



HAL
open science

Modafinil and modafinil analogues: free radical mechanism of the eugeroic and cognitive enhancement effect

Clifford W Fong

► **To cite this version:**

Clifford W Fong. Modafinil and modafinil analogues: free radical mechanism of the eugeroic and cognitive enhancement effect. [Research Report] Eigenenergy. 2018. hal-01933737

HAL Id: hal-01933737

<https://hal.science/hal-01933737>

Submitted on 24 Nov 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Modafinil and modafinil analogues: free radical mechanism of the eugeroic and cognitive enhancement effect

Clifford W. Fong

Eigenenergy, Adelaide, South Australia.

Keywords: Modafinil, modafinil-like analogues, eugeroic effect, cognitive enhancement, free radicals, quantum mechanics

Abbreviations

Dopamine DA, dopamine transporter DAT, Dissociative electron transfer or attachment DET, Linear free energy relationship LFER, free energy of water desolvation $\Delta G_{\text{desolv,CDS}}$, lipophilicity free energy $\Delta G_{\text{lipo,CDS}}$, 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, standard errors for the estimate (SEE) and standard errors of the variables $SE(\Delta G_{\text{desolv,CDS}})$, $SE(\Delta G_{\text{lipo,CDS}})$, $SE(\text{Dipole Moment})$, $SE(\text{Molecular Volume})$, transition state TS, reactive oxygen species ROS.

Contact: cwfong@internode.on.net

Abstract

Evidence is presented that strongly implicates the involvement of free radical species in the eugeroic and cognitive enhancement ability of modafinil and modafinil-like analogues via a dissociative electron transfer (DET) or attachment mechanism. Examination of modafinil and modafinil-like analogues which have been shown in the literature to exhibit wake promoting and cognitive psychobiological properties are shown in this study to produce free radicals when subject to electron attachment. Such observations are consistent with a free radical oxidative stress eugeroic and cognitive altering mechanism for modafinil and related analogues. It has also been shown that appropriate substitution of the acetamide moiety of modafinil can result in quite different DET behaviour, which holds promise of the ability to design and predict eugeroic and cognitive behaviour. In so far as DAT binding is a determinant of eugeroic and cognitive enhancing behaviour of modafinil-like analogues, a linear free energy relationship with DAT binding can also help identify the molecular properties of analogues which govern binding behaviour.

Introduction

The mechanism of how modafinil exerts its eugeroic or wake-promoting effect is controversial. [1,2,3][Zare 2016, Kim 2012, Gerrard 2007] Modafinil is known to weakly but selectively bind with the dopamine transporter (DAT) and possibly exert some of its eugeroic effect by disrupting the transport effect of DAT and hence raising extracellular concentrations of dopamine (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. Modafinil is also thought to be dependent on catecholaminergic (dopaminergic and adrenergic) signaling for its wake-promoting effects. [4,5][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. [6,7][Dunn 2012] Another study found that modafinil has a long duration of action, with its wake promoting properties largely arising from dopaminergic activity. [8][Turner 2013] Other possible mechanisms for the eugeroic effect besides the dopamine reuptake inhibition include activation of the orexin system. [9][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 by directly affecting antioxidant enzymes and decreasing free radical production. ROS affect ATP production, so if modafinil can decrease ROS levels, ATP synthesis can be increased, reducing the need for sleep, as ATP metabolism causes energy reduction and the need for sleep. Cytochrome enzymes in the inner mitochondrial membrane transport chain may be involved with modafinil's electron accepting ability from superoxide species in this environment. Modafinil is known to suppress the CYP2C9 enzyme and hence reactive oxygen species in the brain, and hence possibly promoting better mitochondrial function and wakefulness. [3][Gerrard 2007] There is evidence that modafinil causes oxidative damage in the amygdala, hippocampus, and striatum of rats at high doses. Modafinil caused behavioural changes, particularly increased locomotor activity, depending on the administered dose. [10][Ornell 2014] It seems likely that the eugeroic effect of modafinil and modafinil-like analogues has a multi-factor mechanism.

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. [11][Villafuerte 2015]

The neuroprotective antioxidant properties of modafinil may arise from inhibitory interaction with the enzymes (eg mitochondrial CYP-450 family, including CYP2C) responsible for the free radical scavenging in the brain. Increased levels of mitochondrial free radicals may lead to increased levels of adenosine, a sleep promoting factor, in the brain. Mitochondrial activity may be a major factor in sleep inducing activity. [1][Zare 2016]

Modafinil has been shown to have cognitive enhancement properties independent of its effect on sleep disorders, a "smart drug". The cognitive enhancement was observed in non-sleep-deprived individuals. [12][Battleday 2015] Modafinil is thought to exert its neurochemical and cognitive altering ability by primarily affecting catecholamines, as well as serotonin, glutamate, gamma amino-butyric acid, orexin, and histamine systems in the brain. [13][Minzenberg 2007]

We have previously developed a structure activity model of the binding (K_i nM) of 20 modafinil-like analogues to DAT (equation 1), where $\Delta G_{\text{desolv,CDS}}$ is the free energy of desolvation of the analogues, $\Delta G_{\text{lipo,CDS}}$ is the free energy of lipophilicity or hydrophobicity

in n-octane, DM is the dipole moment in water, and Vol is the molecular volume in water. The general form of this linear free energy relationship (LFER) 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 transporters, cyclin-dependent kinase inhibitors and HIV-1 protease inhibitors, and the penetration of drugs into tumours. [14-21][Fong 2016-18]

Eq 1

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

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

It is noted that the LFER eq 1 includes substituted modafinil analogues $(XC_6H_4)CH-S(=O)-CH_2-C(=O)-NR_1R_2$ as well as the sulphide analogues $(XC_6H_4)CH-S-CH_2-C(=O)-NR_1R_2$. [21,22][Fong 2018, Cao 2011] However while eq 1 is of interest to how the properties modafinil is related to binding to DAT, the focus of this paper is on the molecular mechanism of how modafinil and its analogues may actually exert their eugeroic and cognitive effects.

