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CONVENIENT SYNTHESIS OF NOVEL PHENYLPYRIMIDO[1,2-*c*]-THIENOPYRIMIDINONES AS IL-6/STAT3 INHIBITORS

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Abstract – New phenylpyrimido[1,2-*c*]thienopyrimidinones **4A** and **4B** were easily prepared in good yields by the one-pot reaction of formamidine derivatives **2** of 4-aminothienopyrimidine **1** with phenylacetyl chlorides. The application of this convenient and reliable method could be used for the synthesis of a variety of pyrimido[1,2-*c*]thienopyrimidinone derivatives of biological importance. Some of the compounds synthesized showed strong IL-6/STAT3 inhibition.

Amongst pro-inflammatory cytokines, interleukin-6 (IL-6) plays a key role in the induction of initiation and extension of the inflammatory process and immune response.[1] And, an over-production of IL-6 is responsible for several diseases such as rheumatoid arthritis, psoriasis, inflammatory bowel disease, osteoarthritis, multiple myeloma and also for human atherosclerotic plaque.[2] IL-6 binds to its receptor and leads to the activation of the Janus kinase (Jak)/Signal Transducer and Activator of Transcription-3 (STAT3).[3] STAT3 is also frequently over-expressed or persistently activated in most tumors and cancer, and activated STAT3 was found to suppress tumor-immune surveillance.[4] Therefore, the inhibition of STAT3 activation pathway stimulated by IL-6 represents a useful therapeutic strategy for discovery of new anti-inflammatory and anticancer drugs and is currently under intense investigation.[5]

In the other hand, thienopyrimidines, pyrido[1,2-*a*]pyrimidinones and pyrimido[1,6-*a*]pyrimidinones have been reported to have a wide range of biological properties, and their structural motif is present in the antitumor (**I**),[6] antiplatelet agent (**II**),[7] anxiolytic agent (**III**)[8] and antiviral agent (**IV**).[9] As part of a programme to discover novel IL-6/STAT3 inhibitors containing thienopyrimidines and to synthesize

thienopyrimidine derivatives,[10] we focused our attention on the new heterocyclic scaffold, phenylpyrimido[1,2-*c*]thienopyrimidinones **4A** and **4B** which are incorporated thiophene moiety into pyrimidopyrimidinones in attempt to improve the IL-6/STAT3 inhibitory activity. Herein, we describe a convenient and reliable method for the preparation of these compounds having biological activity.

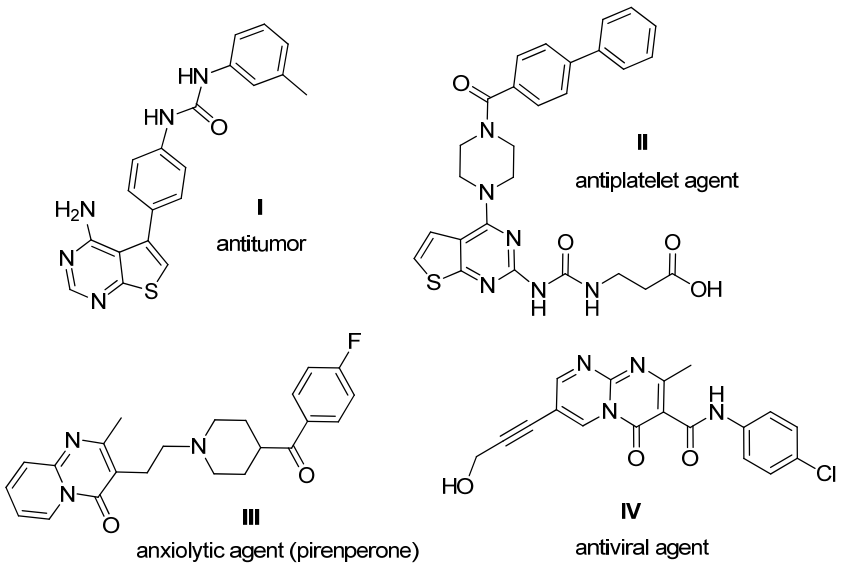
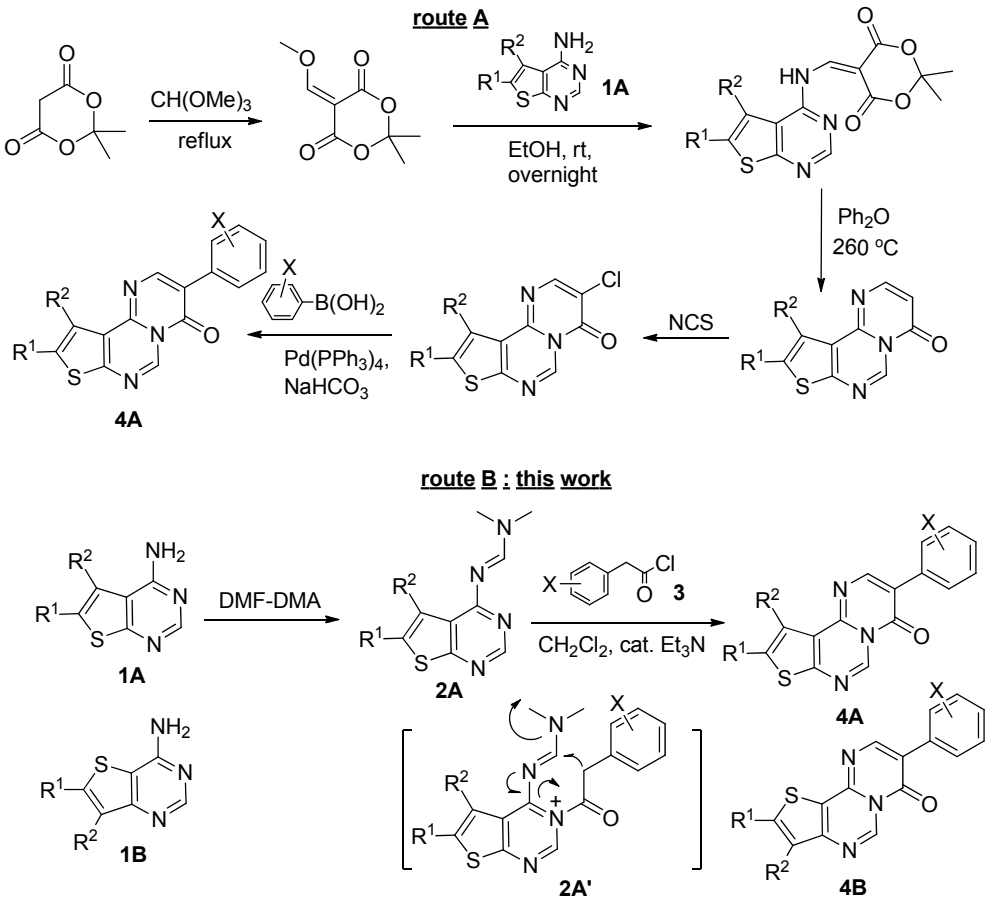


