About OMICS Group

OMICS Group is an amalgamation of Open Access Publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 500 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 500 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

OMICS International Conferences

OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

Design, Synthesis and Investigation of Aryl Derivatives of Alaptide

as Potential Transdermal Permeation Enhancers

Josef Jampílek

Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic


MAPQUEST
Norwegian Sea
Sweden
Finland
Tórshavn
Oslo
Tallinn
Atlantic Ocean
North Sea
Glasgow
Copenhagen
Riga
Russia
Moscow
Hamburg
Minsk
Berlin
Tula
Ryazan
London
Belarus
Brussels
Prague
Kiev (Kyiv)
Paris
Bratislava
Donetsk
Bern
Ukraine
France
Bucharest
Krasnodar
Turin
Ljubljana
Black Sea
Rome
Tirane
Skopje
Tbilisi
Madrid
Ankara
Naples
Istanbul
Yerevan
Sevilla
Spain
Algiers
Tunis
Turkey
Valletta
Izmir
Nicosia
Rabat
Algeria
Tripoli
Mediterranean Sea
Alexandria
Beirut
© 2006 MapQuest, Inc.


Labe R.
GERMANY
Liberec
Ústí nad Labem
POLAND
Trutnov
Litoměřice
Teplice
Mladá Boleslav
Odra R.
Karlovy Vary
Louny
Mělník
Labe R.
Hradec Králové
Sokolov
Cheb
Prague
Pardubice
50° N
Rakovník
Krnov
Beroun
Kutná Hora
Šumperk
Opava
Orlová
Karviná
Plzeň
BOHEMIA
Benešov
Ostrava
Havířov
Žďár nad Sázavou
MORAVIA
Třinec
Vltava R.
Havlíčkův Brod
Olomouc
Frýdek-Místek
Prostějov
Klatovy
Přerov
Tábor
Jihlava
Zlín
Strakonice
Písek
Kroměříž
Brno
Otrokovice
Třebíč
České Budějovice
Jindřichův Hradec
Uherské Hradiště
Hodonín
Znojmo
SLOVAKIA
Český Krumlov
Břeclav
AUSTRIA
0 40 Miles
0 40 Kilometers
Danube R.



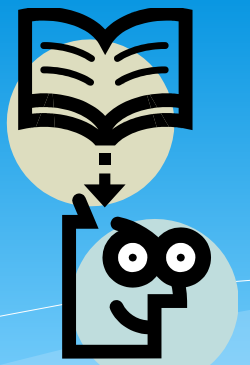




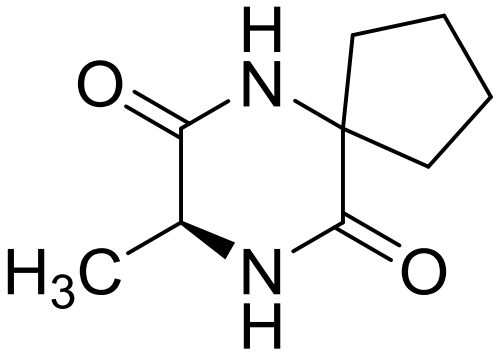




Content



* Transdermal administration
* Structure of skin
* Transport through skin
* Chemical penetration/permeation enhancers
* Alaptide derivatives as CPEs



Transdermal administration

The advantages of transdermal administration include:

* good pharmacokinetic properties of application systems
* the ability to maintain long-lasting steady-state plasma concentration of active substances (including drugs with short biological half-lives)
* reduction of undesirable side effects occurring as a result of considerable fluctuations of drug plasma concentration
* protection from presystemic elimination of the applied drug (first-pass effect) and from pH change in GIT and interactions with other simultaneously applied pharmaceutics or food
* possibility to apply drugs with a narrow therapeutic window and to interrupt drug delivery into the system immediately when an undesirable effect occurs
* very simple and painless application
* non-invasive alternative to parenteral, subcutaneous and intramuscular injections

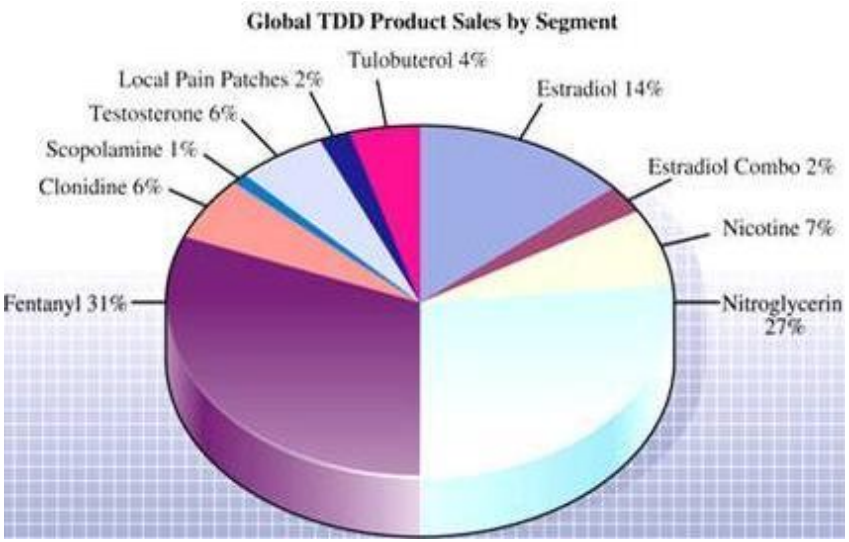
Transdermal administration

The disadvantages of transdermal administration include:

* possibility of local skin irritation or allergisation by an active substance or excipient
* variable intra and interindividual absorption depending on skin conditions and on the place of application (differences in skin structure and thickness on different body parts cause absorption diversity)
* long-term transdermal application on the same place can damage the skin by affecting its microflora and enzymes
* longer time of effect onset connected with the need to overcome the skin barrier

Drugs in transdermal therapeutic systems approved by FDA

Under development: paroxetine, physostigmine, flurbiprofen and other NSAIDs



Drug (molecule)	Approved (year)
scopolamine	1979
glycerol trinitrate	1981
clonidine	1984
estradiol	1986
fentanyl	1990
nicotine	1991
testosteron	1993
estradiol/noretisterone acetate	1998
norelgestromin/ethinyl estradiol	2001
estradiol/levonorgestrel	2003
oxybutinin	2003
selegiline	2006
metylfenidate	2006
rotigotine	2007
rivastigmine	2007
granisetron	2008
buprenorphine	2010
sumatriptan	2013

Transdermal administration

The number of drugs that can be applied transdermally is limited by some requirements on the drug.

