 (t, *J*=7.4 Hz, 1H), 7.20 (t, *J*=7.9 Hz, 1H), 7.32 (t, *J*=7.9 Hz, 2H), 7.73 (d, *J*=7.9 Hz, 2H), 8.57 (s, 1H), 8.76 (s, 1H), 9.17 (s, 1H), 9.33 (s, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ u: 175.5, 164.8, 162.7, 157.7, 145.3, 145.1, 139.3, 101.2, 62.39; d: 138.4, 129.7, 128.7, 124.9, 123.5, 119.4, 117.8, 114.8, 113.6, 55.0, 17.2; HRMS calcd for $C_{22}H_{21}N_3O_4S$ (M^+) 423.1253, found 423.1237.

*5.1.1.5.2. (E)-4-(2,4,6-Trimethylphenylamino)-4-oxo-2-buten-1-yl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5b**).* White solid, 31% yield; mp 132–134 °C; TLC R_f=0.48 (MTBE/CH_2Cl_2 4:6); IR (neat, cm^{-1}) 3232 (br), 2920, 1675, 1640, 1566, 1484, 1460, 1276, 1106, 700; ^1H NMR (400 MHz, CD_3COCD_3) δ 2.16 (s, 6H), 2.25 (s, 3H), 2.50 (s, 3H), 4.85 (d, *J*=4.4 Hz, 2H), 5.45 (d, *J*=3.6 Hz, 1H), 6.28 (dt, *J*=15.6, 2.0 Hz, 1H), 6.77–6.87 (m, 1H), 6.88–6.91 (m, 5H), 7.20 (t, *J*=8.2 Hz, 1H), 8.41 (s, 1H), 8.52 (s, 1H), 8.74 (s, 1H), 9.32 (s, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ u: 175.5, 164.8, 162.7, 157.6, 145.3, 145.1, 136.0, 135.2, 132.3, 101.2, 62.4; d: 137.5, 129.8, 128.3, 124.5, 117.8, 114.8, 113.6, 55.0, 20.0, 17.7, 17.2; HRMS calcd for $C_{25}H_{27}N_3O_4S$ (M^+) 465.1722, found 465.1720.

*5.1.1.5.3. (E)-4-(4-Fluorophenylamino)-4-oxo-2-buten-1-yl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5c**).* Yellow solid, 31% yield; mp 158–160 °C; TLC R_f=0.22 (MTBE/CH_2Cl_2 2:8); IR (neat, cm^{-1}) 3257 (br), 2925, 1676, 1645, 1558, 1508, 1184, 1108, 835, 785; ^1H NMR (400 MHz, DMSO-d_6) δ, 2.35 (s, 3H), 4.72 (dd, *J*=16.1, 2.9 Hz, 1H), 4.85 (dd, *J*=17.7, 4.4 Hz, 1H), 5.17 (d, *J*=3.6 Hz, 1H), 6.19 (d, *J*=15.5 Hz, 1H), 6.65–6.69 (m, 3H), 6.79 (dt, *J*=15.5, 4.5 Hz, 1H), 7.11–7.16 (m, 2H), 7.18 (d, *J*=8.9 Hz, 1H), 7.67 (dd, *J*=9.1, 5.0 Hz, 2H), 9.49 (s, 1H), 9.75 (s, 1H), 10.17 (s, 1H), 10.45 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ 174.64, 165.24, 163.03, 157.97, 146.19, 145.06, 138.87, 135.86, 130.05, 124.83, 121.56, 121.48, 117.43, 115.95, 115.73, 115.15, 113.63, 100.60, 62.93, 54.32, 40.57, 31.19, 18.06; HRMS calcd for $C_{22}H_{20}FN_3O_4S$ (M^+) 441.1159, found 441.1128.

