SPECIES DIFFERENCES IN PREGNANE X RECEPTOR (PXR) ACTIVATION: EXAMINATION OF COMMON LABORATORY ANIMAL SPECIES

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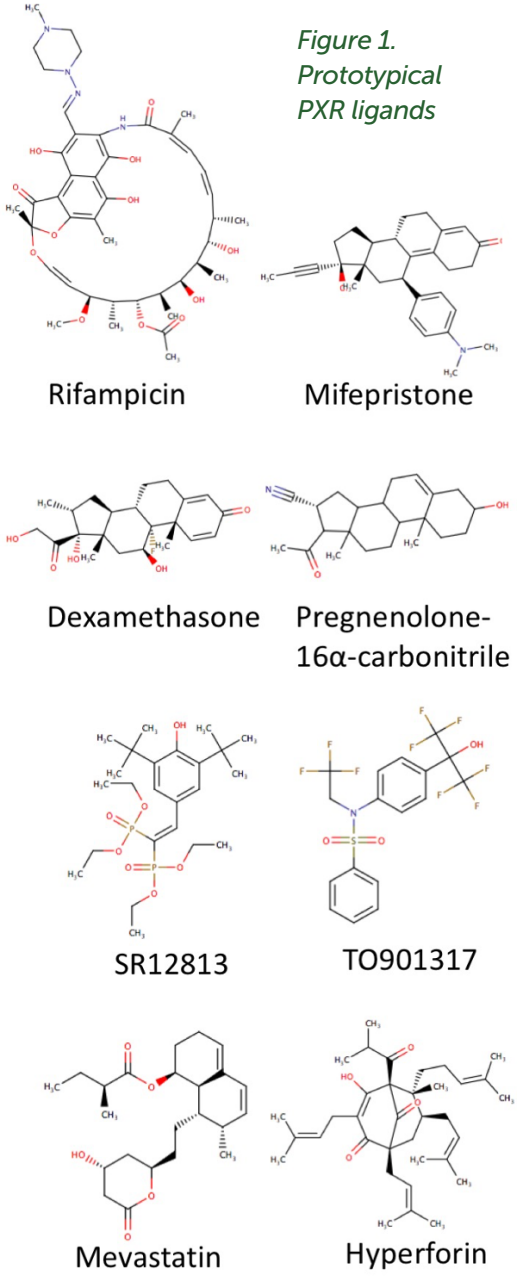
1. Introduction

Nuclear receptors (NRs) are ligand-dependent transcription factors found in many species that regulate the expression of important target genes involved in a spectrum of developmental and physiological processes. In addition to ligand binding, the transcriptional activities of NRs are also modulated through a range of protein-protein interactions with coregulatory proteins, either with coactivator or corepressor functions (1-4). The ligand binding domain (LBD) of NRs is responsible for both ligand recognition and regulation of protein-protein interactions, notably with coregulatory factors. Upon agonist binding, conformational changes are induced within the LBD, particularly the activation function-2 (AF-2) region, which leads to the dissociation of corepressors and recruitment of the coactivator complex, ultimately leading to transcriptional activation from specific DNA response elements.

NRs represent important targets for therapeutic interventions for diseases including cancer, inflammation, and metabolic diseases. Understanding xenobiotic interactions with NRs is also important in the context of endocrine disruptors and environmental toxicity assessment. One NR with the ability to associate with a wide range of xenobiotics, including pharmaceutical agents, natural products, and environmental chemicals is pregnane X receptor (PXR). Activation of PXR regulates the expression of xenobiotic metabolizing enzymes such as cytochrome P450 enzymes (CYP3A4, CYP2B6, and CYP2C8/9) and glutathione-S-transferases, as well as important drug transporters (P-glycoprotein, multidrug resistance protein, as well as others). Since the CYP enzymes metabolize the majority of clinically important drugs, inadvertent up-regulation by PXR agonists may increase metabolism and excretion of other co-administered therapeutic agents and cause undesirable drug-drug interactions or the generation of toxic drug metabolites. Thus, it is important to identify molecules that interact with PXR early in the drug development process (1). The PXR LBD is unusually divergent across species, compared to other NRs, with only 50% sequence identity between mammalian and non-mammalian PXR sequences; other NRs tend to have corresponding sequence identities at least 10-20% higher (5). The ability to extrapolate PXR activity across laboratory animal species to humans is an important aspect of addressing the potential of drug-drug interactions with a new drug lead; this is made more difficult with the dramatic species differences in LBD structure noted above. In this study, we examined a small group of known PXR agonists (see Figure 1) for their ability to regulate the activity of human, monkey, dog, rat, and mouse orthologues of this receptor. We show that there is indeed a great deal of species difference in both potency and efficacy of chemicals examined, with a unique profile observed for each orthologue.