Study objectives:

Investigate whether modafinil could be involved in free radical processes when exerting its eugeroic and cognitive effects.

Investigate how structural variations to modafinil and its analogues can be used to predict eugeroic and cognitive enhancing capacity to design better modafinil-like analogues.

Results

Free radical eugeroic and cognitive enhancement mechanism of modafinil and modafinil-like analogues

The possibility of modafinil exerting its eugeroic and cognitive behavioural effect by a free radical mechanism has been investigated by examining the behaviour of modafinil after transfer or 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(a). This *dissociative electron transfer (DET)* or attachment reaction [23,24][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 $(C_6H_5)_2-\underline{C}H-$ 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 modafinil in water. The free energy of activation ΔG^*_{act} for the water solvated modafinil transition state (TS), C---S bond length 3.02 Å, shown in Figure 1(a) is estimated to be about -15.4 kcal/mol assuming a ΔG for the hydrated electron of -34.6 kcal/mol. [25][Zhan 2003] The ΔG^*_{act} for the vertical electron addition to the starting conformation of modafinil in water is -3.9 kcal/mol, followed by the adiabatic relaxation of the radical anion with a ΔG^*_{act} of -11.5 kcal/mol, resulting in an overall ΔG^*_{act} of -15.4 kcal/mol for the TS shown in Figure 1(a). The elongated benzylic methine C---S bond is 3.02

Å in Figure 1(a) compared to the starting compound with the bond length of 1.93 Å before dissociative electron attachment. The ease of formation of a TS for modafinil by electron attachment is dependent on the particular conformation, particularly the orientation of the –S(=O)-CH₂-C(=O)NH₂ moiety to the almost coplanar (C₆H₅)₂CH- moiety. For example, in Figure 1(b) the ΔG^*_{act} for the vertical electron addition to the starting conformation of modafinil in water is -3.9 kcal/mol, whereas the conformation in Figure 1(c) has a value of -1.7 kcal/mol. The HOMO in Figure 1(b) more effectively spans the radical anion than it does in Figure 1(c) indicating greater delocalized electron stabilization, and effectively decreased stabilization of 1(c), so increasing the ease of bond elongation of the C--S bond in the vertical TS for conformation 1(c). The conformation in Figure 1(c) where the C---S bond elongation upon adiabatic relaxation is 3.02 Å has a ΔG^*_{act} which is lower by -12.9 kcal/mol for the equivalent conformation shown in Figure 1(b) with a C---S bond elongation of 3.02 Å.

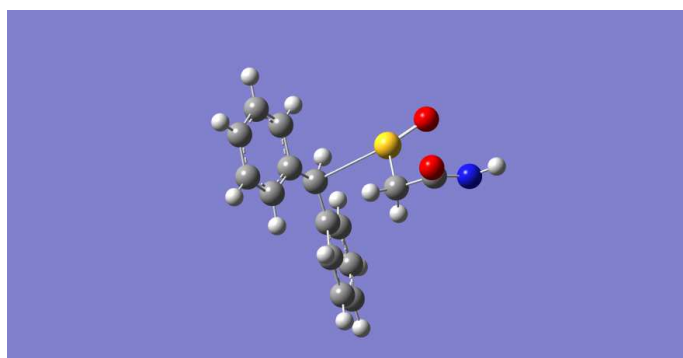
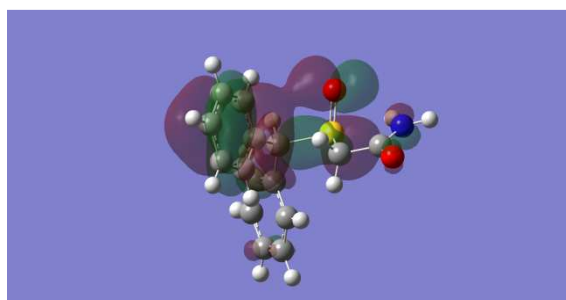
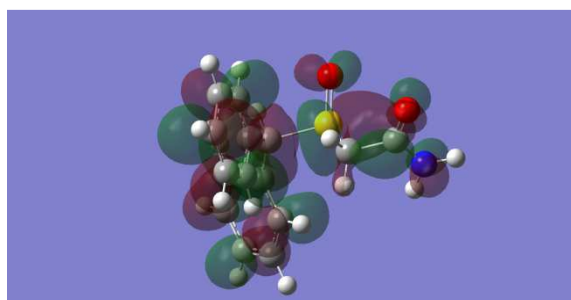


Figure 1(a). Adiabatic transition state for dissociative electron attachment of modafinil in water



Figures 1(b) and 1(c). Vertical transition states for different conformations of modafinil radical anion showing the HOMOs.