*5.1.1.5.4. (E)-4-(4-Cyanophenylamino)-4-oxo-2-buten-1-yl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5d**).* Yellowish white solid, 38% yield; mp 137–139 °C; TLC R_f=0.20 (MTBE/CH_2Cl_2 2:8); IR (neat, cm^{-1}) 3311 (br), 2958, 2924, 2854, 2226, 1699, 1684, 1593, 1558, 1457, 1183, 1107, 839; ^1H NMR (400 MHz, CD_3COCD_3) δ 2.49 (s, 3H), 4.82 (ddd, *J*=16.4, 4.4, 1.9 Hz, 1H), 4.89 (ddd, *J*=16.4, 4.4, 1.9 Hz, 1H), 5.44 (d, *J*=3.4 Hz, 1H), 6.26 (dt, *J*=15.3, 1.9 Hz, 1H), 6.76–6.79 (m, 1H), 6.80–6.84 (m, 1H), 6.85–6.91 (m, 2H), 7.00 (dt, *J*=15.3, 4.6 Hz, 1H), 7.19 (t, *J*=7.8 Hz, 1H), 7.73 (d, *J*=8.8 Hz, 2H), 7.94 (d, *J*=8.8 Hz, 2H), 8.79 (s, 1H), 9.36 (s, 1H), 9.69 (s, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ u: 175.5, 164.7, 163.3, 157.7, 145.3, 145.1, 143.3, 118.6, 106.2, 101.2, 62.3; d: 139.9, 133.1, 129.8, 124.1, 119.5, 117.8, 114.8, 113.6, 54.9, 17.3; HRMS calcd for $C_{23}H_{20}N_4O_4S$ (M^+) 448.1205, found 448.1210.

*5.1.1.5.5. (E)-4-(4-(Ethoxycarbonyl)phenylamino)-4-oxo-2-buten-1-yl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5e**).* Yellow solid, 47% yield; mp 155–157 °C; TLC R_f=0.25 (MTBE/CH_2Cl_2 2:8); IR (neat, cm^{-1}) 3395 (br), 2950, 1686, 1646, 1598, 1532, 1275, 1180, 1108, 771, 698; ^1H NMR (400 MHz, CD_3COCD_3) δ 1.37 (t, *J*=,7.2 Hz, 3H), 2.50 (s, 3H), 4.34 (t, *J*=7.1 Hz, 2H), 4.83 (ddd, *J*=16.2, 4.6, 2.0 Hz, 1H), 4.90 (ddd, *J*=16.2, 4.6, 2.0 Hz, 1H), 5.44 (d, *J*=3.6 Hz, 1H), 6.24 (dt, *J*=15.3, 2.0 Hz, 1H), 6.77–6.80 (m, 1H), 6.87–6.89 (m, 2H), 6.99 (dt, *J*=15.3, 4.4 Hz, 1H), 7.20 (t, *J*=8.0 Hz, 1H), 7.85 (d, *J*=8.8 Hz, 2H), 7.99 (d, *J*=8.8 Hz, 2H), 8.53 (s, 1H), 8.76 (s, 1H), 9.34 (s, 1H), 9.48 (s, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ u: 175.5, 165.4, 164.7, 163.1, 157.7, 145.3, 145.1, 143.4, 125.3, 101.2, 62.3, 60.3, d: 139.4, 130.3, 129.8, 124.4, 118.6, 117.8, 114.8, 113.5, 55.0, 17.2, 13.7; HRMS calcd for $C_{25}H_{26}N_3O_6S$ $(M+H)^+$ 496.1542, found 496.1542.

*5.1.1.5.6. (E)-4-[(4-Methoxyphenyl)amino]-4-oxo-2-buten-1-yl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5f**).* Yellow solid, 32% yield; mp 136–138 °C; TLC R_f=0.40 (MTBE/CH_2Cl_2 4:6); IR (neat, cm^{-1}) 3403 (br), 2950, 1674,

