chemistry proceedings

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Benzyl Carbamates of 4-Aminosalicylanilides as Possible BACE1 Modulators †

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† Presented at the 26th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2022; Available online: https://sciforum.net/event/ecsoc-26.

Abstract: Recently, a series of thirty-eight 4-{[(benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid amides designed as potential acetyl- and butyrylcholinesterase (AChE/BChE) inhibitors were described as potential drugs to alleviate the symptoms of Alzheimer's disease (AD). Some of these compounds have shown promise for inhibiting either AChE or BChE. Since these compounds are structurally similar to agents inhibiting beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), the aim of the contribution was to verify how our compounds were able to affect this enzyme, which, when inhibited, blocks the formation of amyloid-β, but whose inhibition is associated with significant adverse effects in humans. At a concentration of 10 μM, only benzyl {4-[(4-fluorophenyl)carbamoyl]-3-hydroxyphenyl}carbamate was found to show approximately 28% inhibition of BACE1 activity.

Keywords: 4-aminosalicylanilides; BACE1; carbamates; modulation

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Citation: Majerova, P.; Pindjakova, D.; Jankech, T.; Gerhardtova, I.; Kos, J.; Kovac, A.; Jampilek, J. Benzyl Carbamates of 4-Aminosalicylanilides as Possible BACE1 Modulators. *Chem. Proc.* **2022**, *12*, 40. https://doi.org/10.3390/ecsoc-26-13680

Academic Editor: Julio A. Seijas

Published: 17 November 2022

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is clinically manifested by memory loss, speech impairment, and general disorientation in space and time [1]. AD is a multifactorial disease in which both genetic and environmental factors contribute to its pathogenesis [2]; but the exact cause is unknown. Risk factors for AD include age, vascular disease, traumatic brain injury, epilepsy, depression and lifestyle [3]. In 2015, over 46 million people were living with dementia worldwide. This number is estimated to increase to 131.5 million by 2050. Moreover, AD is ranked as the fifth leading cause of death worldwide [4]. An estimated 6.5 million Americans aged 65 and older are living with AD today. This number could grow to 13.8 million by 2060, barring the development of medical breakthroughs to prevent, slow or cure AD [5]. AD is currently incurable and the drugs used bring only relatively little benefit to patients [6]. Acetyl/butyrylcholinesterase inhibitors (tacrine, rivastigmine, galantamine, and donepezil) reduce the degradation of acetyl/butyrylcholine, which compensates for the losses caused by the degeneration of cholinergic centers in the brain. Another drug used is memantine from the group of NMDA receptor inhibitors [6–11]. Medication is supplemented by psychosocial support for patients and care for the sick.

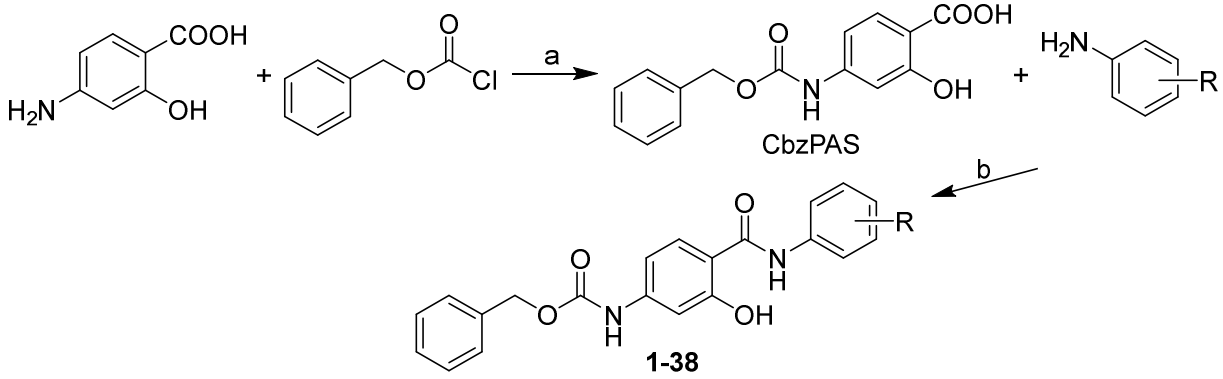
AD is characterized by several neuropathological hallmarks such as amyloid-β (Aβ) plaques, tau-rich neurofibrillary tangles (NFTs) composed from hyperphosphorylated and truncated tau protein, inflammation, synaptic impairment, and neuronal loss [3,12]. Aβ plaques are derived from the sequential proteolytic cleavage of the amyloid-β precursor

