Will 1,2-dihydro-1,2-azaborine-based drugs resist metabolism by cytochrome P450 compound I?

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Abstract: 1,2-dihydro-1,2-azaborine is a structural and electronic analogue of benzene which is able to occupy benzene-binding pockets in T4 lysozyme and has been proposed as suitable arene-mimicking group for biological and pharmaceutical applications. Its applicability in a biological context requires it to be able to resist modification by xenobiotic-degrading enzymes like the P450 cytochromes. Quantum chemical computations described in this work show that 1,2-dihydro-1,2-azaborine is much more prone to modification by these enzymes than benzene, unless steric crowding of the ring prevents it from reaching the active site, or otherwise only allows reaction at the very sluggish C_4-position. This novel heterocyclic compound is therefore expected to be of limited usefulness as an aryl bioisostere.

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Introduction

1,2-dihydro-1,2-azaborine (abbreviated in this paper as "azaborine") is a structural and electronic analogue of benzene which, like benzene, undergoes classical electrophilic aromatic substitution(Pan, Kampf & Ashe, 2007) but, in contrast to benzene, also readily undergoes nucleophilic aromatic substitution under mild reaction conditions(Lamm et al., 2011). Computational studies have shown azaborines to be generally much more reactive towards one-electron oxidation and electrophilic substitution than their corresponding benzene analogues(Silva & Ramos, 2009).Azaborines are generally stable in water and react sluggishly with oxygen when substituted on their boron atoms with electron-withdrawing substituents (Lamm & Liu, 2009). These benzene isosteres are able to occupy benzene-binding pockets in T4 lysozyme(Liu et al., 2009) and have been proposed as suitable arene-mimicking groups for biological and pharmaceutical applications(Marwitz et al., 2007). Their deployment as useful components of drug scaffolds requires, however, that they are stable in the presence of drug-metabolizing enzymes such as the P450 cytochromes which hydroxylate the related benzene ring (Guengerich, 2003, 2008).

The active oxidant species of cytochrome P450 (Compound I) is a thiolate-bound heme compound which possesses two unpaired electrons in its Fe=O moiety and one unpaired electron delocalized throughout the porphyrin ring and the thiolate ligand (Schöneboom et al., 2002 and references therein). Depending on the orientation of this lone spin relative to the Fe=O-localized spins, compound I may exist in a doublet (S=1/2) or a quartet (S=3/2) state, which have very similar energies(Rydberg, Sigfridsson & Ryde, 2004 and references therein). Extensive experimental and computational investigations on the reaction of compound I towards benzene and other aromatic compounds (Guroff et al., 1967; Jerina et al., 1968; Burka, Plucinski & Macdonald, 1983; Koop, Laethem & Schnier, 1989; Korzekwa, Swinney & Trager, 1989; Koerts et al., 1998; de Visser & Shaik, 2003; Bathelt et al., 2003; Bathelt, Mulholland & Harvey, 2008) have shown that the initial formation of a σ-adduct between

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compound I and the aromatic compound is endergonic and that the subsequent formation of different products (arene oxides, phenols, or ketones) is ruled by a complex potential energy surface, which is sensitive to the reaction environment and to the mode of attack of the benzene (either perpendicular or parallel to the plane of the porphyrin ring). In this paper, we analyze the metabolic stability of 1,2-azaborines towards P450 enzymes through the computational investigation of their reactions with "compound I".