1642, 1510, 1460, 1184, 1108, 700; ^{1}H NMR (400 MHz, CD_3COCD_3) δ 2.49 (s, 3H), 3.78 (s, 3H), 4.79 (ddd, J=16.0, 4.4, 1.8 Hz, 1H), 4.86 (ddd, J=16.0, 4.4, 1.8 Hz, 1H), 5.45 (d, J=3.2 Hz, 1H), 6.21 (dt, J=15.2, 1.6 Hz, 1H), 6.77–6.79 (m, 1H), 6.88–6.93 (m, 5H), 7.13–7.21 (m, 1H), 7.51 (d, J=8.8 Hz, 1H), 7.65 (d, J=9.2 Hz, 2H), 8.79 (s, 1H), 9.08 (s, 1H), 9.34 (s, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ u: 175.5, 164.8, 162.4, 157.7, 156.0, 145.2, 145.1, 132.4, 101.2, 62.4; d: 137.8, 129.8, 125.1, 120.9, 117.7, 114.8, 113.8, 113.6, 54.9, 54.7, 17.2; HRMS calcd for $C_{23}H_{23}N_3O_5S$ (M^+) 453.1358, found 453.1378.

*5.1.1.5.7. (E)-4-[[4-(4-Methoxyphenoxy)phenyl]amino]-4-oxo-2-buten-1-yl 4-(3-hydroxy phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5g**).* Yellow solid, 33% yield; mp 140–142 °C; TLC R_f=0.31 (MTBE/CH_2Cl_2 2:8); IR (neat, cm^{-1}) 3273 (br), 2925, 1688, 1652, 1558, 1497, 1220, 1182, 1102, 831, 667; ^{1}H NMR (400 MHz, CD_3COCD_3) δ 2.48 (s, 3H), 3.80 (s, 3H), 4.81 (d, J=15.8 Hz, 2H), 5.43 (d, J=3.3 Hz, 1H), 6.20 (d, J=15.4 Hz, 1H), 6.76–6.80 (m, 1H), 6.86–6.93 (m, 5H), 6.95–6.98 (m, 4H), 7.20 (d, J=7.6 Hz, 1H), 7.69 (d, J=8.9 Hz, 2H), 8.57 (s, 1H), 8.79 (s, 1H), 9.17 (s, 1H), 9.36 (s, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ u: 175.4, 164.8, 162.6, 157.6, 156.0, 154.4, 150.5, 145.2, 145.1, 134.2, 101.2, 62.4; d: 138.2, 129.8, 124.8, 121.0, 120.2, 117.9, 117.8, 114.8, 113.6, 55.0, 17.3; HRMS calcd for $C_{29}H_{27}N_3O_6S$ (M^+) 545.1621, found 545.1603.

*5.1.1.5.8. (E)-4-[Ethyl(phenyl)amino]-4-oxo-2-buten-1-yl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5h**).* Yellow solid, 34% yield; mp: 142–145 °C; TLC R_f=0.26 (MTBE/CH_2Cl_2 2:8); IR (neat, cm^{-1}) 3232 (br), 2974, 2934, 1665, 1619, 1589, 1453, 1278, 1183, 1108, 700, 574; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 (t, J=7.2 Hz, 3H), 1.27 (s, 1H), 2.15 (s, 3H), 3.78–3.89 (m, 2H), 4.49 (dd, J=15.2, 4.8 Hz, 1H), 4.58 (dd, J=15.2, 4.8 Hz, 1H), 5.16 (d, J=2.4 Hz, 1H), 5.82 (d, J=15.6 Hz, 1H), 6.71–6.75 (m, 2H), 6.77–6.85 (m, 2H), 7.09–7.12 (m, 1H), 7.14–7.18 (m, 2H), 7.37–7.46 (m, 3H), 8.27 (s, 1H), 8.64 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 174.1, 165.2, 164.7, 156.7, 144.0, 143.7, 141.1, 102.1, 63.3, 44.7; d: 138.1, 130.1, 129.8, 129.6, 128.3, 123.5, 118.1, 115.7, 114.0, 55.5, 18.3, 12.9; HRMS calcd for $C_{24}H_{25}N_3O_4S$ (M^+) 451.1566, found 451.1581.

