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Khadiga M. Abu-Zied, Tahia K. Mohamed, Omar K. Al-Duiaj and Magdi E.A. Zaki*

A simple approach to fused pyrido[2,3-*d*] pyrimidines incorporating khellinone and trimethoxyphenyl moieties as new scaffolds for antibacterial and antifungal agents

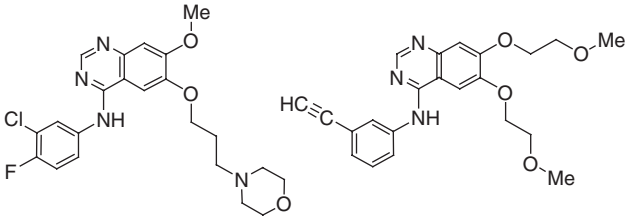
Abstract: 2-Amino-3-cyanopyridine is a simple precursor for the synthesis of analogues of Egyptian natural products visnagin and khellin. Fused pyrido[2,3-*d*]pyrimidines were prepared under mild reaction conditions. The detailed syntheses and spectroscopic data of the synthesised compounds are reported. Some isolated compounds show antibacterial and antifungal activity.

Keywords: 2-amino-3-cyanopyridine; antibacterial activity; antifungal activity; khellinone; *N*-pyrazolyl derivatives; pyrido[2,3-*d*]pyrimidine; trimethoxyphenyl.

***Corresponding author: Magdi E.A. Zaki,** National Research Centre, Photochemistry Department, Cairo, Egypt; and Chemistry Department, College of Science, IMSIU (Al-Imam Mohammad Ibn Saud Islamic University), Riyadh 11623, Kingdom of Saudi Arabia, e-mail: mezaki@imamu.edu.sa
Khadiga M. Abu-Zied: National Research Centre, Photochemistry Department, Cairo, Egypt
Tahia K. Mohamed: National Research Centre, Chemistry of Natural Compounds Department, Cairo, Egypt
Omar K. Al-Duiaj: Chemistry Department, College of Science, IMSIU (Al-Imam Mohammad Ibn Saud Islamic University), Riyadh 11623, Kingdom of Saudi Arabia

Introduction

Bicyclic nitrogen-containing heterocyclic compounds such as purines [1–3], quinazolines [4–6], pteridines and pyrido-pyrimidines [7, 8] are well-known pharmacophores in drug discovery. Gefitinib and erlotinib are examples of market drugs with a bicyclic core structure, which are quinazoline derivatives acting as tyrosine kinase inhibitors and both are used for treatment of non-small cell lung cancer.



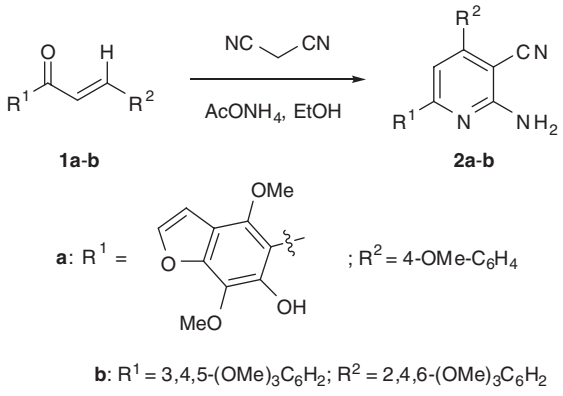
Gefitinib **Erlotinib**

Pyrido[2,3-*d*]pyrimidines, as analogues of quinazoline, have been intensively investigated. This scaffold is associated with a wide range of biological activities including dihydrofolate reductase (DHFR) inhibition, antitumor activity [9–11], adenosine kinase inhibition [12] and tyrosine kinase inhibition [13, 14], among other properties [15–18]. Part of our ongoing interest is to explore new scaffolds based on analogues of Egyptian natural products such as khellinone and its analogues.

Results and discussion

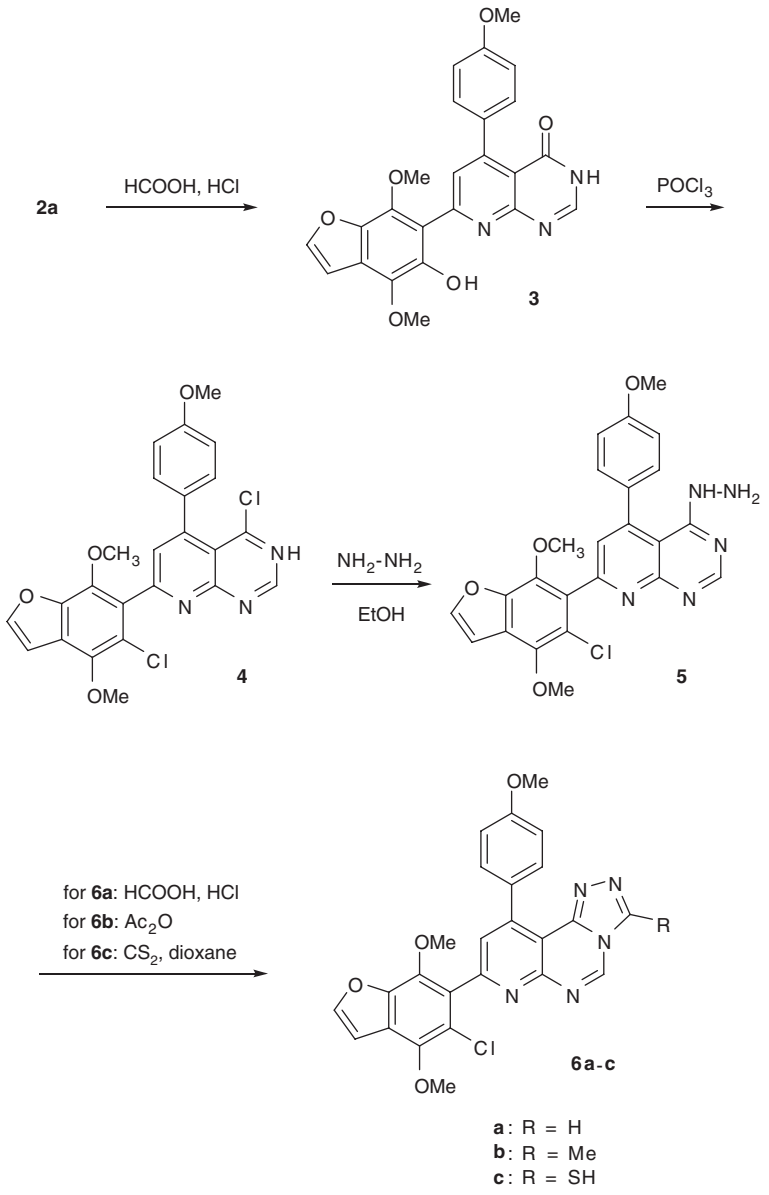
Chemistry

The interesting biological activity of khellinone derivatives [19] prompted us to continue our investigation of pyrido[2,3-*d*]pyrimidine incorporating 4,7-dimethoxy-1-benzofuran-5-ol moiety or trimethoxyphenyl moiety. A facile synthesis of 2-amino-3-cyanopyridine derivatives in a one-pot reaction was achieved using aromatic aldehyde, methyl ketone, malononitrile and ammonium acetate [20] or by preparing α,β-unsaturated ketone followed by reaction with malononitrile in the presence of ammonium acetate [21, 22]. 2-Amino-3-cyanopyridine is considered a simple and convenient precursor for the synthesis of pyrido[2,3-*d*]pyrimidine incorporating some new khellinone derivatives and its analogues.



