

# Synthesis, Biological Evaluation, and Computational Studies of Tri- and Tetracyclic Nitrogen-Bridgehead Compounds as Potent Dual-Acting AChE Inhibitors and $hH_3$ Receptor Antagonists

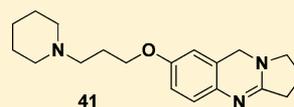
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## Supporting Information

**ABSTRACT:** Combination of AChE inhibiting and histamine  $H_3$  receptor antagonizing properties in a single molecule might show synergistic effects to improve cognitive deficits in Alzheimer's disease, since both pharmacological actions are able to enhance cholinergic neurotransmission in the cortex. However, whereas AChE inhibitors prevent hydrolysis of acetylcholine also peripherally, histamine  $H_3$  antagonists will raise acetylcholine levels mostly in the brain due to predominant occurrence of the receptor in the central nervous system. In this work, we designed and synthesized two novel classes of tri- and tetracyclic nitrogen-bridgehead compounds acting as dual AChE inhibitors and histamine  $H_3$  antagonists by combining the nitrogen-bridgehead moiety of novel AChE inhibitors with a second *N*-basic fragment based on the piperidinypropoxy pharmacophore with different spacer lengths. Intensive structure–activity relationships (SARs) with regard to both biological targets led to compound **41** which showed balanced affinities as *h*AChE inhibitor with  $IC_{50} = 33.9$  nM, and *h* $H_3$ R antagonism with  $K_i = 76.2$  nM with greater than 200-fold selectivity over the other histamine receptor subtypes. Molecular docking studies were performed to explain the potent AChE inhibition of the target compounds and molecular dynamics studies to explain high affinity at the *h* $H_3$ R.



$K_i$  ( $hH_3$ ) = 76.2 nM  
 $IC_{50}$  (*h*AChE) = 33.9 nM  
 $IC_{50}$  (*Ee*AChE) = 66.5 nM

Dual-acting AChE inhibitor /  $hH_3$  antagonist

**KEYWORDS:** Acetylcholinesterase inhibitors, histamine  $H_3$  antagonists, Alzheimer's disease, docking, MD simulation

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative brain disorder and the most common form of dementia. It is estimated that about 6% of the population worldwide aged over 65 currently suffer from AD.<sup>1</sup> Worldwide, AD afflicts more than 35 million people.<sup>2,3</sup> The occurrence of dementia increases exponentially with increasing age, and it is expected to more than triple during the next 30 years.<sup>4</sup> This alarming prognosis demands further effort to elucidate the molecular mechanisms of AD and search for effective treatment methods.

The cholinergic hypothesis, proposed by Davies and Maloney in 1976, is one of the earliest and most extensively researched theories regarding AD pathogenesis.<sup>5</sup> It has led to the introduction of tacrine as the first drug to be approved for the treatment of AD, and subsequently galantamine, donepezil, and rivastigmine, respectively. All these compounds represent inhibitors of acetylcholinesterase (AChE), the inhibition of which increases the amount of the neurotransmitter acetylcholine (ACh) in the synaptic cleft. Clinically this leads to a symptomatic, but significant improvement of cognitive deficits in early stages of AD. Together with the symptomatically acting *N*-methyl-D-aspartate (NMDA) blocker memantine, these compounds represent the only therapeutic treatment of AD currently available.

The histamine  $H_3$  receptor is postulated as a G protein-coupled receptor (GPCR) drug target for several cognitive disorders including attention deficit hyperactivity disorder (ADHD), AD, and schizophrenia.<sup>6</sup> The  $H_3$  receptor had originally been described as a presynaptic autoreceptor that inhibits histamine release in the brain,<sup>7</sup> and was subsequently shown also to regulate the release of other important neurotransmitters such as acetylcholine, glutamate, serotonin, dopamine, norepinephrine, gamma-aminobutyric acid (GABA), and others via a parallel role as a heteroreceptor.<sup>8,9</sup> Therefore, blocking these receptors could be promising for potential treatment of central nervous system (CNS) disorders such as AD,<sup>9</sup> since blocking the central *h* $H_3$  receptor can induce the release of procognitive neurotransmitters like acetylcholine (ACh). Due to their multiple therapeutic possibilities including cognitive deficits, there has been a very considerable and remarkable progress in the development of *h* $H_3$ R antagonists both in academia and especially pharmaceutical industry.<sup>8,10</sup> Johnson & Johnson have reported on dual-acting  $H_3$  antagonists and serotonin transporter inhibitors, and Hoff-

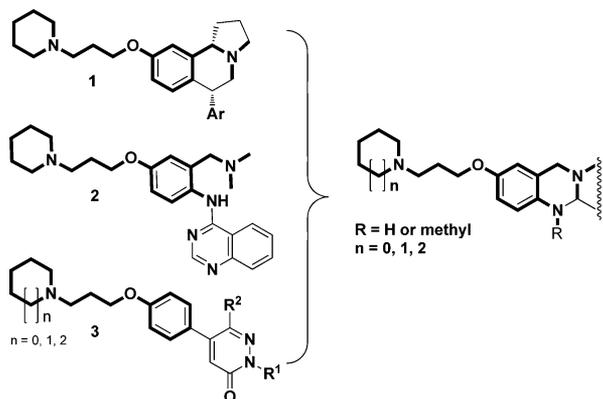
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mann-LaRoche as well as Hudkines et al. on H<sub>3</sub> ligands with piperidinylpropoxyphenyl pharmacophores (Chart 1).<sup>11–14</sup>

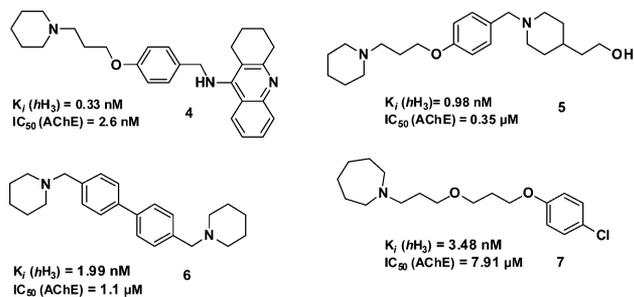
**Chart 1. Schematic Illustration of the Merging Design Methodology for Synthesizing Dual Acting AChE inhibitors/H<sub>3</sub> Antagonist Hybrid Structures**<sup>11–13</sup>



The complexity and multiple etiologies of AD make single-target strategies difficult to achieve desirable therapeutic effects and make the development of multitarget-directed ligands (MTDL) a more effective strategy, as intensively applied in recent years using very diverse biological targets.<sup>14–17</sup>

Recently, dual-acting AChE inhibitors and hH<sub>3</sub> receptor antagonists have been developed, since such multitarget-directed ligands might be able to achieve a more specific cognition-enhancing level of cholinergic neurotransmission than either an AChE inhibitor or histamine H<sub>3</sub> receptor antagonist alone. For example, such dual-acting compounds have been obtained from H<sub>3</sub> receptor antagonists that additionally contain a tacrine-based molecular structure. These compounds act at hH<sub>3</sub>, N-methyltransferase (HMT), AChE, and butyrylcholinesterase (BChE), respectively.<sup>18</sup> The most effective compound FUB-833 4 (Chart 2) displayed good

**Chart 2. Previously Described Acetylcholinesterase Inhibitors/Histamine H<sub>3</sub> Receptor Antagonists**<sup>18,21–23</sup>



affinity for hH<sub>3</sub> receptors, AChE and HMT.<sup>18</sup> Despite these exceptional activities, tacrine-based compounds always bear the risk of severe hepatotoxicity, the reason why it was withdrawn from the market.<sup>14,19,20</sup>

Several groups have also reported on interesting dual AChE inhibitors and H<sub>3</sub> antagonists.<sup>22–24</sup> Unfortunately, these compounds act at much lower concentration (nM) on H<sub>3</sub> than on AChE ( $\mu$ M).

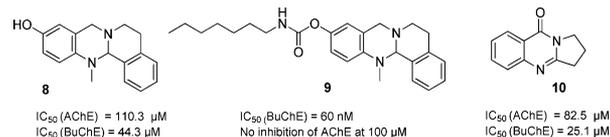
Significant amount of knowledge with regard to structure–activity relationships (SARs) of H<sub>3</sub> antagonists, including the group of piperidinylpropoxyphenyl pharmacophore containing

compounds, is available. In some cases, it was also possible to synthesize dual acting H<sub>3</sub>/AChE compounds, but despite the fact that the principle of combination seems to work, it has not yet been possible neither to avoid large molecular weight tacrine-containing compounds nor to describe compounds with high affinities in the same concentration range at both targets, a core problem of hybrid and dual acting compounds albeit rarely discussed and addressed in the literature.<sup>15</sup> Gaining balanced affinities is a very demanding task, since compounds can follow completely diverse SARs at the different targets to be addressed.

Previous SARs on numerous H<sub>3</sub> ligands described show that the phenol ethers in *meta* position to tertiary benzyl amine possess high H<sub>3</sub> receptor binding affinity and antagonistic potency, for example, compounds 1, 2 (Chart 1); similarly, the presence of an anilinic amine in *para* position to the phenolic ether is favorable, e. g. compound 2 (Chart 1). Finally, connecting the basic cyclic amine through an alkyl spacer of variable length to a phenolic OH group of heterocyclic and/or aromatic rings is a key feature to get H<sub>3</sub> receptor binding affinity and antagonistic potency (Charts 1 and 2).

We have previously synthesized a series of novel tri- and tetracyclic N-bridgehead AChE inhibiting lead structures with moderate activity (i.e., two digit micromolar) characterized by a phenolic hydroxyl group in the *para* position to a tertiary anilinic and in the *meta* position to the basic N-bridgehead atom and used them as starting points for the development of novel butyrylcholinesterase selective inhibitors (compound 8) (Chart 3).<sup>24</sup> The cholinesterase inhibitory activities of these

**Chart 3. Tri- and Tetracyclic Compounds As Starting Points for H<sub>3</sub>/AChE Dual Acting Compounds' SARs**<sup>24</sup>



classes of compounds and their selectivity toward BChE were dramatically improved by introducing a carbamate group at the phenolic hydroxyl group (compound 9) (Chart 3).<sup>24</sup> The chemical features of these two moieties (tri- and tetracyclic) served as the starting point for our SAR studies in order to synthesize a novel series of dual acting compounds as AChE inhibitors/H<sub>3</sub> receptor antagonists due to their similarity to the aromatic and/or heterocyclic parts of the above-described H<sub>3</sub> ligands (Chart 1) and then, combining the phenolic heterocycle as an ether to tertiary amines.

With the aim to investigate SARs of this class of cholinesterase inhibitors, we synthesized novel heterocyclic templates consisting of basic tri- and tetracyclic N-bridgehead molecules with phenolic ether connected to the basic cyclic amine moieties by different carbon spacer lengths. The ability of these structural templates to antagonize selectively the H<sub>3</sub> receptor and the effect of structural modifications on cholinesterase inhibitory activity were intensively investigated with the ultimate goal to find structures with high affinity/activity at both targets (i.e., balanced activities).

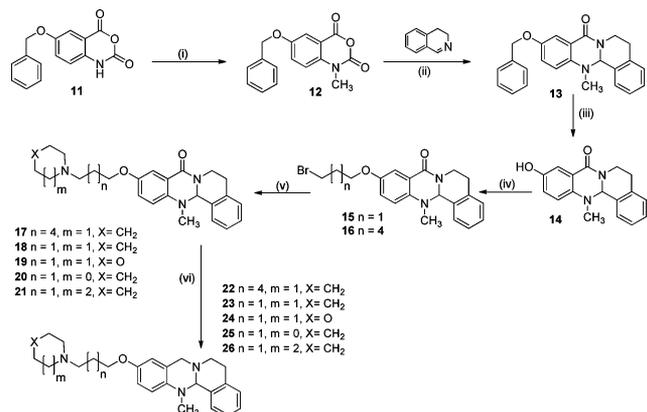
The results of SARs at AChE were used for computational docking studies of the inhibitors on AChE. Additionally, the binding mode of one of the most potent target compounds at the hH<sub>3</sub> was investigated by molecular dynamics (MD) studies.

We intended to synthesize both tri- and tetracyclic compounds, different spacer lengths, different basicities within the heterocycle, various structures of the tertiary amines, and related structural features to cover systematically a range of chemical structures for investigating SARs at both targets.

## CHEMISTRY

Scheme 1 shows the synthesis of the tetracyclic target compounds. The starting material 6-(benzyloxy)-1*H*-benzo[*d*]-

### Scheme 1. Synthesis of Tetracyclic *N*-Bridgehead Compounds<sup>a</sup>



<sup>a</sup>Reagents: (i)  $\text{CH}_3\text{I}$ , *N,N*-diisopropylethylamine, *N,N*-dimethylacetamide, 40 °C, 24 h; (ii) toluene/reflux, 24 h; (iii)  $\text{H}_2$ , Pd/C, ethanol, rt, 24 h; (iv) 1,3-dibromopropane or 1,6-dibromohexane,  $\text{K}_2\text{CO}_3$ , KI, acetonitrile, 90 °C, 3 h; (v) piperidine, morpholine, pyrrolidine, or azepane,  $\text{K}_2\text{CO}_3$ , KI, acetonitrile, 90 °C, 3 h; (vi)  $\text{LiAlH}_4$ , THF, 70 °C, 3 h.

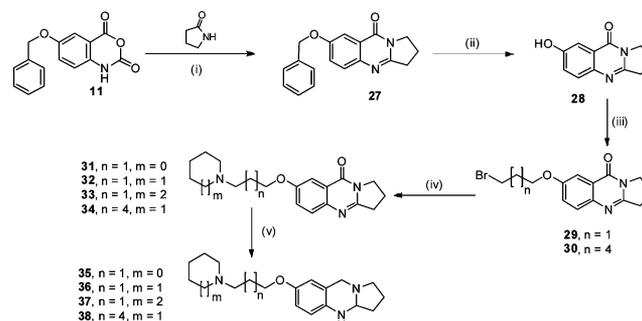
[1,3]oxazine-2,4-dione **11** was synthesized in four steps with almost quantitative overall yield, starting from 2-amino-5-hydroxybenzoic acid. The resulting isatoic anhydride represents an activated form of anthranilic acid with the benzylated phenolic-OH, the benzylation of the phenolic group in compound **11** is necessary to avoid methylation of the phenolic group, and to improve the solubility of the resulting isatoic anhydride.<sup>25</sup>

10-Hydroxy-13-methyl-13,13*a*-dihydro-5*H*-isoquinolino[1,2-*b*]quinazolin-8(6*H*)-one **14** was synthesized as shown in Scheme 1: *N*-Methylation of 6-(benzyloxy)-1*H*-benzo[*d*][1,3]-oxazine-2,4-dione **11** in the presence of *N,N*-diisopropylethylamine as a base and iodomethane as an alkylating agent in *N,N*-dimethylacetamide as a solvent afforded compound **12**.<sup>26</sup> Compound **12** was fused with 3,4-dihydroisoquinoline in toluene under reflux overnight to afford compound **13** in an acceptable yield (40%). After benzyl deprotection by hydrogenation over Pd/C in ethanol at room temperature, compound **14** was obtained in quantitative yield. Reaction of compound **14** with 1,3-dibromopropane or 1,6-dibromohexane in the presence of potassium carbonate as a base with a catalytic amount of potassium iodide in acetonitrile as solvent for 3 h under reflux yielded compounds **15** or **16**, respectively. The last synthetic step was nucleophilic substitution by reaction of bromo spacer compounds **15** or **16** with different cyclic amines using again potassium carbonate as a base with a catalytic amount of potassium iodide in acetonitrile as a solvent to yield the first series of quinazolinone target compounds **17–21**. The second set of *N*-bridgehead target compounds was synthesized

by reduction of the amide group of compounds **17–21** using  $\text{LiAlH}_4$  to give compounds **22–26**.

Tricyclic target compounds were synthesized by a related synthetic procedure as for the tetracyclic compounds as shown in Scheme 2. Solventless fusion of pyrrolidin-2-one with

### Scheme 2. Synthesis of Tricyclic *N*-Bridgehead Compounds (quinazolinones and diamines)<sup>a</sup>

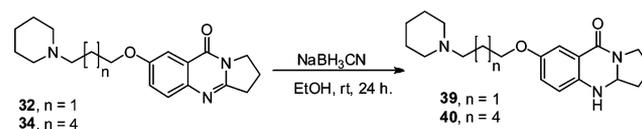


<sup>a</sup>Reagents: (i) MW, 200 W, 220 °C, 1 h; (ii)  $\text{H}_2$ , Pd/C, ethanol, rt, 24 h; (iii) 1,3-dibromopropane or 1,6-dibromohexane,  $\text{K}_2\text{CO}_3$ , KI, acetonitrile, 90 °C, 3 h; (iv) pyrrolidine, piperidine, or azepane,  $\text{K}_2\text{CO}_3$ , KI, acetonitrile, 90 °C, 3 h; (v)  $\text{LiAlH}_4$ , THF, 70 °C, 3 h.

compound **11** under microwave conditions with 200 W irradiation power at 220 °C for 1 h gave compound **27**, which was then debenzylated by catalytic hydrogenation over Pd/C in ethanol at room temperature to give compound **28**. Reaction of compound **28** with 1,3-dibromopropane or 1,6-dibromohexane in a Williamson ether synthesis gave compounds **29** or **30**, respectively. Reaction of different cyclic amine with compounds **29** or **30** under similar reaction conditions as for compounds **17–21** yielded the series of tricyclic quinazolinone target compounds **31–34**. Tricyclic compounds **35–38** were synthesized using the same reduction conditions used for the preparation of tetracyclic compounds **22–26** ( $\text{LiAlH}_4$ ).

