

# Design and Synthesis of the First Generation of Novel Potent, Selective, and in Vivo Active (Benzothiazol-2-yl)acetonitrile Inhibitors of the c-Jun N-Terminal Kinase

Pascale Gaillard,<sup>†</sup> Isabelle Jeanclaude-Etter,<sup>†</sup> Vittoria Ardisson,<sup>#</sup> Steve Arkinstall,<sup>‡</sup> Yves Cambet,<sup>||</sup> Montserrat Camps,<sup>‡</sup> Christian Chabert,<sup>‡</sup> Dennis Church,<sup>†</sup> Rocco Cirillo,<sup>#</sup> Denise Gretener,<sup>||</sup> Serge Halazy,<sup>†</sup> Anthony Nichols,<sup>||</sup> Cedric Szyndralewicz,<sup>§</sup> Pierre-Alain Vitte,<sup>§</sup> and Jean-Pierre Gotteland<sup>\*,†</sup>

Serono Pharmaceutical Research Institute, 14 Chemin des Aulx, 1228 Plan-Les-Ouates, Geneva, Switzerland, Serono Research Institute, 1-Technology Drive, Rockland, Massachusetts 02370, and LCG-RBM, Serono International S.A., 10010 Colletterto Giacosa, Turin, Italy

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Several lines of evidence support the hypothesis that c-Jun N-terminal kinase (JNKs) plays a critical role in a wide range of diseases including cell death (apoptosis)-related disorders (neurodegenerative diseases, brain, heart, and renal ischemia, epilepsy) and inflammatory disorders (multiple sclerosis, rheumatoid arthritis, inflammatory bowel diseases). Screening of our internal compound collection for inhibitors of JNK3 led to the identification of (benzothiazol-2-yl)acetonitrile derivatives as potent and selective JNK1, -2, -3 inhibitors. Starting from initial hit **1** (AS007149), the chemistry and initial structure–activity relationship (SAR) of this novel and unique kinase inhibitor template were explored. Investigation of the SAR rapidly revealed that the benzothiazol-2-ylacetonitrile pyrimidine core was crucial to retain a good level of potency on rat JNK3. Therefore, compound **6** was further optimized by exploring a number of distal combinations in place of the chlorine atom. This led to the observation that the presence of an aromatic group, two carbons away from the aminopyrimidine moiety and bearing substituents conferring hydrogen bond acceptor (HBA) properties, could improve the potency. Further improvements to the biological and biopharmaceutical profile of the most promising compounds were performed, resulting in the discovery of compound **59** (AS601245). The in vitro and in vivo anti-inflammatory potential of this new JNK inhibitor was investigated and found to demonstrate efficacy per oral route in an experimental model of rheumatoid arthritis (RA).

## Introduction

The c-Jun N-terminal kinases (JNKs) (also known as “stress-activated protein kinases”) are members of the mitogen-activated protein kinase (MAPK) family along with p38 mitogen-activated protein kinases (p38 kinases) and extracellular signal-regulated kinases (ERKs).

MAP kinases are serine/threonine kinases that are activated by dual phosphorylation of threonine and tyrosine residues of the Thr-X-Tyr motif in a loop located adjacent to the active site.<sup>1,2</sup> Phosphorylation of each MAP kinase is carried out by specific kinases. Activated MAP kinases then phosphorylate various substrates, including transcription factors, which in turn regulate the expression of specific sets of genes and thus mediate a specific response to the stimulus.

Members of the JNK family of kinases are activated by proinflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) as well as environmental stress, such as anisomycin, UV irradiation, hypoxia, and osmotic shock.<sup>3</sup> Three distinct genes encoding JNKs (jnk1, jnk2, and jnk3) have been identified, and at least 10 different splice variants exist in mammalian cells.<sup>4</sup> The downstream substrates of JNKs include the transcription factors c-Jun, ATF-2, Elk1, NFAT, p53, and a cell death domain protein.<sup>5–8</sup> Each JNK isoform binds to these substrates with different affinities, suggesting a regulation of signaling pathways by substrate specificity of different JNKs in vivo.<sup>9</sup> JNK1 and JNK2 are widely expressed in a variety of tissues. In contrast, JNK3 is selectively expressed in the brain and to a lesser extent in the heart and testis.<sup>10,11</sup>

Mice lacking jnk1 or jnk2 exhibit deficits in T-helper (CD4) cell function.<sup>12–14</sup> Double knockout animals are embryonic lethal, although fibroblasts from these animals are viable in vitro and exhibit a remarkable resistance to radiation-induced apoptosis.<sup>15</sup> The jnk3 knockout mouse exhibits resistance to kainic acid induced apoptosis in the hippocampus and to subsequent seizures.<sup>16</sup> Therefore, JNK activity seems to be critical for both the immune response and for programmed cell death,<sup>17</sup> and therapeutic inhibition<sup>18</sup> of

\* To whom correspondence should be addressed. Phone: +41227069824. Fax: +41227946965. E-mail: jean-pierre.gotteland@serono.com.

<sup>†</sup> Department of Chemistry, Serono Pharmaceutical Research Institute.

<sup>#</sup> Serono International S.A.

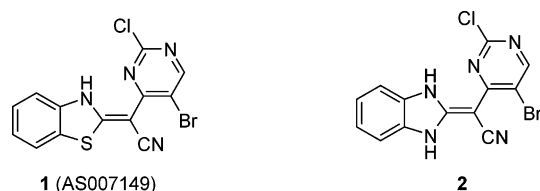
<sup>‡</sup> Serono Research Institute.

<sup>||</sup> Department of Screening, Serono Pharmaceutical Research Institute.

<sup>§</sup> Department of Signaling, Serono Pharmaceutical Research Institute.

<sup>§</sup> Department of Pharmacology, Serono Pharmaceutical Research Institute.

## Chart 1



JNK may provide clinical benefit in a wide range of apoptosis-related and inflammatory disorders (e.g., neurodegenerative diseases,<sup>19</sup> ischemia reperfusion injuries, multiple sclerosis, rheumatoid arthritis) although recent evidence also supports the application of JNK inhibitors in vascular, metabolic,<sup>20</sup> and oncological diseases.<sup>21</sup>

Development of JNK inhibitors has gained increasing interest in recent years.<sup>18</sup> The anthrapyrazolone SP600125 is an ATP competitive JNK1, -2, and -3 inhibitor for which a pharmacological profile has been reported<sup>22</sup> with a moderate selectivity over a range of Ser/Thr and Tyr specific protein kinases.<sup>23</sup> Important contribution to the discovery of JNK inhibitors has been made very recently by Merck researchers who have published, for the first time, the JNK3 crystal structure complex with various JNK small-molecule inhibitors.<sup>24</sup>

A proprietary drug discovery program at SeroPharmaceutical Research Institute was initiated with the aim of identifying and characterizing small-molecule JNK inhibitors as novel new chemical entities. We have reported very recently the identification of (benzoylaminomethyl)thiophene sulfonamide inhibitors such as AS600292 as the first potent and selective JNK inhibitor of this class that demonstrates a protective action against neuronal cell death induced by growth factor and serum deprivation.<sup>25</sup>

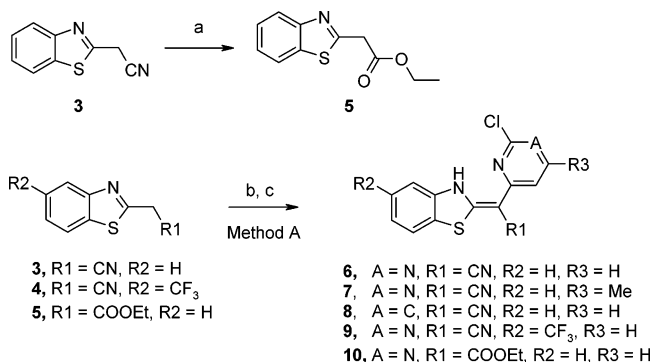
We now report the discovery of inhibitors of JNK isoforms, based on the benzothiazol-2-ylacetonitrile pyrimidine core, as a novel and unique kinase scaffold. The structure–activity relationship (SAR) carried out on the initial hit compound **1**, identified by high-throughput screening and leading to the discovery of compound **59**, and its antiinflammatory pharmacological properties are the subject of this paper.

## Chemistry Section

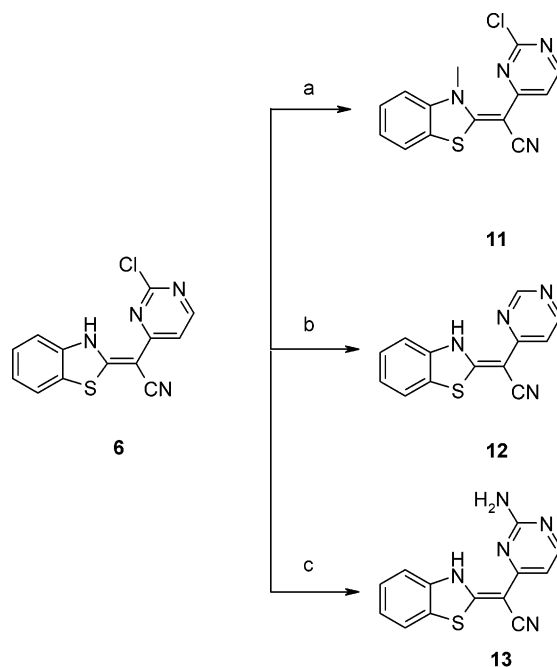
Screening of our internal compound collection for the inhibition of rat JNK3 resulted in the identification of compounds belonging to the benzothiazol-2-ylacetonitrile series. (3*H*-Benzothiazol-2-ylidene)(6-bromo-2-chloropyrimidin-4-yl)acetonitrile **1** was qualified as a hit and was the starting point of our medicinal chemistry efforts (Chart 1).

To investigate the effects on potency induced by small changes in the structure of **1**, cornerstone compounds (with the exception of the commercially available (1*H*-benzimidazol-2-yl)acetonitrile analogue **2**) were readily synthesized from commercially available 1,3-benzothiazol-2-ylacetonitrile **3** or (6-trifluoromethylbenzothiazol-2-yl)acetonitrile **4** (Scheme 1). Benzothiazol-2-ylacetic acid ethyl ester **5** was obtained by treatment of **3** at room temperature for 2 days in a 1/1 mixture of concentrated HCl and EtOH (Scheme 1).<sup>26</sup>

Derivatives **6–10** were obtained using method A by reacting the corresponding dichloropyrimidine or di-

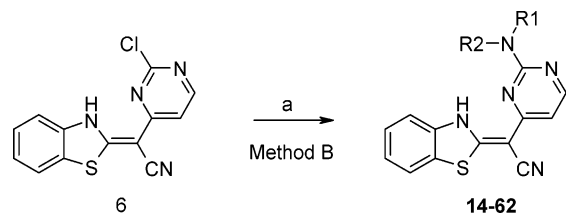
Scheme 1<sup>a</sup>

<sup>a</sup> Reagents: (a) concentrated HCl/EtOH [1/1], 2 days, room temp; (b) (1) THF, NaH, 0 °C, 1 h; (2) 2,4-dichloroheteroaryl, THF, room temp, ON; (c) aminoethylpolystyrene resin, Et<sub>3</sub>N, dioxane/DMA [3/1], 70 °C.

Scheme 2<sup>a</sup>

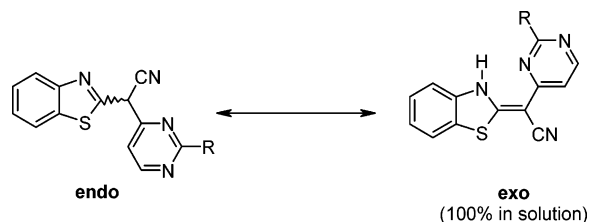
<sup>a</sup> Reagents: (a) MeI, K<sub>2</sub>CO<sub>3</sub>, DMSO, room temp, 2 days; (b) H<sub>2</sub>/Pd, NaOAc, AcOH, 70 °C, 3 bar, 3 h; (c) 2 M NH<sub>3</sub> in EtOH, 150 °C, 3 h.

chloropyrimidine in THF at room temperature under inert atmosphere with the anion of **3** generated using sodium hydride in THF (Scheme 1). The substitution of the benzothiazole nitrogen to obtain compound **11** was obtained by treating **6** with an excess of methyl iodide in the presence of potassium carbonate in DMSO (Scheme 2). The low yield obtained (5%) was due to the low nucleophilicity of this nitrogen. Removal of the chlorine atom at position 2 of the pyrimidine, leading to compound **12**, was performed by catalytic hydrogenation of compound **6** using a combination of sodium acetate and palladium on charcoal as a catalyst in acetic acid (Scheme 2). An optimal yield of only 13% was obtained by heating the suspension at 70 °C for 3 h under 3 bar. The low yield observed was due to degradation of either the product or the starting material. The lack of efficiency of this reaction was probably due to the presence of the sulfur atom (benzothiazole moiety) poisoning the catalyst.

Scheme 3<sup>a</sup>

<sup>a</sup> Reagents: (a)  $R_1R_2NH$ ,  $Et_3N$ , EtOH, 70 °C, 1–2 days.

## Chart 2



Compounds **13**–**62** were synthesized by nucleophilic substitution using the common intermediate **6**. The replacement of the chlorine by  $NH_2$  was performed under pressure by reacting compound **6** with a 2 M solution of ammonia in EtOH at 150 °C in a Parr vessel, affording compound **13** in 51% yield (Scheme 2).

Compounds **14**–**62** were obtained by displacing the chlorine atom in position 2 of the pyrimidine moiety with various amines using the conditions of method B. All the reactions were performed in ethanol at 70 °C using  $Et_3N$  to scavenge the HCl formed (Scheme 3).

It is noteworthy that compounds **1**, **2**, **6**–**10**, and **12**–**62**, described in this paper, exist as tautomers. Extensive studies were performed to characterize these compounds (unpublished results). The  $^1H$  NMR in deuterated DMSO showed that in all cases only the form with the exocyclic double bond exists in solution, most likely in configuration E (Chart 2). Indeed, the presence of a broad exchangeable signal between 10 and 12 ppm on one hand and the absence of signal in the range of 4–6 ppm on the other hand could correspond only to the form with the exocyclic double bond. This broad signal accounting for one proton in the case of the benzothiazole derivatives (**1**, **6**–**10**, and **12**–**62**) and two protons in case of the benzimidazoles **2** could only correspond to the proton of the enamine.

