Engineering, Environment and Materials in Process Industry

CHROMATOGRAPHIC PARAMETERS AS PREDICTORS OF PHENYLACETAMIDE DERIVATIVES' BIOLOGICAL ACTIVITY

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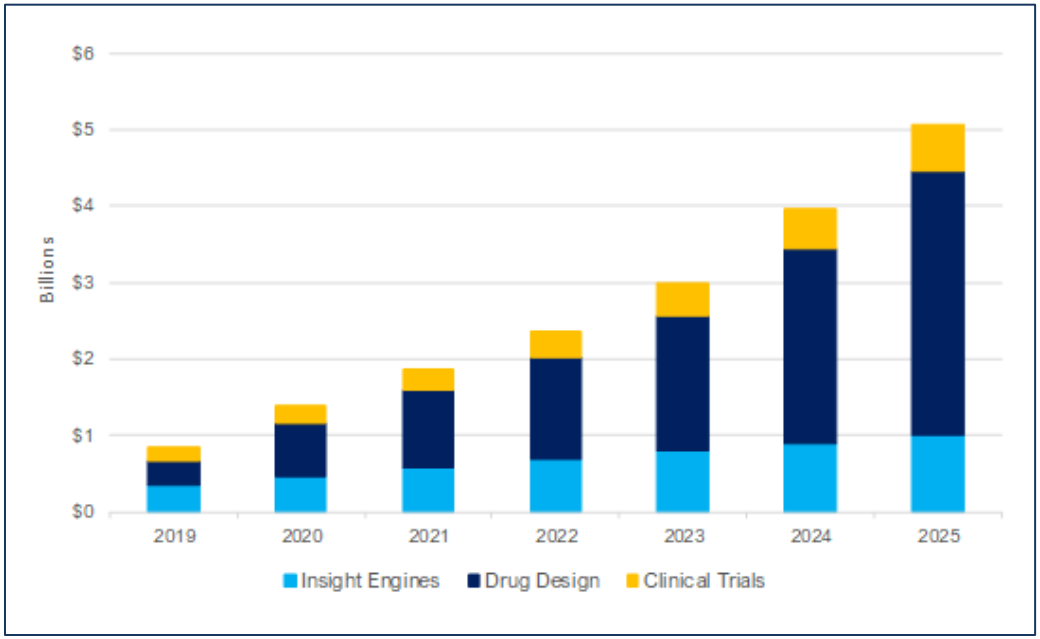
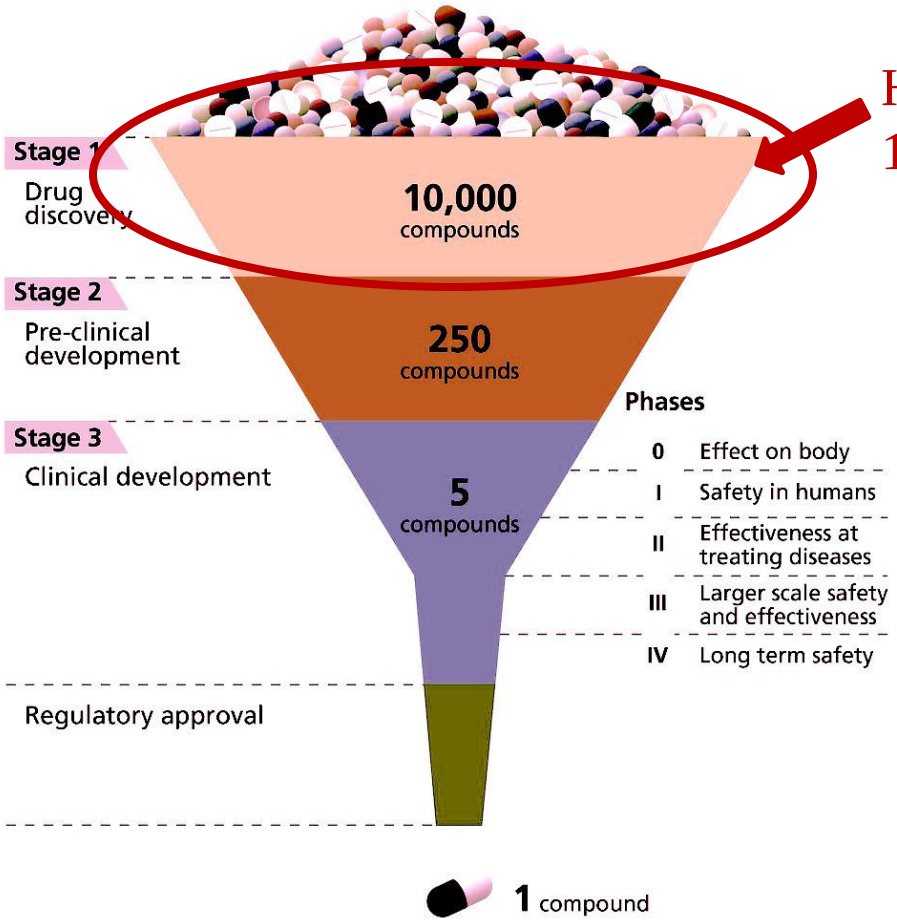
Drug development

- expensive ~ 2.8 billion $
- lengthy process ~ 15 years



https://emersioninsights.io

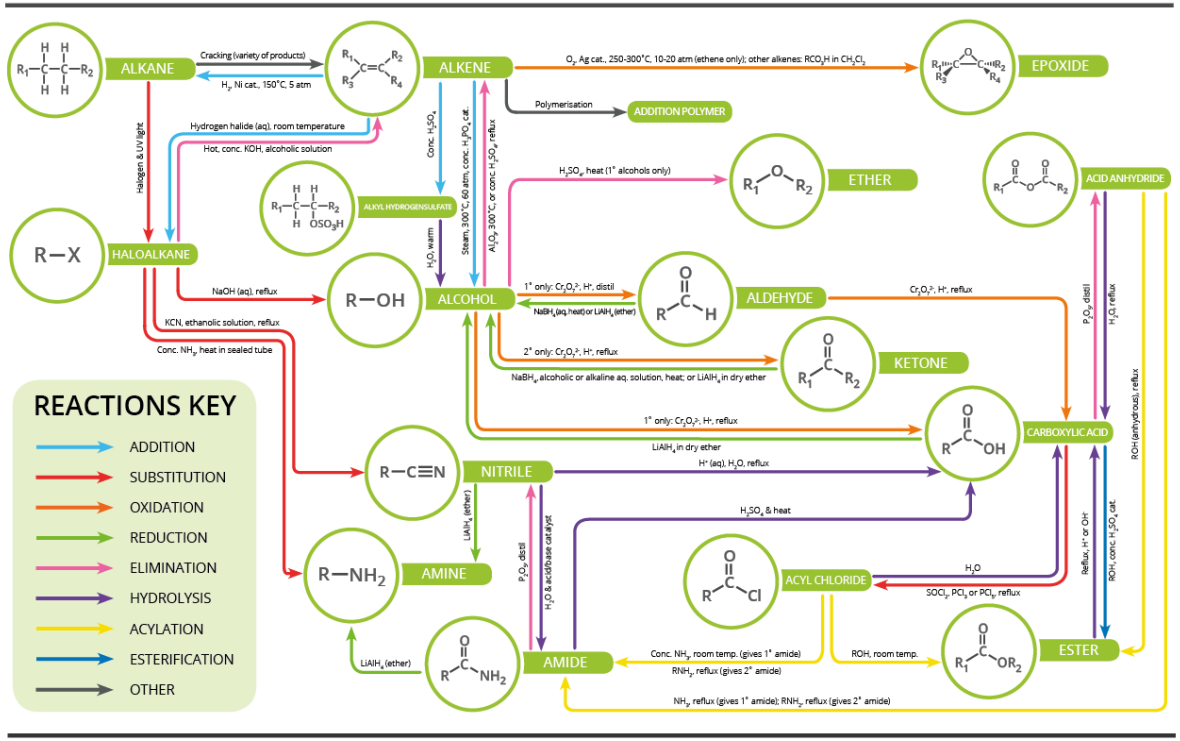
Drug development

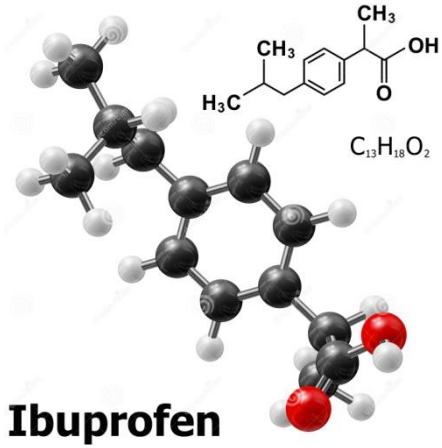


How to choose from 10,000 compounds?