Zhu [26] has examined the stimulatory effect of a number of modafinil analogues on mice, and found that 2-[(diphenylmethyl)thio]N-(3-chlorophenyl)acetamide (see Figure 2(b)) had a slightly better stimulatory response than modafinil over prolonged periods of up to 3 hours. Of the other analogues tested, only compound 3(d) in Figure 3 showed a significant stimulatory effect which was less potent than modafinil. However, 2-[(diphenylmethyl)sulfinyl]-N-(3-chlorophenyl)acetamide, compound 2(a) in Figure 2, was not tested in Zhu's biological assay.

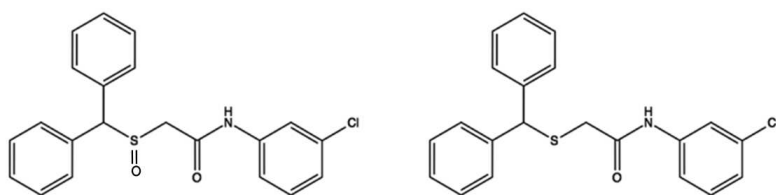


Figure 2. 2-[(diphenylmethyl)sulfinyl]-N-(3-chlorophenyl)acetamide Figure 2(a) left, 2-[(diphenylmethyl)thio]-N-(3-chlorophenyl)acetamide Figure 2(b) right

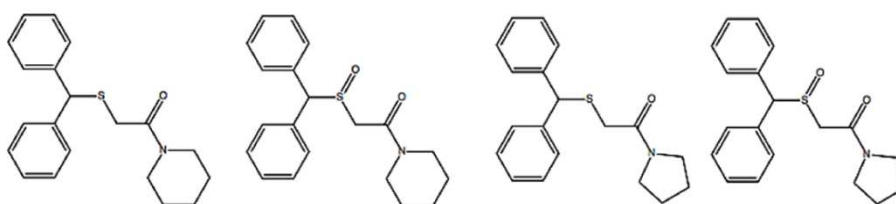


Figure 3. Compounds 3(a) - 3(d) left to right

Lari [27] has examined the effect of modafinil and its analogue 2-[(diphenylmethyl)sulfinyl]-N-(3-chlorophenyl)acetamide (compound 2(a) in Figure 2) on wakefulness using the phenobarbital-induced loss of the righting reflex (LORR) and other psychobiological tests (exploratory activity, depression, anxiogenic and anxiolytic like effects) in albino mice. It was found that 2-[(diphenylmethyl)sulfinyl]-N-(3-chlorophenyl)acetamide had a comparable but weaker efficacy compared to modafinil against these tests.

The enhanced stimulatory effect found by Zhu for 2-[(diphenylmethyl)thio]-N-(3-chlorophenyl)acetamide over that for modafinil has been found to be due to the elongation of the S---CH₂ bond upon electron addition, whereas modafinil undergoes elongation of the benzylic methine C---S bond. Figure 4 shows the dissociative electron attachment of 2-[(diphenylmethyl)thio]-N-(3-chlorophenyl)acetamide in water. The S---CH₂ bond length is 2.60 Å, the benzylic methine C(H)---S bond is 1.88 Å in the radical anion TS, compared to values of 1.845 Å and 1.88 Å respectively in the neutral starting compound. It was found that different conformations of the S-CH₂-C(=O)NH(3Cl-C₆H₄) moiety had little effect on the ease of S---CH₂ bond elongation upon electron attachment. The ΔG_{act}^* for the TS state shown in Figure 4 was -17.0 kcal/mol.

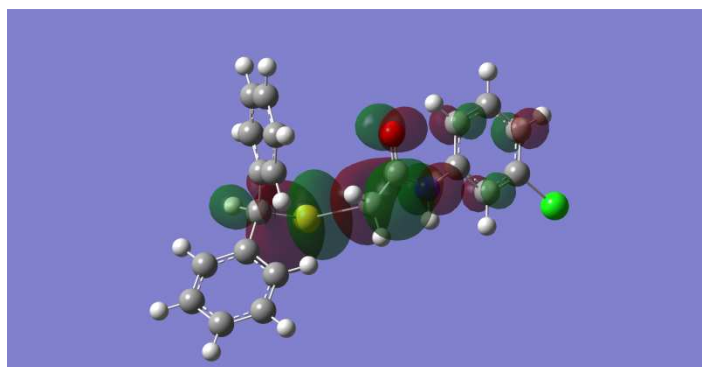


Figure 4. Dissociative electron attachment of 2-[(diphenylmethyl)thio]-N-(3-chlorophenyl)acetamide in water showing the HOMO mainly located over the S-CH₂-C(=O)NH(3Cl-C₆H₄) moiety.

The behaviour of 2-[(diphenylmethyl)sulfinyl]-N-(3-chlorophenyl)acetamide (compound 2(a) in Figure 2) upon adiabatic electron attachment in water is dependent upon the conformation of the sulphinyl-N-(3-chlorophenyl)acetamide moiety with respect to the 2-[(diphenylmethyl)sulfinyl] moiety. Figure 5(b) shows when -S-N-(3-chlorophenyl)acetamide moiety is coplanar, dissociative electron attachment results in elongation of the benzylic diphenylmethyl C(H)---S(=O) bond, (2.48 Å, with CH₂-S bond length essentially unchanged from the starting compound) whereas as shown in Figure 5(a) when the -S-N-(3-chlorophenyl)acetamide moiety is not coplanar, elongation of the S(=O)---CH₂ bond (2.97 Å with (C₆H₅)₂CH-S bond length essentially unchanged from starting compound) occurs instead. The location of the delocalized HOMOs clearly shows why this behaviour occurs, since in Figure 5(b) the excess electron HOMO is delocalized over the diphenylmethylsulphinyl group, whereas in Figure 5(a) the excess electron HOMO is delocalized over the -S-N-(3-chlorophenyl)acetamide moiety. These results show that the electron attachment dissociative bond lability of the benzylic methine C(H)---S(=O) and the S(=O)---CH₂ bonds are quite similar.