Figure 1. Thienopyrimidine (**I**, **II**), pyrido- (**III**) and pyrimidopyrimidinone (**IV**) compounds

The first synthetic route to target **4A** (route A) is described in Scheme 1. The multi-step synthetic approach was investigated by using the conventional method previously described in the literature for the synthesis[11] (Gould-Jacob type reaction) and the functionalization[12] (Suzuki-Miyaura reaction) of pyridopyrimidinone analogues. However, the synthetic reaction for the precursor of **4Aa** (R^1, R^2 = X = H), pyrimido[1,2-*c*]thienopyrimidinone skeleton, gave a mixture of inseparable products in 25% yield under high temperature (>260 °C) and longer reaction time. Modified methods using the condensation of 4-aminothienopyrimidine **1** with 3-dimethylamino-2-arylpropenoates,[13] and using microwave assisted synthesis[14] were not also effective. Deniaud reported recently the synthetic method of pyrimido[1,2-*a*]pyrimidine-2,6-diones by using formamidines and acetyl chloride.[15] In this literature, however, only methoxy or methoxycarbonyl group was introduced on the 7-position of pyrimido[1,2-*a*]pyrimidine-2,6-diones that were formed by cyclization reaction, and the scope and generality of the reaction to other heterocycles was not examined.

With the purpose of devising a milder and more convenient method of synthesizing **4A** and **4B**, another synthetic route B (this work) was examined by the cyclization reaction of formamidine derivative, (*E*)-*N,N*-dimethyl-*N*-(thieno[2,3-*d*]pyrimidin-4-yl)formimidamide **(2Aa**, R^1, R^2 = H**)**, of **1Aa** with

2-phenylacetyl chloride **(3a)** via **2Aa'**. The key reactant **2Aa** was easily prepared in quantitative yield by the reaction of **1Aa** with dimethylformamide dimethyl acetal (DMF-DMA), and used without isolation for the next reaction with 2-phenylacetyl chloride. We indeed observed that the reaction proceeded smoothly in THF at room temperature affording the desired compound **4Aa** as the only product in 48% yield (Table 1, entry 1). As shown in Table 1, reaction optimization for **4Aa** was investigated at the various conditions such as different catalyst, reaction temperature and solvent. Use of ethanol or DMF as solvent resulted in inferior yield of product (Table 1, entries 3, 4, 10, 11). When the same reaction was carried out in dichloromethane in the presence of 0.1 equiv Et_3N as a catalyst at room temperature, **4Aa** was obtained in best yield within 2 h (Table 1, entry 6). The solid product was easily isolated with filtration, washing and drying. Notably, it was found that the stoichiometric usage of Et_3N did not increased the yield as compared to its catalytic amount (Table 1, entry 7), and longer reaction time also did not affect the yield of **4Aa** (Table 1, entry 8).



Scheme 1. Synthesis of phenylpyrimido[1,2-*c*]thienopyrimidinone derivatives, **4A** and **4B**

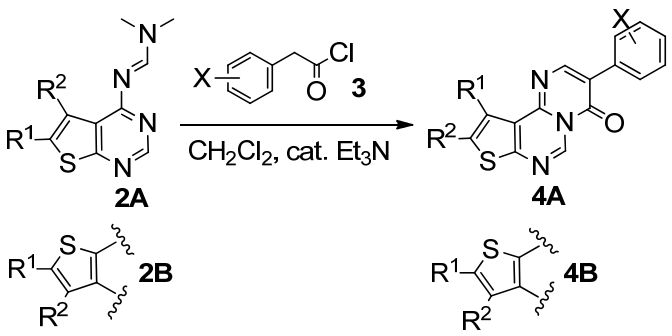
Table 1. Optimization for the reaction of **2Aa** (R^1, R^2 = H) with **3a** (X = H)[a]

entry	base (equiv)	solvent	time (h)	temp	yield (%)[b]
1		THF	6	rt	48
2	Et_3N (0.1)	THF	6	rt	58
3	NaOEt (0.1)	EtOH	10	rt	15
4	Et_3N (0.1)	EtOH	5	reflux	30
5		CH_2Cl_2	2	rt	64
6	Et_3N (0.1)	CH_2Cl_2	2	rt	82
7	Et_3N (1.0)	CH_2Cl_2	5	rt	79
8	Et_3N (0.1)	CH_2Cl_2	12	rt	80
9	Et_3N (0.1)	$CHCl_3$	2	rt	70
10	Et_3N (0.1)	DMF	4	rt	42
11	K_2CO_3 (1.0)	DMF	4	reflux	38

[a] mole ratio of **2Aa**:**3a** = 1.0:1.2. [b] isolated yield.

