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Cyclic acyl guanidines bearing carbamate moieties allow potent and dirigible cholinesterase inhibition of either acetyl- or butyrylcholinesterase

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ABSTRACT

A series of cyclic acyl guanidine with carbamate moieties have been synthesized and evaluated in vitro for their AChE and BChE inhibitory activities. Structure–activity relationships identified compound **23** as a nanomolar and selective BChE inhibitor, while compound **32** exhibited nanomolar and selective AChE inhibition, selectivity depending on both the structure of the carbamate substituent as well as the position of guanidines-*N* substitution. The velocity of enzyme carbamoylation was analyzed and showed similar behavior to physostigmine. Phenolic compounds formed after carbamate transfer to the active site of cholinesterases showed additional neuroprotective properties on a hippocampal neuronal cell line (HT-22) after glutamate-induced intracellular reactive oxygen species generation.

1. Introduction

Alzheimer's disease (AD) is the most common dementia occurring among elderly people. It is a progressive and neurodegenerative disorder affecting regions of the brain that control cognition, memory, language, speech and awareness.[1,2] The major pathological hallmarks of AD are formation of beta-amyloid aggregates that form senile plaques, neurofibrillary tangles of hyperphosphorylated τ-protein and progressive loss of cholinergic neural transmission.[3,4]

Acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine (ACh); the second cholinesterase in the brain of mammals is butyrylcholinesterase (BChE, or pseudo-cholinesterase), which is less substrate specific than AChE. Its expression level is more abundant in the peripheral system. However, both cholinesterases are found in neurons and glial cells as well as in neuritic plaques and tangles in AD patients.[5]

Both AChE and BChE are involved in the breakdown of acetylcholine in the brain. It is demonstrated that AChE has a key role in the acceleration of Aβ-peptide deposition and promoting the formation of Aβ-plaques in Alzheimer's brain.[6] Recent studies suggest that BChE is present in key brain areas and may also influence the aggregation of neuritic β-amyloid (Aβ) plaques.[7,8] For clinical purposes, it is particularly important to consider the fact that while brain AChE activity continuously declines, BChE activity might stay the same or even increase during disease progression.[9–11]

To date, only four drugs have been approved and licensed to treat AD. The NMDA antagonist memantine, and three AChE inhibitors (rivastigmine, donepezil and galanthamine). Although these drugs share the same therapeutic target, they differ in their molecular mode of action and pharmacokinetics and possess different degrees of side effects. While galanthamine and donepezil are reversible ChE inhibitors, rivastigmine (**1**, Fig. 1)—a compound derived from the alkaloid physostigmine (**2**, Fig. 1)—represents an irreversible inhibitor transferring a carbamate moiety to the serine unit in the catalytic active site (CAS) of AChE and BChE. The carbamates' mode of action is termed more exactly pseudoirreversible since the carbamate moiety slowly hydrolyses off the enzyme, yielding the active enzyme again, in contrast to irreversibly inhibiting organophosphates. Rivastigmine is of special interest because in clinical and pharmacological investigations it was shown to be of high effectiveness, which some authors attributed to its ability to inhibit BChE also with high efficiency (Fig. 1).[12,13]

The guanidine group possesses chemical and physicochemical properties relevant for many compounds of medicinal interest and guanidine-containing derivatives constitute an important class of therapeutic agents suitable for the treatment of a wide spectrum of diseases.[14] Recently, acyl guanidine derivatives were applied to

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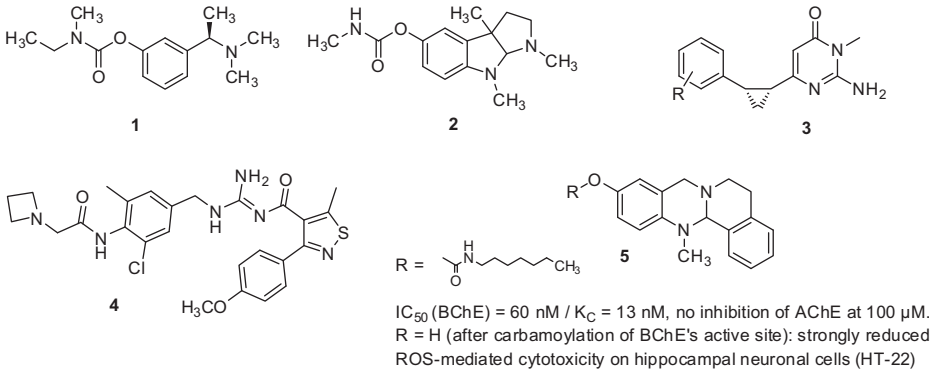


Figure 1. Structures of AD drug rivastigmine **1**, the ChE inhibiting alkaloid physostigmine **2**, acyl guanidine inhibitors of β-secretase (BACE-1) **3** and **4**, and the tetracyclic pseudoirreversible BChE inhibitor lead structure **5**.[15–17]

target AD as β-secretase (BACE-1) inhibitors (compounds **3** and **4**, Fig. 1).[15,16]

We had previously synthesized a series of novel tri- and tetracyclic *N*-bridgehead ChE inhibiting structures based on a quinazolinone moiety.[18] In 2012, we modified one lead structure by introducing a phenolic hydroxyl group in the *para* position to the tertiary anilinic amine, and in the *meta* position to the basic *N*-bridgehead atom (compound **5**, Fig. 1) and used it as a starting point for the development of novel BChE selective inhibitors. The cholinesterase inhibitory activities and their selectivity towards BChE were dramatically improved by introducing a carbamate group at the phenolic hydroxy group (compound **5**, Fig. 1). The enzyme carbamoylation process of this class of inhibitors were studied kinetically, and the hydrolyzed inhibitor released after carbamate transfer to the serine residue at the catalytic active site of the ChEs is a phenolic compound with pronounced neuroprotective properties on a hippocampal neuronal cell line (HT-22), in which reactive oxygen species (ROS) are formed intracellularly after extracellular glutamate challenge (see the neuroprotectivity chapter for more details). The phenolic compounds showed also antioxidant capacities ranging from 1.3 to 1.6 trolox equivalents, meaning that they are more potent than the positive control trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) a water soluble vitamin E derivative.[17] Recently, we identified another neuroprotective and BChE-selective inhibitor derived from the natural quinazolinone alkaloid evodiamine by connecting the carbonyl reduced analog of evodiamine with heptyl carbamate. The heptyl carbamate heterocycle exhibited an IC_{50} at BChE of 77 nM with no inhibition of AChE at 10 μM. The heptyl carbamate and its corresponding phenolic structure showed again pronounced antioxidant capacity and noticeable neuroprotective properties on HT-22 cells.[19]

These properties might become important for future AD therapeutics since it has been proven experimentally that dysregulation of the redox state strongly participates in an early stage of AD by stimulating and activating multiple cell signaling pathways that contribute to the initial progression of the neurodegenerative process.[20] Additionally, it was observed that ROS and reactive nitrogen species (RNS) are mediators of injury in AD patients' brains.[21] Numerous studies showed that levels of oxidative markers of proteins, lipids, carbohydrates, and nucleic acids are increased in AD.[22–24] Furthermore, levels of antioxidant enzymes were found to be altered in brain regions of AD patients.[25] All of the aforementioned findings support the 'oxidative stress hypothesis' of AD at least as one component of neuronal cell damage in AD.[26,27]

Low basicity of the quinazolinone moiety besides its very poor water solubility and low to moderate inhibitory activities made structural modifications of the heterocycle necessary for further development. Modifications of quinazolinones e. g. to mono- and bivalent quinazolinimines and *N*-bridgehead tri- and tetracyclic moieties proved to yield more potent ChE inhibitors.[18,28,29] The quinazolinones' modifications were based on an increase of the basicity of the heterocycle by (a) reduction of the amide carbonyl group to the corresponding quinazoline (Fig. 2),[18] (b) imine formation by Schiff-base reaction of thioquinazolinones with different aliphatic and aromatic amines in presence of heavy metal salts.[29,30]

Quinazolinone modification presented in this work was based on increasing the basicity of the core ring by introducing a nitrogen atom in the fused aliphatic cycle to the core ring of the lead quinazolinone to yield tricyclic acyl guanidine structures. Besides, introduction of suitable carbamate groups to the same phenolic hydroxyl group had been described before to improve inhibitory activities at BChE and to increase in BChE selectivity (Fig. 2).[17]

Here, several sets of cyclic acyl guanidine compounds connected with different carbamates groups were synthesized. SARs were investigated with regards to the guanidine group by introducing different substituents at its two unsubstituted nitrogen atoms. Inhibitory activities of the synthesized pseudoirreversible inhibitors were evaluated at both cholinesterases and the carbamoylation process studied kinetically for the most potent inhibitors.

1.1. Chemistry

6-(Benzyloxy)-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (**6**) was synthesized in four steps with almost quantitative overall yield, starting from 2-amino-5-hydroxybenzoic acid.[31,32] This benzylated isatoic anhydride was used as a key intermediate for synthesis of all target compounds, the benzylated phenolic group helped to avoid the solubility problem of the isatoic heterocycle. 2-(Methylthio)-1,4,5,6-tetrahydropyrimidine was prepared according to a literature procedure starting from 1,4,5,6-tetrahydropyrimidine-2-thiol in quantitative yield (Scheme 1).[33]

The unsubstituted (*N*H)-cyclic acyl guanidine **7** (8-(benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one) was employed as a synthetic precursor for different substituted cyclic guanidine moieties and it was obtained by fusion reaction of the benzylated isatoic anhydride **6** with the with 2-(methylthio)-1,4,5,6-tetrahydropyrimidine in *N*,*N*-dimethylformamide (DMF) at 100 °C (Scheme 1A).

Alkylation of the *N*H group of the resulting cyclic acyl guanidine with iodomethane, benzylbromide, or 1-(3-bromopropyl)-piperidine, respectively, using sodium hydride in tetrahydrofurane (THF) gave the corresponding alkylated cyclic acyl guanidine compounds **8**, **9**, and **10** (Scheme 1A). Quantitative debenzylation by catalytic hydrogenation using Pd/C in ethanol at room temperature gave the corresponding phenolic cyclic acyl guanidine compounds

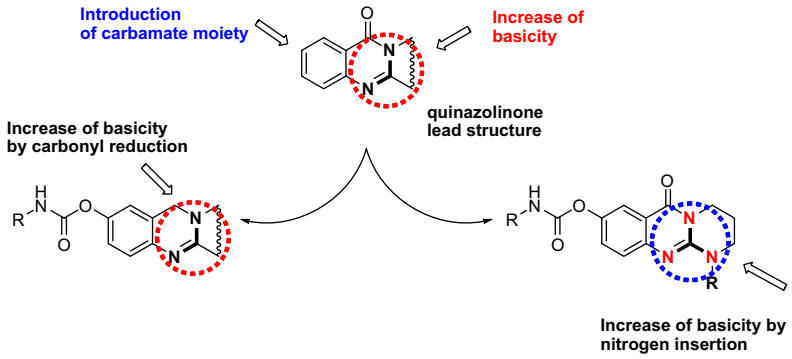


Figure 2. Design strategies and SARs at the quinazolinone core ring to improve ChE inhibition.

11, **12**, and **13**, respectively, (Scheme 1A). The carbamoylated target compounds (compounds **14**–**25**) were obtained by reaction of different isocyanates with the corresponding phenolic compounds **11**–**13** in dichloromethane with triethylamine as base (Scheme 1A).

N-Methylation of the benzylated isatoic anhydride gave another isomeric set of methylated (at position 11) cyclic acyl guanidines. The methylated isatoic anhydride compound **26** was obtained by reaction of the isatoic anhydride with iodomethane in *N*,*N*-dimethyl acetamide (DMAc) as solvent using diisopropyl ethylamine (DIPEA) as base.[17] Fusion reaction of the methylated isatoic anhydride with the methylthio compound in DMF at 100 °C gave 11-methylated cyclic acyl guanidine **27** (8-(benzyloxy)-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one) (Scheme 1B). Quantitative debenzylation by catalytic hydrogenation using Pd/C gave compound **28**, and finally carbamate synthesis from isocyanates—as described for the first set of target compounds—yielded the target compounds **29**–**33** (Scheme 1B).