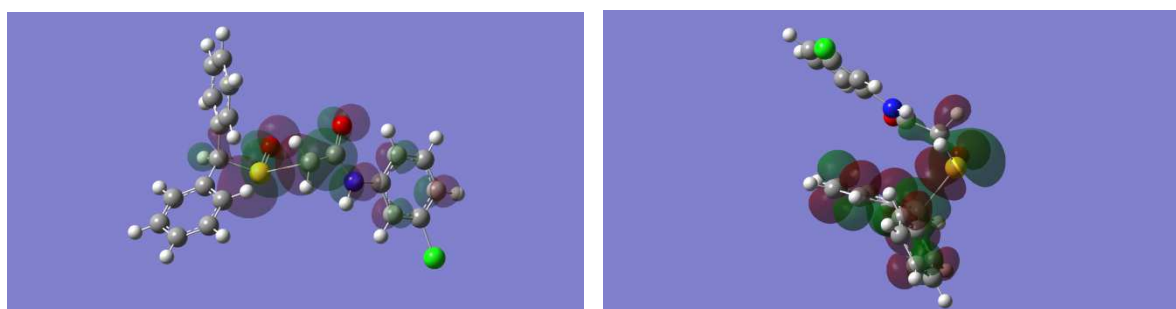


Figure 5. Dissociative electron attachment of 2-[(diphenylmethyl)sulphinyl]-N-(3-chlorophenyl)acetamide in water: (a) the left conformation with the non planar -S-N-(3-chlorophenyl)acetamide moiety shows the HOMO mainly located over the -S-N-(3-chlorophenyl)acetamide moiety and elongation of the S(=O)---CH₂ bond, (b) the right conformation with the planar -S-N-(3-chlorophenyl)acetamide moiety shows the HOMO mainly located over the diphenylmethylsulphinyl moiety and elongation of the benzylic methine C(H)---S(=O) bond.

Examination of the substituted modafinil analogue shown in Figure 6 shows the same behaviour upon electron attachment as that for modafinil with elongation of benzylic C---S bond. This analogue has a high DAT binding affinity ($K_i = 2.5$ nM) [28][Cao 2016] compared to modafinil ($K_i = 3800$ nM), and it was thought that the atypical DAT inhibitory behaviour might be useful in psychostimulant abuse medication.

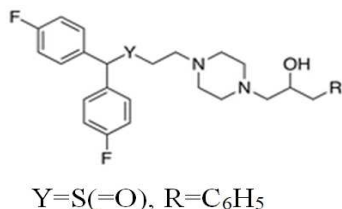


Figure 6

Fluorenyl analogues of modafinil

Another analogue which shows quite different behaviour to modafinil is the compound 9-fluorenyl-S(=O)-CH₂-C(=O)-(4N-piperazine-1N)-C(=O)-CH₃ [6,7][Dunn 2012] which shows a weak DAT inhibitory effect compared to modafinil but a potent eugeroic effect. Figure 7 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-sulphoxide moiety, with the LUMO delocalized over the fluorenyl group. The free energy of ΔG^*_{act} for the bond elongation (2.50 Å compared to the starting compound 1.90 Å) shown for the fluorenyl analogue in Figure 7 is estimated to be about -23.1 kcal/mol, similar to that shown for modafinil in Figure 1(a) where the ΔG^*_{act} for the bond elongation to 3.02 Å was -15.4 kcal/mol. The results are indicative and not directly comparable since ΔG^*_{act} are somewhat dependent on the conformations of the modafinil and the fluorenyl analogue.

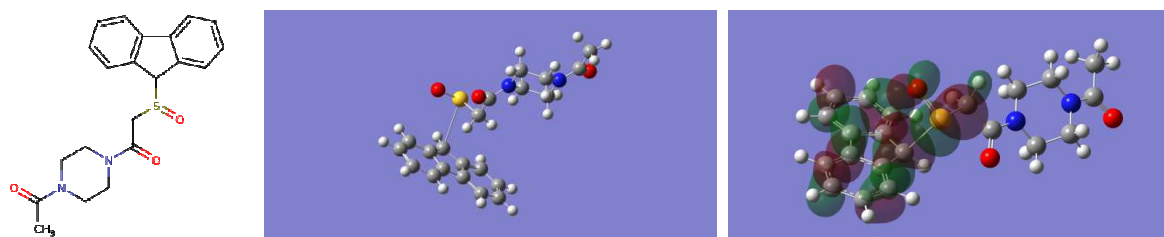


Figure 7 9-fluorenyl-S(=O)-CH₂-C(=O)-(4N-piperazine-1N)-C(=O)-CH₃ and the transition state for dissociative electron attachment in water showing the HOMO located over the 9-fluorenyl-S(=O)- moiety

