MOLECULAR DETERMINANTS OF SOLUBILITY IN BIORELEVANT FLUIDS FOR DIBENZOTHIEPINE COMPOUNDS

CAMELIA ELENA STECOZA[1], DRAGOŞ CIOLAN[1], FLAVIAN-ŞTEFAN RĂDULESCU[2]*, GEORGE MIHAI NIŢULESCU[1], MAGDALENA MAJEKOVA[4], DALIA SIMONA MIRON[3]

University of Medicine and Pharmacy „Carol Davila" Bucharest, Faculty of Pharmacy, 6 Traian Vuia street, 020956, Bucharest, Romania
[1]Department of Pharmaceutical Chemistry
[2]Department of Drug Industry and Pharmaceutical Biotechnologies
[3]Department of Pharmaceutical Physics and Informatics
[4]Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dubravska cesta 9, 84104, Bratislava, Slovakia
**corresponding author:* flavian_stefan@yahoo.com

Abstract

The solubility profile of ten benzothiepine compounds was screened using the currently recommended fasted and fed state simulated gastric and intestinal fluids, as part of the *in vitro* evaluation of their biopharmaceutical properties and of the developability potential. The experimental data was compared with the previously reported *in silico* predictions generated on ADMET Predictor platform. The results indicated a considerable impact of the endogenous tensioactives, with maximum absorbable doses varying between 20 and 200 mg. The non-correlation between the *in vitro* evaluated and *in silico* estimates were significant, especially for the gastric media. The global solubility profile seems to indicate that the evaluated compounds have dissolution-rate limited characteristics and belong to the sub-class IIa of the Biopharmaceutical Classification System.

Rezumat

A fost evaluat profilul solubilităţii pentru zece compuşi cu structură dibenzotiepinică utilizând medii fiziologice simulate gastrice şi intestinale, pre- şi postprandial, ca parte a analizei *in vitro* a proprietăţilor biofarmaceutice şi a potenţialului de dezvoltare farmaceutică. Datele experimentale au fost comparate cu predicţiile *in silico* raportate anterior, generate în cadrul platformei ADMET Predictor. Rezultatele indică un impact major al compuşilor tensioactivi endogeni, doza maximă absorbabilă variind între 20 şi 200 mg. Non-corelările între datele generate *in vitro* şi estimările *in silico* au fost semnificative, în special pentru mediul gastric. Profilul global al solubilităţii pare să indice o limitare cinetică a dizolvării şi apartenenţa la clasa IIa a sistemului de clasificare biofarmaceutică.

Keywords: dibenzothiepine, biorelevant fluids, solubility, maximum absorbable doses, molecular descriptors.

Introduction

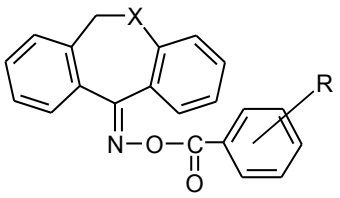
The solubility is a key thermodynamic property [1], with critical impact on absorption profile after oral administration and consequent *in vivo* exposure [2, 3]. It determines the fraction of dissolved drug available in the gastro-intestinal lumen, subject to passive diffusion or carrier-mediated transport. The introduction of simulated gastro-intestinal fluids marked a turning point in the biorelevant screening of candidate molecules. For the entities from the class II of the Biopharmaceutical Classification System [4, 5], the interplay of endogenous tensioactive substances generates fractions absorbed higher than predicted from the aqueous solubility in the pH interval of the gastro-intestinal tract, based on formation of mixed micelles [6]. Correlation between the fraction released *in vitro* and *in vivo* can be obtained, except for the cases where the oral dose is very high [1, 7]. Moreover, the standardized composition of simulated fluids simulate both fast and fed conditions of administration, for either gastric or intestinal environment (Fasted / Fed State Simulated Gastric / Intestinal Fluids, Fa/eSSG/IF [8]). Several types of interactions described between their components and specific chemical structures, mainly dependent upon the lipophilic character and acido-basic properties [9]. A previous report indicated that impact for these interactions and their concentration dependent profile are altered by the hydrodynamic parameters used during *in vitro* dissolution studies [10]. When the gastric compartment is considered, two particularities must be outlined, which concerns the utility of associated simulated fluids. The solubility values for a molecular entity reaching this level must be correlated with the prospected variations in pH-values, volumes, composition, surface tension and buffering capacity of the fluids resident in the consecutive intestinal segments. In several instances, e.g. weak bases, a high value in acidic environments generates supersaturated systems at the site of absorption [11]. Secondly, the gastric level has a minor contribution to the total amount of drug absorbed, but significantly contributes to the disintegration of the solid pharmaceutical formulations [10].