https://expert.cheekyscientist.com

Traditional approaches in drug discovery phase





Computer-aided drug design

https://www.sciencedirect.com

Assessment of physico-chemical properties and prediction of activity

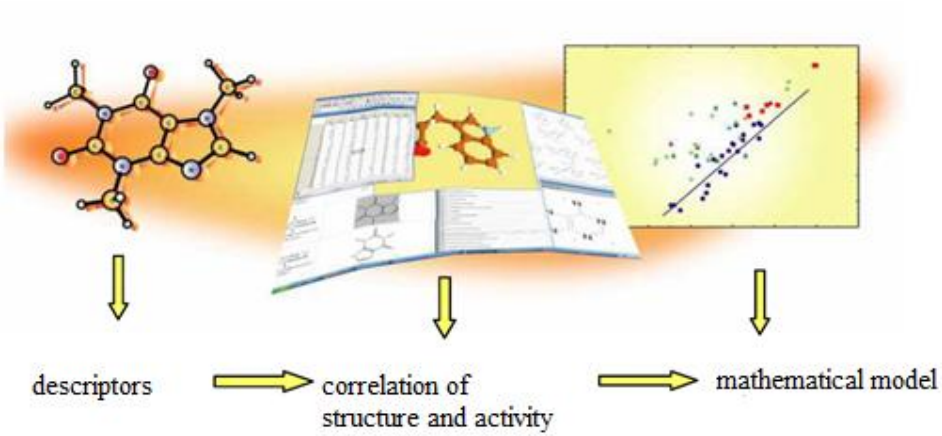
Quantitative Structure Property Relationship, QSPR

relevant physico-chemical property

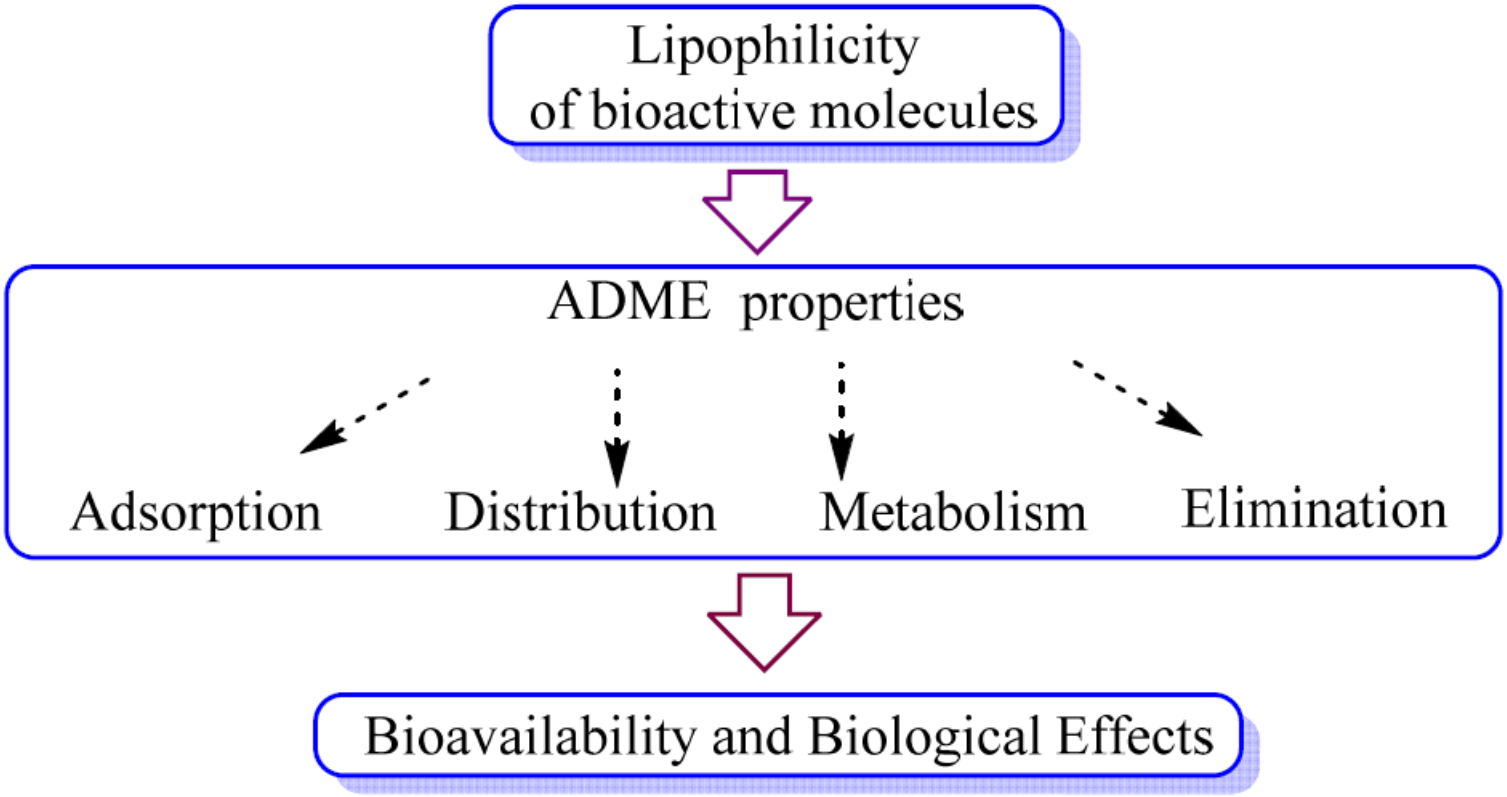
quantification of the structural change's effect

bioactivity

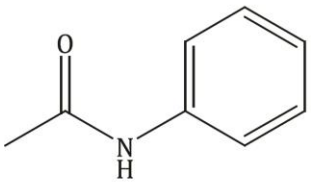
Quantitative Structure Activity Relationship, QSAR



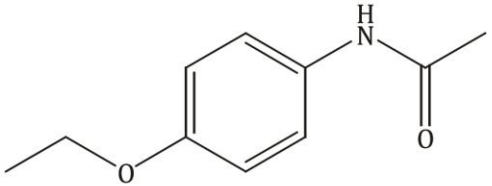
Lipophilicity



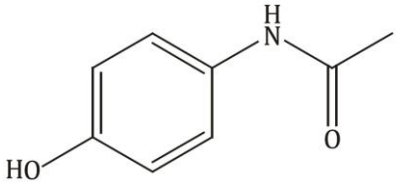
Phenylacetamides



a)



b)



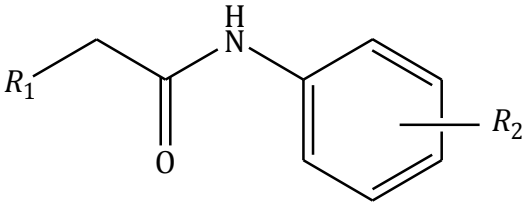
c)

a) Acetanilide
b) Phenacetin
c) Acetaminophen

Phenylacetamide derivatives have number of other biological properties:

- Anticonvulsants
- Antiarrhythmics
- Antidepressants
- Antituberculotics
- Bactericides
- Insecticides
- new anti-HIV drugs
- Antitumor agents
- Theranostics

