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Design, Synthesis and Bioactivities of Novel Diarylthiophenes: Inhibitors of Tumor Necrosis Factor-α (TNF-α) Production

Masakazu Fujita,* Tetsuya Hirayama and Naoko Ikeda

Pharmaceutical Research Laboratories, Nikken Chemicals Co., Ltd., 1-346, Kitabukuro-cho, Saitama-shi, Saitama 330-0835, Japan

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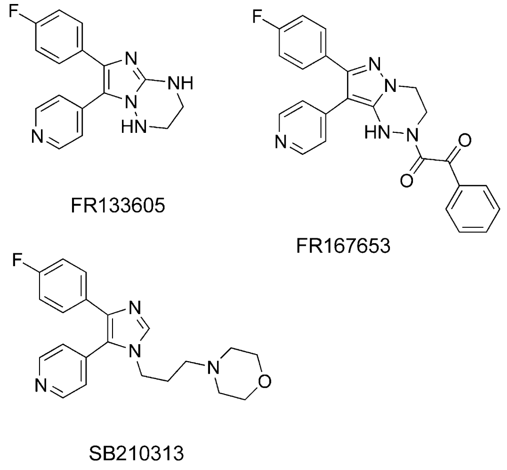
Abstract—The design, synthesis and SAR of novel diarylthiophene derivatives were performed. These compounds were designed by structural hybridization of TNF-α production inhibitors bearing 4-fluorophenyl and 4-pyridyl groups such as FR133605, FR167653 and SB210313, and 6-acetyl-3-ethoxycarbonyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (**1**) found previously by us. As a result, several compounds were more potent in vitro than FR133605 against TNF-α production stimulated with lipopolysaccharide (LPS).

Introduction

Under normal conditions, the production of cytokines helps clear viral or bacterial infections and damaged cells from injured tissues. However, the overproduction of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) is known to cause immune and inflammatory diseases.[1] In particular, the overproduction of TNF-α has been strongly implicated in the pathogenesis of such diseases as septic shock,[2] rheumatoid arthritis (RA),[3] inflammatory bowel disease (IBD),[4] multiple sclerosis,[5] type II diabetes[6] and AIDS.[7] In addition, TNF-α is a potent inducer of IL-1, IL-6, IL-8, GM-CSF and so on.[8] Therefore, agents which can inhibit the production of TNF-α have attracted much attention as potential therapies for the direct or indirect treatment of these diseases. Recently, in a clinical trial, the TNF-α antibodies, Remicade (infliximab),[9] and the soluble TNF receptor, Enbrel (etanercept)[3,10] have been reported to have excellent efficacy in RA and Crohn's patients. However, these biopharmaceuticals are expensive and inconvenient to patients. Given these backgrounds, currently, small molecule anti-TNF-α agents are being exploited through multiple approaches.[11]

In a previous paper we have found that 6-acetyl-3-ethoxycarbonyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (**1**) indicate more excellent inhibitory activity for TNF-α production by oral dosing and some excellent profiles in adjuvant-induced arthritic model (AIA) of rat as a candidate for antirheumatic drugs.[12] On the other hand, recently, a number of excellent TNF-α production inhibitors bearing 4-fluorophenyl and 4-pyridyl groups such as FR133605,[13] FR167653[14] and SB210313[15] have been reported (Fig. 1).[11] These results and reports encouraged us to discover more excellent candidates. Therefore, by structural hybridization of these TNF-α production inhibitors and **1**, we designed and synthesized 2-diethylcarbamoylamino-3-ethoxycarbonyl-4-(4-fluorophenyl)-5-(4-pyridyl)thiophene (**9c**), further 2-ethyl-



FR133605

FR167653

SB210313

Figure 1.

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*Corresponding author. Fax: +81-48-641-5749; e-mail: mfujita-nikken@nifty.com

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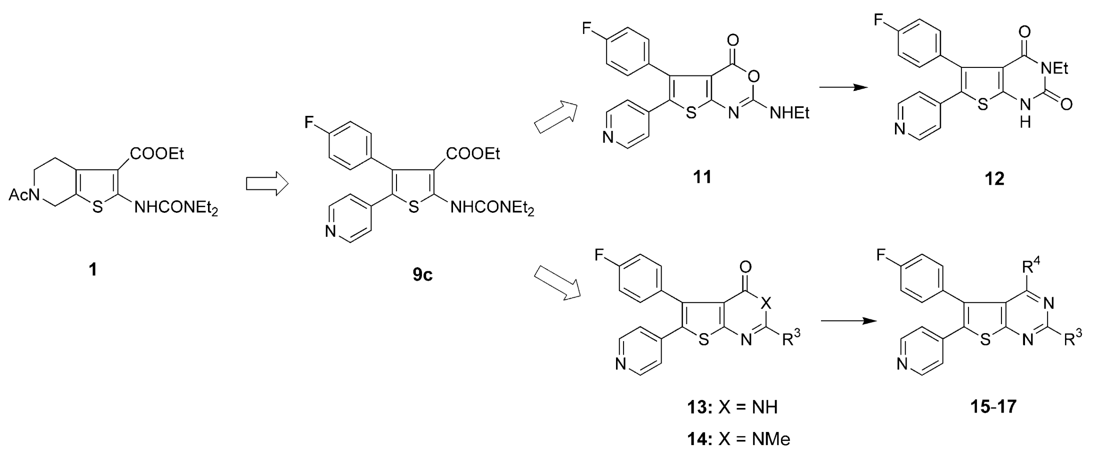


Figure 2.

amino-5-(4-fluorophenyl)-6-(4-pyridyl)-4*H*-thieno[2,3-*d*][1,3]oxazin-4-one **11** and 5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidines **12**–**17** (Fig. 2). We report here the design, synthesis and SAR of these compounds.

Chemistry and Biology

A series of the compounds listed in Tables 1–3 were synthesized by the methods illustrated in Scheme 1–3.

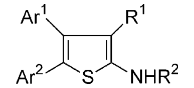
The syntheses of compounds **9** and **10** were prepared via amines **7** and **8** from ketones **3** and **6**, respectively, using synthetic sequence previously reported by us.[16] Compounds **3** and **6** were synthesized from compounds **2** and **4**, respectively, by the method of Bender et al.[17] (Scheme 1).

Oxazine (**11**) was provided by deesterification and cyclocondensation of urea **9d** with a mixture of trifluoroacetic acid and trifluoroacetic anhydride in 96% yield. 3-ethyl-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine-2,4(1*H*, 3*H*)-dione (**12**) was afforded by sodium ethoxide-promoted Dimroth rearrangement of **11** in 88% yield (Scheme 2).[18]

The reaction of **7c** with triethyl orthoformate provided 5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-d]pyrimidin-4(3*H*)-one (**13a**) in 86% yield. Pyrimidones **13b**–**c** were given by acylation of **7c** followed by cyclocondensation with sodium methoxide in 34–51% yield. Compounds **14a** and **14b** were obtained by methylation of **13b** and **13c**, respectively. Compounds **15** were prepared by chlorination of **13** with phosphorus oxychloride. Subsequently, compounds **15a** and **15b** were converted to **16** and **17** by amination, respectively (Scheme 3).

As previously described, these compounds were evaluated for their ability to inhibit LPS-stimulated production of TNF-α in rat whole blood. IC_{50} of TNF-α production was determined by comparison of yield with a control to which no test compound was added. FR133605 that showed the most potent in vivo activity in our research into many published TNF-α inhibitors and Dexamethasone were used as the positive control. Furthermore, representative compounds which possess

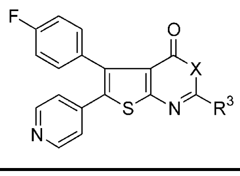
Table 1. In vitro inhibitory activity of TNF-α production for compounds **7**–**10**



Compd	Ar^1	Ar^2	R^1	R^2	In vitro IC_{50} (μM)[a]
7a	4-FPh	4-Pyridyl	COOEt	H	56
8a	4-Pyridyl	4-FPh	COOEt	H	55
7b	4-FPh	4-Pyridyl	COOH	H	> 100
7c	4-FPh	4-Pyridyl	$CONH_2$	H	> 100
7d	4-FPh	4-Pyridyl	CN	H	> 100
9a	4-FPh	4-Pyridyl	COOEt	$COCHMe_2$	36
10a	4-Pyridyl	4-FPh	COOEt	$COCHMe_2$	37
9b	4-FPh	4-Pyridyl	$CONH_2$	$COCHMe_2$	> 100
9c	4-FPh	4-Pyridyl	COOEt	$CONEt_2$	2.6
10b	4-Pyridyl	4-FPh	COOEt	$CONEt_2$	1.7
9d	4-FPh	4-Pyridyl	COOtBu	CONHEt	5.4
9e	4-FPh	4-Pyridyl	CN	$CONEt_2$	24
1	$-(CH_2)_2N(Ac)CH_2-$		COOEt	$CONEt_2$	7.1 ± 1.3
FR133605					10.2 ± 1.4
Dexamethasone					0.02

[a] IC_{50} of LPS-stimulated TNF-α production in rat whole blood.