Figure 1. Prototypical PXR ligands



2. Methods

Chimeric PXR LBD-Gal4 DNA binding domain, Gal4-Luciferase reporter assays were utilized, as they are predictive of subsequent CYP3A4 induction (6) but are more specific and robust than either full length PXR constructs or primary hepatocyte systems. Human (hPXR, catalog number IB07001), Cynomolgus Monkey (cynPXR, C07001), Beagle Dog (dPXR, D07001), Rat (rPXR, R07001), and Mouse (mPXR, M07001) complete assay kits from INDIGO Biosciences, Inc. (State College, PA) were used in these studies.

3. Results and Discussion

The amino acid sequences of human, monkey, dog, rat, and mouse PXR were compared (Figure 2). As mentioned above, there is more species divergence among the PXR sequences than seen with other nuclear receptors with the majority of the differences seen in the A/B domain (hypervariable domain, residues 1-40) as well as the E/F (LBD, residues 143-427)(Figure 2, top panel). The DNA binding domain (residues 40-127) show the highest similarity. Overall, the monkey and human, as well as the mouse and rat, PXRs were more similar to each other while the dog was more divergent (bottom panel).

The ligand-dependent transactivation of PXR was examined using LBD-Gal4 chimeric receptor systems, as described in Methods. Each prototypical agonist was examined at 10 doses with dilutions prepared using a maximally tolerated dose with subsequent 1:3 dilutions, in triplicate. Following 24-hour treatment, luciferase activity was measured and expressed relative to the DMSO (vehicle control) treated wells (signal/background S/B). Following non-linear regression analysis, maximum activity for each receptor (MaxSystem) was determined and the data for each compound was transformed using the following formula:

$$((S/B_n - S/B_{DMSO})/Max_{System})*100$$

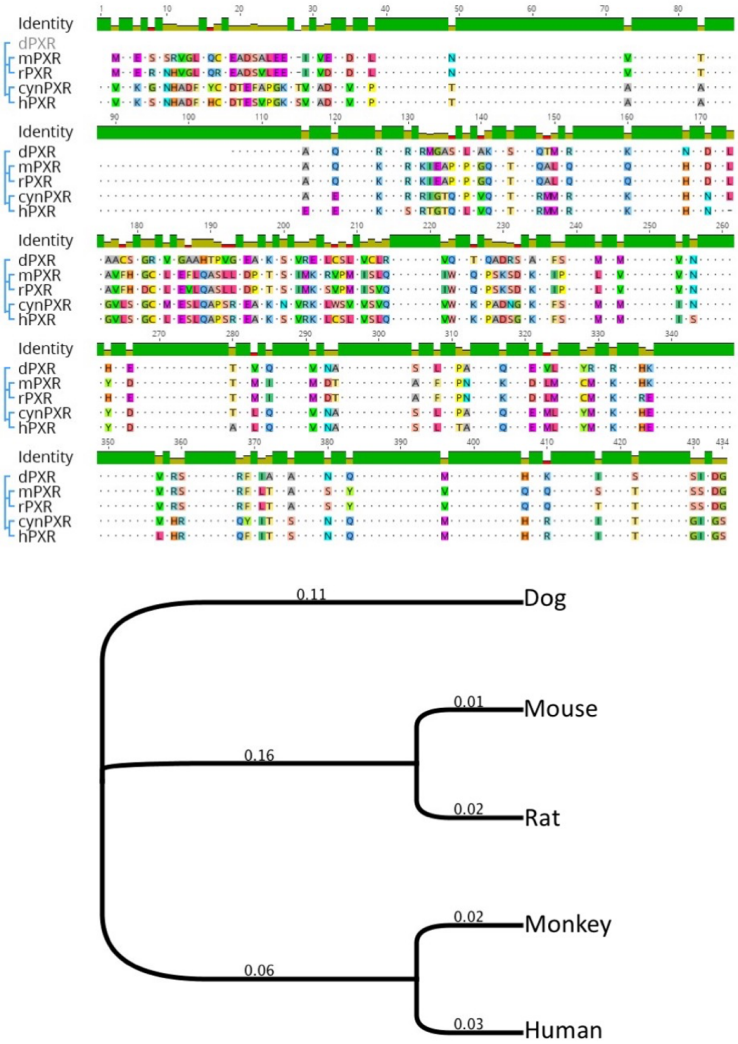


Figure 2. Alignment of PXR amino acid sequences. Full length PXR amino acid sequences were aligned using the ClustalW algorithm (Geneious 9.1.3, BioMatters, Auckland NZ). Top panel depicts discrepancies in the amino acid sequences. Bottom panel shows the overall phylogenic similarity.

Using this MaxSystem approach, we can then compare the relative potency and efficacy of each compound across species. The dose-response relationships are shown in Figure 3 and the non-linear regression analysis is presented in Table 1. Compounds were evaluated based on potency (logEC50, logKA) and efficacy (Span, logtau) following non-linear regression using the following models:

Model 1 (Log agonist vs. response)

Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)))

Model 2 (Operational Model)

operate= (((10^logKA)+(10^X))/(10^(logtau+X)))^n

<A> Y = Basal + (Effectmax-Basal)/(1+10^((LogEC50-X)*n))

<~A> Y = Basal + (Effectmax-Basal)/(1+operate)

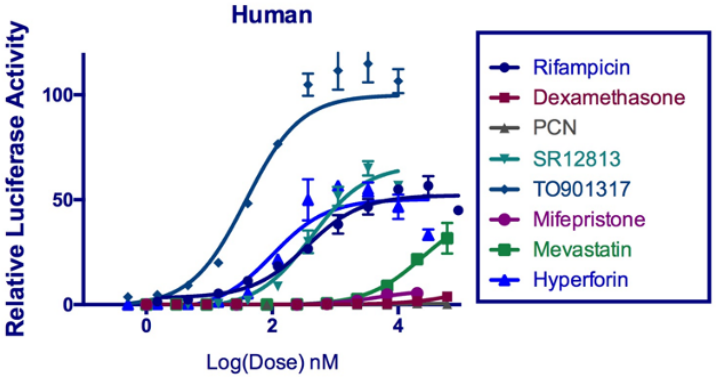
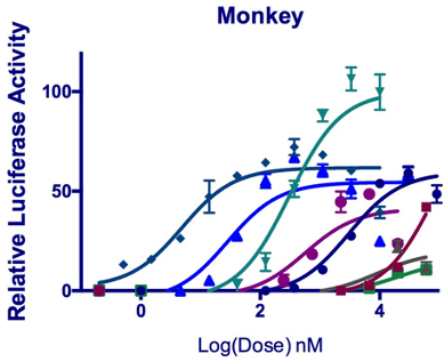
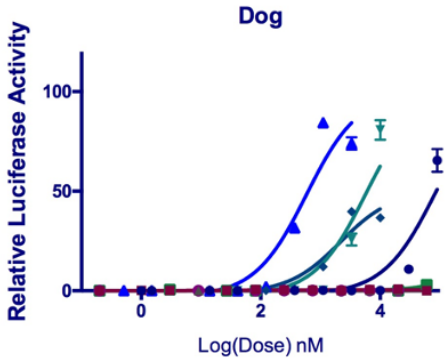
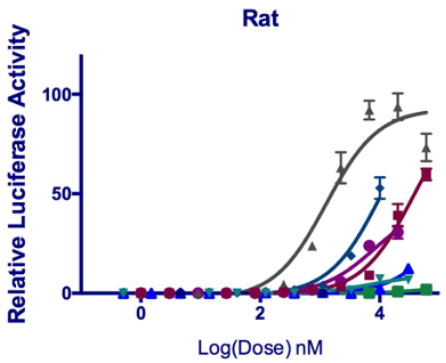


Figure 3. Dose response relationships of prototypical PXR ligands. PXR assays from INDIGO Biosciences, Inc (State College, PA) were utilized using the manufacturer's instructions. Each compound was examined in 8-10 doses (n=3) and luciferase activity expressed relative to DMSO control and normalized to the highest observed effect for each PXR following curve fitting (GraphPad Prizm).







