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A Structure–Permeability Study of Small Drug-like Molecules

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Abstract—A systematic structure–permeability relationship study on a set of small drug-like molecules with log D values in the range −2.5 to 3 and carrying a diverse array of functionality reveals that the compounds with log D > 0 and < 3 are highly permeable. Surprisingly, several tetrazole derivatives were found to be substrates for efflux pump(s).

The Caco-2 cell model is widely used as an indicator of oral drug absorption[1] and permeability data generated in this assay has been published for various sets of structurally diverse compounds and drugs.[2] We have previously used the Caco-2 cell model to evaluate the cell permeability of peptidomimetic carboxylic acid derivatives designed as ligands for the p56lck SH2 domain,[3] and found that the data was useful in guiding medicinal chemistry efforts to design molecules active in cellular assays. Based on this experience we were interested in applying the model to a broader exploration of structure–permeability relationships, particularly the effect on cell permeability of some polar groups commonly used in lead optimization efforts. The introduction of such groups is sometimes necessary to improve the molecular potency or physicochemical properties of lead molecules but the effect on cellular activity can be inconsistent. The few published[4] systematic studies of structure–permeability relationships have generally been restricted in scope either because of a limited number of compounds studied or a focus on peptide-like structures. Here, we present a systematic exploration of the effect on cell permeability of some commonly used functional groups attached to the drug-like[5] core structures shown in Scheme 1.

The compounds[6] evaluated in this study were prepared as shown in Scheme 1. Compounds **1**–**19** were derived from the appropriately substituted benzyl bromide by *N*-alkylation of imidazole, benzimidazole or pyrazole, respectively. For the synthesis of **14** and **15** the benzyl bromides substituted with a sulfonic acid or sulfonamide group were obtained from the sulfonyl chloride by hydrolysis or by treatment with ammonia.[7] Pyrimidines **20**, **21** and **22**, were obtained by reaction of 2-chloropyrimidine with activated zinc followed by nickel catalyzed coupling[8,9] with the appropriate benzyl bromide. Carboxylic acids **23**–**27** were obtained by hydrolysis of the corresponding methyl esters, while, alternatively, treatment of the esters with ammonia gave amides **28**–**31**. Reduction of nitriles **8**, **16**, or **20** with hydrogen/Raney–Ni gave the amines **32**–**34**. The amidine **35** was prepared from nitrile **20** by the Pinner reaction.[10] The tetrazoles **36**–**39** were formed from the corresponding nitriles by treatment with sodium azide and ammonium chloride.[11] The methyl sulfonamide **41** was prepared by stannous chloride reduction of nitro derivative **13** in DMF[12] followed by reaction of the aniline **40** with methanesulfonyl chloride.

We evaluated compound permeability[13] in the Caco-2 model following a standard protocol[2a] and the data is presented in Table 1. Figure 1 illustrates graphically the permeability data presented in Table 1. Compounds are arranged according to the substituent attached to the different core structures, and are categorized as having high, moderate, or low permeability based on their permeability coefficients. For compounds displaying low apical to basolateral ($P_{A\rightarrow B}$) permeability we also measured permeability in the basolateral to apical direction

