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Synthesis and c-Src inhibitory activity of imidazo[1,5-*a*]pyrazine derivatives as an agent for treatment of acute ischemic stroke

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Abstract—We synthesized and evaluated a series of C-5 substituted imidazo[1,5-*a*]pyrazine derivatives to identify potent c-Src inhibitors as potential therapeutic agents for acute ischemic stroke. Among these compounds, compound **14c·HCl** demonstrated remarkable central nervous system (CNS) penetration and significant neuroprotective efficacy in vivo in rat models.

1. Introduction

The protein tyrosine kinase c-Src is prototypical of the eight members of the kinase family found in vertebrates, namely, c-Src, Fyn, Yes, Fgr, Hck, Lyn, Lck, and Blk. Widely found in mammalian cells, c-Src has particularly high concentrations in the brain, platelets, and osteoclasts,[1] acting as a common signal mediator to a broad spectrum of physiological responses.[2] In particular, c-Src regulates the activity of the *N*-methyl-D-aspartate (NMDA) receptor, which induces a large and prolonged Ca^{2+} influx into the neurons after cerebral ischemia[3,4] that culminates in neuronal damage. The c-Src kinase also plays a predominant role in cytokine release and superoxide production in neutrophils,[5,6] in addition to mediating the signaling events in response to vascular endothelial growth factor (VEGF),[7] which modulates vascular permeability and contributes to cerebral edema.[8,9]

Mice lacking $pp60^{c\text{-}Src}$ show both a reduction in infarct volumes and VEGF-mediated vascular permeability after brain ischemia.[10] Additionally, treatment of wild-type mice with PP1, a c-Src selective inhibitor (Fig. 1), reduces infarct size and decreases edema.[10–12] An inhibitor selectively active against tyrosine kinase c-Src would hold promise as an effective therapeutic medication against stroke.

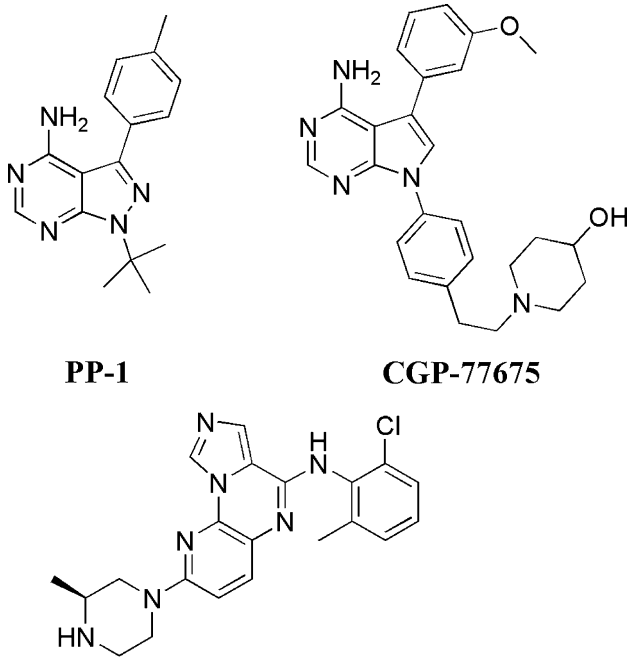

Figure 1. Structures of the Src family of kinase inhibitors.

Keywords: c-Src; Brain ischemia; Imidazo[1,5-*a*]pyrazine; Central nervous system (CNS) penetration; CH–π interaction.
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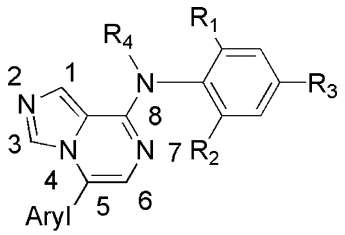

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Figure 2.

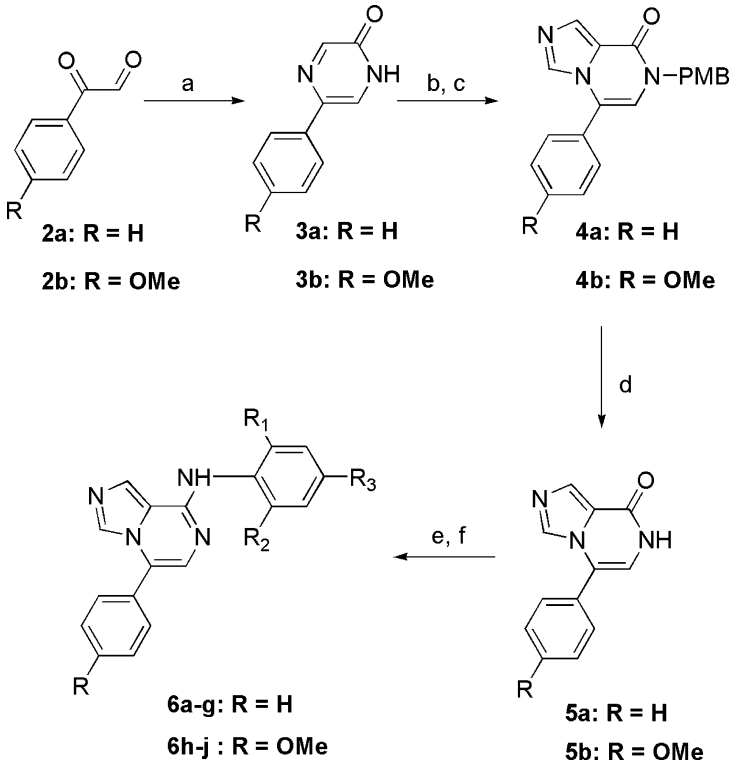
Several kinase inhibitors in the Src family, such as PP1,[12] CGP-77675 (c-Src inhibitors),[13] and BMS-279700 (Lck inhibitor),[14] act at the catalytic site (see Fig. 1). We evaluated whether these kinase inhibitors could reduce cerebral infarct volume after middle cerebral artery (MCA) occlusion in rats. PP1 reduced brain injury with cerebral infarct volume (23%, data not shown) 24 h after MCA occlusion. BMS-279700, an effective inhibitor of Lck, has both excellent in vivo anti-inflammatory effects in mice and superior c-Src inhibitory activity compared to PP1. However, it has poor central nervous system (CNS) penetration (brain/plasma = 0.6, Table 5). Consequently, we sought to improve CNS penetration by designing and synthesizing compounds based on the core structure of BMS-279700, resulting in a series of C-5 substituted imidazo[1,5-*a*]pyrazine derivatives, represented by the general formula **1** (Fig. 2).

We explore the activity of an exciting new compound, compound **14c·HCl**, which has better CNS penetration and c-Src inhibitory activity compared to BMS-279700, in addition to significant neuroprotective efficacy in vivo in rats. We describe the synthesis and structure–activity relationship (SAR) of the imidazo[1,5-*a*]pyrazine derivatives in detail, and report the pharmacological profile and biological evaluation of compound **14c·HCl**.

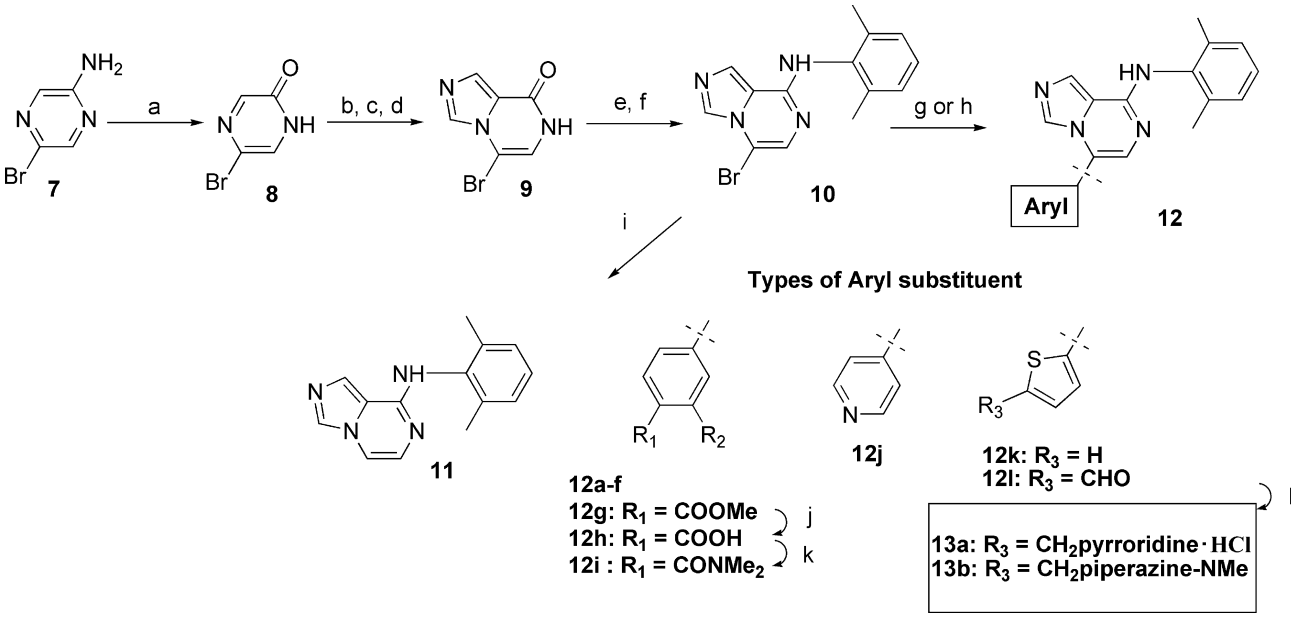
2. Chemistry

2.1. Synthesis of the C-5 aryl imidazo[1,5-*a*]pyrazine derivatives

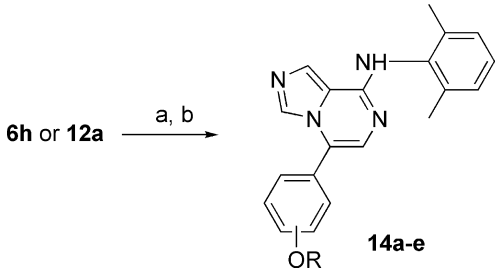
The C-5 aryl imidazo[1,5-*a*]pyrazine derivatives **6a–j** were prepared as shown in Scheme 1. The pyrazine-2-one derivatives (**3a** and **b**) were prepared by regioselective condensation of the phenylglyoxal derivatives (**2a** and **b**) with glycinamide hydrochloride.[15] After protection of the nitrogen in the pyrazine ring with *p*-methoxybenzyl chloride (PMB-Cl), the imidazo[1,5-*a*]pyrazine core **4a** and **b** was synthesized via condensation with tosylmethyl isocyanide (Tos-MIC) according to the procedure of Chen et al.[16] Cleavage of the *p*-methoxybenzyl group with TfOH/TFA and treatment of **5a** and **b** with $POCl_3$ yielded the 8-chloroimidazo[1,5-*a*]pyrazine derivatives. Finally, the chloride at the 8-position was substituted with various anilines in the presence of sodium bis(trimethylsilyl)amide (NaHMDS) to produce the C-5 aryl imidazo[1,5-*a*]pyrazine derivatives **6a–j**.



Scheme 1. Reagents: (a) NaOH, glycinamide hydrochloride; (b) PMB-Cl, NaH, *n*-$Bu_4N^+I^-$; (c) Tos-MIC, NaH; (d) TfOH, TFA, anisol; (e) $POCl_3$, cat. DMF; (f) aniline analogues, NaHMDS.



Scheme 2. Reagents: (a) $NaNO_2$; (b) PMB-Cl, NaH, n-$Bu_4N^+I^-$; (c) Tos-MIC, NaH; (d) TfOH, TFA, anisol; (e) $POCl_3$, (cat.)DMF; (f) aniline analogues, NaHMDS; (g) Aryl-$B(OH)_2$, Pd(II)$(dppf)_2Cl_2$, K_2CO_3; (h) 5-n-Bu_3Sn-thiophen-2-CHO, $(PPh_3)_2PdCl_2$; (i) n-BuLi; (j) NaOH; (k) 1 M Me_2NH in THF, WSC·HCl, HOBt, NEt_3; (l) amines, Ti(Oi-Pr)$_4$, $NaBH_4$.

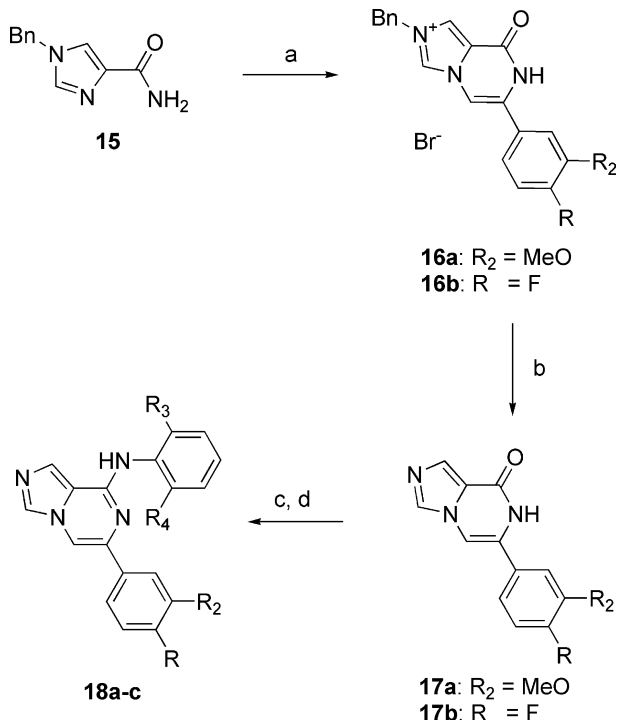


Scheme 3. Reagents: (a) 1 M BBr_3; (b) aminoalcohol derivatives, DEAD, PPh_3.

