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Preparation and Hydro-Lipophilic Properties of Novel Fluorinated Benzyl Carbamates of 4-Aminosalicylanilides †

Timotej Jankech [1,2], Dominika Pindjakova [1], Jiri Kos [3,*], Milan Hutta [1] and Josef Jampilek [1,3]

[1] Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Ilkovicova 6, 842 15 Bratislava, Slovakia; timotej.jankech@gmail.com (T.J.); pindjakova.dominika@gmail.com (D.P.); milan.hutta@uniba.sk (M.H.); josef.jampilek@gmail.com (J.J.)
[2] NT-LAB o.z., Teplicka 35, 921 01 Piestany, Slovakia
[3] Regional Centre of Advanced Technologies and Materials, Faculty of Science, Palacky University, Slechtitelu 27, 783 71 Olomouc, Czech Republic
* Correspondence: jiri.kos@upol.cz
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Abstract: A series of seven fluorinated benzyl carbamates of 4-aminosalicylanilides and unsubstituted benzyl [3-hydroxy-4-(phenylcarbamoyl)phenyl]carbamate designed as agents with the expected anticholinesterase and anti-inflammatory activity were prepared and characterized. As lipophilicity significantly influences the biological activity of compounds, the hydro-lipophilic properties of these mono-, di-, and tri-substituted carbamates were investigated in this study. All the discussed derivatives of 4-{[(benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid were analyzed using reversed-phase high performance liquid chromatography to measure lipophilicity. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using an end-capped non-polar C_{18} stationary reversed-phase column. In the present study, the correlations between the logarithm of the capacity factor *k*, the logarithm of the distributive parameter *D* at pH 7.4, and log *P*/Clog *P* values calculated in various ways as well as the relationships between the lipophilicity and the chemical structure of the studied compounds are discussed.

Keywords: 4-aminosalicylanilides; carbamates; synthesis; lipophilicity determinations; structure–lipophilicity relationships

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1. Introduction

One of major prerequisites for pharmacological screening and drug development is the prediction of absorption, e.g., the transport of a molecule through membranes. Drugs most frequently cross biological barriers by passive transport, which strongly depends on lipophilicity. Therefore, hydro-lipophilic properties are one of the most important physical characteristics of biologically active compounds [1,2]. The thermodynamic parameter characterized by the partition (log *P*) coefficient describes the partitioning of a compound between an aqueous and an organic phase [3]. Classical methods for the determination of these constants are time consuming and not always sufficiently reliable. Therefore, reversed-phase high performance liquid chromatography (RP-HPLC) methods have become popular and widely used for lipophilicity measurement. A general procedure is the measurement of directly accessible retention time under isocratic conditions with varying amounts of an organic modifier in the mobile phase using end-capped non-polar C_{18} stationary RP columns and calculating the capacity factor *k* [4–8]. Log *k*, calculated from the capacity factor *k*, is used as the lipophilicity index converted to log *P* scale [4].

Nevertheless, the log *P* values include only the neutral form of compounds and are independent of ionization under physiological conditions [9]. However, if the molecule

contains basic or acidic groups, it is ionized, and its distribution in the octanol/water mixture is pH-dependent. At physiological pH, many basic or acidic drugs are ionized [10]. It is estimated that 95% of all drugs are ionizable [9]. Therefore, the second descriptor of lipophilicity is the distribution coefficient expressed as D (D_{pH}) or its logarithm (log D). This descriptor is dependent on the pH of the medium and is used for ionizable molecules, and, therefore, its value includes the contribution of all ionized forms present at a given pH. If only neutral (non-ionizable) molecules are studied, log P = log D [11]. The distribution coefficient, which takes into account ionization, is a more reliable expression of lipophilicity at physiological pH, and log $D_{7.4}$ values (at pH 7.4) are of particular importance because they resemble actual physiological values. This descriptor is considered to be the most important lipophilicity descriptor and is preferred in the ADME study [12].

Aminosalicylic acids belong to the large family of salicylic acids. Salicylic acid itself is known for its anti-inflammatory, antibacterial, and cosmetic properties. However, due to the free phenolic moiety, it has a significant irritability and, therefore, is used only locally. Even in medical cosmetics, it is used only in units of percent. Salicylic acid is also an important signaling component in plant immunity. In addition, it has been shown to be able to affect acidic ion channels, which are the most sensitive molecular sensors for changes in extracellular pH in mammals. These channels are in the membranes of cells, where these molecules are involved in different important regulatory functions, such as synaptic plasticity, learning, memory, and nociception as well as in various pathological states. Salicylic acid-based structures show a wide range of biological activities, although the appropriate mechanism of action responsible for the overall biological activities of these compounds has not been proposed so far [13–16]. There are two *para*-aminosalicylic acids: (*i*) 5-aminosalicylic acid (mesalazine), which has anti-inflammatory activity and is used as one of the possible drugs for treatment of inflammatory bowel disease (IBD) [17]; (*ii*) 4-aminosalicylic acid (PAS), which is a bacteriostatic antituberculous drug [18] and also a drug for IBD treatment [19,20].