Table 2. In vitro inhibitory activity of TNF-α production for compounds **11**–**14**



Compd	R^3	X	In vitro IC_{50} (μM)[a]
11	NHEt	O	> 100
13a	H	NH	> 100
13b	Me	NH	> 100
13c	$CHMe_2$	NH	> 100
14a	Me	NMe	> 100
14b	$CHMe_2$	NMe	> 100
12[b]			20

[a] IC_{50} of LPS-stimulated TNF-α production in rat whole blood.
[b] See Figure 2.

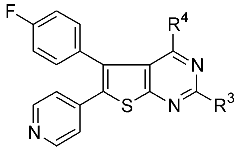
excellent in vitro activity were tested in vivo after oral administration. TNF-α inhibitory activity was assessed by in vivo inhibition of serum TNF-α production in the rat.[16]

Results and Discussion

According to our previous report, it suggests that chemical modification of the pyridine moiety of 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine analogues makes little difference to the activity.[12] Therefore, we designed **9c** by structural hybridization, that is, changing the pyridine moiety to 4-fluorophenyl and 4-pyridyl groups. Furthermore, bicyclic compounds **11–17**, which were provided by cyclization of the analogues of **9c**, were synthesized and evaluated for their ability to inhibit lipopolysaccharide (LPS)-stimulated production of TNF-α in rat whole blood.

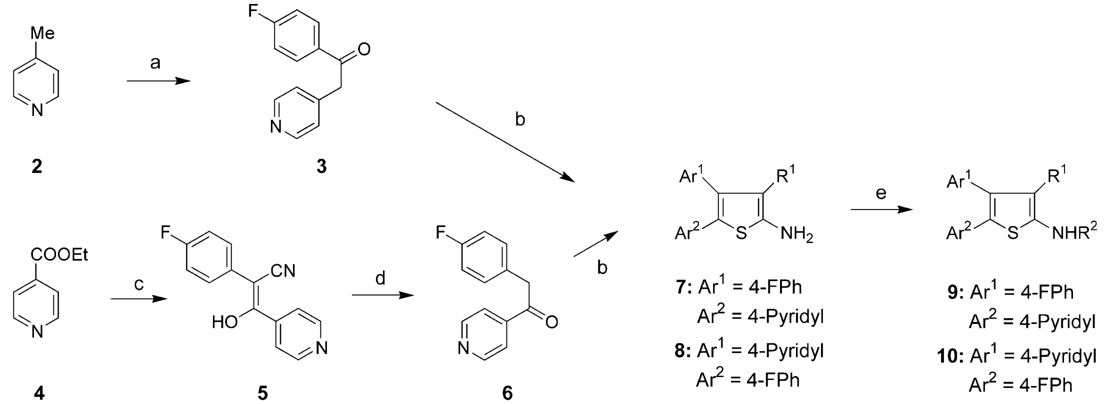
As we expected, compound **9c** exhibited 4-fold more potent activity than FR133605. Based on our previous SAR of 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines, we initially investigated in vitro SAR of **9c** (Table 1). Compound having basic amino group at the 2-position was weaker active. Further, carboxylic acid, carboxamide and cyanide analogues were inactive (see **7a–d**). In the case of acylation of their amino groups, the carbamide analogue was similarly inactive, while the cyanide analogue indicated increased activity (**7c** vs **9b** and **7d** vs **9e**). Isobutyrylation of **7a** and **8a** showed slightly more potent activity (**9a** and **10a**). *tert*-Butylester **9d** showed excellent in vitro activity, but was essentially inactive in the in vivo activity (data not shown). This modification in the above cases afforded very similar results as the previous. On the other hand, the in vitro activities for regioisomers were found to be equipotent (**7a**, **9a**, **9c** vs **8a**, **10a**, **10b**, respectively). However, as these compounds were tested in vivo after oral administration, the inhibitions of **9a**, **10a**, **9c** and **10b** at 50 mg/

Table 3. In vitro inhibitory activity of TNF-α production for compounds **15–17**

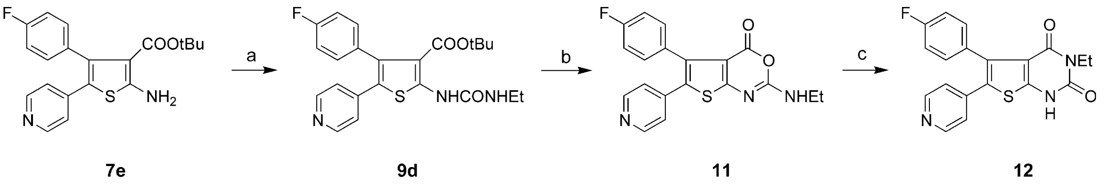


Compd	R^3	R^4	In vitro IC_{50} (μM)[a]
15a	H	Cl	2.9
15b	Me	Cl	14
15c	$CHMe_2$	Cl	15
16a	H	NHEt	40
16b	H	$NHCH_2Ph$	14
17a	Me	$NHCH_2Ph$	15
16c	H	$NHCH_2$(4-Cl-Ph)	66
16d	H	NH-Piperonyl	29
16e	H	$NH(CH_2)_2OH$	29
17b	Me	$NH(CH_2)_2OH$	17
16f	H	$NH(CH_2)_2OEt$	10
17c	Me	$NH(CH_2)_2OEt$	20
16g	H	$NHCH_2CH(OEt)_2$	>100
16h	H	$NH(CH_2)_2O(CH_2)_2OH$	10
17d	Me	$NH(CH_2)_2O(CH_2)_2OH$	13
16i	H	$NH(CH_2)_2NH_2$	88
16j	H	$NHCH_2COOH$	>100
16k	H	$NHCH_2COOEt$	>100
16l	H	NHCH(Me)COOEt	>100
16m	H	NEt_2	>100
16n	H	$N((CH_2)_2OEt)_2$	40

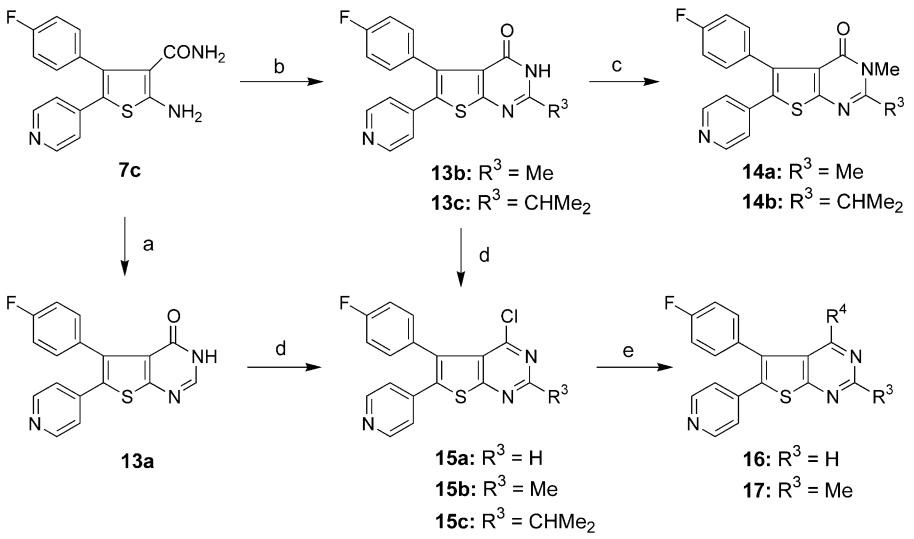
[a] IC_{50} of LPS-stimulated TNF-α production in rat whole blood.