The ^{1}H NMR spectra of both constitutional isomers **8** and **27** showed slight differences in the chemical shift of the singlet N-CH_3 peak. The singlet of N-CH_3 in compound **27** (8-(benzyloxy)-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one) is at $\delta = 3.46$ ppm, while in compound **8** (8-(benzyloxy)-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one) the singlet of N-CH_3 is upfield shifted to $\delta = 3.24$ ppm.

Reduction of the carbonyl group of the core ring of the acyl guanidine heterocycle using $NaBH_4$ and $AlCl_3$ gave a set of cyclic guanidine compounds; debenzylation yielded the corresponding phenolic compound **35** and subsequent carbamate formation gave target compounds **36** and **37** (Scheme 2).

In order to evaluate the influence of the guanidine substitution in general on inhibitory activities at both ChEs, compound **39** was synthesized from its corresponding phenolic compound **38** in which the guanidine group is unsubstituted (*N*H), (Scheme 3).

1.2. Pharmacology

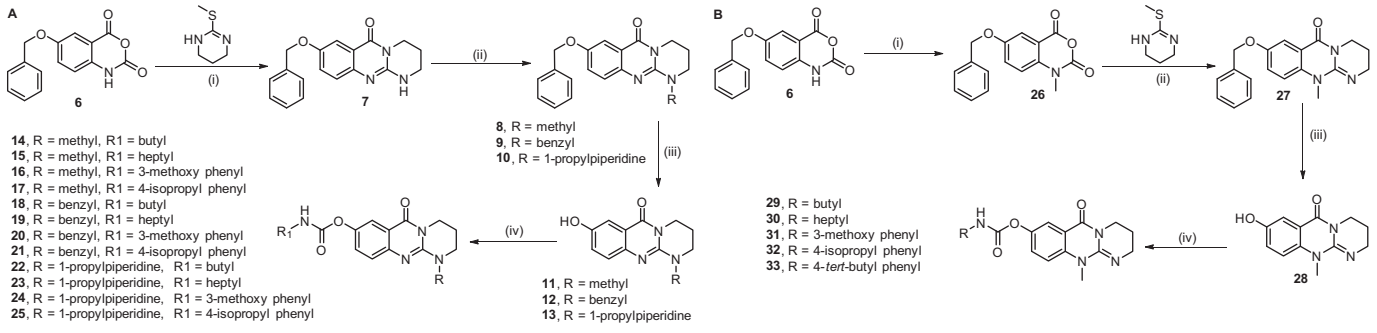
1.2.1. AChE and BChE inhibition and kinetic study

All target compounds were tested for their ability to inhibit acetylcholinesterase (*ee*AChE, EC.3.1.1.7 from electric eel) and butyrylcholinesterase (*eq*BChE, EC.3.1.1.8 from equine serum) (Table 1). A sequence alignment showed that both enzyme isoforms exhibit very high amino acid sequence homology with regard to the human ones (88% and 84% sequence identity, respectively). The electric eel and equine enzymes are heavily used for screening compound libraries due to cost reduction and the stability of the enzymes also.[17,32,34]

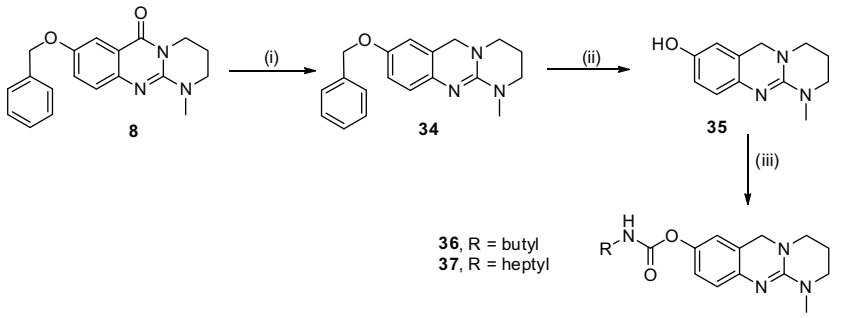
In addition to determination of inhibitory activities, representative compounds were selected for kinetic studies in order to get more detailed information about the time-course of inhibition and therefore more specific data about the equilibrium constant of inhibition. The equilibrium constant of the inhibitor–ChE complexes (K_C) and the carbamoylation rate constants (k_3) of two representative target compounds **23** (as BChE selective inhibitor, **32** as AChE selective inhibitor), and physostigmine as positive control, respectively, were determined (Table 2 and Fig. 3).

1.2.2. Antioxidant potency and neuroprotection

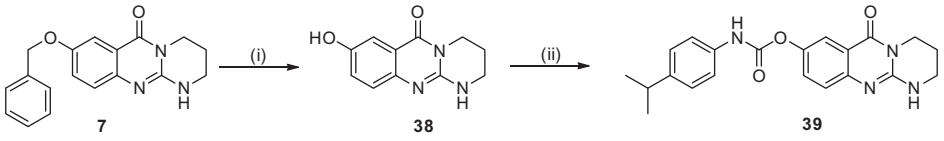
According to the assumption of pseudoirreversible inhibition, the phenolic compounds' antioxidant and neuroprotective properties were determined in two different assays. First, the oxygen radical absorbance capacity (ORAC) assay measures the ability of compounds to protect fluorescein from destruction by peroxyl radicals and, therefore, directly assesses their antioxidant physicochemical properties.[17] The second assay is cell-based and uses murine HT-22 hippocampal cells that lack ionotropic glutamate receptor to study intracellular oxidative stress-induced neurotoxicity.[35–38] In this neuronal oxidative stress-induced toxicity (oxytosis), cell death is induced by extracellular glutamate challenge in a



Scheme 1. (A) Synthesis of 1-alkylated cyclic acyl guanidine carbamates, reagents and conditions: (i) DMF, 100 °C, 6 h (ii) NaH, THF, methyliodide, or benzylbromide, or 1-(3-bromopropyl)piperidine, rt, 24 h (iii) H_2, Pd/C, ethanol, rt, 24 h (iv) isocyanates, CH_2Cl_2, Et_3N, rt, 2 h. (B) Synthesis of 11-alkylated cyclic acyl guanidine carbamates, reagents and conditions: (i) DIPEA, DMAc, 70 °C, 8 h (ii) 1,4-dioxane, 120 °C, 4 h (iii) H_2, Pd/C, ethanol, rt, 24 h (iv) isocyanates, CH_2Cl_2, Et_3N, rt, 2 h.



Scheme 2. Synthesis of *N*-bridgehead cyclic guanidine carbamates, reagents and conditions: (i) $AlCl_3$, $NaBH_4$, diglyme, 0–90 °C, 3 h (ii) H_2, Pd/C, ethanol, rt, 4 h (iv) isocyanates, CH_2Cl_2, Et_3N, rt, 2 h.



Scheme 3. Synthesis of 1-*N*H cyclic guanidine carbamate **39**, reagents and conditions: (i) H_2, Pd/C, ethanol, rt, 4 h (ii) 4-isopropyl phenyl isocyanate, CH_2Cl_2, Et_3N, rt, 2 h.

nonreceptor-mediated 'oxidative' pathway. Glutamate toxicity is mediated by the cystine/glutamate antiporter system, which—after exposure to high extracellular glutamate concentrations—results in inhibition of cystine uptake, leading to intracellular cysteine and therefore glutathione depletion, which induce ROS accumulation and cell injuries.[36–38] Administration of antioxidants such as flavonoids can effectively prevent oxidative neuronal death in this cell line.[39,40]

Not only the phenolic compounds formed after the carbamoylation process were assayed. Neuroprotection profiles and antioxidant capacities of two carbamates (compound **23** and **32**) were also evaluated. Furthermore, the unsubstituted cyclic acyl guanidine (compound **40**) was synthesized (Table 1, for synthesis cf. experimental part) to evaluate the influence of the presence and absence of the phenolic group of this class of compounds on antioxidant capacity as well as the neuroprotection profile of this set of compounds.

2. Results and discussion

SARs of carbamates have been investigated before[41,42] and could be to some degree confirmed on quinazoline compounds.[17,41,42] Heptyl carbamates lead to BChE selectivity and 4-isopropylphenyl carbamates can increase selectivity in the opposite direction.[17,41,42]

Interestingly, the two isomeric sets of carbamates (**14**–**17** and **29**–**33**) (Scheme 1A and B) show different biological profiles. *N*-1 Methyl substituted compounds (**14**–**17**) show moderate inhibitory activities at both enzymes ranging from one to two digit micromolar activities without significant selectivity. The IC_{50} values at AChE range from 5.5 μM (compound **16**) to 100 μM (compound **17**) while the IC_{50} values at BChE range from 1.8 μM for compound **15** to 44.3 μM for compound **14** (Table 1). The isomeric set of *N*-11 methyl substituted compounds (**29**–**33**) show different activities at both ChEs besides different selectivity profiles: cyclic acyl guanidine with heptylcarbamate (**30**) showed high BChE inhibitory activity (IC_{50} = 140.1 nM) and 26fold selectivity over AChE ([(IC_{50}(AChE)/IC_{50}(BChE)]) (Table 1). Interestingly, the 4-isopropyl phenyl carbamate compound **32** showed a noticeable activity and selectivity at AChE with IC_{50} (AChE) = 83.7 nM and 39 fold selectivity over BChE (Table 1). For further investigation an analogous compound of **32** with 4-*tert*-butyl phenyl carbamate (**33**) was synthesized to prove the assumption that the more bulky *para* substituents will maintain the AChE inhibitory activity and the selectivity profile of compound **32**. Compound **33** showed a similar behavior to compound **32** with inhibitory activity at AChE with IC_{50} (AChE) = 200 nM and 500fold selectivity over BChE ([(IC_{50}(BChE)/IC_{50}(AChE)]) (Table 1).

N-1 benzylated compounds **18**–**21** exhibited moderate to very weak inhibitory activity at both ChEs (Table 1).

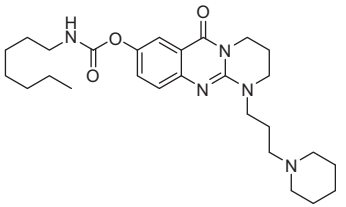
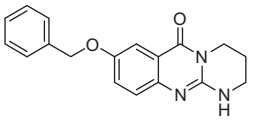
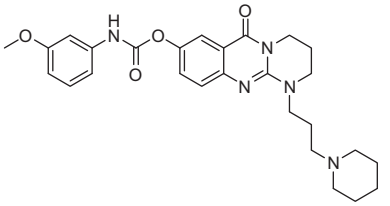
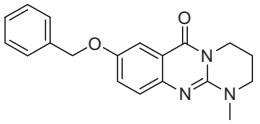
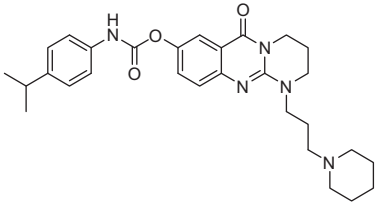
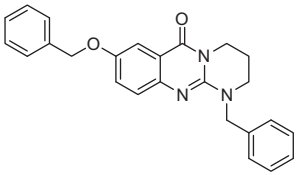
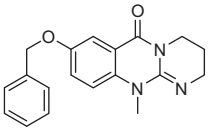
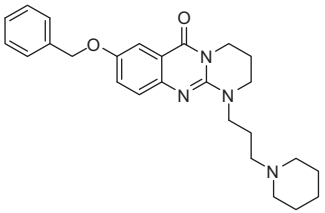
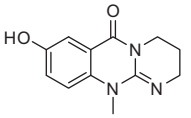
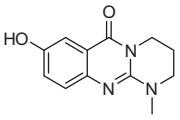
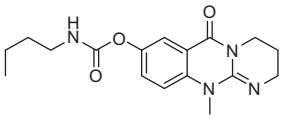
Remarkably, substituting the *N*H at position 1 by a flexible basic center, such as 1-propylpiperidine (compounds **22**–**25**) increased inhibitory activity at both enzymes to the submicromolar range (Table 1). The heptylcarbamate **23** showed a pronounced inhibitory activity at BChE with IC_{50} (BChE) = 24.7 nM and 325-fold selectivity over AChE ([(IC_{50}(AChE)/IC_{50}(BChE)]) (Table 1). This increase in both activity at BChE and selectivity over AChE, is common for the heptyl carbamate and has been previously described for several related heterocycles.[17,41,42]

To increase the basicity of the heterocyclic template, reduced analogs of compounds **14**, **15** (butyl and heptyl carbamates of *N*-1 methylated tricycle) were synthesized via carbonyl reduction to yield compounds **36** and **37** (Scheme 2 and Table 1). *N*-Unsubstituted 4-isopropylphenyl carbamate **39** loses the potency at both enzymes with only 34% inhibitory activity of AChE at 50 μM and 19% inhibitory activity of BChE at 50 μM. Therefore, substitution is necessary for inhibitory activity (Scheme 2 and Table 1).

