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Pyrazole derivatives as new potent and selective 20-hydroxy-5,8,11,14-eicosatetraenoic acid synthase inhibitors

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Abstract—Improvement of the physical properties of pyrazole derivative **1**, which we reported previously as a potent and selective 20-HETE synthase inhibitor (IC_{50} 5.7 nM), is described. Introduction of a sufficient substituted-amino group on the side chain enhanced the water-solubility of **1** (0.014 mg/mL at pH 6.8). Among the products, 2-piperazinoethoxy derivatives **3e** and **6b** showed solubility suitable for injection and potent inhibitory activity toward 20-HETE synthase (IC_{50} 21.2 and 14.0 nM, respectively).

1. Introduction

20-Hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) is a major metabolite of arachidonic acid (AA) produced in the kidney.[1] Its biological properties have recently been extensively studied. 20-HETE is synthesized from AA by oxidation with cytochrome P450 (CYP) 4A isozymes (CYP4A1, 4A2, 4A3, and 4A8) in rat kidney,[2] and with CYP4A11 and 4F2 in human liver and kidney.[3] 20-HETE plays an important role in the regulation of renal vascular and tubular functions.[4–6] 20-HETE contributes to the control of arterial blood pressure.[7] More recent studies have indicated that 20-HETE is also produced in the brain, where it regulates vascular tone and contributes to the autoregulation of cerebral blood flow.[8] Therefore, the inhibition of 20-HETE is now considered to be a promising new target for the treatment of renal and cerebral diseases. Some compounds are known to inhibit the production of 20-HETE (Fig. 1), however, these compounds lack sufficient potency and specificity for further investigation as therapeutics. 1-Aminobenzotriazole (1-ABT)[9] has been reported to be a 20-HETE synthase inhibitor with an IC_{50} value of 5 μM, however, it inhibits drug-metabolizing enzymes at almost the same concentration. 17-Octadecynoic acid (17-ODYA),[10,11] *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS),[12] 12,12-dibromododec-11-enoic acid (DBDD)[12] and sodium 10-undecynyl sulfate (10-SUYS)[13] deactivate 20-HETE synthase at micromolar concentrations by forming a covalent bond at the active site via their chemically-reactive oxidized product. In previous papers, we reported HET0016 (*N*-hydroxy-*N*′-(4-*n*-butyl-2-methylphenyl)formamidine) as the first potent and selective 20-HETE synthase inhibitor.[14] HET0016 inhibited the formation of 20-HETE by

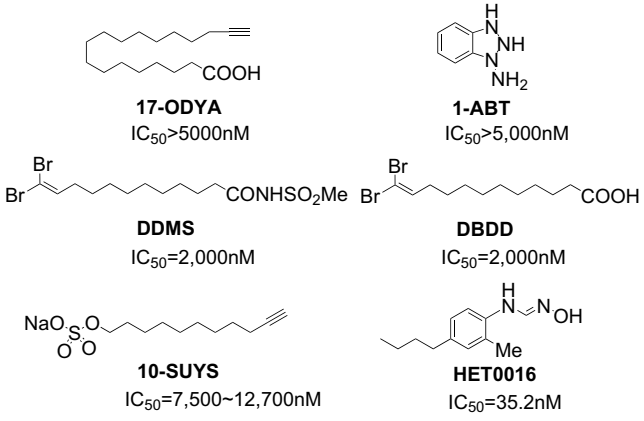

Figure 1. Structures of 1-ABT, 17-ODYA, DDMS, DBDD, 10-SUYS and HET0016. IC_{50} values are for 20-HETE production from AA by rat renal microsome.

Keywords: 20-HETE; Pyrazole.
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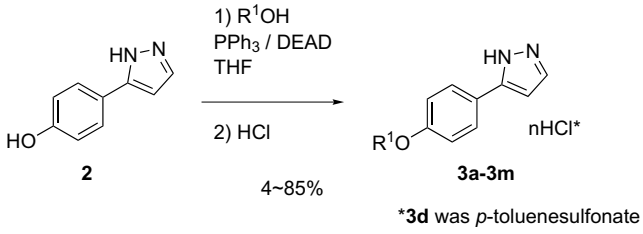
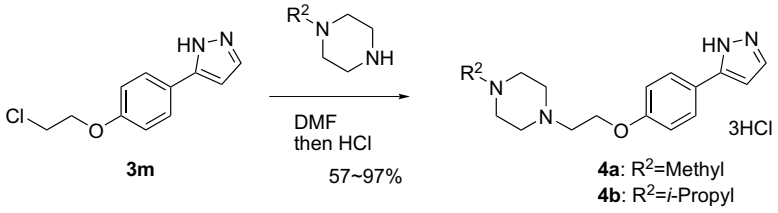
rat renal microsomes ($IC_{50} = 35.2 \pm 4.4$ nM) and by human renal microsomes ($IC_{50} = 8.9 \pm 2.7$ nM),[15] with remarkable selectivity against CYP2C9, CYP2D6, CYP3A4, and cyclooxygenases (COX-1,COX-2).[15] HET0016 prevented the acute fall in cerebral blood flow following subarachnoid hemorrhage (SAH) in the rat when administrated by intravenous injection.[16] However, despite its promising pharmacological properties, the therapeutic potency of HET0016 as an injectable formulation is limited due to its low water-solubility under neutral conditions (0.0037 mg/mL at pH 6.8) and instability under acidic conditions. Replacement of the *N*-hydroxyformamidine moiety of HET0016 by a pyrazole ring gave compound **1**, which retains potent 20-HETE synthase inhibitory activity ($IC_{50} = 26.2$ nM) while showing improved solubility and stability under acidic conditions. However, its water-solubility under neutral conditions (0.026 mg/mL at pH 6.8) is still inadequate for injectable formulations.[17] In this study, we investigated the introduction of a polar substituent the hydrophilic side chain of compound **1** to improve its water-solubility.

2. Chemistry

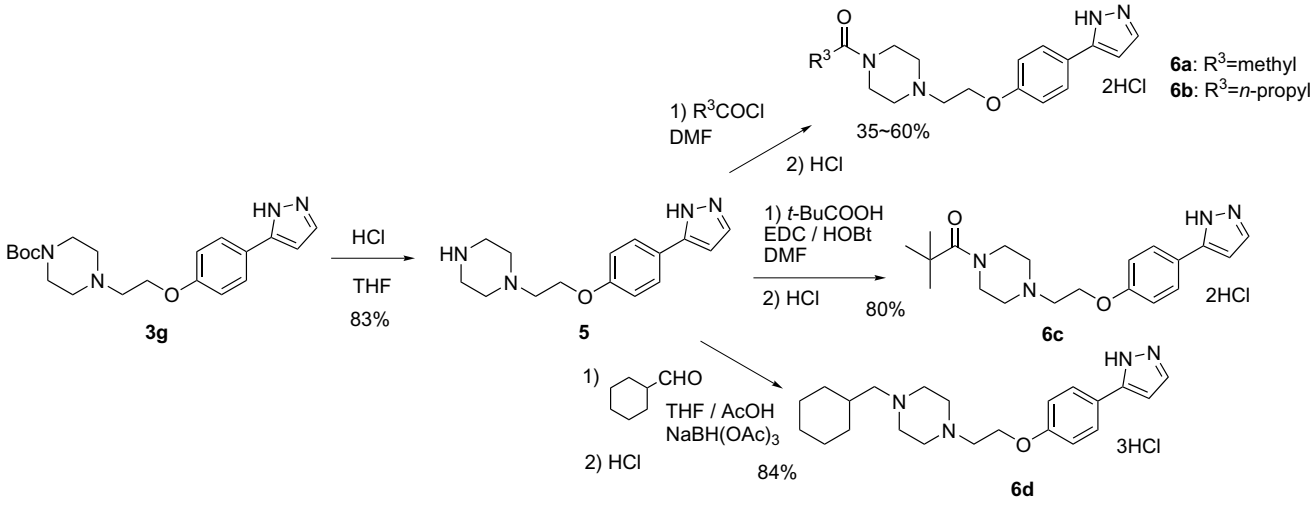
Compounds **3a**–**m** were prepared in yields of 4–85% by the alkylation of 4-(3-pyrazolyl)phenol **2** with corresponding alcohols by the Mitsunobu reaction, as reported for **1** (Scheme 1). Compounds **4a**,**b** were prepared by the alkylation of *N*-methylpiperazine and *N*-isopropylpiperazine by **3m** with Et_3N in DMF at 120 °C (Scheme 2). Deprotection of 1-[2-(4-*tert*-butoxycarbonylpiperazino)ethoxy]-4-(3-pyrazolyl)benzene **3g** with HCl and successive treatment of the resulting mono-alkyl piperazine derivative **5** with acyl chlorides or condensation with carboxylic acid gave amide derivatives **6a**–**c**, respectively. Reductive alkylation of **5** by cyclohexanecarboxaldehyde with $NaBH(OAc)_3$ gave **6d** (Scheme 3). Introduction of a 4- or 5- amino-substituted



