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Theoretical study of phototoxic reactions of psoralens

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Abstract

The phototoxic reactions between a large set of furocoumarin compounds and molecular oxygen are explored using density functional theory, using a time-dependent formalism for excited states (TD-DFT) and a continuum model to include effects of bulk solvation. The computed singlet and triplet excitation energies throughout agree very well (to within 0.2 eV) with experimental data. It is concluded that the furocoumarin compounds do display phototoxic reactions with molecular oxygen, and that they are able to generate superoxide anions as well as singlet oxygen. However, given the right conditions the generated superoxide anions will in turn serve as reducing agents for triplet psoralens, thereby efficiently scavenging the generation of reactive oxygen species. Direct electron transfer between the drugs and oxygen are also explored, as well as the effect of triplet excitation on autionisation reactions between furocoumarins.

Keywords: Furocoumarins; Psoralens; TD-DFT; Phototoxicity; Excitation energies; Molecular oxygen

1. Introduction

Psoralens, or furocoumarins, form a class of heterocyclic aromatic compounds utilised in photochemotherapy treatment of a variety of skin diseases such as psoriasis, vitiligo, mycosis fungoides, polymorphous light eruption, and more [1–4]. The compounds are present in numerous plants throughout the world. In photochemotherapy, the drug is either applied topically or orally administered, where after the patient is irradiated with UV-A light (320–400 nm). In some cases visible light can also be utilised [5]. The increased aromaticity of the systems, as compared with smaller heterocycles, also allows for transitions with strong bands in the 250–300 nm range. These wavelengths are however too energetic to provide photoactivation, and instead lead to photodegradation reactions.

Upon absorption of the UV-A/vis radiation the psoralen may undergo several different reactions (Scheme 1) [6–8], that can be outlined as follows.

In oxygen-dependent type I reactions the compound is first raised from the ground state (S_0) to the first excited singlet state (S_1). It may then through intersystem crossing come to reside in the relatively long-lived first excited triplet state (T_1), from which the photosensitised compound readily may carry out redox chemistry. The most common reactions are reduction of the excited triplet by an electron donor (e.g. one of the DNA bases), followed by electron transfer from the reduced photosensitiser to molecular oxygen resulting in formation of reactive superoxide anion radicals and fragmenting substrate cations. One could also imagine a direct ionisation of the psoralen by radiation, and electron uptake by molecular oxygen ("direct electron transfer" in Scheme 1).

In oxygen-dependent type II reactions (electron-exchange type mechanism) the excitation energy of the first excited triplet state of the drug is transferred to molecular oxygen. The excited singlet molecular oxygen ($^1\Delta_g$ state) can in turn react rapidly and essentially without discrimination with a wide variety of biomaterials, and thus cause severe damage. Type II reactions do however pose some constraints on the sensitizer, such that the triplet state must be very long-lived, its triplet de-excitation energy must lie above the triplet → singlet excitation energy of oxygen (ca. 22.1 kcal/mol), and that the drug itself must not be susceptible to attack by the generated singlet oxygen. In addition, it has as yet not been satisfactorily proven that in situ formation of singlet molecular oxygen actually occurs inside biological cells.

In the oxygen-independent type III reactions, also termed photobinding reactions, the excited/sensitised psoralen donates its excitation energy directly to, or reacts with, the target compound. This occurs if the substrate and the target compound (e.g. DNA) are already in close proximity or intercalated. The reactions will proceed very rapidly via

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(*i*) *Formation of excited states*:

$$Ps(S_0) \xrightarrow{h\nu} Ps(S_1) \qquad \text{(Excitation)}$$

$$Ps(S_1) \longrightarrow Ps(T_1) \qquad \text{(Intersystem crossing)}$$

(*ii*) *Pathways of photosensitization*:

Type I. *Electron transfer mechanism*: Photosensitizer triplet state reacts with an electron donor D.

$$Ps(T_1) + D \longrightarrow Ps^{\bullet -} + D^{\bullet +} \qquad \text{(Reduction)}$$

$$Ps^{\bullet -} + {}^3O_2 \longrightarrow Ps + O_2^{\bullet -} \qquad \text{(Electron transfer, ET)}$$

$$D^{\bullet +} + O_2^{\bullet -} \longrightarrow D(O_2) \qquad \text{(Back ET)}$$