Scheme 1. Reagents and conditions: (a) 4-FPhCOOEt, LHMDS, THF, 0 °C, quant.; (b) $NCCH_2R^1$, S, base/EtOH or pyridine, 60 °C, 39–72%; (c) 4-$FPhCH_2CN$, NaOEt, EtOH, reflux; (d) 48% HBr, reflux, 23% (two steps); (e) for **9a–b** and **10a**: $Me_2CHCOCl$, pyridine, 12%–quant. For **9c–e** and **10b**: Et_2NH or $EtNH_2$, triphosgene, Et_3N, CH_2Cl_2, 0 °C–rt, 47%–quant.



Scheme 2. Reagents and conditions: (a) $EtNH_2$, triphosgene, Et_3N, CH_2Cl_2, 71%; (b) $(CF_3CO)_2O$, TFA, 0 °C–rt, 96%; (c) NaOEt, EtOH, reflux, 88%.



Scheme 3. Reagents and conditions: (a) $CH(OEt)_3$, PhMe, reflux, 86%; (b) (i) AcCl or $Me_2CHCOCl$, pyridine; (ii) NaOMe, MeOH, reflux, 34–51%; (c) MeI, NaH, DMF, 40–79% (d) $POCl_3$, 100 °C, 71–93%; (e) amines, Et_3N/CH_2Cl_2 or NaOH/THF, rt–reflux, 65%–quant.

kg were 22.1, 12.3, 53.1 and 34.2%, respectively. Namely, it demonstrates that 4-(4-fluorophenyl)-5-(4-pyridyl)thiophene analogues are desirable.

Next, we investigated the activities for bicyclic compounds **11**–**17**.

Oxazinone **11** and a series of pyrimidones **13**–**14** were inactive despite pyrimidinedione **12** revealed weaker activity (Table 2).

The results of pyrimidines **15**–**17** are summarized in Table 3. Keeping the substituents at the 4-position, modification of 2-substituents suggests that hydrogen atom is preferred to methyl and isopropyl groups (e.g., **15a** vs **15b** vs **15c**). Next, to see the substituent effect of the 4-position, the introductions of *N*-substituted amino groups were examined. Ethylamino group had an IC_{50} value of 40 μM (**16a**). A hydroxyl group was oriented to the rear of the ethyl, resulting in increased activity (**16e**). Further, extending the alkyl group outward by capping the hydroxyl with an ethyl group and repeating them led to increased potency up to 10 μM (**16f** and **16h**). *N*-disubstituted amino groups had diminished potency (**16f** vs **16n** and **16a** vs **16m**). Incorporation of an acid substituent (**16j**), a basic substituent (**16i**) or polar groups such as ethoxycarbonyl (**16k**–**l**) and diethylacetal (**16g**) groups were found to be deleterious toward activity. Aryl residues showed the decrease in activity in the order of 4-Cl-benzyl < piperonyl < benzyl (**16b**–**d**).

Table 4. In vivo inhibitory activities of TNF-α production for representative compounds

Compd	Inhibition (%) at 50 mg/kg, po[a]
9c	53.1
9d	4.8
10b	34.2
15a	20.1
15b	15.8
15c	17.1
16b	69.2
16f	50.1
16h	58.9
17a	58.1
17b	78.3
17d	45.1
1	85.1 ± 2.8
FR133605	88.1 ± 3.5

[a] Inhibition of LPS-stimulated serum TNF-α production in the rat.

Representative compounds that possess excellent in vitro activity were tested in vivo after oral administration at 50 mg/kg (Table 4). TNF-α inhibitory activity was assessed by in vivo inhibition of serum TNF-α production in the rat.[16] As a result, compound **17b** was slightly weaker than **1**.

Conclusion

We performed the design, synthesis and SAR of novel diarylthiophene derivatives. Several compounds exhibited more potent in vitro activity than **1** and FR133605 but they could not exceed the two by oral dosing at 50 mg/kg. However, these results suggest that further SAR of novel diarylthiophene derivatives lead to the discovery of orally excellent agents. At present, a number of novel diaryl substituted heterocyclic compounds that possess more potent TNF-α inhibitory activity than **1** and FR133605 are synthesized, and it continues. In addition, interestingly, although diaryl substituted heterocyclic inhibitors of p38 MAP kinase have been reported,[19] fortunately, many of our compounds have not almost shown inhibition of p38 MAP kinase. On the other hand, it is very important to understand the action mechanism of them. Therefore, our studies are currently in progress toward the goal and a complete report containing them will appear elsewhere.

Experimental

Chemistry

General procedures. Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR SPECTRUM 2000 using KBr pellets. ^{1}H NMR spectra were obtained on a Jeol JNM-AL 400. Chemical shifts are reported in parts per million relative to TMS as internal standard. Melting points were determined using Yanaco MP-500D and are uncorrected. Mass spectra (MS) and high-resolution mass spectra (HRMS) were measured on a ThermoQuest FINNIGAN AQA and a Micromass VG-70VSE, respectively. When air- or moisture-sensitive reagents were used, reactions were run under argon. All reagents and solvents were of commercial quality and used without further purification unless indicated otherwise. Column chromatography was performed on columns of E. Merck silica gel 60 (230–400 mesh).

1-(4-Fluorophenyl)-2-(4-pyridyl)ethanone (3). To a mixture of 4-methylpyridine (**2**) (5.0 g, 53.7 mmol) and ethyl 4-fluorobenzoate (9.0 g, 53.5 mmol) in THF (40 mL) was added dropwise lithium bis(trimethylsilyl)amide (53.5 mL, 1.0 M solution in THF) at 0 °C. After stirring for 1 h, the reaction mixture was allowed to warm to room temperature, triturated with hexane (138 mL) and filtered. The solid was dissolved in 3 N HCl (50 mL). The solution was neutralized with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The combined organic layers were washed with H_2O followed by brine, dried with $MgSO_4$ and evaporated to afford **3** (10.5 g, y. 91%) as a light yellow solid; ^{1}H NMR ($CDCl_3$) δ: 4.27 (2H, s, CH_2), 7.11–7.21 (4H, m, Pyridyl + Ph), 8.00–8.05 (2H, m, Ph), 8.57 (2H, dd, *J* = 2.0, 4.8 Hz, Pyridyl).

2-(4-Fluorophenyl)-1-(4-pyridyl)ethanone (6). To a mixture of ethyl isonicotinate (**4**) (99.5 g, 0.66 mol) and 4-fluorophenylacetonitrile (100 g, 0.74 mol) in EtOH (300 mL) was added sodium ethoxide (59 g, 0.87 mol) at room temperature. After reflux for 24 h, the cooled reaction mixture was neutralized with concd. HCl and extracted with AcOEt. The combined organic layers were washed with H_2O followed by brine, dried with $MgSO_4$ and evaporated to afford 2-(4-fluorophenyl)-3-hydroxy-3-(4-pyridyl)acrylonitrile (**5**). A solution of **5** in concd. HBr (100 mL) was heated to reflux for 4 h. The reaction solution was kept at room temperature for 1 h, neutralized with K_2CO_3 and extracted with AcOEt. The combined organic layers were washed with H_2O, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $CHCl_3$/MeOH (50:1 v/v) to afford **6** (35.7 g, y. 23%) as a light yellow solid; ^{1}H NMR ($CDCl_3$) δ: 4.26 (2H, s, CH_2), 6.98–7.04 (2H, m, Ph), 7.17–7.22 (2H, m, Ph), 7.74 (2H, dd, *J* = 2.0, 4.4 Hz, Pyridyl), 8.78 (2H, dd, *J* = 2.0, 4.4 Hz, Pyridyl).

2-Amino-3-ethoxycarbonyl-4-(4-fluorophenyl)-5-(4-pyridyl)thiophene (7a). To a mixture of **3** (6.0 g, 27.9 mmol), sulfur (1.1 g, 34.3 mmol) and ethyl cyanoacetate (4.1 g, 36.2 mmol) in EtOH (50 mL) was added triethylamine (5.6 g, 55.3 mmol). After stirring at 60 °C for 3 days, the reaction mixture was evaporated. Then H_2O was added and the solution was extracted with AcOEt. The combined organic layers were washed with brine, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $CHCl_3$/MeOH (15:1 v/v) followed by crystallization from Et_2O afforded **7a** (3.7 g, y. 39%); mp: 210–213 °C; ^{1}H NMR ($CDCl_3$) δ: 1.02 (3H, t, *J* = 7.2 Hz, CH_3(Et)), 4.07 (2H, q, *J* = 7.2 Hz, CH_2(Et)), 5.74 (2H, br s, NH_2), 6.09 (2H, t, *J* = 8.8 Hz, Ph), 7.16 (2H, dd, *J* = 4.4, 8.8 Hz, Ph), 7.28 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl), 8.62 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $C_{18}H_{15}FN_2O_2S$ 342.0838, found 342.0841.

