The pharmacology of chocolate
Desktop virtual screening to determine the pharmacological potential of theobromine

Chocolate has a wonderful collection of chemical components that provide a delicious taste, a sense of well-being and a low level of addictiveness. Dark chocolate is widely promoted as being a health food. It is claimed to be a mood enhancer, a cough suppressant, and a key nutritional supplement for cardiovascular health. There are a few hundred pharmacologically active compounds in chocolate, including, but not limited to:

- Theobromine and caffeine — both central nervous system (CNS) stimulants;
- Salsolinol — dopaminergically active, and possibly responsible for chocolate addiction (1);
- Anandamide — an endogenous cannabinoid neurotransmitter;
- Phenylethylamine — the alleged “love chemical”, another endogenous neurotransmitter.

Even from this short list of components, from their CNS activity, it’s easy to see that chocolate is indeed mood-altering.

Focus on theobromine
It is thought that theobromine is the compound responsible for many of chocolate’s pharmacological effects and its bitter taste. Theobromine is part of the methylxanthine class of compounds and has a similar structure to caffeine. Pharmacologically, theobromine is a known antagonist of both Adenosine A1 and A2a receptors, as well as an inhibitor of cAMP-specific-3',5'-cyclic phosphodiesterase 4B (PDE4B) (2). (See note on PDE4B inhibition.)

Virtual screening is a computational technique of comparing molecules to find other molecules that may be biologically active in the same way. The experiments reported here used the software applications torch V10 and forge V10 from Cresset to analyze the molecular fields of compounds in order to compare them in a way that’s biologically relevant (3).

Comparing theobromine and adenosine
Two peer-reviewed articles report that a shot of adenosine during anesthesia and surgery reduces the amount of opioid pain-kills required for post-operative recovery (4).

Figure 1 – Theobromine and adenosine; adenine and N9-methyladenine. Blue field points are negative, red field points are positive, yellow field points denote shape, and orange field points denote hydrophobicity. The numbers are the similarity scores, as assigned by torchV10.

Figure 2 – Theobromine and codeine compared in field point space. The low similarity score leads us to hypothesize that these molecules are likely to be binding to different receptors or alternate areas of the same receptor responsible for relaxation of the vagus nerve.
Pharmacologically, theobromine binds to the A1 and A2a receptors, and thus it is plausible that theobromine may have a similar, albeit less pronounced effect (see notes on Adenosine A1 and A2 Antagonism).

Given this pharmacological binding pattern, a computational comparison was carried out between theobromine and adenosine. The software torch V10 analyzes the molecular fields of compounds and expresses the field pattern as a series of field points. These are computationally manageable for large amounts of data. Comparing the field points results in the calculation of a similarity score.

A computational comparison of the biological activity of theobromine and adenosine using torch V10 resulted in a low similarity score (Figure 1). The low similarity is likely to be a result of the molecules’ different sizes.

Adenine and modified N9-methyladenine are the pharmacologically active components of theobromine and adenosine, respectively. When we look at the similarity scores for adenine and the modified N9-methyladenine, the scores suggest higher similarity for the fragments. This suggests that theobromine is likely to be accessing the same part of the adenosine receptors’ binding site as the purine piece of the natural ligand, adenosine.

THEOBROMINE’S ANTI-TUSSIVE PROPERTIES

In a recent publication from National Heart and Lung Institute (London, UK) (5), it was demonstrated that theobromine has significant anti-tussive (cough suppressing) properties via inhibiting action potentials in the vagus nerve.

Theobromine’s activity was compared against codeine, which is the gold standard in cough suppression, and was found to be as good as codeine, and without the adverse side effects or addiction risk. BC1035 (theobromine) is currently being developed by SEEK and Pernix Therapeutics and has just begun Phase III trials.

A comparison of theobromine and codeine in field point space using torch V10 results in a low similarity score (Figure 2). This leads us to hypothesize that these molecules are likely to be binding to different receptors or alternate areas of the same receptor responsible for relaxation of the vagus nerve.

A VIRTUAL SCREEN OF THEOBROMINE

The plethora of information about the pharmacology of chocolate and the amount of research interest in it indicates that there is a great deal of pharmacological potential in theobromine and its other components.

Virtual screening is a good technique of evaluating how similar a compound is to other compounds in a database. Therefore, a virtual screen of theobromine against the Drug Bank’s databases (6) of approved drugs will reveal how similar theobromine is to existing drugs, and will help to evaluate its ‘druggability’.