Previously we have conducted an *in silico* evaluation of a group of new molecular entities with dibenzothiepine structures [12]. The study emphasized on the contribution of the considerable lipophilic character to the distribution profile, especially the penetration of highly specialized biological interfaces, e.g. blood-brain barrier. The predicted solubility values in simulated gastro-intestinal fluids indicated a considerable impact of sodium taurocholate and lecithin. The current paper presents the results

of the *in vitro* evaluation of these parameters and possible correlations between estimated and experimental values.

Materials and Methods

Ten chemical entities with dibenzothiepine structures (Figure 1) [13-15] were subject to *in vitro* evaluation of solubility in fast and fed state simulated gastric and intestinal fluid, prepared according to previously reported methodology [10]. Briefly, an excess of drug substances was added in 2 mL round bottom polypropylene vials, containing 1 mL of FaSSGF, FeSSGF, FaSSIF or FeSSIF. The vials were stirred for 30 seconds at 5000 rpm on an IKA Vortex-3 shaker (IKA, Germany) and maintained under agitation for 12 hours at 25°C, using a RZR 2020 stirrer (Heidolph Instruments, Germany). The evaluations were performed in triplicate.



Compound	X	R
SH12	SO_2	4-C_2H_5
SH23	SO_2	2-F
TH10	S	3-CH_3
TH12	S	4-C_2H_5
TH15	S	2-OCH_3
TH16	S	3-OCH_3
TH23	S	2-F
TH25	S	4-F
TH28	S	3-Cl
TH32	S	3,4-Cl_2

Figure 1

The molecular structure of dibenzothiepine compounds, subject of *in vitro* solubility evaluation

The probes were centrifuged at 10000 rpm, 25°C, for 10 minutes (using a Sigma 2-16K centrifuge, Sartorius, Germany). Samples of 0.75 mL were collected from the supernatant and an equal volume of methanol was added, in order to induce micelle de-structure and to avoid further precipitation. The quantitative evaluation of the amounts dissolved was performed using spectrophotometric methods, on an 8453 Pharmaceutical UV-visible system, 8453 UV-Visible Spectrophotometer, Agilent Technologies. The control samples being prepared according to the same protocol.

All reagents used were of analytical grade and used as purchased (sodium hydroxide pellets - Riedel-de Haen; sodium phosphate dibasic dihydrate, sodium phosphate monobasic monohydrate, sodium chloride, acetic acid 100%, sodium acetate - Sigma Aldrich; sodium taurocholate

hydrate - Fluka; lecithin - Roth. Purified was obtained using a SGW Ultraclear UV Plus^{TM} system.

The experimental values of solubility were used for the calculation of maximum absorbable dose (MAD), as part of the Developability Classification System (DCS) [2], according to the following formula:

$$MAD = P_{eff} \cdot S_i \cdot A \cdot T_i$$

where P_{eff} is the effective jejunal permeability (cm/sec, estimated using ADMET Predictor, SimulationPlus Inc., USA), S_i is the solubility in the intestinal fluid, either FaSSIF or FeSSIF (μg/mL), A is the surface available for passive diffusion ($7.54 \cdot 10^4$ cm^2, without absorption windows or involvement of active transport systems) and T_i intestinal transit time (3.32 hours).

Results and Discussion

The experimental results confirm the contribution of the endogenous tensioactives on the solubility and consequently, on the biopharmaceutical profile of the highly lipophilic dibenzotiepine compounds. The fast state gastric media (FaSSGF) generates the highest discrepancies between the estimated and *in vitro* determined values, notably for SH12, SH23 and TH25 structures. In the first two cases, the prediction software overestimates the dissolved quantity by 30 and, respectively 7 times (Figure 2). For pH values lower than 2, the protonated species dominate, according to estimated profile of distribution coefficient (LogD). Error in estimation of ionization constants (pKa) could explain, at least partially these non-correlations. On the other hand, TH25 generates apparently supersaturated solutions, possible due to molecular aggregates.

