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Preparation of dihydrotetrazolo[1,5-*a*]pyrimidine derivatives from Biginelli 3,4-dihydropyrimidine-2-thiones

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ABSTRACT

Dihydropyrimidines (DHPM) with a fused tetrazole ring have been synthesized via a smooth and efficient three-step protocol involving *N*,*S*-dimethylated and 2-hydrazinyl-dihydropyrimidine intermediates, respectively. The described protocol is applicable to electron rich and electron poor DHPM precursors as well. The insertion of tetrazole ring in monastrol type dihydropyrimidines makes it interesting for pharmacological investigations.

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1. Introduction

Pyrimidines are an integral part of DNA and RNA, and have diverse pharmacological properties.[1] In addition, other heterocycles, in association with the pyrimidine nucleus, have shown an essential role in several biological processes and have a considerable chemical and pharmacological importance (Fig. 1). Notably, pyrimidines of the Biginelli type (DHPMs) represent one of the most active classes of pyrimidine derivatives, possessing a wide spectrum of biological activities. For example, they show significant in vitro activity against DNA and RNA viruses,[2] including polio and herpes viruses,[2] and have diuretic,[3] anti-HIV (**1**),[4] and antitumor activities (**2**).[5] Furthermore, pyrimidines of this type are known antihypertensive agents (**3**),[6] have anti-epileptic (**4**),[7] and antitubercular activity (**5**),[8] and are inhibitors for the propagation of the malarial parasite (**6**)[9] (Fig. 1). In search of more potent and effective medicinally important molecules, numerous Biginelli dihydropyrimidines and related annulated or multi-functionalized pyrimidines heterocycles have been investigated or tested against different diseases.[10]

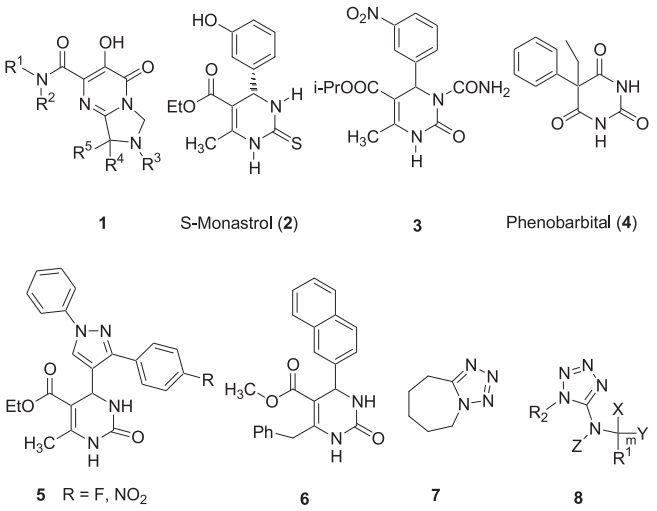

Fig. 1. Important drug molecules containing DHPM and tetrazole structural motifs.

On the other hand the tetrazole structural sub unit has been identified as an active pharmaceutical scaffold and thus is an important structural descriptor in the methodology of new drug design. Compounds containing the tetrazole structural unit have hypotensive, antimicrobial, antiviral, antiallergic, cytostatic, nootropic, and other biological activities.[11] The introduction of the

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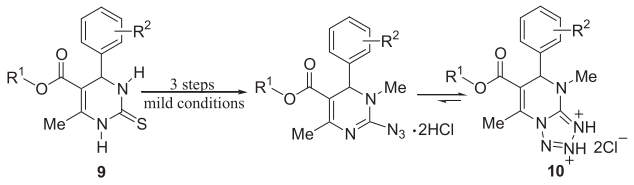
tetrazole ring into an organic substrate quite often leads not only to an increase in the efficacy but also to an increase in the prolongation of drug action. Additionally 1,5-disubstituted tetrazoles have a notable drug recognition,[1a,b,12] for example, tetrazoloazepine Cardiazol (**7**) has long been used as anticonvulsant and central nervous system stimulant.[1a,b] Moreover, aminotetrazole analogues of type **8** have been disclosed for the treatment/prevention of a variety of pain states, e.g., chronic inflammatory pain, neuropathic pain, spinal cord injury, neuro-degeneration, or depression.[11]

Therefore, the heterocyclic system of Biginelli DHPMs, linked to another medicinally important substructure (i.e., the tetrazole ring), is expected to exhibit interesting pharmacological properties.

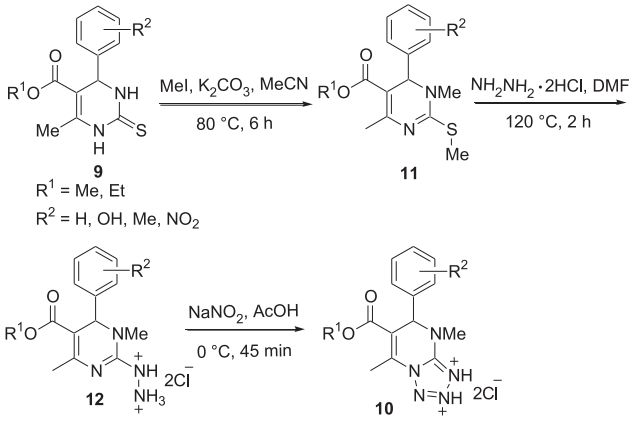
In the view of the facts, mentioned above, and as part of our initial efforts to discover potentially bioactive new compounds, we have synthesized some new derivatives of dihydropyrimidines annulated at the 1/2 position with a tetrazole ring of type **10** (Scheme 1). Herein, we describe a novel approach for the synthesis of hitherto unknown dihydrotetrazolo[1,5-*a*]pyrimidine dihydrochloride derivatives of type **10** by utilizing Biginelli DHPMs **9** as a precursor via a three-step sequence.