*5.1.1.5.9. (E)-4-(4-Acetylphenylamino)-4-oxo-2-buten-1-yl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5i**).* Yellow solid, yield 33%; mp 118–120 °C; TLC R_f=0.27 (MTBE/CH_2Cl_2 4:6); IR (neat, cm^{-1}) 3392 (br), 2920, 1653, 1593, 1559, 1275, 1181, 1107, 668; ^{1}H NMR (400 MHz, CD_3COCD_3) δ 2.49 (s, 3H), 2.55 (s, 3H), 2.90 (s, 1H), 3.14 (s, 1H), 4.83 (ddd, J=,16.1, 4.5, 1.9 Hz, 1H), 4.89 (ddd, J=16.2, 4.5, 1.8 Hz, 1H), 5.43 (d, J=3.2 Hz, 1H), 6.24 (dt, J=15.3, 1.8 Hz, 1H), 6.77–6.80 (m, 1H), 6.88–6.89 (m, 1H), 6.99 (dt, J=1.5.2, 4.6 Hz, 1H), 7.20 (t, J=8.0 Hz, 1H), 7.85 (d, J=8.8 Hz, 2H), 7.98 (d, J=8.8 Hz, 2H), 8.54 (s, 1H), 8.76 (s, 1H), 9.33 (s, 1H), 9.47 (s, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ u: 195.7, 175.5, 164.8, 163.1, 157.7, 145.3, 145.1, 143.4, 132.5, 101.2, 62.4; d: 139.5, 130.5, 129.8, 129.4, 118.7, 117.8, 114.8, 113.5, 55.0, 26.6, 17.3; HRMS calcd for $C_{24}H_{23}N_3O_5S$ (M^+) 465.1358, found 465.1349.

*5.1.1.5.10. 2-Propen-1-yl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**6**).* A mixture of 2-propenyl 3-oxobutanoate (0.5 g, 3.52 mmol), 3-hydroxybenzaldhyde (0.43 g, 3.52 mmol), thiourea (0.32 g, 4.22 mmol), and conc aqueous HCl (0.15 mL) in ethanol (5.0 mL) were combined. The mixture was heated under reflux for 3 h. The reaction mixture was chromatographed to give the product as a white fluffy solid (0.67 g, 63% yield); mp 148–150 °C; TLC R_f=0.24 (MTBE/PE 2.5:7.5); IR (neat, cm^{-1}) 3197, 2985, 1695, 1652, 1575, 1558, 1457, 1273, 1185, 1111, 699; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.16 (s, 1H), 2.32 (s, 3H), 4.51–4.61 (m, 2H), 5.15–5.19 (m, 2H), 5.33 (d, J=10.0 Hz, 1H), 5.82 (ddt, J=15.7, 11.0, 5.6 Hz, 1H), 6.74–6.83 (m, 3H), 7.12 (t, J=7.6 Hz, 1H), 8.18 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ u: 173.8, 165.3, 156.2, 143.8, 143.6, 118.4, 102.5, 65.4; d: 131.8, 130.2, 118.9, 115.7, 113.8, 55.7, 18.4; HRMS calcd for $C_{15}H_{16}N_2O_3S$ (M^+) 304.0898, found 304.0898.

