COMPUTERS IN MEDICINAL CHEMISTRY. MOLECULAR MODELING AND CALCULATION OF THE STRUCTURE-CORRELATED PARAMETERS DESCRIBING SKIN PERMEABILITY OF THEAFLAVONOIDS

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Abstract

The application of computational technique for predicting biological activity of chemicals plays an important role in development and design of new drugs. Being now a routine approach in medicinal chemistry, the method still encounters difficulties in teaching medical science students.
In this paper the project of introduction the students to the computational tools and approaches usually used in pharmacy and medicine is presented. The project involves estimation of the skin permeability of theaflavonoids in respect to their usefulness in topical application. Topical application of flavonoids for inhibition of skin tumor is preferred because flavonoids are poorly absorbed from intestines and are decomposed by intestinal microorganisms.
Key words: *theaflavonoids, bioactivity, QSAR, molecular modeling.*

Introduction

In recent years there is increasing interest in the health benefits of tea *Camelia sinensis*, particularly as a protecting agent against certain forms of cancer (Yang et al., 2000; Dufresne & Farnworth, 2001). Teas contain poliphenolic compounds, composition of which varies due to the different kind of tea preparation. Green tea is prepared by dehydratation of tea leaves, which does not lead to the oxidation of constituent polyphenols. Therefore, green tea contains high concentrations of monomeric polyphenols from catechin group. For black tea the *Camelia sinensis,* leaves are kept warm for 6 hours and the polyphenols, especially the catechins, are oxidized and condensed. The third form, oolong tea is an intermediate one between green and black tea and is produced by heating leaves in air during one or two hours.

It was proved (Wang et al., 1992; Katiyar et al., 1997; Record & Dreosti, 1998; Lu et al., 2005) that formation of skin tumors was inhibited by oral or topical administration of the tea constituents.

Topical application of flavonoids for inhibition of skin tumor is preferred because flavonoids are poorly absorbed from intestines and are decomposed by intestinal microorganisms (Formica & Regelson, 1995).

Numerous studies of green and black teas and skin cancer have been recently reviewed (Katyar, Ahmad & Mukhtar, 2000). It was found that topical application of green tea exerts a photoprotective effect on human skin (Huang et al., 1992; Elmets et al., 2001). Also theaflavins from black tea are active components in chemoprevention (Nomura et al., 2000). Several studies have proved that topical application of black tea constituents can decrease UV-B-induced erythema, inhibit tumor initiation and act as antitumor-promoter (Javed, Mehrotra & Shukla, 1998; Zhao et al., 1999).

Stratum corneum, the outermost thick layer (15-20 μm) of epidermis, is the main barrier that controls permeation of topically applied substances. There is little evidence suggesting any active processes involved in skin penetration. Therefore, the underlaying transport processes is assumed to be controlled by simple passive diffusion. The measurement of the skin permeability of the all constituents of green or black tea, using in vivo or in vitro methods, was very expensive, time consuming and requires sufficient quantity of pure compounds which separation from tea is analytically difficult.

The application of computational technique for predicting biological activity of these compounds based on their structure allows reduce the number of experimental studies required for compound selection. Computational modeling provides an inexpensive and fast way to asses the potential for skin permeability of molecule before its isolation from natural plant and enables prioritization of molecules for in vitro and in vivo studies.

Although these software may have limited utility in structure activity relationships of an individual compound, it is useful in categorizing collections of compound and projecting their biological activity.

Computational methods

Lipophilicity

Lipophilicity is a significant molecular descriptor that correlates well with bioactivity of compounds (Leo and Hansh, 1999; Jóźwiak, Szumiło & Soczewiński, 2001; Cronin et al., 2002). It can be usually described by log P (the logarithm of partition coefficient between n-octanol and water) which reflects the equilibrium of partitioning a molecule between apolar and polar (aqueous) phase.

Partition coefficient can be measured experimentally by several methods ranging from simple "shake flask" technique to chromatographic and electrometric methods (Sangster, 1997). Experimental determination of partition coefficient is time and material consuming and can be done when the compound is available in pure form only. Therefore, much easer, faster and cheaper method to predict log P is using the calculation procedure based on the chemical structure of investigated compound.