Scheme 3 shows the reaction conditions for reduction of the imine group of compounds **32** and **34** to the corresponding

### Scheme 3. Selective Reduction of Quinazolinone Imine Bond to the Corresponding Amine



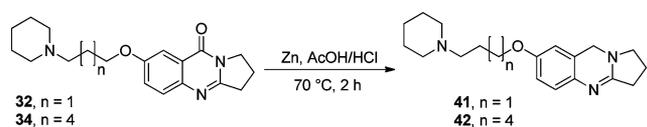
amines using sodium cyanoborohydride in ethanol at room temperature for 24 h to yield compounds **39** and **40**, respectively.<sup>27</sup>

The carbonyl group of quinazolinones (compounds **32** and **34**) could be selectively reduced using Zn metal powder in the presence of acetic acid as a solvent to give compounds **41** and **42**, respectively. The reaction mixture was then heated at 70 °C for 2 h after a few drops of conc. hydrochloric acid had been added as shown in Scheme 4.<sup>28</sup>

## RESULTS AND DISCUSSION

A novel and potent class of dual acting AChE inhibitor/ $\text{H}_3$  antagonist hybrid compounds was developed by connecting the tri- and tetracyclic *N*-bridgehead structural templates via a

#### Scheme 4. Selective Reduction of Quinazolinones by Clemmensen Reduction



hydroxyl group, in *para* position to anilinic *N*, with different cyclic amines spaced by three or six carbon atom linkers. A detailed look at the respective SARs reveals several interesting insights, also of importance to both the development of AChE inhibitors as well as  $H_3$  antagonists individually.

All target compounds were tested for their ability to inhibit acetylcholinesterase (*EeAChE*, EC.3.1.1.7 from electric eel) and butyrylcholinesterase (BChE, EC.3.1.1.8 from equine serum). Both enzyme isoforms exhibit very high amino acid sequence homology with regard to the human ones (88% and 90%, respectively).<sup>29,30</sup> Inhibitory activity at BChE, a plasma-soluble isoform of AChE, has recently gained attention for dementia-related drug development since the amount of AChE decreases during the course of the disease, whereas this seems not to be the case for BChE. Therefore, BChE inhibition might compensate for the lack of efficiency of administration of AChE inhibitors in later stages of AD.<sup>24</sup>

Additionally, selected compounds were tested for their inhibitory activity on human acetylcholinesterase *hAChE* (E.C.3.1.1.7, acetylcholinesterase from human erythrocytes) to confirm binding profiles and be able to compare the data with the docking results on the human enzyme.

**AChE Inhibitor/ $H_3$  Antagonist Tetracyclic Hybrid Compounds.** With regard to the tetracyclic template, we had previously synthesized a series of potent and selective carbamate-based (pseudo)irreversible BChE inhibitors. The free phenolic tetracyclic compound **8** displayed a low inhibitory activity at both cholinesterases ( $IC_{50}$  (*EeAChE*) = 110.3  $\mu$ M,  $IC_{50}$  (BuChE) = 44.3  $\mu$ M) (Table 1). The low activity was attributed to low basicity and poor solubility of this tetracyclic moiety. However, in this project, the same template unexpectedly showed submicromolar inhibitory activity toward AChE and one digit micromolar inhibitory activity toward BChE, simply due to connection with different cyclic amines spaced by three carbon atoms (**18**, **20**, and **21**). For example, compound **18** ( $IC_{50}$  (*EeAChE*) = 0.55  $\mu$ M) showed 200-fold higher inhibitory activities than those for the free phenolic tetracyclic lead structure compound **8** (Chart 3) ( $IC_{50}$  (*EeAChE*) = 110.3  $\mu$ M). Interestingly, the morpholino compound **19** has an  $IC_{50}$  (*EeAChE*) = 29.81  $\mu$ M and  $IC_{50}$  (BChE) = 8.5  $\mu$ M, being the only compound with higher BChE than AChE inhibitory activity in this set of compounds (Table 1), albeit with lower inhibitory activity at AChE.

According to our previously published work, we expected that the inhibitory activity of this heterocycle will increase by amide reduction through increasing the basicity of the core ring, but surprisingly, the AChE inhibition activity of this set of the tetracyclic compounds (**22–26**) is lower than that of the same set of compounds before reduction (**17–21**), but still in the micromolar range (from 0.53  $\mu$ M for compound **23** to 19.8  $\mu$ M for compound **25**). Therefore, selectivity and activity profiles for these sets of compounds depend more on the structure of the alicyclic *N*-ring connected to the heterocycle than the heterocycle itself. We investigated this feature in the

molecular docking studies (see below) with regard to the orientation of the molecule within the enzyme.

As we had planned from the compound design,  $H_3$  binding data for these quinazolinone compounds with an amide carbonyl group (compounds **17–21**) (Scheme 1), showed binding affinity in the micromolar range. The corresponding reduced tetracyclic compounds (**23**, **25**, and **26**) (Scheme 1) displayed a moderate inhibitory activity toward AChE in the micromolar range, while this set of compounds showed high binding affinity at  $H_3$  in two digit nanomolar range ( $K_i$  ( $hH_3$ ) = 57.7, 46.9, and 17.5 nM respectively) (Table 1).

Interestingly, the tetracyclic compounds connected with morpholine as a basic center showed poor cholinesterase inhibition affinities, compound **19** with  $IC_{50}$  (*EeAChE*) = 29.81  $\mu$ M and  $IC_{50}$  (BChE) = 8.5  $\mu$ M, and its corresponding reduced form compound **24** with  $IC_{50}$  (*EeAChE*) = 13.1  $\mu$ M and  $IC_{50}$  (BChE) = 27.5  $\mu$ M. Both compounds showed also poor binding affinities toward the  $H_3$  receptor (Table 1).

We also evaluated the influence of the carbon spacer length on the biological activity for the tetracyclic moiety by connecting the tetracyclic moiety with piperidine spaced by six carbon atoms (compound **17**), and the corresponding reduced form (compound **22**). Compound **22** showed inhibitory activity in submicromolar range ( $IC_{50}$  (*EeAChE*) = 0.57  $\mu$ M) toward AChE, and binding toward  $hH_3R$  in the same range ( $K_i$  ( $hH_3$ ) = 0.64  $\mu$ M). In contrast, the corresponding three carbon atom spacer length compound **23** showed also submicromolar inhibition activity range toward AChE ( $IC_{50}$  (*EeAChE*) = 0.53  $\mu$ M), but significantly improved the binding affinity toward  $hH_3R$  ( $K_i$  ( $hH_3$ ) = 57.7 nM). So, a three carbon spacer length is better for  $H_3$  affinity with regard to the tetracyclic moiety (Table 1).

**AChE Inhibitor/ $H_3$  Antagonist Tricyclic Hybrid Compounds.** The tricyclic quinazolinone compound **10** (Chart 3) showed moderate inhibitory activities toward both enzymes ( $IC_{50}$  (*EeAChE*) = 82.5  $\mu$ M and  $IC_{50}$  (BChE) = 25.1  $\mu$ M).<sup>31</sup> Surprisingly, the same template showed two digit nanomolar inhibitory activities toward AChE and one digit micromolar toward BChE by connecting it with different cyclic amines spaced by three C atoms (compounds **31**, **32**, and **33** depicted in Scheme 2). This fact and the fact that these compounds did not show high binding affinity toward the  $H_3$  receptor (compound **32** with  $K_i$  ( $hH_3$ ) = 1.56  $\mu$ M) (Table 1) intensively made us study the binding mode of this class of compounds toward AChE in a separate project by synthesis of additional compounds and computational studies. Results will be reported separately.

We also evaluated the influence of the spacer length for this moiety, and again three carbon spacer length is better for achieving dual acting compound from the tricyclic moiety, when compound **32** with three carbon spacer length showed  $IC_{50}$  (*EeAChE*) = 69.2 nM and ( $hH_3$ ) = 1.6  $\mu$ M, while the same moiety with six carbon spacer length compound **34** showed  $IC_{50}$  (*EeAChE*) = 390 nM and ( $hH_3$ ) = 0.92  $\mu$ M (Table 1).

For this moiety, we evaluated the effect and biological consequences of the amide oxygen, the double bond, and the resulting basicity differences of the core ring of tricyclic quinazolinone compounds (**31–34**). First, total reduction of the core carbonyl and imine groups using  $LiAlH_4$  afforded the diamine class of compounds **35–38**; these amines showed good inhibitory activity toward AChE (submicromolar to one digit micromolar range) and showed a high binding affinity at the  $H_3$  receptor (two digit nanomolar range) for compounds

Table 1. Chemical Structures, ChE Inhibition Results, and  $hH_3R$  Binding Data for Tri- and Tetracyclic Synthesized Compounds

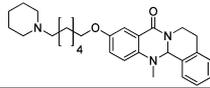
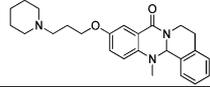
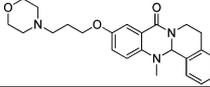
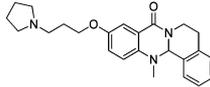
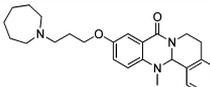
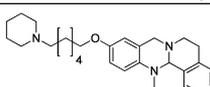
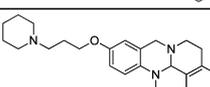
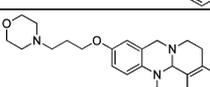
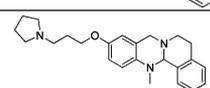
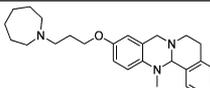
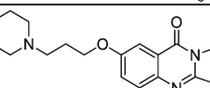
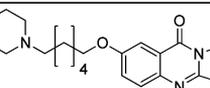
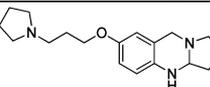
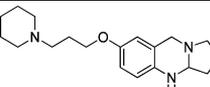
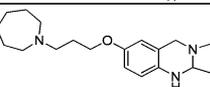
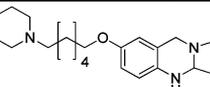
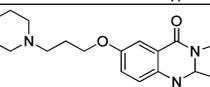
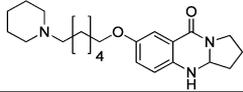
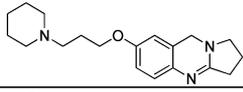
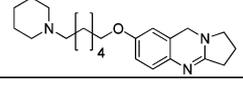
Cpd	Structure	$IC_{50}$ ( $pIC_{50} \pm SEM$ ) <sup>a</sup>			$K_i$ ( $hH_3$ ) <sup>b</sup> ( $pK_i \pm SEM$ )
		<i>hAChE</i>	<i>EeAChE</i>	<i>EqBChE</i>	
17		n.d. <sup>c</sup>	0.23 $\mu$ M (6.64 $\pm$ 0.16)	1.82 $\mu$ M (5.74 $\pm$ 0.11)	361.4 nM (6.44 $\pm$ 0.17)
18		n.d.	0.55 $\mu$ M (6.26 $\pm$ 0.08)	2.14 $\mu$ M (5.67 $\pm$ 0.02)	116.6 nM (6.93 $\pm$ 0.08)
19		3.40 $\mu$ M (5.47 $\pm$ 0.09)	29.81 $\mu$ M (4.53 $\pm$ 0.12)	8.52 $\mu$ M (5.07 $\pm$ 0.03)	733.2 nM (6.14 $\pm$ 0.13)
20		n.d.	0.72 $\mu$ M (6.14 $\pm$ 0.03)	1.04 $\mu$ M (5.98 $\pm$ 0.01)	1362.0 nM (5.87 $\pm$ 0.29)
21		n.d.	0.25 $\mu$ M (6.61 $\pm$ 0.10)	0.43 $\mu$ M (6.37 $\pm$ 0.06)	1804.0 nM (5.74 $\pm$ 0.31)
22		n.d.	0.57 $\mu$ M (6.24 $\pm$ 0.04)	0.41 $\mu$ M (6.39 $\pm$ 0.07)	639.6 nM (6.19 $\pm$ 0.07)
23		1.98 $\mu$ M (5.70 $\pm$ 0.09)	0.53 $\mu$ M (6.28 $\pm$ 0.04)	1.80 $\mu$ M (5.73 $\pm$ 0.07)	57.7 nM (7.24 $\pm$ 0.07)
24		n.d.	13.10 $\mu$ M (4.88 $\pm$ 0.06)	27.50 $\mu$ M (4.56 $\pm$ 0.03)	1532.0 nM (5.81 $\pm$ 0.13)
25		7.95 $\mu$ M (5.10 $\pm$ 0.07)	19.77 $\mu$ M (4.70 $\pm$ 0.27)	3.30 $\mu$ M (5.48 $\pm$ 0.02)	46.9 nM (7.33 $\pm$ 0.12)
26		n.d.	6.77 $\mu$ M (5.17 $\pm$ 0.06)	1.07 $\mu$ M (5.97 $\pm$ 0.02)	17.6 nM (7.76 $\pm$ 0.07)
32		n.d.	69.2 nM (7.16 $\pm$ 0.15)	122.30 $\mu$ M (3.91 $\pm$ 0.15)	1559.0 nM (5.81 $\pm$ 0.17)
34		n.d.	0.39 $\mu$ M (6.41 $\pm$ 0.05)	8.6 $\mu$ M (5.07 $\pm$ 0.01)	924.8 nM (6.03 $\pm$ 0.11)
35		9.42 $\mu$ M (5.03 $\pm$ 0.08)	8.91 $\mu$ M (5.05 $\pm$ 0.09)	19.87 $\mu$ M (4.70 $\pm$ 0.21)	31.9 nM (7.50 $\pm$ 0.07)
36		0.67 $\mu$ M (6.18 $\pm$ 0.11)	0.51 $\mu$ M (6.29 $\pm$ 0.09)	1.59 $\mu$ M (5.80 $\pm$ 0.06)	37.0 nM (7.43 $\pm$ 0.08)
37		n.d.	4.78 $\mu$ M (5.32 $\pm$ 0.07)	5.07 $\mu$ M (5.30 $\pm$ 0.03)	36.0 nM (7.44 $\pm$ 0.12)
38		91.5 nM (7.04 $\pm$ 0.11)	0.40 $\mu$ M (6.40 $\pm$ 0.04)	1.00 $\mu$ M (5.99 $\pm$ 0.02)	352.8 nM (6.45 $\pm$ 0.09)
39		n.d.	6.92 $\mu$ M (5.16 $\pm$ 0.12)	44.60 $\mu$ M (4.35 $\pm$ 0.05)	2334.0 nM (5.63 $\pm$ 0.07)

Table 1. continued

Cpd	Structure	IC <sub>50</sub> (pIC <sub>50</sub> ± SEM) <sup>a</sup>			K <sub>i</sub> (hH <sub>3</sub> ) <sup>b</sup> (pK <sub>i</sub> ± SEM)
		hAChE	EeAChE	EqBChE	
40		n.d.	3.33 μM (5.48 ± 0.14)	3.80 μM (5.42 ± 0.03)	1394.0 nM (5.86 ± 0.16)
41		33.9 nM (7.47 ± 0.06)	66.5 nM (7.18 ± 0.05)	8.20 μM (5.09 ± 0.01)	76.2 nM (7.12 ± 0.08)
42		39.1 nM (7.41 ± 0.14)	88.4 nM (7.05 ± 0.05)	1.30 μM (5.89 ± 0.04)	113.4 nM (6.95 ± 0.09)

<sup>a</sup>AChE, BChE: data are the means of three independent determinations. <sup>b</sup>hH<sub>3</sub>R binding affinity: data are means ± standard deviation of triplicate independent experiments. <sup>c</sup>n.d.: Not determined.

35, 36, and 37 ( $K_i$  (hH<sub>3</sub>) = 31.9, 37.0, 36.0 nM), respectively. Second, reducing the imine group in the quinazolinone core ring using NaBH<sub>3</sub>CN yielded its corresponding amine (39, 40), but these compounds lost their AChE inhibitory activity and showed very weak binding at H<sub>3</sub> receptor (compound 39 with IC<sub>50</sub> (EeAChE) = 6.92 μM and ( $hH_3$ ) = 2.3 μM, and compound 40 with IC<sub>50</sub> (EeAChE) = 3.33 μM and ( $hH_3$ ) = 1.4 μM). Third, selective reduction of the carbonyl group was achieved by Zn/AcOH reduction (Clemmensen reaction) and left the imine double bond as it is (41, 42). This set of compounds showed a balanced activity toward our two targets; they act as balanced acetylcholinesterase inhibitors/hH<sub>3</sub> antagonists, with compound 41 showing IC<sub>50</sub> (hAChE) = 34 nM, IC<sub>50</sub> (EeAChE) = 67 nM, and  $K_i$  (hH<sub>3</sub>) = 76 nM. Similarly, compound 42 showed IC<sub>50</sub> (hAChE) = 39 nM, IC<sub>50</sub> (EeAChE) = 88 nM, and  $K_i$  (hH<sub>3</sub>) = 113 nM (Table 1).

Balanced activity, that is, compound activity and affinity, respectively, at the relevant targets in the same or at least a similar concentration range, is a therapeutically highly relevant issue, that is very rarely properly addressed in research efforts on hybrid and multitarget compounds. This is not surprising, since two, often distinct and independent, SARs have to be investigated at each target, and even if a balanced activity is reached for one compound, it is rarely the highest affinity/activity of the set of compounds described.

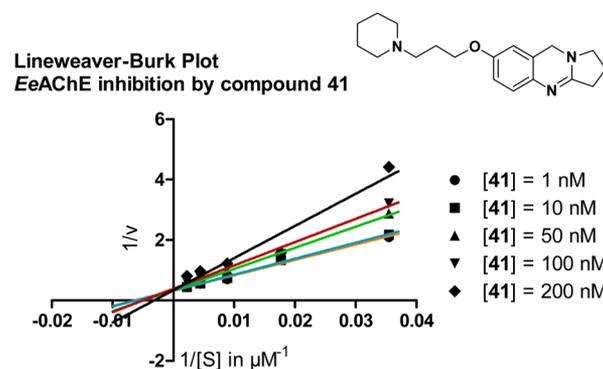
In general, all synthesized target compounds represent moderate or potent AChE inhibitors with inhibitory activities ranging from 30 μM (compound 19) down to the two digit nanomolar range (67 nM for compound 41) with roughly 500-fold higher activity. This compound is in the range of the “gold-standard” of AChE inhibition, tacrine.