Select phenyl groups were introduced at position C-5 as described above. We also introduced various aryl groups at the C-5 position by Suzuki- or Stille-coupling of the 5-bromo intermediate **10**, as shown in Scheme 2. Compound **7** was converted to pyrazine-2-one (**8**) with sodium nitrite.[17] The major intermediate **10** was prepared from pyrazine-2-one (**8**) in five steps, as noted above. Reaction of intermediate **10** with aryl boronic acid derivatives in the presence of Pd$(dppf)_2Cl_2$ provided the C-5 aryl substituted imidazo[1,5-*a*]pyrazine derivatives **12a**–**g**, **j**, and **k**. *N*,*N*-Dimethyl amide **12i** was prepared by hydrolysis of the ester **12g**, followed by condensation with dimethylamine. Treatment of intermediate **10** with 5-tributylstannylthiophen-2-carbaldehyde produced **12l**; reductive amination formed the amine derivatives **13a** and **b**. The methyl groups were removed from compounds **6h**, **12a**, and Mitsunobu-coupling with several amino-alcohols produced derivatives **14a**–**e** with various polar functionalities, as shown in Scheme 3.

2.2. Synthesis of the C-6 aryl substituted imidazo[1,5-*a*] pyrazine

Synthesis of the derivatives with C-6 substituents is illustrated in Scheme 4.[18] Treatment of imidazole (**15**) with



Scheme 4. Reagents: (a) 2-bromoacetophenone derivatives; (b) imidazole; (c) $POCl_3$, cat. DMF; (d) aniline analogues, NaHMDS.

2-bromoacetophenone derivatives produced imidazopyrazinium salt derivatives (**16a** and **b**). The benzyl groups were deprotected with imidazole to yield C-6 aryl substituted imidazo[1,5-*a*]pyrazine-8-one derivatives **17a** and **b**. Subsequently, the C-6 aryl substituted imidazo[1,5-*a*]pyrazine derivatives **18a**–**c** were prepared through coupling reactions with anilines via the chloroimidate derivatives.

3. Binding model

We examined the binding model of compound **6a** to the c-Src kinase domain (PDB code. 1YOL)[19] using a docking program (Quanta/Charm™),[20] which is analogous to the X-ray crystal structure of the complex between **6a** and Lck kinase.[21] As previously reported,[22–24] compound **6a** interacted with several amino acid residues to enhance its binding affinity with the ATP-binding site (Fig. 4). For example, a hydrogen bond formed between the amino group of the aniline and the hydroxyl group (acceptor) of Thr 340; the N-2 nitrogen (acceptor) of imidazo[1,5-*a*]pyrazine interacted with the backbone NH (donor) of Met 343; and there was a strong hydrogen bonding interaction between C-1-H (donor) of imidazo[1,5-*a*]pyrazine and the backbone CO (acceptor) of Glu 341. Furthermore, the aniline phenyl ring formed a CH–π interaction[25] with the side chains of Thr 340 and Lys 297 in the hydrophobic pocket. In the deep cleft, CH–π interactions formed between the imidazo[1,5-*a*]pyrazine core and the side chains of Val 283 and Leu 395, as well as between the C-5 phenyl ring and the side chains of Leu 275.

4. Results and discussion

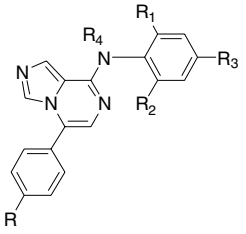
4.1. SAR for the c-Src kinase and c-Src cellular assays

The c-Src inhibitory activity of imidazo[1,5-*a*]pyrazine derivatives was evaluated by both coupled spectrophotometric enzyme assay and enzyme-linked immunosorbent assay (ELISA), using COS7 cells as an intracellular assay (Tables 1–4).

The SAR (Table 1) around the aniline phenyl ring at the C-5 position of the imidazo[1,5-*a*]pyrazine nucleus was evaluated for compounds **6a**–**j**. Modifying the substituents (R_1–R_3) on the aniline phenyl ring led to a dramatic change in inhibitory activity against c-Src kinase. The IC_{50} for the derivatives with unsubstituted aniline phenyl rings **6b** and **j** were 5.5 and 3.7 μM, respectively. The *ortho*-methyl group **6i** significantly enhanced c-Src inhibitory activity. Furthermore, the 2,6-dimethyl compounds **6a** and **h** showed greater inhibitory activity than the *ortho*-methyl compound **6i**. The methyl group could be replaced by chloride without any loss of activity (**6a** vs **6c**). However, smaller substituents, such as fluoride (**6d**), or bulkier methoxy groups (**6f**) decreased activity. Addition of a substituent to the 4-position of the aniline phenyl ring **6e** (R_3 = Me) did not alter activity, whereas *N*-methyl substitution in **6g** showed less activity. This suggests that aniline N–H plays an important inhibitory role.

We used a binding model and conformation analysis to analyze the importance of the two substituents on the aniline phenyl ring. In the binding model of **6a** to the c-Src kinase domain, the amino group of the aniline formed a hydrogen bond with the side-chain hydroxyl group of Thr 340 (Fig. 4); this interaction is highly conserved in the tyrosine kinase family. The model revealed that the 2,6-dimethyl substituted aniline phenyl ring is about 90° to the plane of the imidazo[1,5-*a*]pyrazine nucleus, suggesting CH–π interactions between the aniline phenyl ring and hydrogen atoms of both the methylene and methyl moieties (Lys 297 and Thr 340, respectively). The model also suggested that a twisted conformation, formed by repulsion of the methyl group

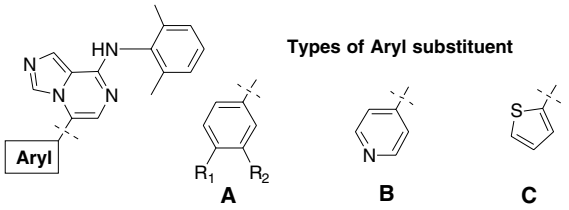
Table 1. The c-Src inhibitory activity of imidazo[1,5-*a*]pyrazine derivatives substituted with various aniline groups attached to C-8



Compound	R	R_1	R_2	R_3	R_4	c-Src inhibition	
						Enzyme IC_{50}[a] (μM)	ELISA % at 1 μM[b]
6a	H	Me	Me	H	H	0.013	10.6
6b	H	H	H	H	H	5.5	0
6c	H	Cl	Cl	H	H	0.042	25.6
6d	H	F	F	H	H	0.490	0
6e	H	Me	Me	Me	H	0.038	0.2
6f	H	Me	MeO	H	H	0.693	0
6g	H	H	H	H	Me	1000	NT[c]
6h	MeO	Me	Me	H	H	0.015	12.5
6i	MeO	Me	H	H	H	0.260	22.7
6j	MeO	H	H	H	H	3.7	NT[c]

[a] IC_{50} values for inhibition of c-Src were determined in duplicate.
[b] Inhibition (%) of intracellular c-Src kinase with 1 μM each compound; determined in duplicate.
[c] NT means not tested.

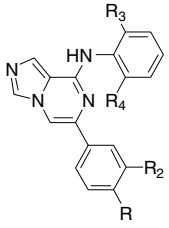
Table 2. The c-Src inhibitory activity of C-5 aryl substituted imidazo[1,5-*a*]pyrazine derivatives (Part I)



Compound	Type	R_1	R_2	c-Src inhibition	
				Enzyme IC_{50}[a] (μM)	ELISA % at 1 μM[b]
6a	A	H	H	0.013	10.6
6h	A	MeO	H	0.015	12.5
11	—			3.3	NT[c]
12a	A	H	MeO	0.011	18.9
12b	A	CN	H	0.235	0
12c	A	MeO	MeO	0.023	16.4
12d	A	F	H	0.042	0.20
12e	A	CF_3	H	0.235	7.0
12f	A	NMe_2	H	0.019	25.7
12h	A	COOH	H	0.235	12.0
12i	A	4′-$CONMe_2$	H	0.052	0.0
12j	B	H		0.108	0
12k	C	H		0.012	22.4
BMS-279700	—			0.096	NT[c]

[a] IC_{50} values for inhibition of c-Src were determined in duplicate.
[b] Inhibition (%) of intracellular c-Src kinase with 1 μM each compound; determined in duplicate.
[c] NT means not tested.

Table 3. The c-Src inhibitory activity of C-6 aryl substituted imidazo[1,5-*a*]pyrazine derivatives



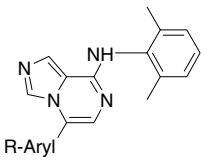
Compound	R	R_2	R_3	R_4	c-Src inhibition	
					Enzyme IC_{50}[a] (μM)	ELISA % at 1 μM[b]
18a	H	OMe	Cl	Me	0.059	0
18b	F	H	Cl	Me	0.145	5.8
18c	F	H	Me	Me	0.403	7.0

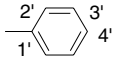
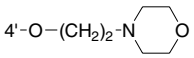
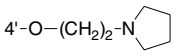
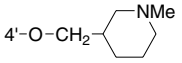
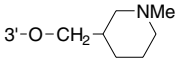
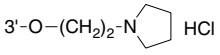
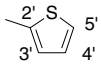
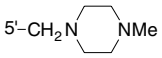
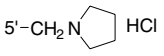
[a] IC_{50} values for inhibition of c-Src were determined in duplicate.
[b] Inhibition (%) of intracellular c-Src kinase with 1 μM each compound; determined in duplicate.

on the aniline phenyl ring, was responsible for increased potency.

For conformation analysis, we calculated the conformational energy of each of the 2,6-dimethyl, *o*-monomethyl, and unsubstituted anilines bound to imidazo[1,5-*a*]pyrazine in accordance with the method of Snow et al.[26] Each conformational energy value was calculated using ab initio quantum mechanics (Hartree–Fock method with a 6-31G**) implemented in SPARTAN™.[27] Conformational analysis of the torsion angle (θ_2) around the N–Ph bond was performed in 30° increments (Fig. 3a and b). There are two planar conformers to the plane of imidazo[1,5-*a*]pyrazine (Fig. 3 top). The torsion angles (θ_1) are 0° for conformer A and 180° for conformer B. The binding model indicated that the position of conformer A ($\theta_1 = 0°$) was clearly unfavorable to hydrogen bonding with amino acids in the hinge region. With respect to conformer B ($\theta_1 = 180°$), the 2,6-dimethyl analogue was markedly stable when the torsion angle (θ_2) was 90°. Conversely, an energy difference ($\Delta E = 2.9$ kcal/mol) between the two suggests the *o*-methyl

Table 4. The c-Src inhibitory activity of C-5 aryl substituted imidazo[1,5-*a*]pyrazine derivatives (Part II)



Compound	Aryl	R	c-Src inhibition		
			Enzyme IC_{50}[a] (μM)	ELISA IC_{50}[b] (μM)	% at 1 μM[c]
14a			0.037	0.70	57.3
14b			0.015	0.95	51.6
14c			0.020	0.53	91.6
14d			0.019	0.74	59.9
14e			0.055	1.00	49.1
13a			0.305	2.30	28.7
13b			0.052	0.82	57.6

[a] IC_{50} values for inhibition of c-Src were determined in duplicate.
[b] IC_{50} values for inhibition of intracellular c-Src were determined in duplicate.
[c] Inhibition (%) of intracellular c-Src kinase at 1 μM compound; determined in duplicate.

analogue favors a planar conformation ($\theta_2 = 0°$) over a perpendicular one ($\theta_2 = 90°$). Furthermore, the unsubstituted analogue was more stable ($\Delta E = 4.6$ kcal/mol) in the planar conformations than the twisted conformation.

These results strongly suggest that 2,6-dimethyl aniline adopts a conformation that is perpendicular to the plane of imidazo[1,5-*a*]pyrazine. Hence, the 2,6-disubstituent may play an important role in stabilizing the binding conformation with CH–π interactions. Conversely, the low inhibitory activity of the monomethyl and unsubstituted analogues could be due to the loss of hydrogen bonds and CH–π interactions. Our findings agree well with similar studies by Snow[26] and Chen.[14]

Based on these results, we optimized the aryl ring at the C-5 position of the imidazo[1,5-*a*]pyrazine by adding highly favorable 2,6-dimethyl groups to the aniline phenyl ring. Table 2 lists the results of those analogues. Introducing a phenyl ring at the C-5 position of the imidazo[1,5-*a*]pyrazine clearly enhanced inhibitory activity compared to non-substituted compounds (**6a** vs **11**). Several compounds were evaluated to examine the effects of varying substituents on the phenyl ring at the C-5 position. Adding electron-donating substituents, such as 3′- and/or 4′-methoxy (**6h**, **12a**, and **c**), *N*,*N*-dimethylamino **12f**, or an electron-rich aryl ring (thienyl group) **12k**, did not adversely affect inhibition. In contrast, introducing an electron-withdrawing group to the phenyl ring considerably reduced activity against c-Src (**12b**, **d**, **e**, and **h**). Similarly, electron-deficient aryl groups, such as the 4-pyridinyl group **12j**, showed decreased activity. These results underscore the importance of electron-rich functional groups in c-Src inhibition. Indeed, the C-6 substituted compounds (Table 3) were less potent than the C-5 aryl ring compounds (**18c** vs **12d**), suggesting that C-5 aryl groups interact more readily with the cleft region of the c-Src enzyme, forming CH–π interactions with Leu 275 (Fig. 4). Moreover, compounds with electron-rich aryl groups, such as **6h** and **12a**, showed superior inhibition of BMS-279700 in the enzyme assay. Thus, the electron-rich ring of the C-5 imidazo[1,5-*a*]pyrazine seems to afford greater CH–π interactions with the cleft region than BMS-279700.

