Aminopyridazines as Acetylcholinesterase Inhibitors

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Following the discovery of the weak, competitive and reversible acetylcholinesterase (AChE)-inhibiting activity of minaprine (**3c**) (IC_{50} = 85 μM on homogenized rat striatum AChE), a series of 3-amino-6-phenylpyridazines was synthesized and tested for inhibition of AChE. A classical structure−activity relationship exploration suggested that, in comparison to minaprine, the critical elements for high AChE inhibition are as follows: (i) presence of a central pyridazine ring, (ii) necessity of a lipophilic cationic head, (iii) change from a 2- to a 4-5-carbon units distance between the pyridazine ring and the cationic head. Among all the derivatives investigated, 3-[2-(1-benzylpiperidin-4-yl)ethylamino]-6-phenylpyridazine (**3y**), which shows an IC_{50} of 0.12 μM on purified AChE (electric eel), was found to be one of the most potent anti-AChE inhibitors, representing a 5000-fold increase in potency compared to minaprine.[1]

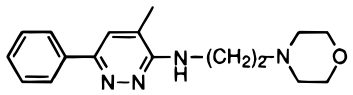
Introduction

Senile dementia of the Alzheimer's type (SDAT) is a neurodegenerative disease affecting mainly aging populations. A deficit in cholinergic neurotransmission is considered to be one of the major causes of disturbances in learning and memory in SDAT patients.[2] Muscarinic M_1 agonists[3] can enhance cholinergic transmission, and acetylcholinesterase (AChE) inhibitors[4] such as tacrine, donepezil, and rivastigmine are already used as therapeutic agents for the palliative treatment of SDAT.[5,6]

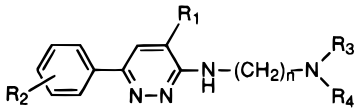
In the search for original AChE inhibitors with potential central bioavailability, we concentrated our research on an aminopyridazine, minaprine (**3c**) (Chart 1), which possesses favorable characteristics. Effectively, minaprine, in addition to its original antidepressive properties, exhibits cholinomimetic activities. Thus, an in vivo administration of minaprine (30 mg/kg po) to rats significantly increases acetylcholine (ACh) levels in the hippocampus (38%) and striatum (60%).[7] In vitro, minaprine presents a weak, competitive, and reversible AChE inhibitive activity on homogenized rat striatum (IC_{50} = 85 μM)[8] and a very weak activity on electric eel AChE (IC_{50} = 600 μM). On the other hand, minaprine exhibits a weak but highly selective affinity for muscarinic M_1 receptor (IC_{50} = 17 μM, [^{3}H]pirenzepine).[9−12] Assuming that the tertiary amino function (protonated in vivo) can mimic the quaternary group of ACh and that the *exo-endo* amidino function can be considered as bioisosteric to the ACh ester function,[8] we hypothesized that minaprine can as well be recognized by the muscarinic M_1 receptors as by the enzyme AChE. These findings and the excellent central bioavailability of minaprine prompted us to investigate further around this lead compound.

In the present study, we describe the synthesis and the biochemical evaluation of a series of 3-amino-6-arylpyridazine derivatives (Chart 1) based on the structure of minaprine.

Chart 1



minaprine **3c**



3a - 3y

Chemistry

A general synthesis of 3-aminoalkyl-substituted pyridazines [13−15] (Tables 2 and 4) is shown in Scheme 1. The key intermediates in their preparation are imino chlorides **2** which can be readily obtained from the corresponding 3(2*H*)-pyridazinones **1** by the action of phosphorus oxychloride. Condensation of the imino chlorides **2** with various primary amines **12** (Table 1) gave the final products **3**. Most of the 3(2*H*)-pyridazinones **1** were prepared according to the literature.[16−20] The 4-trifluoromethyl-6-phenyl-3(2*H*)-pyridazinone (**1j**) (Scheme 2) was obtained by Michael addition of KCN to 4,4,4-trifluoro-1-phenyl-2-buten-1-one (**13**),[21] followed by hydrolysis of the nitrile **14**, esterification, and cyclization with hydrazine hydrate. It was converted to **3j** by the previously described procedure (Scheme 1). The synthesis of 4-amino-6-phenylpyridazine (**3k**) (Scheme 2) involved a particular procedure. A nucleophilic displacement of the 1,1-bis(thiomethyl)-2-nitroethylene (**15**), first by 1-aminoethylmorpholine and then by hydrazine hydrate, followed by condensation with phenylglyoxal, allowed us to obtain **16**. The catalytic hydrogenation (H_2, Pd/C) of **16** gave **3k**.

The preparation of the non-pyridazinic heterocyclic compounds **4**−**6** (Table 3) utilizes the general route

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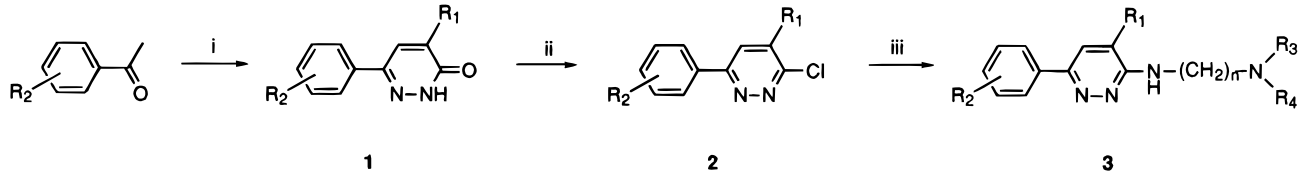
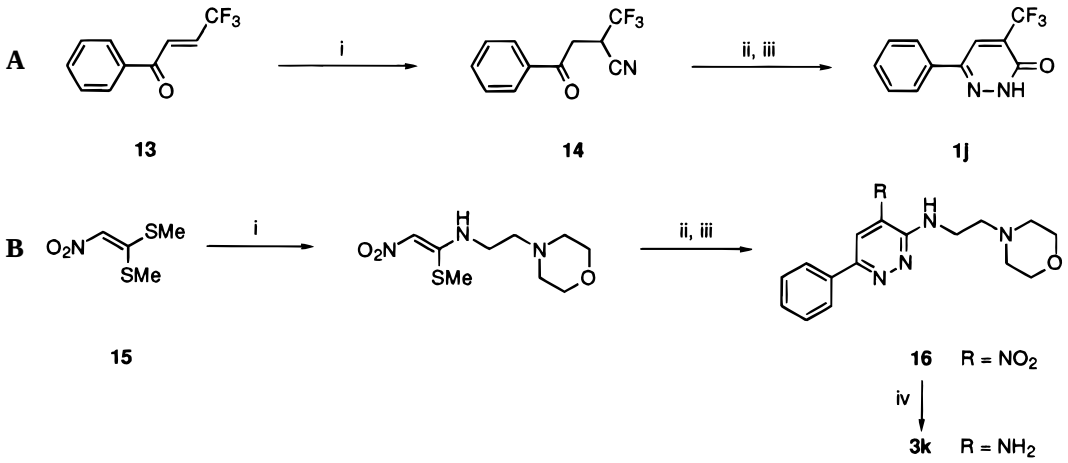
Scheme 1[a]



1 **2** **3**

[a] Reagents and conditions: (i) R_1-CO-COOH, NH_2NH_2, 100 °C; (ii) $POCl_3$, 75 °C, 4 h; (iii) H_2N-$(CH_2)_n$-NR_3R_4 (**12**), NH_4Cl, *n*-BuOH, Δ, 48 h.

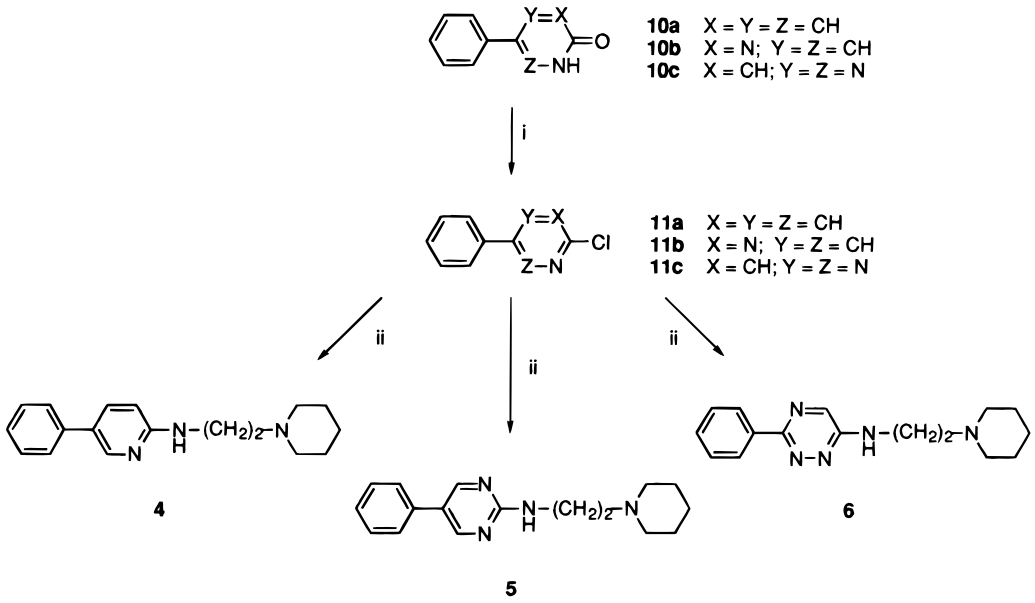
Scheme 2[a]



13 **14** **1j** **15** **16** R = NO_2 **3k** R = NH_2

[a] A. Reagents and conditions: (i) KCN; (ii) H_2SO_4, EtOH; (iii) H_2N-NH_2, H_2O, 100 °C. B. Reagents and conditions: (i) aminoethylmorpholine, Δ, 3 h; (ii) H_2N-NH_2, EtOH, 0.5 h; (iii) C_6H_5COCHO, room temperature, 5 h; (iv) H_2, Pd/C, MeOH, 70 psi, 3 h.