Studied compounds



R_1 = CN		R_1 = Cl		R_1 = C_6H_5	
Deriv.	R_2	**Deriv.**	R_2	**Deriv.**	R_2
CN1	H	CL1	H	DF1	H
CN2	CH_3	CL2	CH_3	DF2	CH_3
CN3	I	CL3	OCH_3	DF3	C_2H_5
CN4	Br	CL4	Cl	DF4	OH
CN5	NO_2	CL5	4Br	DF5	Cl
CN6	OH	CL6	F	DF6	Br
CN7	COOH	CL7	I	DF7	F
CN8	$COCH_3$	CL8	$COCH_3$	DF8	CN
CN9	C_2H_5	CL9	OH	DF9	$COOCH_3$
		CL10	3CN	DF10	$COCH_3$
		CL11	4CN		
		CL12	3Br		

Compatibility of the studied chloro-phenylacetamide derivatives and the “drug-likeness” rules

R	AClog*P*	Alog*P*	AlogP_s	Mlog*P*	milog*P*	*kowwin*	log*P*ch.s	XlogP_3
H	1.77	1.70	1.73	1.95	1.72	1.68	1.64	1.63
4CH_3	2.08	2.18	1.8			23	2.31	1.99
4OCH_3	1.66	1.68	1.6			76	1.86	1.67
4Cl	2.38	2.36	2.3			32	2.59	2.26
4Br	2.47	2.44	2.4			57	2.79	2.32
4F	1.83	1.90	2.0			38	1.91	1.73
4I	2.70	2.72	2.9			35	3.21	2.28
4$COCH_3$	1.69	1.43	1.5			36	1.65	1.86
4OH	1.47	1.43	0.9			35	1.32	1.27
3CN	1.58	1.57	1.5			78	1.50	1.82
4CN	1.58	1.57	1.54	1.59	1.48	1.78	1.52	1.35
3Br	2.47	2.44	2.42	2.66	2.51	2.57	2.78	2.94

Lipinski’s rule:
MW ≤ 500;
nOHNH ≤ 5;
nON ≤ 10 (2·5);
log*P* ≤ 5

Ghose’s rule:
160 ≤ MW ≤ 180;
-0.4 ≤ log*P* ≤ 5.6;
20 ≤ natoms ≤ 70;
40 ≤ MR ≤ 130

MW- molecular weight; nON- number of hydrogen bond acceptor;
nOHNH- number of hydrogen bond donor; natoms-the total number of atoms in molecule; MR- molar refractivity

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4OCH_3	1.66	1.68	1.69	1.68	1.78	1.76	1.86	1.67
4Cl	2.38	2.36	2.39	2.52	2.40	2.32	2.59	2.26
4Br	2.47	2.44	2.42	2.66	2.53	2.57	2.79	2.32
4F	1.83	1.90	2.00	2.37	1.88	1.88	1.91	1.73
4I	2.70	2.72	2.94	2.81	2.81	2.85	3.21	2.28
4$COCH_3$	1.69	1.43	1.59	1.89	1.62	1.36	1.65	1.86
4OH	1.47	1.43	0.97	1.38	1.24	0.85	1.32	1.27
3CN	1.58	1.57	1.53	1.59	1.45	1.78	1.50	1.82
4CN	1.58	1.57	1.54	1.59	1.48	1.78	1.52	1.35
3Br	2.47	2.44	2.42	2.66	2.51	2.57	2.78	2.94

Chromatographic measurements

Stationary phase

- commercial plates RPTLC C18/UV254s, (Macherey–Nagel, Germany)
- commercial plates TLC 60CN F254s (Merck, Darmstadt, Germany)

Mobile phase

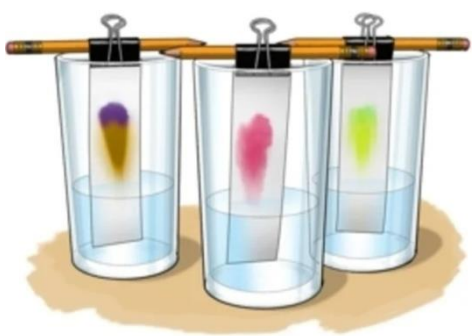
- water – methanol ($\varphi_{(\text{methanol})}$ = 0,34 – 0,56)
- water – ethanol ($\varphi_{(\text{ethanol})}$ = 0,32 – 0,52)
- water – *n*-propanole ($\varphi_{(n\text{-propanole})}$ = 0,32 – 0,52)
- water – *i*-propanole ($\varphi_{(i\text{-propanole})}$ = 0,36 – 0,52)
- water – acetone ($\varphi_{(\text{acetone})}$ = 0,36 – 0,52)
- water – acetonitrile ($\varphi_{(\text{acetonitrile})}$ = 0,36 – 0,52)
- water – tetrahidrofuran ($\varphi_{(\text{thf})}$ = 0,36 – 0,56)
- water – dioxane ($\varphi_{(\text{dioxane})}$ = 0,32 – 0,52)

$$R_f = \frac{l_r}{l_f}$$

$$R_M = \log\left(\frac{1}{R_f} - 1\right)$$

$$R_M = R_M^0 + m\varphi$$

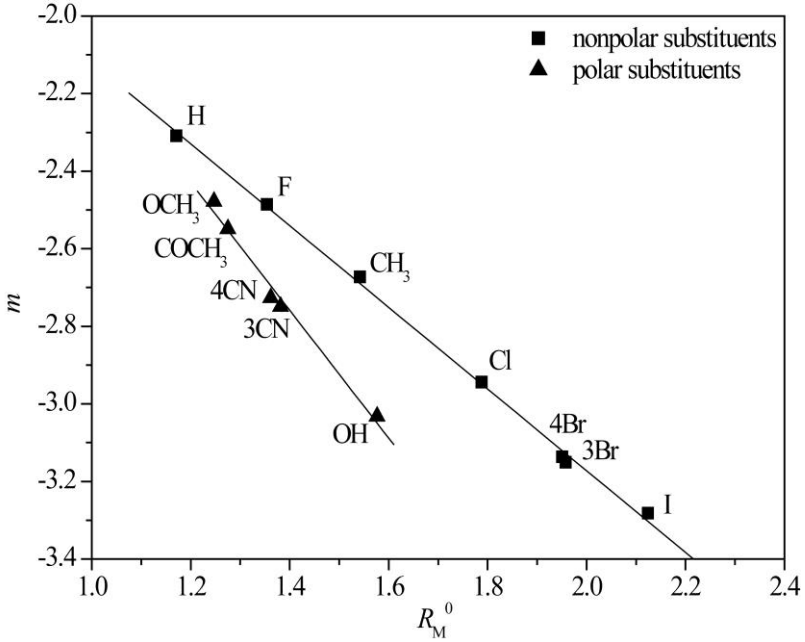
$$C_0 = -\frac{R_M^0}{m}$$



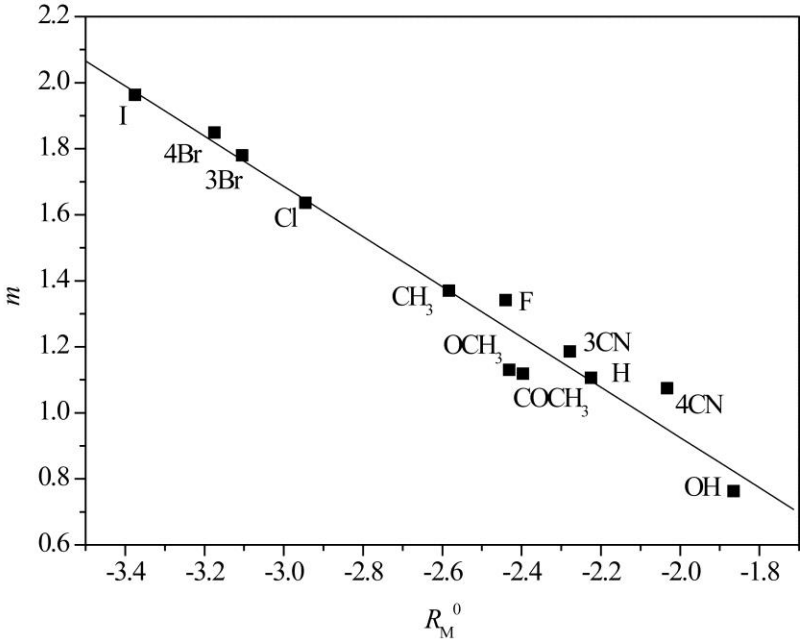
Chromatographic results of chloro-phenylacetamides

