Modafinil mechanism of action: Inhibition of the dopamine monoamine transporter DAT by modafinil like and piperidine-based analogues and the role of free radicals in the eugeroic effect

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Keywords: Modafinil analogues, eugeroic effect, dopamine monoamine transporter, free radicals

Abbreviations
Dopamine DA, dopamine transporter DAT, free energy of water desolvation $\Delta G_{desolv,CDS}$, lipophilicity free energy $\Delta G_{lipo,CDS}$, cavity dispersion solvent structure of the first solvation shell CDS, highest occupied molecular orbital HOMO, lowest unoccupied molecular orbital LUMO, multiple correlation coefficient R^2, the F test of significance, standards errors for the estimate (SEE) and standard errors of the variables SE($\Delta G_{desolCDS}$), SE($\Delta G_{lipoCDS}$), SE(Dipole Moment), SE (Molecular Volume), SE(AEA), transition state TS, reactive oxygen species ROS.

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Abstract

A structure activity model which incorporates the desolvation, lipophilicity, dipole moment and molecular volume of a series of modafinil like analogues has been compared to a series of saturated heterocyclic analogues in their ability to inhibit the dopamine transporter (DAT). It has been found that hydrophilicity or lipophilicity has a larger inhibitory effect for the heterocyclic analogues than the modafinil like analogues, but the heterocyclic inhibitors show no dependence on dipole moment unlike the modafinil like analogues. The modafinil like analogues have a higher desolvation requirement than the heterocyclic analogues prior to binding with DAT as expected for the more polar structures.

Evidence is presented that strongly implicates the involvement of free radical species in the eugeroic ability of modafinil like and 9-fluorene analogues via a dissociative electron attachment mechanism. This eugeroic ability is largely separate from a DAT inhibition mechanism.

Introduction

The neurotransmitter dopamine (DA) determines many body functions including cognition, mood, movement, and reward. The levels of DA in the brain is modulated by the dopamine transporter (DAT), which is a plasma membrane protein that actively transfers released DA from the extracellular space into the presynaptic neuron. The DAT is a target for addictive drugs including cocaine, amphetamines and for drugs prescribed for the treatment of attention deficit hyperactivity disorder (ADHD), depression, and other dopamine imbalance diseases such as Parkinson's disease, Alzheimer's disease, bipolar disorder, depression and alcoholism. Cocaine binds to the protein and inhibits transport, while amphetamines are transported and stimulate reverse transport (efflux) of intracellular DA. Other related drugs include nocaine (CPCA) originally developed as a less potent substitute for cocaine, and modafinil (Provigil or Nuvigil) a wake promoting agent used for the treatment of narcolepsy.

A number of comprehensive studies have examined the structure activity relationships of modafinil analogues with the DAT. [1-4][Zhou 2004, Cao 2011, 2016, Kalaba 2017]

DAT belongs to the SLC6 family of transporters that couple inward solute transport to downhill movement of Na^+ and Cl^-. It is thought that Na^+ ions bind to the extracellular domain of the transporter before dopamine can bind. After dopamine binds, the protein undergoes a conformational change, which allows both sodium and dopamine to unbind on the intracellular side of the membrane. [5][Sonders 1997]

While many drugs are routinely used to treat DA disorders, there is no clear consensus about the mechanism of how these drugs affect the action of DAT. In particular the mechanism of how modafinil exerts its wake-promoting effect is controversial. Modafinil is known to weakly but selectively bind with DAT and exert its eugeroic effect by disrupting the transport effect of DAT and hence raising extracellular concentrations of DA, which results in wakefulness. Modafinil is thus thought to be a dopamine reuptake inhibitor. DAT knockout mice are known to be unresponsive to modafinil. [6,7][Wisor 2001, 2013] However, there is significant evidence that the eugeroic action of modafinil includes other processes beside acting as a dopamine reuptake inhibitor. For example, a structure activity study of modafinil analogues found that DAT inhibition did not correlate with wakefulness-promoting effects in animals, and a number of analogues without any significant inhibition of the DAT still produced wakefulness-promoting effects. [8,9][Dunn 2012] Other possible mechanisms for the eugeroic effect besides the dopamine reuptake inhibition include activation of the orexin system. [10][Mereu 2013]

However there is also evidence that free radicals may be related to sleep induction as well as cellular damage, and that modafinil has the ability to oppose both of these effects. It is thought that modafinil could directly act on enzymes in the brain's free-radical scavenging system (eg. glutathione peroxidase or superoxide dismutase) and hence directly reduce free-radical levels. This may account for modafinil's known ability to increase the cortical creatine-phosphocreatine pool. Cytochrome enzymes in the inner mitachrondrial membrane transport chain may be involved with modafinil's electron accepting ability from superoxide species in this environment. Modifinil is known to suppress the CYP2C9 enzyme and hence reactive oxygen species in the brain, and hence possibly promoting better mitachrondrial function and wakefulness. [11][[Gerrard 2007] There is evidence that modifinil causes oxidative damage in the amygdala, hippocampus, and striatum of rats at high doses. [12][Ornell 2014]

