Articles

Design, Synthesis, and Structure−Activity Relationships of a Series of 3-[2-(1-Benzylpiperidin-4-yl)ethylamino]pyridazine Derivatives as Acetylcholinesterase Inhibitors

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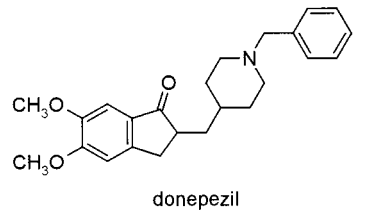
Starting from the 3-[2-(1-benzylpiperidin-4-yl)ethylamino]-6-phenylpyridazine **1**, we performed the design, the synthesis, and the structure−activity relationships of a series of pyridazine analogues acting as AChE inhibitors. Structural modifications were achieved on four different parts of compound **1** and led to the following observations: (i) introduction of a lipophilic environment in the C-5 position of the pyridazine ring is favorable for the AChE-inhibitory activity and the AChE/BuChE selectivity; (ii) substitution and various replacements of the C-6 phenyl group are possible and led to equivalent or slightly more active derivatives; (iii) isosteric replacements or modifications of the benzylpiperidine moiety are detrimental to the activity. Among all derivatives prepared, the indenopyridazine derivative **4g** was found to be the more potent inhibitor with an IC_{50} of 10 nM on electric eel AChE. Compared to compound **1**, this represents a 12-fold increase in potency. Moreover**,** 3-[2-(1-benzylpiperidin-4-yl)-ethylamino]-5-methyl-6-phenylpyridazine **4c**, which showed an IC_{50} of 21 nM, is 100-times more selective for human AChE (human BuChE/AChE ratio of 24) than the reference compound tacrine.

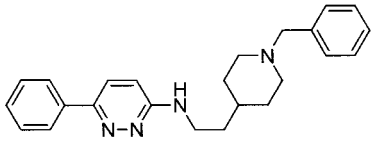
Introduction

The neurodegenerative Alzheimer's disease (AD) affecting mainly aging populations in industrialized nations is characterized by three major pathological signs: β-amyloid plaques, neurofibrillary tangles, and synaptic loss.[1,2] A deficiency in cholinergic neurotransmission is considered to be one of the major causes of memory impairments in patients.[3,4] A palliative treatment of AD is possible by the use of agents that restore the level of acetylcholine.[5] Acetylcholinesterase (AChE) inhibitors such as tacrine,[6] donepezil[7] (Chart 1), galanthamine,[8] and rivastigmine[9] are able to enhance memory in AD patients. Recent studies[10,11] have shown that AChE inhibitors, which interact with both peripheral and active sites of the enzyme,[12,13]could in addition to AChE inhibition act as potential inhibitors of the formation of βA4-amyloid protein (βAP). Thus, such AChE inhibitors with central bioavailabality represent still a promising approach to the treatment of AD. In a previous paper,[14] we reported the synthesis and the biochemical evaluation of a series of 3-aminoalkyl-6-arylpyridazine derivatives based on the structure of minaprine,[15] an original lead compound with antidepressive properties. Among all the derivatives investigated, 3-[2-(1-benzylpiperidin-4-yl)ethylamino]-6-phenylpyridazine **1** (Chart 1) was found to be one of the most potent AChE inhibitors (IC_{50} = 120 nM, electric eel) with a selectivity profile more favorable than tacrine but less than donepezil.

Chart 1



donepezil



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In the present study, we describe the design, the synthesis (Schemes 1−4), and the structure−activity relationships (SAR) (Tables 1−5) of a series of 3-[2-(1-benzylpiperidin-4-yl)ethylamino]pyridazine analogues prepared with the aim of increasing the potency of compound **1** and its selectivity toward AChE inhibition versus BuChE inhibition.

Design

The crystallographic structure of AChE from *Torpedo californica*[16] has been well-known for 10 years. More-

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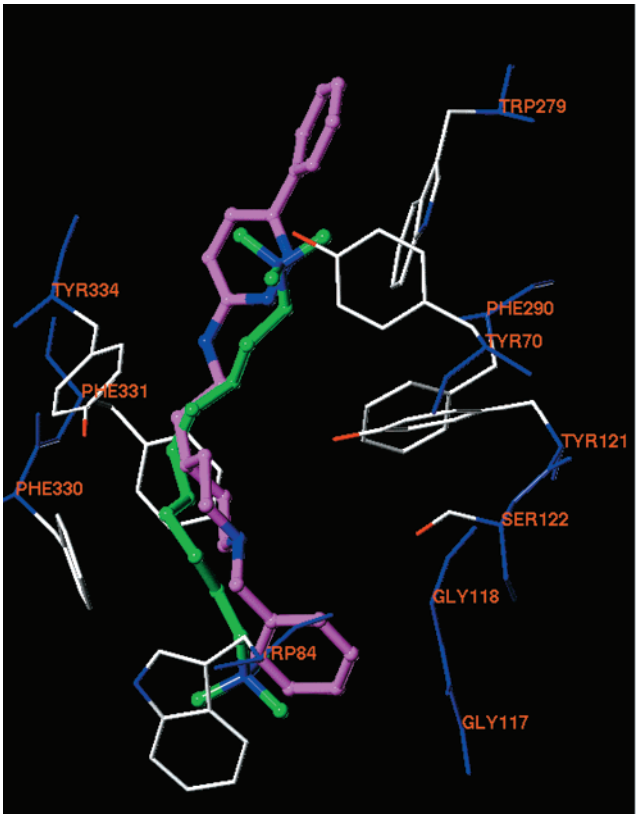
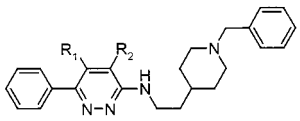


Figure 1. Comparison between the predicted position of compound **1** (magenta) and the X-ray structure of decamethonium (green) are shown. (Nitrogen atoms are colored blue.) Only the amino acid residues of the binding pocket are displayed for clarity.

over, X-ray structures of the enzyme complexed with inhibitors made the binding site characterization possible.[17−20] These studies showed that AChE possesses two distinct binding sites for the substrate acetylcholine: an active site (catalytic site) and a peripheral site (allosteric site). The peripheral site is localized at the entrance of a deep and narrow channel (about 20 Å long and as narrow as 4.5 Å). This gorge, which is lined with aromatic residues (ca. 40% of the residues present in the binding pocket), leads to the active site near the bottom. We used the X-ray structure of AChE to investigate the possible binding modes of our inhibitors. A preliminary molecular docking study was carried out (as described in the Computational Methods section) mainly on the previously described inhibitor **1**. Thus, Figure 1 shows the calculated and predicted position of compound **1** (magenta) in the binding pocket of the AChE-decamethonium crystallographic structure[17](decamethonium shown in green). The results of the docking studies allowed the identification of some features of the aminopyridazine **1** binding mode. The positively charged piperidine nitrogen achieves a cation-π-cloud interaction with Phe330 and Trp84, residues involved in the "anionic" part of the enzyme active site. We observed no direct hydrogen bond between the polar groups of the inhibitor and the binding site. However, a water-bridged hydrogen bond may occur between the piperidine nitrogen of the inhibitor and Tyr121 or Ser122 (as detected in the AChE-donepezil crystallographic structure[20]). Other comparable water-bridged hydrogen bonds have been

Table 1. Anticholinesterase Activity of 4- and 5-Substituted-6-aryl-3-benzylpiperidinylethylaminopyridazines



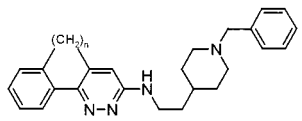
Compnd	R_1	R_2	Mp, °C [a]	Formula	IC_{50} (nM) [b]
1[c]	H	H	268	$C_{24}H_{28}N_4$. 2 HCl. H_2O	120 ± 10
4a	H	Me	246	$C_{25}H_{30}N_4$. 2 HCl. 0.5 H_2O	320 ± 20
4b	H	*i*-Pr	103	$C_{27}H_{34}N_4$. 2 HCl. 2 H_2O	430 ± 14
4c	Me	H	135	$C_{25}H_{30}N_4$. 2 HCl. 1.2 H_2O	21 ± 3
4d	Et	H	270	$C_{26}H_{32}N_4$. 2HCl. H_2O	27 ± 2
4e	Pr	H	177	$C_{27}H_{34}N_4$. 2 HCl. 1.5 H_2O	61 ± 10
4f			206	$C_{28}H_{30}N_4$. 2 HCl. 3 H_2O	440 ± 42

[a] All melting points refer to hydrochlorides. [b] Electric eel acetylcholinesterase. [c] Reference 14.

observed in the X-ray structure of AChE complexes.[21] Hydrophobic and van der Waals interactions are also evident for the protein−inhibitor model. Inhibitor **1** takes advantage of the aromatic residues present in the gorge by making a variety of interactions. van der Waals interactions of the piperidine ring occur with the phenyl rings of Phe331 and Tyr334. The benzyl ring of derivative **1** displays $\pi-\pi$ stacking with the aromatic ring of Trp84. It occupies the binding site for the quaternary ligands, such as edrophonium or decamethonium.[17] The phenyl-aminopyridazine part of **1** is located at the entrance of the gorge and interacts with two aromatic residues (Trp279 and Tyr70) which are involved in the AChE peripheral site. The phenyl ring, which is not coplanar with the pyridazine heterocycle, seems to display a $\pi-\pi$ stacking with the aromatic ring of Trp279. Thus, the aminopyridazine ring occupies the same region in space as the quaternary trimethylammonium group of decamethonium in the corresponding X-ray structure.[17] In general, our inhibitor **1** seems to interact with both peripheral and active sites of the enzyme. Huperzine A dimer[22] and bis-tetrahydroaminacrine[23] were found to have the same binding mode.

Although this molecular modeling study provided us some interesting information for the design of potent inhibitors, this computational work was performed after we started a classical structure activity strategy. Thus, four types of structural modifications were investigated in order to improve the anti-AChE activity of **1**. First, substitution of pyridazine ring at C-4 and C-5 positions resulted in a series of **1** analogues (**4a**−**f** in Table 1). Second, introduction of a methylene bridge between the C-5 pyridazine position and the phenyl group gave tricyclic analogues of **1** (**4g**−**j** in Table 2). Third, replacement of the phenyl moiety by nonaromatic and aromatic groups was also investigated (**4k**, **5**, **6a**−**o**, and **7** in Table 3). Finally, benzylpiperidinyl-ethylamine

Table 2. Anticholinesterase Activity of Tricyclic-6-aryl-3-benzylpiperidinylethylaminopyridazines



Compnd	n	Mp, °C [a]	Formula	IC_{50} (nM) [b]
1[c]	0	268	$C_{24}H_{28}N_4$. 2 HCl. H_2O	120 ± 10
4g	1	dec	$C_{25}H_{28}N_4$. 2 HCl. 1.5 H_2O	10 ± 3
4h	2	270	$C_{26}H_{30}N_4$. 2 HCl. 2 H_2O	39 ± 5
4i		269	$C_{26}H_{28}N_4$. 2 HCl. 3.5 H_2O	22 ± 3
4j	3	160	$C_{27}H_{32}N_4$. 2 HCl. H_2O	22 ± 2

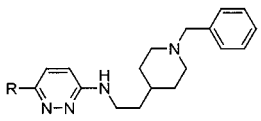
[a] All melting points refer to hydrochlorides. [b] Electric eel acetylcholinesterase. [c] Reference 14.

chain analogues of compound **1** were also prepared (**10**, **12**, **14a**−**c**, **16a**−**b**, and **18a**−**b** in Table 4).