Computational methods

The geometries of every molecule described were optimized using B3LYP(Lee, Yang & Parr, 1988; Becke, 1993; Hertwig & Koch, 1995). Autogenerated delocalized coordinates(Baker, Kessi & Delley, 1996) were used in geometry optimizations performed with 6-31G(d)(Ditchfield, Hehre & Pople, 1971; Hehre, Ditchfield & Pople, 1972) for all elements except for Fe, which used the SBKJ VDZ(Stevens et al., 1992) basis set in combination with the SBKJ pseudo-potential(Stevens et al., 1992) for the inner shells corresponding to the (1s2s2p) core of Fe. Single-point energies of the DFT-optimized geometries were then calculated using the same functional using the 6-311+G(2d,p)(Hariharan & Pople, 1973; Krishnan et al., 1980; Clark et al., 1983; Frisch, Pople & Binkley, 1984) basis set for all elements except Fe, which used the s6-31G* basis set, specifically developed by Swart *et al.* to afford more reliable spin-state splittings (Swart et al., 2010). Zero-point vibrational effects (ZPVE) were computed using a scaling factor of 0.9804 for the computed frequencies. Atomic charge and spin density distribu- tions were calculated with a Mulliken population analysis(Mulliken, 1955) based on symmetrically orthogonalized orbitals(Löwdin, 1970). All energy values described in the text include solvation effects (ε=10) computed using the Polarizable Continuum Model (Tomasi & Persico, 1994; Mennucci & Tomasi, 1997; Cossi et al., 1998) implemented in Firefly. All computations were performed with the Firefly(Granovsky, 2013) quantum chemistry package, which is partially based on the GAMESS (US)(Schmidt et al., 1993) source code. Intra- and inter-molecular dispersion effects on the energies of

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the optimized species were computed with the DFT-D3 formalism developed by Grimme *et al.* (Grimme et al., 2010).

Results

The experimental rates of benzene hydroxylation by the thiolate-bound compound I present in cytochrome P450 and haloperoxydases range from 4.6 min^{-1} (Koop et al., 1989) to 8 s^{-1} (Karich et al., 2013), which translate to activation free energies from 16.9 $kcal \cdot mol^{-1}$ to 19.8 $kcal \cdot mol^{-1}$. The computationally-derived activation energies vary from 12 $kcal \cdot mol^{-1}$ to 21 $kcal \cdot mol^{-1}$, depending on the theory level, model size, and inclusion (or not) of ZPVE, dispersion effects, or solvation (Table 1). Analysis of the susceptibility of 1,2-dihydro-1,2-azaborine to attack by compound I therefore required us to start our investigation by determining the influence of our theory level on the energetic barrier of the analogous reaction of benzene.

In the doublet potential energy surface (Figure 1), we observed that the electronic structure of the reaction product depends on the aryl mode of attack: when benzene approaches the doublet state of compound I perpendicularly to the porphyrin ring ("side-on" in the nomenclature of (Bathelt et al., 2004)), half an electron is transferred from the benzene to the Fe ligands (porphyrin and thiolate) with concomitant spin rearrangements, which lead to the loss of one spin from the Fe-O moiety , mostly to the thiolate ligand (0.52 spin) and substrate (0.32 spin). In contrast, a parallel mode of attack ("face-on" in the nomenclature of (Bathelt et al., 2004)) yields the transfer of almost a full spin (0.86) (but no charge) from the thiolate and porphyrin to the benzene. These results are similar to the observation of a cation-like and a radical-like adduct by (Bathelt et al., 2004), though these workers were able (unlike us) to find both adducts with either attack mode.

Without taking into account zero-point vibrational effects, the quartet state of compound I lies only 0.4 $kcal \cdot mol^{-1}$ above the doublet state, and the quartet portential energy surface is therefore very accessible.

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In this spin state, no dramatic differences in electronic structure were found between both attack modes, which always yield a radical-like adduct on the benzene. In the perpendicular attack mode, the quartet state has the same energetic barrier has the doublet state, but produces a more stable product. Such a competitive benzene hydroxylation in the quartet state has not been found by earlier workers, whose studies on the subsequent rearrangement of the compound I/benzene adduct to yield phenol, ketone or epoxide (Bathelt et al., 2008) focused only on the doublet surface due to the higher activation energies they observed for the formation of the compound I/benzene adduct in the quartet state.