Although the compounds listed in Tables 1–3 were active against the enzyme in vitro, none of them demonstrated a significant level of intracellular c-Src inhibition ($IC_{50} < 1$ μM). Therefore, we focused on enhancing the compound's solubility to improve cell penetration in the cellular assay (ELISA). The binding model (Fig. 4)

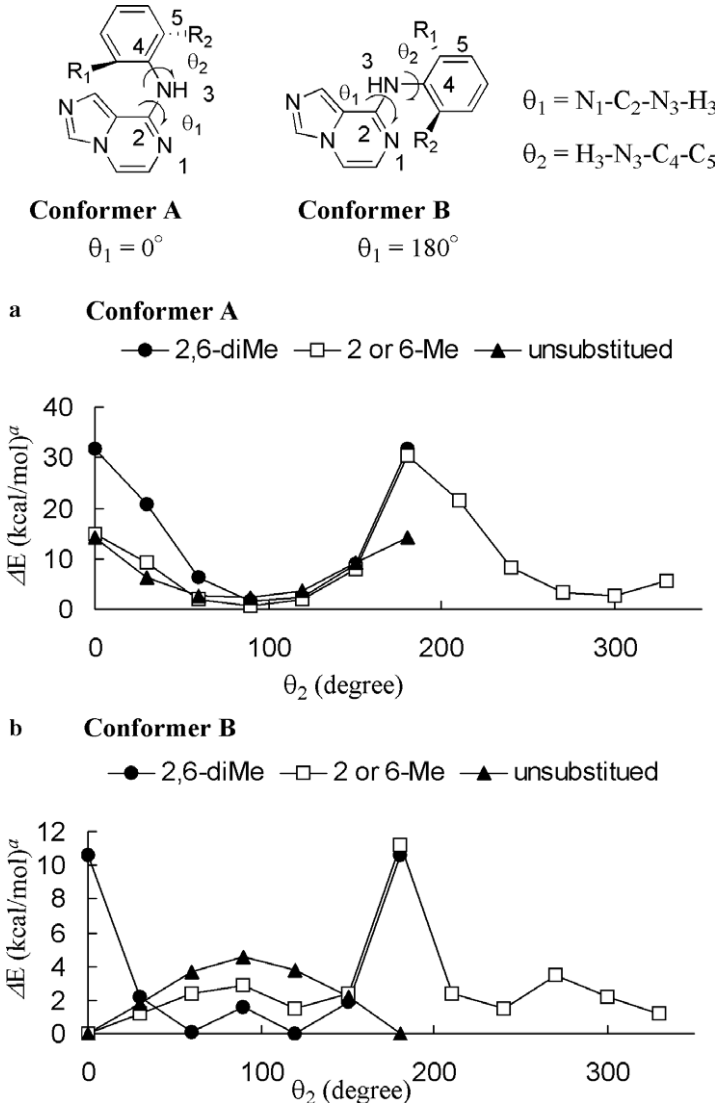


Figure 3. Energy versus torsion angle (θ_2) of the aniline phenyl ring H_3–N_3–C_4–C_5 moiety. The illustration at the top compares the two conformers. The energy in each conformer was computed using ab initio quantum mechanics (Hartree–Fock 6-31G**) implemented in SPARTAM™. [a] Each ΔE is relative to the minimum energy for each compound.

indicated that the 3- and/or 4-substituents on the phenyl ring at the C-5 position were exposed to the solvent side of the cleft in the c-Src kinase, creating the possibility for additional substitution (see Table 4 for the results of those derivatives). The carboxyl group **12h** showed poor inhibition in both the enzyme and cell assays. Replacing the carboxyl group **12h** with an amide group **12i** lowered c-Src inhibition, probably due to electrostatic repulsion with Asp 350. We then selectively introduced cyclic tertiary amine analogues at the 3′- or 4′-position to improve CNS penetration.[28,29] The pyrrolidine derivatives (**14b** and **e**) were shown to be more effective inhibitors of c-Src cellular activity than the methoxy derivatives (**6h** and **12a**). Moreover, introducing the piperidine derivative at the 3′- or 4′-position (**14c** and **d**) produced the best results in both the enzyme and cell assays. The addition of a pyrrolidine derivative to the 5-position of thienyl group **13a** did not alter the activity in comparison to compound **12k**, whereas the piperazinyl derivative **13b** enhanced the inhibition activity in the cellular assay.

4.2. Compound concentrations in the brain and plasma

The concentrations of compounds **14c·HCl** and **14d·HCl** in the brain and plasma were determined to estimate whether these compounds would have sufficient exposure levels in the brain to treat acute ischemic stroke. Each compound was administered at a dose of 3 mg/kg/h by intravenous infusion, and concentration levels were measured after 3 h (results shown in Table 5). Substitution of the cyclic tertiary amino group at the 4′-position of **14c·HCl** significantly enhanced CNS penetration compared to the substituent at the 3′-position of **14d·HCl**, with brain/plasma ratios of 1.1 and 0.3, respectively. In addition, compound **14b** also showed a significant brain/plasma ratio (1.7), but **14e** had poor CNS penetration. The pharmacokinetic profile of compound **14c·HCl** justified advanced efficacy studies in vivo. Table 6 shows the rat PK parameters of compound **14c·HCl**. Compound **14c·HCl** was also highly soluble (>5 mg/mL) in aqueous solution.

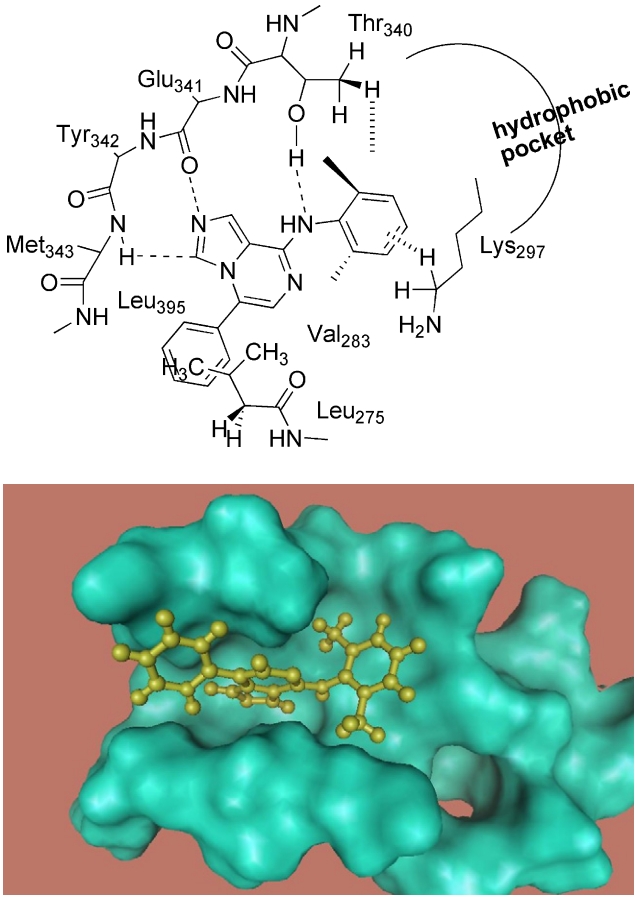


Figure 4. Views of **6a** in relationship to the cleft region, and binding orientations of **6a** to the ATP-binding site of c-Src kinase.

4.3. The selectivity profile of compound 14c·HCl against other kinase families

Selectivity against other kinase families is important to circumvent undesirable side effects. Compound **14c·HCl** for c-Src kinase was evaluated against various protein kinases (Table 7). Poor inhibition was observed with EGFR, Zap70, syk, PKCβ2, and KDR. However, the inhibition of Lck was comparable to that of c-Src due to high structural homology. These assays suggest that compound **14c·HCl** is more selective toward the Src family of kinases compared to other kinase families.

4.4. Neuroprotective effect in the rat MCA occlusion model

The neuroprotective effect of compound **14c·HCl** was examined using the photochemically induced middle cerebral artery thrombosis (PIT) model in rats, reported by K. Umemura et al.[30] Compound **14c·HCl** was delivered by intravenous infusions for 6 h after MCA occlusion to evaluate its effect on infarct size following stroke. Infarct volumes were significantly reduced with inhibition, by 20% at 3 mg/kg/h to a maximum of 29% at 10 mg/kg/h (Figs. 5 and 6).

5. Conclusion

We designed and synthesized several novel C_5-selective substituted imidazo[1,5-*a*]pyrazine derivatives (**6a–j**, **12a–k**) as potent c-Src inhibitors. SAR and binding model analyses indicated that a twisted conformation

Table 5. CNS penetration of select c-Src kinase inhibitors in rats[a]

Compound[b]	Plasma[c] $C_{3\ h}$ (ng/mL)	Brain[c] $C_{3\ h}$ (ng/g)	Ratio (brain/plasma)
14c·HCl	457 ± 33	501 ± 47	1.1 ± 0.2
14d·HCl	520 ± 50	133 ± 31	0.3 ± 0.1
14b	368 ± 42	631 ± 90	1.7 ± 0.1
14e	242 ± 15	229 ± 17	0.9 ± 0.0
BMS-279700	139 ± 35	81 ± 35	0.6 ± 0.3

[a] Results are shown as means ± SE ($n = 3$).
[b] Each compound was administered at a dose of 3 mg/kg/h by intravenous infusion.
[c] Concentration levels were measured after 3 h.

Table 6. Pharmacokinetic parameters of c-Src inhibitor **14c·HCl** in rats[a]

Pharmacokinetic parameters (iv (3 mg/kg))	AUC[b] (μM h)	$t_{1/2}$ (min)	Cl (mL/min/kg)	V_{ss} (L/kg)
Compound **14c·HCl**	1.25	63	91	7.64

[a] Results are shown as a mean value.
[b] The area under the curves represents 0 h to infinity.

Table 7. Selectivity profile of compound **14c·HCl** for various kinases

Kinase	c-Src	Lck	Syk	Zap	PKCβ2	EGFR	KDR
IC_{50}[a] (μM)	0.020	0.008	>10	>10	>10	2.730	>10

[a] IC_{50} values for inhibition of each kinase were determined in duplicate.

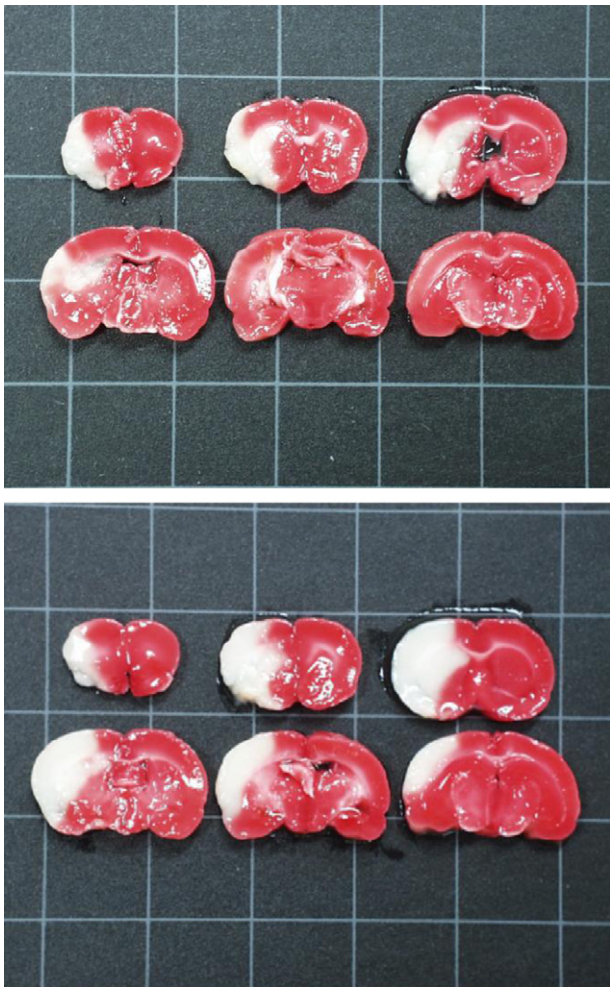


Figure 5. Representative images of six 2-mm-thick slices of rat brain 24 h after MCA occlusion. Compound **14c·HCl** or saline was administered as a continuous infusion for 6 h. The sections were incubated in 1% TTC. The infarct area appears as a white region. (n = 6–12) Top: compound **14c·HCl** (10 mg/kg/h); bottom: saline.