Next, as depicted in Table 2, the reaction of **2Aa** with **3b-h** was further evaluated under optimized reaction condition. Desired products **4Ab-g** were obtained in good yields without side product. The results also indicated that **3b-f** having electron-withdrawing group on phenyl ring gave products in slightly better yields (Table 2, entries 2-6) as compared with **3g** having electron-donating group (Table 2, entry 7). No desired product **4Ah**, however, was formed when the reaction was carried out with **3h** (Table 2, entry 8). This could be due to the steric hindrance of two methyl groups at *ortho* position on phenyl of **3h**. The reaction of **2Ba** with **3a-g** was also successfully applied to the synthesis of **4Ba-g** as products. Notably, the reaction with **2Aa** gave the corresponding products in little higher yield than that of **2Ba**. The cyclohexyl- and cyclopentylthiophene derivatives, **2Ab** and **2Ac**, also performed well, affording the respective **4Ai-o** and **4Ap-t** in good yield (Table 2, entries 17-28). All compounds were characterized from their spectroscopic data.

Table 2. Results of phenylpyrimido[1,2-*c*]thienopyrimidinone synthesis

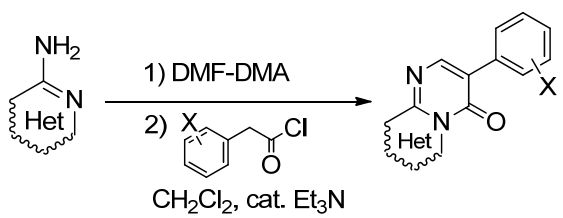


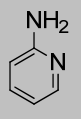
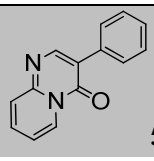
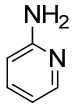
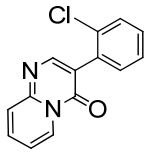
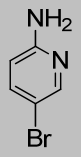
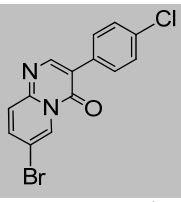
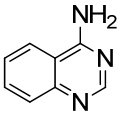
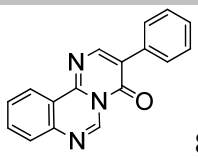
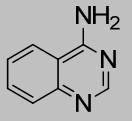
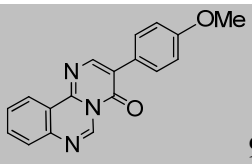
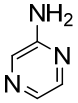
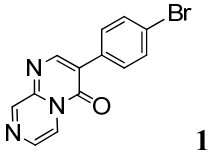
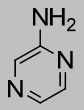
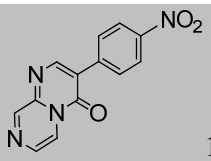
entry	**2A**	**3** (X)	**4A/4B**	yield (%)[a]
1	**2Aa** (R^1, R^2 = H)	**3a** (H)	**4Aa**	82
2	**2Aa**	**3b** (4-Br)	**4Ab**	85
3	**2Aa**	**3c** (2-Cl)	**4Ac**	84
4	**2Aa**	**3d** (3-Cl)	**4Ad**	89
5	**2Aa**	**3e** (4-Cl)	**4Ae**	92
6	**2Aa**	**3f** (4-NO_2)	**4Af**	90
7	**2Aa**	**3g** (4-OMe)	**4Ag**	72
8	**2Aa**	**3h** (2,5-*di*Me)	**4Ah**	0
9	**2Ba** (R^1, R^2 = H)	**3a** (H)	**4Ba**	77
10	**2Ba**	**3b** (4-Br)	**4Bb**	75
11	**2Ba**	**3c** (2-Cl)	**4Bc**	78
12	**2Ba**	**3d** (3-Cl)	**4Bd**	79
13	**2Ba**	**3e** (4-Cl)	**4Be**	83
14	**2Ba**	**3f** (4-NO_2)	**4Bf**	88
15	**2Ba**	**3g** (4-OMe)	**4Bg**	70
16	**2Ba**	**3h** (2,5-*di*Me)	**4Bh**	0
17	**2Ab** (R^1, R^2 = cyclohexyl)	**3a** (H)	**4Ai**	77
18	**2Ab**	**3b** (4-Br)	**4Aj**	82
19	**2Ab**	**3c** (2-Cl)	**4Ak**	80
20	**2Ab**	**3d** (3-Cl)	**4Al**	88
21	**2Ab**	**3e** (4-Cl)	**4Am**	90
22	**2Ab**	**3f** (4-NO_2)	**4An**	91
23	**2Ab**	**3g** (4-OMe)	**4Ao**	75
24	**2Ac** (R^1, R^2 = cyclopentyl)	**3a** (H)	**4Ap**	78
25	**2Ac**	**3b** (4-Br)	**4Aq**	80
26	**2Ac**	**3c** (2-Cl)	**4Ar**	77
27	**2Ac**	**3e** (4-Cl)	**4As**	88
28	**2Ac**	**3g** (4-OMe)	**4At**	72

[a] isolated yield.

To explore the diversity of this methodology, various heterocyclic amines were used in cyclization reaction. Under the same reaction condition, phenylpyrido[1,2-*a*]pyrimidinones, phenylpyrimido[1,2-*c*]-quinazolinones and phenylpyrazino[1,2-*a*]pyrimidinones **5 – 11** were also obtained in good yield (Table 3).

Table 3. Cyclization reaction of various heterocyclic amines



entry	amine	X	product	yield (%)[a]
1		H	 **5**	78
2		2-Cl	 **6**	85
3		4-Cl	 **7**	82
4		H	 **8**	92
5		4-OMe	 **9**	85
6		4-Br	 **10**	76
7		4-NO_2	 **11**	81

[a] isolated yield.

