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Membrane permeability related physicochemical properties of a novel γ -secretase inhibitor

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Abstract

Pharmaceutical profiling studies were conducted on a novel prototype γ -secretase inhibitor, to determine the potential of its oral absorption. Such compounds can be of use in the treatment of Alzheimer's disease (AD). The studies included determination of solubility, dissociation constant (pK_a), octanol/water partition coefficient ($\log P$) and the capacity factor (k'_{IAM}) on immobilized artificial membrane (IAM) chromatographic columns. The compound is very slightly soluble in water ($120 \pm 50 \mu\text{g/mL}$) but the solubility increased considerably in basic medium ($270 \pm 60 \mu\text{g/mL}$). The compound exhibited pK_a of (10.36 ± 0.11) ; and $\log P$ of (3.36 ± 0.16) determined by shake-flask method and (3.31 ± 0.01) determined by high performance liquid chromatography (HPLC). The experimentally determined $\log P$ values correlated well with the calculated one of 3.44. The observed k'_{IAM} value of (2.79 ± 0.04) indicates that the compound can reasonably be expected to have high membrane permeability, and therefore, good absorption profile if taken orally. This conclusion is also supported by other parameters determined.

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive loss of memory and cognition. Secretases (β - and γ -) have been characterized to be key enzymes involved with processing amyloid precursor protein (APP) to give A β 40–A β 43 fragments. A β 42 is thought to trigger the peptides to form fibrillar deposits in the surrounding neurons, and progression of AD (Dominguez and De Strooper, 2002; Citron, 2002). The γ -secretase inhibitory ac-

tivities of several compounds synthesized in our laboratory were conducted. Sulfonamide 1 (Fig. 1) has emerged as a prototype member of its class. The compound is an adamantane based sulfonamide and was obtained as a pure white crystalline solid. Since the target site of action is the brain and oral formulation is generally preferred, such compounds, therefore, have to cross two biological barriers namely, intestinal membrane and blood brain barrier to reach the central nervous system and elicit action if taken orally.

Physicochemical properties are useful in the quest to evaluate abilities of drugs to cross biological membranes and oral absorption (Horter and Dressman, 2001). Aqueous solubility as a function of pH along with pK_a determination are important and

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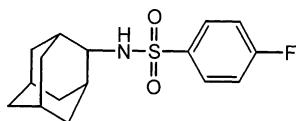


Fig. 1. Structure of sulfonamide 1.

fundamental in determining the degree of dissolution (for solid dosage forms) and subsequent permeation through cell membrane. Prior to drug administration, they are of value in drug design, selection of formulation, and development processes. Octanol/water partition coefficient has been used as a measure of lipophilicity of compounds and correlates well with biological availability (Paschke et al., 2001). Recently, a chromatographic technique referred to as immobilized artificial membrane phosphatidylcholine chromatography (IAM-PC) (Pidgeon et al., 1995) utilizing columns packed with phosphatidylcholine bound to silica support has been developed and validated as a model to predict intestinal permeability.

The objectives of this study are to evaluate selected physicochemical properties of sulfonamide 1, and to estimate its ability to cross biological membranes with a view as to determining its suitability for oral administration. The properties evaluated are solubility, dissociation constant, partition coefficient and IAM partitioning. These properties are very relevant to membrane permeability and for prediction oral absorption.

2. Materials and methods

2.1. Materials

Sulfonamide 1 (4-Fluoro-N-(adamantan-2-yl)benzenesulfonamide) was synthesized in our laboratory (Wells, 2003). Confirmation of structure and purity were obtained using NMR, MS, IR, elemental analysis and high performance liquid chromatography (HPLC). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Solubility

Equilibrium solubility of sulfonamide 1 in water and buffers (Accumet pH Meter 915, Fisher Scien-

tific, PA, USA) were determined at $23.0 \pm 1.0^\circ\text{C}$. Phosphate buffers, 50 mM, were used in the pH range 3.00–11.50 adjusted to constant ionic strength of 0.15 with NaCl. Appropriate amount of the compound was added to 2 mL of the solvent into screw-capped vials. Suspensions were shaken (American Rotator R4140, American Dade, Division of American Hospital Supply Corp., FL, USA) for 48 h at 220 rpm until equilibrium was attained. Preliminary experiments showed that equilibrium was achieved in this period of time. Samples were filtered through a Cameo 3N-syringe filter, nylon, 0.22 μm (Osmonics Inc., Minnetonka, MN, USA). Concentration of sulfonamide 1 in the filtrate was appropriately diluted with the suitable solvent and determined spectrophotometrically (Lambda 40 UV/Vis Spectrometer, Perkin Elmer Analytical Instruments, CT, USA) at 222 nm, against blank.