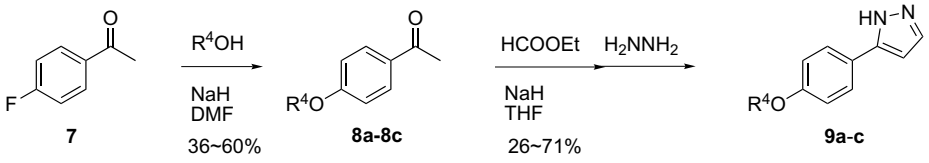
Scheme 1.



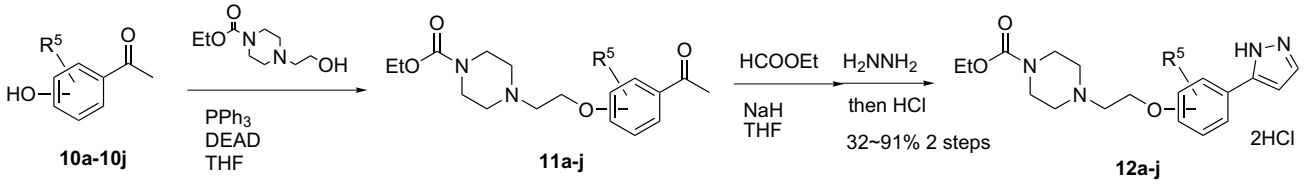
Scheme 2.



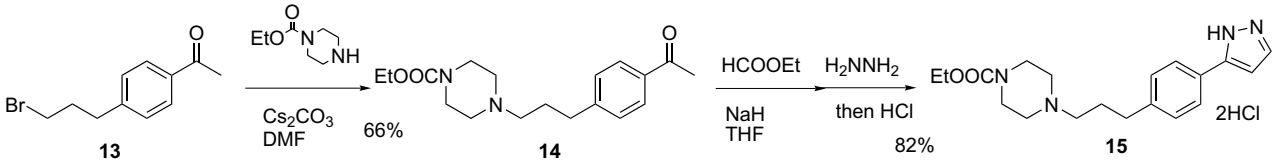
Scheme 3.



Scheme 4.



Scheme 5.



Scheme 6.

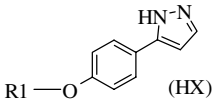
alkyl group to phenol **2** failed, since intramolecular nucleophilic attack of the nitrogen atom took the place of the formation of intermolecular ether. Therefore, compounds **9a**–**c** were prepared as shown in Scheme 4. Nucleophilic substitution of 4-fluoroacetophenone **7** with 3 equiv of the corresponding alkoxide in *N*,*N*-dimethylformamide at room temperature afforded **8a**–**c** in moderate yields, which were then treated with ethyl formate and sodium hydride, and then hydrazine to give corresponding pyrazole derivatives **9a**–**c** in yields of 26–58%.[17] Compounds **12a**–**j** were prepared as shown in Scheme 5. The reaction of hydroxyacetophenone derivatives **10a**–**j** with 2-(4-ethoxycarbonylpiperazino)ethanol under Mitsunobu conditions afforded **11a**–**j**, which were converted to the corresponding pyrazole derivatives **12a**–**j** by the same method as used in the synthesis of **9a**–**c**. Compound **15** was prepared as shown in Scheme 6. The reaction of 1-bromo-3-(4-acetylphenyl)propane **13**[18] with *N*-ethoxycarbonylpiperazine gave amino derivative **14**, which was converted to the corresponding pyrazole derivative **15** in the same way.

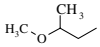
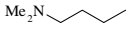
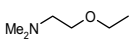
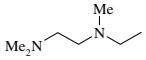
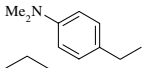
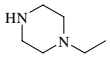
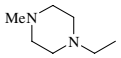
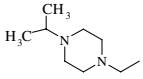
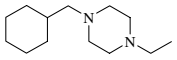
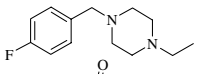
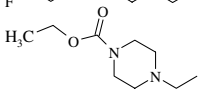
3. Results and discussion

All of the compounds synthesized were evaluated with regard to their inhibitory activity against human renal microsomal synthesis of 20-HETE[17] and water-solubility at pH 6.8. The obtained IC_{50} values are shown in Tables 1–3 along with the water-solubility values. We took particular note between the inhibitory activity toward 20-HETE synthase and lipophilicity of the compounds, therefore, we calculated ACD-log *D* values and drew upon them for drug design. Calculated ACD-log *D* values were also shown in Tables 1–3.

The introduction of a polar functional group such as an amino group or ether group to the hydrophobic side chain of **1** improved its water-solubility (**3a**, **3c**, **4a**, **4b**, **5**, **9a**, **9b**, and **9c**, Table 1) as expected. The solubility of compounds with an acyclic aliphatic amino substituent (**9a**, **9b**, and **9c**) and a cyclic aliphatic amino substituent (**3c**, **4a**, **4b**, **5**) was about 100-fold better than that of simple alkoxy derivative **1**, however, this was accompanied by a great loss of activity. In contrast, methoxy derivative **3a** and some 4-substituted piperazine derivatives (**3d**, **3e**, and **6d**) maintained potent activity with improved solubility. Among these compounds, the substituent at the 4-position of the piperazine ring obviously affects their activities. While nonsubstituted (**5**), methyl (**4a**), and isopropyl (**4b**) derivatives did not show activity at 100 μM, cyclohexylmethyl (**6d**), 4-fluorobenzyl (**3d**), and ethoxycarbonyl (**3e**) derivatives showed potent activities. These results suggest that a rather bulky substituent for the R group is necessary for potent activity. However, this hypothesis does not explain the difference between **3b** and **4b**. There are slight differences between the bulkiness of the 4-dimethylaminophenyl group of **3b** and the 4-isopropylpiperazino group of **4b**. Another possible explanation is the difference in the lipophilicity of these compounds. All of the ACD-log *D* values of the active compounds shown in Table 1 were greater than 2, and those of the inactive compounds were less than 1. With regard to their ability to reduce the lipophilicity of xenobiotic chemicals by oxidation, drug-metabolizing CYPs generally have

Table 1. Inhibition of AA metabolism human 20-HETE synthesizing enzyme by new heterocyclic compounds and their calculated ACD-log *D* values (1)



Compound	R^1	HX	IC_{50} (nM)[a]	Solubility (mg/mL) at pH 6.8	ACD-log *D*[b] at pH 6.8
1	*n*-Bu		26.2	0.014	3.63
3a		HCl	67.9	0.573	2.42
9a			111.0	0.945	−0.03
9b			>300	>1.05	−0.90
9c			>300	0.935	0.41
3b			5.7	0.009	4.22
3c		HCl	>300	>1.02	1.06
5			>300	>1.12	−0.87
4a		3HCl	>300	>0.978	0.18
4b		3HCl	>300	>1.02	0.95
6d		3HCl	32.8	>1.12	2.88
3d		*p*-TsOH	38.2	0.240	2.37
3e		2HCl	21.2	0.783	2.51

[a] IC_{50} value for 20-HETE production from AA by human renal microsome ($n = 2$).
[b] ACD-log *D* was calculated by ACD/log *P* DB version 5.0, Advanced Chemistry Development Inc.