Type II. *Electron-exchange type mechanism*.

$$Ps(T_1) + {}^3O_2({}^3\Sigma) \longrightarrow Ps(S_0) + {}^1O_2({}^1\Delta)$$

Type III. *Photobinding to, e.g., DNA*.

$$Ps(S_1) + DNA(S_0) \longrightarrow Ps = DNA \qquad (C_4\text{-cycloadduct})$$

(*iii*) *Direct electron transfer*.

$$Ps(S_0) + {}^3O_2 \xrightarrow{h\nu} Ps^{\bullet +} + O_2^{\bullet -}$$

(*iv*) *Autoionization*.

$$Ps(T_1) + Ps(S_0) \longrightarrow Ps^{\bullet -} + Ps^{\bullet +}$$

$$Ps(T_1) + Ps(T_1) \longrightarrow Ps^{\bullet -} + Ps^{\bullet +}$$

Scheme 1. Possible reactions of psoralen compounds upon absorption of UV-A/Vis radiation.

the first excited singlet state of the drug, in which the 3,4- and the 4′,5′ π-bonds of the pyrone and furan moieties, respectively, can undergo C_4-cyclization reactions with, e.g. unsaturated bonds of lipids, or the C_5=C_6 double bonds of thymine in DNA. In reactions with DNA, the psoralen is believed to intercalate with DNA in the dark, whereafter irradiation at 400 nm in general leads to furan-side 4′,5′-monoadduct formation, whereas irradiation at 350 nm increases the formation of cross-links in which both the furan and pyrone rings form cycloadducts to thymines on opposite strands [9]. Subsequent irradiation of the 4′,5′-monoadducts at 350 nm leads to formation of cross-links as well as a conversion into pyrone-side 3,4-monoadducts. Shorter wavelengths (<320 nm) may lead to photoreversal of formed adducts and degradation of non-intercalated psoralens. For the frequently utilised 8-MOP compound this is particularly efficient at $\lambda = 300$ nm [10,11].

Other possible reactions of this family of compounds are auto-ionisation reactions, in which one sensitiser in its T_1 state is reduced by another T_1 or S_0 state sensitiser, thereby forming a radical anion–radical cation couple. The psoralen may finally become ionised (oxidised), either directly or via an excited state, and react with the target compound in form of a radical cation.

Furocoumarins are also employed in treatment of cutaneous T-cell lymphoma and some infections connected with AIDS, through so-called photopheresis processes [4,10,12,13]. In this case, peripheral blood is exposed to, e.g., photoactivated (sensitised) 8-methoxypsoralen, 8-MOP, in an extracorporeal flow system. This is also the idea behind the recently developed pathogen eradication technology (PET), in which viruses, bacteria and parasites are removed from blood products by adding riboflavin (vitamin B_2) and exposing the mixture to visible light. In this case, the process is speculated to proceed through UV induced electron ejection from DNA to the (sensitised) first excited triplet state of the flavin ring [14].

Exactly which biochemical moieties the activated psoralen compounds (or the thereby activated molecular oxygen) bind to or interact with in order to function as specific