The synthesized compounds **4A** and **4B** were tested for their inhibitory activities on STAT3-dependent luciferase activity induced by IL-6, according to the reported method.[16] The inhibitory activities of compounds were investigated as compared to genistein was used as a positive control which inhibited the STAT3-dependent luciferase activity with an IC_{50} value of 15 μM in this assay system.[17] Among these compounds, some of strong IL-6/STAT3 inhibitors are listed in Table 4.

Table 4. Inhibitory effects of compounds on IL-6-induced STAT3 activation[a]

Compound	IC_{50}(μM)[b]
4Ac	6.82
4Ag	7.41
4Bc	5.28
4Bd	2.01
4Bg	5.57
4Ai	1.39
4Ak	0.52
4Al	0.65
4Ao	0.81
4Ap	0.68
4Ar	0.60
4At	0.67
Genistein[c]	15.0

[a]Data mean ± standard error values of three replications.
[b]IC_{50}: mean (50%) value of inhibition concentration.
[c]Genistein was used as a positive control.

Phenylpyrimido[1,2-*c*]thieno[2,3-*e*]pyrimidinone analogues (**4Bc**, **4Bd**, **4Bg**) have slightly potent inhibitory activities than phenylpyrimido[1,2-*c*]thieno[3,2-*e*]pyrimidinone analogues (**4Ac**, **4Ag**). The presence of cyclohexane or cyclopentane fused thiophene ring resulted in the improved activity of compounds (**4Ai-4At**). The most potent compound **4Ak** showed inhibitory activity (IC_{50} value of 0.52 μM) with 30-fold when compared to genistein. None of the compounds tested had any cytotoxicity in Hep3B cells with MTT assay (data not shown).

In summary, we have showed the convenient synthesis of novel phenylpyrimido[1,2-*c*]-thienopyrimidinone derivatives, and investigated preliminary biological activity for them. This simple and reliable one-pot synthetic method may allow the preparation of a larger library of these compounds of biological importance. Further investigations on their biological evaluation on IL-6/STAT3 inhibition and the application of methodology to other heterocyclic scaffold are currently underway.

EXPERIMENTAL

Melting points were determined in capillary tubes on Büchi apparatus and are uncorrected. Reactions were checked and monitored on thin-layer chromatography of Merck Kieselgel $60F_{254}$. The ^1H NMR spectra were recorded on Unity Inova 400NB FT NMR spectrometer (400 MHz) with Me_4Si as internal

standard and chemical shifts are given in ppm (δ). Mass spectra were recorded on a HP 59580 B spectrometer. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

General procedure for the preparation of (*E*)-*N,N*-dimethyl-*N*-(thieno[2,3-*d*]pyrimidin-4-yl)-formimidamide (2A) and (2B).

A mixture of thieno[2,3-*d*]pyrimidin-4-amine **1A** or **1B** (30 mmol) and dimethylformamide dimethyl acetal (DMF-DMA) (36 mmol) was heated to 110 °C for 8 h. The solution was evaporated to dryness, and the crude product was used without isolation for the next reaction.

(*E*)-*N,N*-Dimethyl-*N*-(thieno[2,3-*d*]pyrimidin-4-yl)formimidamide (2Aa).

Yield: 95%; mp 96-97 °C (recrystallized from *n*-hexane); ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.89 (s, 1H), 8.55 (s, 1H), 7.64 (d, 1H, J = 5.8 Hz), 7.48 (d, 1H, J = 5.8 Hz), 3.20 (s, 6H); MS (ESI): *m/z* 206.63 (M^+); *Anal*. Calcd for $C_9H_{10}N_4S$: C, 52.41; H, 4.89; N, 27.16. Found: C, 52.23; H, 4.77; N, 27.02.

(*E*)-*N,N*-Dimethyl-*N'*-(5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)formimidamide (2Ab).

Yield: 98%; mp 136-137 °C (recrystallized from *n*-hexane); ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.35 (s, 1H), 3.03 (s, 6H), 2.99 (s, 2H), 2.68 (s, 2H), 1.72 (s, 4H); MS (ESI): *m/z* 261.1 (M^++1); *Anal*. Calcd for $C_{13}H_{16}N_4S$: C, 59.97; H, 6.19; N, 21.52. Found: C, 59.89; H, 6.10; N, 21.40.

(*E*)-*N'*-(6,7-Dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-*N,N*-dimethylformimidamide (2Ac).

Yield: 97%; mp 145-146 °C (recrystallized from *n*-hexane); ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.52 (s, 1H), 3.18-3.11 (m, 8H), 2.99 (s, 2H), 2.46 (s, 2H); MS (ESI): *m/z* 246.8 (M^+); *Anal*. Calcd for $C_{12}H_{14}N_4S$: C, 58.51; H, 5.73; N, 22.74. Found: C, 58.64; H, 5.61; N, 22.86.

(*E*)-*N,N*-Dimethyl-*N*-(thieno[3,2-*d*]pyrimidin-4-yl)formimidamide (2Ba).

Yield: 95%; mp 107-108 °C (recrystallized from $CHCl_3$ and petroleum ether); ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.78 (s, 1H), 7.82 (d, 1H, J = 5.8 Hz), 7.48 (d, 1H, J = 5.8 Hz), 3.22 (s, 6H); MS (ESI): *m/z* 206.57 (M^+); *Anal*. Calcd for $C_9H_{10}N_4S$: C, 52.41; H, 4.89; N, 27.16. Found: C, 52.52; H, 4.78; N, 27.09.

General procedure for the preparation of phenylpyrimido[1,2-*c*]thieno[3,2-*e*]pyrimidinone (4Aa-t) and phenylpyrimido[1,2-*c*]thieno[2,3-*e*]pyrimidinone (4Ba-g).