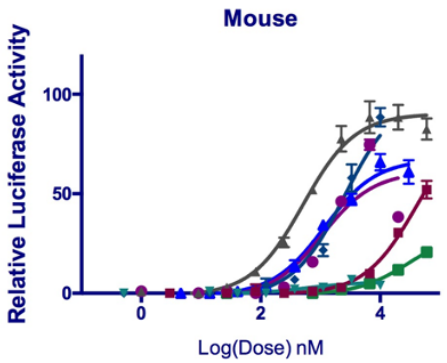


Table 1. Curve Fitting of dose-response relationships of prototypical PXR ligands

		Rifampicin	Dexameth.	PCN	SR12813	TO901317	Mifepristone	Mevastatin	Hyperfoin
Human	**LogEC50**	2.54	5.25	4.12	2.62	1.58	3.68	4.37	2.00
	Rank	3	8	6	4	1	5	7	2
	Span	48.91	14.68	0.54	68.38	100.80	7.47	44.86	53.10
	Rank	4	6	8	2	1	7	5	3
	Agonism	19.23	2.80	0.13	26.10	68.72	2.03	10.27	26.50
	Rank	4	6	8	3	1	7	5	2
	logKA	2.571	4.964	18.93	2.906	(not used)	3.671	4.425	2.207
	Rank	2	6	7	3		4	5	1
	logtau	-0.07	-0.84	-4.63	0.13	(not used)	-0.99	-0.18	-0.08
	Rank	2	5	7	1		6	4	3
Monkey	**LogEC50**	3.48	4.95	3.95	2.49	0.69	2.75	4.32	1.45
	Rank	5	8	6	3	1	4	7	2
	Span	61.79	102.80	22.09	104.50	60.12	44.44	17.20	59.86
	Rank	3	2	7	1	4	6	8	5
	Agonism	17.75	20.76	5.59	41.98	87.13	16.15	3.98	41.28
	Rank	5	4	7	2	1	6	8	3
	logKA	3.456	5.171	3.737	(not used)	0.6181	2.665	23.22	1.482
	Rank	4	6	5		1	3	7	2
	logtau	0.07	0.46	-0.34	(not used)	0.09	-0.09	4.31	0.03
	Rank	4	2	7		3	6	1	5
Dog	**LogEC50**	4.92	1.40	3.43	3.76	3.33	1.79	6.33	2.77
	Rank	7	1	5	6	4	2	8	3
	Span	101.90	0.38	0.19	102.50	51.47	0.18	100.10	102.70
	Rank	3	6	7	2	5	8	4	1
	Agonism	20.70	0.27	0.06	27.26	15.44	0.10	15.82	37.13
	Rank	3	6	8	2	5	7	4	1
	logKA	1.573	37.14	47.04	8.573	-0.3177	44.71	51.35	(not used)
	Rank	2	4	6	3	1	5	7	
	logtau	3.44E-04	-4.80	-5.93	5.05	2.79E-05	-5.89	-4.63	(not used)
	Rank	2	5	7	1	5	6	4	
Rat	**LogEC50**	0.85	4.56	3.10	3.87	4.02	3.91	4.75	5.35
	Rank	1	6	2	3	5	4	7	8
	Span	-0.12	98.87	95.83	9.51	101.20	45.59	4.15	100.20
	Rank	8	3	4	6	1	5	7	2
	Agonism	-0.13	21.67	30.88	2.46	25.16	11.67	0.87	18.72
	Rank	8	3	1	6	2	5	7	4
	logKA	13.92	4.611	11.93	43.6	(not used)	3.663	53.5	15.66
	Rank	4	2	3	6		1	7	5
	logtau	-9.51	0.42	8.83	10.19	(not used)	-0.04	2.01	10.77
	Rank	7	5	3	2		6	4	1
Mouse	**LogEC50**	4.63	4.62	2.71	2.78	3.41	3.03	4.49	3.06
	Rank	8	7	1	2	5	3	6	4
	Span	-8.67	89.55	91.54	6.08	101.80	63.53	32.29	68.45
	Rank	8	3	2	7	1	5	6	4
	Agonism	-1.87	19.40	33.80	2.19	29.89	20.97	7.20	22.34
	Rank	8	5	1	7	2	4	6	3
	logKA	-13.43	4.666	3.247	2.524	(not used)	3.197	4.282	3.208
	Rank	1	7	5	2		3	6	4
	logtau	-1.89E+11	0.27	0.59	-0.82	(not used)	0.13	-0.28	0.19
	Rank	7	2	1	6		4	5	3