However Dunn [6,7] reported that fluoreneol which is the 9-hydroxyfluorene derivative formed from 9-Fluorenyl-S(=O)-CH₂-C(=O)-(4N-piperazine-1N)-C(=O)-CH₃ (see Figure 8) itself shows a stronger eugeroic effect but a 59% weaker DAT binding ability than modafinil. This result is unusual since fluoreneol is vastly structurally different from modafinil indicating a quite different mechanism to that of modafinil. Since the fluorene moiety can easily

accommodate electron attachment, an investigation of electron attachment to fluorenil (hydrafnil) 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)-CH₂-C(=O)-(4N-piperazine-1N)-C(=O)-CH₃. However, an examination of electron attachment to the protonated fluorenil species 9-fluoreneOH₂⁺ did show fluorene---OH₂⁺ bond elongation 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. [29][Magnotta 2012] The electroreduction of the bond cleavage of the C-OH bond of 9-fluorenil has been previously shown to be initiated by electron transfer.[30][Mendkovich 2016]

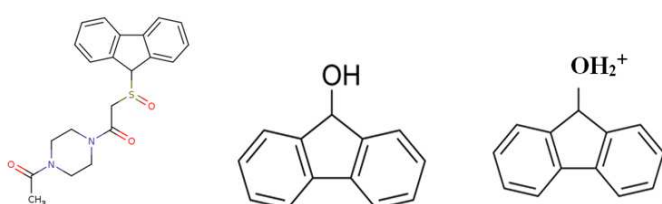


Figure 8 showing the 9-fluorenyl-S(=O)-CH₂-C(=O)-(4N-piperazine-1N)-C(=O)-CH₃, 9-fluorenil and protonated 9-fluorenil

Biphenyl analogues of modafinil

Dunn 2012 [6,7] and Louvet 2012 [31] 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 9). 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 (C₆H₅)₂CH- and 9-fluorenyl moieties (see Figures 1(a) and 7) can better stabilize electron density than can the benzylic moieties in Figure 9, and the TSs for CH₂---S bond dissociation in these two drugs requires a second electron to be attached to activate the CH₂---S bond. The free energy of activation ΔG^*_{act} for the water solvated ether analogue transition state shown in Figure 9 is estimated to be about -44 kcal/mol, which compares to the TS shown in Figure 1(a) for modafinil of -15.4 kcal/mol.

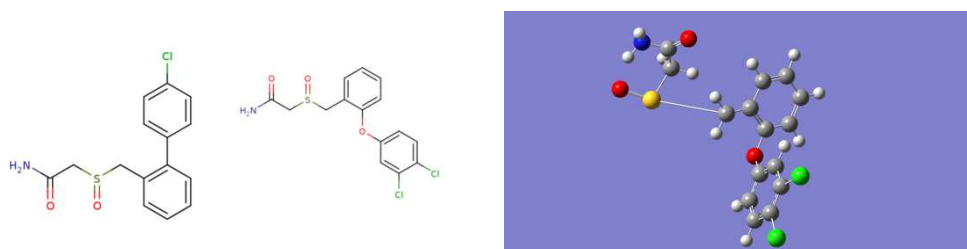


Figure 9 Showing biphenyl and ether analogues and transition state for two electron dissociative electron attachment to the ether compound.

Sulphide analogue of modafinil

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. [32][Farnia 1978] Since the sulphide modafinil analogues 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 modafinil. It was found that $(C_6H_5)_2CH-S-CH_2C(=O)NH_2$ 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 10 is estimated to be -25.5 kcal/mol in water which can be compared to a value of -15.4 kcal/mol for the modafinil TS in Figure 1(a). The same dissociative behaviour was observed in dimethylformamide as in water.

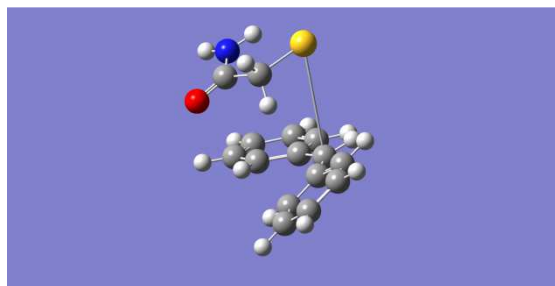


Figure 10. Transition state for dissociative electron attachment of $(C_6H_5)_2CH-S-CH_2C(=O)NH_2$ in water

Discussion

The effectiveness of eugeroic drugs and their cognition altering neurochemical action is dependent on the bioavailability of the drug, particularly its ability to enter brain and other cells. The ability of modafinil analogues to cross the blood brain barrier and cell membranes, and bind to the DAT, has been previously examined using equation 1. The modafinil-like analogues used to construct eq 1 are closely structurally related to modafinil itself.