A suspension of **2A** or **2B** (4 mmol) and **3** (4.8 mmol) in the presence of a catalytic amount of triethylamine in CH_2Cl_2 (10 mL) was stirred at rt for 2 h. The solid product was filtered, washed with EtOAc and dried. The crude product was recrystallized from EtOH to give **4A** or **4B**.

3-Phenyl-4*H*-pyrimido[1,2-*c*]thieno[3,2-*e*]pyrimidin-4-one (4Aa).

Yield: 82%; mp 231-232 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.69 (s, 1H), 8.59 (s, 1H), 8.04 (d, 1H, J =

5.8 Hz), 7.90 (d, 1H, *J* = 5.8 Hz), 7.80 (d, 2H, *J* = 7.8 Hz), 7.47 (t, 2H, *J* = 7.8 Hz), 7.38 (t, 1H, *J* = 7.8 Hz); MS (ESI): *m/z* 279.82 (M^+); *Anal*. Calcd for $C_{15}H_9N_3OS$: C, 64.50; H, 3.25; N, 15.04 Found: C, 64.31; H, 3.14; N, 15.20.

3-(4-Bromophenyl)-4*H*-pyrimido[1,2-*c*]thieno[3,2-*e*]pyrimidin-4-one (4Ab).

Yield: 85%; mp 253-254 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.61 (s, 1H), 8.02 (d, 1H, *J* = 5.8 Hz), 7.88 (d, 1H, *J* = 5.8 Hz), 7.76 (d, 2H, *J* = 8.0 Hz), 7.73 (d, 2H, *J* = 8.0 Hz); MS (ESI): *m/z* 358.4 (M^+); *Anal*. Calcd for $C_{15}H_8BrN_3OS$: C, 50.29; H, 2.25; N, 11.73. Found: C, 50.12; H, 2.19; N, 11.61.

3-(2-Chlorophenyl)-4*H*-pyrimido[1,2-*c*]thieno[3,2-*e*]pyrimidin-4-one (4Ac).

Yield: 84%; mp 243-244 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 8.36 (s, 1H), 8.04 (d, 1H, *J* = 5.8 Hz), 7.89 (d, 1H, *J* = 5.8 Hz), 7.46 (d, 1H, *J* = 8.0 Hz), 7.40-7.30 (m, 3H); MS (ESI): *m/z* 313.5 (M^+); *Anal*. Calcd for $C_{15}H_8ClN_3OS$: C, 57.42; H, 2.57; N, 11.30. Found: C, 57.30; H, 2.50; N, 11.41.

3-(3-Chlorophenyl)-4*H*-pyrimido[1,2-*c*]thieno[3,2-*e*]pyrimidin-4-one (4Ad).

Yield: 89%; mp 259-260 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.59 (s, 1H), 8.65 (s, 1H), 8.04 (d, 1H, *J* = 5.8 Hz), 7.90 (m, 2H), 7.76 (d, 1H, *J* = 8.0 Hz), 7.49-7.43 (m, 2H); MS (ESI): *m/z* 313.8 (M^+); *Anal*. Calcd for $C_{15}H_8ClN_3OS$: C, 57.42; H, 2.57; N, 11.30. Found: C, 57.50; H, 2.50; N, 11.44.

3-(4-Chlorophenyl)-4*H*-pyrimido[1,2-*c*]thieno[3,2-*e*]pyrimidin-4-one (4Ae).

Yield: 92%; mp 255-256 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.61 (s, 1H), 8.02 (d, 1H, *J* = 5.8 Hz), 7.88 (d, 1H, *J* = 5.8 Hz), 7.83 (d, 2H, *J* = 7.8 Hz), 7.50 (d, 2H, *J* = 7.8 Hz); MS (ESI): *m/z* 313.9 (M^+); *Anal*. Calcd for $C_{15}H_8ClN_3OS$: C, 57.42; H, 2.57; N, 11.30. Found: C, 57.55; H, 2.48; N, 11.24.

3-(4-Nitrophenyl)-4*H*-pyrimido[1,2-*c*]thieno[3,2-*e*]pyrimidin-4-one (4Af).

Yield: 90%; mp 344-345 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 8.77 (s, 1H), 8.28 (d, 2H, *J* = 8.0 Hz), 8.13 (d, 2H, *J* = 8.0 Hz), 8.06 (d, 1H, *J* = 5.8 Hz), 7.91 (d, 1H, *J* = 5.8 Hz); MS (ESI): *m/z* 324.8 (M^+); *Anal*. Calcd for $C_{15}H_8N_4O_3S$: C, 55.55; H, 2.49; N, 17.28. Found: C, 55.44; H, 2.40; N, 17.38.

3-(4-Methoxyphenyl)-4*H*-pyrimido[1,2-*c*]thieno[3,2-*e*]pyrimidin-4-one (4Ag).

Yield: 72%; mp 208-209 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.56 (s, 1H), 8.52 (s, 1H), 8.00 (d, 1H, *J* = 5.8 Hz), 7.85 (d, 1H, *J* = 5.8 Hz), 7.73 (d, 2H, *J* = 8.0 Hz), 7.00 (d, 2H, *J* = 8.0 Hz), 3.77 (s, 3H); MS (ESI): *m/z* 309.1 (M^+); *Anal*. Calcd for $C_{16}H_{11}N_3O_2S$: C, 62.12; H, 3.58; N, 13.58. Found: C, 62.01; H, 3.55; N, 13.38.

3-Phenyl-9,10,11,12-tetrahydro-4*H*-benzo[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4-one (4Ai).

