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# Synthesis and in vitro evaluation of new derivatives of 2-substituted-6-fluorobenzo[*d*]thiazoles as cholinesterase inhibitors

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ABSTRACT

A series of novel cholinesterase inhibitors based on 2-substituted 6-fluorobenzo[*d*]thiazole were synthesised and characterised by IR, $^{1}H$, $^{13}C$ and $^{19}F$ NMR spectroscopy and HRMS. Purity was checked by elemental analyses. The novel carbamates were tested for their ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The toxicity of the most active compounds was investigated using a standard in vitro test with HepG2 cells, and the ratio between biological activity and toxicity was determined. In addition, the toxicity of the most active compounds was evaluated against MCF7 cells using the xCELLigence system. Structure–activity relationships reflecting the dependence of cholinesterase inhibitors on the lipophilicity of the compounds as well as on the Taft polar and steric substituent constants are discussed. The specific orientation of the inhibitors in the binding site of acetylcholinesterase was determined using molecular docking of the most active compound.



## 1. Introduction

Two important enzymes from the group of serine hydrolases, acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8; also known as pseudocholinesterase), are usually defined as cholinesterases (ChEs). Structurally, these serine hydrolases belong to the class of proteins known as the esterase/lipase family within the α/β-hydrolase fold superfamily.[1] The major role of AChE is to catalyse the hydrolysis of acetylcholine (ACh) in cholinergic synapses, whereas the function of BChE is less clearly defined because it can hydrolyse ACh as well as other esters.[2,3] The inhibition of these enzymes causes an increase in the concentration of ACh in cholinergic synapses and can subsequently affect a number of pathogenic processes.[2] ChE inhibitors (ChEIs) are used in the treatment of various neuromuscular disorders and have provided the first generation of drugs for the treatment of Alzheimer's disease,[4–9] myasthenia gravis[10,11] and glaucoma.[12,13] An increase in the concentration of ACh can result in an alleviation of the symptoms of these diseases. Thus, research on new ChEIs may be valuable for further progress in the treatment of these diseases.

(*R*)-1-(6-Fluorobenzo[*d*]thiazol-2-yl)ethanamine is a basic scaffold for antimicrobials, herbicides, plant desiccants and defoliant compounds.[14] Generally, benzothiazoles have been reported as a class of compounds with a wide range of biological activities, including antibacterial and antimicrobial,[15] anticancer,[16–18] antitumour,[19] antiviral,[20] anthelmintic,[21] antiparasitic,[22] anti-inflammatory,[23] fungicidal[24] and antioxidant[25] activity. The benzothiazole scaffold has provided several promising candidates, which were investigated as a clinical antitumor candidates.[26] Also other interesting bioactivities of these compounds were described in recent years, such as protein inhibitors[27,28] or biosensors.[29] The benzothiazole ring is also present in various natural marine and terrestrial compounds which have interesting biological activities.[30–32] The moiety of these compounds can serve as a unique and versatile scaffold for experimental drug design. Benzothiazole has an important place in research, especially in synthetic and pharmaceutical chemistry, due to its potent pharmacological activities. Studies on structure–activity relationships have interestingly revealed that a change in the structure of the substituent group at the $C_{(2)}$ position commonly results in a change in bioactivity. Such derivatives

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have received considerable attention due to their potential biological activity.[33]

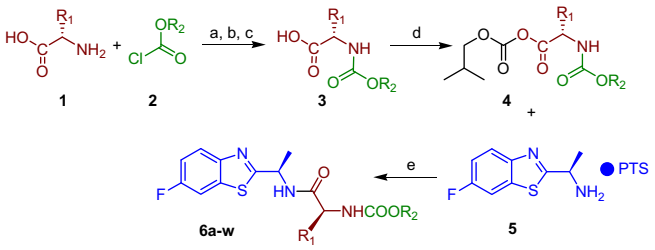
Substituted carbamates are characterised as pseudosubstrate inhibitors or active site-directed irreversible inhibitors of ChEs that, in the presence of the substrate, compete for the AChE active site via nucleophilic attack of the hydroxyl moiety of Ser residues in the enzyme;[2,34–37] nevertheless; non-covalent ChEIs have also been described.[38–40] This publication is a follow-up paper to our previous articles[40,41] dealing with the synthesis and cholinesterase inhibiting ability of novel 2-substituted-6-fluorobenzo[*d*]thiazole derivatives. In the context of research on benzothiazoles previously described by us, new modifications of the 6-fluorobenzothiazole moiety that can trigger interesting biological activity were investigated. Reaching the inhibitory site of action and binding to the active site cleft are influenced by the hydro/lipophilic properties of compounds; therefore, the lipophilicity of the target compounds was experimentally determined using RP-HPLC. The structure–activity relationships between the chemical structure, physical properties and biological activities of the evaluated compounds are discussed. The specific orientation of the inhibitors in the AChE binding site was determined using molecular docking.

One of the main goals in drug discovery is to evaluate the safety of potential therapeutic agents. Cytotoxicity assays are among the first in vitro methods used to predict the toxicity of novel compounds. In vitro cytotoxicity assays can also be used to predict human toxicity and for the general (scheme 1) screening of chemicals.[42] For this reason, the studied compounds were also evaluated using two standardised in vitro cytotoxicity assays.

## 2. Chemistry

The synthesis of the desired compounds can be described as a step-by-step synthesis. The first part of synthetic pathway is the two-step preparation of activated 2-[(alkoxycarbonyl)amino] carboxylic acid. At this stage, the amino acid is combined with a selected chloroformate, and the obtained acid is then transformed to a reactive derivate using isobutyl chloroformate. This reactive intermediate reacts with (*R*)-1-(6-fluorobenzo[*d*]thiazol-2-yl) ethanamine, which is released from its *p*-toluene sulphonate salt in situ. All synthetic steps are described in detail in the following paragraphs.

One of the advantages of the applied method of synthesis is the simple preparation of all intermediates. The desired products **6a**–**w** were obtained by filtration or vacuum concentration of the organic phase. On the other hand, the isolation of compounds **3a** or **4w** was possible and not problematic. Finally, the method is applicable to various amino acids and a number of chloroformates.




**Scheme 1.** Synthesis of reactive intermediates **4a**–**4w**. Reagents and conditions: (a) NaOH, *T* <10 °C, 30 min; (b) parallel addition of chloroformate and NaOH, *T* <10 °C, 60 min; (c) HCl, pH 7; (d) TOL, DMBA, isobutyl chloroformate, *T* <10 °C, 15 min, *T* = RT, 45 min; (e) NaOH, *T* = RT, 6 h.

## 3. Liphophilicity

The calculated hydrophobicity (log*P*/C log*P* values) of compounds **6a**–**w** was obtained using two commercial programs, and their experimental lipophilicities were additionally measured by RP-HPLC to determine their capacity factors (*k*) with the subsequent calculation of their log*k* values. This involved the measurement of compound retention times using isocratic conditions with methanol as an organic modifier in the mobile phase. The capacity factor *k* was calculated using a non-polar $C_{18}$ stationary RP column and the corresponding log *k* value was used as a lipophilicity index converted to the log*P* scale.[43] The results are shown in Table 1. A graphical illustration and comparison of the experimentally determined and calculated lipophilicity values is shown and commented on in detail in the Supplementary data associated with this article.

The compounds under investigation could be divided into two groups based on their chemical structure: *Group 1* includes $R^1$ = *n*-propyl substituted compounds **6a**–**k** and *Group 2* contains $R^1$ = isopropyl substituted compounds **6l**–**w**. Generally, it can be stated that the compounds with a branched alkyl substituent showed lower lipophilicity, that is, *Group 1* possessed higher lipophilicity than *Group 2*, and within the individual series, the isopropyl or isobutyl derivatives **6d**,**f**,**o**,**q** showed less lipophilicity than the unbranched derivatives **6c**,**e**,**n**,**p**. The substitution of the alkyl chain by fluorine atoms is noteworthy. According to the assumption, the $-CF_3$ substituted compounds **6i**,**u** possessed less lipophilicity than the $-CCl_3$ derivatives **6h**,**t**. The order of all the fluorine substituted compounds is the following: $-CH_2CF_2CHF_2$ (**6j**,**v**) < $-CH_2CF_3$ (**6i**,**u**) < $-CH_2CF_2CF_2CF_3$ (**6k**,**w**). Thus, it can be concluded that the log *k* data specify lipophilicity within the series of compounds.

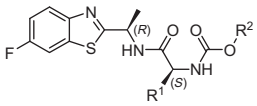
## 4. In vitro evaluation of cholinesterase/anticholinesterase activity

All the prepared compounds were tested for their inhibition potency using AChE from *Electrophorus electricus* (Sigma–Aldrich) and BChE from equine serum (Sigma–Aldrich). The activities of the compounds were compared with the internal standards rivastigmine (RIV, Exelon®) and galanthamine (GLT, Reminyl®) (Table 1). These standards were chosen due to their different structures. While rivastigmine is a classical acylating pseudo-reversible carbamate cholinesterase inhibitor that inhibits both AChE and BChE, galanthamine is a non-acylating competitive reversible cholinesterase inhibitor as well as an allosteric ligand at nicotinic acetylcholine receptors. The choice of these reference drugs with different mechanisms of action can provide relevant results. The results are summarised in Table 1 and are expressed as 50% inhibitory concentration ($IC_{50}$ [μmol/L]). The $IC_{50}$ represents the concentration of inhibitor required for 50% inhibition of the mentioned enzymes.

Based on these results (see Table 1), it is evident that effective AChE inhibitors are different from BChE inhibitors. Both ChEs differ from each other by the distribution of amino acids in the cavity and the peripheral sites of the enzymes. Some aromatic amino acids typical for AChE (e.g., phenylalanine) are replaced in the structure of BChE with aliphatic amino acids (e.g., valine, leucine), and the active site of both ChEs is thus sterically accessible to substrates differing in terms of bulkiness or the ability to interact with amino acids within the peripheral site.[2,3,45–48]

All the discussed carbamate-like compounds demonstrated activity comparable with or higher than that of the standard rivastigmine and slightly lower than that of the standard galanthamine. In general, *Group 1* showed higher activity, particularly in

**Table 1**
List of prepared derivates **6a**–**6w** and their inhibitory activity against AChE and BChE expressed as $IC_{50}$ [μmol/L] in comparison with standards rivastigmine (RIV) and galanthamine (GLT)



| Compd | $R^1$ | $R^2$ | $IC_{50}$ (μmol/L) | | log*k* | log*P*/C log*P* | log*P* | $\sigma^{*44}$ | $E_S^{44}$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | AChE | BChE | | ChemOffice | ACD/LogP | | |
| **6a** | (*S*)-$(CH_2)_2CH_3$ | $-CH_3$ | 48.80 ± 0.35 | 20.42 ± 0.20 | 0.4166 | 3.19/2.64611 | 3.20 ± 0.69 | 0 | 0 |
| **6b** | (*S*)-$(CH_2)_2CH_3$ | $-C_2H_5$ | 34.77 ± 0.40 | 14.10 ± 0.13 | 0.4873 | 3.52/3.17511 | 3.73 ± 0.69 | −0.10 | −0.07 |
| **6c** | (*S*)-$(CH_2)_2CH_3$ | $-(CH_2)_2CH_3$ | 28.54 ± 0.25 | 19.25 ± 0.18 | 0.5873 | 4.01/3.70411 | 4.26 ± 0.69 | −0.115 | −0.36 |
| **6d** | (*S*)-$(CH_2)_2CH_3$ | $-CH(CH_3)_2$ | 21.31 ± 0.20 | 32.20 ± 0.29 | 0.5752 | 3.84/3.48411 | 4.08 ± 0.69 | −0.19 | −0.47 |
| **6e** | (*S*)-$(CH_2)_2CH_3$ | $-(CH_2)_3CH_3$ | 28.00 ± 0.21 | 19.58 ± 0.18 | 0.7231 | 4.43/4.23311 | 4.80 ± 0.69 | −0.13 | −0.39 |
| **6f** | (*S*)-$(CH_2)_2CH_3$ | $-CH_2CH(CH_3)_2$ | 20.22 ± 0.25 | 26.13 ± 0.23 | 0.7063 | 4.41/4.10311 | 4.61 ± 0.69 | −0.125 | −0.93 |
| **6g** | (*S*)-$(CH_2)_2CH_3$ | $-CH_2CH_2Cl$ | 35.38 ± 0.32 | 54.11 ± 0.55 | 0.5140 | 3.90/3.23811 | 4.00 ± 0.70 | 0.385 | NF |
| **6h** | (*S*)-$(CH_2)_2CH_3$ | $-CH_2CCl_3$ | 28.59 ± 0.27 | 62.69 ± 0.63 | 0.8297 | 4.91/4.95411 | 5.77 ± 0.77 | NF | NF |
| **6i** | (*S*)-$(CH_2)_2CH_3$ | $-CH_2CF_3$ | 29.26 ± 0.27 | 59.00 ± 0.48 | 0.5825 | 4.16/3.43911 | 4.50 ± 0.87 | NF | NF |
| **6j** | (*S*)-$(CH_2)_2CH_3$ | $-CH_2CF_2CHF_2$ | 30.71 ± 0.32 | 43.95 ± 0.38 | 0.5415 | 4.11/3.73611 | 4.61 ± 0.91 | NF | NF |
| **6k** | (*S*)-$(CH_2)_2CH_3$ | $-CH_2CF_2CF_2CF_3$ | 46.01 ± 0.45 | 59.75 ± 0.55 | 0.9463 | 5.37/4.89911 | 6.54 ± 1.07 | NF | NF |
| **6l** | (*S*)-$CH(CH_3)_2$ | $-CH_3$ | 91.23 ± 0.89 | 32.00 ± 0.29 | 0.4113 | 3.17/2.51611 | 3.02 ± 0.69 | 0 | 0 |
| **6m** | (*S*)-$CH(CH_3)_2$ | $-C_2H_5$ | 153.0 ± 1.52 | 35.60 ± 0.33 | 0.4834 | 3.51/3.04511 | 3.55 ± 0.69 | −0.10 | −0.07 |
| **6n** | (*S*)-$CH(CH_3)_2$ | $-(CH_2)_2CH_3$ | 108.6 ± 1.15 | 70.45 ± 0.64 | 0.5851 | 3.99/3.57411 | 4.08 ± 0.69 | −0.115 | −0.36 |
| **6o** | (*S*)-$CH(CH_3)_2$ | $-CH(CH_3)_2$ | 95.04 ± 0.99 | 41.10 ± 0.35 | 0.5725 | 3.82/3.35411 | 3.90 ± 0.69 | −0.19 | −0.47 |
| **6p** | (*S*)-$CH(CH_3)_2$ | $-(CH_2)_3CH_3$ | 68.05 ± 0.66 | 62.50 ± 0.58 | 0.7135 | 4.41/4.10311 | 4.61 ± 0.69 | −0.13 | −0.39 |
| **6q** | (*S*)-$CH(CH_3)_2$ | $-CH_2CH(CH_3)_2$ | 49.26 ± 0.52 | 41.10 ± 0.45 | 0.7041 | 4.39/3.97311 | 4.43 ± 0.69 | −0.125 | −0.93 |
| **6r** | (*S*)-$CH(CH_3)_2$ | $-CH_2CH_2Cl$ | 47.50 ± 0.42 | 54.20 ± 0.62 | 0.5014 | 3.88/3.10811 | 3.82 ± 0.70 | 0.385 | NF |
| **6s** | (*S*)-$CH(CH_3)_2$ | $-CH_2CH_2Br$ | 47.15 ± 0.40 | ND | 0.5418 | 4.00/3.24811 | 4.00 ± 0.69 | NF | NF |
| **6t** | (*S*)-$CH(CH_3)_2$ | $-CH_2CCl_3$ | 30.30 ± 0.32 | 26.56 ± 0.20 | 0.8043 | 4.89/4.82411 | 5.59 ± 0.77 | NF | NF |
| **6u** | (*S*)-$CH(CH_3)_2$ | $-CH_2CF_3$ | 49.40 ± 0.45 | 72.00 ± 0.80 | 0.5643 | 4.15/3.30911 | 4.32 ± 0.88 | NF | NF |
| **6v** | (*S*)-$CH(CH_3)_2$ | $-CH_2CF_2CHF_2$ | 28.97 ± 0.25 | 33.80 ± 0.32 | 0.5237 | 4.09/3.60611 | 4.43 ± 0.91 | NF | NF |
| **6w** | (*S*)-$CH(CH_3)_2$ | $-CH_2CF_2CF_2CF_3$ | 85.51 ± 0.77 | 83.20 ± 0.75 | 0.9218 | 5.35/4.76911 | 4.62 ± 1.06 | NF | NF |
| | **RIVastigmine** (Exelon®) | | 501 ± 3.08 | 19.95 ± 0.20 | | 2.36/2.099 | | | |
| | **GaLanThamin**e (Reminyl®) | | 4 ± 0.13 | 7.96 ± 0.59 | | 1.41/1.025 | | | |