Kinetic studies gave the equilibrium constants of the inhibitor–ChE complexes (K_C) and the carbamoylation rate constants (k_3) of two representative target compounds **23** and **32** (Fig. 3 and Table 2). The K_C and k_3 values of physostigmine (positive control) had been determined in a previous study.[17] For compound **23**, the actual K_C value at BChE is even smaller than the IC_{50} value with K_C = 1.3 nM (IC_{50} = 24.7 nM). The k_3 carbamoylation rate constant of compound **23** is 0.125 min^{-1} which is considered rapid carbamoylation, albeit slower than for physostigmine (k_3 = 0.067 min^{-1}) (Table 2).

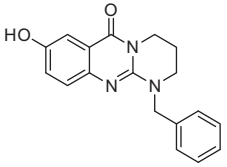
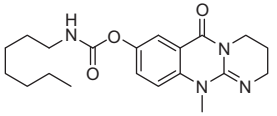
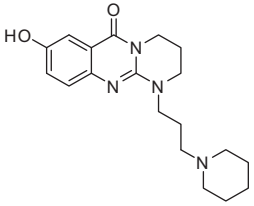
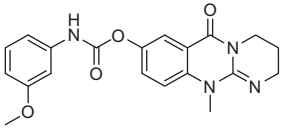
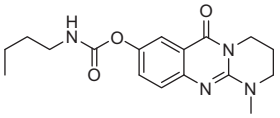
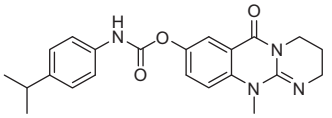
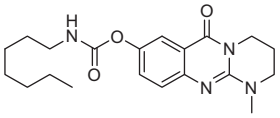
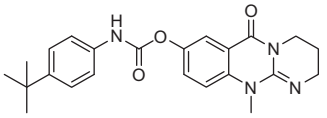
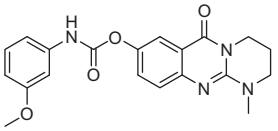
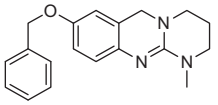
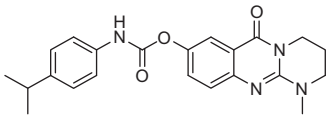
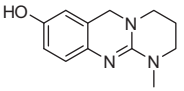
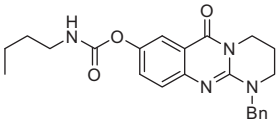
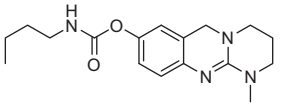
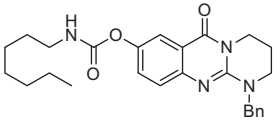
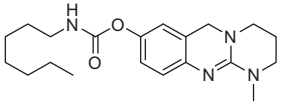
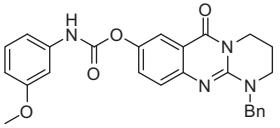
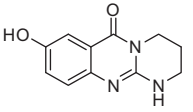
Kinetic studies were also exemplary investigated for compound **32** on *h*AChE (cf. supporting information, Fig. S1). Interestingly, although human and electric eel AChE high structural homology the results are significantly different.[43] When *h*AChE was preincubated in a short time frame of only 2 min with compound **32**, fast inhibition is observed. Extending the preincubation time to 20 min led to a 3–4 times lower inhibition (in%). Therefore, two

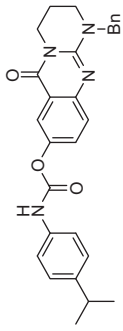
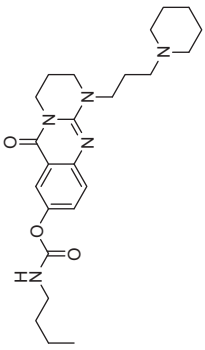
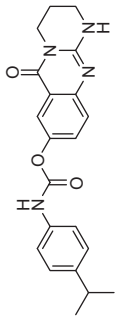
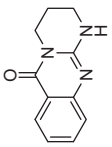
Table 1
AChE and BChE inhibition results and resulting ChE selectivity

Compd	Structure	IC_{50} (pIC_{50} ± SEM)[a]		BChE selectivity[b]	Compd	Structure	IC_{50} (pIC_{50} ± SEM)[a]		BChE selectivity.[b]
		AChE	BChE				AChE	BChE	
	Physostigmine	0.09 μM	0.04 μM	2.14	**23**		**7.95 μM (5.10 ± 0.06)**	**24.7 nM (7.61 ± 0.03)**	322
7		19.31 μM (4.72 ± 0.23)	20% inhibition at 50 μM	0.19	**24**		7.30 μM (5.14 ± 0.08)	0.45 μM (4.59 ± 0.05)	16.2
8		15.61 μM (4.81 ± 0.16)	81.72 μM (4.09 ± 0.04)	0.21	**25**		0.78 μM (6.11 ± 0.01)	0.25 μM (6.61 ± 0.03)	3.1
9		20% inhibition at 100 μM	10% inhibition at 100 μM		**27**		0.70 μM (6.16 ± 0.01)	21.40 μM (4.67 ± 0.0)	0.03
10		1.31 μM (5.87 ± 0.04)	0.81 μM (6.09 ± 0.01)	1.60	**28**		11.75 μM (4.93 ± 0.01)	55.53 μM (4.26 ± 0.01)	0.21
11		61% inhibition at 50 μM	9% inhibition at 50 μM		**29**		3.84 μM (5.42 ± 0.03)	43.91 μM (4.36 ± 0.02)	0.09

(*continued on next page*)

Table 1 (*continued*)

Compd	Structure	IC_{50} (pIC_{50} ± SEM)[a]		BChE selectivity[b]	Compd	Structure	IC_{50} (pIC_{50} ± SEM)[a]		BChE selectivity.[b]
		AChE	BChE				AChE	BChE	
12		41% inhibition at 50 μM	40% inhibition at 50 μM		**30**		3.64 μM (5.44 ± 0.01)	140 nM (6.85 ± 0.08)	26.1
13		22.92 μM (4.64 ± 0.04)	22.72 μM (4.64 ± 0.06)	1.0	**31**		2.90 μM (5.54 ± 0.04)	52.22 μM (4.28 ± 0.04)	0.06
14		16.31 μM (4.788 ± 0.137)	44.32 μM (4.353 ± 0.034)	0.37	**32**		**83.7 nM (7.08 ± 0.05)**	**3.26 μM (5.49 ± 0.16)**	**0.03**
15		5.98 μM (5.224 ± 0.014)	1.75 μM (5.757 ± 0.137)	3.41	**33**		**200 nM (6.700 ± 0.053)**	**22% inhibition at 50 μM**	**<0.004**
16		5.46 μM (5.263 ± 0.013)	12.64 μM (4.898 ± 0.052)	0.33	**34**		0.8255 μM (6.08 ± 0.01)	4.172 μM (5.38 ± 0.03)	0.20
17		50% inhibition at 100 μM	17.70 μM (4.75 ± 0.13)	>5.6	**35**		15.92 μM (4.80 ± 0.04)	53% inhibition at 50 μM	<0.3
18		41% inhibition at 50 μM	8% inhibition at 50 μM		**36**		3.13 μM (5.50 ± 0.07)	1.34 μM (5.41 ± 0.05)	2.31
19		88.82 μM (4.05 ± 0.12)	25.31 μM (4.597 ± 0.37)	3.51	**37**		3.36 μM (5.47 ± 0.06)	0.63 μM (6.20 ± 0.003)	5.31
20		35% inhibition at 50 μM	11% inhibition at 50 μM		**38**		55.62 μM (4.26 ± 0.13)	24% inhibition at 100 μM	0.28

21		30% inhibition at 50 μM	6% inhibition at 50 μM	6.91 μM (5.16 ± 0.04)	1.10 μM (5.97 ± 0.01)	6.35
22		34% inhibition at 50 μM	19% inhibition at 50 μM	29.62 μM (4.53 ± 0.07)	47% inhibition at 100 μM	0.30
39		34% inhibition at 50 μM	19% inhibition at 50 μM	29.62 μM (4.53 ± 0.07)	47% inhibition at 100 μM	0.50
40						

[a] $pIC_{50} = -\log IC_{50}$, AChE from electric eel, BChE from equine serum, data are the means of at least three independent determinations.
[b] Selectivity ratio $[(IC_{50}(AChE)/IC_{50}(BChE)]$.

Table 2
The stability constants of the inhibitor-ChE complex (K_C) and the rate constants of the carbamoyl-ChE formation (k_3) of **23**, **32**, and physostigmine, respectively[17]

Compd	k_3 (min^{-1})		K_C (nM)	
	AChE	BChE	AChE	BChE
23	nd	0.125 ± 0.05	nd	1.3 ± 0.1
32	0.090 ± 0.04	nd	43.5 ± 1.2	nd
Phys.	0.123 ± 0.03	0.067 ± 0.01	34.3 ± 4.2	41.1 ± 5.7

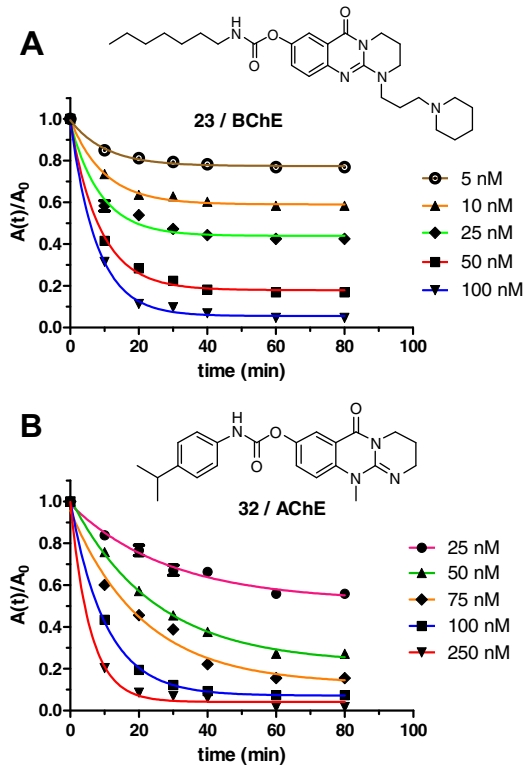


Figure 3. (A) Time-dependent pattern of *eq*BChE inhibition by compound **23** (5 nM–100 nM). (B) Time-dependent pattern of *ee*AChE inhibition by compound **32** (25 nM–250 nM).

conclusions can be drawn: first, a high k_3 value of compound **32** on *h*AChE is the reason for a fast carbamoylation thereby resulting in a high inhibition of the enzyme during a short incubation time. Second, it can be assumed that decarbamoylation of the transferred group is rapidly taking place as a longer incubation time up to 20 min showed a significant reconstitution of enzymatic activity. These differences in the kinetic data were already described for several carbamates.[43] Nevertheless, such short duration of action is not taking place for BChE, the main target for our compounds (Fig. 3 and Table 2).[19,28–30] In contrast, after 80 min no significant decarbamoylation could be observed.

Compound **32** shows a K_C value at AChE similar to the IC_{50} value (K_C = 43.5 nM and IC_{50} = 83.7 nM). The velocity of carbamoylation was fairly rapid with k_3 = 0.09 min^{-1} compared to k_3 = 0.123 min^{-1} for physostigmine (Table 2).

The heptyl carbamate **23** represents a highly potent and selective pseudo-irreversible BChE inhibitor, with K_C = 1.3 nM and 322fold selectivity over *ee*AChE. The 4-isopropyl carbamate **32** is a highly potent and selective pseudo-irreversible AChE inhibitor, with K_C = 43.5 nM and 39fold selectivity over BChE.

Both inhibitors showed a time-dependent pattern of inhibition characterized by an increase until a steady state after 60 to 80 min (Fig. 3).