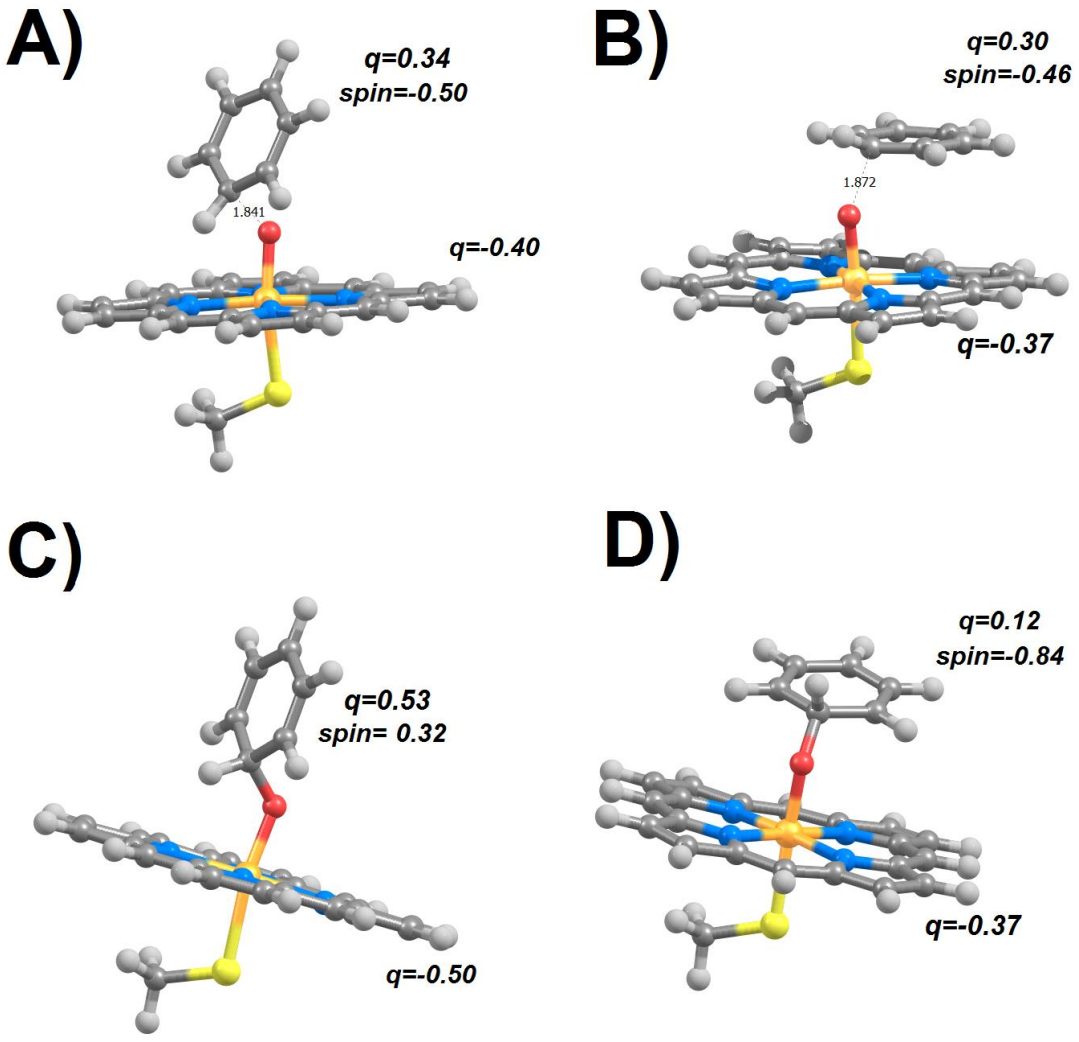

Figure 1: Transition states (A and B) and products (C and D) arising from perpendicular (A and C) or parallel (B and D) attack of benzene by compound I in the doublet (S=1/2) state. Charges (and spins) on the substrate moiety and on the porphyrin ring are highlighted.

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Table 1: Energies (in kcal·mol^{-1}, *vs.* the reactant state) of the transition states (^{2}TS and ^{4}TS) and products (^{2}product and ^{4}product) of direct attack benzene by compound I. Species preceded by ^{2} are in the doublet (S=1/2) state, whereas those preceded by ^{4} are in the quartet state (S=3/2). These values cannot be directly compared to the experimental barriers due to the neglect of vibrational/rotational/translational contributions to entropy. Inclusion of entropic effects increases barriers by 4-6.5 kcal·mol^{-1} due to the loss of vibrational entropy in the transition state (see Supporting Information).