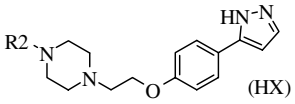
higher affinity for more lipophilic compounds.[19] Accordingly, 20-HETE synthase, which is a member of the CYP family, should require appropriate lipophilicity for its substrate and inhibitors. On the other hand, it is well known that increased lipophilicity is generally associated with a decrease in water-solubility.[20] This information suggests that a suitable range of lipophilicity is needed to improve of the water-solubility of **1** while retaining 20-HETE synthase inhibitory activity. The preferable lipophilicity of the inhibitor is estimated to have an ACD-log *D* value of between 2 and 3.

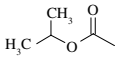
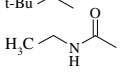
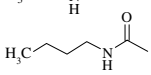
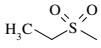
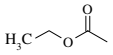
As the next step in the optimization of the compounds, the substituent at the 4-position of the piperazine ring of **3e** was examined. The ACD-log *D* values of the compounds were calculated before synthesis, and compounds with an ACD-log *D* value between 2 and 3 were examined.

The solubilities of the isopropyl carbamate (**3f**) and *tert*-butyl carbamate (**3g**) derivatives were slightly less than that of the ethyl carbamate derivative (**3e**) in accordance with the slight increase in lipophilicity, however, these compounds showed drastically decreased activity. The slight difference in the lipophilicity of these compounds can not explain these results, and accordingly the introduction of a bulky group at this position should be responsible for the loss of activity. In the case of amide derivatives **6a**–**c**, the introduction of a bulky substituent resulted in a loss of activity as in the case of carbamate derivatives. The introduction of a pivaloyl group (**6c**) resulted in an almost complete loss of activity, while acetyl (**6a**) and butyryl (**6b**) derivatives maintained potent activity. These results indicate that the introduction of a branched alkyl group around the 4-position of the piperazine ring should result in a decrease in activity, and suggest that there should be steric restriction around the corresponding 20-HETE synthase active site. Substitution of the carbamate group of **3e** with a group other than an amide group, such as a urea (**3h**,**i**) or sulfonamide (**3j**) group, was also shown to be associated with potent activity, even though the potency of the urea derivatives was less than that of the corresponding carbamate derivatives due to the decrease in lipophilicity.

Further modification of **3e** was performed, and the results are shown in Table 3. While extension of the 1,2-ethylene group linkage between the benzene ring and the piperazine ring of **3e** to a 1,3-propylene group (**3k**) did not strongly affect activity, further extension to a

Table 2. Inhibition of AA metabolism human 20-HETE synthesizing enzyme by new heterocyclic compounds and their calculated ACD-log *D* values (2)



Compound	R^2	HX	IC_{50} (nM)[a]	Solubility (mg/mL) at pH 6.8	ACD-log *D*[b] at pH 6.8
3f		2HCl	231.0	0.684	2.86
3g			>300	0.119	3.21
6a		2HCl	22.4	1.15	1.31
6b		2HCl	14.0	0.890	2.40
6c		2HCl	>300	>1.15	2.53
3h		2HCl	>300	>1.21	0.94
3i		2HCl	49.9	0.980	2.01
3j		2HCl	72.5	0.101	1.97
3e		2HCl	21.2	0.783	2.51

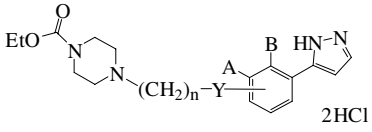
[a] IC_{50} value for 20-HETE production from AA by human renal microsome ($n = 2$).
[b] ACD-log *D* was calculated by ACD/log *P* DB version 5.0, Advanced Chemistry Development Inc.

1,4-butylene group (**3l**) resulted in a complete loss of activity. These results indicate that the distance between the benzene ring and the piperazine ring of **3e** strongly affects the activity and a methylene chain number of 2 or 3 is acceptable. The position of the piperazinoethoxy group on the benzene ring of **3e** was also found to be critically important for the activity, as in the case of related isoxazole derivatives we have reported previously,[17] based on a complete loss of activity upon moving the substituent from the 4-position to the 3-position (**12a**) or 2-position (**12b**).

Finally, the introduction of a substituent on the benzene ring of **3e** was examined. The introduction of a methyl group to the 3-position (position A) of **3e** increased the activity (**12e**; $IC_{50} = 3.2$ nM)), however, the introduction of a chlorine atom at the same position decreased the activity (**12d**; $IC_{50} = 5.6$ nM), and a fluorine atom and a methoxy group resulted in a complete loss of activity (**12c**, **12f**). The effects of the introduction of these substituents were rather complicated. These results cannot be simply explained by electrostatic ability, the size of the substituent, or the lipophilicity of the compounds. Introduction of the same substituents at the 2-position (position B) of **3e** resulted in a great decrease in activity in all of the compounds (**12g–j**). These results indicate that the spatial tolerance around the benzene ring of **3e** has a narrow range in coordination to the enzyme, especially around the B-position. In addition to the above examinations, the ether linkage of the 4-position of the benzene ring was substituted with a methylene group (**15**). In contrast to our previous results with *N*-hydroxyformamidine derivatives,[14] the activity of **15** ($IC_{50} = 502$ nM) was decreased to 1/30 of that of the corresponding ether derivative **3e**. This result also suggests the activity is sensitive to the electrostatic ability of the substituent on the benzene ring of these compounds.

Among the compounds synthesized, compounds **3e** and **6b** were selected for further investigations based to their potent activity and good solubility (Table 4). Both of

Table 3. Inhibition of AA metabolism human 20-HETE synthesizing enzyme by new heterocyclic compounds and their calculated ACD-log *D* values (3)



Compound	Position	n	Y	A	B	IC_{50} (nM)[a]	ACD-log *D*[b]
3k	4-	3	O	H	H	16.3	2.91
3l	4-	4	O	H	H	>300	3.26
12a	3-	2	O	H	H	>300	2.46
12b	2-	2	O	H	H	>300	2.36
12c	4-	2	O	F	H	>1000	2.49
12d	4-	2	O	Cl	H	85.6	3.03
12e	4-	2	O	CH_3	H	3.2	2.97
12f	4-	2	O	CH_3O	H	>300	2.19
12g	4-	2	O	H	F	>1000	3.15
12h	4-	2	O	H	Cl	>1000	3.22
12i	4-	2	O	H	CH_3	372	2.97
12j	4-	2	O	H	CH_3O	>1000	2.06
15	4-	2	CH_2	H	H	502	3.35
3e	4-	2	O	H	H	21.2	2.51

[a] IC_{50} value for 20-HETE production from AA by human renal microsome ($n = 2$).
[b] ACD-log *D* was calculated at pH 6.8 by ACD/log *P* DB version 5.0, Advanced Chemistry Development Inc.