Scheme 1

Thus, refluxing substrate **1a** [21, 22] or **1b** with anhydrous ammonium acetate and malononitrile in ethanol for 8 h led to the formation of the product **2a** or **2b**, respectively, in good yield (Scheme 1). Treatment of **2a** with formic acid yielded pyrido[2,3-*d*]pyrimidine-4(3*H*)-one **3** in a moderate yield (Scheme 2). Treatment of **3** with phosphorus oxychloride in dioxane led to the formation of a pyrido[2,3-*d*] pyrimidine derivative **4** in poor yield. We believe that the formation of a hydrochloride salt of the pyrido[2,3-*d*] pyrimidine substrate may be responsible for the low yield. The dichloro-pyrido[2,3-*d*]pyrimidine **4** was allowed to react with hydrazine hydrate to give derivative **5** in low yield. Compound **5** was transformed into triazolo[4,3-*c*]



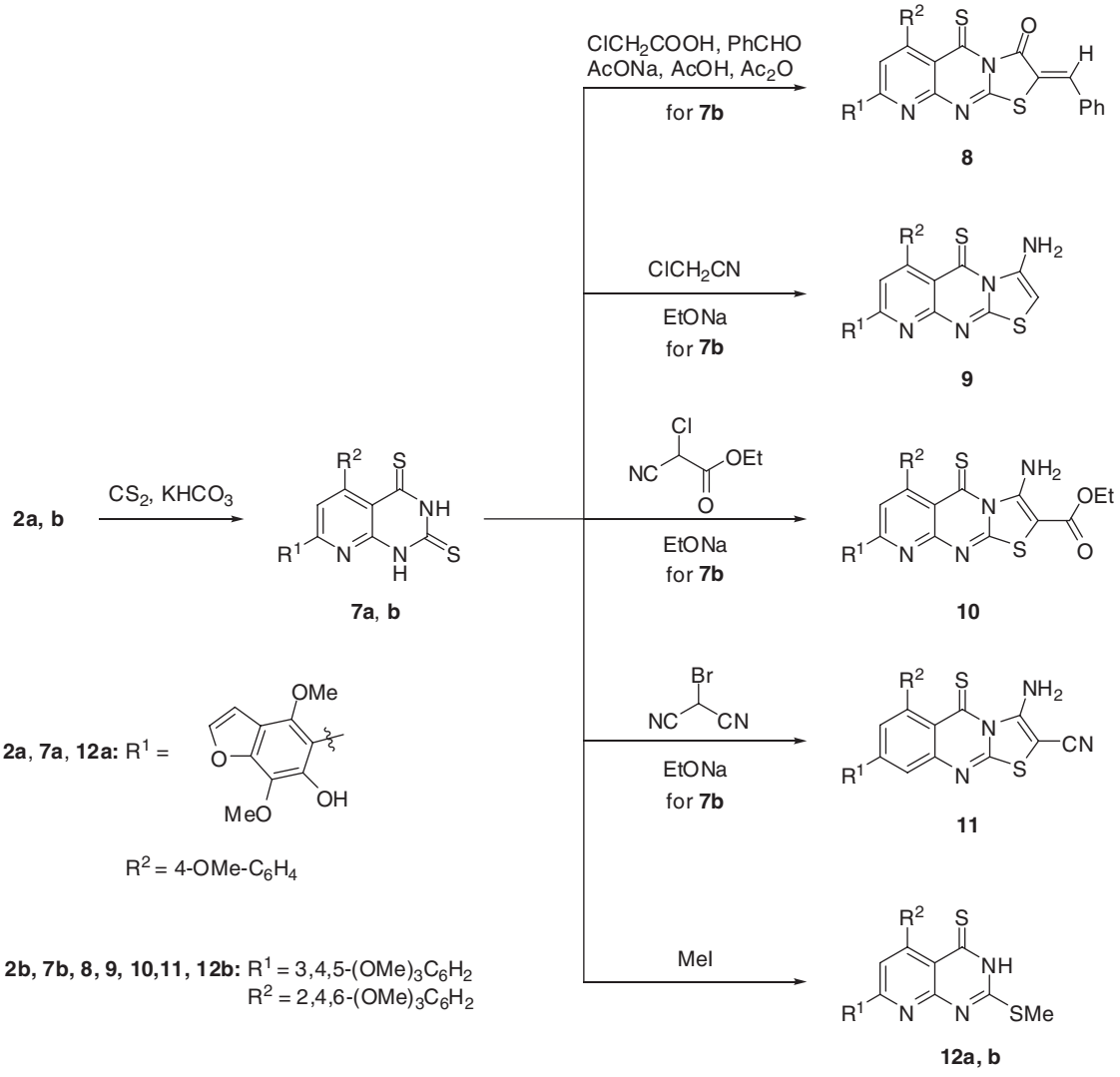
Scheme 2

pyrimidines **6a–c** (Scheme 2), which may possess pharmacological properties similar to theophylline [23–26].