Level of theory	^{2}TS	^{2}Product	^{4}TS	^{4}Product	Reference
B3LYP (ε=5.7)	17.5-18.1	12.3-13.5	20.6	14.0	(de Visser & Shaik, 2003)
B3LYP (ε=4.0)	15.6-17.9	6.1-6.9	n.d	n.d	(Bathelt et al., 2004)
B3LYP (gas phase only, including ZPVE)	20.7	n.d.	21.1	n.d.	(Rydberg, Ryde & Olsen, 2008)
QM/MM B3LYP/CHARMM27	20.4	n.d.	20.4	n.d.	(Lonsdale, Harvey & Mulholland, 2012)
QM/MM B3LYP-D2/CHARMM27	13.5	n.d.	11.9	n.d.	(Lonsdale et al., 2012)
PBE0 (gas phase only, no ZPVE)	18.8	8.8	24.4	n.d.	(Tomberg et al., 2015)
B3LYP-D3//B3LYP (ε=10.0) (including ZPVE) parallel attack	**16.1**	**7.6**	**21.6**	**7.9**	This work
B3LYP-D3//B3LYP (ε=10.0) (including ZPVE) perpendicular attack	**16.9**	**9.4**	**16.9**	**5.9**	This work

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The energy of the reactant state of compound I towards benzene is mostly independent of the spin state of compound I and of the parallel/perpendicular orientation of benzene. In contrast, the perpendicular orientation of 1,2-dihydro-1,2-azaborine is almost 8 kcal·mol^{-1} more favorable than the parallel orientation, due to the stabilization provided by hydrogen binding between the nitrogen-bound hydrogen and the compound I oxygen in the perpendicular orientation. This difference is not, by any means, the most dramatic when comparing the reactivity of benzene towards that of azaborine, as a large variety of products, transition states and activation energies is observed when compound I is made to react with azaborine, as described in the next paragraphs.

Attack on the azaborine nitrogen atom (Figure 2) is kinetically viable only in the doublet state and with a perpendicular orientation, yielding an azaborine peroxide product (activation energy=9 kcal·mol^{-1}; reaction energy 5 kcal·mol^{-1}). With a parallel orientation, reaction is slow (activation energy=33.4 kcal·mol^{-1}) and yields a high energy intermediate bearing an unusual interaction between the boron moiety of the substrate and one of the porphyrin nitrogens. Surprisingly, reaction in the quartet state yields (like that in the doublet state) an azaborine peroxide product, though with a higher barrier activation energy (18.6 kcal·mol^{-1}). In contrast, attack on the boron atom is extremely fast (with activation energies between 5.5 and 7.7 kcal·mol^{-1}), regardless of the spin state and initial orientation of the substrate (Table 2).

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Table 2: Energies (in kcal·mol^{-1}, *vs.* the most stable reactant state) of the transition states (^{2}TS and ^{4}TS) and products (^{2}product and ^{4}product) for the direct attack of the heteroatoms in 1,2-dihydro-1,2-azaborine by compound I. Species preceded by ^{2} are in the doublet (S=1/2) state, whereas those preceded by ^{4} are in the quartet state (S=3/2). All energy values include solvation effects (ε=10.0), zero-point vibrational energy and dispersion effects. Transition states with activation energies above the activation energy of the reaction of compound I towards benzene are highlighted in bold. Unless otherwise noted, all products are σ-adducts of the substrate. [a]:peroxide product.

	^{2}TS	^{2}Product	^{4}TS	^{4}Product
N (parallel orientation)	**33.4**	20.0	absent	Absent
N (perpendicular orientation)	9.0	5.0[a]	**18.6**	11.0[a]
B (parallel orientation)	5.9	-6.2	5.5	-1.8
B (perpendicular orientation)	7.6	-3.8	6.9	-16.2