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 modafinil-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. [26][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 $R = Et, piperidine$ or *morpholine* these compounds were found to be sedatives. For the series $(C_6H_4X)_2CHS(=O)CH_2C(=O)NH_2$ where $(X,X) = (4-F,4-H), (4-Cl,4-H)$ and $(4-F,4-H)$, these analogues were also found to be stimulants. These CNS activities were measured using electrically-evoked tritiated serotonin ($[^3H]5-HT$) efflux from rat cortical slices. [32][De Risi 2008] It is noted that the modafinil-like analogues studied by Lari [27][Lari

2013] and De Risi [33][De Risi 2008] all showed similar DET behaviour to modafinil upon electron attachment. These in-vivo results overall suggest that substitutions at the amide N group or at the phenyl groups adjacent to the sulphoxide moiety have an effect on CNS activity, with the majority of these substitutions resulting 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. The animal cognition altering studies of Lari [27] and De Risi [33] on modafinil-like analogues suggests that modafinil-like analogues may have human cognitive enhancement properties similar to those found for modafinil [12][Battleday] if the appropriate substitutions are made to the basic modafinil framework. It has been observed that there is a relationship between the administered modafinil dose and the emotional modulation of working memory performance of stressed mice, ie there was a decrease in the efficiency performance threshold of administered modafinil under stress. [34][Pierard] Recent reviews have examined mitochondrial stress transduction and the links between psychosomatic medicine, psychoneuroendocrinology, psychoneuroimmunology, and mitochondrial ROS and the electron transport chain. [35,36][Picard 2018]

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. [1,3,10, 11, 37][Zare 2016, Gerrard 2007, Ornell 2014, Villafuerte 2015, 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. [38,39][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, with cleavage of the methine C---S bond. [32][Farnia 1978] This observation 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 methine C---S bond cleavage in water and dimethylformamide.

The dissociative electron attachment behaviour shown by modafinil and modafinil-like analogues is strongly indicative of a free radical mechanism for eugeroic and 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 quite 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 the aryl groups.

However, as shown by the DET behaviour of 2-[(diphenylmethyl)sulfinyl]-N-(3-chlorophenyl)acetamide, 2-[(diphenylmethyl)thio]-N-(3-chlorophenyl)acetamide, the substitution of appropriate groups at the acetamide moiety can lead to quite different eugeroic capacity when the sulphinyl group is replaced by the sulphide group (see Figure 4). The enhanced stimulatory effect for 2-[(diphenylmethyl)thio]-N-(3-chlorophenyl)acetamide over that for modafinil has been found to be due to the elongation of the S---CH₂ bond upon electron addition, whereas modafinil undergoes elongation of the benzylic methine C---S bond (see Figure 4). It was also shown in Figure 5(a) and (b) that 2-

[(diphenylmethyl)sulphonyl]-N-(3-chlorophenyl)acetamide that substituted N-acetamide groups in the appropriate conformation can give completely different DET behaviour. These observations can allow appropriate ab initio design of eugeroic behaviours in modafinil-like molecules. For example, substitution of the $-C(=O)NH_2$ group of modafinil by a $-C(=O)-NH-R$ group where the R group can accommodate the excess electron after DET (by delocalizing the HOMO over the $-C(=O)-NH-R$ group) can completely alter the resultant dissociative behaviour and the resultant eugeroic effect.

The observation that 9-Fluorenyl-S(=O)-CH₂-C(=O)-(4N-piperazine-1N)-C(=O)-CH₃ 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. [6,7][Dunn 2012]

Conclusions

Evidence is presented that strongly implicates the involvement of free radical species in the eugeroic and cognitive altering ability of modafinil and modafinil-like analogues via a dissociative electron transfer or attachment mechanism. Examination of modafinil and modafinil-like analogues which have been shown *in the literature* to exhibit wake promoting and cognitive psychobiological properties are shown in this study to produce free radicals when subject to electron attachment. Such observations are consistent with a free radical oxidative stress eugeroic and cognitive altering mechanism for modafinil and related analogues. It has also been shown that appropriate substitution of the acetamide moiety of modafinil can result in quite different DET behaviour, which holds promise of the ability to design and predict eugeroic and cognitive behaviour. In so far as DAT binding is a determinant of eugeroic and cognitive enhancing behaviour of modafinil-like analogues, the LFER with DAT binding can also help identify the molecular properties which govern binding behaviour.

Experimental

All calculations were carried out using the Gaussian 09 package. Energy optimisations for the various drugs 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. Anion optimisations, energy and thermodynamic calculations were conducted at the DFT/B3LYP/6-31⁺G(d) (6d, 7f) level of theory with optimised geometries in water, using the IEFPCM/SMD solvent model. Similar results were obtained at the DFT/B3LYP/6-311⁺G(d,p) (6d, 7f) and DFT/wB97XD/6-31⁺G(d) (6d,7f) levels of theory. It was found that the free energy of ΔG^* for bond elongations was not only dependent upon the particular conformation of modafinil and the studied analogues, but also on the level of theory. That is, ΔG^* determined at the DFT/B3LYP/6-31⁺G(d) (6d, 7f) level of theory were lower than those conducted at a DFT/B3LYP/6-31G(d,p) (6d, 7f) level of theory. This observation is consistent with a greater delocalization of the excess electron in the HOMOs using diffuse basis sets, and as shown in the results it is the location of the HOMO that determines bond elongation in the transition state upon

electron attachment. The modest dependency on basis set and diffuse functions in DET processes have been previously noted. [40][Soriano] However, only the degree (%) of elongation of bonds in the TSs of the DET reactions are dependent on basis sets or functionals. The wB97XD functional is known to better account for long range dispersion effects than the B3LYP functional.

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. [41][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. [42,43][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 (C₆H₅)₂CH ion. [44][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 equation have much higher experimental uncertainties than the calculated molecular properties. The statistical analyses include the multiple correlation coefficient R², the F test of significance, standards errors for the estimates (SEE) and each of the variables SE($\Delta G_{\text{desolCDS}}$), SE($\Delta G_{\text{lipoCDS}}$), SE(Dipole Moment), SE (Molecular Volume), as calculated from “t” distribution statistics. Residual analysis was used to identify outliers.