The ideal properties of a molecule that effectively penetrates through the skin include drug:

* solubility
* penetrability (drug gets into the skin)
* permeation (drug passing through the skin)
* resorption in blood or lymphatic vessels

Transdermal administration

Requirements to the drug:

* applied drug dosage <25 mg/day
* biological half-life <10 h
* molecular weight <500 Da
* log *P* 1– 3 (ideal 2.5)
* melting temperature <200°C
* saturated aqueous solution pH 5–9
* should not cause any skin irritation or immunity reactions

Only about 5% of drugs are suitable for administration through the skin.

Skin

* anatomically and physiologically specialized barrier
* forms integrated external surface of human body
* skin surface of adult is 1.6-2 m^2, thickness is 1.5–4 mm, its weight is 3 kg
* contains ca 72% of water

The skin has a number of different functions; the most important is protection from:

* excessive water loss
* mechanical, chemical, microbial and physical impacts.

Skin

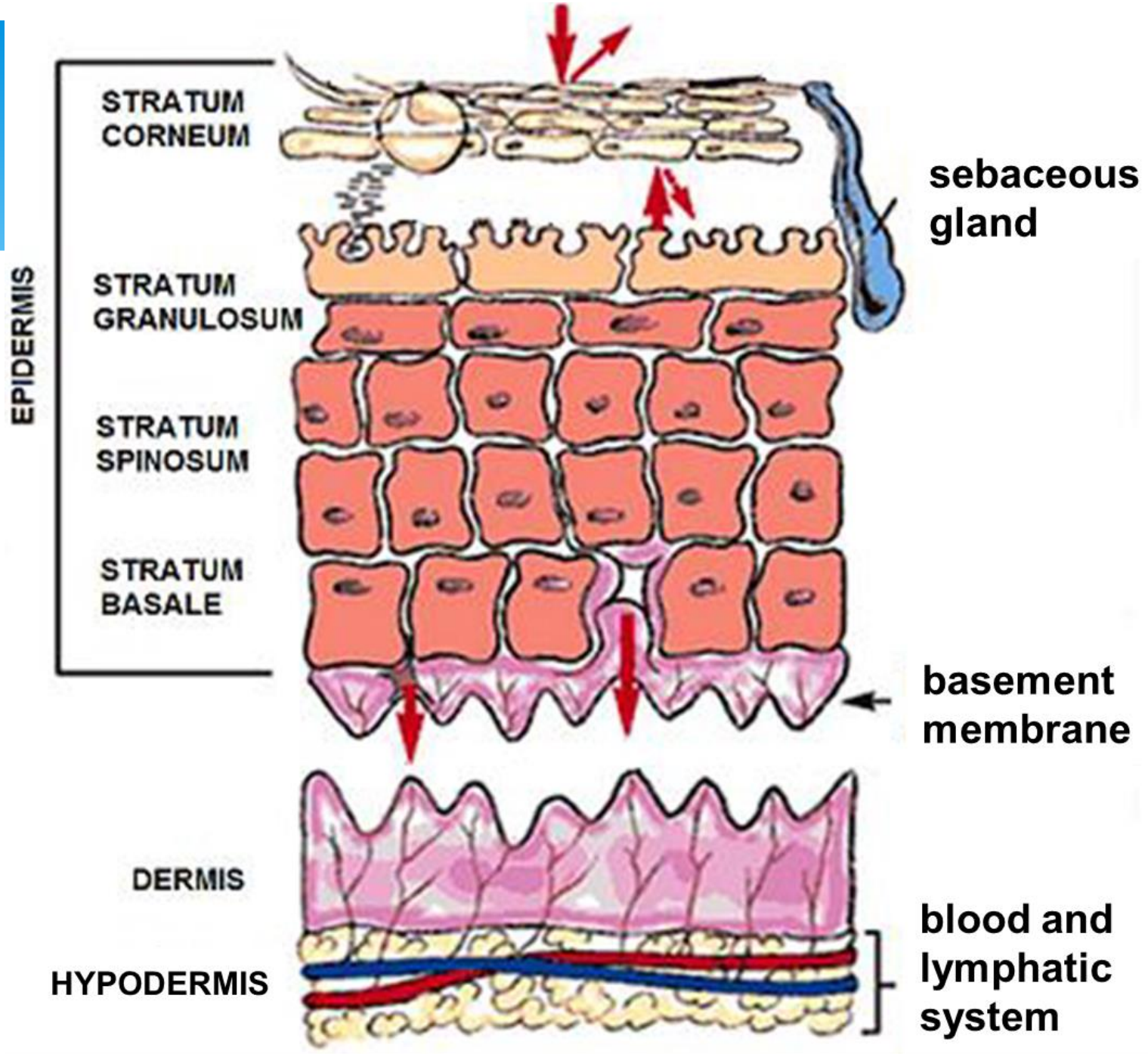
The skin consists of three basic functional layers:

* epidermis (the upper layer) – external corneous layers
  - impermeable to water and chemically inert, primary barrier against mechanical damage, drying and penetration of microbes, xenobiotics
  - consists of four layers: *stratum basale*, *stratum spinosum*, *stratum granulosum*, ***stratum corneum***

* dermis (curium) – is formed by fibroblasts and extracellular matrix and is rich in capillaries and nerve endings; fibroelastic layer ensures mechanical strength, elasticity, tensibility and tensile strength thanks to tangled structure of collagen fibres

* hypodermis (subcutaneous tissue)

Skin


EPIDERMIS
STRATUM CORNEUM
STRATUM GRANULOSUM
STRATUM SPINOSUM
STRATUM BASALE
sebaceous gland
basement membrane
DERMIS
HYPODERMIS
blood and lymphatic system

Skin

Stratum corneum (horned layer):

* final product of differentiation of epidermal cells
* outermost layer of skin (responsible for barrier function)
* formed by 18–21 cell layers and intercellular substance consisting of specific lipids (50% ceramides, 25% cholesterol, 10% free fatty acids, cholesterol esters, cholesterol sulfate and glucosylceramides)
* have "**brick and mortar**" structure, where corneocytes represent "bricks" and lipid matrix in intercellular space represents "mortar"
* horned layer is formed by proteins (75-80%), lipids (5-15%), other organic compounds and water