2-Amino-4-(4-fluorophenyl)-3-hydroxycarbonyl-5-(4-pyridyl)thiophene (7b). This was prepared as described in the synthesis of **7a**; mp: 221–223 °C; ^{1}H NMR ($CDCl_3$) δ: 6.75 (2H, d, *J* = 5.6 Hz, Pyridyl), 7.12–7.25 (4H, m, Ph), 7.79 (2H, br s, NH_2), 8.25 (2H, d, *J* = 5.6 Hz, Pyridyl), 11.91 (1H, br s, COOH); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $C_{16}H_{11}FN_2O_2S$ 314.0525, found 314.0520.

2-Amino-3-carbamoyl-4-(4-fluorophenyl)-5-(4-pyridyl)thiophene (7c). This was prepared as described in the synthesis of **7a**; mp: 243–246 °C; ^{1}H NMR ($CDCl_3$) δ: 5.02 (2H, br s, NH_2), 6.17 (2H, br s, $CONH_2$), 7.04 (2H, t, *J* = 8.8 Hz, Ph), 7.23–7.28 (2H, m, Ph), 7.54 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl), 8.91 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $C_{16}H_{12}FN_3OS$ 313.0685, found 313.0692.

2-Amino-3-cyano-4-(4-fluorophenyl)-5-(4-pyridyl)thiophene (7d). To a mixture of **3** (4.0g, 18.6 mmol), sulfur (0.8 g, 25.0 mmol) and malononitrile (1.4 g, 21.2 mmol) in pyridine (40 mL) was added morpholine (0.8 g, 9.2 mmol). After stirring at 60 °C for 3 days, the reaction mixture was evaporated. Then H_2O was added and the solution was extracted with AcOEt. The combined organic layers were washed with brine, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $CHCl_3$/MeOH (100:1 followed by 15:1 v/v) to afford **7d** (2.1 g, y. 39%); mp: 254–256 °C; ^{1}H NMR ($CDCl_3$) δ: 6.97 (2H, d, *J* = 4.8 Hz, Pyridyl), 7.11 (2H, t, *J* = 8.8 Hz, Ph), 7.24–7.33 (2H, m, Ph), 8.33 (2H, d, *J* = 4.8 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $C_{16}H_{10}FN_3S$ 295.0579, found 295.0571.

2-Amino-3-ethoxycarbonyl-5-(4-fluorophenyl)-4-(4-pyridyl)thiophene (8a). This was prepared as described in the synthesis of **7a** with **6** instead of **3**; mp: 161–163 °C; ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, *J* = 7.6 Hz, CH_3 (Et)), 4.05 (2H, q, *J* = 7.6 Hz, CH_2 (Et)), 6.24 (2H, s, NH_2), 7.00–7.04 (2H, m, H_{arom}), 7.13 (2H, t, *J* = 8.4 Hz, Ph), 7.27–7.40 (2H, m, H_{arom}), 8.35 (2H, dd, *J* = 1.6, 4.8 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $C_{18}H_{15}FN_2O_2S$ 342.0838, found 342.0837.

3-Ethoxycarbonyl-2-isobutyrylamino-4-(4-fluorophenyl)-5-(4-pyridyl)thiophene (9a). To a solution of **7a** (1.0 g, 2.92 mmol) in pyridine (20 mL) was added dropwise isobutyryl chloride (0.34 mL, 3.25 mmol) at room temperature. After stirring for 2 h at room temperature, MeOH (10 mL) was added. The reaction mixture was stirred for 30 min at room temperature and evaporated. Then saturated aqueous $NaHCO_3$ was added and the solution was extracted with AcOEt. The combined organic layers were washed with H_2O followed by brine, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $CHCl_3$/MeOH (30:1 v/v) followed by crystallization from Et_2O afforded **9a** (190 mg, y. 16%); mp: 167–169 °C; ^1H NMR ($CDCl_3$) δ: 1.01 (3H, t, *J* = 7.2 Hz, CH_3 (Et)), 1.33 (6H, d, *J* = 6.8 Hz, CH_3 (*i*-Bu)×2), 2.61–2.75 (1H, m, CH (*i*-Bu)) 4.10 (2H, q, *J* = 7.2 Hz, CH_2 (Et)), 6.92 (2H, t, *J* = 8.8 Hz, Ph), 7.20–7.40 (4H, m, Ph and Pyridyl),8.66 (2H, d, *J* = 5.2 Hz, Pyridyl), 9.85 (1H, br s, NH); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{22}H_{21}FN_2O_3S$ 412.1257, found 412.1260.

3-Carbamoyl-2-isobutyrylamino-4-(4-fluorophenyl)-5-(4-pyridyl)thiophene (9b). This was prepared as described in the synthesis of **9a** with **7c** instead of **7a**; mp: 243–246 °C; ^1H NMR ($CDCl_3$) δ: 1.32 (6H, d, *J* = 6.8 Hz, CH_3 (*i*-Bu)×2), 2.60–2.71 (1H, m, CH (*i*-Bu)), 7.05 (2H, t, *J* = 8.4 Hz, Ph), 7.31–7.42 (4H, m, Ph and Pyridyl), 8.08–8.25 (1H, m, NH), 8.68 (2H, d, *J* = 4.4 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{20}H_{18}FN_3O_2S$ 383.1104, found 383.1094.

2-Diethylcarbamoylamino-3-ethoxycarbonyl-4-(4-fluorophenyl)-5-(4-pyridyl)thiophene (9c). To a solution of **7a** (140 mg, 0.41 mmol) in CH_2Cl_2 (7 mL) was added dropwise triphosgene (122 mg, 0.41 mmol), followed by dropwise triethylamine (0.09 mL, 0.65 mmol) at 0 °C. After stirring for 2 h at room temperature, diethylamine (0.34 mL, 3.28 mmol) was added. In addition, after stirring for 1 h, H_2O was added. The solution was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried with Na_2SO_4 and evaporated. The residue was recrystallized from hexane/Et_2O to afford **9c** (151 mg, y. 84%); mp: 141–143 °C; ^1H NMR ($CDCl_3$) δ: 0.98 (3H, t, *J* = 6.8 Hz, CH_3CH_2O), 1.30 (6H, t, *J* = 7.2 Hz, $(CH_3CH_2)_2N$), 3.45 (4H, q, *J* = 7.2 Hz, $(CH_3CH_2)_2N$), 4.08 (2H, q, *J* = 6.8 Hz, CH_3CH_2O), 6.90 (2H, t, *J* = 8.4 Hz, Ph), 7.26–7.33 (4H, m, Ph and Pyridyl), 8.64 (2H, dd, *J* = 1.6, 4.8 Hz, Pyridyl), 9.55 (1H, br s, NH); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{23}H_{24}FN_3O_3S$ 441.1522, found 441.1513.

3-*tert*-Butoxycarbonyl-2-ethylcarbamoylamino-4-(4-fluorophenyl)-5-(4-pyridyl)thiophene (9d). **7d** was prepared as described in the synthesis of **7a** with *t*-butyl cyanoacetate instead of ethyl cyanoacetate. Then **9d** was prepared as described in the synthesis of **9c** with ethylamine instead of diethylamine; mp: 223–225 °C; ^1H NMR ($CDCl_3$) δ: 1.16 (9H, s, CH_3 (*t*-Bu)×3), 1.24 (3H, t, *J* = 7.2 Hz, CH_3 (Et)), 3.36–3.44 (2H, m, CH_2 (Et)), 4.91 (1H, br s, NH), 6.90 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl), 7.03 (2H,dt, *J* = 2.0, 8.8 Hz, Ph), 7.10–7.53 (2H, m, Ph), 8.33 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{23}H_{24}FN_3O_3S$ 441.1522, found 441.1525.

3-Cyano-2-diethylcarbamoylamino-4-(4-fluorophenyl)-5-(4-pyridyl)thiophene (9e). This was prepared as described in the synthesis of **9c** with **7d** instead of **7a**; mp: 147–149 °C; ^1H NMR ($CDCl_3$) δ: 1.28 (6H, t, *J* = 7.2 Hz, CH_3 (Et)×2), 3.46 (4H, q, *J* = 7.2 Hz, CH_2 (Et)×2), 7.05 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl), 7.10 (2H, t, *J* = 8.8 Hz, Ph), 7.26–7.35 (2H, m, Ph), 7.70 (1H, s, NH), 8.45 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{21}H_{19}FN_4OS$ 394.1264, found 394.1277.