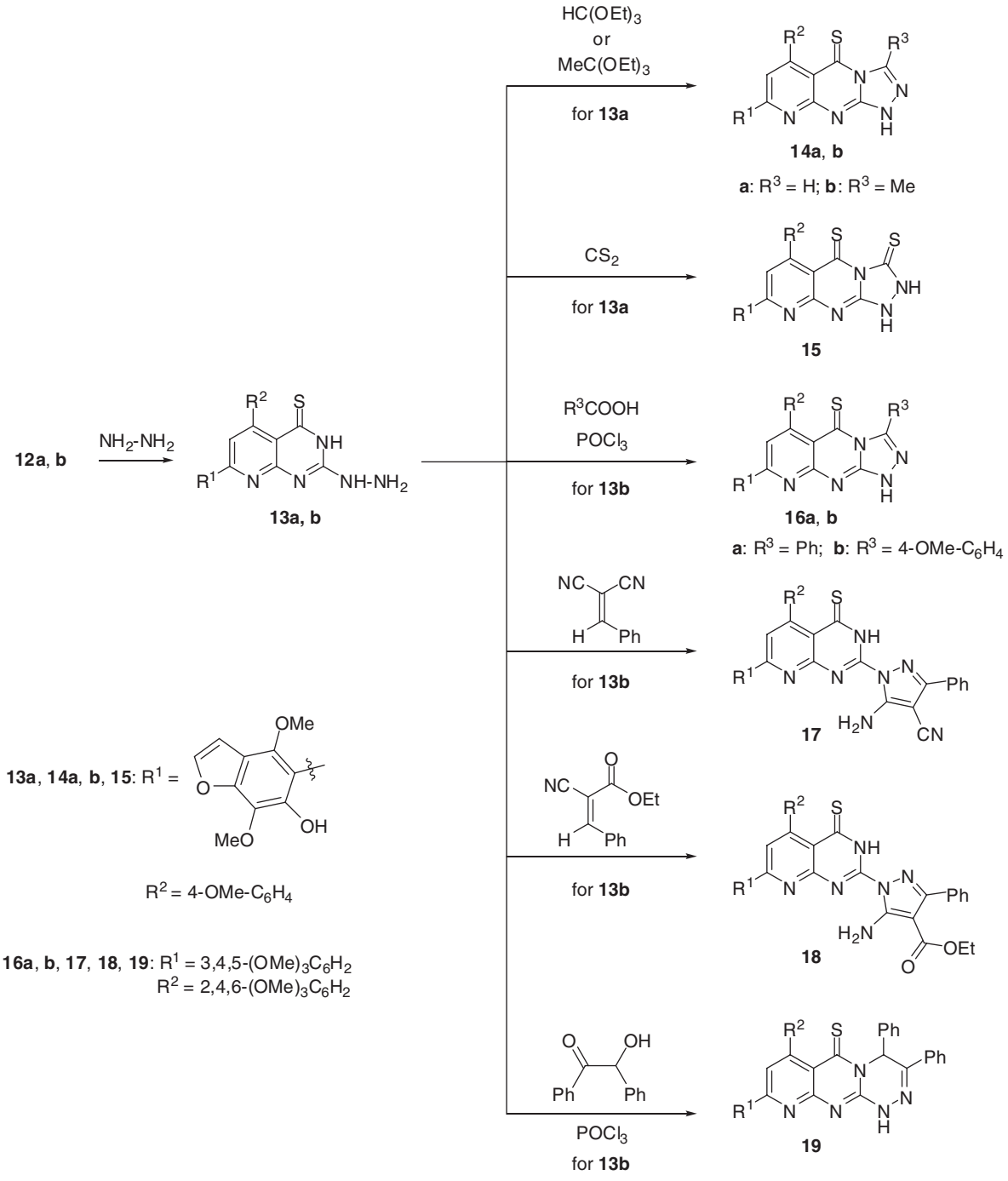
The poor yield was a problem in the synthesis of a variety of fused pyridopyrimidine derivatives. Herein, we explored a method that can offer a better yield of pyridopyrimidine products. As can be seen from Scheme 3, compounds **2a,b** were allowed to react with carbon disulphide in the presence of hydrogen sodium carbonate yielding the respective dithiones **7a** and **7b** in good yield. Compound **7b** was allowed to react with chloroacetic acid and benzaldehyde in the presence of sodium acetate under reflux in a mixture of acetic acid and acetic anhydride to afford the product **8** in a moderate yield.

The alkylation of compound **7b** with the appropriate functionalised halo compounds, followed by intramolecular cyclisation, led to the formation of the corresponding pyrido[2,3-*d*][1,3]thiazolo[3,2-*a*]pyrimidine derivatives **8–11.**

The introduction of a good leaving group was pursued in another approach. Thus, compounds **7a,b** were treated with iodomethane leading to formation of the respective methylthio derivatives **12a,b** in good yield. The nucleophilic substitution of the methylthio group took place by treatment of compounds **12a,b** with hydrazine hydrate leading to the formation of hydrazino derivatives **13a,b** (Scheme 4). Compound **13a** was allowed to react with two ortho esters and carbon disulphide to furnish the respective thiones **14a**, **14b** and **15**. Yet, another approach to the construction of pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine derivatives utilises the reaction of compound **13b** with appropriate carboxylic acid in the presence of phosphorus oxychloride. Compounds **16a,b** were synthesised



Scheme 3



Scheme 4

in this way. A different methodology for the preparation of unfused biheterocyclic products **17** and **18** involves conjugate addition of the hydrazine derivative **13b** with appropriate α,β-unsaturated nitriles in the presence of piperidine as a catalyst. Compound **13b** was also allowed to react with benzoin in the presence of phosphorus oxychloride (Scheme 4). This reaction led to the formation of the fused tricyclic thione **19**.

Biological activity

Some of the synthesised compounds were evaluated for their antimicrobial activity. The results were compared with the activity of well-known antimicrobial and antifungal standards. The results of the preliminary screening tests are given in Table 1. As can be seen, some khellinone derivatives show higher antimicrobial activity than the standard.

Table 1 Antimicrobial activity of selected compounds.

Tested compounds and standards (μg/mL)	Inhibition zone diameter (mm/mg sample)				
	Escherichia coli (G^-)	*Staphylococcus aureus* (G^+)	*Bacillus subtilis* (G^+)	*Candida albicans* (yeast)	*Aspergillus niger* (fungi)
1	++	++	+++	–	–
2a	++	++	++	–	–
3	+++	++	+++	++	–
4	+++	++	++	++	+++
5	+++	+++	++	++	++
6a	+	+	+	++	–
6b	++	++	++	++	++
6c	++	++	++	++	+++
7a	++	++	++	++	++
12a	++	++	++	+++	++
13a	++	++	++	++	++
14a	+++	++	++	++	++
14b	++	+	+	+	++
14c	++	+++	++	+	+++
Levofloxacin[a]	+++	+++	+++	-	–
Nystatin[b]	–	–	–	+++	+++

+++, Highly active (21–25 mm); ++, fairly active (16–20 mm); +, slightly active (15–10 mm); –, not active.
[a]Levofloxacin is an anti-Gram positive and anti-Gram negative antibiotic.
[b]Nystatin is an antifungal antibiotic.

Experimental

General

Melting points are uncorrected. Microanalyses were carried out at the Microanalytical Unit, National Research Centre and Faculty of Science, Cairo University. The IR spectra were recorded in KBr pellets on an FT-IR NEXCES spectrophotometer (Shimadzu, Japan). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured with a Jeol ECA 500 (Japan) in DMSO-d_6 or $CDCl_3$. Mass spectra (EI) were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo Instrument System Incorporation, USA). All reactions were followed up by TLC analysis.

Biological evaluation

The antimicrobial activity was determined by the cup plate technique method with some modifications. Levofloxacin and nystatin were used as reference antibiotics. The medium was nutrient agar for bacteria, potato dextrose for fungi, and the tested microorganisms were Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, Gram-negative bacteria *Escherichia coli*, yeast (single cell fungus) *Candida albicans* and multicellular fungus *Aspergillus niger*. Data were obtained according to the following procedure. A volume of 40 mL of the medium (at 55–60°C) was inoculated with 200 μL of the prepared test microorganism suspensions and poured into 15 cm diameter plates, mixed well and allowed to solidify. After solidification, holes (0.9 cm diameter) were made in the agar plates with the aid of a sterile cork-borer. In the holes, 50 μL of the dissolved sample was placed using an automatic micropipette. The Petri dishes were left at 5°C for 1 h to allow diffusion of the antibiotic through the agar medium prior to the growth of the test organism, and then they were incubated at 30°C for 24 h. The antimicrobial data are compiled in Table 1.