Chemistry

The 3-[2-(1-benzylpiperidin-4-yl)ethylamino]pyridazines **4a**−**k** (Tables 1, 2, and 3) were synthesized according to Scheme 1. Condensation of the iminochlorides **3** with the 2-(1-benzylpiperidin-4-yl)ethylamine[24] gave the final pyridazines **4** as described previously.[14] Iminochlorides **3** were readily obtained from the corresponding 3(2*H*)-pyridazinones **2** by action of phosphorus oxychloride. Most of the 3(2*H*)-pyridazinones **2** were prepared according to the literature.[25−31] The synthesis of 5-alkyl-6-phenyl-3(2*H*)-pyridazinones **2c**−**e** was realized by using the one-pot procedure described by Coates et al.[32] Condensation of the 3-chloro-5,6-dihydrobenzo[*h*]cinnoline **3h**[33] with the benzylpiperidinyl-ethylamine by using acid condition (NH_4Cl) gave the benzocinnoline **4i** (Table 2) in 25% yield. The dihydrobenzocinnoline derivative **4h** was obtained in neutral condition without solvent (e condition, Scheme 1). Suzuki coupling of available aryl boronic acids with 6-chloro-3-[2-(1-benzylpiperidin-4-yl)ethylamino]pyridazine **5** described previously[34] was used to prepare 6-aryl-3-[2-(1-benzylpiperidin-4-yl)ethylamino]pyridazines **6a**−**o** with good yields (Scheme 2, Table 3). Nucleophilic displacement of compound **5** by NaOMe afforded methoxypyridazine **7** in 50% yield (Scheme 2). The preparation of the isosteric compounds **10** and **12** (Table 4) is shown in Scheme 3. A slightly modified method described by Dutta et al.[35] allowed us to obtain the 1-benzyl-4-[(ethoxycarbonyl)-methylene]piperidine **8** in 84% yield, which gave compound **9** after reduction with $LiAlH_4$. Condensation of the sodium alcoolate of **9** with 3-chloro-6-phenylpyridazine[36] provided the alkoxypyridazine derivative **10**. Treatment of **9** with thionyl chloride allowed 1-benzyl-4-(2-chloroethyl)piperidine[37] **11** to be obtained which is condensed with 3-mercapto-6-phenylpyridazine[38] to give the alkylthiopyridazine derivative **12**. The acetamidopyridazine derivatives **14a**−**c** (Scheme 4, Table 4) were prepared by coupling the activated carboxylic acids **13a**−**c** with the 3-amino-6-phenylpyridazine. The 2-(1-benzylpiperidin-4-yl)acetic acid **13a** was obtained by the hydrolysis product of 2-(1-benzylpiperidin-4-yl)acetonitrile.[14] Alkylation of commercial 1-benzylpiperazine and 4-benzylpiperidine by means of ethyl chloroacetate followed by saponification gave, respectively, the 2-(4-benzylpiperazin-1-yl)acetic acid **13b** and the 2-(4-benzylpiperidin-1-yl)acetic acid **13c**. The diamine chains **15a** and **15b** were synthesized, respectively, by alkylation with halogenonitrile of the secondary amine (1-benzylpiperazine and 4-benzylpiperidine, respectively) followed by reduction. Gabriel synthesis with the commercially available bromoalkylphthalimide can also be used for the preparation of **15a** and **15b** (Scheme 4). The carboxyamine chains **17a** and **17b** were prepared starting from the corresponding commercially available secondary amines by alkylation with chloroacetic chloride followed by a Gabriel synthesis (Scheme 4). Condensation of the 3-chloro-6-phenylpyridazine[36] with primary amines **15a**−**b** and **17a**−**b** yielded, respectively, compounds **16a**−**b** (acid conditions) and compounds **18a**−**b** (basic conditions) (Scheme 4).

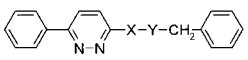
Table 3. Anticholinesterase Activity of 6-Substituted-3-benzylpiperidinylethylaminopyridazines



Compnd	R	Mp, °C [a]	Formula	IC_{50} (nM) [b]
1[c]	Ph	268	$C_{24}H_{28}N_4$. 2 HCl. H_2O	120 ± 10
4k	H	120	$C_{18}H_{24}N_4$. 2 HCl. 1.5 H_2O	240 ± 10
5	Cl	dec	$C_{18}H_{23}ClN_4$. 2 HCl. H_2O	73 ± 4
7	MeO	120	$C_{19}H_{26}N_4O$. 2 HCl. 2 H_2O	220 ± 10
6a[d]	2-Me-Ph	95	$C_{25}H_{30}N_4$. 2 HCl. H_2O	90 ± 5
6b	2-Et-Ph	115	$C_{26}H_{32}N_4$. 2 HCl. 2,5 H_2O	87 ± 7
6c[d]	2,4,6-$(Me)_3$-Ph	dec	$C_{27}H_{34}N_4$. 2 HCl. 1.5 H_2O	3 000 ± 200
6d[d]	2-MeO-Ph	128	$C_{25}H_{30}N_4O$. 2 HCl. 3 H_2O	110 ± 10
6e	2-Cl-Ph	153	$C_{24}H_{27}ClN_4$. 2HCl. 2,5 H_2O	80 ± 6
6f[d]	3,5-$(CF_3)_2$-Ph	220	$C_{26}H_{26}N_4F_6$. 2 HCl	56 ± 3
6g[d]	2-naphthyl	205	$C_{25}H_{26}N_4$. 2 HCl. 1.25 H_2O	56 ± 10
6h	3-AcNH-Ph	236	$C_{26}H_{31}N_5O$. 2 HCl. 1.5 H_2O	58 ± 2
6i	3-Ac-Ph	243	$C_{26}H_{30}N_4O$. 2 HCl. H_2O	54 ± 4
6j	3,4-OCH_2O-Ph	279	$C_{25}H_{28}N_4O_2$. 2 HCl. H_2O	73 ± 3
6k	4-CN-Ph	247	$C_{25}H_{27}N_5$. 2 HCl. 1.75 H_2O	100 ± 13
6l	4-F-Ph	253	$C_{24}H_{27}FN_4$. 2 HCl. 0.5 H_2O	350 ± 30
6m	4-(NMe_2)-Ph	145	$C_{26}H_{32}N_5$. 3 HCl. 3.5 H_2O	210 ± 10
6n	2-thiophenyl	227	$C_{22}H_{26}N_4S$. 2 HCl. 2 H_2O	97 ± 3
6o	3-pyridinyl	131	$C_{23}H_{27}N_5$. 3 HCl. 3 H_2O	57 ± 4

[a] All melting points refer to hydrochlorides. [b] Electric eel acetylcholinesterase. [c] Reference 14. [d] Reference 34.

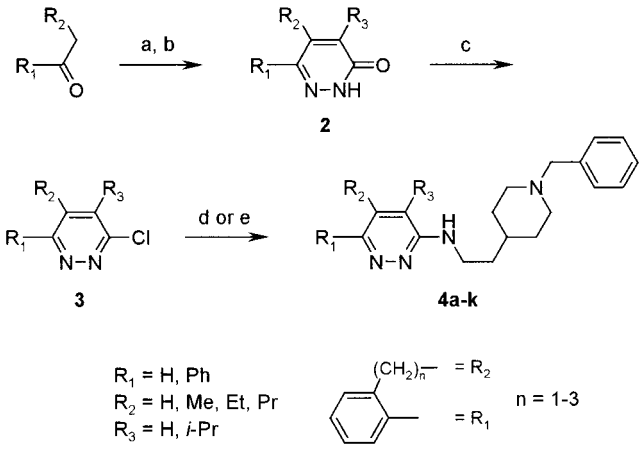
Table 4. Anticholinesterase Activity of 6-Phenylpyridazines with Various Side Chains



Compnd	X	Y	Mp, °C [a]	Formula	IC_{50} (μM) [b]
1[c]	$NHCH_2CH_2$		268	$C_{24}H_{28}N_4$. 2 HCl. H_2O	0.12 ± 0.01
10	OCH_2CH_2		155	$C_{24}H_{27}N_3O$. 2 HCl. 0.5 H_2O	0.14 ± 0.02
12	SCH_2CH_2		210	$C_{24}H_{27}N_3S$. 2 HCl	0.063 ± 0.003
14a	$NHCOCH_2$		117	$C_{24}H_{26}N_4O$. 2 HCl. 5 H_2O	4.2 ± 1.6
16a	$NHCH_2CH_2$		263	$C_{23}H_{27}N_5$. 3 HCl. H_2O	1.5 ± 0.1
14b	$NHCOCH_2$		241	$C_{23}H_{25}N_5O$. 2 HCl. 2 H_2O	17 ± 2
18a	$NHCH_2CO$		225	$C_{23}H_{25}N_5O$. 2 HCl. 1.5 H_2O	15 ± 1
16b	$NHCH_2CH_2$		186	$C_{24}H_{28}N_4$. 2 HCl. 1.5 H_2O	24 ± 1
14c	$NHCOCH_2$		234	$C_{24}H_{26}N_4O$. 2 HCl. 0.5 H_2O	18 ± 1
18b	$NHCH_2CO$		215	$C_{24}H_{26}N_4O$. HCl. 0.5 H_2O	120 ± 10

[a] All melting points refer to hydrochlorides. [b] Electric eel acetylcholinesterase. [c] Reference 14.

Scheme 1[a]



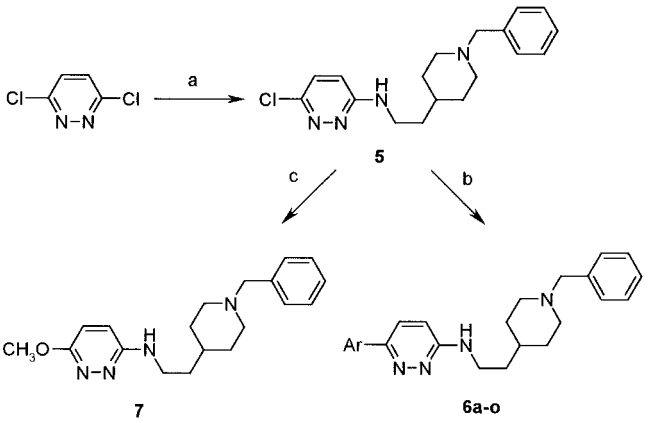
[a] Reagents and conditions: (a) R_3-CO-CO_2H, rt; (b) $NH_2NH_2 \cdot H_2O$, reflux; (c) $POCl_3$, 75 °C; (d) 2-(1-benzylpiperidin-4-yl)ethylamine, NH_4Cl, *n*-BuOH, 130 °C; (e) 2-(1-benzylpiperidin-4-yl)ethylamine, 100 °C.

Results and Discussion

The pyridazine derivatives were tested for in vitro inhibition of acetylcholinesterase on the commercially available electric eel AChE (Tables 1−4). The results obtained with this enzyme preparation allowed us to investigate molecular modeling studies (described in another paper[39]) using the published AChE structure which is also derived from the electric eel enzyme.[16] In addition to electric eel AChE, we used human erythrocytes as a source of human AChE and human serum as a source of human butyrylcholinesterase (BuChE) to determine the selectivity profile of the most potent inhibitors (Table 5). The esterase activity was determined according to the method of Ellman et al.[40]

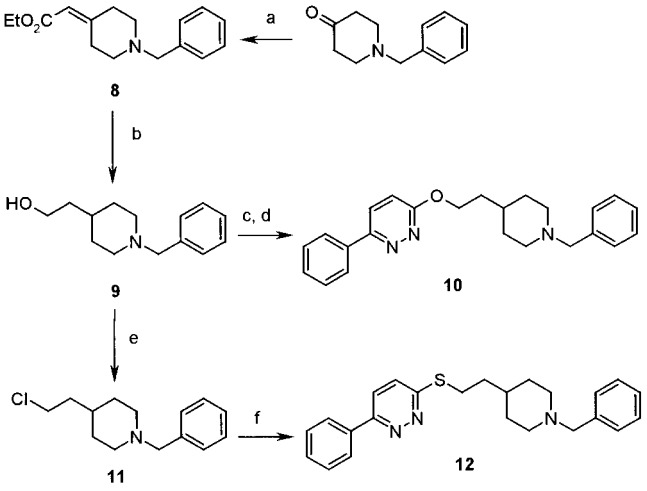
We observed that introduction of a lipophilic and aliphatic group in the C-4 position of the pyridazine ring

Scheme 2[a]



[a] Reagents and conditions: (a) 2-(1-benzylpiperidin-4-yl)ethylamine, HCl/H_2O/acetone, 100 °C; (b) $ArB(OH)_2$, base, solvent, 100 °C; (c) MeONa, DMF, 130 °C.