3-Ethoxycarbonyl-2-isobutyrylamino-5-(4-fluorophenyl)-4-(4-pyridyl)thiophene (10a). This was prepared as described in the synthesis of **9a** with **8a** instead of **7a**; mp: 154–156 °C; ^1H NMR ($CDCl_3$) δ: 1.00 (3H, t, *J* = 7.2 Hz, CH_3 (Et)), 1.34 (6H, d, *J* = 6.8 Hz, CH_3 (*i*-Bu)×2), 2.68–2.73 (1H, m, CH (*i*-Bu)) 4.09 (2H, q, *J* = 7.2 Hz, CH_2 (Et)), 7.12–7.21 (4H, m, H_{arom}), 7.26–7.33 (2H, m, H_{arom}), 8.42 (2H, d, *J* = 6.4 Hz, Pyridyl), 9.93 (1H, s, NH); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{22}H_{21}FN_2O_3S$ 412.1257, found 412.1250.

2-Diethylcarbamoylamino-3-ethoxycarbonyl-5-(4-fluorophenyl)-4-(4-pyridyl)thiophene (10b). This was prepared as described in the synthesis of **9c** with **8a** instead of **7a**; mp: 140–142 °C; ^1H NMR ($CDCl_3$) δ: 0.97 (3H, t, *J* = 6.8 Hz, CH_3CH_2O), 1.30 (6H, t, *J* = 6.8 Hz, $(CH_3CH_2)_2N$), 3.47 (4H, q, *J* = 6.8 Hz, $(CH_3CH_2)_2N$), 4.06 (2H, q, *J* = 6.8 Hz, CH_3CH_2O), 7.10–7.19 (4H, m, H_{arom}), 7.26–7.33 (2H, m, H_{arom}),8.38 (2H, dd, *J* = 2.0, 4.8 Hz, Pyridyl), 9.65 (1H, s, NH); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{23}H_{24}FN_3O_3S$ 441.1522, found 441.1521.

2-Ethylamino-5-(4-fluorophenyl)-6-(4-pyridyl)-4*H*-thieno[2,3-*d*][1,3]oxazin-4-one (11). **9d** (300 mg, 0.68 mmol) was treated dropwise with a mixture of trifluoroacetic acid (3 mL) and trifluoroacetic anhydride (140 μL, 1.02 mmol) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and for an additional 16 h at room temperature. The mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The combined organic layers were washed with H_2O followed by brine, dried with $MgSO_4$ and evaporated to an oil. Trituration from AcOEt afford **11** (239 mg, y. 96%); mp: 219–221 °C; IR (KBr, cm^{-1}): 1743 (C=O); ^1H NMR ($CDCl_3$) δ: 1.31 (3H, t, *J* = 7.2 Hz, CH_3), 3.45–3.61 (2H, m, CH_2), 5.18 (1H, br s, NH), 7.01 (2H, d, *J* = 6.0 Hz, Pyridyl), 7.07 (2H, t, *J* = 8.8 Hz, Ph), 7.20–7.30 (2H, m, Ph), 8.44 (2H, d, *J* = 6.0 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{19}H_{14}FN_3O_2S$ 367.0791, found 367.0799.

3-Ethyl-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (12). To a solution of **11** (200 mg, 0.54 mmol) in EtOH (20 mL) was added sodium ethoxide (37 mg, 0.54 mmol) at room temperature. After reflux for 1 h, the reaction mixture was allowed to cool to room temperature. Furthermore, the reaction mixture was cooled, neutralized with 1 N HCl and evaporated. The residue was diluted with H_2O and extracted with AcOEt. The combined organic layers were washed with brine, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $CHCl_3$/MeOH (30:1 v/v) to afford **12** (175 mg, y. 88%) as a yellow solid; mp: 291–293 °C; IR (KBr, cm^{-1}): 1668, 1714 (C=O); ^1H NMR ($CDCl_3$) δ: 1.23 (3H, br t, *J* = 7.2 Hz, CH_3), 4.04 (2H, br q, *J* = 7.2 Hz, CH_2), 5.30 (1H, s, NH), 6.99 (2H, d, *J* = 5.2 Hz, Pyridyl), 7.09 (2H, t, *J* = 8.4 Hz, Ph), 7.20–7.31 (2H, m, Ph), 8.46 (2H, br d, *J* = 5.2 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{19}H_{14}FN_3O_2S$ 367.0791, found 367.0790.

5-(4-Fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (13a). To a solution of **7c** (1.0 g, 3.2 mmol) in toluene (20 mL) was added dropwise triethyl orthoformate (2.4 g, 16.2 mmol) at room temperature. After reflux for 6 h, the reaction mixture was allowed to cool to room temperature. The resulting precipitate was collected, washed with AcOEt, $CHCl_3$ followed by MeOH and dried in vacuo to afford **13a** (887 mg, y. 86%) as a white solid; mp: 325–327 °C; ^1H NMR ($CDCl_3 + CD_3OD$) δ: 7.07 (2H, t, *J* = 8.8 Hz, Ph), 7.41–7.62 (4H, m, Ph and Pyridyl), 8.00 (1H, s, H_{arom}), 8.58 (2H, d, *J* = 6.0 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{17}H_{10}FN_3OS$ 323.0529, found 323.0541.

5-(4-Fluorophenyl)-2-methyl-6-(4-pyridyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (13b). To a solution of **7c** (5.0 g, 16.0 mmol) in pyridine (20 mL) was added dropwise acetyl chloride (1.25 mL, 17.6 mmol) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was evaporated. The residue was diluted with MeOH (100 mL). To the solution was added sodium methoxide (1.7 g, 31.5 mmol) at room temperature. After reflux for 4 h, the reaction mixture was allowed to cool to room temperature. Furthermore, the reaction mixture was cooled, neutralized with concd. HCl and evaporated. The residue was diluted with H_2O and extracted with AcOEt. The combined organic layers were washed with brine, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $CHCl_3$/MeOH (30:1 v/v) followed by crystallization from Et_2O afforded **13b** (2.1 g, y. 40%); mp: 319–321 °C; ^1H NMR ($CDCl_3$) δ: 2.55 (3H, s, CH_3), 7.05 (2H, t, *J* = 8.4 Hz, Ph), 7.48–7.53 (4H, m, Ph and Pyridyl), 8.66 (2H, d, *J* = 6.0 Hz, Pyridyl), 11.38 (1H, br s, NH); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{18}H_{12}FN_3OS$ 337.0685, found 337.0680.

5-(4-Fluorophenyl)-2-isopropyl-6-(4-pyridyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (13c). This was prepared by treatment of **9b** with sodium methoxide as described in the synthesis of **13b**; mp: 311–312 °C; ^1H NMR ($CDCl_3$) δ: 1.38 (6H, d, *J* = 7.2 Hz, $CH_3 \times 2$), 2.93–3.02 (1H, m, CH), 7.06 (2H, t, *J* = 8.4 Hz, Ph), 7.49–7.57 (4H, m, Ph and Pyridyl), 8.65 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl), 11.92 (1H, s, NH); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{20}H_{16}FN_3OS$ 365.0998, found 365.1001.

5-(4-Fluorophenyl)-2,3-dimethyl-6-(4-pyridyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (14a). Sodium hydride (119 mg, 60% dispersion, 3.0 mmol) was added to a stirred solution of **13b** (500 mg, 1.5 mmol) in DMF (5 mL) at 0 °C. After stirring for 1 h at room temperature, methyl iodide (140 μL, 2.25 mmol) was added at 0 °C. The reaction mixture was stirred for 2 h at room temperature and after adding H_2O for an additional 30 min at room temperature. Then the solution was extracted with AcOEt. The combined organic layers were washed with H_2O followed by brine, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $CHCl_3$/MeOH (30:1 v/v) followed by crystallization from Et_2O afforded **14a** (341 mg, y. 66%); mp: 238–240 °C; ^1H NMR ($CDCl_3$) δ: 2.69 (3H, s, CH_3), 3.61 (3H, s, CH_3*N*), 7.03 (2H, t, *J* = 8.4 Hz, Ph), 7.42–7.52 (4H, m, Ph and Pyridyl), 8.66 (2H, d, *J* = 5.6 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{19}H_{14}FN_3OS$ 351.0842, found 351.0847.