Table 4. Effects of **3e** and **6b** on cytochrome P450 (CYP) enzymes

Compound	IC_{50} (nM) for CYP enzymes				
	1A2	2C9	2C19	2D6	3A4
3e	>100,000	>100,000	98,860	38,990	86,060
6b	>100,000	49,770	>100,000	>100,000	>100,000

them showed more than 1000-fold selectivity against major drug-metabolizing CYPs. They did not inhibit the activity of COX-1 and COX-2 at 100 μM. The log *D* value of **3e** was 2.60, and the log *D* value of **6b** was 2.22. These values corresponded with the ACD-log *D* values of them (**3e**: 2.51; **6b**: 2.40). Along with these results, pharmacological examinations were conducted in animal models of cerebrovascular disease and these indicated that **3e** and **6b** possess attractive characteristics as potential therapeutic agents. The detailed findings regarding the pharmacological properties of these compounds will be reported in a separate paper.

4. Conclusion

The introduction of a polar functional group to a prototype pyrazole derivative, the 20-HETE synthase inhibitor **1**, led to some promising compounds with potent activity and good solubility. Among them, compounds **3e** and **6b** were selected for further investigation for the treatment of cerebrovascular diseases.

5. Experimental

Melting points were determined on a Mettler FP-61 or a Yanaco MP-500D melting point apparatus. NMR spectra were recorded at 200 MHz or 300 MHz using a Varian Instruments Gemini 2000 or a Varian Instruments INOVA 300 with tetramethylsilane as an internal standard. Electron impact (EI) mass spectra were taken on a Perkin Elmer Sciex API-300 mass spectrometer. Electrospray ionization (ESI) mass spectra were taken on a Micromass Platform LC mass spectrometer. Elemental analyses were performed on EA2400 elemental analyzers, and the results were within 0.4% of calculated values. Reactions were monitored by TLC analysis using Merck silica gel 60F-254 thin-layer plates. Column chromatography was carried out on silica gel Wako Pure Chemical C-200 and NH silica gel Fuji Silicia chromatorex DM1020.

5.1. 3-[4-(3-Methoxybutoxy)phenyl]pyrazole hydrochloride (3a)

To a mixture of **2** (0.25 g, 1.57 mmol) and 3-methoxybutanol (0.294 g, 2.83 mmol; 1.8 equiv) in THF (7.5 mL) was added a mixture of triphenylphosphine (0.823 g, 3.14 mmol; 2 equiv) and diethylazodicarboxylate (0.494 mL, 3.14 mmol; 2 equiv), and the mixture was stirred at room temperature for 16 h. The reaction mixture was evaporated in vacuo and then purified by silica gel column chromatography to give free base of **3a** (0.220 g, yield: 47%), which was treated with 4 M HCl in EtOAc to give **3a** as a colorless powder: mp 133.5–136.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.14 (d, J = 6.1 Hz, 3H), 1.79–1.93 (m, 2H), 3.23 (s, 3H), 3.46–3.55 (m, 1H), 4.07 (t, J = 6.8 Hz, 2H), 6.75 (d, J = 2.3 Hz, 1H), 7.01 (d, J = 8.9 Hz, 2H) 7.77 (d, J = 8.9 Hz, 1H), 7.85 (d, J = 2.3 Hz, 1H), 9.81 (br s, 1H); MS (ESI) *m/z* 247 (M+H); Anal. ($C_{14}H_{18}N_2O_2$·HCl) C, H, N. Compounds **3b**–**m** were synthesized with the corresponding alcohols in the same way.

5.2. 3-(4-Dimethylaminophenethyloxyphenyl)pyrazole (3b)

Yield: 76%, a colorless powder: mp 112.5–113.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.94 (s, 6H), 3.02 (t, J = 7.3 Hz, 2H), 4.16 (t, J = 7.3 Hz, 2H), 6.54 (d, J = 2.2 Hz, 1H), 6.74 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 7.18 (d, J = 8.8 Hz, 2H), 7.60 (d, J = 2.2 Hz, 1H), 7.65 (d, J = 8.8 Hz, 2H); MS (ESI) *m/z* 330 (M+Na); Anal. ($C_{19}H_{21}N_3O$) C, H, N.

5.3. 3-{4-[2-(1-Piperidino)ethoxy]phenyl}pyrazole hydrochloride (3c)

Yield: 34%, a colorless powder: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38–1.68 (m, 6H), 2.38–2.59 (m, 4H), 2.80 (t, J = 6.0 Hz, 2H), 4.15 (t, J = 6.0 Hz, 2H), 6.53 (d, J = 2.3 Hz, 1H), 6.95 (d, J = 8.9 Hz, 2H), 7.59 (d, J = 2.3 Hz, 1H), 7.65 (d, J = 8.9 Hz, 2H); MS (ESI) *m/z* 272 (M+H); Anal. ($C_{16}H_{21}N_3O$·HCl·3/2H_2O) C, H, N.

5.4. 1-(4-Fluorobenzyl)-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine *p*-toluenesulfonate (3d)

Yield: 47%, a colorless powder: ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.38 (s, 3H), 6.55 (d, J = 2.2 Hz, 1H), 6.87 (d, J = 8.8 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H), 7.20–7.40 (m, 4H), 7.60 (d, J = 2.2 Hz, 1H), 7.66 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.4 Hz, 2H); MS (ESI) *m/z* 381 (M+H); Anal. ($C_{22}H_{25}FN_4O$·$C_7H_8O_3S$·3/2H_2O) C, H, N.

5.5. 1-Ethoxycarbonyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (3e)

Yield: 73%, a colorless powder: mp 217.0–218.0 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (t, J = 7.1 Hz, 3H), 2.58 (m, 4H), 2.86 (m, 2H), 3.54 (m, 4H), 4.11–4.20 (m, 2H), 4.15 (q, J = 7.1Hz, 2H), 6.55 (d, J = 2.4 Hz, 1H), 6.97 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 2.4 Hz, 1H), 7.68 (d, J = 8.6 Hz, 2H); MS (ESI) *m/z* 345 (M+H); Anal. ($C_{18}H_{24}N_4O_3$·2HCl·H_2O) C, H, N.

5.6. 1-Isopropoxycarbonyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (3f)

Yield: 43%, a colorless powder: mp 206.0–208.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (d, J = 6.2 Hz,

6H), 3.04–3.22 (m, 2H), 3.26–3.44 (m, 2H), 3.49–3.65 (m, 4H), 4.05 (d, J = 13.4 Hz, 2H), 4.48 (t, J = 4.8 Hz, 2H), 4.77–4.85 (m, 1H), 6.72 (d, J = 2.2 Hz, 1H), 7.07 (d, J = 8.9 Hz, 2H), 7.77–7.83 (m, 3H), 11.47 (br s, 1H); MS (ESI) m/z 359 (M+H); Anal. ($C_{19}H_{26}N_4O_3$·2HCl) C, H, N.

5.7. 1-*tert*-Butoxycarbonyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine (3g)

Yield: 26%, a colorless powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.53–2.56 (m, 4H), 2.84 (t, J = 5.8 Hz, 2H), 3.45–3.48 (m, 4H), 4.15 (t, J = 5.8 Hz, 2H), 6.53 (d, J = 2.3 Hz, 1H), 6.96 (m, 2H), 7.60 (d, J = 2.3 Hz, 1H), 7.67 (m, 2H); MS (ESI) m/z 373 (M+H); Anal. ($C_{20}H_{28}N_4O_3$·1/2H_2O) C, H, N.

5.8. 1-Ethylaminocarbonyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (3h)

Yield: 50%, a colorless powder: mp 138.0–144.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.02 (t, J = 7.2 Hz, 3H), 2.98–3.08 (m, 2H), 3.06 (q, J = 7.2 Hz, 2H), 3.25 (t, J = 12.4 Hz, 2H), 3.46–3.61 (m, 4H), 4.07 (d, J = 13.8 Hz, 2H), 4.49 (m, 2H), 6.80 (d, J = 2.3 Hz, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.84 (d, J = 8.9 Hz, 2H), 7.88 (d, J = 2.3 Hz, 1H), 11.45 (br s, 1H); MS (ESI) m/z 344 (M+H); Anal. ($C_{18}H_{25}N_5O_2$·2HCl·2H_2O) C, H, N.