Scheme 3[a]

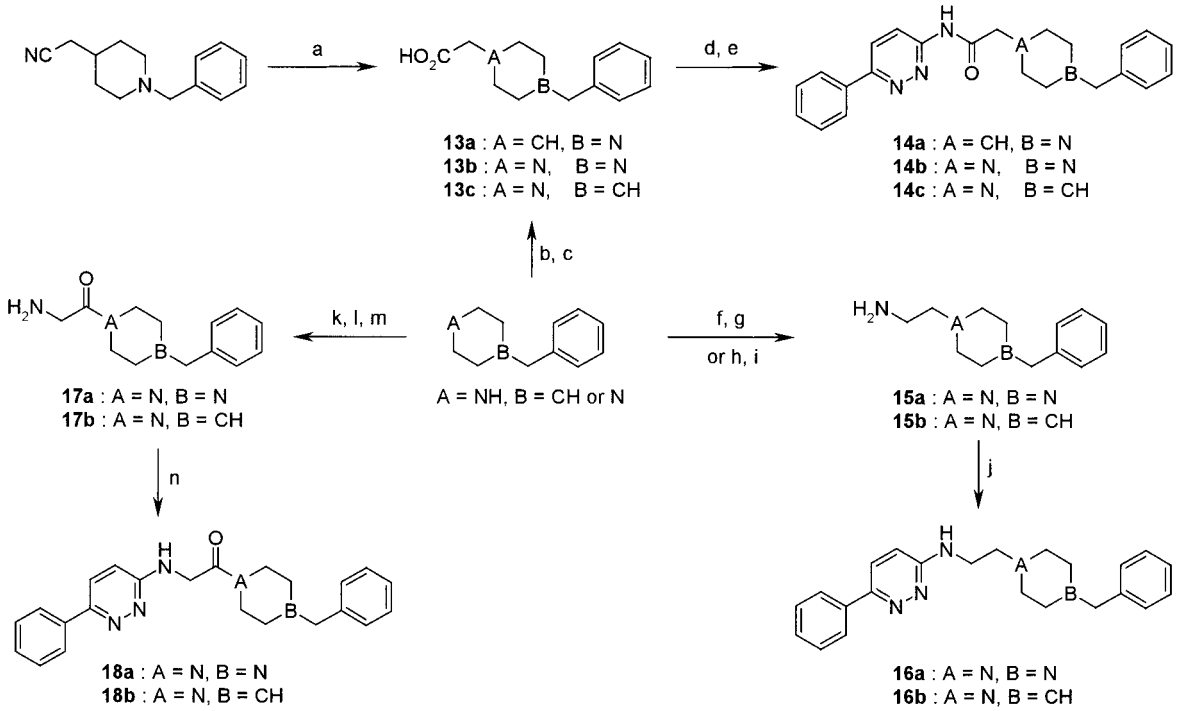


[a] Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$, THF, K_2CO_3, reflux; (b) $LiAlH_4$, THF, reflux; (c) Na, THF; (d) 3-chloro-6-phenylpyridazine, reflux; (e) $SOCl_2$, CH_2Cl_2, reflux; (f) 6-phenyl-3-thiolpyridazine, EtONa, EtOH, reflux.

led to compounds showing weaker activities (Table 1). Thus, isopropyl derivative **4b** was found to be 3 times less potent than the lead compound **1**. On the other hand, the presence of an alkyl group in the C-5 position (**4c**−**e**) increased the anti-AChE potency. Compound **4c** was the best compound from this series with an IC_{50} = 21 nM (a 6-fold increase in activity compared to **1**). Slight increases were observed for derivatives with an ethyl (**4d**) or propyl (**4e**) group. The combined introduction of an aromatic environment in the C-4 and C-5 position (phthalazine ring) gave a less potent derivative (**4f**). In general, it seems that the C-5 substitution with an aliphatic group is particularly favorable for anti-AChE activity.

All the constrained tricyclic pyridazines (**4g**−**j**) showed an increased inhibitory activity (Table 2). With an IC_{50} value of 10 nM, the indenopyridazine derivative **4g** was found to be 12 times more potent than compound **1**. The benzocinnoline compound **4i** showed a slight increase in activity compared to its saturated analogue **4h**. The ring extension (n = 3) led to the benzocycloheptapy-

Scheme 4[a]



[a] Reagents and conditions: (a) NaOH, EtOH, reflux; (b) $ClCH_2CO_2Et$, toluene, TEA, reflux; (c) NaOH, MeOH, reflux; (d) $(COCl)_2$, rt; (e) 3-amino-6-phenylpyridazine, CH_2Cl_2, TEA, rt; (f) $NCCH_2Cl$, K_2CO_3, reflux; (g) $LiAlH_4$, THF, rt; (h) *N*-bromoethylphthalimide, xylene, 130 °C; (i) $NH_2NH_2 \cdot H_2O$, EtOH, reflux; (j) 3-chloro-6-phenylpyridazine, NH_4Cl, *n*-BuOH, 130 °C; (k) $ClCH_2COCl$, CH_2Cl_2, reflux; (l) phthalimide-K, DMF, rt; (m) $NH_2NH_2 \cdot H_2O$, EtOH, Δ; (n) 3-chloro-6-phenylpyridazine, pyridine, reflux.

Table 5. Comparative Activity on Various Cholinesterases

Compnd	activity IC_{50} (nM)			ratio of IC_{50}
	tAChE [a]	hAChE [b]	hBuChE [c]	(hBuChE / hAChE)
1	120 ± 10	140 ± 10	700 ± 30	5.0
4c	21 ± 3	39 ± 3	940 ± 60	24
4d	27 ± 2	66 ± 8	920 ± 20	14
4g	10 ± 3	74 ± 3	230 ± 20	3.1
4h	39 ± 5	190 ± 10	280 ± 10	1.5
4i	22 ± 3	190 ± 20	270 ± 10	1.4
4j	22 ± 2	120 ±10	510 ± 20	4.2
10	140 ± 20	710 ± 50	1600 ± 100	2.2
12	63 ± 3	470 ± 10	480 ± 10	1.0
tacrine	39 ± 4	95 ± 6	21 ± 2	0.22
donepezil	48 ± 6	16 ± 1	8200 ± 200	510

[a] From electric eel. [b] From human erythrocytes. [c] From human serum.

ridazine **4j** which is comparable in activity to compound **4i** (IC_{50} = 22 nM on electric eel AChE). The phenyl ring of compound **4g** is coplanar with the pyridazine heterocycle contrary to derivative **4j**. It seems that the correlation between the uncoplanar phenyl ring observed and the $\pi-\pi$ stacking with Trp279 is not so evident. However, the introduction of a flexible (**4c**−**e**) or constraint (**4g**−**j**) lipophilic environment in the C-5 pyridazine position seems to be critical for the anti-AChE activity. A hydrophobic and van der Waals interaction with the enzyme binding site is probably responsible for the potency increase.

Surprisingly, the deletion of the phenyl moiety (Table 3) induces only a 2-fold decrease in activity (**4k**, IC_{50} = 240 nM). This finding could suggest the slight importance of the phenyl ring in the binding (hydrophobic interaction) with the peripheral site. The replacement of the phenyl group by a halogen atom (**5**) produces a weak increase of the anti-AChE activity whereas the replacement by a methoxy (**7**) leads to a less active compound (compared to **1**). The isosteric substitution of the C-6 phenyl ring with thiophene (**6n**) and pyridine (**6o**) gives, respectively, comparable and 2 times more potent derivatives. Phenyl ring substitution with electrostatic or hydrophobic groups leads, in most of the cases, to comparable or slightly more active compounds (**6a**−**6m**) than **1**. Derivative **6c** showed a very weak anti-cholinesterase activity (IC_{50} = 3 μM). Among the 6-arylpyridazines investigated, **6g** and **6i** show, respectively, IC_{50} values of 56 and 54 nM, which represent a 2-fold increase in potency. On the other hand, the *p*-dimethylaminophenyl derivative **6m** was found to be 2 times less active than **1**. The weak modification (2-fold maximum) produced by the C-6 pyridazine substitution could suggest rather a $\pi-\pi$ stacking between the pyridazine ring (instead of the phenylpyridazine moiety) and the indole part of Trp279.

The isosteric replacement of the exo nitrogen atom of compound **1** by an oxygen and sulfur atom yield, respectively, the ethoxypyridazine **10** and the ethylthiopyridazine **12** (Table 4). Derivative **10** was comparable to **1** whereas **12** showed a 2-fold increase activity with an IC_{50} of 63 nM. The 4-benzylpiperazine **16a** and

4-benzylpiperidine **16b** analogues were, respectively, 10 and 200 times less potent than the 1-benzylpiperidine derivative **1**. The position of the nitrogen atom in the aliphatic heterocycle appears to be important for the anti-AChE activity. The introduction of a carbonyl group in the ethyl chain was realized in order to obtain an additional interaction with the enzyme. In all cases (**14a**−**c** and **18a**−**b**), a decrease of the activity in comparison to the non-carbonyl analogues (**1**, **16a**, and **16b**) was observed. All of these observations confirm that the 1-benzylpiperidine linked to a hydrophobic chain (as shown previously[14]) is favorable for the interaction with the enzyme.

The most active and representative compounds, as well as two reference inhibitors, tacrine and donepezil, were then compared in order to determine their relative inhibitory effects (Table 5) toward human acetyl and butyrylcholinesterase (ratio of IC_{50} hBuChE/hAChE). Compound **4g**, which was the most active derivative on electric eel AChE, and compound **4j** have comparable BuChE/AChE ratios remaining, however, inferior to that of compound **1**. The other tricyclic derivatives (**4h** and **4i**) did not present a selective profile (ratio close to 1). The 5-alkylpyridazines **4c** and **4d** were found to be, respectively, 3- and 5-fold more selective for hAChE than the reference compound **1**. Thus, 3-[2-(1-benzylpiperidin-4-yl)ethylamino]-5-methyl-6-phenylpyridazine **4c** was the more selective inhibitor with a ratio of 24 (IC_{50} of 39 nM for hAChE). The O- (**10**) and S-isosteric derivatives (**12**) present a weak selectivity profile despite their satisfactory anti-AChE activity. Compared to tacrine, compound **4c** is about 100 times more selective but remains less selective than donepezil.

Conclusion

The synthesis and biochemical evaluation of a series of compound **1** analogues led to the design of potent and selective AChE inhibitors. The most potent inhibitor, **4g**, which presents an IC_{50} of 10 nM on electric eel AChE, is 60 000 times more active than our initial lead compound, minaprine,[14] and 12 times more active than **1**. Structure−activity relationships on four different parts of **1** indicated that (i) introduction of a flexible or constraint lipophilic environment in the C-5 pyridazine ring causes an increase of the potency and human selectivity; (ii) replacement or substitution of C-6 phenyl group seems to be less determining for the anticholinesterase activity; (iii) isosteric replacement or modification of the 1-benzylpiperidine ethylamine chain does not improve the inhibition or the selectivity. Among all derivatives investigated, 3-[2-(1-benzylpiperidin-4-yl)ethylamino]-5-methyl-6-phenylpyridazine **4c** was found to be one of the most potent and selective AChE inhibitors: this derivative presents an IC_{50} of 21 nM on electric eel AChE and a human selective ratio of 24 (100 more selective than tacrine).