Affinity at the H<sub>3</sub> receptor turned out to be very high as planned and desired from the compounds' design. Since none of our structures had been tested for H<sub>3</sub> affinity before, not even our ChE inhibiting lead structures, this fact proved our assumptions concerning ligand design. With regard to the H<sub>3</sub> affinities, they ranged from the (this time one digit) micromolar range ( $K_i$  (hH<sub>3</sub>) = 2.3 μM for compound 39) to the nanomolar range ( $K_i$  (hH<sub>3</sub>) = 18 nM for compound 26) covering a range of 2 orders of magnitude. Not surprisingly, SARs follow different routes than for AChE inhibition as described in detail above.

## PHARMACOLOGY

In addition to determination of inhibitory activities, compound 41 was selected for kinetic measurements, in order to

understand the mode of inhibition and binding of this inhibitor at EeAChE. The mechanism of inhibition was analyzed by recording substrate–velocity curves in the presence of different concentrations of compound 41 using 50–450 μM acetylthiocholine (ATC) substrate EeAChE. Figure 1 shows the

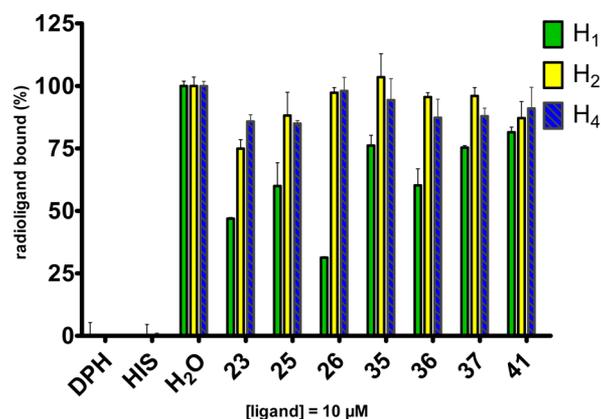


**Figure 1.** Lineweaver–Burk plot resulting from substrate–velocity curves of EeAChE activity with different substrate (ATC) concentrations (50–450 μM) in the presence of 1, 10, 50, 100, and 200 nM compound 41.

Lineweaver–Burk plot (reciprocal rates versus reciprocal substrate concentrations for the different inhibitor concentrations resulting from the substrate–velocity curves for EeAChE). In this Lineweaver–Burk plot, the  $K_m$  values differ with the different inhibitor concentrations, while the  $v_{max}$  value remains unchanged. The Lineweaver–Burk plot for EeAChE shows reversible and competitive inhibition by compound 41.

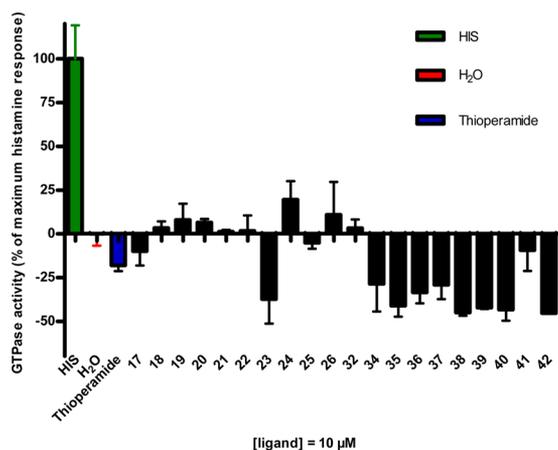
Affinity toward the human histamine H<sub>3</sub> receptor was determined by displacement of specific [<sup>3</sup>H]-histamine binding to H<sub>3</sub> receptor binding sites in Sf9 insect cell membranes<sup>32</sup> (Table 1).

Putative affinity at other hH receptor subtypes, that is, H<sub>1</sub>, H<sub>2</sub>, and H<sub>4</sub>, respectively, was determined by testing high concentration (10 μM) of the most potent compounds at hH<sub>3</sub>R for displacement of [<sup>3</sup>H]mepyramine (hH<sub>1</sub>R), [<sup>3</sup>H]UR-DE257 (hH<sub>2</sub>R),<sup>33</sup> and [<sup>3</sup>H]-histamine (hH<sub>4</sub>R) (Figure 2). Generally, all of the tested compounds exhibited a very good selectivity profile, since none of the tested compounds have affinity at hH<sub>4</sub>R and hH<sub>2</sub>R even at a concentration of 10 μM. At 10 μM, some of the tested compounds showed to slightly interact with hH<sub>1</sub>R (Figure 2).



**Figure 2.** Binding assay results toward  $hH_1R$ ,  $hH_2R$ , and  $hH_4R$ , applying a  $10 \mu\text{M}$  concentration of the most potent  $hH_3R$  antagonist compounds. Diphenhydramine (DPH) as  $hH_1R$  standard ligand and histamine (HIS) as  $hH_2R$ , and  $hH_4R$  standard ligand.

Determination of whether the synthesized compounds possess antagonist, agonist, or inverse agonist properties was investigated by steady-state GTPase activity assay. The functional assay (GTPase) was performed at  $10 \mu\text{M}$  concentration of all synthesized compounds. As a reference  $hH_3R$  antagonist,  $10 \mu\text{M}$  concentration of thioperamide was used (Figure 3). Almost all the tri- and tetracyclic synthesized



**Figure 3.** Functional assay (GTPase) results at the  $hH_3$  receptor applying a  $10 \mu\text{M}$  concentration of all synthesized compounds with water as a baseline and thioperamide as control (antagonist).<sup>34</sup>

compounds are  $hH_3R$  antagonists or inverse agonists; only compound **24**, which is connected with morpholine by three carbon spacer length, showed slight agonist properties at  $hH_3R$ .

## COMPUTATIONAL DOCKING STUDIES ON HUMAN AChE

Docking studies were carried out on AChE to investigate the putative preferred binding mode of the compounds in the inhibitor library. Docking studies with the structure of human AChE (PDB 4EY7)<sup>36</sup> and consideration of conserved water molecules (cf. Methods) suggest two possible modes of inhibition: a “classical” binding mode corresponding to the placement of the tri- or tetracyclic scaffold near the catalytic active site (CAS) at the bottom of the binding gorge; and an “inverted” binding mode showing the protonated tertiary amine instead of the aromatic scaffold deep in the binding gorge. In

the latter, cation- $\pi$  interactions are formed with mostly Trp86 or Tyr337 ( $hAChE$  numbering), residues known for attracting the tertiary amine of the natural substrate acetylcholine.<sup>21</sup>

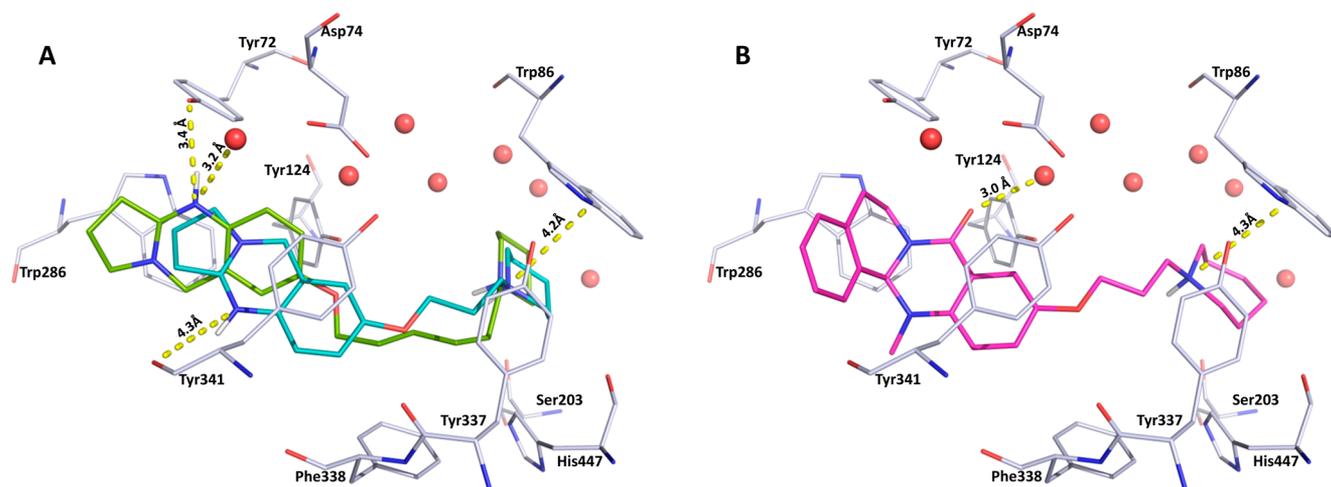
The ligands were docked in their expected ionization state at pH 8. This means protonation at the tertiary amine of all compounds, consistent with their  $pK_a$  values between 10.13 and 9.58 as calculated with MoKa.<sup>37</sup> A  $pK_a$  of 7.66 was calculated for the morpholine substituent. The aminal structure in compounds **35–38** is protonated due to a calculated  $pK_a$  of 8.73. Similarly, the amidine moiety was protonated in the inhibitors **41** and **42** with a calculated  $pK_a$  of 10.72 for both scaffolds resulting in double protonation of these compounds.

A very clear preference for the inverted binding mode is shown by the most potent compounds, for which good clustering of the docking results in the top poses was obtained. This includes the nonchiral tricyclic inhibitors **32**, **34**, **41**, and **42**, but also the chiral tricyclic compounds **36** and **38**, which are closely related to **41** and **42**, respectively (Figure 4). The quinazolinone compounds **31** and **33** also fit excellently in here; the binding as well as the docking results for a whole set of quinazolines will be presented in a subsequent publication. The piperidinyl ring of **41** and **42** is placed near the key residue of the choline binding site, Trp86. The alkyl chains point away from the water molecules in the binding site, so that the tricyclic ring systems are placed near the peripheral anionic site (PAS) interacting with the Tyr72 hydroxyl group or the backbone carbonyl of Tyr341. All of the mentioned compounds (except **34**: 3.8) achieve a per-atom-score (GoldScore/no. heavy atoms) of  $>4$ , indicating influential contributions of most atoms to the overall interaction. In contrast, the weaker inhibitors generally show lower per-atom-scores and/or inconsistent binding-orientation preferences for the two enantiomeric forms. Yet, for the most active tetracyclic compounds with a carbonyl function (**17** and **21**) the inverted binding mode appears to be preferred over the classical binding mode for both enantiomers with comparable score. In this binding mode, a 3.0–3.6 Å hydrogen distance bond can be observed between the carbonyl oxygen and a conserved water molecule (Figure 4).

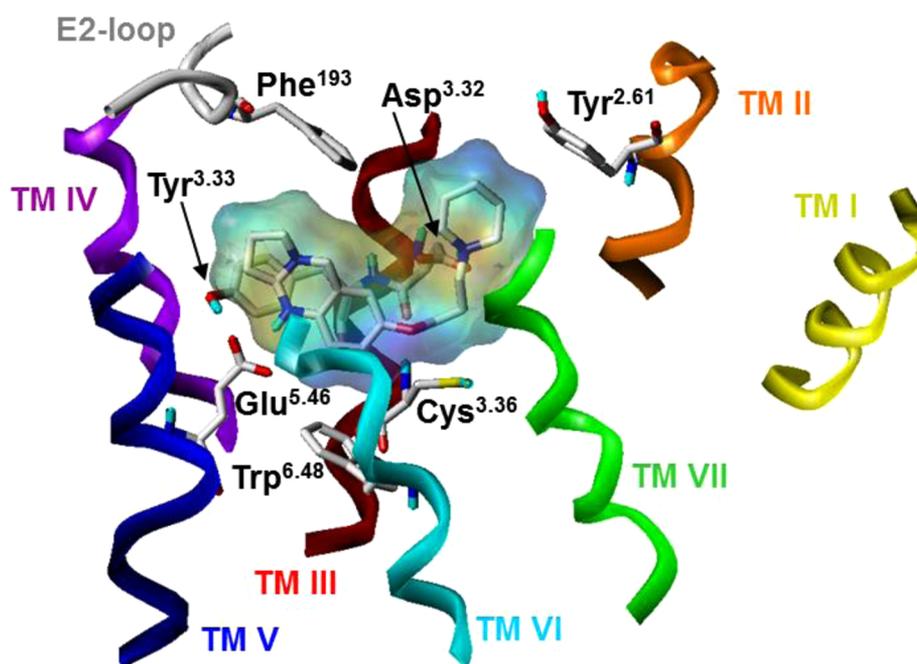
The docking results suggest that the inverted binding mode is favored for most of the inhibitors tested in this study. Especially for strong inhibitors ( $IC_{50} < 0.1 \mu\text{M}$ ), the scores show a clear preference for this binding mode. The main interactions consist of a cation- $\pi$  interaction with Trp86 and hydrogen bonds with conserved water molecules. Weaker inhibitors show lower per-atom-scores and in most cases less clear preference of the inverted binding mode versus the classical binding mode for the two enantiomeric forms.

## THE BINDING MODE OF COMPOUND 41 AT $hH_3R$

The molecular dynamics (MD) simulation revealed a stable binding mode of the double protonated form of compound **41** (the most potent compound at both targets investigated) in the binding pocket of the inactive  $hH_3R$  (Figures 5, 6A). During the whole productive phase of the MD simulation, a stable electrostatic interaction between the positively charged piperidine moiety of compound **41** and the highly conserved Asp<sup>3.32</sup> was observed (Figure 5). The mean distances between the hydrogen of the piperidine nitrogen of compound **41** and the oxygens of the Asp<sup>3.32</sup> side chain were fairly well maintained over time at about 0.22 nm (Figure 6A). A second stable interaction during the MD simulation was observed between Glu<sup>5.46</sup> and the positively charged amidine moiety of **41**



**Figure 4.** (A) Docking pose showing the inverted binding mode of inhibitor **41** (cyan) with a 4.2 Å cation– $\pi$  interaction to Trp86 and potential hydrogen bonds to a water molecule (3.2 Å) and Tyr72 (3.4 Å). Inhibitor **42** (green) shows the same placement of the piperidine ring near the CAS (4.2 Å to Trp86) and a distance of 4.3 Å to the Tyr341 backbone carbonyl. (B) Selected docking pose of the tetracyclic inhibitor **21** (pink, *R*-enantiomeric form) showing the inverted binding mode. The inhibitor is stabilized by a 3.0 Å interaction of the scaffold carbonyl oxygen with a water molecule and a 4.3 Å cation– $\pi$  interaction of the piperidine with Trp86 near the CAS. Color code: red, conserved water molecules; gray, *hAChE* binding site; catalytic active site, Ser203, His447 (Glu334 not shown here); peripheral anionic site (PAS), Tyr72, Trp286. Both figures were created using Pymol.<sup>35</sup>



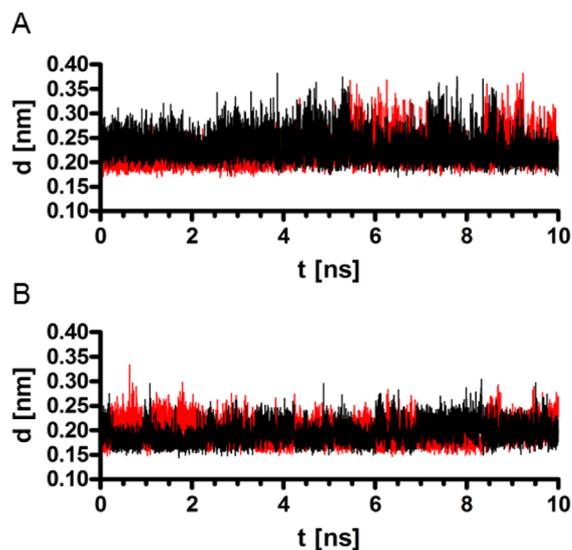
**Figure 5.** Compound **41** embedded in the binding pocket of the inactive *hH3R*, obtained as a snapshot of MD simulation. The translucent surface of the ligand is colored according to the electrostatic potential.

(Figures 5, 6B). Here, the mean distance between the hydrogen and the oxygens of Glu<sup>5.46</sup> is about 0.20 nm.

The tricyclic partial structure of compound **41** remained stable in a pocket formed by the amino acids Leu<sup>3.29</sup>, Tyr<sup>3.33</sup>, Cys<sup>3.36</sup>, Trp<sup>6.48</sup>, Tyr<sup>6.51</sup>, Met<sup>6.55</sup>, and Phe<sup>193</sup> (E2-loop) (Figure 5). Furthermore, the hydrophobic part of the piperidine moiety of compound **41** is in close contact to Tyr<sup>2.61</sup> and Leu<sup>7.42</sup> (Figure 5). Besides, the MD simulations revealed that water molecules occupy parts of the binding pocket in presence of compound **41**. An aromatic interaction was observed between Tyr<sup>3.33</sup> and the aromatic partial structure of compound **41**.

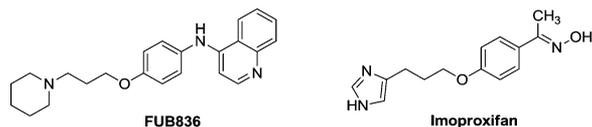
The observed binding mode based on modeling studies of compound **41** at *hH3R* is similar to the binding mode of the *hH3R* ligands FUB836,<sup>38</sup> imoproxifan<sup>39,40</sup> (Chart 4), and compound **6**<sup>41</sup> (Chart 2). The binding mode described for compound **6** at *hH3R* is very similar to that we observed for compound **41** within this study: both positively charged moieties were observed to interact electrostatically with Asp<sup>3.32</sup> and Glu<sup>5.46</sup>.