Yield: 77%; mp 214-215 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.50 (s, 1H), 7.78 (d, 2H, *J* = 7.8 Hz), 7.50 (t, 2H, *J* = 7.8 Hz), 7.41 (d, 1H, *J* = 8.0 Hz), 3.29-3.27 (m, 2H), 2.95-2.92 (m, 2H), 1.98-1.94 (m, 4H); MS (ESI): *m/z* 334.2 (M^+); *Anal*. Calcd for $C_{19}H_{15}N_3OS$: C, 68.45; H, 4.53; N, 12.60. Found: C, 68.34; H, 4.42; N, 12.72.

3-(4-Bromophenyl)-9,10,11,12-tetrahydro-4*H*-benzo[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4-one (4Aj).

Yield: 82%; mp 211-212 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 8.41 (s, 1H), 7.60 (d, 2H, *J* = 8.0 Hz), 7.53 (d, 2H, *J* = 8.0 Hz), 3.20-3.19 (m, 2H), 2.88-2.87 (m, 2H), 1.89-1.87 (m, 4H); MS (ESI): *m/z* 413.9 (M^+); *Anal*. Calcd for $C_{19}H_{14}BrN_3OS$: C, 55.35; H, 3.42; N, 10.19. Found: C, 55.22; H, 3.52; N, 10.08.

3-(2-Chlorophenyl)-9,10,11,12-tetrahydro-4*H*-benzo[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4-one (4Ak).

Yield: 80%; mp 234-235 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 8.24 (s, 1H), 7.43 (d, 1H, *J* = 7.6 Hz), 7.36 (d, 1H, *J* = 7.6 Hz), 7.27-7.25 (m, 2H), 3.19-3.17 (m, 2H), 2.85-2.83 (m, 2H), 1.85-1.84 (m, 4H); MS (ESI): *m/z* 367.3 (M^+); *Anal*. Calcd for $C_{19}H_{14}ClN_3OS$: C, 62.04; H, 3.84; N, 11.42. Found: C, 61.95; H, 3.77; N, 11.30.

3-(3-Chlorophenyl)-9,10,11,12-tetrahydro-4*H*-benzo[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4-one (4Al).

Yield: 88%; mp 238-239 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 8.42 (s, 1H), 7.72 (s, 1H), 7.60 (d, 1H, *J* = 7.9 Hz), 7.35-7.28 (m, 2H), 3.21-3.19 (m, 2H), 2.88-2.87 (m, 2H), 1.88-1.87 (m, 4H); MS (ESI): *m/z* 367.1(M^+); *Anal*. Calcd for $C_{19}H_{14}ClN_3OS$: C, 62.04; H, 3.84; N, 11.42. Found: C, 62.11; H, 3.79; N, 11.33.

3-(4-Chlorophenyl)-9,10,11,12-tetrahydro-4*H*-benzo[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4-one (4Am).

Yield: 90%; mp 230-231 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.67 (s, 1H), 8.48 (s, 1H), 7.74 (d, 2H, *J* = 8.0 Hz), 7.45 (d, 2H, *J* = 8.0 Hz), 3.28-3.26 (m, 2H), 2.94-2.93 (m, 2H), 1.98-1.95 (m, 4H); MS (ESI): *m/z* 368.2 (M^+); *Anal*. Calcd for $C_{19}H_{14}ClN_3OS$: C, 62.04; H, 3.84; N, 11.42. Found: C, 62.16; H, 3.77; N, 11.36.

3-(4-Nitrophenyl)-9,10,11,12-tetrahydro-4*H*-benzo[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4-one (4An).

Yield: 91%; mp 300-301 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.63 (s, 1H), 8.52 (s, 1H), 7.94 (d, 2H, *J* = 8.0 Hz), 7.27 (d, 2H, *J* = 8.0 Hz), 3.22 (s, 2H), 2.89 (s, 2H), 1.89 (s, 4H); MS (ESI): *m/z* 378.1 (M^+); *Anal*. Calcd for $C_{19}H_{14}N_4O_3S$: C, 60.31; H, 3.73; N, 14.81. Found: C, 60.20; H, 3.66; N, 14.70.

3-(4-Methoxyphenyl)-9,10,11,12-tetrahydro-4*H*-benzo[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4-one (4Ao).

Yield: 75%; mp 210-211 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 8.38 (s, 1H), 7.66 (d, 2H, *J* = 8.0 Hz), 6.94 (d, 2H, *J* = 8.0 Hz), 3.79 (s, 3H), 3.20 (s, 2H), 2.87 (s, 2H), 1.88 (s, 4H); MS (ESI): *m/z* 363.8

(M^+); *Anal*. Calcd for $C_{20}H_{17}N_3O_2S$: C, 66.10; H, 4.71; N, 11.56. Found: C, 66.01; H, 4.63; N, 11.44.

3-Phenyl-10,11-dihydrocyclopenta[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4(9*H*)-one (4Ap).

Yield: 78%; mp 205-206 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.50 (s, 1H), 8.36 (s, 1H), 7.62 (d, 2H, *J* = 8.0 Hz), 7.26 (t, 2H, *J* = 8.0 Hz), 7.11 (d, 1H, *J* = 8.0 Hz), 3.14 (s, 2H), 2.97 (s, 2H), 2.43 (s, 2H); MS (ESI): *m/z* 320.1 (M^+); *Anal*. Calcd for $C_{18}H_{13}N_3OS$: C, 67.69; H, 4.10; N, 13.16. Found: C, 67.59; H, 4.01; N, 13.31.

3-(4-Bromophenyl-10,11-dihydrocyclopenta[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4(9*H*)-one (4Aq).

Yield: 80%; mp 185-186 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 8.21 (s, 1H), 7.43 (d, 2H, *J* = 8.0 Hz), 7.16 (d, 2H, *J* = 8.0 Hz), 3.56 (s, 2H), 2.98 (s, 2H), 2.53 (s, 2H); MS (ESI): *m/z* 397.8 (M^+); *Anal*. Calcd for $C_{18}H_{12}BrN_3OS$: C, 54.28; H, 3.04; N, 10.55. Found: C, 54.10; H, 3.13; N, 10.41.

