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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3658–3664
Bioorganic & Medicinal Chemistry Letters

Oral delivery of G protein-coupled receptor modulators: An explanation for the observed class difference

Kevin Beaumont,* Esther Schmid and Dennis A. Smith

Department of Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK

Received 23 March 2005; revised 3 May 2005; accepted 11 May 2005
Available online 27 June 2005

Abstract—G protein-coupled receptors (GPCRs) represent important targets for drug intervention. However, analysis of GPCR modulator drugs exhibits an important class difference, with many drugs available against aminergic GPCR targets, but relatively few against non-aminergic targets. The reason for this is that commonly drugs mimic the physicochemistry of the receptor ligand. Aminergic ligands generally exhibit physicochemical properties (molecular weight, lipophilicity and hydrogen bonding potential) that are consistent with extensive oral absorption. In contrast, non-aminergic ligands generally exhibit physicochemical properties that are at odds with oral delivery. Thus, combining required potency versus the receptor, with oral delivery potential is a significant challenge, and drug discovery becomes a question of finding the exceptional compound that lies at the edge of ADME space.

1. Introduction

The total human genome consists of approximately 30,000 genes. Further analysis[1] suggests that approximately 10% of these genes could be targets for drug intervention in the treatment of disease. These approximately 3000 genes include those encoding for nuclear receptors, ion channels, enzyme inhibitors and G protein-coupled receptors (GPCRs).

Thus, the GPCRs represent an important group of targets for pharmacological intervention (Fig. 1). Indeed, there are many drugs on the market that are modulators of GPCRs. However, an in-depth analysis of marketed GPCR modulating drugs (Table 1) indicates an important class distinction within the GPCRs between aminergic and non-aminergic liganded receptors. Thus, there are a large number of drugs on the market that modulate the aminergic GPCRs. The same cannot be said of the non-aminergic GPCRs (especially the peptidic class) with a relative paucity of drugs modulating these receptors (Table 1). Since non-aminergic GPCRs represent one of the most important drug intervention targets for the pharmaceutical industry, it is important to understand the reasons for this apparent class distinction. This paper puts forward a potential explanation for this question and suggests a method whereby drugs for non-aminergic GPCRs can be discovered.

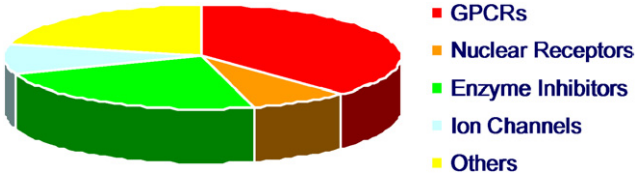

Figure 1. GPCRs represent a significant portion of the known druggable targets.

2. Properties that determine drug potential

Most drugs need to be delivered by the oral route. In order to be orally delivered a drug must be amenable to absorption from the GI tract and avoid extensive hepatic first-pass extraction. The physicochemical requirements required for oral absorption have been established and are best described by the 'Rule of 5' mnemonic established by Lipinski et al.[2] Effectively, a compound is most likely to exhibit significant oral absorption if it possesses a log P of less than 5, a molecular weight of less than 500, less than 5 hydrogen bond donors and less than 10 hydrogen bond acceptors. These parameters are important as they determine the ability of the compound

* Corresponding author. E-mail: kevin.beaumont@pfizer.com

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doi:10.1016/j.bmcl.2005.05.042