The positively charged imidazole moiety of imoproxifan was found to interact electrostatically with Asp<sup>3.32</sup>, and the remaining part of imoproxifan, including the aromatic moiety, was embedded in a pocket, similar as described for compound



**Figure 6.** (A) Time evolution of the distances between the hydrogen of the protonated piperidine of **41** and the oxygens of the Asp<sup>3.32</sup> side chain during the 10 ns productive MD. (B) Time evolution of the distances between the hydrogen of the protonated amidine of **41** and the oxygens of the Glu<sup>5.46</sup> side chain during the 10 ns productive MD.

#### Chart 4. FUB836<sup>38</sup> and Imoproxifan<sup>39,40</sup>



**41.** Surprisingly, imoproxifan is described to act, in contrast to compound **41**, as an agonist at *hH<sub>3</sub>R*.<sup>40</sup> A reason for that difference may be the imidazole moiety (as present in the full agonist histamine) in imoproxifan, instead of the piperidine partial structure in compound **41**. The pharmacological data suggest that the introduction of an amide moiety into the tricycle leads to a decrease in affinity to *hH<sub>3</sub>R*, as shown by direct comparison of compound **41** with compound **32** (Table 1). This may be explained by a steric hindrance of the amide with the *hH<sub>3</sub>R* or with the differences in electronic distribution between the two compounds. Phe<sup>193</sup>, located in the E2-loop, points into the binding pocket and shows an interaction with the ligand. This is in good accordance to data obtained for the histamine H<sub>4</sub> receptor.<sup>42</sup> For the histamine H<sub>4</sub> receptor, it was described that Phe<sup>169</sup>, which corresponds to Phe<sup>193</sup> in the *hH<sub>3</sub>R*, is responsible for differences in binding of agonists between *hH<sub>4</sub>R* and *mH<sub>4</sub>R*.<sup>42</sup> As presented in Figure 2, compound **41** shows no affinity to *hH<sub>1</sub>R*, *hH<sub>2</sub>R*, and *hH<sub>4</sub>R*. It is not surprising that compound **41** shows no affinity to *hH<sub>1</sub>R* or *hH<sub>2</sub>R*, because the structural profile of this compound does not fit to the typical *hH<sub>1</sub>R* or *hH<sub>2</sub>R* ligand structure.<sup>43</sup> A comparison of the most important amino acids in the binding pocket of the four human histamine receptor subtypes (Figure 7) shows that only the highly conserved Asp<sup>3.32</sup>, Trp<sup>6.48</sup>, and Tyr<sup>6.51</sup> are identical, which corresponds to an identity of only 20% (with respect to the 15 compared amino acids, Figure 7). A complete comparison of the different histamine receptor subtypes and species can be found in literature.<sup>44</sup> In contrast, between *hH<sub>3</sub>R* and *hH<sub>4</sub>R* about 60% of the amino acids (comparison of 15 amino acids, Figure 7) are identical. A large number of ligands with affinity or potency at *hH<sub>3</sub>R* and *hH<sub>4</sub>R*

	hH <sub>1</sub> R	hH <sub>2</sub> R	hH <sub>3</sub> R	hH <sub>4</sub> R	hH <sub>1</sub> R	hH <sub>2</sub> R	hH <sub>3</sub> R	hH <sub>4</sub> R	hH <sub>1</sub> R	hH <sub>2</sub> R	hH <sub>3</sub> R	hH <sub>4</sub> R	hH <sub>1</sub> R	hH <sub>2</sub> R	hH <sub>3</sub> R	hH <sub>4</sub> R	hH <sub>1</sub> R	hH <sub>2</sub> R	hH <sub>3</sub> R	hH <sub>4</sub> R	
hH <sub>1</sub> R	Asn	Asp	Tyr	Ser	Ile	Trp	Phe	Lys	Thr	Ala	Asn	Trp	Tyr	Phe	Gly						
hH <sub>2</sub> R	Ser	Asp	Val	Cys	Ile	Leu	Tyr	Gly	Asp	Gly	Thr	Trp	Tyr	Phe	Gly						
hH <sub>3</sub> R	Tyr	Asp	Tyr	Cys	Ala	Leu	Phe	Leu	Ala	Ser	Glu	Trp	Tyr	Thr	Leu						
hH <sub>4</sub> R	Tyr	Asp	Tyr	Cys	Val	Val	Ile	Leu	Thr	Ser	Glu	Trp	Tyr	Ser	Gln						

**Figure 7.** Comparison of selected amino acids, located in the binding pocket of the four human histamine receptor subtypes; green, highly conserved amino acids within all subtypes or identical amino acids between *hH<sub>3</sub>R* and *hH<sub>4</sub>R*, red: amino acids being different between *hH<sub>3</sub>R* and *hH<sub>4</sub>R*.

are known, for example, proxifan, thioperamide, UR-PI294, and UR-AK381.<sup>43</sup> Arylbenzimidazols were described to show affinity to *hH<sub>4</sub>R*.<sup>45</sup> The 1-methyl-4-(3-phenoxypropyl) piperazine partial structure of these compounds is similar to the 1-(3-phenoxypropyl) piperidine partial structure of compound **41**. However, a reason for selectivity of compound **41** toward *hH<sub>3</sub>R* (compared to *hH<sub>4</sub>R*) may be the exchange of the *N*-methylpiperazine moiety into a piperidine moiety. The *N*-methylpiperazine moiety is typical for a large number of H<sub>4</sub>R ligands,<sup>46</sup> its basic amine is relevant for interaction with Asp<sup>3.32</sup>, and it is located at a different position compared to the piperidine. It might be suggested that the sequence difference at position 7.42 (*hH<sub>3</sub>R*, Leu; *hH<sub>4</sub>R*, Gln) (Figure 7) would be responsible, since this amino acid side chain is in direct neighborhood to compound **41**. For the *hH<sub>3</sub>R* and *rH<sub>3</sub>R*, it was shown that species differences between *hH<sub>3</sub>R* and *rH<sub>3</sub>R* at positions 3.37 and 3.40 (*hH<sub>3</sub>R*: Thr<sup>3.37</sup>, Ala<sup>3.40</sup>; *rH<sub>3</sub>R*: Ala<sup>3.37</sup>, Val<sup>3.40</sup>) are responsible for pharmacological differences of imoproxifan.<sup>39</sup> Thus, it could also be speculated that the differences in amino acids between *hH<sub>3</sub>R* and *hH<sub>4</sub>R* at position 3.40 (Figure 7) may be involved in subtype differences. However, in order to obtain a more detailed insight into the interactions with *hH<sub>3</sub>R*, site-directed mutagenesis studies combined with further molecular modeling studies have to be performed.

In summary, merging the piperidinylpropoxy phenyl and related *hH<sub>3</sub>R* pharmacophores into heterocyclic templates with AChE inhibiting properties yielded reversible and competitive AChE inhibitors with antagonizing properties at *hH<sub>3</sub>R*. SARs led to highly potent compounds with balanced two digit nanomolar affinities at both targets. The compounds show excellent selectivity over all other *hHR* receptor subtypes. Docking studies could explain the pronounced influence of the tertiary amine on SARs onto AChE inhibitory activity by an “inverted” binding mode locating the tertiary amine into the CAS. MD simulation confirmed the compound design strategy for *hH<sub>3</sub>R* affinity by identifying a binding mode related to other highly potent *hH<sub>3</sub>R* antagonists.

## METHODS

**Construction of Inactive-State Model of *hH<sub>3</sub>R*.** The crystal structure of *hH<sub>1</sub>R* 3RZE<sup>47</sup> representing the inactive conformation of the *hH<sub>1</sub>R*, was used as a template to construct the inactive state of *hH<sub>3</sub>R*. Lysozyme which was artificially cocrystallized in 3RZE was deleted. The *N*-terminus, which is missing in the crystal structure, was added using SYBYL 7.0 (Tripos Inc.). The “Loop-Search” module of SYBYL 7.0 was used to complete the E2-loop. Due to the lack of information concerning the conformation of the long I3-loop of the *hH<sub>3</sub>R* (more than 100 amino acids) the amino acids Ala<sup>239</sup> to Arg<sup>347</sup> were not included in the model. To close the gap between the intracellular parts of TM V and TM VI eight alanines were inserted instead. Since internal water molecules were shown to play an important role in stabilization or activation of aminergic GPCRs these internal water molecules were included into the model of inactive

$hH_3R$ <sup>48,49</sup> Using SYBYL7.0 the compound **41** was docked manually into the binding pocket of  $hH_3R$  in such manner that the positively charged piperidine moiety interacts electrostatically with the highly conserved Asp<sup>332</sup>, similarly to previous studies addressing other  $H_3R$  ligands.<sup>38,40</sup> The tricyclic partial structure of compound **41** was embedded in a pocket between TM III, TM V and TM VI. The resulting complex was energetically minimized. Afterward, the minimized ligand–receptor complex was embedded in a POPC lipid bilayer. Intracellular and extracellular water, as well as an appropriate number of sodium and chloride ions were added into the simulation box and the molecular dynamics simulation, using GROMACS 4.0.2 (<http://www.gromacs.org>), was performed as described.<sup>50,51</sup> The parametrization for the ligand was obtained from the PRODRG server (<http://davapc1.bioch.dundee.ac.uk/prodrg/>), but the partial charges were adopted based on the Gasteiger–Hückel partial charges, calculated by SYBYL 7.0. For the POPC lipid, the force field parameters available at an appropriate online source ([http://moose.bio.ucalgary.ca/index.php?page=Structures\\_and\\_Topologies](http://moose.bio.ucalgary.ca/index.php?page=Structures_and_Topologies)) were used. Subsequent to a 5 ns equilibration phase (force constants (250 kJ/(mol nm<sup>2</sup>) for the first 2.5 ns) and 100 kJ/(mol nm<sup>2</sup>) for the second 2.5 ns) were put onto the backbone atoms of the TM domains of  $hH_3R$ ), a 10 ns productive phase was performed.

#### Computational Docking of Inhibitors to hAChE Binding Site.

Docking was carried out with GOLD v5.1 and GOLD v5.2 (GOLDSUITE 5.1 and 5.2, CCDC Software, <http://www.ccdc.cam.ac.uk>)<sup>52</sup> in the 4EY7 crystal structure of human AChE. The protein was set up for docking by removing all water molecules and nonprotein atoms and protonation of the amino acids was performed with MOE 2011.10<sup>53</sup> using Protonate 3D at standard conditions. The search region was focused on the binding area comprising the CAS, the binding gorge, and the PAS. The binding site was defined by the following residues: Tyr72, Asp74, Trp86, Asn87, Gly120, Gly121, Tyr124, Ser125, Gly126, Ala127, Tyr133, Glu202, Ser203, Trp236, Trp286, Phe295, Phe297, Glu334, Tyr337, Phe338, Tyr341, His447, Gly448, and Tyr449. The ligand structures were either built in MOE (compounds 17–26, 32 and 34–42) and the chiral compounds in their R- and S-forms) or extracted from the crystal structure (ligands for redocking experiments, cf. below). The protonation for the ligands was set according to calculations performed with the program MoKa.<sup>37</sup> The inhibitor structures were energy minimized in MOE with the MMFF94x force field to an rms-gradient of 0.001 kcal/(mol·Å). Using default settings in GOLD, the best-suited scoring function was selected based on redocking experiments in the *Torpedo californica* AChE binding site, with the galanthamine derivative of PDB structure 1W4L and donepezil of 1EVE. Another redocking experiment was performed using the donepezil ligand of 4EY7 and (–)-huperzine A of 4EY5, both human AChE structures. With each of the four scoring functions available in GOLD (ASP, CHEMPLP, ChemScore, GoldScore), 50 ligand poses were generated with the default number of operations of 100 000. In all cases, the top-ranked docking pose was deviating less than 1.07 Å with GoldScore.<sup>54,55</sup> By raising the number of operations in the GOLD GA settings to 500 000, thereby prolonging the optimization time, the top-pose showed a significantly lower rmsd to the crystal pose of 0.55 Å (compared to 1.04 Å with GA 100 000) for redocking using 4EY7 and 0.63 Å for 1EVE (compared to 0.65 Å with GA 100 000). In all cases, a top-ranked docking pose deviating less than 0.91 Å from the crystal structure was obtained with GoldScore. Based on a docking study for AChE selective compounds [manuscript in preparation], the GoldScore function<sup>54,55</sup> was best able to reflect the affinity and, hence, chosen for this project, too. All docking poses were clustered with a 1.5 Å cluster-cutoff by applying the complete linkage method. Of the five best-scored poses, the pose associated with the biggest cluster was selected for further pose analysis. The described redocking experiments showed that the largest cluster always contained the pose with the lowest rmsd to the crystal structure and the top-scoring pose. Seven conserved water molecules (HOH722, 729, 731, 737, 881, 952, 954 form the structure 4EY7) were chosen from an alignment of 1EVE, 4EY7, and the apoprotein structure 4EY4. The usage of these selected water molecules was validated using the water-“toggle” and water-“on” mode in the docking program. In 9 out

of 12 analyzed docking runs, all water molecules were accepted in the “toggle”-mode and kept for generating ligand binding modes. In only three cases, one water molecule, located at the entrance of the binding site, was excluded from the docking process. Docking studies were thus carried out with the selected conserved water molecules in the water-“on” mode. For the donepezil ligand in 1EVE, the redocking with water gave an rmsd of 0.52 Å for the pose most similar to the crystal structure and 47 poses in the cluster. For the ligand in 4EY7, an rmsd of 0.35 Å was found (49 poses in the cluster).

**Enzyme Inhibition. Acetyl- and Butyrylcholinesterase Inhibition Assay.** The assay has been previously described in detail:<sup>14,24</sup> AChE (E.C.3.1.1.7, Type VI-S, from electric eel), BChE (E.C.3.1.1.8, from equine serum), and hAChE (E.C.3.1.1.7, acetylcholinesterase from human erythrocytes) were purchased from Sigma-Aldrich (Steinheim, Germany); DTNB (Ellman’s reagent), ATC, and BTC iodides were obtained from Fluka (Buchs, Switzerland).

The assay was performed as described in the following procedure: stock solutions of the tested compounds were prepared in ethanol/water (1:4 ratio), 100  $\mu$ L of which gave a final concentration of  $10^{-3}$  M when diluted to the final volume of 3.32 mL. The highest concentration of the tested compounds applied in the assay was  $10^{-4}$  M (10% EtOH in the stock solution did not influence enzyme activity). In order to obtain an inhibition curve, at least five different concentrations (normally  $10^{-4}$ – $10^{-9}$  M) of the test compound were measured at 25 °C and 412 nm, each concentration in triplicate.

For buffer preparation, 1.36 g of potassium dihydrogen phosphate (10 mmol) was dissolved in 100 mL of water and adjusted with NaOH to pH = 8.0  $\pm$  0.1. Enzyme solutions were prepared to give 2.5 units mL<sup>-1</sup> in 1.4 mL aliquots. Furthermore, 0.01 M DTNB solution and 0.075 M ATC and BTC solutions were used. A cuvette containing 3.0 mL of phosphate buffer, 100  $\mu$ L of the respective enzyme, and 100  $\mu$ L of the test compound solution was allowed to stand for 4.5 min, then 100  $\mu$ L of DTNB was added, and the reaction was started by addition of 20  $\mu$ L of the substrate solution (ATC/BTC). The solution was mixed immediately, and exactly 2.5 min after substrate addition the absorption was measured. For the reference value, 100  $\mu$ L of water replaced the test compound solution. For determining the blank value, additionally 100  $\mu$ L of water replaced the enzyme solution. The inhibition curve was obtained by plotting the percentage enzyme activity (100% for the reference) versus logarithm of test compound concentration.

**Radioligand Binding Assays.** For the binding experiments, three different batches of Sf9 insect cell membranes were generated and prepared as described in literature.<sup>33,56–59</sup> The accordant membranes were thawed and sedimented by a 10 min centrifugation at 4 °C and 13 000g. Membranes were resuspended in binding buffer (12.5 mM MgCl<sub>2</sub>, 1 mM EDTA, 75 mM Tris/HCl, pH 7.4). Each well (total volume 100  $\mu$ L) contained 40  $\mu$ g ( $hH_1R$ ), 60  $\mu$ g ( $hH_2R$ ), 50  $\mu$ g ( $hH_3R$ ), or 90  $\mu$ g ( $hH_4R$ ) of protein. Competition binding experiments were performed in attendance of 5 nM [<sup>3</sup>H]mepyramine ( $K_D$  = 4.49 nM)<sup>56</sup> ( $hH_1R$ ) (American Radiolabeled Chemicals, St. Louis, MO), 30 nM [<sup>3</sup>H]UR-DE257 ( $K_D$  = 31.33 nM)<sup>33</sup> ( $hH_2R$ ), or 15 nM [<sup>3</sup>H]histamine ( $K_D$  = 39.53 nM ( $hH_3R$ );  $K_D$  = 15.88 nM ( $hH_4R$ ))<sup>60</sup> (American Radiolabeled Chemicals, St. Louis, MO), increasing concentrations of unlabeled ligands in  $hH_3R$  binding assay and for  $hH_1R$ ,  $hH_2R$ , and  $hH_4R$  experiments, a single concentration (10  $\mu$ M) of unlabeled ligand was used. The assays were conducted in 96-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany) and incubated for 60 min at 25 °C under shaking at 250 rpm using a Heidolph Titramax 101 instrument (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Bound radioligand was separated from free radioligand by filtration through GF/C filters, which were pretreated with 0.3% polyethyleneimine solution. Subsequently, three washing steps with cold binding buffer (4 °C) using a Brandel Harvester instrument (Brandel, Gaithersburg, MD) were followed. Filter-bound radioactivity was detected after an equilibration phase of at least 12 h by liquid scintillation counting in 96-well plates (PerkinElmer, Waltham, MA).

