

Finding pharmaceutical targets using ToxWiz: channels for pain, GPCRs for inflammation

For further information:

Email: info@camcellnet.com

Tel: +44 1223 703 137 Fax: +44 1223 858 794

Suggesting targets for a new class of disease can be a bewildering exercise, particularly when there is a vast selection of biomedical literature to trawl through. Cambridge Cell Networks (CCNet) has introduced a new search feature inside the ToxWiz browser that enables very fast, molecule-focused searches through the literature. Essentially, this search lets you do pubmed style searches of any complexity, and then retrieves our set of molecules – genes, proteins, chemicals – that have been linked to whatever literature is retrieved. The more evidence for an association of a molecule with your search terms, the higher it appears in the list of possible molecules. Here, we illustrate how this feature can be used to find possible targets for particular disease indications quickly

Pain and possible ion channel targets

The goal here was to identify quickly channels that make interesting targets for chemical intervention to treat pain. We searched using the PubMed search terms 'pain AND channel'. Inside of PubMed itself, this search finds more than 4000 abstracts, and inside of ToxWiz these searches find 1554 molecules in total, though most of these (1524) are chemicals (drugs, metabolites, etc.). Among the 30 proteins, returned are several known and putative ion channel targets for pain (Figure 1).

		<p>PMID: 17018028 J Neurochem 2006 Nov;99(4):1088-102 TRPV1b overexpression specifically regulates TRPV1 responsiveness to capsaicin, heat, and low pH in HEK293 cells.</p> <p>PMID: 15211926 J Biol Chem 2005 Dec 22;280(51):4211-23 NGF causes increases membrane expression of TRPV1 heat-activated ion channels.</p> <p>PMID: 15691846 J Biol Chem 2005 Apr 8;280(14):13424-32 Regulation of Ca²⁺-dependent desensitization in the vanilloid receptor TRPV1 by calcitonin and cAMP-dependent protein kinase.</p> <p>PMID: 15844492 Mol Pharmacol 2005 Apr;67(4):1119-27 TRPV1b, a functional human vanilloid receptor splice variant.</p> <p>PMID: 15574747 J Neurosci 2004 Dec 22;24(48):12074-9 Acid-sensory ion and its modulation in humans.</p>
	TRPV1 (21)	
		<p>PMID: 17135418 J Neurosci 2006 Nov 29;26(48):12566-75 NaV1.7 mutant A833P in erythromelalgia: effects of altered activation and steady-state inactivation on excitability of nociceptive dorsal root ganglion neurons.</p> <p>PMID: 17008310 J Biol Chem 2006 Nov 24;281(47):36229-35 Sicx matters: Erythromelalgia mutation S241L in Nav1.7 alters channel gating.</p> <p>PMID: 15980869 Neurology 2006 Nov 14;67(10):1562-7 Inherited erythromelalgia: limb pain from an S4 charge-neutral Na channelopathy.</p> <p>PMID: 16392115 Ann Neurol 2008 Mar;63(3):553-8 Genetic onset of erythromelalgia: a gain-of-function mutation in Nav1.7.</p>
	SCN9A (16)	
		<p>PMID: 12292854 J Clin Invest 2002 Oct;110(10):1495-80 Anionic, bicubic acid-sensing ion channels are heparan acid sensors expressed in human osteoclasts.</p> <p>PMID: 11512022 J Neurosci Arch 2001 Aug;44(25):668-74 Characterization of a human acid-sensing ion channel (hASIC1a) endogenously expressed in HEK293 cells.</p> <p>PMID: 11448963 J Biol Chem 2001 Sep 7;276(36):33762-7 Molecular and functional characterization of acid-sensing ion channel (ASIC1b).</p> <p>PMID: 10790388 Neuron 2000 Apr;26(1):133-41 Neuropeptide Y and P2U7 modulate potassium currents from sensory neurons and proton-activated DEG/TrpA channels.</p> <p>PMID: 9062189 Nature 1997 Mar 13;386(6621):173-7</p>
	ACCN2 (6)	

Figure 1 Top 3 of 30 protein targets found when searching for 'pain AND channel' inside the ToxWiz database.

The best among these is the transient receptor potential cation channel (TRPV1), which was discovered recently as a good target for various pain indications (Amaya et al, 2006; Palazzo et al, 2008), particular those related to thermal sensitivity. Indeed, the system readily finds Capsazepine (Figure 2), the capsaicin analogue as a chemical to target this channel (Ugawa et al 2002).