## Results and Discussion

Our starting point compound **1** exhibited an  $IC_{50}$  on rJNK3 of 350 nM as shown in Table 1. To investigate which parts of the scaffold were necessary for JNK3 activity, cornerstone compounds, like the benzimidazole, were purchased (analogue **2**) or synthesized (compounds **6**–**12**). As shown in Table 1, among the combinations tested, the only transformation tolerated by the enzyme involved the removal of the bromine on position 6 of the pyrimidine moiety, giving rise to compound **6**, which exhibited an  $IC_{50}$  of 250 nM. The replacement of the bromine by a methyl group at the same position on the pyrimidine (compound **7**) induced a complete loss of activity. The result obtained with compound **6** was a real improvement (from a chemistry point of view) because it did not affect the reactivity of the remaining chlorine atom but led to an increase in solubility of these

Table 1. Cornerstone Modifications from Screening Hit 1

compd	A	X	R1	R2	R3	R4	R5	rJNK3 $IC_{50}$ (nM) <sup>a</sup>
<b>1</b>	N	S	CN	H	Br	H	Cl	350
<b>2</b>	N	NH	CN	H	Br	H	Cl	>5000
<b>6</b>	N	S	CN	H	H	H	Cl	250
<b>7</b>	N	S	CN	H	Me	H	Cl	>5000
<b>8</b>	C	S	CN	H	H	H	Cl	>5000
<b>9</b>	N	S	CN	CF <sub>3</sub>	H	H	Cl	993
<b>10</b>	N	S	COOEt	H	H	H	Cl	>5000
<b>11</b>	N	S	CN	H	H	Me	Cl	>5000
<b>12</b>	N	S	CN	H	H	H	H	>5000

<sup>a</sup> All values in triplicate.

compounds in organic solvents, extending the scope of chemical transformations that could be investigated.

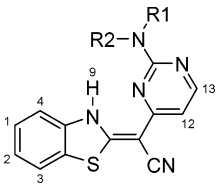
Going from the benzothiazole group to a benzimidazole group led to a drastic drop in activity. The same effect was seen with the replacement of the cyano group by an ethyl ester (compound **10**) or when a methyl group was substituted on the nitrogen of the benzothiazole moiety (compound **11**). This latter observation demonstrates that the benzothiazole acetonitrile moiety binds to the ATP pocket via its exo tautomeric form because an intramolecular hydrogen bond is likely to form between the NH group of the benzothiazole moiety and one of the nitrogens of the pyrimidine ring (Chart 2). The outcome of the introduction of a trifluoromethyl group at position 6 of the benzothiazole moiety was less drastic **9**, although it did induce a loss of potency (990 nM).

The changes investigated in the pyrimidine moiety were also detrimental to JNK3 potency, as shown by compound **8**. The replacement of one of the pyrimidine nitrogens at position 4 afforded the inactive pyridine analogue **8**. In addition, the presence of the chlorine atom at position 2 of the pyrimidine moiety seemed necessary to retain JNK3 activity as shown by compound **12**.

Because none of the cornerstone changes attempted on the scaffold led to any real improvements in JNK3 potency, compound **6**, exhibiting an improved potency and solubility, was considered as the new starting point to further investigate the SAR.

All the results obtained by the displacement of the chlorine at position 2 of the pyrimidine moiety are gathered in Table 2. Starting with alkylamines and cycloalkylamines (**12**–**35**), we observed that tertiary amines were less potent than secondary amines, as shown by the potency loss observed between compounds **15** and **16** (10-fold), thereby suggesting that the presence of the pyrimidine “NH” was crucial for activity on JNK3. This optimized atom arrangement could correspond to the well-described bidentate interaction, commonplace in several publications on ATP-competitive kinase inhibitors.<sup>27,28</sup> The results obtained with the cyclic amines (**17**–**21**) corroborated this hypothesis because all these compounds exhibited an  $IC_{50}$  of greater than 2500 nM. In addition, the stronger the donating capacity of the substituents on the pyrimidine moiety, the weaker was the potency on rJNK3. Any substituent



**Table 2.** Substitution at the Pyrimidinyl-2-chloro Position


compd	R1	R2	rJNK3 IC <sub>50</sub> (nM) <sup>a</sup>
13	H	H	7500
14	H	NH <sub>2</sub>	500
15	H	Me	950
16	Me	Me	9400
17		piperazinyl	6600
18		4-Me-piperazinyl	6800
19		morpholinyl	2900
20		pyrrolidinyl	>5000
21		4-OH-piperidinyl	7200
22	H	(CH <sub>2</sub> ) <sub>2</sub> N(Me) <sub>2</sub>	1300
23	H	(CH <sub>2</sub> ) <sub>2</sub> NH <sub>2</sub>	1490
24	H	(CH <sub>2</sub> ) <sub>2</sub> OMe	820
25	H	(CH <sub>2</sub> ) <sub>2</sub> OH	510
26	H	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	>5000
27	H	(CH <sub>2</sub> ) <sub>2</sub> N-piperidinyl	3740
28	H	(CH <sub>2</sub> ) <sub>2</sub> N-morpholinyl	760
29	H	(CH <sub>2</sub> ) <sub>3</sub> N(Me) <sub>2</sub>	1644
30	H	(CH <sub>2</sub> ) <sub>3</sub> NH <sub>2</sub>	707
31	H	(CH <sub>2</sub> ) <sub>3</sub> OH	660
32	H	(CH <sub>2</sub> ) <sub>3</sub> N-morpholinyl	407
33	H	(CH <sub>2</sub> ) <sub>3</sub> N-(4-Me-piperazinyl)	473
34	H	(CH <sub>2</sub> ) <sub>3</sub> N-pyrrolidinyl-2-one	1340
35	Me	(CH <sub>2</sub> ) <sub>3</sub> NHMe	1324
36	H	CH <sub>2</sub> Ph	6500
37	H	CH <sub>2</sub> -pyridin-2-yl	650
38	H	CH <sub>2</sub> -pyridin-3-yl	337
39	H	CH <sub>2</sub> -pyridin-4-yl	340
40	H	CH <sub>2</sub> -tetrazol-2-yl	>5000
41	H	(CH <sub>2</sub> ) <sub>2</sub> Ph	>5000
42	H	(CH <sub>2</sub> ) <sub>2</sub> -Ph-2-F	273
43	H	(CH <sub>2</sub> ) <sub>2</sub> -Ph-3-F	1810
44	H	(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-F	>5000
45	H	NH(CH <sub>2</sub> ) <sub>2</sub> -Ph-3-Cl	>5000
46	H	NH(CH <sub>2</sub> ) <sub>2</sub> -Ph-3,4-diCl	>5000
47	H	NH(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-Br	>5000
48	H	(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-Me	>5000
49	H	(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-OH	3500
50	H	(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-OMe	3080
51	H	(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-NH <sub>2</sub>	80
52	H	(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-SO <sub>2</sub> NH <sub>2</sub>	41
53	H	(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-NO <sub>2</sub>	600
54	H	(CH <sub>2</sub> ) <sub>2</sub> -indolyl	458
55	H	(CH <sub>2</sub> ) <sub>2</sub> -imidazol-4-yl	80
56	H	(CH <sub>2</sub> ) <sub>2</sub> -imidazol-4N-Me	65
57	H	(CH <sub>2</sub> ) <sub>2</sub> -imidazol-2N-Me	143
58	H	(CH <sub>2</sub> ) <sub>2</sub> -pyridin-2-yl	250
59	H	(CH <sub>2</sub> ) <sub>2</sub> -pyridin-3-yl	120
60	H	(CH <sub>2</sub> ) <sub>2</sub> -N-1,2,4-triazolyl	397
61	H	(CH <sub>2</sub> ) <sub>3</sub> -N-imidazolyl	147
62	H	(CH <sub>2</sub> ) <sub>3</sub> -N-pyrazolyl	583

<sup>a</sup> All values in triplicate.

that enhanced the electron-deficient character of the pyrimidine decreased the potency of the inhibitors within this series (N(Me)<sub>2</sub> < NHMe < NHNH<sub>2</sub> < Cl). Further analysis of these moderately active compounds allowed the extraction of the following information: the presence of polar groups (OR or NR<sub>1</sub>R<sub>2</sub>) within the lipophilic chains, cyclic or not (**17–19**, **21**, and **35**), contributed to improving the potency if compared with compounds **20** and **26** bearing, respectively, a pyrrolidinyl and *n*-propyl moiety.

The length of the linker, between the pyrimidine and this polar group identified as being important for activity, was investigated. The results obtained suggested the presence of a second interaction at the binding site two to three carbons away from the main interaction reached by the pyrimidine-NH group. Indeed, most of the compounds exhibited potency on JNK3 ranging from 400 to 4000 nM. The best potency was obtained with compounds **32** and **33**, bearing morpholinyl or 4-methylpiperazinyl moieties linked to the pyrimidine group by an aminopropyl chain.

Although none of the substitutions described above led to compounds better than our initial compound **6**, features critical for activity were identified, i.e., the presence of the pyrimidine-NH (main interaction) and distal heteroatom (putative second interaction) separated by a two to three carbon linker. The influence on the potency of a number of aromatic or heteroaromatic groups combining these structural features was then investigated.

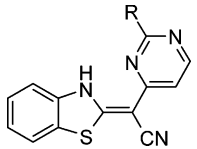
Substitution of the pyrimidine by aromatic or heteroaromatic amines with different linker lengths led to compounds **36–62**. The results obtained are in accordance with our previous observations. Among compounds **36–40** bearing a one-carbon linker, only the pyridine derivatives **37–39**, with a preference for 3- and 4-pyridines, allowed us to retain the potency observed with the best alkylamines, previously described (compounds **32** and **33**). The benzylamine derivative **36** was 20 times less active, confirming the need of a heteroatom in this part of the molecule, although heteroaromatic groups having an acidic character like the tetrazole derivative **40** were not tolerated by the enzyme. These results most probably suggested that the required heteroatom acts as a hydrogen bond acceptor (HBA), explaining the lack of activity of the tetrazolyl analogue.

Increasing the length of the linker by up to two carbons with the substituted phenethylamine derivatives **41–53** led to potency below 100 nM, particularly in the case where the phenethylamine was substituted in the para position with an amine group (**51**) or a primary sulfonamide (**52**). On one hand, naked phenethylamines and phenethylamines substituted with electron-withdrawing or electron-donating lipophilic groups (4-F, 3-Cl, 3,4-di-Cl, 4-Br, 4-Me) led to inactive compounds (**44** and **45–48**). On the other hand, the introduction of polar groups (4-OH, 4-OMe, 4-NH<sub>2</sub>, 4-SO<sub>2</sub>NH<sub>2</sub>, 4-NO<sub>2</sub>) led to compounds exhibiting a potency ranging from 3500 to 40 nM (**49–53**). Interestingly, the 2- and 3-fluorophenethylamine derivatives **42** and **43** showed a potency of 273 and 1810 nM, respectively, on the enzyme probably because of the HBA properties of the fluorine atom, thereby corroborating the hypothesis drawn previously.

From these observations, it was clearly shown that the potency on rJNK3 was improved by the presence of an aromatic group two carbons away from the amino pyrimidine moiety and bearing substituents conferring HBA properties able to reach a second interaction in the ATP binding pocket.

Phenethyl-like heteroaromatic amine derivatives (**54–60**) were further examined for their ability to inhibit the purified enzyme. As shown in Table 2, *N*-ethyl-imidazolyl derivatives, substituted or not (**55** to **57**), and

Table 3

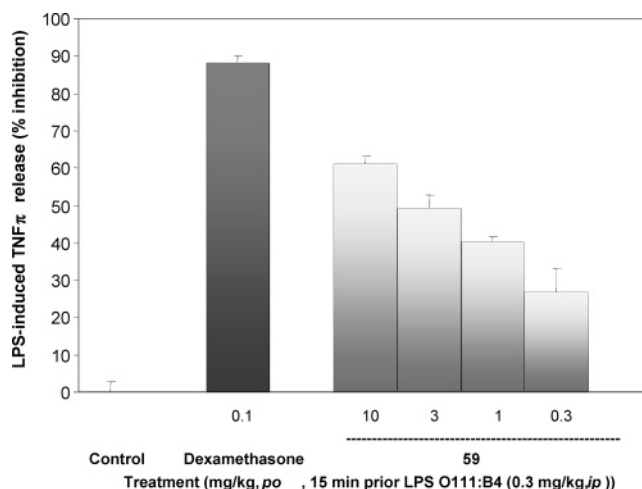
compd	R		
		rJNK3 IC <sub>50</sub> (nM)	Jurkat cells % inhibition @10 $\mu$ M
<b>6</b>	Cl	250	90
<b>33</b>	NH(CH <sub>2</sub> ) <sub>3</sub> N-(4-Me-piperazinyl)	473	83
<b>38</b>	NHCH <sub>2</sub> -pyridin-3-yl	337	95
<b>39</b>	NHCH <sub>2</sub> -pyridin-4-yl	340	nd <sup>a</sup>
<b>44</b>	NH(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-F	>5000	0
<b>42</b>	NH(CH <sub>2</sub> ) <sub>2</sub> -Ph-2-F	273	90
<b>51</b>	NH(CH <sub>2</sub> ) <sub>2</sub> -Ph-4-NH <sub>2</sub>	80	80
<b>55</b>	NH(CH <sub>2</sub> ) <sub>2</sub> -imidazol-4-yl	80	80
<b>56</b>	NH(CH <sub>2</sub> ) <sub>2</sub> -imidazol-4N-Me	65	nd <sup>a</sup>
<b>57</b>	NH(CH <sub>2</sub> ) <sub>2</sub> -imidazol-2N-Me	143	100
<b>58</b>	NH(CH <sub>2</sub> ) <sub>2</sub> -pyridin-2-yl	250	60
<b>59</b>	NH(CH <sub>2</sub> ) <sub>2</sub> -pyridin-3-yl	120	90
<b>61</b>	NH(CH <sub>2</sub> ) <sub>3</sub> -N-imidazolyl	147	31

<sup>a</sup> Not determined.