References

- [1] Zare B, Moosavi B, Torabi-Nami M. Wake-Promoting Agents, Insights into Clinical Use and Molecular Perspectives. *J Adv Med Sci Appl Technol (JAMSAT)*, 2016, 2:129-139
- [2] Kim D, Practical Use and Risk of Modafinil, a Novel Waking Drug, *Environmental Health and Toxicology*, 2012, 27: Article ID: e2012007
- [3] Gerrard P, Malcolm R. Mechanisms of modafinil: A review of current research. *Neuropsychiatric Disease Treatment*, 2007, 3: 349–364
- [4] Wisor J. Modafinil as a catecholaminergic agent: empirical evidence and unanswered questions, *Frontiers Neurology*, 2013, 4: 1-10
- [5] Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM. Dopaminergic role in stimulant-induced wakefulness. *J Neurosci*. 2001, 1: 1787-94.
- [6] Dunn D, Hostetler G, Iqbal M, Marcy VR, Lin, Yin G, Jones B, Aimone LiD, Gruner J, Ator MA, Bacon ER, Chatterjee S. Wake promoting agents: Search for next generation modafinil, lessons learned: Part III. *Bioorg Med Chem Lett*. 2012, 22: 3751–3753.
- [7] Dunn D, Hostetler G, Iqbal M, Messina-McLaughlin P, Reiboldt A, Lin YG,

- Gruner J, Bacon ER, Ator MA, Chatterjee S, Wake-promoting agents: Search for next generation modafinil: Part I. *Bioorganic & Medical Chemistry Letters* 2012, 22: 2312-2314.
- [8] Turner C, Belyavin A, Nicholson A. Duration of activity and mode of action of modafinil: Studies on sleep and wakefulness in humans. *J. Psychopharmacol.* 2013, 3:643-654.
- [9] Mereu M, Bonc, A, Hauck Newman Amy, Tanda G. The neurobiology of modafinil as an enhancer of cognitive performance and a potential treatment for substance use disorders. *Psychopharmacology*, 2013, 229: 415–434
- [10] Ornell F, Valvassori SS, Steckert AV, Deroza PF, Resende WR, Varela RB, Quevedo J. Modafinil Effects on Behavior and Oxidative Damage Parameters in Brain of Wistar Rats. *Behav Neurol*, Volume 2014, 7 pages, Article ID 917246, <http://dx.doi.org/10.1155/2014/917246>
- [11] Villafuerte G, Miguel-Puga A, Rodríguez EM, Machado S, Manjarrez E, Arias-Carrión O. Sleep Deprivation and Oxidative Stress in Animal Models: A Systematic Review, *Oxidat Med Cell Longevity*, Volume 2015, Article ID 234952, 15 pages <http://dx.doi.org/10.1155/2015/234952>
- [12] Battleday RM, Brem AK, Modafinil for cognitive neuroenhancement in healthy non-sleep-deprived subjects: a systematic review, *Eur Neuropsychopharmacology*, 2015, 25:1865-81
- [13] Minzenberg MJ, Carter CS, Modafinil: A Review of Neurochemical Actions and Effects on Cognition, *Neuropsychopharmacology*, 2007:1–26, doi:10.1038/sj.npp.1301534
- [14] Fong CW, Permeability of the Blood–Brain Barrier: Molecular Mechanism of Transport of Drugs and Physiologically Important Compounds. *J Membr Biol.* 2015, 248: 651-69.
- [15] Fong CW, Drug discovery model using molecular orbital computations: tyrosine kinase inhibitors. HAL Archives, hal-01350862v1, 2016
- [16] Fong CW, The extravascular penetration of tirapazamine into tumours: a predictive model of the transport and efficacy of hypoxia specific cytotoxic analogues and the potential use of cucurbiturils to facilitate delivery. *Int. J. Comput Biol Drug Design*, 2017, 10: 343-373
- [17] Fong CW, The effect of desolvation on the binding of inhibitors to HIV-1 protease and cyclin-dependent kinases: Causes of resistance. *Bioorg Med Chem Lett.* 2016, 26: 3705–3713.
- [18] Fong CW, Predicting PARP inhibitory activity – A novel quantum mechanical based model. HAL Archives, hal-01367894v1, 2016
- [19] Fong CW, A novel predictive model for the anti-bacterial, anti-malarial and hERG cardiac QT prolongation properties of fluoroquinolones. HAL Archives, hal-01363812v1, 2016
- [20] Fong CW, Statins in therapy: Cellular transport, side effects, drug-drug interactions and cytotoxicity - the unrecognized role of lactones. HAL Archives, hal-01185910v1, 2016
- [21] Fong, CW, 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. HAL Archives, hal-01693225v2, 2018
- [22] Cao J, Prisinzano TE, Okunola OM, Kopajtic T, Shook M, Katz JL, Hauck Newman A. SARs at the Monoamine Transporters for a Novel Series of Modafinil Analogues. *ACS Med. Chem. Lett.* 2011, 2: 48–52
- [23] Saveant JM, Dissociative electron transfer. *Adv Electron Transfer Chem*, 1994, 4: 53-116
- [24] Maran F, Workentin MS. Dissociative electron transfer. *Electrochemical Society Interface Winter* 2002, 44-49
- [25] Zhan CG, Dixon DA. The Nature and Absolute Hydration Free Energy of the Solvated Electron in Water. *J. Phys. Chem. B*, 2003, 107: 4403–4417