ChE inhibitions are expressed as mean ± SD ($n$ = 3 experiments). Comparison of calculated lipophilicities (log*P*/C log*P*) with experimentally determined log*k* values, Taft polar substituent constants ($\sigma^*$) and Taft steric substituent constants ($E_S$).ND—not determined, NF—not found.

case of non-halogenated $R^2$ substituents in comparison with *Group 2*. Based on this fact, it can be assumed that $R^1$ substitution by the unbranched *n*-propyl chain plays a crucial role for AChE/BChE inhibition (see Figs. 1 and 2). Whereas the *n*-propyl chain in position $R^1$ is necessary for both types of AChE/BChE inhibitors, for higher AChE-inhibiting activity, $R^2$ branched long-chain alkyl substitution of the carbamate ester seems to be preferred (see Fig. 1) in contrast to BChE inhibitors, where a short unbranched alkyl chain at position $R^2$ is more favoured (see Fig. 2). Within a halogenated $R^2$ chain, $R^1$ substitution seems to be not so important. Based on the docking study (see below), it can be stated that the studied compounds could act as 'bulky' blockers of entrance into the active site cleft; this observation agrees with the above-mentioned fact that, due to different distribution of amino acids in the peripheral site of both ChEs, BChE is resistant to bulky inhibitors. Isobutyl-(*S*)-1-[(*R*)-1-(6-fluorobenzo[*d*]thiazol-2-yl)ethylcarbamoyl]butyl-carbamate (**6f**) and isopropyl-(*S*)-1-[(*R*)-1-(6-fluorodrobenzo[*d*]thiazol-2-yl)ethylcarbamoyl]butyl carbamate (**6d**) were the most active AChE-inhibiting compounds, while ethyl-(*S*)-1-[(*R*)-1-(6-fluoro benzo[*d*]thiazol-2-yl)ethylcarbamoyl]butylcarbamate (**6b**) was the most active BChE inhibitor.

The shapes of the biological response curves shown in Figures 1 and 2 are typical of *n*-alkyl homologous series, when activity can alternate with the number of carbon atoms (with a change in a long side/straight chain). The inhibition ability of carbamates (binding to the active site of AChE/BChE) is influenced by the bulkiness of the substituents, due to the difficulty of larger inhibitors to enter the narrow enzyme active site cleft, as well as the strictly balanced hydro/lipophilic properties of the inhibitors, taking into account the fact that hydrophobic substituents accelerate inhibitor entry into the active site cleft of the enzyme.[6] The rate of carbamylation depends on a delicate balance between the length of the residue and its degree of freedom of rotation.[4]

A number of papers refer to the fact that an increase in *N*-alkyl chain length results in ChE inhibition. This fact is in compliance with the observation that the introduction of bulky hydrophobic

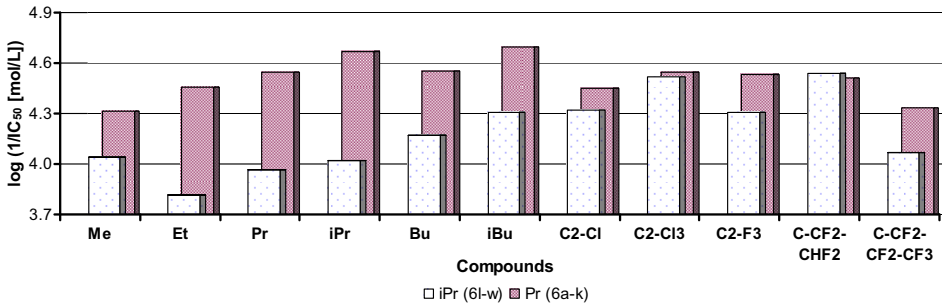



**Figure 1.** Dependence of AChE inhibition (log 1/$IC_{50}$ [mol/L]) on long alkyl chain of $R^2$ substitution within *Group 1* (Pr) and *Group 2* (*i*Pr).

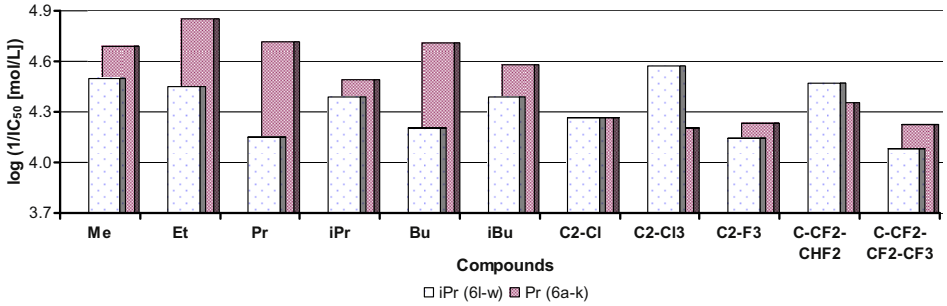



**Figure 2.** Dependence of BChE inhibition ($\log 1/IC_{50}$ [mol/L]) on long alkyl chain of $R^2$ substitution within *Group 1* (Pr) and *Group 2* (*i*Pr).

groups at the carbamoyl nitrogen leads to compounds with better AChE inhibition[2,34,37,49] and, vice versa, to compounds with low BChE inhibition.[46] It was also observed that activity increases with increasing electron-withdrawing effect.[50] From these facts, it can be concluded that ChE inhibition correlates with lipophilicity, electronic, inductive or polar properties and steric effects.[43]

The dependence of AChE inhibition ($\log 1/IC_{50}$ [mol/L]) on $\log k$ within *Group 1* is illustrated in Figure 3A. In the set of 11 tested alkyl- and/or halogeno-substituted compounds, a quasi-parabolic dependence was found. It can be concluded that, for optimum activity, the range of lipophilicity expressed as $\log k$ 0.6–0.7 and the branched alkyl chain are advantageous. Also, a multihalogenated $C_2$ or $C_3$ chain seems to be advantageous. Within *Group 2*, the dependence of AChE inhibition on $\log k$ did not seem to be as clear (lipophilicity was only a secondary parameter) but, generally, AChE-inhibiting activity increases with prolongation of the chain and an increasing number of halogens in the chain (see Table 1). The substitution with a branched alkyl at the $R^2$ position positively affected the activity as well. Universal advantageous $R^2$ substituents for both groups were $-CH_2CCl_3$ and $-CH_2CF_2CHF_2$, because **6h**/**t**, **6j**/**v** expressed similar activity (see Table 1). From the data shown in Table 1, it follows that lipophilicity for both groups was only a secondary parameter for BChE inhibition. Nevertheless, in both cases, activity decreased with increasing lipophilicity and, similarly to AChE inhibition, halogenated $R^2$ substituents seemed to be more advantageous in *Group 2* than in *Group 1*.

ChE inhibition activity was also correlated with the Taft polar and steric substituent constants $\sigma^*$ and $E_S$. Values $\sigma^*$ represent field, inductive and resonance contributions of the substituent and $E_S$ values represent the bulkiness of substituent. Both Taft parameters are listed in Table 1. These parameters were not found in the literature for this series of halogenated substituents. From the data in Table 1, it is evident that AChE inhibition activity within both groups was particularly influenced by the bulkiness of the substituent, that is, the Taft steric substituent constant $E_S$, which is illustrated Figure 4. For *Group 1* and *Group 2*, it can be stated that AChE inhibition dramatically decreased with decreasing bulkiness. The AChE inhibition activity of these compounds was also positively influenced by a negative polar parameter $\sigma^*$.

The dependences of AChE inhibition ($\log 1/IC_{50}$ [mol/L]) on the Taft steric substituent constant $E_S$ is illustrated in Figure 4. It is evident that AChE inhibition of compounds **6a**–**f** was positively influenced by a negative polar parameter $\sigma^*$ as well as by a bulky substituent. AChE inhibition of compounds **6l**–**q** was again positively influenced by a bulky substituent (Fig. 4B), but the effect of the $\sigma^*$ parameter seemed to be only secondary.

As discussed above, different BChE inhibition results were observed for the two groups. The dependence of BChE inhibition ($\log 1/IC_{50}$ [mol/L]) on the Taft polar and steric parameters $\sigma^*$ and $E_S$ within *Group 1* is illustrated in Figure 5A and B; the results for *Group 2* are illustrated in Figure 6A and B. Figure 5A and B show the bilinear dependence of BChE inhibiting activity on both Taft parameters with a preferred polarity value of −0.10 (compound **6b**) and bulkiness expressed as $E_S = -0.07$ (ethyl, compound **6b**). Within *Group 2*, the least active carbamates/outliers, that is, **6n** and **6p**, were eliminated; the dependence of BChE inhibition ($\log 1/IC_{50}$, [mol/L]) on $\sigma^*$ or $E_S$ is presented in Figure 6A and B. In both cases, the dependence of activity on the Taft polar and steric parameters was linear with the correlation coefficient $R = 0.9198$ or $R = 0.8610$, respectively. Therefore, it can be stated that within the $R^2$ substituents, minimal steric (Fig. 6B) and polar (Fig. 6A) effects seem to be important for the inhibition of BChE.

Different ChE inhibition results were observed for halogenated compounds. In general, it can be concluded that halogenated $R^2$ substituents showed fewer differences between AChE/BChE activity in comparison with non-halogenated alkyl chain substituents, and in the context of these facts, the highly electronegative $-CCl_3$ moiety (compounds **6h**,**t**) showed high AChE and BChE inhibition activity within *Groups 1* and *2*.