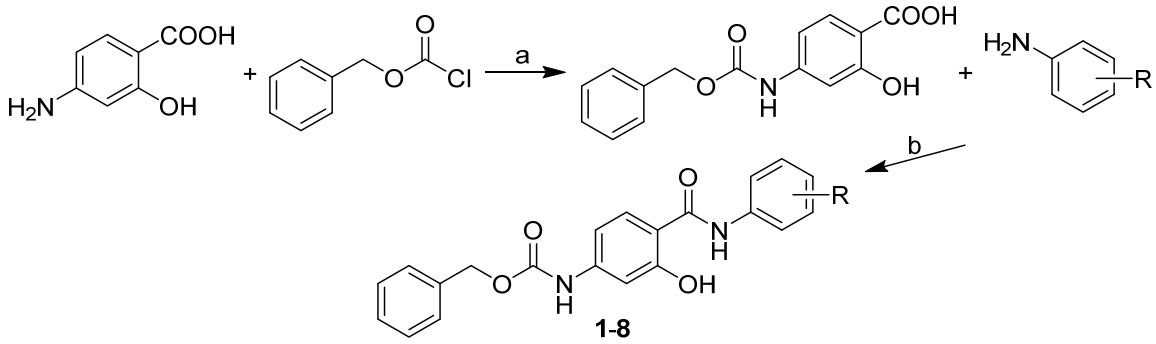
Carbamates are prepared to modify ADME (especially bioavailability) properties by blocking the hydrophilic and often unstable/easily metabolizable amino moiety and may extend the biological potential of molecules by inserting a new peptide-binding fragment capable of modulating inter- and intramolecular interactions with target enzymes or receptors [3,21,22].

Thus, based on previous experience with carbamates derived from various types of compounds [16,23–32], 4-{[(benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid was prepared from 4-aminosalicylic acid, from which a series of ring-substituted benzyl [3-hydroxy-4-(phenyl-carbamoyl)phenyl]carbamates was prepared, and lipophilicity (log k, log D) and structure–lipophilicity relationships of these compounds were investigated.

2. Results and Discussion

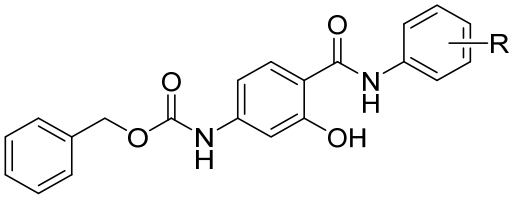
The synthesis of the carbamates of 4-aminosalicylanilides was carried out in two steps (see Scheme 1). The primary amino moiety of 4-aminosalicylic acid was protected by a reaction with benzyl chloroformate in an alkaline medium to form 4-{[(benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid that was subsequently condensed with appropriate substituted anilines using phosphorus trichloride in dry chlorobenzene under microwave conditions to give a series of investigated benzyl carbamates of 4-aminosalicylanilides **1**–**8**.

The lipophilicities (log P/Clog P data) of all eight compounds were calculated using commercially available programs such as ACD/Percepta ver. 2012 and ChemBioDraw Ultra 13.0. In addition, the lipophilicity of the studied compounds was investigated by means of RP-HPLC determination of capacity factors k with a subsequent calculation of log k and determination of distribution coefficient $D_{7.4}$ with a subsequent calculation of log $D_{7.4}$. 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. All the results are shown in Table 1.



Scheme 1. Synthesis of ring-substituted benzyl carbamates of 4-aminosalicylanilides **1**–**8**. *Reagents and conditions*: (**a**) MeOH, $NaHCO_3$, room temperature, 24 h; (**b**) PCl_3, chlorobenzene, MW, 130 °C, 30 min.

Table 1. Structure of ring-substituted benzyl [3-hydroxy-4-(phenylcarbamoyl)phenyl]carbamates **1**–**8**, calculated lipophilicities (log *P*/Clog *P*), and experimentally determined log *k* and log $D_{7.4}$ values of investigated compounds.



Comp.	R	log *k*	log $D_{7.4}$	log *P* [a]	log *P*/Clog *P* [b]
1	H	0.1160	0.1896	4.62	3.68/3.4412
2	3-F	0.2303	0.2465	4.91	3.83/3.8418
3	2,4-F	0.1912	0.2218	4.83	3.99/3.4749
4	2,5-F	0.4012	0.3974	4.95	3.99/3.4749
5	2,6-F	−0.1205	−0.0086	4.70	3.99/2.8749
6	3,5-F	0.4072	0.3788	5.15	3.99/4.0749
7	2,4,6-F	−0.0131	0.0861	4.81	4.15/3.0495
8	3,4,5-F	0.5169	0.4895	5.26	4.15/4.1095

[a] ACD/Percepta ver. 2012, [b] ChemBioDraw ver. 13.0.