A meta analysis of published studies on sleep deprivation and oxidative stress in the brain was used to test the hypothesis that that sleep is a dynamic-resting state with antioxidative properties. Wakefulness is thought to involve high neuronal metabolism and neuronal electrical potentials, and requires high oxygen levels, and therefore oxidants. Sleep is a state with an increased antioxidant activity which promotes a brain protection against free radicals by lowering oxidant production. ROS and other oxidative stress markers can accumulate in the brain during wakefulness, and so behave as sleep promoters. [13][Villafuerte 2015]

We have previously developed a structure activity model that has been shown to apply to the transport and anti-cancer and metabolic efficacy of various drugs. The four parameter general model is based on establishing linear free energy relationships between the four drug

molecular properties and various biological processes. The equation has been previously applied to passive and facilitated diffusion of a wide range of drugs crossing the ***blood brain barrier***, the active competitive transport of tyrosine kinase inhibitors by the hOCT3, OATP1A2 and OCT1 transporters, cyclin-dependent kinase inhibitors and HIV-1 protease inhibitors, and the penetration of drugs into tumours. The model also applies to PARP inhibitors, the anti-bacterial and anti-malarial properties of fluoroquinolones, and active organic anion transporter drug membrane transport, and some competitive statin-CYP enzyme binding processes. There is strong independent evidence from the literature that $\Delta G_{desolvation}$, $\Delta G_{lipophilicity/hydrophobicity}$, the dipole moment and molecular volume are good inherent indicators of the transport or binding ability of drugs. As such the model differs from docking studies or molecular dynamic studies of the inhibitor-transporter interaction, in that the model represents how the inhibitor-ligand undergoes (de)solvation (within the transporter environment) just prior to the actual binding interaction but after the inhibitor leaves the bulk solvent. The model allows the prediction of new analogues in a structure activity series using easily available quantum mechanical molecular properties and allows insights into molecular mechanisms. [14-20][Fong 2015-17]

Study objectives:
Apply the previously developed structure activity model to two widely different series of modafinil analogs using available literature DAT inhibitor data to gain mechanistic insights into the inhibitory process and possibly the wakefulness promotion processes.
Investigate whether modafinil could be involved in free radical processes when exerting its eugeroic effect.

Results

(a) Structure activity DAT inhibitory models of modafinil analogues

Table 1 shows the structures of the substituted modifinil like analogue series of Cao 2011, [2] and Table 2 shows the substituted heterocyclic piperidine analogues series of Zhou 2004 [1] Table 3 shows the DAT binding data and their calculated -$\mathbf{\Delta G_{desolv,CDS}}$ (free energy of desolvation in water, as calculated by $\mathbf{\Delta G_{cds,w}}$), $\mathbf{\Delta G_{lipo,CDS}}$ (free energy of lipophilicity or hydrophobicity in n-octane, as calculated by $\mathbf{\Delta G_{cds,o}}$), **DM** (dipole moment in water), **Volume** (molecular volume in water) for the two series of DAT inhibitors.

The DAT binding data has been analysed according to the equations below which has been previously shown to apply to a wide range of transport or binding of drugs to proteins, and in particular transport of drugs across the blood brain barrier:

The free energy of water desolvation ($\Delta G_{desolv,CDS}$) and the lipophilicity free energy ($\Delta G_{lipo,CDS}$) where CDS represents the non-electrostatic first solvation shell solvent properties. These molecular properties may be a better approximation of the cybotactic environment around the drug approaching or within the protein receptor pocket, or the cell membrane surface or the surface of a drug transporter, than the bulk water environment outside the receptor pocket or cell membrane surface. The CDS includes dispersion, cavitation, and covalent components of hydrogen bonding, hydrophobic effects. Desolvation of water from the drug ($\Delta G_{desolv,CDS}$) before binding in the receptor pocket is required, and hydrophobic interactions between the drug and protein ($\Delta G_{lipo,CDS}$) is a positive contribution to binding. $\Delta G_{lipo,CDS}$ is calculated from the solvation energy in n-octane.

Application of the general equation to the 20 DAT modafinil analogues gives the following equation 1, where the molecular volumes have been multiplied by 0.03 to normalize the volumes to being similar in magnitude to the other three independent variables to allow direct comparison of the relative contributions of the variables.