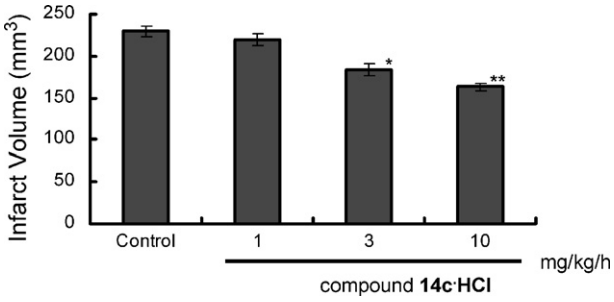


Figure 6. Neuroprotective effects of compound **14c·HCl** (intravenous infusion, 6 h) in an MCA occlusion model. **$p < 0.01$, *<0.05 versus control (Dunnett-type test; n = 6–12).

is stabilized by the 2,6-dimethyl substituents, and the C-5 electron-rich aryl groups play an important role in enhancing inhibition. We also found that the 3′- or 4′-methoxy substituted compounds **6h** and **12a** had excellent inhibitory activity. Furthermore, introduction of tertiary amines **14a**–**e** on the phenyl ring at the C-5 position increased cellular inhibitory potency and provided good CNS penetration. Among these compounds, compound **14c·HCl** was highly soluble in aqueous solution, and significantly reduced infarct volume after brain ischemia. This compound holds promise as a new therapeutic modality for acute ischemic stroke.

6. Experimental

6.1. Chemistry

Melting points were taken on a Yanako MP-3S Micro melting point apparatus and are uncorrected. Infrared spectra were measured on a Nicolet 510 FT-IR spectrophotometer and are reported in reciprocal centimeters. Proton NMR spectra were recorded at 400 or 500 MHz with a Bruker AMX 400 or DRX 500 instrument, and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as the internal standard. The peak patterns are shown as the following abbreviations: br, broad; d, doublet; m, multiplet; s, singlet; t, triplet; q, quartet. The mass spectra (MS) were carried out with a Thermo Quest FINNIGAN AQA electrospray ionization mass spectrometer. Elemental analyses were performed on an Elementar Vario EL analyzer (C, H, and N). The analytical results obtained were within ±0.4% of the theoretical values unless otherwise stated. Silica gel $60F_{254}$ precoated plates on glass from Merck KGaA or aminopropyl silica gel (APS) precoated NH plates from Fuji Silysia Chemical Ltd were used for thin-layer chromatography (TLC). Flash or medium-pressure liquid column chromatography (MPLC) was performed on silica gel 60 N (particle size 40–50 μm) from Kanto Chemical Co., Inc. or APS Daisogel IR-60 (particle size 25–40 μm) from Daiso Co., Ltd. All reagents and solvents were commercially available unless otherwise indicated.

6.1.1. 5-Phenylpyrazine-2(1*H*)-one (3a). To a suspension of α-aminoacetamide hydrochloride (15 g, 0.14 mol) in MeOH (125 mL)–water (31 mL) was added 12.5 M aqueous NaOH (16.5 mL, 0.20 mol) solution at −30 °C, and then a solution of NaOH (5.43 g, 0.14 mol) in MeOH (61 mL) was added to the mixture. A solution of **2a** in MeOH (111 mL) was added to the mixture at −20 °C, and then the resulting suspension was stirred for 2 h at same temperature, and the stirring continued for 1 h at room temperature. After cooling with ice water, the mixture was acidified with AcOH. Collection of the resulting precipitates by filtration gave 17.0 g of **3a** (73%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6) δ 7.25–7.35 (1H, m), 7.40–7.45 (2H, m), 7.85–7.90 (2H, m), 8.07 (1H, br s), 8.12 (1H, d, J = 1.3 Hz), 12.51 (1H, br s).

6.1.2. 5-(4-Methoxyphenyl)pyrazine-2(1*H*)-one (3b). This title compound was prepared from **2b** in the same manner as described above, and obtained as a pale yellow solid (61%): ^{1}H NMR (DMSO-d_6) δ 6.98 (2H, d, J = 9.1 Hz), 7.80 (2H, d, J = 9.1 Hz), 7.99 (1H, br s), 8.08 (1H, d, J = 1.3 Hz), 12.32 (1H, br s).

6.1.3. 7-(4-Methoxybenzyl)-5-phenylimidazo[1,5-*a*]pyrazin-8(7*H*)-one (4a). To a suspension of NaH (60% w/w dispersion in mineral oil, 2.69 g, 67.3 mmol) in DMF (56 mL) was added a suspension of **3a** (10.1 g, 58.5 mmol) in DMF (28 mL) and THF (28 mL) at 0 °C. After stirring for 30 min, 4-methoxybenzyl chloride (8.7 mL, 64 mmol) and tetrabutylammonium iodide (2.2 g, 5.8 mmol) were added to the reaction mixture. The mixture was stirred for 12 h at room temperature and stirring continued for 1 h at 70 °C. The reaction mixture was quenched with 10% aqueous NH_4Cl solution and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo to give 9.3 g (54%) of 1-(4-methoxybenzyl)-5-phenylpyrazine-2(1*H*)-one as a brown solid: mp 111–112 °C (EtOAc/diisopropylether); IR (KBr) 3052, 1653, 1606, 1596, 1515 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.72 (3H, s), 5.08 (2H, s), 6.91 (2H, d, J = 8.8 Hz), 7.30–7.35 (1H, m), 7.35–7.50 (4H, m), 7.84 (2H, dd, J = 8.4, 1.1 Hz), 8.15 (1H, d, J = 1.3 Hz), 8.49 (1H, d, J = 1.3 Hz); MS *m*/*z*: 293 $(M+H)^+$.

To a suspension of 60% NaH (2.4 g, 60.2 mmol) in dry THF (30 mL) was added a solution of 1-(4-methoxybenzyl)-5-phenylpyrazine-2(1*H*)-one (8.0 g, 27.4 mmol) and tosylmethyl isocyanide (5.9 g, 30.1 mmol) in THF (34 mL) at 0 °C. The mixture was stirred for 30 min at same temperature, and stirring continued for 1 h at room temperature. Water (300 mL) was poured into the resulting suspension and collection of the precipitates gave 9.90 g (100%) of **4a** as a pale brown solid: mp 111–112 °C; IR (KBr) 1642, 1515, 1251 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.72 (3H, s), 5.00 (2H, s), 6.90 (2H, d, J = 8.8 Hz), 7.12 (1H, s), 7.37 (2H, d, J = 8.8 Hz), 7.50–7.70 (5H, m), 7.90 (1H, s), 8.13 (1H, s); MS *m*/*z*: 332 $(M+H)^+$.

6.1.4. 7-(4-Methoxybenzyl)-5-(4-methoxyphenyl)imidazo[1,5-*a*]pyrazin-8(7*H*)-one (4b). 1-(4-Methoxybenzyl)-5-(4-methoxyphenyl)pyrazine-2(1*H*)-one was prepared from **3b** in the same manner described above, and obtained as a pale brown solid (57%): mp 104–105 °C; IR (KBr) 3056, 1647, 1612, 1515, 1250, 838 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.72 (3H, s), 3.78 (3H, s), 5.06 (2H, s), 6.91 (2H, d, J = 8.8 Hz), 7.00 (2H, d, J = 8.8 Hz), 7.40 (2H, d, J = 8.8 Hz), 7.76 (2H, d, J = 8.8 Hz), 8.11 (1H, d, J = 1.3 Hz), 8.34 (1H, d, J = 1.3 Hz); MS *m*/*z*: 323 $(M+H)^+$.

Compound **4b** was prepared from 1-(4-methoxybenzyl)-5-(4-methoxyphenyl)pyrazine-2(1*H*)-one in the same manner described above, and obtained as a pale yellow solid (65%): mp 106–107 °C; IR (KBr) 3055, 1646, 1595, 1515, 1249, 828 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.79 (3H, s), 3.86 (3H, s), 5.03 (2H, s), 6.29 (1H, s), 6.88 (2H, d, J = 8.8 Hz), 7.00 (2H, d, J = 8.8 Hz), 7.30 (2H, d, J = 8.8 Hz), 7.38 (2H, d, J = 8.8 Hz), 7.86 (1H, d, J = 0.9 Hz), 8.05 (1H, d, J = 0.9 Hz); MS *m*/*z*: 362 $(M+H)^+$.

6.1.5. 5-Phenylimidazo[1,5-*a*]pyrazin-8(7*H*)-one (5a). To a solution of **4a** (9.62 g, 29.0 mmol) and anisole (57 mL, 0.52 mol) in trifluoroacetic acid (134 mL) was added trifluoromethanesulfonic acid (31 mL, 0.35 mol) at 0 °C. The mixture was stirred for 30 min at room temperature, and stirring continued for 6 h at 40 °C. The solvent was evaporated in vacuo, and the residue was poured into saturated aqueous $NaHCO_3$ solution. Collection of the resulting precipitates by filtration gave 3.49 g (57%) of **5a** as an off-white solid: mp 290–291 °C (MeOH); IR (KBr) 3119, 2904, 1666, 1350, 928 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.87 (1H, s), 7.65–7.90 (5H, m), 8.07 (1H, s), 8.31 (1H, s), 11.14 (1H, br s); MS *m*/*z*: 212 $(M+H)^+$. Anal. Calcd for $C_{12}H_9N_3O{\cdot}0.2H_2O$: C, 67.09; H, 4.41; N, 19.56. Found: C, 67.41; H, 4.11; N, 19.55.

6.1.6. 5-(4-Methoxyphenyl)imidazo[1,5-*a*]pyrazin-8(7*H*)-one (5b). This title compound was prepared from **4b** in the same manner described above, and obtained as a white solid (63%): mp 268–269 °C; IR (KBr) 3041, 1652, 1515, 1255, 823 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.83 (3H, s), 6.59 (1H, d, J = 5.7 Hz), 7.09 (2H, d, J = 8.8 Hz), 7.56 (2H, d, J = 8.8 Hz), 7.86 (1H, s), 8.05 (1H, s), 10.88 (1H, br s); MS *m*/*z*: 242 $(M+H)^+$. Anal. Calcd for $C_{13}H_{11}N_3O_2{\cdot}0.25H_2O$: C, 63.54; H, 4.72; N, 17.10. Found: C, 63.87; H, 4.45; N, 17.02.

6.1.7. *N*-(2,6-Dimethylphenyl)-5-phenylimidazo[1,5-*a*]pyrazine-8-amine (6a). A suspension of **5a** (107 mg, 0.51 mmol) in phosphorus oxychloride (1.2 mL) was stirred for 1.5 h at reflux. The mixture was evaporated in vacuo to give 116 mg of 8-chloro-5-phenylimidazo[1,5-*a*]pyrazine as a crude mixture. To a suspension of 8-chloro-5-phenylimidazo[1,5-*a*]pyrazine and 2,6-dimethylaniline (0.16 mL, 1.36 mmol) in THF (1.0 mL) was added sodium bis(trimethylsilyl)amide (NaHMDS, 1 M in THF; 3.0 mL) at room temperature under an argon atmosphere, and the mixture was stirred for 2 h at 60 °C. A saturated aqueous NH_4Cl was added to the stirring suspension, and the resulting suspension extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the residue by flash column chromatography on APS (eluent: hexane/EtOAc, 1:2) gave 75 mg (47%) of **6a** as an off white solid: mp 196–197 °C (CH_3CN); IR (KBr) 3133, 3039, 1516, 1435, 763 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.19 (6H, s), 6.97 (1H, s), 7.15 (3H, s), 7.40–7.60 (3H, m), 7.67 (2H, d, J = 8.0 Hz), 8.06 (1H, br s), 8.33 (1H, s), 9.05 (1H, s); MS *m*/*z*: 315 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}N_4{\cdot}0.1H_2O$: C, 75.97; H, 5.80; N, 17.72. Found: C, 76.16; H, 5.86; N, 17.34.

6.1.8. *N*-Phenyl-5-phenylimidazo[1,5-*a*]pyrazin-8-amine (6b). This title compound was prepared from **5a** in the same manner as described in **6a**, and obtained as a pale yellow solid (44%): mp 203–204 °C (CH_3CN); IR (KBr) 3056, 1516, 1496, 1447 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.00–7.10 (1H, m), 7.18 (1H, s), 7.30–7.40 (2H, m), 7.45–7.60 (3H, m), 7.65–7.75 (2H, m), 7.90–8.00 (2H, m), 8.19 (1H, s), 8.36 (1H, s), 9.47 (1H, s); MS *m*/*z*: 287 $(M+H)^+$. Anal. Calcd for $C_{18}H_{14}N_4{\cdot}0.15H_2O$: C, 74.80; H, 4.99; N, 19.38. Found: C, 75.05; H, 4.94; N, 19.00.

6.1.9. *N*-(2,6-Dichlorophenyl)-5-phenylimidazo[1,5-*a*]pyrazin-8-amine (6c). This title compound was prepared from **5a** in the same manner as described in **6a**, and obtained as a white solid (46%): mp 266–267 °C (CH_3CN); IR (KBr) cm^{-1}: 3139, 1635, 1130, 769 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.00 (1H, s), 7.35–7.75 (8H, m), 8.07 (1H, s), 8.37 (1H, s), 9.61 (1H, s); MS *m*/*z* (relative intensity): 357 (0.67), 355 $(M+H)^+$, 320 (0.41). Anal. Calcd for $C_{18}H_{12}Cl_2N_4$: C, 60.86; H, 3.41; N, 15.77. Found: C, 60.92; H, 3.40; N, 15.54.