Scheme 3[a]



10a X = Y = Z = CH
10b X = N; Y = Z = CH
10c X = CH; Y = Z = N

11a X = Y = Z = CH
11b X = N; Y = Z = CH
11c X = CH; Y = Z = N

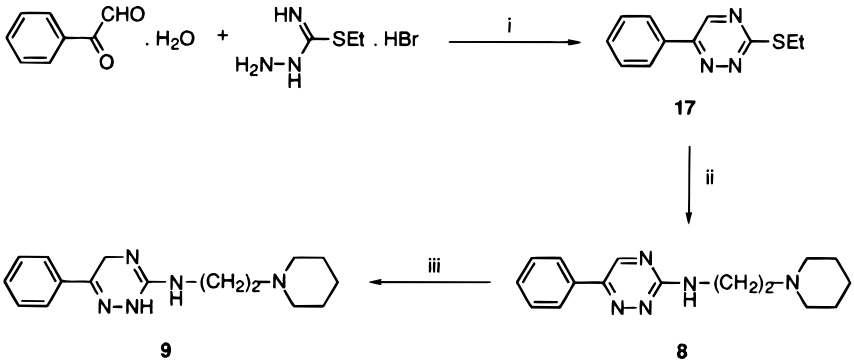
4 **5** **6**

[a] Reagents and conditions: (i) $POCl_3$, 75 °C, 4 h; (ii) aminoethylpiperidine, NH_4Cl, *n*-BuOH, Δ, 48 h.

depicted in Scheme 3. The 5-phenylpyridinone **10a**, starting material for the synthesis of **4**, was obtained according to the literature[22−24] as well as the 1-methyl-5-phenyl-2(1*H*)-pyrimidinone (**10b**),[24−26] starting material for the synthesis of **5**. To obtain **6**, the synthesis of 3-phenyl-1,2,4-triazinone (**10c**), was accomplished by a slightly modified version of the procedure described by Lawesson:[27] thionation of ethyl hippurate by means of P_4S_{10}, cyclization with hydrazine hydrate, followed by an oxidation of the obtained 3-phenyl-4,5-dihydrotriazinone with MnO_2.[28,29] The synthesis of 2-[2-(piperidin-1-yl)ethylamino]-5-phenyl-1,3,4-thiadiazole (**7**) (Table 3) involves the nucleophilic displacement of the corresponding imino chloride with 2-aminoethylpiperidine. The necessary imino chloride was prepared by a Sandmeyer reaction applied to 2-amino-5-phenyl-1,3,4-thiadiazole.[30]

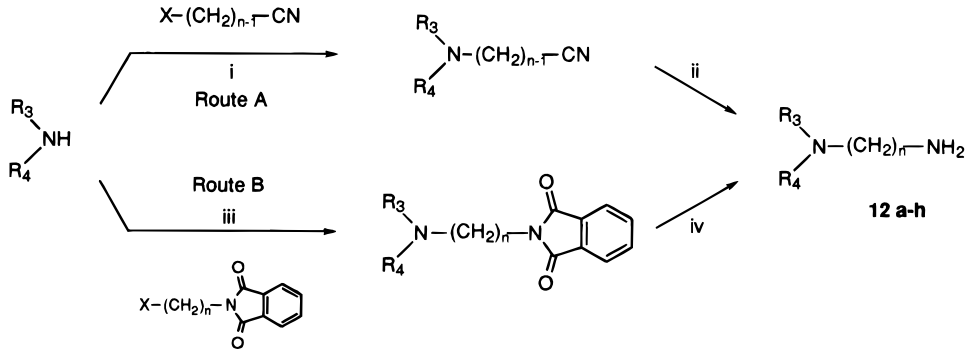
The intermediate 3-ethylthiotriazine (**17**), necessary for the synthesis of the 6-phenyl-3-[2-(piperidin-1-yl)-ethylamino]triazine (**8**), was prepared (Scheme 4) by a modification of the Paudler et al.[31] procedure by reacting *S*-ethylthiosemicarbazide hydrobromide with phenylg-

Scheme 4[a]



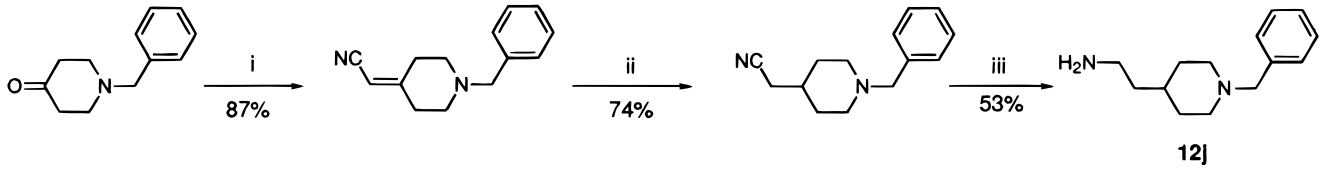
[a] Reagents and conditions: (i) EtOH, Δ; (ii) aminoethylpiperidine, 125 °C; (iii) H_2, Pd/C, MeOH, 50 psi.

Scheme 5[a]



[a] Reagents and conditions: Route A (i) toluene, K_2CO_3, Δ; (ii) $LiAlH_4$, THF, room temperature; route B (iii) xylene, 130 °C; (iv) H_2N-NH_2, EtOH.

Scheme 6[a]



[a] Reagents and conditions: (i) $(EtO)_2P(O)CH_2CN$, THF, K_2CO_3, Δ, 12 h; (ii) Mg, MeOH, room temperature, 4 h; (iii) $LiAlH_4$, THF, 0 °C, 1 h.

lyoxal in refluxing ethanol and condensing the obtained *S*-ethylthiotriazine with 2-aminoethylpiperidine. Catalytic hydrogenation (H_2, Pd/C) of the triazine **8** afforded the dihydrotriazine **9**.

The diamines **12a**−**12h** (Table 1) were prepared by conventional methods (Scheme 5): either alkylation of the secondary amines by means of *ω*-halogenonitriles (route A) or Gabriel syntheses starting from the commercially available *ω*-bromoalkylphthalimides[32] (route B). A slightly modified method described by Dutta et al.[33] allowed us to obtain 2-(1-benzylpiperidin-4-yl)-ethylamine (**12j**) (Scheme 6) after reduction of the intermediate 2-(1-benzyl-4-piperidylidene)acetonitrile.[34]

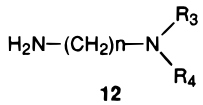
Results and Discussion

The minaprine analogues were initially tested for in vitro inhibition of acetylcholinesterase in homogenized rat striatum (Tables 2 and 3) and, for more extensive studies, on the commercially available electric eel AChE (Table 4). The results obtained with the latter enzyme preparations allow molecular modeling studies using the published AChE structure which is also derived from the electric eel enzyme.[35] The esterase activity was determined according to the method of Ellman et al.[36]

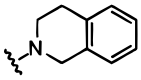
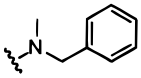
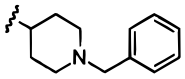
We observed that rather simple molecular variations of the lead structure **3c** can drastically modify AChE inhibition potency (Table 2). Thus, the replacement of the terminal morpholino (**3a**, **3c**) to a piperidino group (**3b**, **3d**) is associated with a noticeable increase in AChE inhibition (>75-fold for **3b** and 15-fold for **3d**). The presence of a methyl group in the 4 position of the pyridazine ring seems to be determinant for anti-AChE activity. Compounds **3a** and **3b** were found to be respectively 12 and 3 times less potent than compounds **3c** and **3d**. Substituents other than methyl or hydrogen in the 4-position are detrimental (**3h**−**3l**). Substitution of the phenyl ring was also investigated. Slight increases were observed for derivatives **3e**−**3g**.

To select the most suitable central heterocycle, we examined a set of pyridazine bioisosteres (Table 3). The replacement of the pyridazine ring (**3b**) by pyridine (**4**), pyrimidine (**5**), 1,2,4-thiadiazole (**7**), and triazine (**6** and **8**) rings yielded compounds showing weaker activities. The comparison shows clearly that the central py-

Table 1. Diamines Synthesized



12

Compnd	n		Conditions [a]	Yield (%)
12a	2		A	28
12b	3		B	85
12c	4		A	18
12d	5		A	50
12e	2		A	56
12f	3		B	75
12g	4		B	75
12h	5		A	66
12i[b]	1		/	45
12j[c]	2		/	34

[a] A: (i) toluene, K_2CO_3, Δ; (ii) $LiAlH_4$, THF, room temperature. B: (iii) xylene, 130 °C; (iv) H_2N-NH_2, EtOH. [b] References 47−49. [c] References 33−34.

ridazine ring (**3b**) is particularly favorable, all the other ring systems being 4 to 5 times less potent.