Scheme 1. Dihydrotetrazolo[1,5-*a*]pyrimidine dihydrochloride derivatives of type **10**.

2. Results and discussion

According to the general strategy outlined in Scheme 1, our synthesis of the required tetrazole-annulated DHPM commenced with the preparation of DHPM precursors **9**, which were synthesized in good to excellent yields on 20 mmol scale by the cyclization of three components, e.g., arylaldehydes, thiourea, and substituted β-ketoesters in acetonitrile via reflux or microwave heating following the literature (Biginelli reaction).[13] The initially formed dihydropyrimidine-2-thiones **9a**–**f** were subsequently converted to dihydrotetrazolo[1,5-*a*]pyrimidine dihydrochloride **10a**–**f** by a three-step sequence following dimethylation, hydrazination and then cyclization (Scheme 2).

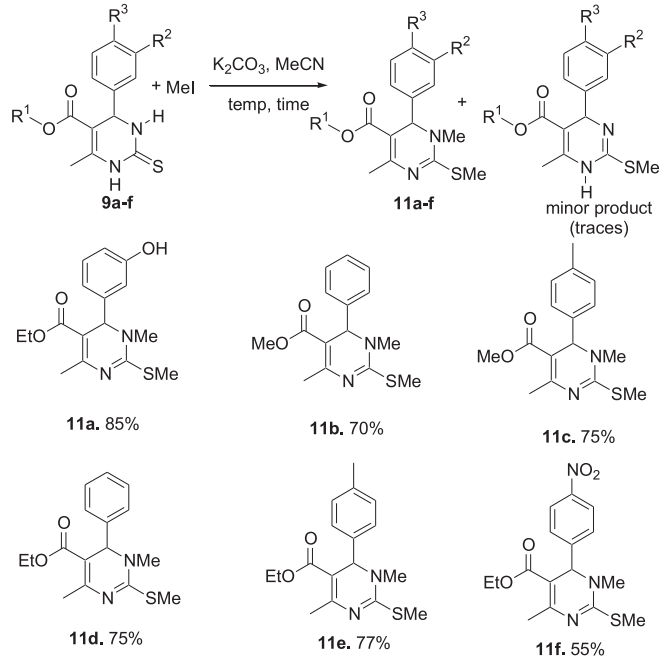


Scheme 2. Three-step synthetic sequence of **10**.

We optimized reaction conditions for the three-step sequence for the 4-phenyl DHPM precursor. Thereafter, on successful completion of the reaction sequence, the same strategy was applied to other electron rich and electron poor DHPMs and as well as monastrol because of the medicinal importance of dihydropyrimidine (±)-**2** (monastrol and its sub-derivatives) in human mitotic kinesin, e.g., **5**,[14] good overall yields were observed.

As a first step in three-step sequence, the initially formed dihydropyrimidine-2-thiones **9a**–**f** were subjected to double methylation.[15] For the desired double methylation at N-1 and C(2)=S positions of the DHPM scaffold, we initially performed model studies on phenyl-2-thione by employing microwave sealed vessel conditions (100 °C, 5 min).[16] It was quickly realized that for the required double methylation, K_2CO_3 as base is required along with methyl iodide. However, with K_2CO_3 under microwave conditions at 100 °C a mixture of three products, identified as mono *S*-methylated, double methylated at N-3 and S positions, and a product with triple methylation on all available positions at N-1, C-2, and N-3 was obtained. Ultimately, the reaction proceeded by heating 1 equiv of thione, 2 equiv of K_2CO_3 with 5 equiv of methyl iodide in acetonitrile as solvent under conventional oil bath reflux heating at 80 °C for 6 h under inert conditions. This provided 85% of isolated yield of the desired product (**11d**) after flash chromatography along with traces of the mono *S*-methylated product. With the optimized conditions, successful double *N*,*S*-methylation was achieved with the other four DHPMs with overall good yields along with mono *S*-methylated product as minor byproduct (Table 1).

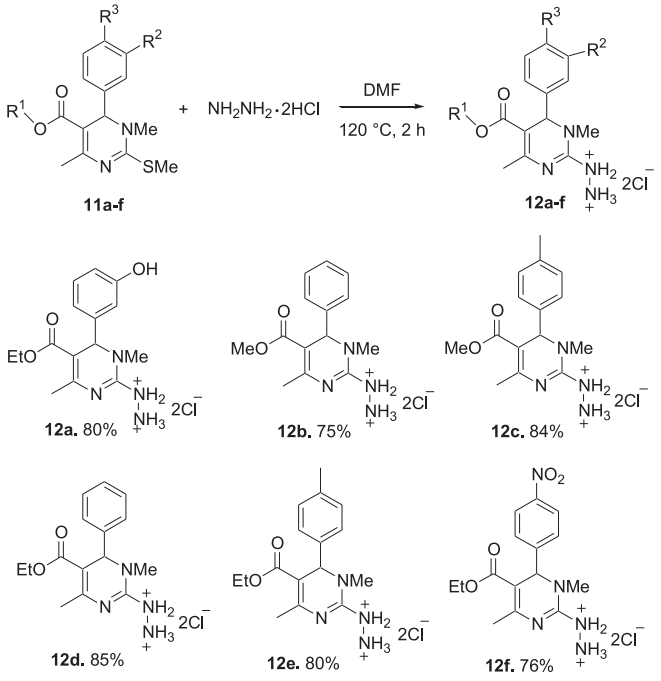
Table 1
N,S-Dimethylation step of DHPMs[a,b]



[a] Reaction conditions: 0.25 mmol DHPM **9a**–**f**, 2.0 equiv K_2CO_3, 3.0 equiv MeI, 1.5 mL MeCN, N_2 atmosphere, 80 °C for 6 h. [b] Isolated yields of *N*,*S*–dimethylated product by flash chromatography.