A UV scan between 400 and 200 nm for sulfonamide 1 in 1:1 acetonitrile to water mixture gave two peaks. The first is at $\lambda = 264$ nm and the second is at $\lambda = 222$ nm. The peak at 264 nm is very small and unsuitable for analysis unless a very high concentration of the compound is used whether the method of analysis is UV or HPLC with UV detection. Therefore, we decided to use $\lambda = 222$ nm taking into account that many solvents could give absorbance at this level. For the solvents utilized in this study, water, acetonitrile, and phosphate buffers, the UV cut off points were at 200 nm or lower. Pure octanol has UV absorbance at 230 nm. However, when sulfonamide 1 was to be analyzed, a few or up to hundred μL of the octanolic solutions of the compound were diluted more than 20-times with acetonitrile before spectrophotometric measuring of the compound. In addition, measurements were conducted against blanks which were identical as the test samples except for sulfonamide 1. Therefore, there was no solvent interference of the UV analysis of sulfonamide 1. In literature, a good number of drugs have been analyzed spectrophotometrically at or below 222 nm (Scott et al., 1981; de Villiers and Bergh, 2000; Erk, 2002; El-Gindy et al., 2003).

We measured the absorbance of sulfonamide 1 at different pH values, but the readings were almost same. This did not allow us to use spectrophotometric analysis to determine $\text{p}K_a$ of the compound though it is easier experimentally and reported as an efficient method. However, we did not compare absorbance of compound in water versus octanol as it is not

particularly relevant to this study. For the standard calibration curve, a 1:1 mixture of acetonitrile/water was used to make a stock solution of the compound. From this stock solution, dilutions were made and the samples were analyzed. To avoid any error due to volatilization of acetonitrile and changing the concentration of compound in the stock solution, dilutions and UV measurements were conducted right after preparation of the stock solution.

2.2.2. pK_a and $\log P$ determinations by shake-flask method

The ionization constant was determined by partitioning between octanol and 0.5 M phosphate buffer as earlier described (Golumbic et al., 1949) with slight modification. First, mutual saturation of octanol and buffer solutions of pH 5.61 and 11.07 were made by shaking equal portions of both phases at 40 rpm for 8 h. After shaking, the solutions were then left overnight for separation. A 4.8 mg of sulfonamide 1 was dissolved in 10 mL octanol. Octanolic solution (1 mL) was introduced into 10 mL of pH 5.61 and 11.07 buffers in 60 mL separatory funnel and gently shaken by hand for 2 min. Solutions were then placed in the shaker and mechanically shaken for 0.5 h at 180 rpm. The solutions were left overnight for equilibration and separation of the two phases. Then the aqueous phases were removed and centrifuged at 6000 rpm for 30 min. The experiment was repeated by shaking for 2, 10, and 24 h. There was no difference in sulfonamide 1 partition coefficient, which indicated that a 0.5 h period was sufficient for equilibration. Partitioning of sulfonamide 1 between octanol and water was determined using the same procedure to determine its $\log P$. The concentration of sulfonamide 1 was determined in the organic and aqueous phases spectrophotometrically at 222 nm. Eq. (1) was used to calculate $\log P$:

$$\log P = \log \left(\frac{C_o}{C_w} \right) \quad (1)$$

where C_o and C_w are the molar concentrations of sulfonamide 1 in octanol and aqueous phases, respectively. Eq. (2) was used to determine pK_a after calculating amount of sulfonamide 1 in the octanol and aqueous buffers using Eq. (1):

$$\log \hat{K} = -\text{pH} + pK_a + \log k \quad (2)$$

where \hat{K} is the observed partition coefficient of weak acids (HA) at pH 11.07. This observed partition coefficient is given by Eq. (3) and is a resultant of two equilibria for weak acids: (i) partition of the un-dissociated acid between the immiscible phases, and (ii) ionization of the acid in the aqueous phase. The intrinsic partition coefficient at pH 5.61 is represented by k , which results from partitioning of the unionized form only between the immiscible phases.