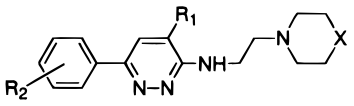
We chose to conserve the phenylpyridazine moiety and to examine the importance of the nature and the length of the side chain (Table 4). The replacement of the hydrophilic morpholine by a lipophilic monocyclic side chain (**3a** → **3b** or **3n**) enhanced AChE inhibition 10-fold when the distance *n* between the *exo*-amidinic and the tertiary nitrogens corresponds to two methylene units. The addition of an aromatic ring close to the cationic nitrogen, such as found in the *N*-benzyl-*N*-methyl derivative **3s** or its constraint 1,2,3,4-tetrahydroisoquinolinyl derivative **3o**, did not modify dramatically the inhibition potency, at least for an $n = 2$ value. Augmenting the distance between the *exo*-amidinic nitrogen and the tertiary nitrogen atom ($n = 3$, 4, and 5) entails a regular increase in potency: an 8-fold increase for the passage from **3o** to **3r** and a 230-fold increase for the passage from **3s** to **3v**.The flexibility of the *N*-benzyl group seems to be critical for the anti-AChE activity when the *n* distance corresponds to 4−5 methylene groups. Thus, compound **3v** is 14 times more active than compound **3r**. Inserting the side-chain methylenes in a piperidine ring (**3w**−**3y**) yields compounds which were more potent than the open equivalent *N*-benzyl-*N*-methyl derivatives. Within this series the inhibition potency increases with the side-chain length. Thus, the 3-[2-(1-benzylpiperidin-4-yl)ethylamino]-6-phenylpyridazine (**3y**) was found to be respectively 46 and 540 times more active than the shorter analogues **3x** and **3w**. It is also 6 times more potent than the corresponding open analogue equivalent **3v**.

Among all the compounds examined **3y** was the most potent inhibitor with an $IC_{50} = 0.12$ μM on electric eel AChE. It appears thus that the ideal inter-nitrogen distance corresponds to a 4- or 5-carbon chain and that the most favorable lipophilic side chain is the ethyl *N*-benzylpiperidinyl chain which is also present in donepezil. The three compounds **3r**, **3v**, and **3y** as well as two references inhibitors, tacrine[37] and donepezil,[38,39] were then compared in order to determine their relative inhibitory effects to acetyl and butyrylcholinesterase (IC_{50} BuChE/AChE). In addition to electric eel AChE, we used human erythrocytes as a source of AChE and human serum as a source of butyrylcholinesterase (Table 5). Compound **3y** is comparable to tacrine; however, its BuChE/AChE ratio is about 10 times more favorable. Compared to donepezil, compound **3y** is about 10 times less potent and clearly less selective toward BuChE. Surprisingly the tetrahydroisoquinolinyl analogue **3r** shows an inversed profile, being 150 times more selective for butyrylcholinesterase than for acetylcholinesterase. As such it might present some interest as a research tool.

Conclusion

With respect to minaprine, chosen as lead compound, we were able to prepare AChE inhibitors with a 5000-fold increased potency. Structure−activity relationship studies indicated that at least three factors are favorable

Table 2. Substitution Effects in Morpholinoethyl- and Piperidinoethylaminopyridazines



Compnd	X	R_1	R_2	Mp,°C [a]	Formula	IC_{50} (μM)[b]
3a	O	H	H	231	$C_{16}H_{20}N_4O$. 2 HCl	> 1000
3b	CH_2	H	H	282	$C_{17}H_{22}N_4$. 2 HCl. 0.5 H_2O	13 ± 2
3c[c]	O	CH_3	H	182	$C_{17}H_{22}N_4$. 2 HCl	85 ± 17
3d[d]	CH_2	CH_3	H	246	$C_{18}H_{24}N_4$. 2 HCl	5.4 ± 0.8
3e[d]	O	CH_3	*p*-Cl	222	$C_{17}H_{21}N_4OCl$. 2 HCl. H_2O	34 ± 4
3f	CH_2	CH_3	*p*-Cl	221[e]	$C_{18}H_{23}ClN_4$. $C_2H_2O_4$	1.3 ± 0.5
3g	CH_2	CH_3	3,4-(OCH_2O)	205	$C_{19}H_{24}N_4O_2$. 2 HCl. H_2O	1.0 ± 0.3
3h[d]	O	CH_2OH	H	190	$C_{17}H_{22}N_4O_2$. 2 HCl. 2 H_2O	440 ± 30
3i[d]	O	CN	H	144	$C_{17}H_{19}N_5$. 2 HCl. 2 H_2O	> 100
3j	O	CF_3	H	218	$C_{17}H_{19}F_3N_4O$. 2 HCl	740 ± 80
3k	O	NH_2	H	275	$C_{16}H_{21}N_5O$. 2 HCl. H_2O	270 ± 80
3l[d]	O	C_6H_5	H	214	$C_{22}H_{24}N_4O$. 2 HCl	260 ± 30

[a] All melting points refer to hydrochlorides unless otherwise indicated. [b] Homogenized rat striatum. [c] Reference 1. [d] Reference 9. [e] Oxalate.

for potency: (i) presence of a central pyridazine ring, (ii) presence of a lipophilic cationic head, (iii) change from a 2- to a 4−5-carbon units distance between the amidinic function and the tertiary amine. Among the different side chains tested, it is remarkable to notice that the highest potency, together with the best discrimination between AChE and BuChE inhibition, is associated with the *N*-benzylpiperidine ethyl moiety. The replacement of the *N*-benzylpiperidine ethyl side chain by a tetrahydroisoquinolinyl side chain induces surprisingly an inversion of the AChE/BuChE inhibition profile thus leading to a BuChE-selective inhibitor.

Experimental Section

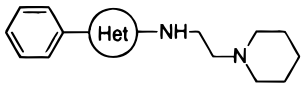
Chemistry. ^{1}H NMR spectra were recorded on a Bruker WP 80 (80 MHz), a Bruker AC 200 (200 MHz), or a Bruker DPX 300 (300 MHz) spectrophotometer at room temperature. Chemical shifts are given in ppm (δ) relative to $SiMe_3$ as internal standard. Coupling constants (J) are in hertz (Hz), and signals are designated as follows: s, singlet; d, doublet; t, triplet; brs, broad singlet; m, multiplet; etc. Melting points were determined with a Mettler FP62 apparatus and are uncorrected. Elemental analyses were performed by the departement 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/$(NH_4)_6Mo_7O_{24}$·$4H_2O$/$(NH_4)_2$−$Ce(SO_4)_4$·$2H_2O$, 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.

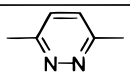
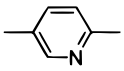
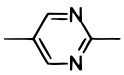
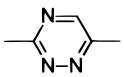
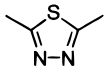
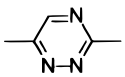
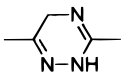
(I) 3(2*H*)-Pyridazinones. The 3(2*H*)-pyridazinones necessary for the synthesis of compounds **3a**−**3f**, **3h**, **3i**, and **3l** are already known and were prepared according to literature procedures.[16−20]

4-Trifluoromethyl-6-phenyl-3(2*H*)-pyridazinone (1j). (a) 3-Benzoyl-2-trifluoromethylpropionitrile (14). To a chilled solution of 1 g (5 mmol) of (*E*)-4,4,4-trifluoro-1-phenyl-2-buten-1-one[21] (**13**) dissolved in 10 mL of EtOH, were added 0.34 g (5.7 mmol) of acetic acid and then a solution of KCN (0.38 g, 6 mmol) in water (1 mL). The mixture was stirred at 0 °C during 12 h. The solvent was evaporated, and water was added to the residue. After extraction with EtOAc, the crude product was purified by flash chromatography (hexane−EtOAc, 85:15): yield 22%; mp 86 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.57 (d, 1H, J = 6.8 Hz), 3.61 (d, 1H, J = 7.2 Hz), 4.18 (m, 1H), 7.4−7.7 (m, 3H), 7.9−8.1 (m, 2H).

(b) Ethyl 3-Benzoyl-2-trifluoromethylpropionate. A 2.9 g portion (27 mmol) of 3-benzoyl-2-trifluoromethylpropionitrile was added to a mixture of 5 mL of sulfuric acid and 5 mL of EtOH. The solution was stirred overnight at room temperature. EtOH was removed under reduced pressure, and the residue was poured onto ice. The aqueous layer was extracted with EtOAc (3 × 10 mL); the organic phase was washed with 20% K_2CO_3, dried over Na_2SO_4, and purified by flash chromatography (hexane−EtOAc, 85:15): yield 80%; mp 48 °C; ^{1}H

Table 3. Influence of the Heterocycle



Compnd	Het	Mp,°C [a]	Formula	IC_{50} (μM) [b]
3b		95	$C_{17}H_{22}N_4$	13 ± 2
4		/ [c]	$C_{18}H_{23}N_3$	70 ± 8
5		78	$C_{17}H_{22}N_4$	> 100
6		105	$C_{16}H_{21}N_5 \cdot H_2O$	57 ± 7
7		100	$C_{15}H_{20}N_4S$	54 ± 7
8		201 [d]	$C_{16}H_{21}N_5 \cdot C_2H_2O_4$	60 ± 5
9		195 [d]	$C_{16}H_{23}N_5 \cdot C_2H_2O_4 \cdot H_2O$	47 ± 6

[a] All melting points refer to free bases unless otherwise indicated. [b] Homogenized rat striatum. [c] Colorless oil. [d] Oxalate.

NMR (200 MHz, $CDCl_3$) δ 1.3 (t, 3H, J = 7.2 Hz), 3.0−4.5 (m, 1H), 4.25 (q, 2H, J = 7.2 Hz), 7.3−7.5 (m, 3H), 7.8−8.1 (m, 2H).

