Comparison of binding and functional data on a set of safety-related GPCRs

Jolas, T[1], Migeon, J[2], Fouchaq, B[1]
[1]Eurofins Cerep SA, Le bois l'Evêque, 86600 Celle l'Evescault, France
[2]Eurofins Panlabs Inc, 15318 NE 95th Street, Redmond WA 98052, USA

Eurofins Pharma Discovery Services

Introduction

Getting a better idea of the safety fingerprint of lead compounds early in the discovery process may reduce the risk of off-target interactions leading to adverse drug reactions (ADRs) (Bowes *et al.*, 2012*). In vitro* pharmacology profiling is a classical approach that has been used for this purpose.

For a set of 282 to 616 pharmacologically relevant compounds, we compared binding and functional data obtained on several GPCRs selected for their cardiovascular ADRs. Those GPCRs were part of the targets listed by Bowes *et al*. The objective of this study was to compare the sensitivity of the two different methods using dose-response determinations and assess how the binding and functional assays were related to one another.

Methods

Target	# cpds	Function measure	G protein	Agonist	Radioligand
5-HT$_{1A}$	465	cAMP	Gi	8-OH-DPAT	8-OH-DPAT (agonist)
5-HT$_{2B}$	616	Ca^{2+} flux	Gq	serotonin	DOI (agonist)
α_{1A} Adrenoceptor	494	Ca^{2+} flux	Gq	epinephrine	prazosin
α_{2A} Adrenoceptor	395	Impedance	Gi	epinephrine	RX821002
α_{2B} Adrenoceptor	490	cAMP	Gi	dexmedetomidine	RX821002
A_{2A} Adenosine	371	cAMP	Gs	NECA	CGS21680 (agonist)
β_1 Adrenoceptor	282	cAMP	Gs	isoproterenol	CGP12177 (agonist)
β_2 Adrenoceptor	424	cAMP	Gs	isoproterenol	CGP12177 (agonist)
D_1 Dopamine	540	cAMP	Gs	dopamine	SCH 23390
H_1 Histamine	460	Ca^{2+} flux	Gq	Histamine	pyrilamine
H_2 Histamine	491	cAMP	Gs	Histamine	APT
M_2 Muscarinic	517	cAMP	Gi	Acetylcholine	AF-DX384

Radioligand binding was assessed at equilibrium, using the ligands indicated in the table, at recombinantly expressed human receptors.

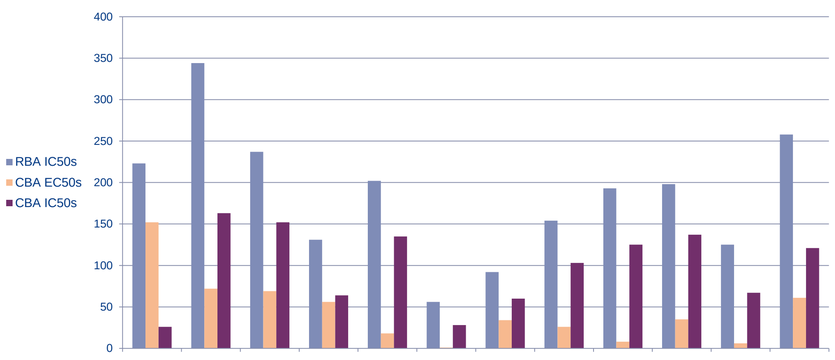
Functional assessment was by:
•HTRF - *homogeneous time resolved fluorescence*
• Ca^{2+} flux – *fluorescent calcium sensitive dye*
• CDS – *cellular dielectric spectroscopy, impedance*

In functional cell-based assays, compounds were assessed for effect on their own (compound agonism) and for ability to inhibit the response to the reference agonists shown in the table (compound antagonism). Compounds were tested over 8 concentrations from a maximum of 100µM or 30µM.

Compounds were selected using the BioPrint database (Eurofins Cerep) as being marketed or withdrawn drugs and pharmacological reference compounds

Results

Radioligand Binding Assays are more sensitive than Cell-Based Assays
More antagonists are detected than agonists



Targets	5-HT$_{1A}$	5-HT$_{2B}$	α_{1A}	α_{2A}	α_{2B}	A_{2A}	β_1	β_2	D_1	H_1	H_2	M_2
Cpds tested	465	616	494	395	490	371	282	424	540	460	491	517
RBA IC$_{50}$s	223	344	237	131	202	56	92	154	193	198	125	258
CBA EC$_{50}$s	152	72	69	56	18	1	34	26	8	35	6	61
CBA IC$_{50}$s	26	163	152	64	135	28	60	103	125	137	67	121