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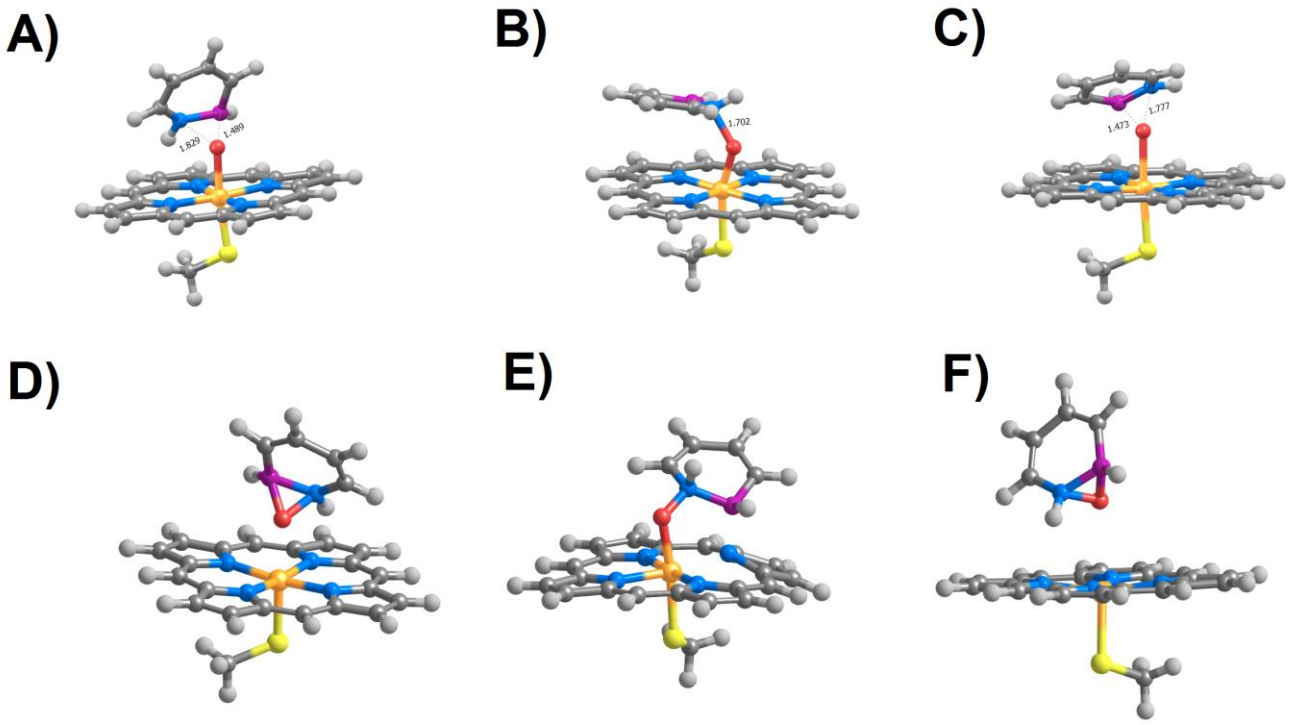

Figure 2: Transition states (A-C) and products (D-F) arising from attack of the nitrogen atom in azaborine by compound I. A and C: S=1/2, perpendicular attack; B and D: S=1/2, parallel attack; E and F: S=3/2.

Previous computational (Silva & Ramos, 2009) and experimental studies (Pan et al., 2007) ascertained that the most reactive carbon positions in azaborine towards classical electrophilic agents are its C_3 and C_5 atoms. Our computations show that the same is true regarding its reaction with the doublet state of compound I: the reaction is spontaneous by at least 47.8 kcal·mol^{-1} at C_3, and by 19 kcal·mol^{-1} at C_5. The reaction products are, however, quite different in both instances: attack on C_3, yields a novel heptagonal ring (3*H*-1,3,2-Oxazaborepine) containing a N-B-O-C moiety, whereas reaction in C_5 must overcome a 13-15 kcal·mol^{-1} barrier and yields epoxides over the C_5-C_6 bond. Both these products assume very similar conformations relative to the heme regardless of the initial orientation of the substrate (parallel or perpendicular) relative to the porphyrin plane (Figure 3).