Many methods for calculation of log P, based on a chemical structure of the compounds, have been proposed, e.g. Leo, Hansh & Elkins (1971), Leo (1993), Hansh & Leo (1997), divided a compound into basic fragments and calculated its log P by the summation of the hydrophobic constituents of these fragments. Similar procedures, using fragment constants have been developed by Rekker (1977), Suzuki & Kudo (1990), Klopman & Wang (1991), Moriguchi et al. (1992), and Kloppman et al. (1994). Different approach for computation of log P was proposed by Broto, Moreau & Vandycke (1984). This method, based on additive atomic contributions, assumed, that the parameters used in the log P calculation can be obtained classifying atoms into different atom types according to their different topological environments, which contribute differently to global log P value. Similar procedure was developed by Ghose & Crippen (1986), Ghose, Prittchet & Crippen (1988) and Viswanadhan et al., (1989). The other method, based on neural network was proposed by Tetko, Tanchuk & Villa (2001) and Tetko & Tanchuk (2002).

In this paper values of log P for four groups of compounds present in green and black teas was estimated by use of various calculation methods:

- first group – catechins:
  - epigallocatechin gallate
  - epicatechin gallate

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- epigallocatechin
- gallocatechin
- catechin
- gallocatechin gallate

- second group – theaflavines:
  - theaflavine
  - theaflavine 3-gallate
  - theaflavine 3'-gallate
  - theaflavine 3,3'-gallate
- third group – flavonoids:
  - kempferol
  - quercetin
  - rutin
- forth group – phenolic acids:
  - caffeic acid
  - gallic acid
  - quinic acid

The results are presented in Table 1.

Table 1. The values of log P for selected theaflavonoids calculated by different methods.

Compound	log P						
	1	2	3	4	5	6	7
Epigallocatechin gallate	2.07	3.02	1.46	2.39	1.49	2.73	2.56
Epicatechin gallate	2.46	3.30	1.85	2.55	2.16	3.12	2.62
Epigallocatechin	1.11	1.83	0.41	0.71	-0.13	1.54	1.12
Gallocatechin	1.11	1.83	0.41	0.71	-0.13	1.54	1.12
Catechin	1.50	2.11	0.80	1.02	0.53	1.92	1.18
Gallocatechin gallate	2.07	3.02	1.46	2.39	1.49	2.73	2.56
Theaflavine	0.31	1.30	0.31	1.84	0.89	2.66	2.60
Theaflavine 3-gallate	1.27	2.49	1.36	3.02	2.52	3.86	4.04
Theaflavine 3'-gallate	1.27	2.49	1.36	3.02	2.52	3.86	4.04
Theaflavine 3,3'-digallate	2.62	3.96	2.80	3.98	3.87	5.44	4.78
Kempferol	0.74	1.07	1.75	1.99	2.10	2.36	1.96
Quercetin	0.35	0.78	1.36	1.81	1.50	1.98	1.48
Rutin	-2.28	-0.86		0.15	-1.36	-0.90	-1.11
Caffeic acid	1.15	1.58	1.18	1.67	0.98	1.38	1.11
Gallic acid	0.42	0.89	0.06	1.17	0.43	0.60	0.86
Quinic acid	-2.44	-1.86	-3.80	-2.47	-3.01	-2.27	-2.45

1. Crippen fragmentation method (Ghose & Crippen, 1986)
2. Visvanadhan fragmentation method (Viswanadhan et al., 1989)
3. Broto method (Broto, Moreau & Vandycke, 1984)
4. www.vcclab.org/lab/alogps
5. www.biobyte,com/bb/prod/clogp40.html
6. www.molinspiration.com/cgi-bin/properties
7. www.syrres.com/esc/kowwin.htm

The transport of a substance across biological membranes such as skin is a complex phenomenon comprising physical, chemical and biological interaction (Handgraft, 2001 and 2004). *Stratum corneum*, the outermost layer of epidermis, is the primary barrier against permeation of topically applied drugs. The interior of cornified cells consists of cross-linked keratin filoments, while the intercellular space is filled with materials arranged in multilamellar bilayers. That is why, the lipophilicity of compounds is a critical parameter for transdermal delivery of drugs. The ability of the compound to pass through the skin is usually characterized by dermal permeability coefficient (K_p) defined by equation:

$$K_p = K_m \cdot \frac{D_m}{h}$$

where: h – the thickness of *stratum corneum*
D_m – permeant diffusivity in the membrane
K_m – partition coefficient between *stratum corneum* and vehicle

Quantitative structure activity relationship (QSAR) method attempt to relate statistically skin permeation of compounds to their structural descriptors or physicochemical parameters. Many of these compounds reveal a linear relationship between permeability and lipophilicity descriptor K_m, which in calculation is usually substituted for by octanol-water partition coefficient (P).