Table 1. Known drugs against G protein-coupled receptors

GPCR class		Receptor types	Lowest MW	PK properties in human	Example Drugs (incl. non-selective)	Comments
Aminergic	Muscarinic	Receptors 1–5	199	Mainly oral	Atropine, Scopolamine, Tolterodine, Darifenacin, Tiotropium, Pirenzepine, Cevimeline	No M3 selective agonist, no M5 selective agonists or antagonists
	Histamine	Receptors 1–4	125	Mainly oral	Cimetidine, Ranitidine, Diphenhydramine, Loratadine, Cipralisant	Many drugs
	Dopamine	Receptors 1–5	285	Mainly oral	Clozapine, Sumanirole, Pimozide, Apomorphine, Dopexamine, Methysergide, Olanzapine, Sulpiride	Many drugs
	Serotonin	Receptors 1A–F; 2A–C; 4–7	230	Mostly tools, few selective, oral drugs	Sumatriptan, Eletriptan, Ondansetron, Tropisetron, Nefazodone, Buspirone, Flibanserin, Tegaserod, Cisapride, Alosetron	Many drugs, but many subtypes without selective drugs or tools
	α,β-Adrenergic	α1A–D; α2A–C; β1–3	252	Oral, inhaled	Clonidine, Salbutamol, Propranolol, Prazosin, Atenolol, Ephedrine, Salmeterol	Many drugs, but several subtypes without selective drugs
Non-aminergic	Cannabinoid	Cannabinoid R1–2	386	Oral	Rimonabant (Ph3)	Drugs close to market
	Eicosanoid	Leukotriene R1–2 (Cysteinyl); LTB4 R1–2	428	Oral	Montelukast, Zafirlukast	Many subtypes without selective drugs or tools
	Eicosanoid	Prostanoid R. DP, FP, EP1–4, IP, TP)	358	Most agents in preclinical stage	Carboprost, Ramatroban, Treprostinil	Many subtypes without selective drugs or tools
	Glycoprotein hormone	FSH, LSH, TSH, MCH Receptors	644	Only 1 small molecule drug	Chlordiazepoxide	Many subtypes without selective drugs or tools
	Lipid	Lysosphingolipid R. (EDG1–8)	—	No drugs	—	Few tools
	Melatonin	Melatonin R1–3	259	Few drugs	Melatonin, Ramelteon	Subtypes without selective drugs or tools
	Nucleotide	Adenosine R1, 2A, 2B, 3	243	Mainly iv	Caffeine, Theophylline, Binodenoson (Ph3)	Several drugs and many tools
	Nucleotide	Purinoceptors P2Y1,5,6,9,10,11,12	321	Few oral drugs	Ticlopidine, Clopidogrel	Most subtypes without tools or drugs
	Peptide	Angiotensin R.1–2	440	oral	Candesartan, Losartan, Irbesartan, Valsartan	Several drugs only for one receptor subtype
	Peptide	Bombesin R. BRS-3, GRP-Bombesin R, NMB-R	736	Mainly tools	—	Few tools, no drugs
	Peptide	Bradykinin R.1–2	757	Mainly tools	Labradamil, Icadibant	Only iv drugs
	Peptide	Chemokine R. C3a, C5a, CCR1–11, CXCR1–6, CX3CR1, fmlp	352	Mainly preclinical agents	UK-427857 (Phase 3)	Few oral agents
	Peptide	Cholecystokinin RA–B	398	Oral	Loxiglumide	Some oral drugs (marketed and in development),

(*continued on next page*)

Table 1 (*continued*)

GPCR class	Receptor types	Lowest MW	PK properties in human	Example Drugs (incl. non-selective)	Comments
Peptide	Endothelin RA–B	510	Mainly peptidic	Bosentan, Atrasentan	Few oral drugs
Peptide	Galanin R1–3	—	Mainly tools	—	No drugs, only peptide tools
Peptide	Melanocortin R1–5	1024	Mainly tools	Melanotan, SEMAX	Peptide drugs and tools
Peptide	Motilin R.	2681	Mainly tools	—	Peptide tools
Peptide	Neuropeptide R1–6	512	Mainly tools	—	Preclinical agents
Peptide	Opioid R. delta, kappa, mu, ORL-1	351	Oral	Oxycodone, Roxanol, Morphine, Naloxone,	Several oral drugs, but few selective drugs
Peptide	Orexin R1–2	—		—	No drugs
Peptide	Proteinase activated R1–4	—		—	No drugs
Peptide	Somatostatin R1–5	556	Mainly peptidic	—	No drugs
Peptide	Tachykinin R1–3	382	Oral	Aprepitant	Few drugs
Peptide	Urotensin R.	516	Oral	—	No drugs
Peptide	Vasopressin R1A, 1B, 2	621	Oral	Vasopressin, Desmopressin, Conivaptan	Some drugs
Metabotropic	mGlu R1–8	201	Oral	—	Many tools and preclinical/ clinical agents