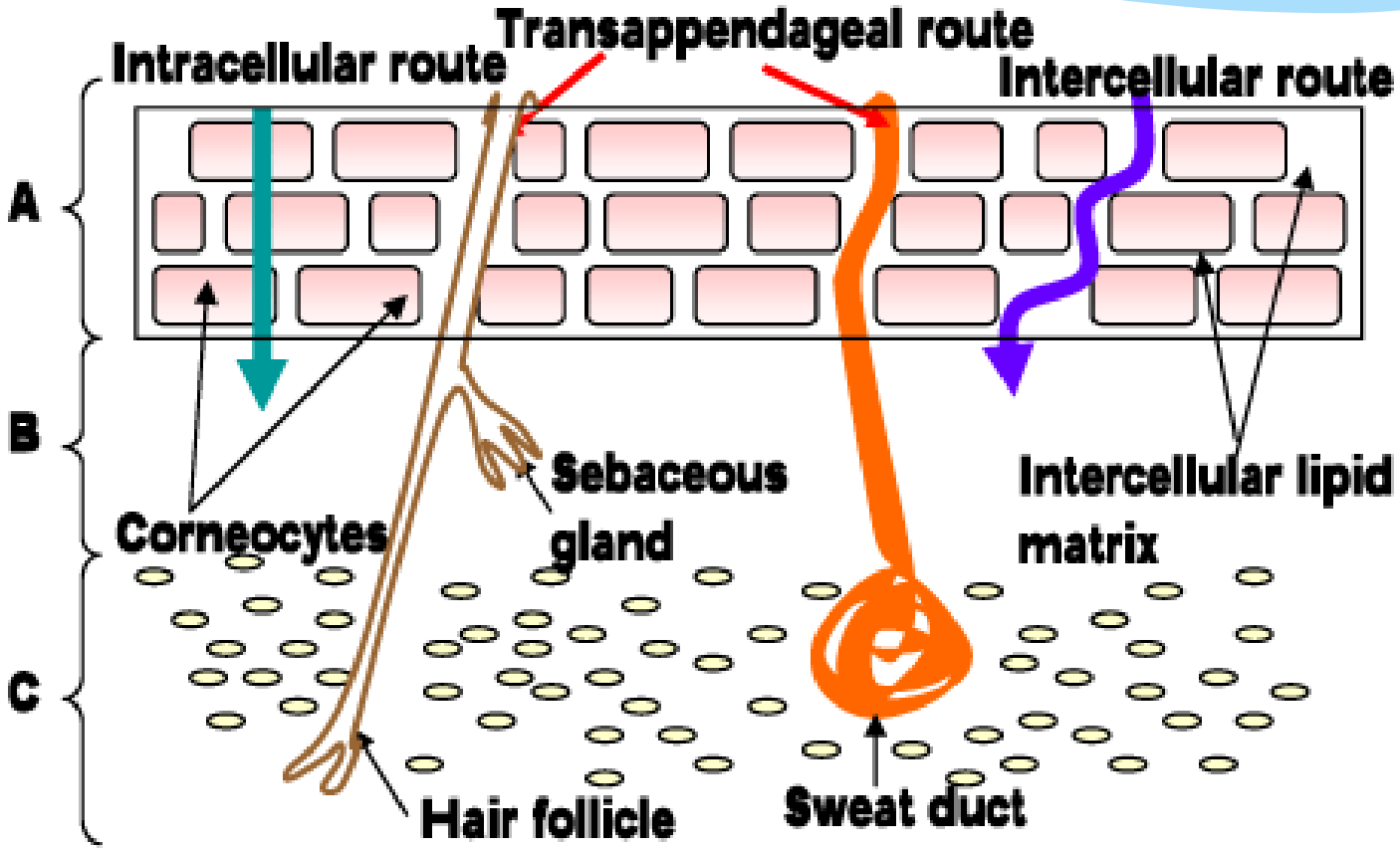
Skin

Drugs can permeate to internal environment by:

* **transcellular** way (through cell bodies)
* **intercellular** way (through intercellular space)

} **transepidermally**

* through **accessory skin organs** (follicles, sebaceous/ sweat glands)



A) Epidermis (S. corneum), B) Dermis, C) subcutaneous layer

Skin

The permeation through the least permeable layer, the SC, is limiting.

To solve this critical issue various approaches for overcoming the skin barrier were developed.

These approaches can be classified as:

* **chemical** (modification of drugs (prodrugs), using transdermal chemical permeation enhancers)
* **physical** (modification of drug particles size to nanosize, physical enhancement techniques).

Skin

Another classification can be based on:

1. **optimization of drug/vehicle**
2. **SC modification.**

Optimization of drug/vehicle consists in preparation/application of:

* lipophilic prodrugs or ion pairs
* eutectic systems
* complexes of drugs with cyclodextrins
* liposomes and other vesicles (transfersomes, ethosomes, niosomes, etc.)
* solid lipid nanoparticles and other nanoparticles and/or nanodelivery systems
* saturated and supersaturated solutions

Skin

Another classification can be based on:

1. **optimization of drug/vehicle**
2. **SC modification.**

Modification (i.e., hydration/lipid fluidization/disrupting) of SC, means:

* application of transdermal chemical permeation enhancers
* overall optimization of formulation using non-hydrophobic excipients
* application of physical enhancement techniques (electrically assisted methods), such as iontophoresis, electroporation, acoustic methods – ultrasound (phonophoresis, sonophoresis), microneedles, magnetophoresis or photomechanical wave

Chemical penetration/ permeation enhancers (CPEs)

–compounds (excipients) specifically affecting intercellular space between corneocytes or modifying corneocytes by hydration or denaturation of keratin.

CPEs can be divided into 10 categories:

1. anionic surfactants (e.g. sodium lauryl sulfate)
2. cationic surfactants (e.g. alkyl ammonium bromides)
3. zwitterionic surfactants (e.g. dodecyl betain)
4. non-ionic surfactants (e.g. polysorbate, poloxamers)
5. fatty acids (e.g. oleic acid, palmitic acid)
6. sodium salts of fatty acids (e.g. sodium octyl sulfate)
7. fatty esters (e.g. isopropyl myristate)
8. fatty amines (e.g. long-chain alkyl amines)
9. Azone-like compounds
10. others (e.g. menthol, urea, cyclodextrins)

CPEs

The heterogeneity of molecular structures of CPEs limits simple explanation of their action.

Several possible mechanisms of action were hypothesized, but exact mechanisms have not been elucidated. It is almost certain that CPEs exhibit multiple effects:

* interact with the intercellular lipid matrix (especially ceramides)
* interact with proteins (influencing the conformation of keratin in corneocytes or proteins in desmosomes)
* promote partitioning (influencing the SC nature leads to raising the penetrant concentration gradient and thus increasing the flux, i.e. increasing the concentration of the drug in the skin)