Inhibitory activities of all phenolic compounds (released after carbamoylation of the enzymes' active center) were also evaluated and showed that they still act as ChEIs with moderate to weak inhibitory activities (23 μM at both ChEs for compound **13** and 12 μM (AChE) and 56 μM (BChE) for compound **28**) (Table 1). To obtain physiologically relevant data of the antioxidant compounds with regard to neuroprotection, the neuronal cell line HT-22 was chosen as a model system.[35,39,40,44]

All phenolic compounds released after carbamoylation (**11**, **12**, **13**, **28**, **35**, and **38**), two carbamates (**23** and **32**) and one un-substituted compound (**40**) were selected and evaluated at different concentrations ranging from 1 to 25 μM for their neuroprotective potential towards neuronal HT-22 cells and their putative neurotoxic effects towards the HT-22 neurons to elucidate possible self-toxic influences of tested compounds toward the cells. Of the six phenolic compounds tested, there are three *N*-1 substituted cyclic acyl guanidine moieties (compounds **11**, **12** and **13**) and the *N*-1 unsubstituted compound **38**. Among those, only compound **12** and **38** exhibit slight neurotoxic properties at the highest test concentration of 25 μM while compounds **11** and **12** do not reduce the viability of HT-22 cells (Fig. 4A). Considering the neuroprotective potential, compound **12** (with *N*-1 benzyl substituent) is the most potent neuroprotective compound with significant protection from 5 μM onward, which nearly reached to the extent of the positive control quercetin. Compound **11** (with methyl substituent) behaves similarly and reduces the glutamate induced toxicity from 10 μM onward (Fig. 4B).

A 1-propylpiperidine *N*-1 substituent led to a decrease in the neuroprotective effects as demonstrated by compound **13** showing lower neuroprotection compared to compounds **11** and **12** starting from 25 μM. The *N*-1 unsubstituted compound **38** has no significant neuroprotective potential leading to the assumption that *N*-1 substitution is necessary for neuroprotective properties of these compounds. Neuroprotective effects of compound **28**—a constitutional isomer of compound **11**—are completely lost in comparison to compound **11** indicating that substitution at the *N*-1 position is required for relevant neuroprotectivity towards HT-22 cells (Fig. 4B). The reduction of the amide carbonyl group of compound **11** to compound **35** led to a decrease in the neuroprotective effect as it can only be observed at 25 μM (Fig. 4B). Although phenolic compounds **28** and **35** did not show any noticeable neuroprotection properties compared to compound **11**, they also did not show any neurotoxic effects (Fig. 4A).

Two carbamates, namely the heptyl carbamate **23** and the 4-isopropylphenyl derivative **32**, were included in the testing as it was shown before that carbamates can act directly as neuroprotectants in their own regard even though a neuroprotective effect had not been expected.[17] Neither **23** nor **32** exhibited neurotoxic or neuroprotective effects (Fig. 4A and B). The same observation can be made for compound **40**, an exemplary structure which lacks

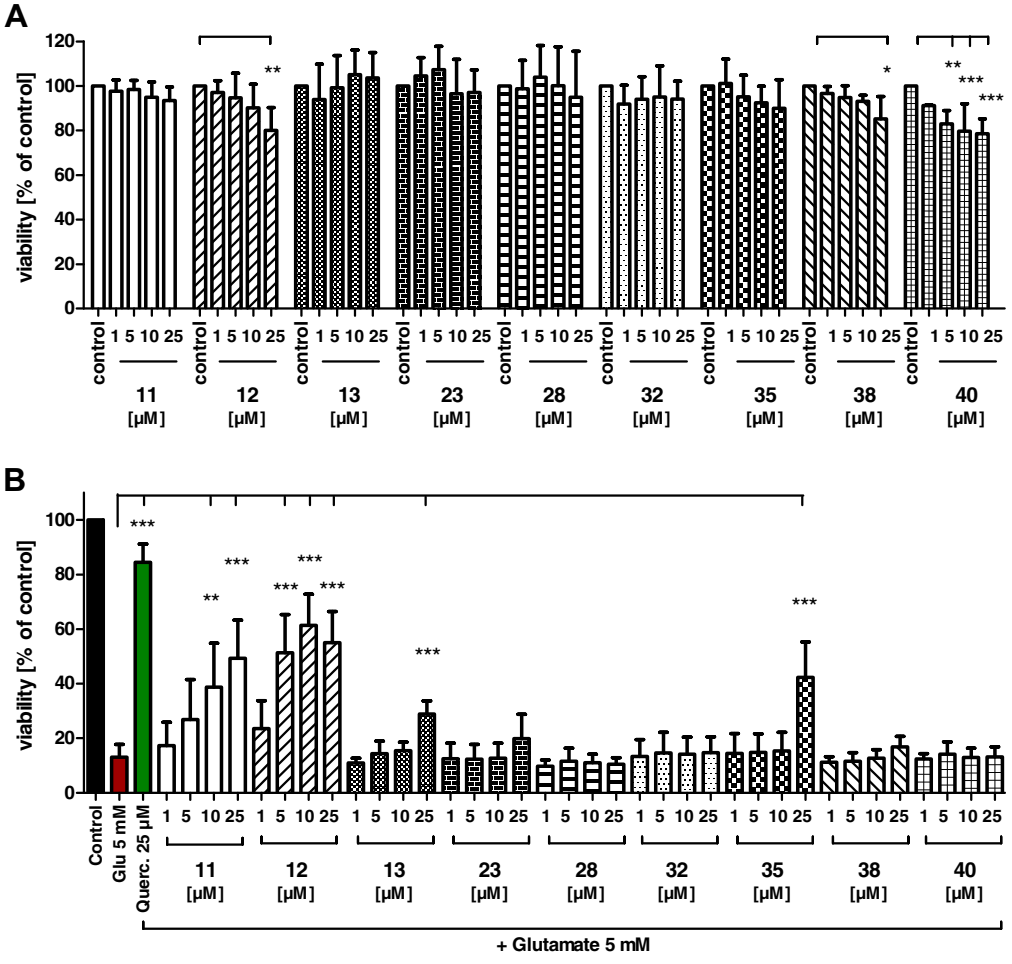
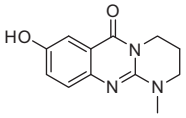
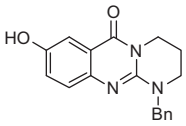
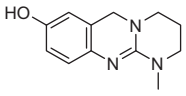


Figure 4. (A) Compounds **11**, **12, 13, 23, 28, 32, 35, 38** and **40** tested on neuronal HT-22 cells for neurotoxic effects and (B) neuroprotection against glutamate induced oxidative stress. Results of the modified MTT test are presented as means ± SD and refer to untreated control cells which were set as 100% values.[17,34] Data are expressed as means ± SD of at least 3 different independent experiments. Data were subjected to one way ANOVA followed by Dunnett's multiple comparison post test using GraphPad Prism 5 Software. (Levels of significance $^{*}p$ <0.05; $^{**}p$ <0.01; $^{***}p$ <0.001).

Table 3
Antioxidant capacities expressed as trolox equivalents (concentration range, 1.0–5.0 μM)

Compd	Structure	Trolox equiv
11		1.54 ± 0.19
12		1.56 ± 0.15
35		1.34 ± 0.12

the free hydroxyl group, and the *N*-substitution. As expected, compound **40** revealed no neuroprotective effects and furthermore exhibited neurotoxic effects starting at a low concentration of 5 μM (Fig. 4A). These observations lead to the assumption that a free hydroxyl group and a small lipophilic substituent (ideally a methyl group) on the *N*-1 of the cyclic acyl guanidine moiety are necessary and sufficient for the neuroprotective properties and the lack of the self-toxic effects toward HT-22 neuronal cells.

For evaluation of physicochemical antioxidant potencies, radical scavenging capacities were determined by means of their ability to reduce the amount of peroxyl radicals (oxygen radical absorbance capacity assay ORAC assay) (Table 3). The ORAC-fluorescein assay was performed for three phenolic compounds **11**, **12**, **35** identified before as neuroprotectants in the cell based assay. All of the three tested phenolic compounds showed a good antioxidative capacity, even higher than the water soluble vitamin E derivative trolox. The *N*-1 substituent cyclic acyl guanidine e.g. compound **11** (*N*-1 methyl substituent) and **12** (*N*-1 benzyl substituent), showed no substituent influence on the antioxidant capacity of the whole phenolic moiety. The antioxidant capacity of compound **35** (the reduced analog of compound **11)** is slightly lower measured in trolox equivalents showing that the amide's carbonyl group reduction lowers antioxidant capacity (Table 3).

3. Conclusion

Cyclic acyl guanidine carbamate-based ChEIs templates were synthesized and tested on both ChEs. Heptyl carbamate compound **23** with 1-propylpiperidine substituent at *N*-1 showed high inhibitory activity and selectivity towards BChE with IC_{50} (BChE) = 24.7 nM, and IC_{50} (AChE) = 7.95 μM with a pronounced selectivity (325 fold). The kinetic study of this compound proved the carbamoylation process of BChE, and the calculated K_C of this heptyl carbamate is 1.3 nM. *N*-11 methylated compound **32** bearing a 4-isopropyl phenyl carbamate moiety showed potent inhibitory activity and selectivity at *ee*AChE with IC_{50} (AChE) = 83.7 nM and IC_{50} (BChE) = 3.3 μM. The kinetic study results of this carbamate compound gave a K_C value of 43.5 nM. Phenolic compound formed after the carbamoylation process at the CAS of the ChEs showed additional neuroprotective properties and antioxidant capacity, especially compounds **11** and **12**. Therefore, the cyclic acyl guanidine template seems to be an excellent starting point for the development of AChE as well as BChE inhibitors depending on the carbamate structure and *N*-substitution. The heterocycle also bears the possibility for pronounced neuroprotectivity and antioxidant capacity; all activities are highly dependent on the heterocycles' chemical structure.

4. Experimental part

4.1. Enzyme inhibition

4.1.1. Acetyl- and butyrylcholinesterase inhibition assay

The assay has been previously described in detail:[17,35] AChE (E.C.3.1.1.7, Type VI-S, from electric eel), BChE (E.C.3.1.1.8, from equine serum) and *h*AChE (E.C.3.1.1.7, 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 μL 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) were dissolved in 100 mL of water and adjusted with NaOH to pH = 8.0 ± 0.1. Enzyme solutions were prepared to give 2.5 units mL^{-1} in 1.4 mL aliquots. Furthermore, 0.01 M DTNB solution, 0.075 M ATC and BTC solutions, respectively, were used. A cuvette containing 3.0 mL of phosphate buffer, 100 μL of the respective enzyme, and 100 μL of the test compound solution was allowed to stand for 4.5 min, then 100 μL of DTNB were added, and the reaction was started by addition of 20 μL 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 μL of water replaced the test compound solution. For determining the blank value, additionally 100 μL 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.

4.2. Neuroprotection and neurotoxicity

HT-22[44,45] cells were derived from murine hippocampal tissue,[46] and were kindly provided by the Max Planck Institute of Psychiatry, Munich. They are grown in high glucose Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Karlsruhe, Germany) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) (Biochrom, Berlin, Germany). Cells were kept under standard cell culture conditions at 37 °C under 5% CO_2 in a humidified incubator. Cells were subcultured every 2 days.

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay[47] as described previously.[17,35,40] Briefly, cells were seeded in 96-well plates at a density of 5×10^3 per well and cultured for 24 h. Subsequently cells were incubated for another 24 h either with medium, compounds, or solvent only in presence (neuroprotection assay) or absence (neurotoxicity assay) of 5 mM glutamate (Monosodium-L-glutamate, Merck, Darmstadt, Germany). Quercetin (Sigma, Steinheim, Germany) in a concentration of 25 μM served as positive control in the neuroprotection assay. MTT (Sigma, Steinheim, Germany) solution (4 mg/mL in PBS) was diluted 1:10 with medium and the mixture was added to the wells after removal of previous medium. The plates were then incubated for another 3 h. Afterwards, supernatants were removed and 100 μL of lysis buffer (10% SDS) was added to the wells. Absorbance at 560 nM was determined on the next day with a multi-well plate photometer (Spectra Fluor Plus, Crailsheim, Germany).