2-Amino-6-(5-hydroxy-4,7-dimethoxy-1-benzofuran-6-yl)-4-(4-methoxyphenyl)nicotinonitrile (2a) Compound **1** (1 mmol), malononitrile (1.1 mmol) and anhydrous ammonium acetate (2 mmol) were heated under reflux in absolute ethanol (15 mL) for 8 h. The reaction mixture was cooled to room temperature and the precipitate was collected upon filtration, washed several times with water, dried and crystallised from ethanol to give compound **2a** as yellow crystals in 53% yield; mp 201–203°C; IR (cm^{-1}, ν): 3440 (br, OH), 3256, 3253 (NH_2), 2224 (CN); ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.1 (s, 3H, OCH_3), 7.11 (d, J = 7.25 Hz, 1H, CH), 7.45 (d, J = 8.4 Hz, 2H, ArH), 7.69 (d, J = 8.4 Hz, 2H, ArH), 7.73 (s, 1H, ArH), 7.78 (d, J = 7.3 Hz 1H, CH) and 8.2 (br, s, 2H, NH_2, D_2O exchangeable); ^{13}C NMR ($CDCl_3$): δ 46.6, 46.8, 52.7, 99.1, 111.3, 128.1, 134, 138, 143, 145, 146.2, 147.1, 147.4, 147.7, 147.8, 148.6, 148.9, 149.2, 149.6, 149.8,151.3; MS: m/z 414 (M^+, 100%). Anal. Calcd for $C_{23}H_{19}N_3O_5$: C, 66.18; H, 4.59; N, 10.07. Found: C, 66.35; H, 4.78; N, 10.19.

2-Amino-4-(2,4,6-trimethoxyphenyl)-6-(3,4,5-trimethoxyphenyl)nicotinonitrile (2b) A mixture of **1** (1 mmol), malononitrile (1.2 mmol) and anhydrous ammonium acetate (80 mmol) in absolute ethanol was heated on a water bath for 8 h. After cooling, the obtained product was filtered and washed several times with water, dried and then crystallised from ethanol affording **2b** in 78% yield; mp 256°C; IR (cm^{-1}, ν): 3266, 3242 (NH_2), 2231 (CN); ^{1}H NMR (DMSO-d_6): δ 3.62 (s, 6H, 2 OCH_3), 3.71 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.86 (s, 6H, 2 OCH_3), 6.81 (s, 2H, ArH), 7.01 (s, 2H, ArH), 7.75 (s, 1H, CH), 8.07–8.10 (br, 2H, NH_2, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6): δ 55.1, 55.9, 57.1,

59.7, 92.4, 97.6, 103.6, 114.2, 118.1, 119.9, 124.1, 147.4, 151.9, 152.4, 158.4, 158.9, 159.3, 164.7. Anal. Calcd for $C_{24}H_{25}N_3O_6$: C, 63.85; H, 5.58; N, 9.31. Found: C, 63.69; H, 5.78.; N, 9.19.

7-(5-Hydroxy-4,7-dimethoxybenzofuran-6-yl)-5-(4-methoxyphenyl)pyrido[2,3-*d*]pyrimidine-4(3*H*)-one (3) A mixture of compound **2** (1 mmol) and formic acid (5 mL) was heated and stirred for 8 h. The precipitate, isolated after neutralisation with sodium hydroxide solution, was crystallised from ethanol to furnish compound **3** as shiny yellow crystals in 55% yield; mp 188°C; IR (cm^{-1}, ν): 3443 (br, OH), 3358, (NH), 1668 (C=O); ^1H NMR ($CDCl_3$): δ 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.1(s, 3H, OCH_3), 7.15 (d, *J* = 7.3 Hz, 1H, CH), 7.45 (d, *J* = 8.4 Hz, 2H, ArH), 7.69 (d, *J* = 8.4 Hz, 2H, ArH), 7.74 (s, 1H, ArH), 7.78 (d, *J* = 7.3 Hz, 1H, CH), 8.31 (s, 1H, ArH), 8.49 (br, s, 1H, NH, D_2O exchangeable); MS: m/z 417 (M^+, 100%). Anal. Calcd for $C_{24}H_{19}N_3O_6$: C, 64.72; H, 4.30; N, 9.43. Found: C, 64.59; H, 4.08; N, 9.23.

4-Chloro-7-(6-chloro-4,7-dimethoxy-1-benzofuran-6-yl)-5-(4-methoxyphenyl)pyrido[2,3-*d*]pyrimidine (4) A mixture of compound **3** (5 mmol) and phosphorus oxychloride (10 mL) in dry dioxane (15 mL) was heated under reflux for 1 h, then the mixture was cooled to room temperature and poured into crushed ice. The resultant precipitate was filtered, washed with ice water and dried to afford compound **4** in 56% yield; mp 175°C; IR (cm^{-1}, ν): 3349 (NH); ^1H NMR ($CDCl_3$): δ 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 7.22 (d, *J* = 6.7 Hz, 1H, CH), 7.45 (d, *J* = 8.7 Hz, 2H, ArH), 7.73 (d, *J* = 8.7 Hz, 2H, ArH), 7.77 (d, *J* = 6.7 Hz, 1H, CH), 7.69 (s, 1H, CH), 8.28 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 43.6, 46.8, 52.8 (3 OCH_3), 99.1, 111.3, 128.1, 134.0, 138.0, 143.0, 145.0, 146.2, 147.4, 147.7, 147.8, 148.3, 148.6, 148.7, 148.9, 149.2, 149.6, 149.8, 151.3. Anal. Calcd for $C_{24}H_{17}Cl_2N_3O_4$: C, 59.77; H, 3.55; N, 8.71. Found: C, 56.59; H, 3.87; N, 8.58.

7-(5-Chloro-4,7-dimethoxy-1-benzofuran-6-yl)-4-hydrazino-5-(4-methoxyphenyl)pyrido[2,3-*d*]pyrimidine (5) A solution of compound **4** (5 mmol) and hydrazine hydrate (0.15 mL) in ethanol (15 mL) was stirred under reflux for 10 h (under TLC control) whereby a greenish yellow precipitate was formed. The precipitate was filtered, washed with water, dried and crystallised from ethanol to furnish **5** in a yield of 48%; mp 204–206°C; IR (cm^{-1}, ν): 3344 (NH), 3277, 3270 (NH_2); ^1H NMR (DMSO-d_6): δ 3.74 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.1 (s, 3H, OCH_3), 6.65 (br, s, 2 H, NH_2, D_2O exchangeable), 7.40 (d, *J* = 6.7 Hz, 1H, CH), 7.55 (d, *J* = 8.6 Hz, 2H, ArH), 7.77 (d, *J* = 8.6 Hz, 2H, ArH), 7.81 (s, 1H, CH), 7.88 (d, *J* = 6.7 Hz 1H, CH), 8.56 (s, 1H, Ar-H), 9.12 (br, s, H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 43.6, 46.8, 52.8 (3 OCH_3), 99.1, 111.3, 128.1, 134.0, 138.0, 143.0, 145.0, 146.2, 147.1, 147.4, 147.7, 147.8, 148.3, 148.6, 148.7, 148.9, 149.2, 149.6, 149.8 and 151.3. Anal. Calcd for $C_{24}H_{20}ClN_5O_4$: C, 60.30; H, 4.22; N, 14.65. Found: C, 59.98; H, 4.57; N, 14.43.