3-(2-Chlorophenyl-10,11-dihydrocyclopenta[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4(9*H*)-one (4Ar).

Yield: 77%; mp 254-255 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.19 (s, 1H), 7.35 (d, 1H, *J* = 8.0 Hz), 7.33 (d, 1H, *J* = 7.9 Hz), 7.28 (m, 2H), 3.13 (s, 2H), 2.96 (s, 2H), 2.43 (s, 2H); MS (ESI): *m/z* 354.1 (M^+); *Anal*. Calcd for $C_{18}H_{12}ClN_3OS$: C, 61.10 H, 3.42; N, 11.88. Found: C, 61.00; H, 3.35; N, 11.75.

3-(4-Chlorophenyl-10,11-dihydrocyclopenta[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4(9*H*)-one (4As).

Yield: 88%; mp 255-256 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 8.38 (s, 1H), 7.61 (d, 2H, *J* = 8.0 Hz), 7.33 (d, 2H, *J* = 8.0 Hz), 3.18 (s, 2H), 3.01 (s, 2H), 2.47 (s, 2H); MS (ESI): *m/z* 353.9 (M^+); *Anal*. Calcd for $C_{18}H_{12}ClN_3OS$: C, 61.10 H, 3.42; N, 11.88. Found: C, 61.22; H, 3.49; N, 11.79.

3-(4-Methoxyphenyl-10,11-dihydrocyclopenta[4,5]thieno[3,2-*e*]pyrimido[1,2-*c*]pyrimidin-4(9*H*)-one (4At).

Yield: 72%; mp 206-207 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ 9.53 (s, 1H), 8.36 (s, 1H), 7.60 (d, 2H, *J* = 8.0 Hz), 6.89 (d, 2H, *J* = 8.0 Hz), 3.75 (s, 3H), 3.17 (s, 2H), 3.00 (s, 2H), 2.46 (s, 2H); MS (ESI): *m/z* 350.2 (M^+); *Anal*. Calcd for $C_{19}H_{15}N_3O_2S$: C, 65.31 H, 4.33; N, 12.03. Found: C, 65.25 H, 4.26; N, 12.12.

8-Phenyl-7*H*-pyrimido[1,2-*c*]thieno[2,3-*e*]pyrimidin-7-one (4Ba).

Yield: 77%; mp 257-258 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.62 (s, 1H), 8.56 (s, 1H), 8.42 (d, 1H, *J* = 5.8 Hz), 7.81 (d, 2H, *J* = 8.0 Hz), 7.73 (d, 1H, *J* = 5.8 Hz), 7.48 (t, 2H, *J* = 8.0 Hz), 7.40 (t, 1H, *J* = 8.0 Hz) ; MS (ESI): *m/z* 279.95 (M^+); *Anal*. Calcd for $C_{15}H_9N_3OS$: C, 64.50; H, 3.25; N, 15.04 Found: C, 64.66; H, 3.34; N, 15.17.

8-(4-Bromophenyl)-7*H*-pyrimido[1,2-*c*]thieno[2,3-*e*]pyrimidin-7-one (4Bb).

Yield: 75%; mp 237-238 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.56 (s, 1H), 8.39 (d, 1H, *J* =

5.8 Hz), 7.74 (d, 2H, *J* = 8.0 Hz), 7.69 (d, 1H, *J* = 5.8 Hz), 7.63 (d, 2H, *J* = 8.0 Hz); MS (ESI): *m/z* 358.8 (M^+); *Anal*. Calcd for $C_{15}H_8BrN_3OS$: C, 50.29; H, 2.25; N, 11.73. Found: C, 50.19; H, 2.15; N, 11.79.

8-(2-Chlorophenyl)-7*H*-pyrimido[1,2-*c*]thieno[2,3-*e*]pyrimidin-7-one (4Bc).

Yield: 78%; mp 203-204 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.40 (d, 1H, *J* = 5.8 Hz), 8.31 (s, 1H), 7.69 (d, 1H, *J* = 5.8 Hz), 7.56-7.54 (m, 1H), 7.46-7.40 (m, 3H); MS (ESI): *m/z* 313.3 (M^+); *Anal*. Calcd for $C_{15}H_8ClN_3OS$: C, 57.42; H, 2.57; N, 11.30. Found: C, 57.50; H, 2.49; N, 11.21.

8-(3-Chlorophenyl)-7*H*-pyrimido[1,2-*c*]thieno[2,3-*e*]pyrimidin-7-one (4Bd).

Yield: 79%; mp 335-336 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.60 (s, 1H), 8.40 (d, 1H, *J* = 5.8 Hz), 7.87 (s, 1H), 7.75 (d, 1H, *J* = 7.8 Hz), 7.70 (d, 1H, *J* = 5.8 Hz), 7.49-7.40 (m, 2H); MS (ESI): *m/z* 313.8 (M^+); *Anal*. Calcd for $C_{15}H_8ClN_3OS$: C, 57.42; H, 2.57; N, 11.30. Found: C, 57.34; H, 2.56; N, 11.40.

8-(4-Chlorophenyl)-7*H*-pyrimido[1,2-*c*]thieno[2,3-*e*]pyrimidin-7-one (4Be).

Yield: 83%; mp 229-230 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.52 (s, 1H), 8.35 (d, 1H, *J* = 5.8 Hz), 7.77 (d, 2H, *J* = 8.0 Hz), 7.65 (d, 1H, *J* = 5.8 Hz), 7.45 (d, 2H, *J* = 8.0 Hz); MS (ESI): *m/z* 313.9 (M^+); *Anal*. Calcd for $C_{15}H_8ClN_3OS$: C, 57.42; H, 2.57; N, 11.30. Found: C, 57.49; H, 2.51; N, 11.41.