Eq 1

DAT Binding = -4604.8 $\Delta G_{desolv,CDS}$ -4588.9 $\Delta G_{lipo,CDS}$ -3244.1 DM -2668.9 Vol -26413.5

Where R^2 = 0.518, SEE = 8332, SE($\Delta G_{desolvCDS}$) = 1687.8, SE($\Delta G_{lipoCDS}$) = 1.413.2, SE(Dipole Moment) = 1679.4, SE(Vol) = 1379.2, F=4.022, Significance=0.020

Analysis of the 26 heterocyclic piperidine analogues gave equation 2 (after omitting modafinil, cocaine, and Z4, Z17D, Z17F, and Z19 as large outliers, and normalizing the volumes by 0.03.

Eq 2

DAT Binding = 23.2 $\Delta G_{desolv,CDS}$ +35.6 $\Delta G_{lipo,CDS}$ +24.4 Vol +291.6

Where R^2 = 0.250, SEE = 86.3, SE($\Delta G_{desolvCDS}$) = 21.1, SE($\Delta G_{lipoCDS}$) = 18.2, SE(Vol) = 23.5, F=2.44, Significance=0.090

Eq 2 is a fairly poor precision equation, compared to eq 1, and particularly shows no correlation with dipole moment, with the lipophilicity coefficient being the most significant, and the desolvation and volume coefficients of lower significance and relative importance. Most notable is the change of signs for dependence on lipophilicity (and desolvation and volume) between eqs 1 and 2, and no dependence on dipole moment for the piperidine analogues in eq 2.

(b) **Free radical eugeroic mechanism of modafinil analogues**

The possibility of modafinil exerting its eugeroic effect by a free radical mechanism has been investigated by examining the behaviour of modafinil after attachment of an electron in water. This bioreduction process may arise in the brain from superoxide species and or mitochondrial sources or cytochrome sources. The anion radical formed from the attachment of an electron to modafinil is shown to exhibit extension and then cleavage of the diphenylmethyl carbon to sulphoxide (C---S) bond, as shown in the transition state in Figure 1. This dissociative electron attachment reaction [21,22][Malan 2002, Saveant 1994] also occurs in the substituted modafinil analogues $(C_6H_4X)_2$-CH-S(=O)-Z and is driven by the delocalized resonance stabilization of the excess electron over the $(C_6H_4X)_2$-CH-S(=O)- moiety in the HOMO. There is a decrease of negative charge on the benzylic $(C_6H_5)_2$-**C**H- atom and a decrease of positive charge on the S atom when elongation of the C---S bond occurs upon the attachment of an electron to modifinil in water. The free energy of activation ΔG^* for the water solvated modafinil transition state shown in Figure 1 is estimated to be about -30 kcal/mol assuming a ΔG for the hydrated electron of -34.6 kcal/mol. [23][Zhan 2003]

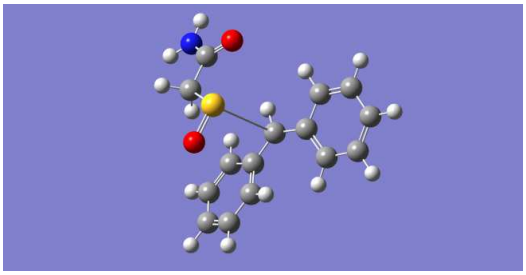
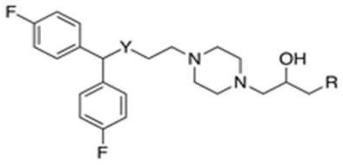


Figure 1. Transition state for dissociative electron attachment of Modafinil in water

Examination of the analogue (11B in Table 3) where X = 4-F and Z = $-(CH_2)_2$-(4N-piperazine-1N)-CH_2-CH(OH)-C_6H_5 (Figure 2) which has a high DAT binding affinity (K_i = 2.5 nM) [3][Cao 2016] compared to modafinil (K_i = 3800 nM) shows the same behaviour upon electron attachment as that for modafinil. Similarly substitution of the X groups in $(C_6H_4X)_2$-CH-S(=O)-CH_2-C(=O)NH_2 shows the same behaviour of benzylic C---S bond elongation upon electron attachment.



Y=S(=O), R=C_6H_5

Figure 2

Another analogue which shows quite different behaviour to modafinil is the compound 9-fluorenyl-S(=O)-CH_2-C(=O)-(4N-piperazine-1N)-C(=O)-CH_3 [8,9][Dunn 2012] which shows a weak DAT inhibitory effect compared to modafinil but a potent eugeroic effect. Figure 3 shows the effect of an electron attachment to this compound with elongation of the fluorenyl-9 carbon to S(=O) bond, very similar to the anion radical of modafinil. The HOMO is delocalized over the fluorenyl and sulphoxide groups, with the LUMO delocalized over the fluorenyl group. The free energy of $\mathbf{\Delta G}^*$ for the bond elongation shown for the fluorenyl analogue in Figure 3 is estimated to be about -29 kcal/mol, similar to that shown for modafinil.