***Agonism defined as Span/LogEC50**

A convenient manner to examined a compound's agonism activity is to determine Span/logEC50 with the highest value generally indicative of a full agonist (Table 2). TO901317 was the most efficacious human PXR agonist with an Span of 100 and EC50 of 40 nM. Hyperforin, rifampicin, SR12813, and mevastatin are partial human PXR agonists. Dexamethasone, mifepristone, and PCN had little effect on human PXR activity. In contrast, SR12813 was the most efficacious monkey PXR agonist, although TO901317 was the most potent (EC50 5 nM). In addition to TO901317, SR12813, hyperforin, dexamethasone, rifampicin, and mifepristone were partial agonists. PCN and mevastatin had the lowest agonism of monkey PXR. Dog PXR exhibited the highest amount of absolute luciferase activity (data not shown), but was generally seen at higher doses of each compound (high logEC50 and logKA); the peak activity of dog PXR was harder to discern due to lack of a plateau in the luciferase activity at higher doses. Hyperforin was considered a full agonist with SR12813, rifampicin, and TO901317 acting as partial agonists. Very little activity was seen with mifepristone, mevastatin, dexamethasone, and PCN. Rat PXR was maximally activated by PCN with T0901317 and dexamethasone also predicted to be full agonists (albeit with higher logEC50 and logKA). Rifampicin, SR12813, and mevastatin exhibited the least amount of rat PXR agonism. Similar to the rat PXR, full agonists of mouse PXR include PCN, T0901317, and dexamethasone; however hyperforin was a better mPXR agonist than a rPXR agonist.