6.1.10. *N*-(2,6-Difluorophenyl)-5-phenylimidazo[1,5-*a*]pyrazin-8-amine (6d). This title compound was prepared from **5a** in the same manner as described in **6a**, and obtained as a white solid (48%): mp 256–257 °C (CH_3CN); IR (KBr) 3141, 3048, 1623, 1517, 1470, 1001, 769 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.03 (1H, s), 7.15–7.30 (2H, m), 7.30–7.45 (1H, m), 7.45–7.60 (3H, m), 7.65–7.75 (2H, m), 8.08 (1H, s), 8.37 (1H, s), 9.43 (1H, s). MS *m*/*z*: 323 $(M+H)^+$. Anal. Calcd for $C_{18}H_{12}F_2N_4$: C, 67.08; H, 3.75; N, 17.38. Found: C, 67.01; H, 3.64; N, 17.24.

6.1.11. *N*-(5-Phenylimidazo[1,5-*a*]pyrazin-8-yl)-2,4,6-trimethylphenylamine (6e). This title compound was prepared from **5a** in the same manner as described in **6a**, and obtained as a pale brown solid (43%): mp 197–198 °C (CH_3CN); IR (KBr) 3129, 1538, 1432, 765 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.14 (6H, s), 2.28 (3H, s), 6.95 (3H, s), 7.40–7.50 (1H, m), 7.50–7.60 (2H, m), 7.60–7.70 (2H, m), 8.32 (1H, s), 8.96 (1H, s); MS *m*/*z*: 329 $(M+H)^+$. Anal. Calcd for $C_{21}H_{20}N_4 \cdot 0.5H_2O$: C, 74.75; H, 6.27; N, 16.60. Found: C, 74.89; H, 6.04; N, 16.21.

6.1.12. *N*-(2-Methoxy-6-methylphenyl)-5-phenylimidazo[1,5-*a*]pyrazin-8-amine (6f). This title compound was prepared from **5a** in the same manner as described in **6a**, and obtained as a pale brown solid (20%): mp 118–119 °C (CH_3CN); IR (KBr) 3134, 2964, 1506, 1085, 764 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.17 (3H, s), 3.72 (3H, s), 6.85–7.00 (3H, m), 7.15–7.25 (1H, m), 7.40–7.50 (1H, m), 7.50–7.60 (2H, m), 7.60–7.70 (2H, m), 8.31 (1H, s), 8.88 (1H, s); MS *m*/*z*: 331 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 19.96. Found: C, 72.51; H, 5.40; N, 16.88.

6.1.13. *N*-Methyl-*N*-phenyl-5-phenylimidazo[1,5-*a*]pyrazin-8-amine (6g). This title compound was prepared from **5a** in the same manner as described in **6a**, and obtained as a pale brown solid (43%): mp 114–115 °C (CH_3CN); IR (KBr) 3130, 1495, 1394, 701 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.51 (3H, s), 5.65–5.75 (1H, m), 7.23 (1H, s), 7.40–7.60 (8H, m), 7.60–7.70 (2H, m), 8.15–8.20 (1H, m); MS *m*/*z*: 301 $(M+H)^+$. Anal. Calcd for $C_{19}H_{16}N_4 \cdot 0.25H_2O$: C, 74.85; H, 5.46; N, 18.38. Found: C, 75.07; H, 5.28; N, 18.11.

6.1.14. *N*-(2,6-Dimethylphenyl)-5-(4-methoxyphenyl)imidazo[1,5-*a*]pyrazin-8-amine (6h). This title compound was prepared from **5b** in the same manner as described in **6a**, and obtained as an off white solid (83%): mp 190–191 °C (CH_3CN); IR (KBr) 3134, 3041, 1607, 1515, 1437, 769 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (6H, s), 3.82 (3H, s), 6.88 (1H, s), 7.05–7.15 (2H, m), 7.14 (3H, s), 7.55–7.65 (2H, m), 8.26 (1H, s), 8.97 (1H, s); MS *m*/*z*: 345 $(M+H)^+$. Anal. Calcd for $C_{21}H_{20}N_4O \cdot 0.25H_2O$: C, 72.29; H, 5.92; N, 16.06. Found: C, 72.25; H, 5.82; N, 15.89.

6.1.15. *N*-[5-(4-Methoxyphenyl)imidazo[1,5-*a*]pyrazin-8-yl]-*o*-tolylamine (6i). This title compound was prepared from **5b** in the same manner as described in **6a**, and obtained as a pale yellow solid (53%): mp 183–184 °C (CH_3CN); IR (KBr) 3041, 1607, 1516, 1457, 749 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.26 (3H, s), 3.84 (3H, s), 6.90 (1H, s), 7.12 (2H, d, J = 8.8 Hz), 7.25–7.45 (4H, m), 7.61 (2H, d, J = 8.8 Hz), 8.13 (1H, br s), 8.39 (1H, s), 9.95 (1H, br s); MS *m*/*z*: 331 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.39; H, 5.43; N, 16.93.

6.1.16. *N*-[5-(4-Methoxyphenyl)imidazo[1,5-*a*]pyrazin-8-yl]phenylamine (6j). This title compound was prepared from **5b** in the same manner as described in **6a**, and obtained as an off white solid (52%): mp 182–183 °C (CH_3CN); IR (KBr) 3312, 3134, 1609, 1516, 1444, 749 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.84 (3H, s), 7.00–7.15 (4H, m), 7.30–7.40 (2H, m), 7.64 (2H, d, J = 8.5 Hz), 7.92 (2H, d, J = 8.5 Hz), 8.21 (1H, s), 8.35 (1H, s), 9.55 (1H, br s); MS *m*/*z*: 317 $(M+H)^+$. Anal. Calcd for $C_{19}H_{16}N_4O$: C, 72.13; H, 5.10; N, 17.71. Found: C, 72.05; H, 5.02; N, 17.48.

6.1.17. 5-Bromopyrazin-2(1*H*)-one (8). To a solution of **7** (50.5 g, 0.29 mol) in 50% aqueous acetic acid (450 mL) and dioxane (50 mL) was added a solution of sodium nitrite (22.0 g, 0.32 mol) in 50% aqueous acetic acid (20 mL) at 4 °C, and the mixture was stirred for 10 min at same temperature. The mixture was neutralized with 5 M NaOH and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and removed in vacuo. The residue was recrystallized from EtOAc to give 29.8 g (59%) of **8** as a pale brown solid: ^1H NMR (DMSO-d_6) δ 7.92 (1H, s), 8.09 (1H, s), 12.22 (1H, br s).

6.1.18. 5-Bromoimidazo[1,5-*a*]pyrazin-8(7*H*)-one (9). To a suspension of 60% NaH (6.41 g, 0.16 mol) in DMF (75 mL) was added a suspension of **8** (24.4 g, 0.14 mol) in DMF (75 mL) and THF (75 mL) under ice cooling, and the mixture was stirred for 30 min at same temperature. A solution of 4-methoxybenzyl chloride (20.8 mL, 0.15 mol) in THF (75 mL) was added to the mixture, which was followed by addition of tetrabutylammonium iodide (5.14 g, 13.9 mmol). The mixture was stirred for 12 h at room temperature and stirring continued for 1 h at 70 °C. After cooling with ice water, the mixture was quenched with 10% aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the residue by MPLC on silica gel (eluent: hexane/EtOAc = 2:1) gave 26.3 g (64%) of 5-bromo-1-(4-methoxybenzyl)pyrazin-2(1*H*)-one as an amorphous oil: IR (KBr) 3052, 1654, 1577, 1513, 1250 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.73 (3H, s), 4.97 (2H, s), 6,92

(2H, d, J = 8.8 Hz), 7.34 (2H, d, J = 8.8 Hz), 7.88 (1H, s), 8.18 (1H, s).

To a suspension of 60% NaH (7.9 g, 0.20 mol) in mineral oil in dry THF (100 mL) was added a solution of 5-bromo-1-(4-methoxybenzyl)pyrazin-2(1*H*)-one (26.3 g, 89.1 mmol) and tosylmethyl isocyanide (19.1 g, 98.0 mmol) in THF (160 mL) at 0 °C. The mixture was stirred for 30 min at same temperature, and stirring continued for 1 h at room temperature. The resulting mixture was poured into water and extracted with EtOAc. The organic layer was washed successively with water and brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the residue by MPLC on silica gel (eluent: hexane/EtOAc = 1/1:1/3) gave 23.7 g (80%) of 5-bromo-7-(4-methoxybenzyl)imidazo[1,5-*a*]pyrazin-8(7*H*)-one as a pale brown solid: mp 150–151 °C (MeOH); IR (KBr) 3073, 1669, 1512 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.72 (3H, s), 4.94 (2H, s), 6,90 (2H, d, J = 8.8 Hz), 7.32 (2H, d, J = 8.8 Hz), 7.43 (1H, s), 7.94 (1H, s), 8.32 (1H, s); MS m/z: 335 $(M+H)^+$. Anal. Calcd for $C_{14}H_{12}BrN_3O_2$: C, 50.32; H, 3.62; N, 12.57. Found: C, 50.36; H, 3.53; N, 12.49.

To a solution of 5-bromo-7-(4-methoxybenzyl)imidazo[1,5-*a*]pyrazin-8(7*H*)-one (23.2 g, 69.5 mmol) and anisole (135 mL, 1.25 mol) in trifluoroacetic acid (321 mL) was added trifluoromethanesulfonic acid (74 mL, 0.83 mol) at 0 °C. The mixture was stirred for 30 min at room temperature, and stirring continued for 1 h at 40 °C. The solvent was evaporated in vacuo, and the residue was poured into saturated aqueous $NaHCO_3$ solution. Collection of the resulting precipitates by filtration gave 12.4 g (83%) of **9** as an off-white solid: mp 256–257 °C (dec.); IR (KBr) 3021, 2902, 1669, 1512, 1148 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.04 (1H, s), 7.92 (1H, d, J = 0.9 Hz), 8.32 (1H, d, J = 0.9 Hz), 11.05 (1H, br s); MS m/z: 215 $(M+H)^+$. Anal. Calcd for $C_6H_4BrN_3O$: C, 33.67; H, 1.88; N, 19.63. Found: C, 33.56; H, 1.76; N, 19.50.

6.1.19. *N*-(5-Bromoimidazo[1,5-*a*]pyrazin-8-yl)-2,6-dimethylphenylamine (10). This title compound was prepared from **9** in the same manner as described in **6a**, and obtained as an off white solid (33%): mp 151–152 °C (MeOH); IR (KBr) 3214, 1608, 1513, 1260, 1148 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.15 (6H, s), 7.10–7.20 (4H, m), 8.14 (1H, br s), 8.43 (1H, s), 9.18 (1H, s); MS m/z: 318 $(M+H)^+$. Anal. Calcd for $C_{14}H_{13}BrN_4$: C, 53.01; H, 4.13; N, 17.66. Found: C, 53.08; H, 4.07; N, 17.70.

6.1.20. *N*-(2,6-Dimethylphenyl)imidazo[1,5-*a*]pyrazin-8-amine (11). To a solution of **10** (237 mg, 0.75 mmol) in THF (1 mL) was added 2.62 M *n*-BuLi (2.62 M in *n*-hexane; 0.6 mL, 1.57 mmol) at −78 °C. After stirring for 30 min at same temperature, saturated aqueous NH_4Cl was added to the reaction mixture. The resulting mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the residue by flash column chromatography on APS (eluent: hexane/EtOAc, 1:2) gave 98 mg (55%) of **11** as a colorless amorphous: IR (KBr) 3201, 1624, 1519, 1465, 1209, 772 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.14 (6H, s), 6.91 (1H, d, J = 4.7 Hz), 7.10–7.15 (3H, m), 7.66 (1H, d, J = 4.7 Hz), 7.90 (1H, br s), 8.35 (1H, s), 8.91 (1H, s); MS m/z: 239 $(M + H)^+$. Anal. Calcd for $C_{14}H_{14}N_4$: C, 70.57; H, 5.92; N, 14.96. Found: C, 70.62; H, 5.89; N, 14.93.

6.1.21. *N*-(2,6-Dimethylphenyl)-5-(3-methoxyphenyl)imidazo[1,5-*a*]pyrazin-8-amine (12a). A mixture of **10** (506 mg, 1.60 mmol), 3-methoxyphenylboronic acid (255 mg, 1.67 mmol), 1,1′-bis(diphenylphosphino)ferrocene palladium(II)dichloride dichloromethane (65.1 mg, 0.08 mmol), and potassium carbonate (309 mg, 2.23 mmol) in DMF (0.5 mL), water (2.5 mL), and toluene (2.5 mL) was stirred for 15 h at 90 °C. The mixture was diluted with EtOAc, and the organic layer was separated, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the residue by flash column chromatography on APS (eluent: hexane/EtOAc = 1:1) gave 572 mg (100%) of **12a** as an off white solid: mp 177–178 °C (CH_3CN); IR (KBr) 3141, 2950, 1620, 1539, 1535, 1229, 770 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (6H, s), 3.82 (3H, s), 6.99 (1H, s), 7.03 (1H, dd, J = 8.0, 2.5 Hz), 7.14 (3H, s), 7.15–7.25 (2H, m), 7.44 (1H, t, J = 8.0 Hz), 8.08 (1H, br s), 8.37 (1H, s), 9.05 (1H, s); MS m/z: 345 $(M+H)^+$. Anal. Calcd for $C_{21}H_{20}N_4O$: C, 73.23; H, 5.85; N, 16.27. Found: C, 72.98; H, 5.81; N, 16.26.

