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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4284–4289

Synthesis and structure–activity relationship of imidazo(1,2-*a*)thieno(3,2-*e*)pyrazines as IKK-β inhibitors

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Received 17 April 2007; revised 8 May 2007; accepted 9 May 2007
Available online 16 May 2007

Abstract—The identification of a potent series of IKK-β selective inhibitors based on an imidazothienopyrazine template and the oral efficacy of one such analog (**22j**) in the LPS-induced TNF-α release mouse model are described.

Nuclear Factor-κB (NF-κB) is a family of closely related proteins that regulate the transcription of numerous genes implicated in the induction of inflammatory and immune responses and in the prevention of apoptosis.[1] NF-κB resides in the cytoplasm of unstimulated cells as a silent complex with a family of proteins known as inhibitor of kappa B (IκB). In response to specific external stimuli, the IκB component of the complex is phosphorylated and degraded, resulting in the translocation of the NF-κB into the nucleus and the induction of gene transcription. The enzyme responsible for the phosphorylation of the IκB protein is IκB kinase (IKK), a multisubunit complex that contains two catalytic units (IKK-α and -β) and a regulatory unit (IKK-γ or NEMO). Various studies indicate that IKK-β plays the dominant role in the proinflammatory signal-induced phosphorylation of the IκB protein.[2,3]

Reducing the production and/or activities of cytokines is an approach that is being actively explored by the biotech and pharmaceutical industries to discover therapies for various immune/inflammatory disorders.[4] A number of groups have reported IKK-β selective inhibitors that decrease lipopolysaccharide (LPS)-induced tumor necrosis factor-α (TNF-α) release both in vitro and in vivo.[5] Amine **1** is one such example culminating from an earlier structure–activity relationship (SAR) investigation which was conducted around a broad screening lead compound. Based on the results of enzyme kinetic studies, **1** appears to interact with an allosteric site of IKK-β.[6a] Proof of concept studies carried out with **1** in murine inflammation models, such as collagen-induced arthritis and dextran sodium sulfate-induced colitis, revealed dose-dependent reductions in the incidence and severity of the respective clinical symptoms.[6]

Preliminary SAR studies conducted on the imidazoquinoxaline core of **1** have indicated tolerance to modifications at C-7 and C-8. Moreover, tetracyclic analog **2** exhibited improved enzyme and cell activity (Table 1).[7] However, the extended fused aromatic core of **2** was expected to lead to poor solubility and possible toxicological problems.[8] In order to closely mimic the topological disposition of the tetracyclic template of **2**, an aryl substituted [5,6,5] thiophene tricyclic template, exemplified in structure **12**, was envisioned. Herein, we describe the synthesis, SAR, and PK/PD profiles of such a series which was built upon the preliminary SAR observations noted above.

We began the SAR investigation around C-7 of **12** while keeping an ethylenediamine side chain at C-4

Keywords: IKK; NF-κB; TNF-α; Imidazothienopyrazine.
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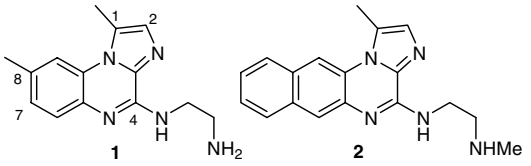
doi:10.1016/j.bmcl.2007.05.031