CPEs

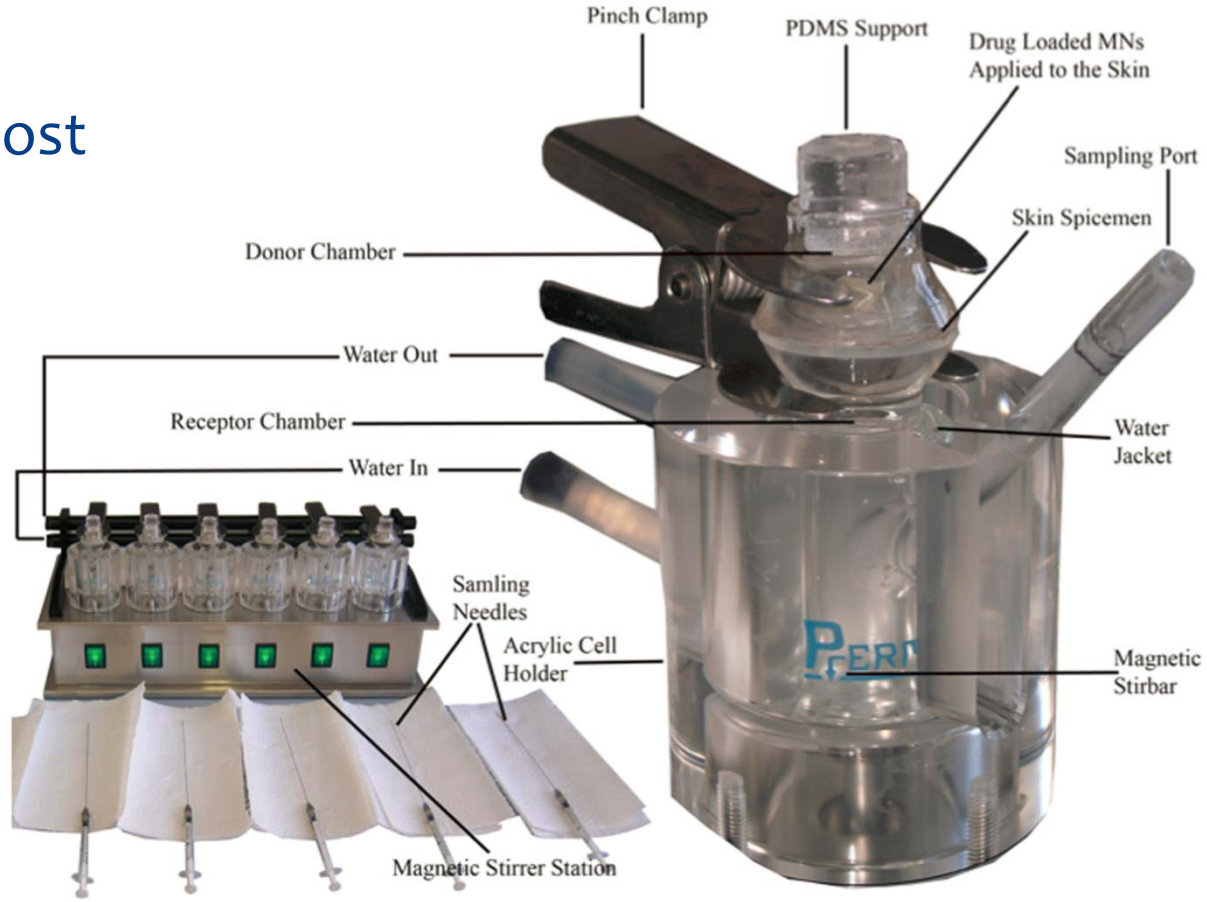
Ideal enhancer should:

* be nontoxic, non-irritating and cause no allergic reactions
* have reversible influence on the skin barrier
* possess rapid onset of action, predictable and repeatable effect
* be pharmacologically and chemically inert
* act one way (ensuring drug entrance to the body)
* be physically and chemically compatible with drugs and other excipients
* be cosmetically acceptable with convenient organoleptic properties
* have uncomplicated and inexpensive synthesis
* meet recently imposed additional biodegradability requirements

Testing of CPEs

Primary *in vitro* (*ex vivo*) screening is performed using:

* Franz diffusion cell
* skin of different animals (snake, hairless mice, rat, rabbit, guinea pig, pig, monkey) or human skin
* different drugs (theophylline – the most frequent model drug; hydrocortisone, indomethacin, 5-fluoruracil, antipsychotics, cardiovascular drugs, etc.)
* HPLC



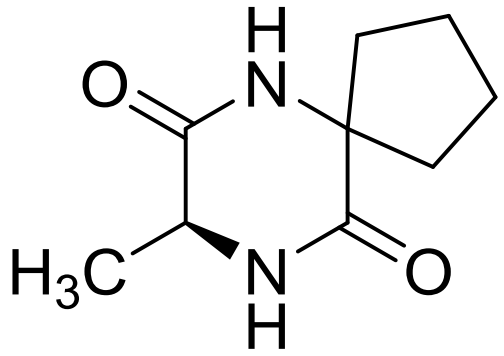
Testing of CPEs

* skin samples obtained from porcine ear
* Franz diffusion cell (SES – Analytical Systems, Germany), donor surface area of 0.635 cm^2 and receptor volume of 5.2 ml
* receptor compartment filled with phosphate buffered saline (pH 7.4), 37±0.5 °C
* donor samples were prepared by dissolving the tested enhancer, theophylline or drug in medium (propylene glycol/water (1:1), formulations)
* control sample was prepared in the same manner without enhancers
* samples (0.5 ml) of the receptor phase withdrawn at time intervals 1, 2, 4, 6, 8, 12 and 24 h and the cell refilled with an equivalent amount of fresh buffer solution
* five determinations were performed using skin fragments from 2 animals for each compound

Testing of CPEs

* analysis of samples for theophylline/drug content was performed using an Agilent 1200 series HPLC system, equipped with a diode array detection (DAD) system; data acquisition was performed using ChemStation chromatography software
* Waters Symmetry® C8 5 µm, 4.6×250 mm (Waters Corp., Milford, MA, USA) chromatographic column was used
* The cumulative amounts of theophylline/drug that penetrated through the skin into the receptor compartment [µg/cm^2] were corrected for sample removal and plotted against time [h].
* Steady state fluxes [µg/cm^2/h] were calculated using the linear region of the plots.
* Enhancement ratios (ERs) were calculated as ratios of theophylline flux with and without the enhancer.

Why alaptide?



Alaptide



(S)-8-Methyl-6,9-diazaspiro[4.5]decan-7,10-dione

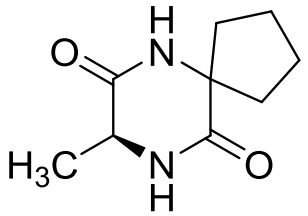
- piperazine-2,5-dione / spirocyclic cyclodipeptide
- synthesized at the Research Institute for Pharmacy and Biochemistry (former Czechoslovakia, now the Czech Republic) in the 80s of the 20th century
- designed as analogues of melanocyte-stimulating hormone release-inhibiting factor (MIF-1, known as L-prolyl-L-leucylglycinamide)
- showed significant curative effect in different therapeutic areas
- demonstrated very low acute toxicity; no subchronic and chronic toxicity, genotoxic, teratogenic and embryotoxic effects were observed
- its enantiomers do not induce the biotransformation enzymes of the cytochrome P450 superfamily 1A1, 1A2 and 1B1 in hepatocytes

Kasafírek E. et al. CS 231227, 1986
Kasafírek E. et al. Drugs Fut. 1990, 15, 445-447.
Jampílek J. et al. WO/2014/019556 A1.