(c) 4-Trifluoromethyl-6-phenyl-3(2*H*)-pyridazinone (1j). A 2.8 g portion (10 mmol) of ethyl 3-benzoyl-2-trifluoromethylpropionate was refluxed for 6 h with 0.55 g (11 mmol) of hydrazine hydrate and 50 mL of EtOH. After cooling, the precipitate formed was collected by filtration, and the filtrate was concentrated by evaporation. Water was added; the mixture was extracted with EtOAc. The organic layer was separated and dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude dihydropyridazinone (1.75 g, 7.2 mmol) was heated at 80 °C with 3.5 mL of AcOH, and a solution of Br_2 (0.37 mL, 7.2 mmol) in AcOH (1.5 mL) was added. After cooling, the solvent was evaporated, and water was added. The solution was alkalinized with 20% K_2CO_3, and the precipitate was collected by filtration and washed with water; 1.7 g of beige powder was obtained: yield 72%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.5−7.7 (m, 3H), 7.7−8.0 (m, 4H), 12.17 (s, 1H).

4-Methyl-6-(3,4-methylenedioxyphenyl)-3(2*H*)-pyridazinone (1g). The pyridazinone was prepared according to Maghioros et al.[16] and was recrystallized in *i*-PrOH: yield 11%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.3 (s, 3H), 6.0 (s, 2H), 6.8 (m, 1H), 7.3 (m, 2H), 7.9 (s, 1H), 12.6 (m, 1H).

(II) General Procedure for 3-chloropyridazines. The appropriate substituted 3(2*H*)-pyridazinone was heated at 75 ± 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 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 hexane−EtOAc as eluent. The 3-chloropyridazines are already known and were prepared according to literature procedures.[16−20]

3-Chloro-4-trifluoromethyl-6-phenylpyridazine (2j). The chloropyridazine was purified by flash chromatography (hexane−EtOAc 85:15): yield 53%; mp 152 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.5−7.6 (m, 3H), 8.1−8.3 (m, 2H), 8.7 (s, 1H).

3-Chloro-4-methyl-6-(3,4-methylenedioxyphenyl)pyridazine (2g). The chloropyridazine (yellow crystals) was purified by recrystallization in *i*-PrOH: yield 85%; mp undeterminated (unstable); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.4 (s, 3H), 6.0 (s, 2H), 6.9 (m, 1H), 7.5 (m, 1H), 7.6 (m, 1H), 7.7 (s, 1H).

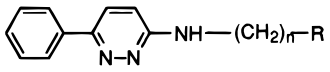
(III) Preparation of the Amines 12. Most of the aminoalkyl chains used in this work were obtained from commercial suppliers (aminoethylmorpholine, aminoethylpiperidine, aminopropylmorpholine, aminomethylpyrrolidine, aminobenzylpiperidine) or prepared according to literature procedures.

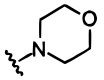
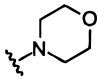
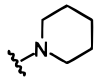
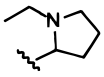
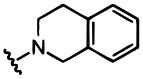
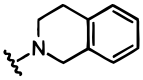
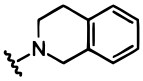
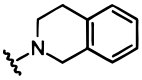
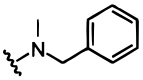
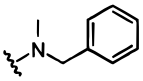
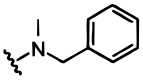
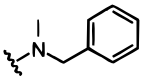
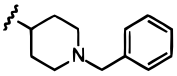
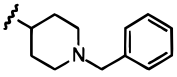
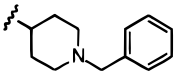
Route A. 2-(1-Benzyl-1-methylamino)ethylamine (12e).[40] **(a) 2-(1-Benzyl-1-methylamino)acetonitrile.**[41] To a solution of chloroacetonitrile (60 mmol) and either triethylamine (60 mmol) or K_2CO_3 (60 mmol) in toluene (30 mL) was added *N*-benzyl-*N*-methylamine (50 mmol) in toluene (10 mL) at room temperature. The mixture was heated at 100 °C for 2 h and cooled and water added. The reaction mixture was extracted with EtOAc and the organic layer, dried over Na_2SO_4, was evaporated under reduced pressure to give colorless oil: yield 75%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.30 (s, 3H), 3.30 (s, 2H), 3.50 (s, 2H), 7.20 (m, 5H).

(b) 2-(1-Benzyl-1-methylamino)ethylamine (12e). An aqueous 33% NH_4OH solution (36 mL) and 1 g of Raney Ni were added to a solution of 2-(1-benzyl-1-methylamino)acetonitrile (37 mmol) in EtOH (100 mL). The mixture was placed on Parr hydrogenation apparatus and left under a pressure of hydrogen (70 psi) for 6 h. The catalyst was filtered off, and the filtrate was evaporated under reduced pressure. The resulting colorless oil was purified by distillation under reduce pressure (bp 140 °C at 1.4 mbar): yield 75%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.20 (s, 3H), 2.40 (m, 2H), 2.80 (m, 2H), 3.50 (s, 2H), 7.20 (m, 5H).

5-(1-Benzyl-1-methylamino)pentylamine (12h). (a) 5-(1-Benzyl-1-methylamino)pentanenitrile:[42] yellow oil, yield

Table 4. 3-Aminoalkylamino Chain Modifications



Compnd	n	R	Mp,°C [a]	Formula	IC_{50} (μM)[b]
3a	2		231	$C_{16}H_{20}N_4O$. 2 HCl	800[c] ± 30
3m	3		276	$C_{17}H_{22}N_4O$. 2 HCl. 0.5 H_2O	410 ± 40
3b	2		262	$C_{17}H_{22}N_4$. 2 HCl. 0.5 H_2O	71 ± 10
3n	1		219	$C_{17}H_{22}N_4$. 2 HCl. 0.5 H_2O	62 ± 14
3o	2		288	$C_{21}H_{22}N_4$. 2 HCl. H_2O	83 ± 9
3p	3		230	$C_{22}H_{24}N_4$. 2 HCl. 2 H_2O	35 ± 5
3q	4		162	$C_{23}H_{26}N_4$. 2 HCl. 2 H_2O	15 ± 1
3r	5		95	$C_{24}H_{28}N_4$. 2 HCl. H_2O	10 ± 1
3s	2		201	$C_{20}H_{22}N_4$. 2 HCl. 0.5 H_2O	170 ± 90
3t	3		193	$C_{21}H_{24}N_4$. 2 HCl. 0.5 H_2O	13 ± 1
3u	4		92	$C_{22}H_{26}N_4$. 2 HCl. H_2O	11 ± 2
3v	5		108	$C_{23}H_{28}N_4$. 2 HCl. H_2O	0.74 ± 0.03
3w	0		286	$C_{22}H_{24}N_4$. 2 HCl. 1.5 H_2O	65 ± 1
3x	1		280	$C_{23}H_{26}N_4$. 2 HCl	5.6 ± 1.0
3y	2		268	$C_{24}H_{28}N_4$. 2 HCl. H_2O	0.12 ± 0.01

[a] All melting points refer to hydrochlorides. [b] Electric eel (*Torpedo californica*). [c] Minaprine (**3c**): IC_{50} = 600 ± 50 μM on electric eel AChE.

82%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.79−1.89 (m, 4H), 2.20 (s, 3H), 2.40−2.52 (m, 4H), 3.51 (s, 2H), 7.27−7.34 (m, 5H).

(b) 5-(1-Benzyl-1-methylamino)pentylamine (12h): To a suspension of $LiAlH_4$ (15 mmol) in dry THF (20 mL) was added 5-(1-benzyl-1-methylamino)pentanenitrile (10 mmol) in dry THF (10 mL) at 0 °C under inert atmosphere. The mixture was stirred at 0 °C for 1 h, and water was added. The precipitate was filtered and washed with EtOAc. The filtrate was decanted and extracted with EtOAc. The organic layer was washed, dried over Na_2SO_4, and evaporated under reduced pressure: yellow oil, yield 80%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37−1.55 (m, 6H), 2.19 (s, 3H), 2.35 (m, 2H), 2.69 (m, 2H), 3.48 (s, 2H), 7.28−7.34 (m, 5H).

2-(1,2,3,4-Tetrahydroisoquinolin-2-yl)ethylamine (12a). (a) 2-(1,2,3,4-Tetrahydroisoquinolin-2-yl)acetonitrile: col-

Table 5. Anticholinesterase Activity of **3r**, **3v**, **3y**, and Reference Compounds (in Vitro)

	activity IC_{50}, μM			ratio of IC_{50}[d] (BuChE/ AChE)
Compnd	AChE[a]	AChE[b]	BuChE[c]	
3r	10 ± 1	47 ± 30	0.31 ± 0.02	0.0066
3v	0.74 ± 0.03	0.93 ± 0.09	0.34 ± 0.03	0.36
3y	0.12 ± 0.01	0.14 ± 0.01	0.70 ± 0.03	5.0
tacrine	0.039 ± 0.004	0.095 ± 0.006	0.021 ± 0.002	0.22
donepezil	0.048 ± 0.006	0.016 ± 0.001	8.2 ± 0.2	510

[a] From electric eel. [b] From human erythrocytes. [c] From human serum. [d] Human BuChE/human AChE.

orless oil, yield 70%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.85−3.20 (m, 4H), 3.72 (s, 2H), 3.80 (s, 2H), 7.04−7.20 (m, 4H).

(b) 2-(1,2,3,4-Tetrahydroisoquinolin-2-yl)ethylamine (12a):[43] reduction by means of $LiAlH_4$ in THF gave a yellow oil, yield 40%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.83 (brs, 2H), 2.59 (t, 2H, J = 5.1 Hz), 2.74 (t, 2H, J = 5.1 Hz), 2.81−2.93 (m, 4H), 3.64 (s, 2H), 7.00−7.15 (m, 4H).