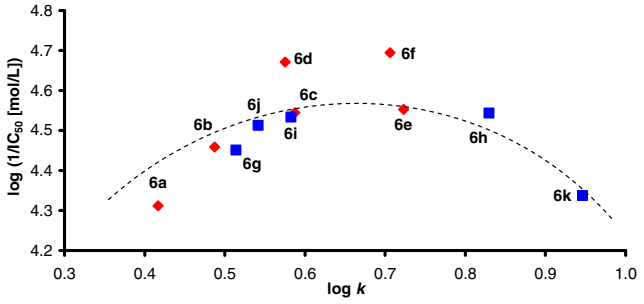



**Figure 3.** Dependence of AChE inhibition ($\log 1/IC_{50}$ [mol/L]) on the lipophilicity of compounds **6a**–**k**.

## 5. Molecular docking

Structural analysis of AChE revealed that the active site is placed near the bottom of a narrow gorge imbedded halfway into the protein and 14 aromatic residues lining a substantial portion of the surface of the gorge. This cavity was named the 'active site gorge' and, further at the gorge mouth a peripheral anionic binding site (PAS) was found. The active site of AChE contains: (i) an esteratic site (ES) comprising the catalytic triad Ser-His-Glu, which is located at the bottom of the gorge; (ii) an oxyanion hole (OAH) that stabilizes the tetrahedral intermediate binding of the carbamate carbonyl group; (iii) an acyl binding site (ABS) that binds the acetyl group of ACh or the alkyl moiety of carbamate inhibitors; and (iv) an anionic substrate binding site (AS) that contains a small number of negative charges but many aromatic residues, where the quaternary ammonium pole of ACh and of various active site ligands binds through a preferential interaction of quarternary nitrogens

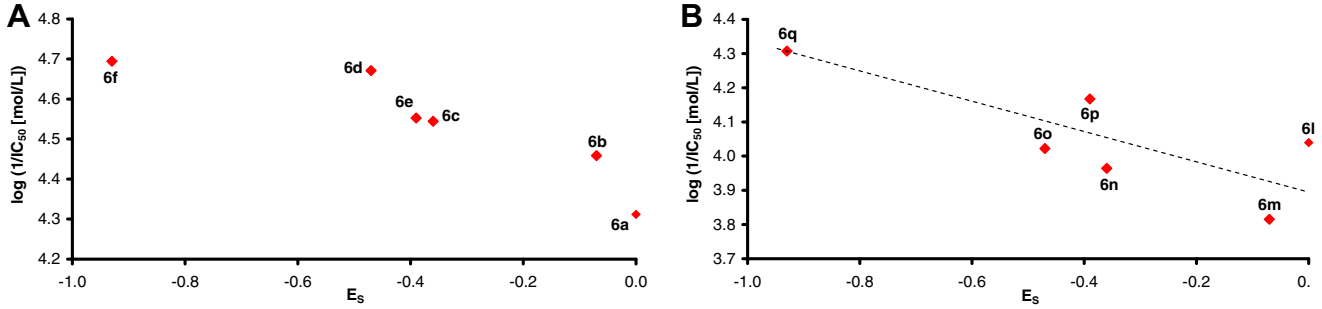



**Figure 4.** Dependence of AChE inhibition ($\log 1/IC_{50}$ [mol/L]) on Taft steric substituent constants expressed as $E_S$: Figure 4A—compounds **6a–f**; Fig. 4B—compounds **6l–q**.

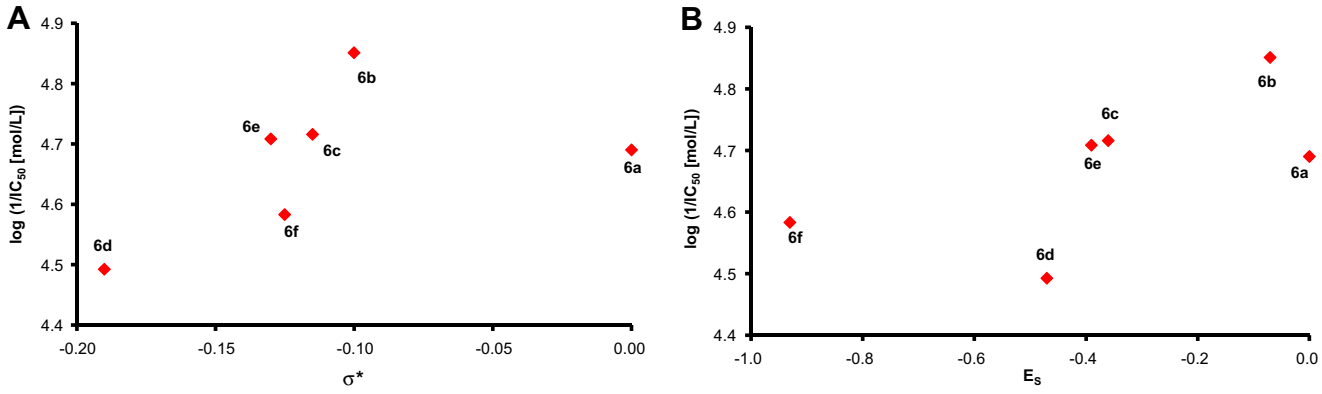



**Figure 5.** Dependence of BChE inhibition ($\log 1/IC_{50}$ [mol/L]) of compounds **6a–f** on the Taft polar $\sigma^*$ (**5A**) and steric $E_S$ (**5B**) substituent constants.

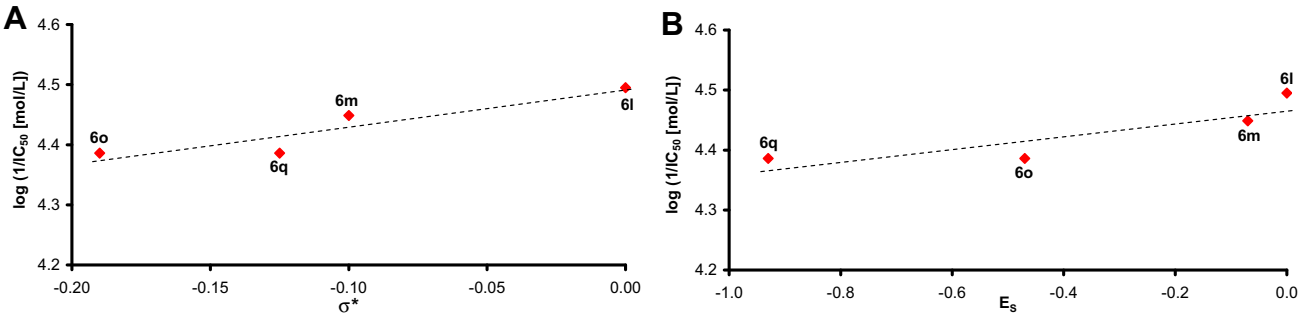



**Figure 6.** Dependence of BChE inhibition ($\log 1/IC_{50}$ [mol/L]) of compounds **6l–q** on the Taft polar $\sigma^*$ (**6A**) and steric $E_S$ (**6B**) substituent constants. Compounds **6n** and **6p** were excluded and are not plotted.

or a partial positive charge generated by electron-withdrawing moieties.[35,38,51]

Molecular docking was carried out by Gold 5.0.1 (CCDC, UK) using the primary GoldScore function and ASP (Astex Statistical Potential) function for re-scoring the docking results to produce the optimal conformations for the docked ligands. Additionally, the GoldScore values were used to formulate the QSAR theory between the results of AChE inhibition studies and the structural parameters. The majority of best ranked conformations for the applied ligands (18 out of 22, while other 2 results comply with the proposed arrangement of ligands when using the second best ASP score) are based on the same arrangement of the molecules within the active site gorge characterized, similarly as reported by Paz et al.,[52] by insignificant direct interaction with the catalytic triad. On the other hand, they are able to bind in the middle part of the peripheral anionic site (PAS) by means of hydrogen bonds between the amide moiety (either by the NH group or the oxygen atom) and the Tyr121 side chain (O–O = 2.576–3.924 Å, or N–O = 2.632–3.696 Å) and between the amide oxygen and the Tyr334 side chain (O–O = 2.917–2.945 Å). Moreover, the possible interactions of the carbamate moiety with the Tyr70 OH moiety (O–O = 3.816–3.881 Å) or the amide nitrogen from Phe288 (O–N = 2.913–2.973 Å) were considered as probable. Other interactions, like π–π interaction between the benzothiazole moiety and the Trp84 side chain (centroid–centroid ≈3.384 Å) and F–H–N/O hydrogen bond formation, which was identified in our molecular docking experiments between the fluorine heterocyclic substitution and Glu199 (F–O = 2.558–3.315 Å), Tyr130 (F–O = 2.626–3.618 Å) or the NH moiety from the backbone of Ser124 (F–O = 2.880–3.408 Å), see Figure 7, could also play an important role in positioning and stabilizing the ligands inside the active site gorge. The low-energy conformations of all investigated compounds confirmed that the non-polar parts of the molecules are oriented to the outer part of the active site gorge. The graphical

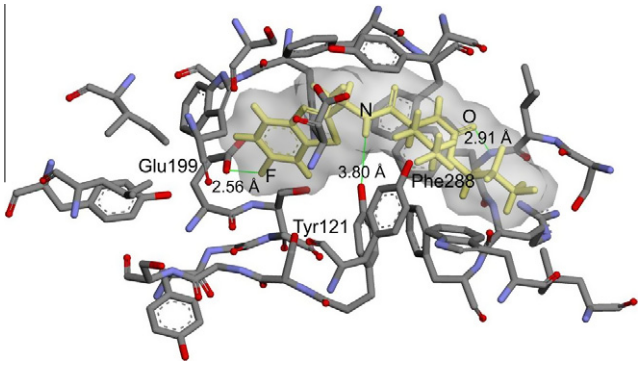



**Figure 7.** Example of interactions between docked molecule of **6c** (coloured in yellow and shown with solvent accessible surface) and amino acid residues from active site gorge, within range of 3 Å. Interacting atoms from ligand and amino acid residues are depicted and corresponding distances between non-hydrogen atoms are shown.

model of the AChE molecule, involving the AChE inhibitors and demonstrating the conformations of docked ligands within the active site gorge, is shown in Figure 8. On the basis of such orientation and the set of interactions, which were identified between the ligands and the protein residues within the gorge, it can be hypothesized that the studied compounds could act as 'bulky'-blockers the entrance of the normal ionic substrate (ACh) into the active site gorge.

## 6. In vitro cytotoxicity assay

To evaluate the potential harmful effects of the tested compounds on human cells, a standard in vitro method using the human liver cell line HepG2 was used. Representative members of the investigated group (**6d**, **6f**, **6m** and **6n**) were tested for their in vitro cytotoxicity. It was observed that **6d** and **6f** at concentrations of >10 μmol/L, **6n** at concentrations >100 μmol/L and **6m** only at concentrations of >1000 μmol/L were effective in inhibiting the growth of HepG2 cells. Compounds **6d** and **6f** at concentrations ranging from 0.001 to 10 μmol/L did not exhibit a dose-dependent growth inhibitory effect. Similarly, **6n** at concentrations <100 μmol/L and **6m** even at concentrations <1000 μmol/L showed dramatically decreased efficacy against the growth of HepG2 cells. To evaluate the effect of **6d**, **6f**, **6n** and **6m** on the cell viability of HepG2 cells, the MTS assay was performed. Cytotoxicity was also expressed as $IC_{50}$, that is, the concentration at which the viability of cells decreased to 50% of the maximal viability (Table 2). The $IC_{50}$ was determined for **6d**, **6f** and **6n**. Only an estimate could be determined for compound **6m**, because the course of the toxicity curve did not allow for a valid calculation of the $IC_{50}$ value. A graphical representation of this assay is shown in the supplementary data for this paper.

**Table 2**
Cytotoxicity of tested compounds in HepG2 and MCF7 cells

| Compd | $IC_{50}$ (μmol/L) | |
|---|---|---|
| | CellTiter96 (HepG2) | RTCA SP (MCF7) |
| **6d** | 273 | 1213 |
| **6f** | 275 | 612 |
| **6n** | 1734 | 4393 |
| **6m** | >5000 | >4393 |

The xCELLigence RTCA SP system was used for real-time and time-dependent analysis of the cellular response of MCF7 cells. A parameter termed Cell Index (CI) was derived to represent cell status based on the measured electrical impedance. It was observed that a reduction in the CI value occurred immediately after treatment with 1000 and 2500 μmol/L of **6d**, **6f**, **6n** and **6m**. These data suggest that **6d**, **6f**, **6n** and **6m**, at the concentrations 1000 and 2500 μmol/L, inhibit the proliferation of MCF7 cells. However, treatment with 1000 μmol/L of **6n** and **6m** and 2500 μmol/L of **6m** initially inhibited MCF7 cell proliferation, but the cells eventually recovered to the level of the vehicle control treated cells. MCF7 cells treated at lower concentrations (0.01–100 μmol/L) of investigated compounds **6d**, **6f**, **6n** and **6m** proliferated in parallel to cells treated with the vehicle control, as indicated by an increase in the CI values. These results correlate with the data obtained from the MTS assay. However, the $IC_{50}$ values of MCF7 cells (Table 2) were higher than those of HepG2 cells, suggesting the selective cytotoxicity of **6d**, **6f**, **6n** and **6m** towards HepG2 cells. The course of a measurement for each compound is shown in the separate part of the Supplementary data section.

## 7. Conclusion

In this study, twenty-two original derivates of (*R*)-1-(6-fluoro benzo[*d*]thiazol-2-yl)ethanamine were designed, synthesised and fully characterised. The synthetic pathway was quick and effective. All the compounds were evaluated in vitro for their ability to inhibit acetylcholinesterase and butyrylcholinesterase. The anticholinesterase activities were compared with those of rivastigmine and galanthamine as the standards. Isobutyl-(*S*)-1-[(*R*)-1-(6-fluorobenzo[*d*]thiazol-2-yl)ethylcarbamoyl]butylcarbamate (**6f**) and isopropyl-(*S*)-1-[(*R*)-1-(6-fluoro benzo[*d*]thiazol-2-yl)ethylcarbamoyl]butylcarbamate (**6d**) expressed the highest AChE-inhibiting

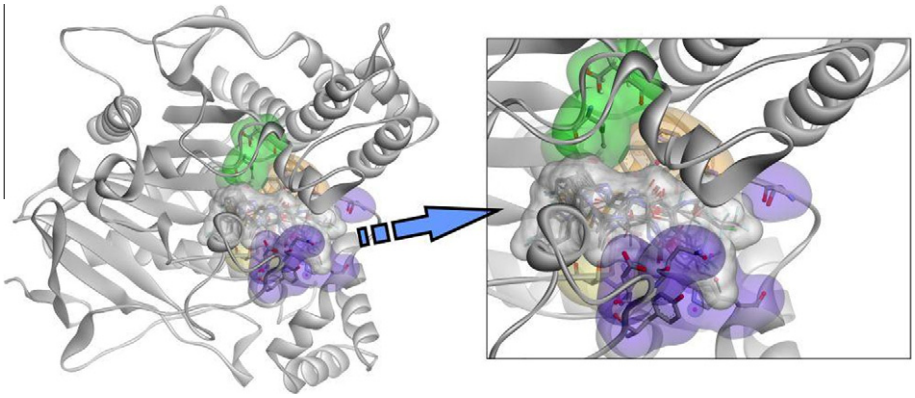


**Figure 8.** Schematic visualization of basic parts of AChE active site gorge, showing catalytic triad (green), oxanion hole (yellow), acyl binding site (orange), anionic substrate binding site (blue) and highest ranked conformations of studied compounds with solvent-accessible surface (grey). Insert (on the right) contains enlarged view at active site part of AChE molecule.

activity, while ethyl-(*S*)-1-[(*R*)-1-(6-fluoro benzo[*d*]thiazol-2-yl)ethylcarbamoyl]butylcarbamate (**6b**) showed the highest BChE inhibition. The cytotoxicity of the selected compounds was determined using HepG2 and MCF7 cells. The tested compounds showed low cytotoxicity. It can be assumed that $R^1$ substitution with an *n*-propyl chain is more advantageous for high AChE/BChE inhibition. $R^2$ substitution with a branched long-chain alkyl is crucial for potent AChE inhibition, while $R^2$ substitution by a short unbranched alkyl chain seems to be preferred for potent BChE inhibition. AChE/BChE inhibition was especially dependent on the Taft constants. In the case of halogenated $R^2$ chains, $R^1$ substitution seemed to be not as important, and it seemed that the $CCl_3$ moiety was a universal non-selective substituent of carbamate esters for AChE/BChE inhibition. Based on the comprehensive docking study, it can be proposed that the studied compounds could act as 'bulky' blockers of ACh entry into the active site cleft. Although primary screening of the discussed compounds confirmed medium or rather moderate activity, these noteworthy compounds surely deserve further attention, because their chemical analogues and alterations of their physico-chemical properties may confer some positive changes in terms of biological effect.