$$\hat{K} = \frac{[\text{HA}]_0}{[\text{HA}]_w + [\text{A}^-]_w} \quad (3)$$

where $[\text{HA}]_0$ is the concentration of the acid in the octanolic phase, $[\text{HA}]_w$ is the concentration in the aqueous phase and A^-_w is the ionized form of the acid in the aqueous layer. The partition coefficients in the systems of octanol–0.5 M phosphate buffers of pH 11.07 and 5.61 as shown in Table 1 were used for \hat{K} and k , respectively.

2.2.3. $\log P$ determination by HPLC

A reversed-phase HPLC using isocratic elution mode at room temperature was used for determination of $\log P$ of sulfonamide 1. A liquid chromatograph equipped with UV detector (L-4000 UV, Hitachi Ltd, Japan) operating at 222 nm was used. The stationary phase was an octadecyl (ODS) C_{18} column (5 μm , 25 cm \times 4.6 mm i.d., Phenomenex, CA, USA). The mobile phase, acetonitrile (ACN)/water mixture, was pumped (L-6200A Intelligent Pump, Hitachi Ltd, Japan) at a flow rate of 0.75 mL/min. Data were recorded and integrated using Spectra-Physics

Table 1
Physicochemical properties of sulfonamide 1

M_w^a	309.40
mp ^b (°C)	150–152
Solubility (23.0 \pm 1.0 °C) (μg/mL)	
Water	120 \pm 50
Phosphate buffer (pH 7.4)	200 \pm 30
Phosphate buffer (pH 11.50)	270 \pm 60
Log partition coefficients	
Observed (\hat{K})	2.80 \pm 0.06
Intrinsic (k)	3.52 \pm 0.11
pK_a	10.36 \pm 0.11

Values shown represent average (\pm S.D.; $n = 5$).

^a Molecular weight.

^b Melting point.

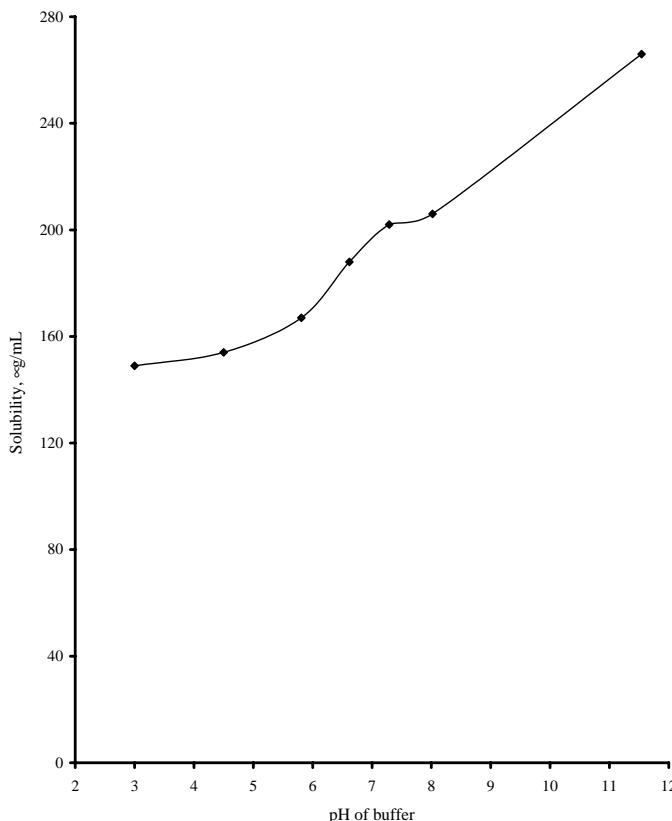


Fig. 2. Aqueous solubility of sulfonamide **1** with respect to buffer pH.