5.9. 1-Butylaminocarbonyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (3i)

Yield: 85%, a colorless powder: mp 202.0–204.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, J = 7.23 Hz, 3H), 1.09–1.53 (m, J = 133.05 Hz, 4H), 2.99–3.23 (m, 6H), 3.51–3.63 (m, 4H), 3.97–4.14 (m, 2H), 4.40–4.46 (m, 2H), 6.64 (d, J = 2.18 Hz, 1H), 7.05 (d, J = 8.86 Hz, 2H), 7.69 (d, J = 2.33 Hz, 1H), 7.76 (d, J = 8.70 Hz, 2H); MS (ESI) m/z 372 (M+H); Anal. ($C_{20}H_{29}N_5O_2$·2HCl·H_2O) C, H, N.

5.10. 1-Ethylsulfonyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (3j)

Yield: 69%, a colorless powder: mp 196.0–198.0 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.23 (t, J = 7.4 Hz, 3H), 3.13–3.84 (m, 10H), 3.19 (q, J = 7.4 Hz, 2H), 4.44–4.51 (m, 2H), 6.69 (d, J = 2.3 Hz, 1H), 7.07 (d, J = 8.9 Hz, 2H), 7.74 (d, J = 2.2 Hz, 1H), 7.78 (d, J = 8.9 Hz, 2H), 11.45 (br s, 1H); MS (ESI) m/z 387 (M+Na); Anal. ($C_{17}H_{24}N_4O_3S$·2HCl·H_2O) C, H, N.

5.11. 1-Ethoxycarbonyl-4-{3-[4-(pyrazol-3-yl)phenyl]propoxy}piperazine dihydrochloride (3k)

Yield: 22%, a colorless powder: mp 202.0–203.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (t, J = 7.2 Hz, 3H), 3.64 (br t, J = 4.8 Hz, 2H), 4.08 (q, J = 7.2 Hz, 2H), 4.56 (br t, J = 4.8 Hz, 2H), 6.81 (d, J = 2.2 Hz, 1H), 7.10 (m, 1H), 7.20 (d, J = 7.7 Hz, 1H), 7.40 (m, 1H), 7.79 (dd, J = 1.8, 7.7 Hz, 1H), 7.84 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 381 (M+Na); Anal. ($C_{19}H_{26}N_4O_3$·2HCl·1/2H_2O) C, H, N.

5.12. 1-Ethoxycarbonyl-4-{4-[4-(pyrazol-3-yl)phenyl]butoxy}piperazine dihydrochloride (3l)

Yield: 4%, colorless amorphous: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.2 Hz, 3H), 3.17 (m, 2H), 3.35 (m, 2H), 3.56 (m, 4H), 4.05–4.10 (m, 2H), 4.08 (q, J = 7.2 Hz, 2H), 4.48 (t, J = 5.0 Hz, 2H), 6.74 (d, J = 2.2 Hz, 1H), 6.94 (dd, J = 1.6, 8.0 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 7.44–7.46 (m, 2H), 7.74 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 373 (M+H). HPLC: Purity 95.08%.

5.13. 3-[4-(2-Chloroethoxy)phenyl]pyrazole (3m)

72% yield, colorless powder: ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.85 (t, J = 5.5 Hz, 2H), 4.28 (t, J =5.5 Hz, 2H), 6.58 (d, J = 2.0 Hz, 1H), 6.98 (m, J_{AB} = 9.5 Hz, 2H), 7.61 (d, J = 2.0 Hz, 1H), 7.69 (m, J_{AB} = 9.5 Hz, 2H).

5.14. 3-{4-[2-(4-Methylpiperazino)ethoxy]phenyl}pyrazole trihydrochloride (4a)

To a solution of **3m** (0.3 g, 0.35 mmol) in DMF (3 mL) was added *N*-methylpiperazine (0.406 g, 4.06 mmol, 3 equiv) and triethylamine (0.411 g, 4.06 mmol, 3 equiv) and the mixture was stirred for 9 h at 120 °C. The reaction mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 1:5) to give 0.374 g (yield: 97%) of free base of **4a** as a colorless oil, which was treated with 4 M HCl in EtOAc to give **4a** as a colorless powder: mp 159.0–163.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.84 (s, 3H), 3.43–3.92 (m, 10H), 4.42–4.51 (m, 2H), 6.69 (d, J = 2.2 Hz, 1H), 7.08 (d, J = 8.9 Hz, 2H), 7.74 (d, J = 2.2 Hz, 1H), 7.78 (d, J = 8.9 Hz, 2H), 11.95 (br s, 1H); MS (ESI) m/z 287 (M+H); Anal. ($C_{16}H_{22}N_4O$·3HCl·3H_2O) C, H, N.

5.15. 1-Isopropyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine trihydrochloride (4b)

Yield: 57%, a colorless powder: mp 215.0–220.0 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 (d, J = 6.5 Hz, 6H), 3.48–3.93 (m, 11H), 4.41–4.50 (m, 2H), 6.70 (d, J = 2.2 Hz, 1H), 7.09 (d, J = 9.0 Hz, 2H), 7.76 (d, J = 2.3 Hz, 1H), 7.79 (d, J = 8.9 Hz, 2H), 11.96 (br s, 1H); MS (ESI) m/z 315 (M+H); Anal. ($C_{18}H_{26}N_4O$·3HCl·2H_2O) C, H, N.

5.16. 1-{2-[4-(Pyrazol-3-yl)phenyl]ethoxy}piperazine (5)

To a solution of **3g** (5 g, 13.42 mmol, 1 equiv) in THF (25 mL) was added 4 M HCl in EtOAc (33.6 mL, 10 equiv). The resulting mixture was stirred at room temperature for 4 h, and 5 N NaOH (26 mL) was then added to the reaction mixture, and extracted with chloroform. The organic layer was dried over magnesium sulfate and evaporated in vacuo. The residue was recrystallized from diethyl ether to give 3.04 g (yield: 83%) of **5** as a slightly red powder. Mp 148.0–151.0 °C; ^{1}H NMR

(200 MHz, $CDCl_3$) δ 2.55–2.59 (m, 4H), 2.82 (t, J = 5.9 Hz, 2H), 2.91–2.96 (m, 4H), 4.15 (t, J = 5.9 Hz, 2H), 6.54 (d, J = 2.2 Hz, 1H), 6.96 (m, 2H), 7.60 (d, J = 2.2 Hz, 1H), 7.66 (m, 2H); MS (ESI) m/z 273 (M+H); Anal. ($C_{15}H_{20}N_4O\cdot 1/4H_2O$) C, H, N.

5.17. 1-Acetyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine (6a)

To a solution of **5** (0.25 g, 0.925 mmol) in DMF (7.5 mL) was added acetyl chloride (0.087 g, 1.02 mmol, 1.1 equiv), and the mixture was stirred at 0 °C for 2 h. To the reaction mixture was added 5 N NaOH (2 mL), and the mixture was extracted with toluene. The organic layer was dried over magnesium sulfate, evaporated in vacuo, and then purified by NH silica gel column chromatography (eluent: chloroform/methanol = 10:1) to give free base of **6a** (0.10 g, yield: 35%) as a colorless amorphous, which was treated with 4 M HCl in EtOAc to give **6a** as a colorless powder. Mp 221.5–225.0 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.05 (s, 3H), 2.96–3.28 (m, 3H), 3.47–3.67 (m, 5H), 3.93–4.07 (m, 1H), 4.35–4.47 (m, 1H), 4.48 (t, J = 4.9 Hz, 2H), 6.72 (d, J = 2.3 Hz, 1H), 7.06 (d, J = 9.0 Hz, 2H), 7.76–7.83 (m, 3H), 11.52 (br s, 1H); MS (ESI) m/z 315 (M+H); Anal. ($C_{17}H_{22}N_4O_2\cdot 2HCl\cdot H_2O$) C, H, N.