The stock solutions of all tested compounds were prepared by dissolving the free base compound in equivalent ethanolic HCl and

water. The dilution series of all stock solutions were prepared by using water (the highest concentration (10  $\mu$ M) of all tested compounds contains less than 0.01% ethanol (v/v)).

**Steady-State GTPase Activity Assay.** GTPase activity assays were operated as described for the  $hH_1R$ ,  $hH_2R$ ,  $hH_3R$ , and  $hH_4R$ <sup>33,56–59</sup>  $hH_1R$  assays: Sf9 insect cell membranes coexpressing the  $hH_1R$  and RGS4 were employed;  $hH_2R$  assays: Sf9 insect cell membranes expressing the  $hH_2R$ -GsaS fusion protein were employed;  $hH_3R$  assays: Sf9 insect cell membranes coexpressing the  $hH_3R$ , mammalian *Gia2*, and *G $\beta$ 1 $\gamma$ 2* were employed;  $hH_4R$  assays: Sf9 insect cell membranes coexpressing the  $hH_4R$ -RGS19 fusion protein, mammalian *Gia2*, and *G $\beta$ 1 $\gamma$ 2* were employed. The relative membranes were thawed and then centrifuged for 10 min at 4 °C and 13 000g.

Membranes were resuspended in 10 mM Tris/HCl, pH 7.4. Each assay tube contained Sf9 membranes expressing the respective histamine-receptor subtype (10  $\mu$ g protein/tube), MgCl<sub>2</sub> ( $hH_1R$ ,  $hH_2R$  assays: 1.0 mM,  $hH_3R$ ,  $hH_4R$  assays: 5.0 mM), 100  $\mu$ M EDTA, 100  $\mu$ M ATP, 100 nM GTP, 100  $\mu$ M adenylyl imidophosphate, 5 mM creatine phosphate, 40  $\mu$ g creatine kinase, 0.2% (w/v) bovine serum albumin in 50 mM Tris/HCl (pH 7.4), and the investigated ligands at various concentrations. All  $hH_4R$  assays additionally included 100 mM NaCl.

Reaction mixtures (80  $\mu$ L) were incubated for 2 min at 27.5 °C. After the addition of 20  $\mu$ L of [ $\gamma$ -<sup>33</sup>P]GTP (0.05  $\mu$ Ci/tube), reaction mixtures were incubated for 20 min at 27.5 °C. The reactions were stopped by the addition of 900  $\mu$ L of slurry consisting of 5% (w/v) activated charcoal and 50 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 2.0. Charcoal absorbs nucleotides but not <sup>33</sup>P. Charcoal-quenched reaction mixtures were centrifuged for 7 min at 4 °C and 13 000g. A volume of 600  $\mu$ L of the supernatant was taken, and the activity of <sup>33</sup>P<sub>i</sub> was determined by liquid scintillation counting.

Spontaneous [ $\gamma$ -<sup>33</sup>P]GTP degradation was determined in tubes containing all components described above, plus a high concentration of unlabeled GTP (1 mM) that, due to competition with [ $\gamma$ -<sup>33</sup>P]GTP, prevents [ $\gamma$ -<sup>33</sup>P]GTP hydrolysis by enzymatic activities in the presence of Sf9 membranes. Spontaneous [ $\gamma$ -<sup>33</sup>P]GTP degradation was <1% of the total amount of radioactivity added. The experimental conditions chosen ensured that less than 10% of the total amount of [ $\gamma$ -<sup>33</sup>P]GTP added was converted to <sup>33</sup>P<sub>i</sub>. For all the experiments, three different batches of Sf9 membranes were prepared and used. All experimental data were analyzed by nonlinear regression using Prism 5 program (GraphPad Software, San Diego, CA).

**Chemistry. General Methods.** Melting points are uncorrected and were measured in open capillary tubes, using a Barnstead Electro-thermal IA9100 melting point apparatus. <sup>1</sup>H and <sup>13</sup>C NMR spectral data were obtained from a Bruker Advance spectrometer (300 and 75 MHz, respectively). TLC was performed on silica gel on aluminum foils with fluorescent indicator 254 nm (Merck). For detection, iodine vapor or UV light (254 nm) was used. ESI-MS samples were analyzed using electrospray ionization ion-trap mass spectrometry in nanospray mode using a Thermo Finnigan LCQ Deca instrument. The CHN analyses were undertaken using Perkin-Elmer elemental analyzer PE2400CHNS. In case purity was determined by elemental analysis, small amounts of solvent had to be taken into account which had been applied in previous column chromatography and appeared in the corresponding NMR spectra. For column chromatography, silica gel 60, 230–400 mesh (Merck) was used.

**Benzyl 2-Amino-5-benzyloxy-N-ethoxycarbonylbenzoate (I).**<sup>25</sup> 2-Amino-5-hydroxybenzoic acid (0.765 g, 5.0 mmol) and ethyl chloroformate (0.814 g, 0.72 mL, 7.5 mmol) were heated in dry dioxane (25 mL) at reflux temperature for 2 h. The solvent was evaporated, the residue dissolved in dry DMF (10 mL), K<sub>2</sub>CO<sub>3</sub> (2.07 g, 0.015 mol), and benzyl bromide (1.88 g, 1.31 mL, 0.011 mol) added and the reaction stirred under N<sub>2</sub> for 2 h at ambient temperature. The reaction was diluted with water (50 mL) and extracted with ether (3  $\times$  20 mL). The ether extract was concentrated and purified by column chromatography using 10:1 (EtOAc/hexanes) as eluent system to afford the title compound (1.63 g, 80%) as a white solid, mp 65–67 °C (lit. mp 64 °C). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 10.17 (s, 1H, NH), 8.37 (d,  $J$  = 9.3 Hz, 1H, arom.), 7.63 (d,  $J$  = 3.1 Hz, 1H, arom.),

7.50–7.27 (m, 10H, arom.), 7.19 (dd,  $J$  = 9.2, 3.1 Hz, 1H, arom.), 5.35 (s, 2H, OCH<sub>2</sub>Ph), 5.04 (s, 2H, OCH<sub>2</sub>Ph), 4.22 (d,  $J$  = 7.1 Hz, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 1.37–1.25 (m, 3H, OCH<sub>2</sub>CH<sub>3</sub>). Lit. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 10.2 (s, 1H, NH), 8.35 (d, 1H,  $J$  = 9 Hz, H3), 7.62 (d, 1H,  $J$  = 3 Hz, H6), 7.40 (m, 10H, OCH<sub>2</sub>Ph), 7.19 (dd, 1H,  $J$  = 3 and 9 Hz, H4), 5.34 (s, 2H, OCH<sub>2</sub>Ph), 5.04 (s, 2H, OCH<sub>2</sub>Ph), 4.21 (q, 2H,  $J$  = 7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 1.31 (t, 3H,  $J$  = 7 Hz, OCH<sub>2</sub>CH<sub>3</sub>).

**6-Amino-3-benzyloxy-N-ethoxycarbonylbenzoic Acid (II).**<sup>25</sup> The benzyl ester I (0.405 g, 1.0 mmol) and KOH (0.112 g, 2.0 mmol) were heated at reflux in MeOH/H<sub>2</sub>O (10 mL, 3:1) for 2 h. The reaction was cooled and acidified with 1 N HCl (2 mL). The precipitate was filtered and air-dried to afford the title compound (0.281 g, 89%) as a white solid, mp 157–160 °C (lit. mp 159–160 °C). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 9.94 (s, 1H, CO<sub>2</sub>H), 8.40 (d,  $J$  = 9.3 Hz, 1H, arom.), 7.69 (d,  $J$  = 3.1 Hz, 1H, arom.), 7.51–7.18 (m, 7H, arom.), 5.08 (s, 2H, OCH<sub>2</sub>Ph), 4.24 (q,  $J$  = 7.1 Hz, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 1.34 (s, 3H, OCH<sub>2</sub>CH<sub>3</sub>). Lit. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 9.94 (s, 1H, CO<sub>2</sub>H), 8.39 (d, 1H,  $J$  = 9 Hz, H3), 7.69 (d, 1H,  $J$  = 3 Hz, H6), 7.37 (m, 5H, OCH<sub>2</sub>Ph), 7.24 (dd, 1H,  $J$  = 3 and 9 Hz, H4), 5.07 (s, 2H, OCH<sub>2</sub>Ph), 4.24 (q, 2H,  $J$  = 7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 1.34 (t, 3H,  $J$  = 7 Hz, OCH<sub>2</sub>CH<sub>3</sub>).

**6-Benzyloxyisatoic Anhydride (11).**<sup>25</sup> The acid II (0.472 g, 1.5 mmol) was dissolved in dry THF (15 mL), oxalyl chloride (1 mL) added, and the reaction heated at reflux temperature for 2 h. The solvent was evaporated, ether (10 mL) added to the residue, and the mixture heated under reflux for 10 min. The precipitate was filtered and washed with ether (10 mL) to afford the title compound (0.350 g, 87%) as a white solid, mp 220–222 °C (lit. mp 218–220 °C). <sup>1</sup>H NMR (300 MHz, DMSO)  $\delta$ : 11.62 (s, 1H), 7.39 (ddd,  $J$  = 19.4, 13.6, 7.6 Hz, 7H), 7.11 (d,  $J$  = 8.6 Hz, 1H), 5.17 (s, 2H). Lit. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$ : 11.60 (s, 1H, NH), 7.40 (m, 7H, CH<sub>2</sub>Ph, H7 and H5), 7.12 (d, 1H,  $J$  = 8.5 Hz, H4), 5.16 (s, 2H, OCH<sub>2</sub>Ph).

**6-(Benzyloxy)-1-methyl-1H-benzo[d][1,3]oxazine-2,4-dione (12).** To a solution of 6-benzyloxyisatoic anhydride compound 11 (500 mg, 1.86 mmol) in 5 mL of DMAc (*N,N*-dimethyl acetamide), diisopropylethylamine (370  $\mu$ L, 2.23 mmol) was added. The solution was stirred for 10 min prior to addition of methyl iodide (140  $\mu$ L, 2.23 mmol). The solution was heated at 60 °C for 6 h. The solution was allowed to cool to room temperature, and 15 mL of cooled water was added. The resulting suspension was stirred vigorously for 30 min, filtered, and washed with 20 mL of water and 20 mL of *n*-hexane to afford the title compound (490 mg, 93%) as a yellow solid, mp 248–251 °C. ESI-MS: 284.18  $m/z$  [M]<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, DMSO)  $\delta$ : 7.61–7.29 (m, 8H, arom.), 5.22 (s, 2H, CCH<sub>2</sub>C), 3.44 (s, 3H, NCH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, DMSO)  $\delta$ : 158.73 (CO), 153.85 (CO), 147.68 (arom.), 136.42 (arom.), 136.39 (arom.), 128.39 (arom.), 127.87 (arom.), 127.58 (arom.), 125.61 (arom.), 116.52 (arom.), 112.08 (arom.), 112.02 (arom.), 69.68 (CCH<sub>2</sub>C), 31.65 (NCH<sub>3</sub>).

**10-(Benzyloxy)-13-methyl-13,13a-dihydro-5H-isoquinolino[1,2-*b*]quinazolin-8(6H)-one (13).** A suspension of 6-(benzyloxy)-1-methyl-1H-benzo[d][1,3]-oxazine-2,4-dione 12 (300 mg, 1.45 mmol) and 3,4-dihydroisoquinoline (228 mg, 1.74 mmol) in 50 mL of toluene was refluxed for 24 h. After cooling, the solvent was removed under reduced pressure and the residue was purified by column chromatography using (100:1) CH<sub>2</sub>Cl<sub>2</sub>/MeOH as eluent system to afford the title compound (150 mg, 35%) as yellow thick oil. HRESIMS (C<sub>24</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>+H)<sup>+</sup>,  $m/z$  calcd: 371.1754; found: 371.1758. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.68 (d,  $J$  = 2.7 Hz, 1H), 7.51–7.21 (m, 9H), 7.18–7.07 (m, 2H), 5.73 (s, 1H, NCHN), 5.19–5.05 (m, 2H, OCH<sub>2</sub>C), 4.66 (ddd,  $J$  = 12.8, 4.8, 3.1 Hz, 1H), 3.26 (ddd,  $J$  = 12.8, 11.4, 3.9 Hz, 1H), 3.10–2.79 (m, 2H), 2.37 (d,  $J$  = 4.5 Hz, 3H, NCH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 164.12 (CO), 155.04 (arom.), 145.30 (arom.), 136.93 (arom.), 136.86 (arom.), 132.02 (arom.), 128.62 (arom.), 128.57 (arom.), 128.42 (arom.), 128.25 (arom.), 128.05 (arom.), 127.65 (arom.), 127.12 (arom.), 124.11 (arom.), 123.65 (arom.), 122.06 (arom.), 111.82 (arom.), 71.80 (NCHN), 70.46 (OCH<sub>2</sub>C), 38.85 (NCH<sub>2</sub>CH<sub>2</sub>), 36.29 (NCH<sub>3</sub>), 28.72 (CH<sub>2</sub>CH<sub>2</sub>C).

**10-Hydroxy-13-methyl-13,13a-dihydro-5H-isoquinolino-[1,2-*b*]quinazolin-8(6H)-one (14).** To a mixture of compound 13 (300 mg, 0.81 mmol) and 10% Pd–C (20 mg) was added 10 mL of ethanol.

The mixture was stirred at room temperature and under an atmosphere of H<sub>2</sub> for 4 h. The mixture was filtered over celite to afford the title compound (190 mg, 84%) as greenish yellow solid, mp 189–192 °C. ESI-MS: 281.0 *m/z* [M]<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 2.33 (s, 3H, NCH<sub>3</sub>), 2.86 (m, 1H, CCH<sub>2</sub>CH<sub>2</sub>), 2.99 (m, 1H, CCH<sub>2</sub>CH<sub>2</sub>), 3.37–3.23 (m, 1H, NCH<sub>2</sub>CH<sub>2</sub>), 4.66 (m, 1H, NCH<sub>2</sub>CH<sub>2</sub>), 5.75 (s, 1H, NCHN), 7.13–7.02 (m, 2H, arom.), 7.25–7.19 (m, 1H, arom.), 7.39–7.27 (m, 2H, arom.), 7.55–7.41 (m, 1H, arom.), 7.97 (s, 1H, arom.) ppm. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 28.69 (CCH<sub>2</sub>CH<sub>2</sub>), 36.31 (NCH<sub>3</sub>), 38.99 (NCH<sub>2</sub>CH<sub>2</sub>), 71.98 (NCHN), 114.37 (arom.), 121.72 (arom.), 123.85 (arom.), 123.88 (arom.), 127.19 (arom.), 128.27 (arom.), 128.51 (arom.), 128.51 (arom.), 131.78 (arom.), 136.75 (arom.), 144.44 (arom.), 153.43 (COH), 164.63 (CO) ppm.

**10-(3-Bromopropoxy)-13-methyl-13,13a-dihydro-5H-isoquinolino[1,2-b]quinazolin-8(6H)-one (15).** 1,3-Dibromopropane (505 mg, 2.5 mmol), compound 14 (350 mg, 1.25 mmol), potassium carbonate (260 mg, 1.9 mmol), and potassium iodide (21 mg, 0.125 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl, and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography giving the title compound (400 mg, 80%) as a yellow thick oil. HRESIMS (C<sub>20</sub>H<sub>21</sub>BrN<sub>2</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 401.0859; found: 401.0855. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.60–7.53 (m, 1H), 7.47 (dd, *J* = 7.9, 5.1 Hz, 1H), 7.36–7.26 (m, 2H), 7.24–7.18 (m, 1H), 7.12–7.01 (m, 2H), 5.72 (s, 1H), 4.75–4.56 (m, 1H), 4.14–4.06 (m, 2H), 3.67–3.54 (m, 2H), 3.33–3.16 (m, 1H), 3.07–2.77 (m, 2H), 2.36 (s, 3H), 1.31–1.20 (m, 2H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 164.10 (CO), 154.92 (arom.), 145.27 (arom.), 136.90 (arom.), 132.00 (arom.), 128.55 (arom.), 128.37 (arom.), 128.24 (arom.), 127.10 (arom.), 124.09 (arom.), 123.62 (arom.), 121.67 (arom.), 111.58 (arom.), 71.82 (NCHN), 65.83, 38.86, 36.29 (NCH<sub>3</sub>), 32.34, 29.96, 28.69.

**10-((6-Bromohexyloxy)-13-methyl-13,13a-dihydro-5H-isoquinolino[1,2-b]quinazolin-8(6H)-one (16).** 1,6-Dibromohexane (610 mg, 2.5 mmol), compound 14 (350 mg, 1.25 mmol), potassium carbonate (260 mg, 1.9 mmol) and potassium iodide (21 mg, 0.125 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl, and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over sodium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography giving the title compound (390 mg, 70%) as a yellow thick oil. HRESIMS (C<sub>23</sub>H<sub>27</sub>BrN<sub>2</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 443.12829; found: 443.1283. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.54 (d, *J* = 2.5 Hz, 1H), 7.48 (dd, *J* = 7.9, 5.3 Hz, 1H), 7.37–7.26 (m, 2H), 7.21 (dd, *J* = 7.8, 5.1 Hz, 1H), 7.12–7.00 (m, 2H), 5.72 (s, 1H), 4.65 (ddd, *J* = 12.8, 4.8, 3.1 Hz, 1H), 4.11–3.90 (m, 2H), 3.42 (t, *J* = 6.8 Hz, 2H), 3.34–3.16 (m, 1H), 3.07–2.77 (m, 2H), 2.35 (s, 3H), 2.01–1.73 (m, 4H), 1.57–1.43 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 164.17 (CO), 155.36 (arom.), 145.01 (arom.), 136.91 (arom.), 132.05 (arom.), 128.53 (arom.), 128.4 (arom.), 128.20 (arom.), 127.10 (arom.), 124.13 (arom.), 123.65 (arom.), 121.78 (arom.), 111.32 (arom.), 71.82 (NCHN), 68.25, 38.81, 36.29 (NCH<sub>3</sub>), 33.84, 32.72, 29.07, 28.73, 27.95, 25.30.