Table 1. Enzyme and cell activities of program leads



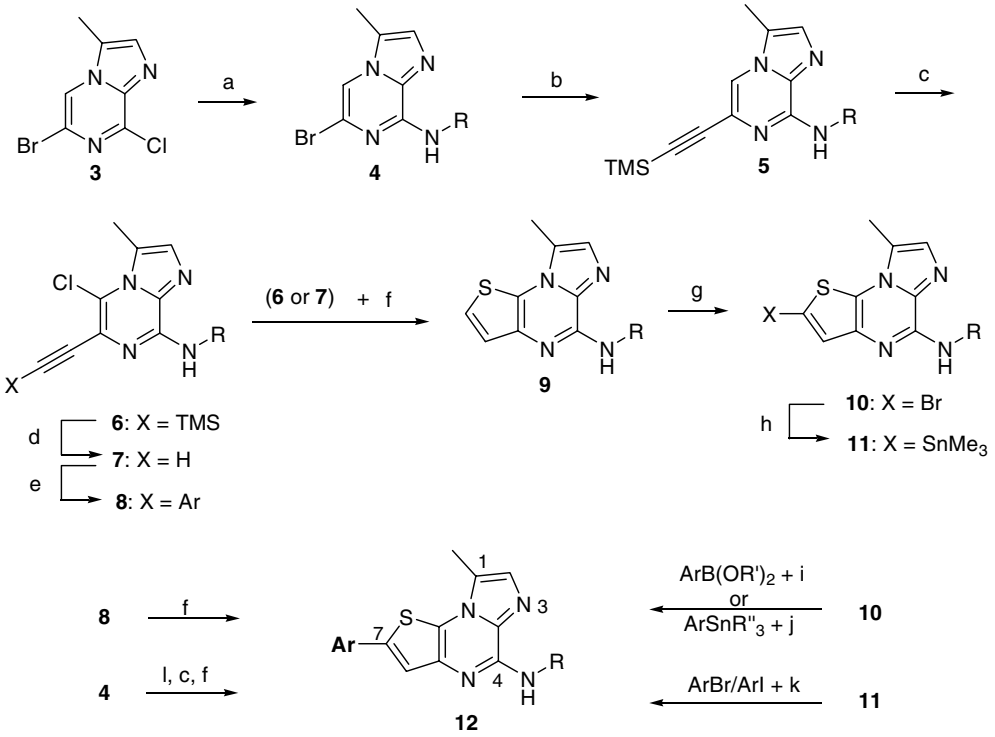
Compound	IC_{50} (μM)		Jurkat cell EC_{50}/CC_{50} (μM)
	IKK-α	IKK-β	
1	7.4	0.15	13.3/100
2	0.42	0.023	0.85/NT[a]

[a] NT, not tested.

fixed, using complementary synthetic approaches devised for this purpose (Scheme 1). Selective aminolysis of the chloride of **3**, followed by a Sonogashira coupling with ethynyltrimethylsilane and chlorination with NCS, afforded **6**, which was elaborated to **12** via one of two pathways.[9,10] In the first approach, **6** was desilylated and then coupled with an arylhalide under Sonogashira conditions to afford **8**, which was cyclized to give compound **12** by employing Na_2S.[11] In the second approach, which was found to be more efficient for SAR investigation, the cyclization step was effected before the introduction of the aryl moiety. The cyclization could be carried out on either of alkynes **6** or **7**, and the resultant product (**9**) was subsequently brominated and stannylated. Both bromide **10** and stannane **11** served as substrates for Pd-assisted couplings to prepare a diverse family of C-7 aryl-substituted analogs. The incoming aromatic substrates were obtained either from commercial sources or prepared by employing modifications of literature protocols.[12] Carbamates **9** and **12** were deprotected with TFA/CH_2Cl_2 to afford the final products (**13a–j**, **14a–g**). During the scale-up synthesis of certain analogs, where the corresponding arylalkyne (ArC≡CH) was commercially available, it was more efficient to prepare the desired **12** directly from **4** by employing a three-step protocol: Sonogashira coupling with ArC≡CH, followed by NCS chlorination and sulfide cyclization.

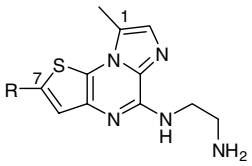
Compounds were screened for their ability to inhibit the IKK-α or IKK-β-catalyzed phosphorylation of glutathione *S* transferase (GST)-IκB-α and the TNF-α-induced degradation of the β-lactamase/IκB-α fusion protein in Jurkat cells.[13] The cytotoxicity of compounds was assessed in an Alamar blue assay.[14]

The imidazothienopyrazine core structure (**13a**) had an IC_{50} comparable to that of **1** and an IKK-α/IKK-β IC_{50} ratio of ~70 (Table 2). The introduction of a phenyl group increased the potency against both enzymes significantly, while maintaining >10-fold selectivity for IKK-β (see **13b**). Changing the phenyl group to a heteroaryl moiety gave mixed results: while the IKK-β inhibitory potency of the pyrazole analog (**13g**) increased by about twofold, the potency of most of the