Log *P* values calculated by the ChemBioDraw software for individual anilide positional isomers, i.e., for compounds **4**–**6** (R = di-F) and **7**, **8** (R = tri-F) are not distinguished; therefore, these values are listed only in Table 1 without other discussion. Clog *P* values were not distinguished only for compounds **3** (R = 2,4-F) and **4** (R = 2,5-F). The matches of all experimental (log *k*, log $D_{7.4}$) and predicted values of log *P* (ACD/Percepta) and Clog *P* (ChemBioDraw) of the substituted compounds are illustrated in Figure 1. All calculated lipophilicity values for compounds **1**–**8** have only moderate consensus with experimentally determined log *k* (correlation coefficients r = 0.8532, 0.8912 for $n = 8$), log $D_{7.4}$ (r = 0.8398, 0.8722 for $n = 8$); see Figure 1. On the other hand, the consensus of both experimentally determined parameters log *k* and log $D_{7.4}$ is very high (r = 0.9972, for $n = 8$); see Figure 2. Thus, based on the experimental results, benzyl {4-[(2,6-difluorophenyl)carbamoyl]-3-hydroxyphenyl}carbamate (**5**) is the least lipophilic compound, while benzyl {3-hydroxy-4-[(3,4,5-trifluorophenyl)carbamoyl]phenyl}- carbamate (**8**) is the most lipophilic. Contrary to the predictions, unsubstituted benzyl [3-hydroxy-4-(phenylcarbamoyl)phenyl]carbamate (**1**) is the third-least-lipophilic compound.

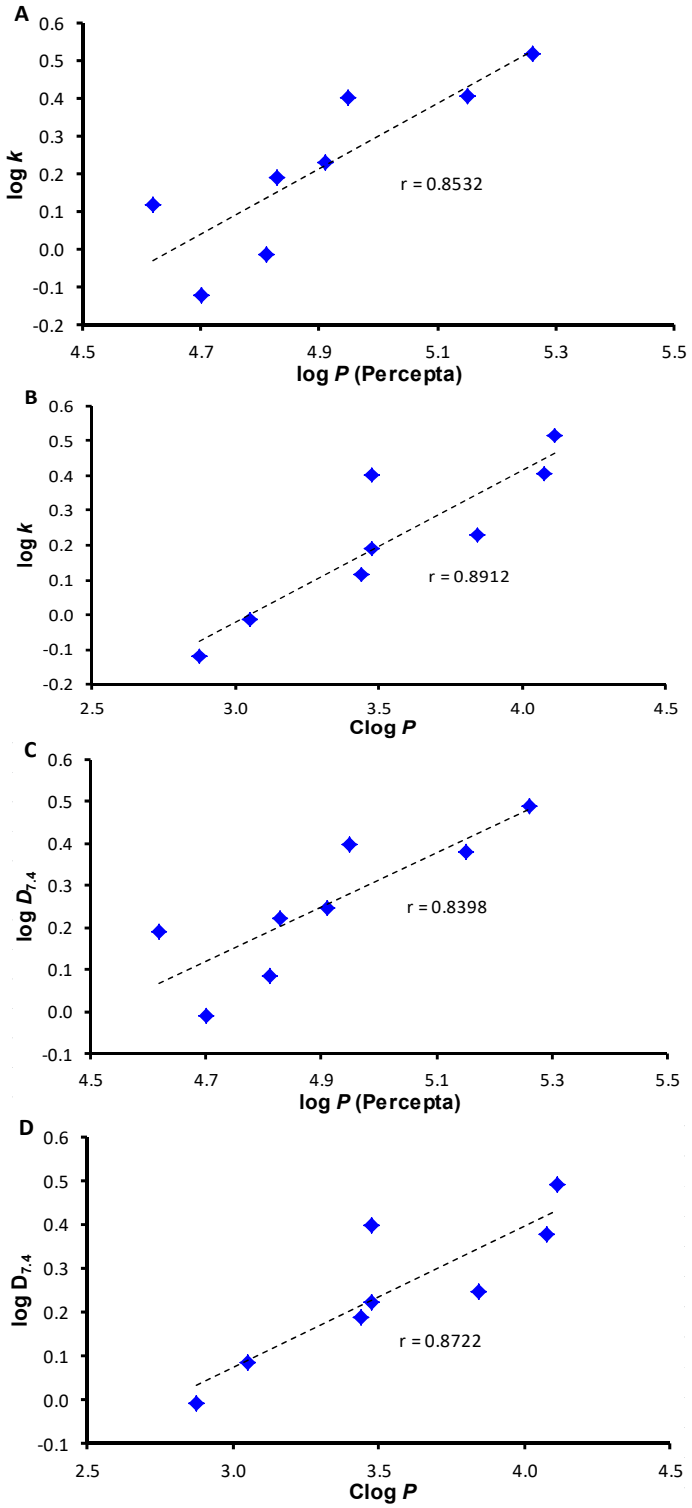


Figure 1. Comparison of experimentally found log *k* values with predicted log *P* (ACD/Percepta, (**A**) and Clog *P* (ChemBioDraw, (**B**) and log $D_{7.4}$ values with predicted log *P* (ACD/Percepta, (**C**) and Clog *P* (ChemBioDraw, (**D**) of ring-substituted benzyl carbamates of 4-aminosalicylanilides **1**–**8**.