*5.1.1.5.11. Ethane-1,2-diyl bis [4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2, 3,4-tetrahydro pyrimidine-5-carboxylate] (**7**).* A mixture of ethane-1,2-diylbis(3-oxobutanoate) (0.50 g, 2.17 mmol), 3-hydroxybenzaldhyde (0.53 g, 4.34 mmol), thiourea (0.40 g, 5.21 mmol), and conc aqueous HCl (0.20 mL) in ethanol (5.0 mL) were combined. The mixture was heated under reflux for 20 h. The reaction mixture was chromatographed using PE/wet EtOAc to give the homodimer as a yellow solid (0.863 g, 72% yield); mp 164–165 °C; TLC R_f=0.11 (P.E/Wet EtOAc 6:4); IR (neat, cm^{-1}) 3306 (br), 1698, 1651, 1569, 1186; ^{1}H NMR (400 MHz, (CD_3COCD_3) δ 2.39 (s, 3H), 2.42 (s, 3H), 4.24–4.33 (m, 4H), 5.29 (d, J=3.6 Hz, 1H), 5.35 (d, J=3.2 Hz, 1H), 6.74–6.78 (m, 2H), 6.80–6.83 (m, 4H), 7.13 (td, J=8.0, 2.8 Hz, 2H), 8.47 (s, 2H), 8.72 (s, 2H), 9.29 (s, 2H); ^{13}C NMR (100 MHz, (CD_3COCD_3) δ u: 175.4, 175.3, 165.1, 165.0, 157.6, 145.1, 145.0, 145.0, 101.4, 101.4, 61.9, 61.8; d: 129.6, 117.8, 114.8, 113.4, 113.4, 55.0, 17.2, 17.1; HRMS calcd for $C_{26}H_{26}N_4O_6S_2$ (M^+) 554.1294, found 554.1270.

Acknowledgements

We thank Jesse McAtee for high resolution mass spectrometry financially supported by NSF CHE-1229234, and Dr. Shi Bai for NMR spectra financially supported by NSF CRIF:MU, CHE 080401. We thank John A. Beutler of NCI for the 60 cell results.

Supplementary data

Results from 60 cell screen, and ^{1}H and ^{13}C NMR spectra for all new compounds. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.11.022.

References and notes

1. Orru, R. V. A.; De Greef, M. *Synthesis* **2003**, *10*, 1471–1499.
2. Tejedor, D.; González-Cruz, D.; Santos-Expósito, A.; Marrero-Tellado, J. J.; de Armas, P.; García-Tellado, F. *Chem.—Eur. J.* **2005**, *11*, 3502–3510.
3. Dömling, A. *Chem. Rev.* **2006**, *106*, 17–89.
4. Guillena, G.; Ramón, D. J.; Yus, M. *Tetrahedron: Asymmetry* **2007**, *18*, 693–700.
5. Toure, B. B.; Hall, D. G. *Chem. Rev.* **2009**, *109*, 4439–4486.
6. Spring, D. R. *Org. Biomol. Chem.* **2003**, *1*, 3867–3870.
7. Burke, M. D.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 46–58.
8. Spandl, R. J.; Bender, A.; Spring, D. R. *Org. Biomol. Chem.* **2008**, *6*, 1149–1158.