## 8. Experimental

### 8.1. Chemistry

#### 8.1.1. Instrumentation and chemicals

All reagents and solvents were purchased from commercial sources (Sigma–Aldrich, Merck, Acros Organics). Phosgene was purchased from Synthesia (Pardubice, Czech Republic). Fluorinated alcohols were purchased from Fluorochem UK. Commercial grade reagents were used without further purification. Reactions were monitored by thin layer chromatography plates coated with 0.2 mm silica gel 60 $F_{254}$ (Merck). TLC plates were visualized by the UV irradiation (254 nm).

All melting points were determined on a Melting Point B-545 apparatus (Buchi, Switzerland) and are uncorrected. Infrared spectra (KBr pellets) were recorded on FT-IR spectrometer Nicolet 6700 FT-IR in the range of 400–4000 $cm^{-1}$. The NMR spectra were measured in DMSO-$d_6$ solutions at ambient temperature on a Bruker Avance III 400 spectrometer (400 MHz for $^1H$, 100 MHz for $^{13}C$ and 376.5 MHz for $^{19}F$). Coupling constants are expressed in Hz. Proton chemical shifts in $CDCl_3$ are related to the internal TMS ($\delta = 0.0$) and in DMSO-d6 to the middle of the solvent multiplet ($\delta = 2.50$). $^{13}C$ NMR spectra were measured using APT pulse sequence optimized to $^{1J}(^{13}C, ^1H) = 145$ Hz. Carbon chemical shifts are referenced to the middle of the solvent's multiplet ($\delta = 77.0$ or 39.5 in $CDCl_3$ or DMSO-$d_6$, respectively). Fluorine-19 NMR spectra were measured using Waltz-16 proton decoupling and were standardised against fluorobenzene as the secondary external standard ($\delta = -113.1$ against $CFCl_3$ as the primary standard[44]) td 64 k zero filled to 128 k. Chemical shifts for the AB systems were calculated as weighed average of the line positions weighted by the peak intensities. Elemental analyses (C, H, N) were performed on an automatic microanalyser EA1110CE (Fisons Instruments, Milano, Italy). High resolution mass spectra were measured using a LTQ Orbitrap Hybrid Mass Spectrometer (Thermo Electron Corporation, USA) with direct injection into APCI source (400 °C) in the positive mode.

#### 8.1.2. General experimental procedure for synthesis of chloroformates 2[42]

Phosgene (0.5 mol) was condensed in anhydrous diethyl ether (150 mL) at ice bath temperature ($T$ <10 °C). A selected alcohol (0.2 mol) was added to diethyl ether solution for 15 min. An anhydrous triethylamine or DIEA (0.22 mol) was added dropwise to the reaction mixture. Temperature was kept between 4 and 12 °C, and addition of a base was accomplished over a 30 min period. Reaction mixture was stirred for 3 h at room temperature. After that the excess of phosgene was removed from the reaction mixture with a fine stream of argon. The outgoing gas was discharged through the water solution of sodium hydroxide. The precipitated hydrochloride salt was collected by filtration. An appropriate chloroformate was obtained by fractional distillation as a colourless liquid.

#### 8.1.3. Characterization of prepared chloroformates

**8.1.3.1. Characterization data of 2,2,2-trifluoroethyl chloroformate 2a.** Colourless liquid; yield 76%; bp 75–76 °C. $^1H$ NMR ($CDCl_3$) $\delta = 4.63$ (q, 2H, $^3J(^1H,^{19}F) = 8$). $^{13}C$ NMR ($CDCl_3$) $\delta = 150.6$, 121.9 (q, $^1J(^{19}F,^{13}C) = 280.8$ Hz), 65.5 (q, $^2J(^{19}F,^{13}C) = 37.8$ Hz). $^{19}F$ NMR ($CDCl_3$) $\delta = -74.7$.

**8.1.3.2. Characterization data of 2,2,3,3-tetrafluoropropyl chloroformate 2b.** Colourless liquid; yield 78.0%; bp 112–113 °C. $^1H$ NMR ($CDCl_3$) $\delta = 5.92$ (tt, 1H, $^2J(^1H,^{19}F) = 53.2$, $^3J(^1H,^{19}F) = 3.2$, H-3), 4.67 (tt, 2H, $^3J(^1H,^{19}F) = 12.4$, $^4J(^1H,^{19}F) = 1.2$, H-1). $^{13}C$ NMR ($CDCl_3$) $\delta = 150.5$, 113.3 (tt, $^1J(^{19}F,^{13}C) = 250.2$ Hz, $^2J(^{19}F,^{13}C) = 28.5$ Hz), 109.1 (tt, $^1J(^{19}F,^{13}C) = 251.5$ Hz, $^2J(^{19}F,^{13}C) = 38.1$ Hz), 64.8 (t, $^2J(^{19}F,^{13}C) = 29.3$ Hz). $^{19}F$ NMR ($CDCl_3$) $\delta = -123.7$ (t, $^3J(^{19}F,^{19}F) = 3.8$, F-2), -137.3 (t, $^3J(^{19}F,^{19}F) = 3.8$, F-3).

**8.1.3.3. Characterization data of 2,2,3,3,4,4,4-heptafluorobutyl chloroformate 2a.** Colourless liquid; yield 82%; bp 108–109 °C. $^1H$ NMR ($CDCl_3$) $\delta = 4.75$ (tt, $^3J(^1H,^{19}F) = 12.8$, $^4J(^1H,^{19}F) = 1.2$). $^{13}C$ NMR ($CDCl_3$) $\delta = 150.6$, 117.4 (qt, $^1J(^{19}F,^{13}C) = 287.0$ Hz, $^2J(^{19}F,\ ^{13}C) = 32.6$ Hz), 113.1 (tt, $^1J(^{19}F,^{13}C) = 258.7$ Hz, $^2J(^{19}F,\ ^{13}C) = 30.4$ Hz), 108.5 (tq, $^1J(^{19}F,\ ^{13}C) = 263.3$ Hz, $^2J(^{19}F,^{13}C) = 38.4$ Hz), 64.4 (t, $^2J(^{19}F,\ ^{13}C) = 26.5$ Hz). $^{19}F$ NMR ($CDCl_3$) $\delta = -81.0$ (t, $^4J(^{19}F,\ ^{19}F) = 11.3$, F-4), −120.7–120.89 (m, F-2), −127.7–127.8 (m, F-3).

#### 8.1.4. General experimental procedure for the synthesis of compounds 6a-6w

Amino acid **1** (8.65 mmol) was dissolved in 2.1 mL of distilled water and 2.4 g of 23% water solution of NaOH. The mixture was stirred for 30 min, and during this time it was cooled under 10 °C. Chloroformate **2** (13.63 mmol) and 2.4 g of 23% water solution of NaOH were added concurrently to the amino acid sodium salt solution for 15 min. Reaction mixture was then stirred for 45 min at 10 °C. After that, the pH of the reaction mixture was adjusted with HCl (approx. 2.4 g of 10% water solution). Product **3** was formed, and it was used directly in the next step of synthesis.

Toluene (20 mL) and dimethyl benzylamine (DMBA, 0.155 mmol) were added to the reaction mixture at temperature under 10 °C. Isobutyl chloroformate (8.60 mmoL) was added to the reaction mixture for 15 min. Reaction mixture was then heated to 25 °C and stirred for 45 min. Distilled water (35 mL) was added to the reaction mixture, and the toluene layer was separated. Product **4** was formed in the organic layer.

To the separated organic phase toluene (20 mL) and *p*-toluene sulfonate salt of (*R*)-1-(6-fluorobenzo[*d*]thiazole)ethanamine **5** (8.60 mmol) were added. A solution of sodium hydroxide was added dropwise to the reaction mixture to reach pH 9.7 (approx. 4.5 g of 10% solution). Reaction mixture was stirred for 5 h at room temperature. The pH was maintained on 9.7 with addition of a sodium hydroxide solution.

After that the isolation of final product **6** followed. Two isolation techniques were used for isolation of desired products:

**8.1.4.1. Isolation method A.** This method was used for experiments where a solid compound precipitated during stirring of the

reaction mixture. The crude product was filtered off by vacuum filtration. Crystals were dissolved in hot chloroform, and non-soluble impurities (PTS) were filtered off by simple hot filtration. The clear solution was evaporated till dryness. The residue was crystallized using a chloroform/*n*-hexane mixture.

**8.1.4.2. Isolation method B.** This method was used for experiments where a solid compound did not precipitated from the reaction mixture. Distilled water was added to the reaction mixture and heated to 60–70 °C, then the toluene layer was separated, cooled down and dried with sodium sulphate. The $Na_2SO_4$ was collected by filtration, toluene was partially evaporated under vacuum, and the mixture was left for crystallization at 4 °C overnight. Products were filtered off and dried under vacuum at room temperature. The residue was crystallized using a chloroform/*n*-hexane mixture

**8.1.5. Characterization data of prepared compounds 6a–6w**

**8.1.5.1. Methyl-(*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]butylcarbamate 6a.** White solid; yield 45.7% (isolation method A); mp 180–181 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.85 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.6 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.97 (1H, dd, $J$ = 8.9 Hz, $^4J(^{19}F, ^1H)$ = 4.9 Hz, H4), 7.36 (1H, dt, $J$ = 2.6 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.30 (1H, d, $J$ = 8.3 Hz, N*H*-COOMe), 5.23 (1H, kv, $J$ = 7.2 Hz, H8), 4.05 (1H, m, H10), 3.53 (3H, s, H14), 1.59 (2H, m, H11), 1.55 (3H, d, $J$ = 7.2 Hz, H9), 1.34 (2H, m, H12), 0.88 (3H, m, H13). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.7 (d, $^4J(^{19}F, ^{13}C)$ = 3.2 Hz), 172.2, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 240.9 Hz), 156.5, 149.6 (d $^5J(^{19}F, ^{13}C)$ = 1.5 Hz), 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.9 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.3 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 25.4 Hz), 108.6 (d, $^2J(^{19}F,^{13}C)$ = 27.1 Hz), 54.4, 51.5, 47.4, 34.1, 20.6, 18.7, 13.7. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.6. IR (ATR): 3273, 3287, 3006, 2928, 1691 (CO—carbamate), 1647 (CO—amide), 1541, 1457, 1264, 1236, 1196, 1059, 965, 918, 850, 824, 797, 755, 701, 689 cm$^{-1}$. Anal. Calcd for $C_{16}H_{20}FN_3O_3S$ (353.121): C, 54.38; H, 5.70; N, 11.89. Found: C, 54.69; H, 6.02; N, 11.91. HR-MS: for $C_{16}H_{21}FN_3O_3S$ [M+H$^+$] calcd 354.1282 *m*/*z*, found 354.1282 *m*/*z*.

**8.1.5.2. Ethyl-(*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl] butylcarbamate 6b.** White solid; yield 56.7% (isolation method A); mp 182–184 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.82 (1H, d, $J$ = 7.7 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.6 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.97 (1H, dd, $J$ = 8.9 Hz, $^4J(^{19}F, ^1H)$ = 4.9 Hz, H4), 7.36 (1H, dt, $J$ = 2.8 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.22 (1H, d, $J$ = 8.1 Hz, N*H*-COOMe), 5.23 (1H, kv, $J$ = 7.2 Hz, H8), 4.00 (3H, m, H10, H14), 1.59 (2H, m, H11), 1.55 (3H, d, $J$ = 7.2 Hz, H9), 1.51 (2H, m, H15), 1.34 (2H, m, H12), 0.88 (3H, m, H13). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.7 (d, $^4J(^{19}F, ^{13}C)$ = 3.4 Hz), 172.3, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.7 Hz), 156.1, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.6 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.8 Hz), 108.6 (d, $^2J(^{19}F,^{13}C)$ = 27.4 Hz), 59.8, 54.3, 47.4, 34.0, 20.1, 18.8, 14.6, 13.6. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.6. IR (ATR): 3274, 3255, 1684 (CO—carbamate), 1648 (CO—amide), 1540, 1457, 1373, 1276, 1259, 1238, 1150, 1019, 898, 846, 821, 668 cm$^{-1}$. Anal. Calcd for $C_{17}H_{22}FN_3O_3S$ (367.137): C, 55.57; H, 6.03; N, 11.44. Found: C, 55.69; H, 6.08; N, 11.78. HR-MS: for $C_{17}H_{23}FN_3O_3S$ [M+H$^+$] calcd 368.1439 *m*/*z*, found 368.1440 *m*/*z*.