5-(4-Fluorophenyl)-2-isopropyl-3-methyl-6-(4-pyridyl)thieno[2,3-d]pyrimidin-4(3*H*)-one (14b). This was prepared as described in the synthesis of **14a** with **13c** instead of **13b**; mp: 223–225 °C; ^1H NMR ($CDCl_3$) δ: 1.36 (6H, d, *J* = 7.2 Hz, $CH_3 \times 2$), 3.10–3.19 (1H, m, CH), 3.62 (3H, s, CH_3*N*), 7.03 (2H, t, *J* = 8.4 Hz, Ph), 7.43–7.55 (4H, m, Ph and Pyridyl), 8.65 (2H, dd, *J* = 1.6, 4.8 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{21}H_{18}FN_3OS$ 379.1155, found 379.1156.

4-Chloro-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (15a). A mixture of **13a** (470 mg, 1.5 mmol) and phosphorus oxychloride (5 mL) was heated to 100 °C for 7 h, cooled to room temperature and poured into H_2O (50 mL) at 0 °C. The pH was adjusted to 8 with saturated aqueous $NaHCO_3$. Then the solution was extracted with AcOEt. The combined organic layers were washed with H_2O followed by brine, dried with $MgSO_4$ and evaporated. The residue was crystallized from Et_2O to afford **15a** (459 mg, y. 93%); mp: 157–159 °C; ^1H NMR ($CDCl_3$) δ: 7.06 (2H, t, *J* = 8.4 Hz, Ph), 7.43 (2H, d, *J* = 5.2 Hz, Pyridyl), 7.50–7.62 (2H, m, Ph), 8.78 (2H, d, *J* = 5.2 Hz, Pyridyl), 8.80 (1H, s, H_{arom}); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{17}H_9ClFN_3S$ 341.0190, found 341.0199.

4-Chloro-5-(4-fluorophenyl)-2-methyl-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (15b). This was prepared as described in the synthesis of **15a** with **13b** instead of **13a**; mp: 153–155 °C; ^1H NMR ($CDCl_3$) δ: 2.83 (3H, s, CH_3), 7.04 (2H, dt, *J* = 2.0, 8.4 Hz, Ph), 7.41 (2H, d, *J* = 5.6 Hz, Pyridyl), 7.49–7.54 (2H, m, Ph), 8.77 (2H, d, *J* = 5.6 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{18}H_{11}ClFN_3S$ 355.0346, found 355.0356.

4-Chloro-5-(4-fluorophenyl)-2-isopropyl-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (15c). This was prepared as described in the synthesis of **15a** with **13c** instead of **13a**; mp: 139–141 °C; ^1H NMR ($CDCl_3$) δ: 1.42 (6H, d, *J* = 6.8 Hz, $CH_3 \times 2$), 3.25–3.35 (1H, m, CH), 7.04 (2H, t, *J* = 8.4 Hz, Ph), 7.42 (2H, d, *J* = 5.6 Hz, Pyridyl), 7.53 (2H, dd, *J* = 5.6, 8.4 Hz, Ph), 8.77 (2H, d, *J* = 5.6 Hz, Pyridyl); HRMS (FAB^+) *m*/*z* exact mass calcd for $C_{20}H_{15}ClFN_3S$ 383.0659, found 383.0651.

4-Ethylamino-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (16a). To a mixture of **15a** (400 mg, 1.2

mmol) and triethylamine (486 mg, 4.8 mmol) in CH_2Cl_2 (40 mL) was added ethylamine hydrochloride (196 mg, 2.4 mmol). After reflux for 10 h, the reaction mixture was cooled to room temperature, diluted with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The combined organic layers were washed with brine, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $CHCl_3$/MeOH (50:1 v/v) to afford **16a** (360 mg, y. 88%) as a light brown solid; mp: 181–183 °C; ^1H NMR ($CDCl_3$) δ: 1.13 (3H, t, J = 7.2 Hz, CH_3), 3.42–3.61 (2H, m, CH_2),4.50 (1H, s, NH), 7.01 (2H, t, J = 8.8 Hz, Ph), 7.40–7.51 (4H, m, Ph and Pyridyl), 8.45 (1H, s, H_{arom}), 8.81 (2H, d, J = 6.0 Hz, Pyridyl); HRMS (FAB^+) m/z exact mass calcd for $C_{19}H_{15}FN_4S$ 350.1001, found 350.1003.

4-Benzylamino-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (16b). To a mixture of **15a** (200 mg, 0.59 mmol) and NaOH (48 mg, 1.2 mmol) in THF (20 mL) was added benzylamine (129 mg, 1.2 mmol). After reflux for 16 h, the reaction mixture was cooled to room temperature, diluted with H_2O and extracted with AcOEt. The combined organic layers were washed with brine, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $CHCl_3$:MeOH (100:1 v/v) to afford **16b** (182 mg, y. 76%) as a white solid; mp: 199–201 °C; ^1H NMR ($CDCl_3$) δ: 4.70 (2H, d, J = 5.6 Hz, CH_2), 4.87 (1H, br, NH), 7.00 (2H, t, J = 8.8 Hz, FPh), 7.18 (2H, d, J = 6.8 Hz, $\mathit{CH_2}$Ph), 7.22–7.35 (3H, m, $\mathit{CH_2}$Ph), 7.40 (2H, d, J = 5.6 Hz, Pyridyl), 7.46 (2H, dd, J = 5.6, 8.8 Hz, FPh), 8.47 (1H, s, H_{arom}), 8.71 (2H, d, J = 5.6 Hz, Pyridyl); HRMS (FAB^+) m/z exact mass calcd for $C_{24}H_{17}FN_4S$ 412.1158, found 412.1170.

4-(4-Chlorobenzylamino)-5-(4-fluorophenyl)-6-(4-pyridyl)-thieno[2,3-*d*]pyrimidine (16c). This was prepared as described in the synthesis of **16a** with 4-chlorobenzylamine instead of ethylamine hydrochloride; mp: 198–201 °C; ^1H NMR ($CDCl_3$) δ: 4.67 (2H, d, J = 6.4 Hz, CH_2), 4.83 (1H, br t, NH), 7.01 (2H, t, J = 5.2 Hz, Ph), 7.14 (2H, d, J = 8.4 Hz, Ph), 7.27–7.29 (2H, m, Ph), 7.40 (2H, dd, J = 1.6, 4.4 Hz, Pyridyl), 7.41–7.48 (2H, m, Ph), 8.50 (1H, s, H_{arom}), 8.74 (2H, dd, J = 1.6, 4.4 Hz, Pyridyl); HRMS (FAB^+) m/z exact mass calcd for $C_{24}H_{16}ClFN_4S$ 446.0768, found 446.0776.

5-(4-Fluorophenyl)-4-piperonylamino-6-(4-pyridyl)-thieno[2,3-*d*]pyrimidine (16d). This was prepared as described in the synthesis of **16a** with piperonylamine instead of ethylamine hydrochloride; mp: 164–166 °C; ^1H NMR ($CDCl_3$) δ: 4.42 (2H, d, J = 4.8 Hz, $CH_2\mathit{Ph}$), 4.78 (1H, br, NH), 5.96 (2H, s, $\mathit{OCH_2O}$), 6.52 (1H, d, J = 7.6 Hz, Ph), 6.53 (1H, s, Ph), 6.72 (1H, d, J = 7.6 Hz, Ph), 7.06 (2H, d, J = 4.8 Hz, Pyridyl), 7.09 (2H, t, J = 8.4 Hz, FPh), 7.28–7.35 (2H, m, FPh), 8.46 (2H, d, J = 4.8 Hz, Pyridyl), 8.53 (1H, s, H_{arom}); HRMS (FAB^+) m/z exact mass calcd for $C_{25}H_{17}FN_4O_2S$ 456.1056, found 456.1050.

5-(4-Fluorophenyl)-4-(2-hydroxyethylamino)-6-(4-pyridyl)-thieno[2,3-*d*]pyrimidine (16e). This was prepared as described in the synthesis of **16a** with 2-aminoethanol instead of ethylamine hydrochloride; mp: 191–193 °C; ^1H NMR ($CDCl_3$) δ: 3.64 (2H, q, J = 4.8 Hz, $\mathit{NH}CH_2\mathit{CH_2OH}$), 3.73 (1H, br s, OH), 3.77 (2H, br s, $\mathit{NHCH_2}CH_2\mathit{OH}$), 5.15 (1H, br s, NH), 7.01 (2H, t, J = 8.4 Hz, Ph), 7.35–7.58 (4H, m, Ph and Pyridyl), 8.41 (1H, s, H_{arom}), 8.77 (2H, d, J = 5.2 Hz, Pyridyl); HRMS (FAB^+) m/z exact mass calcd for $C_{19}H_{15}FN_4OS$ 366.0951, found 366.0942.