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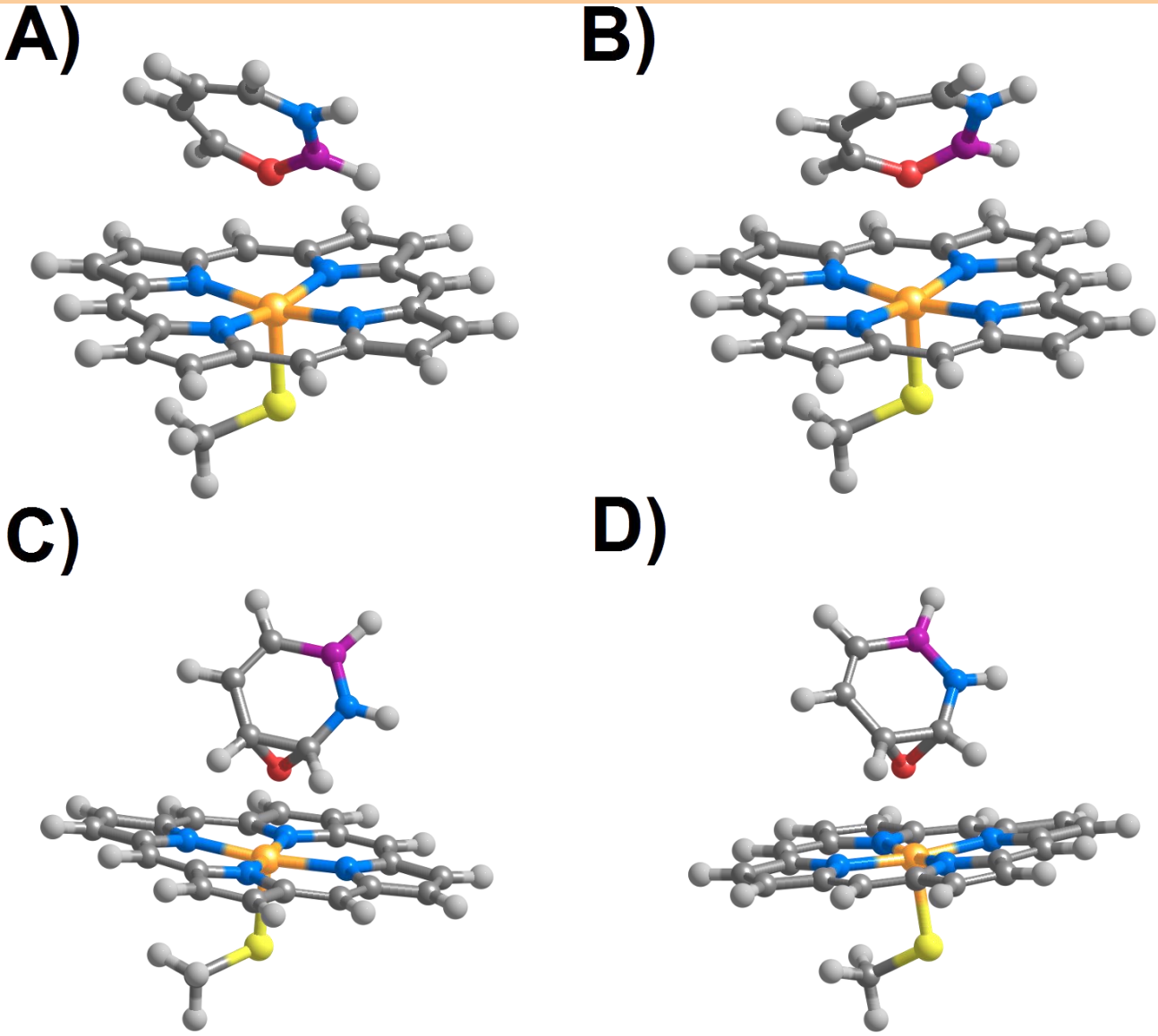

157
158 Figure 3: Products arising from perpendicular (A and C) or parallel (B and D) attack of positions C_3 (A
159 and B) and C_5 (C and D) in 1,2-dihydro-1,2-azaborine by compound I in the doublet (S=1/2) state.

160

161 The search for a transition state for the attack on C_3 showed that the formation of 3*H*-1,3,2-
162 oxazaborepine cannot occur directly from the isolated reactants, as no transition state connects this
163 product to the reactant state: instead, 3*H*-1,3,2-oxazaborepine is formed from the boron-bound
164 azaborine-compound I adduct, after surmounting a small barrier (Figure 4). A second intermediate
165 bearing a C_3-compound I bond was found to be thermodynamically stable (Figure 4, upper right),
166 though kinetically inaccessible due to the absence of any transition state linking it to the isolated

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reactants: it can only be formed (upon crossing an activation barrier above 40 kcal·mol^{-1}) through rearrangement of the extraordinarily stable oxazaborepine.