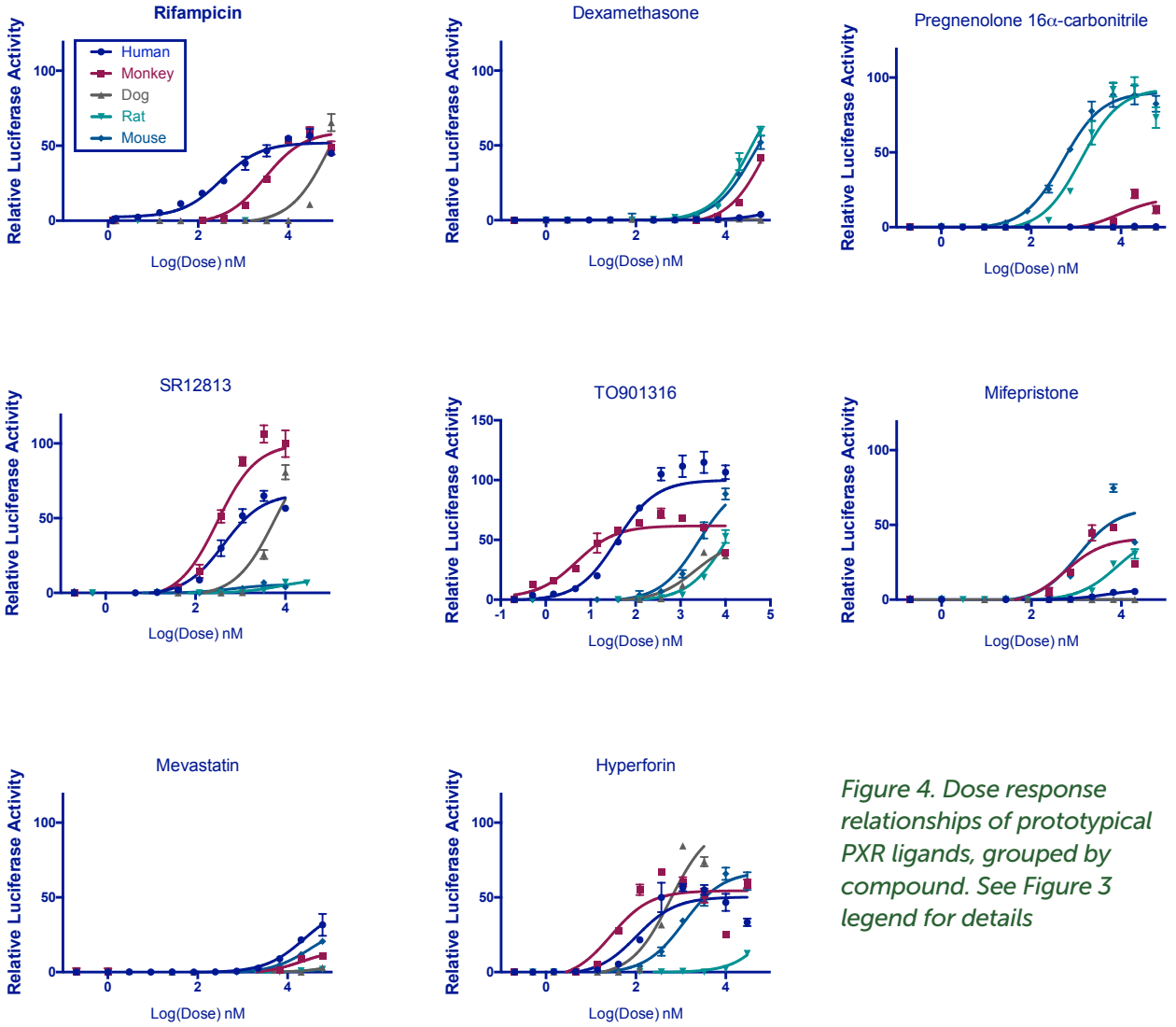


Figure 4. Dose response relationships of prototypical PXR ligands, grouped by compound. See Figure 3 legend for details

This data is also depicted in Figure 4 where each compound is grouped and the species differences are easier to visualize. Rifampicin as well as SR12813 are human, monkey, and dog agonists, while PCN and dexamethasone are rat and mouse agonists with some monkey PXR activation. SR12813, TO901316, and hyperforin are more promiscuous PXR activators, although there are species differences in both efficacy and potency across species. Hierarchical clustering of the agonism (Span/logEC50, Figure 5) reveals that, at least with this small group of compounds, the common laboratory rodents (rat and mouse) have distinct activities from human.

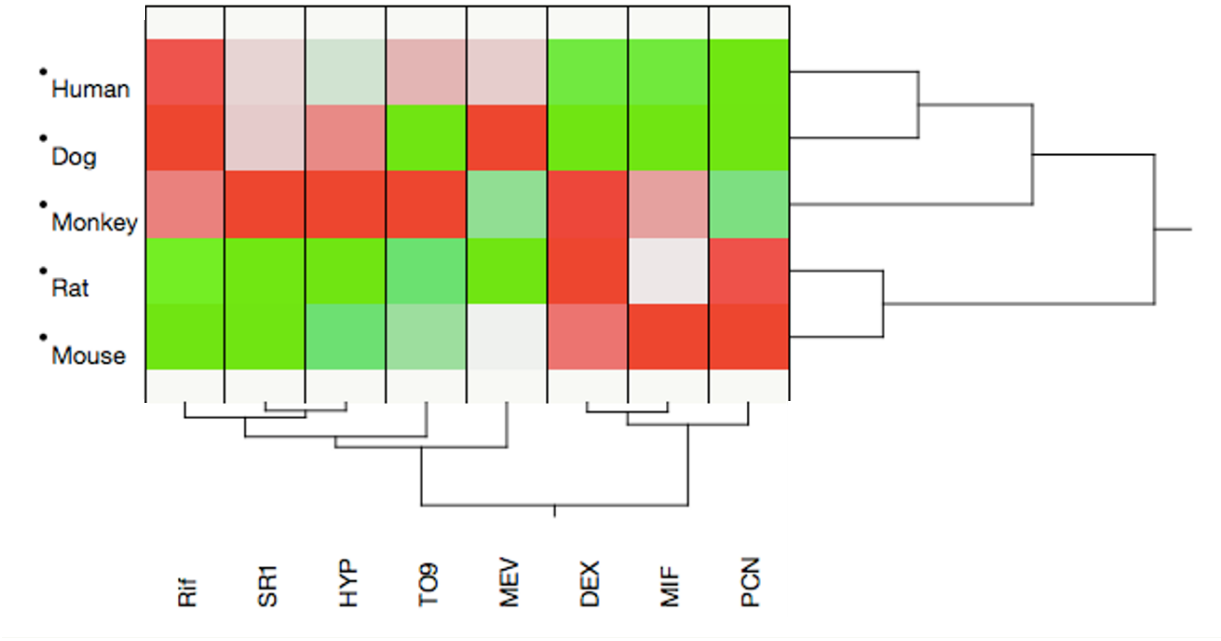


Figure 5. Hierarchical clustering of agonism (Span/logEC50)

4. References

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