R	methanol			acetone		
	R_M^0	*m*	r	R_M^0	*m*	r
H	1.146	-2.225	0.984	1.272	-2.153	0.992
4CH$_3$	1.370	-2.583	0.989	1.715	-2.785	0.999
4OCH$_3$	1.130	-2.431	0.989	1.149	-1.975	0.989
4Cl	1.636	-2.945	0.997	2.032	-3.408	0.994
4Br	1.849	-3.175	0.994	2.152	-3.595	0.999
4F	1.341	-2.440	0.998	1.551	-2.515	0.997
4I	1.963	-3.375	0.990	2.269	-3.726	0.990
4COCH$_3$	0.995	-2.396	0.992	1.075	-1.881	0.996
4OH	0.763	-1.865	0.998	0.785	-1.375	0.998
3CN	1.066	-2.278	0.991	1.228	-2.079	0.996
4CN	1.054	-2.033	0.996	1.195	-2.033	0.995
3Br	1.780	-3.105	0.991	2.155	-3.525	0.999

Chromatographic results of chloro-phenylacetamides

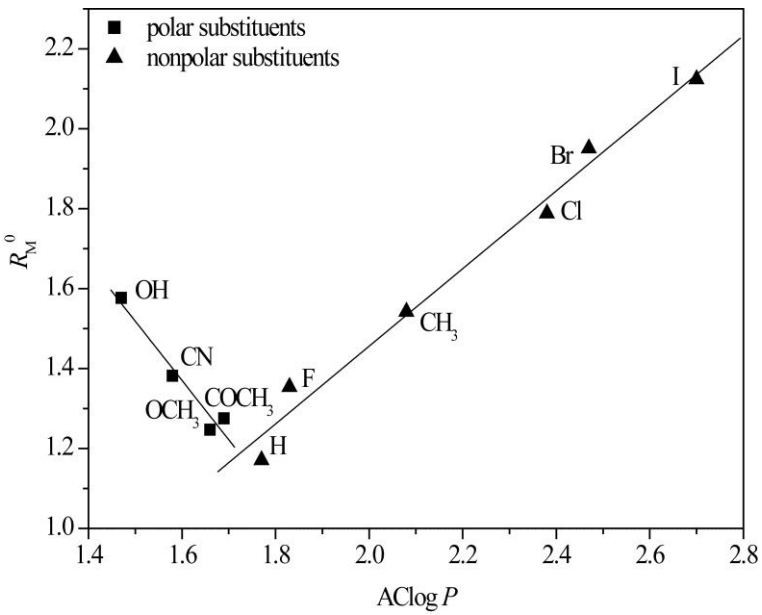


R_M^0-m correlation obtained in methanol

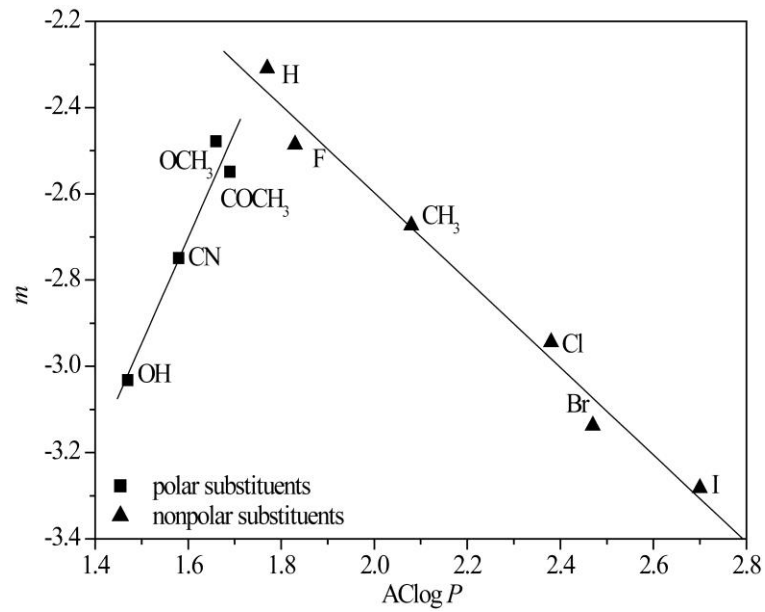


R_M^0-m correlation obtained in acetone

Chromatographic parameters as alternative measure of lipophilicity of chloro-phenylacetamides



R_M^0 – AClog$P$ relationship in methanol



m – AClog$P$ relationship in methanol

approximately $r > 0.870$

R_M^0 and m valid measures of the lipophilicity of the chloro-phenylacetamides

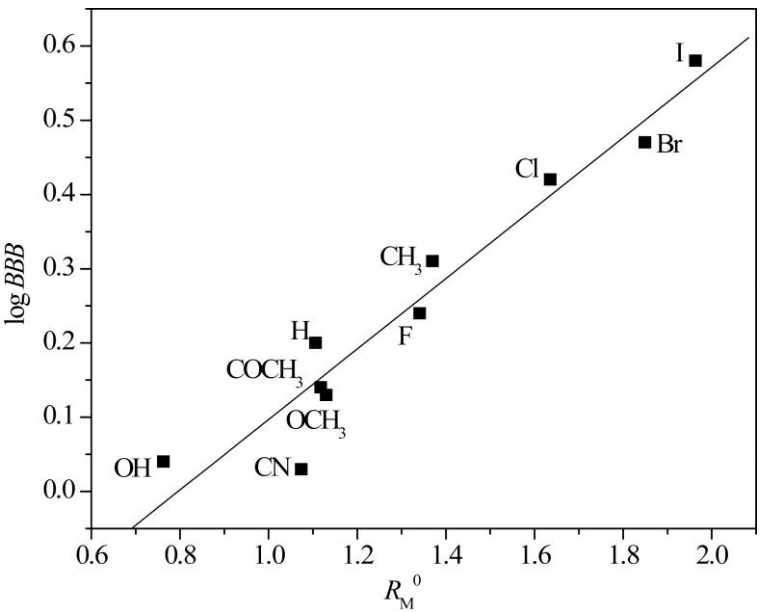
Relationship between the alternative measure of lipophilicity and selected pharmacokinetic predictors

Molinspiration,
SimulationPlus,
PreADMET

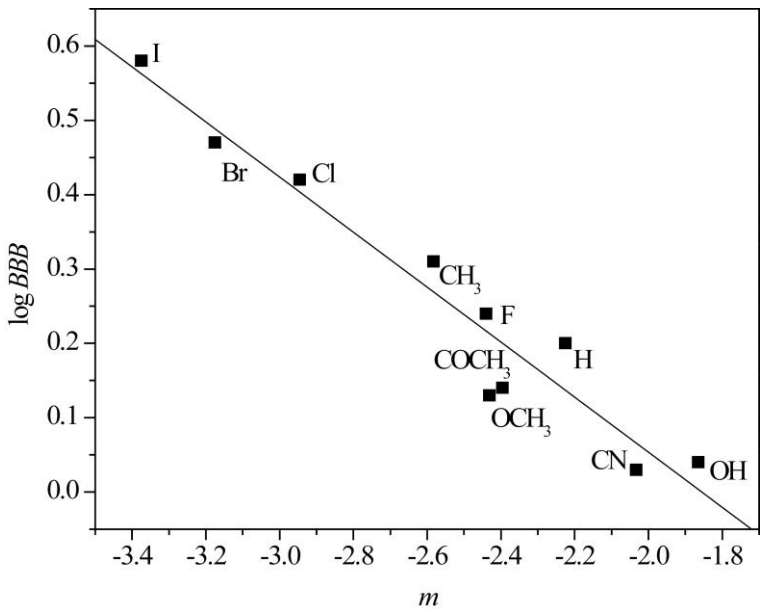
R	P_{eff}	*PPB*	log*BBB*
H	3.536	45.46	0.20
$4CH_3$	4.243	76.38	0.31
$4OCH_3$	3.254	64.10	0.13
4Cl	4.857	80.00	0.42
4Br	5.213	84.80	0.47
4F	4.559	63.51	0.24
4I	5.579	91.06	0.58
$4COCH_3$	3.679	61.70	0.14
4OH	1.956	41.59	0.04
3CN	2.366	62.42	0.03
4CN	3.102	62.42	0.03
3Br	4.546	84.80	0.47