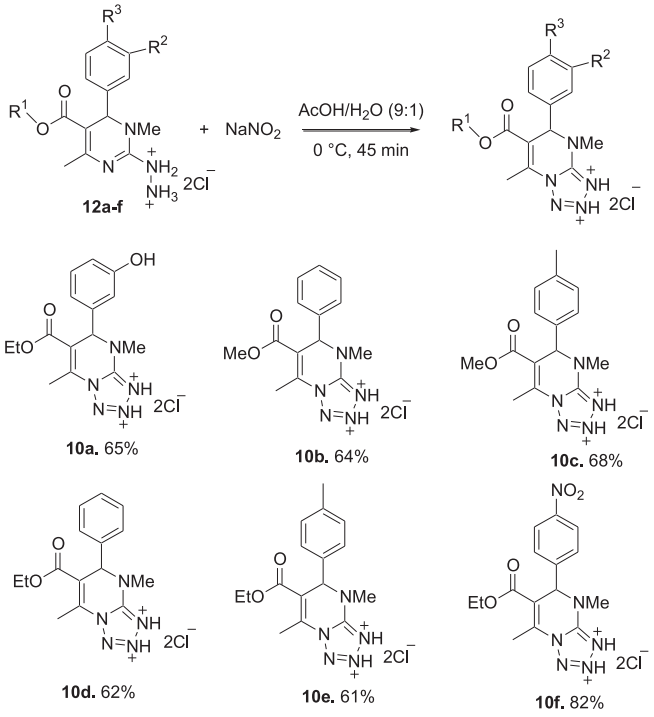
For the conversion of *N*,*S*-dimethylated dihydropyrimidines to the corresponding hydrazino derivatives, different reaction conditions were investigated involving, hydrazinating reagents, solvent and temperature variations. In our hands the most suitable protocol was the use of 4 equiv of hydrazinum dihydrochloride in DMF as solvent under sealed vessel oil bath heating for 2 h at 120 °C. All the dimethylated adducts **11a**–**f** were successfully transformed to the corresponding hydrazine dihydrochloride derivatives **12a**–**f** in excellent yields after flash column chromatography (Table 2).

Table 2
Hydrazino derivatives of DHPM[a,b]



[a] Reaction conditions: 0.25 mmol DHPM **11a–f**, 4.0 equiv hydrazinum dihydrochloride, 3.0 mL DMF, N_2 atmosphere, sealed vessel heated at 120 ºC for 2 h. [b] Isolated yields by flash chromatography

Table 3
Tetrazolo derivatives of DHPM core[a,b]



[a] Reaction conditions: 0.25 mmol DHPM **12a–f**, 3.5 equiv $NaNO_2$, 6 mL (9:1) $AcOH/H_2O$, N_2 atmosphere, sealed vessel at ice bath (0 ºC) for 45 min. [b] Product refers to isolated yields by flash chromatography.

The conversion of hydrazine derivatives **12a**–**f** to tetrazolo dihydrochloride derivatives **10a**–**f**, as the final step in sequence, was readily accomplished by using sodium nitrite as a reducing agent under an inert atmosphere at 0 °C. Acetic acid of 90% was found to be the best solvent for this transformation while less than 90% acetic acid provided incomplete conversions due to poor solubility of hydrazine derivatives. The reaction needed 45 min to completion and provided moderate to good yields of the tetrazolo derivatives of DHPM **10** (Table 3).

DHPM compounds **10a**–**f** may exist in their azide and/or tetrazole forms, respectively.[17] The proton NMR spectrums of **10a**–**f** recorded in MeOD/$CDCl_3$ displayed only single set of signals, which indicates that only one tautomer of both is present. Since a strong azido absorption band (2160–2120 cm^{-1}) in the IR spectrum is absent, it appears evident that compounds **10a**–**f** are present in its tetrazolo form.

The new derivatives were characterized by spectral data and these compounds are currently under investigation for their antioxidant, anti-inflammatory, antimicrobial, calcium channel blocking activity, anti-HIV, diabetes, and for treating and/or preventing diseases of the lungs and cardiovascular system in our laboratories.

3. Conclusions

In conclusion, we have developed a smooth and efficient protocol for the synthesis of novel DHPM tetrazolo derivatives. The conversion of Biginelli 3,4-dihydropyrimidine-2-thiones to dihydrotetrazolo[1,5-*a*]pyrimidine derivatives by the described protocol is applicable for electron rich and electron poor DHPMs as well and provides good overall yields for the tetrazolo adducts. These derivatives were designed, inspired by highly potent bicyclic DHPM derivatives, which have been described earlier in the literature. Due to the fact that tetrazole is a component in different bioactive compounds, the insertion of tetrazole ring in monastrol type DHPMs makes it interesting for pharmacological investigations. Our ongoing interest is to screen these potentially biological active molecules and continue our efforts to design novel DHPM for drug discovery.

4. Experimental section

4.1. General experimental details

NMR spectra were recorded on Bruker AV-III, Bruker AV-500 and Bruker AV-600 instruments. One-dimensional (1D) ^1H NMR spectra were acquired using a 300 and 500 MHz spectrometers while ^{13}C NMR at 75, 100, 125, and 150 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. The letters s, d, t, q, and m are used to indicate singlet, doublet, triplet, quadruplet, and multiplet. Coupling constants (*J* values) are quoted in hertz (Hz).

Electron ionization (EI), electrospray ionization (ESI), and fast atom bombardment (FAB) were measured on JEOL (JMS-600H), Applied Biosystems (QSTAR XL MS/MS System), and JEOL (JMS-HX110), respectively. High-resolution mass spectra were obtained by ESI method using time-of-flight (TOF) analyzer or FAB or EI methods using high-resolution magnetic sector analyzer mass spectrometer. FTIR spectra were recorded on Shimadzu (FTIR-8900) and Bruker (Vector-22).