QSARs considers also descriptors characterizing the influence of molecular size on the diffusion process, such as molecular weight (MW) or molecular volume (MV). Molecular weight is not an ideal measure of molecular size because it does not take into account the shape of the molecule. Therefore, in some QSAR equations, calculated molecular volume is preferred. Using the Titan 1.05 software it was possible to construct the molecule of the investigated compounds. Molecules were optimized by reducing the energy of initial structure until minimum-energy conformer was found. Minimalization process can correct unfavorable bond length, bond angles, torsion angles and nonbounded interactions in starting structure, creating most stable conformation which volume is taken then into calculation of permeability coefficient. The results are presented in Table 2.

The general equation for calculation of permeability coefficient taking into account these parameters is given below:

$$\log K_p = a\,(\text{hydrophobilicity}) - b\,(\text{molecular size}) + c$$

The equations of this type proposed by Fitzpatrick, Corish and Hayes (2004) are:

$$\log K_p = -2,19 + 0,781 \log P - 0,0115 MW \quad (1)$$

and Barrat (1995) [35]:

$$\log K_p = -2,771 + 0,769 \log P - 0,00734 MV \quad (2)$$

obtained results are presented in Tables 3 and 4.

The results show moderate permeation rate of investigated compounds through the skin, that is in accordance with Lipiński et al., (1997) "rule of five".

In the compounds under consideration:

- Index of H-bond donor character, calculated (according to Lipiński) by simply adding the number of NH bonds and OH bonds was above 5 for the most of investigated compounds.
- The calculation of H bond accepting ability as a sum of Ns i Os in considered compounds is not nearly as good as the OH and NH count. That is due to far

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more variations in hydrogen bond acceptor than donor abilities across the atom types. Estimated index of bond acceptor character not exceeds 10 for the majority of studied compounds.

- The index of molecular size described by molecular weight – for all compounds (exceptions theaflavine and theaflavine gallate and rutine) was below 500
- Values of log P was below 5 (in range -2,5 to 3, 5).

Table2. Molecular weight and molecular volume of selected theaflavonoids.

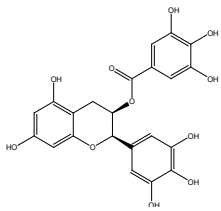
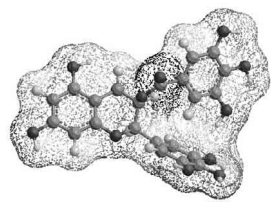
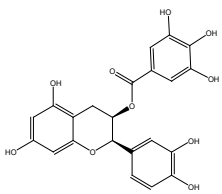
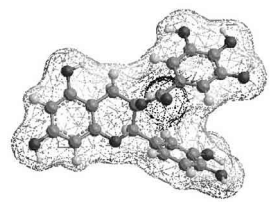
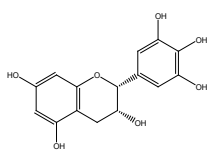
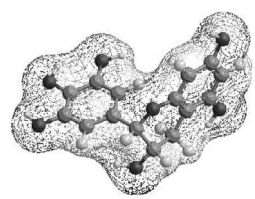
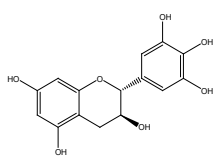
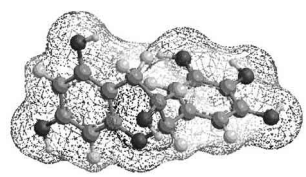
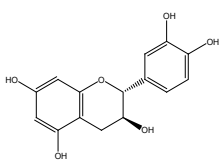
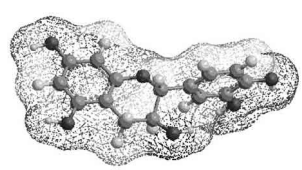
Compound			MW	MV [Å^3]
Epigallocatechin gallate			458.373	457.63
Epicatechin gallate			442.374	455.64
Epigallocatechin			306.269	314.32
Gallocatechin			306.269	314.96
Catechin			290.27	303.77

Table2. Molecular weight and molecular volume of selected theaflavonoids, continued.