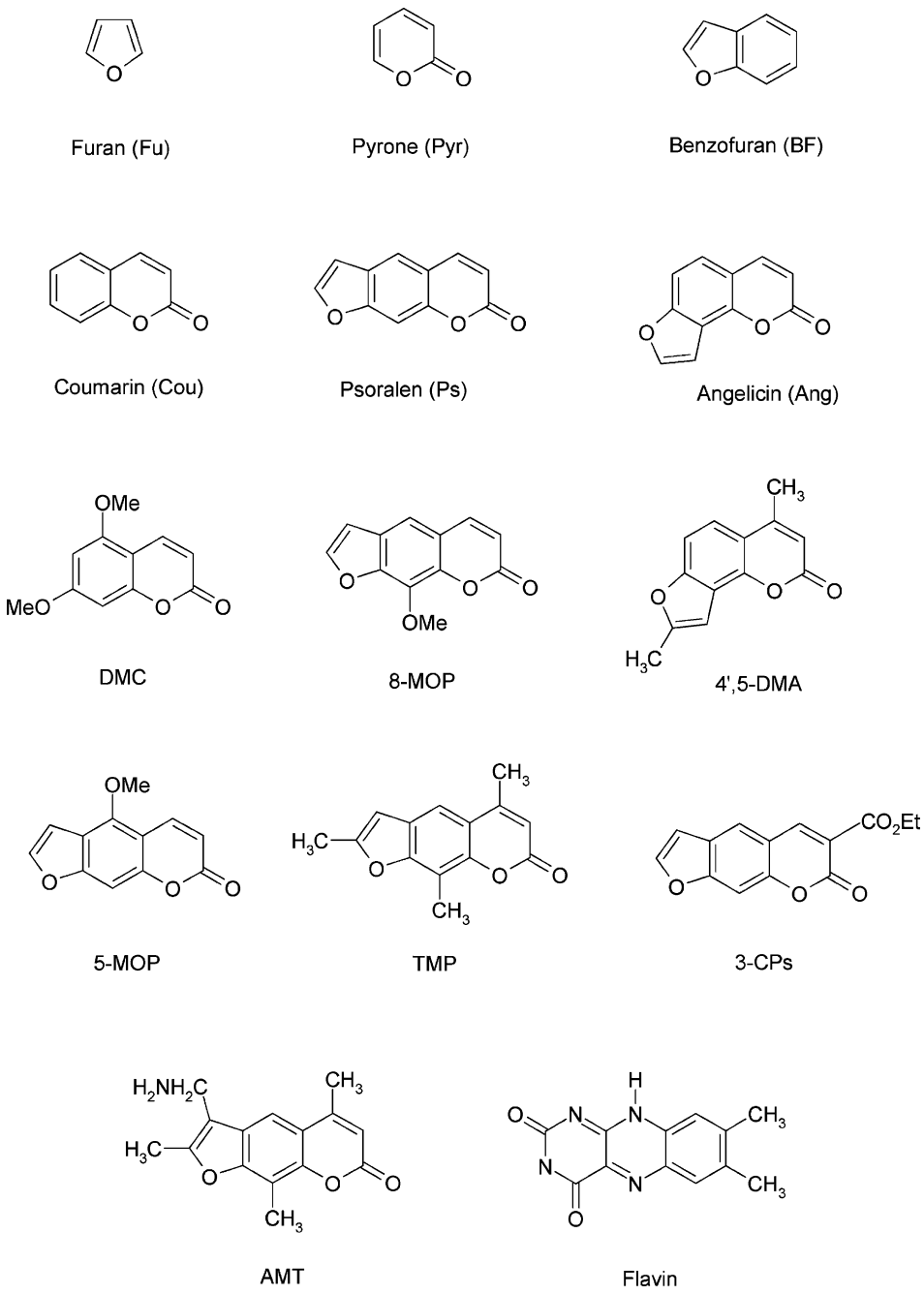

Fig. 1. Furocoumarins and related compounds investigated in the present work.

drugs in the wide variety of diseases listed earlier is however not fully understood at present. It is for example known that photoactivated psoralens can interact with membrane and cytoplasmic receptors [15,16], that they form C_4-cycloadducts with unsaturated fatty acids and lecithins in lipid membranes [17–19], that they form cross-links with DNA (primarily by C_4-cycloadduct formation to the two thymine residues of opposite strands in an AT sequence) [20–22], and that they inactivate enzymes and ribosomes [23]. In particular, certain psoralens are known to inhibit the epidermal growth factor (EGF) receptor upon exposure of the receptor–psoralen complex to UV-A light [16,24]. The biological effects the drugs have on skin disorders has been attributed to this particular observation [25]. Psoralens are also used in nucleic acid research aiming to a better understanding of DNA damage and repair processes [26,27]. The fact that psoralen compounds bind to DNA is also a complicating factor in treatment of skin disorders, as long-time exposure can cause mutations and development of cancer [28–30].

We present here some basic theoretical photochemistry of a set of furocoumarin compounds, starting from the basic building blocks furan and pyrone, as shown in Fig. 1. In

this study, we also incorporate flavin, the active component of vitamin B_2. Comparison is made with the corresponding data for molecular oxygen, in order to explore the ability of the different compounds to undergo the phototoxic oxygen-dependent type I or type II reactions.