9. Weber, L. *Curr. Med. Chem.* **2002**, *9*, 2085–2093.
10. Hulme, C.; Gore, V. *Curr. Med. Chem.* **2003**, *10*, 51–81.
11. (a) Biginelli, P. *Gazz. Chim. Ital.* **1893**, *23*, 360–414 Despite its age, the Biginelli reaction is still under active investigation. For recent reviews, see: (b) de Graaff, C.; Ruijter, E.; Orru, R. V. A. *Chem. Soc. Rev.* **2012**, *41*, 3969–4009; (c) Suresh; Sandhu, J. S. *ARKIVOC* **2012**, 66–133; (d) Wan, J.-P.; Liu, Y. *Synthesis* **2010**, 3943–3953 For more recent reports, see: (e) Abranyi-Balogh, P.; Dancso, A.; Frigyes, D.; Volk, B.; Keglevich, G.; Milen, M. *Tetrahedron* **2014**, *70*, 5711–5719; (f) Rahman, M.; Sarkar, A.; Ghosh, M.; Majee, A.; Hajra, A. *Tetrahedron Lett.* **2014**, *55*, 235–239; (g) Safari, J.; Zarnegar, Z. *New J. Chem.* **2014**, *38*, 358–365; (h) Kolvari, E.; Koukabi, N.; Armandpour, O. *Tetrahedron* **2014**, *70*, 1383–1386; (i) Balan, B.; Bahulayan, D. *Tetrahedron Lett.* **2014**, *55*, 227–231; (j) Ladole, C. A.; Salunkhe, N. G.; Bhaskar, R. S.; Aswar, A. S. *Eur. J. Chem.* **2014**, *5*, 122–126; (k) Svetlik, J.; Pronayova, N.; Svorc, L.; Frecer, V. *Tetrahedron* **2014**, *70*, 8354–8360; (l) Siddiqui, A. B.; Trivedi, A. R.; Kataria, V. B.; Shah, V. H. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1493–1495.
12. (a) Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937–6963; (b) Kappe, C. O. *Acc. Chem. Res.* **2000**, *33*, 879–888.
13. Atwal, K. S.; Rovnyak, G. C.; Kimball, S. D.; Floyd, D. M.; Moreland, S.; Swanson, B. N.; Gougoutas, J. Z.; Schwartz, J.; Smillie, K. M.; Malley, M. F. *J. Med. Chem.* **1990**, *33*, 2629–2635.
14. Kappe, C. O. *Eur. J. Med. Chem.* **2000**, *35*, 1043–1052.
15. Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J. *Science* **1999**, *286*, 971–974.
16. Kumar, B. R. P.; Sankar, G.; Baig, R. B. N.; Chandrashekaran, S. *Eur. J. Med. Chem.* **2009**, *44*, 4192–4198.
17. Gartner, M.; Sunder-Plassmann, N.; Seiler, J.; Utz, M.; Vernos, I.; Surrey, T.; Giannis, A. *ChemBioChem* **2005**, *6*, 1173–1177.
18. Sarli, V.; Huemmer, S.; Sunder-Plassmann, N.; Mayer, T. U.; Giannis, A. *ChemBioChem* **2005**, *6*, 2005–2013.