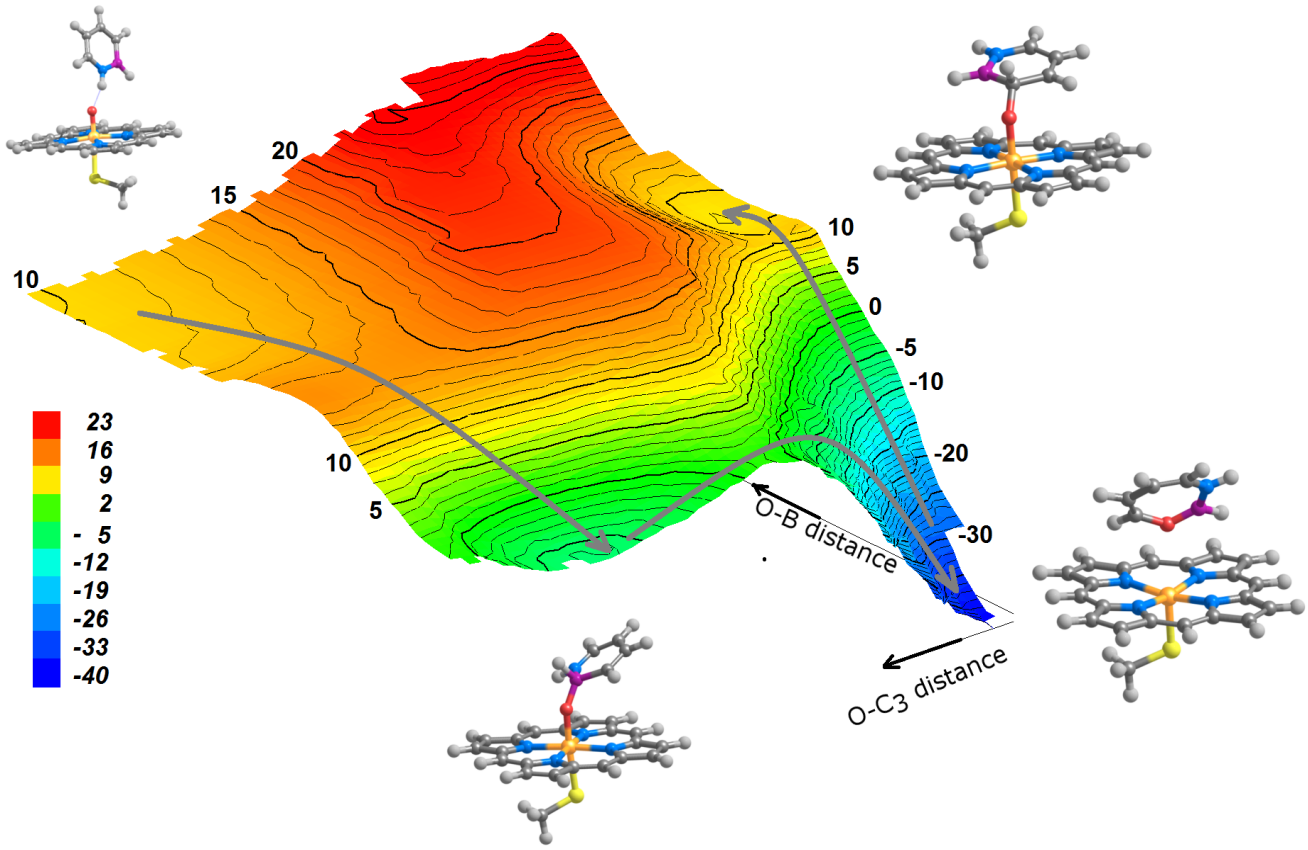

Figure 4: Potential energy surface obtained as B_2 and C_3 approach the reactive oxygen in compound I, computed using B3LYP with the 6-31G(d) basis set for all elements except for Fe, which used the SBKJ VDZ basis set in combination with the SBKJ pseudo-potential for the inner shells corresponding to the (1s2s2p) core of Fe. No solvation or dispersion effects are included. Isoenergetic lines are depicted at 1 kcal·mol^{-1} intervals. Separated reactants with a perpendicular arrangement (corresponding to 0 kcal·mol^{-1}) would lie far to the upper left corner of this depiction of the potential energy surface. Grey arrows show the sequence of transformations allowed as B_2/C_3 atoms approach compound I. 3*H*-1,3,2-oxazaborepine (lower right) is only accessible after the boron-bound adduct (lower left) has been formed, and the C_3-bound compound I intermediate (upper right) is shown to be kinetically inaccessible.