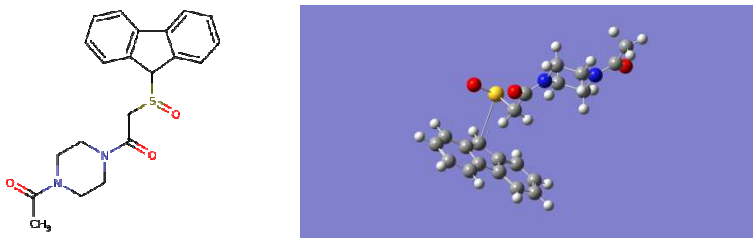


Figure 3 9-fluorenyl-S(=O)-CH_2-C(=O)-(4N-piperazine-1N)-C(=O)-CH_3 and the transition state for dissociative electron attachment in water

However Dunn [8,9] reported that fluorenol which is the 9-hydroxyfluorene derivative formed from 9-Fluorenyl-S(=O)-CH_2-C(=O)-(4N-piperazine-1N)-C(=O)-CH_3 itself shows a

stronger eugeroic effect but a 59% weaker DAT binding ability than modifinil. This result is unusual since fluorenol is vastly structurally different from modifinil indicating a quite different mechanism to that of modafinil. Since the fluorene moiety can easily accommodate electron attachment, an investigation of electron attachment to fluorenol showed no evidence of bond elongation of the fluorene C9—OH bond ruling out formation of a fluorene radical anion species similar to that shown by 9-fluorenyl-S(=O)-CH_2-C(=O)-(4N-piperazine-1N)-C(=O)-CH_3. However, an examination of electron attachment to the protonated fluorenol species 9-fluoreneOH_2^+ did show fluorene---OH_2^+ bond elongation as illustrated in Figure 4, indicating a dissociative electron attachment reaction. This proposed process may have validity since the brain pH varies from slightly acidic to neutral, and the brain pH environment is known to vary regularly with acidic surges. [24][Magnotta 2012] The electroreduction of the bond cleavage of the C-OH bond of 9-fluorenol has been previously shown to be initiated by electron transfer.[25][Mendkovich 2016]

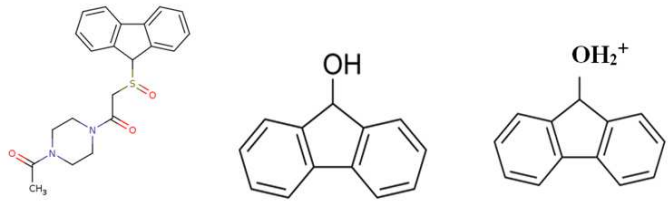


Figure 4 showing the 9-fluorenyl-S(=O)-CH_2-C(=O)-(4N-piperazine-1N)-C(=O)-CH_3, 9-fluorenol and protonated 9-fluorenol

Dunn 2012 [8,9] and Louvet 2012 [26] have also reported that biphenyl analogues and diphenylether derivative of modafinil also exhibit similar eugeroic effects and DAT binding to modafinil. These compounds all possess benzylic carbon atoms adjacent to the sulphoxide group. Figure 5. Both of these drugs did not show dissociative electron attachment upon 1 electron attachment, but did show dissociation of the benzylic C---S bond upon 2 electron attachment. It is clear from examination of the HOMOs that the $(C_6H_4X)_2CH$- and 9-fluorenyl moieties (see Figures 1 and 2) can better stabilize electron density than can the benzylic moieties in Figure 5, and the TSs for CH_2---S bond dissociation in these two drugs requires a second electron to be attached to activate the CH_2---S bond. The free energy of activation $\mathbf{\Delta G^*}$ for the water solvated ether analogue transition state shown in Figure 5 is estimated to be about -44 kcal/mol, which compares to the TS shown in Figure1 for modafinil of -30 kcal/mol.

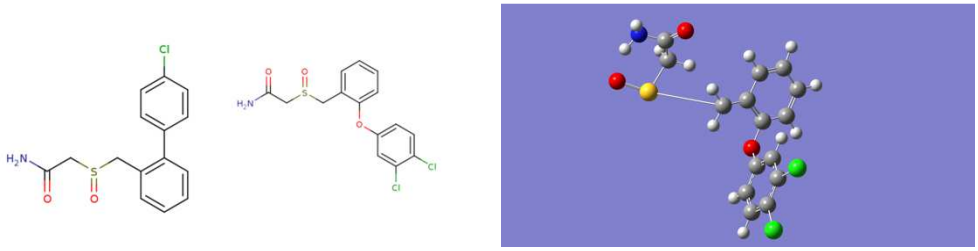


Figure 5 Showing biphenyl and ether analogues and transition state for two electron dissociative electron attachment to the ether compound.