4-(1,2,3,4-Tetrahydroisoquinolin-2-yl)butylamine[43] **(12c). (a) 4-(1,2,3,4-Tetrahydroisoquinolin-2-yl)butyronitrile:** yellow oil, yield 60%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.93 (q, 2H, J = 7.0 Hz), 2.48 (t, 2H, J = 7.0 Hz), 2.64 (t, 2H, J = 6.7 Hz), 2.74 (t, 2H, J = 5.6 Hz), 2.91 (t, 2H, J = 5.6 Hz), 3.63 (s, 2H), 7.01−7.17 (m, 4H).

(b) 4-(1,2,3,4-Tetrahydroisoquinolin-2-yl)butylamine (12c): reduction by $LiAlH_4$ in THF gave a yellow oil, yield 30%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.48−1.62 (m, 4H), 1.72 (brs, 2H) 2.49 (t, 2H, J = 7.3 Hz), 2.70 (m, 4H), 2.88 (t, 2H, J = 5.8 Hz), 3.59 (s, 2H), 6.98−7.09 (m, 4H).

5-(1,2,3,4-Tetrahydroisoquinolin-2-yl)pentylamine (12d). (a) 5-(1,2,3,4-Tetrahydroisoquinolin-2-yl)butyronitrile: colorless oil, yield 68%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72−1.80 (m, 4H), 2.41 (m, 2H), 2.55 (m, 2H), 2.72 (t, 2H, J = 6.0 Hz), 2.91 (t, 2H, J = 6.0 Hz), 3.62 (s, 2H), 7.01−7.16 (m, 4H).

(b) 5-(1,2,3,4-Tetrahydroisoquinolin-2-yl)pentylamine (12d): reduction by $LiAlH_4$ in THF gave a yellow oil, yield 73%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31−1.66 (m, 6H), 2.50 (t, 2H, J = 7.6 Hz), 2.67 (m, 2H), 2.71 (t, 2H, J = 5.7 Hz), 2.90 (t, 2H, J = 5.7 Hz), 3.61 (s, 2H), 6.99−7.12 (m, 4H).

Route B. 3-[(1-Benzyl-1-methylamino)propylamine (12f).[44,45] *N*-(4-Bromopropyl)phthalimide (6 g, 22.4 mmol) in xylene (60 mL) was added dropwise to a warm (oil bath temperature ca. 70 °C) solution of *N*-benzyl-*N*-methylamine (6.2 mL, 48 mmol) in xylene (60 mL). The reaction mixture was stirred at 120−130 °C for 24 h and after cooling to 0 °C. The solid material was collected by filtration and washed with xylene. Evaporation of the filtrate afforded an oil (6 g). A solution of hydrazine hydrate (1.1 mL, 22.4 mmol) was added dropwise to a stirred solution of the previous oil dissolved in EtOH (5 mL). The reaction mixture was maintained under reflux for 1 h, then allowed to cool to room temperature, and treated with an additional amount of EtOH (5 mL) and 37% HCl (22.4 mmol). The mixture was refluxed again for 1 h. The precipitate was filtered off and washed with water and the filtrate concentrated under reduced pressure. The residue was rendered alkaline with 10% K_2CO_3 and extracted with Et_2O. The organic layer, dried over Na_2SO_4, was evaporated under reduced pressure to give yellow oil: yield 75%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.63 (quint., 2H, J = 10.4 Hz), 2.18 (s, 3H), 2.28 (brs, 2H), 2.43 (t, 2H, J = 10.4 Hz), 2.72 (m, 2H), 3.46 (s, 2H), 7.29 (m, 5H).

3-(1,2,3,4-Tetrahydroisoquinolin-2-yl)propylamine[46] **(12b):** colorless oil, yield 85%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60−1.79 (m, 4H), 2.57 (m, 2H), 2.70−2.82 (m, 4H), 2.90 (t, 2H, J = 5.3 Hz), 3.63 (s, 2H), 6.98−7.10 (m, 4H).

4-(1-Benzyl-1-methylamino)butylamine[47] **(12g):** yellow oil; yield 75%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.63 (quint., 2H, J = 10.4 Hz), 2.18 (s, 3H), 2.28 (brs, 2H), 2.43 (t, 2H, J = 10.4 Hz), 2.72 (m, 2H), 3.46 (s, 2H), 7.29 (m, 5H).

1-(1-Benzylpiperidin-4-yl)methylamine (12i). (a) 1-Benzyl-4-carboxamidopiperidine. [48−50] To a mixture of 25 g (0.19 mol) of 4-carboxamidopiperidine and 29.4 g (0.35 mol) of $NaHCO_3$ in toluene (400 mL) was added 25.5 mL (0.21 mol) of benzyl bromide. The reaction mixture was refluxed under N_2 atmosphere for 5 h and filtered, and the precipitate was washed with hexane. White crystals were obtained after recrystallization in a mixture of EtOAc−hexane (75:25): yield 50%; mp 161 °C (lit.[48] 161−162 °C); ^{1}H NMR (200 MHz, MeOH-d_4) δ 1.62−1.80 (m, 4H), 2.03 (td, 2H, J_1 = 12.0 Hz, J_2 = 4.0 Hz), 2.20 (m, 1H), 2.93 (brd, 2H, J = 12.0 Hz), 3.51 (s, 2H), 7.23−7.33 (m, 5H).

(b) 1-(1-Benzylpiperidin-4-yl)methylamine (12i).[48] A solution of 1-benzyl-4-carboxamidopiperidine (5 g, 23 mmol) in dry Et_2O (20 mL) was added to a suspension of 1.3 g (34.3 mmol) of $LiAlH_4$ in dry Et_2O (50 mL). The mixture was stirred under reflux and N_2 atmosphere for 8 h. After cooling, water was added to the mixture, and the precipitate was collected by filtration and washed with Et_2O. The organic layer, dried over Na_2SO_4, was evaporated under reduced pressure: colorless oil, yield 91%; ^{1}H NMR (200 MHz, MeOH-d_4) δ 1.10−1.40 (m, 3H), 1.72 (brd, 2H, J = 11.0 Hz), 2.00 (td, 2H, J_1 = 11.7 Hz, J_2 = 2.5 Hz), 2.48 (d, 2H, J = 5.8 Hz), 2.90 (brd, 2H, J = 12.0 Hz), 3.50 (s, 2H), 7.23−7.32 (m, 5H).

2-(1-Benzylpiperidin-4-yl)ethylamine (12j). (a) 1-Benzylpiperidin-4-ylideneacetonitrile.[51] A mixture of 6.5 g (47 mmol) of K_2CO_3 and 10 g (56.4 mmol) of diethyl cyanomethylphosphonate in dry THF (10 mL) was stirred at room temperature for 15 min and then refluxed for 20 min. After cooling 8.3 mL (47 mmol) of 1-benzyl-4-piperidone was added, and the mixture was heated under reflux for 12 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 reduced pressure. The crude product was purified by flash chromatography (EtOAc−hexane, 5:5). A white solid was obtained: yield 87%; mp 85 °C (lit.[51] mp 87−89 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37−2.41 (m, 2H), 2.51−2.66 (m, 6H), 3.56 (s, 2H), 5.11 (s, 1H), 7.26−7.38 (m, 5H).

(b) 2-(1-Benzylpiperidin-4-yl)acetonitrile. To a solution of 11 g (51.8 mmol) of 1-benzylpiperidin-4-ylideneacetonitrile in MeOH (500 mL) was added 50.4 g (2.1 mol) of magnesium turnings progressively with cooling.[34,52] The reaction mixture was stirred at room temperature for 4 h. The magnesium salts were dissolved by addition of concentrated hydrochloric acid, and the mixture was rendered alkaline with 10 N NaOH. The precipitate was filtered and washed with water and EtOAc. The filtrate was extracted with EtOAc, and the combined organic layers, dried over Na_2SO_4, were concentrated: yellow oil; yield 74%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (td, 2H, J_1 = 11.7 Hz, J_2 = 3.4 Hz), 1.65−1.85 (m, 3H), 1.99 (td, 2H, J_1 = 11.7 Hz, J_2 = 2.3 Hz), 2.28 (d, 2H, J = 6.4 Hz), 2.91 (brd, 2H, J = 11.7 Hz), 3.50 (s, 2H), 7.24−7.35 (m, 5H).

(c) 2-(1-Benzylpiperidin-4-yl)ethylamine Dihydrochloride.[53] To a suspension of $LiAlH_4$ (2.66 g, 69 mmol) in dry THF (40 mL) was added, at 0 °C, 10 g (46 mmol) of 2-(1-benzylpiperidin-4-yl)acetonitrile in dry THF (10 mL). The mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into water, and the precipitate was filtered and washed with EtOAc. The filtrate was extracted with EtOAc, and the combined organic layers, dried over Na_2SO_4, were evaporated under reduced pressure to give a colorless oil: yield 53%; mp (dihydrochloride) 137 °C (lit.[54] mp 175−178 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.18−1.45 (m, 5H), 1.59−1.64 (m, 2H), 1.85−1.96 (m, 2H), 2.18 (brs, 2H), 2.63−2.71 (m, 2H), 2.80−2.86 (m, 2H), 3.44 (s, 2H), 7.18−7.29 (m, 5H).

(IV) General Procedure for the Substitution of the Imino Chloride by Diamines. All the final 3-(aminoalkyl)-aminopyridazines were prepared by substituting heterocyclic imino chlorides with the suitable aminoalkylamines. 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 recrystallized in *i*-PrOH with Et_2O or $(i\text{-Pr})_2O$.