5.18. 1-Propionyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (6b)

Yield: 60%, a colorless powder: mp 204.0–207.0 °C (dec); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.97 (t, J = 7.5 Hz, 3H), 1.66 (sext, J = 7.5 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.54–2.64 (m, 4H), 2.85 (t, J = 5.6 Hz, 2H), 3.51 (t, J = 5.0 Hz, 2H), 3.67 (t, J = 5.0 Hz, 2H), 4.15 (t, J = 5.6 Hz, 2H), 6.54 (d, J = 2.3 Hz, 1H), 6.95 (m, 2H), 7.60 (d, J = 2.3 Hz, 1H), 7.67 (m, 2H); MS (ESI) m/z 343 (M+H); Anal. ($C_{19}H_{26}N_4O_2\cdot 2HCl\cdot 3/2H_2O$) C, H, N.

5.19. 1-Pivaloyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine (6c)

To a mixture of **5** (0.248 g, 0.918 mmol), pivaric acid (0.094 g, 0.918 mmol, 1 equiv) and HOBt 0.149 g (1.10 mmol, 1.2 equiv) in DMF (1.5 mL) was added WSC·HCl (0.211 g, 10 mmol, 1.2 equiv), and the mixture was stirred at room temperature for 19 h. To the reaction mixture was added 5 N NaOH solution (2 mL), and the mixture was extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, evaporated in vacuo, and then purified by silica gel column chromatography (eluent: chloroform/methanol = 10:1) to give free base of **6c** (0.26 g, yield: 80%) as a colorless powder, which was treated with 4 M HCl in EtOAc to give **6c** as a colorless powder: mp 272.0–273.0 °C (dec); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.210 (s, 9H), 3.01–3.65 (m, 8H), 4.34–4.52 (m, 4H), 6.69 (d, J = 2.2 Hz, 1H), 7.07 (m, J_{AB} = 8.8 Hz, 2H), 7.75 (d, J = 2.2 Hz, 1H), 7.78 (m, J_{AB} = 8.8 Hz, 2H), 11.43 (br s, 1H); MS (ESI) m/z 379 (M+Na); Anal. ($C_{20}H_{28}N_4O_2\cdot 2HCl\cdot 1/2H_2O$) C, H, N.

5.20. 1-Cyclohexylmethyl-4-{2-[4-(pyrazol-3-yl)phenyl]ethoxy}piperazine trihydrochloride (6d)

To a mixture of **5** (0.25 g, 0.925 mmol), acetic acid 0.167 g (2.77 mmol, 3 equiv) and THF (1.5 mL) were added in succession cyclohexanecarbaldehyde (0.156 g, 1.39 mmol, 1.5 equiv) and sodium triacetoxyborohydride (0.588 g, 2.77 mmol, 3 equiv), and the mixture was then stirred at room temperature for 2.5 h. To the reaction mixture was added 5 N NaOH (2 mL), and the mixture was extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, evaporated in vacuo, and purified by silica gel column chromatography (eluent: chloroform/methanol = 10:1) to give free base of **6d** (0.285 g, yield: 84%) as a colorless powder, which was treated with 4 M HCl in EtOAc to give **6d** as a colorless powder: mp 272.0–273.0 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86–1.04 (m, 2H), 1.07–1.31 (m, 3H), 1.55–1.96 (m, 6H), 3.00 (br s, 2H), 3.36–3.92 (m, 10H), 4.45–4.51 (m, 2H), 6.72 (d, J = 2.2 Hz, 1H), 7.08 (d, J = 8.9 Hz, 2H), 7.78 (d, J = 2.2 Hz, 1H), 7.80 (d, J = 9.0 Hz, 2H), 11.38 (br s, 1H); MS (ESI) m/z 369 (M+H); Anal. ($C_{22}H_{32}N_4O\cdot 3HCl\cdot 2H_2O$) C, H, N.

5.21. 4′-(4-Dimethylaminobutoxy)acetophenone (8a)

To a stirred suspension of sodium hydride (60% in oil, 1.74 g, 43.5 mmol, 3 equiv) in DMF (29 mL) was added a solution of 4-dimethylamino-1-butanol (5.10 g, 43.5 mmol, 3 equiv) in DMF (7.2 mL) at room temperature. The reaction mixture was stirred at room temperature for 30 min, and 4′-fluoroacetophenone (2 g, 14.5 mmol, 1 equiv) was then added to the mixture at 0 °C. After stirring for 3 h, the reaction mixture was diluted with water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, evaporated in vacuo, and purified by NH silica gel column chromatography (eluent: hexane/ethyl acetate = 2:1) to give 2.03 g (yield: 60%) of **8a** as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.56–1.94 (m, 4H), 2.24 (s, 6H), 2.33 (t, J = 7.0 Hz, 2H), 2.56 (s, 3H), 4.05 (t, J = 6.0 Hz, 2H), 6.93 (m, J_{AB} = 9.5 Hz, 2H), 7.93 (m, J_{AB} = 9.5 Hz, 2H). Compounds **8b** and **8c** were synthesized in the same way.

5.22. 4′-[2-(2-Dimethylaminoethoxy)ethoxy]acetophenone (8b)

Yield: 36%, a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.28 (s, 6H), 2.55 (t, J = 5.5 Hz, 2H), 2.58 (s, 3H), 3.66 (t, J = 6.0 Hz, 2H), 3.85 (t, J = 5.0 Hz, 2H), 4.21 (t, J = 5.0 Hz, 2H), 6.95 (m, J_{AB} = 8.5 Hz, 2H), 7.93 (m, J_{AB} = 8.5 Hz, 2H).

5.23. 4′-{2-[*N*,*N*-(2-Dimethylaminoethyl)methylamino]ethoxy}acetophenone (8c)

Yield: 43%, a light yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.25 (s, 6H), 2.38–2.49 (m, 2H), 2.39 (s, 3H), 2.54–2.68 (m, 2H), 2.56 (s, 3H), 2.83–2.93 (m, 2H), 4.14 (t, J = 6.0 Hz, 2H), 6.94 (m, J_{AB} = 9.5 Hz, 2H), 7.94 (m, J_{AB} = 9.5 Hz, 2H).

5.24. 3-[4-(4-Dimethylaminobutoxy)phenyl]pyrazole (9a)

Ethyl formate (0.378 g, 5.10 mmol; 4 equiv) was added to a suspension of sodium hydride (60% in oil, 0.101 g, 2.54 mmol, 2 equiv) in THF (1 mL) at room temperature. After stirring for 10 min, a solution of **8a** (0.3 g, 1.27 mmol, 1 equiv) in THF (2 mL) was added, and the mixture was stirred for an additional 30 min. To the reaction mixture was then added 1 M HCl (2.7 mL), and the mixture was separated. The aqueous layer was washed with diethyl ether, hydrazine monohydrate (0.636 g, 12.7 mmol, 10 equiv) was added, and the mixture was stirred for 30 min. The reaction mixture was made alkaline by adding of 6 N NaOH (2 mL) and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, evaporated in vacuo, and purified by NH silica gel column chromatography (eluent: ethyl acetate) to give a colorless solid, which was recrystallized from hexane/ethyl acetate to give 0.19 g (yield: 58%) of **9a** as a colorless powder. Mp 76.0–78.0 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.60–1.91 (m, 4H), 2.25 (s, 6H), 2.34 (t, J = 7.3 Hz, 2H), 4.02 (t, J = 6.2 Hz, 2H), 6.54 (d, J = 2.2 Hz, 1H), 6.95 (m, J_{AB} = 8.8 Hz, 2H), 7.60 (d, J = 2.2 Hz, 1H), 7.65 (m, J_{AB} = 8.6 Hz, 2H); MS (ESI) m/z 260 (M+H); Anal. ($C_{15}H_{21}N_3O\cdot1/4H_2O$) C, H, N. Compounds **9b** and **9c** were prepared in the same way.