2. Theoretical approach

All structures were geometry optimised at the B3LYP/6-31G(d,p) level [31–33]. The data for ionisation potentials were extracted at this level of theory, whereas for the anionic systems and excited states, diffuse functions [34] are known to be essential for accurate structures and energetics [35,36]. Excitation energies and electron affinities were thus evaluated at the single-point B3LYP/6-31+G(d,p)//B3LYP/6-31G(d,p) level of theory. No zero-point vibrational effects (ZPEs) were included. The effect of bulk water as solvent was included through single-point calculations using the polarised continuum model (PCM) of Tomasi and coworkers [37]. Excitation energies were computed within the time-dependent DFT (TD-DFT) formalism [38,39]. TD-DFT calculations in the gas phase are at the present level known to be accurate to within ca. 0.2 eV (5 kcal/mol) [36]. In its present formulation of the PCM, in which only the nuclear or 'slow' part of the reaction field is included, the effects of bulk solvation are negligible for excitations and thus, for computational simplicity, excluded in the calculations of excitation energies.

For molecular oxygen, diffuse functions were incorporated already in the geometry optimisations. The data obtained for this system in vacuum agrees to within 0.15 eV with experimental data (calculated vertical electron affinity: 0.593 eV, exp 0.451 [40]; computed excitation energies to $^1\Delta_g$ and $^1\sum_g^+$ levels: 1.06 and 1.67 eV, respectively, exp 0.98 and 1.64 eV [41]) cf. Table 1. The effects of bulk solvation on the one-electron properties include a lowering of the ionisation potential, by ca. 4 eV, and a significant stabilisation of the radical anion, by 3.5 eV. For reasons discussed earlier, the excitation energies are essentially unaffected by the solvent. It should also be noted that an accurate treatment of the molecular oxygen singlet states require the use of a multi-configuration approach. However, a good estimate of the excitation energy for such cases is possible using Landés interval rules, as outlined by Noodleman and Case [42].

All calculations were performed with the Gaussian'98 program package [43].

3. Results and discussion

3.1. Formation of excited states

As mentioned earlier, the photosensitiser reactions are initiated by excitation to the first excited singlet and intersystem crossing to the first excited triplet state of the drug (cf. Scheme 1). In Table 2 we report the excitation energies and probability coefficients for the six lowest singlet excitations of the various furocoumarins, and compare with experimental data as available. From the table we note that the excitation spectra of both furan (Fu) and pyrone (Pyr) lie above that of the UV-A region (320–400 nm) in energy. Fusing the systems to a benzene ring (BF, Cou) lowers the excitation energies considerably, especially for furan, and we are now starting to approach the UV-A active regime. The calculated lowest singlet excitation for coumarin, 4.13 eV, is close to that observed experimentally (3.96 eV). The deviation between computed and experimental value (0.17 eV, or ca. 4 kcal/mol) is representative of the accuracy of the method.

For psoralen (Ps) and angelicin (Ang), the lowest singlet excitation is somewhat lower than for coumarin, and occurs at 320–330 nm. This excitation does however have rather low probability, as compared to the second lowest excited state which lies ca. 0.5 eV higher in energy. A transition with very high probability is also found at 5.1 eV, which is well reproduced by theory. It can also be noted that the excitation spectra of the two isomers Ps and Ang are very similar, both in wavelengths and in transition probabilities. Differences in their photochemical behaviour can thus primarily be attributed to the differences in geometry. In angelicin, the photoactive double bond in the furan ring lies on the opposite side to that of the pyrone moiety. This can explain why most psoralen derivatives such as 5-MOP, 8-MOP and trimethyl psoralen (TMP) are known to form diadducts to adjacent thymines in an AT sequence of DNA, whereas coumarin and angelicin derivatives generally are monofunctional [44,45]. The same holds for 3-CPs, where the pyrone

Table 1
Computed[a] one-electron properties (eV) of molecular oxygen, in vacuum and in water (using polarised continuum model)

System	Excitation[b] $^3\sum_g^- \rightarrow {}^1\Delta_g$	Excitation $^3\sum_g^- \rightarrow {}^1\sum_g^+$	Adiabatic electron affinity (AEA)	Adiabatic ionisation potential (AIP)
O_2 in vacuum	1.06 (0.98[c])	1.67 (1.64[c])	0.59 (0.45[d])	12.73 (12.07)[c]
O_2 in water	1.05	1.65	3.91	8.93

Experimental data in parentheses.
[a] Computed at the B3LYP/6-31+G(d,p) level no. ZPE effects are included.
[b] Obtained using Lande's interval rules (see text) [42].
[c] [41].
[d] [40].