During our preliminary molecular docking study, we were able to show that our inhibitors interact with both the cation-π part of the catalytic site and the peripheral site of the enzyme. After we had performed our modeling studies and before we had finished our structure−activity approach, experimental data were published which supported the applied docking strategy. The crystal structure of the AChE complexed with the benzylpiperidine derivative donepezil has been made available.[20] Like donepezil, our most potent inhibitors contain a benzylpiperidine moiety which shows a similar position and orientation when compared to the reported X-ray structure (illustrated with compound **4j** in Figure 2). Both kind of inhibitors adopt a comparable conformation and position in the narrow binding pocket. The indanone ring of donepezil stacks against the indole ring of Trp279, in the peripheral binding site, by a classical $\pi-\pi$ interaction. A similar interaction was found for the arylpyridazine part of our inhibitors. However, experimental results showed that the pyridazine ring is probably the part of the molecule which interacts with Trp279.

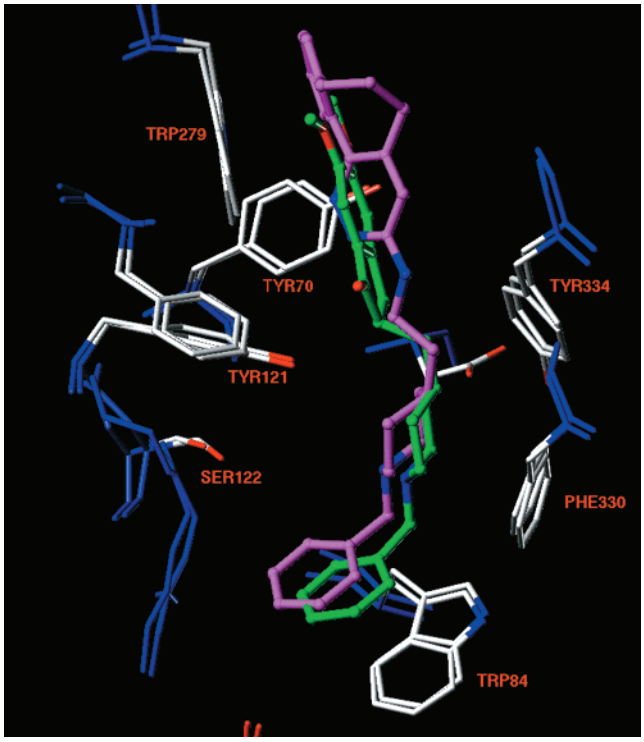


Figure 2. Comparison of the experimentally determined AChE-donepezil complex and the predicted AChE-aminopyridazine complex (magenta). The aminopyridazine inhibitor **4j** (magenta) adopts a conformation similar to that of donepezil (green) in the corresponding complex.

At the present time, we consider synthesizing new pyridazine derivatives according to the SAR observations and to a complete molecular modeling study.[39] The combination of the C-5 methyl substitution with, for instance, the appropriate phenyl substitution should allow us to obtain more potent inhibitors. Unfortunately, the synthesis of such compounds is not so evident. Indeed, the use of the Suzuki reaction for the 6-aryl-5-methyl-3-[2-(1-benzylpiperidin-4-yl)ethylamino]pyridazines preparation is impossible. Moreover, the synthesis of 6-aryl-5-methyl-3-chloropyridazine by the classical route (Scheme 1) is in most of the cases very difficult (low yields for some substituents on the phenyl ring).

Experimental Section

Chemistry. ^{1}H NMR were recorded on a Bruker AC 200 (200 MHz) or a Bruker DPX 300 (300 MHz) spectrophotometer at room temperature. Chemical shifts are given in ppm (δ) relative to $SiMe_3$ as internal standard. Coupling constants (J)

are in hertz (Hz), and signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; quint., quintuplet; m, multiplet; brs, broad singlet; etc. The mass spectra were obtained on a Mariner API-TOF. Melting points were determined with a Mettler FP62 apparatus and are uncorrected. Elemental analyses were performed by the CNRS department of microanalysis (CNRS, Vernaison, France) and are indicated only by the elemental symbols within ±0.4% of the theoretical values unless otherwise noted. All chemicals and solvents were obtained from commercial suppliers and used without purification. THF and ethyl ether (Et_2O) were freshly distilled from sodium benzophenone ketyl. Flash chromatography was carried out on Geduran silica gel Si 60 (40−63 μm, Merck). Thin-layer chromatography was carried out using plates silica gel 60 F_{254} (Merck). Spots were visualized either under UV light (λ = 254 nm) or by spraying with molybdate reagent (H_2O/concentrated H_2SO_4/$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/$(NH_4)_2-Ce(SO_4)_4\cdot 2H_2O$, 90/10/25/1, v/v/w/w) and charring at 140 °C for a few minutes. All chemical yields are unoptimized and generally represent the result of a single experiment.

(I) 3(2*H*)-Pyridazinones. The 3(2*H*)-pyridazinones **2** necessary for the synthesis of compounds **4a**−**k** are already known and were prepared according to literature procedures.[25−31] The 3(2*H*)-pyridazinone **2k** is commercially available. Pyridazinones **2c**−**e** and **2j** were synthesized by using the one-pot procedure described by Coates et al.[32]

5-Methyl-6-phenyl-3(2*H*)-pyridazinone (2c). A stirred mixture of glyoxylic acid monohydrate (54 mmol) and propiophenone (163 mmol) was heated at 100−105 °C for 2 h and allowed to cool to 40 °C, and then H_2O (20 mL) was added followed by concentrated aqueous NH_4OH (4 mL). The mixture was extracted with CH_2Cl_2 (4 × 25 mL). The ammoniac solution was stirred with hydrazine hydrate (54 mmol) and heated under reflux for 2 h. After cooling, the precipitate formed was collected by filtration and washed with H_2O to give **2c**: yield 70%; mp 210 °C (lit.[32] 217−219 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.19 (s, 3H), 6.89 (s, 1H), 7.44 (m, 5H), 12.57 (brs, 1H).

5-Ethyl-6-phenyl-3(2*H*)-pyridazinone (2d). Yield 35%; mp 181 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.09 (t, 3H, J = 7.6 Hz), 2,48 (q, 2H, J = 7.4 Hz), 6.89 (s, 1H), 7.38−7.45 (m, 5H).

6-Phenyl-5-propyl-3(2*H*)-pyridazinone (2e). Yield 27%; mp 128 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 7.0 Hz), 1.26 (m, 2H), 3.58 (m, 2H), 7.45−7.48 (m, 3H), 8.67 (s, 1H), 11.22 (brs, 1H).

2,5,6,7-Tetrahydro-3*H*-benzo[6,7]cyclohepta[1,2-*c*]pyridazin-3-one (2j). Yield 21%; mp 243 °C (lit.[30] 235−239 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.03 (quint., 2H, J = 7.3 Hz), 2.35 (t, 2H, J = 6.9 Hz), 2.54 (t, 2H, J = 6.9 Hz), 6.74 (s, 1H), 7.10−7.32 (m, 3H), 7.42−7.46 (m, 1H), 12.21 (brs, 1H).

(II) General Procedure for 3-Chloropyridazines. The appropriate substituted 3-(2*H*)-pyridazinone **2** was heated at 80 ± 5 °C for 4 h with an excess (10 equiv) of phosphorus oxychloride ($POCl_3$). The excess of $POCl_3$ was removed by distillation under reduced pressure, and the residue was carefully poured onto ice. The water was rendered alkaline with 20% NaOH solution and extracted with EtOAc. The crude 3-chloropyridazine was purified by recrystallization in EtOH or *i*-PrOH or by flash chromatography using a mixture of hexanes−EtOAc as eluant. The 3-chloropyridazines **3** are already known and were prepared according to literature procedures.[28,31−33,36,41−43]

3-Chloro-4-isopropyl-6-phenylpyridazine (3b). Yield 94%; mp 72 °C (lit.[44] 71−74 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36 (d, J = 6,9 Hz, 6H), 3.35 (sept., 1H, J = 6.9 Hz), 7.51−7.55 (m, 3H), 7.71 (s, 1H), 8.02−8.07 (m, 2H).

3-Chloro-5-methyl-6-phenylpyridazine (3c). Yield 95%; mp 123 °C (lit.[32,45] 123−124 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37 (s, 3H), 7.42 (s, 1H), 7.48−7.57 (m, 5H).

3-Chloro-5-ethyl-6-phenylpyridazine (3d). Yield 89%; mp 64 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, 3H, J = 7.5 Hz), 2.68 (q, 2H, J = 7.5 Hz), 7.46 (s, 1H), 7.49 (brs, 5H).

3-Chloro-6-phenyl-5-propylpyridazine (3e). Yield 59%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 7.5 Hz), 1.55 (m, 2H), 2.62 (t, 2H, J = 7.9 Hz), 7.42 (s, 1H), 7.49 (brs, 5H).

3-Chloro-2,5,6,7-tetrahydro-3*H*-benzo[6,7]cyclohepta-[1,2-*c*]pyridazine (3j). Yield 93%; mp 158 °C (lit.[31] 155−156 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.27 (quint., 2H, J = 7.2 Hz), 2.53 (t, 2H, J = 7.2 Hz), 2.57 (t, 2H, J = 7.2 Hz), 7.27−7.30 (m, 1H), 7.39 (s, 1H), 7.43−7.46 (m, 2H), 7.81−7.84 (m, 1H).

(III) Preparation of the Amines. The 2-(1-benzylpiperidin-4-yl)ethylamine[24] chain used in this work was obtained from a commercial supplier as described in our previous paper.[14] The diamine chain **15a** was prepared by alkylation with an halogenonitrile followed by reduction whereas **15b** was obtained by Gabriel synthesis. These two procedures were reported previously.[14] The carboxyamine chains **17a** and **17b** were synthesized starting from commercially secondary amines according to literature procedure.

2-(1-Benzylpiperazin-4-yl)ethylamine (15a). (a) 2-(1-Benzylpiperazin-4-yl)acetonitrile. Brown oil; yield 96%; mp (dihydrochloride) 219 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.49−2.53 (m, 4H), 2.59−2.65 (m, 4H), 3.49 (s, 2H), 3.51 (s, 2H), 7.24−7.33 (m, 5H).

(b) 2-(1-Benzylpiperazin-4-yl)ethylamine[46] (15a). Yellow oil; yield 75%; mp (dihydrochloride) 247 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (brs, 2H), 2.42 (t, 2H, J = 6.2 Hz), 2.48 (m, 8H), 2.78 (t, 2H, J = 6.2 Hz), 3.51 (s, 2H), 7.24−7.33 (m, 5H).

2-(4-Benzylpiperidin-1-yl)ethylamine (15b). Brown oil; yield 85%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.18−1.38 (m, 2H), 1.50−1.68 (m, 3H), 1.81−2.01 (m, 4H), 2.35 (t, 2H, J = 6.2 Hz), 2.57 (t, 2H, J = 6.6 Hz), 2.75 (t, 2H, J = 6.2 Hz), 2.85 (brd, 2H, J = 11.7 Hz), 7.14−7.30 (m, 5H).

2-Amino-1-(4-benzylpiperazin-1-yl)ethan-1-one (17a). (a) 1-(4-Benzylpiperazin-1-yl)-2-chloroethan-1-one. To a solution of chloroacetic chloride (13.8 mmol) in CH_2Cl_2 (20 mL) was added, at 0 °C, 1-benzylpiperazine (11.5 mmol). The reaction mixture was stirred at room temperature for 30 min. H_2O (100 mL) was added, and the mixture was rendered alkaline with a 10% $NaHCO_3$ solution and extracted with EtOAc. The organic layer, dried over Na_2SO_4, was evaporated under reduce pressure to give a yellow oil: yield 95%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.47 (quint., 4H, J = 4.9 Hz), 3.50 (t, 2H, J = 4.9 Hz), 3.53 (s, 2H), 3.63 (t, 2H, J = 4.9 Hz), 4.06 (s, 2H), 7.25−7.32 (m, 5H).