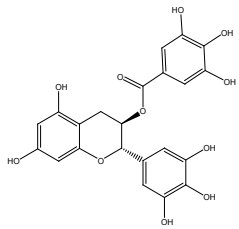
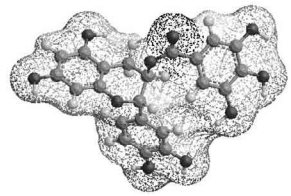
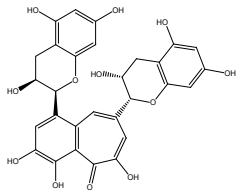
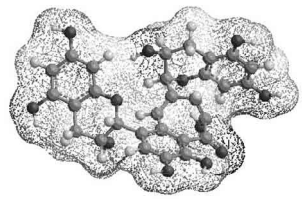
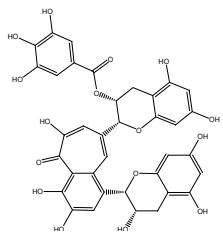
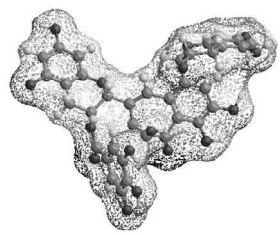
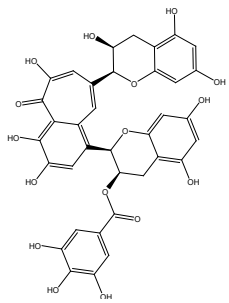
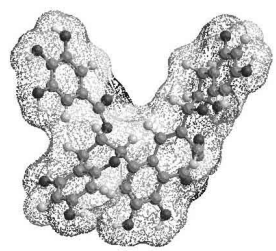
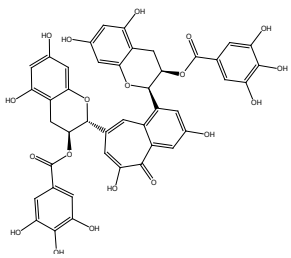
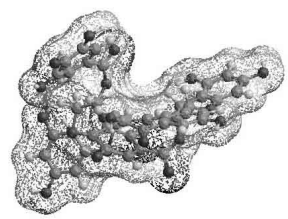
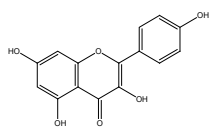
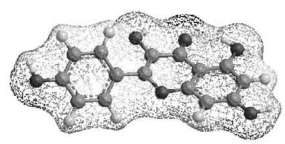
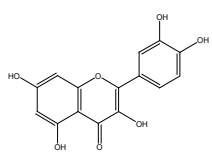
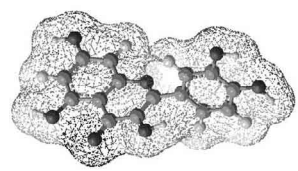
Gallocatechin gallate			458.38	456.41
Theaflavine			564.5	563.88
Theaflavine 3-gallate			716.601	706.45
Theaflavine 3'-gallate			716.601	710.84
Theaflavine 3.3'-digallate			852.72	851.45
Kempferol			286.24	289.5
Quercetin			302.24	300.44

Table2. Molecular weight and molecular volume of selected theaflavonoids, continued.