protein (AβPP) by β- and γ-secretases [13]. β-Secretase 1 is also known as beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) [14,15]. In addition to the above-mentioned medications, attention has been paid to BACE1, which seemed to be a suitable target for the design of new drugs, because its inhibition actually reduced the formation of Aβ peptide in vivo. BACE1 inhibitors [16], such as verubecestat, lanabecestat, atabecestat, LY2886721, elenbecestat, umibecestat, were designed but their clinical trials were terminated in phase II or III, because the effect of reducing the formation of Aβ peptide did not appear in humans, but significant side effects (liver abnormalities, impaired muscle (motor) coordination) occurred [17–24]. However, many scientists did not give up after these failures and new highly selective BACE1 inhibitors were designed. Despite these efforts, clinical trial data suggest that their toxicity is caused by the inhibition of BACE1 itself and that the only potential future for BACE1 inhibitors lies in careful titration of highly selective compounds in early populations where the amyloid burden is still minimal as a prophylactic therapy or as an affordable oral maintenance therapy following amyloid-clearing therapies [16,17].

Recently, a series of fifty 4-{[(benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid amides designed as potential acetyl- and butyrylcholinesterase (AChE/BChE) inhibitors have been described [25]. Some of these compounds have shown promise for inhibiting either AChE or BChE. Known BACE1 inhibitors are poly(hetero)aromatic amide scaffolds structurally similar to these compounds; therefore, the aim of this contribution is to verify how our compounds are able to modulate this enzyme.

2. Results and Discussion

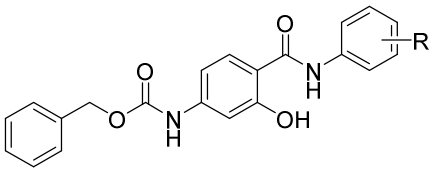
The synthesis of the carbamates of 4-aminosalicylanilides was performed in two steps (see Scheme 1). The primary amino moiety of 4-aminosalicylic acid was protected by a reaction with benzyl chloroformate in an alkaline medium to form 4-{[(benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid (CbzPAS). The carboxyl group of CbzPAS was subsequently activated in dry chlorobenzene with phosphorous chloride and, using microwave-assisted synthesis, the resulting acyl chloride provided the target benzyl carbamates of 4-aminosalicylanilides with a suitable aniline [25], see Table 1.



Scheme 1. Synthesis of 4-{[(benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid (CbzPAS) and corresponding anilides **1–38**. *Reagents and conditions:* (a) MeOH, $NaHCO_3$, room temperature, 24 h; (b) PCl_3, chlorobenzene, microwave reactor, 130 °C, 30 min. [25].

The lipophilicity and electronic relations in the molecule are among the important parameters of the compounds that influence binding to the active centers of enzymes. The lipophilicity of the studied compounds was determined using RP-HPLC as logarithm of capacity factor *k*. The retention times of individual compounds were determined under isocratic conditions with methanol as an organic modifier in the mobile phase using end-capped non-polar C18 stationary RP columns. Electronic σ parameters of the whole substituted anilide ring characterizing the ability to withdraw or donate electrons to the molecule system were predicted by ACD/Percepta software. Both data are presented in Table 1.

Table 1. Structure of ring-substituted benzyl [4-(arylcarbamoyl)phenyl-3-hydroxy]carbamates **1**–**38**, experimentally determined log *k* of investigated compounds, electronic σ parameters of substituted anilide ring and in vitro reduction of BACE1 activity [%] in comparison with starting 4-{[(benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid (CbzPAS).



Comp.	R	log *k*	σ_{Ar} [1]	Reduction of BACE1 Activity [%]
1	H	0.1160	0.60	0
2	2-OCH_3	0.3368	0.01	0.7
3	3-OCH_3	0.128	0.66	0
4	4-OCH_3	0.1176	0.36	2.6
5	2-OCF_3	0.6227	0.09	0
6	3-CH_3	0.2725	0.48	0
7	2-F	0.3577	1.02	5.1
8	3-F	0.2303	0.82	0
9	4-F	0.3924	0.62	27.5
10	2-Cl	0.5188	1.02	0
11	3-Cl	0.6411	0.85	0
12	4-Cl	0.6399	0.75	0
13	3-CF_3	0.4477	0.89	0
14	4-CF_3	0.7408	0.95	0
15	2,3-F	0.4705	1.24	0
16	2,4-F	0.1912	1.04	0
17	2,5-F	0.4012	1.24	0
18	3,5-F	0.4072	1.12	0
19	2,3-Cl	0.5497	1.22	0
20	2,5-Cl	0.6138	1.22	0
21	2,6-Cl	0.8125	1.33	0
22	3,4-Cl	0.6664	1.19	0
23	3,5-Cl	0.9084	1.11	0
24	2,4-Br	0.7366	1.11	0
25	3,5-CF_3	0.9667	1.05	0
26	2,4,6-F	-0.0131	1.46	0
27	3,4,5-F	0.5169	1.64	0
28	2,4,5-Cl	0.9360	1.56	0
29	2,4,6-Cl	0.3679	1.48	0
30	2,4,6-Br	0.4427	1.47	0
31	2-OCH_3-5-NO_2	0.0914	1.32	0
32	2-CH_3-5-F	0.2694	0.81	0
33	2-F-3-CF_3	0.4423	1.24	0
34	3-F-4-CF_3	0.5541	1.11	0
35	2-Cl-5-CF_3	0.6137	1.19	1.5
36	3-Br-5-CF_3	0.8632	1.08	0
37	2-CF_3-4-Br	0.6270	1.05	0
38	2-CF_3-4-NO_2	0.4907	1.45	0
CbzPAS	–	–	–	3.4