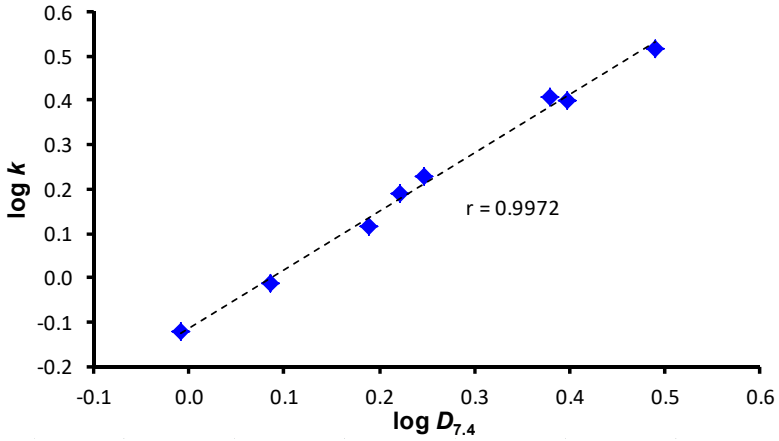


Figure 2. Comparison of experimentally found log *k* values with log $D_{7.4}$ values of ring-substituted benzyl carbamates of 4-aminosalicylanilides **1**–**8**.

Although the correlation coefficient of both experimental values is high, a discrepancy for compound **6** (R = 3,5-F) can be found. While log *k* of compound **6** is 0.4072, and it is found between those of compound **4** (R = 2,5-F, log *k* = 0.4012) and compound **8** (R = 3,4,5-F, log *k* = 0.5169), the order of log $D_{7.4}$ values is reversed for compounds **6** (log $D_{7.4}$ = 0.3788) and **4** (log $D_{7.4}$ = 0.3974).

The biggest discrepancy between the experimental and predicted values, apart from the above unsubstituted derivative **1**, is the completely different lipophilicity of di-*ortho*-substituted derivatives **2** (R = 2,6) and **7** (R = 2,4,6-F) as well as the abovementioned derivative **4**. The differences between the order of lipophilicity of the predicted and experimental results are shown in Figure 3. It can be assumed that substitution in *ortho* positions significantly modifies lipo-hydrophilic properties, which none of the used programs is able to reflect. However, these subtle nuances often affect the biological activity of the compounds. These differences, which are particularly pronounced in aqueous/buffered media, are due to intra- and intermolecular interactions of specific substituents such as –F, $–CF_3$, $–OCH_3$, –OH, and $–NH_2$ and spatially close amide/carbamate bonds [25,28,30,33–37].

Distributive π parameters describe the lipophilicity contribution of individual moieties substituted in some skeleton [38,39]. These π parameters characterizing the hydrophobicity of individual substituents were calculated according to the formula $\pi = \log k_S - \log k_U$ where log k_S is the determined capacity factor logarithm of individual substituted compounds and log k_U denotes the determined capacity factor logarithm of unsubstituted compound **1**; this means π = 0. The same applies to the values of the distribution coefficient *D*. The π values of individual substituted anilide rings (π_{Ar}) of the discussed compounds are shown in Table 2, where it is possible to see discrepancies between experimental and calculated π_{Ar} values (mutual order of values) especially for compounds **5** (R = 2,6-F), **6** (R = 3,5-F), and **7** (R = 2,4,6-F). These observed discrepancies between π_{Ar} values calculated by ACD/Percepta (the order of log *P* data of which fully corresponds to experimentally determined log *k*; see Table 1) are only lipophilicity contributions without possible interactions of substituents in the *ortho* position with a carboxamide group within one molecule, while π_{Ar} values based on experimentally determined log *k*/log $D_{7.4}$ data carry these interactions in them. It should be noted that the π_{Ar} values calculated from the experimental log *k* and log $D_{7.4}$ differ insignificantly from each other in contrast to the values obtained from ACD/Percepta.