(b) *N*-[2-(4-Benzylpiperazin-1-yl)-2-oxoethyl]phthalimide. A mixture of 1-(4-benzylpiperazin-1-yl)-2-chloroethan-1-one (45.1 mmol) and potassium phthalimide (45.1 mmol) in DMF (60 mL) was stirred at room temperature for 5 h. A saturated NH_4Cl solution (400 mL) was added, and the reaction mixture was extracted with EtOAc. The organic layer was washed with brine solution, dried over Na_2SO_4, and evaporated under reduce pressure to give a white solid: yield 95%; mp 152 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.43−2.56 (m, 4H), 3.55 (s, 2H), 3.57−6.65 (m, 4H), 4.49 (s, 2H), 7.29−7.35 (m, 5H), 7.70−7.74 (m, 2H), 7.84−7.89 (m, 2H).

(c) 2-Amino-1-(4-benzylpiperazin-1-yl)ethan-1-one (17a). A mixture of *N*-[2-(4-benzylpiperazin-1-yl)-2-oxoethyl]phthalimide (8.2 mmol) and hydrazine hydrate (12.4 mmol) in EtOH (25 mL) was refluxed for 30 min. The precipitate was filtered off and washed with EtOH, and the filtrate was concentrated under reduced pressure. Et_2O was added to the residue, and the precipitate was filtered. The filtrate, dried over Na_2SO_4, was evaporated to give a colorless oil: yield 98%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.30−2.36 (m, 6H), 3.27 (t, 2H, J = 4.7 Hz), 3.33 (s, 2H), 3.43 (s, 2H), 3.55 (t, 2H, J = 4.7 Hz), 7.22−7.26 (m, 5H).

2-Amino-1-(4-benzylpiperidin-1-yl)ethan-1-one (17b). (a) 1-(4-Benzylpiperidin-1-yl)-2-chloroethan-1-one. Orange oil; yield 83%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.71−1.90 (m, 5H), 2.51−2.66 (m, 3H), 3.05 (m, 1H), 3.81−3.86 (m, 1H), 4.08 (s, 2H), 4.52−4.62 (m, 1H), 7.13−7.28 (m, 5H).

(b) *N*-[2-(4-Benzylpiperidin-1-yl)-2-oxoethyl]phthalimide. White solid; yield 75%; ^{1}H NMR (200 MHz, $CDCl_3$) δ

1.15−1.37 (m, 2H), 1.38−1.86 (m, 3H), 2.53−5.62 (m, 3H), 3.03−3.12 (m, 1H), 3.83 (brd, 1H, J = 13.5 Hz), 4.48 (s, 2H), 4.54 (m, 1H), 7.14−7.33 (m, 5H), 7.70−7.74 (m, 2H), 7.84−7.88 (m, 2H).

(c) 2-Amino-1-(4-benzylpiperidin-1-yl)ethan-1-one (17b). Yellow oil; yield 91%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.96−1.20 (m, 2H), 1.56−1.75 (m, 3H), 1.84 (brs, 2H), 2.38−2.51 (m, 3H), 2.78 (td, 1H, J_1 = 13.3 Hz, J_2 = 1.2 Hz), 3.33 (s, 2H), 3.56 (brd, 1H, J = 13.5 Hz), 4.50 (brd, 1H, J = 13.2 Hz), 7.02−7.12 (m, 5H).

(IV) General Procedure for the Substitution of the Iminochlorides by Diamines. All the final 3-aminoalkylpyridazines were prepared by substituting the iminochlorides **3** with the suitable amines. Compounds **4a**−**g**, **4i**−**k**, **16a**, and **16b** were obtained by using acid conditions (d condition, Scheme 1) described in our previous paper.[14] Compound **4h** was synthesized in neutral conditions (e condition, Scheme 1) whereas aminoethanone pyridazine derivatives **18a** and **18b** were prepared in basic conditions (n condition, Scheme 4).

The corresponding hydrochlorides were prepared by treating the free base dissolved in Et_2O and/or EtOAc with gaseous hydrogen chloride or with 2.1 equiv of 37% HCl. The collected solids were crystallized in *i*-PrOH with Et_2O or $(i\text{-Pr})_2O$.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-4-methyl-6-phenylpyridazine (4a). A mixture of 3-chloro-4-methyl-6-phenylpyridazine **3a**[41](0.63 g, 3.1 mmol), 2-(1-benzylpiperidin-4-yl)ethylamine[14](1.34 g, 6.2 mmol), and ammonium chloride (0.17 g, 3.1 mmol) in 1-butanol (10 mL) was refluxed for 48 h. The solvent was removed by evaporation, and the residue was diluted with 10% K_2CO_3 (100 mL) and extracted with EtOAc. The organic layer was washed with a 10% citric acid solution, and the combined aqueous phases were extracted with EtOAc. The aqueous layer was rendered alkaline with K_2CO_3 and then extracted with EtOAc. After drying over Na_2SO_4 and removing the solvent by evaporation, the free base (yellow oil) was purified by flash chromatography (EtOAc−MeOH, 9:1 with 2% (v) TEA): yield 19%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21−1.40 (m, 3H), 1.66−1.77 (m, 4H), 1.96 (brt, 2H, J = 11.3 Hz), 2.17 (s, 3H), 2.88 (brd, 2H, J = 12.0 Hz), 3.49 (s, 2H), 3.65−3.75 (m, 2H), 4.19 (brt, 1H, J = 5.1 Hz), 7.31−7.45 (m, 9H), 7.97−8.01 (m, 2H).

Dihydrochloride (*i*-PrOH): mp 246 °C. Anal. ($C_{25}H_{30}N_4$·2HCl·0.5H_2O) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-4-isopropyl-6-phenylpyridazine (4b). The free base (brown oil) was purified by flash chromatography (EtOAc with 2% (v) TEA): yield 13%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30−1.45 (m, 3H), 1.32 (d, 6H, J = 6.9 Hz), 1.59−1.78 (m, 4H), 1.97 (brt, 2H, J = 10.9 Hz), 2.73 (m, 1H), 2.89 (brd, 2H, J = 10.9 Hz), 3.50 (s, 2H), 3.66−3.76 (m, 2H), 4.30 (brs, 1H), 7.22−7.31 (m, 5H), 7.39−7.41 (m, 4H), 7.97−8.02 (m, 2H).

Dihydrochloride (*i*-PrOH): mp 103 °C. Anal. ($C_{27}H_{34}N_4$·2HCl·2H_2O) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-5-methyl-6-phenylpyridazine (4c). The free base (yellow oil) was purified by flash chromatography (EtOAc−MeOH, 9:1 and then EtOAc with 2% (v) TEA): yield 22%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28−1.43 (m, 3H), 1.56−1.63 (m, 2H), 1.69 (brd, 2H, J = 11.7 Hz), 1.93 (brt, 2H, J = 11.3 Hz), 2.20 (s, 3H), 2.87 (brd, 2H, J = 11.7 Hz), 3.38−3.45 (m, 2H), 3.48 (s, 2H), 5.15 (brt, 1H, J = 5.6 Hz), 6.50 (s, 1H), 7.22−7.32 (m, 5H), 7.35−7.46 (m, 3H), 7.50−7.54 (m, 2H).

Dihydrochloride (*i*-PrOH): mp 135 °C. Anal. ($C_{25}H_{30}N_4$·2 HCl·1.2H_2O) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-5-ethyl-6-phenylpyridazine (4d). The free base (yellow oil) was purified by flash chromatography (EtOAc−MeOH, 9:1): yield 28%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (t, 3H, J = 7.5 Hz), 1.26−1.47 (m, 3H), 1.57−1.64 (m, 2H), 1.70 (brd, 2H, J = 11,7 Hz), 1.95 (brt, 2H, J = 11.7 Hz), 2.55 (q, 2H, J = 7.2 Hz), 2.88 (brd, 2H, J = 11.7 Hz), 3.40−3.47 (m, 2H), 3.49 (s, 2H), 5.08 (brt, 1H, J = 5.2 Hz), 6.53 (s, 1H), 7.23−7.35 (m, 5H), 7.39−7.45 (m, 5H).

Dihydrochloride (*i*-PrOH): mp 270 °C. Anal. ($C_{26}H_{32}N_4$·2HCl·H_2O) C, H; N: calcd, 11.40; found, 10.93.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]- 6-phenyl-5-propyl-pyridazine (4e). The free base (colorless oil) was purified by flash chromatography (EtOAc and then EtOAc with 2% (v) TEA): yield 10%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 7.3 Hz), 1.29−1.76 (m, 9H), 1.98 (brt, 2H, J = 10.8 Hz), 2.55 (t, 2H, J = 7.9 Hz), 2.91 (brd, 2H, J = 11.4 Hz), 3.40−3.50 (m, 2H), 3.52 (s, 2H), 5.08 (brs, 1H), 6.53 (s, 1H), 730−7.36 (m, 5H), 7.43−7.49 (m, 5H).

Dihydrochloride (*i*-PrOH): mp 177 °C. Anal. ($C_{27}H_{34}N_4$·2HCl·1.5H_2O) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-phenylphthalazine (4f). The free base (yellow solid) was purified by flash chromatography (EtOAc−MeOH, 9:1): yield 59%; mp 170 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17−1.29 (m, 3H), 1.55−1.67 (m, 4H), 1.81 (brt, 2H, J = 10.9 Hz), 2.75 (brd, 2H, J = 10.6 Hz), 3.39 (s, 2H), 3.73 (m, 2H), 6.37 (brs, 1H), 7.19−7.27 (m, 5H), 7.38−7.46 (m, 3H), 7.57−7.69 (m, 4H), 7.84−7.87 (m, 1H), 8.10−8.13 (m, 1H).

Dihydrochloride (*i*-PrOH): mp 206 °C. Anal. ($C_{28}H_{30}N_4$·2HCl·3H_2O) C, H; N: calcd, 10.19; found, 9.65.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-5*H*-indeno-[1,2-*c*]pyridazine (4g). The free base (beige solid) was purified by flash chromatography (EtOAc−MeOH, 9:1 with 2% (v) TEA): yield 13%; mp 186 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29−1.39 (m, 3H), 1.57−1.73 (m, 4H), 1.95 (brt, 2H, J = 11.4 Hz), 2.88 (brd, 2H, J = 11.7 Hz), 3.40−3.47 (m, 2H), 3.49 (s, 2H), 3.82 (s, 2H), 4.83 (brt, 1H, J = 5.3 Hz), 6.74 (s, 1H), 7.24−7.32 (m, 5H), 7.36−7.50 (m, 3H), 8.11−8.15 (m, 1H).

Dihydrochloride (*i*-PrOH): mp dec. Anal. ($C_{25}H_{28}N_4$·2HCl·1.5H_2O) C, H; N: calcd, 11.57; found, 11.15.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-5,6-dihydrobenzo[*h*]cinnoline (4h). The free base (white solid) obtained after 24 h at 100 °C was purified by flash chromatography (EtOAc with 2% (v) NH_4OH): yield 15%; mp 113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26−1.47 (m, 3H), 1.59−1.73 (m, 4H), 2.81−2.93 (m, 6H), 3.42−3.47 (m, 2H), 3.49 (s, 2H), 4.47 (brt, 1H, J = 5.2 Hz), 6.43 (s, 1H), 7.18−7.38 (m, 8H), 8.40−8.43 (m, 1H).

Dihydrochloride (*i*-PrOH): mp 270 °C. Anal. ($C_{26}H_{30}N_4$·2HCl·2H_2O) C, H; N: calcd, 11.04; found, 11.57.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]benzo[*h*]cinnoline (4i). The free base (yellow solid) was purified by flash chromatography (EtOAc): yield 25%; mp 160 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26−1.45 (m, 3H), 1.54−1.76 (m, 4H), 1.98 (brt, 2H, J = 11.2 Hz), 2.90 (brd, 2H, J = 12.2 Hz), 3.41−3.49 (m, 2H), 3.50 (s, 2H), 5.08 (brs, 1H), 6.78 (s, 1H), 7.29−7.39 (m, 6H), 7.58−7.81 (m, 4H), 9.33−9.36 (m, 1H).