6.1.22. 4-[8-(2,6-Dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]benzonitrile (12b). This title compound was prepared from **10** in the same manner as described in **12a**, and obtained as a white solid (47%): mp 138–139 °C (CH_3CN); IR (KBr) 2228, 1617, 1507, 1161, 772 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (6H, s), 7.10–7.20 (4H, m), 7.90 (2H, d, J = 8.5 Hz), 7.97 (2H, d, J = 8.5 Hz), 8.13 (1H, br s), 8.48 (1H, br s), 9.25 (1H, br s); MS m/z: 340 $(M+H)^+$. Anal. Calcd for $C_{21}H_{17}N_5 \cdot 0.25\ H_2O$: C, 73.34; H, 5.13; N, 20.36. Found: C, 73.53; H, 5.01; N, 20.15.

6.1.23. *N*-[5-(3,4-Dimethoxyphenyl)imidazo[1,5-*a*]pyrazin-8-yl]-2,6-dimethylphenylamine (12c). This title compound was prepared from **10** in the same manner as described in **12a**, and obtained as a white solid (45%): mp 190–191 °C (CH_3CN); IR (KBr) 3124, 2928, 1623, 1602, 1505, 1429, 774 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (6H, s), 3.81 (3H, s), 3.82 (3H, s), 6.92 (1H, s), 7.09 (1H, d, J = 8.5 Hz), 7.10–7.20 (4H, m), 7.22 (1H, d, J = 1.9 Hz), 8.05 (1H, br s), 8.37 (1H, s), 9.00 (1H, s); MS m/z: 375 $(M+H)^+$. Anal. Calcd for $C_{22}H_{22}N_4O_2$: C, 70.57; H, 5.92; N, 14.96. Found: C, 70.62; H, 5.89; N, 14.93.

6.1.24. *N*-(2,6-Dimethylphenyl)-5-(4-fluorophenyl)imidazo[1,5-*a*]pyrazin-8-amine (12d). This title compound was prepared from **10** in the same manner as described in **12a**, and obtained as a white solid (84%): mp 174–175 °C (CH_3CN); IR (KBr) 3121, 3044, 1539, 1514, 1433, 1228, 772 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (6H, s), 6.94 (1H, s), 7.10–7.20 (3H, m), 7.30–7.40 (2H, m), 7.65–7.75 (2H, m), 8.08 (1H, br s), 8.31 (1H,

s), 9.07 (1H, s); MS *m*/*z*: 333 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}FN_4$: C, 72.27; H, 5.16; N, 16.86. Found: C, 72.13; H, 5.12; N, 16.83.

6.1.25. *N*-(2,6-Dimethylphenyl)-5-(4-trifluoromethylphenyl)imidazo[1,5-*a*]pyrazin-8-amine (12e). This title compound was prepared from **10** in the same manner as described in **12a**, and obtained as a white solid (99%): mp 205–206 °C (CH_3CN); IR (KBr) 3123, 3040, 1617, 1540, 1327, 1128, 778 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.19 (6H, s), 7.09 (1H, s), 7.10–7.20 (3H, m), 7.87 (2H, d, J = 8.5 Hz), 7.92 (2H, d, J = 8.5 Hz), 8.11 (1H, br s), 8.47 (1H, s), 9.21 (1H, s); MS *m*/*z*: 383 $(M+H)^+$. Anal. Calcd for $C_{21}H_{17}F_3N_4$: C, 65.96; H, 4.48; N, 14.65. Found: C, 66.13; H, 4.47; N, 14.62.

6.1.26. *N*-[5-(4-Dimethylaminophenyl)imidazo[1,5-*a*]pyrazin-8-yl]-2,6-dimethylphenylamine (12f). This title compound was prepared from **10** in the same manner as described in **12a**, and obtained as a white solid (45%): mp 227–228 °C (CH_3CN); IR (KBr) 3154, 1609, 1518, 1196, 772 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (6H, s), 2.97 (6H, m), 6.84 (1H, s), 6.84 (2H, d, J = 8.8 Hz), 7.10–7.20 (3H, m), 7.45 (2H, d, J = 8.8 Hz), 7.94 (1H, br s), 8.26 (1H, s), 8.89 (1H, s); MS *m*/*z*: 358 $(M+H)^+$. Anal. Calcd for $C_{22}H_{23}N_5$: C, 73.92; H, 6.49; N, 19.59. Found: C, 73.65; H, 6.44; N, 19.44.

6.1.27. Methyl 4-[8-(2,6-dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]benzoate (12g). This title compound was prepared from **10** in the same manner as described in **12a**, and obtained as a white solid (56%): mp 202–203 °C (MeOH); IR (KBr) 3126, 1715, 1539, 1434, 1284, 771 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.19 (6H, s), 3.89 (3H, s), 7.05–7.20 (4H, m), 7.85 (2H, d, J = 8.2 Hz), 8.05–8.20 (3H, m), 8,47 (1H, s), 9.21 (1H, s); MS *m*/*z*: 373 $(M+H)^+$. Anal. Calcd for $C_{22}H_{20}N_4O_2$: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.95; H, 5.30; N, 15.26.

6.1.28. *N*-(2,6-Dimethylphenyl)-5-pyridin-4-ylimidazo[1,5-*a*]pyrazin-8-amine (12j). This title compound was prepared from **10** in the same manner as described in **12a**, and obtained as a yellow solid (30%): mp 246–247 °C (CH_3CN); IR (KBr) 3193, 3010, 1613, 1596, 1497, 1210, 764 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (6H, s), 7.05–7.25 (4H, m), 7.65–7.75 (2H, m), 8.14 (1H, br s), 8.57 (1H, s), 8.60–8.70 (2H, m), 9.30 (1H, s); MS *m*/*z*: 316 $(M+H)^+$. Anal. Calcd for $C_{19}H_{17}N_5 \cdot 0.4H_2O$: C, 70.74; H, 5.56; N, 21.71. Found: C, 71.14; H, 5.58; N, 21.39.

6.1.29. *N*-(2,6-Dimethylphenyl)-5-thiophen-2-ylimidazo[1,5-*a*]pyrazin-8-amine (12k). This title compound was prepared from **10** in the same manner as described in **12a**, and obtained as an off white solid (83%): mp 223–224 °C (CH_3CN); IR (KBr) 3199, 1621, 1553, 1519, 1252, 777 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (6H, s), 7.10–7.20 (4H, m), 7.45–7.55 (1H, m), 7.70–7.80 (1H, m), 7.90–8.25 (2H, m), 8.46 (1H, s), 9.05 (1H, s); MS *m*/*z*: 321 $(M+H)^+$. Anal. Calcd for $C_{18}H_{16}NP_4S$: C, 67.47; H, 5.03; N, 17.49. Found: C, 67.35; H, 5.04; N, 17.48.

6.1.30. 4-[8-(2,6-Dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]benzoic acid (12h). To a suspension of **12g** (177 mg, 0.48 mmol) in THF (1 mL) and EtOH (1 mL) was added 2 M NaOH (0.52 mL, 1.05 mmol), and the mixture was stirred for 2 h at reflux. After cooling at room temperature, the mixture was neutralized with 2 M HCl (0.52 mL), extracted with EtOAc, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was recrystallized from EtOAc, and collection of the resulting precipitate gave 165 mg (97%) of **12 h** as a white solid: mp 288–289 °C (dec.); IR (KBr) 3127, 1684, 1540, 1289, 777 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.19 (6H, s), 7.09 (1H, s), 7.10–7.20 (3H, m), 7.81 (2H, d, J = 8.2 Hz), 8.12 (2H, d, J = 8.2 Hz), 8.47 (1H, s), 9.19 (1H, br s), 13.11 (1H, br s); MS *m*/*z*: 359 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}N_4O_2$: C, 70.38; H, 5.06; N, 15.63. Found: C, 70.03; H, 5.00; N, 15.55.

6.1.31. 4-[8-(2,6-Dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]-*N*,*N*-dimethylbenzamide (12i). A mixture of **12 h** (59.0 mg, 0.17 mmol), dimethylamine (1 M in THF; 0.17 mL, 0.17 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl, 38.0 mg, 0.2 mmol), 1-hydroxybenzotriazole (HOBt, 31.0 mg, 0.2 mmol), and triethylamine (69 μL, 0.5 mmol) in THF (1 mL) was stirred for 15 h at 50 °C. The mixture was diluted with EtOAc, and the organic layer was washed successively with 1 M HCl and brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the residue by flash column chromatography on APS (eluent: hexane/EtOAc = 1:1) gave 52 mg (81%) of **12i** as a white solid: mp 231–232 °C (CH_3CN); IR (KBr) 3306, 1613, 1514, 1160, 770 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.19 (6H, s), 2.99 (3H, s), 3.01 (3H, s), 7.03 (1H, s), 7.10–7.20 (3H, m), 7.54 (2H, d, J = 8.2 Hz), 7.73 (2H, d, J = 8.2 Hz), 8.09 (1H, br s), 8.41 (1H, s), 9.10 (1H, s); MS *m*/*z*: 386 $(M+H)^+$. Anal. Calcd for $C_{23}H_{23}N_5O$: C, 71.67; H, 6.01; N, 18.17. Found: C, 71.36; H, 6.03; N, 17.82.

6.1.32. 5-[8-(2,6-Dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]thiophene-2-carbaldehyde (12l). A mixture of **10** (303 mg, 0.96 mmol), 5-tributylstannylthiophen-2-carbaldehyde (498 mg, 1.24 mmol), and bis(triphenylphosphine)palladium(II) dichloride (67.0 mg, 0.10 mmol) in dioxane (3 mL) was stirred for 15 h at reflux. The mixture was diluted with EtOAc, and the resulting suspension was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the residue by flash column chromatography on silica gel (eluent: hexane/EtOAc = 1:1) gave 253 mg (76%) of **12l** as a yellow solid: mp 250–251 °C (CH_3CN); IR (KBr) 3128, 1659, 1534, 1437, 1060 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.17 (6H, s), 7.10–7.20 (3H, m), 7.42 (1H, s), 7.82 (1H, d, J = 4.1 Hz), 8,12 (1H, d, J = 4.1 Hz), 8.18 (1H, br s), 8.72 (1H, s), 9.46 (1H, s), 9.95 (1H, s); MS *m*/*z*, 349 $(M+H)^+$. Anal. Calcd for $C_{19}H_{16}N_4OS$: C, 65.50; H, 4.63; N, 16.08. Found: C, 65.34; H, 4.56; N, 16.17.

6.1.33. *N*-(2,6-Dimethylphenyl)-5-(5-pyrrolidin-1-ylmethylthiophen-2-yl)imidazo[1,5-*a*]pyrazin-8-amine hydrochloride (13a). To a mixture of **12l** (62.0 mg, 0.18 mmol) and pyrrolidine (30.0 μL, 0.35 mmol) in EtOH (0.7 mL) was

added titanium isopropoxide (0.10 mL, 0.35 mmol). After stirring for 15 h at room temperature, sodium borohydride (33.0 mg, 0.88 mmol) was added to the mixture, and stirring continued for 3 h at room temperature. The mixture was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the residue by flash column chromatography on APS (eluent, EtOAc) gave 36 mg (51%) of *N*-(2,6-dimethylphenyl)-5-(5-pyrrolidin-1-ylmethylthiophen-2- yl)imidazo[1,5-*a*]pyrazin-8-amine as a colorless amorphous oil: ^{1}H NMR (DMSO-d_6) δ 1.65–1.75 (4H, m), 2.17 (6H, s), 2.45–2.60 (4H, m), 3.81 (2H, s), 7.05 (1H, d, J = 3.8 Hz), 7.11 (1H, s), 7.10–7.20 (3H, m), 7.40 (1H, d, J = 3.8 Hz), 8,10 (1H, br s), 8.56 (1H, s), 9.17 (1H, s); MS *m*/*z*: 404 $(M+H)^+$.

To a solution of *N*-(2,6-dimethylphenyl)-5-(5-pyrrolidin-1-ylmethylthiophen-2- yl)imidazo[1,5-*a*]pyrazin-8-amine (24.0 mg, 0.06 mmol) in EtOAc (1 mL) was added 4 M HCl in EtOAc (33.0 μL, 0.13 mmol) at 4 °C. Collection of the resulting precipitates by filtration gave 20 mg (76%) of **13a** as a yellow solid: mp 269–270 °C (dec.); IR (KBr) 3398, 3198, 1617, 1534, 1502, 1208, 772 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.85–2.10 (4H, m), 2.23 (6H, s), 3.05–3.20 (2H, m), 3.40–3.50 (2H, m), 4.60–4.70 (2H, m), 7.10–7.20 (3H, m), 7.47 (1H, d, J = 3.8 Hz), 7.57 (1H, d, J = 3.8 Hz), 8.19 (1H, br s), 8.60 (1H, s), 9.39 (1H, br s), 10.77 (1H, br s); Anal. Calcd for $C_{23}H_{25}N_5S{\cdot}HCl{\cdot}H_2O$: C, 60.31; H, 6.10; N, 15.29. Found: C, 60.56; H, 6.04; N, 14.95.