General procedure for pyrido[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine derivatives 6a–c

A mixture of compound **5** (1 mmol), formic acid (1 mL) and concentrated hydrochloric acid (1 mL) in the case of **6a** or acetic anhydride (5 mL) in the case of **6b** or carbon disulphide (1 mL) in the case of **6c**, was heated gently on a water bath for 6–8 h (under TLC control). The reaction mixture was cooled to room temperature, poured into cold water (100 mL), and the resultant precipitate was collected by filtration, washed several times with water, dried and crystallised from ethanol.

8-(5-Chloro-4,7-dimethoxy-1-benzofuran-6-yl)-10-(4-methoxyphenyl)pyrido[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine (6a) This compound was isolated in 61% yield; mp 161–162°C; ^1H NMR ($CDCl_3$): δ 3.78 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.10 (s, 3H, OCH_3), 7.20 (d, *J* = 6.7 Hz, 1H, CH), 7.55 (d, *J* = 8.7 Hz, 2H, ArH), 7.66 (s, 1H, CH), 7.77 (d, *J* = 8.7 Hz, 2H, ArH), 7.88 (d, *J* = 6.7 Hz, 1H, CH), 8.56 (s, 1H, CH, ArH), 8.68 (s, 1H, CH, ArH). Anal. Calcd for $C_{25}H_{18}ClN_5O_4$: C, 61.54; H, 3.72; N, 14.35. Found: C, 61.38; H, 3.57; N, 14.21.

8-(5-Chloro-4,7-dimethoxy-1-benzofuran-6-yl)-10-(4-methoxyphenyl)-3-methylpyrido[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine (6b) This compound was isolated in 58% yield; mp 183–184°C; yellow crystals; ^1H NMR ($CDCl_3$): δ 2.21 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 7.20 (d, *J* = 6.7 Hz, 1H, CH), 7.55 (d, *J* = 8.7 Hz, 2H, ArH), 7.78 (d, *J* = 8.7 Hz, 2H, ArH), 7.84 (s, 1H, CH, ArH), 7.88 (d, *J* = 6.7 Hz, 1H, CH), 8.66 (s, 1H, CH, ArH). Anal. Calcd for $C_{26}H_{20}ClN_5O_4$: C, 62.22; H, 4.02; N, 13.95. Found: C, 61.95; H, 3.77; N, 14.21.

8-(5-Chloro-4,7-dimethoxy-1-benzofuran-6-yl)-10-(4-methoxyphenyl)pyrido[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine-3-thiol (6c) This compound was isolated in 67% yield; mp 211–212°C; IR (cm^{-1}, ν): 3440 (br, NH); ^1H NMR (DMSO-d_6): δ 1.77 (s, 1H, SH), 3.78 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.07 (s, 3H, OCH_3), 7.20 (d, *J* = 6.7 Hz, 1H, CH), 7.57 (d, *J* = 8.7 Hz, 2 H, ArH), 7.77 (d, *J* = 8.7 Hz, 2 H, ArH), 7.81 (s, 1H, CH, ArH), 7.88 (d, *J* = 6.7 Hz, 1H, CH), 8.56 (s, 1H, CH, ArH). Anal. Calcd for $C_{26}H_{18}ClN_5O_4S$: C, 57.75; H, 3.49; N, 13.47. Found: C, 57.57; H, 3.77; N, 13.19.

7-(5-Hydroxy-4,7-dimethoxybenzofuran-6-yl)-5-(4-methoxyphenyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dithione (7a) A mixture of compound **2a** (10 mmol) and sodium bicarbonate solution [0.084 g of sodium bicarbonate dissolved in 15 mL water/ethanol mixture (2:3)] was heated under reflux for 2 h and then cooled to room temperature. Carbon disulphide (0.08 mL) was then added and the heating was continued on a water bath for an additional 2 h. The formed precipitate was isolated after neutralisation with diluted hydrochloric acid, washed with water, dried and crystallised from an ethanol/dioxane mixture (3:1) affording **7a** in 71% yield; mp 304–305°C; IR (cm^{-1}, ν): 3430 (OH), 3345 (NH), 1555 (SH); ^1H NMR ($CDCl_3$): δ 1.60 (s, 1H, SH), 3.74 (s, H, OCH_3), 3.86 (s, H, OCH_3), 4.04 (s, 3H, OCH_3), 7.25 (d, *J* = 7.2 Hz, 1H, CH), 7.65 (d, *J* = 8.4 Hz, 2 H, ArH), 7.74 (d, *J* = 8.4 Hz, 2 H, ArH), 7.77 (s, 1H, CH, ArH), 7.79 (d, *J* = 7.2 Hz, 1H, CH), 8.60 (br, s, 1H, NH, D_2O exchangeable) and 10.60 (br, s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$): δ 46.6, 48.8, 52.8 (3 OCH_3), 100.3, 113.4, 128.6, 133.8, 138.6, 145.7, 145.8, 146.8, 147.6, 147.9, 148.3, 148.6, 148.8, 149, 150, 150.6, 151, 151.6, 153.1, 166.8, 168.9; MS: m/z 493 (M^+, 100%). Anal. Calcd for $C_{24}H_{19}N_3O_5S_2$: C, 58.40; H, 3.88; N, 8.51. Found: C, 58.57; H, 4.12; N, 8.72.

5-(2,4,6-Trimethoxyphenyl)-7-(3,4,5-trimethoxyphenyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dithione (7b) Compound **2b** (1 mmol) was subjected to the procedure described above. Product **7b** was obtained as yellowish brown powder; yield 78%; mp 295–296°C; IR (cm^{-1}, ν): 3349 (NH) and 1561 (SH); ^1H NMR (DMSO-d_6): δ 3.64 (s, 6H, 2 OCH_3), 3.73 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.92 (s, 6H, 2 OCH_3), 6.92 (s, 2H, ArH), 7.04 (s, 2H, ArH), 7.76 (s, 1H, CH), 8.84–8.79 ppm (br, 1H, NH, D_2O exchangeable), 9.60 (br, s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 169.2, 164.7, 154.2, 152.3, 151.1, 147.7, 138.7, 128.1, 127.6, 126.2, 112.1, 103.5, 92.1, 59.3, 55.9, 55.8, 54.8. Anal. Calcd for $C_{25}H_{25}N_3O_6S_2$: C, 56.91; H, 4.78; N, 7.96. Found: C, 56.79; H, 4.87; N, 7.68.