This table summarises major GPCRs and their ligands. The GPCRs are categorised into aminergic and non-aminergics. The lowest molecular weight of randomly selected, subtype-selective ligands (agonists and antagonists) are listed against each receptor type. PK properties are based on whether the agents are orally available. The 'Example drugs' column lists a non-comprehensive list of drugs that are currently on the market. Red, amber, and green assignation was performed based on the number of marketed drugs, and the availability of subtype-selective agonist and antagonist agents.

to dissolve in the aqueous environment of the GI tract lumen ($\log P$) and to cross the lipophilic environment of the gut wall cell membrane (molecular weight and hydrogen bonding potential). Hepatic extraction of compounds is often driven by a metabolic component, with the majority of metabolism being due to cytochrome P450s (CYPs). The major human drug metabolising CYP is CYP3A4, which has a preference for metabolism at electron-rich sites in lipophilic molecules.[3,4] Thus, one strategy for avoiding hepatic first-pass extraction of drugs is to modulate the lipophilicity of these compounds.

Overall, the strategy to optimise the oral delivery of candidate drugs is to modulate the physicochemistry. This includes balancing lipophilicity within a range that allows the compound to dissolve in an aqueous environment but still cross the gut wall membrane and avoid extensive hepatic metabolism. In addition, molecular weight and hydrogen bonding potential need to be limited. Thus, the combination of three physicochemical properties (lipophilicity, molecular weight and hydrogen bonding potential) determines the potential of a drug to be delivered by the oral route, and define what can be called 'ADME space' (see later). More importantly for approaches towards non-aminergic GPCR modulator drugs, there are exceptions to the 'rules' governing oral delivery potential.

3. Properties of GPCRs and their ligands

The GPCRs share seven hydrophobic transmembrane segments of 20–25 amino acid residues. The GPCR super family has been classically divided into subfamilies A, B and C based on amino acid sequence. Subfamily A are activated by ligands that bind to a crevice formed by transmembrane regions 3, 5, 6 and 7 as characterised by the aminergic receptors[5] in Table 1 (e.g., muscarinic, adrenergic, etc.). Subfamily A also includes non-aminergic receptors (e.g., glycoprotein receptors) characterised by the ligand often binding to the extracellular region of the receptor. Family B receptors are activated by peptides (also classified as non-aminergic in Table 1) and, like family C (metabotropic-like) receptors, the activating ligand binds to the extracellular domain. Allosteric modulation of GPCRs occurs and in these cases binding may represent a combination of transmembrane and extracellular binding. Binding into a transmembrane crevice gives a maximal accessible hydrophobic surface area and when combined with polar interactions allows small ligands to bind with high affinity. Extracellular binding sites require larger molecular weight ligands due to the much lower relative accessible surface area. It can be expected, therefore, that the ligands for the GPCRs will show an increase in molecular size in moving from aminergic to non-aminergic receptors (Fig. 2).

The structures of the aminergic and non-aminergic (peptidic) GPCR ligands are exemplified in Figure 3. The aminergic GPCR ligands (adrenaline, histamine and dopamine) are small (low molecular weight), moderately lipophilic molecules that tend to exhibit minimal

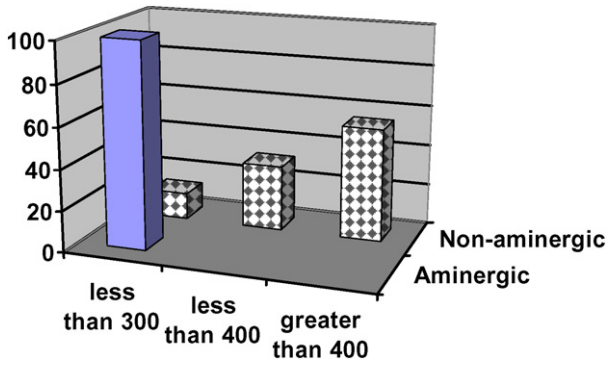

Figure 2. Molecular weights of aminergic and non-aminergic GPCR ligands.