Scheme 1. Reagents and conditions for R=CH_2CH_2NHBoc: (a) $NH_2CH_2CH_2NHBoc$, Et_3N, THF; (b) TMSC≡CH, $Pd(Ph_3P)_4$, CuI, DMF, Et_3N, 64 °C; (c) NCS, THF, 64 °C; (d) K_2CO_3, MeOH; (e) ArI or ArBr, $Pd(Ph_3P)_4$, CuI, DMF, Et_3N, 64 °C; (f) $Na_2S{\cdot}9H_2O$, DMF, 100 °C; (g) NBS, THF, 0 °C–rt; (h) $(Me_3Sn)_2$, $Pd(Ph_3P)_4$, Et_3N, toluene, 100 °C; (i) $Pd(Ph_3P)_4$, MeOH, toluene, satd $NaHCO_3$, 80 °C; (j) $Pd(Ph_3P)_4$, LiCl, dioxane, 90 °C; (k) $PdCl_2(Ph_3P)_2$, KF, DMF, 90 °C; (l) ArC≡CH, $Pd(Ph_3P)_4$, CuI, DMF, Et_3N, 66 °C.

Table 2. SAR of C-7 aromatic substituted imidazothienopyrazines (**13**)



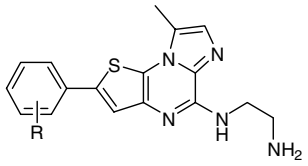
Compound	R	IC_{50}[a] (μM)		Jurkat cell[a] EC_{50}/CC_{50} (μM)
		IKK-α	IKK-β	
13a	H	12.1	0.17	>100/>100
13b	Phenyl	0.91	0.022	>100/77
13c	2′-Thiazolyl	1.11	0.23	18.6/5.9
13d	3′-Furanyl	0.94	0.029	4.53/>100
13e	4′-Imidazolyl	3.86	0.45	>100/38
13f	2′-Pyrrolyl	1.26	0.25	7.96/7.9
13g	4′-Pyrazolyl	1.45	0.01	8.02/46
13h	2′-Pyridinyl	1.24	0.19	>100/13
13i	3′-Pyridinyl	2.57	0.14	29.5/49
13j	4′-Pyridinyl	2.93	0.27	59.6/>100

[a] Single experiment, except for IC_{50} of **13b** (IKK-α: $n > 10$, SD = 0.41 μM; IKK-β: $n > 10$, SD = 0.013 μM).

other analogs dropped by more than sixfold. Moreover, the gain in the intrinsic potency of analogs such as **13b** and **13g** did not translate to enhanced cellular activity, when compared with that of **1**. It is noteworthy that with respect to the cellular activity, only **13d** looked modestly encouraging.

At this juncture, in order to probe the steric and polarity preferences of the enzyme with the goal, in part, of enhancing cellular activity, we chose to investigate substituted phenyl analogs of **13b** (Table 3). From two sets of scans that were conducted using a methoxy or a hydroxymethyl group, the *meta* position of the phenyl group appeared to be the most tolerant site (see **14b** and **14e**). The enzyme and cell activities of **14g** were very encouraging, and the emerging SAR suggested the presence of an open channel adjacent to the *meta* position of the C-7 phenyl group. Many of the analogs discussed thus far exhibited appreciable cytotoxicities, and a significant difference between the CC_{50} values of close regioisomers was observed [e.g., **14d** (>100 μM) and **14e** (7 μM)].

Table 3. SAR of regioisomeric substituents on the phenyl group of **13b**



Compound	R	IC_{50}[a] (μM)		Jurkat cell[a] EC_{50}/CC_{50} (μM)
		IKK-α	IKK-β	
14a	2′-Methoxy	13.0	0.28	>100/3.5
14b	3′-Methoxy	0.69	0.044	29.2/3.5
14c	4′-Methoxy	2.80	0.095	>30.0/>100
14d	2′-(CH_2OH)	4.40	0.13	15.6/>100
14e	3′-(CH_2OH)	0.45	0.008	2.4/7.0
14f	4′-(CH_2OH)	2.33	0.023	40.9/14
14g	3′-(C_2H_4OH)	0.91	0.021	0.95/13