*N*-ethyl-2- and -3-pyridines (**58** and **59**) led to compounds with a potency ranging from 250 to 65 nM. The results obtained with the *N*-ethylimidazolyl derivatives confirmed that the presence of a nitrogen with HBA properties was necessary to get a potency below 100 nM, as shown by the difference in activity exhibited by compounds **55** and **56** on one hand and compound **57** on the other hand, in which the HBA was forced into the position 4 of the imidazole moiety by introduction of a methyl group at position 2. This observation was emphasized by the weaker activity shown by the aminoethylindolyl derivatives **54** where the nitrogen behaves as a hydrogen bond donor (HBD). The marked decrease in potency noticed for the *N*-ethyltriazole derivative **60** (400 nM) might suggest that decreasing the electron density of the nitrogen HBA is detrimental for activity, thus weakening this second interaction. Finally, extending the linker to three carbons (**61** and **62**) did not further influence the activity.

The introduction of these *N*-ethyl heteroaromatic substituents allowed access to potent JNK3 inhibitors with improved physicochemical properties, hence improving the druglike properties of the compounds of this series compared to our hit compound **6**. Indeed, the introduction of another protonable center allowed a dramatic increase in the solubility in aqueous media of these compounds, as shown by compound **59**, which is soluble at over 100 mg/mL in saline as a dimesylate salt. As anticipated for the high identity of the ATP binding region of the different JNK isoforms, none of the compounds mentioned above demonstrated specificity when tested against the rat or the human versions of JNK1, -2, or -3 (results not shown).

The activity of the most potent compounds was then assessed in a Jurkat T-cell assay (Table 3) where JNK has been reported to regulate the transcription of the IL-2 gene.<sup>29,30</sup> Jurkat cells from a human T lymphoblast cell line were treated with phorbol-12 myristate-13 acetate (PMA) plus ionomycin to induce IL-2 production. Compounds **6**, **33**, **42**, **57**, and **59** demonstrated almost complete inhibition at 10  $\mu$ M IL-2 secretion in this assay (no cell toxicity was observed at this concentration as



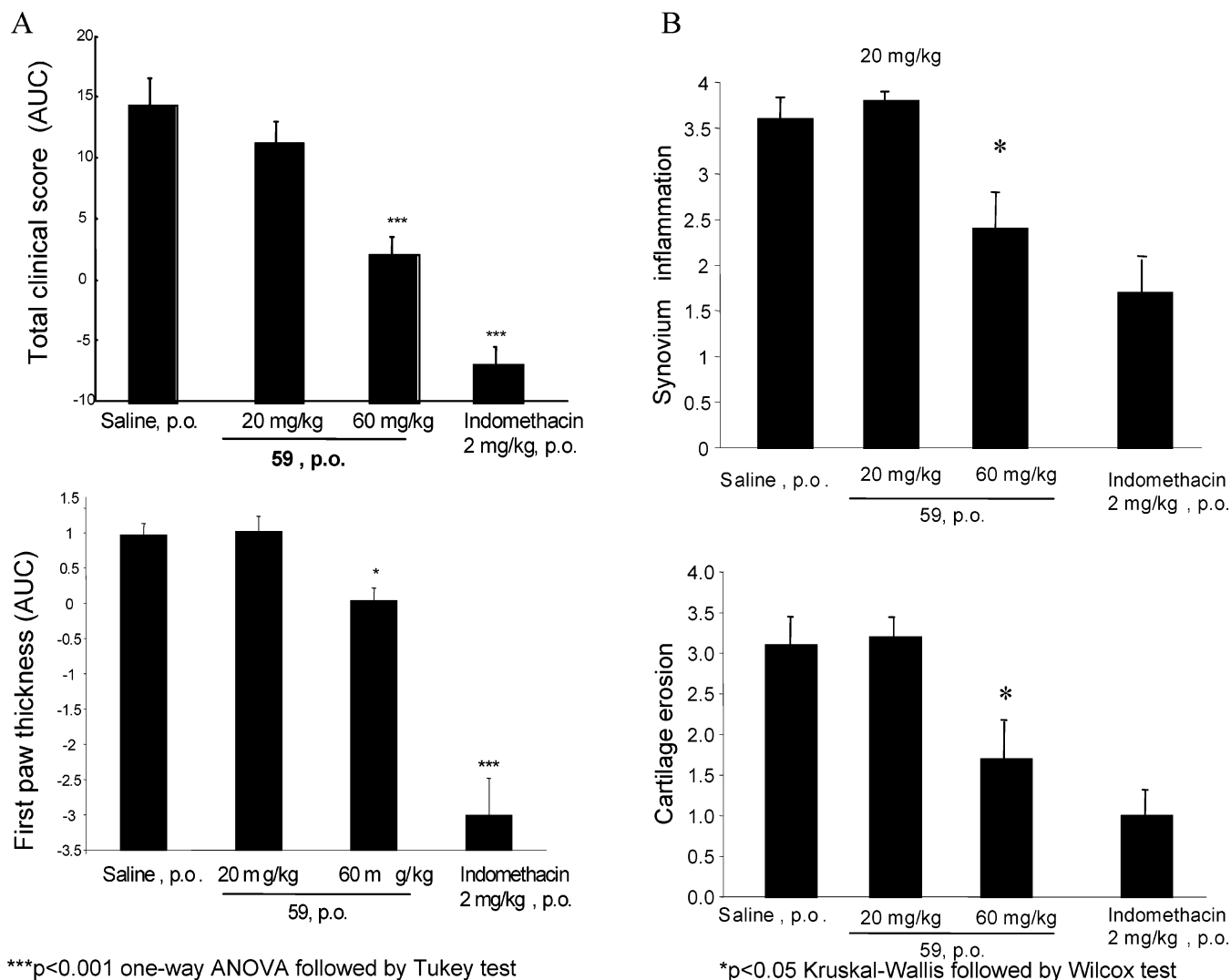
**Figure 1.** Effect of compound **59** on LPS induced TNF- $\alpha$  release.

monitored by MTS). The observed increase in IC<sub>50</sub> values between the biochemical assay and cell-based assay is most likely a reflection of high ATP levels present in the cell, although equally we cannot exclude that this may be due to low cellular permeability of the compounds. Further IC<sub>50</sub> determinations led us to select the most effective compounds in cells to further characterized their pharmacokinetics properties and in particular their oral bioavailability. Compound **59** exhibited an acceptable bioavailability in rat (*F<sub>z</sub>* = 38%) and was selected as our model compound to further evaluate, in vivo, the anti-inflammatory efficacy of this new class of JNK inhibitors.

Compound **59** was tested in a proof of concept in vivo model of inflammation that measured its ability to reduce TNF $\alpha$  release induced by lipopolysaccharide (LPS) in mice in comparison with the broad anti-inflammatory drug dexamethasone as a positive control. Compound **59** exhibited a dose-dependent decrease of TNF $\alpha$  plasma level with an ED<sub>50</sub> of 3 mg/kg when administered by oral route (Figure 1). In light of this result, **59** could have a high potential in disease models of inflammation. Indeed, overproduction of proinflammatory cytokines in rheumatoid arthritis (RA), such as TNF $\alpha$ , leads to persistent up-regulation of various molecules,<sup>31</sup> such as metalloproteases, responsible for the inflammation and destructive processes in the joints. Inhibition of the signal transduction pathways that either lead to production of proinflammatory cytokines or are responsible for their downstream effects could be an effective route for the treatment of RA. Because the role of JNK activation and JNK inhibition in the pathology of RA has already been demonstrated,<sup>32–34</sup> compound **59** was tested in an experimental model of RA.

Compound **59** induced significant anti-inflammatory effects when orally administered in a therapeutic dosing regimen (i.e., after disease onset) in a mouse model of collagen induced adjuvant (CIA) rheumatoid arthritis. The compound elicited significant depression of paw swelling and a clinical score at 60 mg/kg (Figure 2), while the reference compound indomethacin completely inhibited paw swelling.

As well as reducing joint swelling, histopathological analysis revealed that **59** preserved joint areas (de-



\*\*\*p<0.001 one-way ANOVA followed by Tukey test

**Figure 2.** Effect of compound **59** on the clinical course (A) and joint histology (B) in an experimental CIA model.

**Table 4.** Compound **59** Kinase Selectivity Profile<sup>a</sup>

kinases	IC <sub>50</sub> (μM)	kinases	IC <sub>50</sub> (μM)
JNK3	0.07	MKK7b	>10
JNK2	0.22	MKK4	>10
JNK1	0.15	MAPKAP-K2	>10
		ERK1	>10
c-SRC	1.1	MEK1	>10
c-Raf	1.2	PI3Kg	>10
CDK2/CycA	1.4	PDK1	>10
P38a	4.8	AKT	>10
		p70S6K	>10
MKK6	5–10	P56Lck	>10
RSK-2	5–10	CHK1	>10
ROCK-II	5–10	EGF	>10
Blk	5–10	IKKb	>10
MSK1	5–10	PKC	>10
SGK	5–10	PRAK	>10

<sup>a</sup> [ATP] = 10 μM.

creasing cartilage erosion) and reduced synovium inflammation in a significant manner at 60 mg/kg. This clearly demonstrates the potential of JNK inhibitors for the treatment of anti-inflammatory diseases. Selectivity of compound **59** was tested against a large panel of kinases (Table 4). It exhibited 10- to 20-fold selectivity over c-src, CDK2, and c-Raf and above 50- to 100-fold selectivity over a range of Ser/Thr and Tyr protein kinases. Although **59** was established as moderately

selective, we can assume that its in vivo effects are mainly due to the selective inhibition of the JNK isoforms, as we have demonstrated in other in vivo models of ischemia reperfusion injuries such as myocardial infarction<sup>35</sup> or cerebral ischemia.<sup>36</sup>

## Conclusion

Altogether, we have identified a novel class of (benzothiazol-2-yl)acetonitrile derivatives as potent and selective JNK1, -2, -3 inhibitors. SAR studies initially led to the discovery of compound **6**, which was further optimized. It was clearly shown that the potency on rJNK3 was improved by the presence of an aromatic group, two carbons away from the aminopyrimidine moiety and bearing substituents conferring HBA properties able to reach a second interaction in the ATP binding pocket. The introduction of aminoethyl heteroaromatic substituents allowed access to very potent JNK3 inhibitors with improved physicochemical properties, thereby improving the druglike properties of compounds of this chemical series to provide **59**. This compound demonstrated in vivo reduction of TNF-α production and arthritis severity, confirming the ever increasing potential of JNK inhibitors to effectively act as anti-inflammatory agents.



We have further concentrated our efforts on the identification of compounds with an improved biopharmaceutical profile and kinase selectivity profile to fully dissect the pharmacological potential of this new class of JNK inhibitors for the treatment of inflammatory diseases,<sup>37</sup> and this will be the subject of a future paper.

## Experimental Section

**General Experimental Methods. Procedures.** Melting points were measured with a Büchi B-545 melting point apparatus and were uncorrected. <sup>1</sup>H NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer. The following data were reported: chemical shift  $\delta$  in ppm using residual DMSO-*d*<sub>6</sub> as internal standard (2.49 ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet), coupling constant (s) in hertz, and integration. MS data provided were obtained using a Perkin-Elmer API 150 EX (APCI) mass spectrometer. Analytical HPLC was performed using an HPLC Waters Symmetry C8 50 mm  $\times$  4.6 mm column. Conditions were the following: (a) MeCN/H<sub>2</sub>O, 0.09% TFA, from 0% to 100% (10 min); (b) MeCN/H<sub>2</sub>O, 0.09% TFA, from 0% to 100% (20 min); (c) MeCN/H<sub>2</sub>O, 0.09% TFA, from 5% to 100% (10 min), max plot 230–400 nm; (d) MeCN/H<sub>2</sub>O, from 5% to 100% (10 min), max plot 230–400 nm.

In the experimental procedures, "purification by preparative HPLC" refers to dissolving the crude product in DMF and in the following purifying solvents: solvent A, 0.1% TFA in acetonitrile; solvent B, 0.1% TFA in water; 0–100% solvent A over 50 min (flow rate 50 mL/min; UV monitored at 240 nm). Preparative HPLC was performed on a Water Prep LC 4000 system using two different column sizes depending on the quantity to purify: small column (up to 200 mg), Xterra Prep MS C8, 10  $\mu$ m, 50 mm  $\times$  30 mm; large column (from 200 to 1000 mg), Xterra Prep MS C8, 10  $\mu$ m, 50 mm  $\times$  30 mm. Signals were detected using a Waters 2487 absorbance detector with dual wavelength.

Elemental analyses were performed with a VarioEL CHN analyzer from Elementar.

**Benzothiazol-2-ylacetic Acid Ethyl Ester (5).** A solution of benzothiazol-2-ylacetonitrile (**3**) in a mixture of EtOH and concentrated HCl [1/1] (30 mL) was stirred at room temperature. After 2 days, EtOH was evaporated. The acidic aqueous phase was extracted (3 $\times$ ) with AcOEt. Then the organics were washed with brine, and the solvent was evaporated. The oily residue was dried in a vacuum at 40 °C overnight.

**Method A: 1,3-Benzothiazol-2-yl-(2-chloro-4-pyrimidin-yl)acetonitrile (6).** To a stirred suspension of NaH (60% in oil, 9.2 g, 0.23 mol) in dry THF (200 mL) was added dropwise under inert atmosphere a solution of 1,3-benzothiazol-2-ylacetonitrile (20 g, 0.15 mol) in dry THF (200 mL). After the mixture was stirred for 1 h and 30 min at room temperature, a solution of 2,4-dichloropyrimidine (17.1 g, 0.15 mol) in dry THF (200 mL) was added dropwise. The reaction mixture was allowed to stir under inert atmosphere at room temperature until complete disappearance of the starting material. The reaction was quenched by addition of water, and the THF was evaporated. Water was added, and the suspension was slightly acidified with aqueous 1 M HCl to pH~4.0. The precipitate obtained was filtered off, washed thoroughly with water until neutral pH was attained, and then washed with hexane to remove the oil. The crude solid was dried under vacuum at 40 °C, affording 28 g (84%) of the title compound (**6**) as a brown powder: mp 246 °C dec; MS *m/z* 286.8 (M + 1); HPLC (condition a, 268 nm) 97%, *t*<sub>R</sub> = 5.66 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  13.25 (br s, 1H, exchangeable, H9), 8.09 (d, *J* = 4.14 Hz, 1H, H13), 7.90 (d, *J* = 7.53 Hz, 1H, H3), 7.61 (d, *J* = 7.92 Hz, 1H, H4), 7.39–7.34 (m, 1H, H2), 7.20–7.15 (m, 1H, H1), 6.96 (br d, 1H, H12). Anal. (C<sub>13</sub>H<sub>7</sub>ClN<sub>4</sub>S) C, H, N.