2-Benzylidene-5-thioxo-6-(2,4,6-trimethoxyphenyl)-8-(3,4,5-trimethoxyphenyl)-5*H*-pyrido[2,3-*d*][1,3]thiazolo[3,2-*a*]pyrimidin-3(2*H*)-one (8) A mixture of compound **7b** (1 mmol), chloroacetic acid (1.2 mmol), benzaldehyde (1.2 mmol) and anhydrous sodium acetate (4 mmol) in a mixture of acetic acid and acetic anhydride (1:1) was heated under reflux for 8 h. The formed solid was collected and washed with acetic acid and ethanol several times yielding **8** in 76% yield; mp 310–311°C; ^{1}H NMR (DMSO-d_6): δ 3.61 (s, 6H, 2 OCH_3), 3.75 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.95 (s, 6H, 2 OCH_3), 6.96 (s, 2H, ArH), 7.03 (s, 2H, ArH), 7.28–7.36 (m, 5H, ArH), 8.32 (s, CH), 8.71 (s, CH); ^{13}C NMR (DMSO-d_6): δ 54.7, 55.2, 55.8, 59.3, 94.1, 96.3, 107.3, 113.7, 121.1, 124.2, 127.9, 128.3, 129.8, 131.7, 138.4, 139.9, 143.4, 145.6, 148.2, 152.4, 154.8, 159.7, 162.6, 163.9, 172.4. Anal. Calcd for $C_{33}H_{26}N_3O_7S_2$: C, 61.86; H, 4.09; N, 6.56. Found: C, 61.48; H, 4.37; N, 6.78.

3-Amino-6-(2,4,6-trimethoxyphenyl)-8-(3,4,5-trimethoxyphenyl)-5*H*-pyrido[2,3-*d*][1,3]thiazolo[3,2-*a*]pyrimidine-5-thione (9) A mixture of compound **7b** (1 mmol) and sodium methoxide (1.5 mmol) in methanol was stirred at room temperature for 1 h. Chloroacetonitrile (1.2 mmol) was added to the reaction mixture, followed by gentle heating for 3 h. The formed solid was filtered and washed several times with water, affording **9** in 67% yield; mp 283–284°C; ^{1}H NMR (DMSO-d_6): δ 3.63 (s, 6H, 2 OCH_3), 3.72 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.97 (s, 6H, 2 OCH_3), 5.8 (s, 1H), 6.43–6.76 (br, 2H, NH_2, exchangeable with D_2O), 6.96 (s, 2H, ArH), 7.02 (s, 2H, ArH), 8.25 (s, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 54.8, 55.1, 55.9, 59.4, 93.4, 95.6, 108.2, 115.1, 117.2, 122.6, 124.1, 138.1, 147.1, 149.4, 151.3, 153.9, 157.6, 159.1, 159.9, 162.2, 170.3. Anal. Calcd for $C_{27}H_{26}N_4O_6S_2$: C, 57.23; H, 4.62; N, 9.89. Found: C, 57.06; H, 4.79; N, 9.57.

Ethyl 3-amino-5-thioxo-6-(2,4,6-trimethoxyphenyl)-8-(3,4,5-trimethoxyphenyl)-5*H*-pyrido[2,3-*d*][1,3]thiazolo[3,2-*a*]pyrimidine-2-carboxylate (10) A mixture of compound **7b** (1 mmol) and sodium methoxide (1.5 mmol) in methanol was stirred at room temperature for 1 h, then treated with ethyl chloro(cyano)acetate (1.2 mmol), and the mixture was heated for 5 h. The resultant solid was filtered and washed several times with water, affording **10** in 63% yield; mp 292–293°C; ^{1}H NMR (DMSO-d_6): δ 1.34 (t, 3H, J = 7.1 Hz, CH_2-CH_3), 3.61 (s, 6H, 2 OCH_3), 3.75 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 4.21 (q, 2H, J = 7.1 Hz, CH_2-CH_3), 6.43–6.76 (br, 2H, NH_2, exchangeable with D_2O), 6.98 (s, 2H, ArH), 7.06 (s, 2H, ArH), 8.29 (s, CH). Anal. Calcd for $C_{30}H_{30}N_4O_8S_2$: C, 56.41; H, 4.73; N, 8.72. Found: C, 56.24; H, 4.93; N, 8.59.

3-Amino-5-thioxo-6-(2,4,6-trimethoxyphenyl)-8-(3,4,5-trimethoxyphenyl)-5*H*-pyrido [2,3-*d*][1,3]thiazolo[3,2-*a*]pyrimidine-2-carbonitrile (11) A mixture of compound **7b** (1 mmol) and sodium methoxide (1.5 mmol) in methanol was stirred at room temperature for 1 h, then treated with bromomalononitrile (1.2 mmol), and the mixture was heated for 5 h. The resultant solid was filtered and washed several times with water, affording **11** in 69% yield; mp 284–285°C; ^{1}H NMR (DMSO-d_6): δ 3.61 (s, 6H, 2 OCH_3), 3.75 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 5.98–6.11 (br, 2H, NH_2, exchangeable with D_2O), 6.93 (s, 2H, ArH), 7.08 (s, 2H, ArH), 8.36 (s, CH). Anal. Calcd for $C_{28}H_{25}N_5O_6S_2$: C, 56.84; H, 4.26; N, 11.84. Found: C, 56.69; H, 4.53; N, 11.72.

7-(5-Hydroxy-4,7-dimethoxybenzofuran-6-yl)-5-(4-methoxyphenyl)-2-(methylthio)-2,3-dihydropyrido[2,3-*d*]pyrimidine-4(1*H*)-thione (12a) A mixture of ethanolic sodium hydrogen carbonate solution [0.084 g of sodium bicarbonate dissolved in 15 mL water/ethanol mixture (2:3)] and compound **4** (1 mmol) was stirred for 4 h at room temperature, then heated under reflux for 3 min, and cooled to room temperature. Iodomethane (1.4 mmol) was added to sodium salt **4** and heating was continued for an additional 2 h. The reaction mixture was cooled to room temperature and poured onto cold water (25 mL). The resultant precipitate was filtered, washed well with water, dried and crystallised from an ethanol/dioxane mixture (3:2) to furnish **12a** as pale yellow powder in 55% yield; mp 251–252°C; IR (cm^{-1}, ν): 3430 (br, OH), 3345 (NH); ^{1}H NMR ($CDCl_3$): δ 2.63 (s, 3H, SCH_3), 3.78 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.1 (s, 3H, OCH_3), 7.25 (d, J = 7.2 Hz, 1H, CH), 7.65 (d, J = 8.7 Hz, 2H, ArH), 7.74 (d, J = 8.7 Hz, 2H, ArH), 7.77 (s, 1H, CH, ArH), 7.79 (d, J = 7.2 Hz, 1H, CH), 9.70 (br, s, 1H, NH, D_2O exchangeable); MS: m/z 521 (M^+, 100%). Anal. Calcd for $C_{25}H_{21}N_3O_5S_2$: C. 59.16; H, 4.17; N, 8.28. Found: C, 59.39; H, 4.41; N, 8.35.