Analytical HPLC analysis was carried out on Shimadzu (LC-20) using a C^{18} reversed-phase column (RP) (150×4.6 mm, particle size 5 mm) at 25 °C. Mobile phase A (water/acetonitrile 90:10 (v/v)+0.1% TFA) and B (MeCN+0.1% TFA) at a flow rate of 1.0 mL/min were used. The following gradient was applied: linear increase from solution 30% B to 100% B in 8 min, hold at 100% solution B for

7 min. The synthesized compounds were purified via flash chromatography on silica gel or Biotage SP-1 automated flash chromatography system using cartridges packed with KP-SIL, 60 Å (32–63 μm particle size). TLC analyses were performed on precoated (silica gel 60 HF_{254}) plates. All anhydrous solvents (stored over molecular sieves) and chemicals were obtained from standard commercial vendors and were used without any further purification.

4.2. Microwave irradiation experiments

Microwave-assisted synthesis was carried out in an Initiator-8 single-mode microwave instrument producing controlled irradiation at 2.450 GHz (Biotage AB, Uppsala), including proprietary Workflow Manager Software (version 2.1). Experiments were carried out in sealed microwave microwave (2–5 mL, 10–20 mL filling volume) process vials utilizing the standard absorbance level (400 W maximum power). Reaction times under microwave conditions refer to hold times at the temperatures indicated, not to total irradiation times. The temperature was measured with an IR sensor on the outside of the reaction vessel.

4.3. General procedure for the synthesis of dihydropyrimidines precursors (9a–f)

A mixture containing 10 mmol of the corresponding benzaldehyde (**1a**), 15 mmol of corresponding methyl **2a** or ethyl acetoacetate **2b**, 761 mg (10 mmol, 1.0 equiv) of thiourea **3a**, and 620 mg (1.0 mmol, 10 mol %) of $Yb(OTf)_3$ or 1.27 mL (10 mmol, 1.0 equiv) of TMSCl was suspended in 10 mL of anhydrous acetonitrile under a nitrogen atmosphere in a 20 mL microwave vial (Pyrex) equipped with a magnetic stirring bar. The vial was sealed and stirred for 2 min at room temperature to allow homogenization then the mixture was heated for 40 min at 120 °C by microwave irradiation. After cooling the reaction mixture was poured onto 500 mL of ice water and allowed to stir for 1 h in order to obtain maximum precipitation. Filtration then delivers the pure DHPMs. Where needed the product was directly purified by gradient dry flash chromatography using appropriate solvents. The physical and spectroscopic data of DHPM (**9a**–**f**) were found in agreement with the literature.[13a,b]

4.4. General procedure for the synthesis of dimethylated dihydropyrimidines (11a–f)

A mixture of 0.25 mmol of the corresponding DHPMs **9a**–**f**, 69.17 mg (0.50 mmol, 2.0 equiv) of potassium carbonate, and 78 μL (1.25 mmol, 5.0 equiv) of methyl iodide was suspended in 1.2 mL of anhydrous acetonitrile in a 5 mL microwave vial equipped with magnetic stirrer. The reaction vessel was sealed and flushed with nitrogen then heated for 6 h at 80 °C using a conventional oil bath. Thereafter, the solvent was removed under reduced pressure. The product was purified by automated flash chromatography (hexane/ethyl acetate) to provide the desired 2-substituted dihydropyrimidines **11a**–**f** as yellow solids.

*4.4.1. Ethyl 6-(3-hydroxyphenyl)-1,4-dimethyl-2-(methylthio)-1,6-dihydropyrimidine-5-carboxylate (**11a**).* Yellow solid; 68.0 mg (85%); mp 150–152 °C (ethanol); R_f (15% EtOAc/hexane) 0.24; IR (KBr) ν_{max} 3278, 1664, 1230, 1106 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.14 (t, J=7.8 Hz, 1H), 6.78–6.86 (m, 1H), 5.19 (s, 1H), 4.09 (q, J=5.2 Hz, 2H), 3.08 (s, 3H), 2.70 (s, 3H), 2.45 (s, 3H), 1.20 (t, J=7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1, 163.9, 158.8, 143.2, 138.0, 129.9, 118.80, 118.0, 112.8, 107.5, 64.6, 61.4, 39.4, 17.7, 16.9, 14.1; MS (positive EI) m/z 321 (5, M+1), 320 (29, M), 319 (3, M−1), 247 (16, M−73), 227 (100, M−93); HRMS (ESI-TOF) m/z calcd for $C_{16}H_{20}N_2O_3S$ $[M+H]^+$=321.1273, found 321.1274.

*4.4.2. Methyl 1,4-dimethyl-2-(methylthio)-6-phenyl-1,6-dihydropyrimidine-5-carboxylate (**11b**).* Yellow solid; 50.5 mg (70%); mp 100–102 °C (ethanol); R_f (10% EtOAc/hexane) 0.39; IR (KBr) ν_{max} 2924, 2854, 1699, 1621, 1363, 1161, 1067 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.24–7.29 (m, 3H), 7.10–7.13 (m, 2H), 5.57 (s, 1H), 3.77 (s, 3H), 3.55 (s, 3H), 3.47 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.7, 166.1, 147.7, 139.5, 128.9 (2C), 128.0, 125.8 (2C), 105.8, 61.3, 51.6, 43.0, 38.1, 16.9; MS (positive ESI) m/z 291 (5, M+1), 290 (100, M); HRMS (ESI-TOF) m/z calcd for $C_{15}H_{18}N_2O_2S$ $[M+H]^+$=291.1167, found 291.1187.