hydrogen bonding potential. Indeed, these compounds show all the characteristics required for oral delivery. In contrast, the peptidic GPCR ligands display properties that are distinctly at odds with oral delivery. These are exemplified in Figure 3 by neurokinin A. Neurokinin A is a 14 amino acid peptide, which exhibits a very high molecular weight, significant lipophilicity and hydrogen bonding potential. Other examples are the endothelin ligands, which are both 21 amino acid peptides and clearly show similar physicochemical properties to neurokinin A.

4. How do drugs emerge from ligands?

One medicinal chemistry strategy to generate potential drug-like molecules is to use the receptor ligand as the molecular lead. This strategy has a significant likelihood of generating compounds that are potent modulators of the target receptor. This is because molecules generated in this manner will share characteristics of the ligand and will have the potential to displace the ligand competitively from that receptor. This is exemplified for the adrenergic β-receptors in Figure 4. Both the β-agonists (isoprenaline and salbutamol) and the β-blockers (propanolol and atenolol) share significant chemical features with the β-ligand, adrenaline. As discussed above, the aminergic GPCR ligands display physicochemical properties that support oral delivery. Therefore, it is not surprising that drugs arising from these ligands also share the physicochemical properties that promote oral delivery. Thus, aminergic GPCR modulator drugs are well represented on the pharmaceutical market (Table 1).

In contrast, a medicinal chemistry strategy to modify non-aminergic GPCR ligands to produce oral drugs is fraught with difficulty. For example, any modification of neurokinin A to produce a potent antagonist of the neurokinin receptors is most unlikely to lead to a compound that exhibits the physicochemical characteristics in line with oral delivery. Faced with these constraints (i.e., no small molecular weight ligand/lead) many industrial medicinal chemistry strategies turn to high throughput screening of large compound files against a particular non-aminergic GPCR. Not surprisingly, the hits that emerge often maintain many of the characteristics of the ligand. Whilst they tend not to be peptidic in nature, they do exhibit significant molecular weight, hydrogen bonding potential and lipophilicity. Often the medicinal chemistry strategy to exploit these hits is to improve binding energy to the receptor by appropriately positioned lipophilic interactions. The net outcome of this strategy is highly potent receptor modulators, exhibiting high molecular weight and significant lipophilicity in addition to the hydrogen bonding capacity of the original hit. These are the properties we have described as being at odds with oral delivery potential. Thus, given the properties of the non-aminergic GPCR ligands, it is perhaps not surprising that there are only very few examples of marketed drugs from this important receptor class.

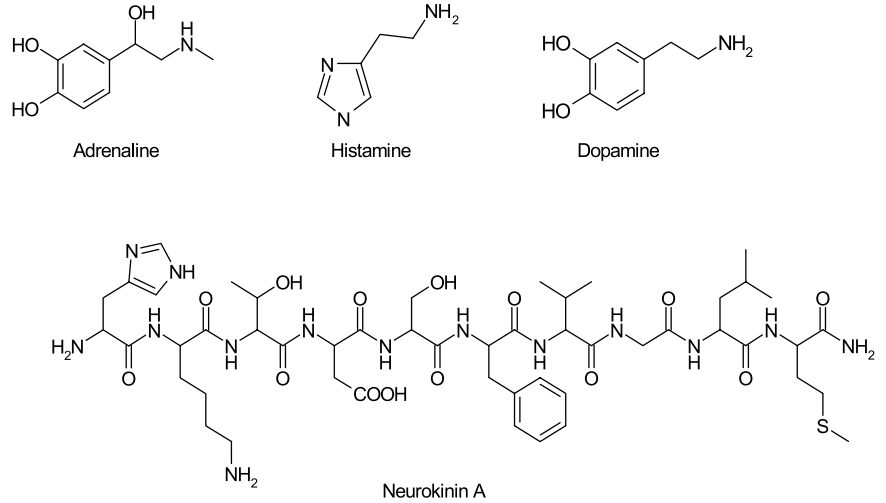

Figure 3. Structural examples of aminergic and non-aminergic GPCR ligands.