Dihydrochloride (*i*-PrOH): mp 269 °C. Anal. ($C_{26}H_{28}N_4$·2HCl·3.5H_2O) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-2,5,6,7-tetrahydro-3*H*-benzo[6,7]cyclohepta[1,2-*c*]pyridazine (4j). The free base (colorless oil) was purified by flash chromatography (EtOAc−MeOH, 9:1): yield 30%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24−1.45 (m, 3H), 1.54−1.69 (m, 4H), 1.91 (brt, 2H, J = 11.3 Hz), 2.11 (quint., 2H, J = 7.1 Hz), 2.36 (t, 2H, J = 7.1 Hz), 2.51 (t, 2H, J = 7.1 Hz), 2.84 (brd, 2H, J = 11.6 Hz), 3.40−3.44 (m, 2H), 3.45 (s, 2H), 5.05 (brt, 1H, J = 5.2 Hz), 7.15−7.36 (m, 8H), 7.73−7.76 (m, 1H).

Dihydrochloride (*i*-PrOH): mp 160 °C. Anal. ($C_{27}H_{32}N_4$·2HCl·H_2O) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]pyridazine (4k). The free base (brown oil) was purified by flash chromatography (EtOAc−MeOH, 9:1 with 2% (v) TEA): yield 11%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28−1.46 (m, 3H), 1.61−1.74 (m, 4H), 1.98 (brt, 2H, J = 11.1 Hz), 2.91 (brd, 2H, J = 11.8 Hz), 3.37−3.49 (m, 2H), 3.52 (m, 2H), 4.92 (t, 1H, J = 5.0 Hz), 6.64 (dd, 1H, J_1 = 9.0 Hz, J_2 = 1.3 Hz), 7.15 (dd, 1H, J_1 = 9.0 Hz, J_2 = 4.5 Hz), 7.26−7.35 (m, 5H), 8.54 (dd, 1H, J_1 = 4.4 Hz, J_2 = 1.3 Hz).

Dihydrochloride (*i*-PrOH): mp 120 °C. Anal. ($C_{18}H_{24}N_4$·2HCl·1.5H_2O) C, H; N: calcd, 14.14; found, 13.72.

3-[2-(4-Benzylpiperazin-1-yl)ethylamino]-6-phenylpyridazine (16a). The free base (white solid) was purified by flash chromatography (EtOAc−MeOH, 9:1 with 2% (v) TEA):

yield 22%; mp 140 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.52 (m, 8H), 2.68 (t, 2H, J = 6.2 Hz), 3.52 (s, 2H), 3.53−3.60 (m, 2H), 5.36 (brs, 1H), 6.73 (d, 1H, J = 9.1 Hz), 7.28−7.34 (m, 5H), 7.42−7.47 (m, 3H), 7.59 (d, 1H, J = 9.5 Hz), 7.-95−8.00 (m, 2H).

Dihydrochloride (*i*-PrOH): mp 263 °C. Anal. ($C_{23}H_{27}N_5$·3HCl·H_2O) C, H, N.

3-[2-(4-Benzylpiperidin-1-yl)ethylamino]-6-phenylpyridazine (16b). The free base (white solid) was purified by flash chromatography (EtOAc−MeOH, 9:1 with 2% (v) TEA): yield 22%; mp 135 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32−1.36 (m, 2H), 1.65−1.69 (m, 3H), 2.05 (m, 2H), 2.37 (t, 2H, J = 6.5 Hz), 2.75 (d, 2H, J = 6.9 Hz), 3.03 (m, 2H), 3.62 (m, 2H), 7.05 (d, 1H, J = 9.4 Hz), 7.27−7.60 (m, 8H), 7.92 (d, 1H, J = 9.0 Hz), 8.09 (m, 2H).

Dihydrochloride (*i*-PrOH): mp 186 °C. Anal. ($C_{24}H_{28}N_4$·2HCl·1.5H_2O) C, H, N.

3-[2-(4-Benzylpiperazin-1-yl)ethan-1-one-2-amino3]-6-phenylpyridazine (18a). The free base (white solid) obtained after 24 h reflux in pyridine was purified by flash chromatography (EtOAc and then EtOAc with 2% (v) TEA): yield 10%; mp dec; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.42−2.50 (m, 4H), 3.49−3.53 (m, 2H), 3.54 (s, 2H), 3.71 (t, 2H, J = 5.1 Hz), 4.39 (d, 2H, J = 3.6 Hz), 5.84 (brs, 1H), 6.86 (d, 1H, J = 9.1 Hz), 7.31−7.33 (m, 5H), 7.39−7.49 (m, 3H), 7.60 (d, 1H, J = 9.4 Hz), 7.95−7.98 (m, 2H).

Dihydrochloride (*i*-PrOH): mp 225 °C. Anal. ($C_{23}H_{25}N_5O$·2HCl·2H_2O) C, H, N.

3-[2-(4-Benzylpiperidin-1-yl)ethan-1-one-2-amino3]-6-phenylpyridazine (18b). The free base (white solid) obtained after 24 h reflux in pyridine was purified by flash chromatography (EtOAc and then EtOAc with 2% (v) TEA): yield 16%; mp 144 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.18−1.31 (m, 2H), 1.73−1.88 (m, 3H), 2.58 (d, 2H, J = 6.7 Hz), 2.63−2.71 (m, 1H), 3.01 (brt, 1H, J = 12.6 Hz), 3.87 (brd, 1H, J = 13.5 Hz), 4.41 (t, 2H, J = 3.3 Hz), 4.64 (brd, 1H, J = 13.3 Hz), 6.05 (t, 1H, J = 3.6 Hz), 6.89 (d, 1H, J = 9.3 Hz), 7.13−7.36 (m, 5H), 7.40−7.45 (m, 3H), 7.59 (d, 1H, J = 9.3 Hz), 7.96−8.05 (2H, m).

Dihydrochloride (*i*-PrOH): mp 215 °C. Anal. ($C_{24}H_{26}N_4O$·HCl·0.5H_2O) C, H; N: calcd, 12.97; found, 12.52.

(V) Special Procedures. The 6-chloro-3-[2-(1-benzylpiperidin-4-yl)ethylamino]pyridazine **5** necessary for the synthesis of compounds **6a**−**o** and **7** was prepared according to a literature procedure.[34] Aryl boronic acids were commercially available except for the 2-ethylphenylboronic acid which was synthesized. Substituted 3-amino-6-arylpyridazines **6a**−**o** were synthesized by using the Suzuki procedure described in our previous paper[34] where the 6-arylpyridazines **6a**, **6c**, **6d**, **6f**, and **6g** were already prepared.

General Procedure for the Preparation of 6-Aryl-3-[2-(1-benzylpiperidin-4-yl)ethylamino]pyridazines. Argon was passed through a suspension of **5** (3.46 mmol, 1 equiv), arylboronic acid (3.98 mmol, 1.15 equiv), 2 M sodium carbonate (3.7 mL, 7.34 mmol, 2.12 equiv), toluene (20 mL), and possibly EtOH for 30 min. Tetrakis(triphenylphosphine)palladium(0) (0.10 mmol, 0.031 equiv) was added, and the mixture was heated at 110 °C for 24 h. The toluene was removed in vacuo. The residue was diluted with H_2O and extracted with EtOAc (3 × 5 mL). The organic layer was washed with H_2O (3 × 5 mL) and concentrated in vacuo. The crude product was purified by chromatography with a mixture of EtOAc, MeOH, and TEA. The corresponding hydrochlorides were prepared using the same procedure described previously.

Compounds obtained by this method are listed below.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-(2-ethylphenyl)pyridazine (6b). (a) 2-Ethylphenylboronic Acid. A solution of 2-bromoethyl-benzene (17.1 mmol, 1 equiv) in THF was added dropwise to a solution of Mg turnings (20.5 mmol, 1.2 equiv) in THF. The mixture was stirred at 20 °C over 1 h, and then after cooling at −78 °C, triisopropylborate (51.4 mmol, 3 equiv) was added dropwise. The solution was stirred at −78 °C for 1 h and allowed to warm to room temperature over 48 h. Then, the mixture was hydrolyzed with water (25 mL, stirred 1 h, and extracted with EtOAc (3 × 25 mL). The organic layer, dried over Na_2SO_4, was evaporated under reduce pressure to give the boronic acid, purified by trituration in a mixture Et_2O/hexane: yield 80%; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.15 (t, 3H, J = 7.5 Hz), 2.68 (q, 2H, J = 7.6 Hz), 7.24 (m, 4H), 7.55 (s, 2H); ES-MS m/z 150 $[M + H]^+$ (100%).

(b) 3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-(2-ethylphenyl)pyridazine (6b). The free base (colorless oil) was purified by flash chromatography (EtOAc−MeOH, 9:1 with 2% (v) TEA): yield 40%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.22 (m, 6H), 1.66 (m, 4H), 1.99 (brt, 2H, J = 9.0 Hz), 2.84 (m, 4H), 3.54 (m, 4H), 5.27 (s, 1H), 6.78 (d, 1H, J = 9.0 Hz), 7.30 (m, 9H), 7.68 (d, 1H, J = 9.0 Hz).

Dihydrochloride (*i*-PrOH): mp 115 °C. Anal. ($C_{26}H_{32}N_4$·2HCl·2.5H_2O) C, H; N: calcd, 10.81; found, 10.29.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-(2-chlorophenyl)pyridazine (6e). The free base (colorless oil) was purified by flash chromatography (EtOAc−MeOH, 9:1 with 2% (v) TEA): yield 50%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (m, 3H), 1.62 (m, 4H), 1.94 (brt, 2H, J = 9.0 Hz), 2.84 (m, 4H), 3.49 (m, 4H), 5.48 (s, 1H), 6.73 (d, 1H, J = 9.4 Hz), 7.08 (d, 1H, J = 9.3 Hz), 7.31−7.57 (m, 6H), 7.68 (d, 1H, J = 9.0 Hz), 7.58 (m, 1H), 7.82 (m, 1H), 7.84 (s, 1H).

Dihydrochloride (*i*-PrOH): mp 153 °C. Anal. ($C_{24}H_{27}ClN_4$·2HCl·2.5H_2O) C, H, N.

6-(3-Acetamidophenyl)-3-[2-(1-benzylpiperidin-4-yl)-ethylamino]pyridazine (6h). The free base (brown oil) was purified by flash chromatography (EtOAc−MeOH, 9:1): yield 30%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24−1.37 (m, 3H), 1.55−1.68 (m, 4H), 2.05 (brt, 2H, J = 12.4 Hz), 2.17 (s, 3H), 2.93 (brd, 2H, J = 11.3 Hz), 3.36−3.42 (m, 2H), 3.58 (s, 2H), 5.15 (brs, 1H), 6.69 (d, 1H, J = 9.4 Hz), 7.28−7.37 (m, 6H), 7.51 (d, 1H, J = 9.4 Hz), 7.62 (d, 1H, J = 7.9 Hz), 7.69 (d, 1H, J = 7.9 Hz), 8.06 (s, 1H), 8.31 (s, 1H).

Dihydrochloride (*i*-PrOH): mp 243 °C. Anal. ($C_{26}H_{31}N_5O$·2HCl·1.5H_2O) C, H, N.

6-(3-Acetylphenyl)-3-[2-(1-benzylpiperidin-4-yl)ethylamino]pyridazine (6i). The free base (white solid) was purified by flash chromatography (CH_2Cl_2−MeOH, 9:1): yield 80%; mp 108 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20−1.45 (m, 3H), 1.59−1.72 (m, 4H), 1.98 (brt, 2H, J = 10.9 Hz), 2.65 (s, 3H), 2.90 (brd, 2H, J = 11.6 Hz), 3.45−3.48 (m, 2H), 3.51 (s, 2H), 5.09 (brs, 1H), 6.74 (d, 1H, J = 9.4 Hz), 7.24−7.31 (m, 5H), 7.53 (t, 1H, J = 7.9 Hz), 7.63 (d, 1H, J = 9.4 Hz), 7.96 (d, 1H, J = 7.9 Hz), 8.17 (d, 1H, J = 7.9 Hz), 8.55 (s, 1H).