A study of the possibility of free radical species being involved in the heterocyclic piperidine series studied for inhibition of DAT showed as expected that electron attachment to compound 16A (see Table 2) did not produce any possible reactive intermediates such as those discussed above for the modafinil and its analogues, or fluorenyl analogues. This can be anticipated since the heterocyclic compounds do not have benzylic type structures that can stabilize electron attachment.

It has been shown that diphenylmethyl *p*-nitrophenyl sulphide (DNPS) in dimethylformamide undergoes an electrochemical one-electron transfer to form the radical anion of DNPS (as identified by ESR spectrometry), followed by fission of the activated C–S bond to form the *p*-nitrothiophenolate anion and diphenylmethyl radical. The dissociation of the radical anion is the rate determining step of the electrochemical processes. [27][Farnia 1978] Since the sulphide modafinil analogs (4b, 4c, 4d, 6a, 6b, 6c, 6d in Table 3) are known to inhibit DAT and show some eugeroic behaviour, it was instructive to see if these drugs also underwent dissociative electron attachment like the parent modifinil. It was found that $(C_6H_5)_2CH\text{-}S\text{-}CH_2C(=O)NH_2$ also undergoes dissociative electron attachment with elongation of the methine –(H)C---S bond, and the free energy of activation for the TS shown in Figure 6 is estimated to be -25.5 kcal/mol in water which can be compared to a value of -30 kcal/mol for the modafinil TS in Figure 1. The same dissociative behaviour was observed in dimethylformamide as in water.

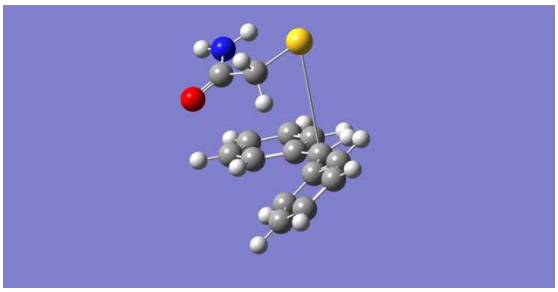


Figure 6. Transition state for dissociative electron attachment of $(C_6H_5)_2CH\text{-}S\text{-}CH_2C(=O)NH_2$ in water

Discussion

The effectiveness of eugeroic drugs is dependent on the bioavailability of the drug, particularly its ability to enter brain cells as well as its cognition altering neurochemical action.

The ability of modafinil analogues to cross the blood brain barrier, and bind to the DAT, has been examined using equations 1 and 2. The modafinil like analogues used to construct eq 1 are closely related to modifinil itself, whereas the analogues used in eq 2 are structurally different, with higher proportions of saturated carbons and higher hydrophobicity or lipophilicity. The relative magnitudes of the coefficients in eq 1 and 2 give insights into the

molecular factors influencing DAT binding in the two series of analogues. The three ratios $\{\Delta G_{desolv,CDS} / \Delta G_{lipo,CDS}\}$, $\{DM / \Delta G_{lipo,CDS}\}$, $\{Volume / \Delta G_{lipo,CDS}\}$ measure the importance of the molecular properties relative to hydrophobicity or lipophilicity. Eq 1 gives values of **1.0** *(-4604.8/-4588.9*), **0.7** (*-3244.1/-4588.9*), and **0.6** *(-2668.9/-4588.9*) respectively, while eq 2 gives values of **0.65** (*23.2/35.6*), no dependence on DM, and **0.7** (*24.4/35.6*) respectively. What is noteworthy is the change of signs for the coefficients in the two equations, showing that hydrophobicity has a major and opposite effect on DAT binding for modafinil like analogues, whereas the heterocyclic saturated analogues behave completely differently driven by their higher hydrophobicity. Also the heterocyclic series shows no dependency on dipole moment, indicating no dipole-dipole interaction between the DAT and the heterocyclic analogues. This is opposite to that found for the modafinil like analogues in eq 1 where the substituted phenyl groups can exert significant $\boldsymbol{\pi}$ (di)polar effects. The larger desolvation ratio of 1.0 for the modafinil like analogues compared to the value of 0.65 for the heterocyclic analogues is also consistent with an expected stronger desolvation effect to be operating for the more polar modafinil like analogues in water.