**8.1.5.3. Propyl-(*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl] butylcarbamate 6c.** White solid; yield 61.8% (isolation method A); mp 177.5–178.5 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.82 (1H, d, $J$ = 7.5 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.6 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H7), 7.97 (1H, dd, $J$ = 9.1 Hz, $^4J(^{19}F, ^1H)$ = 4.9 Hz, H4), 7.37 (1H, dt, $J$ = 2.7 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.22 (1H, d, $J$ = 8.2 Hz, N*H*-COOMe), 5.24 (1H, kv, $J$ = 7.2 Hz, H8), 4.05 (1H, m, H10), 3.90 (2H, t, $J$ = 6.3 Hz, H14), 1.56 (7H, m, H9,H11, H15), 1.34 (2H, m, H12), 0.88 (6H, m, H13, H16). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.2 Hz), 172.3, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 240.9 Hz), 156.2, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.9 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.5 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.9 Hz), 108.6 (d, $^2J(^{19}F,^{13}C)$ = 27.3 Hz), 65.4, 54.3, 47.4, 34.0, 22.0, 20.1, 18.8, 13.6, 10.3. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ -116.6. IR (ATR): 3282, 2949, 2927, 1680 (CO—carbamate), 1649 (CO—amide), 1537, 1455, 1381, 1276, 1259, 1136, 1066, 926, 907, 854, 821, 780, 745, 677 cm$^{-1}$. Anal. Calcd for $C_{18}H_{24}FN_3O_3S$ (381.15): C, 56.67; H, 6.34; N, 11.02. Found: C, 56.77; H, 6.43; N, 11.15. HR-MS: for $C_{18}H_{25}FN_3O_3S$ [M+H$^+$] calcd 382.1595 *m*/*z*, found 382.1591 *m*/*z*.

**8.1.5.4. Isopropyl-(*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl] butylcarbamate 6d.** White solid; Yield 88.1% (isolation method A); mp 178–178.5 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.80 (1H, d, $J$ = 7.8 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.6 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.97 (1H, dd, $J$ = 8.9 Hz, $^4J(^{19}F, ^1H)$ = 4.7 Hz, H4), 7.36 (1H, dt, $J$ = 2.6 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.11 (1H, d, $J$ = 8.2 Hz, N*H*-COOMe), 5.24 (1H, kv, $J$ = 7.2 Hz, H8), 4.74 (1H, m, $J$ = 6.3 Hz, H14), 4.03 (1H, m, H10), 1.59 (2H, m, H11), 1.55 (3H, d, $J$ = 7.2 Hz, H9), 1.34 (2H, m, H12), 1.16 (6H, d, $J$ = 6.2 Hz, H15), 0.88 (3H, m, H13). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.7 (d, $^4J(^{19}F, ^{13}C)$ = 3.5 Hz), 172.3, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 240.9 Hz), 155.7, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.7 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.8 Hz), 108.6 (d, $^2J(^{19}F,^{13}C)$ = 26.9 Hz), 67.0, 54.3, 47.4, 34.0, 22.0, 20.1, 18.8, 13.6. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ -116.6. IR (ATR): 3300, 3267, 2971, 2940, 1678 (CO—carbamate), 1644 (CO—amide), 1568, 1537, 1457, 1385, 1277, 1263, 1239, 1218, 1195, 1180, 1112, 1062, 1041, 1024, 960, 847, 820 cm$^{-1}$. Anal. Calcd for $C_{18}H_{24}FN_3O_3S$ (381.152): C, 56.67; H, 6.34; N, 11.02. Found: C, 56.80; H, 6.57; N, 11.31. HR-MS: for $C_{18}H_{25}FN_3O_3S$ [M+H$^+$] calcd 382.1595 *m*/*z*, found 382.1594 *m*/*z*.

**8.1.5.5. Butyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl] butylcarbamate 6e.** White solid; yield 72.3% (isolation method A); mp 177.5–178.5 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.82 (1H, d, $J$ = 7.5 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.6 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H7), 7.97 (1H, dd, $J$ = 9.1 Hz, $^4J(^{19}F, ^1H)$ = 4.9 Hz, H4), 7.37 (1H, dt, $J$ = 2.7 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.22 (1H, d, $J$ = 8.2 Hz, N*H*-COOMe), 5.24 (1H, kv, $J$ = 7.2 Hz, H8), 4.05 (1H, m, H10), 3.90 (2H, t, $J$ = 6.3 Hz, H14), 1.56 (7H, m, H9,H11, H15), 1.34 (2H, m, H12), 0.88 (6H, m, H13, H16). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.2 Hz), 172.3, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 240.9 Hz), 156.2, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.9 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.5 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.9 Hz), 108.6 (d, $^2J(^{19}F,^{13}C)$ = 27.3 Hz), 65.4, 54.3, 47.4, 34.0, 22.0, 20.1, 18.8, 13.6, 10.3. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.6. IR (ATR): 3282, 2949, 2927, 1680 (CO—carbamate), 1649 (CO—amide), 1537, 1455, 1381, 1276, 1259, 1136, 1066, 926, 907, 854, 821, 780, 745, 677 cm$^{-1}$. Anal. Calcd for $C_{18}H_{24}FN_3O_3S$ (381.15): C, 56.67; H, 6.34; N, 11.02. Found: C, 56.77; H, 6.43; N, 11.15. HR-MS: for $C_{18}H_{25}FN_3O_3S$ [M+H$^+$] calcd 382.1595 *m*/*z*, found 382.1591 *m*/*z*.

**8.1.5.6. Isobutyl- (S)-1-[(R)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl] butylcarbamate 6f.** White solid; Yield 52.1% (isolation method A); mp 134-135 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.81 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.7 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H7), 7.97 (1H, dd, $J$ = 8.9 Hz, $^4J(^{19}F, ^1H)$ = 5.0 Hz, H4), 7.36 (1H, dt, $J$ = 2.7 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.22 (1H, d, $J$ = 8.2 Hz, N*H*-COOMe), 5.23 (1H, kv, $J$ = 7.2 Hz, H8), 4.02 (1H, m, H10), 3.72 (2H, d, $J$ = 6.3 Hz, H14),

1.82 (2H, m, $J$ = 6.6 Hz, H15), 1.58 (2H, m, H11), 1.54 (3H, d, $J$ = 7.2 Hz, H9), 1.33 (2H, m, H12), 0.87 (9H, m, H13, H16). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.7 (d, $^4J(^{19}F, ^{13}C)$ = 3.5 Hz), 172.3, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.6 Hz), 156.2, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.7 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.8 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 25.0 Hz), 108.6 (d, $^2J(^{19}F,^{13}C)$ = 27.0 Hz), 69.8, 54.3, 47.4, 34.0, 27.7, 20.1, 19.9, 18.8, 13.6. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ -116.6 IR (ATR): 3297, 3233, 1683 (CO—carbamate), 1648 (CO—amide), 1542, 1397, 1275, 1363, 1237, 1148, 1068, 985, 917, 845, 785, 667 cm$^{-1}$. Anal. Calcd for $C_{19}H_{26}FN_3O_3S$ (395.168): C, 57.70; H, 6.63; N, 10.62. Found: C, 57.87; H, 6.43; N, 10.80. HR-MS: for $C_{19}H_{27}FN_3O_3S$ [M+H$^+$] calcd 396.1752 $m/z$, found 396.1752 $m/z$.

**8.1.5.7. 2-Chloroethyl-(*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl] butylcarbamate 6g.** White solid; yield 32.8% (isolation method B); mp 162.5–163 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.83 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.98 (1H, dd, $^4J$ = 2.6 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H7), 7.97 (1H, dd, $J$ = 9.1 Hz, $^4J(^{19}F, ^1H)$ = 4.7 Hz, H4), 7.48 (1H, d, $J$ = 8.3 Hz, N*H*-COOMe), 7.36 (1H, dt, $J$ = 2.7 Hz, $J$ = 8.9 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H5), 5.24 (1H, kv, $J$ = 7.2 Hz, H8), 4.21 (2H, m, H15), 4.05 (1H, m, H10), 3.78 (2H, t, $J$ = 5.2 Hz, H14), 1.60 (2H, m, H11), 1.56 (3H, d, $J$ = 7.2 Hz, H9), 1.34 (2H, m, H12), 0.90 (3H, t, $J$ = 7.3 Hz, H13). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.7 (d, $^4J(^{19}F, ^{13}C)$ = 3.5 Hz), 172.1, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 240.1 Hz), 155.6, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.6 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.5 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.8 Hz), 108.5 (d, $^2J(^{19}F,^{13}C)$ = 27.0 Hz), 64.2, 54.4, 47.4, 43.1, 33.9, 20.1, 18.8, 13.6. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ -116.6. IR (ATR): 3271, 2965, 1689 (CO—carbamate), 1646 (CO—amide), 1539, 1483, 1399, 1376, 1276, 1238, 1197, 1149, 1132, 1108, 1050, 918, 853, 823, 685, 665 cm$^{-1}$. Anal. Calcd for $C_{17}H_{21}ClFN_3O_3S$ (401.098): C, 50.81; H, 5.27; N, 10.46. Found: C, 51.05; H, 5.39; N, 10.57. HR-MS: for $C_{17}H_{22}ClFN_3O_3S$ [M+H$^+$] calcd 402.1049 $m/z$, found 402.1048 $m/z$.

**8.1.5.8. 2,2,2-Trichloroethyl-(*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]butylcarbamate 6h.** White solid; yield 41.2% (isolation method A); mp 116.4–117.0 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.89 (1H, d, $J$ = 7.3 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.5 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.97 (1H, dd, $J$ = 8.9 Hz, $^4J(^{19}F, ^1H)$ = 4.7 Hz, H4), 7.91 (1H, d, $J$ = 8.2 Hz, N*H*-COOMe), 7.37 (1H, dt, $J$ = 2.6 Hz, $J$ = 8.9 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H5), 5.24 (1H, kv, $J$ = 7.2 Hz, H8), 4.82 (2H, m, H14), 4.09 (1H, m, H10), 1.62 (2H, m, H11), 1.56 (3H, d, $J$ = 7.2 Hz, H9), 1.36 (2H, m, H12), 0.89 (3H, t, $J$ = 7.3 Hz, H13). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.0 Hz), 171.7, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.6 Hz), 154.4, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.7 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.4 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.8 Hz), 108.5 (d, $^2J(^{19}F,^{13}C)$ = 27.0 Hz), 96.3, 73.5, 54.7, 47.4, 33.8, 20.1, 18.8, 13.3. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ -116.6. IR (ATR): 3336, 3311, 1710 (CO—carbamate), 1647 (CO—amide), 1566, 1528, 1453, 1375, 1251, 1226, 1197, 1162, 1133, 1108, 1086, 1046, 1022, 952, 911, 859, 822, 788, 767, 752, 716, 682 cm$^{-1}$. Anal. Calcd for $C_{17}H_{19}Cl_3FN_3O_3S$ (469.02): C, 43.37; H, 4.07; N, 8.93. Found: C, 43.42; H, 4.20; N, 9.05. HR-MS: for $C_{17}H_{20}Cl_3FN_3O_3S$ [M+H$^+$] calcd 470.0269 $m/z$, found 470.0273 $m/z$.

**8.1.5.9. 2,2,2-Trifluoroethyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl] butylcarbamate 6i.** White solid; yield 59% (isolation method A); mp 171–173 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.91 (1H, d, $J$ = 7.7 Hz, N*H*-CO), 7.99 (1H, dd, $J$ = 9,0 Hz, $^4J(^{19}F, ^1H)$ = 4.5 Hz, H4), 7.97 (H, dd, $^4J$ = 2.4 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.90 (1H, d, $J$ = 8.1 Hz, N*H*-COOMe),7.37 (1H, dt, $J$ = 2.6 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 5.24 (1H, kv, $J$ = 7.2 Hz, H8), 4.65 (2H, m, H14), 4.07 (1H, m, H10), 1.62 (2H, m, H11), 1.56 (3H, d, $J$ = 7.0 Hz, H9), 1.33 (2H, m, H12), 0.90 (3H, t, $J$ = 7.2 Hz, H13). $^{13}$C NMR (100.62 MHz, DMSO-$d6$): $\delta$ 175.6 (d, $^4J(^{19}F, ^{13}C)$ = 2.8 Hz), 171.7, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.2 Hz), 154.3, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.4 Hz), 123.8 (tt, $^1J(^{19}F,^{13}C)$ = 277.9 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.4 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.5 Hz), 108.6 (d, $^2J(^{19}F,^{13}C)$ = 27.0 Hz), 59.7 (q, $^2J(^{19}F,^{13}C)$ = 34.4 Hz), 54.6, 47.4, 33.8, 20.1, 18.8, 13.5. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −72.7 (s, $CF_3$), -116.6 (s, Ar-F). IR (ATR): 3306, 3063, 2950, 1698 (CO—carbamate), 1651 (CO—amide), 1541, 1459, 1283, 1256, 1237, 1168, 957, 858, 822, 771, 683 cm$^{-1}$. Anal. Calcd for $C_{17}H_{19}F_4N_3O_3S$ (421,108): C, 48.45; H, 4.54; N, 9.97. Found: C, 48.76; H, 4.60; N, 10.16. HR-MS: for $C_{17}H_{20}F_4N_3O_3S$ [M+H$^+$] calcd 422.1157 $m/z$, found 422.1157 $m/z$.