Dihydrochloride (*i*-PrOH): mp 243 °C. Anal. ($C_{26}H_{30}N_4O$·2HCl·H_2O) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-(3,4-methylenedioxyphenyl)pyridazine (6j). The free base (white solid) was purified by flash chromatography (EtOAc−MeOH, 9:1): yield 66%; mp 160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26−1.43 (m, 3H), 1.59−1.73 (m, 4H), 1.96 (brt, 2H, J = 10.9 Hz), 2.89 (brd, 2H, J = 11.7 Hz), 3.43−3.48 (m, 2H), 3.50 (s, 2H), 4.71 (brs, 1H), 6.01 (s, 2H), 6.66 (d, 1H, J = 9.0 Hz), 6.87 (d, 1H, J = 8.3 Hz), 7.24−7.39 (m, 6H), 7.50 (d,, 1H J = 9.4 Hz), 7.56 (d, 1H, J = 1.5 Hz).

Dihydrochloride (*i*-PrOH): mp 279 °C. Anal. ($C_{25}H_{28}N_4O_2$·2HCl·H_2O) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-(4-cyanophenyl)pyridazine (6k). The free base (yellow oil) was purified by flash chromatography (EtOAc−MeOH, 9:1): yield 50%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (m, 3H), 1.72 (m, 4H), 1.98 (t, 2H, J = 8.0 Hz), 2.91 (d, 2H, J = 11.0 Hz), 3.49 (m, 4H), 4.32 (s, 1H), 6.69 (d, 1H, J = 9.0 Hz), 7.25 (m, 5H), 7.91 (m, 4H), 8.16 (d, 1H, J = 9.0 Hz). ES-MS m/z: 398 $[M + H]^+$ (100%).

Dihydrochloride (*i*-PrOH): mp 247 °C. Anal. ($C_{25}H_{27}N_5$·2HCl·1.75H_2O) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-(4-fluorophenyl)pyridazine (6l). The free base (yellow oil) was purified by flash chromatography (EtOAc−MeOH, 9:1): yield 45%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (m, 3H), 1.67 (m, 3H), 1.67 (m, 4H), 1.81 (t, 2H, J = 8.0 Hz), 2.75 (d, 2H, J = 11.0

Hz), 3.50 (m, 4H), 4.73 (s, 1H), 6.84 (d, 1H, J = 9.4 Hz), 7.75 (m, 7H), 7.98 (d, 1H, J = 9.4 Hz), 8.01 (m, 2H). ES-MS m/z: 391 $[M + H]^+$ (100%).

Dihydrochloride (*i*-PrOH): mp 253 °C. Anal. ($C_{24}H_{27}FN_4 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-(4-dimethylaminophenyl)pyridazine (6m). The free base (yellow oil) was purified by flash chromatography (EtOAc−MeOH, 9:1): yield 65%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (m, 3H), 1.71 (m, 4H), 1.95 (t, 2H, J = 8.0 Hz), 2.84 (d, 2H, J = 11.0 Hz), 3.48 (m, 4H), 4.96 (s, 1H), 6.81 (m, 3H), 7.32 (m, 5H), 7.71 (d, 2H, J = 8.6 Hz), 7.84 (d, 1H, J = 9.2 Hz). ES-MS m/z: 416 $[M + H]^+$ (100%).

Trihydrochloride (*i*-PrOH): mp 145 °C. Anal. ($C_{26}H_{32}N_5 \cdot 3HCl \cdot 3.5H_2O$) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-(thiophen-2-yl)pyridazine (6n). The free base (white solid) was purified by flash chromatography (CH_2Cl_2−MeOH, 95:5): yield 22%; mp 164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55−1.59 (m, 3H), 1.75−1.88 (m, 4H), 2.17−2.22 (m, 2H), 3.07 (brd, 2H, J = 10.9 Hz), 3.60−3.66 (m, 2H), 3.69 (s, 2H), 4.83 (brs, 1H), 6.76 (d, 1H, J = 9.1 Hz), 7.20−7.23 (m, 1H), 7.39−7.47 (m, 6H), 7.56−7.57 (m, 1H), 7.62 (d, 1H, J = 9.3 Hz).

Dihydrochloride (*i*-PrOH): mp 227 °C. Anal. ($C_{22}H_{26}N_4S \cdot 2HCl \cdot 2H_2O$) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-(pyridin-3-yl)pyridazine (6o). The free base (colorless oil) was purified by flash chromatography (EtOAc and then EtOAc−MeOH, 9:1): yield 24%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37−1.42 (m, 3H), 1.62−1.72 (m, 4H), 1.97−2.05 (m, 2H), 2.93 (brd, 2H, J = 11.5 Hz), 3.44−3.51 (m, 2H), 3.54 (s, 2H), 5.22 (brs, 1H), 6.75 (d, 1H, J = 9.0 Hz), 7.27−7.39 (m, 6H), 7.59 (d, 1H, J = 9.3 Hz), 8.32 (dt, 1H, J_1 = 8.1 Hz, J_2 = 1.8 Hz), 8.60 (dd, 1H, J_1 = 4.6 Hz, J_2 = 1.6 Hz), 9.10 (d, 1H, J = 1.8 Hz).

Trihydrochloride (*i*-PrOH): mp 131 °C. Anal. ($C_{23}H_{27}N_5 \cdot 3HCl \cdot 3H_2O$) C, H; N: calcd, 13.04; found, 12.08.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-methoxypyridazine (7). To a solution of 3-[2-(1-benzylpiperidin-4-yl)-ethylamino]-6-chloropyridazine **5** [34] (1.6 mmol) in DMF (10 mL) was added at room temperature a solution of MeONa (Na, 3.2 mmol) in MeOH (5 mL). The mixture was heated at 130 °C for 20 h. After cooling, the reaction mixture was poured onto H_2O (100 mL), rendered alkaline with a 10% K_2CO_3 solution, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, concentrated under reduce pressure, and purified by flash chromatography (EtOAc−MeOH, 9:1 with 2% (v) TEA) to give a yellow oil: yield 50%; ^{1}H NMR (200 MHz, acetone-d_6) δ 1.70−1.90 (m, 7H), 2.60 (m, 2H), 3.21 (brd, 2H, J = 10.9 Hz), 3.50−3.55 (m, 2H), 3.98 (s, 2H), 6.05 (s, 1H), 6.83 (d, 1H, J = 9.1 Hz), 7.05 (d, 1H, J = 9.1 Hz), 7.41−7.47 (m, 3H), 7.67−7.72 (m, 3H).

Dihydrochloride (*i*-PrOH): mp 120 °C. Anal. ($C_{19}H_{26}N_4O \cdot 2HCl \cdot 2H_2O$) C, H, N.

3-[2-(1-Benzylpiperidin-4-yl)ethoxy]-6-phenylpyridazine (10). (a) 1-Benzyl-4-[(ethoxycarbonyl)methylene]piperidine[35] (8). A mixture of K_2CO_3 (84.7 mmol) and triethyl phosphonoacetate (84.7 mmol) in dry THF (40 mL) was stirred at room temperature for 15 min and then refluxed for 20 min. After cooling, 1-benzyl-4-piperidone (56.4 mmol) was added, and the mixture was heated under reflux for 48 h. After cooling, a 10% K_2CO_3 solution (100 mL) was added, and the mixture was extracted with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated under reduce pressure. The crude product was purified by flash chromatography (EtOAc−hexane, 1:9). A colorless oil was obtained: yield 84%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, 3H, J = 7.2 Hz), 2.31−2.36 (m, 2H), 2.53 (t, 4H, J = 5.6 Hz), 2.98 (t, 2H, J = 5.6 Hz), 3.53 (s, 2H), 4.15 (q, 2H, J = 7.2 Hz), 5.64 (s, 1H), 7.17−7.27 (m, 5H).

(b) 1-Benzyl-4-(2-hydroxyethyl)piperidine [35] (9). To a suspension of $LiAlH_4$ ((38.5 mmol) in dry THF (30 mL) was added, at 0 °C, 1-benzyl-4-[(ethoxycarbonyl)methylene]piperidine **8** (77.1 mmol) in dry THF (10 mL). The mixture was refluxed for 20 h, and after cooling unreacted $LiAlH_4$ was quenched by careful addition of 10% NaOH solution (60 mL). The solution was filtered and washed with H_2O and EtOAc. The filtrate was extracted with EtOAc, and the combined organic layers, dried over Na_2SO_4, were concentrated to give a colorless oil: yield 95%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34−1.53 (m, 5H), 1.95 (brd, 2H, J = 11.8 Hz), 1.98 (brt, 2H, J = 11.4 Hz), 2.90 (brd, 2H, J = 11.7 Hz), 3.52 (s, 2H), 3.70 (t, 2H, J = 6.6 Hz), 7.30−7.36 (m, 5H).

(c) 3-[2-(1-Benzylpiperidin-4-yl)ethoxy]-6-phenylpyridazine (10). A mixture of sodium (7.2 mmol) and 1-benzyl-2-(hydroxyethyl)piperidine) **9** (7.2 mmol) in dry THF (10 mL) was stirred vigorously at 50 °C for 1 h. 3-Chloro-6-phenylpyridazine[36] (7.2 mmol), dissolved in dry THF (10 mL), was added dropwise, and the mixture was refluxed for 1 h. After cooling, the mixture was hydrolyzed with H_2O, and THF was removed under reduced pressure. The residue was extracted with EtOAc, and the organic layer, dried over Na_2SO_4, was evaporated under reduce pressure. The crude product was purified by flash chromatography (EtOAc) to obtain a white solid: yield 10%; mp 149 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.23−1.52 (m, 3H), 1.64−1.86 (m, 4H), 1.90−2.03 (m, 2H), 2.89 (brd, 2H, J = 11.7 Hz), 3.49 (s, 2H), 4.62 (t, 2H, J = 6.7 Hz), 7.02 (d, 1H, J = 9.2 Hz), 7.28−7.34 (m, 5H), 7.44−7.51 (m, 3H), 7.78 (d, 1H, J = 9.2 Hz), 7.99−8.03 (m, 2H).

Dihydrochloride (*i*-PrOH): mp 155 °C. Anal. ($C_{24}H_{27}N_3O \cdot 2HCl \cdot 0.5H_2O$) C, H; N.

3-[2-(1-Benzylpiperidin-4-yl)ethylthio]-6-phenylpyridazine (12). (a) 1-Benzyl-4-(2-chloroethyl)piperidine[37] (11). To a solution of 1-benzyl-4-(2-hydroxyethyl)piperidine **9** (4.3 mmol) in CH_2Cl_2 (10 mL), was added dropwise $SOCl_2$ (8.6 mmol) with ice cooling. The mixture was refluxed for 2 h and then evaporated. The residue was rendered alkaline with 10% K_2CO_3 solution and extracted with EtOAc. The organic layer, dried over Na_2SO_4, was evaporated under reduce pressure to give a yellow oil: yield 81%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21−1.34 (m, 2H), 1.48−1.52 (m, 1H), 1.65−1.76 (m, 4H), 1.96 (td, 2H, J_1 = 11.5 Hz, J_2 = 2.1 Hz), 2.88 (brd, 2H, J = 11.5 Hz), 3.50 (s, 2H), 3.57 (t, 2H, J = 7.2 Hz), 7.25−7.32 (m, 5H).