2-(Methylthio)-5-(2,4,6-trimethoxyphenyl)-7-(3,4,5-trimethoxyphenyl)pyrido[2,3-*d*]pyrimidine-4(3*H*)-thione (12b) A mixture of compound **7b** (1 mmol) and sodium methoxide (1.5 mmol) in methanol was stirred at room temperature for 1 h. Iodomethane (1.2 mmol) was added to the reaction mixture, followed by gentle heating on a water bath for 2 h. The reaction mixture was cooled and poured onto cold water. The resultant precipitate was filtered and washed several times with water yielding **12b** in 83% yield, mp 212–213°C; ^{1}H NMR (DMSO-d_6): δ 2.72 (s, 3H, CH_3), 3.61 (s, 6H, 2 OCH_3), 3.75 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 6.96 (s, 2H, ArH), 7.01 (s, 2H, ArH), 8.26 (s, CH), 9.57 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{26}H_{27}N_3O_6S_2$: C, 57.65; H, 5.02; N, 7.76. Found: C, 57.45; H, 5.23; N, 7.54.

2-Hydrazinyl-7-(5-hydroxy-4,7-dimethoxybenzofuran-6-yl)-5-(4-methoxyphenyl)pyrido[2,3-*d*]pyrimidine-4(3*H*)-thione (13a) A mixture of compound **6** (1 mmol) and hydrazine hydrate (99%, 1.5 mL) in ethanol (10 mL) was heated on a water bath for 8 h (monitored by TLC) whereby a yellow precipitate was formed. The precipitate was filtered, washed well with water, dried and crystallised from an ethanol/dioxane mixture (3:1) to afford **13a** in 80% yield; mp 291–292°C; IR (cm^{-1}, ν): 3445 (br, OH), 3325 (NH), 3240, 3244(NH_2); ^{1}H NMR (DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 7.25 (d, J = 7.2 Hz, 1H, CH), 7.65 (d, J = 8.4 Hz, 2H, ArH), 7.74 (d, J = 8.4 Hz, 2H, ArH), 7.77 (s, 1H, CH, ArH), 7.79 (d, J = 7.2 Hz, 1H, CH), 8.15 (br, s, 2H, NH_2, D_2O exchangeable), 9.42 (s, 1H, NH, exchangeable with D_2O), 10.70 (br, s, 1H, NH, D_2O exchangeable); MS: m/z 491 (M^+, 100%). Anal. Calcd for $C_{24}H_{21}N_5O_5S$: C, 58.65; H, 4.31; N, 14.25. Found: C. 58.45; H, 4.13; N, 14.41.

2-Hydrazino-5-(2,4,6-trimethoxyphenyl)-7-(3,4,5-trimethoxyphenyl)pyrido[2,3-*d*]pyrimidine-4(3*H*)-thione (13b) A mixture of compound **12b** (1 mmol) and hydrazine hydrate (99%, 1.5 mmol) in ethanol (10 mL) was stirred on a water bath for 8 h. A yellow precipitate was formed after all the starting material was consumed (monitoring by TLC). The precipitate was filtered, washed well with water, dried and crystallised from an ethanol/dioxane mixture affording **13b** in 86% yield; mp 303–305°C; IR (cm^{-1}, ν): 3325 (NH), 3240, 3244 (NH_2); ^{1}H NMR (DMSO-d_6) δ ppm: 3.63 (s, 6H, 2 OCH_3), 3.79 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.02 (s, 6H, 2 OCH_3), 6.98 (s, 2H, ArH), 7.04 (s, 2H, ArH), 8.26 (s, CH, ArH), 8.39 (br, 2H, NH_2, D_2O exchangeable), 9.47 (s, 1H, NH, exchangeable with D_2O), 10.32 (br, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{25}H_{27}N_5O_6S$: C, 57.13; H, 5.18; N, 13.33. Found: C, 57.03; H, 5.42.; N, 13.08.

General procedure for pyrido[2,3-*d*][1,2,4]-triazolo[4,3-*a*]pyrimidine-5(1*H*)-thiones 14a,b

A mixture of compound **12a** (1 mmol) and trimethyl orthoformate (5 mL for **14a**) or triethyl orthoacetate (5 mL for **14b**) was heated gently on a water bath for 6–8 h (under TLC control). The formed precipitate was collected upon filtration, dried and crystallised from ethanol.

8-(5-Hydroxy-4,7-dimethoxybenzofuran-6-yl)-6-(4-methoxyphenyl)pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-5(1*H*)-thione (14a) This compound was isolated as pale yellow crystals; yield 53%; mp 188–190°C; IR (cm^{-1}, ν): 3400 (br, OH), 3333 (NH); ^{1}H NMR (DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 7.40 (d, *J* = 6.7 Hz, 1H, CH), 7.55 (d, *J* = 8.7 Hz, 2H, ArH), 7.77 (d, *J* = 8.7 Hz, 2H, ArH), 7.81 (s, 1H, CH, ArH), 7.88 (d, *J* = 6.7 Hz, 1H, CH), 8.23 (s, 1H), 9.12 (br, s, H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 43.6, 46.8, 52.7 (3 CH_3), 99.1, 111.3, 128.1, 134, 138, 143, 145, 146.2, 147.1, 147.4, 147.7, 147.8, 148.3, 148.6, 148.7, 148.9, 149.2, 149.6, 149.8, 151.3; MS: m/z 501 (100%).

8-(5-Hydroxy-4,7-dimethoxybenzofuran-6-yl)-6-(4-methoxyphenyl)-3-methylpyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-5(1*H*)-thione (14b) This compound was isolated as shiny yellow crystals in 61% yield; mp 206–208°C; IR (cm^{-1}, ν): 3408 (br, OH), 3335 (NH); ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.40 (d, *J* = 6.7 Hz, 1H, CH), 7.57 (d, *J* = 8.7 Hz, 2H, ArH), 7.77 (d, *J* = 8.7 Hz, 2H, ArH), 7.80 (d, *J* = 6.7 Hz, 1H, CH), 7.86 (s, 1H, CH, ArH) and 10.11 (br, s, 1H, NH, D_2O exchangeable); MS: m/z 515 (100%), 516 (31%).

8-(5-Hydroxy-4,7-dimethoxybenzofuran-6-yl)-3-mercapto-6-(4-methoxyphenyl)pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-5(1*H*)-thione (15) To a warm ethanolic sodium hydroxide solution [prepared by dissolving sodium hydroxide (1 mmol) in ethanol (10 mL)], compound **12a** (1 mmol) and carbon disulphide (3 mL) were added. The mixture was heated on a water bath at 80°C under reflux for 8 h, and then cooled, poured into cold water (25 mL) and neutralised with dilute acetic acid. The formed precipitate was filtered, dried and crystallised from ethanol/dioxane (15 mL, 2:1) to yield **15** as yellow powder in 52% yield; mp 223–225°C; IR (cm^{-1}, ν): 3408 (br, -OH), 3335 (NH); ^{1}H NMR (DMSO-d_6): δ 1.82 (s, 1H, SH), 3.78 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.40 (d, *J* = 6.7 Hz, 1H, CH), 7.55 (d, *J* = 8.4 Hz, 2H, ArH), 7.77 (d, *J* = 8.4 Hz, 2H, ArH), 7.80 (d, *J* = 6.7 Hz, 1H, CH), 7.86 (s, 1H, CH, ArH), 10.11 (br, s, 1H, NH, D_2O exchangeable); MS: m/z 533.08 (100%), 534.09 (27%).