Integrator (Model SP4270, Autolab Division, CA, USA). The column was conditioned by making several injections of sulfonamide **1** until identical retention times were obtained. The dead time was determined with acetone. Solution of sulfonamide **1** was prepared at $\sim 3 \times 10^{-4}$ M and a sample of 5 μ L was injected (AS-4000 Intelligent Autosampler, Hitachi Ltd, Japan) into the column. The logarithm of the retention time parameter, $\log k_{rt}$, which correlates linearly with $\log P$ and is also used as a lipophilicity index, was determined using Eq. (4) (McCall, 1975)

$$\log k_{rt} = \log \left(\frac{t_R - t_0}{t_0} \right) \quad (4)$$

where; t_R is the retention time of the retained compound peak and t_0 is the retention time of an unretained peak. The $\log k_{rt}$ was determined at different concentrations of ACN in the mobile phase. The resultant $\log k_{rt}$ values were plotted versus percentage

of ACN and then extrapolated to 100% water mobile phase to yield $\log k_{rtw}$ as shown in Fig. 2. Data presented in Table 2 were used to calculate $\log P$.

2.2.4. IAM partition chromatography

All chromatographic conditions and procedures under $\log P$ determination by HPLC study were applied. Different column, IAM·PC·DD·2^{C10/C3} (12 μ m, 300 \AA , 3 cm \times 4.6 mm i.d., Regis Technologies, Inc., IL, USA) was used, preceded by a 3 cm ODS guard column. The retention time of a solute molecule on IAM·PC columns is also used to calculate its capacity factor, k'_{IAM} , as follows (Ong et al., 1996):

$$\log k'_{IAM} = \log \left(\frac{t_R - t_0}{t_0} \right) \quad (5)$$

$\log k'_{IAM}$ at 100% aqueous mobile phase was obtained as described earlier under $\log P$ determination by HPLC.

Table 2
The averaged retention parameters ($\log k_{\text{rt}}$, non-aqueous $\log k'_{\text{IAM}}$) of sulfonamide **1** at different eluent compositions (water/ACN) along with their extrapolated and $\log P$ values

Retention parameter	Percent of ACN					Extrapolated value	$\log P$	
		90	80	70	60		Cal. ^a	SFM ^b
$\log k_{\text{rt}}$		-0.35 ± 0.00	0.04 ± 0.00	0.36 ± 0.02	0.69 ± 0.00	1.11 ± 0.00	1.64 ± 0.01	3.09 ± 0.01^c
Non-aqueous $\log k'_{\text{IAM}}$		-0.65 ± 0.08	-0.28 ± 0.02	0.07 ± 0.13	0.40 ± 0.01	0.78 ± 0.02	1.35 ± 0.03	2.79 ± 0.04^d

Values shown represent average (\pm S.D.; $n = 5$).

^a Calculated.
^b Shake-flask method.
^c $\log k_{\text{rw}}$.
^d $\log k'_{\text{IAM}}$.

3. Results and discussion

3.1. Solubility

Solubility of sulfonamide **1** in water along with other physicochemical properties are given in Table 1. Fig. 1 shows solubility as a function of buffers of pH values between 3 and 8, which simulate physiological conditions, and at pH 11.50. The compound shows very slightly soluble characteristics in water. Low solubility was observed at low pH values, which indicates that the amide group is un-dissociated. When the pH is high and above the pK_a of the compound the solubility increases due to increased water solubility of the ionized form of sulfonamide **1**. However, the solubility profile of the compound is not as expected. Below pK_a of the compound, there is some increase of the solubility and above pK_a the solubility does not increase to reach asymptote as expected. Although this compound is categorized as a sulfonamide, it contains the adamantane group, which may give it specific characteristics different from common sulfonamides. The saturated polycyclic hydrocarbon structure of adamantane is expected to affect the solubility of the compound. It has been found that adamantane crystallizes in a face-centered cubic lattice. This is extremely unusual for an organic compound. The molecule, therefore, should be completely free from both angle and torsional strain. At the beginning of growth, crystals of adamantane show only cubic and octahedral faces. The effects of this unusual structure upon physical properties are striking (<http://tigger.cc.uic.edu/~mansoori/Diamondoids.html>). Based on that, we believe that abnormal physical characteristics of this compound could be observed. In addition it is possible for pseudopolymorphism to occur in the solubilizing process. It seems that the amide group is not the only factor that controls the solubility profile of the compound. The hydrocarbon part of the compound as well as steric considerations may have significant effects. Therefore, further studies need to be conducted to be able to understand why the solubility curve deviated from the expected pattern. Solid state characterizations of the compound before and after solubility determination and also prior and after equilibrium is attained need to be studied. The counter ion in the buffer might have an effect; therefore, using another buffer system might give some explanations.