P_{eff} - Human effective permeability in jejunum;
PPB - Plasma protein binding; log*BBB* - Blood-brain barierr passage

Relationship between the alternative measure of lipophilicity and selected pharmacokinetic predictors



R_M^0 – log*BBB* relationship in acetone

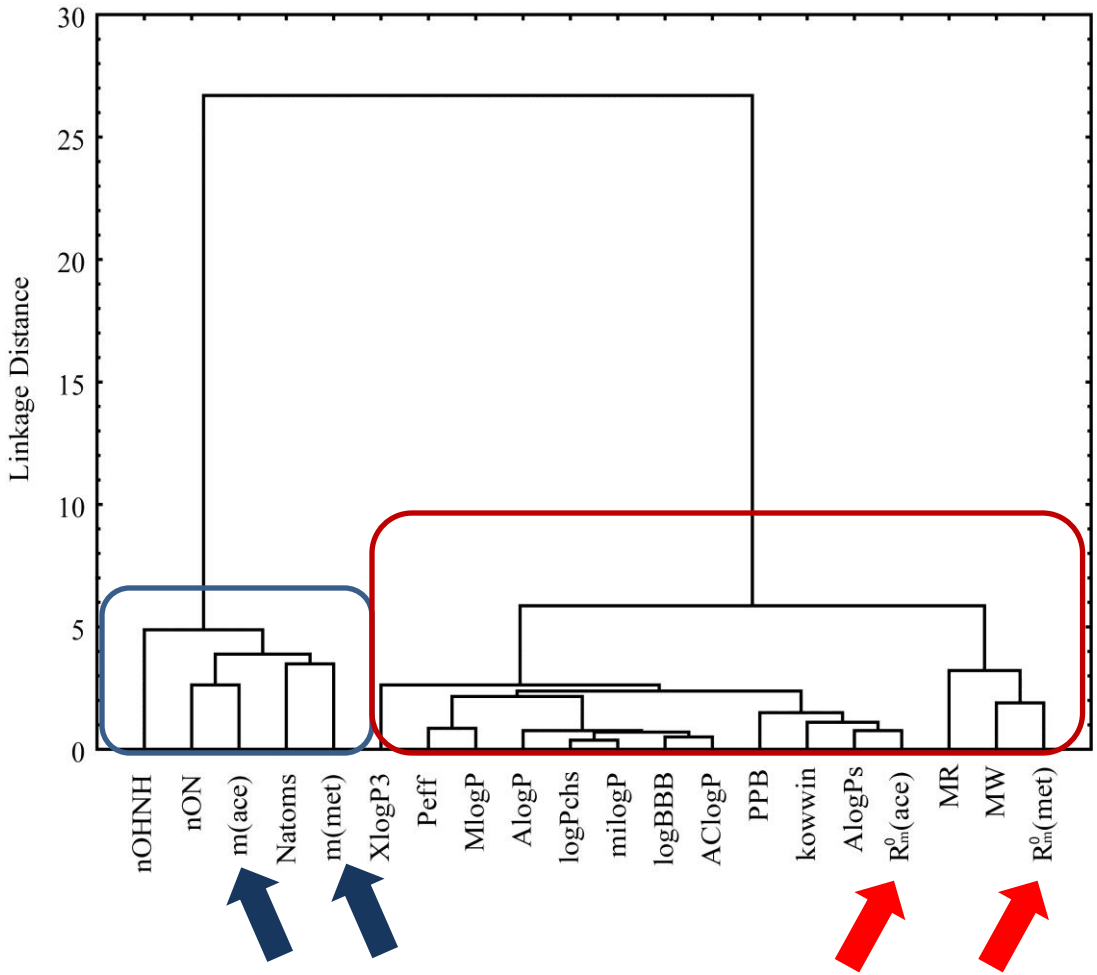


m – log*BBB* relationship in acetone

approximately $r > 0.910$

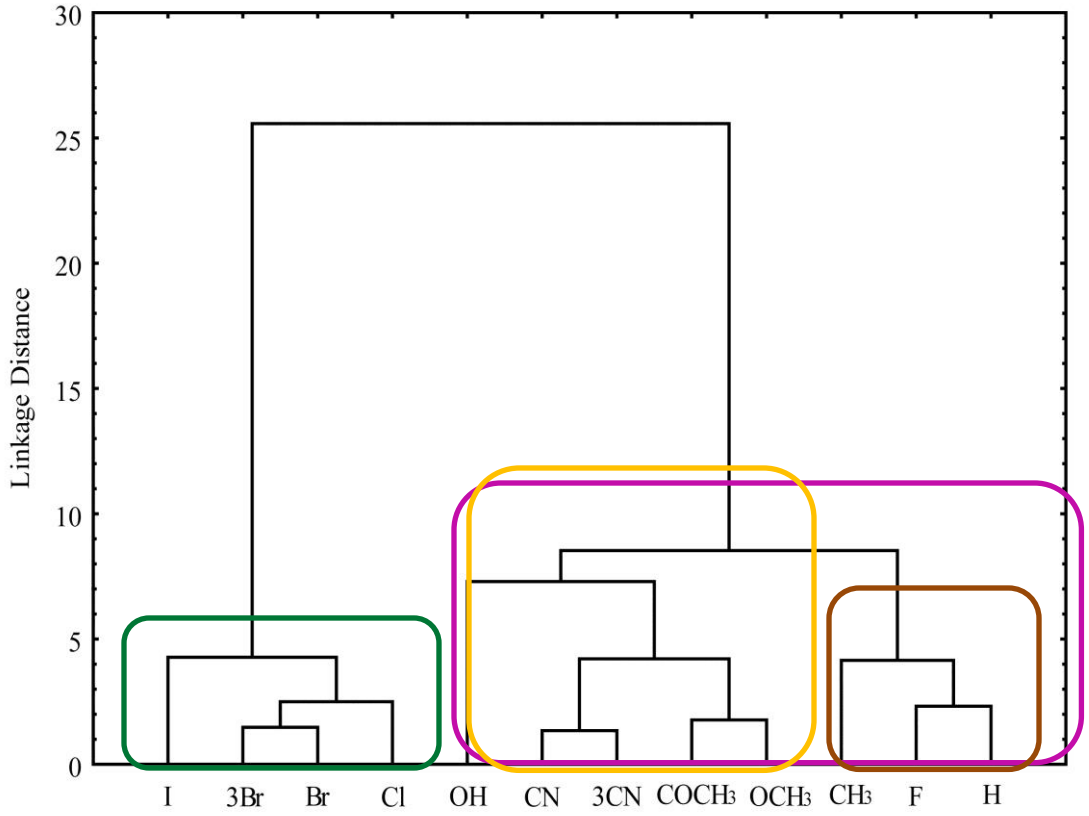
R_M^0 and *m* reliably use to assess the pharmacokinetic behavior of the chloro-phenylacetamides