With respect to the cognition altering neurochemical action of modafinil and analogues, there is little systematic investigation available. The in vivo CNS activity of various modifinil like analogues has also been evaluated. For the series $(C_6H_5)_2CHS(=O)CH_2C(=O)NH(C_6H_4X)$ where X = H, 3-Cl, 4-Cl, 4-Et, 3,4-Cl, 4-NO_2, 4-Br, all these analogues were CNS stimulants, except where X = H. The psychological performances of mice for wakefulness, exploratory activity, depression and anxiogenic and anxiolytic like effects were measured. [28][Lari 2013] These results were similar to those previously found by De Risi for the series $(C_6H_5)_2CHS(=O)CH_2C(=O)NH$-R where R = Me, iPr, tBu were found to be stimulants, but where X = Et, piperidine or morpholine were found to be sedatives. For the series $(C_6H_4X)_2CHS(=O)CH_2C(=O)NH_2$ where X = 4-F, 4-Cl,4-H and 4-F,4-H, these analogues were also found to be stimulants. These CNS activities were measured using electrically-evoked tritiated serotonin ([^{3}H]5-HT) efflux from rat cortical slices. [29][De Risi 2008] These in-vivo results overall suggest that substitutions at the amide N group or at the phenyl groups adjacent to the sulphoxide moiety have little effect on CNS activity, since the majority of these substitutions result in stimulatory outcomes. These observations are consistent with a common and dominant cognition altering neurochemical mechanism which may be modulated by smaller steric effects at the amide N atom.

It has also been reported that modafinil exhibits antioxidant and neuroprotective properties, while also increasing the cortical phosphocreatine pool, and that there is evidence of the involvement of free radicals. [11,30][Gerrard 2007, Pierard 1995] Dimethylsulfoxide (DMSO) is a well known free radical scavenger, and has been recommended as a treatment for endotoxemia and systemic inflammatory response syndrome in horses because of its anti-inflammatory and reactive oxygen species (ROS)–scavenging benefits. DMSO was found to be a scavenger of hydroxyl radicals and an effective inhibitor of platelet aggregation in an in-vivo mouse model of pial arteriolar injury. [31,32][Rosenblum 1982, Sprayberry 2015] Hence there is literature evidence that sulphoxides can form free radicals.

It has been shown that diphenylmethyl *p*-nitrophenyl sulphide (DNPS) undergoes electrochemical dissociative electron attachment in dimethylformamide, [27][Farnia 1978] which is consistent with this study which shows that the sulphide analogue of modafinil $(C_6H_5)_2CH$-S-$CH_2C(=O)NH_2$ also undergoes the same process in water.

The dissociative electron attachment behaviour shown by modafinil like analogues is strongly indicative of a free radical mechanism for cognition altering neurochemical action of modafinil and analogues in the CNS. Similarly the eugeroic abilities of 9-fluorenyl derivatives can also be explained by a free radical mechanism. Since the modafinil like analogues are vastly different in structure from the 9-fluorenyl derivatives, but both have a common basis in that both possess benzylic carbon moieties that can form stable radicals which can delocalize electron density over aryl groups.

The observation that 9-Fluorenyl-S(=O)-CH_2-C(=O)-(4N-piperazine-1N)-C(=O)-CH_3 which has been shown to exert a potent eugeroic effect but is a weak inhibitor of DAT shows a strong dissociation of the C9---S bond upon electron attachment is indicative of a free radical eugeroic effect that is largely independent of acting as a dopamine reuptake inhibitor. Previous structure activity studies of modafinil analogues found that DAT inhibition did not correlate with wakefulness-promoting effects in animals, and a number of analogues without any significant inhibition of the DAT still produced wakefulness-promoting effects. [8,9][Dunn 2012]

Conclusions

A structure activity model which incorporates the desolvation, lipophilicity, dipole moment moment and molecular volume of a series of modafinil like analogues has been compared to a series of saturated heterocyclic analogues in their ability to inhibit the dopamine transporter (DAT). It has been found that hydrophilicity or lipophilicity has a larger inhibitory effect for the heterocyclic analogues than the modafinil like analogues, but the heterocyclic inhibitors show no dependence on dipole moment unlike the modafinil like analogues. The modafinil like analogues have a higher desolvation requirement than the heterocyclic analogues prior to binding with DAT as expected for the more polar structures.

Evidence is presented that strongly implicates the involvement of free radical species in the eugeroic ability of modafinil like and 9-fluorene analogues via a dissociative electron attachment mechanism. This eugeroic ability is largely separate from a DAT inhibition mechanism.