5.25. 3-{4-[2-(2-Dimethylaminoethoxy)ethoxy]phenyl}pyrazole (9b)

Yield: 71%, a brown powder: mp 130.0–138.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.76 (s, 3H), 2.78 (s, 3H), 3.28 (q, J = 5.2 Hz, 2H), 3.78–3.89 (m, 4H), 4.15–4.24 (m, 2H), 6.75 (d, J = 2.2 Hz, 1H), 7.02 (m, J_{AB} = 8.8 Hz, 2H), 7.79 (m, J_{AB} = 8.6 Hz, 2H), 7.84 (d, J = 2.2 Hz, 1H), 10.52 (br s, 1H); MS (ESI) m/z 276 (M+H); Anal. ($C_{15}H_{21}N_3O_2\cdot2HCl\cdot1/2H_2O$) C, H, N.

5.26. 3-{4-[2-(N-Methyl-2-dimethylaminoethylamino)ethoxy]phenyl}pyrazole (9c)

Yield: 26%, a colorless powder: mp 38.5–41.0 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.30 (s, 6H), 2.39 (s, 3H), 2.49 (m, 2H), 2.65 (m, 2H), 2.87 (t, J = 5.9 Hz, 2H), 4.13 (t, J = 5.9 Hz, 2H), 6.55 (d, J = 2.2 Hz, 1H), 6.96 (d, J = 8.8 Hz, 2H), 7.60 (d, J = 2.2 Hz, 1H), 7.66 (d, J = 8.8 Hz, 2H); MS (ESI) m/z 311 (M+Na); Anal. ($C_{16}H_{24}N_4O\cdot1/2H_2O$) C, H, N.

5.27. 1-Ethoxycarbonyl-4-{3-[4-(pyrazol-3-yl)phenyl]propyl}piperazine dihydrochloride (15)

Yield: 82%, a light yellow powder: mp 175.0–177.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.20 (t, J = 7.03 Hz, 3H), 1.91–2.19 (m, 2H), 2.67 (t, J = 7.47 Hz, 2H), 2.85–3.15 (m, 4H), 3.23–3.55 (m, 4H), 3.93–4.07 (m, 2H), 4.08 (q, J = 7.18 Hz, 2H), 6.72 (d, J = 2.20 Hz, 1H), 7.30 (d, J = 8.35 Hz, 2H), 7.73–7.81 (m, 3H), 11.16 (br s, 1H); MS (ESI) m/z 343 (M+H); Anal. ($C_{19}H_{26}N_4O_2\cdot2HCl\cdot H_2O$) C, H, N.

5.28. 1-Ethoxycarbonyl-4-{2-[3-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12a)

A mixture of **11a** (0.50 g, 1.56 mmol), which was prepared from **10a** by Mitsunobu alkylation, and dimethylaminomethyl-*tert*-butylether (1.36 g, 7.8 mmol; 5 equiv) was stirred at 90 °C for 30 min. The reaction mixture was then cooled to room temperature and immediately purified by silica gel column chromatography. The resulting yellow oil was diluted with THF (10 mL) and hydrazine monohydrate (0.32 g, 6.39 mmol; 4.1 equiv) was added. The mixture was then stirred at room temperature for 16 h. The reaction mixture was diluted with brine and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, evaporated in vacuo, and purified by NH silica gel column chromatography to give free base of **12a** (0.325 g, yield: 61%), which was treated with 4 M HCl in EtOAc to give **12a** as a colorless powder: mp 133.5–136.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.2 Hz, 3H), 3.05–3.63 (m, 8H), 4.01–4.10 (m, 2H), 4.08 (q, J = 7.2 Hz, 2H), 4.44–4.52 (m, 2H), 6.74 (d, J = 2.2 Hz, 1H), 6.87–6.98 (m, 1H), 7.36 (t, J = 8.0 Hz, 2H), 7.42–7.48 (m, 2H), 7.71–7.76 (m, 1H), 11.10 (br s, 1H); MS (ESI) m/z 345 (M+H); Anal. ($C_{18}H_{24}N_4O_3\cdot2HCl\cdot 3/2H_2O$) C, H, N. Compounds **12b–j** were synthesized in the same way.

5.29. 1-Ethoxycarbonyl-4-{2-[2-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12b)

Yield: 42%, a colorless powder: ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.20 (t, J = 7.2 Hz, 3H), 3.01–3.98 (m, 8H), 3.64 (br t, J = 4.8 Hz, 2H), 4.08 (q, J = 7.2 Hz, 2H), 4.56 (br t, J = 4.8 Hz, 2H), 6.81 (d, J = 2.2 Hz, 1H), 7.10 (m, 1H), 7.20 (d, J = 7.7 Hz, 1H), 7.35–7.44 (m, 1H), 7.79 (dd, J = 1.8, 7.7 Hz, 1H), 7.84 (d, J = 2.2 Hz, 1H), 11.75 (br s, 1H); MS (ESI) m/z 367 (M+H); Anal. ($C_{18}H_{24}N_4O_3\cdot2HCl\cdot1/2H_2O$) C, H, N.

5.30. 1-Ethoxycarbonyl-4-{2-[2-fluoro-4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12c)

Yield: 83%, a colorless powder: mp 207.0–208.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.07 Hz, 3H), 3.07–3.66 (m, 8H), 3.99–4.14 (m, 2H), 4.09 (q, J = 7.15 Hz, 2H), 4.51–4.59 (m, 2H), 6.74 (d, J = 2.33 Hz, 1H), 7.29 (t, J = 8.70 Hz, 1H), 7.61–7.77 (m, 3H), 11.49 (br s, 1H); MS (ESI) m/z 363 (M+H); Anal. ($C_{18}H_{23}FN_4O_3\cdot2HCl$) C, H, N.

5.31. 1-Ethoxycarbonyl-4-{2-[2-chloro-4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12d)

Yield: 59%, a colorless powder: mp 202.0–203.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.2 Hz, 3H), 3.13–3.48 (m, 4H), 3.53–3.66 (m, 4H), 4.00–4.14 (m, 4H), 4.57 (br t, J = 4.8 Hz, 2H), 6.76 (d, J = 2.3 Hz, 1H), 7.26 (d, J = 8.7 Hz, 1H), 7.75 (d, J = 2.2 Hz, 1H), 7.78 (dd, J = 2.2, 8.5 Hz, 1H), 7.91 (d, J = 2.0 Hz, 1H); MS (ESI) m/z 379 (M+H); Anal. ($C_{18}H_{23}ClN_4O_3\cdot2HCl\cdot H_2O$) C, H, N.

5.32. 1-Ethoxycarbonyl-4-{2-[2-methyl-4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12e)

Yield: 32%, a colorless powder: mp 203.5–204.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.2 Hz, 3H), 2.24 (s, 3H), 3.10–3.45 (m, 4H), 3.50–3.65 (m, 4H), 4.03–4.16 (m, 4H), 4.44 (t, J = 4.5 Hz, 2H), 6.64 (d, J = 2.2 Hz, 1H), 7.01 (d, J = 8.2 Hz, 2H), 7.58–7.65 (m, 2H), 7.70 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 359 (M+H); Anal. ($C_{19}H_{26}N_4O_3\cdot 2HCl\cdot H_2O$) C, H, N.