By use of method A described above and the appropriate starting material and reagents, compounds **7–9** could be obtained.

**1,3-Benzothiazol-2-yl-(2-chloro-6-methyl-4-pyrimidin-yl)acetonitrile (7).** Yield = 42.8%; MS *m/z* 300.8 (M + 1);

HPLC (condition b, 254 nm) 92%, *t*<sub>R</sub> = 13.91 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  13.22 (br s, 1H, exchangeable, H9), 7.96 (d, *J* = 7.79 Hz, 1H, H3), 7.63 (d, *J* = 8.17 Hz, 1H, H4), 7.48–7.42 (m, 1H, H2), 7.30–7.25 (m, 1H, H1), 7.07 (s, 1H, H12), 2.39 (s, 3H, CH<sub>3</sub> pyrimidine).

**1,3-Benzothiazol-2-yl(6-chloro-2-pyridyl)acetonitrile (8).** Yield = 36.2%; HPLC (314 nm) 99.6%, *t*<sub>R</sub> = 7.02 min (10 min TFA); MS *m/z* (APCI+) 286.0; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  12.44 (s, 1H, H9), 7.64 (d, *J* = 7.54 Hz, 1H, H3), 7.57 (t, *J* = 7.91 Hz, 1H, H pyridine), 7.31 (d, *J* = 7.91 Hz, 1H, H pyridine), 7.20–7.14 (m, 1H, H2), 7.07 (d, *J* = 8.29 Hz, 1H, H pyridine), 7.00–6.96 (m, 1H, H5), 6.86 (d, *J* = 7.54 Hz, 1H, H4). Anal. (C<sub>14</sub>H<sub>8</sub>ClN<sub>3</sub>S) C, H, N.

**(2-Chloropyrimidin-4-yl)[5-(trifluoromethyl)-1,3-benzothiazol-2-yl]acetonitrile (9).** Yield = 58.6%; MS *m/z* 352.6 (M – 1); HPLC (condition c, max plot) 99.8%, *t*<sub>R</sub> = 5.96 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  13.40 (s, 1H, H9), 8.37 (d, *J* = 5.66 Hz, 1H, H13), 8.21 (d, *J* = 8.29 Hz, 1H, H3), 7.87 (s, 1H, H4), 7.60 (d, *J* = 7.91 Hz, 1H, H2), 7.23–7.19 (m, 1H, H12). Anal. (C<sub>14</sub>H<sub>6</sub>ClF<sub>3</sub>N<sub>4</sub>S) C, H, N.

**1,3-Benzothiazol-2-yl(2-chloro-pyrimidin-4-yl)acetic Acid Ethyl Ester (10).** To a suspension of NaH (60% in oil, 0.039 g, 0.94 mmol) in dry THF (1 mL) was added dropwise a solution of benzothiazol-2-ylacetic acid ethyl ester (**5**) (0.104 mg, 0.47 mmol) in dry THF (1 mL), and the suspension was stirred for 1 h at room temperature under an inert atmosphere. A solution of 2,4-dichloropyrimidine (0.070 g, 0.47 mmol) in THF (1 mL) was added dropwise. After the mixture was stirred for 30 min at room temperature under argon, the reaction was stopped by addition of water. The product was extracted with AcOEt (4 $\times$ ). Then the organics were washed with water and brine (4 $\times$ ) until neutral pH was attained and dried over MgSO<sub>4</sub> before removal of the solvent. The solid obtained was dried overnight in a vacuum. The solid was taken up in ether and warmed. The precipitate was filtered off and washed with ether and dried at 40 °C in a vacuum to give 44 mg of the title compound as a beige powder. Yield = 28%; HPLC (247 nm) 97.8% *t*<sub>R</sub> = 6.76 min (10 min TFA); MS *m/z* (APCI–) 332.0; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  13.62 (br s, 1H, H9), 8.42 (br d, 1H, H13), 7.96–7.93 (m, 2H, H3, H4), 7.87–7.84 (m, 1H, H12), 7.49–7.44 (m, 1H, H2), 7.34–7.29 (m, 1H, H1), 4.36 (q, *J* = 7.16 Hz, 2H, COOCH<sub>2</sub>CH<sub>3</sub>), 1.37 (t, *J* = 7.16 Hz, 3H, COOCH<sub>2</sub>CH<sub>3</sub>).

**(2-Chloro-4-pyrimidinyl)(3-methyl-1,3-benzothiazol-2(3H)-ylidene)ethanenitrile (11).** To a solution of **6** (0.1 g, 0.35 mmol) in dry DMSO (1 mL) was added dry K<sub>2</sub>CO<sub>3</sub> (48 mg, 0.35 mmol) and then methyl iodide (0.02 mL, 0.35 mmol). The suspension was shaken at room temperature for 2 days. The precipitate formed after addition of water, was filtered off, and then washed with water until neutral pH was attained. The crude residue, dried under vacuum at 40 °C, was triturated in warm acetonitrile, filtered off, and then dried under vacuum at 40 °C, affording 5.6 mg (5%) of the title compound as a yellow powder. MS *m/z* 623 (2M + Na); HPLC (condition a, 388 nm) 99%, *t*<sub>R</sub> = 5.31 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  8.01 (d, *J* = 7.7 Hz, 1H, H3), 7.83 (d, *J* = 8.0 Hz, 1H, H4), 7.79 (d, *J* = 7.4 Hz, 1H, H13), 7.45–7.40 (m, 1H, H2), 7.31–7.24 (m, 1H, H1), 6.81 (d, *J* = 7.4 Hz, 1H, H12), 3.67 (s, 3H, CH<sub>3</sub>).

**1,3-Benzothiazol-2-yl(pyrimidin-4-yl)acetonitrile (12).** To a solution of **6** (0.1 g, 0.35 mmol) in acetic acid was added sodium acetate (29 mg, 0.35 mmol) and palladium on charcoal (20 mg). The suspension was heated to 70 °C under hydrogen at 3.5 bar for 3 h. After cooling to room temperature, the suspension was filtered through Celite and the acetic acid was evaporated. The bright-yellow powder was taken up in AcOEt and 10% aqueous NaOH. After three extractions, the organic phases were thoroughly washed with brine and then dried over MgSO<sub>4</sub> and concentrated to dryness. After purification by preparative HPLC and drying under vacuum at 50 °C, 12 mg (13%) of the title compound were obtained as a yellow powder. MS *m/z* 253.2 (M + 1); HPLC (condition c, max plot) 98%, *t*<sub>R</sub> = 3.35 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  11.24 (br s, H9), 8.61 (s, 1H, H pyrimidine), 8.00–7.73 (m, 3H, H13, H3, H4), 7.44–7.39 (m, 1H, H2), 7.29–7.23 (m, 1H, H1), 6.90 (br d, 1H, H12).

**(2-Aminopyrimidin-4-yl)(1,3-benzothiazol-2-yl)acetonitrile (13).** A suspension of **6** (0.1 g, 0.35 mmol) in a 2 M solution of ammonia in EtOH (10 mL) was heated to 150 °C in a Parr vessel for 3 h. The solution was cooled to room temperature, and the yellow precipitate formed was filtered off and then washed thoroughly with 1:1 EtOH/water and water. The precipitate was dried in a vacuum at 40 °C, affording 48 mg (51%) of the title compound as a yellow powder. MS *m/z* 268.0 (*M* + 1); HPLC (condition c, max plot) 95%, *t<sub>R</sub>* = 3.20 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 10.92 (br s, 1H, exchangeable, H9), 7.79 (d, *J* = 7.16 Hz, 1H, H3), 7.70 (d, *J* = 7.01 Hz, 1H, H4), 7.42–7.15 (m, 5H, H13, H1, H2, pyrimidine NH2 exchangeable), 6.34 (d, *J* = 7.54 Hz, 1H, H12).

**Method B: 1,3-Benzothiazol-2-yl[2-{[2-(1H-imidazol-4-yl)ethyl]amino}-4-pyrimidinyl]acetonitrile (Bis(trifluoroacetate)) (55).** To a suspension of **6** (0.1 g, 0.35 mmol) in dry EtOH (3 mL) was added Et<sub>3</sub>N (0.05 mL, 0.35 mmol) and histamine (0.078 g, 0.70 mmol). After sonication, the yellow solution was shaken at 70 °C for 3 days. The yellow precipitate formed was filtered off and washed with H<sub>2</sub>O (2×) and then with EtOH (3×) and dried under vacuum at 40 °C, affording 47 mg (37%) of the title compound as a bright-yellow powder: mp 257–258 °C. This powder was taken up in a mixture of DCM and TFA. The yellow fluffy solid formed by addition of Et<sub>2</sub>O was filtered off, washed with Et<sub>2</sub>O (3×), then dried under vacuum at 40 °C, affording 36 mg (29%) of the title compound **55** as a yellow powder: mp 247–249 °C; MS *m/z* 362.0 (*M* + 1); HPLC (condition a, 265 nm) 98%, *t<sub>R</sub>* = 2.87 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 14.25 (br s, 2H, exchangeable), 11.05 (br s, 1H, exchangeable), 9.03 (s, 1H), 7.94–7.87 (m, 1H), 7.74–7.71 (m, 2H), 7.57–7.52 (m, 2H), 7.42–7.37 (m, 1H), 7.24–7.19 (m, 1H), 6.40 (d, *J* = 7.1 Hz, 1H), 3.97–3.55 (m, 3H), 3.11–3.05 (m, 2H). Anal. (C<sub>18</sub>H<sub>15</sub>N<sub>7</sub>S·2C<sub>2</sub>H<sub>3</sub>F<sub>3</sub>O<sub>2</sub>) C, H, N.

By use of method B described above for compound **55** and the appropriate starting material and reagents, the following compounds could be obtained:

**1,3-Benzothiazol-2-yl[2-(2-hydrazino-4-pyrimidinyl)acetonitrile (Trifluoroacetate) (14).** Yield = 60%; MS *m/z* 283.0 (*M* + 1); HPLC (271 nm) 98%, *t<sub>R</sub>* = 3.17 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 9.78 (br s, 1H, exchangeable, H9), 7.89–7.75 (m, 4H, H13, H4, H3, pyrimidineNHNH2), 7.48–7.43 (m, 1H, H2), 7.32–7.27 (m, 1H, H1), 6.53 (br d, 1H, H12), 4.25–3.40 (m, 1H, CF<sub>3</sub>CO<sub>2</sub>H).

**1,3-Benzothiazol-2-yl[2-(methylamino)-4-pyrimidinyl]acetonitrile (Trifluoroacetate) (15).** Yield = 11%; MS *m/z* 282.0 (*M* + 1); HPLC (condition a, 270 nm) 97%, *t<sub>R</sub>* = 3.39 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.70 (v br s, 1H, exchangeable, H9), 8.15–7.90 (m, 2H [1 + 1 exchangeable], H3, pyrimidine NH), 7.85–7.55 (m, 2H, H4, H13), 7.46–7.41 (m, 1H, H2), 7.30–7.25 (m, 1H, H1), 6.43 (d, *J* = 6.0 Hz, 1H, H12), 4.81–3.78 (m, 1H), 3.10 (s, 3H, NHCH<sub>3</sub>).

**1,3-Benzothiazol-2-yl[2-(dimethylamino)-4-pyrimidinyl]acetonitrile (16).** Yield = 12%; MS *m/z* 295.8 (*M* + 1); HPLC (condition a, 270 nm) 99%, *t<sub>R</sub>* = 3.50 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.20 (br s, 1H, exchangeable, H9), 7.88 (d, *J* = 7.76 Hz, 1H, H3), 7.69 (d, *J* = 8 Hz, 1H, H4), 7.50–7.40 (m, 1H, H13), 7.38–7.33 (m, 1H, H2), 7.20–7.16 (m, 1H, H1), 6.38 (d, *J* = 6.9 Hz, 1H, H12), 3.26 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>). Anal. (C<sub>15</sub>H<sub>13</sub>N<sub>5</sub>S·0.2H<sub>2</sub>O) C, H, N.

**1,3-Benzothiazol-2-yl[2-(1-piperazinyl)-4-pyrimidinyl]acetonitrile (Bis(trifluoroacetate)) (17).** Yield = 37%; MS *m/z* 337.2 (*M* + 1); HPLC (condition a, 271 nm) 96%, *t<sub>R</sub>* = 2.58 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 9.13 (br s, NH-piperazine, CF<sub>3</sub>COOH), 7.96 (br d, 1H, H13), 7.90 (d, *J* = 7.74 Hz, 1H, H3), 7.65 (d, *J* = 8.0 Hz, 1H, H4), 7.45–7.40 (m, 1H, H2), 7.29–7.24 (m, 1H, H1), 6.58 (d, *J* = 6.0 Hz, 1H, H12), 4.70–3.60 (m, 5H, CH<sub>2</sub>-piperazine, CF<sub>3</sub>COOH), 3.38–3.20 (m, 4H, CH<sub>2</sub>-piperazine).

**1,3-Benzothiazol-2-yl[2-(4-methyl-1-piperazinyl)-4-pyrimidinyl]acetonitrile (Bis(trifluoroacetate)) (18).** Yield = 30%; MS *m/z* 351.0 (*M* + 1); HPLC (condition a, 271 nm) 99%, *t<sub>R</sub>* = 2.54 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 10.10 (br s, 1H, exchangeable, H9), 8.05 (br d, 1H, H13), 7.88 (d, *J* = 7.8 Hz, 1H, H3), 7.63 (d, *J* = 8.0 Hz, 1H, H4), 7.45–7.40 (m, 1H, H2),

7.29–7.24 (m, 1H, H1), 6.60 (d, *J* = 5.8 Hz, 1H, H12), 4.95–4.70 (m, 2H, CH<sub>2</sub>-piperazine), 4.42–3.68 (m, 1H, CF<sub>3</sub>CO<sub>2</sub>H), 3.67–3.50 (m, 2H, CH<sub>2</sub>-piperazine), 3.48–3.31 (m, 2H, CH<sub>2</sub>-piperazine), 3.26–3.05 (m, 2H, CH<sub>2</sub>-piperazine), 2.86 (s, 3H, NCH<sub>3</sub>).