4-(2-Ethoxyethylamino)-5-(4-fluorophenyl)-6-(4-pyridyl)-thieno[2,3-*d*]pyrimidine (16f). This was prepared as described in the synthesis of **16a** with 2-ethoxyethylamine instead of ethylamine hydrochloride; mp: 160–162 °C; ^1H NMR ($CDCl_3$) δ: 1.10 (3H, t, J = 6.8 Hz, $CH_3\mathit{CH_2O}$), 3.33 (2H, q, J = 6.8 Hz, $\mathit{CH_3}CH_2\mathit{O}$), 3.39 (2H, t, J = 4.8 Hz, $\mathit{NH}CH_2\mathit{CH_2OEt}$), 3.58 (2H, q, J = 4.8 Hz, $\mathit{NHCH_2}CH_2\mathit{OEt}$), 5.17 (1H, br s, NH), 7.04 (2H, d, J = 6.0 Hz, Pyridyl), 7.20 (2H, t, J = 8.4 Hz, Ph), 7.37 (2H, dd, J = 4.8, 8.4 Hz, Ph), 8.46 (2H, d, J = 6.0 Hz, Pyridyl), 8.46 (1H, s, H_{arom}); HRMS (FAB^+) m/z exact mass calcd for $C_{21}H_{19}FN_4OS$ 394.1264, found 394.1270.

4-(2,2-Diethoxyethylamino)-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (16g). This was prepared as described in the synthesis of **16a** with 2,2-diethoxyethylamine instead of ethylamine hydrochloride; mp: 161–163 °C; ^1H NMR ($CDCl_3$) δ: 1.13 (6H, t, J = 6.8 Hz, $CH_3\mathit{CH_2O}\times2$), 3.43–3.50 (2H, m, $\mathit{NH}CH_2\mathit{CH}$), 3.58–3.67 (4H, m, $\mathit{CH_3}CH_2\mathit{O}\times2$), 4.52 (1H, t, J = 5.2 Hz, $\mathit{NHCH_2}CH$), 4.91 (1H, br t, NH), 7.00 (2H, t, J = 8.4 Hz, Ph), 7.42–7.48 (4H, m, Ph and Pyridyl), 8.43 (1H, s, H_{arom}), 8.79 (2H, d, J = 5.6 Hz, Pyridyl); HRMS (FAB^+) m/z exact mass calcd for $C_{23}H_{23}FN_4O_2S$ 438.1526, found 438.1519.

5-(4-Fluorophenyl)-4-[2-(2-hydroxyethoxy)ethylamino]-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (16h). This was prepared as described in the synthesis of **16a** with 2-(2-aminoethoxy)ethanol instead of ethylamine hydrochloride; mp: 161–163 °C; ^1H NMR ($CDCl_3$) δ: 1.87 (1H, t, J = 4.4 Hz, OH), 3.43 (2H, t, J = 4.8 Hz, $\mathit{NH}CH_2\mathit{CH_2O}$), 3.49 (2H, t, J = 4.8 Hz, $\mathit{NHCH_2}CH_2\mathit{O}$), 3.55–3.75 (4H, m, $\mathit{O}CH_2\mathit{CH_2OH}$), 5.09 (1H, s, NH), 7.04 (2H, d, J = 6.0 Hz, Pyridyl), 7.22 (2H, t, J = 8.4 Hz, Ph), 7.37 (2H, dd, J = 5.2, 8.4 Hz, Ph), 8.46 (2H, d, J = 6.0 Hz, Pyridyl), 8.49 (1H, s, H_{arom}); HRMS (FAB^+) m/z exact mass calcd for $C_{21}H_{19}FN_4O_2S$ 410.1213, found 410.1217.

4-(2-Aminoethylamino)-5-(4-fluorophenyl)-6-(4-pyridyl)-thieno[2,3-*d*]pyrimidine (16i). This was prepared as described in the synthesis of **16a** with ethylenediamine instead of ethylamine hydrochloride; mp: 167–169 °C; ^1H NMR ($CDCl_3$) δ: 1.26 (2H, br, $\mathit{NHCH_2CH_2}NH_2$), 2.84 (2H, t, J = 5.2 Hz, $\mathit{NHCH_2}$-$CH_2\mathit{NH_2}$), 3.49 (2H, t, J = 5.2 Hz, $\mathit{NH}CH_2\mathit{CH_2NH_2}$), 5.34 (1H, s, $NH\mathit{CH_2CH_2NH_2}$), 7.01 (2H, t, J = 8.4 Hz, Pyridyl), 7.43–7.49 (4H, m, Ph), 8.43 (1H, s, H_{arom}), 8.77 (2H, d, J = 8.4 Hz, Pyridyl); HRMS (FAB^+) m/z exact mass calcd for $C_{19}H_{16}FN_5S$ 365.1110, found 365.1121.

5-(4-Fluorophenyl)-4-[(hydroxycarbonylmethyl)amino]-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (16j). This was prepared as described in the synthesis of **16b** with glycine ethyl ester hydrochloride and 2 N NaOH instead of benzylamine and NaOH, respectively; mp: 235–238 °C; ^{1}H NMR (DMSO-d_6) δ: 4.05 (2H, d, *J* = 5.6 Hz, $\mathrm{CH_2}$), 6.00 (1H, t, *J* = 5.6 Hz, NH), 7.25 (2H, t, *J* = 8.8 Hz, Ph), 7.42–7.46 (2H, m, Ph), 7.51 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl), 8.36 (1H, s, $\mathrm{H_{arom}}$), 8.72 (2H, dd, *J* = 1.6, 4.4 Hz, Pyridyl), 11.05 (1H, br, COOH); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $\mathrm{C_{19}H_{13}FN_4O_2S}$ 380.0743, found 380.0741.

4-[(Ethoxycarbonylmethyl)amino]-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (16k). This was prepared as described in the synthesis of **16a** with glycine ethyl ester hydrochloride instead of ethylamine hydrochloride; mp: 136–138 °C; ^{1}H NMR ($\mathrm{CDCl_3}$) δ: 1.27 (3H, t, *J* = 7.2 Hz, $\mathrm{CH_3}\mathit{CH_2O}$), 4.15–4.26 (4H, m, $\mathit{CH_3}\mathrm{CH_2}\mathit{O}$ and $\mathit{NH}\mathrm{CH_2}$), 5.20 (1H, br t, NH), 7.02 (2H, t, *J* = 8.4 Hz, Ph), 7.47–7.51 (4H, m, Ph and Pyridyl), 8.45 (1H, s, $\mathrm{H_{arom}}$), 8.82 (2H, d, *J* = 7.2 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $\mathrm{C_{21}H_{17}FN_4O_2S}$ 408.1056, found 408.1055.

4-[(1-Ethoxycarbonylethyl)amino]-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (16l). This was prepared as described in the synthesis of **16a** with alanine ethyl ester hydrochloride instead of ethylamine hydrochloride; mp: 171–173 °C; ^{1}H NMR ($\mathrm{CDCl_3}$) δ: 1.26 (3H, t, *J* = 7.2 Hz, $\mathrm{CH_3}\mathit{CH_2O}$), 1.40 (3H, d, *J* = 7.2 Hz, $\mathrm{CH_3}\mathit{CH}$), 4.18 (2H, q, *J* = 7.2 Hz, $\mathit{CH_3}\mathrm{CH_2O}$), 4.84 (1H, quint, *J* = 7.2 Hz, $\mathit{NH}\mathrm{CH}$), 5.28 (1H, d, *J* = 7.2 Hz, NH), 7.02 (2H, t, *J* = 8.4 Hz, Ph), 7.43–7.51 (4H, m, Ph and Pyridyl), 8.43 (1H, s, $\mathrm{H_{arom}}$), 8.82 (2H, d, *J* = 5.6 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $\mathrm{C_{22}H_{19}FN_4O_2S}$ 422.1213, found 422.1201.