**8.1.5.10. 2,2,3,3-Tetrafluoropropyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[*d*]thiazole)ethylcarbamoyl]butylcarbamate 6j.** White solid; yield 58% (isolation method A); mp 163–165 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.9 (1H, d, $J$ = 7.7 Hz, N*H*-CO), 8.0 (1H, dd, $J$ = 8,8 Hz, $^4J(^{19}F, ^1H)$ = 4.7 Hz, H4), 7.98 (H, dd, $^4J$ = 3.0 Hz, $^3J(^{19}F, ^1H)$ = 8.8 Hz, H7), 7.8 (1H, d, $J$ = 8.2 Hz, N*H*-COOMe),7.37 (1H, dt, $J$ = 2.6 Hz, $J$ = 9.0 Hz, $^3J(^{19}F, ^1H)$ = 9.0 Hz, H5), 6.5 (1H, t, $^2J(^{19}F, ^1H)$ = 52.3 Hz, $^3J(^{19}F, ^1H)$ = 5.3 Hz, H15), 5.2 (1H, kv, $J$ = 7.2 Hz, H8), 4.5 (2H, m, $^3J(^{19}F, ^1H)$ = 14.1 Hz, H14), 4.1 (1H, m, H10), 1.6 (2H, m, H11), 1.5 (3H, d, $J$ = 7.2 Hz, H9), 1.3 (2H, m, H12), 0.9 (3H, t, $J$ = 7.3 Hz, H13). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.6 (d, $^4J(^{19}F, ^{13}C)$ = 2.8 Hz), 171.7, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.0 Hz), 154.5, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.7 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 114.8 (tt, $^1J(^{19}F,^{13}C)$ = 249.3 Hz, $^2J(^{19}F,^{13}C)$ = 25.1 Hz), 114.6 (d, $^2J(^{19}F,^{13}C)$ = 24.8 Hz), 109.3 ($^1J(^{19}F,^{13}C)$ = 248.4 Hz, $^2J(^{19}F,^{13}C)$ = 33.1 Hz), 108.5 (d, $^2J(^{19}F,^{13}C)$ = 27.1 Hz), 59.5 (t, $^2J(^{19}F,^{13}C)$ = 26.7 Hz), 54.6, 47.4, 33.9, 20.1, 18.7, 13.5. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.6, −124.9 (2F,dt, $^2J(^{19}F, ^1H)$ = 40.7 Hz, $^3J(^{19}F, ^1H)$ = 5.5 Hz)), −138.8 (2F,dt, $^2J(^{19}F, ^1H)$ = 22.7 Hz, $^3J(^{19}F, ^1H)$ = 5.5 Hz)). IR (ATR): 3306, 3064, 2950, 1698 (CO—carbamate), 1646 (CO—amide), 1607, 1551, 1456, 1394, 1274, 1239, 1110, 993, 825, cm$^{-1}$. Anal. Calcd for $C_{18}H_{20}F_5N_3O_3S$ (453,4267): C, 47.68; H, 4.45; N, 9.27. Found: C, 47.76; H, 4.47; N, 9.30. HR-MS: for $C_{18}H_{21}F_5N_3O_3S$ [M+H$^+$] calcd 454.1218 $m/z$, found 454.1221 $m/z$.

**8.1.5.11. 2,2,3,3,4,4,4-Heptafluorobutyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]butylcarbamate 6k.** White solid; yield 68% (isolation method A); mp 125–126 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.91 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.98 (1H, dd, $J$ = 9,2 Hz, $^4J(^{19}F, ^1H)$ = 4.8 Hz, H4), 7.96 (H, dd, $^4J$ = 2.9 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.93 (1H, d, $J$ = 8.2 Hz, N*H*-COOMe),7.36 (1H, dt, $J$ = 2.7 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 5.24 (1H, kv, $J$ = 7.2 Hz, H8), 4.78 (2H, m, $^3J(^{19}F, ^1H)$ = 15.6 Hz, H14), 4.09 (1H, m, H10), 1.62 (2H, m, H11), 1.56 (3H, d, $J$ = 7.0 Hz, H9), 1.35 (2H, m, H12), 0.90 (3H, t, $J$ = 7.5 Hz, H13). $^{13}$C NMR (100.62 MHz, DMSO-$d6$): $\delta$ 175.6 (d, $^4J(^{19}F, ^{13}C)$ = 2.9 Hz), 171.7, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 241.4 Hz), 154.2, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.6 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 117.3 (qt, $^1J(^{19}F,^{13}C)$ = 286.4 Hz, $^2J(^{19}F,^{13}C)$ = 33.7 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 25.0 Hz), 114.4 (tt, $^1J(^{19}F,^{13}C)$ = 254.1 Hz, $^2J(^{19}F,^{13}C)$ = 30.0 Hz), 108.5 (d, $^2J(^{19}F,^{13}C)$ = 27.2 Hz), 108.0 (tq, $^1J(^{19}F,^{13}C)$ = 275.8.0 Hz, $^2J(^{19}F,^{13}C)$ = 37.8 Hz), 58.7 (t, $^2J(^{19}F,^{13}C)$ = 25.6 Hz), 54.7, 47.4, 33.7, 20.1, 18.7, 13.5. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −80.5 (t, $^4J(^{19}F,^{19}F)$ = 9.5, $CF_3$), −116.7 (s, Ar-F), −119.9 (brq, $^3J(^{19}F,^{19}F)$ = 9.5, $CH_2CF_2$), −127.4 (s $CF_2$). IR (ATR): 3312, 3269, 2966, 1706 (CO—carbamate), 1659 (CO—amide), 1543, 1456, 1234, 1216, 1175, 1150, 1020, 923, 846, 818, 761, 709 cm$^{-1}$. Anal. Calcd for $C_{19}H_{19}F_8N_3O_3S$ (521,102): C, 43.77; H,

3.67; N, 8.06. Found: C, 44.00; H, 3.88; N, 8.24. HR-MS: for $C_{19}H_{20}F_8N_3O_3S$ [M+H$^+$] calcd 522.1083 *m*/*z*, found 522.1096 *m*/*z*.

**8.1.5.12. Methyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6l.** White solid; yield 26.1% (isolation method **B**); mp 188.8–189.1 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.87 (1H, d, $J$ = 7.2 Hz, N*H*-CO), 8.00 (1H, dd, $^4J$ = 2.5 Hz, $^3J(^{19}F, ^1H)$ = 8.8 Hz, H7), 7.97 (1H, dd, $^3J$ = 8.9 Hz, $^4J(^{19}F, ^1H)$ = 4.6 Hz, H4), 7.36 (1H, dt, $^4J$ = 2.5 Hz, $^3J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.20 (1H, d, $J$ = 8.9 Hz, N*H*-COOMe), 5.28 (1H, kv, $J$ = 6.8 Hz, H8), 3.91 (1H, t, $J$ = 8.0 Hz, H10), 3.55 (3H, s, H14), 1.98 (1H, m, H11), 1.56 (3H, d, $J$ = 7.2 Hz, H9), 0.89 (6H, d, H12). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 177.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.4 Hz), 171.4, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 239.0 Hz), 156.7, 149.6 (d, $^5J(^{19}F, ^{13}C)$ = 1.6 Hz), 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.8 Hz), 123.6 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.9 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.4 Hz), 60.2, 51.5, 47.2, 20.1, 19.2, 18.4. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ -116.56. IR (ATR): 3269, 3068, 2962, 2872, 1693 (CO—carbamate), 1650 (CO—amide), 1567, 1537, 1456, 1250, 1197, 1044 cm$^{-1}$. Anal. Calcd for $C_{16}H_{20}FN_3O_3S$ (353.121): C, 54.38; H, 5.70; N, 11.89. Found: C, 54.64; H, 5.78; N, 11.99. HR-MS: for $C_{16}H_{20}FN_3O_3S$ [M+H$^+$] calcd 354.1283 *m*/*z*, found 354.1282.

**8.1.5.13. Ethyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6m.** White solid; yield 35.8% (isolation method B); mp 188.8–189.1 °C. $^1$H NMR (400.13 MHz, DMSO- $d_6$): $\delta$ 8.84 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 8.00 (1H dd, $^4J$ = 2.6 Hz, $^3J(^{19}F, ^1H)$ = 8.8 Hz, H7), 7.99 (1H, dd, $^3J$ = 8.8 Hz, $^4J(^{19}F, ^1H)$ = 4.9 Hz, H4), 7.37 (1H, dt, $^4J$ = 2.5 Hz, $^3J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.12 (1H, d, $J$ = 9.2 Hz, N*H*-COOMe), 5.28 (1H, kv, $J$ = 7.2 Hz, H8), 4.00 (2H, q, $J$ = 7.2 Hz, H14), 3.90 (1H, t, $J$ = 7.8 Hz, H10), 1.98 (1H, m, H11), 1.56 (3H, d, $J$ = 7.2 Hz, H9), 1.17 (3H, t, $J$ = 7.2 Hz, H15), 0.89 (6H, t, H12). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.4 Hz), 171.4, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.0 Hz), 156.3, 149.6, 136.0 (d, $^3J(^{19}F, ^{13}C)$ = 11.8 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.5 Hz), 114.6 (d, $^2J(^{19}F,^{13}C)$ = 24.8 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.0 Hz), 60.2, 59.9, 47.3, 30.3, 20.1, 19.2, 18.4, 14.7. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.57. IR (ATR): 3289, 3256, 3064, 2981, 2962, 2931, 2909, 2871, 1683 (CO carbamate), 1645 (CO amide), 1541, 1456, 1244, 1227, 1196, 1044 cm$^{-1}$. Anal. Calcd for $C_{17}H_{22}FN_3O_3S$ (367.137): C, 55.57; H, 6.03; N, 11.44. Found: C, 55.39; H, 5.94; N, 10.57. HR-MS: for $C_{17}H_{22}FN_3O_3S$ [M+H$^+$] calcd 368.1439 *m*/*z*, found 368.1438 *m*/*z*.

**8.1.5.14. Propyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6n.** White solid; yield 33.2% (isolation method B); mp 175.8–176.2 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.83 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.7 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.97 (1H, dd, $^3J$ = 8.8 Hz, $^4J(^{19}F, ^1H)$ = 5.0 Hz, H4), 7.37 (1H, dt, $^4J$ = 2.6 Hz, $^3J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.12 (1H, d, $J$ = 8.9 Hz, N*H*-COOMe), 5.27 (1H, kv, $J$ = 7.2 Hz, H8), 3.90 (3H, m, H10, H14), 1.98 (1H, m, H11), 1.56 (5H, m, H9, H15), 0.89 (9H, m, H12, H16). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.5 (d, $^4J(^{19}F, ^{13}C)$ = 2.9 Hz), 171.4, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.0 Hz), 156.4, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.6 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.8 Hz), 114.6 (d, $^2J(^{19}F,^{13}C)$ = 24.6 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.1 Hz), 65.4, 60.2, 47.3, 30.3, 22.1, 20.1, 19.2, 18.4, 10.2. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.56. IR (ATR): 3280, 2959, 2932, 1685 (CO—carbamate), 1643 (CO—amide), 1605, 1535, 1455, 1376, 1287, 1244, 1226, 1193, 1151, 1047, 917, 845, 824, 800, 669 cm$^{-1}$. Anal. Calcd for $C_{18}H_{24}FN_3O_3S$ (381.152): C, 56.67; H, 6.34; N, 11.02. Found: C, 56.72; H, 6.40; N, 11.11. HR-MS: for $C_{18}H_{24}FN_3O_3S$ [M+H$^+$] calcd 382.1595 *m*/*z*, found 382.1595 *m*/*z*.

**8.1.5.15. Isopropyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazol-2-yl) ethylcarbamoyl]-2-methylpropylcarbamate 6o.** White solid; yield 93% (isolation method A); mp 166.9–168.0 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.83 (1H, d, $J$ = 7.2 Hz, N*H*-CO), 7.98 (1H, dd, $J$ = 8.9 Hz, $^4J(^{19}F, ^1H)$ = 4.8 Hz, H4),7.96 (1H, dd, $^4J$ = 2.6 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.36 (1H, dt, $J$ = 2.6 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.02 (1H, d, $J$ = 8.9 Hz, N*H*-COO-iPr), 5.27 (1H, kv, $J$ = 7.2 Hz, H8), 4.75 (1H, m, $J$ = 6.2 Hz, H11), 3.88 (1H, m, H10), 1.96 (1H, m, H13), 1.55 (3H, d, $J$ = 7.2 Hz, H9), 1.16 (6H, d, $J$ = 6.2 Hz, 2H, H14), 0.89 (3H, d, $J$ = 6.5 Hz, H12), 0.87 (3H, d, $J$ = 6.5 Hz, H12). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.0 Hz), 171.5, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.1 Hz), 155.9, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.9 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.5 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.9 Hz), 108.5 (d, $^2J(^{19}F,^{13}C)$ = 27.1 Hz), 67.0, 60.2, 47.2, 30.3, 22.0, 22.1, 20.1, 19.2, 18.4. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.5. IR (ATR): 3288, 3264, 3061, 2975, 2928, 2871, 1686 (CO—carbamate), 1647 (CO—amide), 1567, 1537, 1456, 1383, 1372, 1344, 1287, 1247, 1225, 1194, 1178, 1111, 1073, 1037, 919, 841, 824, 815, 801, 710, 679, 650, 603, 557, 538, 472, 431 cm$^{-1}$. Anal. Calcd for $C_{18}H_{24}FN_3O_3S$ (381.15): C, 56.67; H, 6.34; N, 11.02. Found: C, 56.91; H, 6.20; N, 10.95. HR-MS: for $C_{18}H_{25}FN_3O_3S$ [M+H$^+$] calcd 382.1595 *m*/*z*, found 382.1597 *m*/*z*.