The second best match from this search, the type IX alpha (Nav1.7) sodium channel (SCN9A) is another well-studied target for pain. The associated manuscripts reveal that human mutations in this channel are correlated with insensitivity to pain (e.g. Cox et al, 2006), and analysis of putative interacting chemicals (Figure 2) readily shows that it is Tetrodotoxin sensitive (e.g. Jo et al, 2004).

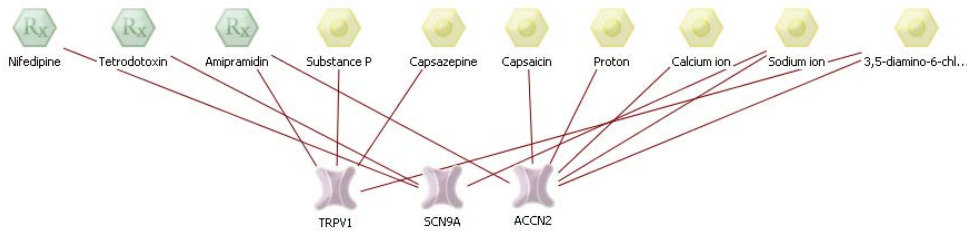


Figure 2 Some of the interactions involving chemicals (hexagons, top) and three of the pain-associated channels (bottom) inside the system.

Overall, this quick search demonstrates how fast ToxWiz can find what is known related to particular targets for a particular indication.

Inflammation & possible novel G-protein coupled receptor targets

Here the goal was to find novel G-protein coupled receptors (GPCRs) suitable for indications related to inflammatory processes. This is the sort of scenario that might arise in a company with a compound library rich in GPCR targeting compounds, and which is searching for new opportunities to exploit them.

As above, we performed a similar search for possible GPCRs for inflammation. Specifically, we looked for “Inflammation AND G-protein”. This finds some 833 molecules, of which 175 are proteins and at least 27 are G-protein coupled receptors. This is not surprising as GPCRs are popular targets for many indications, including inflammation (e.g. chemokine receptors like CCR3; e.g. Suzuki et al, 2008). However, among them are GPCRs that are not well understood (e.g. GPR4, GPR15, GPR65, GPR68, GPR77, GPR126, GPR132), and among these are only emerging signs of an association with inflammation. For instance, GPR4 plays a role in lysophosphatidylcholine inflammatory responses (Qiao et al, 2006), GPR77 (C5L2) appears to limit the pro-inflammatory response to the anaphylatoxin (Gerard et al, 2005), and GPR126 (VIGR) has roles in cell-adhesion that argue for a role in mediation of inflammatory responses.

As for the above example, there are also many putative chemical-protein interactions inside the ToxWiz database – including natural ligands, metabolites, or drugs – that might be inroads to lead molecules targeting some of these receptors. The sequence similarities between these receptors and better studied GPCRs also suggests numerous compounds that might be also be effective (results not shown).

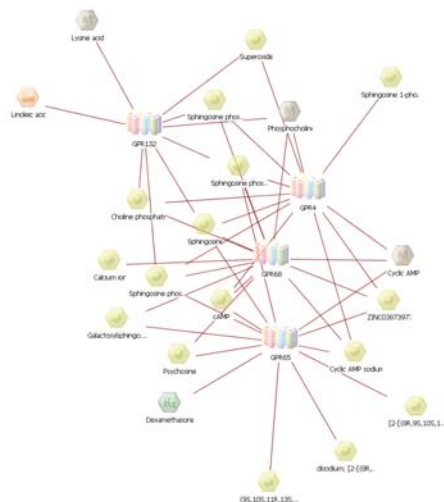


Figure 3 Some of the interactions involving chemicals (hexagons) and four of the potential inflammation associated novel GPCRs (multi-coloured 7TM icons) inside associated channels (bottom) inside the system.

Summary

All of the above searches took only a few minutes using the ToxWiz system. Obviously a more careful investigation can confirm or deny the various targets suggested, but this case study serves to demonstrate how quickly this system can be used to get molecular insights from what can be complex questions.

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The Company was founded in 2002 by eminent scientific figures from the European Bioinformatics Institute in Cambridge, UK, the University of Cambridge and the European Molecular Biology Laboratory in Heidelberg, Germany. CCNet has facilities in three countries and is staffed by a team of expert biochemists, pharmacologists, bioinformaticians, chemists and industrial toxicologists.