There is literature precedence for weak acids not exhibiting expected profiles. Examples include flurbiprofen which has pK_a of 4.9 and its solubility increases from 8 $\mu\text{g}/\text{mL}$ at pH of 1.2 to 12 $\mu\text{g}/\text{mL}$ at pH 2 to 15 $\mu\text{g}/\text{mL}$ at pH 3 (Herzfeldt and Kummel, 1983). Another example is naproxen which has pK_a of 4.1 and its solubility increases from 13 $\mu\text{g}/\text{mL}$ at pH of 1.2 to 22 $\mu\text{g}/\text{mL}$ at pH 2. While at pH higher than pK_a of the drug by almost one unit, the solubility only increased from 29 $\mu\text{g}/\text{mL}$ at pH 4 to 35 $\mu\text{g}/\text{mL}$ at pH of 5 (Herzfeldt and Kummel, 1983).

It is well known that solubility is a prime factor in the dissolution process of solid dosage forms before partitioning into the GI wall. It has also been reported (Horter and Dressman, 2001) that if the solubility of a compound is lower than 100 $\mu\text{g}/\text{mL}$ such compound would have poor absorption profile from the GI lumen due to poor dissolution rate. For sulfonamide 1, the solubility is higher than 100 $\mu\text{g}/\text{mL}$, which indicates that the compound may show reasonable dissolution rate in the aqueous GI environment. Solubility of sulfonamide 1 was also determined in some organic solvents and found to be very soluble in chloroform, methanol, acetone and ACN.

3.2. pK_a determination

The observed and intrinsic partition coefficients of sulfonamide 1 determined by partitioning between octanol and phosphate buffers of pH 11.07 and 5.61 along with the calculated ionization constant are shown in Table 1. At higher pH, sulfonamide 1 dissolved in organic phase partitions into the aqueous buffer and, thus, equilibrium between immiscible phases is a result of two equilibria as shown in Eq. (3). Whereas at lower pH the equilibrium between phases is a function of the unionized form. Based on such principle, Eq. (3) can be converted into a relationship that relates \bar{K} , k , the acidity constant, K_a , and the hydrogen ion concentration $[\text{H}^+]$ of the aqueous phase as follows (Gombic et al., 1949):

$$\bar{K} = \frac{k}{1 + K_a/[\text{H}^+]} \quad (6)$$

When the aqueous phase contains a buffer of sufficient alkalinity so that $[\text{H}^+] \ll K_a$, the unity term in the denominator becomes negligible in compari-

son with other term; hence Eq. (6) can be written as follows:

$$\bar{K} = \frac{k[\text{H}^+]}{K_a} \quad (7)$$

Taking logarithm of Eq. (7) gives rise to Eq. (2). The method has the advantages of simplicity and applicability to insoluble compounds. Compared with other sulfonamides of similar structures, the pK_a value of sulfonamide 1 is quite reasonable. The initial concentration of sulfonamide 1 in the organic phase was suitable since dilute solution may affect the resultant pK_a while high concentration might lead to association and dimer formation. The pK_a is a fixed property of a drug or compound and is of importance in understanding its absorption and disposition. Based on the determined pK_a for sulfonamide 1 and the pH-partition theory, it can be concluded that sulfonamide 1 should be in the absorbed form (unionized) along the whole GI pas-sageway.