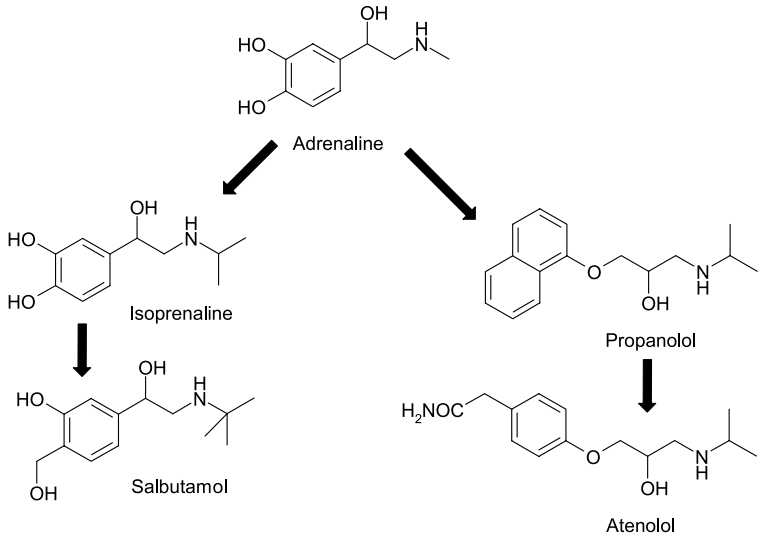

Figure 4. Evolution of β-adrenergic receptor modulator drugs from the GPCR ligand.

5. The concept of ADME (absorption, distribution, metabolism and excretion) and target physicochemical space

The difference between the aminergic and non-aminergic GPCRs as potential targets to yield drugs against disease is explained in Figure 5. As stated previously, oral delivery potential can effectively be described by three physicochemical parameters (molecular weight, hydrogen bonding capacity and lipophilicity). If we consider these as three separate axes in three dimensions, these properties describe a cube, into which drug-like molecules can be mapped. When the physicochemical properties required to modulate the receptor (i.e., target physicochemical space) are encompassed within ADME space, then oral delivery of potent agents is readily achievable. The aminergic GPCR modulators exemplify this situation. However, when the physicochemical requirements for potency versus the target are at odds with those required for oral delivery, target space may only overlap with ADME space. Non-aminergic GPCR modulators exemplify this situation. Unfortunately, ADME and target space often do not overlap with disease space (targets clearly linked to a human disease phenotype, whilst providing sufficient safety index), further limiting small molecule options. This makes it even more paramount to maximise ADME–target space exploitation. An example of this would be the nucleotide GPCRs (Table 1), which are eminently druggable, but where either safety index limits disease space or the link to human disease has only recently emerged. Therefore, when faced with this problem, industrial discovery scientists are charged with finding the exception to the ADME physicochemical space rules, in order to deliver a potent compound.

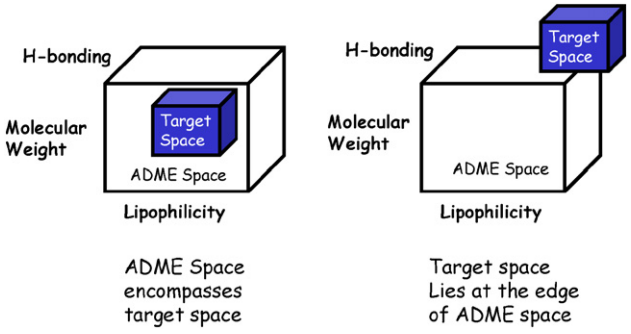

Figure 5. The concept of ADME and target physicochemical space for both aminergic and non-aminergic GPCR modulators.