**8.1.5.16. Butyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6p.** White solid; yield 27.8% (isolation method B); mp 152.4–153.1 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.85 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.5 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.97 (1H, dd, $^3J$ = 8.8 Hz, $^4J(^{19}F, ^1H)$ = 4.3 Hz, H4), 7.36 (1H, dt, $^4J$ = 2.6 Hz, $^3J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 7.12 (1H, d, $J$ = 8.8 Hz, N*H*-COOMe), 5.28 (1H, kv, $J$ = 7.2 Hz, H8), 3.96 (2H, t, $J$ = 6.4 Hz, H14), 3.90 (1H, t, $J$ = 8.4 Hz, H10), 1.99 (1H, m, H11), 1.56 (3H, d, $J$ = 7.2 Hz, H9), 1.52 (2H, m, H15), 1.33 (2H, m, H16), 0.88 (9H, m, H12, H17). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 177.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.3 Hz), 171.4, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.1 Hz), 156.4, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.7 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.5 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.8 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.1 Hz), 63.6, 60.2, 47.3, 30.8, 30.3, 20.1, 19.2, 18.6, 18.4, 13.6. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ = −116.58. IR (ATR): 3288, 3067, 2962, 2931, 2871, 2359, 1686 (CO—carbamate), 1646 (CO—amide), 1539, 1458, 1246, 1229, 1196, 1046 cm$^{-1}$. Anal. Calcd for $C_{19}H_{26}FN_3O_3S$ (395.168): C, 57.70; H, 6.63; N, 10.62. Found: C, 57.82; H, 6.83; N, 10.90. HR-MS: for $C_{19}H_{26}FN_3O_3S$ [M+H$^+$] calcd 396.1752 *m*/*z*, found 396.1751 *m*/*z*.

**8.1.5.17. Isobutyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6q.** White solid; yield 10.0% (isolation method B); mp 198.9–199.2 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.86 (1H, d, $J$ = 7.2 Hz, N*H*-CO), 7.98 (2H, m, H4, H7), 7.37 (1H, dt, $J$ = 2.4 Hz, $J$ = 8.8 Hz, $^3J(^{19}F, ^1H)$ = 8.8 Hz, H5), 7.14 (1H, d, $J$ = 8.8 Hz, N*H*-COOMe), 5.28 (1H, kv, $J$ = 7.2 Hz, H8), 3.90 (1H, t, $J$ = 8.4 Hz, H10), 3.76 (2H, d, $J$ = 6.8 Hz, H14), 1.99 (1H, m, H11), 1.84 (1H, m, H15), 1.56 (3H, d, $J$ = 7.2 Hz, H9), 0.88 (12H, m, H12, H16). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.4 Hz), 171.4, 159.6 (d, $^1J(^{19}F,^{13}C)$ = 241.8 Hz), 156.4, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.9 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 114.6 (d, $^2J(^{19}F,^{13}C)$ = 24.9 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.2 Hz), 69.9, 60.3, 47.2, 30.3, 27.7, 20.1, 19.2, 18.9, 18.8, 18.4. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.55. IR (ATR): 3283, 3241, 2959, 1685 (CO—carbamate), 1644 (CO—amide), 1605, 1533, 1457, 1379, 1287, 1245, 1196, 1150, 1129, 1108, 1041, 917, 844, 798, 777, 668 cm$^{-1}$. Anal. Calcd for $C_{19}H_{26}FN_3O_3S$ (395.168): C, 57.70; H, 6.63; N, 10.62. Found: C, 57.84; H, 6.72; N, 10.77. HR-MS: for $C_{19}H_{26}FN_3O_3S$ [M+H$^+$] calcd 396.1752 *m*/*z*, found 396.1751 *m*/*z*.

**8.1.5.18. 2-Chloroethyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6r.** White solid; yield 37.0% (isolation method B); mp 193.4–193.6 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.85 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.6 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.97 (1H, dd, $^3J$ = 8.9 Hz, $^4J(^{19}F, ^1H)$ = 4.3 Hz, H4), 7.40 (1H, d, $J$ = 9.0 Hz, N*H*-COOMe), 7.36 (1H, dt, $^4J$ = 2.5 Hz, $^3J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 5.28 (1H, kv, $J$ = 7.2 Hz, H8), 4.24 (2H, t, $J$ = 5.4 Hz, H15), 3.90 (1H, t, $J$ = 8.0 Hz, H10), 3.80 (2H, t, $J$ = 5.0 Hz, H14), 2.00 (1H, m, $J$ = 6.9 H11), 1.57 (3H, d, $J$ = 7.2 Hz, H9), 0.90 (6H, m, H12). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.7 Hz), 171.2, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.1 Hz), 155.8, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.6 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.5 Hz), 114.6 (d, $^2J(^{19}F,^{13}C)$ = 24.9 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.0 Hz), 64.2, 60.4, 47.3, 43.1, 30.2, 20.1, 19.2, 18.6. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.56. IR (ATR): 3283, 3242, 2960, 1690 (CO—carbamate), 1641 (CO—amide), 1605, 1542, 1455, 1396, 1375, 1286, 1244, 1227, 1193, 1153, 1080, 1032, 916, 844, 822, 794, 756, 708, 687, 667 cm$^{-1}$. Anal. Calcd for $C_{17}H_{21}ClFN_3O_3S$ (401.098): C, 50.81; H, 5.27; N, 10.46. Found: C, 50.91; H, 5.52; N, 10.56. HR-MS: for $C_{16}H_{20}FN_3O_3S$ [M+H$^+$] calcd 402.1049 $m/z$, found 402.1048 $m/z$.

**8.1.5.19. 2-Bromoethyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6s.** White solid; yield 32.0% (isolation method B); mp 174.9–175.2 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.86 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.7 Hz, $^3J(^{19}F, ^1H)$ = 8.9 Hz, H7), 7.97 (1H, dd, $^3J$ = 8.9 Hz, $^4J(^{19}F, ^1H)$ = 4.7 Hz, H4), 7.40 (1H, d, $J$ = 9.6 Hz, N*H*-COOMe), 7.36 (1H, dt, $J$ = 2.5 Hz, $J$ = 9.2 Hz, $^3J(^{19}F, ^1H)$ = 9.2 Hz, H5), 5.28 (1H, kv, $J$ = 6.8 Hz, H8), 4.30 (2H, t, $J$ = 5.6 Hz, H15), 3.90 (1H, t, $J$ = 8.4 Hz, H10), 3.65 (2H, t, $J$ = 5.6 Hz, H14), 1.99 (1H, m, H11), 1.57 (3H, d, $J$ = 7.2 Hz, H9), 0.91 (3H, d, $J$ = 6.5 Hz, H12), 0.89 (3H, d, $J$ = 6.5 Hz, H12). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.5 (d, $^4J(^{19}F, ^{13}C)$ = 3.7 Hz), 171.2, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.2 Hz), 155.8, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.6 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 114.6 (d, $^2J(^{19}F,^{13}C)$ = 24.9 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.0 Hz), 64.0, 60.4, 47.3, 31.4, 30.2, 20.1, 19.2, 18.9. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.55. IR (ATR): 3284, 3242, 2968, 2930, 1691 (CO—carbamate), 1642 (CO—amide), 1605, 1565, 1541, 1455, 1395, 1375, 1291, 1226, 1245, 1193, 1154, 1133, 1075, 1006, 997, 916, 905, 844, 828, 819, 713, 675 cm$^{-1}$. Anal. Calcd for $C_{17}H_{21}BrFN_3O_3S$ (445.047): C, 45.75; H, 4.74; N, 9.41. Found: C, 45.95; H, 4.80; N, 9.45. HR-MS: for $C_{16}H_{20}FN_3O_3S$ [M+H$^+$] calcd 446.0544 $m/z$, found 446.0544 $m/z$.

**8.1.5.20. 2,2,2-Trichloroethyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6t.** White solid; yield 25.1% (isolation method B); mp 107.8–108.5 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.93 (1H, d, $J$ = 7.5 Hz, N*H*-CO), 7.99 (1H, dd, $^4J$ = 2.2 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H7), 7.97 (1H, dd, $^3J$ = 9.1 Hz, $^4J(^{19}F, ^1H)$ = 4.7 Hz, H4), 7.85 (1H, d, $J$ = 8.9 Hz, N*H*-COOMe), 7.37 (1H, dt, $^4J$ = 2.6 Hz, $^3J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 5.28 (1H, kv, $J$ = 7.2 Hz, H8), 4.84 (1H, H14), 4.83 (1H, H14), 3.92 (1H, t, $J$ = 8.5 Hz, H10), 2.02 (1H, m, H11), 1.56 (3H, d, $J$ = 7.2 Hz, H9), 0.92 (6H, d, $J$ = 7.0 Hz, H12). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.4 (d, $^4J(^{19}F, ^{13}C)$ = 3.0 Hz), 170.9, 159.6 (d, $^1J(^{19}F,^{13}C)$ = 239.0 Hz), 154.5, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.7 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 114.6 (d, $^2J(^{19}F,^{13}C)$ = 24.6 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.2 Hz), 96.3, 73.5, 60.8, 47.3, 30.2, 20.1, 19.2, 18.6. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.52. IR (ATR): 3265, 2962, 2938, 1682 (CO—carbamate), 1648 (CO—amide), 1598, 1537, 1453, 1382, 1288, 1249, 1238, 1222, 1201, 1194, 1148, 1050, 916, 845, 833, 825, 806, 671 cm$^{-1}$. Anal. Calcd for $C_{17}H_{19}Cl_3FN_3O_3S$ (469.02): C, 43.37; H, 4.07; N, 8.93. Found: C, 43.42; H, 4.15; N, 9.15. HR-MS: for $C_{17}H_{19}Cl_3FN_3O_3S$ [M+H$^+$] calcd 470.0269 $m/z$, found 470.0269 $m/z$.

**8.1.5.21. 2,2,2-Trifluoroethyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6u.** White solid; yield 52% (isolation method A); mp 202.9–203.8 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.93 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.98 (2H, m, H4, H7), 7.83 (1H, d, $J$ = 8.8 Hz, N*H*-COOMe), 7.37 (1H, dt, $J$ = 2.4 Hz, $J$ = 9.2 Hz, $^3J(^{19}F, ^1H)$ = 9.2 Hz, H5), 5.28 (1H, kv, $J$ = 7.2 Hz, H8), 4.67 (2H, m, H14), 3.91 (1H, t, $J$ = 8.4 Hz, H10), 2.02 (1H, m, C*H*-$(CH_3)_2$), 1.56 (3H, d, $J$ = 7.2 Hz, H9), 0.92 (6H, d, $J$ = 6.4 Hz, H9). $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 175.4 (d, $^4J(^{19}F, ^{13}C)$ = 3.2 Hz), 170.9, 159.6 (d, $^1J(^{19}F,^{13}C)$ = 242.4 Hz), 154.5, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.8 Hz), 123.8 (d, $^1J(^{19}F,^{13}C)$ = 278.1 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 114.6 (d, $^2J(^{19}F,^{13}C)$ = 24.8 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.1 Hz), 60.7, 59.8 (q, $^2J(^{19}F,^{13}C)$ = 34.5 Hz), 47.3, 30.2, 20.1, 19.2, 18.6. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −116.6 (F-6), -72.8 ($CF_3$). IR (ATR): 3295, 3068, 2969, 1708 (CO—carbamate), 1650 (CO—amide), 1541, 1456, 1241, 1173, 1062 cm$^{-1}$. Anal. Calcd for $C_{17}H_{19}F_4N_3O_3S$ (421.11): C, 48.45; H, 4.54; N, 9.97. Found: C, 48.55; H, 4.59; N, 10.15. HR-MS: for $C_{17}H_{19}Cl_3FN_3O_3S$ [M+H$^+$] calcd 422.1173 $m/z$, found 422.1157 $m/z$.

**8.1.5.22. 2,2,3,3,-Tetrafluoropropyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6v.** White solid; yield 55% (isolation method A); mp 179.2–180.8 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.94 (1H, d, $J$ = 7.4 Hz, N*H*-CO), 7.98 (1H, dd, $J$ = 8,8 Hz, $^4J(^{19}F, ^1H)$ = 4.9 Hz, H4), 7.96 (H, dd, $^4J$ = 2.4 Hz, $^3J(^{19}F, ^1H)$ = 8.8 Hz, H7), 7.72 (1H, d, $J$ = 8.9 Hz, N*H*-COOMe), 7.37 (1H, dt, $J$ = 2.6 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 6.55 (1H, tt, $^2J(^{19}F, ^1H)$ = 52.1 Hz, $^3J(^{19}F, ^1H)$ = 5.5 Hz, H15), 5.28 (1H, kv, $J$ = 7.2 Hz, H8), 4.54 (2H, m, H14), 3.91 (1H, t, $J$ = 8.2 Hz, H10), 2.00 (1H, m, $J$ = 7.0 Hz C*H*-$(CH_3)_2$), 1.55 (3H, d, $J$ = 7.0 Hz, H9), 0.90 (6H, d, $J$ = 6.4 Hz, H9). $^{13}$C NMR (100.62 MHz, DMSO-*d*6): $\delta$ 175.4 (d, $^4J(^{19}F, ^{13}C)$ = 3.0 Hz), 170.9, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.5 Hz), 154.7, 149.6, 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.8 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.4 Hz), 114.7 (tt, $^1J(^{19}F,^{13}C)$ = 252.5 Hz, $^2J(^{19}F,^{13}C)$ = 25.0 Hz), 114.5 (d, $^2J(^{19}F,^{13}C)$ = 24.7 Hz), 109.3 ($^1J(^{19}F,^{13}C)$ = 248.3 Hz, $^2J(^{19}F,^{13}C)$ = 29.5 Hz), 108.6 (d, $^2J(^{19}F, ^{13}C)$) = 27.0 Hz), 60.5, 59.5 (t, $^2J(^{19}F,^{13}C)$ = 26.5 Hz), 47.3, 30.3, 20.1, 19.1, 18.4. $^{19}$F NMR (376.46 MHz, DMSO-$d_6$): $\delta$ -116.6, (arom.F), −139.0 (AB system, $^2J(^{19}F,^{19}F)$ = 303.0, $^3J(^{19}F,^{19}F)$ = 5.5, C$F_a$F$_b$), −138.9 (AB system, $^2J(^{19}F,^{19}F)$ = 303.0, $^3J(^{19}F,^{19}F)$ = 5.4, CF$_a$F$_b$), −125.1 (AB system, $^2J(^{19}F,^{19}F)$ = 269.0, $^3J(^{19}F,^{19}F)$ = 5.5, CH$F_a$F$_b$), −124.9 (AB system, $^2J(^{19}F,^{19}F)$ = 269.0, $^3J(^{19}F,^{19}F)$ = 5.4, CHF$_a$F$_b$). IR (ATR): 3310, 3300, 2966, 1703 (CO—carbamate), 1655 (CO—amide), 1541, 1450, 1247, 1207, 1128, 918, 797, 818, 710 cm$^{-1}$. Anal. Calcd for $C_{18}H_{20}F_5N_3O_3S$ (453,4267): C, 47.68; H, 4.45; N, 9.27. Found: C, 47.75; H, 4.46; N, 9.30. HR-MS: for $C_{18}H_{21}F_5N_3O_3S$ [M+H$^+$] calcd 454.1218 $m/z$, found 454,1220 $m/z$.