3.3. Determination of $\log P$

Computed and experimental $\log P$ values determined by shake-flask method and HPLC are shown in Table 2. ChemDraw Ultra® software program was used to determine $\log P$ of sulfonamide 1 (Wells, 2003). Log P determined by shake-flask was calculated using Eq. (1) and found to be 3.36 ± 0.16 . In the last several decades, HPLC has been used for the determination of log octanol/water partition coefficient (Mirrlees et al., 1976; Donovan and Pescatore, 2002). Log P determination by HPLC is mainly based on determining log retention time, previously described in this paper, and log retention volume, $\log V_R$, defined as $\log V_R = \log[(t_R - t_0)(\text{flow rate})]$ (Mirrlees et al., 1976). In this study, log retention time was used for determining $\log P$ of sulfonamide 1 as it is more common and independent of the flow rate, which might be a problem in the $\log V_R$ method. The retention time of sulfonamide 1 was prolonged considerably by increasing the water content of the mobile phase. Log k_{rt} value of the sample compound was calculated from its retention value by the application of Eq. (4). The ACN concentration in the mobile phase was varied to obtain peaks with reasonable time. Plot of $\log k_{rt}$ versus percentage of ACN gives a good linear relationship ($r = 0.996$) as shown in Fig. 3. The Figure

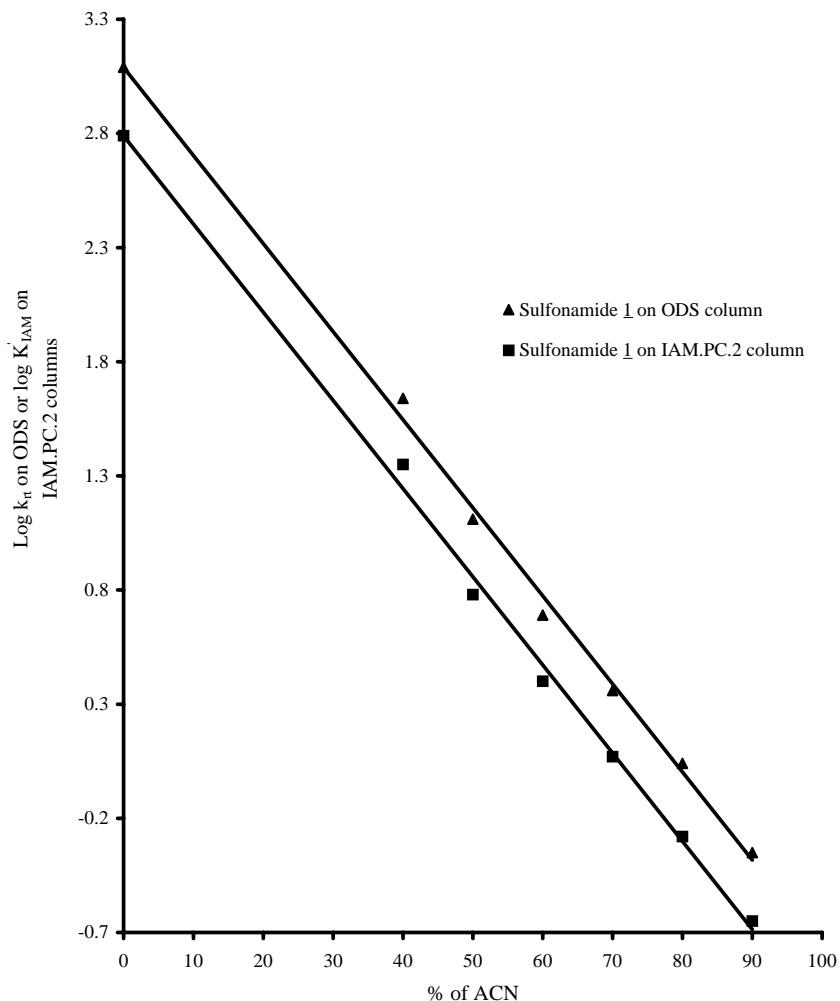


Fig. 3. Extrapolated $\log k_{rt}$ (on ODS column) and non-aqueous $\log k'_{IAM}$ (on IAM-PC-2 column) values of sulfonamide 1 to 0% ACN.