Figure 1. Number of concentration-response curves generated using Radioligand Binding Assays (RBA) (IC_{50}) and the functional Cell-Based Assays (CBA) (IC_{50} for antagonists and EC_{50} for agonists) across a panel of 12 GPCR targets

Abbreviations:
5HT$_{1A}$, serotonin 5-HT$_{1A}$ receptor
5HT$_{2B}$, serotonin 5-HT$_{2A}$ receptor
α_{1A}, α_{1A} adrenoceptor
α_{2A}, α_{2A} adrenoceptor
A_{2A}, adenosine A_{2A} receptor
A_{2B}, adenosine A_{2B} receptor
β_1, β_1 adrenoceptor
β_2, β_2 adrenoceptor
D_1, dopamine D_1 receptor
H_1, histamine H_1 receptor
H_2, histamine H_2 receptor
M_2, muscarinic M_2 receptor

- For each target tested, radioligand binding assays gave the greatest number of actives (defined IC_{50} value).
- In functional assays, generally, more antagonists than agonists were detected; 5-HT$_{1A}$ was the single exception.

Agonists ➔ Binding IC50s and functional EC50s do not correlate for most targets

Figure 2. Comparison of pIC_{50} values from RBA (x-axis) and pEC_{50} from CBA (y-axis) for a subset of BioPrint compounds across a panel of 12 GPCR targets.

Compounds inactive in one or other assay are shown with an arbitrary pIC_{50} or pEC_{50} value of 1 (blue dots).

- Correlation between binding IC_{50}s and functional EC_{50}s with a R2>0.6 was obtained in 2 out of the 12 targets

Antagonists ➔ Binding IC50s and functional IC50s correlate for most targets

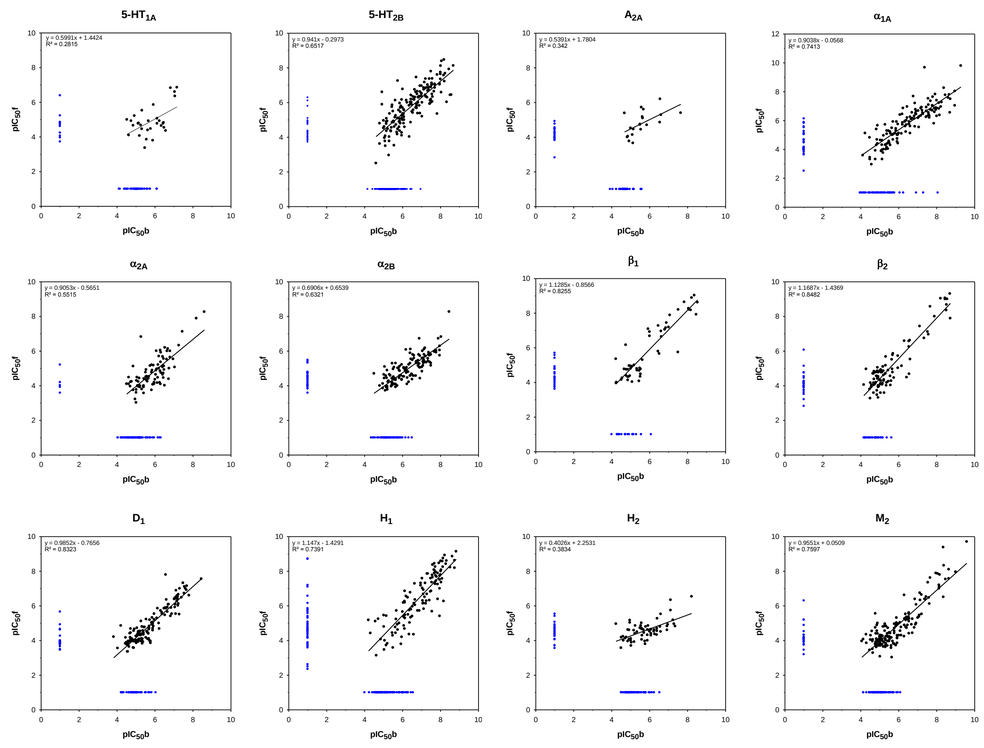


Figure 3. Comparison of pIC_{50} values from RBA (x-axis) and pIC_{50} from CBA (y-axis) for a subset of BioPrint compounds across a panel of 12 GPCR targets.

Compounds inactive in one or other assay are shown with an arbitrary pIC_{50} value of 1 (blue dots).

- Correlation between binding and functional IC_{50}s with a R2>0.6 was obtained in 8 out of the 12 targets.
- For targets with good correlation, between 88% and 100% of the test compounds with a binding IC_{50} below 1µM have a calculated IC_{50} in the functional assay.