3-[2-(1-Benzylpiperidin-4-yl)ethylamino]-6-phenylpyridazine (3y). A mixture of 3-chloro-6-phenylpyridazine (0.59 g, 3.1 mmol), 4-(2-aminoethyl)-1-benzylpiperidine (**12j**) (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; 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, the obtained crude free base was purified by flash chromatography using two different eluents (EtOAc and then a mixture of EtOAc−MeOH, 9:1, with 2% (v) triethylamine): yield 32%; mp 281 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26−1.43 (m, 3H), 1.60−1.73 (m, 4H), 1.95 (m, 2H), 2.88 (m, 2H), 3.49 (s, 2H), 3.50 (m, 2H), 4.96 (t, 1H,

J = 5.3 Hz), 6.70 (d, 1H, J = 9.2 Hz), 7.21−7.33 (m, 5H), 7.39−7.49 (m, 3H), 7.58 (d, 1H, J = 9.2 Hz), 7.97 (m, 2H). Dihydrochloride (*i*-PrOH): mp 268 °C. Anal. Calcd for $C_{24}H_{28}N_4$·2HCl·H_2O: C, 62.19; H, 6.97; N, 12.09. Found: C, 62.46; H, 6.98; N, 11.90.

3-[2-(Morpholin-4-yl)ethylamino]-6-phenylpyridazine (3a). The free base was purified by recrystallization in (*i*-Pr)$_2$O: yield 80%; mp 107 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.42 (m, 4H), 2.58 (m, 2H), 3.52 (m, 2H), 3.62−3.67 (m, 4H), 5.54 (s, 1H), 6.71 (d, 1H, J = 9.1 Hz), 7.31−7.44 (m, 3H), 7.50 (d, 1H, J = 9.1 Hz), 7.90 (m, 2H). Dihydrochloride (*i*-PrOH): mp 231 °C. Anal. Calcd for $C_{16}H_{20}N_4O$·2HCl: C, 53.81; H, 6.16; N, 15.69. Found: C, 53.77; H, 6.17; N, 15.69.

3-[2-(Piperidin-1-yl)ethylamino]-6-phenylpyridazine (3b). The free base (white solid) was purified by flash chromatography (EtOAc−MeOH, 9:1, with 2% (v) triethylamine): yield 81%; mp 110 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45−1.65 (m, 6H), 2.38−2.50 (m, 4H), 2.63 (t, 2H, J = 5.8 Hz), 3.56 (m, 2H), 5.45 (s, 1H), 6.74 (d, 1H, J = 9.1 Hz), 7.35−7.51 (m, 3H), 7.59 (d, 1H, J = 9.1 Hz), 7.95−8.00 (m, 2H). Dihydrochloride (*i*-PrOH): mp 262 °C. Anal. Calcd for $C_{17}H_{22}N_4$·2HCl·0.5H_2O: C, 56.04; H, 6.93; N, 15.38. Found: C, 56.12; H, 6.95; N, 15.37.

4-Methyl-6-(4-chlorophenyl)-3-[2-(piperidin-1-yl)ethylamino]pyridazine (3f). The free base was purified by crystallization in *i*-PrOH: yield 80%; ^{1}H NMR (200 MHz, D_2O) δ 1.63−2.09 (m, 6H), 2.40 (s, 3H), 3.19 (m, 2H), 3.58 (t, 2H, J = 5.9 Hz), 3.76−3.82 (m, 2H), 4.06 (t, 2H, J = 5.9 Hz), 7.61 (d, 2H, J = 8.4 Hz), 7.79 (d, 2H, J = 8.4 Hz), 7.81 (s, 1H). Oxalate (*i*-PrOH): mp 221 °C. Anal. Calcd for $C_{18}H_{23}ClN_4$·$C_2H_2O_4$: C, 57.06; H, 5.98; N, 13.31. Found: C, 56.77; H, 5.96; N, 13.30.

4-Methyl-6-(3,4-methylenedioxyphenyl)-3-[2-(piperidin-1-yl)ethylamino]pyridazine (3g). The free base (oil) was purified by flash chromatography (EtOAc−MeOH, 9:1, with 2% (v) triethylamine): yield 11%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.5 (m, 6H), 2.1 (s, 3H), 2.4 (m, 4H), 2.7 (m, 2H), 3.6 (m, 2H), 5.4 (m, 1H), 5.9 (s, 2H), 6.8 (d, 1H), 7.3 (m, 2H), 7.5 (s, 1H). Dihydrochloride (EtOH): mp 205 °C. Anal. Calcd for $C_{19}H_{24}N_4O_2$·2HCl·H_2O: C, 52.90; H, 6.30; N, 12.90. Found: C, 52.75; H, 6.24; N, 12.10.

4-Trifluoromethyl-3-[2-(morpholin-4-yl)ethylamino]-6-phenylpyridazine (3j). The free base was purified by flash chromatography (EtOAc): yield 94%; mp 82 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.6−3.0 (m, 6H), 3.7−4.1 (m, 6H), 6.17 (s, 1H), 7.5−7.7 (m, 3H), 7.92 (s, 1H), 8.0−8.2 (m, 2H). Dihydrochloride (*i*-PrOH): mp 218 °C. Anal. Calcd for $C_{17}H_{19}F_3N_4O$·2HCl: C, 48.01; H, 4.98; N, 13.17. Found: C, 47.74; H, 5.15; N, 12.93.

5-Phenyl-2-[2-(piperidin-1-yl)ethylamino]pyridine (4). The free base (colorless oil) was purified by flash (EtOAc−MeOH, 9:1, with 2% (v) triethylamine): yield 39%; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.53−1.71 (m, 6H), 2.30−2.50 (m, 6H), 3.10 (m, 2H), 5.10 (s, 1H), 6.40 (s, 1H), 7.20−7.50 (m, 5H), 8.26 (m, 2H).

5-Phenyl-2-[2-(piperidin-1-yl)ethylamino]pyrimidine (5). The free base (white crystals) was purified by flash chromatography (EtOAc with 2% (v) triethylamine): yield 65%; mp 78 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.56−1.58 (m, 6H), 2.43−2.60 (m, 6H), 3.51 (t, 2H, J = 5.8 Hz), 5.86 (s, 1H), 7.26−7.46 (m, 5H), 8.52 (s, 2H). Anal. Calcd for $C_{17}H_{22}N_4$: C, 72.31; H, 7.85; N, 19.84. Found: C, 72.28; H, 7.90; N, 19.75.

3-Phenyl-6-[2-(piperidin-1-yl)ethylamino]-1,2,4-triazine (6). The free base (brown solid) was purified by flash chromatography (EtOAc−MeOH, 8:2, with 2% (v) triethylamine): yield 30%; mp 105 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.47−1.52 (m, 2H), 1.56−1.66 (m, 4H), 2.45 (m, 4H), 2.64 (t, 2H, J = 5.8 Hz), 3.60 (q, 2H, J = 5.5 Hz), 5.71 (s, 1H), 5.86 (s, 1H), 7.43−7.50 (m, 3H), 8.12 (s, 1H), 8.32−8.37 (m, 2H). Anal. Calcd for $C_{16}H_{21}N_5$·H_2O: C, 63.75; H, 7.69; N, 23.23. Found: C, 63.66; H, 7.31; N, 22.71.

5-Phenyl-2-[2-(piperidin-1-yl)ethylamino]-1,3,4-thiadiazole (7).[30] The free base (white crystals) was purified by flash chromatography (EtOAc with 2% (v) triethylamine): yield 60%; mp 100 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.56−1.58 (m, 6H), 2.43−2.60 (m, 6H), 3.46 (t, 2H, J = 5.8 Hz), 6.25 (s, 1H), 7.36−7.40 (m, 3H), 7.75−7.76 (m, 2H). Anal. Calcd for $C_{15}H_{20}N_4S$: C, 62.47; H, 6.98; N, 19.42. Found: C, 62.68; H, 7.06; N, 19.39.

3-[3-(Morpholin-4-yl)propylamino]-6-phenylpyridazine (3m). The free base (white solid) was purified by flash chromatography (EtOAc−MeOH, 9:1, with 2% (v) triethylamine): yield 55%; mp 138 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.87 (quint., 2H, J = 6.6 Hz), 2.51 (m, 6H), 3.58 (m, 2H), 3.75 (t, 4H, J = 4.5 Hz), 5.97 (brs, 1H), 6.70 (d, 1H, J = 9.3 Hz), 7.38−7.50 (m, 3H), 7.59 (d, 1H, J = 9.3), 7.97 (m, 2H). Dihydrochloride (*i*-PrOH): mp 276 °C. Anal. Calcd for $C_{17}H_{22}N_4O$·2HCl·0.5H_2O: C, 53.68; H, 6.64; N, 14.73. Found: C, 53.67; H, 6.66; N, 14.66.

3-[1-(1-Ethylpyrrolidin-2-yl)methylamino]-6-phenylpyridazine (3n). The free base (brown oil) was purified by flash chromatography (EtOAc−MeOH, 9:1, with 2% (v) triethylamine): yield 49%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.12 (t, 3H, J = 7.3 Hz), 1.73−1.75 (m, 4H), 2.20−2.29 (q, 4H, J = 7.3 Hz), 2.72−2.92 (m, 5H), 3.23 (m, 1H), 3.35−3.75 (m, 2H), 5.38 (brs, 1H), 6.74 (d, 1H, J = 9.5 Hz), 7.37−7.49 (m, 3H), 7.58 (d, 1H, J = 9.5 Hz), 7.97 (m, 2H). Dihydrochloride (*i*-PrOH): mp 219 °C. Anal. Calcd for $C_{17}H_{22}N_4$·2HCl·0.5H_2O: C, 56.03; H, 6.93; N, 15.38. Found: C, 55.94; H, 7.05; N, 15.19.