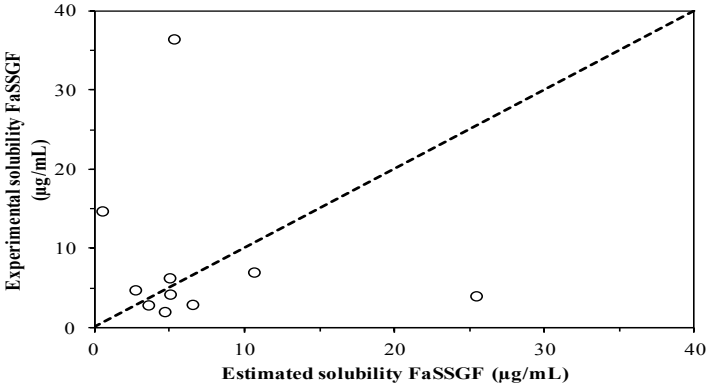


Figure 2

The estimated and experimental solubility of selected dibenzothiepines in fasted state simulated gastric fluid (FaSSGF)

For the fed state, a large interval of variation for the experimental solubility in the gastric fluids was noticed (6.76 - 179.16 µg/mL), with significantly lower values for the SH series. The *in silico* platform didn't provide any prediction, probably due to the difficulty in estimating the role of lipophilicity and molecular descriptors in dissolution processes occurring in such complex micellar systems (based on mixture of aqueous buffers and milk). Noteworthy, the values are considerably higher compared to the fasted state, but this prospected food-effect must be scaled with the reduced residence times [16]. The combined impact of pH, composition and concentration of tensioactives could be subject to high inter-individual variability.

The best correlations were obtained for solubility in the intestinal media, although all the values were overestimated (Figure 3). A possible limitation of the methodology is the short duration (12 hours). Longer evaluation periods can lead to chemical and / or microbiological alteration of media. The overall solubility pattern indicates that the selected dibenzothiepine possible belong to the BCS class IIa, i.e. they have a dissolution-rate limited profile [17]. Therefore, depending on the effective dose regimens, the pharmaceutical formulation will play an important role in the determination of bioavailable fraction.

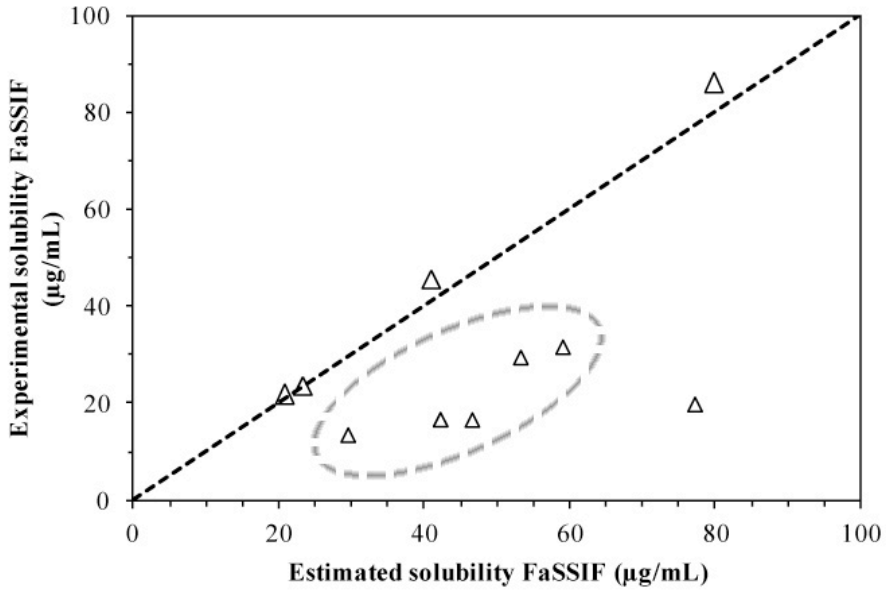


Figure 3a
Correlation between the estimated and experimental values of the solubility in simulated intestinal fluids - fasted state