Results of cell viability are expressed as percentage to untreated control cells. All compounds were dissolved in DMSO and diluted

with fresh medium. DMSO concentration in final dilutions was ⩽0.1%. Statistical Analysis: Data are expressed as means ± SD of at least 3 different independent experiments. Data were subjected to one way ANOVA followed by Dunnett's multiple comparison post test using GraphPad Prism 5 Software. (Levels of significance $^{*}p$ <0.05; $^{**}p$ <0.01; $^{***}p$ <0.001).

4.3. Antioxidant capacity

The antioxidant activity was determined by the oxygen radical absorbance capacity—fluorescein (ORAC-FL) assay[48,49] as described previously.[17,19] The ORAC-assay measures antioxidant scavenging activity against peroxyl radicals induced by 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) at 37 °C. The reaction was carried out in 75 mM phosphate buffer (pH 7.4) and the final reaction mixture was 200 μL. Antioxidant (20 μL) and fluorescein (120 μL, 300 nM final concentration) were placed in the wells of a 96 well plate and the mixture was incubated for 15 min at 37 °C. Then AAPH (Sigma, Steinheim, Germany) solution (60 μL; 12 mM final concentration) was added rapidly. The plate was immediately placed into a Spectrafluor Plus plate reader (Tecan, Crailsheim, Germany) and the fluorescence was measured every 60 seconds for 90 minutes with excitation at 485 nm and emission at 535 nm. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma, Steinheim Germany) was used as standard (1–8 μM, final concentration). A blank (FL+AAPH) using phosphate buffer instead of antioxidant and Trolox calibration were carried out in each assay. The samples were measured at different concentrations (1.0–5.0 μM). All reaction mixtures were prepared fourfold and at least four independent runs were performed for each sample. Fluorescence measurements were normalized to the curve of the blank (without antioxidant).

4.4. Chemistry

General methods: Melting points are uncorrected and were measured in open capillary tubes, using a Barnstead Electrothermal IA9100 melting point apparatus. ^{1}H and ^{13}C NMR spectral data were obtained from Bruker Advance spectrometers (300 MHz and 75 MHz or 400 and 101 MHz, respectively). TLC was performed on silica gel on aluminium foils with fluorescent indicator 254 nm (Merck). For detection iodine vapor, or UV light (254 nm), were used. ESI-MS samples were analyzed using electrospray ionisation ion-trap mass spectrometry in nanospray mode using a Thermo Finnigan LCQ Deca. The CHN analyses were undertaken using Perkin Elmer Elemental Analyser 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. For column chromatography, silica gel 60, 230–400 mesh (Merck) was used.

6-(Benzyloxy)-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (**6**) was synthesized in four steps with almost quantitative overall yield, starting from 2-amino-5-hydroxybenzoic acid.[31,32]

4.4.1. 8-(Benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (7)

To a stirred solution of 6-(benzyloxy)-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (**6**, 1.0 g, 3.7 mmol) in DMF (*N*,*N*-dimethylformamide, 40 mL) was added 2-methylsulfanyl-1,4,5,6-tetrahydropyrimidine (485 mg, 3.7 mmol). The mixture was heated to 100 °C for 6 h and a stream of nitrogen was bubbled continuously through the mixture. The mixture was cooled and concentrated under reduced pressure, the crude mixture was then dissolved in dichloromethane and purified by column chromatography using (50:1) dichloromethane: methanol as eluent system gave the title compound as a white solid, mp = 215–217 °C. (600 mg, 53%). HRESIMS ($C_{18}H_{17}N_3O_2$+H)$^{+}$, *m*/*z* calcd: 308. 1394; found: 308.1401. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, *J* = 2.9 Hz, 1H), 7.44–7.36 (m, 2H), 7.36–7.16 (m, 4H), 7.08 (d, *J* = 8.9 Hz, 1H), 5.03 (s, 2H), 4.15–3.90 (m, 2H), 3.42 (t, *J* = 5.8 Hz, 2H), 2.13–1.90 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.0, 153.9, 149.1, 144.1, 136.7, 128.6, 128.1, 127.7, 125.5, 124.7, 116.6, 107.7, 70.4, 40.0, 39.3, 20.6 ppm.

4.4.2. General synthetic procedure (I) for compounds 8, 9, and 10

8-(Benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**7**, 500 mg, 1.6 mmol) was dissolved in anhydrous THF (50 mL) at room temperature. Sodium hydride (136 mg, 3.4 mmol, 60% dispersion in oil) was added slowly to the stirred solution, followed by benzylbromide or iodomethane or 1-(3-bromopropyl)piperidine (2.0 mmol). The mixture was stirred for 24 h. The mixture was diluted with iced water (20 mL) and saturated aqueous sodium carbonate (20 mL) was added. The mixture was extracted with dichloromethane (2 × 100 mL), the organic phase was dried over magnesium sulfate and the solvent evaporated in vacuo. The residue was then purified by column chromatography using dichloromethane: methanol (20:1) as eluent system to afford the title compounds.

4.4.3. 8-(Benzyloxy)-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (8)

Starting from 8-(benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**7**, 500 mg, 1.6 mmol) and iodomethane (280 mg, 2.0 mmol). The title compound was obtained as a white solid, mp = 121–124 °C. (450 mg, 87%). HRESIMS ($C_{19}H_{19}N_3O_2$+H)$^{+}$, *m*/*z* calcd: 322.155; found: 322.1558. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, *J* = 3.0 Hz, 1H), 7.46 (m, 2H), 7.42–7.21 (m, 5H), 5.08 (s, 2H), 4.13 (dd, *J* = 13.3, 7.3 Hz, 2H), 3.50–3.38 (m, 2H), 3.24 (s, 3H), 2.20–2.00 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.2, 154.1, 148.3, 147.1, 136.9, 128.6, 128.0, 127.7, 126.1, 125.4, 116.3, 107.6, 70.4, 47.9, 40.0, 37.6, 20.9 ppm.

4.4.4. 1-Benzyl-8-(benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (9)

Starting from 8-(benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**7,** 500 mg, 1.6 mmol) and benzylbromide (340 mg, 2.0 mmol). The title compound was obtained as a white solid, mp = 127–129 °C. (520 mg, 82%). HRESIMS ($C_{25}H_{23}N_3O_2$+H)$^{+}$, *m*/*z* calcd: 398.1863; found: 398.1866. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (m, 1H), 7.48 (dd, *J* = 7.7, 1.1 Hz, 2H), 7.44–7.24 (m, 10H (OBn+NBn)), 5.12 (s, 2H), 4.97 (s, 2H), 4.18–4.03 (m, 2H), 3.34 (t, *J* = 5.9 Hz, 2H), 2.13–1.94 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.5, 154.1, 148.1, 144.4, 137.7, 136.9, 128.7, 128.6, 128.1, 128.0, 127.7, 127.5, 126.5, 125.4, 116.4, 107.5, 70.4, 52.3, 45.1, 40.4, 20.8 ppm.

4.4.5. 8-(Benzyloxy)-1-(3-(piperidin-1-yl)propyl)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (10)

Starting from 8-(benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**7,** 500 mg, 1.6 mmol) and 1-(3-bromopropyl)piperidine (415 mg, 2.0 mmol). The title compound was obtained as yellow oil. (570 mg, 82%). HRESIMS ($C_{26}H_{30}N_4O_2$+H)$^{+}$, *m*/*z* calcd: 333.2601; found: 333.2601. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (dd, *J* = 2.3, 0.9 Hz, 1H), 7.42 (dd, *J* = 8.2, 1.3 Hz, 2H), 7.39–7.17 (m, 5H), 5.05 (s, 2H), 4.03 (dd, *J* = 15.8, 9.7 Hz, 2H), 3.67 (t, *J* = 7.2 Hz, 2H), 3.44–3.40 (m, 2H), 2.64–2.43 (m, 6H), 2.11–1.88 (m, 4H), 1.73–1.58 (m, 4H), 1.55–1.37 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.4, 153.9, 147.7, 144.4, 136.9, 128.6, 128.0, 127.7, 126.2, 125.2, 116.2, 107.5, 70.4, 56.2, 54.3, 47.8, 46.0, 40.3, 25.2, 23.9, 23.9, 20.8 ppm.

4.4.6. General synthetic procedure (II) for phenolic compounds 11, 12, 13, 28, 35 and 38

To a mixture of the benzylated compound (**8**, **9**, **10**, **27**, **34** or **7**, respectively) and 10% Pd-C ethanol was added. The mixture was stirred at room temperature and under an atmosphere of H_2 for 4 h. The mixture then was filtered over celite and then the solvent removed under reduced pressure to afford the title phenolic compounds.

4.4.7. 8-Hydroxy-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (11)

Starting from 8-(benzyloxy)-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**8**, 500 mg, 1.56 mmol) and 10% Pd-C (50 mg). The title compound was obtained as a yellow solid. Mp = 223–227 °C. (320 mg, 89%). HRESIMS ($C_{12}H_{13}N_3O_2$+H)$^+$, *m*/*z* calcd: 332.1081; found: 332.1079. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (s, 1H (OH)), 7.31–7.00 (m, 3H), 3.94 (dd, *J* = 13.8, 7.8 Hz, 2H), 3.43–3.36 (m, 2H), 3.09 (s, 3H), 2.06–1.88 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.0, 151.9, 147.6, 142.4, 125.6, 123.8, 116.1, 108.9, 47.0, 39.6, 36.9, 20.1 ppm.

4.4.8. 1-Benzyl-8-hydroxy-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (12)

Starting from 1-benzyl-8-(benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one **9** (500 mg, 1.3 mmol) and 10% Pd-C (50 mg). The title compound was obtained as a yellow solid. Mp = 148–150 °C. (370 mg, 92%). HRESIMS ($C_{18}H_{17}N_3O_2$+H)$^+$, *m*/*z* calcd: 308.1396; found: 308.1394. ^{1}H NMR (300 MHz, MeOD) δ 7.68–6.83 (m, 8H), 4.75 (s, 2H), 4.14–3.98 (m, 2H), 3.47–3.32 (m, 2H), 2.06 (m, 2H) ppm. ^{13}C NMR (75 MHz, MeOD) δ 164.5, 153.7, 149.1, 144.7, 138.8, 129.9, 129.2, 128.7, 127.4, 126.0, 117.7, 110.5, 53.6, 46.5, 42.0, 22.0 ppm.

4.4.9. 8-Hydroxy-1-(3-(piperidin-1-yl)propyl)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (13)

Starting from 8-(benzyloxy)-1-(3-(piperidin-1-yl)propyl)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**10**, 500 mg, 1.16 mmol) and 10% Pd-C (50 mg). The title compound was obtained as yellow thick oil. (300 mg, 76%). HRESIMS ($C_{19}H_{24}N_4O_2$+H)$^+$, *m*/*z* calcd: 343.2130; found: 343.2130. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H (OH)), 7.26–7.19 (m, 1H), 7.06 (d, *J* = 1.7 Hz, 2H), 4.00–3.88 (m, 2H), 3.64–3.53 (m, 2H), 3.45–3.37 (m, 2H), 2.29 (m, 6H), 2.07–1.93 (m, 2H), 1.84–1.71 (m, 2H), 1.49 (dt, *J* = 10.6, 5.4 Hz, 4H), 1.38 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.1, 151.8, 146.9, 142.6, 125.5, 123.9, 116.0, 108.8, 55.7, 53.8, 47.3, 45.4, 40.0, 25.5, 24.1, 23.5, 20.2 ppm.

4.4.10. 8-Hydroxy-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one (28)

Starting from 8-(benzyloxy)-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one **(27**, 500 mg, 1.56 mmol) and 10% Pd-C (50 mg). The title compound was obtained as yellow solid. Mp = 261–264 °C. (310 mg, 86%). HRESIMS ($C_{12}H_{13}N_3O_2$+H)$^+$, *m*/*z* calcd: 332.1081; found: 332.1081. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 1H (OH)), 7.30–7.27 (m, 1H), 7.10–6.97 (m, 2H), 3.92–3.74 (m, 2H), 3.40 (t, *J* = 5.5 Hz, 2H), 3.33 (s, 3H), 1.84–1.67 (m, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.5, 150.9, 141.7, 135.4, 123.0, 114.5, 113.6, 111.8, 43.0, 40.4, 30.4, 20.2 ppm.