Cluster analysis of chloro-phenylacetamide series



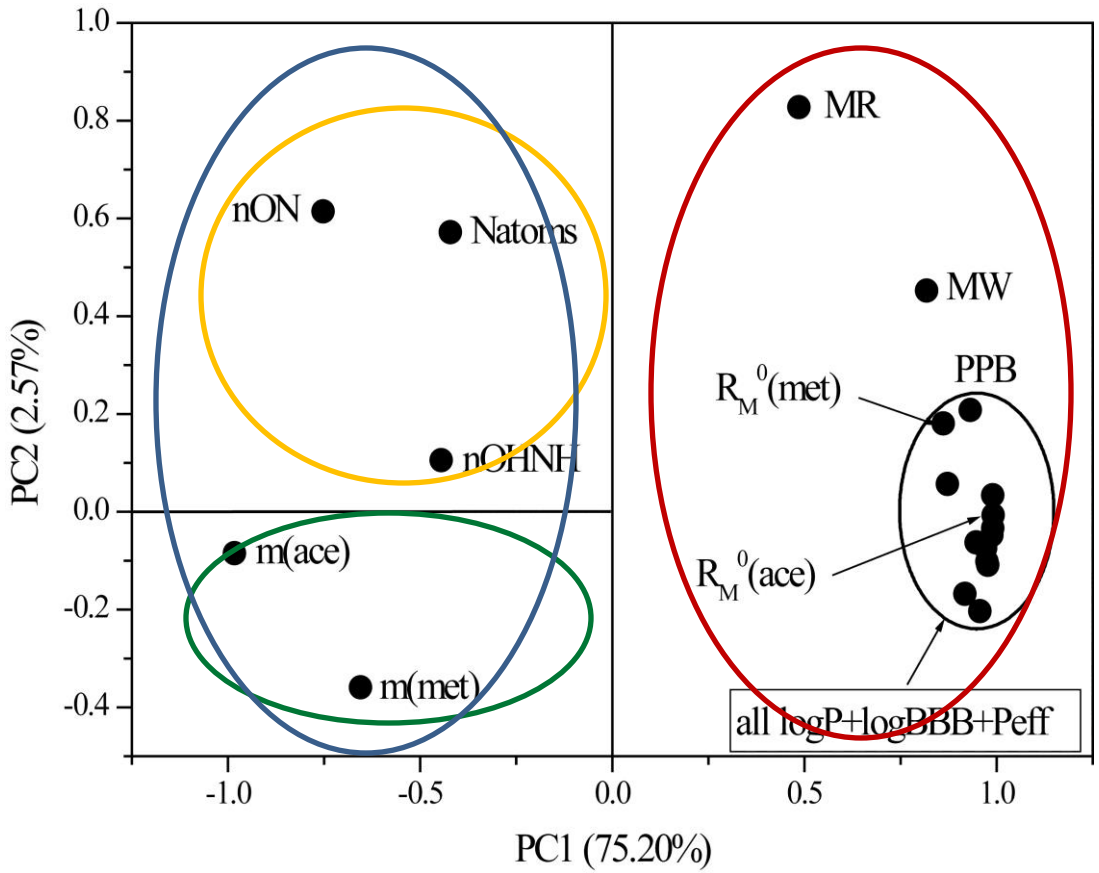
Dendrogram of bioactivity parameters of examined chloro-phenylacetamides

Cluster analysis of chloro-phenylacetamide series



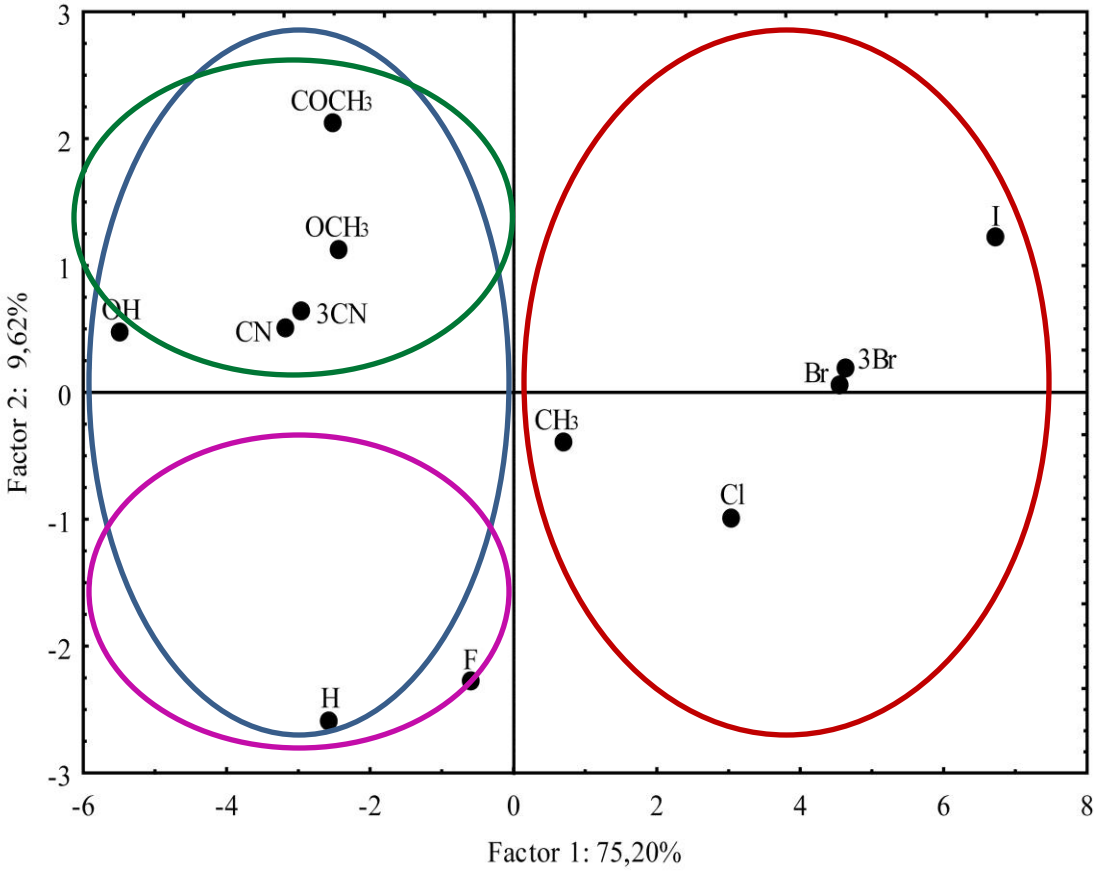
Dendrogram of examined derivatives chloro-phenylacetamides based on their parameters of bioactivity

Principal Component Analysis (PCA) of chloro-phenylacetamide series



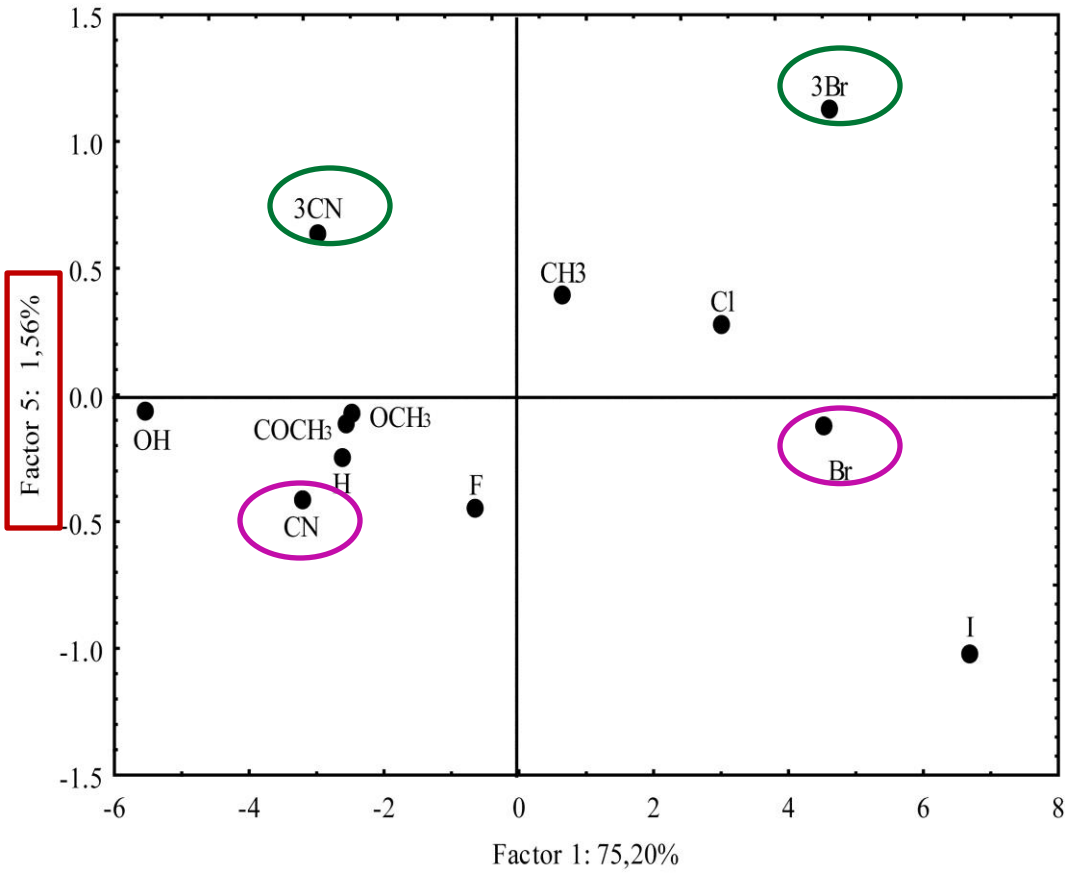
Loading plots as a result of PC1 versus PC2