*4.4.3. Methyl 1,4-dimethyl-2-(methylthio)-6-(p-tolyl)-1,6-dihydropyrimidine-5-carboxylate (**11c**).* Yellow solid; 57.0 mg (75%); mp 74–76 °C; R_f (10% EtOAc/hexane) 0.43; IR (KBr) ν_{max} 2925, 1697, 1599, 1506, 1233, 1100 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.05–7.18 (m, 4H), 5.15 (s, 1H), 3.59 (s, 3H), 2.95 (s, 3H), 2.48 (s, 3H), 2.34 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 162.7, 154.5, 138.8, 137.6, 129.1 (2C), 126.8 (2C), 103.3, 63.0, 50.6, 36.4, 23.0, 21.0, 14.0; MS (positive ESI) m/z 305 (10, M+1), 304 (100, M); HRMS (ESI-TOF) m/z calcd for $C_{16}H_{20}N_2O_2S$ $[M+H]^+$=305.1324, found 305.1322.

*4.4.4. Ethyl 1,4-dimethyl-2-(methylthio)-6-phenyl-1,6-dihydropyrimidine-5-carboxylate (**11d**).* Yellow solid; 57.0 mg (75%); mp 90–92 °C; R_f (10% EtOAc/hexane) 0.41; IR (KBr) ν_{max} 2989, 2503, 1757, 1615, 1381, 1160, 790 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.25–7.28 (m, 4H), 7.06–7.20 (m, 1H), 5.19 (s, 1H), 3.98–4.14 (m, 2H), 2.97 (s, 3H), 2.49 (s, 3H), 2.34 (s, 3H), 1.17 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.3, 161.2, 155.8, 142.9, 129.6 (2C), 129.2, 128.1 (2C), 104.8, 64.2, 60.8, 43.5, 22.9, 14.5, 11.7; MS (positive ESI) m/z 305 (5, M+1), 304 (100, M); HRMS (ESI-TOF) m/z calcd for $C_{16}H_{20}N_2O_2S$ $[M+H]^+$=305.1324, found 305.1331.

*4.4.5. Ethyl 1,4-dimethyl-2-(methylthio)-6-(p-tolyl)-1,6-dihydropyrimidine-5-carboxylate (**11e**).* Yellow solid; 61.0 mg (77%); mp 92–94 °C (ethanol); R_f (10% EtOAc/hexane) 0.48; IR (KBr) ν_{max} 2925, 1690, 1600, 1507, 1384, 1233, 1103 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.17 (d, J=8.0 Hz, 2H), 7.07 (d, J=7.9 Hz, 2H), 5.15 (s, 1H), 4.02–4.09 (m, 2H), 2.96 (s, 3H), 2.48 (s, 3H), 2.34 (s, 3H), 2.28 (s, 3H), 1.18 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.4, 165.1, 155.7, 140.1, 139.2, 130.2 (2C), 128.1 (2C), 105.1, 64.2, 60.8, 37.1, 30.7, 23.0, 21.1, 14.6; MS (direct probe, positive EI) m/z 318 (100, M), 317 (20, M−1); HRMS (ESI-TOF) m/z calcd for $C_{17}H_{22}N_2O_2S$ $[M+H]^+$=319.1480, found 319.1481.

*4.4.6. Ethyl 1,4-dimethyl-2-(methylthio)-6-(p-nitro)-1,6-dihydropyrimidine-5-carboxylate (**11f**).* Yellow solid; 48.0 mg (55%); mp 176–178 °C (ethanol); R_f (15% EtOAc/hexane) 0.37; IR (KBr) ν_{max} 3412, 2976, 2924, 1677, 1519, 1367, 1337, 1237, 1105, 822 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.14 (d, J=8.7 Hz, 2H), 7.46 (d, J=8.7 Hz, 2H), 5.32 (s, 1H), 4.03–4.14 (m, 2H), 2.99 (s, 3H), 2.50 (s, 3H), 2.33 (s, 3H), 1.20 (t, J=7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5, 163.3, 155.3, 148.8, 147.6, 127.8 (2C), 123.9 (2C), 102.9, 62.8, 59.9, 36.8, 23.2, 14.3, 14.2; MS (direct probe, positive EI) m/z 349 (10, M), 320 (15, M−29), 227 (100, M−122); HRMS (EI^+) m/z calcd for $C_{16}H_{19}N_3O_4S$ $[M]^+$=349.1096, found 349.1095.

4.5. General procedure for the synthesis of hydrazinyl dihydropyrimidines dihydrochloride (12a–f)

A mixture of 0.25 mmol of the corresponding *N*,*S*-dimethylated DHPMs **11a**–**f** and 105 mg (1.0 mmol, 4.0 equiv) of $NH_2NH_2 \cdot 2HCl$ was suspended in 3 mL of anhydrous DMF in a 5 mL microwave vial equipped with magnetic stirrer. The reaction vessel was sealed and

flushed with nitrogen then heated for 2 h at 120 °C in a conventional oil bath. Thereafter, the solvent was removed under reduced pressure. The product was purified by automated flash chromatography using (hexane and ethyl acetate) solvents to provide the desired 2-substituted dihydropyrimidines dihydrochloride **12a**–**f** as white solids.

*4.5.1. Ethyl 2-hydrazinyl-6-(3-hydroxyphenyl)-1,4-dimethyl-1,6-dihydropyrimidine-5-carboxylate dihydrochloride (**12a**).* White solid; 61.0 mg (80%); mp 168–170 °C; R_f (25% EtOAc/hexane) 0.50; IR (KBr) ν_{max} 3383, 3209, 1682, 1482, 1442, 1244, 1112 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.17 (t, J=7.9 Hz, 1H), 6.74–6.89 (m, 3H), 5.35 (s, 1H), 4.13 (q, J=5.4 Hz, 2H), 3.31 (s, 3H), 2.30 (s, 3H), 1.24 (t, J=7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 165.3, 156.1, 142.3, 142.0, 130.1, 119.3, 115.5, 113.6, 102.3, 62.9, 60.5, 40.5, 18.4, 14.2; MS (positive ESI) m/z 307 (5, M+1), 306 (100, M), 233 (2, M−73); HRMS (EI^+) m/z calcd for $C_{15}H_{22}N_4O_3$ $[M]^+$=306.1692, found 306.1720.