- [26] Zhu X, Tang Y, Goa C, Zhang LQ, Huang WL. Synthesis and Biological Evaluation of Novel Diphenylthio-Acetamide Derivatives. *Acta Pharmaceutica Sinica* 2013, 48:371-376.
- [27] Lari A, Karimi I, Adib H, Aliabadi A, Firoozpour L, Foroumadi A. Synthesis and psychobiological evaluation of modafinil analogs in mice. *DARU J Pharmaceut Sci* 2013, 21:67-75
- [28] Cao J, Slack RD, Bakare OM, Burzynski C, Rais R, Slusher BS, Kopajtic T, Bonifazi A, Ellenberger MP, Yano H, He Yi, Bi GH, Xi ZX, Loland CJ, Hauck Newman A. Novel and High Affinity 2-[(Diphenylmethyl)sulfinyl]acetamide (Modafinil) Analogues as Atypical Dopamine Transporter Inhibitors. *J. Med. Chem.*, 2016, 59: 10676–10691
- [29] Magnotta VA, Heo HY, Dlouhy BJ, Dahdaleh NS, Follmer RL, Thedens DR, Welsh MJ, Wemmie JA. Detecting activity-evoked pH changes in human brain. *PNAS*, 2012, 109: 8270-8273
- [30] Mendkovich AS, Syroeshkin MA, Nasybullina DV, Mikhailov MN, Rusakov AI, C-OH bond cleavage initiated by electron transfer: Electroreduction of 9-fluoreno. *Electrochimica Acta* 2016, 191: 962-973.
- [31] Louvet P, Schweizer D, Gourdel ME, Riguet E, Yue C, Marcy VR, Lin Y, Gruner J, Lesur B, Bacon ER, Chatterjee S. Wake-promoting agents: Search for next generation modafinil: Part IV. *Eur. J. Med. Chem.*, 2012, 54: 949-951
- [32] Farnia G, Severin MG, Capobianco G, Vianello E. Electrochemical reduction mechanism of organic sulphides. Diphenylmethyl *p*-nitrophenyl sulphide. *J. Chem. Soc., Perkin Trans. 2*, 1978, 0:1-8
- [33] De Risi C, Ferraro L, Pollini GP, Tanganelli S, Valente F, Veronese AC, Efficient synthesis and biological evaluation of two modafinil analogs. *Bioorg Med Chem* 2008, 16, 9904–9910.
- [34] Pierard C, Liscia P, Valleau M, Drouet I, Chauveau F et al. Modafinil-induced modulation of working memory and plasma corticosterone in chronically-stressed mice. *Pharmacology Biochemistry and Behavior / Pharmacology Biochemistry and Behaviour*, 2006, 83:1-8.
- [35] Picard M, McEwen BS. Psychological Stress and Mitochondria: A Conceptual Framework. *Psychosom Med.* 2018, 80:126–140
- [36] Picard M, McEwen BS. Psychological Stress and Mitochondria: A Systematic Review. *Psychosom Med.* 2018; 80: 141–153.
- [37] Piérard C, Satabin P, Lagarde D, Barrère B, Guezennec CY, Menu JP, Pérès M. Effects of a vigilance-enhancing drug, modafinil, on rat brain metabolism: a 2D COSY 1H-NMR study. *Brain Res.* 1995, 693: 251-6
- [38] Rosenblum WI, El-Sabban F. Dimethyl sulfoxide (DMSO) and glycerol, hydroxyl radical scavengers, impair platelet aggregation within and eliminate the accompanying vasodilation of injured mouse pial arterioles. *Stroke* 1982, 13: 35-9.
- [39] Sprayberry K A, Robinson NE, Robinson's Current Therapy in Equine Medicine, (Seventh Edition), Elsevier 2015, Ch 177, Section XVI, p 744, ISBN: 978-1-4557-4555-5
- [40] Soriano A, Silla E, Tunon I, A quantum mechanics-molecular mechanics study of dissociative electron transfer: The methylchloride radical anion in aqueous solution, *J Chem Phys*, 2002, 116:6102-6110.
- [41] Marenich AV, Cramer CJ, Truhlar DJ. Universal Solvation Model Based on Solute Electron Density and on a Continuum Model of the Solvent Defined by the Bulk Dielectric Constant and Atomic Surface Tensions. *J Phys Chem B*, 2009, 113: 6378 -96
- [42] Rayne S, Forest K. Accuracy of computational solvation free energies for neutral and ionic compounds: Dependence on level of theory and solvent model. *Nature Proceedings*, 2010, <http://dx.doi.org/10.1038/npre.2010.4864.1>.

- [43] Rizzo RC, Aynechi T, Case DA, Kuntz ID. Estimation of Absolute Free Energies of Hydration Using Continuum Methods: Accuracy of Partial Charge Models and Optimization of Nonpolar Contributions. *J Chem Theory Comput.* 2006, 2: 128-139.
- [44] Dubey S, Ahi S, Reddy I M, Kaur T, Beotra A, Jain S. A novel study of screening and confirmation of modafinil, adrafinil and their metabolite modafinilic acid under EI-GC-MS and ESI-LC-MS-MS ionization. *Indian J Pharmacol* 2009, 41: 278-83