**1,3-Benzothiazol-2-yl[2-(4-morpholinyl)-4-pyrimidinyl]acetonitrile (Trifluoroacetate) (19).** Yield = 55%; MS *m/z* 338.0 (*M* + 1); HPLC (condition a, 270 nm) 99%, *t<sub>R</sub>* = 3.51 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 7.94 (d, *J* = 7.8 Hz, 1H, H3), 7.79 (br d, 1H, H13), 7.70 (d, *J* = 8.0 Hz, 1H, H4), 7.45–7.40 (m, 1H, H2), 7.30–7.25 (m, 1H, H12), 6.54 (d, *J* = 6.3 Hz, 1H, H12), 4.40–3.65 (m, 9H, CH<sub>2</sub> morpholine and CF<sub>3</sub>CO<sub>2</sub>H).

**1,3-Benzothiazol-2-yl[2-(1-pyrrolidinyl)-4-pyrimidinyl]acetonitrile (20).** Yield = 27%, mp 270–272 °C; MS *m/z* 322.0 (*M* + 1); HPLC (condition a, 372 nm) 98%, *t<sub>R</sub>* = 3.90 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.30 (v br s, 1H, exchangeable, H9), 7.86 (d, *J* = 7.2 Hz, 1H), 7.66 (d, *J* = 7.9 Hz, 1H, H3), 7.46 (d, *J* = 6.8 Hz, 1H, H13), 7.35–7.30 (m, 1H, H2), 7.17–7.12 (m, 1H, H1), 6.33 (d, *J* = 6.8 Hz, 1H, H12), 3.90–3.45 (m, 4H, CH<sub>2</sub>-pyrrolidine), 2.08–1.94 (m, 4H, CH<sub>2</sub>-pyrrolidine).

**1,3-Benzothiazol-2-yl[2-(4-hydroxy-1-piperidinyl)-4-pyrimidinyl]acetonitrile (Trifluoroacetate) (21).** Yield = 14%; MS *m/z* 352 (*M* + 1); HPLC (condition a, 271 nm) 97%, *t<sub>R</sub>* = 3.21 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 7.95 (d, *J* = 7.79 Hz, 1H, H3), 7.72–7.65 (m, 2H, H4 and H13), 7.45–7.40 (m, 1H, H2), 7.28–7.23 (m, 1H, H1), 6.48 (d, *J* = 6.07 Hz, 1H, H12), 4.60–3.75 (m, 5H, CH<sub>2</sub>piperidine, HOCH<sub>2</sub>-piperidine, CF<sub>3</sub>CO<sub>2</sub>H), 3.58–3.51 (m, 2H, CH<sub>2</sub>-piperidine), 1.95–1.82 (m, 2H, CH<sub>2</sub>-piperidine), 1.55–1.42 (m, 2H, CH<sub>2</sub>-piperidine).

**1,3-Benzothiazol-2-yl[2-{[2-(dimethylamino)ethyl]amino}-4-pyrimidinyl]acetonitrile (Bis(trifluoroacetate)) (22).** Yield = 30%; MS *m/z* 339.0 (*M* + 1); HPLC (condition a, 270 nm) 99%, *t<sub>R</sub>* = 2.69 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.85 (v br s, 1H, exchangeable, H9), 9.59 (br s, 1H), 7.90 (br d, 2H, H3 and NH-pyrimidine), 7.73 (d, *J* = 7.9 Hz, 1H, H4), 7.60 (br d, 1H, H13), 7.43–7.38 (m, 1H, H2), 7.27–7.22 (m, 1H, H1), 6.43 (d, *J* = 6.8 Hz, 1H, H12), 4.25–3.70 (m, 3H, NCH<sub>2</sub>CH<sub>2</sub> and CF<sub>3</sub>CO<sub>2</sub>H), 3.51–3.41 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 2.87 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>).

**1,3-Benzothiazol-2-yl[2-{[2-(2-aminoethyl)amino]pyrimidin-4-yl}]acetonitrile (Bis(trifluoroacetate)) (23).** Yield = 86%; MS *m/z* 311.0 (*M* + 1); HPLC (condition a, 382 nm) 95%, *t<sub>R</sub>* = 2.64 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.60 (v br s, 1H, exchangeable, H9), 7.95–7.86 (m, 3H, H3, NH-pyrimidine and CF<sub>3</sub>CO<sub>2</sub>H), 7.73 (d, *J* = 7.91 Hz, 1H, H4), 7.59 (br d, 1H, H13), 7.43–7.37 (m, 1H, H2), 7.28–7.22 (m, 1H, H1), 6.43 (d, *J* = 7.16 Hz, 1H, H12), 5.50–4.00 (m, 3H, exchangeable, CH<sub>2</sub>NH<sub>2</sub> and CF<sub>3</sub>CO<sub>2</sub>H), 3.90–3.70 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 3.25–3.10 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>).

**1,3-Benzothiazol-2-yl[2-{[2-(methoxyethyl)amino]-4-pyrimidinyl]acetonitrile (24).** Yield = 54%; MS *m/z* 326.0 (*M* + 1); HPLC (condition a, 273 nm) 99%, *t<sub>R</sub>* = 3.66 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 10.83 (s, 1H, H9), 7.85 (d, *J* = 7.54 Hz, 1H, H3), 7.72 (d, *J* = 7.91 Hz, 1H, H4), 7.60 (br s, 1H, NH-pyrimidine), 7.44 (d, *J* = 6.78 Hz, 1H, H13), 7.38–7.33 (m, 1H, H2), 7.22–7.16 (m, 1H, H1), 6.33 (d, *J* = 7.16 Hz, 1H, H12), 3.82–3.74 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 3.62 (t, *J* = 5.27 Hz, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 3.31 (s, 3H, OCH<sub>3</sub>).

**1,3-Benzothiazol-2-yl[2-{[2-(2-hydroxyethyl)amino]-4-pyrimidinyl]acetonitrile (25).** Yield = 80%; MS *m/z* 312.2 (*M* + 1); HPLC (condition a, 273 nm) 99%, *t<sub>R</sub>* = 3.16 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 10.85 (s, 1H, H9), 7.86 (d, *J* = 7.91 Hz, 1H, H3), 7.71 (d, *J* = 7.91 Hz, 1H, H4), 7.57 (br s, 1H, NH-pyrimidine), 7.44 (d, *J* = 7.16 Hz, 1H, H13), 7.38–7.32 (m, 1H, H2), 7.21–7.16 (m, 1H, H1), 6.32 (d, *J* = 7.16 Hz, 1H, H12), 4.92 (br s, 1H, OH), 3.68–3.45 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>OH).

**1,3-Benzothiazol-2-yl[2-(propylamino)-4-pyrimidinyl]acetonitrile (26).** Yield = 81%; MS *m/z* 310.0 (*M* + 1); HPLC (condition a, 273 nm) 95%, *t<sub>R</sub>* = 4.04 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 10.91 (br s, 1H, H9), 7.84 (d, *J* = 7.54 Hz, 1H, H3), 7.71 (d, *J* = 8.29 Hz, 1H, H4), 7.62 (br s, 1H, NH-pyrimidine), 7.42 (d, *J* = 6.78 Hz, 1H, H13), 7.38–7.32 (m, 1H, H2), 7.21–7.16 (m, 1H, H1), 6.31 (d, *J* = 7.53 Hz, 1H, H12), 3.42–3.33 (m,



2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.71–1.64 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.02–0.97 (m, 3H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>).

**1,3-Benzothiazol-2-yl[2-[(2-piperidin-1-ylethyl)amino]pyrimidin-4-yl]acetonitrile (Bis(trifluoroacetate)) (27).** Yield = 12%; mp 228–230 °C; MS *m/z* 379.0 (M + 1); HPLC (condition a, 270 nm) 99.9%, *t<sub>R</sub>* = 2.84 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 9.18 (br s, 1H, H9), 7.89–7.87 (m, 2H, H3 and H13), 7.74 (d, *J* = 7.91 Hz, 1H, H4), 7.62 (br s, 1H, NH-pyrimidine), 7.44–7.39 (m, 1H, H2), 7.29–7.24 (m, 1H, H1), 6.45 (d, *J* = 7.17 Hz, 1H, H12), 5.25–4.30 (m, 1H, CF<sub>3</sub>CO<sub>2</sub>H), 4.05–3.93 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>N-piperidine), 3.63–3.50 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>N-piperidine), 3.48–3.37 (m, 2H, piperidine), 3.07–2.92 (m, 2H, piperidine), 1.90–1.30 (m, 6H, piperidine).

**1,3-Benzothiazol-2-yl[2-[(2-morpholin-4-ylethyl)amino]pyrimidin-4-yl]acetonitrile (Bis(trifluoroacetate)) (28).** Yield = 53%; MS *m/z* 381.0 (M + 1); HPLC (condition a, 254 nm) 99.5%, *t<sub>R</sub>* = 2.80 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 10.9 (v br s, 1H, exchangeable, H9), 8.00–7.98 (m, 2H, H3 and H13), 7.73 (d, *J* = 7.9 Hz, 1H, H4), 7.58 (br s, 1H, NH-pyrimidine), 7.42–7.37 (m, 1H, H2), 7.27–7.22 (m, 1H, H1), 6.55 (d, *J* = 7.15, 1H, H12), 4.24–3.18 (m, 12H, NCH<sub>2</sub>CH<sub>2</sub>N-morpholine); <sup>1</sup>H NMR (D<sub>2</sub>O) δ 7.59 (d, *J* = 7.9 Hz, 1H), 7.39–7.29 (m, 2H), 7.20–7.15 (m, 2H), 6.20 (d, *J* = 6.8, 1H), 3.89–3.82 (m, 6H), 3.45–3.32 (m, 6H).

**1,3-Benzothiazol-2-yl[2-[(3-(dimethylamino)propyl)amino]pyrimidin-4-yl]acetonitrile (Bis(trifluoroacetate)) (29).** Yield = 54%; mp 204–205 °C; MS *m/z* 353.0 (M + 1); HPLC (condition a, 272 nm) 98%, *t<sub>R</sub>* = 2.75 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.4 (v br s, 1H), 9.43 (s, 1H), 7.94 (d, *J* = 7.91 Hz, 1H), 7.90 (br s, 1H), 7.73 (d, *J* = 7.91 Hz, 1H), 7.55 (br d, 1H), 7.43–7.37 (m, 1H), 7.27–7.22 (m, 1H), 6.39 (d, *J* = 7.14 Hz, 1H), 4.80–4.00 (m, 1H), 3.71–3.60 (m, 2H), 3.24–3.13 (m, 2H), 2.78 (d, *J* = 4.14 Hz, 6H), 2.11–1.99 (m, 2H). Anal. (C<sub>18</sub>H<sub>20</sub>N<sub>6</sub>S·2C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub>·0.3H<sub>2</sub>O) C, H, N.

**[2-[(3-Aminopropyl)amino]pyrimidin-4-yl][1,3-benzothiazol-2-yl]acetonitrile (Bis(trifluoroacetate)) (30).** Yield = 62%; MS *m/z* 325.0 (M + 1); HPLC (condition a, 382 nm) 90.0%, *t<sub>R</sub>* = 2.67 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.60 (v br s, 1H, exchangeable, H9), 7.99 (br s, 1H, exchangeable, CF<sub>3</sub>CO<sub>2</sub>H), 7.90 (d, *J* = 7.92 Hz, 1H, H3), 7.75–7.49 (m, 3H, H13 and H4 and NH-pyrimidine), 7.44–7.37 (m, 1H, H2), 7.27–7.22 (m, 1H, H1), 6.39 (d, *J* = 7.16 Hz, 1H, H12), 5.50–4.00 (m, 3H, exchangeable, NH<sub>2</sub> and CF<sub>3</sub>CO<sub>2</sub>H), 3.80–3.5 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 3.00–2.80 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 2.10–1.80 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>).

**1,3-Benzothiazol-2-yl[2-[(3-hydroxypropyl)amino]pyrimidin-4-yl]acetonitrile (31).** Yield = 44%; MS *m/z* 326.0 (M + 1); HPLC (condition c, max plot) 99%, *t<sub>R</sub>* = 3.26 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 10.81 (br s, 1H, exchangeable, H9), 7.84 (d, *J* = 7.54 Hz, 1H, H13), 7.71 (d, *J* = 8.29 Hz, 1H, H3), 7.49 (br s, 1H, exchangeable, NH-pyrimidine), 7.43–7.32 (m, 2H, H2 and H4), 7.21–7.15 (m, 1H, H1), 6.32 (d, *J* = 7.20 Hz, 1H, H12), 4.65–4.50 (br s, 1H, exchangeable, OH), 3.80–3.50 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OH), 1.90–1.70 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OH). Anal. (C<sub>16</sub>H<sub>15</sub>N<sub>5</sub>OS·0.2H<sub>2</sub>O) C, H, N.

**1,3-Benzothiazol-2-yl[2-[(3-morpholin-4-ylpropyl)amino]pyrimidin-4-yl]acetonitrile (Bis(trifluoroacetate)) (32).** Yield = 73.9%; MS *m/z* 408.2 (M + 1); HPLC (condition a, 272 nm) 99.6%, *t<sub>R</sub>* = 2.77 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 9.66 (br s, 1H), 7.94 (d, *J* = 7.91 Hz, 1H), 7.86 (br s, 1H), 7.73 (d, *J* = 7.91 Hz, 1H), 7.55 (br d, 1H), 7.43–7.38 (m, 1H), 7.27–7.22 (m, 1H), 6.40 (d, *J* = 7.17 Hz, 1H), 4.15–3.52 (m, 7H [6 + 1]), 3.49–3.38 (m, 2H), 3.29–3.19 (m, 2H), 3.16–3.00 (m, 2H), 2.15–2.01 (m, 2H). Anal. (C<sub>20</sub>H<sub>22</sub>N<sub>6</sub>OS·2C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub>·0.5H<sub>2</sub>O) C, H, N.