4.4.11. 1-Methyl-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-ol (35)

Starting from 8-(benzyloxy)-1-methyl-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazoline (**34**, 250 mg, 0.81 mmol) and 10% Pd-C (25 mg). The title compound was obtained as yellow thick oil. (155 mg, 88%). HRESIMS ($C_{12}H_{15}N_3O$+H)$^+$, *m*/*z* calcd: 218.1288; found: 218.1285. ^{1}H NMR (300 MHz, MeOD) δ 7.06 (d, *J* = 8.7 Hz, 1H), 6.71 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.57 (d, *J* = 2.6 Hz, 1H), 4.47 (s, 2H), 3.37–3.28 (m, 4H), 3.21 (s, 3H), 2.23–2.09 (m, 2H) ppm. ^{13}C NMR (75 MHz, MeOD) δ 155.9, 150.8, 126.5, 120.7, 118.5, 116.9, 113.1, 63.8, 51.5, 39.0, 22.0, 9.6 ppm.

4.4.12. 8-Hydroxy-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (38)

Starting from 8-(benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**7**, 250 mg, 0.8 mmol) and 10% Pd-C (25 mg). The title compound was obtained as yellow thick oil. (155 mg, 89%). HRESIMS ($C_{11}H_{11}N_3O_2$+H)$^+$, *m*/*z* calcd: 218.0931; found: 218.0932. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.40–7.37 (m, 1H), 7.36–7.29 (m, 1H), 7.18 (d, *J* = 8.9 Hz, 1H), 4.11–3.97 (m, 2H), 3.49 (t, *J* = 5.6 Hz, 2H), 2.17–1.96 (m, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.1, 159.9, 156.9, 144.2, 137.6, 131.4, 126.4, 117.2, 42.1, 39.7, 20.3 ppm.

4.4.13. General synthetic procedure (III) for target carbamates 14–25, 29–33, 36–37, 39

Triethylamine (1.1 equiv) was added to a stirred solution of phenolic compound 8-hydroxy-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one **11**, 1-benzyl-8-hydroxy-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**12**), 8-hydroxy-1-(3-(piperidin-1-yl)propyl)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**13**), 8-hydroxy-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one (**28**), 1-methyl-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-ol (**35**), or 8-hydroxy-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**38**), respectively, (1 equiv) in dichloromethane and stirred at ambient temperature for 15 min. Isocyanate (1.1 equiv) was added and stirred for another 1 to 3 h at room temperature. Upon completion (TLC), the reaction mixture was diluted with dichloromethane, washed with water, washed with brine, dried and evaporated under reduced pressure. The crude compound was purified by preparative TLC (dichloromethane: MeOH, 30:1) to get the carbamate compounds **14**–**25**, **29**–**33**, **36**–**37**, or **39**, respectively.

4.4.14. 1-Methyl-6-oxo-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl butylcarbamate (14)

Starting from 8-hydroxy-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one **11** (100 mg, 0.43 mmol) and 1-isocyanatobutane (45 mg, 0.45 mmol). The title compound was obtained as colourless oil. (77 mg, 54%). HRESIMS ($C_{17}H_{22}N_4O_3$+H)$^+$, *m*/*z* calcd: 331.1765; found: 331.1766. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 1.4 Hz, 1H), 7.34–7.27 (m, 2H), 4.04 (dd, *J* = 16.3, 10.3 Hz, 2H), 3.38 (t, *J* = 6.1 Hz, 2H), 3.22 (dd, *J* = 12.9, 7.3 Hz, 2H), 3.19 (s, 3H), 2.11–2.00 (m, 2H), 1.35–1.21 (m, 4H), 0.91 (t, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 158.8, 154.9, 149.0, 145.7, 128.9, 125.6, 118.4, 116.3, 47.9, 40.1, 39.9, 37.6, 32.5, 20.1, 13.8, 13.7 ppm. Elem. Anal. ($C_{17}H_{22}N_4O_3$ - × 0.65 EtOH) Calcd: C, 61.00; H, 7.25; N, 15.55; O, 16.21; Found: C, 60.65; H, 7.67; N, 16.06.

4.4.15. 1-Methyl-6-oxo-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl heptylcarbamate (15)

Starting from 8-hydroxy-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**11**, 100 mg, 0.43 mmol) and isocyanatoheptane (65 mg, 0.45 mmol). The title compound was obtained as colourless oil. (100 mg, 62%). HRESIMS ($C_{20}H_{28}N_4O_3$+H)$^+$, *m*/*z* calcd: 373.2234; found: 373.2241. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 2.4 Hz, 1H), 7.35–7.22 (m, 2H), 5.41 (m, 1H (*NH*)), 4.14–3.93 (m, 2H), 3.49–3.31 (m, 2H), 3.27–3.13 (m, 4H), 2.13–1.96 (m, 2H), 1.65–1.45 (m, 2H), 1.27 (m, 8H), 0.91–0.81 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 154.8, 149.0, 146.0, 145.7, 129.0, 125.6, 118.4, 116.3, 47.9, 41.3, 39.9, 37.5, 31.7, 29.8, 29.0, 26.7, 22.6, 20.7, 14.1 ppm. Elem. Anal.

($C_{20}H_{28}N_4O_3 \times 0.11\ CH_2Cl_2$) Calcd: C, 63.26; H, 7.45; Cl, 2.04; N, 14.67; O, 12.57; Found: C, 63.57; H, 7.80; N, 14.28.

4.4.16. 1-Methyl-6-oxo-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl (3-methoxyphenyl)carbamate (16)

Starting from 8-hydroxy-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one **11** (100 mg, 0.43 mmol) and 1-isocyanato-3-methoxybenzene (67 mg, 0.45 mmol). The title compound was obtained as white solid. Mp = 198–202 °C. (110 mg, 61%). HRESIMS ($C_{20}H_{20}N_4O_4$+H)$^+$, *m*/*z* calcd: 381.1557; found: 381.1558. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.23 (s, 1H(*N*H)), 7.62 (d, *J* = 2.8 Hz, 1H), 7.42 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.21 (m, 3H), 7.07 (d, *J* = 7.7 Hz, 1H), 6.62 (m, 1H), 4.07–3.90 (m, 2H), 3.72 (s, 3H), 3.52–3.38 (m, 2H), 3.15 (s, 3H), 2.12–1.93 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.3, 164.9, 157.2, 148.4, 147.9, 145.1, 143.8, 134.2, 133.2, 124.8, 119.2, 118.8, 116.3, 113.1, 107.4, 57.2, 49.3, 41.2, 34.9, 24.4 ppm. Elem. Anal. ($C_{20}H_{20}N_4O_4 \times 0.35\ CHCl_3$) Calcd: C, 57.89; H, 4.86; N, 13.27; O, 15.16; Found: C, 57.96; H, 5.16; N, 13.47.

4.4.17. 1-Methyl-6-oxo-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl(4-isopropylphenyl)carbamate (17)

Starting from 8-hydroxy-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**11,** 100 mg, 0.43 mmol) and 1-isocyanato-4-isopropylbenzene (75 mg, 0.45 mmol). The title compound was obtained as white solid. Mp = 197–199 °C. (105 mg, 62%). HRESIMS ($C_{22}H_{24}N_4O_3$+H)$^+$, *m*/*z* calcd: 393.1921; found: 393.1924. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H (*N*H)), 7.39 (m, 4H), 7.26 (s, 1H), 7.23–7.14 (m, 2H), 4.23–3.99 (m, 2H), 3.46 (t, *J* = 6.1 Hz, 2H), 3.26 (s, 3H), 2.88 (hept, *J* = 6.9 Hz, 1H), 2.21–1.92 (m, 2H), 1.24 (d, *J* = 6.9 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 149.1, 145.4, 144.6, 135.3, 128.9, 127.0, 127.0, 125.6, 119.1, 118.6, 116.4, 113.8, 48.0, 40.0, 37.8, 33.5, 24.1, 20.7 ppm. Elem. Anal. ($C_{22}H_{24}N_4O_3 \times 0.1\ CH_2Cl_2$) Calcd: C, 66.20; H, 6.08; N, 13.97; O, 11.97; Found: C, 66.00; H, 6.30; N, 13.61.

4.4.18. 1-Benzyl-6-oxo-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl butylcarbamate (18)

Starting from 1-benzyl-8-hydroxy-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**12,** 100 mg, 0.33 mmol) and 1-isocyanatobutane (36 mg, 0.36 mmol). The title compound was obtained as white solid. Mp = 168–171 °C. (98 mg, 73%). HRESIMS ($C_{23}H_{26}N_4O_3$+H)$^+$, *m*/*z* calcd: 407.2078; found: 407.2078. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.46–7.23 (m, 7H), 5.13 (t, *J* = 5.7 Hz, 1H (*N*H)), 4.99 (s, 2H), 4.19–4.02 (m, 2H), 3.37 (t, *J* = 5.9 Hz, 2H), 3.27 (dd, *J* = 13.1, 6.9 Hz, 2H), 2.13–1.98 (m, 2H), 1.66–1.47 (m, 2H), 1.39 (dq, *J* = 14.1, 7.1 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.45, 154.8, 148.8, 145.8, 137.3, 129.0, 128.7, 128.1, 127.6, 126.3, 125.7, 118.4, 116.4, 52.6, 45.2, 41.0, 40.3, 31.9, 20.7, 19.9, 13.8 ppm. Elem. Anal. ($C_{23}H_{26}N_4O_3$) Calcd: C, 67.96; H, 6.45; N, 13.78; O, 11.81; Found: C, 67.73; H, 6.51; N, 13.87.

4.4.19. 1-Benzyl-6-oxo-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl heptylcarbamate (19)

Starting from 1-benzyl-8-hydroxy-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**12,** 100 mg, 0.33 mmol) and isocyanatoheptane (51 mg, 0.36 mmol). The title compound was obtained as white solid. Mp = 161–164 °C. (110 mg, 74%). HRESIMS ($C_{26}H_{32}N_4O_3$+H)$^+$, *m*/*z* calcd: 449.2547; found: 449.2554. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86–7.73 (m, 1H), 7.41–7.26 (m, 7H), 4.99 (s, 2H), 4.09 (dd, *J* = 14.3, 8.4 Hz, 2H), 3.39 (dd, *J* = 14.2, 8.3 Hz, 2H), 3.33–3.20 (m, 2H), 2.08 (m, 2H), 1.71–1.45 (m, 2H), 1.44–1.33 (m, 8H), 0.93–0.84 (m, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.9, 160.7, 148.8, 137.3, 136.6, 132.4, 130.8, 128.7, 128.1, 127.6, 125.7, 118.5, 41.3, 40.3, 31.8, 29.9, 29.8, 29.0, 29.0, 26.7, 22.6, 20.7, 14.1 ppm. Elem. Anal. ($C_{26}H_{32}N_4O_3$) Calcd: C, 69.62; H, 7.19; N, 12.49; O, 10.70; Found: C, 69.66; H, 7.21; N, 12.51.

4.4.20. 1-Benzyl-6-oxo-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl (3-methoxy-phenyl)carbamate (20)

Starting from 1-benzyl-8-hydroxy-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**12**, 100 mg, 0.33 mmol) and 1-isocyanato-3-methoxybenzene (54 mg, 0.36 mmol). The title compound was obtained as white solid. Mp = 149–151 °C. (98 mg, 73%). HRESIMS ($C_{26}H_{24}N_4O_4$+H)$^+$, *m*/*z* calcd: 457.187; found: 457.1869. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81(s, 1H (*N*H)), 7.02–7.21 (m, 3H), 7.22–7.55 (m, 9H), 5.05 (s, 2H), 4.32–4.01 (m, 2H), 3.72 (s, 3H), 3.41 (m, 2H), 2.23–1.99 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.0, 154.6, 150.3, 147.4, 146.6, 140.4, 139.2, 131.4, 130.0, 129.9, 129.6, 128.8, 128.3, 127.9, 121.4, 119.7, 119.5, 116.4, 54.3, 40.4, 37.5, 35.6, 24.1 ppm. Elem. Anal. ($C_{26}H_{24}N_4O_4$) Calcd: C, 68.41; H, 5.30; N, 12.27; O, 14.02; Found: C, 68.56; H, 5.42; N, 12.33.