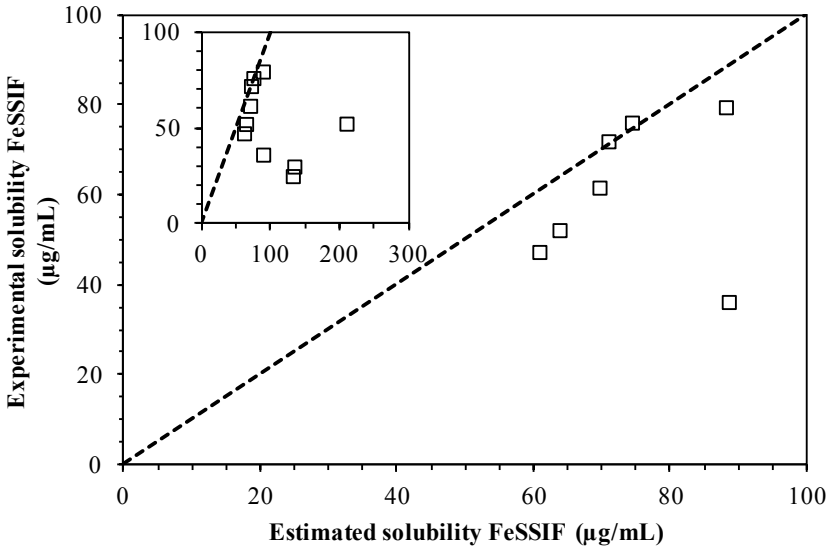


Figure 3b
Correlation between the estimated and experimental values of the solubility in simulated intestinal fluids - fed state.

The values of maximum absorbable doses (MAD) are more homogenously distributed within the considered group of compounds, despite the considerable differences in terms of solubility (Table I). The estimated effective permeability, a direct consequence of lipophilicity since only the passive diffusion processes were considered, is the dominant property.

Table I
Estimation of maximum absorbable doses (MAD), based on experimental values of the solubility (S) in simulated intestinal fluids

Compound	P_{eff}* (cm/sec 10^{-4})	S_{FaSSIF} (µg/mL)	S_{FeSSIF} (µg/mL)	MAD_{FaSSIF} (mg/mL)	MAD_{FeSSIF} (mg/mL)
SH12	2.01	58.96	74.35	55.26	69.69
SH23	1.58	53.16	70.91	49.82	66.46
TH10	3.52	23.31	69.60	21.84	65.23
TH12	3.79	77.12	209.45	72.28	196.31
TH15	2.76	42.12	131.57	39.48	123.31
TH16	3.46	46.49	133.75	43.57	125.35
TH23	3.64	41.01	60.80	38.43	56.98
TH25	4.27	79.78	88.11	74.77	82.58
TH28	3.98	20.83	63.75	19.52	59.75
TH32	3.92	29.41	88.53	27.57	82.98

* - estimated jejunal effective permeability, ADMET Predictor, Simulation Plus Inc.

Preliminary evaluations of the partition coefficient determined in binary systems using *n*-octanol and cyclohexane as the model biorelevant organic solvents confirmed the potential penetration of blood-brain barrier for similar compounds. Added to previously estimated MAD interval of 20 to 200 mg, this further increases the developability potential for this newly designed entities as psychotropic drugs. The extent and nature of biotransformation, as well as the affinity for the efflux transporters, both associated to lipophilic xenobiotics, must be adequately screened as significant interfering processes.

Conclusions

The solubility of ten new molecular entities with dibenzothiepine structure in fasted and fed state simulated gastric and intestinal fluids was assessed, based on a previously developed abbreviated protocol. The experimental data was correlated with previously reported *in silico* estimates. The results proved a significant contribution of the endogenous tensioactive to the biopharmaceutical profile of these highly lipophilic entities. Possible interferences of supersaturating phenomenon or dissolution-rate limited solubility profiles were suggested as potential sources for non-correlation.