Antagonists ➔ Screening at 10 µM does not detect as many active compounds

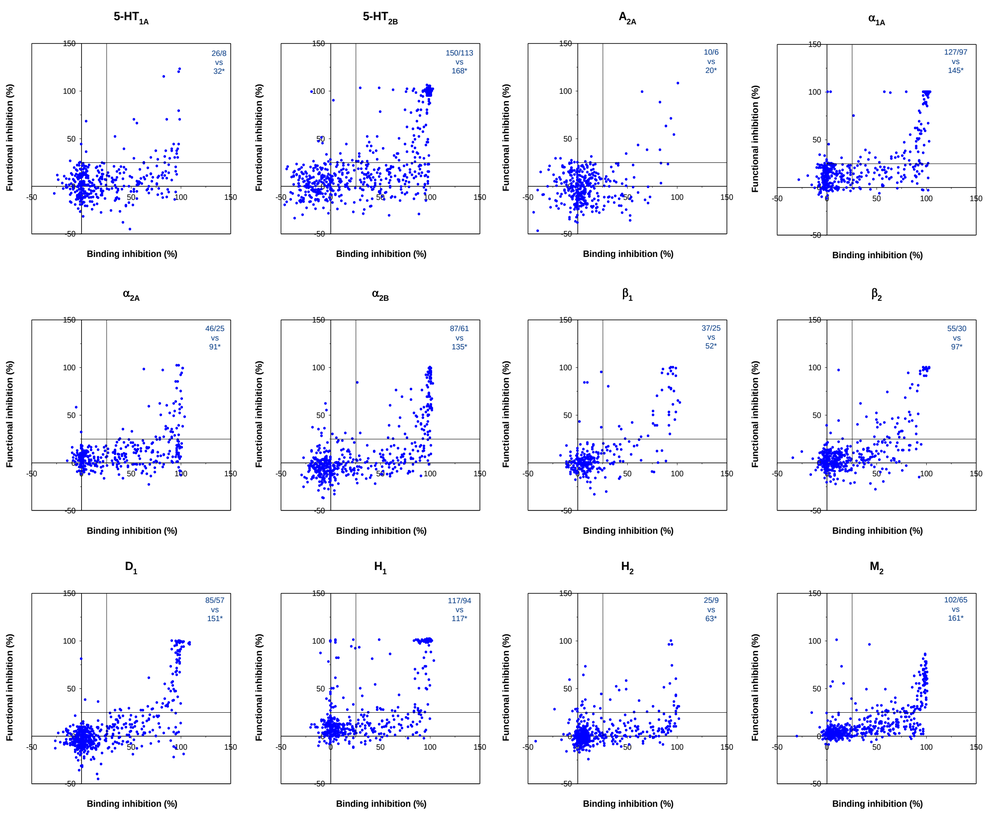


Figure 4. Comparison of binding (x-axis) and antagonist properties (y-axis) for a subset of BioPrint compounds across a panel of 12 GPCR targets. Compounds were tested at 10 µM.

Black lines indicate 25% inhibition that in average is considered to be the limit of noise level.

Numbers in the upper corner of each graph indicate number of compounds with binding and functional effects above 25% / number of compounds with binding and functional effects above 50% versus number of compounds with binding and functional IC_{50}s

- Single concentration screening does not detect as many compounds active in binding and inhibition functional assays when compared to testing at several concentrations.
- Comparison between binding and functional data is more complex when performing single concentration screening.

Conclusions

- Radioligand binding assays detect more interactions between compound and receptor than functional cell-based assays.
- For most targets, a poor correlation was found between agonist EC_{50} and binding IC_{50} whereas a good correlation was found between antagonist IC_{50} and binding IC_{50}, but correlation is a property of the individual tests and the functional mode being run.
- Most test compounds (88-100%) showing strong binding properties (IC_{50} < 1µM) have a calculable IC_{50} in the functional CBA assay. For binding IC_{50}s higher than 1 µM, a population of compounds had binding interactions without detectable cellular effects. Similarly, a population of compounds had agonist or antagonist effects without binding interactions. Those populations need to be further assessed for selectivity of the response, sensitivity of the assay and allosteric modulation.

In conclusion,
- affinity (binding) and functional (agonist/antagonist) data,
- multi-concentration testing,

are required to provide an integrated assessment of compound interaction with secondary targets.

References
Bowes et al (2012) Reducing safety-related drug attrition: the use of in vitro pharmacological profiling. Nat Rev Drug Discov. 2012 Dec;11(12):909-22

Acknowledgments
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