*4.5.2. Methyl 2-hydrazinyl-1,4-dimethyl-6-phenyl-1,6-dihydropyrimidine-5-carboxylate dihydrochloride (**12b**).* White solid; 51.5 mg (75%); mp 178–180 °C; R_f (15% EtOAc/hexane) 0.39; IR (KBr) ν_{max} 3328, 3180, 2925, 1709, 1436, 1240, 1106, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28 (s, 5H), 5.39 (s, 1H), 3.66 (s, 3H), 3.29 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 165.7, 142.7, 140.3, 128.9 (2C), 128.4, 126.8 (2C), 102.2, 63.2, 51.4, 40.5, 18.3; MS (positive ESI) m/z 277 (5, M+1), 276 (100, M); HRMS (EI^+) m/z calcd for $C_{14}H_{20}N_4O_2$ $[M]^+$=276.1586, found 276.1612.

*4.5.3. Methyl 2-hydrazinyl-1,4-dimethyl-6-(p-tolyl)-1,6-dihydropyrimidine-5-carboxylate dihydrochloride (**12c**).* White solid; 60.5 mg (84%); mp 154–156 °C (ethanol); R_f (10% EtOAc/hexane) 0.24; IR (KBr) ν_{max} 3219, 2949, 1709, 1545, 1382, 1242, 1111, 769 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.09–7.17 (m, 4H), 5.33 (s, 1H), 3.66 (s, 3H), 3.28 (s, 3H), 2.30 (d, J=3.4 Hz, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.8, 167.5, 145.1, 139.4, 139.3, 130.3 (2C), 127.9 (2C), 102.8, 63.9, 51.6, 40.5, 21.1, 17.4; MS (positive ESI) m/z 291 (5, M+1), 290 (100, M); HRMS (EI^+) m/z calcd for $C_{15}H_{22}N_4O_2$ $[M]^+$=290.1743, found 290.1764.

*4.5.4. Ethyl 2-hydrazinyl-1,4-dimethyl-6-phenyl-1,6-dihydropyrimidine-5-carboxylate dihydrochloride (**12d**).* White solid; 61.0 mg (85%); mp 136–138 °C (ethanol); R_f (10% EtOAc/hexane) 0.38; IR (KBr) ν_{max}: 3436, 3185, 2922, 1707, 1552, 1485, 1239, 1105, 699 cm^{-1}; ^1H NMR (300 MHz, DMSO–d6) δ 7.23–7.38 (m, 5H), 5.41 (s, 1H), 4.01 (q, J=3.9 Hz, 2H), 3.16 (s, 3H), 2.25 (s, 3H), 1.12 (t, J=7.0, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.9, 167.0, 145.1, 142.5, 129.7 (2C), 129.3, 128.1 (2C), 102.9, 64.2, 61.3, 40.6, 17.4, 14.5; MS (positive ESI) m/z 291 (5, M+1), 290 (100, M), 258 (6, M−32); HRMS (EI^+) m/z calcd for $C_{15}H_{22}N_4O_2$ $[M]^+$=290.1743, found 290.1731.

*4.5.5. Ethyl 2-hydrazinyl-1,4-dimethyl-6-(p-tolyl)-1,6-dihydropyrimidine-5-carboxylate dihydrochloride (**12e**).* White solid; 60.5 mg (80%); mp 140–142 °C (ethanol); R_f (10% EtOAc/hexane) 0.28; IR (KBr) ν_{max}: 3217, 2923, 1701, 1638, 1554, 1477, 1234, 1102, 784 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.09–7.17 (m, 4H), 5.33 (s, 1H), 4.08–4.14 (m, 2H), 3.28 (s, 1H), 2.30 (d, 6H, 4.0 Hz), 1.22-1.25 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.8, 167.1, 144.9, 139.5, 139.3, 130.3 (2C), 128.1 (2C), 103.0, 64.0, 61.2, 40.5, 21.1, 17.4, 14.5; MS (positive ESI) m/z 305 (5, M+1), 304 (100, M); HRMS (EI^+) m/z calcd for $C_{16}H_{24}N_4O_2$ $[M]^+$=304.1899, found 304.1930.

*4.5.6. Ethyl 2-hydrazinyl-1,4-dimethyl-6-(p-nitro)-1,6-dihydropyrimidine-5-carboxylate dihydrochloride (**12f**).* White solid; 77.0 mg (76%); mp 183–185 °C (ethanol); R_f (25% EtOAc/hexane) 0.52; IR (KBr) ν_{max}: 3219, 1699, 1641, 1522, 1476, 1349, 1238, 1118 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, J=8.6 Hz, 2H), 7.48 (d, J=8.6 Hz, 2H), 5.53 (s, 1H), 4.10–4.25 (m, 2H), 3.31 (s, 3H), 2.33 (s, 3H), 1.26 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, CD Cl_3) δ 175.4, 164.9, 147.8, 147.2, 143.2, 127.7 (2C), 124.2 (2C), 101.4, 62.5, 60.7, 40.6, 18.5, 14.2; MS (direct probe, positive EI) m/z 336 (15, M+1), 335 (60, M), 213 (100, M−122); HRMS (ESI-TOF) m/z calcd for $C_{15}H_{21}N_5O_4$ $[M+H]^+$=336.1672, found 336.1646.