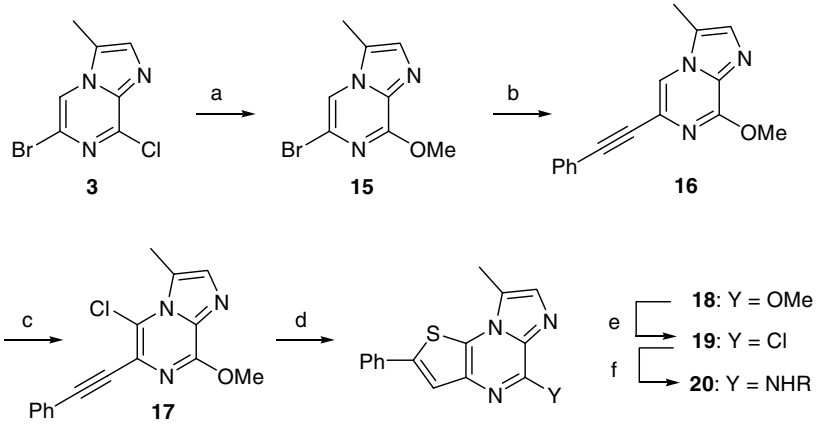
[a] Single experiment.

Concurrent with the above study, the SAR of the C-4 side chain was investigated while keeping a phenyl group at C-7. The original synthetic approach was modified so as to allow introduction of the C-4 side chain at the final step through aminolysis of chloride **19** (Scheme 2). Since the chloride moiety of **3** was unlikely to survive the construction of the thiophene ring, it was converted to methyl ether for the intermediate steps and then regenerated at the penultimate step.

As illustrated in Table 4, replacing the ethylenediamine moiety of **13b** with a methylamine improved the cellular potency and cytotoxicity index, while maintaining the intrinsic potency against IKK-β. Changing the methylamine side chain of **20a** to either OMe (**18**) or NH_2 (**20b**) caused a significant erosion in the enzymatic activity. The lack of activity for **18** suggests that the 'NH' moiety of the side chain might be involved in a critical H-bond interaction with the enzyme.[15] The homologation of the side chain from a methylamine (**20a**) to an ethylamine (**20c**) also caused a drop in the enzymatic activity. However, the addition of an amino group to **20c** caused a rebound in the enzyme potency (see **13b**). Various analogs of the ethylenediamine side chain, exemplified by **20d–i**, had weaker intrinsic potency than that of the parent compound (**13b**), some more so than others. Although the ethylenediamine side chain is desirable from a solubility perspective, its NH_2 group appeared to correlate with the cell cytotoxicity associated with several analogs (see Tables 3 and 4). It is noteworthy that among the C-4 analogs highlighted in Table 4, the two analogs with a basic side chain, **13b** and **20h**, are the only ones with $CC_{50} < 100$ μM.

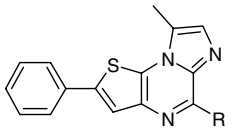
Since the C-4 methylamine side chain had the best combination of enzyme and cellular assay potency and cytotoxicity profile, it was chosen for a more detailed investigation of the *meta* position of the C-7 phenyl group SAR (Table 5). The synthesis of these analogs was conducted according to the procedures outlined in Scheme 1, by employing NH_2CH_3 instead of $NH_2CH_2CH_2NHBoc$. In addition to potency optimization, other deficiencies needed to be addressed at this stage of our investigation, including the lack of aqueous solubility (<1 μg/mL in pH 6.5 sodium phosphate buffer) and the poor in vitro metabolic stability (human and mouse microsomal clearance rate: 0.144 and 0.219 nmol/min/mg-protein, respectively) of **20a**. It was hypothesized that, for instance, introducing flexible polar moieties to the C-7 phenyl group might improve the aqueous solubility of the series, in part by preventing the π-stacking interactions of the core.

As was the case with the ethylenediamine side chain, the presence of a phenyl group at C-7 made a significant difference in the intrinsic potency of the tricyclic core (compare **20a** and **21a**, Table 5). A wide array of substituents at the *meta* position were tolerated by the enzyme, and a



Scheme 2. Reagents and conditions: (a) NaOMe, MeOH; (b) PhC≡CH, $Pd(Ph_3P)_4$, CuI, DMF, Et_3N, 70 °C; (c) NCS, THF, 55 °C; (d) $Na_2S{\cdot}9H_2O$, DMF, 90 °C; (e) $POCl_3$, 90 °C; (f) NH_2R, THF or THF/DMSO/(*i*-Pr)$_2$EtN, 85–120 °C.