**1,3-Benzothiazol-2-yl[2-[[3-(4-methylpiperazin-1-yl)propyl]amino]pyrimidin-4-yl]acetonitrile (Tris(trifluoroacetate)) (33).** Yield = 65.2%; MS *m/z* 408.0 (M + 1); HPLC (condition a, 272 nm) 99.2%, *t<sub>R</sub>* = 2.67 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 7.94–7.91 (m, 2H), 7.74 (d, *J* = 7.92 Hz, 1H), 7.56 (br d, 1H), 7.43–7.38 (m, 1H), 7.27–7.22 (m, 1H), 6.40 (d, *J* = 7.17 Hz, 1H), 4.95–4.05 (m, 1H), 3.71–3.60 (m, 2H), 3.54–3.15 (m, 4H), 3.02–2.86 (m, 4H), 2.75 (s, 3H), 2.03–1.91 (m, 2H). Anal. (C<sub>21</sub>H<sub>25</sub>N<sub>7</sub>S·3C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub>·0.5H<sub>2</sub>O) C, H, N.

**1,3-Benzothiazol-2-yl[2-[[3-(2-oxopyrrolidin-1-yl)propyl]amino]pyrimidin-4-yl]acetonitrile (trifluoroacetate) (34).** Yield = 65%; MS *m/z* 393.0 (M + 1); HPLC (condition a, 254 nm) 98%, *t<sub>R</sub>* = 3.52 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.50 (v br s, 1H, exchangeable), 8.15–8.02 (br s, 1H), 7.95–7.60 (m, 4H), 7.46–7.41 (m, 1H), 7.31–7.25 (m, 1H), 6.45 (br s, 1H), 3.70–3.50 (m, 2H), 3.48–2.28 (m, 4H), 2.23–2.17 (m, 2H), 1.92–1.84 (m, 4H). Anal. (C<sub>20</sub>H<sub>20</sub>N<sub>6</sub>O<sub>3</sub>S) C, H, N.

**1,3-Benzothiazol-2-yl[2-[[methyl[3-(methylamino)propyl]amino]pyrimidin-4-yl]acetonitrile (Bis(trifluoroacetate)) (35).** Yield = 11%; MS *m/z* 353.2 (M + 1); HPLC (condition a, 272 nm) 96%, *t<sub>R</sub>* = 2.88 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.30 (v br s, 1H, exchangeable, H9), 8.50–8.25 (br s, 2H, H3 and CF<sub>3</sub>CO<sub>2</sub>H), 7.92 (d, *J* = 7.91 Hz, 1H, H4), 7.72–7.68 (m, 2H, H12 and NH-pyrimidine), 7.43–7.37 (m, 1H, H2), 7.27–7.21 (m, 1H, H1), 6.46 (d, *J* = 6.8 Hz, 1H, H12), 4.00–3.65 (m, 2H, NH and CF<sub>3</sub>CO<sub>2</sub>H), 3.22 (s, 3H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHCH<sub>3</sub>), 3.10–2.85 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHCH<sub>3</sub>), 2.63–2.51 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHCH<sub>3</sub>), 2.10–1.80 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHCH<sub>3</sub>).

**1,3-Benzothiazol-2-yl[2-(benzylamino)pyrimidin-4-yl]acetonitrile (Trifluoroacetate) (36).** Yield = 78%; MS *m/z* 358.0 (M + 1); HPLC (condition a, 254 nm) 99.2%, *t<sub>R</sub>* = 4.40 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.30 (br t, 1H), 7.84 (d, *J* = 7.53 Hz, 1H), 7.71 (d, *J* = 7.91 Hz, 1H), 7.65 (br d, 1H), 7.46–7.34 (m, 5H), 7.28–7.22 (m, 2H), 6.47 (d, *J* = 7.14 Hz, 1H), 5.2–4.5 (m, 1H), 4.86 (br d, 2H). Anal. (C<sub>20</sub>H<sub>15</sub>N<sub>5</sub>S·C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub>·0.2H<sub>2</sub>O) C, H, N.

**1,3-Benzothiazol-2-yl[2-[(2-pyridinylmethyl)amino]-4-pyrimidinyl]acetonitrile (Bis(trifluoroacetate)) (37).** Yield = 52%; mp = 250 °C dec; MS *m/z* 359.0 (M + 1); HPLC (condition a, 266 nm) 99%, *t<sub>R</sub>* = 2.84 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.66 (d, *J* = 4.9 Hz, 1H), 8.38 (br s, 1H), 7.99–7.94 (m, 1H), 7.82 (d, *J* = 7.91 Hz, 1H), 7.71–7.69 (m, 2H), 7.64 (d, *J* = 7.91 Hz, 1H), 7.45–7.38 (m, 2H), 7.28–7.23 (m, 1H), 6.48 (d, *J* = 6.78 Hz, 1H), 5.00 (br s, 2H), 5.15–4.05 (m, 2H). Anal. (C<sub>19</sub>H<sub>14</sub>N<sub>6</sub>S·2C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub>) C, H, N.

**1,3-Benzothiazol-2-yl[2-[(pyridin-3-ylmethyl)amino]pyrimidin-4-yl]acetonitrile (Bis(trifluoroacetate)) (38).** Yield = 46%; MS *m/z* 359.0 (M + 1); HPLC (condition c, max plot) 99.7%, *t<sub>R</sub>* = 2.56 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.87 (s, 1H), 8.64 (d, *J* = 4.9 Hz, 1H), 8.29–8.26 (m, 2H), 7.84–7.63 (m, 4H), 7.41–7.36 (m, 1H), 7.25–7.20 (m, 1H), 6.46 (d, *J* = 7.16 Hz, 1H), 4.97 (br d, 2H). Anal. (C<sub>19</sub>H<sub>14</sub>N<sub>6</sub>S·2C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub>·0.5H<sub>2</sub>O) C, H, N.

**1,3-Benzothiazol-2-yl[2-[(pyridin-4-ylmethyl)amino]pyrimidin-4-yl]acetonitrile (Bis(trifluoroacetate)) (39).** Yield = 46.5%; MS *m/z* 359.0 (M + 1); HPLC (condition c, max plot) 99%, *t<sub>R</sub>* = 2.55 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.77 (d, *J* = 6.4 Hz, 1H), 8.33 (br t, 1H), 7.95–7.93 (br d, 2H), 7.79 (d, *J* = 7.54 Hz, 1H), 7.69–7.62 (m, 2H), 7.39–7.34 (m, 1H), 7.24–7.19 (m, 1H), 6.43 (d, *J* = 7.17 Hz, 1H), 5.05 (br d, 2H), 5.6–4.4 (br s, 1H). Anal. (C<sub>19</sub>H<sub>14</sub>N<sub>6</sub>S·2C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub>·0.4H<sub>2</sub>O) C, H, N.

**1,3-Benzothiazol-2-yl[2-[(1H-tetrazol-5-ylmethyl)amino]pyrimidin-4-yl]acetonitrile (Trifluoroacetate) (40).** Yield = 31%; MS *m/z* 447.8 (M – 1); HPLC (condition c, max plot) 99%, *t<sub>R</sub>* = 2.60 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.40–8.29 (br d, 1H, exchangeable), 7.78–7.60 (m, 4H), 7.41–7.36 (m, 1H), 7.27–7.21 (m, 1H), 6.47 (d, *J* = 6.78 Hz, 1H), 5.25–5.05 (m, 2H). Anal. (C<sub>15</sub>H<sub>11</sub>N<sub>9</sub>S·C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub>·0.5H<sub>2</sub>O) C, H, N.

**1,3-Benzothiazol-2-yl[2-[(2-phenylethyl)amino]-4-pyrimidinyl]acetonitrile (Trifluoroacetate) (41).** Yield = 46.1%; mp = 256 °C dec; MS *m/z* 371.8 (M + 1); HPLC (condition a, 270 nm) 99%, *t<sub>R</sub>* = 4.64 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 11.04 (br s, 1H), 7.71 (d, *J* = 8.29 Hz, 1H), 7.64–7.61 (m, 2H), 7.45 (d, *J* = 7.16 Hz, 1H), 7.37–7.32 (m, 5H), 7.29–7.26 (m, 1H), 7.20–7.16 (m, 1H), 6.33 (d, *J* = 7.17 Hz, 1H), 3.94–3.81 (m, 2H), 2.99 (t, *J* = 7.54 Hz, 2H). Anal. (C<sub>21</sub>H<sub>17</sub>N<sub>5</sub>S·C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub>·0.3H<sub>2</sub>O) C, H, N.

**1,3-Benzothiazol-2-yl[2-[[2-(2-fluorophenyl)ethyl]amino]pyrimidin-4-yl]acetonitrile (42).** Yield = 76%; MS *m/z* 390.2 (M + 1); HPLC (condition c, max plot) 99.7%, *t<sub>R</sub>* = 4.41 min; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 7.88 (br t, 1H), 7.75–7.68 (m, 2H), 7.60 (br d, 1H), 7.45–7.40 (m, 2H), 7.33–7.16 (m, 4H),

6.44 (d,  $J = 7.17$  Hz, 1H), 4.20–3.60 (m, 3H), 3.04 (t,  $J = 7.14$  Hz, 2H). Anal. ( $C_{21}H_{16}FN_5S$ ) C, H, N.

**1,3-Benzothiazol-2-yl(2-([2-(3-fluorophenyl)ethyl]amino)pyrimidin-4-yl)acetonitrile (Bis(trifluoroacetate)) (43).** Yield = 34%; MS  $m/z$  390.0 ( $M + 1$ ); HPLC (condition c, max plot) 98.4%,  $t_R = 4.72$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.90–7.67 (m, 3H, H3 and H4 and H13), 7.57 (br d, 1H, NH-pyrimidine), 7.44–7.36 (m, 2H, 3-F-Ph), 7.27–7.16 (m, 3H, H2 and 3-F-Ph), 7.12–7.06 (m, 1H, H1), 6.42 (d,  $J = 7.14$  Hz, 1H, H12), 4.50–3.70 (m, 3H,  $CH_2CH_2N$  and  $CF_3CO_2H$ ), 3.05–3.00 (m, 2H,  $CH_2CH_2N$ ).

**1,3-Benzothiazol-2-yl(2-([2-(4-fluorophenyl)ethyl]amino)pyrimidin-4-yl)acetonitrile (44).** Yield = 73%; MS  $m/z$  389.8 ( $M + 1$ ); HPLC (condition c, max plot) 99.6%,  $t_R = 4.72$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.75–7.70 (m, 3H, H3 and H4 and H13), 7.59 (br d, 1H, NH-pyrimidine), 7.45–7.34 (m, 3H, H2 and 4-F-Ph), 7.29–7.24 (m, 1H, H1), 7.20–7.14 (m, 2H, 4-F-Ph), 6.43 (d,  $J = 7.17$  Hz, 1H, H12), 4.32–3.65 (m, 3H [2 + 1],  $CH_2CH_2N$  and  $CF_3CO_2H$ ), 2.99 (t,  $J = 7.16$  Hz, 2H,  $CH_2CH_2N$ ).

**1,3-Benzothiazol-2-yl(2-([2-(3-chlorophenyl)ethyl]amino)pyrimidin-4-yl)acetonitrile (Bis(trifluoroacetate)) (45).** Yield = 70%; MS  $m/z$  406.0 ( $M + 1$ ); HPLC (condition c, max plot) 99.7%,  $t_R = 4.91$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.85–7.73 (m, 3H), 7.60 (br d, 1H), 7.41–7.24 (m, 7H), 6.44 (d,  $J = 7.17$  Hz, 1H), 3.98–3.50 (m, 3H), 3.04–2.99 (m, 2H). Anal. ( $C_{21}H_{16}ClN_5S \cdot C_2HF_3O_2$ ) C, H, N.

**1,3-Benzothiazol-2-yl(2-([2-(3,4-dichlorophenyl)ethyl]amino)pyrimidin-4-yl)acetonitrile (Trifluoroacetate) (46).** Yield = 56%; MS  $m/z$  440.0 ( $M + 1$ ); HPLC (condition c, max plot) 99.6%,  $t_R = 5.15$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.75–7.73 (m, 3H), 7.62–7.58 (m, 3H), 7.45–7.40 (m, 1H), 7.33–7.25 (m, 2H), 6.44 (d,  $J = 7.17$  Hz, 1H), 4.20–3.60 (m, 3H), 3.01 (t,  $J = 6.78$  Hz, 2H). Anal. ( $C_{12}H_{15}Cl_2N_5S \cdot C_2HF_3O_2$ ) C, H, N.

**1,3-Benzothiazol-2-yl(2-([2-(4-bromophenyl)ethyl]amino)pyrimidin-4-yl)acetonitrile (Trifluoroacetate) (47).** Yield = 96%; MS  $m/z$  450.0 ( $M + 1$ ); HPLC (condition c, max plot) 99.6%,  $t_R = 5.01$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.81–7.68 (m, 3H), 7.60 (br d, 1H), 7.53 (d,  $J = 8.28$  Hz, 2H), 7.45–7.40 (m, 1H), 7.30–7.25 (m, 3H), 6.43 (d,  $J = 7.16$  Hz, 1H), 4.12–3.55 (m, 3H), 3.01–2.96 (m, 2H). Anal. ( $C_{21}H_{16}BrN_5S \cdot C_2HF_3O_2$ ) C, H, N.

**1,3-Benzothiazol-2-yl(2-([2-(4-methylphenyl)ethyl]amino)pyrimidin-4-yl)acetonitrile (Trifluoroacetate) (48).** Yield = 72%; MS  $m/z$  386.0 ( $M + 1$ ); HPLC (condition c, max plot) 100%  $t_R = 4.66$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.87 (br s, 1H, H13), 7.76–7.67 (m, 3H, H3, H4 and NH-pyrimidine), 7.47–7.42 (m, 1H, H2), 7.32–7.27 (m, 1H, H1), 7.23–7.14 (m, 4H, 4-CH<sub>3</sub>-Ph), 6.47 (d,  $J = 7.14$  Hz, 1H, H12), 4.20–3.55 (m, 3H,  $CH_2CH_2N$  and  $CF_3CO_2H$ ), 2.98–2.93 (m, 2H,  $CH_2CH_2N$ ), 2.30 (s, 3H, 4-CH<sub>3</sub>-Ph).