6.1.34. *N*-(2,6-Dimethylphenyl)-5-[5-(4-methylpiperazin-1-ylmethyl)thiophen-2-yl]imidazo[1,5-*a*]pyrazin-8-amine (13b). This title compound was prepared from **12l** in the same manner as described above, and obtained as an off white solid: mp 186–187 °C (dec.); IR (KBr) 3133, 2940, 2806, 1533, 1287, 1163 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.15 (3H, s), 2.17 (6H, s), 2.20–2.50 (8H, m), 3.70 (2H, s), 7.60 (1H, d, J = 3.8 Hz), 7.10–7.20 (4H, m), 7.41 (1H, d, J = 3.8 Hz), 8.09 (1H, br s), 8.54 (1H, s), 9.15 (1H, s); Anal. Calcd for $C_{24}H_{28}N_6S{\cdot}0.5H_2O$: C, 65.28; H, 6.62; N, 19.03. Found: C, 65.56; H, 6.37; N, 18.64.

6.1.35. *N*-(2,6-Dimethylphenyl)-5-[4-(2-morpholin-4-ylethoxy)phenyl]imidazo[1,5-*a*]pyrazin-8-amine (14a). To a suspension of **6h** (731 mg, 2.12 mmol) in dichloromethane (CH_2Cl_2, 5 mL) was added BBr_3 (1 M in CH_2Cl_2; 8.5 mL, 8.50 mmol) at −78 °C, and the mixture was stirred for 5 h. A solution of triethanolamine (3.17 g, 21.2 mmol) in THF (10 mL) was added to the mixture at 0 °C, and the mixture was stirred for 15 h at reflux. The mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo to give 613 mg (87%) of 4-[8-(2,6-dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]phenol as a pale brown solid: mp > 300 °C (CH_3CN); IR (KBr) 3408, 2985, 1608, 1517, 1278, 838 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.18 (6H, s), 6.85 (1H, s), 6.90 (2H, d, J = 8.5 Hz), 7.10–7.20 (3H, m), 7.45 (2H, d, J = 8.5 Hz), 8.00 (1H, br s), 8.26 (1H, s), 8.95 (1H, br s), 9.81 (1H, s); MS *m*/*z*, 331 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}N_4O{\cdot}0.2H_2O$: C, 71.92; H, 5.55; N, 16.78. Found: C, 72.12; H, 5.58; N, 16.69.

To a mixture of 4-[8-(2,6-dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]phenol (71.0 mg, 0.21 mmol), *N*-(2-hydroxyethyl)morpholine (104 μL, 0.86 mmol), and triphenylphosphine (113 mg, 0.43 mmol) in THF (1 mL) was added 40% diethyl azodicarboxylate solution in toluene (228 μL) at room temperature, and the mixture was stirred for 15 h at 50 °C. The mixture was evaporated in vacuo, and purification of the residue by flash column chromatography on APS (eluent: hexane/EtOAc = 2:1) gave 69 mg (72%) of **14a** as a white solid: mp 195–196 °C; IR (KBr) 2955, 1607, 1514, 1250, 771 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.18 (6H, s), 2.72 (2H, t, J = 5.7 Hz), 3.55–3.65 (8H, m), 4.16 (2H, t, J = 5.7 Hz), 6.88 (1H, s), 7.09 (2H, d, J = 8.8 Hz), 7.10–7.20 (3H, m), 7.57 (2H, d, J = 8.8 Hz), 8.05 (1H, br s), 8.27 (1H, s), 8.99 (1H, s); MS *m*/*z*, 444 $(M+H)^+$. Anal. Calcd for $C_{26}H_{29}N_5O_2$: C, 70.41; H, 6.59; N, 15.79. Found: C, 70.27; H, 6.52; N, 15.63.

6.1.36. *N*-(2,6-Dimethylphenyl)-5-[4-(2-pyrrolidin-1-ylethoxy)phenyl]imidazo[1,5-*a*]pyrazin-8-amine (14b). This title compound was prepared from 4-[8-(2,6-dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]phenol in the same manner as described in **14a**, and obtained as an off white solid: mp 222–223 °C; IR (KBr) 3124, 2964, 1607, 1514, 1251, 770 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.65–1.75 (4H, m), 2.18 (6H, s), 2.50–2.60 (4H, m), 2.81 (2H, t, J = 6.0 Hz), 4.13 (2H, t, J = 6.0 Hz), 6.89 (1H, s), 7.08 (2H, d, J = 8.8 Hz), 7.10–7.15 (3H, m), 7.57 (2H, d, J = 8.8 Hz), 8.05 (1H, br s), 8.27 (1H, s), 8.98 (1H, s); Anal. Calcd for $C_{26}H_{29}N_5O{\cdot}0.25H_2O$: C, 72.28; H, 6.68; N, 16.21. Found: C, 72.63; H, 6.96; N, 16.03.

6.1.37. *N*-(2,6-Dimethylphenyl)-5-[4-(1-methylpiperidin-3-ylmethoxy)phenyl]imidazo[1,5-*a*]pyrazin-8-amine (14c). This title compound was prepared from 4-[8-(2,6-dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]phenol in the same manner as described in **14a**, and obtained as an off white solid: mp 191–192 °C (CH_3CN); IR (KBr) 2953, 1606, 1514, 1247, 772 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.10–1.15 (1H, m), 1.40–1.55 (1H, m), 1.60–1.85 (3H, m), 1.85–1.95 (1H, m), 1.95–2.10 (1H, m), 2.16 (3H, s), 2.18 (6H, s), 2.55–2.70 (1H, m), 2.75–2.85 (1H, m), 3.85–4.00 (2H, m), 6.88 (1H, s), 7.07 (2H, d, J = 8.8 Hz), 7.10–7.20 (3H, m), 7.56 (2H, d, J = 8.8 Hz), 8.05 (1H, br s), 8.27 (1H, s), 8.99 (1H, s); MS *m*/*z*, 442 $(M+H)^+$. Anal. Calcd for $C_{27}H_{31}N_5O{\cdot}0.2H_2O$: C, 72.85; H, 7.11; N, 15.73. Found: C, 73.02; H, 7.02; N, 15.73.

6.1.38. *N*-(2,6-Dimethylphenyl)-5-[3-(1-methylpiperidin-3-ylmethoxy)phenyl]imidazo[1,5-*a*]pyrazin-8-amine (14d). 3-[8-(2,6-Dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]phenol was prepared from **12a** in the same manner as described in **14a**, and obtained as an off white solid: mp 157–158 °C (MeOH); IR (KBr) 3325, 3050, 1624, 1517, 1451, 1221, 775 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.18 (6H, s), 6.86 (1H, dd, J = 8.2, 2.2 Hz), 6.94 (1H,

s), 6.95–7.05 (1H, m), 7.08 (1H, d, J = 7.6 Hz), 7.10–7.20 (3H, m), 7.32 (1H, t, J = 8.2 Hz), 8.07 (1H, br s), 8.33 (1H, s), 9.05 (1H, s), 9.75 (1H, s); MS m/z, 331 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}N_4O{\cdot}0.2H_2O$: C, 71.92; H, 5.55; N, 16.78. Found: C, 72.24; H, 5.52; N, 16.86.

This title compound was prepared from 3-[8-(2,6-dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]phenol in the same manner as described in **14a**, and obtained as an off white solid: mp 173–174 °C (CH_3CN); IR (KBr) 3398, 2936, 1543, 1515, 1437, 771 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.00–1.15 (1H, m), 1.40–1.55 (1H, m), 1.55–1.85 (3H, m), 1.85–1.95 (1H, m), 1.95–2.05 (1H, m), 2.14 (3H, s), 2.19 (6H, s), 2.55–2.65 (1H, m), 2.75–2.85 (1H, m), 3.85–4.00 (2H, m), 6.98 (1H, s), 7.02 (1H, d, J = 8.2 Hz), 7.10–7.25 (5H, m), 7.42 (1H, t, J = 8.2 Hz), 8.05 (1H, br s), 8.36 (1H, s), 9.04 (1H, s); MS m/z, 442 $(M+H)^+$. Anal. Calcd for $C_{27}H_{31}N_5O$: C, 73.44; H, 7.08; N, 15.86. Found: C, 73.09; H, 7.02; N, 15.73.

6.1.39. *N*-(2,6-Dimethylphenyl)-5-[3-(2-pyrrolidin-1-ylethoxy)phenyl]imidazo[1,5-*a*]pyrazin-8-amine hydrochloride (14e). This title compound was prepared from 3-[8-(2,6-dimethylphenylamino)imidazo[1,5-*a*]pyrazin-5-yl]phenol in the same manner as described in **14a**, and obtained as an off white solid: mp 282–283 °C (dec.); IR (KBr) 3412, 3212, 1538, 1507, 1220 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.80–2.10 (4H, m), 2.18 (6H, s), 3.00–3.20 (2H, m), 3.50–3.70 (4H, m), 4.41 (2H, t, J = 4.4 Hz), 7.00 (1H, s), 7.05–7.20 (4H, m), 7.25–7.35 (2H, m), 7.48 (1H, t, J = 7.9 Hz), 8.11 (1H, br s), 8.41 (1H, s), 9.13 (1H, br s), 10.28 (1H, br s); Anal. Calcd for $C_{26}H_{29}N_5O{\cdot}HCl{\cdot}0.5H_2O$: C, 66.02; H, 6.61; N, 14.81. Found: C, 65.94; H, 6.64; N, 14.68.

6.1.40. 2-Benzyl-6-(3-methoxyphenyl)-8-oxo-7,8-dihydroimidazo[1,5-*a*]pyrazin-2-ium bromide (16a). A mixture of **15** (563 mg, 2.80 mmol) and 2-bromo-3′-methoxyacetophenone (673 mg, 2.94 mmol) in acetonitrile (5 mL) and DMF (1.6 mL) was stirred for 14 h at 90 °C. After cooling to room temperature, collection of the insoluble material by filtration gave 1.04 g (90%) of **16a** as a white solid: mp 267–268 °C; IR (KBr) 3417, 3100, 1680, 1599 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.84 (3H, s), 5.66 (2H, s), 7.05–7.15 (1H, m), 7.20–7.30 (2H, m), 7.35–7.55 (6H, m), 8.00 (1H, s), 8.85(1H, d, J = 0.7 Hz), 9.66 (1H, d, J = 1.6 Hz), 11.84 (1H, s); Anal. Calcd for $C_{20}H_{18}BrN_3O_2$: C, 58.26; H, 4.40; N, 10.19. Found: C, 58.05; H, 4.40; N, 10.17.

6.1.41. 2-Benzyl-6-(4-fluorophenyl)-8-oxo-7,8-dihydroimidazo[1,5-*a*]pyrazin-2-ium bromide (16b). This title compound was prepared from **15** in the same manner as described above, and obtained as an off white solid (37%): mp 277–278 °C; IR (KBr) 3041, 1673, 1520, 1247, 1120 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.66 (2H, s), 7.35–7.55 (7H, m), 7.65–7.80 (2H, m), 7.94 (1H, s), 8.85 (1H, s), 9.68 (1H, s), 11.88 (1H, s); Anal. Calcd for $C_{19}H_{15}BrFN_3O$: C, 57.02; H, 3.78; N, 10.50. Found: C, 56.98; H, 3.77; N, 10.56.

6.1.42. 6-(3-Methoxyphenyl)imidazo[1,5-*a*]pyrazin-8(7*H*)-one (17a). A mixture of **16a** (903 mg, 2.19 mmol) and imidazole (3.73 g, 54.8 mmol) was stirred for 14 h at 180 °C. After cooling to 100 °C, ice water was added to the mixture. Collection of the insoluble material by filtration gave 264 mg (50%) of **17a** as a pale brown solid: mp 275–276 °C; IR (KBr) 3100, 1644, 1597 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.84 (3H, s), 6.95–7.05 (1H, m), 7.20–7.30 (2H, m), 7.35–7.45 (1H, m), 7.75–7.80 (1H, m), 7.80–7.90 (1H, m), 8.27 (1H, d, J = 0. 6 Hz), 10.99 (1H, s); Anal. Calcd for $C_{13}H_{11}N_3O_2{\cdot}0.1H_2O$: C, 64.24; H, 4.64; N, 17.29. Found: C, 64.27; H, 4.60; N, 17.30.

6.1.43. 6-(4-Fluorophenyl)imidazo[1,5-*a*]pyrazin-8(7*H*)-one (17b). This title compound was prepared from **16b** in the same manner as described above, and obtained as an off white solid (55%): mp > 300 °C (MeOH); IR (KBr) 3131, 3069, 1681, 1108 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.30–7.40 (2H, m), 7.65–7.75 (2H, m), 7.75–7.85 (2H, m), 8.28 (1H, d, J = 0.6 Hz), 11.05 (1H, s); Anal. Calcd for $C_{12}H_8FN_3O{\cdot}0.1H_2O$: C, 62.39; H, 3.58; N, 18.19. Found: C, 62.44; H, 3.54; N, 18.31.