Table 4. SAR of selected C-4 side chain analogs of **13b**



Compound	R	IC_{50}[a] (μM)		Jurkat cell[a,b] EC_{50} (μM)
		IKK-α	IKK-β	
13b	$NHCH_2CH_2NH_2$	0.91	0.022	>100
18	OMe	>100	>1.0	>100
20a	$HNCH_3$	1.09	0.027	1.35
20b	NH_2	6.5	0.37	17.0
20c	$HNCH_2CH_3$	1.99	0.26	5.73
20d	$HNCH_2CH_2N(CH_3)_2$	1.17	0.095	>30.0
20e	$HNCH_2CONH_2$	>25	0.36	>100
20f	$HNCH_2CH_2NHCHO$	7.99	0.23	3.16
20g	$HNCH_2CH_2NHAc$	7.03	0.29	10.2
20h	HN-(2-(Piperidin-1-yl)ethyl)	3.13	0.040	13.4
20i	$HNCH_2CH_2OH$	3.48	0.085	7.17

[a] Single experiment, except for **20a** (IKK-α: $n > 10$, SD = 0.82 μM; IKK-β: $n > 10$, SD = 0.010) and **20g** (IKK-α and -β, $n = 2$).
[b] CC_{50} > 100 μM, except for **13b** (77 μM) and **20h** (15 μM).

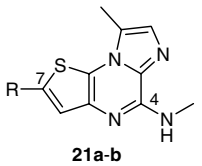
subset of these analogs had improved cellular potency that was not reflected in the enzymatic activity. For example, although amine **22a** had potent enzyme inhibitory activity (IC_{50} = 8.5 nM), it had an EC_{50} of 5.9 μM in the cell assay. However, derivatization of the benzylamine moiety as a urea (**22c**) or a sulfamide (**22d**) resulted in submicromolar cellular activity, albeit with some loss of enzyme activity for **22c**. It is noteworthy that benzylamine **22a** was cytotoxic, whereas a number of its non-basic derivatives (**22b–d**) were not. Moreover, glycinamide **22e**, which is expected to be less basic than amine **22a**, had a better cytotoxicity profile (a CC_{50} of >100 μM vs 19 μM). It appears that cytotoxicity is associated with the presence of a basic moiety in the molecule, an observation that is consistent with the relative cytotoxicities of the side-chain analogs **13b** and **20a**. Analogs **22f–j**, which are homologs of **22a–e**, also showed improved cellular activity and cytotoxicity profiles when the amine group of **22f** was elaborated similarly. However, in the homologous case, derivatization of the amine group also improved enzymatic activity.

Despite the wide tolerance for polarity and size at the *meta* position, there were some limitations. For example, the introduction of an acid or an amide group to **20a** caused a substantial drop in enzymatic and cellular assay activity (see **22l** and **22m**). Interestingly, the homologation of these functional groups with a methylene or an ethylene linker restored activity, where the amide-containing analogs had a good combination of enzyme and cell potencies (see analogs **22n–p**). The addition of an amino group to the *meta* appendages of **22o** or **22p** had mixed results: amino acid **22q** had no cell activity, presumably due to poor membrane-permeability, whereas amino amide **22r** (and its enantiomer, **22s**) had IKK-β and cell potencies that are within a factor of two of that of amide **22p**, albeit accompanied by a reduced enzyme selectivity. Further homologation of the amino-amide moiety to a methylamide (**22t**) or an ethylamide (**22u**) was tolerated.[16]

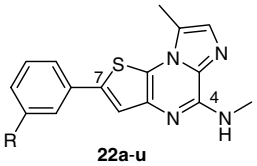
Through the investigation of the *meta* position of the C-7 phenyl ring, the aqueous solubility and metabolic stability of the series was improved. For example, the TFA salts of **22t** and **22u** had aqueous solubilities of 42 and 44 μg/mL, respectively, in pH 6.5 sodium phosphate buffer. As illustrated in Table 6, the stability of a selected set of analogs in human and mouse liver microsomes was better than that of the parent compound **20a**, vide supra, and a subset of these analogs exhibited very encouraging systemic exposure in the mouse after oral dosing.