**1,3-Benzothiazol-2-yl(2-([2-(4-hydroxyphenyl)ethyl]amino)pyrimidin-4-yl)acetonitrile (Trifluoroacetate) (49).** Yield = 76%; mp 258–261°C; MS  $m/z$  388.0 ( $M + 1$ ); HPLC (condition a, 272 nm) 98.8%,  $t_R = 4.00$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  9.25 (br s, 1H), 7.75–7.69 (m, 2H), 7.59 (br d, 1H), 7.45–7.40 (m, 1H), 7.29–7.24 (m, 1H), 7.12 (d,  $J = 8.29$  Hz, 1H), 6.74 (d,  $J = 8.29$  Hz, 1H), 6.43 (d,  $J = 6.78$  Hz, 1H), 3.89–3.71 (m, 2H), 2.90–2.85 (m, 2H). Anal. ( $C_{21}H_{17}N_5OS \cdot C_2HF_3O_2 \cdot 0.7H_2O$ ) C, H, N.

**1,3-Benzothiazol-2-yl(2-([2-(4-methoxyphenyl)ethyl]amino)pyrimidin-4-yl)acetonitrile (Bis(trifluoroacetate)) (50).** Yield = 69%; MS  $m/z$  402.0 ( $M + 1$ ); HPLC (condition c, max plot) 99.6%,  $t_R = 4.33$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.75–7.69 (m, 3H), 7.61 (br d, 1H), 7.40–7.35 (m, 1H), 7.24–7.17 (m, 3H), 6.86 (d,  $J = 8.28$  Hz, 2H), 6.38 (d,  $J = 7.16$  Hz, 1H), 4.15–3.65 (m, 3H), 3.69 (s, 3H), 2.9–2.85 (m, 2H). Anal. ( $C_{22}H_{19}N_5OS \cdot 2C_2HF_3O_2 \cdot 0.4H_2O$ ) C, H, N.

**(2-([2-(4-aminophenyl)ethyl]amino)pyrimidin-4-yl)-(1,3-benzothiazol-2-yl)acetonitrile (Bis(trifluoroacetate)) (51).** Yield = 73% (salt); MS  $m/z$  387.2 ( $M + 1$ ); HPLC (condition c, max plot) 98.3%,  $t_R = 3.02$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  7.93 (br d, 1H), 7.75–7.66 (m, 2H), 7.55 (br d, 1H), 7.43–7.36 (m, 3H), 7.26–7.17 (m, 3H), 6.40 (d,  $J = 7.16$  Hz, 1H),

4.9–4.2 (v br s, 1H), 3.94–3.82 (m, 2H), 2.99 (t,  $J = 7.16$  Hz, 2H). Anal. ( $C_{21}H_{18}N_6S \cdot 2C_2HF_3O_2 \cdot 0.7H_2O$ ) C, H, N.

**4-[2-([4-[1,3-Benzothiazol-2-yl(cyano)methyl]pyrimidin-2-yl]amino)ethyl]benzenesulfonamide (52).** Yield = 80%; MS  $m/z$  449.0 ( $M - 1$ ); HPLC (condition c, max plot) 99%,  $t_R = 3.28$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  11.20 (v br s, 1H, exchangeable, H9), 7.81–7.65 (m, 5H, H13, H3, H4 and 4-H<sub>2</sub>N<sub>2</sub>SO<sub>2</sub>-Ph), 7.57–7.50 (m, 3H, H<sub>2</sub>NSO<sub>2</sub> and NH-pyrimidine), 7.44–7.22 (m, 4H, H1, H2 and 4-H<sub>2</sub>N<sub>2</sub>SO<sub>2</sub>-Ph), 6.43 (d,  $J = 6.78$  Hz, 1H, H12), 4.10–3.80 (m, 2H,  $CH_2CH_2N$ ), 3.20–3.00 (m, 2H,  $CH_2CH_2N$ ).

**1,3-Benzothiazol-2-yl(2-([2-(2-[4-hydroxy(oxido)amino]phenyl)ethyl]amino)pyrimidin-4-yl)acetonitrile (53).** Yield = 33.8%; MS  $m/z$  417.2 ( $M + 1$ ); HPLC (condition c, max plot) 98.7%,  $t_R = 4.21$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  8.21 (d,  $J = 8.66$  Hz, 2H, 4-NO<sub>2</sub>-Ph), 7.88 (br s, 1H, H13), 7.75–7.68 (m, 2H, H3 and H4), 7.61 (d,  $J = 8.66$  Hz, 2H, 4-NO<sub>2</sub>-Ph), 7.54 (br d, 1H, NH-pyrimidine), 7.42–7.37 (m, 1H, H2), 7.26–7.21 (m, 1H, H1), 6.41 (d,  $J = 7.17$  Hz, 1H, H12), 3.85–3.70 (m, 2H,  $CH_2CH_2N$ ), 3.55–3.10 (m, 1H,  $CF_3CO_2H$ ), 3.18–3.14 (m, 2H,  $CH_2CH_2N$ ).

**1,3-Benzothiazol-2-yl(2-([2-(1H-indol-3-yl)ethyl]amino)pyrimidin-4-yl)acetonitrile (TFA) (54).** Yield = 60.6%; MS  $m/z$  411.0 ( $M + 1$ ); HPLC (condition a, 272 nm) 99.9%,  $t_R = 4.94$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  11.25 (very br s, 1H), 10.98 (s, 1H), 7.92–7.79 (m, 1H), 7.71 (d,  $J = 7.92$  Hz, 1H), 7.63–7.57 (m, 2H), 7.42–7.37 (m, 3H), 7.28 (br d, 1H), 7.21–7.16 (m, 1H), 7.10–7.05 (m, 1H), 6.97–6.92 (m, 1H), 6.44 (d,  $J = 7.17$  Hz, 1H), 4.60–3.70 (m, 3H [2 + 1]), 3.12 (t,  $J = 7.15$  Hz, 2H). Anal. ( $C_{23}H_{18}N_6S \cdot C_2HF_3O_2 \cdot 0.4H_2O$ ) C, H, N.

**1,3-Benzothiazol-2-yl(2-([2-(1-methyl-1H-imidazol-4-yl)ethyl]amino)pyrimidin-4-yl)acetonitrile (56).** Yield = 58.8%; MS  $m/z$  376.0 ( $M + 1$ ); HPLC (condition a, 272 nm) 99.3%,  $t_R = 3.09$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  11.20 (very br s, 1H, H9), 9.00 (s, 1H, imidazolyl-4-CH<sub>3</sub>), 7.90 (br s, 1H, H13), 7.78–7.72 (m, 2H, H3 and H4), 7.58–7.54 (m, 2H, imidazolyl-4-CH<sub>3</sub> and NH-pyrimidine), 7.43–7.38 (m, 1H, H2), 7.27–7.22 (m, 1H, H1), 6.41 (d,  $J = 7.17$  Hz, 1H, H12), 4.60–4.10 (m, 1H,  $CF_3CO_2H$ ), 3.93–3.85 (m, 2H,  $CH_2CH_2N$ ), 3.83 (s, 3H, imidazolyl-4-CH<sub>3</sub>), 3.11–3.02 (m, 2H,  $CH_2CH_2N$ ).

**1,3-Benzothiazol-2-yl(2-([2-(1-methyl-1H-imidazol-5-yl)ethyl]amino)pyrimidin-4-yl)acetonitrile (Bis(trifluoroacetate)) (57).** Yield = 46%; mp 219–220 °C; MS  $m/z$  376.0 ( $M + 1$ ); HPLC (condition a, 270 nm) 99.8%,  $t_R = 2.73$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  11.15 (br s, 1H, H9), 9.03 (s, 1H, imidazole), 7.83 (br s, 1H, H13), 7.74–7.64 (m, 3H, H3 and H4 and imidazole), 7.54 (br d, 1H, NH-pyrimidine), 7.41–7.36 (m, 1H, H2), 7.24–7.18 (m, 1H, H1), 6.41 (d,  $J = 7.14$  Hz, 1H, H12), 4.50–3.88 (m, 3H,  $CH_2CH_2N$  and  $CF_3CO_2H$ ), 3.78 (s, 3H, imidazolyl-2-CH<sub>3</sub>), 3.10–3.05 (m, 2H,  $CH_2CH_2N$ ).

**1,3-Benzothiazol-2-yl(2-([2-(2-pyridinyl)ethyl]amino)-4-pyrimidinyl)acetonitrile (Bis(trifluoroacetate)) (58).** Yield = 80%; mp = 247°C dec; MS  $m/z$  373.2 ( $M + 1$ ); HPLC (condition a, 266 nm) 99%,  $t_R = 2.85$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  8.70 (d,  $J = 5.28$  Hz, 1H), 8.15–8.10 (m, 1H), 7.88 (br s, 1H), 7.75–7.72 (m, 2H), 7.67 (d,  $J = 7.91$  Hz, 1H), 7.61–7.50 (m, 2H), 7.44–7.39 (m, 1H), 7.28–7.23 (m, 1H), 6.44 (d,  $J = 7.14$  Hz, 1H), 4.65–3.60 (m, 3H), 3.32–3.28 (m, 2H). Anal. ( $C_{20}H_{16}N_6S \cdot 2C_2HF_3O_2$ ) C, H, N.

**1,3-Benzothiazol-2-yl(2-([2-(3-pyridinyl)ethyl]amino)-4-pyrimidinyl)acetonitrile (Bis(trifluoroacetate)) (59).** Yield = 74%; MS  $m/z$  373.0 ( $M + 1$ ); HPLC (condition a, 263 nm) 99%,  $t_R = 2.92$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  8.81 (d,  $J = 1.13$  Hz, 1H, H-pyridine), 8.71 (dd,  $J = 5.27$  Hz,  $J = 1.13$  Hz, 1H, H-pyridine), 8.31 (d,  $J = 7.91$  Hz, 1H, H-pyridine), 7.94 (br s, 1H, NHCH<sub>2</sub>), 7.85–7.73 (m, 3H, H3/H4/H13), 7.60 (br d, 1H, H-pyridine), 7.45–7.40 (m, 1H, H1), 7.29–7.24 (m, 1H, H2), 6.43 (d,  $J = 7.14$  Hz, 1H, H12), 6.00–4.40 (m, 2H,  $CF_3CO_2H$ ), 4.05–3.87 (m, 2H, NCH<sub>2</sub>), 3.19–3.15 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>). Anal. ( $C_{23}H_{18}N_6S \cdot 2C_2HF_3O_2 \cdot 1H_2O$ ) C, H, N.

**1,3-Benzothiazol-2-yl(2-([2-(1H-1,2,4-triazol-1-yl)ethyl]amino)pyrimidin-4-yl)acetonitrile (60).** Yield = 77.6%; MS  $m/z$  361.2 ( $M - 1$ ); HPLC (condition c, max plot) 99.4%,  $t_R = 2.79$  min;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  8.59 (s, 1H, triazole), 8.09 (s,



1H, triazole), 7.98–7.82 (m, 2H, H13, H3), 7.75 (d,  $J$  = 7.91 Hz, 1H, H4), 7.67 (br d, 1H, NH-pyrimidine), 7.48–7.43 (m, 1H, H2), 7.33–7.28 (m, 1H, H1), 6.51 (d,  $J$  = 7.16 Hz, 1H, H12), 5.05–4.25 (m, 3H [2 + 1], CH<sub>2</sub>CH<sub>2</sub>N and CF<sub>3</sub>CO<sub>2</sub>H), 4.10–3.98 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>N).

**1,3-Benzothiazol-2-yl(2-{[3-(1H-imidazol-1-yl)propyl]-amino}-4-pyrimidinyl)acetonitrile (Trifluoroacetate) (61).** Yield = 57%; MS  $m/z$  376.0 ( $M$  + 1); HPLC (condition a, 270 nm) 98%,  $t_R$  = 2.80 min; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  11.60 (v br s, 1 H, exchangeable, H9), 9.18 (s, 1H, imidazole), 8.13 (br s, 1H, NH-pyrimidine), 7.92–7.85 (m, 2H, H4 and imidazole), 7.74–7.59 (m, 3H, H3, H13 and imidazole), 7.43–7.39 (m, 1H, H2), 7.28–7.23 (m, 1H, H1), 6.40 (d,  $J$  = 7.2 Hz, 1H, H12), 5.05–4.20 (m, 1H, CF<sub>3</sub>CO<sub>2</sub>H), 4.35 (t,  $J$  = 6.8 Hz, 2H, NCH<sub>2</sub>-CH<sub>2</sub>CH<sub>2</sub>), 3.70–3.45 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.35–2.10 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>).

**1,3-Benzothiazol-2-yl(2-{[3-(1H-pyrazol-1-yl)propyl]-amino}pyrimidin-4-yl)acetonitrile (62).** Yield = 70%; MS  $m/z$  374.0 ( $M$  – 1); HPLC (condition c, max plot) 94.8%,  $t_R$  = 3.40 min; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  8.01–7.85 (m, 2H, H3, NH-pyrimidine), 7.78–7.73 (m, 2H, H4 and pyrazole), 7.62 (br d, 1H, H13), 7.46–7.43 (m, 2H, H2 and pyrazole), 7.31–7.26 (m, 1H, H1), 6.45 (d,  $J$  = 7.17 Hz, 1H, H12), 6.22 (s, 1H, pyrazole), 4.30–3.85 (m, 3H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub> and CF<sub>3</sub>CO<sub>2</sub>H), 3.62–3.48 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.21–2.06 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>).