6.1.44. *N*-(2-Chloro-6-methylphenyl)-6-(3-methoxyphenyl)imidazo[1,5-*a*]pyrazin-8-amine (18a). The title compound was prepared from **17a** in the same manner as described in **6a**, and obtained as an off white solid (32%): mp 277–278 °C; IR (KBr) 3204, 3115, 2955, 1609, 1580, 1540, 1505, 780 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (3H, s), 3.70 (3H, s), 6.80-6.90 (1H, m), 7.20–7.40 (5H, m), 7.40–7.50 (1H, m), 7.86 (1H, br s), 8.40 (1H, s), 8.41 (1H, s), 9.35 (1H, s); MS m/z, 365 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}ClN_4O{\cdot}0.2H_2O$: C, 65.20; H, 4.76; N, 15.21. Found: C, 65.43; H, 4.61; N, 15.30.

6.1.45. *N*-(2-Chloro-6-methylphenyl)-6-(4-fluorophenyl)-imidazo[1,5-*a*]pyrazin-8-amine (18b). The title compound was prepared from **17b** in the same manner as described in **6a**, and obtained as an off white solid: mp 215–216 °C; IR (KBr) 3184, 3107, 1617, 1545, 1473, 1436, 1223, 776 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.27 (3H, s), 7.15–7.25 (2H, m), 7.25–7.40 (2H, m), 7.40–7.50 (1H, m), 7.65–7.80 (2H, m), 7.84 (1H, br s), 8.36 (1H, s), 8.40 (1H, s), 9.35 (1H, s); MS m/z, 353 $(M+H)^+$. Anal. Calcd for $C_{19}H_{14}ClFN_4{\cdot}0.2H_2O$: C, 64.03; H, 4.07; N, 15.72. Found: C, 64.35; H, 3.89; N, 15.70.

6.1.46. *N*-(2,6-Dimethylphenyl)-6-(4-fluorophenyl)imidazo-[1,5-*a*]pyrazin-8-amine (18c). The title compound was prepared from **17b** in the same manner as described in **6a**, and obtained as an off white solid: mp 228–229 °C; IR (KBr) 3186, 3107, 1616, 1547, 1515, 1223, 776 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.21 (6H, s), 7.10–7.25 (5H, m), 7.50–7.90 (2H, m), 7.96 (1H, br s), 8.32 (1H, s), 8.38 (1H, s), 9.07 (1H, s); MS m/z, 333 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}FN_4{\cdot}0.2H_2O$: C, 71.50; H, 5.22; N, 16.68. Found: C, 71.57; H, 5.14; N, 16.37.

6.1.47. *N*-(2,6-Dimethylphenyl)-5-[4-(1-methylpiperidin-3-ylmethoxy)phenyl]imidazo[1,5-*a*]pyrazin-8-amine hydrochloride (14c·HCl). To a solution of **14c** (856 mg, 1.94 mmol) in EtOAc (9 mL) was added 4 M HCl in

EtOAc (1.93 mL, 3.88 mmol) at 5 °C. Collection of the resulting precipitates by filtration gave 766mg (83%) of **14c·HCl** as a white solid: mp 187–188 °C (CH_3CN–H_2O); IR (KBr) 3416, 2954, 1624, 1512, 1250 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.20–1.40 (1H, m), 1.80–1.95 (3H, m), 2.24 (6H, s), 2.35–2.50 (1H, m), 2.50–2.90 (5H, m), 3.35–3.45 (1H, m), 3.45–3.55 (1H, m), 3.90–3.95 (1H, m), 4.00–4.10 (1H, m), 6.78 (1H, br s), 7.15 (2H, d, J = 8.8 Hz), 7.20–7.40 (3H, m), 7.65 (2H, d, J = 8.8 Hz), 8.54 (1H, br s), 8.82 (1H, br s), 10.74 (1H, br s), 12.00 (1H, br s); Anal. Calcd for $C_{27}H_{31}N_5O·HCl·H_2O$: C, 65.38; H, 6.91; N, 14.12. Found: C, 65.25; H, 6.79; N, 14.10.

6.1.48. *N*-(2,6-Dimethylphenyl)-5-[3-(1-methylpiperidin-3-ylmethoxy)phenyl]imidazo[1,5-*a*]pyrazin-8-amine hydrochloride (14d·HCl). The title compound was prepared from **14d** in the same manner as described above, and obtained as an off white solid: mp 188–189 °C (CH_3CN–H_2O); IR (KBr) 3421, 2949, 1636, 1587, 1472 cm^{-1}; ^1H NMR (CD_3OD) δ 1.40–1.55 (1H, m), 1.80–2.10 (3H, m), 2.25–2.50 (7H, m), 2.80–3.05 (5H, m), 3.53 (1H, d, J = 11.7 Hz), 3.70 (1H, d, J = 11.7 Hz), 3.95–4.05 (1H, m), 4.05–4.15 (1H, m), 6.81 (1H, s), 7.15–7.25 (1H, m), 7.25–7.45 (5H, m), 7.54 (1H, t, J = 8.0 Hz), 8.55 (1H, br s), 8.60 (1H, br s); Anal. Calcd for $C_{27}H_{31}N_5O·HCl·H_2O$: C, 65.38; H, 6.91; N, 14.12. Found: C, 65.29; H, 6.84; N, 13.91.

6.2. X-ray crystallography

A binary complex of purified human Lck protein and compound **6a** was formed by the hanging-drop co-crystallization method. Crystals grew over 7 days to a maximum dimension of $0.3 \times 0.5 \times 0.7$ mm. Prior to data collection, crystals were quickly dipped in 20% (v/v) glycerol containing a cryosolution and flash-cooled in liquid nitrogen. X-ray diffraction intensity was measured under cryogenic conditions using an R-AXIS IV diffractometer (Rigaku) operating at 50 kV and 108 mA. The data were processed using MOSFLM Ver.6.2.3.[31] The crystal structure diffracted to 2.55 Å resolution and conformed to the space group $P3_121$ with one molecule per asymmetric unit. The structure of the binary complex was refined and modeled against the published structure (PDB code: 3LCK)[21] in the absence of ligand, using the CNX program (Accerlys). A rigid-body rotation–translation refinement was performed to accurately place the model structure into the new unit cell. Further crystallographic refinement was done using conjugated-gradient minimization. Individual B factor refinement was done with CNX, and model/ligand building was performed using QUANTA (Accerlys). The final model included 271 residues and 184 water molecules, with an R factor of 21.6% and an R_f of 28.3%.

6.3. Molecular modeling

The structure of inhibitor **6a** was extracted from our X-ray structure of Lck and manually docked into the ATP binding site of c-Src (PDB code. 1YOL)[19] using CHARM/Quanta™.

We used the configuration of the inhibitor in Lck as the initial structure to build the c-Src model because both kinases have high sequence homology, particularly within the ATP binding site, and to ensure internal consistency in the enzymatic assay, among other reasons.

6.4. Biology

6.4.1. Kinase assays. A coupled spectrophotometric assay was used wherein ADP generated by Src kinase was converted to ATP by pyruvate kinase (PK), with concomitant production of pyruvate from phosphoenolpyruvate (PEP). LDH reduces pyruvate to lactate by oxidizing NADH. NADH depletion was monitored at 340 nm using a microplate reader (Spectra Max 250, Molecular Device) at 30 °C for 20 min. Reactions were performed at 30 °C in 100 mM Hepes buffer (pH 7.6), containing 20 mM $MgCl_2$ and 10% glycerol, initiated by adding ATP. PK (100 μg/mL), LDH (50 μg/mL), PEP (2 mM), and NADH (140 μM) were also added. Kinase activity was measured by adding 100 μM Src optimal peptide substrate (peptide sequence: AEEEIYGEFEAKKKK, Sawady, Tokyo).

6.4.2. Intracellular kinase inhibition assay. The kinase domain of human Src kinase (NM_005417, base# 790-1650) was cloned by PCR amplification, using the linker-containing primers 5′-AAACTTAAGCTTCATATG TCCAAGCCGCAGAC-3′ and 5′-CTGCAGATATC CCTAGAAGTAGTCCTCCAGGAA-3′. The gene for the Src kinase domain was integrated between the *Hin*dIII and *Eco*RV restriction sites within the multiple cloning site of the pcDNA3.1(+) expression vector. The vector was transfected into COS7 cells according to the calcium phosphate method.[32] Subsequently, 8.8 μg DNA was mixed with 220 μL of 1 mM Tris–HCl, 0.1 mM EDTA buffer (pH 8.0), 250 μL 2× Hepes-buffered saline, and 31 μL of 2 M $CaCl_2$. The solution was incubated at room temperature for 15 min and then used to resuspend a pellet of 10^6 COS7 cells. The cell suspension was incubated at room temperature for 15 min; then, 4.5 mL Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics was added, and the solution was pre-warmed to 37 °C. Cells were seeded into 96-well multi-well plates with 2×10^4 cells/well/100 μL. A day after transfection, the culture media were replaced with media containing fixed concentrations of test compounds. On the third day, the cellular phosphotyrosine contents were determined using a commercially available phosphotyrosine ELISA kit (Upstate Biotechnology, NY, USA. Cellular Phosphotyrosine ELISA Kit™, Catalog #17-182). Any basal phosphotyrosine content unrelated to c-Src activity was screened for using vector-transfected cells. Intracellular c-Src inhibition was expressed as the percentage of specific phosphotyrosine levels in drug-treated cells compared to the levels in control cells without drug. The toxicity of each chemical was determined with another replica plate using a colorimetric MTT assay kit (Chemicon International, CA, USA. Cat. #CT02).

6.4.3. Analysis of c-Src inhibitors in the brain and plasma. To inhibit c-Src kinase in the brain, we first determined the brain and plasma levels of the test compounds. Sprague–Dawley rats (300–350 g: $n = 3$/study) were anesthetized with 25% urethane (1.1 g/kg, sc), and the femoral vein was cannulated to deliver the test compounds. Tracheal intubation was performed for artificial respiration. The animal's body temperature was maintained at 37.5 °C with a heating-pad. Test compounds were administered by intravenous infusion at 3 mg/kg/h. Three hours later, blood samples were taken from the aorta and centrifuged to separate the plasma. Brain tissue was homogenized with a 3-fold volume of saline.

Plasma and brain tissue homogenate (50 μL) was mixed with 200 μL of 100% acetonitrile, 100 μL of 0.1% heptafluorobutyric acid, and 200 μL internal standard solution. After centrifugation at 3300 rpm for 20 min, the supernatant was removed and transferred to the column. Samples were quantified using liquid chromatography-tandem mass spectrometry (LC–MS/MS). The LC–MS/MS system consisted of an Alliance HPLC (Waters 2690) with a SYNERGI MAX-RP 80A column (4 μm, 50 × 4.6 mm, Phenomenex, Torrance, CA), and a Sciex API 365 mass spectrometer (Perkin–Elmer Sciex, Toronto, Canada) equipped with a turbo ion spray source. The mobile phase consisted of 100% acetonitrile and 0.1% acetic acid in water (50:50 v/v). The column was maintained at 50 °C with a constant flow rate of 0.4 mL/min. The data were processed using Mass Chrom 1.1 software. The standard curves plotted for each test compound demonstrated good linearity (coefficient of determination >0.99). The limits of quantification of the compounds in plasma and brain were 50 ng/mL.

6.4.4. Photochemically induced middle cerebral artery occlusion in rats. Male Sprague–Dawley rats weighing 260–320 g were anesthetized with enflurane. The animal's body temperature was maintained at 37.5 °C with a heating pad. We performed middle cerebral artery (MCA) thrombosis as previously described.[30] A catheter was inserted into the femoral vein to administer the drug and rose bengal dye. The scalp and temporalis muscle were folded over. A subtemporal craniotomy was performed using a dental drill under an operating microscope to open a 3-mm-diameter oval bony window. The window was irradiated with green light (540 nm wavelength) using a xenon lamp (L4887, Hamamatsu Photonics, Hamamatsu, Japan), with both heat-absorbing and green filters. The irradiation was directed by a 3-mm-diameter optic fiber mounted on a micromanipulator. The head of the optic fiber was placed on the window in the skull base at a distance of 2 mm above the vessel, providing an irradiation dose of 0.62 W/cm^2. Rose bengal (20 mg/kg) was injected intravenously. Photo-irradiation was continued for another 10 min. Compound **14c·HCl** was administered at doses of 1, 3, and 10 mg/kg/h, continuous infusion for 6 h, starting immediately after the MCA occlusion. Twenty-four hours after surgery, the rats were sacrificed by administering an overdose of pentobarbital, and their brains were quickly removed. The cerebrum was separated from the other parts of the brain and cut into six 2-mm-thick slices using a Brain Matrix (Muromachi Kikai, Japan). Each slice was incubated in 1% tetratrihydrochloride (TTC) solution at room temperature for 30 min and then photographed. The area of infarction was measured for each slice using a computerized image analysis system (Mac scope, Japan), and the ratio of infarction size was calculated by dividing the whole area by the region of cerebral ischemic damage. All animal studies were approved by the Kissei Pharmaceutical Co., Ltd. Committee on Ethics of Animal Experimentation, and special care was taken to prevent animal suffering.

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