4.4.21. 1-Benzyl-6-oxo-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl (4-isopropyl-phenyl)carbamate (21)

Starting from 1-benzyl-8-hydroxy-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one **12** (100 mg, 0.33 mmol) and 1-isocyanato-4-isopropylbenzene (58 mg, 0.36 mmol). The title compound was obtained as white solid. Mp = 131–133 °C. (98 mg, 73%). HRESIMS ($C_{28}H_{28}N_4O_3$+H)$^+$, *m*/*z* calcd: 469.2234; found: 469.2231. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (s, 1H (*N*H)), 7.40 (m, 3H), 7.37–7.32 (m, 4H), 7.32–7.06 (m, 5H), 5.01 (s, 2H), 4.21–3.99 (m, 2H), 3.37 (dd, *J* = 13.7, 7.8 Hz, 2H), 2.94–2.83 (m, 1H), 2.16–1.97 (m, 2H), 1.23 (d, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.0, 151.7, 148.9, 144.6, 144.2, 138.3, 137.3, 128.7, 128.1, 127.9, 127.6, 127.0, 125.8, 119.0, 119.0, 118.6, 116.4, 45.3, 40.4, 33.6, 33.5, 24.1, 20.6 ppm. Elem. Anal. ($C_{28}H_{28}N_4O_3$) Calcd: C, 71.78; H, 6.02; N, 11.96; O, 10.24; Found: C, 71.80; H, 6.21; N, 11.91.

4.4.22. 6-Oxo-1-(3-(piperidin-1-yl)propyl)-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl butylcarbamate (22)

Starting from 8-hydroxy-1-(3-(piperidin-1-yl)propyl)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**13**, 100 mg, 0.43 mmol) and 1-isocyanatobutane (45 mg, 0.45 mmol). The title compound was obtained as colourless oil. (77 mg, 54%). HRESIMS ($C_{24}H_{35}N_5O_3$+H)$^+$, *m*/*z* calcd: 442.2813; found: 442.2818. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.33–7.15 (m, 2H), 5.51–5.47 (m, 1H (*N*H)), 4.00 (dd, *J* = 13.8, 7.9 Hz, 2H), 3.72–3.58 (m, 2H), 3.44–3.35 (m, 2H), 3.20 (dd, *J* = 13.2, 6.9 Hz, 2H), 2.55–2.24 (m, 6H), 2.09–1.97 (m, 2H), 1.94–1.79 (m, 2H), 1.62–1.48 (m, 6H), 1.45–1.28 (m, 4H), 0.87 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 155.0, 148.3, 147.2, 145.5, 128.9, 125.7, 118.2, 116.2, 56.4, 54.5, 48.2, 46.1, 41.0, 40.3, 31.9, 25.8, 24.3, 24.2, 20.7, 19.9, 13.7 ppm. Elem. Anal. ($C_{24}H_{35}N_5O_3 \times 0.25\ CH_2Cl_2$) Calcd: C, 62.93; H, 7.73; N, 15.13; O, 10.37; Found: C, 62.92; H, 7.99; N, 15.19.

4.4.23. 6-Oxo-1-(3-(piperidin-1-yl)propyl)-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl heptylcarbamate (23)

Starting from 8-hydroxy-1-(3-(piperidin-1-yl)propyl)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one **13** (100 mg, 0.43 mmol) and isocyanatoheptane (65 mg, 0.45 mmol). The title compound was obtained as a colorless oil. (100 mg, 62%). HRESIMS ($C_{27}H_{41}N_5O_3$+H)$^+$, *m*/*z* calcd: 484.3282; found: 484.3289. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (m, 1H), 7.38–7.13 (m, 2H), 5.55–5.44 (m, 1H (*N*H)), 4.06–3.93 (m, 2H), 3.69–3.60 (m, 2H), 3.42 (dd, *J* = 12.2, 6.3 Hz, 2H), 3.20 (dd, *J* = 13.4, 6.8 Hz, 2H), 2.52–2.30 (m, 6H), 2.09–1.97 (m, 2H), 1.95–1.82 (m, 2H), 1.63–1.48 (m, 6H),

1.42 (d, J = 4.6 Hz, 2H), 1.33–1.21 (m, 8H), 0.89–0.81 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 154.9, 148.3, 147.2, 145.6, 128.9, 125.6, 118.2, 116.2, 56.4, 54.5, 48.1, 46.0, 41.3, 40.3, 31.7, 29.8, 28.9, 26.7, 25.7, 24.3, 24.2, 22.6, 20.7, 14.1 ppm. Elem. Anal. ($C_{27}H_{41}N_5O_3 \times 0.12\ CH_2Cl_2$) Calcd: C, 65.96; H, 8.42; N, 14.18; O, 9.72; Found: C, 65.86; H, 8.38; N, 14.18.

4.4.24. 6-oxo-1-(3-(piperidin-1-yl)propyl)-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl (3-methoxyphenyl)carbamate (24)

Starting from 8-hydroxy-1-(3-(piperidin-1-yl)propyl)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one **13** (100 mg, 0.43 mmol) and 1-isocyanato-3-methoxybenzene (67 mg, 0.45 mmol). The title compound was obtained as a white solid. Mp = 194–197 °C. (110 mg, 61%). HRESIMS ($C_{27}H_{33}N_5O_4$+H)$^+$, m/z calcd: 492.2605; found: 492.2615. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.64 (m, 1H (*NH*)), 7.38–6.74 (m, 6H), 6.66–6.46 (m, 1H), 4.11–4.00 (m, 2H), 3.79 (s, 3H), 3.70–3.53 (m, 2H), 3.39– 3.37 (m, 2H), 2.50–2.41 (m, 6H), 2.14–1.98 (m, 3H), 1.93 (m, 1H), 1.63 (m, 4H), 1.42 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.3, 160.3, 152.3, 148.4, 147.5, 144.9, 140.2, 129.8, 128.8, 125.9, 124.6, 118.4, 116.2, 109.7, 105.9, 56.3, 55.3, 54.5, 48.2, 46.1, 40.4, 25.5, 24.1, 20.7 ppm. Elem. Anal. ($C_{27}H_{33}N_5O_4 \times 0.5\ CH_2Cl_2$) Calcd: C, 61.85; H, 6.42; N, 13.11; O, 11.98; Found: C, 61.99; H, 6.37; N, 13.34.

4.4.25. 6-Oxo-1-(3-(piperidin-1-yl)propyl)-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl (4-isopropylphenyl)carbamate (25)

Starting from 8-hydroxy-1-(3-(piperidin-1-yl)propyl)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**13**, 100 mg, 0.43 mmol) and 1-isocyanato-4-isopropylbenzene (75 mg, 0.45 mmol). The title compound was obtained as white solid. Mp = 197–199 °C. (105 mg, 62%). HRESIMS ($C_{29}H_{37}N_5O_3$+H)$^+$, m/z calcd: 504.2969; found: 504.2978. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H (*NH*)), 7.43–7.35 (m, 3H), 7.28 (s, 1H), 7.26 (s, 1H), 7.19 (d, J = 8.5 Hz, 2H), 4.05–4.08 (m, 2H), 3.78–3.61 (m, 2H), 3.54–3.36 (m, 2H), 2.99–2.80 (m, 1H), 2.44 (m, 6H), 2.14–2.02 (m, 2H), 2.01–1.86 (m, 2H), 1.71–1.56 (m, 4H), 1.44–1.41 (m, 2H), 1.26–1.01 (m, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 154.9, 150.5, 148.5, 147.5, 145.1, 128.8, 127.1, 127.0, 125.8, 118.5, 116.3, 60.1, 56.4, 54.5, 48.2, 46.1, 40.3, 39.6, 33.5, 24.3, 24.1, 20.8 ppm. Elem. Anal. ($C_{29}H_{37}N_5O_3 \times 0.15\ CH_2Cl_2$) Calcd: C, 67.80; H, 7.28; N, 13.56; O, 9.30; Found: C, 67.73; H, 7.34; N, 13.69.

4.4.26. 11-Methyl-6-oxo-3,4,6,11-tetrahydro-2*H*-pyrimido[2,1-*b*]quinazolin-8-yl butylcarbamate (29)

Starting from 8-hydroxy-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one (**28,** 100 mg, 0.43 mmol) and 1-isocyanatobutane (45 mg, 0.45 mmol). The title compound was obtained as white solid. Mp = 182–184 °C. (90 mg, 63%). HRESIMS ($C_{17}H_{22}N_4O_3$+H)$^+$, m/z calcd: 331.1765; found: 331.1773. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, J = 2.8 Hz, 1H), 7.33 (dd, J = 9.0, 2.8 Hz, 1H), 6.96 (d, J = 9.1 Hz, 1H), 4.02–3.91 (m, 2H), 3.55 (t, J = 5.6 Hz, 2H), 3.45 (s, 3H), 3.24 (dd, J = 13.1, 7.0 Hz, 2H), 1.95–1.83 (m, 2H), 1.61–1.47 (m, 2H), 1.45–1.35 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 154.6, 144.7, 142.9, 140.1, 129.0, 120.8, 114.4, 113.6, 43.8, 41.1, 41.0, 31.9, 31.4, 20.6, 19.9, 13.7 ppm. Elem. Anal. ($C_{17}H_{22}N_4O_3$) Calcd: C, 61.80; H, 6.71; N, 16.96; O, 14.53; Found: C, 61.47; H, 6.90; N, 17.12.

4.4.27. 11-Methyl-6-oxo-3,4,6,11-tetrahydro-2*H*-pyrimido[2,1-*b*]quinazolin-8-yl heptylcarbamate (30)

Starting from 8-hydroxy-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one **28** (100 mg, 0.43 mmol) and isocyanatoheptane (65 mg, 0.45 mmol). The title compound was obtained as white solid. Mp = 137–139 °C. (120 mg, 75%). HRESIMS ($C_{20}H_{28}N_4O_3$+H)$^+$, m/z calcd: 373.2234; found: 373.2245. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 2.8 Hz, 1H), 7.35 (dd, J = 9.0, 2.8 Hz, 1H), 6.98 (d, J = 9.1 Hz, 1H), 5.14 (m, 1H (*NH*)), 4.08–3.86 (m, 2H), 3.57 (t, J = 5.6 Hz, 2H), 3.48 (s, 3H), 3.30–3.21 (m, 2H), 1.97–1.83 (m, 2H), 1.56 (m, 2H), 1.40–1.21 (m, 8H), 0.89 (t, J = 6.9 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.0, 154.5, 141.3, 140.0, 136.3, 128.9, 120.8, 114.5, 113.6, 43.8, 41.4, 41.1, 31.7, 31.5, 29.8, 28.9, 26.7, 22.6, 20.6, 14.1 ppm. Elem. Anal. ($C_{20}H_{28}N_4O_3 \times 0.05CH_2Cl_2$) Calcd: C, 63.93; H, 7.52; N, 14.87; O, 12.74; Found: C, 63.78; H, 7.47; N, 14.95.

4.4.28. 11-Methyl-6-oxo-3,4,6,11-tetrahydro-2*H*-pyrimido[2,1-*b*]quinazolin-8-yl (3-methoxyphenyl)carbamate (31)

Starting from 8-hydroxy-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one (**28**, 100 mg, 0.43 mmol) and 1-isocyanato-3-methoxybenzene (67 mg, 0.45 mmol). The title compound was obtained as white solid. Mp = 141–145 °C. (105 mg, 64%). HRESIMS ($C_{20}H_{20}N_4O_4$+H)$^+$, m/z calcd: 381.1557; found: 381.1559. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 2.8 Hz, 1H), 7.58–7.43 (m, 1H), 7.30–7.20 (m, 2H), 7.14 (d, J = 9.1 Hz, 1H), 7.10–7.01 (m, 1H), 6.75–6.63 (m, 1H), 4.27 (s, 1H (*NH*)), 4.09–3.97 (m, 2H), 3.84 (s, 3H), 3.62 (t, J = 5.6 Hz, 2H), 3.52 (s, 3H), 2.07–1.89 (m, 2H) ppm.^{13}C NMR (101 MHz, $CDCl_3$) δ 164.3, 164.1, 156.3, 148.5, 147.6, 144.1, 143.1, 133.6, 133.1, 124.6, 118.2, 118.0, 115.1, 113.3, 108.5, 59.0, 47.5, 45.2, 35.5, 24.3 ppm. Elem. Anal. ($C_{20}H_{20}N_4O_4 \times 0.18\ CH_2Cl_2$) Calcd: C, 60.31; H, 5.16; N, 13.98; O, 11.97; Found: C, 60.03; H, 5.55; N, 14.38.