**13-Methyl-10-((6-(piperidin-1-yl)hexyloxy)-13,13a-dihydro-5H-isoquinolino[1,2-b]quinazolin-8(6H)-one (17).** Piperidine (64 mg, 0.75 mmol), compound 16 (222 mg, 0.50 mmol), potassium carbonate (105 mg, 0.75 mmol), and potassium iodide (8 mg, 0.05 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl, and extracted with methylene chloride. The combined organic extracts were washed with brine and

dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography giving the title compound (160 mg, 71%) as a yellow thick oil. HRESIMS (C<sub>28</sub>H<sub>37</sub>N<sub>3</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 448.2959; found: 448.2960. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.53 (d, *J* = 2.4 Hz, 1H), 7.49–7.43 (m, 1H), 7.36–7.27 (m, 2H), 7.24–7.18 (m, 1H), 7.10–7.00 (m, 2H), 5.71 (s, 1H), 4.64 (ddd, *J* = 12.8, 4.8, 3.1 Hz, 1H), 4.18–3.82 (m, 2H), 3.24 (ddd, *J* = 12.8, 11.4, 3.9 Hz, 2H), 3.12–2.68 (m, 2H), 2.47–2.23 (m, 9H), 1.88–1.70 (m, 2H), 1.64–1.30 (m, 12H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 164.19 (CO), 155.45 (arom.), 144.95 (arom.), 136.91 (arom.), 132.05 (arom.), 128.51 (arom.), 128.41 (arom.), 128.18 (arom.), 127.09 (arom.), 124.13 (arom.), 123.66 (arom.), 121.78 (arom.), 111.31 (arom.), 71.81 (NCHN), 68.42, 59.54, 54.65, 38.78 (NCH<sub>3</sub>), 36.27, 29.18, 28.73, 27.52, 26.84, 26.03, 25.95, 24.48. Elem. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>3</sub>O<sub>2</sub> + 0.13 CH<sub>2</sub>Cl<sub>2</sub>) Calcd.: C, 73.66; H, 8.19; N, 9.16; O, 6.98. Found: C, 73.85; H, 8.12; N, 9.14.

**13-Methyl-10-(3-(piperidin-1-yl)propoxy)-13,13a-dihydro-5H-isoquinolino[1,2-b]quinazolin-8(6H)-one (18).** Piperidine (64 mg, 0.75 mmol), compound 15 (200 mg, 0.50 mmol), potassium carbonate (105 mg, 0.75 mmol), and potassium iodide (8 mg, 0.05 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography giving the title compound (136 mg, 67%) as a colorless thick oil. HRESIMS (C<sub>25</sub>H<sub>31</sub>N<sub>3</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 406.2495; found: 406.2501. <sup>1</sup>H NMR (300 MHz, MeOD) δ: 7.50–7.36 (m, 2H), 7.36–7.18 (m, 3H), 7.17–7.08 (m, 2H), 5.73 (s, 1H), 4.66–4.47 (m, 1H), 4.18–3.96 (m, 2H), 3.32–3.17 (m, 2H), 3.08–2.83 (m, 2H), 2.77–2.55 (m, 6H), 2.40 (s, 3H), 2.06 (qd, *J* = 11.8, 6.8 Hz, 2H), 1.70 (ddd, *J* = 16.2, 11.8, 5.9 Hz, 4H), 1.53 (d, *J* = 5.0 Hz, 2H). <sup>13</sup>C NMR (75 MHz, MeOD) δ: 166.00 (CO), 156.25 (arom.), 146.48 (arom.), 138.26 (arom.), 133.47 (arom.), 129.73 (arom.), 129.57 (arom.), 129.21 (arom.), 128.17 (arom.), 124.26 (arom.), 124.23 (arom.), 122.88 (arom.), 112.65 (arom.), 73.38 (NCHN), 67.68, 57.00, 55.40, 49.92, 49.64, 49.36, 49.07, 48.79, 48.51, 48.22, 40.44, 36.71 (NCH<sub>3</sub>), 29.37, 27.07, 26.18, 24.82. Elem. Anal. (C<sub>25</sub>H<sub>31</sub>N<sub>3</sub>O<sub>2</sub> + 0.3 CHCl<sub>3</sub>) Calcd.: C, 68.85; H, 7.15; N, 9.52; O, 7.25. Found: C, 68.74; H, 7.54; N, 9.29.

**13-Methyl-10-(3-(morpholinopropoxy)-13,13a-dihydro-5H-isoquinolino[1,2-b]quinazolin-8(6H)-one (19).** Morpholine (65 mg, 0.75 mmol), compound 15 (200 mg, 0.50 mmol), potassium carbonate (105 mg, 0.75 mmol), and potassium iodide (8 mg, 0.05 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography giving the title compound (144 mg, 71%) as a colorless thick oil. HRESIMS (C<sub>24</sub>H<sub>29</sub>N<sub>3</sub>O<sub>3</sub>+H)<sup>+</sup>, *m/z* calcd: 408.2287; found: 408.2286. <sup>1</sup>H NMR (300 MHz, MeOD) δ: 7.47–7.38 (m, 2H), 7.36–7.22 (m, 3H), 7.14–7.07 (m, 2H), 5.73 (s, 1H), 4.57–4.49 (m, 1H), 4.14–3.98 (m, 2H), 3.73–3.68 (m, 4H), 3.24 (ddd, *J* = 12.7, 10.9, 4.5 Hz, 1H), 3.06–2.81 (m, 2H), 2.61–2.46 (m, 6H), 2.41 (s, 3H), 2.07–1.91 (m, 2H). <sup>13</sup>C NMR (75 MHz, MeOD) δ: 166.03 (CO), 156.40 (arom.), 146.44 (arom.), 138.27 (arom.), 133.46 (arom.), 129.72 (arom.), 129.56 (arom.), 129.24 (arom.), 128.17 (arom.), 124.31 (arom.), 124.27 (arom.), 122.93 (arom.), 112.59 (arom.), 73.38 (NCHN), 67.70, 67.67, 56.79, 54.83, 40.42, 36.69 (NCH<sub>3</sub>), 29.39, 27.24. Elem. Anal. (C<sub>24</sub>H<sub>29</sub>N<sub>3</sub>O<sub>3</sub> + 0.13 CHCl<sub>3</sub>) Calcd.: C, 68.51; H, 6.94; N, 9.93; O, 11.35. Found: C, 68.58; H, 7.13; N, 9.90.

**13-Methyl-10-(3-(pyrrolidin-1-yl)propoxy)-13,13a-dihydro-5H-isoquinolino[1,2-b]quinazolin-8(6H)-one (20).** Pyrrolidine (53 mg, 0.75 mmol), compound 15 (200 mg, 0.50 mmol), potassium

carbonate (105 mg, 0.75 mmol), and potassium iodide (8 mg, 0.05 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography giving the title compound (130 mg, 66%) as a yellow thick oil. HRESIMS ( $C_{24}H_{29}N_3O_2+H$ )<sup>+</sup>, *m/z* calcd: 392.2333; found: 392.2336. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.55 (d, *J* = 2.4 Hz, 1H), 7.50–7.44 (m, 1H), 7.38–7.28 (m, 2H), 7.25–7.17 (m, 1H), 7.12–7.01 (m, 2H), 5.73 (s, 1H), 4.65 (m, 1H), 4.22–3.97 (m, 2H), 3.34–3.20 (m, 1H), 2.84 (m, 6H), 2.36 (s, 3H), 2.16 (d, *J* = 6.7 Hz, 4H), 1.91 (s, 4H). <sup>13</sup>C NMR (75 MHz, MeOD) δ: 166.03 (CO), 156.23, 146.52, 146.13, 138.26, 129.71, 129.56, 129.19, 128.15, 124.23, 122.87, 113.68, 112.68, 73.41 (NCHN), 67.49, 55.17, 54.23, 40.45, 36.70 (NCH<sub>3</sub>), 29.37, 29.03, 24.19. Elem. Anal. ( $C_{24}H_{29}N_3O_2 + 0.8 CH_2Cl_2 + 0.5 H_2O$ ) Calcd.: C, 63.58; H, 6.80; N, 8.97; O, 8.54. Found: C, 63.46; H, 7.14; N, 9.06.

**10-(3-(Azepan-1-yl)propoxy)-13-methyl-13,13a-dihydro-5H-isoquinolino[1,2-b]quinazolin-8(6H)-one (21).** Azepane (75 mg, 0.75 mmol), compound 15 (200 mg, 0.50 mmol), potassium carbonate (105 mg, 0.75 mmol), and potassium iodide (8 mg, 0.05 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography giving the title compound (145 mg, 69%) as a yellow thick oil. HRESIMS ( $C_{26}H_{33}N_3O_2+H$ )<sup>+</sup>, *m/z* calcd: 420.2646; found: 420.2650. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.55 (d, *J* = 2.1 Hz, 1H), 7.47 (d, *J* = 6.2 Hz, 1H), 7.36–7.18 (m, 3H), 7.13–6.99 (m, 2H), 5.73 (s, 1H), 4.78–4.52 (m, 1H), 4.21–3.92 (m, 2H), 3.26 (td, *J* = 12.6, 3.8 Hz, 1H), 3.06–2.67 (m, 8H), 2.33 (s, 3H), 2.12–1.95 (m, 2H), 1.67 (d, *J* = 28.3 Hz, 8H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 166.93 (CO), 156.29, 147.52, 147.23, 138.46, 129.91, 129.76, 129.39, 128.65, 124.27, 122.99, 113.75, 112.75, 71.83 (NCHN), 55.35, 54.80, 38.82, 36.29 (NCH<sub>3</sub>), 32.50, 30.96, 28.72, 27.16, 27.02. Elem. Anal. ( $C_{26}H_{33}N_3O_2 + 1.75 CH_2Cl_2$ ) Calcd.: C, 58.66; H, 6.47; N, 7.40; O, 5.63. Found: C, 58.45; H, 6.84; N, 7.24.

**13-Methyl-10-((6-(piperidin-1-yl)hexyl)oxy)-6,8,13,13a-tetrahydro-5H-isoquinolino[1,2-b]quinazoline (22).** A solution of compound 17 (116 mg, 0.26 mmol) in dry THF was added dropwise to a suspension of lithium aluminum hydride (30 mg, 0.78 mmol) in dry THF under nitrogen atmosphere. The mixture was heated to reflux for 3 h, and then the reaction mixture was poured into ice water, basified with 20% aq. NaOH, and extracted with ethyl acetate. The combined extracts were washed with saturated aq. NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product, which was column chromatographed on a silica gel using CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia solution (20:1:0.1) as an eluent system to afford the title compound (95 mg, 84%) as a dark yellow oil. HRESIMS ( $C_{28}H_{39}N_3O+H$ )<sup>+</sup>, *m/z* calcd: 434.3166; found: 434.3160. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.53–7.43 (m, 1H), 7.25–7.06 (m, 3H), 6.94 (d, *J* = 8.8 Hz, 1H), 6.75 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.56 (d, *J* = 2.8 Hz, 1H), 4.71 (d, *J* = 8.5 Hz, 1H), 4.02–3.78 (m, 4H), 3.35–3.05 (m, 2H), 2.84–2.61 (m, 2H), 2.49 (m, 5H), 2.42–2.35 (m, 2H), 1.84–1.32 (m, 16H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 153.64 (arom.), 142.72 (arom.), 136.29 (arom.), 134.23 (arom.), 128.61 (arom.), 128.46 (arom.), 127.14 (arom.), 126.84 (arom.), 126.00 (arom.), 122.73 (arom.), 114.02 (arom.), 112.04 (arom.), 77.43 (HCHN), 68.23, 59.27, 57.02, 54.44, 48.91, 38.77 (NCH<sub>3</sub>), 29.31, 28.90, 27.43, 26.39, 26.00, 25.48, 24.16. Elem. Anal. ( $C_{28}H_{39}N_3O + 1.1 CHCl_3$ ) Calcd.: C, 61.87; H, 7.15; N, 7.44; O, 2.83. Found: C, 61.90; H, 7.26; N, 7.16.

**13-Methyl-10-(3-(piperidin-1-yl)propoxy)-6,8,13,13a-tetrahydro-5H-isoquinolino[1,2-b]quinazoline (23).** A solution of compound 18

(105 mg, 0.26 mmol) in dry THF was added dropwise to a suspension of lithium aluminum hydride (30 mg, 0.78 mmol) in dry THF under nitrogen atmosphere. The mixture was heated to reflux for 3 h, and then the reaction mixture was poured into ice water, basified with 20% aq. NaOH, and extracted with ethyl acetate. The combined extracts were washed with saturated aq. NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product, which was column chromatographed on a silica gel using CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia solution (20:1:0.1) as an eluent system to afford the title compound (30 mg, 41%) as yellow thick oil. HRESIMS ( $C_{25}H_{33}N_3O+H$ )<sup>+</sup>, *m/z* calcd: 392.2696; found: 392.2697. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.54–7.42 (m, 1H), 7.28–7.07 (m, 3H), 6.93 (dd, *J* = 8.8, 3.5 Hz, 1H), 6.75 (dd, *J* = 8.8, 2.9 Hz, 1H), 6.54 (dd, *J* = 12.9, 2.8 Hz, 1H), 4.72 (s, 1H), 4.05–3.80 (m, 4H), 3.31–3.02 (m, 2H), 2.84–2.41 (m, 11H), 2.04 (td, *J* = 12.4, 6.2 Hz, 2H), 1.76–1.59 (m, 4H), 1.49 (s, 2H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 136.28 (arom.), 128.54 (arom.), 127.15 (arom.), 126.84 (arom.), 125.99 (arom.), 122.67 (arom.), 114.05 (arom.), 112.06 (arom.), 77.43 (NCHN), 66.69, 56.99, 56.02, 54.49, 48.87, 38.74 (NCH<sub>3</sub>), 28.89, 26.50, 25.44, 24.08. Elem. Anal. ( $C_{25}H_{33}N_3O + 0.9 H_2O + 1.8 CH_3CH_2OH$ ) Calcd.: C, 57.24; H, 8.16; N, 7.00; O, 9.87. Found: C, 57.57; H, 7.81; N, 6.72.

**4-(3-((13-Methyl-6,8,13,13a-tetrahydro-5H-isoquinolino[1,2-b]quinazolin-10yl)oxy)propyl)morpholine (24).** A solution of compound 19 (106 mg, 0.26 mmol) in dry THF was added dropwise to a suspension of lithium aluminum hydride (30 mg, 0.78 mmol) in dry THF under nitrogen atmosphere. The mixture was heated to reflux for 3 h, and then the reaction mixture was poured into ice water, basified with 20% aq. NaOH, and extracted with ethyl acetate. The combined extracts were washed with saturated aq. NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product, which was column chromatographed on a silica gel using CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia solution (20:1:0.1) as an eluent system to afford the title compound (38 mg, 42%) as a yellow thick oil. HRESIMS ( $C_{24}H_{31}N_3O_2+H$ )<sup>+</sup>, *m/z* calcd: 394.2489; found: 394.2490. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.51–7.44 (m, 1H), 7.25–7.18 (m, 2H), 7.17–7.10 (m, 1H), 6.94 (d, *J* = 8.8 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.9 Hz, 1H), 6.57 (d, *J* = 2.8 Hz, 1H), 4.72 (s, 1H), 4.04–3.85 (m, 4H), 3.73 (dd, *J* = 8.5, 3.9 Hz, 4H), 3.31–3.05 (m, 2H), 2.85–2.60 (m, 2H), 2.56–2.43 (m, 9H), 2.01–1.87 (m, 2H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 153.52 (arom.), 142.85 (arom.), 136.28 (arom.), 134.21 v, 128.61 (arom.), 128.46 (arom.), 127.16 (arom.), 126.85 (arom.), 126.00 (arom.), 122.69 (arom.), 114.03 (arom.), 112.08 (arom.), 77.40 (NCHN), 67.03, 66.52, 57.00, 55.66, 53.78, 48.89, 38.76 (NCH<sub>3</sub>), 28.89, 26.59. Elem. Anal. ( $C_{24}H_{31}N_3O_2 + 1.15 CH_2Cl_2$ ) Calcd.: C, 50.30; H, 6.09; N, 7.00; O, 5.33. Found: C, 49.91; H, 6.50; N, 6.72.

**13-Methyl-10-(3-(pyrrolidin-1-yl)propoxy)-6,8,13,13a-tetrahydro-5H-isoquinolino[1,2-b]quinazoline (25).** A solution of compound 20 (100 mg, 0.26 mmol) in dry THF was added dropwise to a suspension of lithium aluminum hydride (30 mg, 0.78 mmol) in dry THF under nitrogen atmosphere. The mixture was heated to reflux for 3 h, and then the reaction mixture was poured into ice water, basified with 20% aq. NaOH, and extracted with ethyl acetate. The combined extracts were washed with saturated aq. NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product, which was column chromatographed on a silica gel using CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia solution (20:1:0.1) as an eluent system to afford the title compound (78 mg, 79%) as a light yellow oil. HRESIMS ( $C_{24}H_{31}N_3O+H$ )<sup>+</sup>, *m/z* calcd: 379.2571; found: 379.2570. <sup>1</sup>H NMR (300 MHz, MeOD) δ: 7.52–7.32 (m, 1H), 7.28–7.04 (m, 3H), 6.92 (d, *J* = 8.8 Hz, 1H), 6.80–6.67 (m, 1H), 6.60 (t, *J* = 4.6 Hz, 1H), 4.67 (s, 1H), 4.03–3.75 (m, 4H), 3.27–3.15 (m, 1H), 3.15–3.00 (m, 1H), 2.77–2.61 (m, 8H), 2.48 (s, 3H), 1.98 (dt, *J* = 15.8, 6.1 Hz, 2H), 1.91–1.79 (m, 4H). <sup>13</sup>C NMR (75 MHz, MeOD) δ: 155.00 (arom.), 143.44 (arom.), 137.37 (arom.), 135.22 (arom.), 129.87 (arom.), 129.41 (arom.), 128.62 (arom.), 127.31 (arom.), 123.27 (arom.), 115.37 (arom.), 113.10 (arom.), 78.65 (NCHN), 67.46, 57.48, 55.14, 54.40, 38.99 (NCH<sub>3</sub>), 29.44, 24.23. Elem. Anal.