[1] Calculated using ACD/Percepta ver. 2012 (Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2012).

The lipophilicity of compounds expressed as log *k* is in a wide range of values from −0.0131 to 0.9667. (2*E*)-3-Phenyl-*N*-(2,4,6-trifluorophenyl)prop-2-enamide (**26**) is the least lipophilic derivative, while (2*E*)-*N*-[3,5-bis(trifluoromethyl)phenyl]-3-phenylprop-2-enamide (**25**) is the most lipophilic compound. Substituted anilide rings have rather electron-withdrawing properties, as can be seen from the values of the electronic σ parameters (Table 1). Although (2*E*)-*N*-(2-methoxyphenyl)-3-phenylprop-2-enamide (**2**) and

(2*E*)-3-phenyl-*N*-[2-(trifluoromethoxy)phenyl]prop-2-enamide (**5**) have σ = 0.01 and 0.09, respectively, (2*E*)-*N*-(4-methoxyphenyl)-3-phenylprop-2-enamide (**4**), the third compound in the order, has already σ = 0.36. (2*E*)-3-Phenyl-*N*-(3,4,5-trifluorophenyl)prop-2-enamide (**27**) with σ = 1.64 possesses the most significant electron-withdrawing properties.

All the prepared compounds (Table 1) were subjected to basic preliminary screening for BACE1 inhibition using a commercially available assay [26]. The test performance of the assay is described in Section 3.3. Since the IC_{50} values of known BACE1 inhibitors (including the control compound in the kit [26]) are in the nanomolar range, all the compounds were evaluated at a concentration of 10 μM (see, e.g., [27]). It can be assumed that if there is no activity at 10 μM, the compound most probably does not inhibit BACE1 [27].

Although parent CbzPAS showed 3.4% inhibition of BACE1, its basic unsubstituted (2*E*)-*N*,3-diphenylprop-2-enamide (**1**) lost any ability to modulate BACE1 activity. The ability to inhibit BACE1 was restored by benzyl {4-[(4-fluorophenyl)carbamoyl]-3-hydroxyphenyl}carbamate (**9**), which demonstrated the most significant 27.5% BACE1 inhibition in the series of the discussed compounds. However, this compound showed insignificant inhibition of acetyl (IC_{50} = 47.37 μM) and butyryl- (IC_{50} = 105.82 μM) cholinesterase [25]. (2*E*)-*N*-(2-Fluorophenyl)-3-phenylprop-2-enamide (**7**) and compound **4** (R = 4-OCH_3) already reduced BACE1 activity by only 5.1% and 2.6%, respectively. Compound **2** (R = 2-OCH_3), the most potent BChE inhibitor (IC_{50} = 22.23 μM) with the highest selectivity for BChE (SI = 2.26) [25], showed only 0.7% BACE1 inhibition. (2*E*)-*N*-[2-Chloro-5-(trifluoromethyl)phenyl]-3-phenylprop-2-enamide (**35**), the only one of the disubstituted derivatives, reduced BACE1 activity by 1.5%. The rest of the compounds showed no inhibition.