4-Diethylamino-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-d]pyrimidine (16m). To a solution of **15a** (130 mg, 0.38 mmol) in $\mathrm{CH_2Cl_2}$ (20 mL) was added diethylamine (139 mg, 1.9 mmol). After reflux for 1 day, the reaction mixture was cooled to room temperature, diluted with $\mathrm{H_2O}$ and extracted with AcOEt. The combined organic layers were washed with brine, dried with $\mathrm{MgSO_4}$ and evaporated. The residue was purified by column chromatography on silica gel eluting with a mixture of $\mathrm{CHCl_3}$:MeOH (15:1 v/v) followed by crystallization from $\mathrm{Et_2O}$/hexane afforded **16m** (115 mg, y. 80%); mp: 175–177 °C; ^{1}H NMR ($\mathrm{CDCl_3}$) δ: 0.92 (6H, t, *J* = 7.2 Hz, $\mathrm{CH_3}\mathit{CH_2}\times 2$), 3.20 (4H, q, *J* = 7.2 Hz, $\mathit{CH_3}\mathrm{CH_2}\times 2$), 7.00 (2H, t, *J* = 8.4 Hz, Ph), 7.34–7.41 (4H, m, Ph and Pyridyl), 8.46 (1H, s, $\mathrm{H_{arom}}$), 8.72 (2H, d, *J* = 6.0 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $\mathrm{C_{21}H_{19}FN_4S}$ 378.1314, found 378.1310.

4-Bis(2-ethoxyethyl)amino-5-(4-fluorophenyl)-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (16n). This was prepared as described in the synthesis of **16a** with bis(2-ethoxyethyl)amine instead of ethylamine hydrochloride; mp: 70–72 °C; ^{1}H NMR ($\mathrm{CDCl_3}$) δ: 1.09 (6H, t, *J* = 6.8 Hz, $\mathrm{CH_3}\mathit{CH_2}\times 2$), 3.33–3.40 (12H, m, $\mathit{CH_3}\mathrm{CH_2}\times 2$ and $\mathrm{(CH_2)_2}\mathit{O}\times 2$), 7.02 (2H, t, *J* = 8.4 Hz, Pyridyl), 7.41–7.45 (4H, m, Ph), 8.47 (1H, s, $\mathrm{H_{arom}}$), 8.68 (2H, d, *J* = 8.4 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $\mathrm{C_{25}H_{27}FN_4O_2S}$ 466.1839, found 466.1848.

4-Benzylamino-5-(4-fluorophenyl)-2-methyl-6-(4-pyridyl)-thieno[2,3-*d*]pyrimidine (17a). This was prepared as described in the synthesis of **16b** with **15b** instead of **15a**; mp: 194–197 °C; ^{1}H NMR ($\mathrm{CDCl_3}$) δ: 2.60 (3H, s, $\mathrm{CH_3}$), 4.72 (2H, d, *J* = 5.6 Hz, $\mathrm{CH_2}$), 4.82 (1H, br, NH), 7.01 (2H, t, *J* = 8.8 Hz, FPh), 7.18 (2H, d, *J* = 6.8 Hz, Ph), 7.22–7.37 (3H, m, Ph), 7.40 (2H, d, *J* = 5.6 Hz, Pyridyl), 7.46 (2H, dd, *J* = 5.6, 8.8 Hz, FPh), 8.71 (2H, d, *J* = 5.6 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $\mathrm{C_{25}H_{19}FN_4S}$ 426.1314, found 426.1313.

5-(4-Fluorophenyl)-4-(2-hydroxyethylamino)-2-methyl-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (17b). This was prepared as described in the synthesis of **16e** with **15b** instead of **15a**; mp: 177–180 °C; ^{1}H NMR ($\mathrm{CDCl_3}$) δ: 2.62 (3H, s, $\mathrm{CH_3}$), 3.62 (2H, dt, *J* = 4.4, 5.2 Hz, $\mathit{NH}\mathrm{CH_2}\mathit{CH_2OH}$), 3.77 (2H, t, *J* = 4.4 Hz, $\mathit{NHCH_2}\mathrm{CH_2}\mathit{OH}$), 4.44 (1H, br s, OH), 5.04 (1H, t like, NH), 7.00 (2H, t, *J* = 8.4 Hz, Ph), 7.35–7.55 (4H, m, Ph and Pyridyl), 8.77 (2H, d, *J* = 6.0 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $\mathrm{C_{20}H_{17}FN_4OS}$ 380.1107, found 380.1100.

4-(2-Ethoxyethylamino)-5-(4-fluorophenyl)-2-methyl-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (17c). This was prepared as described in the synthesis of **16f** with **15b** instead of **15a**; mp: 153–155 °C; ^{1}H NMR ($\mathrm{CDCl_3}$) δ: 1.10 (3H, t, *J* = 6.8 Hz, $\mathrm{CH_3}\mathit{CH_2O}$), 2.62 (3H, s, $\mathrm{CH_3}$), 3.32 (2H, q, *J* = 6.8 Hz, $\mathit{CH_3}\mathrm{CH_2O}$), 3.37 (2H, t, *J* = 4.8 Hz, $\mathit{NH}\mathrm{CH_2}\mathit{CH_2OEt}$), 3.58 (2H, q, *J* = 4.8 Hz, $\mathit{NHCH_2}\mathrm{CH_2}\mathit{OEt}$), 5.11 (1H, br s, NH), 7.04 (2H, d, *J* = 6.0 Hz, Pyridyl), 7.20 (2H, t, *J* = 8.4 Hz, Ph), 7.37 (2H, dd, *J* = 4.8, 8.4 Hz, Ph), 8.46 (2H, d, *J* = 6.0 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $\mathrm{C_{22}H_{21}FN_4OS}$ 408.1420, found 408.1433.

5-(4-Fluorophenyl)-4-[2-(2-hydroxyethoxy)ethylamino]-2-methyl-6-(4-pyridyl)thieno[2,3-*d*]pyrimidine (17d). This was prepared as described in the synthesis of **16h** with **15b** instead of **15a**; mp: 158–160 °C; ^{1}H NMR ($\mathrm{CDCl_3}$) δ: 1.92 (1H, t, *J* = 4.4 Hz, OH), 2.61 (3H, s, $\mathrm{CH_3}$), 3.40 (2H, t, *J* = 4.8 Hz, $\mathit{NH}\mathrm{CH_2}\mathit{CH_2O}$), 3.49 (2H, t, *J* = 4.8 Hz, $\mathit{NHCH_2}\mathrm{CH_2}\mathit{O}$), 3.56–3.75 (4H, m, $\mathit{O}\mathrm{CH_2CH_2}\mathit{OH}$), 5.01 (1H, s, NH), 7.03 (2H, d, *J* = 6.0 Hz, Pyridyl), 7.22 (2H, t, *J* = 8.4 Hz, Ph), 7.38 (2H, dd, *J* = 5.2, 8.4 Hz, Ph), 8.46 (2H, d, *J* = 6.0 Hz, Pyridyl); HRMS (FAB$^+$) *m*/*z* exact mass calcd for $\mathrm{C_{22}H_{21}FN_4O_2S}$ 424.1369, found 424.1363.

Biological methods

In vitro TNF-α inhibition assay.[13,20] Whole blood from male SD rats (Charles River Laboratories) was collected into 2% heparinized vacuum tubes. The blood was suspended in RPMI-1640 supplemented with 100 μg/mL streptomycin and 100 U/mL penicillin at a concentration of 50%, and seeded into 24-well plate (950 μL). Vehicle (DMSO) or test compounds in DMSO was added to each well, and the plate was incubated at 37 °C in 5% CO_2 for 30 min. Finally, 10 μg/mL LPS (*Escherichia coli* B055:B5) in RPMI-1640 was added, and plates were incubated at 37 °C in 5% CO_2 for 4 h. Supernatants were then harvested, and assayed for TNF-α using ELISA kits. IC_{50} of TNF-α production was determined by comparison of yield with a control to which no test compound was added ($n = 2$).

In vivo TNF-*α* inhibition assay.[13,20] Male SD rats (6 weeks old, Charles River Laboratories) ($n = 4$) were dosed orally with test compounds suspended in 0.5% CMC. 1 h later, each rat was injected iv with LPS (*E. coli* B055/B5, 0.1 mg/kg). The rats were sacrificed and bled 90 min later, which is a time point of maximal elevation of serum TNF-α activity. Serum TNF-α activity was measured using ELISA kits. Percent inhibition of TNF-α production at 50 mg/kg was determined by comparison of yield with a control to which no test compound was added.

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