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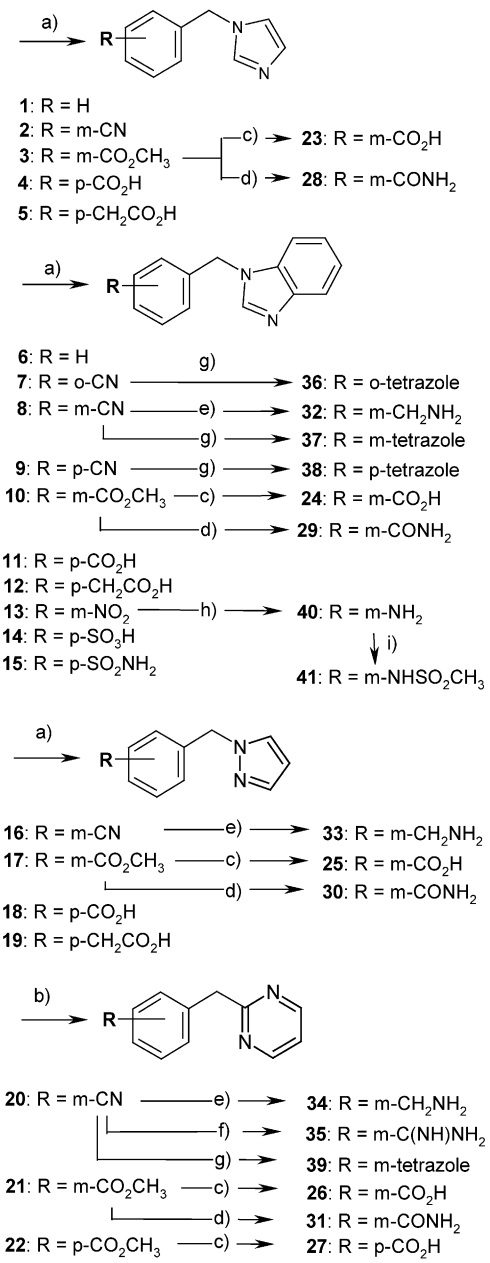
($P_{B\rightarrow A}$) in order to determine whether active transport was occurring. Such compounds were the benzoic acids **4**, **23**, **24**, the amidine **35** and the tetrazoles **36**–**39**. For these compounds, the PDR[14] (permeability directional ratio = $P_{B\rightarrow A}/P_{A\rightarrow B}$) is also shown in Table 1 along with P_t [estimated transcellular permeability = $(P_{A\rightarrow B}+P_{B\rightarrow A})/2$)].[15]

Highly permeable compounds (permeability coefficient $>10\times10^{-6}$ cm/s). This group, contains the core structures (**1** and **6**), nitriles (**2**, **8**, **16** and **20**), carboxamides (**29**, **30** and **31**), and aminomethyl (**32**, **33** and **34**), amino (**40**), methanesulfonylamido (**41**) and sulfonamide derivatives (**15**). Only for the imidazole carboxamide **28**, which falls in the group of moderately permeable compounds, does the nature of the heterocycle influence permeability substantially. This compound has decreased permeability compared to the other carboxamide derivatives. The greater basicity of the imidazole ring compared to the other heterocycles[16] may partially explain this result, but this alone is not sufficient since the benzylamine derivatives **32**–**34** are highly permeable while protonated to greater extent at pH 7.4.



Scheme 1. Reagents: (a) imidazole, benzimidazole or pyrazole, K_2CO_3, acetonitrile or DMF; (b) activated zinc, THF; 2-chloropyrimidine, $(C_6H_5)_2PCH_2CH_2P(C_6H5)_2]NiCl_2$; (c) LiOH, THF/MeOH/$H_2O$; (d) NH_3, MeOH; (e) Raney–Ni, H_2, EtOH/aqueous NH_4OH; (f) HCl/EtOH followed by NH_3/EtOH; (g) NaN_3, NH_4Cl, DMF; (h) $SnCl_2$ DMF; (i) $MeSO_2Cl$, NEt_3, CH_2Cl_2/EtOH.

Moderately permeable compounds (permeability coefficient between 0.3×10^{-6} and 10×10^{-6} cm/s). All the carboxylic acid derivatives fall into this group. The charged nature of these compounds at pH 7.4 is most likely responsible for the moderate permeability. Within each series, the imidazoles (**4**, **5** and **23**) are consistently less permeable than the other heterocycles. There is no substantial difference in permeability for the different carboxylic acids with the same scaffold. We also determined B to A permeability for **4**, **23** and **24**, three of the less permeable carboxylic acids and, while the PDR value for compound **4** is 1, values of 2 and 3 for **23** and **24** indicate that these compounds can be moderate substrates for cellular efflux pumps.

Poorly permeable compounds (permeability coefficients $<0.3\times10^{-6}$ cm/s). This group contains the amidine **35**, tetrazoles **36**–**39** and sulfonic acid **14**. Permeability coefficients are in the range of the low permeable marker mannitol (0.17×10^{-6} cm/s). Since the amidine **35** and the sulfonic acid **14** are completely ionized at pH 7.4, low permeability was expected for these two compounds. We were surprised, however, by the low permeability of the tetrazole derivatives **36**–**39** when compared with the corresponding carboxylic acids.