8-(4-Nitrrophenyl)-7*H*-pyrimido[1,2-*c*]thieno[2,3-*e*]pyrimidin-7-one (4Bf).

Yield: 88%; mp 331-332 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.62 (s, 1H), 8.73 (s, 1H), 8.44 (d, 1H, *J* = 5.8 Hz), 8.29 (d, 2H, *J* = 7.8 Hz), 8.11 (d, 2H, *J* = 7.8 Hz), 7.73 (d, 1H, *J* = 5.8 Hz); MS (ESI): *m/z* 324.1 (M^+); *Anal*. Calcd for $C_{15}H_8N_4O_3S$: C, 55.55; H, 2.49; N, 17.28. Found: C, 55.48; H, 2.41; N, 17.40.

8-(4-Methoxyphenyl)-7*H*-pyrimido[1,2-*c*]thieno[2,3-*e*]pyrimidin-7-one (4Bg).

Yield: 70%; mp 228-229 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 8.47 (s, 1H), 8.34 (d, 1H, *J* = 5.8 Hz), 7.72 (d, 2H, *J* = 8.0 Hz), 7.66 (d, 1H, *J* = 5.8 Hz), 6.99 (d, 2H, *J* = 8.0 Hz), 3.76 (s, 3H); MS (ESI): *m/z* 309.7 (M^+); *Anal*. Calcd for $C_{16}H_{11}N_3O_2S$: C, 62.12; H, 3.58; N, 13.58. Found: C, 62.21; H, 3.48; N, 13.47.

General procedure for the preparation of phenylpyrido[1,2-*a*]pyrimidinones, phenylpyrimido-[1,2-*c*]quinazolinones and phenylpyrazino[1,2-*a*]pyrimidinones 5 – 11.

A suspension of formamidine derivative of various heterocyclic amines (4 mmol) and **3** (4.8 mmol) in the presence of a catalytic amount of triethylamine in CH_2Cl_2 (10 mL) was stirred at rt for 2 h. The solid product was filtered, washed with EtOAc and dried. The crude product was recrystallized from EtOH to give **5 – 11**.

3-Phenyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (5).

Yield: 78%; mp 166-167 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.14 (d, 1H, *J* = 7.6 Hz), 8.63 (s, 1H), 8.02 (t, 1H, *J* = 7.5 Hz), 7.86 (d, 2H, *J* = 7.9 Hz), 7.78 (d, 1H, *J* = 7.6 Hz), 7.49-7.48 (m, 3H), 7.39 (t, 1H, *J* =

8.0 Hz); MS (ESI): *m/z* 223.03 (M^+).

3-(2-Chlorophenyl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (6).

Yield: 85%; mp 160-161 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.04 (d, 1H, *J* = 7.6 Hz), 8.32 (s, 1H), 7.99 (t, 1H, *J* = 7.5 Hz), 7.75 (d, 1H, *J* = 7.5 Hz), 7.53 (d, 1H, *J* = 7.9 Hz), 7.47 (t, 1H, *J* = 8.0 Hz), 7.42 (d, 1H, *J* = 8.0 Hz), 7.40 (t, 2H, *J* = 8.0 Hz); MS (ESI): *m/z* 256.67 (M^+).

7-Bromo-3-(4-chlorophenyl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (7).

Yield: 82%; mp 203-204 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.62 (s, 1H), 8.08 (d, 1H, *J* = 7.3 Hz), 7.85 (d, 2H, *J* = 7.9 Hz), 7.67 (d, 1H, *J* = 7.3 Hz), 7.48 (d, 2H, *J* = 7.9 Hz); MS (ESI): *m/z* 335.58 (M^+).

3-Phenyl-4*H*-pyrimido[1,2-*c*]quinazolin-4-one (8).

Yield: 92%; mp 237-238 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.49 (s, 1H), 8.72 (d, 1H, *J* = 7.6 Hz), 8.60 (s, 1H), 7.97 (t, 2H, *J* = 8.0 Hz), 7.81-7.80 (m, 3H), 7.47 (t, 2H, *J* = 8.0 Hz), 7.39 (t, 1H, *J* = 7.9 Hz); MS (ESI): *m/z* 274.1 (M^+).

3-(4-Methoxyphenyl)-4*H*-pyrimido[1,2-*c*]quinazolin-4-one (9).

Yield: 85%; mp 202-203 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.70 (d, 1H, *J* = 7.8 Hz), 8.55 (s, 1H), 7.94 (t, 2H, *J* = 8.0 Hz), 7.77-7.63 (m, 3H), 7.01 (d, 2H, *J* = 8.0 Hz), 3.76 (s, 3H); MS (ESI): *m/z* 303.31 (M^+).

3-(4-Bromophenyl)-4*H*-pyrazino[1,2-*a*]pyrimidin-4-one (10).

Yield: 76%; mp 254-255 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.22 (s, 1H), 8.90 (s, 1H), 8.83 (d, 1H, *J* = 7.6 Hz), 8.33 (d, 1H, *J* = 7.6 Hz), 8.30 (d, 2H, *J* = 8.0 Hz), 8.18 (d, 2H, *J* = 8.0 Hz); MS (ESI): *m/z* 302.13 (M^+).

3-(4-Nitrophenyl)-4*H*-pyrazino[1,2-*a*]pyrimidin-4-one (11).

Yield: 81%; mp 207-208 °C; ^{1}HNMR (400 MHz, DMSO-d_6) δ 9.15 (s, 1H), 8.76 (d, 1H, *J* = 7.3 Hz), 8.75 (s, 1H), 8.25 (d, 1H, *J* = 7.4 Hz), 7.82 (d, 2H, *J* = 8.0 Hz), 7.65 (d, 2H, *J* = 8.0 Hz); MS (ESI): *m/z* 268.23 (M^+).

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