Acknowledgements

The authors acknowledge the financial support offered by the National Authority for Scientific Research of Romania, through the Joint Research Project Romania-Slovakia 465/2011 and Slovak APVV agency, project SK-RO 0008-10. One of the authors (FSR) acknowledges the financial support offered by CNCSIS-UEFISCSU, project number PN II-RU code 138/2010. Portions of these results were generated by ADMET Predictor™ software provided by Simulations Plus, Inc., Lancaster, California, USA.

References

1. Jain D, Pathak D, Pathak K. Pharmaceutical product development technologies based on the biopharmaceutical classification system. *Die Pharmazie* 2009; 64: 483-90.
2. Butler JM, Dressman JB. The developability classification system: application of biopharmaceutics concepts to formulation development. *J Pharm Sci* 2010; 99: 4940-54.
3. Dahan A, Lennernas H, Amidon GL. The fraction dose absorbed, in humans, and high jejunal human permeability relationship. *Mol Pharm* 2012; 9: 1847-51.
4. Radulescu FS, Voicu V, Miron DS et al. The solubility of oxicams, antifungal azoles and selected psychotropic drugs in simulated gastro-intestinal fluids and the consequences on their biopharmaceutical classification. *Farmacia* 2011; 59: 432-9.
5. Sharma P, Varma MVS, Chawla HPS, Panchagnula R. Relationship between lipophilicity of BCS class III and IV drugs and the functional activity of peroral absorption enhancers. *Il Farmaco* 2005; 60: 870-3.

6. Frank KJ, Westedt U, Rosenblatt KM et al. Impact of FaSSIF on the solubility and dissolution/permeation rate of a poorly water-soluble compound. *Eur J Pharm Sci, in press*
7. Anand O, Yu LX, Conner DP, Davit BM. Dissolution testing for generic drugs: an FDA perspective. *AAPS J* 2011; 13: 328-35.
8. Jantratid E, Dressman JB. Biorelevant Dissolution Media Simulating the Proximal Human Gastrointestinal Tract: An Update, *Diss Tech* 2009; 8: 21-5.
9. Ottaviani G, Gosling DJ, Patissier C et al. What is modulating solubility in simulated intestinal fluids? *Eur J Pharm Sci* 2010; 41: 452-7.
10. Dumitrescu IB, Drăgoicea ID, Lupuleasa D, Draganescu D, Ciolan DF, Miron DS, Rădulescu FS, Adjustments of the biorelevant dissolution testing in case of oxicams oral solid dosage forms, *Farmacia*, 2012, 60(5), 740-748.
11. Psachoulias D, Vertzoni M, Goumas K et al. Precipitation in and supersaturation of contents of the upper small intestine after administration of two weak bases to fasted adults. *Pharm Res* 2011; 28: 3145-58.
12. Stecoza CE, Radulescu FS, Miron DS et al. Integrating the values of molecular descriptors in the prediction of biopharmaceutical properties for new compounds with dibenzothiepine structure, *Farmacia* 2011; 59: 820-9.
13. Stecoza C. E., Căproiu M.T, Drăghici C., Ilie C., Chiriţă I.C., Synthesis and characterization of new dibenzo[b,e]thiepine derivatives. 1, *Revista de Chimie*, 2008, 59(12), 1348-1351
14. Ilie C., Stecoza C. E., Căproiu M.T., Hău R., Guţă R., Nanau-Andreescu D., Synthesis and characterization of new dibenzo[b,e]thiepine derivatives. 2, *Revista de Chimie*, 60,.6, 2009, 588- 591
15. Stecoza C. E., Căproiu M.T, Drăghici C., Ilie C., New 6,11-dihydrodibenzo[b,e]thiepine derivatives. Note VI, *Farmacia*, 2009, 57(5), 616- 624
16. Tsume Y, Amidon GL. The Biowaiver Extension for BCS Class III Drugs: The Effect of Dissolution Rate on the Bioequivalence of BCS Class III Immediate-Release Drugs Predicted by Computer Simulation. *Mol Pharm* 2010; 7: 1235-43.
17. Dressman J, Butler J, Hempenstall J, Reppas C. The BCS: Where do we go from here? *Pharm Tech* 2001; 7: 68-76.

Manuscript recieved: October 16th 2011