Alaptide – effects

- reduced the number and extent of experimental gastric ulcers
- expressed stimulating effect on growth and breeding of human embryonic cells without transformation changes in their morphology
- increased cell proliferation and epidermal regeneration
- regenerated fast *in vivo* experimental skin injury in pigs and accelerated curing of experimental skin injuries in rats

Probable mechanism of action (under investigation): alaptide negatively affects the inhibition of the release of melanocyte-stimulating hormone and thus increases the concentration of melanocytes in epidermis. Melanocytes significantly influence the creation and function of keratinocytes by means of melanosomes.



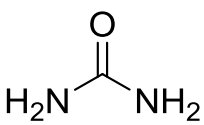
CPEs

- from the chemical point of view CPEs are heterogeneous.

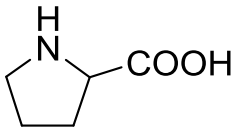
However, it is possible to observe certain common elements in their structure. They **often contain a fragment of the natural moisturizing factor** (**NMF**), which is physiologically present in highly differentiated flattened keratinocytes.

The NMF is a mixture of hygroscopic compounds that help to maintain skin hydration and is capable of retaining water in the horny layer.

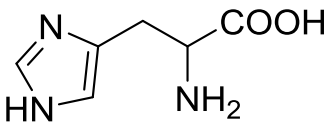
Other substances contained in the NMF with a special water binding capacity are derived from sweat and sebaceous oils, e.g., urea, proline, oxoproline, histidine, glutamine, urocanic acid.



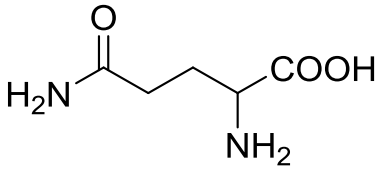
urea



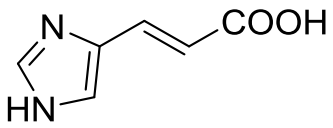
proline



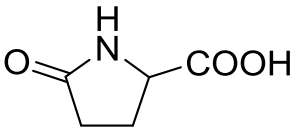
histidine



glutamine



urocanic acid



oxoproline

CPEs

NMF components contain a characteristic fragment of heteroatoms X–CO–N=, where X is –CH_2–, –NH_2 or –NH–.

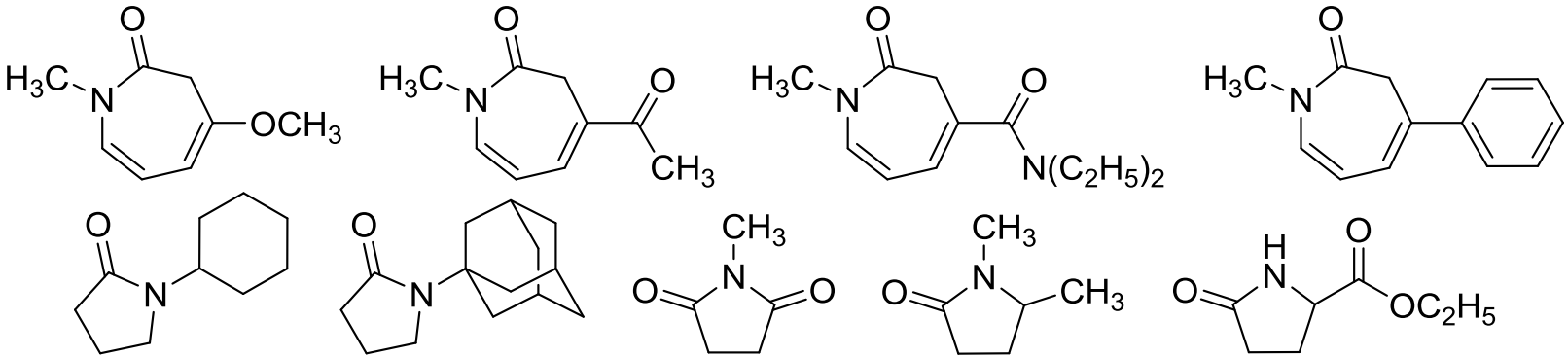
⇨this fragment can be found in many CPEs.

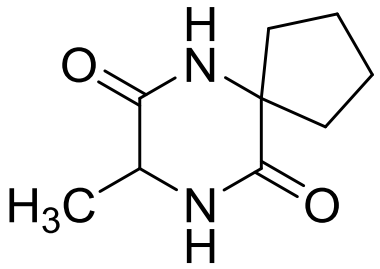
A hypothesis was proposed that small polar molecules may break the intermolecular *H*-bonds that hold the ceramide molecules together in the SC.

Also small molecules derived from NMF components can increase the water binding capacity, and hence moisten the skin and facilitate permeation of compounds through the skin.

CPEs

Based on these characteristics and the knowledge of the structure and properties of NMF and CPEs, the hypotheses of CPE mechanism of action and the previous experience with several other groups of CPEs we decided to evaluate alaptide as a potential transdermal permeation enhancer.





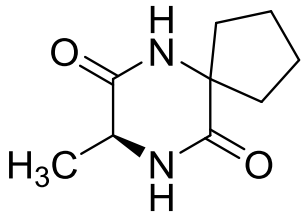
alaptide

Jampílek J. & Brychtová K. *Med. Res. Rev.* **2012,** *32*, 907-947.