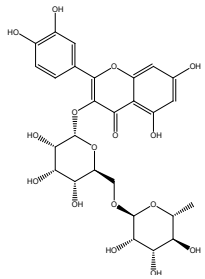
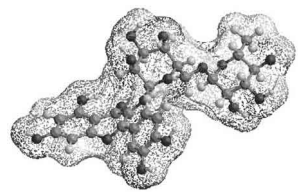
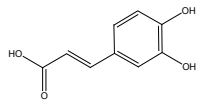
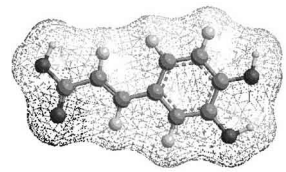
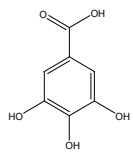
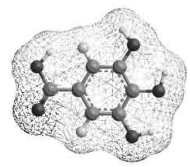
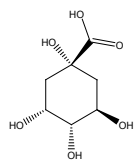
Rutin			610.53	578.65
Caffeic acid			180.16	196.96
Gallic acid			170.119	172.66
Quinic acid			192.166	200.93

Table 3. The values of log K_p for selected theaflavonoids calculated according equation (1).

Compound	Kp (equation 1)							Mean
	1	2	3	4	5	6	7	
Epigallocatechin gallate	$1.42 \cdot 10^{-6}$	$7.84 \cdot 10^{-6}$	$4.76 \cdot 10^{-7}$	$2.53 \cdot 10^{-6}$	$5.02 \cdot 10^{-7}$	$4.66 \cdot 10^{-6}$	$3.43 \cdot 10^{-6}$	$2.98 \cdot 10^{-6}$
Epicatechin gallate	$4.38 \cdot 10^{-6}$	$1.98 \cdot 10^{-5}$	$1.46 \cdot 10^{-6}$	$5.15 \cdot 10^{-6}$	$2.56 \cdot 10^{-6}$	$1.43 \cdot 10^{-5}$	$5.84 \cdot 10^{-6}$	$7.65 \cdot 10^{-6}$
Epigallocatechin	$1.42 \cdot 10^{-5}$	$5.19 \cdot 10^{-5}$	$4.05 \cdot 10^{-6}$	$6.95 \cdot 10^{-6}$	$1.54 \cdot 10^{-6}$	$3.08 \cdot 10^{-5}$	$1.45 \cdot 10^{-5}$	$1.77 \cdot 10^{-5}$
Gallocatechin	$1.42 \cdot 10^{-5}$	$5.19 \cdot 10^{-5}$	$4.05 \cdot 10^{-6}$	$6.95 \cdot 10^{-6}$	$1.54 \cdot 10^{-6}$	$3.08 \cdot 10^{-5}$	$1.45 \cdot 10^{-5}$	$1.77 \cdot 10^{-5}$
Catechin	$4.38 \cdot 10^{-5}$	$1.31 \cdot 10^{-4}$	$1.25 \cdot 10^{-5}$	$1.85 \cdot 10^{-5}$	$7.68 \cdot 10^{-6}$	$9.32 \cdot 10^{-5}$	$2.47 \cdot 10^{-5}$	$4.74 \cdot 10^{-5}$
Gallocatechin gallate	$1.42 \cdot 10^{-6}$	$7.84 \cdot 10^{-6}$	$4.76 \cdot 10^{-7}$	$2.53 \cdot 10^{-6}$	$5.02 \cdot 10^{-7}$	$4.66 \cdot 10^{-6}$	$3.43 \cdot 10^{-6}$	$2.98 \cdot 10^{-6}$
Theaflavine	$3.63 \cdot 10^{-9}$	$2.15 \cdot 10^{-8}$	$3.63 \cdot 10^{-9}$	$5.67 \cdot 10^{-8}$	$1.03 \cdot 10^{-8}$	$2.47 \cdot 10^{-7}$	$2.22 \cdot 10^{-7}$	$8.07 \cdot 10^{-8}$