Compound **22j** was selected, along with the reference compound **1**,[17] in order to evaluate the in vivo efficacy of the series in the murine LPS-induced TNF-α release model. Compounds were dosed either 1 or 4 h before LPS administration, and serum concentrations of TNF-α were determined 1.5 h post-LPS challenge and compared with that of a vehicle-treated group that underwent a similar challenge (Table 7).[18] The serum concentration of the parent compound was also determined 1.5 h post-LPS challenge. Compound **22j** was

Table 5. C-7 SAR of methylamine-containing imidazothieno-pyrazines **21**–**22**



21a-b



22a-u

Compound	R	IC_{50}[a] (μM)		Jurkat cell EC_{50} [a,b](μM)
		IKK-α	IKK-β	
21a	H	4.12	0.52	>3.00
21b	Me	7.83	0.49	>30
22a	CH_2NH_2	0.22	0.0085	5.88
22b	$CH_2NHCOCH_3$	0.77	0.038	2.33
22c	$CH_2HNCONH_2$	0.26	0.043	0.34
22d	$CH_2HNSO_2NH_2$	0.17	0.003	0.27
22e	$CH_2NHCH_2CONH_2$	0.51	0.023	2.61
22f	$C_2H_4NH_2$	0.65	0.045	6.73
22g	$C_2H_4NHCOCH_3$	0.82	0.021	1.97
22h	$C_2H_4HNCONH_2$	0.13	0.018	0.55
22i	$C_2H_4HNSO_2NH_2$	0.44	0.007	0.92
22j	$C_2H_4NHCH_2CONH_2$	0.39	0.013	1.81
22k	$C_2H_4NHCH_2CONHMe$	1.08	0.051	1.90
22l	CO_2H	14.4	0.19	65.7
22m	$CONH_2$	>30	0.29	35.6
22n	CH_2CONH_2	0.68	0.008	0.36
22o	$C_2H_4CO_2H$	1.34	<0.009	4.96
22p	$C_2H_4CONH_2$	1.21	0.019	0.81
22q	$CH_2CH(NH_2)CO_2H$	1.26	0.013	>100
22r	(*R*)-$CH_2CH(NH_2)CONH_2$	0.19	0.027	0.69
22s	(*S*)-$CH_2CH(NH_2)CONH_2$	0.26	0.014	0.45
22t[c]	$CH_2CH(NH_2)CONHMe$	0.33	0.036	0.47
22u[c]	$CH_2CH(NH_2)CONHEt$	1.01	0.059	1.05

[a] Single experiment, except for **21a** and **22b** ($n = 2$).
[b] CC_{50} > 100 μM for all except **22a** (19 μM), **22f** (21 μM), **22k** (50 μM), **22r** (90 μM), **22t** (68 μM), and **22u** (71 μM).
[c] Compounds **22t** and **22u** were tested as a racemic mixture.

efficacious in the 1 h pre-dose study, albeit less than **1**. In the 4 h pre-dose study, only **1** was active and yet the serum concentrations of both compounds were similar at 2.5 and 5.5 h post-dosing. Note that **22j** is more potent than **1** in both the enzyme (IKK-β) and Jurkat cell assays by a factor of ~11.5× and 7.3×, respectively.

An interaction of **22j** with serum proteins is not likely to be the cause of the above observation since retesting **22j** in Jurkat cell assay containing 10% serum gave an EC_{50} of 0.6 μM (vs 1.81 μM without serum). Interestingly, when **1** and **22j** were tested in a human peripheral blood mononuclear cell (PBMC) assay—a primary cell assay that more closely mimics in vivo conditions—for their ability to inhibit LPS-induced TNF-α release, they exhibited EC_{50}'s of 0.8 and 1.37 μM, respectively, values that are closer than what was observed in the Jurkat cell assay [13.3 μM (**1**) and 1.81 μM (**22j**)] (Table 6).[19] Nevertheless, with PBMC activity differing at the most by twofold and considering the similar systemic exposure

Table 6. In vitro metabolic stability, mouse systemic exposure, and inhibition of LPS-induced TNF-α release in PBMC data for selected analogs of the imidazothienopyrazine series and reference compound **1**

Compound	Human and mouse microsomal clearance rate (nmol/min/mg-protein)	po-C_{max}[a] (μM)	po-AUC 1–4 h[a] (μM h)	PBMC EC_{50}[b] (μM)
1	<0.01, 0.016	1.4	3.8	0.80 ± 0.28 (n > 10)
22c	0.066, 0.221	0.15	0.38	0.068 (n = 1)
22d	0.035, 0.231	ND[c]	ND[c]	0.095 (n = 2)
22j	0.017, 0.070	5.2	9.6	1.37 (n = 2)
22n	0.062, 0.13	0.29	0.63	0.065 ± 0.006 (n = 4)
22u	0.069, 0.145	8.6	9.89	0.14 (n = 2)

[a] PK parameters after oral administration of compound (10 mg/kg) to mouse in Tween 80/H_2O (1:9) vehicle; $n = 3$ animals for all except **1** and **22j** ($n = 2$); t_{max} = ~0.5 h for all except **22d** (–) and **22j** (1 h).
[b] Where relevant, data are mean values ± SD, and number of replicates is indicated in parentheses.
[c] ND, not detected.