4.4.29. 11-Methyl-6-oxo-3,4,6,11-tetrahydro-2*H*-pyrimido[2,1-*b*]quinazolin-8-yl (4-isopropylphenyl)carbamate (32)

Starting from 8-hydroxy-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one **28** (100 mg, 0.43 mmol) and 1-isocyanato-4-isopropylbenzene (75 mg, 0.45 mmol). The title compound was obtained as white solid. Mp = 191–194 °C. (115 mg, 68%). HRESIMS ($C_{22}H_{24}N_4O_3$+H)$^+$, m/z calcd: 393.1921; found: 393.1927. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 2.8 Hz, 1H), 7.57 (s, 1H (*NH*)), 7.45–7.34 (m, 3H), 7.20 (s, 1H), 7.17 (s, 1H), 6.99 (d, J = 9.1 Hz, 1H), 4.08–3.93 (m, 2H), 3.58 (t, J = 5.6 Hz, 2H), 3.47 (s, 3H), 2.88 (m, 1H), 1.91 (m, 2H), 1.23 (d, J = 6.9 Hz, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 144.3, 142.9, 140.3, 135.1, 129.0, 127.0, 120.9, 119.0, 114.5, 113.8, 43.8, 41.2, 33.5, 31.4, 24.1, 20.6 ppm. Elem. Anal. ($C_{22}H_{24}N_4O_3 \times 0.1\ CH_2Cl_2$) Calcd: C, 66.20; H, 6.08; N, 13.97; O, 11.97; Found: C, 66.01; H, 6.13; N, 14.24.

4.4.30. 4-(*tert*-Butyl)phenyl (11-methyl-6-oxo-3,4,6,11-tetrahydro-2*H*-pyrimido[2,1-*b*]quinazolin-8-yl)carbamate (33)

Starting from 8-hydroxy-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one (**28**, 100 mg, 0.43 mmol) and 1-(*tert*-butyl)-4-isocyanatobenzene (80 mg, 0.45 mmol). The title compound was obtained as a white solid. Mp = 201–203 °C. (123 mg, 71%). HRESIMS ($C_{23}H_{26}N_4O_3$+H)$^+$, m/z calcd: 407.2078; found: 407.2087. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J = 2.8 Hz, 1H), 7.47–7.19 (m, 5H), 7.13–7.02 (m, 1H), 3.91 (dd, J = 13.7, 8.2 Hz, 2H), 3.47 (t, J = 5.6 Hz, 2H), 3.39 (s, 3H), 3.24 (dt, J = 3.3, 1.6 Hz, 1H), 1.92–1.79 (m, 2H), 1.21 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.3, 156.7, 148.8, 147.9, 143.9, 139.3, 133.2, 129.5, 124.4, 122.6, 118.2, 118.2, 47.3, 45.1, 37.9, 35.4, 34.9, 24.1 ppm. Elem. Anal. ($C_{23}H_{26}N_4O_3 \times 0.3\ CH_2Cl_2$) Calcd: C, 64.79; H, 6.21; N, 12.79; O, 11.11; Found: C, 64.57; H, 6.57; N, 12.64.

4.4.31. 1-Methyl-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl butylcarbamate (36)

Starting from 1-methyl-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-ol (**35,** 100 mg, 0.46 mmol) and 1-isocyanatobutane

(50 mg, 0.51 mmol). The title compound was obtained as brown oil. (84 mg, 58%). HRESIMS ($C_{17}H_{24}N_4O_2$+H)$^+$, *m*/*z* calcd: 317.1972; found: 317.1970. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.20 (m, 1H), 7.18–6.76 (m, 2H), 4.83 (s, 2H), 3.76–3.60 (m, 3H), 3.58–3.35 (m, 6H), 2.52–2.32 (m, 2H), 1.92–1.58 (m, 4H), 1.20 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.2, 154.1, 142.9, 141.1, 123.2, 124.4, 120.8, 117.2, 53.4, 46.8, 47.8, 41.3, 32.5, 27.3, 24.5, 19.7, 13.6 ppm.

4.4.32. 1-Methyl-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl heptylcarbamate (37)

Starting from 1-methyl-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-ol (**35**, 100 mg, 0.46 mmol) and 1-isocyanatoheptane (75 mg, 0.51 mmol). The title compound was obtained as brown oil. (84 mg, 58%). HRESIMS ($C_{20}H_{30}N_4O_2$+H)$^+$, *m*/*z* calcd: 359.2442; found: 492.2615. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (d, *J* = 8.6 Hz, 1H), 6.81 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H), 5.04 (m, 1H (*NH*)), 4.22 (s, 2H), 3.42–3.08 (m, 9H), 2.03 (m, 2H), 1.54 (dd, *J* = 14.9, 7.9 Hz, 2H), 1.26 (m, 8H), 0.87 (t, *J* = 6.7 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.2, 152.3, 144.8, 142.0, 121.7, 121.0, 120.8, 117.6, 51.5, 48.0, 47.8, 41.3, 37.4, 31.8, 29.9, 28.9, 26.7, 22.6, 22.3, 14.1 ppm.

4.4.33. 6-Oxo-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazolin-8-yl (4-isopropylphenyl)carbamate (39)

Starting from 8-hydroxy-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**38,** 100 mg, 0.46 mmol) and 1-isocyanato-4-isopropylbenzene (83 mg, 0.51 mmol). The title compound was obtained as white solid. Mp = 261–265 °C. (105 mg, 0.28 mmol, 61%). HRESIMS ($C_{21}H_{22}N_4O_3$+H)$^+$, *m*/*z* calcd: 379.1765; found: 379.1766. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28–7.14 (m, 4H), 7.04 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.01–6.94 (m, 2H), 3.94–3.86 (m, 2H), 3.85–3.76 (m, 2H), 2.74–2.56 (m, 1H), 2.02–1.87 (m, 2H), 0.99 (d, *J* = 5.5 Hz, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.2, 187.2, 154.9, 148.6, 144.6, 143.0, 138.6, 132.7, 126.6, 126.0, 120.2, 118.1, 110.4, 109.3, 42.1, 41.8, 33.3, 23.5, 20.6 ppm. Elem. Anal. ($C_{21}H_{22}N_4O_3 \times 0.5\ CH_2Cl_2$) Calcd: C, 65.16; H, 5.76; N, 14.39; O, 12.32; Found: C, 65.04; H, 5.98; N, 14.35.

4.4.34. 8-(Benzyloxy)-1-methyl-2,3,4,6-tetrahydro-1*H*-pyrimido[2,1-*b*]quinazoline (34)

To a solution of 8-(benzyloxy)-1-methyl-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (**8,** 500 mg, 1.56 mmol) in 20 mL of diglyme, $AlCl_3$ (226 mg, 1.72 mmol) in 10 mL of diglyme was added drop wise at 0 °C, the reaction mixture allowed to reach to rt. $NaBH_4$ (120 mg, 3.12 mmol) was added to the reaction mixture and then the reaction mixture was heated to 90 °C for extra three hours. The reaction mixture was then cooled to rt and then concentrated in vacuo, extracted with dichloromethane, dried over Na_2SO_4, and the solvent was then evaporated. The crude product was purified by preparative thin layer chromatography (dichloromethane: MeOH: conc. aqueous ammonia solution, 5:1:0.1) to get the title compounds as white solid. Mp = 117–121 °C. (310 mg, 65%). HRESIMS ($C_{19}H_{21}N_3O$+H)$^+$, *m*/*z* calcd: 308.1760; found: 308.176. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–7.27 (m, 5H), 7.15 (d, *J* = 8.7 Hz, 1H), 6.75 (dd, *J* = 8.7, 2.9 Hz, 1H), 6.49 (d, *J* = 2.8 Hz, 1H), 4.93 (s, 2H), 4.20 (s, 2H), 3.23 (t, *J* = 5.9 Hz, 2H), 3.17–3.09 (m, 5H), 2.06–1.92 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.7, 151.3, 137.3, 136.1, 128.5, 127.8, 127.5, 121.4, 120.7, 114.7, 111.2, 70.5, 51.5, 48.0, 47.8, 38.0, 22.0 ppm.

4.4.35. 6-(Benzyloxy)-1-methyl-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (26)

To a solution of 6-benzyloxyisatoic anhydride (**6,** 500 mg, 1.86 mmol) in 5 mL of DMAc (*N*,*N*-dimethyl acetamide), diisopropylethylamine (370 μL, 2.23 mmol) was added. The solution was stirred for 10 min prior to addition of methyl iodide (140 μL, 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 were added. The resulting suspension was stirred vigorously for 30 min, filtered, washed with 20 mL water and 20 mL *n*-hexane to afford the title compound as yellow solid. (490 mg, 93%). Mp 248–251 °C. ESI-MS: 284.18 *m*/*z* [M]$^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.61–7.29 (m, 8H), 5.22 (s, 2H), 3.44 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ: 158.7, 153.9, 147.7, 136.4, 136.4, 128.4, 127.9, 127.6, 125.6, 116.5, 112.1, 112.0, 69.7, 31.7 ppm.[32]

4.4.36. 8-(Benzyloxy)-11-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*]quinazolin-6(11*H*)-one (27)

To a stirred solution of 6-(benzyloxy)-1-methyl-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (**26,** 1.0 g, 3.5 mmol) in DMF (40 mL) was added 2-methylsulfanyl-1,4,5,6-tetrahydropyrimidine (485 mg, 3.7 mmol). The mixture was heated to 100 °C for 6 h and a stream of nitrogen was bubbled continuously through the mixture. The mixture was cooled and concentrated under reduced pressure, the crude mixture was then dissolved in dichloromethane and purified by column chromatography using (20:1:0.1) dichloromethane: methanol: conc. aqueous ammonia solution as an eluent system gave the title compound as a white solid. Mp = 187–189 °C. (570 mg, 51%). HRESIMS ($C_{19}H_{19}N_3O_2$+H)$^+$, *m*/*z* calcd: 322. 1549; found: 322.1549. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, *J* = 3.0 Hz, 1H), 7.50–7.17 (m, 6H), 6.96 (d, *J* = 9.1 Hz, 1H), 5.09 (s, 2H), 3.99 (dd, *J* = 6.6, 5.4 Hz, 2H), 3.57 (t, *J* = 5.6 Hz, 2H), 3.46 (s, 3H), 1.91 (ddd, *J* = 11.6, 7.7, 5.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 152.9, 143.1, 137.3, 136.6, 128.6, 128.1, 127.6, 124.3, 114.3, 114.3, 111.2, 70.6, 43.9, 41.1, 31.2, 20.7 ppm.

4.4.37. 3,4-Dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one (40)

To a stirred solution of isatoic anhydride (1*H*-benzo[*d*][1,3]oxazine-2,4-dione) (0.5 g, 3.1 mmol) in DMF (*N*,*N*-dimethylformamide) (40 mL) was added 2-methylsulfanyl-1,4,5,6-tetrahydropyrimidine (485 mg, 3.7 mmol). The mixture was heated to 100 °C for 6 h and a stream of nitrogen was bubbled continuously through the mixture. The mixture was cooled and concentrated under reduced pressure, the crude mixture was then dissolved in dichloromethane and purified by column chromatography using dichloromethane:methanol (50:1) as eluent system to give the title compound (420 mg, 67%) as off-white solid, Mp = 235–237 °C. lit. Mp = 237–238 °C.[50] ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.77 (s, 1H (*NH*)), 7.54–7.50 (m, 1H), 7.11 (d, *J* = 8.2 Hz, 1H), 7.07 (m, 1H), 4.01 (t, *J* = 5.5 Hz, 2H), 3.31 (t, *J* = 5.5 Hz, 2H), 2.00 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.4, 150.7, 150.1, 133.9, 126.9, 123.7, 120.4, 118.3, 40.1, 38.2, 21.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.70 (s, 1H), 7.54–7.50 (m, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 3.94 (t, *J* = 5.6 Hz, 2H), 3.29 (t, *J* = 5.6 Hz, 2H), 1.96 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.7, 150.4, 150.2, 134.3, 126.5, 123.6, 120.9, 116.1, 39.9, 38.7, 20.2.[50]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.06.010.

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