Theaflavine 3-gallate	$3.63 \cdot 10^{-10}$	$3.25 \cdot 10^{-9}$	$4.26 \cdot 10^{-10}$	$8.41 \cdot 10^{-9}$	$3.43 \cdot 10^{-9}$	$3.80 \cdot 10^{-8}$	$5.25 \cdot 10^{-8}$	$1.52 \cdot 10^{-8}$
Theaflavine 3'-gallate	$3.63 \cdot 10^{-10}$	$3.25 \cdot 10^{-9}$	$4.26 \cdot 10^{-10}$	$8.41 \cdot 10^{-9}$	$3.43 \cdot 10^{-9}$	$3.80 \cdot 10^{-8}$	$5.25 \cdot 10^{-8}$	$1.52 \cdot 10^{-8}$
Theaflavine 3.3'-digallate	$1.12 \cdot 10^{-10}$	$1.24 \cdot 10^{-9}$	$1.54 \cdot 10^{-10}$	$1.28 \cdot 10^{-9}$	$1.05 \cdot 10^{-9}$	$1.77 \cdot 10^{-8}$	$5.40 \cdot 10^{-9}$	$3.84 \cdot 10^{-9}$
Kempferol	$1.25 \cdot 10^{-5}$	$2.25 \cdot 10^{-5}$	$7.64 \cdot 10^{-5}$	$1.18 \cdot 10^{-4}$	$1.43 \cdot 10^{-4}$	$2.29 \cdot 10^{-4}$	$1.11 \cdot 10^{-4}$	$1.02 \cdot 10^{-4}$
Quercetin	$4.05 \cdot 10^{-6}$	$8.76 \cdot 10^{-6}$	$2.48 \cdot 10^{-5}$	$5.57 \cdot 10^{-5}$	$3.19 \cdot 10^{-5}$	$7.56 \cdot 10^{-5}$	$3.08 \cdot 10^{-5}$	$3.31 \cdot 10^{-5}$
Rutin	$1.02 \cdot 10^{-11}$	$1.31 \cdot 10^{-10}$	$6.15 \cdot 10^{-10}$	$8.05 \cdot 10^{-10}$	$5.35 \cdot 10^{-11}$	$1.22 \cdot 10^{-10}$	$8.38 \cdot 10^{-11}$	$2.60 \cdot 10^{-10}$
Caffeic acid	$4.32 \cdot 10^{-4}$	$9.34 \cdot 10^{-4}$	$4.56 \cdot 10^{-4}$	$1.10 \cdot 10^{-03}$	$3.18 \cdot 10^{-4}$	$6.52 \cdot 10^{-4}$	$4.02 \cdot 10^{-4}$	$6.13 \cdot 10^{-4}$
Gallic acid	$1.52 \cdot 10^{-4}$	$3.53 \cdot 10^{-4}$	$7.95 \cdot 10^{-5}$	$5.84 \cdot 10^{-4}$	$1.55 \cdot 10^{-4}$	$2.10 \cdot 10^{-4}$	$3.35 \cdot 10^{-4}$	$2.67 \cdot 10^{-4}$
Quinic acid	$4.98 \cdot 10^{-7}$	$1.41 \cdot 10^{-6}$	$4.33 \cdot 10^{-8}$	$4.72 \cdot 10^{-7}$	$1.79 \cdot 10^{-7}$	$6.75 \cdot 10^{-7}$	$4.89 \cdot 10^{-7}$	$5.38 \cdot 10^{-7}$