Experimental

All calculations were carried out using the Gaussian 09 package. Energy optimisations were at the DFT/B3LYP/6-31G(d,p) (6d, 7f) level of theory for all atoms. Selected optimisations at the DFT/B3LYP/6-311$^+$G(d,p) (6d, 7f) level of theory gave very similar results to those at the lower level. Optimized structures were checked to ensure energy minima were located, with no negative frequencies. Energy calculations were conducted at the DFT/B3LYP/6-31G(d,p) (6d, 7f) level of theory with optimised geometries in water, using the IEFPCM/SMD solvent model. With the 6-31G(d) basis set, the SMD model achieves mean unsigned errors of 0.6 - 1.0 kcal/mol in the solvation free energies of tested neutrals and mean unsigned errors of 4 kcal/mol on average for ions. [33][Marenich 2009] The 6-31G(d,p) basis set has been used to calculate absolute free energies of solvation and compare these data with experimental results for more than 500 neutral and charged compounds. The calculated values were in good agreement with experimental results across a wide range of compounds. [34,35][Rayne 2010, Rizzo 2006] Adding diffuse functions to the 6-31G* basis set (ie 6-31$^+$G**) had no significant effect on the solvation energies with a difference of less than 1% observed in

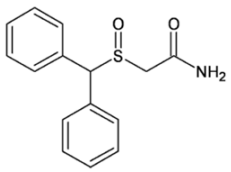
solvents, which is within the literature error range for the IEFPCM/SMD solvent model. HOMO and LUMO calculations included both delocalized and localized orbitals (NBO).

The ease of bond cleavage of the benzylic methine C---S bond of modafinil by dissociative electron attachment was confirmed by observing that this process occurs in the gas phase as well as in water. The electron impact mass spectrometry study of modafinil gives the main ionization fragmentation pathway involving a fragment m/e of 167, ie the $(C_6H_5)_2CH$ ion. [36][Dubey 2009]

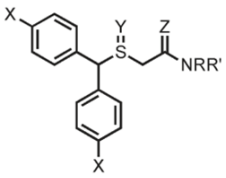
It is noted that high computational accuracy for each species in different environments is not the focus of this study, but comparative differences between various species is the aim of the study. The literature values for DAT Ki used in the multiple regression LFER equations have much higher experimental uncertainties than the calculated molecular properties. The statistical analyses include the multiple correlation coefficient R^2, the F test of significance, standards errors for the estimates (SEE) and each of the variables SE($\Delta G_{desolCDS}$), SE($\Delta G_{lipoCDS}$), SE(Dipole Moment), SE (Molecular Volume), as calculated from "t" distribution statistics. Residual analysis was used to identify outliers.

Racemic mixtures of modafinil like analogues (Table 1, using the same nomenclature as Cao 2011 [2]) were evaluated for DAT binding at the rat brain membrane, K_i (nM). Synaptosomal competitive binding of heterocyclic and nocaine analogues (tested as the free base) and [^{3}H]dopamine by striatum rat brain DAT at pH 7.4 and 37C, K_i (nM). Table 2 shows the analogues evaluated, using the same nomenclature as Zhou 2004. [1]
Table 3 shows the DAT binding data K_i for modafinil and piperidine analogues and the calculated molecular properties in water.

Table 1. Modafinil analogues DAT inhibitors [2][Cao 2011]



Modafinil

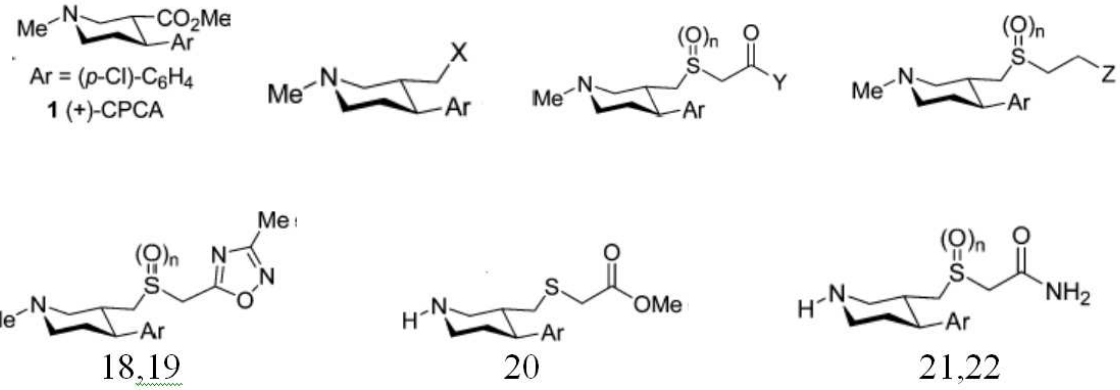


Modafinil like Analogues

Compound	X,Y,Z,R,R' Substituents
(+/-) Modafinil	H,O,O,H,H
4b	F,-,O,H,H
4c	Cl,-,O,H,H
4d	Br,-,O,H,H
5b	F,O,O,H,H
5c	Cl,O,O,H,H
5d	Br,O,O,H,H
6a	H,-,O,Me,Me
6b	F,-,O,Me,Me
6c	Cl,-,O,Me,Me
6d	Br,-,O,Me,Me
7c	Cl,O,O,Me,Me