4.6. General procedure for the synthesis of tetrazolo dihydropyrimidines dihydrochloride (10a–f)

A dry 5 mL microwave vial equipped with a magnetic stirrer was flushed with nitrogen and then charged with 0.25 mmol of the corresponding hydrazinyl dihydropyrimidines dihydrochloride **12a**–**f** and 69 mg (1.0 mmol, 4.0 equiv) of sodium nitrite ($NaNO_2$). The reaction vessel was sealed and flushed with nitrogen and kept at 0 °C in a $CaCl_2$ ice bath. Then 6 mL of 90% cold acetic acid is added slowly in the dark and the reaction mixture was kept at 0 °C for further 45 min under N_2 atmosphere with continuous stirring. Thereafter, the reaction mixture was concentrated and co-evaporated with 3–4 mL of toluene under reduced pressure at room temperature. The product was purified by automated flash chromatography using (chloroform and methanol) solvents to provide the desired 2-substituted dihydropyrimidines dihydrochloride (**10a**–**f**) as oils.

*4.6.1. Ethyl 7-(3-hydroxyphenyl)-6,9-dimethyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carboxylate dihydrochloride (**10a**).* Orange oil; 51.0 mg (65%); R_f (10% MeOH/$CHCl_3$) 0.23; IR (KBr) ν_{max} 3138, 2929, 1711, 1381, 1262, 767 cm^{-1}; ^1H NMR (300 MHz, CD_3OH) δ 7.23 (t, J=7.7 Hz, 1H), 6.80–6.90 (m, 3H, m), 5.48 (s, 1H), 4.08 (q, J=5.8 Hz, 2H), 3.16 (s, 3H), 2.39 (s, 3H), 1.14 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.1, 158.8, 150.8, 146.0, 140.5, 129.5, 119.0, 117.3, 113.8, 105.5, 61.0, 60.6, 39.6, 19.1, 14.1; MS (positive FAB) m/z 318 (10, M+1), 275 (100, M−42); HRMS (ESI-TOF) m/z calcd for $C_{15}H_{19}N_5O_3$ $[M+H]^+$=318.1566, found 318.1587.

*4.6.2. Methyl 6,9-dimethyl-7-phenyl-6,7-dihydrotetrazolo[1,5-a]pyrimidine-8-carboxylate dihydrochloride (**10b**).* Orange oil; 45.5 mg (64%); R_f (5% MeOH/$CHCl_3$) 0.21; IR (KBr) ν_{max} 2950, 2923, 1703, 1625, 1545, 1377, 1254, 1187, 1095, 762, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31–7.33 (m, 5H), 5.31 (s, 1H), 3.60 (s, 3H), 3.06 (s, 3H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 151.9, 151.4, 140.9, 128.8 (2C), 128.7, 127.5 (2C), 104.4, 60.7, 51.1, 38.7, 21.6; MS (positive FAB) m/z 288 (20, M+1), 245 (100, M−42); HRMS (FAB^+) m/z calcd for $C_{14}H_{17}N_5O_2$ $[M+H]^+$=288.1461, found 288.1478.

*4.6.3. Methyl 6,9-dimethyl-7-(p-tolyl)-6,7-dihydrotetrazolo[1,5-a]pyrimidine-8-carboxylate dihydrochloride (**10c**).* Orange oil; 51.0 mg (68%); R_f (5% MeOH/$CHCl_3$) 0.22; ^1H NMR (300 MHz, CD_3OD) δ 7.15–7.25 (m, 4H), 5.31 (s, 1H), 3.56 (s, 3H), 2.95 (s, 3H), 2.31 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 152.0, 151.4, 138.5, 138.2, 129.4 (2C), 127.4 (2C), 104.4, 60.3, 51.0, 38.6, 21.7, 21.1; MS (positive FAB) m/z 302 (10, M+1), 259 (100, M−42); HRMS (FAB^+) m/z calcd for $C_{15}H_{19}N_5O_2$ $[M+H]^+$=302.1617, found 302.1645.

*4.6.4. Ethyl 6,9-dimethyl-7-phenyl-6,7-dihydrotetrazolo[1,5-a]pyrimidine-8-carboxylate dihydrochloride (**10d**).* Orange oil; 46.5 mg (62%); R_f (6% MeOH/$CHCl_3$) 0.28; ^1H NMR (300 MHz, CDCl3) δ 7.03–7.21 (m, 5H), 5.20 (s, 1H), 3.94 (q, J=6.8 Hz, 2H), 2.89 (s, 3H), 2.29 (s, 3H), 1.05–1.09 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 166.5, 152.5, 141.4, 138.3, 130.6, 130.0 (2C), 129.1 (2C), 106.6, 62.2, 61.5, 39.8, 19.5, 14.4; MS (positive FAB) m/z 302 (5, M+1), 259 (100, M−42); HRMS (FAB^+) m/z calcd for $C_{15}H_{19}N_5O_2$ $[M+H]^+$=302.1617, found 302.1638.

*4.6.5. Ethyl 6,9-dimethyl-7-(p-tolyl)-6,7-dihydrotetrazolo[1,5-a]pyrimidine-8-carboxylate dihydrochloride (**10e**).* Orange oil; 47.5 mg

(61%); R_f (5% MeOH/$CHCl_3$) 0.26; ^1H NMR (300 MHz, $CDCl_3$) δ 7.06–7.20 (m, 4H), 5.23 (s, 1H), 4.02 (q, J=4.5 Hz, 3H), 3.00 (s, 3H), 2.37 (s, 3H), 2.29 (s, 3H), 1.13 (t, J=7.1, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 166.5, 152.3, 147.1, 140.5, 138.4, 130.6 (2C), 129.1 (2C), 106.7, 61.9, 61.6, 39.7, 21.2, 19.4, 14.3; MS (positive FAB) m/z 316 (20, M+1), 273 (100, M−42); HRMS (FAB$^+$) m/z calcd for $C_{16}H_{21}N_5O_2$ $[M+H]^+$=316.1774, found 316.1803.