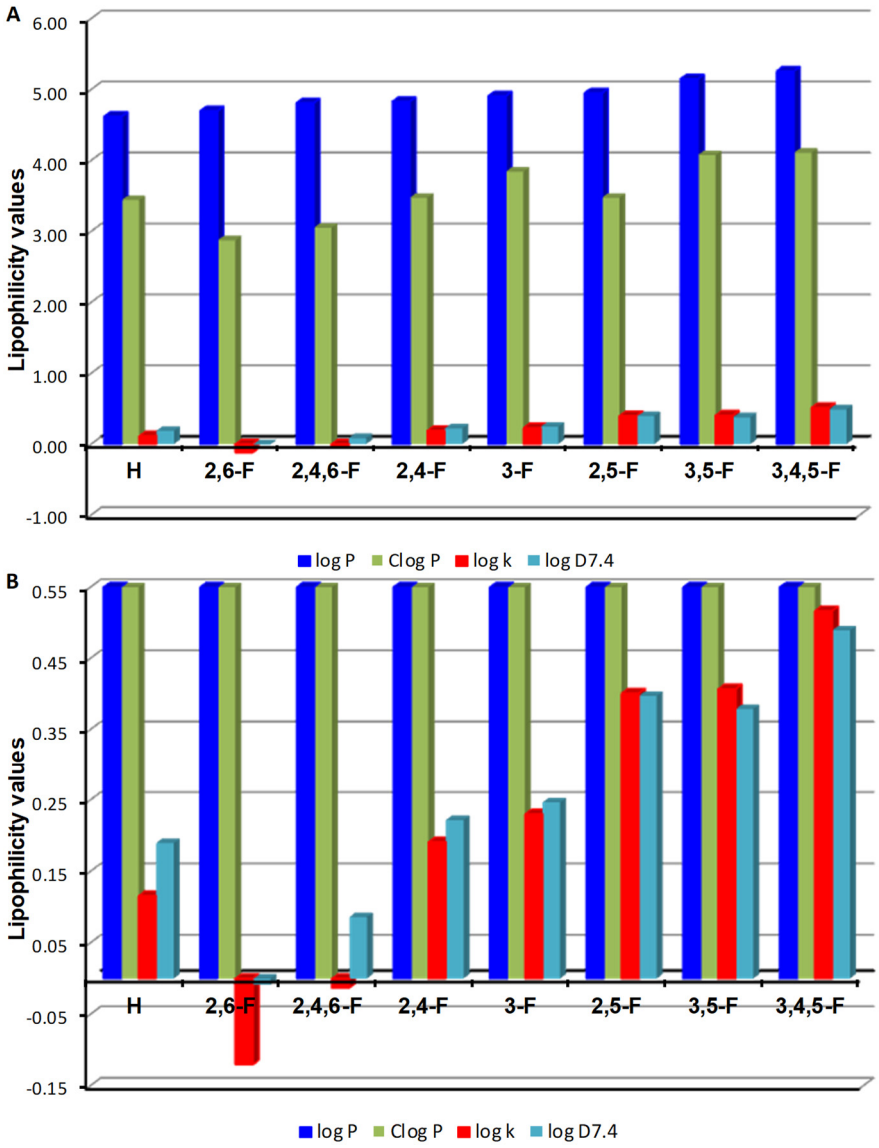


Figure 3. Order of lipophilicity within series of compounds **1**–**8** when they are ordered according to increasing log *P* values ((**A**) = the whole image, (**B**) = frame).

Table 2. Comparison of determined distributive parameters π calculated from log *k* and log $D_{7.4}$ for each individual substituted anilide ring within the investigated series of compounds **1**–**8** and parameters π of individual substituted anilide rings predicted by ACD/Percepta.

Comp.	R	π_{Ar} (exp. log *k*)	π_{Ar} (exp. log $D_{7.4}$)	π_{Ar} (ACD/Percepta)
1	H	0	0	1.76
2	3-F	−0.24	−0.20	2.80
3	2,4-F	−0.13	−0.10	2.81
4	2,5-F	0.08	0.03	2.23
5	2,6-F	0.11	0.06	1.78
6	3,5-F	0.29	0.21	2.35
7	2,4,6-F	0.29	0.19	1.84
8	3,4,5-F	0.40	0.30	1.59

Based on the results of this preliminary study of new fluoro-substituted benzyl [3-hydroxy-4-(phenylcarbamoyl)phenyl]carbamates, it can be assumed that predicted lipophilicity values are in poor agreement with experimentally determined lipophilicity

values and, thus, it will be necessary to use experimentally specified lipophilicity for subsequent investigation of the structure–activity relationships due to strong intra- and intermolecular interactions of these specific substituents with the rest of the molecular pattern or with the second compound or with medium.

3. Experimental

3.1. General

All reagents were purchased from Merck (Sigma-Aldrich, St. Louis, MO, USA) and Alfa (Alfa-Aesar, Ward Hill, MA, USA). Reactions were performed using an Anton Paar Monowave 50 microwave reactor (Graz, Austria). The melting points were determined on a Kofler hotplate apparatus HMK (Franz Kustner Nacht KG, Dresden, Germany) and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet iS5 IR spectrometer (Thermo Scientific, West Palm Beach, FL, USA). The spectra were obtained by the accumulation of 256 scans with 2 cm^{-1} resolution in the region of 4000–450 cm^{-1}. All ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-ECA 600II device (600 MHz for ^{1}H and 150 MHz for ^{13}C, JEOL, Tokyo, Japan) in dimethyl sulfoxide-d_6 (DMSO-d_6). ^{1}H and ^{13}C chemical shifts (δ) are reported in ppm.

3.2. Synthesis

4-{[(Benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid. 4-Aminosylicylic acid (6.0 g, 39.2 mM) was dissolved in methanol (64 mL) at ambient temperature, and benzyl chloroformate (6.8 mL, 47.6 mM) was added dropwise. The reaction mixture was stirred at room temperature for 24 h. The solvent was then removed to dryness under reduced pressure. The crude product was dissolved in the mixture of ethyl acetate (200 mL) and 1 M hydrochloric acid (200 mL). Layers were then divided. The water layer was additionally extracted with ethyl acetate (3 × 50 mL). Afterwards, organic layers were collected and dried with magnesium sulfate. The solvent was then removed to dryness under reduced pressure. Yield 71%; Mp 219–220 °C; IR (ATR, cm^{-1}): 1727, 1623, 1589, 1538, 1440, 1383, 1307, 1270, 1249, 1220, 1204, 1185, 1165, 1104, 1056, 1028, 998, 976, 905, 871, 774, 767, 760, 725, 691, 669, 656, 639, 620, 595, 573, 547, 509, 466, 453; ^{1}H-NMR (DMSO-d_6), δ: 11.39 (brs, 1*H*), 10.13 (s, 1*H*), 7.69 (d, J = 8.7 Hz, 1*H*), 7.46–7.32 (m, 5*H*), 7.16 (d, J = 2.1 Hz, 1*H*), 6.99 (dd, J = 8.8 Hz, 2.2 Hz, 1*H*), 5.17 (s, 2*H*); 13C-NMR (DMSO-d_6), δ: 171.65, 162.20, 153.05, 145.72, 136.23, 131.09, 128.48, 128.25, 128.17, 109.28, 106.96, 104.72, 66.14.