7d	Br,O,O,Me,Me
7f	Cl,O,O,Me,H
7g	Br,O,O,Me,H
7h	H,O,O,H,$(CH_2)_3C_6H_5$
9a	H,O,H,H,$(CH_2)_3C_6H_5$
9d	Cl,O,H,Me,Me
Cocaine	

Table 2. Heterocyclic DAT Inhibitors [1][Zhou 2004]



Compound **Ar = (p-Cl)-C_6H_4**	**X**	**Y**	**Z**	**n**
1(+) CPCA				
4	-OH			
5	-I			
6	-OMe			n=0
7	-OMe			n=1
8			-OH	n=0
9			-OH	n=1
10			-OMe	n=0
11			-OMe	n=1
12			-OC(=O)Me	n=0
13			-OC(=O)Me	n=1
14			-OC(=O)C_6H_5	n=0
15			-OC(=O)C_6H_5	n=1
16(a)		-NH_2		n=0
16(b)		-NH(OH)		n=0
16(c)		-NH(Me)		n=0
16(d)		-NMe_2		n=0
16(e)		-NH(iPr)		n=0
16(f)		-N(-CH_2-)$_5$		n=0
17(a)		-NH_2		n=1
17(b)		-NH(OH)		n=1
17(c)		-NH(Me)		n=1

17(d)		-NMe_2		n=1
17(e)		-NH(iPr)		n=1
17(f)		-$N(-CH_2-)_5$		n=1
18				n=0
19				n=1
20				
21				n=0
22				n=1

Table 3. DAT binding data K_i for modafinil and piperidine analogues and calculated molecular properties in water

	DAT K_i nM	**$\Delta G_{CDS,w}$** kcal/mol	**$\Delta G_{CDS,o}$** kcal/mol	**Dipole Moment** D	**Molec Volume** cm^3/mol
Cao 2011 **Structures in Table 1**					
(+/-) Modafinil	2520	5.98	-6.59	3.7	226
4B	1570	7.59	-5.39	4.84	184
4C	2230	6.37	-7.6	5.41	200
4D	1930	4.8	-9.22	5.28	270
5B	2190	7.34	-5.29	3.65	231
5C	919	6.11	-7.42	3.81	259
5D	600	4.53	-9.06	3.74	272
6A	16500	6.91	-8.3	4.82	220
6B	9150	8.33	-7	5.12	200
6C	4510	6.94	-9.05	5.87	235
6D	2450	5.39	-10.68	6.05	231
7C	34600	6.98	-8.96	3.27	222
7D	21300	5.34	-10.59	3.52	282
7F	24440	6.55	-8.28	4.34	208
7G	1650	4.98	-9.92	4.38	262
7H	26660	8.09	-11.73	4.38	261
9A	194	6.03	-11.24	6.78	316
7H	26660	8.09	-11.73	4.38	261
9A	194	6.03	-11.24	6.78	316
9D	2890	3.5	-8.56	3.77	231
Cocaine	71.8	6.16	-6.8	0.95	159
11B*	2.5	5.85	-11.78	4.55	465
Zhou 2004 **Structures in Table 2**					
Modafinil	3800	5.98	-6.59	3.7	226

Cocaine	423	6.16	-6.8	0.95	159
CPCA	233	4.06	-5.85	6.69	175
Z4	497	1.96	-6.05	5.24	201
Z5	376	1.43	-5.13	4.66	223
Z6	80	4.76	-7.36	3.32	246
Z7	231	4.77	-7.23	6.37	279
Z8	16	2.84	-7.63	3.11	233
Z9	12	2.78	-7.53	7.33	221
Z10	50	3.72	-7.31	3.08	230
Z11	15	3.68	-7.22	7.31	216
Z12	35	4.99	-8.31	6.25	242
Z13	9	4.93	-8.22	9.65	303
Z14	68	5.81	-10.83	6.54	342
Z15	32	5.74	-10.7	9.84	284
Z16A	159	3.68	-6.76	5.33	263
Z16B	85	5.05	-7.47	5.67	247
Z16C	13	3.47	-7.65	4.77	255
Z16D	116	3.94	-8.23	5.08	264
Z16E	1	4.93	-8.98	4.82	282
Z16F	83	3.74	-10.2	5.33	286
Z17A	209	3.76	-6.66	7.2	212
Z17B	55	5.09	-7.4	7.18	257
Z17C	164	3.63	-7.6	5.75	235
Z17D	2884	4.08	-8.06	6.89	224
Z17E	248	4.89	-8.78	6.45	278
Z17F	379	3.8	-10.07	7.09	340
Z18	126	2.17	-7.89	4.78	175
Z19	1653	2.48	-7.87	7.33	229
Z20	51	4.97	-6.46	9.37	246
Z21	114	3.78	-6.08	5.56	191
Z22	108	3.85	-6	7.66	201

Footnote: Compound 11B from Cao 2016 [3]

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