Alaptide

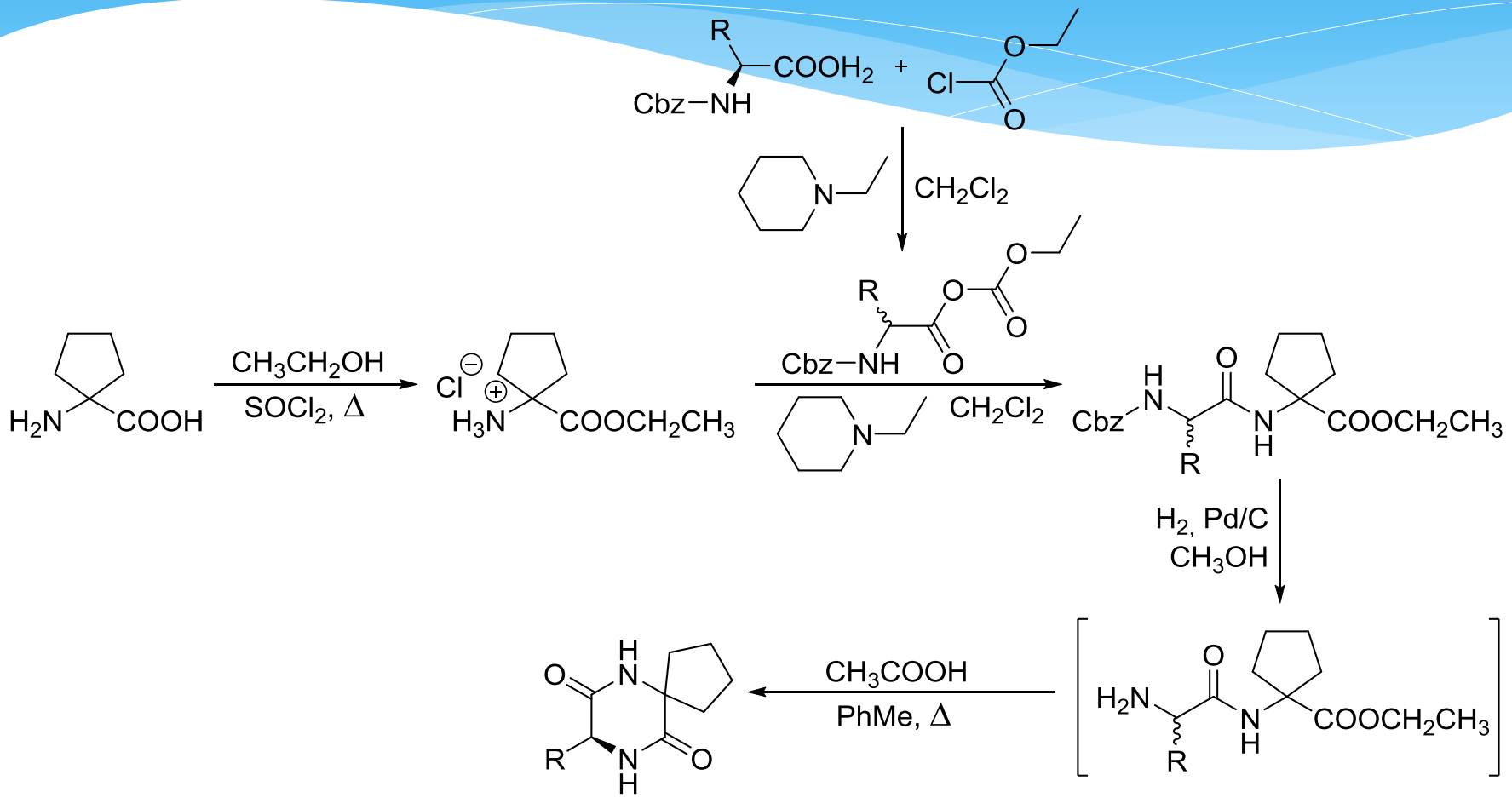
- investigated as a potential CPE of many anti-inflammatory drugs, antimicrobial chemotherapeutics, sex hormones/genital system modulators or drugs of central/vegetative nervous system
- expressed no skin irritability and excellent enhancement effect (till the 3rd hour by several orders of magnitude higher compared with drugs without alaptide)

Jampílek J. et al. Czech Patent PV 2011-232, 2011
Jampílek J. et al. Czech Patent PV 2012-72, 2012.
Jampílek J. et al. Czech Patent PV 2012-511, 2012.
Jampílek J. et al. WO/2013/020527 A1, 2013.
Jampílek J. et al. Czech Patent PV 2013-1000, 2013.
Jampílek J. et al. Czech Patent PV 2013-1001, 2013.
Jampílek J. et al. *Sci. World J.* 2013, Article ID 787283.
Jampílek J. et al. WO/2014/019556 A1, 2014.
Jampílek J. et al. Czech Patent PV 2014-416, 2014.
Jampílek J. et al. *ADMET* 2014, 2, 56-62.
Jampílek J. et al. *Mil. Med. Sci. Lett.* 2014, 83, 34-39.
Jampílek J. et al. *ADMET* 2014, 2, 248-253.
Jampílek J. et al. Czech Patent CZ 304915 B6, 2014.

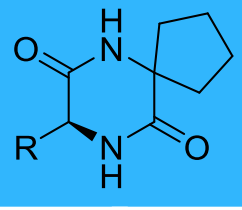


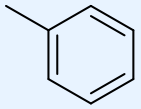
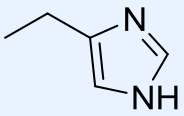
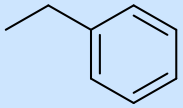
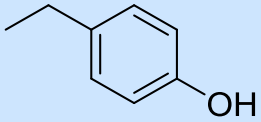
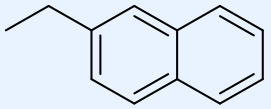
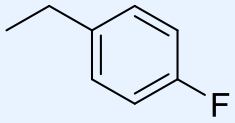
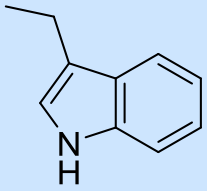
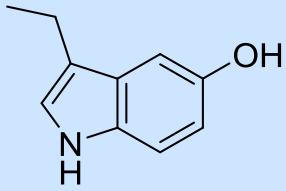
Alaptide

New compounds were prepared by a multi-step synthetic pathway from modified amino acids.