3-[2-(1,2,3,4-Tetrahydroisoquinolin-2-yl)ethylamino]-6-phenylpyridazine (3o). The free base (brown oil) was purified by flash chromatography (EtOAc with 2% (v) triethylamine): yield 10%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.80−2.92 (m, 8H), 3.68 (s, 2H), 5.40 (brs, 1H), 6.70 (d, 1H, J = 8.0 Hz), 7.10−7.20 (m, 4H), 7.30−7.50 (m, 3H), 7.53 (d, 1H, J = 8.0 Hz), 7.98−8.02 (m, 2H). Dihydrochloride (*i*-PrOH): mp 288 °C. Anal. Calcd for $C_{21}H_{22}N_4$·2HCl·H_2O: C, 59.80; H, 6.17; N, 13.29. Found: C, 59.46; H, 6.10; N, 13.31.

3-[3-(1,2,3,4-Tetrahydroisoquinolin-2-yl)propylamino]-6-phenylpyridazine (3p). The free base (colorless oil) was purified by flash chromatography (EtOAc with 2% (v) triethylamine): yield 14%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.96 (m, 2H), 2.64−3.95 (m, 4H), 2.94 (m, 2H), 3.50−3.63 (m, 2H), 3.67 (s, 2H), 6.22 (brs, 1H), 6.61 (d, 1H, J = 9.0 Hz), 7.02 (m, 1H), 7.14 (m, 3H), 7.39−7.51 (m, 4H), 7.97 (m, 2H). Dihydrochloride (*i*-PrOH): mp 230 °C. Anal. Calcd for $C_{22}H_{24}N_4$·2HCl·2H_2O: C, 58.27; H, 6.66; N, 12.36. Found: C, 58.12; H, 6.27; N, 13.05.

3-[4-(1,2,3,4-Tetrahydroisoquinolin-2-yl)butylamino]-6-phenylpyridazine (3q). The free base (colorless oil) was purified by flash chromatography (EtOAc−MeOH, 9:1 with 2% (v) triethylamine): yield 12%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.74−1.90 (m, 4H), 2.59 (t, 2H, J = 6.7 Hz), 2.77 (t, 2H, J = 5.6 Hz), 2.94 (t, 2H, J = 5.6 Hz), 3.51 (m, 2H), 3,65 (s, 2H), 5.72 (brs, 1H), 6.45 (d, 1H, J = 9.5 Hz), 7.02−7.20 (m, 4H), 7.35−7.50 (m, 4H), 7.95 (m, 2H). Dihydrochloride (*i*-PrOH): mp 162 °C. Anal. Calcd for $C_{23}H_{26}N_4$·2HCl·2H_2O: C, 59.09; H, 6.91; N, 11.99. Found: C, 59.23; H, 6.60; N, 11.91.

3-[5-(1,2,3,4-Tetrahydroisoquinolin-2-yl)pentylamino]-6-phenylpyridazine (3r). The free base (white solid) was purified by flash chromatography (EtOAc−MeOH, 9:1): yield 10%; mp 272 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45−1.81 (m, 6H), 2.52 (t, 2H, J = 7.3 Hz), 2.72 (t, 2H, J = 5.8 Hz), 2.91 (t, 2H, J = 5.8 Hz), 3.46 (m, 2H), 3.62 (s, 2H), 5.02 (brs, 1H), 6.64 (d, 1H, J = 9.3 Hz), 6.98−7.15 (m, 4H), 7.30−7.51 (m, 3H), 7.55 (d, 1H, J = 9.3 Hz), 7.96 (m, 2H). Dihydrochloride (*i*-PrOH): mp 95 °C. Anal. Calcd for $C_{24}H_{28}N_4$·2HCl·H_2O: C, 62.19; H, 6.97; N, 12.09. Found: C, 62.56; H, 7.11; N, 11.88.

3-[2-(1-Benzyl-1-methylamino)ethylamino]-6-phenylpyridazine (3s). The free base (colorless oil) was purified by flash chromatography (EtOAc with 2% (v) triethylamine): yield 40%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.20 (s, 3H), 2.70 (m, 2H), 3.50 (m, 4H), 5.30 (brs, 1H), 6.70 (d, 1H, J = 9.1 Hz), 7.30 (m, 3H), 7.40 (m, 5H), 7.60 (d, 1H, J = 9.1 Hz), 8.00 (m, 2H). Dihydrochloride (*i*-PrOH): mp 201 °C. Anal. Calcd for $C_{20}H_{22}N_4$·2HCl·0.5H_2O: C, 60.00; H, 6.29; N, 14.00. Found: C, 59.90; H, 5.92; N, 14.10.

3-[3-(1-Benzyl-1-methylamino)propylamino]-6-phenylpyridazine (3t). The free base (colorless oil) was purified by flash chromatography (EtOAc−MeOH, 9:1, with 2% (v)

triethylamine): yield 20%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87 (m, 2H), 2.25 (s, 3H), 2.55 (m, 2H), 3.51 (s, 2H), 3.55 (m, 2H), 5.88 (brs, 1H), 6.59 (d, 1H, J = 13.7 Hz), 7.20−7.50 (m, 9H), 7.54 (d, 1H, J = 13.7 Hz), 7.95 (m, 2H). Dihydrochloride (*i*-PrOH): mp 193 °C. Anal. Calcd for $C_{21}H_{24}N_4 \cdot 2HCl \cdot 0.5H_2O$: C, 60.87; H, 6.57; N, 13.52. Found: C, 60.96; H, 6.63; N, 13.46.

3-[4-(1-Benzyl-1-methylamino)butylamino]-6-phenylpyridazine (3u). The free base (colorless oil) was purified by flash chromatography (EtOAc−MeOH 9−1): yield 17%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62−1.80 (m, 4H), 2.21 (s, 3H), 2.44 (m, 2H), 3.45 (m, 2H), 3.51 (s, 2H), 5.40 (brs, 1H), 6.60 (d, 1H, J = 14.2 Hz), 7.20−7.47 (m, 8H), 7.56 (d, 1H, J = 14.2 Hz), 7.97 (m, 2H). Dihydrochloride (*i*-PrOH): mp 92 °C. Anal. Calcd for $C_{22}H_{26}N_4 \cdot 2HCl \cdot H_2O$: C, 60.41; H, 6.91; N, 12.81. Found: C, 60.10; H, 7.22; N, 12.54.

3-[5-(1-Benzyl-1-methylamino)pentylamino]-6-phenylpyridazine (3v). The free base (brown oil) was purified by flash chromatography (EtOAc−MeOH; 9:1): yield 18%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47−1.73 (m, 6H), 2.21 (s, 3H), 2.40 (m, 2H), 3.43−3.50 (m, 4H), 4.79 (brs, 1H), 6.69 (d, 1H, J = 9.5 Hz), 7.28−7.51 (m, 8H), 7.62 (d, 1H, J = 9.5 Hz), 7.99 (m, 2H). Dihydrochloride (*i*-PrOH): mp 108 °C. Anal. Calcd for $C_{23}H_{28}N_4 \cdot 2HCl \cdot H_2O$: C, 61.18; H, 7.16; N, 12.41. Found: C, 60.80; H, 7.50; N, 11.54.

3-[(1-Benzylpiperidin-4-yl)amino]-6-phenylpyridazine (3w). The free base (white solid) was purified by flash chromatography (EtOAc with 2% (v) triethylamine): yield 18%; mp 259 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.48−1.70 (m, 2H), 2.10−2.24 (m, 4H), 2.89 (m, 2H), 3.52 (s, 2H), 3.95 (m, 1H), 4.74 (d, 1H, J = 7.7 Hz), 6.67 (d, 1H, J = 9.5 Hz), 7.25−7.50 (m, 8H), 7.56 (d, 1H, J = 9.5 Hz), 7.96 (m, 2H). Dihydrochloride (*i*-PrOH): mp 286 °C. Anal. Calcd for $C_{22}H_{24}N_4 \cdot 2HCl \cdot 1.5H_2O$: C, 59.45; H, 6.59; N, 12.61. Found: C, 59.26; H, 6.53; N, 12.55.

3-[1-(1-Benzylpiperidin-4-yl)methylamino]-6-phenylpyridazine (3x). The free base (beige solid) was purified by flash chromatography (EtOAc−MeOH, 9:1, with 2% (v) triethylamine): yield 41%; mp 183 °C; ^{1}H NMR (200 MHz, MeOH-d_4) δ 1.25−1.43 (m, 2H), 1.60−1.88 (m, 3H), 2.03 (brt, 2H, J = 12.1 Hz), 2.93 (brd, 2H, J = 12.1 Hz), 3.35 (m, 2H), 3.51 (s, 2H), 6.93 (d, 1H, J = 9.5 Hz), 7.20−7.35 (m, 5H), 7.38−7.50 (m, 3H), 7.71 (d, 1H, J = 9.5 Hz), 7.86 (m, 2H). Dihydrochloride (*i*-PrOH): mp 280 °C. Anal. Calcd for $C_{23}H_{26}N_4 \cdot 2HCl$: C, 64.02; H, 6.55; N, 12.99. Found: C, 63.70; H, 6.58; N, 12.80.

Special Procedures. 6-Phenyl-3-[2-(piperidin-1-yl)-ethylamino]-1,2,4-triazine (8). (a) *S*-Ethyl Isothiosemicarbazide Hydrobromide.[55] A solution of 18 g (0.2 mol) of thiosemicarbazide[56] and 32 g (0.3 mol) of ethyl bromide in MeOH (300 mL) was refluxed for 2 h. The MeOH was removed under reduced pressure and Et_2O was added to precipitate the mixture. The precipitate was recrystallized in EtOH−(*i*-Pr)$_2$O to give white crystals: yield 94%; mp 111 °C (lit.[55] mp 120 °C); ^{1}H NMR (80 MHz, DMSO-d_6) δ 1.25 (t, 3H), 3.25 (q, 2H), 7.25 (brs, 2H).