Table 4. The values of log K_p for selected theaflavonoids calculated according equation (2).

Compound	Kp (equation 2)							Mean
	1	2	3	4	5	6	7	
Epigallocatechin gallate	$3.61 \cdot 10^{-7}$	$1.94 \cdot 10^{-6}$	$1.23 \cdot 10^{-7}$	$6.37 \cdot 10^{-7}$	$1.29 \cdot 10^{-7}$	$1.16 \cdot 10^{-6}$	$8.61 \cdot 10^{-7}$	$7.45 \cdot 10^{-7}$
Epicatechin gallate	$7.60 \cdot 10^{-7}$	$3.36 \cdot 10^{-6}$	$2.58 \cdot 10^{-7}$	$8.91 \cdot 10^{-7}$	$4.47 \cdot 10^{-7}$	$2.45 \cdot 10^{-6}$	$1.01 \cdot 10^{-6}$	$1.31 \cdot 10^{-6}$
Epigallocatechin	$2.94 \cdot 10^{-6}$	$1.05 \cdot 10^{-5}$	$8.50 \cdot 10^{-7}$	$1.45 \cdot 10^{-6}$	$3.27 \cdot 10^{-7}$	$6.29 \cdot 10^{-6}$	$2.99 \cdot 10^{-6}$	$3.62 \cdot 10^{-6}$
Gallocatechin	$2.89 \cdot 10^{-6}$	$1.03 \cdot 10^{-5}$	$8.36 \cdot 10^{-7}$	$1.42 \cdot 10^{-6}$	$3.21 \cdot 10^{-7}$	$6.18 \cdot 10^{-6}$	$2.94 \cdot 10^{-6}$	$3.56 \cdot 10^{-6}$
Catechin	$7.75 \cdot 10^{-6}$	$2.28 \cdot 10^{-5}$	$2.24 \cdot 10^{-6}$	$3.31 \cdot 10^{-6}$	$1.39 \cdot 10^{-6}$	$1.63 \cdot 10^{-5}$	$4.40 \cdot 10^{-6}$	$8.31 \cdot 10^{-6}$
Gallocatechin gallate	$3.73 \cdot 10^{-7}$	$2.01 \cdot 10^{-6}$	$1.27 \cdot 10^{-7}$	$6.58 \cdot 10^{-7}$	$1.34 \cdot 10^{-7}$	$1.20 \cdot 10^{-6}$	$8.89 \cdot 10^{-7}$	$7.70 \cdot 10^{-7}$
Theaflavine	$9.61 \cdot 10^{-10}$	$5.55 \cdot 10^{-9}$	$9.61 \cdot 10^{-10}$	$1.44 \cdot 10^{-8}$	$2.68 \cdot 10^{-9}$	$6.16 \cdot 10^{-8}$	$5.54 \cdot 10^{-8}$	$2.02 \cdot 10^{-8}$
Theaflavine 3-gallate	$1.21 \cdot 10^{-10}$	$1.05 \cdot 10^{-9}$	$1.41 \cdot 10^{-10}$	$2.67 \cdot 10^{-9}$	$1.10 \cdot 10^{-9}$	$1.18 \cdot 10^{-8}$	$1.63 \cdot 10^{-8}$	$4.74 \cdot 10^{-9}$
Theaflavine 3'-gallate	$1.07 \cdot 10^{-10}$	$9.31 \cdot 10^{-10}$	$1.26 \cdot 10^{-10}$	$2.38 \cdot 10^{-9}$	$9.82 \cdot 10^{-10}$	$1.05 \cdot 10^{-8}$	$1.45 \cdot 10^{-8}$	$4.22 \cdot 10^{-9}$
Theaflavine 3,3'-digallate	$2.83 \cdot 10^{-11}$	$3.04 \cdot 10^{-10}$	$3.90 \cdot 10^{-11}$	$3.15 \cdot 10^{-10}$	$2.59 \cdot 10^{-10}$	$4.18 \cdot 10^{-9}$	$1.30 \cdot 10^{-9}$	$9.17 \cdot 10^{-10}$
Kempferol	$2.94 \cdot 10^{-6}$	$5.28 \cdot 10^{-6}$	$1.76 \cdot 10^{-5}$	$2.69 \cdot 10^{-5}$	$3.27 \cdot 10^{-5}$	$5.18 \cdot 10^{-5}$	$2.55 \cdot 10^{-5}$	$2.33 \cdot 10^{-5}$
Quercetin	$1.10 \cdot 10^{-6}$	$2.36 \cdot 10^{-6}$	$6.60 \cdot 10^{-6}$	$1.46 \cdot 10^{-5}$	$8.46 \cdot 10^{-6}$	$1.98 \cdot 10^{-5}$	$8.17 \cdot 10^{-6}$	$8.74 \cdot 10^{-6}$
Rutin	$6.63 \cdot 10^{-12}$	$8.19 \cdot 10^{-11}$	$3.75 \cdot 10^{-10}$	$4.90 \cdot 10^{-10}$	$3.38 \cdot 10^{-11}$	$7.63 \cdot 10^{-11}$	$5.26 \cdot 10^{-11}$	$1.59 \cdot 10^{-10}$
Caffeic acid	$7.05 \cdot 10^{-5}$	$1.51 \cdot 10^{-4}$	$7.44 \cdot 10^{-5}$	$1.77 \cdot 10^{-4}$	$5.22 \cdot 10^{-5}$	$1.06 \cdot 10^{-4}$	$6.57 \cdot 10^{-5}$	$9.95 \cdot 10^{-5}$
Gallic acid	$3.68 \cdot 10^{-5}$	$8.47 \cdot 10^{-5}$	$1.95 \cdot 10^{-5}$	$1.39 \cdot 10^{-4}$	$3.75 \cdot 10^{-5}$	$5.07 \cdot 10^{-5}$	$8.03 \cdot 10^{-5}$	$6.41 \cdot 10^{-5}$
Quinic acid	$1.10 \cdot 10^{-7}$	$3.08 \cdot 10^{-7}$	$9.91 \cdot 10^{-9}$	$1.04 \cdot 10^{-7}$	$4.01 \cdot 10^{-8}$	$1.49 \cdot 10^{-7}$	$1.08 \cdot 10^{-7}$	$1.18 \cdot 10^{-7}$