General procedure for synthesis of novel fluorinated benzyl carbamates of 4-aminosalicyl-anilides **1**–**8**: 4-{[(Benzyloxy)carbonyl]amino}-2-hydroxybenzoic acid (0.7 mmol; 0.2 g) was suspended in dry chlorobenzene (6 mL) at ambient temperature and phosphorus trichloride (0.35 mM, 0.5 equation), and the corresponding substituted aniline (0.7 mM, 1 equation) was added dropwise. The reaction mixture was transferred to the microwave reactor, where the synthesis was performed (30 min, 130 °C). Then the mixture was cooled to 40 °C, and then the solvent was removed to dryness under reduced pressure. The residue was washed with hydrochloride acid and water. The crude product was recrystallized from ethanol.

Benzyl [3-hydroxy-4-(phenylcarbamoyl)phenyl]carbamate (**1**). Yield: 69%; Mp 170–172 °C; IR (ATR, cm^{-1}): 3326, 3033, 1742, 1701, 1635, 1618, 1536, 1498, 1446, 1434, 1328, 1298, 1279, 1219, 1191, 1142, 1097, 1076, 1062, 1029, 967, 850, 763, 750, 692, 598, 564, 508, 464; ^{1}H-NMR (DMSO-d_6), δ: 12.14 (s, 1*H*), 10.24 (s, 1*H*), 10.09 (s, 1*H*), 7.93 (d, *J* = 8.3 Hz, 1*H*), 7.67 (d, *J* = 8.3 Hz, 2*H*), 7.45-–7.35 (m, 7*H*), 7.23 (d, *J* = 2.8 Hz, 1*H*), 7.13 (t, *J* = 6.9 Hz, 1*H*), 7.03 (dd, *J*= 8.3 Hz, *J* = 2.8 Hz, 1*H*), 5.18 (s, 2*H*); ^{13}C-NMR (DMSO-d_6), δ: 167.10, 160.54, 153.65, 144.68, 138.66, 136.86, 130.22, 129.26, 129.03, 128.77, 128.71, 124.62, 121.60, 111.43, 109.52, 105.88, 66.59.

Benzyl {4-[(3-fluorophenyl)carbamoyl]-3-hydroxyphenyl}carbamate (**2**). Yield: 63%; Mp 195–196 °C; IR (ATR, cm^{-1}): 3428, 3326, 3038, 1732, 1618, 1604, 1534, 1492, 1437, 1386, 1329, 1302, 1279, 1253, 1201, 1177, 1154, 1109, 1082, 1061, 1029, 991, 980, 948, 889, 858, 839, 773, 762,

733, 695, 677, 635, 597, 547, 520, 466; ^{1}H NMR (DMSO-d_6), δ: 11.92 (s, 1*H*), 10.36 (s, 1*H*), 10.09 (s, 1*H*), 7.89 (d, *J* = 8.8 Hz, 1*H*), 7.69 (dt, *J* = 11.6 Hz, *J*=2.2 Hz, 1*H*), 7.45–7.34 (m, 7*H*), 7.26 (d, *J* = 2.0 Hz, 1*H*), 7.03 (dd, *J* = 8.7 Hz, *J* = 2.1 Hz, 1*H*), 6.97–6.93 (m, 1*H*), 5.17 (s, 2*H*), ^{13}C NMR (DMSO-d_6), δ: 166.46, 162.09 (d, *J* = 241.3 Hz), 159.63, 153.13, 144.29, 140.07 (d, *J* = 10.1 Hz), 136.33, 130.33 (d, *J* = 8.7 Hz), 129.98, 128.49, 128.25, 128.18, 116.49 (d, *J* = 2.3 Hz), 111.16, 110.39 (d, *J* = 20.2 Hz), 109.14, 107.56 (d, *J* = 26.0 Hz), 105.35, 66.09.

Benzyl {4-[(2,4-difluorophenyl)carbamoyl]-3-hydroxyphenyl}carbamate (**3**). Yield: 61%; Mp 218–219 °C; IR (ATR, cm^{-1}): 3325, 3257, 1700, 1651, 1619, 1592, 1573, 1537, 1497, 1455, 1436, 1362, 1346, 1298, 1277, 1244, 1209, 1187, 1142, 1105, 1086, 1066, 1030, 993, 974, 962, 906, 887, 842, 825, 814, 788, 769, 759, 733, 695, 673, 658, 615, 599, 575, 549, 504, 473, 463, 454; ^{1}H-NMR (DMSO-d_6), δ: 12.02 (s, 1*H*), 10.45 (s, 1*H*), 10.1 (s, 1*H*), 8.09 (td, *J* = 9.1 Hz, *J* = 6.5 Hz, 1*H*), 7.91 (d, *J* = 8.9 Hz, 1*H*), 7.46–7.44 (m, 2*H*), 7.42–7.34 (m, 4*H*), 7.33 (d, *J* = 1.4 Hz, 1*H*), 7.14–7.10 (m, 1*H*), 7.03 (dd, *J* = 8.9 Hz, *J* = 2.1 Hz, 1*H*), 5.17 (s, 2*H*); ^{13}C-NMR (DMSO-d_6), δ: 165.00, 158.54, 158.45 (dd, *J* = 242.8 Hz, *J* = 13.0 Hz), 153.82 (dd, *J* = 247.1 Hz, *J* = 13.0 Hz), 153.14, 144.25, 136.33, 130.69, 128.50, 128.25, 128.18, 124.99 (dd, *J* = 10.1 Hz, *J* = 2.9 Hz), 122.73 (dd, *J* = 11.6 Hz, *J* = 4.3 Hz), 111.37 (d, *J* = 2.9 Hz), 111.23, 109.55, 105.22, 104.11 (dd, *J* = 27.5 Hz, *J* = 24.6 Hz), 66.09.