shows a dependence of $\log k_{rt}$ on the ACN content in the mobile phase. When the line was extrapolated to 0% ACN the $\log k_{rtw}$ value was obtained. The relationship between $\log k_{rtw}$ and $\log P$ for sulfonamides (Altomare et al., 1989) is expressed by Eq. (8):

$$\log k_{rtw} = 0.70(\pm 0.09)\log P + 0.77(\pm 0.12) \quad (8)$$

$$n = 20, s = 0.218, r = 0.969$$

Table 2 lists the values of $\log k_{rt}$ and $\log k_{rtw}$ extrapolated to 0% ACN along with the computed and experimentally determined $\log P$ values for sulfonamide 1. The results confirm the usefulness of $\log k_{rtw}$ value

as an expression of the lipophilic index of chemicals and/or a conversion parameter for estimating unknown $\log P$ as previously indicated by many workers (Brent et al., 1983; Yamana et al., 1977). The computed $\log P$ value is higher than observed ones, though not significantly. This higher computed value could be normal and expected since calculation of $\log P$ depends on addition of π -values of the molecule constituents and does not take into account the possible intermolecular or intramolecular interactions. It has been shown (Hansch et al., 1972) that $\log P$ is not entirely additive.

The partition coefficient of a drug molecule governs its absorption, distribution, metabolism and excretion

(Little and Ryan, 1982). Compared with 33 benzene sulfonamide (Altomare et al., 1989) compounds, sulfonamide **1** has the highest $\log P$ value, which favors its permeability through biological membranes. The data are also an incentive for further screening and developmental studies for this compound.

3.4. IAM partition chromatography

Table 2 shows the values of non-aqueous $\log k'_{\text{IAM}}$ and the extrapolated $\log k'_{\text{IAM}}$ to 100% water. Aqueous mobile phase is used to elute compounds on these columns. However, if a compound is highly lipophilic, an organic modifier such as methanol or ACN can be used as co-solvent with the aqueous part. Mobile phase containing 40% ACN was the lowest to elute sulfonamide **1**. Therefore, extrapolated $\log k'_{\text{IAM}}$ at 0% ACN was obtained and used as the capacity factor for sulfonamide **1**. It has been shown that $\log k'_{\text{IAM}}$ of drugs/compounds correlate well with their partitioning into liposomes, permeability through Caco-2 cells, in situ rat intestinal absorption, and percentage of oral absorption in mice (Yang et al., 1996). $\log k'_{\text{IAM}}$ of sulfonamide **1** was compared with those for other drugs and cephalosporines whose values were correlated with oral absorption in mice. The highest $\log k'_{\text{IAM}}$ reported was 3.1 for LY 11193 (Yang et al., 1996), a cephalosporin that gave high log percentage of oral absorption in mice. Since the relationship between $\log k'_{\text{IAM}}$ and log percentage absorption is linear, a value of 2.79 ± 0.04 for sulfonamide **1** is of importance and a positive sign for high intestinal absorption.

4. Conclusions

Pharmaceutical profiling studies have been conducted on a representative γ -secretase inhibitor. The determined physicochemical parameters of sulfonamide **1** indicate that oral formulations can be developed for the compound. It can be absorbed from any part in the GI lumen by passive diffusion since its pK_a is higher than the physiological pH. In the mean time, lipophilicity of the compound, as determined by $\log P$, indicates that absorption extent of sulfonamide **1** could be high and looks comparable to those for highly absorbed drugs (Leo et al., 1971).

Another property which indicates high permeability of sulfonamide **1** is that it seems to have hydrophilic (based on its water solubility) and lipophilic (based on its $\log P$ and $\log k'_{\text{IAM}}$ values) balance that favors its dissolution, as a pre-condition to its absorption from the GI tract followed by permeation through the lipid intestinal membrane.