6. Reconciling the dilemma

Clearly, non-aminergic GPCRs are too important targets for the pharmaceutical industry to ignore. This is especially the case in disease areas where many of the aminergic GPCR targets already have effective drugs against them or where target disease space points to non-aminergic targets as a means to modulate disease (e.g., nucleotide, metabotropic or cannabinoid GPCRs). Therefore, pharmaceutical companies need to find a medicinal chemistry strategy to enable the oral delivery of such agents. Several options exist to address this problem, such as alternative delivery options. However, this letter focuses on the use of high throughput ADME assays. This is exemplified in Figure 6 by the approach taken to discover a potent and orally active antagonist of a peptidic GPCR.

This Discovery programme was initiated by completing an HTS of an entire compound file against the peptidic GPCR in question. This yielded a number of promising 'hit' antagonist series, which did not express acceptable physicochemical properties for oral delivery. Thus, the project mounted an effort to generate more than a thousand analogues, which balanced the required potency against the receptor together with the potential to be delivered orally.

In order to assess potential for oral delivery, each compound was assessed in moderately high throughput in vitro absorption (Caco-2 cell monolayers) and

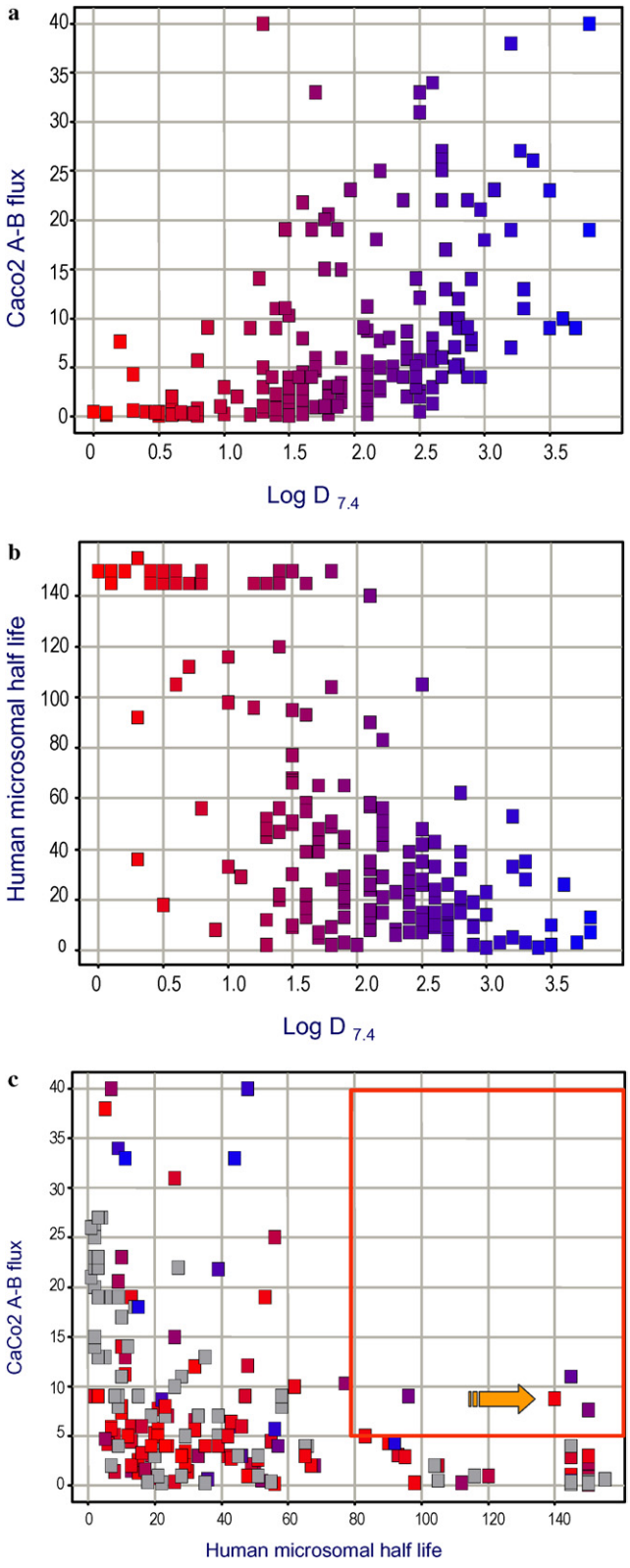

Figure 6. (a) Correlation of Caco-2 cell flux with $\log D_{(7.4)}$ for a series of peptidic GPCR antagonists; (b) correlation of human liver microsomal half-life with $\log D_{(7.4)}$ for the same series of peptidic GPCR antagonists; (c) integration of potency (colour-coded—red most potent) with Caco-2 cell flux and human liver microsomal half-life to define ADME space and identify the candidate.

metabolism (human liver microsomal half-life) assays. Figure 6a shows the relationship between the Caco-2 cell monolayer flux and the $\log D_{(7.4)}$ for this series of receptor antagonists. Not surprisingly, there was a positive relationship between absorptive flux and $\log D_{(7.4)}$, with compounds expressing higher $\log D$ values tending to cross the cells at higher rates. Conversely, there was a strong inverse relationship between human liver microsomal half-life value and $\log D_{(7.4)}$ (Fig. 6b).