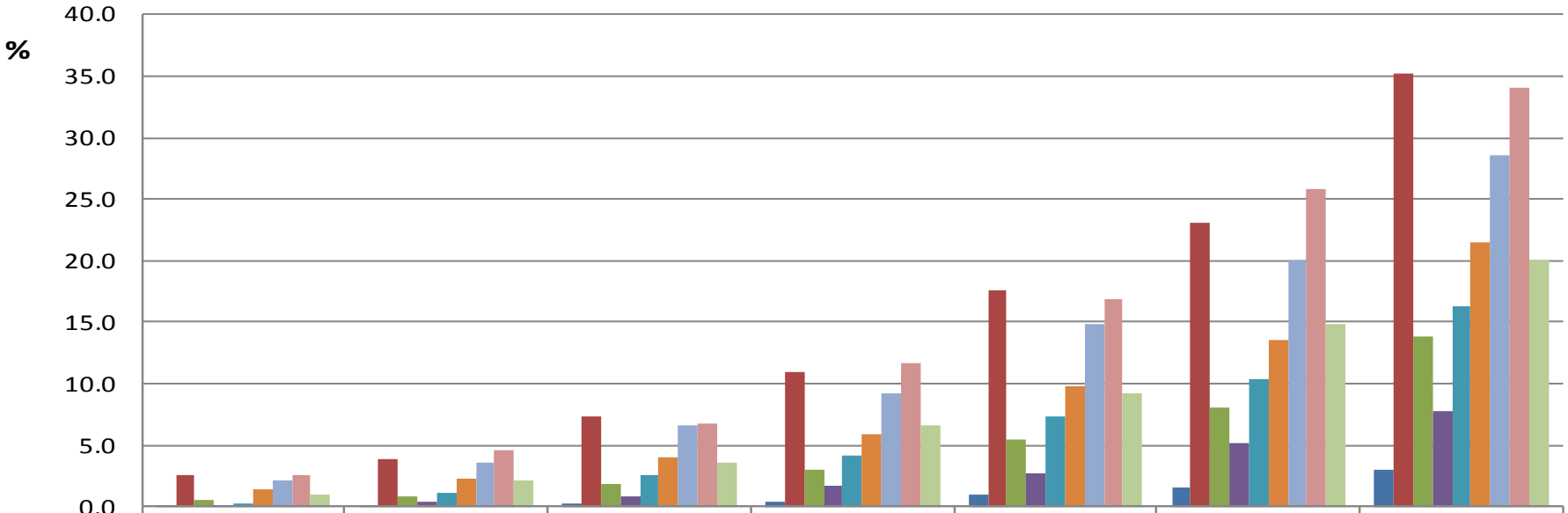
Aryl alaptide derivatives



Comp.	R	$ER_{Theo(24h)}$	Comp.	R	$ER_{Theo(24h)}$
1		56.5±4.07	5		26.5±5.01
2		14.2±5.08	6		42.2±4.13
3		7.0±3.21	7		47.0±3.19
4		16.4±5.17	8		20.8±5.12

Aryl alaptide derivatives

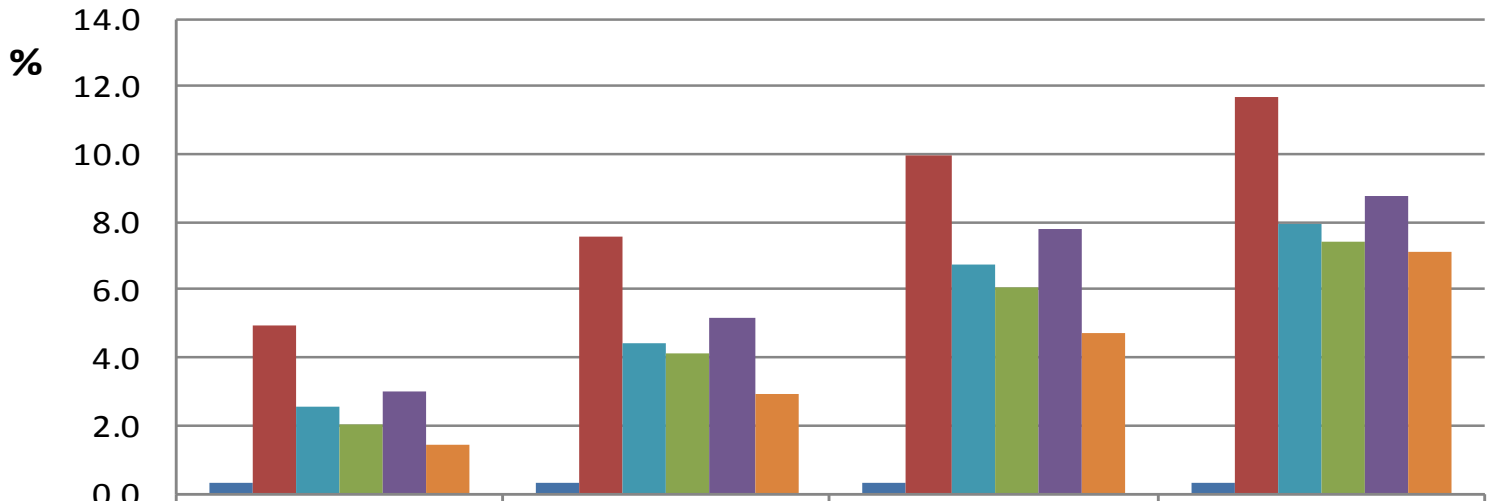
Comparison of the permeation of theophylline (Theo) through the skin from propylene glycol/water (1:1) without and with the presence of 0.1% (in relation to Theo amount) of AADs in time.



	0.5	1	1.5	2	3	4	6
Theo	0.00	0.10	0.24	0.43	0.95	1.52	3.00
Theo+1	2.50	3.92	7.27	10.93	17.55	23.03	35.17
Theo+2	0.48	0.89	1.91	3.05	5.48	8.08	13.82
Theo+3	0.18	0.43	0.78	1.69	2.74	5.11	7.75
Theo+4	0.20	1.19	2.53	4.18	7.36	10.31	16.33
Theo+5	1.41	2.22	3.99	5.84	9.78	13.61	21.53
Theo+6	2.16	3.64	6.55	9.23	14.79	19.98	28.51
Theo+7	2.55	4.58	6.72	11.73	16.88	25.84	34.07
Theo+8	0.96	2.16	3.64	6.55	9.23	14.79	19.98

Aryl alaptide derivatives

Comparison of the permeation of indometacin (IND) through the skin from carbomer gel without and with the presence of 0.1% (in relation to IND amount) of phenyl (**1**), 4-methyl-1*H*-imidazole (**5**), 4-hydroxybenzyl (**6**), 4-fluorobenzyl (**7**) and 3-methyl-1*H*-indol-5-ol (**8**) derivatives in time.