The tetrazole group is frequently used as a carboxylic acid replacement in medicinal chemistry,[17] and we had expected similar permeability properties for the tetrazoles and their carboxylic acid analogues. Since there are no reports describing such a significant detrimental impact of the tetrazole group on cell permeability, we thought it prudent to determine whether the low A to B permeability for these compounds could be due to interactions with cellular efflux pumps. The PDR values in Table 1 (20 for tetrazole **39** and even higher values of 129, 145 and 180 for tetrazoles **38**, **37** and **36**) indicate that these compounds are substrates for efflux pumps. The substrate specificity of efflux pumps is relatively unexplored[18] but these particular tetrazoles are apparently recognized and actively transported. The amidine derivative **35** and the least permeable compound in this study, the sulfonic acid compound **14**, are also moderate substrates for efflux pumps as indicated by PDR values of 3 and 7, respectively. The P_t values for tetrazoles **36** and **37** indicate that these compounds have high intrinsic cell permeability while tetrazoles **38** and **39** are moderately permeable. Even the amidine **35** and sulfonic acid **14** fall in the category of moderately permeable compounds when evaluated in the context of P_t. An alternative plot and reordering of the data in Figure 2 making use of the P_t data gives perhaps a better picture

of the relative effects of the various groups on permeability. It is remarkable that even the most polar compounds synthesized still retain moderate cell permeability.

Table 1. Permeability[a] and log D[b] data

Compd	$P_{Caco\text{-}2} \times 10^6$ (cm/s)	PDR	$P_t \times 10^6$ (cm/s)	Log $D_{oct/PBS}$
	A to B (B to A)			
1	68.1±1.2			1.78±0.25
2	67.8±2.2			1.01±0.04
4	0.67±0.02 (0.82±0.02)	1	0.75	−2.10±0.08
5	0.71±0.05			−2.37±0.11
6	71.1±1.9			2.91±0.05
8	65.8±1.5			2.47±0.01
11	3.19±0.15			−0.61±0.01
12	2.63±0.19			−1.13±0.11
14	0.08±0.00 (0.58±0.02)	7	0.33	−1.78±0.02
15	23.4±0.9			1.04±0.09
16	71.9±4.7			1.74±0.02
18	3.66±0.13			−1.65±0.02
19	2.89±0.17			−1.80±0.08
20	66.7±2.60			1.46±0.03
23	0.65±.05 (1.18±.03)	2	0.92	−2.07±0.10
24	1.56±0.16 (5.21±.012)	3	3.4	−0.72±0.11
25	2.93±0.21			−1.62±0.13
26	6.10±0.47			−1.98±0.12
27	5.39±0.12			−2.20±0.14
28	6.85±0.43			−0.01±0.10
29	25.1±1.2			1.70±0.02
30	44.4±1.3			0.71±0.05
31	34.6±1.0			0.28±0.01
32	27.6±1.1			0.21±0.03
33	28.4±2.1			−0.77±0.00
34	25.3±0.2			−1.11±0.03
35	0.22±0.03 (0.60±0.03)	3	0.41	−1.03±0.03
36	0.13±0.02 (23.4±2.3)	180	12	Not determined[c]
37	0.14±0.01 (20.3±0.5)	145	10	0.27±0.06
38	0.11±0.01 (14.2±0.8)	129	7.2	Not determined[c]
39	0.13±0.00 (2.63±0.11)	20	1.4	−1.28±0.01
40	57.4±3.6			1.92±0.06
41	23.4±0.9			2.08±0.04

[a]Values are means of three experiments±standard deviation.
[b]Log D values were obtained using octanol and phosphate buffered saline at pH 7.4. Values are means of six experiments ± standard deviation.
[c]Compound was not detected in the octanol phase.