(b) 6-Phenyl-3-thiolpyridazine.[38] A mixture of 3-chloro-6-phenylpyridazine (2.6 mmol) and thiourea (2.6 mmol) in EtOH (10 mL) was refluxed for 30 min. The mixture was evaporated, and H_2O (20 mL) was added to the residue, followed by Na_2CO_3 (1.3 mmol). The precipitate was collected by filtration and washed with Et_2O. A yellow solid was obtained: yield 82%; mp 167 °C (lit.[38] 160 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.48−7.55 (m, 4H), 7.79−7.86 (m, 3H).

(c) 3-[2-(1-Benzylpiperidin-4-yl)ethylthio]-6-phenylpyridazine (12). To a solution of 6-phenyl-3-thiolpyridazine (1.6 mmol) and EtONa (1.6 mmol) in EtOH (5 mL) was added at room temperature 1-benzyl-4-(2-chloroethyl)piperidine **11** (1.6 mmol) dissolved in EtOH (5 mL). The mixture was refluxed for 4 h. The solvent was evaporated, H_2O was added, and the residue was extracted with EtOAc. The organic layer, dried over Na_2SO_4, was evaporated under reduced pressure, and the crude product was purified by flash chromatography (EtOAc−hexane, 1:1 and then EtOAc) to give a white solid: yield 20%; mp 152 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.18−1.39 (m, 2H), 1.39−1.57 (m, 1H), 1.66−1.77 (m, 4H), 1.93 (brt, 2H, J = 11.3 Hz), 2.86 (brd, 2H, J = 11.7 Hz), 3.36−3.44 (m, 2H), 3.46 (s, 2H), 7.23−7.35 (m, 6H), 7.45−7.48 (m, 3H), 7.60 (d, 1H, J = 9.0 Hz), 8.00−8.04 (m, 2H).

Dihydrochloride (*i*-PrOH): mp 210 °C. Anal. ($C_{24}H_{27}N_3S \cdot 2HCl$) C, H; N.

3-[2-(1-Benzylpiperidin-4-yl)acetamido]-6-phenylpyridazine (14a). (a) 2-(1-Benzylpiperidin-4-yl)acetic Acid Hydrochloride (13a). A mixture of 2-(1-benzylpiperidin-4-yl)acetonitrile[14] (15.1 mmol) and a 1 M solution of NaOH in ethanol (EtOH−H_2O, 9:1) (30 mL) was refluxed for 28 h. The reaction mixture was concentrated by evaporation, acidified with concentrated HCl, and concentrated again by evaporation. 2-Propanol was added, and the residue was evaporated to give a beige moss: yield 95%; ^{1}H NMR (300 MHz, CD_3OD) δ 1.22−1.34 (m, 2H), 1.66−1.80 (m, 3H), 2.00 (brt, 2H, J = 12.0 Hz), 2.10 (d, 2H, J = 7.1 Hz), 2.87 (brd, 2H, J = 12.0 Hz), 3.49 (s, 2H), 7.22−7.31 (m, 5H).

(b) 3-[2-(1-Benzylpiperidin-4-yl)acetamido]-6-phenylpyridazine (14a). A mixture of 2-(1-benzylpiperidin-4-yl)acetic acid hydrochloride **13a** (5.2 mmol) and $(COCl)_2$ (14 mL) was stirred at room temperature for 30 min. The $(COCl)_2$ excess was evaporated, and a solution of 3-amino-6-phenylpyridazine (2.6 mmol), TEA (7.8 mmol), and CH_2Cl_2 (30 mL) was added to the residue. The reaction mixture was stirred at room temperature for 2 h, and the CH_2Cl_2 was removed in vacuo. A 10% citric acid solution was added to the residue. The aqueous layer was washed with EtOAc, rendered alkaline with K_2CO_3, and then extracted with EtOAc. The organic layer, dried over Na_2SO_4, was concentrated under reduced pressure and purified by flash chromatography (EtOAc−MeOH, 9:1) to give a white solid: yield 15%; mp 293 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39−1.51 (m, 2H), 1.78 (brd, 2H, J = 12.8 Hz), 1.96−2.05 (m, 3H), 2.66 (d, 2H, J = 6.7 Hz), 2.88 (brd 2H, J = 10.9 Hz,), 3.49 (s, 2H), 7.07−7.23 (m, 5H), 7.24−7.52 (m, 3H), 7.91 (d, 1H, J = 9.4 Hz), 8.03−8.06 (m, 2H), 8.65 (d, 1H, J = 9.4 Hz), 10.12 (s, 1H).

Dihydrochloride (*i*-PrOH): mp 117 °C. Anal. ($C_{24}H_{26}N_4O$·2 HCl·5H_2O) H; N; C: calcd, 52.42; found, 52.88.

3-[2-(4-Benzylpiperazin-1-yl)acetamido]-6-phenylpyridazine (14b). (a) Ethyl 2-(4-Benzylpiperazin-1-yl)acetate. A mixture of ethyl chloroacetate (43.3 mmol), 1-benzylpiperazine (28.8 mmol), and TEA (28.8 mmol) in toluene (60 mL) was heated at 80 °C for 7 h. After cooling, the solvent was removed by evaporation and H_2O was added. The reaction mixture was extracted with EtOAc. The organic layer, dried over Na_2SO_4, was concentrated under reduce pressure and purified by flash chromatography (EtOAc) to give a yellow oil: yield 95%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28 (t, 3H, J = 7.0 Hz), 2.54−2.62 (m, 8H), 3.21 (s, 2H), 3.53 (s, 2H), 4.20 (q, 2H, J = 7.0 Hz), 7.27−7.34 (m, 5H).

(b) 2-(4-Benzylpiperazin-1-yl)acetic Acid Dihydrochloride (13b). A mixture of ethyl 2-(4-benzylpiperazin-1-yl)-acetate (28.0 mmol) and 2 M NaOH solution (28 mL) in MeOH (100 mL) was refluxed for 1 h. After cooling, the reaction mixture was concentrated by evaporation, acidified with concentrated HCl, and concentrated again by evaporation. Et_2O was added to the residue, and the solvent was evaporated to give a beige solid: yield 77%; mp 249 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 3.67−3.79 (m, 8H), 4.25 (s, 2H), 4.52 (s, 2H), 7.50−7.65 (m, 5H).

(c) 3-[2-(4-Benzylpiperazin-1-yl)acetamido]-6-phenylpyridazine (14b). This compound was prepared using the same procedure described in the preparation of **14a**. White solid; yield 18%; mp 241 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.62−2.73 (m, 8H), 3.26 (s, 2H), 3.59 (s, 2H), 7.29−7.38 (m, 5H), 7.53−7.57 (m, 3H), 7.92 (d, 1H, J = 9.2 Hz), 8.06−8.11 (m, 2H), 8.59 (d, 1H, J = 9.2 Hz), 10.24 (brs, 1H).

Dihydrochloride (*i*-PrOH): mp 241 °C. Anal. ($C_{23}H_{25}N_5O$·2HCl·3H_2O) C; H; N.

3-[2-(4-Benzylpiperidin-1-yl)acetamido]-6-phenylpyridazine (14c). (a) Ethyl 2-(4-benzylpiperidin-1-yl)acetate. This compound was prepared using the same procedure described in the preparation of ethyl 2-(4-benzylpiperazin-1-yl)acetate. Orange oil; yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H, J = 7.1 Hz), 1.36−1.45 (m, 2H), 1.46−1.54 (m, 1H), 1.61 (brd, 2H, J = 13.9 Hz), 2.09 (brt, 2H, J = 11.3 Hz), 2.53 (d, 2H, J = 6.7 Hz), 2.90 (brd, 2H, J = 11.3 Hz), 3.16 (s, 2H), 4.16 (q, 2H, J = 7.1 Hz), 7.11−7.19 (m, 3H), 7.23−7.28 (m, 2H).

(b) 2-(4-Benzylpiperidin-1-yl)acetic Acid Hydrochloride (13c). This compound was prepared using the same procedure described in the preparation of **13b**. Beige moss; yield 98%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.84 (m, 5H), 2.61 (d 2H, J = 5.7 Hz), 3.03 (m, 2H), 3.68 (brd, 2H, J = 11.3 Hz), 3.81 (s, 2H), 7.12−7.30 (m, 5H), 7.47 (m, 1H).

(c) 3-[2-(4-Benzylpiperidin-1-yl)acetamido]-6-phenylpyridazine (14c). This compound was prepared using the same procedure described in the preparation of **14b**. White solid; yield 20%; mp 160 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15−1.65 (m, 5H), 2.17 (brt, 2H, J = 10.3 Hz), 2.50 (d, 2H, J = 6.0 Hz), 2.85 (brd, 2H, J = 11.8 Hz), 3.10 (s, 2H), 7.06−7.25 (m, 5H), 7.40−7.48 (m, 3H), 7.80 (d, 1H, J = 9.3 Hz), 7.96−8.00 (m, 2H), 8.48 (d, 1H, J = 9.3 Hz), 10.12 (brs, 1H).

Dihydrochloride (*i*-PrOH): mp 234 °C. Anal. ($C_{24}H_{26}N_4O$·2HCl·0.5H_2O) C; H; N.

Biological Studies. In Vitro Measurement of Acetylcholinesterase and Butyrylcholinesterase Inhibition. Acetylcholinesterase and butyrylcholinesterase inhibitory activity were measured by the spectrophotometric method of Ellman et al.[40] Electric eel AChE (Type III, electric eel, Sigma Chemical Co.) and human erythrocytes AChE (Type XIII, human erythrocytes, Sigma Chemical Co.) were used as sources of AChE, and lyophilized human serum (crude powder, Sigma Chemical Co.) was used as source of BuChE. AChE preparations (from electric eel and human erythrocytes) and BuChE (from human serum) were dissolved in 0.1 M potassium phosphate buffer pH 7.2 such as to have an enzyme solution stock with 2.5 units/mL AChE activity. Acetylthiocholine iodide and butyrylthiocholine iodide (Sigma Chemical Co.) were used as the substrates of the enzymatic reaction and 5,5-dithiobis(2-nitrobenzoic) acid (DTNB) for the measurement of cholinesterase activity. In this procedure, 940 μL of 0.1 M potassium buffer pH 8 with 60 mg/500 mL DTNB, 20 μL of test compound solution, and 20 μL of enzyme stock solution (electric eel and human erythrocytes AChE and human serum BuChE) were mixed. After 10 min of preincubation, 20 μL of 10 mM acetylthiocholine/butyrylthiocholine iodide was added to the assay solution. The final assay volume was 1 mL. The change in absorbance at 412 nm was recorded (spectrophotometer SHIMADZU UV-2401PC) during 1 min at 25 °C. The reaction rate was calculated. Different concentrations (range of 10^{-9} M−10^{-3} M) of the test compound were assayed (triplicate), and the percent inhibition due to the presence of test compound was calculated. IC_{50} values were determined graphically from log concentration−inhibition curves.

Computational Methods. (a) Inhibitor Structures. The crystal structure of minaprine retrieved from the Cambridge Structural Database was used as template to construct the inhibitors. All molecules were assumed to be monoprotonated under physiological conditions, and their molecular structures were generated accordingly using the SYBYL 6.5 software (Tripos Associates, St. Louis, MO). The geometry of the inhibitors was optimized using the Tripos force field and the conjugate gradient method until the energy difference between successive cycles was below 0.01 kcal mol^{-1}. Partial atomic charges were calculated using the semiempirical method AM1.

(b) Crystal Structures. The crystal structures of AChE from *T. californica* complexed with decamethonium, edrophonium, huperzine, and tacrine were retrieved from the Brookhaven Protein Database. For the docking studies of the 3-aminopyridazines, we selected the AChE crystal structure complexed with decamethonium, since our developed inhibitors show the closest resemblance with the size and shape of decamethonium. The ligand structure of the complexes was deleted, hydrogen atoms were added, and charges from AMBER [47] were loaded. AM1 [48] charges were calculated for the ligands. All water molecules observed in the receptor crystal structure were deleted, and the enzyme structure was subjected to a minimization using the AMBER force field, keeping all protein backbone atoms at fixed positions.

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