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References

- Altomare, C., Carotti, A., Cellamare, S., Ferappi, M., 1989. Lipophilicity measurements of benzenesulfonamide inhibitors of carbonic anhydrase by reversed-phase HPLC. *Int. J. Pharm.* 56, 273–281.
- Brent, D.A., Sabatka, J.J., Minick, D.J., Henry, D.W., 1983. A simplified high-pressure liquid chromatography method for determining lipophilicity for structure–activity relationships. *J. Med. Chem.* 26, 1014–1020.
- Citron, M., 2002. Emerging Alzheimer's disease therapies: inhibition of beta-secretase. *Neurobiol. Aging* 23, 1017–1022.
- de Villiers, M.M., Bergh, J.J., 2000. Comparing HPLC and UV spectrophotometric analysis methods for determining the stability of sorbic acid in nonionic creams containing lactic acid. *Drug Dev. Ind. Pharm.* 26, 539–547.
- Dominguez, D.I., De Strooper, B., 2002. Novel therapeutic strategies provide the real test for the amyloid hypothesis of Alzheimer's disease. *Trends Pharmacol. Sci.* 23, 324–330.
- Donovan, S.F., Pescatore, M.C., 2002. Method for measuring the logarithm of the octanol–water partition coefficient by using short octadecyl-poly(vinyl alcohol) high-performance liquid chromatography columns. *J. Chromatogr.* 52, 47–61.
- El-Gindy, A., Emara, S., Hadad, G.M., 2003. Spectrophotometric and liquid chromatographic determination of trimebutine maleate in the presence of its degradation products. *J. Pharm. Biomed. Anal.* 33, 231–241.
- Erk, N., 2002. Simultaneous determination of fosinopril and hydrochlorothiazide in pharmaceutical formulations by spectrophotometric methods. *J. Pharm. Biomed. Anal.* 27, 901–912.
- Golumbic, C., Orchin, M., Weller, S., 1949. Partition studies on phenols. 1. Relation between partition coefficient and ionization constant. *J. Am. Chem. Soc.* 71, 2624–2627.
- Hansch, C., Leo, A., Nikaitani, J., 1972. On the additive–constitutive character of partition coefficients. *J. Org. Chem.* 37, 3090–3092.
- Herzfeldt, C.D., Kummel, R., 1983. Dissociation constant, solubilities and dissolution rates of some selected nonsteroidal antiinflammatories. *Drug Dev. Ind. Pharm.* 9, 767–793.

Horter, D., Dressman, J.B., 2001. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Adv. Drug. Deliv. Rev.* 46, 75–87.

Leo, A., Hansch, C., Elkins, D., 1971. Partition coefficients and their uses. *Chem. Rev.* 71, 525–616.

Little, P.J., Ryan, A.J., 1982. Inhibitors of hepatic mixed-function oxidases. 4. Effects of benzimidazole and related compounds on aryl hydrocarbon hydroxylase activity from phenobarbitone and 3-methylcholanthrene induced rats. *J. Med. Chem.* 25, 622–626.

McCall, J.M., 1975. Liquid–liquid partition coefficient by high-pressure liquid chromatography. *J. Med. Chem.* 18, 549–552.

Mirrlees, M.S., Moulton, S.J., Murphy, C.T., Taylor, P.J., 1976. Direct measurement of octanol–water partition coefficients by high-pressure liquid chromatography. *J. Med. Chem.* 19, 615–619.

Ong, S., Liu, H., Pidgeon, C., 1996. Immobilized-artificial-membrane chromatography: measurements of membrane partition coefficient and predicting drug membrane permeability. *J. Chromatogr.* 29, 113–128.

Paschke, A., Manz, M., Schuurmann, G., 2001. Application of different RP-HPLC methods for the determination of the octanol/water partition coefficient of selected tetrachlorobenzyltoluenes. *Chemosphere* 45, 721–728.

Pidgeon, C., Ong, S., Liu, H., Qiu, X., Pidgeon, M., Dantzig, A.H., Munroe, J., Hornback, W.J., Kasher, J.S., Glunz, L., et al., 1995. IAM chromatography: an *in vitro* screen for predicting drug membrane permeability. *J. Med. Chem.* 38, 590–594.

Scott, B.S., Dunn, D.L., Dorsey, E.D., 1981. Analysis of pilocarpine and isopilocarpine in ophthalmic solutions by UV spectrophotometry–polarimetry. *J. Pharm. Sci.* 70, 1046–1048.

Wells, R.M., 2003. Design and Syntheses of Non-Peptide Rigid Sulfonamide gamma-Secretase Inhibitors. MS thesis, Idaho State University.

Yamana, T., Tsuji, A., Miyamoto, E., Kubo, O., 1977. Novel method for determination of partition coefficients of penicillins and cephalosporins by high-pressure liquid chromatography. *J. Pharm. Sci.* 66, 747–749.

Yang, C.Y., Cai, S.J., Liu, H., Pidgeon, C., 1996. Immobilized artificial membrane-screening for drug membrane. *Adv. Drug. Deliv. Rev.* 23, 229–256.