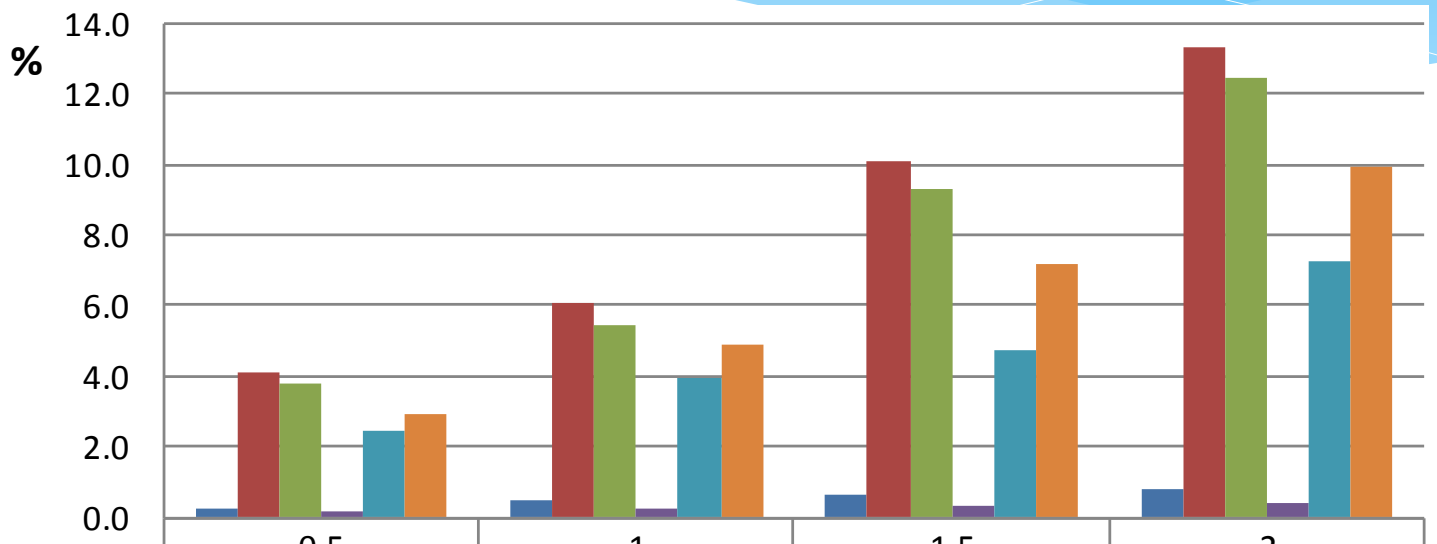


	0.5	1	1.5	2
gel	0.27	0.33	0.30	0.29
gel+1	4.92	7.55	10.01	11.66
gel+5	2.57	4.43	6.73	7.96
gel+6	2.02	4.09	6.03	7.40
gel+7	3.00	5.16	7.77	8.80
gel+8	1.43	2.93	4.69	7.13

Time [h]

Aryl alaptide derivatives

Comparison of the permeation of ibuprofen (Ibu) through the skin from carbomer gel and hydro-cream (Neo-Aquasorb®) without and with the presence of 0.1% (in relation to Ibu amount) of phenyl (**1**) and 4-fluorobenzyl (**7**) derivatives in time.

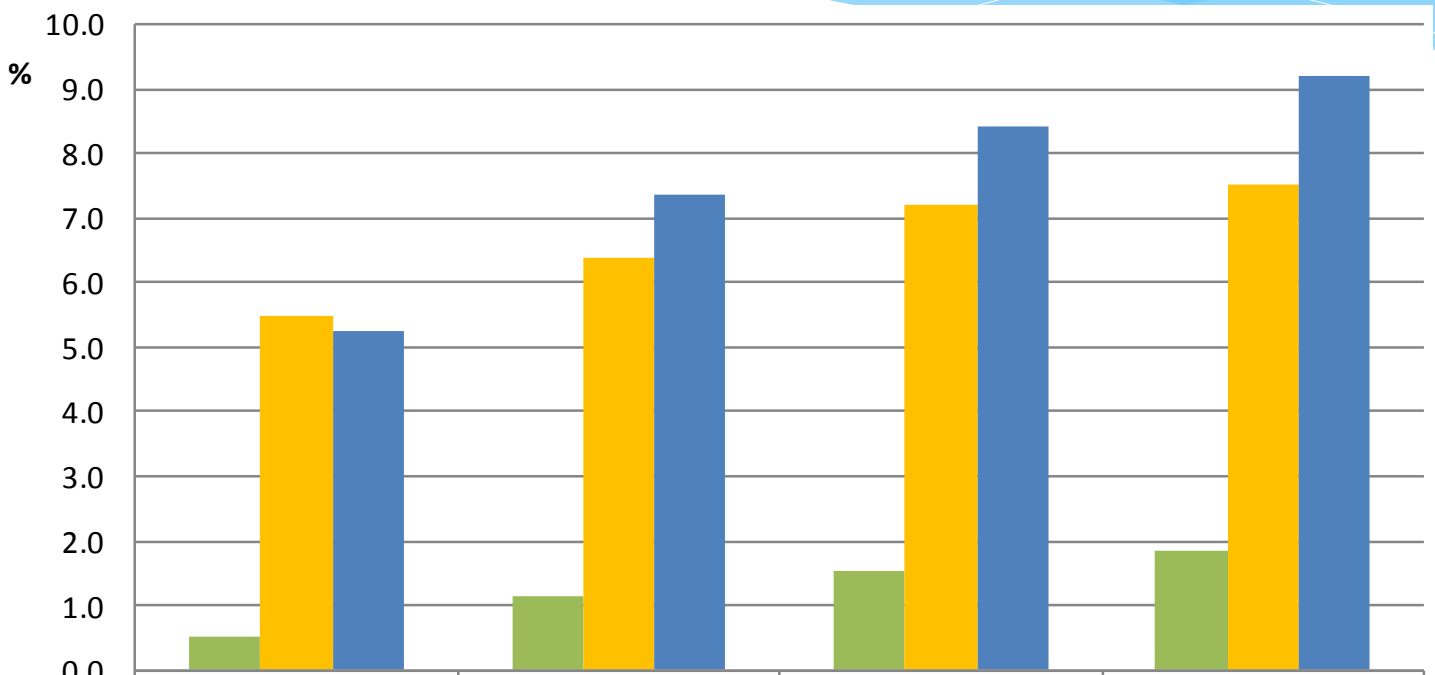


	0.5	1	1.5	2
gel	0.26	0.46	0.62	0.78
gel+1	4.14	6.09	10.08	13.33
gel+7	3.82	5.41	9.34	12.46
cream	0.20	0.25	0.31	0.37
cream+1	2.42	3.98	4.74	7.23
cream+7	2.90	4.87	7.19	9.94

Time [h]

Aryl alaptide derivatives

Comparison of the permeation of sumatriptane (SMT) through the skin from 70% dimethicone (simulation of TTS) without and with the presence of 1% (in relation to SMT amount) of phenyl (**1**) and 4-fluorobenzyl (**7**) derivatives in time.



	0.5	1	2	3
TTS	0.53	1.13	1.54	1.86
TTS+1	5.50	6.40	7.20	7.50
TTS+7	5.26	7.35	8.40	9.20

Time [h]

Conclusion

Series of original (*S*)-8-aryl-6,9-diazaspiro[4.5]decan-7,10-diones were prepared by a multi-step synthetic pathway from modified amino acids and characterized.

All discussed compounds were tested for their *in vitro* transdermal permeation enhancement effect using a vertical Franz diffusion cell and full-thickness pig ear skin.

Some of the tested compounds expressed significant permeation enhancement effect though the skin.

They also expressed no skin irritability (*in vivo* test on mice) and no *in vitro* toxicity (IC50 >50 μmol/l) against cervical cancer cells HeLa, T-lymphoblastic leukaemia CEM, breast adenocarcinoma MCF7, human Caucasian malignant melanoma G361 and human foreskin fibroblasts BJ.

Thank you for your kind attention



This study was supported by the Technology Agency of the Czech Republic TA04010065.

Let us meet again..

We welcome you all to our future conferences of OMICS International

4th Annual Conference on European Pharma Congress

June 18-20,2016, Berlin, Germany.

http://europe.pharmaceuticalconferences.com/