Benzyl {4-[(2,5-difluorophenyl)carbamoyl]-3-hydroxyphenyl}carbamate (**4**). Yield: 65%; Mp 215–216 °C; IR (ATR, cm^{-1}): 3326, 2968, 2880, 2360, 1702, 1643, 1614, 1572, 1540, 1505, 1483, 1436, 1369, 1314, 1287, 1247, 1195, 1170, 1115, 1067, 1030, 1000, 988, 967, 909, 884, 839, 824, 787, 762, 737, 696, 667, 630, 620, 591, 553, 513, 464; ^{1}H-NMR (DMSO-d_6), δ: 11.73 (s, 1*H*), 10.44 (s, 1*H*), 10.10 (s, 1*H*), 7.85 (d, *J* = 8.2 Hz, 1*H*), 7.49 (dd, *J* = 9.6 Hz, *J* = 2.7 Hz, 2*H*), 7.46–7.44 (m, 2*H*), 7.42–7.39 (m, 2*H*), 7.37–7.39 (m, 1*H*), 7.28 (d, *J* = 2.1 Hz, 1*H*), 7.03 (dd, *J* = 8.9 Hz, *J* = 2.1 Hz, 1*H*), 6.97 (tt, *J* = 9.3 Hz, *J* = 2.4 Hz, 1*H*), 5.18 (s, 2H); ^{13}C-NMR (DMSO-d_6), δ: 166.34, 162.41 (d, *J* = 244.2 Hz), 162.31 (d, *J* = 242.8 Hz), 159.23, 153.12, 144.36, 140.98 (t, *J* = 13.0 Hz), 136.31, 130.18, 128.49, 128.24, 128.18, 111.40, 109.24, 105.29, 103.39 (m, 2C), 98.89 (t, *J* = 26,0 Hz), 66.09.

Benzyl {4-[(2,6-difluorophenyl)carbamoyl]-3-hydroxyphenyl}carbamate (**5**). Yield: 69%; Mp 175–178 °C; IR (ATR, cm^{-1}): 3405, 3326, 3035, 1736, 1632, 1615, 1591, 1511, 1497, 1470, 1419, 1372, 1309, 1294, 1270, 1242, 1215, 1198, 1111, 1058, 1012, 990, 963, 915, 848, 826, 776, 760, 741, 721, 700, 650, 628, 558, 528, 509, 483, 455, 444; ^{1}H-NMR (DMSO-d_6), δ: 12.09 (s, 1*H*), 10.14 (s, 1*H*), 10.12 (s, 1*H*), 7.95 (d, *J* = 8.9 Hz, 1*H*), 7.46–7.34 (m, 6*H*), 7.27–7.21 (m, 3*H*), 7.06 (d, *J* = 8.2 Hz, 1*H*), 5.19 (s, 2*H*); ^{13}C-NMR (DMSO-d_6), δ: 167.20, 160.56, 158.11 (dd, *J* = 249 Hz, *J* = 5.8 Hz), 153.16, 144.73, 136.31, 129.75, 128.52, 128.30, 128.20, 114.05 (t, *J* = 17.3 Hz), 111.95 (dd, *J* = 20.2 Hz, *J* = 4.3 Hz), 109.43, 109.22, 105.35, 66.17.

Benzyl {4-[(3,5-difluorophenyl)carbamoyl]-3-hydroxyphenyl}carbamate (**6**). Yield: 57%; Mp 224–225 °C; IR (ATR, cm^{-1}): 3327, 2968, 1708, 1643, 1614, 1573, 1540, 1505, 1483, 1437, 1369, 1314, 1287, 1247, 1195, 1169, 1115, 1067, 1000, 988, 963, 884, 839, 824, 768, 761, 737, 696, 666, 630, 620, 591, 553, 513, 465; ^{1}H-NMR (DMSO-d_6), δ: 11.73 (s, 1*H*), 10.44 (s, 1*H*), 10.10 (s, 1*H*), 7.84 (d, *J* = 8.7 Hz, 1*H*), 7.53–7.31 (m, 6*H*), 7.49 (dd, *J* = 9.8 Hz, *J* = 2.2 Hz, 1*H*), 7.28 (d, *J* = 1.9 Hz, 1*H*), 7.03 (dd, *J* = 8.7 Hz, *J* = 2.1 Hz, 1*H*), 6.96 (tt, *J* = 9.3 Hz, *J* = 2.4 Hz, 1*H*), 5.17 (s, 2*H*); ^{13}C-NMR (DMSO-d_6), δ: 166.34, 162.35 (dd, *J* = 242.8 Hz, *J* = 15.3 Hz), 159.23, 153.10, 144.35, 140.97 (t, *J* = 13.8 Hz), 136.31, 130.16, 128.48, 128.22, 128.16, 111.38, 109.23, 105.30, 103.58–103.20 (m), 98.88 (t, *J* = 26.3 Hz), 66.09.