Although only a small number of compounds demonstrated any ability to modulate BACE1 activity, for interest, a correlation of the decrease in BACE1 activity on the physicochemical parameters of the discussed compounds was performed. The trends of the reduction of BACE1 activity, expressed as BACE1 inhibition [%], on lipophilicity (log *k*) and electronic σ parameters are shown in the graphs in Figure 1. While the effect of lipophilicity (log *k*) on the reduction of BACE1 activity was not apparent, the electronic σ parameters seemed to have a significant effect. Derivatives with minimal electron-withdrawing substituents and, conversely, compounds with significant electron-withdrawing substituents did not reduce BACE1 activity. The greatest inhibition of BACE1 was shown by derivative **9** (R = 4-F) with σ = 0.62. It can also be stated that BACE1 is influenced by derivatives monosubstituted in position $C_{(4)}$′ with small substituents capable of forming hydrogen bonds and possessing σ approx. 0.6. Displacement of the F or OCH_3 group from $C_{(4)}$′ to the $C_{(2)}$′ position caused loss of BACE1 inhibition. Substitution of the *meta* position or multisubstitution resulted in a total inability to modulate BACE1 activity in any way. In summary, none of the compounds capable of inhibiting acetyl/butyrylcholinesterase showed the ability to decrease BACE1 activity.

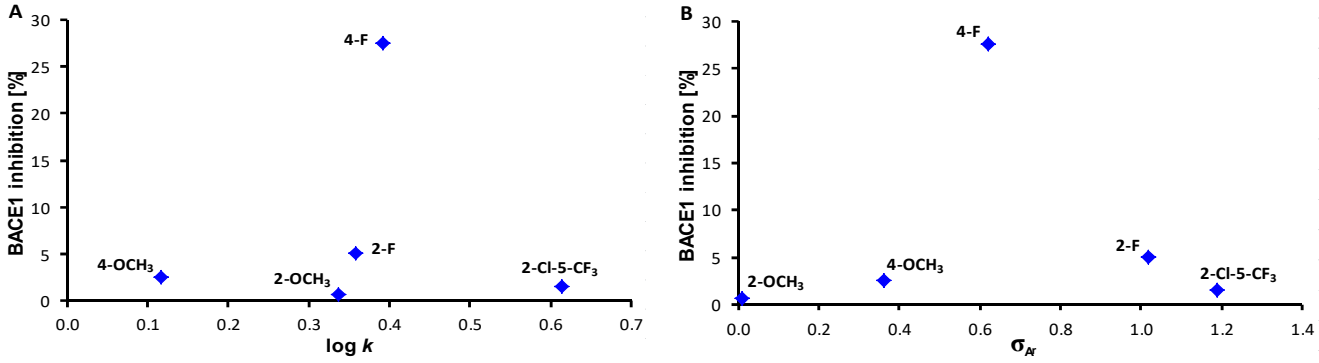


Figure 1. Trends of in vitro reduction of BACE1 activity [%] on lipophilicity expressed as log *k* (**A**) and electronic σ parameters of whole substituted anilides (**B**) of studied compounds.

3. Experimental Section

3.1. Chemistry

All the discussed 4-{[(benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid amides were prepared and characterized previously [25].

3.2. Determination of Lipophilicity by HPLC

The HPLC separation system Agilent 1200 series equipped with a DAD SL (Agilent Technologies, Santa Clara, CA, USA) was used. A chromatographic column Symmetry C18 5 µm, 4.6 × 250 mm, Part No. W21751W016 (Waters Corp., Milford, MA, USA) was applied. The HPLC separation process was monitored by the ChemStation for LC 3D chromatography software (Agilent Technologies). Isocratic elution by a mixture of MeOH p.a. (72%) and H_2O-HPLC Mili-Q grade (28%) as a mobile phase was used for the determination of the capacity factor *k*. The total flow of the column was 1.0 mL/min, injection 20 µL, column temperature 40 °C, and sample temperature 10 °C. The detection wavelength of 210 nm was chosen. A KI methanolic solution was used for the determination of the dead times (t_D). Retention times (t_R) were measured in minutes. The capacity factors *k* were calculated according to the formula $k = (t_R - t_D)/t_D$, where t_R is the retention time of the solute and t_D is the dead time obtained using an unretained analyte. Each experiment was repeated three times. The log *k* values of individual compounds are shown in Table 1.

3.3. Determination of BACE1 Inhibitory Activity

The BACE inhibitory activity was determined by commercial assay according to manufacturer instructions (Merck Life Science, Bratislava, Slovakia) [26]. The principle of the assay is based on the fluorescence resonance energy transfer (FRET) method in which the fluorescence signal enhancement is observed after substrate cleavage by BACE1, meaning that the lower the percentage of BACE activity, the more BACE1 is inhibited by the test compounds. The results are shown in Table 1.

Author Contributions: Conceptualization, A.K. and J.J.; methodology, P.M. and A.K.; investigation, P.M., D.P., T.J., I.G. and J.K.; writing—original draft preparation, A.K. and J.J.; funding acquisition, A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Slovak Research and Development Agency (projects APVV-21-0321, APVV-21-0254) and VEGA 2/0129/21.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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