Solubility

In some measure, the bioactivity of the compound depends on its solubility because the flux of the drug across *stratum corneum* is proportional to its concentration gradient between topically applicated probe of drug and its concentration in the blood. In order to pass through biological membrane, a drug must be water soluble. The solubility of the compound is normally expressed as log S, where S is the concentration of the compound in mol/l for saturated aqueous solution in equilibrium with the most stable form of crystalline material. Aqueous solubility of a given molecule is the result of superposition of several factors ranging from lipophilicity to the energetic cost of disrupt on of the crystal lattice of the solid in order to bring it into solution.

Methods for predicting solubility of drug candidates, at early stage of research, would have a great impact on its future application. There are several approaches to estimating and predicting the solubility of organic compounds (Huuskonen, 2001; Ran, Jain & Yalkowski, 2001; Lipiński et al., 2001; Jorgensen & Duffy, 2002; Ran et al., 2002; Yan & Gasteiger, 2003), but none of presently available methods works accurate for an individual compound. Nevertheless, some of them might be useful for rank-ordering of compound in respect to their solubility. In this paper the molecular weight and electrotopological E-state indices were used to estimate aqueous solubility of investigated compounds (Tetko et al., 2001). The calculation was carried out by use of ALOGPS 2.1 software (http://146.107.217.178/lab/alogps/start.html).

The results are presented in Table 5.

Relatively good solubility of the investigated compounds (in the range 0,05 – 747 mg) shows that they may be well adsorbed, even their moderate or low permeation rate.

Table 5. The values of solubility for selected theaflavonoids.

Compound	log S	Solubility in water [g/dm^3]
	ALOG pS	
Epigallocatechin gallate	-3.78	0.076
Epicatechin gallate	-3.96	0.049
Epigallocatechin	-2.55	0.863
Gallocatechin	-2.55	0.863
Catechin	-2.64	0.665
Gallocatechin gallate	-3.78	0.076
Theaflavine	-3.43	0.210
Theaflavine 3-gallate	-3.81	0.111
Theaflavine 3'-gallate	-3.82	0.108
Theaflavine 3,3'-digallate	-4.24	0.049
Kempferol	-3.22	0.172
Quercetin	-3.06	0.263
Rutin	-2.21	3.764
Caffeic acid	-2.03	1.681
Gallic acid	-1.54	4.906
Quinic acid	0.59	747.628

Conclusions

Modern drug design not only focused on the pharmaceutical activity of compound but also considers its ability to be absorbed and to reach its site of action. Among physicochemical properties used today in the very early stages of drug discovery, lipophilicity has certainly assumed a very important role because of its relevance in governing most stages of drug disposition. However other structural features are important too and more complex calculation approaches are available to attempt to incorporate these.

The obtained results point to potentially wide spectrum of theaflavonoids which may be applied by topical administration route.

Students on biochemistry courses are very well trained to memorize factual information and reproduce that information under examination conditions. In this way of teaching, there is little or no chance for students to discuss the nature of scientific investigations. In contrast, in proposed computer – aided classes, as in real scientific investigations students face problems, which may not have a single correct answer and so require judgments to be made in order to arrive at sensible solutions. The students work on the problems within the small groups but then engage in all groups of students discussion of their answers and are encouraged to explain and justify their answers. By working in a group they solve problems that they would be unlikely to solve alone.

In our opinion it is a best way of encouraging students to think critically, to evaluate and analyze and explore their knowledge in scope of biochemistry to the practical cases. Students are actively engaged in finding the correct solution to the problem proposed and in making sense of the solutions.

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