**Biological Methods. 1. rJNK3 Enzymatic Assay.** GST-JNK3 (1 mg/mL) was incubated overnight at room temperature with 0.05 mg/mL of GST-JNKK2 in a solution containing 200  $\mu$ M ATP- $\gamma$ -S, 1 mM DTT, 10 mM MgCl<sub>2</sub>, and 100  $\mu$ M Na<sub>3</sub>VO<sub>4</sub>, followed by dialysis against 50 mM Tris-HCl, pH 8, 150 mM NaCl, and 5 mM DTT overnight at 4 °C to remove the ATP- $\gamma$ -S.

rJNK3 assays are performed in 96-well low-binding Corning microtiter (MT) plates: 0.5  $\mu$ g of recombinant, preactivated GST-JNK3 were incubated with 1  $\mu$ g of recombinant, biotinylated GST-c-Jun and 2  $\mu$ M <sup>33</sup> $\gamma$ -ATP (2 nCi/ $\mu$ L) in the presence or absence of compounds and in a reaction volume of 50  $\mu$ L containing 50 mM Tris-HCl, pH 8.0, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, and 100  $\mu$ M NaVO<sub>4</sub> for 120 min and at room temperature. The reaction was stopped by the addition of 200  $\mu$ L of a solution containing 250  $\mu$ g of streptavidine-coated SPA beads (Amersham, Inc.), 5 mM EDTA, 0.1% Triton X-100, and 50  $\mu$ M ATP in phosphate saline buffer and further incubated at room temperature for 60 min. After incubation beads were sedimented by centrifugation at 1500g for 5 min, resuspended in 200  $\mu$ L of PBS containing 5 mM EDTA, 0.1% Triton X-100, and 50  $\mu$ M ATP, and the radioactivity was measured in a scintillation  $\beta$  counter after letting the beads settle for an additional 60 min at room temperature.

**2. Jurkat Cell Assay.** Jurkat cells, from a human T cell leukemia cell line (American Type Culture Collection, no. TIB 152), were cultured in RPMI 1640 medium (Gibco, BRL) supplemented with 10% heat-inactivated fetal calf serum, glutamine, and penicillin/streptomycin. The cells were diluted in the medium to give  $2 \times 10^6$  cells/mL, and afterward, they were plated ( $2 \times 10^5$  cells/well) in a 96-well plate containing different concentrations of test compound (final concentration of compounds of 10, 3, 1, 0.3, 0.1  $\mu$ M in 0.1% DMSO). This mixture was incubated for 30 min at 37 °C in a humidified CO<sub>2</sub> atmosphere. Cells were then treated with 10  $\mu$ L of PMA + ionomycin (0.1 and 1  $\mu$ M final concentration) in all wells except the negative control. In wells without compounds, 10  $\mu$ L of RPMI 2% DMSO (=0.1% final) is added. Cells were incubated for 24 h at 37 °C, and then the supernatant was harvested (freeze at –20 °C if not used the same day) prior to performing an IL-2 ELISA test on the supernatant.

IL-2 release into the medium by PMA + ionomycin-stimulated Jurkat cells in the presence or absence of test compounds was assayed by ELISA using a capture monoclonal antihuman IL-2 antibody (MAB602), biotinylated antihuman IL-2 antibody (BAF202, detection), and recombinant human IL-2 (202-IL-010, standard) from R&D Systems.

**3. In Vivo LPS Challenge Assay.** Eight-week-old C3H/HEN mice (IFFA-CREDO, L'arbresles, France) received an oral

treatment with compound **59** (30, 10, 3, and 0.3 mg/kg in 0.5% CMC/0.25% Tween-20/water). Groups of six mice were used. Fifteen minutes later, endotoxins (O111:B4 Sigma, 0.3 mg/kg) were intraperitoneally injected. Heparinized whole blood was collected by decapitation. TNF- $\alpha$  was determined in plasma by ELISA (R & D Systems, Abingdon, U.K.). Control animals received 0.5% CMC/0.25% Tween-20 (10 mL/kg) as the vehicle. Data obtained from experiments were expressed as the mean  $\pm$  SEM and analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's  $t$ -test.

**4. In Vivo Mouse Model of Collagen-Induced Rheumatoid Arthritis.** DBA/1 male mice, 8–12 weeks old, were primed (day 0) by injecting intradermally at the base of the tail 0.2 mL of an emulsion composed of 0.2 mg of bovine type II collagen in complete Freund's adjuvant containing 0.2 mg of mycobacterium tuberculosis. Starting from day 18 of the study, each animal was examined daily and each paw was scored from 0 to 2 for the presence of inflammation in digits, and from 0 to 3 for the presence of oedema in both forepaws and hind paws (paw thickness was measured by means of a precision calliper). The maximum achievable score was 14. Upon appearance of disease signs (score  $\geq$  1.5), the animals were assigned to different treatment groups.

**Treatment.** Compound **59** (bis(trifluoroacetate) salt) was tested at doses of 20 and 60 mg/kg per oral route using saline as vehicle. Indomethacin was included as a reference compound at a dose of 2 mg/kg, orally. All treatments continued for 7 days.

**Histology.** Twenty-four hours after the last treatment, animals were sacrificed by an overdose of anesthetic, and the first limb to become arthritic was removed, identified, fixed in neutral buffered 10% formalin, and decalcified in formic acid solution (50%). This paw was then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Three slides per animal were prepared and scored according to the following scale: for erosion score, 0 = no destruction, 1–2 = localized cartilage erosions, 3 = more extended erosions, 4 = general destruction; for inflammation scores, 0 = no inflammation, 1–2 = slight thickening of lining layer and/or some infiltrating cells in sublining layer, 3 = thickening of lining layer and more pronounced influx of cells in sublining layer with presence of cells in the synovial space, 4 = synovium highly infiltrated with many inflammatory cells.

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**Supporting Information Available:** Results from elemental analysis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Cobb, M. H.; Goldsmith, E. J. How MAP kinases are regulated. *J. Biol. Chem.* **1995**, *270*, 14843–14846.
- (2) Minden, A.; Karin, M. Regulation and function of the JNK subgroup of MAP kinases. *Biochim. Biophys. Acta* **1997**, *1333*, F85–F104.
- (3) Barr, R. K.; Bogoyevitch, M. A. The c-Jun N-terminal protein kinase family of mitogen-activated protein kinases (JNK MAPKs). *Int. J. Biochem. Cell Biol.* **2001**, *33*, 1047–1063.
- (4) Gupta, S.; Barrett, T.; Whitmarsh, A. J.; Cavanagh, J.; Sluss, H. K.; Derijard, B.; Davis, R. J. Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J.* **1996**, *15*, 2760–2770.
- (5) Hibi, M.; Lin, A.; Smeal, T.; Minden, A.; Karin, M. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev.* **1993**, *7*, 2135–2148.
- (6) Whitmarsh, A. J.; Shore, P.; Sharrocks, A. D.; Davis, R. J. Integration of MAP kinase signal transduction pathways at the serum response element. *Science* **1995**, *269*, 403–407.



- (7) Chow, C. W.; Rincon, M.; Cavanagh, J.; Dickens, M.; Davis, R. J. Nuclear accumulation of NFAT4 opposed by the JNK signal transduction pathway. *Science* **1997**, *278*, 1638–1641.
- (8) Milne, D. M.; Campbell, L. E.; Campbell, D. G.; Meek, D. W. p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation-induced protein kinase characteristic of the c-Jun kinase, JNK1. *J. Biol. Chem.* **1995**, *270*, 5511–5518.
- (9) Gupta, S.; Barrett, T.; Whitmarsh, A. J.; Cavanagh, J.; Sluss, H. K.; Derijard, B.; Davis, R. J. Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J.* **1996**, *15*, 2760–2770.
- (10) Gupta, S.; Barrett, T.; Whitmarsh, A. J.; Cavanagh, J.; Sluss, H. K.; Derijard, B.; Davis, R. J. Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J.* **1996**, *15*, 2760–2770.
- (11) Mohit, A. A.; Martin, J. H.; Miller, C. A. p493F12 kinase: a novel MAP kinase expressed in a subset of neurons in the human nervous system. *Neuron* **1995**, *14*, 67–78.
- (12) Dong, C.; Yang, D. D.; Wysk, M.; Whitmarsh, A. J.; Davis, R. J.; Flavell, R. A. Defective T cell differentiation in the absence of Jnk1. *Science* **1998**, *282*, 2092–2095.
- (13) Yang, D. D.; Conze, D.; Whitmarsh, A. J.; Barrett, T.; Davis, R. J.; Rincon, M.; Flavell, R. A. Differentiation of CD4<sup>+</sup> T cells to Th1 cells requires MAP kinase JNK2. *Immunity* **1998**, *9*, 575–585.
- (14) Sabapathy, K.; Hu, Y.; Kallunki, T.; Schreiber, M.; David, J. P.; Jochum, W.; Wagner, E. F.; Karin, M. JNK2 is required for efficient T-cell activation and apoptosis but not for normal lymphocyte development. *Curr. Biol.* **1999**, *9*, 116–125.
- (15) Tournier, C.; Hess, P.; Yang, D. D.; Xu, J.; Turner, T. K.; Nimnual, A.; Bar-Sagi, D.; Jones, S. N.; Flavell, R. A.; Davis, R. J. Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* **2000**, *288*, 870–874.
- (16) Yang, D. D.; Kuan, C. Y.; Whitmarsh, A. J.; Rincon, M.; Zheng, T. S.; Davis, R. J.; Rakic, P.; Flavell, R. A. Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature* **1997**, *389*, 865–870.
- (17) Lin, A. Activation of the JNK signaling pathway: Breaking the brake on apoptosis. *Bioessays* **2003**, *25*, 17–24.
- (18) Manning, A. M.; Davis, R. J. Targeting JNK for therapeutic benefit: from junk to gold? *Nat. Rev. Drug Discovery* **2003**, *2*, 554–565.
- (19) Resnick, L.; Fennell, M. Targeting JNK3 for the treatment of neurodegenerative disorders. *Drug Discovery Today* **2004**, *9*, 932–939.
- (20) Bennett, B. L.; Satoh, Y.; Lewis, A. J. JNK: a new therapeutic target for diabetes. *Curr. Opin. Pharmacol.* **2003**, *3*, 420–425.
- (21) Kennedy, N. J.; Davis, R. J. Role of JNK in tumor development. *Cell Cycle* **2003**, *2*, 199–201.
- (22) Bennett, B. L.; Sasaki, D. T.; Murray, B. W.; O'Leary, E. C.; Sakata, S. T.; Xu, W.; Leisten, J. C.; Motiwala, A.; Pierce, S.; Satoh, Y.; Bhagwat, S. S.; Manning, A. M.; Anderson, D. W. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13681–13686.
- (23) Bain, J.; McLauchlan, H.; Elliott, M.; Cohen, P. The specificities of protein kinase inhibitors: an update. *Biochem. J.* **2003**, *371*, 199–204.
- (24) Scapin, G.; Patel, S. B.; Lisnock, J.; Becker, J. W.; LoGrasso, P. V. The structure of JNK3 in complex with small molecule inhibitors: structural basis for potency and selectivity. *Chem. Biol.* **2003**, *10*, 705–712.
- (25) Ruckle, T.; Biamonte, M.; Grippi-Vallotton, T.; Arkinstall, S.; Cambet, Y.; Camps, M.; Chabert, C.; Church, D. J.; Halazy, S.; Jiang, X.; Martinou, I.; Nichols, A.; Sauer, W.; Gotteland, J. P. Design, synthesis, and biological activity of novel, potent, and selective (benzoylaminomethyl)thiophene sulfonamide inhibitors of c-Jun-N-terminal kinase. *J. Med. Chem.* **2004**, *47*, 6921–6934.
- (26) Kundu, N. G.; Wright, J. A.; Perlman, K. L.; Hallett, W.; Heidelberger, C. Cyclopenta[*f*]isoquinoline derivatives designed to bind specifically to native deoxyribonucleic acid. 1. Synthesis of 3-ethoxy-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinoline. *J. Med. Chem.* **1975**, *18*, 395–399.
- (27) Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **2000**, *351*, 95–105.
- (28) Dumas, J. Protein kinase inhibitors: emerging pharmacophores 1997–2000. *Expert Opin. Ther. Pat.* **2001**, *11*, 405–429.
- (29) Jain, J.; Valge-Archer, V. E.; Rao, A. Analysis of the AP-1 sites in the IL-2 promoter. *J. Immunol.* **1992**, *148*, 1240–1250.
- (30) Faris, M.; Kokot, N.; Lee, L.; Nel, A. E. Regulation of interleukin-2 transcription by inducible stable expression of dominant negative and dominant active mitogen-activated protein kinase kinase in jurkat T cells. Evidence for the importance of Ras in a pathway that is controlled by dual receptor stimulation. *J. Biol. Chem.* **1996**, *271*, 27366–27373.
- (31) Ventura, J. J.; Kennedy, N. J.; Lamb, J. A.; Flavell, R. A.; Davis, R. J. c-Jun NH(2)-terminal kinase is essential for the regulation of AP-1 by tumor necrosis factor. *Mol. Cell. Biol.* **2003**, *23*, 2871–2882.
- (32) Han, Z.; Boyle, D. L.; Aupperle, K. R.; Bennett, B.; Manning, A. M.; Firestein, G. S. Jun N-terminal kinase in rheumatoid arthritis. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 124–130.
- (33) Han, Z.; Boyle, D. L.; Chang, L.; Bennett, B.; Karin, M.; Yang, L.; Manning, A. M.; Firestein, G. S. c-Jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis. *J. Clin. Invest.* **2001**, *108*, 73–81.
- (34) Han, Z.; Chang, L.; Yamanishi, Y.; Karin, M.; Firestein, G. S. Joint damage and inflammation in c-Jun N-terminal kinase 2 knockout mice with passive murine collagen-induced arthritis. *Arthritis Rheum.* **2002**, *46*, 818–823.
- (35) Ferrandi, C.; Ballerio, R.; Gaillard, P.; Giachetti, C.; Carboni, S.; Vitte, P.-A.; Gotteland, J.-P.; Cirillo, R. Inhibition of c-Jun N-terminal kinase decreases cardiomyocyte apoptosis and infarct size after myocardial ischemia and reperfusion in anaesthetized rats. *Br. J. Pharmacol.* **2004**, *142*, 953–960.
- (36) Carboni, S.; Hiver, A.; Szyndralewicz, C.; Gaillard, P.; Gotteland, J. P.; Vitte, P. A. AS601245 (1,3-benzothiazol-2-yl(2-[(2-(3-pyridinyl)ethyl)amino]-4-pyrimidinyl)acetonitrile): a c-Jun NH2-terminal protein kinase inhibitor with neuroprotective properties. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 25–32.
- (37) Potier, V.; Juillard, P.; Camps, M.; Gaillard, P.; Arkinstall, S.; Gotteland, J.-P.; Chvatchko, Y. Efficacy of a novel inhibitor in reducing lung fibrosis. *Inflammation Res.* **2003**, *52* (Suppl. 2), 7.