19. Klein, E.; DeBonis, S.; Thiede, B.; Skoufias, D. A.; Kozielski, F.; Lebeau, L. *Bioorg. Med. Chem.* **2007**, *15*, 6474–6488.
20. Kamal, A.; Shaheer Malik, M.; Bajee, S.; Azeeza, S.; Faazil, S.; Ramakrishna, S.; Naidu, V. G. M.; Vishnuwardhan, M. V. P. S. *Eur. J. Med. Chem.* **2011**, *46*, 3274–3281.
21. Sashidhara, K. V.; Avula, S. R.; Sharma, K.; Palnati, G. R.; Bathula, S. R. *Eur. J. Med. Chem.* **2013**, *60*, 120–127.
22. Russowsky, D.; Canto, R. F. S.; Sanches, S. A. A.; D'Oca, M. G. M.; de Fátima, A.; Pilli, R. A.; Kohn, L. K.; Antônio, M. A.; de Carvalho, J. E. *Bioorg. Chem.* **2006**, *34*, 173–182.
23. Soumyanarayanan, U.; Bhat, V. G.; Kar, S. S.; Mathew, J. A. *Org. Med. Chem. Lett.* **2012**, *2*, 23–33.
24. Prokopcov, H.; Dallinger, D.; Uray, G.; Kristal Kaan, H. Y.; Ulaganathan, V.; Kozielski, F.; Laggner, C.; Kappe, C. O. *ChemMedChem* **2010**, *5*, 1760–1769 For other recent work on variant Biginelli products, see: (b) Dharma Rao, G. B.; Acharya, B. N.; Kaushik, M. P. *Tetrahedron Lett.* **2013**, *54*, 6644–6647; (c) Dharma Rao, G. B.; Anjaneyulu, B.; Kaushik, M. P. *Tetrahedron Lett.* **2014**, *55*, 19–22.
25. Taber, D. F.; Frankowski, K. J. *J. Org. Chem.* **2003**, *68*, 6047–6048.
26. (a) Ragazzini, M.; Vandi, A.; Campadelli, F. *Eur. Polym. J.* **1970**, *6*, 1331–1338; (b) Knapp, S.; Levorse, A. *J. Org. Chem.* **1988**, *53*, 4006–4014.
27. Binot, G.; Zard, S. Z. *Tetrahedron Lett.* **2005**, *46*, 7503–7506.
28. (a) Marson, C.; Grabowska, U.; Walsgrove, T.; Eggleston, D.; Baures, P. *J. Org. Chem.* **1994**, *59*, 284–290; (b) Cacchi, S.; Misiti, D.; La Torre, F. *Synthesis* **1980**, 243–244.
29. Troisi, L.; Granito, C.; Rosato, F.; Videtta, V. *Tetrahedron Lett.* **2010**, *51*, 371–373.
30. Yang, H.; Huang, D.; Wang, K. H.; Xu, C.; Niu, T.; Hu, Y. *Tetrahedron* **2013**, *69*, 2588–2593.
31. Theodorou, V.; Gogou, M.; Philippidou, M.; Ragoussis, V.; Paraskevopoulos, G.; Skobridis, K. *Tetrahedron* **2011**, *67*, 5630–5634.
32. Guha, S. K.; Shibayama, A.; Abe, D.; Sakaguchi, M.; Ukaji, Y.; Inomata, K. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 2147–2157.
33. Imada, Y.; Shibata, O.; Murahashi, S. I. *J. Organomet. Chem.* **1993**, *451*, 183–194.
34. Buchi, G.; Cushman, M.; Wuest, H. *J. Am. Chem. Soc.* **1974**, *96*, 5563–5565.
35. Crandall, J. K.; Apparu, M. *Org. React.* **1983**, *29*, 345–443.
36. Brooks, P. B.; Marson, C. M. *Tetrahedron* **1998**, *54*, 9613–9622.
37. Fava, C.; Galeazzi, R.; Gonzalez-Rosende, E. M.; Orena, M. *Tetrahedron Lett.* **2000**, *41*, 8577–8580.
38. Uchida, T.; Kagoshima, Y.; Konosu, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2013–2017.
39. Rehberg, C. E.; Fisher, C. H. *J. Am. Chem. Soc.* **1944**, *66*, 1203–1207.
40. Bader, A. R.; Cummings, L. O.; Vogel, H. A. *J. Am. Chem. Soc.* **1951**, *73*, 4195–4197.
41. Taber, D. F.; Amedio, J. C., Jr.; Patel, Y. K. *J. Org. Chem.* **1985**, *50*, 3618–3619.
42. Seebach, D.; Thaler, A.; Blaser, D.; Ko, S. Y. *Helv. Chim. Acta* **1991**, *74*, 1102–1118.
43. Seebach, D.; Hungerbühler, E.; Naef, R.; Schnurrenberger, D.; Weidmann, B.; Zügger, M. *Synthesis* **1982**, 138.
44. Ren, Y. M.; Wu, Z. C.; Yang, R. C.; Tao, T. X.; Shao, J. J.; Gao, Y. G.; Zhang, S.; Li, L. *Adv. Mat. Res.* **2013**, *259*, 781–784.
45. Otera, J.; Dan-ho, N.; Nozaki, H. *J. Org. Chem.* **1991**, *56*, 5307–5311.
46. Chavan, S. P.; Shivasankar, K.; Sivappa, R.; Kale, R. *Tetrahedron Lett.* **2002**, *43*, 8583–8586.
47. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
48. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91–109.
49. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
50. http://dtp.nci.nih.gov/branches/btb/ivclsp.html (accessed July 18 2014).
51. Chavan, S. P.; Kale, R. R.; Shivasankar, K.; Chandake, S. I.; Benjamin, S. B. *Synthesis* **2003**, *17*, 2695–2698.
52. Australia patent AU 2008252068 A1 4 Dec 2008.
53. Pistolozzi, M.; Franchini, C.; Corbo, F.; Muraglia, M.; De Giorgi, M.; Felix, G.; Bertucci, C. *J. Pharm. Biomed. Anal.* **2010**, *53*, 179–185.