Benzyl {3-hydroxy-4-[(2,4,6-trifluorophenyl)carbamoyl]phenyl}carbamate (**7**). Yield: 68%; Mp 203–206 °C; IR (ATR, cm^{-1}): 3443, 3363, 1739, 1651, 1606, 1508, 1466, 1448, 1416, 1378, 1363, 1336, 1310, 1252, 1211, 1188, 1172, 1117, 1085, 1052, 1039, 1030, 997, 979, 900, 853, 833, 825, 801, 756, 736, 711, 699, 691, 658, 636, 629, 608, 544, 512, 497; ^{1}H-NMR (DMSO-d_6), δ: 11.97 (s, 1*H*), 10.13 (s, 1*H*), 10.03 (s, 1H), 7.90 (d, *J* = 8.9 Hz, 1*H*), 7.45–7.43 (m, 2*H*), 7.42–7.39 (m, 2*H*), 7.37–7.33 (m, 3*H*), 7.25 (d, *J* = 2.1 Hz, 1*H*), 7.03 (dd, *J* = 8.9 Hz, *J* = 2.1 Hz, 1*H*), 5.18 (s, 2H); ^{13}C-NMR (DMSO-d_6), δ: 167.10, 160.33, 160.29 (dt, *J* = 245.7 Hz, *J* = 14.5 Hz), 158.23 (ddd, *J* = 250 Hz, *J* = 15.9 Hz, *J* = 7.2 Hz), 153.11, 144.70, 136.29, 129.80, 128.50, 128.27, 128.18, 111.1 (td, *J* = 17.3 Hz, *J* = 4.3 Hz), 109.41, 109.0, 105.26, 100.98 (m), 66.13.

Benzyl {3-hydroxy-4-[(3,4,5-trifluorophenyl)carbamoyl]phenyl}carbamate (**8**). Yield: 80%; Mp 206–207 °C**;** IR (ATR, cm^{-1}): 3429, 3334, 3040, 1726, 1616, 1543, 1527, 1438, 1393, 1313, 1285, 1237, 1208, 1182, 1135, 1044, 991, 964, 894, 873, 850, 834, 796, 763, 741, 697, 663, 620, 566, 513, 466, ^{1}H-NMR (DMSO-d_6), δ: 11.72 (s, 1*H*), 10.40 (s, 1*H*), 10.10 (s, 1*H*), 7.83 (d, *J* = 8.9 Hz, 1*H*), 7.73–7.75 (m, 2*H*), 7.46–7.43 (m, 2*H*), 7.42–7.39 (m, 2*H*), 7.36–7.34 (m, 1*H*), 7.28 (d, *J* = 2.1 Hz, 1*H*), 7.03 (dd, *J* = 8.2 Hz, *J* = 2.1 Hz, 1*H*), 5.18 (s, 2*H*); ^{13}C-NMR (DMSO-d_6), δ: 166.30, 159.24, 153.11, 149.97 (ddd, *J* = 244.2 Hz, *J* = 10.1 Hz, *J* = 5.8 Hz), 144.38, 136.31, 135.01 (dt, *J* = 245.7 Hz, *J* = 15.9 Hz), 134.76 (td, *J* = 11.6 Hz, *J* = 4.3 Hz), 130.10, 128.49, 128.23, 128.17, 111.24, 109.23, 105.27, 104.98 (dd, *J* = 20.2 Hz, *J* = 5.8 Hz), 66.09.

3.3. 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 mm × 250 mm, Part No. W21751W016 (Waters Corp., Milford, MA, USA) was used. 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 HPLC-grade Milli-Q H_2O (28%) as a mobile phase was used for the determination of the capacity factor *k*. Isocratic elution by a mixture of MeOH p.a. (72%) and acetate-buffered saline (pH 7.4) (28%) as a mobile phase was used for the determination of the distribution coefficient expressed as $D_{7.4}$. 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. The distribution coefficients $D_{7.4}$ were calculated according to the formula $D_{7.4} = (t_R - t_D)/t_D$. Each experiment was repeated three times. The log *k* values of individual compounds are shown in Table 1.

3.4. Lipophilicity Calculations

Log *P*, i.e., the logarithm of the partition coefficient for *n*-octanol/water, was calculated using the programs ACD/Percepta (Advanced Chemistry Development. Inc., Toronto, ON, Canada, 2012) and ChemBioDraw Ultra 13.0 (CambridgeSoft, PerkinElmer Inc., Cambridge, MA, USA). Clog *P* values (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) were calculated using ChemBioDraw Ultra 13.0 (CambridgeSoft) software. The results are shown in Table 1. The distributive parameters π_{Ar} of individual substituted anilide rings of individual compounds were predicted using ACD/Percepta and are shown in Table 2.

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