Principal Component Analysis (PCA) of chloro-phenylacetamide series



Score plots as a result of PC1 versus PC2

Principal Component Analysis (PCA) of chloro-phenylacetamide series

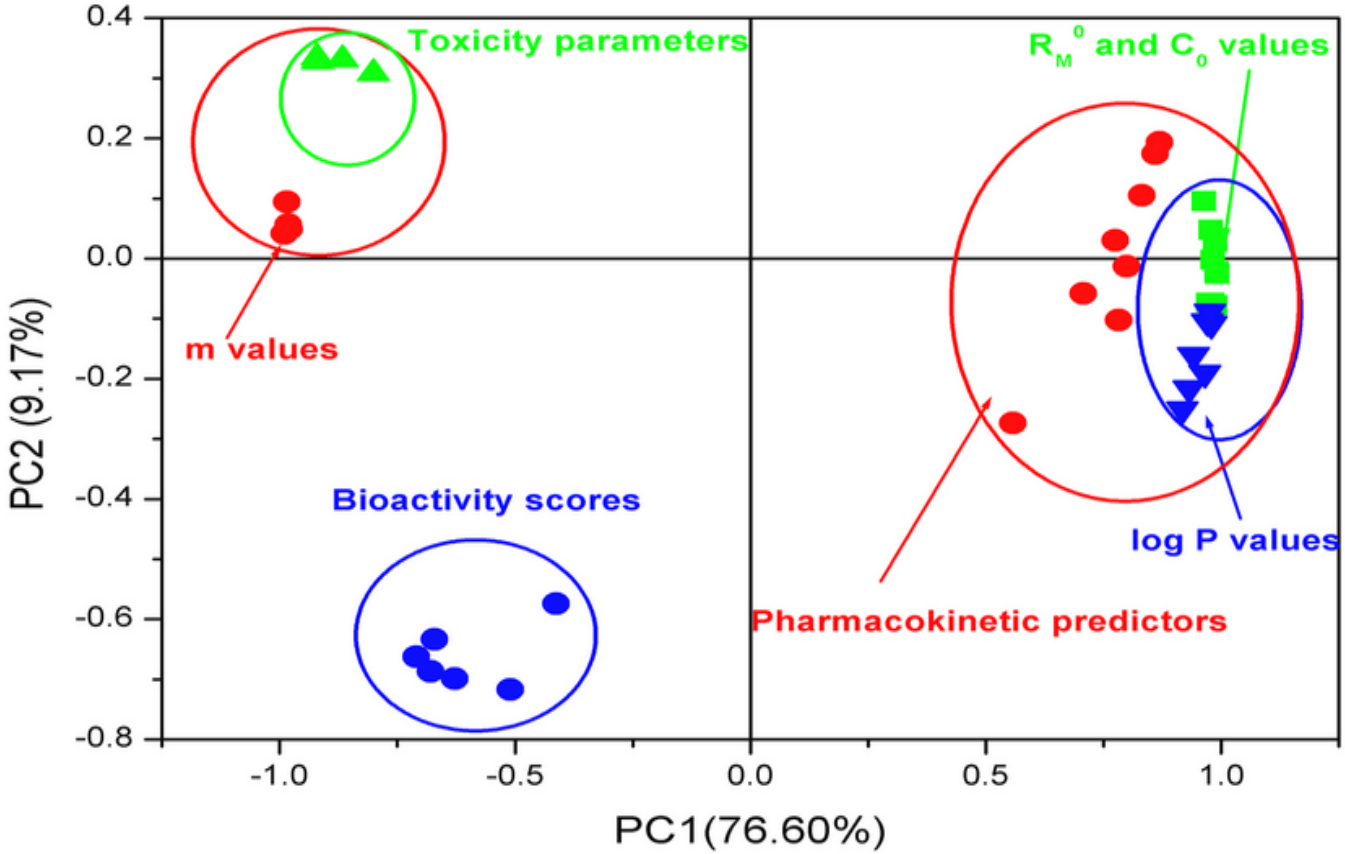


Score plots as a result of PC1 versus PC5

J. Liq. Chromatogr. Relat. Technol. 2015; 38: 1691–1698; Iran. J. Pharm. Res. 2018; 17: 100–114

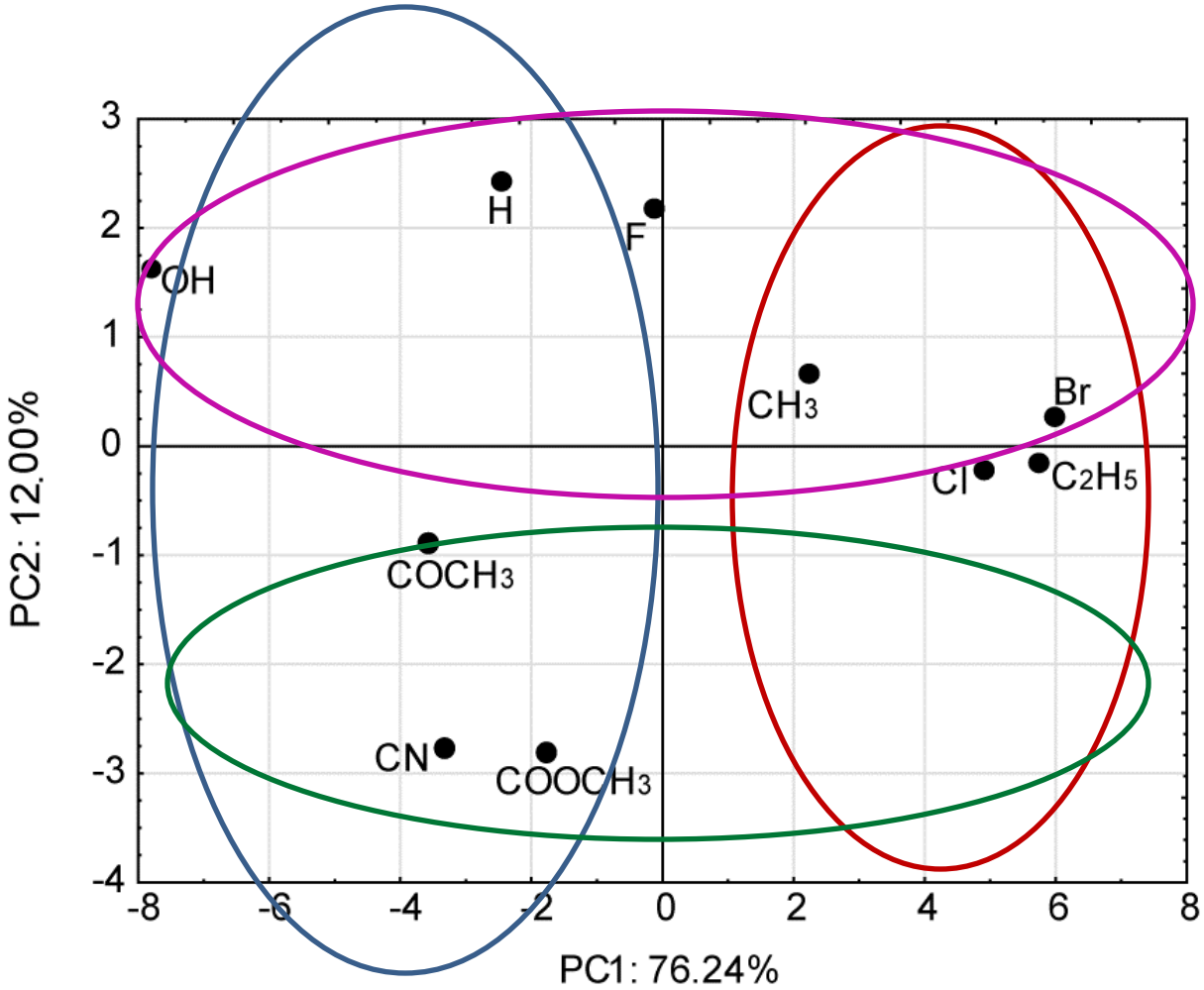
Principal Component Analysis of diphenylacetamides