3-Phenyl-6-(2,4,6-trimethoxyphenyl)-8-(3,4,5-trimethoxyphenyl)pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-5(1*H*)-thione (16a) A mixture of compound **13b** (1 mmol) and benzoic acid (1.2 mmol) in phosphorus oxychloride (5 mL) was stirred under reflux for 2 h. The reaction mixture was cooled and poured onto ice with stirring. The produced precipitate was filtered, washed well with water, dried and crystallised from dioxane, affording **16a** in 81% yield; mp 316–317°C; IR (cm^{-1}, ν): 3325 (NH); ^{1}H NMR (DMSO-d_6): δ 3.62 (s, 6H, 2 OCH_3), 3.75 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 6.96 (s, 2H, ArH), 7.01 (s, 2H, ArH), 7.34–7.53 (m, 5H, ArH), 8.26 (s, CH), 11.12 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ ppm: 173.1, 162.6, 157.2, 156.8, 153.7, 151.1, 149.6, 148.9, 145.7, 141.2, 135.1, 130.3, 129.6, 128.9, 122.3, 119.6, 118.2, 112.9, 94.6, 89.9, 59.6, 55.3, 54.8, 53.9. Anal. Calcd for $C_{32}H_{29}N_5O_6S$: C, 62.84; H, 4.78; N, 11.45. Found: C, 62.66; H, 4.55.; N, 11.28.

3-(4-Methoxyphenyl)-6-(2,4,6-trimethoxyphenyl)-8-(3,4,5-trimethoxyphenyl)pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-5(1*H*)-thione (16b) A mixture of compound **13b** (1 mmol) and anisic acid (1.2 mol) in phosphorus oxychloride (5 mL) was stirred under reflux for 2 h. The reaction mixture was cooled and poured onto ice with stirring. The produced precipitate was filtered, washed well with water, dried and crystallised from dioxane, affording **16b** in 86% yield; mp 327–328°C; IR (cm^{-1}, ν): 3329 (NH); ^{1}H NMR (DMSO-d_6): δ 3.63 (s, 6H, 2 OCH_3), 3.77 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.97 (s, 6H, 2 OCH_3), 6.98 (s, 2H, ArH), 7.05 (s, 2H, ArH), 7.34–7.37 (d, 2H, *J* = 9.0 Hz, ArH), 7.61–7.64 (d, 2H, *J* = 9.0 Hz, ArH), 8.26 (s, CH), 11.22 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6): δ 172.8, 161.9, 159.7, 158.1, 156.4, 153.8, 151.6, 149.7, 148.8, 145.8, 138.9, 131.9, 127.6, 123.4, 119.7, 118.7, 118.1, 116.3, 113.4, 94.7, 90.3, 59.8, 55.8, 55.1, 54.9, 54.2. Anal. Calcd for $C_{33}H_{31}N_5O_7S$: C. 61.77; H, 4.87; N, 10.91. Found: C, 61.51; H, 4.65; N, 10.84.

5-Amino-3-phenyl-1-[4-thioxo-5-(2,4,6-trimethoxyphenyl)-7-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrido[2,3-*d*]pyrimidin-2-yl]-1*H*-pyrazole-4-carbonitrile (17) A mixture of compound **13b** (1 mmol) and benzylidenemalononitrile (1.1 mmol) in ethanol was heated under reflux for 10 h, then concentrated under reduced pressure and the resultant solid was filtered and washed with ethanol and ether, affording **17** in 77% yield; mp 276–277°C; IR (cm^{-1}, ν): 3425–3100 (NH_2), 2210 (CN); ^{1}H NMR (DMSO-d_6): δ 3.62 (s, 6H, 2 OCH_3), 3.75 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 6.2 (br, 2H, NH_2), 6.96 (s, 2H, ArH), 7.04 (s, 2H, ArH), 7.32–7.49 (m, 5H, ArH), 8.26 (s, CH), 9.82 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6): δ 171.3, 159.9, 157.8, 157.1, 155.4, 151.9, 149.6, 148.9, 144.9, 138.6, 131.9, 129.7, 127.2, 126.9, 123.6, 119.7, 118.9, 117.7, 113.8, 92.8, 89.6, 73.2, 59.8, 55.7, 54.8, 53.9. Anal. Calcd for $C_{35}H_{31}N_7O_6S$: C, 62.03; H, 4.61; N, 14.47. Found: C, 61.89; H, 4.75; N, 14.19.

Ethyl 5-amino-3-phenyl-1-[4-thioxo-5-(2,4,6-trimethoxyphenyl)-7-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrido[2,3-*d*]pyrimidin-2-yl]-1*H*-pyrazole-4-carboxylate (18) A mixture of compound **13b** (1 mmol) and ethyl-2-cyano-3-phenylacrylate (1.1 mmol) in ethanol was heated under reflux for 10 h, then concentrated under reduced pressure, and the resultant solid was filtered and washed with ethanol and ether, affording **18** in 69% yield; mp 256–257°C; IR (cm^{-1}, ν): 3425–3100 (NH_2); ^{1}H NMR (DMSO-d_6): δ 1.31 (t, 3H, *J* = 7.2 Hz, CH_2-CH_3), 3.62 (s, 6H, 2 OCH_3), 3.75 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 4.21 (q, 2H, *J* = 7.2 Hz, CH_2-CH_3), 5.96 (br, 2H, NH_2), 6.96 (s, 2H, ArH), 7.01 (s, 2H, ArH), 7.32–7.49 (m, 5H, ArH), 8.26 (s, CH), 9.82 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{37}H_{36}N_6O_6S$: C, 61.31; H, 5.01; N, 11.61. Found: C, 61.11; H, 4.85.; N, 11.49.

3,4-Diphenyl-7-(2,4,6-trimethoxyphenyl)-9-(3,4,5-trimethoxyphenyl)-1,4-dihydro-6*H*-pyrido[2′,3′:4,5]pyrimido[2,1-*c*][1,2,4]triazine-6-thione (19) A mixture of compound **13b** (1 mmol), benzoin (1.1 mmol) and phosphorus oxychloride (5 mL) was heated under reflux for 1 h, cooled and then poured onto ice. The precipitated product was collected and washed several times with water and dried yielding **19** in 68% yield; mp 385–386°C; IR (cm^{-1}, ν): 3227 (NH); ^{1}H NMR (DMSO-d_6): δ 3.62 (s, 6H, 2 OCH_3), 3.75 (s, 3H, OCH_3), 3.89 (s,

3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 5.98 (s, 1H), 6.96 (s, 2H, ArH), 7.01 (s, 2H, ArH), 7.32–7.59 (m, 10 H, ArH), 8.36 (s, CH), 12.18 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6): δ 54.8, 55.9, 56.6, 57.1, 58.9, 89.4, 92.6, 112.9, 114.9, 119.4, 123.3, 126.9, 127.1, 127.9, 128.8, 129.5, 130.1, 136.9, 138.2, 142.8, 144.9, 147.9, 149.7, 151.3, 154.2, 156.1, 157.4, 159.2, 168.1. Anal. Calcd for $C_{39}H_{35}N_5O_6S$: C, 66.75; H, 5.03; N, 9.98. Found: C, 66.53; H, 4.85; N, 9.79.

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