(b) 3-Ethylthio-6-phenyl-1,2,4-triazine (17). To a solution of 7 g (35 mmol) of *S*-ethyl isothiosemicarbazide hydrobromide in EtOH (140 mL) was added 5.3 g (35 mmol) of phenylglyoxal monohydrate. The mixture was refluxed for 10 h, and EtOH was evaporated. The residue was put onto water, alkalinized with 10% Na_2CO_3, and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (hexane−EtOAc−CH_2Cl_2, 5:1:1): yield 39%; mp 84 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.40 (t, 3H, J = 7.3 Hz), 3.30 (q, 2H, J = 7.3 Hz), 7.80 (m, 5H), 8.67 (s, 1H).

(c) 6-Phenyl-3-[2-(piperidin-1-yl)ethylamino]-1,2,4-triazine (8). The product was obtained by using the same protocol as for compound **3y**. The free base (colorless oil) was purified by flash chromatography (EtOAc with 2% (v) triethylamine): yield 74%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.60 (m, 6H), 2.55 (m, 6H), 3.65 (q, 2H), 6.03 (s, 1H), 7.50−7.78 (m, 5H), 8.4 (s, 1H). Oxalate (EtOH): yellow crystals; mp 201 °C. Anal. Calcd for $C_{16}H_{21}N_5 \cdot C_2H_2O_4$: C, 57.89; H, 6.21; N, 18.75. Found: C, 58.10; H, 6.13; N, 18.89.

6-Phenyl-3-[2-(piperidin-1-yl)ethylamino]-(2*H*,5*H*)-dihydro-1,2,4-triazine (9). To a solution of 6-phenyl-3-[2-(piperidin-1-yl)ethylamino]-1,2,4-triazine (1.1 mmol) in MeOH (20 mL) was added 10% Pd/C. The mixture was placed on a Parr hydrogenation apparatus and left under a pressure of hydrogen (50 psi) for 3 h. The catalyst was filtered off, and the filtrate was evaporated under reduced pressure. The residue was dissolved in water, rendered alkaline, and extracted with EtOAc. The organic layers, dried over Na_2SO_4, were removed under reduced pressure. The free base (colorless oil) was purified by flash chromatography (EtOAc with 2% (v) triethylamine): yield 85%; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.60 (m, 6H), 2.50 (m, 6H), 3.45 (q, 2H), 4.40 (s, 2H), 7.50−7.78 (m, 5H). Oxalate (EtOH): mp 195 °C. Anal. Calcd for $C_{16}H_{23}N_5 \cdot C_2H_2O_4 \cdot H_2O$: C, 54.95; H, 6.92; N, 17.80. Found: C, 54.58; H, 6.11; N, 17.60.

4-Hydroxymethyl-3-[2-(morpholin-4-yl)ethylamino]-6-phenylpyridazine (3h). To a solution of 0.72 g (6 mmol) of $CaCl_2$ in EtOH (15 mL) was added dropwise at 0 °C a solution of 0.65 g (1.7 mmol) of $NaBH_4$ in EtOH (27 mL) and a solution of 1.07 g (3 mmol) of 4-carbethoxy-3-[2-(morpholin-4-yl)-ethylamino]-6-phenylpyridazine[19] in EtOH (30 mL). The reaction mixture was stirred at room temperature for 2 h, water was added, and the mixture was acidified with HCl. EtOH was evaporated, and the aqueous layer was extracted with EtOAc. The aqueous phase was rendered alkaline with 10% K_2CO_3 and extracted with EtOAc. The combined organic extracts, dried over Na_2SO_4, were removed under reduced pressure. The free base was purified by flash chromatography (EtOAc−MeOH, 85:15): yield 58%; mp 134 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.8−2.3 (m, 6H), 3.4−3.8 (m, 7H), 4.65 (s, 2H), 5.92 (s, 1H), 7.45 (s, 1H), 7.3−7.6 (m, 3H), 7.7−7.9 (m, 2H). Dihydrochloride (EtOH): mp 190 °C. Anal. Calcd for $C_{17}H_{22}N_4O \cdot 2HCl \cdot 2H_2O$: C, 48.22; H, 6.66; N, 13.23. Found: C, 48.14; H, 6.54; N, 13.42.

4-Amino-3-[2-(morpholin-4-yl)ethylamino]-6-phenylpyridazine (3k).[57,58] **(a) 1,1-Bis(thiomethyl)-2-nitroethylene (15).** To a solution of 40 g (0.65 mol) of nitromethane in EtOH (100 mL) was added 60 mL (1 mol) of carbone disulfide. A solution of KOH (80 g, 1.42 mol) in EtOH (400 mL) was added dropwise to the reaction mixture at 35 °C. Stirring was continued for 30 min at room temperature The red precipitate was filtered and washed with EtOH and Et_2O. The crude product (81.5 g, yield 58%) was used without purification. This salt (40 g, 0.19 mol) was dissolved in MeOH (200 mL) and water (300 mL). The solution was cooled to 0 °C, and 35.5 mL (0.21 mol) of dimethyl sulfate was added dropwise. The reaction mixture was stirred at room temperature for 2 h. Water (1.2 L) was added, and the formed precipitate was collected by filtration, washed with H_2O, and dried under reduced pressure: yield 77%; mp 126 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.57 (s, 6H), 7.10 (s, 1H). Anal. Calcd for $C_4H_7NO_2S_2$: C, 29.07; H, 4.27; N, 8.47. Found: C, 29.19; H, 4.30; N, 8.53.

(b) 1-[2-(Morpholin-4-yl)ethylamino]-1-thiomethyl-2-nitroethylene. A solution containing 0.17 g (1 mmol) of 1,1-bis(thiomethyl)-2-nitroethylene, 0.13 g (1 mmol) of 2-(morpholin-4-yl)ethylamine and EtOH (5 mL) was refluxed for 3 h. The precipitate was filtered and washed with (*i*-Pr)$_2$O, and the filtrate was evaporated. The crude product was purified by flash chromatography (EtOAc−MeOH, 98:2, with 2% NH_4OH): yield 53%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.3−2.8 (m, 9H), 3.3−3.9 (m, 6H), 6.60 (s, 1H). Anal. Calcd for $C_8H_{17}N_3O_3S$: C, 43.70; H, 6.93; N, 16.99. Found: C, 43.97; H, 7.01; N, 16.80.

(c) 4-Nitro-3-[2-(morpholin-4-yl)ethylamino]-6-phenylpyridazine (16). A mixture of 2 g (8 mmol) of 1-[2-(morpholin-4-yl)ethylamino]-1-thiomethyl-2-nitroethylene in EtOH and 0.41 g (8 mmol) of hydrazine hydrate was refluxed for 30 min. The solvent was removed by evaporation, and EtOH (20 mL) was added to the residue. A solution of 1.46 g (9.6 mmol) of phenylglyoxal in EtOH (3 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure, and the crude product was purified by flash chro-

matography (EtOAc): yield 51%; mp 125 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.4−2.9 (m, 6H), 3.6−4.1 (m, 6H), 7.3−7.6 (m, 3H), 7.9−8.4 (m, 4H). Dihydrochloride (*i*-PrOH): mp 182 °C. Anal. Calcd for $C_{16}H_{19}N_5O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 51.26; H, 5.64; N, 18.68. Found: C, 51.70; H, 5.51; N, 18.38.

(d) 4-Amino-3-[2-(morpholin-4-yl)ethylamino]-6-phenylpyridazine (3k). To a solution of 0.2 g (0.5 mmol) of 4-nitro-3-[2-(morpholin-4-yl)ethylamino]-6-phenylpyridazine dihydrochloride in MeOH (25 mL) was added 0.2 g of 10% Pd/C. The mixture was placed on a Parr hydrogenation apparatus and left under a pressure of hydrogen (75 psi) for 3 h. The catalyst was filtered off, and the filtrate was evaporated under reduced pressure. The residue was dissolved in water, rendered alkaline, and extracted with EtOAc. The organic layers, dried over Na_2SO_4, were removed under reduced pressure. The dihydrochloride was obtained starting from the free base (yield 75%): mp 275 °C (dihydrochloride); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.42−2.54 (m, 8H), 2.57 (m, 2H), 3.59 (m, 2H), 5.94 (s, 2H), 5.96 (m, 1H), 6.86 (s, 1H), 7.30−7.47 (m, 3H), 7.83−7.87 (m, 2H). Anal. Calcd for $C_{16}H_{21}N_5O \cdot 2HCl \cdot H_2O$: C, 50.39; H, 6.34; N, 18.37. Found: C, 50.20; H, 6.34; N, 18.21.

Biological Studies. In Vitro Measurement of Acetylcholinesterase and Butyrylcholinesterase Inhibition. Acetylcholinesterase- and butyrylcholinesterase-inhibiting activities were measured by the spectrophotometric method of Ellman et al.[36] Homogenates of rat striatal brain (1:20 w/v in 0.01 M sodium buffer, pH 7.2, with 10 μM tetraisopropylpyrophosphoramide, approximately 1 unit/mL AChE activity), 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) was used 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 (homogenate striatal rat, electric eel, human erythrocyte 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 UVIKON 860) during 1 min at 25 °C. The reaction rate was calculated. Different concentrations (range of 10^{-9}−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.

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