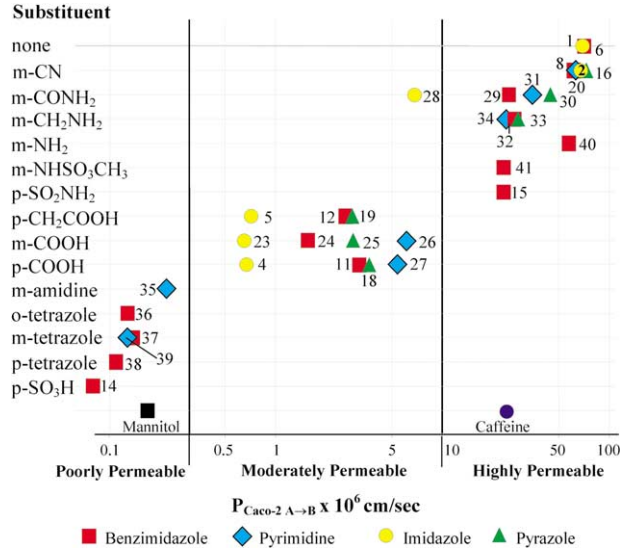


Figure 1.

Figure 3 shows the relationship between cell permeability and experimental distribution coefficients (log D). Because of the nature of the scaffolds and functionality studied here, the log D ranges from −2.4 to 3. P_t is plotted for compounds **14**, **35**, **37** and **39**. Compounds with log D values higher than 0 and less than 3 fall in the highly permeable class. Most of the compounds with log D values below 0 are moderately permeable, with the exception of the amines **33** and **34** which display high permeability. This classification is similar to one previously published for a series of a structurally diverse drugs.[2a]

Using the Caco-2 model we have evaluated the effect on permeability of various functional groups commonly seen in lead optimization efforts. For this set of compounds we find a classification indicating high permeability for compounds having log D values greater than 0 (and less then 3) is seen, while compounds with log D values lower than 0 display variable permeability. Surprisingly, compounds containing the tetrazole functionality were found to be recognized by efflux pumps.

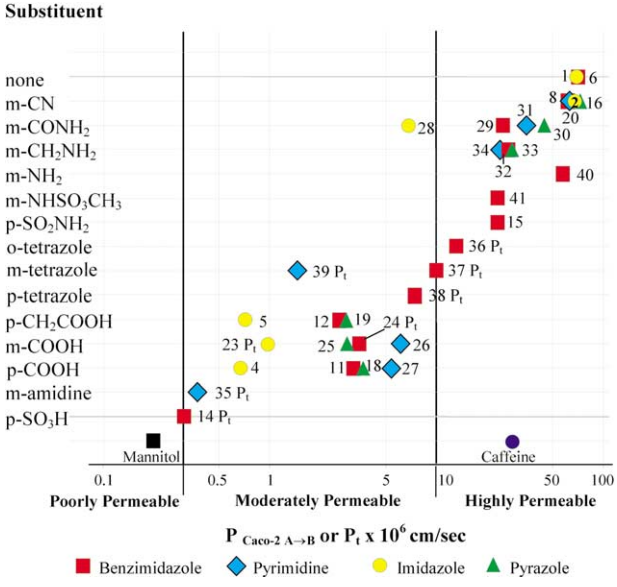


Figure 2.

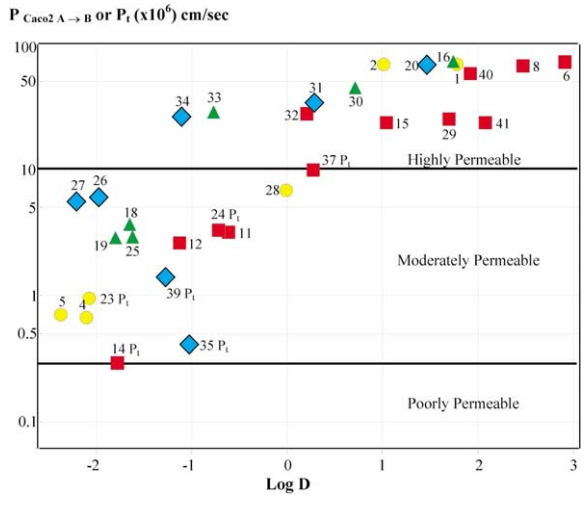


Figure 3.

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