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In the quartet state, attack on C_5 proceeds with a barrier of 17.1 (parallel) or 18.4 kcal·mol^{-1} (perpendicular) and yields epoxides (like the doublet state). In contrast to the doublet state, a parallel attack of the quartet state on C_3 yields a σ–complex similar to that found with benzene. In the perpendicular orientation, the reactivity of the quartet state towards C_3 is, however, identical to that found for the doublet state.

Table 3: Energies (in kcal·mol^{-1}, *vs.* the most stable reactant state) of the transition states (^{2}TS and ^{4}TS) and products (^{2}product and ^{4}product) for the direct attack of carbon atoms in 1,2-dihydro-1,2-azaborine by compound I. Species preceded by ^{2} are in the doublet (S=1/2) state, whereas those preceded by ^{4} are in the quartet state (S=3/2). All energy values include solvation effects (ε=10.0), zero-point vibrational energy and dispersion effects. Transition states with activation energies above the activation energy of the reaction of compound I towards benzene are highlighted in bold. Unless otherwise noted, all products are σ-adducts of the substrate. [a]: formation of 3*H*-1,3,2-oxazaborepine. [b]: formation of a peroxide product.

	^{2}TS	^{2}Product	^{4}TS	^{4}Product
C_3 (parallel orientation)	n.a.	-49.2[a] / 1.2	14.5	2.2
C_3 (perpendicular orientation)	n.a.	-47.8[a] / 1.3	n.a.	-40.2[a] / 1.8
C_4 (parallel orientation)	**21.3**	23.0	**21.8**	11.1
C_4 (perpendicular orientation)	**19.5**	10.8	**20.5**	9.6
C_5 (parallel orientation)	14.8	-19.1[b]	**18.4**	-15.6[b]
C_5 (perpendicular orientation)	13.2	-18.9[b]	**17.1**	-15.4[b]
C_6 (parallel orientation)	13.4	1.5	**21.6**	-0.6
C_6 (perpendicular orientation)	13.1	-2.0	15.2	7.5

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The activation energies for the reactions taking place at the C_4-position are consistently >3 kcal·mol^{-1} higher than the attacks on benzene, regardless of orientation and spin state. In contrast, attacks on C_5 by the doublet state of compound I must surmount a lower barrier than observed for benzene, and yield very stable epoxides over the C_5-C_6 bond. The same products are observed upon attack at C_5 by the quartet state of compound I, though in this instance the activation barriers are 4 kcal·mol-1 above those computed for the doublet state. In spite of its negligible reactivity towards classical electrophiles (Pan et al., 2007; Silva & Ramos, 2009), the C_6-position in azaborine is more susceptible than benzene to attack by the doublet state of compound I in either a parallel or a perpendicular orientation. In the quartet state, the parallel orientation is noticeably less prone to react than the perpendicular orientation, in spite of yielding a more stable intermediate (Table 3).

Discussion

The computations described in this paper show that most ring positions in 1,2-dihydro-1,2-azaborine are much more reactive towards compound I than the benzene ring (for which they have been proposed as biosteres). It is therefore extremely likely that the proposed inclusion of 1,2-dihydro-1,2-azaborine in drug scaffolds will have a very detrimental effect on their ability to remain unscathed in the organism unless measures are taken to ensure that the reactive azaborine portion is sterically unable to reach the active site of P450 enzymes, or that only the very unreactive C_4-position is able to approach compound I.

Supporting Information Geometries and energies of every.intermediate. Input and output files have been deposited in Figshare (https://dx.doi.org/10.6084/m9.figshare.1414338)

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