The virtual screen was carried out on a desktop computer using forge V10. Since theobromine is a fairly rigid flat molecule, it was not necessary to elucidate a binding mode.

Before conducting any experiments, the Drug Bank databases were visually inspected and curated to remove counter ions, waters, etc., leaving only the drug structure. The structures within the approved database ranged from quite small and rigid to quite large with several degrees of rotational freedom.

When aligning molecules under Normal settings within forge V10, it is important to note that the molecules are not aligned simply on structure, but on their field point patterns – it’s well reported that even when the 2D structural similarity is low, it’s possible that two compounds can have similar ‘personalities’ i.e., shape, hydrophobicity, and charge distribution (3).

RESULTS

In the approved drugs database, the top hits were as expected – theobromine (0.998) and caffeine (0.942) (Figure 3). The next best scoring results were a series of PDE inhibitors that result in vasodilation and bronchodilation for the treatment of asthma and COPD.

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Figure 3 – Theobromine and caffeine show a very high similarity score.

Figure 4 – Comparing theobromine to PDE inhibitors used for vasodilation and bronchodilation for the treatment of asthma and COPD.

Figure 5 – Lower scoring comparisons for theobromine. The similarity scores are surprising considering the absence of the xanthine scaffold.
vasodilation and bronchodilation, and are used in treatment for asthma and COPD patients (Figure 4). These are as expected, as the xanthine moiety is conserved through this set of compounds.

The next hits were lower scoring (0.729-0.799), but surprising considering that they don’t share the same xanthine scaffold as theobromine (Figure 5). The known activity of these hits is as follows:

- Azetazolamide is a carbonic anhydrase inhibitor used as an anti-convulsant and to relieve intracranial hypertension;
- Pyridoxal and pyridoxine are forms of Vitamin B6;
- Menadione is Vitamin K3;
- Metharbital is an anti-convulsant barbiturate with similar properties to phenobarbital;
- Stavudine is a nucleoside analog reverse transcriptase inhibitor (NARTI) with some activity against HIV;
- Oxitriptan is a chemical precursor to serotonin and melatonin, and is sold as a nutritional supplement for anti-depression and insomnia.

It is noteworthy that if we look at the rotamers of the hydroxyl groups of pyridoxal and pyridoxine, the alignment score decreases. It’s unlikely that theobromine is likely to be active against all of these indications – a vitamin/anti-depressant truth-serum that helps with high blood pressure and HIV?

While these lower scoring results are on the borderline of what would merit further investigation, it’s interesting to note that there are some similarities to theobromine. It may be that a re-profiling study would reveal valuable activity.

**SUMMARY**

In cases presented above and examining the hits from the desktop virtual screens, it’s clear that the pharmacological activity of theobromine is both exceptional and diverse. Being a small molecule, it’s no surprise that this fragment can access binding sites in a variety of targets to achieve medicinal effects.

To answer the question of whether chocolate is druggable, given the success of the theobromine anti-tussive drug that is currently in Phase III trials, the answer is certainly yes. It is illuminating to see the how virtual screening can reveal many other possible areas of activity for theobromine.

It is always good to have a scientific justification for eating chocolate.

**REFERENCES**

1) J. Ethnopharmacol. 2000, 73, 153-9;
2) Physiol. Behav. 2011, 104 (5), 816-22;
3) http://www.rcsb.org/pdb/ligand/ligandsummary.do?hetId=37T;

**ADDITIONAL NOTES**

- **Adenosine A1 Antagonism**
  The Adenosine A1 receptor is a GPCR receptor for which adenosine is the endogenous ligand, and has been found to be involved in sleep promotion by inhibiting cholinergic receptors in the basal forebrain, and also present in the vascular system’s smooth muscle to regulate myocardial oxygen consumption and blood flow through the heart muscle.

- **Adenosine A2a Antagonism**
  The Adenosine A2a receptor is a GPCR receptor (PDB:3EML) with adenosine as the endogenous ligand. A2a regulates myocardial oxygen consumption by vasodilating coronary arteries, which may result in hypotension (decreased blood pressure). In the brain, it regulates the release of neurotransmitters glutamate and dopamine, and has been indicated as a potential target for treating Parkinson’s Disease, addiction, and mood disorders.

- **PDE4B Inhibition**
  PDE4 inhibitors have been demonstrated as targets for drugs for COPD, asthma, depression, Alzheimer’s and Parkinson’s. Inhibitors block the hydrolysis of cAMP, thus the concentration of cAMP in cells increases.