These relationships are consistent with what is known about the physicochemical properties that govern oral delivery potential. In addition, such data do allow us to further define ADME space (as shown in Fig. 6c). Significant oral absorption is more likely when the Caco-2 cell flux is greater than 5×10^{-6} cm/s and first-pass metabolism is more likely to be limited when the human liver microsomal half-life is above 80 min. Thus, when Caco-2 cell flux and human liver microsomal half-life are plotted against each other, these values describe a box grid that is in effect ADME space. Not surprisingly for a peptidic GPCR antagonist series, very few compounds populate this defined area. When the compounds are colour-coded for potency, the most potent compound within the ADME space box can be selected as a compound for further evaluation.

Given the increase in compound throughput through tactics such as the 'Closed Loop,'[6] which aims to accelerate and increase compound throughput during lead optimisation, ADME strategies will have to be selectively deployed to achieve the greatest leverage via triage (or efficient sampling of ADME space). The ADME space paradigm could be a critical tool in this endeavour.

7. Summary and conclusions

The pharmaceutical industry has traditionally had a great deal of success against aminergic GPCRs, with a significant number of successful marketed drugs against this class of receptor. However, this success has not been repeated against the non-aminergic GPCRs, which remain important targets for drug intervention. We believe that the reason for this difference in success relates back to the physicochemical properties of the natural ligand for each class of GPCR, which drive the physicochemical properties of potential candidate drugs towards higher molecular weight, hydrogen bonding potential and lipophilicity. This problem may be a partial explanation for the observations of Proudfoot,[7] who reported an increase in mean and median molecular of registered oral drugs through the period of 1937–1997. Thus, more difficult druggable targets pose greater physicochemical constraints for potent molecules, leading to a trend to increased molecular weight of drug-like molecules.

Overall, we can conclude that when ligand properties correspond to those required for drug delivery, traditional medicinal chemistry strategies to modulate the ligand towards a drug are most likely to succeed. When ligands exhibit properties that are at odds with drug delivery, alternative strategies are required to select compounds

with properties that balance physicochemical constraints. These strategies can be successful and delivery of drugs against these important drug targets is possible.

Acknowledgments

We gratefully acknowledge the work of Christopher Barber (Discovery Chemistry) for his calculations of the molecular weight of ligand structures. We are also indebted to Bryn Williams-Jones (Discovery Medicinal Technologies) for providing a categorised list of GPCR and non-GPCR targets. In addition, we acknowledge Zoe Smith for help in preparation of the manuscript.

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