(C<sub>24</sub>H<sub>31</sub>N<sub>3</sub>O + 0.57 CH<sub>2</sub>Cl<sub>2</sub>) Calcd.: C, 69.28; H, 7.61; N, 9.87; O, 3.76. Found: C, 69.28; H, 7.65; N, 9.68.

**10-(3-(Azepan-1-yl)propoxy)-13-methyl-6,8,13,13a-tetrahydro-5H-isoquinolino[1,2-b]quinazolin-9(1H)-one (26).** A solution of compound **21** (110 mg, 0.26 mmol) in dry THF was added dropwise to a suspension of lithium aluminum hydride (30 mg, 0.78 mmol) in dry THF under nitrogen atmosphere. The mixture was heated to reflux for 3 h, and then the reaction mixture was poured into ice water, basified with 20% aq. NaOH, and extracted with ethyl acetate. The combined extracts were washed with saturated aq. NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product, which was column chromatographed on a silica gel using CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia solution (20:1:0.1) as an eluent system to afford the title compound (85 mg, 81%) as a light yellow oil. HRESIMS (C<sub>26</sub>H<sub>35</sub>N<sub>3</sub>O+H)<sup>+</sup>, *m/z* calcd: 406.2853; found: 406.2851. <sup>1</sup>H NMR (300 MHz, MeOD) δ: 7.51–7.38 (m, 1H), 7.30–7.09 (m, 3H), 6.92 (d, *J* = 8.9 Hz, 1H), 6.81–6.68 (m, 1H), 6.61 (t, *J* = 4.9 Hz, 1H), 4.69 (s, 1H), 3.98–3.77 (m, 4H), 3.28–3.17 (m, 1H), 1.75–1.60 (m, 8H). <sup>13</sup>C NMR (75 MHz, MeOD) δ: 155.03 (arom.), 143.41 (arom.), 137.36 (arom.), 135.21 (arom.), 129.87 (arom.), 129.40 (arom.), 128.62 (arom.), 127.40 (arom.), 127.18 (arom.), 123.23 (arom.), 115.40 (arom.), 113.12 (arom.), 78.65 (NCHN), 67.59, 64.97, 57.46, 56.63, 56.16, 49.67, 38.95 (NCH<sub>3</sub>), 29.37, 27.99, 27.90, 27.79. Elem. Anal. (C<sub>26</sub>H<sub>35</sub>N<sub>3</sub>O + 0.24 CH<sub>2</sub>Cl<sub>2</sub>) Calcd.: C, 73.99; H, 8.40; N, 9.86; O, 3.76. Found: C, 74.03; H, 8.45; N, 9.66.

**7-(Benzyloxy)-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one (27).** Pyrrolidin-2-one (1.00 g, 12.25 mmol) and 6-benzyloxyisatoic anhydride **11** (1.00 g, 3.71 mmol) were placed in a 10 mL crimp-sealed thick-walled glass tube equipped with a pressure sensor and a magnetic stirrer. The sealed reaction tube was placed inside the cavity of a CEM Discover focused microwave synthesis system, operated at 130 °C (temperature monitored by a built-in infrared sensor), power 10–200 W, and pressure 50–100 psi for 1 h. The residue was purified by column chromatography using (2:1) CH<sub>2</sub>Cl<sub>2</sub>/acetone as eluent system to afford the title compound (450 mg, 78%) as a yellow solid, mp 188–190 °C. ESI-MS: 293.0 *m/z* [MH]<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 2.39–2.20 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.25–3.08 (m, 2H, CCH<sub>2</sub>CH<sub>2</sub>), 4.30–4.11 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 5.17 (s, 2H, COCH<sub>2</sub>), 7.44–7.29 (m, 5H, arom.), 7.47 (dd, *J* = 8.2, 1.2 Hz, 1H, arom.), 7.65–7.58 (m, 1H, arom.), 7.75 (d, *J* = 2.9 Hz, 1H, arom.). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 19.69 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 32.25 (CCH<sub>2</sub>CH<sub>2</sub>), 46.57 (NCH<sub>2</sub>CH<sub>2</sub>), 70.51 (COCH<sub>2</sub>), 107.14 (arom.), 124.90 (arom.), 127.71 (arom.), 128.20 (arom.), 128.24 (arom.), 128.66 (arom.), 131.25 (arom.), 136.34 (arom.), 149.10 (arom.), 157.18 (arom.), 157.41 (NCN), 160.76 (CO).

**7-Hydroxy-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one (28).**<sup>24</sup> 7-(Benzyloxy)-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one **27** (750 mg, 2.57 mmol) and 10% Pd–C (80 mg) were added to 50 mL of ethanol. The mixture was stirred at room temperature and under an atmosphere of H<sub>2</sub> for 4 h. The mixture was filtered over celite to afford the title compound (450 mg, 87%) as beige solid, mp 284–287 °C (lit. mp 289–290 °C). <sup>1</sup>H NMR (300 MHz, DMSO) δ: 2.21–2.10 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.08–2.94 (m, 2H, CCH<sub>2</sub>CH<sub>2</sub>), 4.12–3.94 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 7.23 (dd, *J* = 8.8, 2.9 Hz, 1H, arom.), 7.39 (d, *J* = 2.8 Hz, 1H, arom.), 7.47 (d, *J* = 8.8 Hz, 1H, arom.), 9.95 (s, 1H, COH). Lit. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 250 MHz) δ: 10.50 (br s, OH), 7.65 (1H, d, *J* = 8.8 Hz, H4), 7.45 (1H, d, *J* = 2.5 Hz, H1), 7.38 (1H, dd, *J* = 8.5, 2.5 Hz, H3), 4.09 (2H, t, *J* = 7.0 Hz), 3.26 (2H, d, *J* = 7.6 Hz), 2.23 (2H, quintet, *J* = 7.6 Hz).

**7-(3-Bromopropoxy)-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one (29).** 1,3-Dibromopropane (810 mg, 4.0 mmol), compound **28** (400 mg, 2.0 mmol), and potassium carbonate (415 mg, 3.0 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column

chromatography to afford the title compound (430 mg, 67%) as a yellow thick oil. HRESIMS (C<sub>14</sub>H<sub>15</sub>BrN<sub>2</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 323.0390; found: 323.0392. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.55 (d, *J* = 2.5 Hz, 1H), 7.49 (dd, *J* = 8.9, 1.4 Hz, 1H), 7.29–7.16 (m, 1H), 4.23–4.02 (m, 4H), 3.66–3.47 (m, 2H), 3.08 (t, *J* = 7.9 Hz, 2H), 2.36–2.24 (m, 2H), 1.97 (m, 2H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 160.71 (NCN), 157.41 (COCH<sub>2</sub>), 156.96 (CO), 143.67 (arom.), 128.20 (arom.), 124.38 (arom.), 121.12 (arom.), 106.71 (arom.), 65.81, 46.50, 32.16, 32.14, 29.82, 19.59.

**7-((6-Bromohexyl)oxy)-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one (30).** 1,6-Dibromohexane (980 mg, 4.0 mmol), compound **28** (400 mg, 2.0 mmol), and potassium carbonate (415 mg, 3.0 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography to afford the title compound (420 mg, 58%) as a yellow solid, mp 76–79 °C. HRESIMS (C<sub>17</sub>H<sub>21</sub>BrN<sub>2</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 365.0859; found: 365.0864. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.59 (dd, *J* = 12.3, 5.9 Hz, 2H), 7.31 (dd, *J* = 8.9, 2.9 Hz, 1H), 4.30–4.14 (m, 2H), 4.06 (t, *J* = 6.4 Hz, 2H), 3.48–3.37 (m, 2H), 3.16 (t, *J* = 7.9 Hz, 2H), 2.36–2.21 (m, 2H), 1.97–1.76 (m, 4H), 1.60–1.45 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 160.83 (NCN), 157.48 (COCH<sub>2</sub>), 157.20 (CO), 143.43 (arom.), 128.16 (arom.), 124.68 (arom.), 121.17 (arom.), 106.55 (arom.), 68.33, 46.54, 33.80, 32.68, 32.24, 28.96, 27.93, 25.30, 19.69.

**7-(3-(Pyrrolidin-1-yl)propoxy)-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one (31).** Pyrrolidine (66 mg, 0.93 mmol), compound **29** (200 mg, 0.62 mmol), and potassium carbonate (130 mg, 0.93 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography to afford the title compound (150 mg, 77%) as a yellow thick oil. HRESIMS (C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 314.1863; found: 314.1871. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.58 (d, *J* = 2.9 Hz, 1H), 7.50 (t, *J* = 6.8 Hz, 1H), 7.30–7.22 (m, 1H), 4.12 (dt, *J* = 12.6, 6.7 Hz, 4H), 3.10 (dd, *J* = 10.4, 5.5 Hz, 2H), 2.76–2.67 (m, 2H), 2.67–2.58 (m, 4H), 2.34–2.17 (m, 2H), 2.17–2.01 (m, 2H), 1.86–1.76 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 160.79 (NCN), 157.23 (COCH<sub>2</sub>), 157.19 (CO), 143.62 (arom.), 128.20 (arom.), 124.45 (arom.), 121.14 (arom.), 106.70 (arom.), 66.69, 54.19, 53.07, 46.49, 32.22, 28.25, 23.44, 19.65. Elem. Anal. (C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub> + 0.3 CHCl<sub>3</sub>) Calcd.: C, 62.94; H, 6.73; N, 12.03; O, 9.16. Found: C, 62.69; H, 6.93; N, 12.15.

**7-(3-(Piperidin-1-yl)propoxy)-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one (32).** Piperidine (80 mg, 0.93 mmol), compound **29** (200 mg, 0.62 mmol), and potassium carbonate (130 mg, 0.93 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography to afford the title compound (120 mg, 59%) as a white solid, mp 167–169 °C. HRESIMS (C<sub>19</sub>H<sub>25</sub>N<sub>3</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 328.202; found: 328.202. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 1.42–1.52 (m, 2H), 1.61–1.73 (m, 4H), 2.05–2.14 (m, 2H), 2.22–2.33 (m, 2H), 2.48–2.64 (m, 6H), 3.15 (t, *J* = 7.8 Hz, 2H), 4.11 (t, *J* = 6.3 Hz, 2H), 4.20 (t, *J* = 7.5 Hz, 2H), 7.30 (dd, *J*<sub>1</sub> = 9 Hz, *J*<sub>2</sub> = 3 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.62 (d, *J* = 3 Hz, 1H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 19.7, 22.4, 23.1, 24.4, 32.3, 46.5, 53.7, 55.4, 65.7, 107.1 (arom.), 121.2 (arom.), 124.0 (arom.), 128.5 (arom.), 144.0

(arom.), 156.6 (CO), 157.5 (COCH<sub>2</sub>), 160.7 (NCN). Elem. Anal. (C<sub>19</sub>H<sub>25</sub>N<sub>3</sub>O<sub>2</sub> + 1.33 CHCl<sub>3</sub>) Calcd.: C, 50.22; H, 5.46; N, 8.64; O, 6.58. Found: C, 49.90; H, 5.82; N, 8.93.

**7-(3-(Azepan-1-yl)propoxy)-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one (33).** Azepane (92 mg, 0.93 mmol), compound 29 (200 mg, 0.62 mmol), and potassium carbonate (130 mg, 0.93 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography giving afford the title compound (164 mg, 77%) as a yellow thick oil. HRESIMS (C<sub>20</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 342.2182; found: 342.2176. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.61 (d, *J* = 2.9 Hz, 1H), 7.54 (d, *J* = 8.9 Hz, 1H), 7.33–7.25 (m, 1H), 4.23–4.14 (m, 2H), 4.10 (t, *J* = 6.3 Hz, 2H), 3.21–3.05 (m, 2H), 2.76–2.61 (m, 6H), 2.36–2.19 (m, 2H), 2.07–1.92 (m, 2H), 1.61 (ddd, *J* = 8.7, 6.6, 3.2 Hz, 8H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 160.85 (NCN), 157.43 (COCH<sub>2</sub>), 157.11 (CO), 143.59 (arom.), 128.20 (arom.), 124.54 (arom.), 121.18 (arom.), 106.70 (arom.), 66.91, 55.49, 54.68, 46.49, 36.49, 32.24, 31.42, 27.78, 27.24, 26.99, 19.68. Elem. Anal. (C<sub>20</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub> + 0.56 CHCl<sub>3</sub>) Calcd.: C, 60.48; H, 6.80; N, 10.29; O, 7.84. Found: C, 60.36; H, 6.95; N, 10.02.

**7-((6-(Piperidin-1-yl)hexyl)oxy)-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one (34).** Piperidine (80 mg, 0.93 mmol), compound 30 (226 mg, 0.62 mmol), and potassium carbonate (130 mg, 0.93 mmol) were refluxed in absolute acetonitrile (50 mL) for 2 h. After cooling, the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. The remaining residue was taken up in aq. solution of NaCl and extracted with methylene chloride. The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography to afford the title compound (184 mg, 80%) as a yellow solid, mp 183–187 °C. HRESIMS (C<sub>22</sub>H<sub>31</sub>N<sub>3</sub>O<sub>2</sub>+H)<sup>+</sup>, *m/z* calcd: 370.2489; found: 370.2494. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.51 (dd, *J* = 8.4, 5.9 Hz, 2H), 7.29–7.20 (m, 1H), 4.15 (dd, *J* = 17.6, 10.5 Hz, 2H), 3.98 (t, *J* = 6.3 Hz, 2H), 3.09 (t, *J* = 7.9 Hz, 2H), 2.97 (m, 4H), 2.89–2.74 (m, 2H), 2.32–2.14 (m, 2H), 2.03–1.89 (m, 4H), 1.80 (ddd, *J* = 20.9, 14.3, 7.1 Hz, 4H), 1.61 (d, *J* = 4.4 Hz, 2H), 1.53–1.29 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 160.84 (NCN), 157.20 (COCH<sub>2</sub>), 157.33 (CO), 143.56 (arom.), 128.23 (arom.), 124.51 (arom.), 121.11 (arom.), 106.59 (arom.), 68.14, 57.71, 53.37, 46.52, 32.21, 28.83, 26.69, 25.59, 24.06, 23.03, 22.32, 19.65. Elem. Anal. (C<sub>22</sub>H<sub>31</sub>N<sub>3</sub>O<sub>2</sub>+1.09 CHCl<sub>3</sub>) Calcd.: C, 55.51; H, 6.47; N, 8.41; O, 6.40. Found: C, 55.29; H, 6.67; N, 8.73.

**7-(3-(Pyrrolidin-1-yl)propoxy)-1,2,3,3a,4,9-hexahydro-pyrrolo[2,1-b]quinazoline (35).** A solution of compound 31 (100 mg, 0.32 mmol) in dry THF was added dropwise to a suspension of lithium aluminum hydride (36 mg, 1.0 mmol) in dry THF under nitrogen atmosphere. The mixture was heated to reflux for 3 h, and then the reaction mixture was poured into ice water, basified with 20% aq NaOH, and extracted with ethyl acetate. The combined extracts were washed with saturated aq. NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product, which was column chromatographed on a silica gel using CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia solution (10:1:0.1) as an eluent system to afford the title compound (72 mg, 75%) as a light yellow oil. HRESIMS (C<sub>18</sub>H<sub>27</sub>N<sub>3</sub>O+H)<sup>+</sup>, *m/z* calcd: 302.2227; found: 302.2227. <sup>1</sup>H NMR (300 MHz, MeOD) δ: 6.63 (dt, *J* = 6.4, 3.2 Hz, 1H), 6.61–6.55 (m, 2H), 3.99–3.72 (m, 5H), 3.17–2.99 (m, 1H), 2.77–2.61 (m, 6H), 2.51 (td, *J* = 8.9, 6.9 Hz, 1H), 2.18–2.03 (m, 1H), 2.03–1.88 (m, 4H), 1.88–1.81 (m, 4H), 1.76–1.62 (m, 1H). <sup>13</sup>C NMR (75 MHz, MeOD) δ: 153.57 (COCH<sub>2</sub>), 138.48 (arom.), 122.29 (arom.), 118.43 (arom.), 115.55 (arom.), 114.15 (arom.), 73.95 (NHCHN), 67.73, 55.14, 54.43, 53.06, 51.94, 31.55, 29.52, 24.21, 21.73. Elem. Anal. (C<sub>18</sub>H<sub>27</sub>N<sub>3</sub>O + 0.2 CH<sub>2</sub>Cl<sub>2</sub>) Calcd.: C, 68.65; H, 8.67; N, 13.20; O, 5.02. Found: C, 68.99; H, 8.65; N, 12.75.