5.33. 1-Ethoxycarbonyl-4-{2-[2-methoxy-4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12f)

Yield: 71%, a colorless powder: mp 189.5–191.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.2 Hz, 3H), 3.08–3.25 (m, 2H), 3.28–3.45 (m, 2H), 3.51–3.68 (m, 4H), 3.86 (s, 3H), 4.00–4.15 (m, 4H), 4.44 (br t, J = 4.8 Hz, 2H), 6.75 (d, J = 2.2 Hz, 1H), 7.10 (d, J = 8.2 Hz, 1H), 7.38 (dd, J = 1.9, 8.2 Hz, 1H), 7.49 (d, J = 1.9 Hz, 1H), 7.77 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 375 (M+H); Anal. ($C_{19}H_{26}N_4O_4\cdot 2HCl\cdot H_2O$) C, H, N.

5.34. 1-Ethoxycarbonyl-4-{2-[3-fluoro-4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12g)

Yield: 91%, a colorless powder: mp 199.5–201.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.10 Hz, 3H), 3.04–3.43 (m, 4H), 3.49–3.61 (m, 4H), 3.99–4.05 (m, 2H), 4.09 (q, J = 7.10 Hz, 2H), 4.42–4.51 (m, 2H), 6.55–6.59 (m, 1H), 6.91–7.06 (m, 2H), 7.75 (d, J = 2.18 Hz, 1H), 7.87 (t, J = 8.86 Hz, 1H), 11.02 (br s, 1H); MS (ESI) m/z 363 (M+H); Anal. ($C_{18}H_{23}FN_4O_3\cdot 2HCl\cdot 1/2H_2O$) C, H, N.

5.35. 1-Ethoxycarbonyl-4-{2-[3-chloro-4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12h)

Yield: 85%, a colorless powder: mp 193.0–195.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.07 Hz, 3H), 3.07–3.40 (m, 4H), 3.50–3.63 (m, 4H), 4.03–4.10 (m, 2H), 4.09 (q, J = 7.05 Hz, 2H), 4.44–4.51 (m, 2H), 6.65 (d, J = 2.18 Hz, 1H), 7.07 (dd, J = 8.78, 2.56 Hz, 1H), 7.21 (d, J = 2.64 Hz, 1H), 7.71 (d, J = 8.70 Hz, 1H), 7.75 (d, J = 2.18 Hz, 1H), 10.88 (br s, 1H); MS (ESI) m/z 379 (M+H); Anal. ($C_{18}H_{23}ClN_4O_3\cdot 2HCl\cdot 1/2H_2O$) C, H, N.

5.36. 1-Ethoxycarbonyl-4-{2-[3-methyl-4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12i)

Yield: 83%, a colorless powder: mp 196.5–199.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.15 Hz, 3H), 2.41 (s, 3H), 3.03–3.63 (m, 8H), 4.01–4.10 (m, 2H), 4.09 (q, J = 7.15 Hz, 2H), 4.42 (m, 2H), 6.44 (d, J = 2.18 Hz, 1H), 6.87–6.95 (m, 2H), 7.46 (d, J = 8.39 Hz, 1H), 7.71 (d, J = 2.18 Hz, 1H), 10.89 (br s, 1H); MS (ESI) m/z 359 (M+H); Anal. ($C_{19}H_{26}N_4O_3\cdot 2HCl\cdot H_2O$) C, H, N.

5.37. 1-Ethoxycarbonyl-4-{2-[3-methoxy-4-(pyrazol-3-yl)phenyl]ethoxy}piperazine dihydrochloride (12j)

Yield: 69%, a colorless powder: mp 185.0–186.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (t, J = 7.07 Hz, 3H), 3.04–3.64 (m, 8H), 3.89 (s, 3H), 4.05–4.09 (m, 2H), 4.09 (q, J = 7.10 Hz, 2H), 4.49 (m, 2H), 6.66–6.80 (m, 3H), 7.71–7.79 (m, 2H), 11.32 (br s, 1H); MS (ESI) m/z 375 (M+H); Anal. ($C_{19}H_{26}N_4O_4\cdot 2HCl\cdot H_2O$) C, H, N.

5.38. 1-Ethoxycarbonyl-4-[3-(4-acetylphenyl)propyl]piperazine (14)

N-Ethoxycarbonylpiperazine (1.513 g, 9.56 mmol, 1 equiv) and cesium carbonate (6.40 g, 19.6 mmol, 2.05 equiv) were added to a solution of **13** (2.81 g, 11.66 mmol, 1.22 equiv) in DMF (15 mL), and the mixture was stirred at room temperature for 16 h.The reaction mixture was diluted with ethyl acetate (200 mL) after insoluble material was removed by filtration, washed with brine, concentrated in vacuo, and then purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 4:1 to ethyl acetate) to give 2.00 g (yield: 66%) of **14** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, J = 6.5 Hz, 3H), 1.84 (m, 2H), 2.31–2.44 (m, 6H), 2.59 (s, 3H), 2.70 (t, J = 7.0 Hz, 2H), 3.41–3.56 (m, 4H), 4.14 (q, J = 6.5 Hz, 2H), 7.28 (d, J = 7.5 Hz, 2H), 7.88 (d, J = 8.0 Hz, 2H).

5.39. 4-[3-(*N*′-Ethoxycarbonylpiperazino)propyl]-1-(3-pyrazolyl)benzene (15)

This compound was prepared from **14** (0.732 g, 2.30 mmol) as described in the procedure for synthesizing **9a** to give 0.646 g (1.89 mmol, 82%) of **15** as a light yellow amorphous (2HCl salt, light yellow powder): mp 175.0–177.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.20 (t, J = 7.03 Hz, 3H), 1.91–2.19 (m, 2H), 2.67 (t, J = 7.47 Hz, 2H), 2.85–3.15 (m, 4H), 3.23–3.55 (m, 4H), 3.93–4.07 (m, 2H), 4.08 (q, J = 7.18 Hz, 2H), 6.72 (d, J = 2.20 Hz, 1H), 7.30 (d, J = 8.35 Hz, 2H), 7.73–7.81 (m, 3H), 11.16 (br s, 1H); MS (ESI) m/z 343 (M+H); Anal. ($C_{19}H_{26}N_4O_2\cdot 2HCl\cdot H_2O$) C, H, N.

6. Measurement of the inhibition of 20-HETE formation from AA

Test compounds were dissolved in DMSO, diluted with 50 mM MOPS/5 mM $MgCl_2$/1 mM EDTA (pH 7.4) buffer (final concentration of DMSO: 1%), and incubated with human renal microsome (HCCC, Laurel, MD, USA) (100 μg/mL) at 37 °C for 5 min. [^{3}H]-Arachidonic acid (2 μC/mL) and NADPH (1 mM) were added to the mixture, which was then incubated at 37 °C for 10 min. The reaction was terminated by adding formic acid (pH 3.5). Metabolites of AA were separated on column filled with ODS and the radioactivity of the eluate containing [^{3}H]-20-HETE was measured by a liquid scintillation counter. IC_{50} values were determined by curve-fitting and parameter estimation using Origin 6.0J (OriginLab Corp., MA, USA).

7. Measurement of solubility

About 2 mg of each compound was added to 2 mL of 20 mM phosphate buffer (pH 6.8), which was then shaken at room temperature for 24 h. The suspension was then centrifuged for 10 min at 11,000 rpm. The supernatant was diluted with 50% aqueous acetonitrile for HPLC analysis. The concentration of the compounds was determined by a Shimadzu HPLC system composed of an LC-10AD, SPD-10AV, and SIL-10A. The conditions for HPLC were as follows: mobile phase, 10 mmol/L aqueous ammonium acetate solution/acetonitrile = 45:55; flow rate, 1.0 mL/min; column, reverse-phase (Capcell Pak UG120, 4.6 mm I.D. + 150 mm; Shiseido) at 40 °C; and detection wavelength, 255 nm.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.08.047.

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