**8.1.5.23. 2,2,3,3,4,4,4-Heptafluorobutyl (*S*)-1-[(*R*)-1-(6-fluorobenzo[d]thiazole)ethylcarbamoyl]-2-methylpropylcarbamate 6w.** White solid; yield 62.0% (isolation method A); mp 162.1–162.6 °C. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 8.93 (1H, d, $J$ = 7.6 Hz, N*H*-CO), 7.98 (1H, dd, $J$ = 8,9 Hz, $^4J(^{19}F, ^1H)$ = 4.8 Hz, H4), 7.96 (H, dd, $^4J$ = 2.3 Hz, $^3J(^{19}F, ^1H)$ = 8.7 Hz, H7), 7.87 (1H, d, $J$ = 8.8 Hz, N*H*-COOMe), 7.36 (1H, dt, $^4J$ = 2.5 Hz, $J$ = 9.1 Hz, $^3J(^{19}F, ^1H)$ = 9.1 Hz, H5), 5.28 (1H, kv, $J$ = 7.2 Hz, H8), 4.80 (2H, m, H14), 3.92 (1H, t, $J$ = 8.4 Hz, H10), 2.01 (1H, m, H11), 1.57 (3H, d, $J$ = 6.8 Hz, H1), 0.91 (3H, d, $J$ = 6.6 Hz, H12), 0.90 (3H, d, $J$ = 6.6 Hz, H12). $^{13}$C NMR (100.62 MHz, DMSO-*d*6): $\delta$ 175.4 (d, $^4J(^{19}F, ^{13}C)$ = 2.8 Hz), 170.9, 159.5 (d, $^1J(^{19}F,^{13}C)$ = 242.3 Hz), 154.4, 149.6 (d, $^5J(^{19}F, ^{13}C)$ = 1.7 Hz), 135.9 (d, $^3J(^{19}F, ^{13}C)$ = 11.7 Hz), 123.7 (d, $^3J(^{19}F, ^{13}C)$ = 9.6 Hz), 117.3 (m, $^1J(^{19}F,^{13}C)$ = 288.4 Hz, $^2J(^{19}F,^{13}C)$ = 33.8 Hz), 114.6 (d, $^2J(^{19}F,^{13}C)$ = 24.6 Hz), 114.5 (m, $^1J(^{19}F,^{13}C)$ = 255.0 Hz, $^2J(^{19}F,^{13}C)$ = 30.7 Hz), 108.7 (d, $^2J(^{19}F, ^{13}C)$) = 27.2 Hz),

108.6 (q, $^{1}J(^{19}F,^{13}C)$ = 267.0 Hz), $^{2}J(^{19}F,^{13}C)$ = 33.0 Hz), 60.7, 58.7 (t, $^{3}J(^{19}F,\ ^{13}C)$ = 60.8 Hz), 47.3, 30.2, 20.1, 19.1, 18.4. $^{19}F$ NMR (376.46 MHz, DMSO-$d_6$): $\delta$ −80.5 (t, $^{4}J(^{19}F,^{19}F)$ = 7.5, F-17), −116.6 (s, F-6), −119.9 (dq, $^{4}J(^{19}F15,^{19}F17)$ = 7.5, $^{3}J(^{19}F15,^{19}F16)$ = 3.7, F-15), −127.4 (br s, F-16). IR (ATR): 3307, 3261, 1707(CO—carbamate), 1654 (CO—amide), 1558, 1541, 1457, 1232, 1219, 1198, 1172, 1149, 1131, 1021 $cm^{-1}$. Anal. Calcd for $C_{19}H_{19}F_8N_3O_3S$ (521.102): C, 43.77; H, 3.67; N, 8.06. Found: C, 43.95; H, 3.78; N, 8.26. HR-MS: for $C_{19}H_{19}F_8N_3O_3S$ [M+H$^+$] calcd 522.1093 *m*/*z*, found 522.1089 *m*/*z*.

### 8.2. In vitro evaluation of prepared compounds

All reagents and solvents were purchased from commercial sources (Sigma–Aldrich, Lach-Ner, Penta). The $IC_{50}$ values were determined using the spectrophotometric Ellman's method, which is a simple, rapid and direct method to determine the SH and –S–S– group content in[53] This method is widely used for measuring of cholinesterase activity and effectivity of cholinesterase inhibitors. Cholinesterase activity is measured indirectly by quantifying the concentration of 5-thio-2-nitrobenzoic acid (TNB) ion formed in the reaction between the thiol reagent 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and thiocholine, a product of substrate (i.e., acetylthiocholine, ATCH) hydrolysis by cholinesterase.[54] All tested compounds were dissolved in dioxane (concentration 0.02 M) and then diluted in demineralised water (concentration 0.002 and 0.0002 M). The procedure of determination of $IC_{50}$ is a slightly modified Ellman's method according to Zdrazilova and described in detail in.[55] Into the glass test tubes eight different concentrations of tested compound were placed. All solutions were filled up to 1 mL with phosphate buffer (0.1 M, pH 8) and 0.5 mL of the enzyme preparation (AChE from *Electrophorus electricus* or BChE from equine serum diluted in phosphate buffer). The catalytic activity of enzyme in final reaction mixtures was 0.14 U/mL. The reaction mixtures were homogenized and incubated at 25 °C for 5 min. Then 0.25 mL of indicating solution (0.5 mM 5,5′-dithiobis(2-nitrobenzoic acid), DTNB, purchased from Sigma-Aldrich) and 0.25 mL of substrate (0.25 mM acetylthiocholine dissolved in demineralised water, purchased from Sigma–Aldrich) was added to all test tubes, the mixtures were homogenized and incubated at 25 °C for 10 min. Then the absorbance at 412 nm of all mixtures was measured (in comparative solution the enzyme was replaced by demineralised water). From dependence inhibition in percentages (%I) versus concentration of inhibitor (IC) the $IC_{50}$ value was determined. For determination of $IC_{50}$ values the inhibition was determined for 23 different compound concentrations with 3 replicates.

### 8.3. In vitro cytotoxicity assay

#### 8.3.1. Cell lines

The human hepatocellular liver carcinoma cell line HepG2 (p13) purchased from Health Protection Agency Culture Collections (ECACC, Salisbury, UK) was routinely cultured in MEM (Minimum Essentials Eagle Medium) (Sigma Aldrich) supplemented with 10% foetal bovine serum (PAA), 1% L-Glutamine solution (Sigma Aldrich) and non-essential amino acid solution (Sigma Aldrich) in a humidified atmosphere containing 5% $CO_2$ at 37 °C. The human breast adenocarcinoma cell line MCF7 was a kind gift from Dr. Francoise Farace (Institut Gustave Roussy, Translational Research Laboratory, Villejuif, France). MCF7 (p37) cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% foetal calf serum (Gibco), 1% penicillin-streptomycin antibiotics (Gibco) and 0.01 mg/mL insuline (Gibco) at 37 °C in a humidified 5% $CO_2$ atmosphere.

#### 8.3.2. Cytotoxicity measurement

**8.3.2.1. MTS assay.** The most active compounds **6d**, **6f**, **6n** and **6m** were tested on in vitro cytotoxicity in human liver cell line HepG2 using a standard colorimetric method measuring a tetrazolium salt reduction (CellTiter 96 AQueous One Solution Cell Proliferation Assay,[56] Promega, USA). The cells were seeded in density 10,000 cells per well in a 96-well plate. Next day, the cells were treated with each of the tested substances dissolved in DMSO. Compound **6f** was prepared in 12 incubation concentrations (0.001–5000 μmol/L) in triplicates. Compounds **6d**, **6m**, **6n** were prepared in 12 incubation concentrations (0.001–2500 μmol/L) in triplicates. The following controls were incubated concomitantly: 100% cell viability, 0% cell viability (the cells treated by 10% DMSO), no cell control and vehiculum control. After 24 h incubation in a humidified atmosphere containing 5% $CO_2$ at 37 °C, reagent of the used kit was added into the wells. After 3.5 h incubation at 37 °C, the absorbance was recorded at 490 nm using a plate reader. A standard toxicological parameter $IC_{50}$ (50% inhibitory concentration) was calculated in each of the tested substances using GraphPad Prism software (version 5.02).

**8.3.2.2. Real-time cellular toxicity assay.** Real time growth kinetics of MCF7 cells was examined by the impedance-based xCELLigence RTCA (Real-Time Cell Analysis) SP (Single plate) system (Roche Diagnostic, Mannheim, Germany). The system monitors cellular events in real time without the incorporation of labels. The principle of the system is to monitor the changes in electrode impedance induced by the interaction between testing cells and electrodes.[57,58] The impedance measurement provides quantitative information about the biological status of the cells, including cell number, viability, and morphology. The xCELLigence System was connected and tested by Resistor Plate Verification before the RTCA SP Station was placed inside the incubator at 37 °C and 5% $CO_2$. Cell growth was recorded as cell index (CI), which corresponds to the electrical impedance of a well measured by the xCELLigence system. Cells were seeded at a density of 75,000 cells per well in E-plate 96. Cell proliferation was dynamically monitored at 1 h interval. When the cells entered logarithmic growth phase, they were treated with 10 μL of **6d**, **6f**, **6n** and **6m** dissolved in DMSO at concentrations from 0.01 to 2500 μmol/L in triplicates. Growth curves were normalized to the CI at the last measured time point before compound addition for each well. Cells treated with 0.1% of DMSO were used as a vehicle control and 10% DMSO treated cells were used as a negative control (i.e., 0% viability). Upon incubation with tested compounds the cell status and the cytotoxic effect were visualized using cell characteristic index-time profile. The results were also expressed as $IC_{50}$ after 24 h of incubation with the tested compounds. Evaluations were performed using the RTCA 1.2.1 Software.

### 8.4. Lipophilicity

#### 8.4.1. HPLC determination of liphophilicity (capacity factor k/calculated log*k*)

A Waters Alliance 2695 XE HPLC separation module and a Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, USA) were used. A Symmetry® $C_{18}$ 5 μm, 4.6 × 250 mm, Part No. WAT054275 (Waters Corp., Milford, MA, USA) chromatographic column was used. The HPLC separation process was monitored by Empower™ 2 Chromatography Data Software, Waters 2009 (Waters Corp., Milford, MA, USA). A mixture of MeOH p.a. (70%) and $H_2O$-HPLC – Mili-Q Grade (30%) was used as a mobile phase. The total flow of the column was 1.0 mL/min, injection volume 30 μL, column temperature 45 °C, and sample temperature 10 °C. The detection wavelength of 210 nm was chosen. The KI methanolic solution was used for dead time ($t_D$) determination.

Retention times ($t_R$) were measured in minutes. The capacity factors $k$ were calculated using the Empower™ 2 Chromatography Data Software according to formula $k = (t_R - t_D)/t_D$, where $t_R$ is the retention time of the solute, whereas $t_D$ denotes the dead time obtained using an unretained analyte. Log $k$, calculated from the capacity factor $k$, is used as the lipophilicity index converted to log $P$ scale.[43] The log $k$ values of individual compounds are shown in Table 1.

#### 8.4.2. Liphophilicity calculation

Log $P$ values (i.e., the logarithm of the $n$-octanol/water partition coefficient) were predicted using CS ChemOffice Ultra 10.0 (CambridgeSoft, Cambridge, MA, USA) and ACD/LogP ver. 1.0 software (Advanced Chemistry Development Inc., Toronto, Canada). Clog $P$ values (the logarithm of $n$-octanol/water partition coefficient based on established chemical interactions) were also generated using CS ChemOffice Ultra 10.0 software. The results are shown in Table 1.

### 8.5. Molecular docking

The three-dimensional structure of acetylcholine esterase from *Torpedo californica*, non-aged and conjugated with soman[59], was obtained from Protein Databank (www.pdb.org, PDB code 2wg2). This specific structure has been chosen due to high quality (resolution of 1.95 Å) and its origin (the same type was used in inhibitory activity studies). For molecular docking calculations, the molecules of water, other molecules of crystallization, for example, alpha-l-fucose, *N*-acetyl-l-glucosamine, di(hydroxyethyl)ether or tetraethylene glycol, and the inhibitor soman were deleted, and the Ser200 was dephosphonylated.

To carry out the molecular docking, the GOLD 5.0.1 (CCDC, UK) in 64-bit Linux version was used. The usual procedure consisting in automatic substitution of all hydrogen atoms for GOLD-optimized ones and the standard settings for highly flexible docking (free rotation and flipping of specific functional groups) were used to produce the set of optimal conformations of both the ligand and the protein. The standard GOLD scoring function was used in the primary docking procedure and then the results were rescored using the ASP (Astex Statistical Potential) function to produce the final set of optimized ligand conformations. Prior to docking, the ligands were optimized by semiempirical method RM1 with RMS gradient set to 0.0001 using the MOPAC 2009 package.[60] The interaction site was defined as a cavity around Ser200 with 20 Å diameter, comprising of 14268 fitting points. All molecular graphics materials were prepared using the Discovery Studio 3.1 Client (ver. 3.1.1.11157, Accelrys Software Inc., San Diego, CA, USA).

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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.2013.01.052.

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