Table 7. Activities of **1** and **22j** in murine LPS-induced TNF-α release model[a]

Compound	1 h pre-dose		4 h pre-dose	
	% TNF-α reduction	[Compd][b] at 2.5 h (μM)	% TNF-α reduction	[Compd][b] at 5.5 h (μM)
1	82	7.6 ± 0.8	70	4.2 ± 0.8
22j	49	14.3 ± 5.7	—[c]	2.1 ± 1.2

[a] n = 7–8/group; compounds were dosed at 30 mg/kg in Tween 80/H_2O (1:9) medium; LPS was administered 50 μg/kg, ip.
[b] [Compd] = mean serum concentration of parent compound ± SD.
[c] No statistically significant reduction in TNF-α level was observed.

profiles in mouse after oral dosing, the reason for the modest in vivo performance of **22j** in light of the efficacy of **1** is not apparent.

In summary, a novel series of IKK-2 selective inhibitors endowed with potent in vitro activity in relevant biological assays and efficacious in an acute mouse inflammation model has been discovered. Further lead optimization and detailed in vivo studies are underway to fully understand the PK/PD relation of the series, and these will be the subject of future disclosures.

Acknowledgments

We thank our analytical group for conducting a number of NMR and HRMS analyses, the Preclinical Candidate Optimization group for conducting the metabolic stability studies, Prof. Erick Carreira for suggesting that **6** could be desilylated in situ to **7** if subjected to the Na_2S cyclization protocol, and Lawrence Hamann and Nicholas Meanwell for helpful suggestions regarding the manuscript.

References and notes

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8. It is well documented that the metabolic activation of polycyclic aromatic hydrocarbons results in electrophilic species which could alkylate proteins and DNA. For additional information, see: Skupinska, K.; Misiewicz, I.; Kasprzycka-Guttman, T. *Acta Pol. Pharm. Drug Res.* **2004**, *61*, 233, and ref. cited therein.
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13. (a) For the IKK-α and IKK-β enzyme assays, see Ref. 6a; (b) For the Jurkat cell assay, see: Burke, J. R.; Wang, S. US Patent Appl. 2005/0095616 A1.
14. The Alamar blue assay was conducted according to the protocol described in Zhi-Jun *et al.* (*J. Immunol. Methods* **1997**, *210*, 25) by using reagents obtained from Biosource International.
15. Although enzyme kinetics studies, described in Ref. 6a, indicated that compound **1** is probably an allosteric site inhibitor of IKK-β, the mode of action of the current class has not been elucidated. For a discussion on the role of proximal H-bond acceptor/H-bond donor moieties in determining the interaction of inhibitors with the hinge region of kinases, see: Adams, J. L.; Veal, J.; Shewchuk, L. In *Protein Crystallography in Drug Discovery*; Babine, R. E., Abdel-Meguid, S. S., Eds.; Wiley-VCH: Verlag GmbH & Co. KGaA: Weinheim, 2004 (Chapter 2).
16. Most of the analogs prepared in this effort had IKK-α/IKK-β IC_{50} ratio >10, and no compelling SAR has emerged that could explain the difference in enzyme selectivity among the various analogs. Compounds **13b**, **20a**, and **20h** were screened against a panel of kinases (IKK-ε, p38, Her-1 and 2, LCK, EMT, VEGF, IGF-1R, and PKA) and no appreciable activities were observed (data not shown).
17. For additional PK parameters of **1**, see Ref. 6a.
18. The general procedure of Ghezzi et al. (*Cytokine* **2000**, *12*, 1205), as described in Ref. 6a, was employed to evaluate the effects of compounds on LPS-induced TNF-α release in mice.
19. The PBMC assay was conducted as described by Leftheris et al. *J. Med. Chem.* **2004**, *47*, 6283.