**7-(3-(Piperidin-1-yl)propoxy)-1,2,3,3a,4,9-hexahydropyrrolo[2,1-b]quinazoline (36).** A solution of compound 32 (100 mg, 0.31 mmol) in dry THF was added dropwise to a suspension of lithium aluminum hydride (35 mg, 0.92 mmol) in dry THF under nitrogen atmosphere. The mixture was heated to reflux for 3 h, and then the reaction mixture was poured into ice water, basified with 20% aq NaOH, and extracted with ethyl acetate. The combined extracts were washed with saturated aq. NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product, which was column chromatographed on a silica gel using CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia solution (10:1:0.1) as an eluent system to afford the title compound (75 mg, 77%) as a light yellow oil. HRESIMS (C<sub>19</sub>H<sub>29</sub>N<sub>3</sub>O+H)<sup>+</sup>, *m/z* calcd: 316.2383; found: 316.2379. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 1.42–1.47 (m, 2H), 1.57–1.68 (m, 6H), 1.80–2.00 (m, 5H), 2.08–2.17 (m, 2H), 2.42–2.60 (m, 8H), 3.05–3.12 (m, 1H), 3.89–3.94 (m, 5H), 6.54–6.56 (m, 2H), 6.63 (dd, *J*<sub>1</sub> = 9 Hz, *J*<sub>2</sub> = 3 Hz, 1H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 152.38, 122.08, 117.51, 114.09, 113.27, 72.41, 67.08, 56.11, 54.59, 51.68, 50.91, 31.47, 26.82, 25.82, 24.34, 21.00. Elem. Anal. (C<sub>19</sub>H<sub>29</sub>N<sub>3</sub>O + 0.08 CH<sub>2</sub>Cl<sub>2</sub>) Calcd.: C, 71.11; H, 9.12; N, 13.04; O, 4.96. Found: C, 71.27; H, 8.75; N, 12.82.

**7-(3-(Azepan-1-yl)propoxy)-1,2,3,3a,4,9-hexahydropyrrolo[2,1-b]quinazoline (37).** A solution of compound 33 (100 mg, 0.29 mmol) in dry THF was added dropwise to a suspension of lithium aluminum hydride (33 mg, 0.88 mmol) in dry THF under nitrogen atmosphere. The mixture was heated to reflux for 3 h, and then the reaction mixture was poured into ice water, basified with 20% aq. NaOH, and extracted with ethyl acetate. The combined extracts were washed with saturated aq. NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product, which was column chromatographed on a silica gel using CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia solution (10:1:0.1) as an eluent system to afford the title compound (79 mg, 83%) as a light yellow oil. HRESIMS (C<sub>20</sub>H<sub>31</sub>N<sub>3</sub>O+H)<sup>+</sup>, *m/z* calcd: 330.2540; found: 330.2541. <sup>1</sup>H NMR (300 MHz, MeOD) δ: 6.60 (ddd, *J* = 8.7, 6.1, 1.7 Hz, 3H), 3.82 (tt, *J* = 9.1, 5.8 Hz, 5H), 3.17–2.92 (m, 1H), 2.71 (ddd, *J* = 9.8, 7.6, 5.3 Hz, 6H), 2.50 (td, *J* = 8.9, 6.9 Hz, 1H), 2.19–2.03 (m, 1H), 2.03–1.84 (m, 4H), 1.76–1.58 (m, 9H). <sup>13</sup>C NMR (75 MHz, MeOD) δ: 153.60 (COCH<sub>2</sub>), 138.48 (arom.), 122.29 (arom.), 118.42 (arom.), 115.58 (arom.), 114.19 (arom.), 73.93 (NHCHN), 67.89, 56.64, 56.19, 53.06, 51.94, 31.57, 28.01, 27.88, 21.74. Elem. Anal. C<sub>20</sub>H<sub>31</sub>N<sub>3</sub>O + 0.11 CH<sub>2</sub>Cl<sub>2</sub> Calcd.: C, 71.29; H, 9.29; N, 12.40; O, 4.72. Found: C, 71.39; H, 9.16; N, 12.05.

**7-((6-(Piperidin-1-yl)hexyl)oxy)-1,2,3,3a,4,9-hexahydro-pyrrolo[2,1-b]quinazoline (38).** A solution of compound 24 (100 mg, 0.27 mmol) in dry THF was added dropwise to a suspension of lithium aluminum hydride (31 mg, 0.81 mmol) in dry THF under nitrogen atmosphere. The mixture was heated to reflux for 3 h, and then the reaction mixture was poured into ice water, basified with 20% aq NaOH, and extracted with ethyl acetate. The combined extracts were washed with saturated aq. NaCl solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product, which was column chromatographed on a silica gel using CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ammonia solution (10:1:0.1) as an eluent system to afford the title compound (69 mg, 71%) as a light yellow oil. HRESIMS (C<sub>22</sub>H<sub>35</sub>N<sub>3</sub>O+H)<sup>+</sup>, *m/z* calcd: 358.2853; found: 358.2848. <sup>1</sup>H NMR (300 MHz, MeOD) δ: 6.75–6.68 (m, 2H), 6.66 (d, *J* = 0.8 Hz, 1H), 4.26–4.19 (m, 1H), 3.91 (q, *J* = 6.4 Hz, 2H), 3.33–2.88 (m, 10H), 2.34–2.18 (m, 1H), 2.10 (dt, *J* = 14.5, 8.3 Hz, 3H), 1.92–1.70 (m, 10H), 1.59–1.38 (m, 5H). <sup>13</sup>C NMR (75 MHz, MeOD) δ 154.47 (arom.), 137.10 (arom.), 120.26 (arom.), 119.28 (arom.), 116.55 (arom.), 113.97 (arom.), 75.57, 69.40, 58.32, 54.29, 52.18, 51.98, 30.55, 30.25, 27.52, 26.77, 25.06, 24.35, 22.87, 21.57. Elem. Anal. (C<sub>22</sub>H<sub>35</sub>N<sub>3</sub>O + 0.65 CH<sub>2</sub>Cl<sub>2</sub>) Calcd.: C, 65.91; H, 8.86; N, 10.18; O, 3.88. Found: C, 66.16; H, 9.01; N, 10.08.

**7-(3-(Piperidin-1-yl)propoxy)-2,3,3a,4-tetrahydropyrrolo[2,1-b]quinazolin-9(1H)-one (39).** To a stirred solution of compound 32 (340 mg, 1.04 mmol) in 40 mL of ethanol, NaBCNH<sub>3</sub> (220 mg, 3.11 mmol), 1 N HCl in isopropanol (5 drops), was added. The reaction mixture was stirred at room temperature for 24 h, TLC showed incomplete reaction, and we added NaBCNH<sub>3</sub> (136 mg, 2.16 mmol), 1 N HCl in isopropanol (5 drops) to the reaction mixture until TLC

showed complete reaction. The reaction mixture was quenched by iced-water and basified using 1 N sodium hydroxide solution, extracted by  $\text{CH}_2\text{Cl}_2$ . The organic phase was extracted by  $\text{CH}_2\text{Cl}_2$  ( $3 \times 50$  mL). The combined organic phase was dried over sodium sulfate; the solvents were removed under reduced pressure. The residue was purified by column chromatography using (20:1:0.1)  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{ammonia}$  as eluent system to afford the title compound (310 mg, 90%) as a white solid, mp 277–279 °C. HRESIMS ( $\text{C}_{19}\text{H}_{27}\text{N}_3\text{O}_2 + \text{H}$ )<sup>+</sup>,  $m/z$  calcd: 330.2176; found: 330.2177. <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.28 (d,  $J = 3$  Hz, 1H), 6.96 (dd,  $J = 9$  Hz, 3 Hz, 1H), 6.75 (d,  $J = 9$  Hz, 1H), 4.91 (t,  $J = 6.3$  Hz, 2H), 4.01 (t,  $J = 6.0$  Hz, 2H), 3.58–3.72 (m, 2H), 2.80–2.92 (m, 6H), 2.31–2.42 (m, 1H), 1.93–2.15 (m, 5H), 1.70–1.78 (m, 4H), 1.54–1.65 (m, 2H). <sup>13</sup>C NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ : 164.46, 153.65, 122.90, 117.88, 112.58, 71.87, 67.35, 56.66, 55.07, 49.89, 49.60, 49.32, 49.04, 48.75, 48.47, 48.19, 45.48, 26.45, 25.53, 24.05, 22.85. Elem. Anal. ( $\text{C}_{19}\text{H}_{27}\text{N}_3\text{O}_2 + 0.36 \text{ CH}_2\text{Cl}_2$ ) Calcd.: C, 64.59; H, 7.76; N, 11.67; O, 8.89. Found: C, 64.97; H, 7.73; N, 11.27.

**7-((6-(Piperidin-1-yl)hexyl)oxy)-2,3,3a,4-tetrahydropyrrolo[2,1-b]quinazolin-9(1H)-one (40).** To a stirred solution of compound **34** (200 mg, 0.54 mmol) in 50 mL of ethanol,  $\text{NaBCNH}_3$  (68 mg, 1.8 mmol), 1 N HCl in isopropanol (5 drops), was added. The reaction mixture was stirred at room temperature for 24 h, TLC showed incomplete reaction, and we added  $\text{NaBCNH}_3$  (68 mg, 1.8 mmol), 1 N HCl in isopropanol (5 drops) to the reaction mixture until TLC showed complete reaction. The reaction mixture was quenched by iced-water, basified using 1 N sodium hydroxide solution, and extracted by  $\text{CH}_2\text{Cl}_2$ . The organic phase was extracted by  $\text{CH}_2\text{Cl}_2$  ( $3 \times 50$  mL). The combined organic phase was dried over sodium sulfate; the solvents were removed under reduced pressure. The residue was purified by column chromatography using (20:1:0.1)  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{ammonia}$  as eluent system to afford the title compound (160 mg, 80%) as yellow solid, mp 141–144 °C. HRESIMS ( $\text{C}_{22}\text{H}_{33}\text{N}_3\text{O}_2 + \text{H}$ )<sup>+</sup>,  $m/z$  calcd: 372.2646; found: 372.2648. <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.11 (dd,  $J = 13.2, 2.9$  Hz, 1H), 6.71 (dd,  $J = 8.7, 2.9$  Hz, 1H), 6.53 (d,  $J = 8.7$  Hz, 1H), 4.74 (dd,  $J = 7.1, 5.7$  Hz, 1H), 3.85–3.65 (m, 2H), 3.62–3.36 (m, 2H), 3.32–3.02 (m, 2H), 3.04–2.83 (m, 4H), 2.85–2.69 (m, 2H), 2.14 (ddq,  $J = 16.5, 15.3, 8.2$  Hz, 2H), 1.97–1.81 (m, 2H), 1.73–1.61 (m, 4H), 1.61–1.48 (m, 4H), 1.40–1.21 (m, 4H). <sup>13</sup>C NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ : 166.96 (CO), 156.65 (arom.), 146.10 (arom.), 125.72 (arom.), 122.47 (arom.), 120.85 (arom.), 115.55 (arom.), 74.41 (NHCHN), 72.16, 61.34, 57.30, 48.26, 36.63, 32.65, 30.13, 29.37, 27.87, 27.05, 25.73, 25.61. Elem. Anal. ( $\text{C}_{22}\text{H}_{33}\text{N}_3\text{O}_2 + 1.584 \text{ CH}_2\text{Cl}_2$ ) Calcd.: C, 50.53; H, 6.22; N, 7.50; O, 5.71. Found: C, 50.15; H, 6.51; N, 7.90.

**7-(3-(Piperidin-1-yl)propoxy)-1,2,3,3a,4,9-hexahydropyrrolo[2,1-b]quinazoline (41).** Zn dust (400 mg) was added to compound **32** (100 mg, 0.3 mmol) dissolved in 10 mL of acetic acid, and to the reaction mixture 2 mL of HCl (10 M) was then added. The reaction mixture was then heated up to 70 °C for 2 h. The reaction mixture was allowed to cool to room temperature. The solvent was concentrated under vacuum, and the reaction mixture basified by NaOH (4 N) and extracted by ethylacetate ( $3 \times 100$  mL). The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography using 5:1:0.1  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{ammonia}$  solution mixture as eluent system to afford the title compound (36 mg, 38%) as a yellow thick oil. HRESIMS ( $\text{C}_{19}\text{H}_{27}\text{N}_3\text{O} + \text{H}$ )<sup>+</sup>,  $m/z$  calcd: 314.2232; found: 314.2236. <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 6.95 (t,  $J = 17.1$  Hz, 1H), 6.67 (ddd,  $J = 28.4, 8.6, 2.7$  Hz, 1H), 6.37 (d,  $J = 2.7$  Hz, 1H), 4.45 (s, 2H), 3.87 (t,  $J = 6.3$  Hz, 2H), 3.27 (t,  $J = 6.9$  Hz, 2H), 2.66 (t,  $J = 7.6$  Hz, 2H), 2.41 (m, 6H), 1.99 (dd,  $J = 14.9, 7.4$  Hz, 2H), 1.92–1.82 (m, 2H), 1.60–1.48 (m, 4H), 1.45–1.31 (m, 2H). <sup>13</sup>C NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ : 161.49 (N=C), 155.96 (arom.), 134.84 (arom.), 124.08 (arom.), 119.74 (arom.), 114.16 (arom.), 111.95 (arom.), 66.68, 55.85, 54.46, 51.55, 47.01, 30.88, 26.59, 25.69, 24.25, 18.98. Elem. Anal. ( $\text{C}_{19}\text{H}_{27}\text{N}_3\text{O} + 1.0 \text{ CH}_3\text{COOH} + 0.1 \text{ CH}_2\text{Cl}_2$ ) Calcd.: C, 66.34; H, 8.23; N, 11.00; O, 12.57. Found: C, 66.34; H, 8.01; N, 11.25.

**7-((6-(Piperidin-1-yl)hexyl)oxy)-1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline (42).** Zn dust (400 mg) was added to compound **34** (100 mg, 0.27 mmol) dissolved in 10 mL acetic acid, and to the reaction mixture 2 mL of HCl (10 M) was then added. The reaction mixture was heated up to 70 °C for 2 h. The reaction mixture was allowed to cool to room temperature, the solvent was concentrated under vacuum, and the reaction mixture basified by NaOH (4 N) and extracted by ethylacetate ( $3 \times 100$  mL). The combined organic extracts were washed with brine and dried over magnesium sulfate. The organic solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography using 5:1:0.1  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{ammonia}$  solution mixture as eluent system to afford the title compound (43 mg, 45%) as a yellow semisolid. HRESIMS ( $\text{C}_{22}\text{H}_{33}\text{N}_3\text{O} + \text{H}$ )<sup>+</sup>,  $m/z$  calcd: 356.2702; found: 356.2701. <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 6.94 (d,  $J = 8.6$  Hz, 1H), 6.64 (dd,  $J = 8.6, 2.8$  Hz, 1H), 6.39 (d,  $J = 2.7$  Hz, 1H), 4.48 (s, 2H), 3.92–3.78 (m, 2H), 3.27 (t,  $J = 6.9$  Hz, 2H), 2.73–2.51 (m, 2H), 2.38 (s, 4H), 2.34–2.20 (m, 2H), 2.11–1.90 (m, 2H), 1.82–1.62 (m, 2H), 1.54 (m, 6H), 1.45–1.34 (m, 4H), 1.33–1.25 (m, 2H). <sup>13</sup>C NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ : 161.11 (N=C), 155.96 (arom.), 135.80 (arom.), 124.50 (arom.), 119.97 (arom.), 114.04 (arom.), 112.07 (arom.), 68.14, 59.31, 54.48, 51.42, 47.18, 30.98, 29.24, 27.44, 26.52, 25.99, 25.63, 24.26, 19.09. Elem. Anal. ( $\text{C}_{22}\text{H}_{33}\text{N}_3\text{O} + 1.0 \text{ CH}_3\text{COOH}$ ) Calcd.: C, 69.36; H, 8.97; N, 10.11; O, 11.55. Found: C, 69.53; H, 9.05; N, 10.18.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Additional docking data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Author Contributions

M. Decker was responsible for the supervision and development of the whole project. F. H. Darras performed the chemical syntheses of the majority of the synthesized compounds, performed the kinetic analysis and the *EeChE* and *eqBChE* inhibition studies; G. Huang synthesized compounds **32** and **36** and performed the *hAChE* inhibition study; S. Pockes and M. Nimczick performed the *hH<sub>3</sub>* binding affinity test. S. Pockes performed the selectivity study over *hH<sub>1</sub>R*, *hH<sub>2</sub>R*, and *hH<sub>4</sub>*, as well as the functional GTPase assay; S. Wehle and C. Sotriffer conducted the computational studies on *AChE*. A. Strasser and H. Wittmann performed the molecular simulation analysis at *hH<sub>3</sub>R*.

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### Notes

The authors declare no competing financial interest.

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## DEDICATION

This article is dedicated to Professor John L. Neumeyer on the occasion of his 84th birthday.

## ABBREVIATIONS

ACh, acetylcholine; AChE, acetylcholinesterase; AChEIs, acetylcholinesterase inhibitors; AD, Alzheimer's disease; ADHD, attention deficit hyperactivity disorder; ATC, acetylthiocholine; BTC, butyrylthiocholine; CAS, catalytic active site; ChEs, cholinesterases; ChEIs, cholinesterase inhibitors; CNS, central nervous system; DPH, diphenhydramine; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); *EeAChE*, *Electrophorus electricus* acetylcholinesterase; *eqBuChE*, horse serum butyrylcholinesterase; HIS, histamine; *hAChE*, human recombinant acetylcholinesterase; *hH<sub>1</sub>R*, human histamine receptor type 1; *hH<sub>2</sub>R*, human histamine receptor type 2; *hH<sub>3</sub>R*, human histamine receptor type 3; *hH<sub>4</sub>R*, human histamine receptor type 4; GABA, gamma-aminobutyric acid; GPCR, G protein-coupled receptor; MTDL, multitarget-directed ligands; MD, molecular dynamics; NMDA, *N*-methyl-D-aspartate; PAS, peripheral anionic site; RGS4, regulator of G protein signaling 4; SARs, structure–activity relationships

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