*4.6.6. Ethyl 6,9-dimethyl-7-(p-nitro)-6,7-dihydrotetrazolo[1,5-a]pyrimidine-8-carboxylate dihydrochloride (**10f**).* Orange oil; 85.5 mg (82%); R_f (5% MeOH/$CHCl_3$) 0.20; IR (KBr) ν_{max} 2979, 2923, 1701, 1623, 1523, 1373, 1347, 1260, 1187, 1093, 825 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, J=8.5 Hz, 2H), 7.52 (d, J=8.4 Hz, 2H), 5.38 (s, 1H), 3.98–4.08 (m, 2H), 2.91 (s, 3H), 2.33 (s, 3H), 1.15 (t, J=7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.4, 165.7, 152.8, 151.9, 147.7, 128.5 (2C), 124.0 (2C), 103.7, 60.3, 60.1, 38.9, 21.5, 14.1; MS (positive ESI) m/z 347 (5, M+1), 304 (100, M−42); HRMS (ESI-TOF) m/z calcd for $C_{15}H_{18}N_6O_4$ $[M+H-N_3]^+$=304.1297, found 304.1283.

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Supplementary data

These data include the copies of the ^1H NMR and ^{13}C NMR spectra of all new compounds **10a**–**f**, **11a**–**f**, and **12a**–**f**. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2014.09.069.

References and notes

1. (a) Ostrovskii, V. A.; Trifonov, R. E.; Popova, E. A. *Russ. Chem. Bull., Int. Ed.* **2012**, *61*, 768; (b) Ostrovskii, V. A.; Koldobskii, G. I.; Trifonov, R. E. *Comprehensive Heterocyclic Chemistry III*; Elsevier: Amsterdam, Netherlands, 2008, Vol. 6, pp 257–423; (c) Ramesh, B.; Bhalgat, C. M. *Eur. J. Med. Chem.* **2011**, *46*, 1882.
2. Vaghasia, S. J.; Shah, V. H. *J. Serb. Chem. Soc.* **2007**, *72*, 109.
3. Muenter, K.; Kirchengast, M.; Hergenroeder, S. *Ger. Pat.* DE-19744799-A1, **1999**.
4. Naidu, B. N.; Sorenson, M. E.; Ueda, Y.; Matiskella, J. D.; Walker, M. A. U.S. Patent US-20,070,111,985-A1, 2007.
5. Yadlapalli, R. K.; Chourasia, O. P.; Vemuri, K.; Sritharan, M.; Perali, R. S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2708.
6. Chikhale, R. V.; Bhole, R. P.; Khedekar, P. B.; Bhusari, K. P. *Eur. J. Med. Chem.* **2009**, *44*, 3645.
7. Lewis, R. W.; Mabry, J.; Polisar, J. G.; Eagen, K. P.; Ganem, B.; Hess, G. P. *Biochemistry* **2010**, *49*, 4841.
8. Virsodia, V.; Pissurlenkar, R. R. S.; Manvar, D.; Dholakia, C.; Adlakha, P.; Shah, A.; Coutinho, E. C. *Eur. J. Med. Chem.* **2008**, *43*, 2103.
9. Chiang, A. N.; Valderramos, J. C.; Balachandran, R.; Chovatiya, R. J.; Mead, B. P.; Schneider, C.; Bell, S. L.; Klein, M. G.; Huryn, D. M.; Chen, X. S.; Day, B. W.; Fidock, D. A.; Wipf, P.; Brodsky, J. L. *Bioorg. Med. Chem.* **2009**, *17*, 1527.
10. Kappe, C. O. *Eur. J. Med. Chem.* **2000**, *35*, 1043.
11. Carroll, W.; Perez-Medrano, A.; Florjancic, A.; Nelson, D.; Peddi, S.; Li, T.; Bunnelle, E.; Hirst, G.; Li, B. U.S. Patent US-20,060,052,374-A1, 2006.
12. (a) Osman, A. H.; Aly, A. A. M.; El-Maali, N. A.; Al-Hazmi, G. A. A. *Synth. React. Inorg. Met.-Org. Chem.* **2002**, *32*, 1289; (b) Gaponik, P. N.; Voitekhovich, S. V.; Ivashkevich, O. A. *Russ. Chem. Rev.* **2006**, *75*, 507.
13. (a) Desai, B.; Dallinger, D.; Kappe, C. O. *Tetrahedron* **2006**, *62*, 4651; (b) Kappe, C. O.; Stadler, A. *Org. React.* **2004**, *63*, 1; (c) Kappe, C. O. *Acc. Chem. Res.* **2000**, *33*, 879; (d) Kappe, C. O. In *Multicomponent Reactions*; Zhu, J., Bienaymé, H., Eds.; Wiley-VCH: Weinheim, Germany, 2005, p 95.
14. Klein, E.; DeBonis, S.; Thiede, B.; Skoufias, A. D.; Kozielskib, F.; Lebeau, L. *Bioorg. Med. Chem.* **2007**, *15*, 6474.
15. Kappe, C. O.; Roschger, P. *J. Heterocycl. Chem.* **1989**, *26*, 55.
16. Matloobi, M.; Kappe, C. O. *J. Comb. Chem.* **2007**, *9*, 275.
17. (a) Temple, C., Jr.; Coburn, W. C., Jr.; Thorpe, M. C.; Montgomery, J. A. *J. Org. Chem.* **1965**, *30*, 2395; (b) Nag, S.; Bhowmik, S.; Gauniyal, H. M.; Batra, S. *Eur. J. Org. Chem.* **2010**, 4705; (c) Temple, C., Jr.; Montgomery, J. A. *J. Org. Chem.* **1965**, *30*, 826; (d) Wentrup, C. *Tetrahedron* **1970**, *26*, 4969; (e) Hull, W. E.; Künstlinger, M.; Breitmaier, E. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 924; (f) Hamed, A. A.; Zeid, I. F.; El-Ganzory, H. H.; Aal, M. T. A. *Monatsh. Chem.* **2008**, *139*, 809.