2 different stationary phase additional descriptors



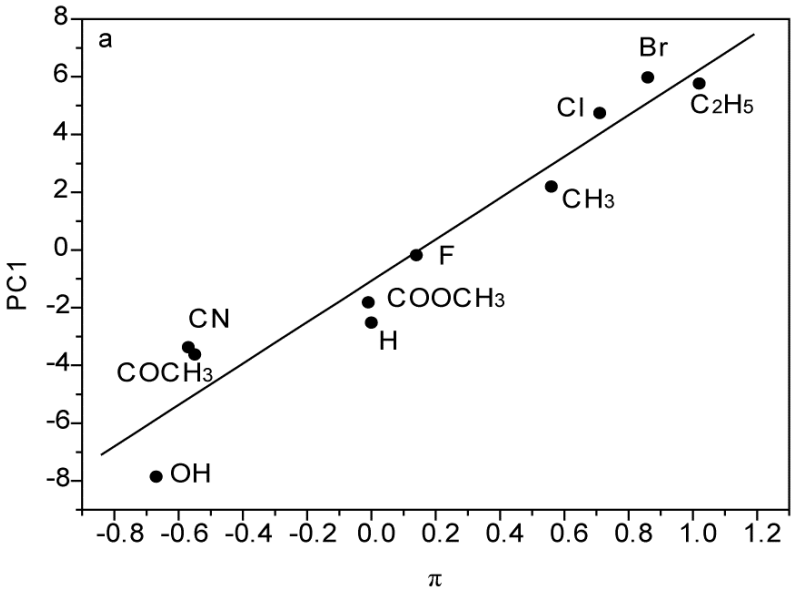
Loading plots as a result of PC1 versus PC2

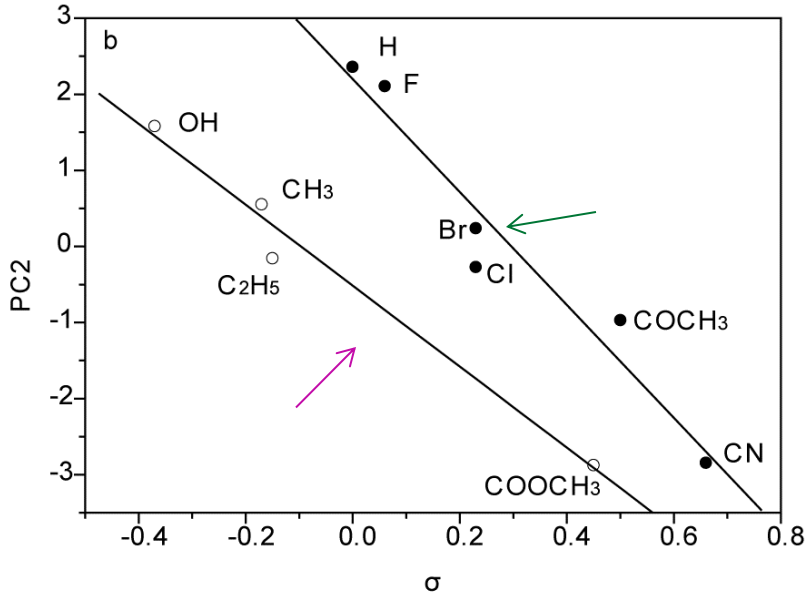
Principal Component Analysis of diphenylacetamides



Score plots as a result of PC1 versus PC2

Principal Component Analysis of diphenylacetamides





$PC1 = -0.931 + 6.247 \cdot \pi$ r = 0.960

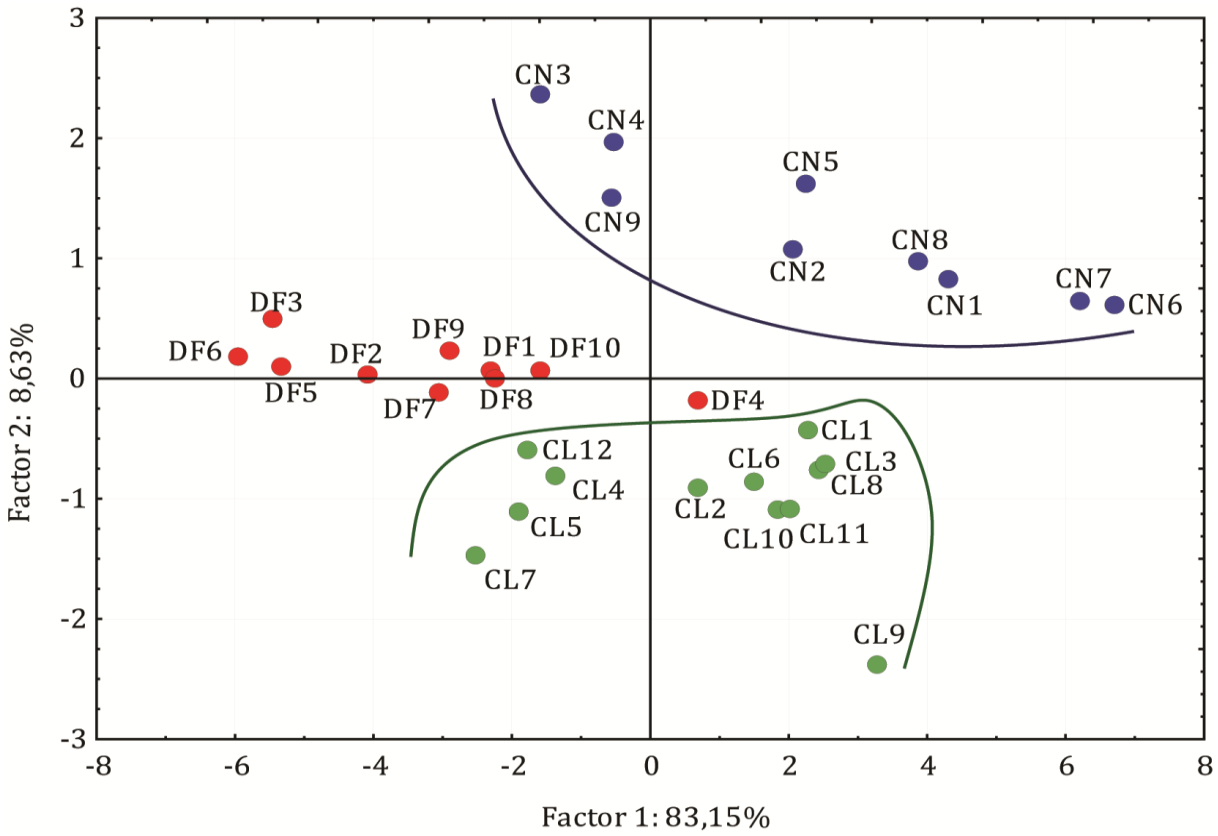
$PC2 = 2.186 - 7.284 \cdot \sigma_{+}$ r = 0.980

$PC2 = -0.784 - 5.960 \cdot \sigma_{-}$ r = 0.990

Examined bioactivity parameters are most influenced by the **polarity** of the substituent and to a lesser extent by its **electronic effects**.

J. Chromatogr. Sci. 2015; 53: 312–319; J. Chemom. 2019; 33: e3091;
J. Liq. Chromatogr. Relat. Technol. 2020; 43: 852–862

Principal Component Analysis of all studied phenylacetamides



Score plots as a result of PC1 versus PC2

PC1 → R_2
PC2 → R_1

Take-home message

The bioactivity parameters of the examined phenylacetamides depend on:

- in large extent by polarity, but also by electronic effect of the present substituent
- in lesser extent by environment

Our current and future research

In vitro and *in vivo* tests of the studied phenylacetamides

THANK YOU FOR YOUR ATTENTION