Table 2
Singlet excitation energies and oscillator strengths f of the psoralen compounds investigated

Fu	$^1E_{0-0}$ (eV)	5.87	6.05	6.31	6.51	6.67	6.94
	λ (nm)	211	205	196	190	186	179
	f	0	0.169	0.032	0	0	0
Pyr	$^1E_{0-0}$ (eV)	4.43	4.57	5.85	6.01	6.11	6.42
	λ (nm)	280	271	212	206	203	193
	f	0.128	0	0.002	0.086	0	0.014
BF	$^1E_{0-0}$ (eV)	4.96	5.11	5.73	5.92	5.96	6.13
	λ (nm)	250	243	217	210	208	202
	f	0.055	0.132	0.009	0.002	0.258	0.008
Cou	$^1E_{0-0}$ (eV)	4.13 (3.96)	4.45	4.59	5.39	5.76	5.96
	λ (nm)	300 (313)	279	270	230	215	208
	f	0.120 (0.056)	0	0.167	0.017	0.117	0.006
Ps	$^1E_{0-0}$ (eV)	3.76 (3.76)	4.34	4.48	4.90	5.14 (5.08)	5.51
	λ (nm)	330 (330)	286	277	253	241 (244)	225
	f	0.076 (−)	0.221	0	0.094	0.401	0.121
Ang	$^1E_{0-0}$ (eV)	3.86	4.30 (4.13)	4.58	4.84	5.09 (5.04)	5.71
	λ (nm)	321	289 (300)	271	256	243 (246)	217
	f	0.042	0.156 (0.173)	0	0.112	0.426	0.010
DMC	$^1E_{0-0}$ (eV)	4.01 (3.79)	4.17	4.59	5.10	5.42	5.52
	λ (nm)	309 (327)	298	270	243	229	225
	f	0.270	0.084	0	0.074	0.003	0.014
5-MOP	$^1E_{0-0}$ (eV)	3.70	4.23 (3.97)	4.49	4.93	4.99	5.34
	λ (nm)	335	293 (312)	276	252	249	232
	f	0.032	0.272 (0.258)	0	0.042	0.448	0.015
8-MOP	$^1E_{0-0}$ (eV)	3.57 (3.54)	4.16 (4.09)	4.51	4.93	4.98 (5.04)	5.05
	λ (nm)	348 (350)	298 (303)	275	252	249 (246)	246
	f	0.022 (0.037)	0.233 (0.218)	0.002	0.194	0.254 (0.381)	0.001
3-CPs	$^1E_{0-0}$ (eV)	3.43 (3.40)	4.04 (3.90)	4.17	4.27	4.89	5.07 (5.02)
	λ (nm)	362 (365)	307 (318)	297	291	254	244 (247)
	f	0.075 (−)	0.389 (0.198)	0	0	0.199	0.292 (−)
TMP	$^1E_{0-0}$ (eV)	3.66 (3.70)	4.28 (4.16)	4.55	4.96	5.12 (4.96)	5.20
	λ (nm)	339 (335)	290 (298)	273	250	242 (250)	239
	f	0.088 (−)	0.198 (0.144)	0	0.178	0.520 (0.272)	0
AMT	$^1E_{0-0}$ (eV)	3.66 (3.69)	4.26 (4.09)	4.57	4.78	4.89	4.98 (4.84)
	λ (nm)	339 (336)	291 (303)	272	260	253	249 (256)
	f	0.071	0.225	0	0	0.038	0.170
Flavin	$^1E_{0-0}$ (eV)	3.00	3.17	3.32	3.80	3.92	4.04
	λ (nm)	413	391	374	326	316	307
	f	0.202	0	0	0.150	0	0.012

Experimental data in aqueous solution given in parentheses [46]. Experimental molar extinction coefficients are converted to oscillator strengths for comparison.

double bond is sterically crowded by the substituent. Monofunctional adducts in general show less genotoxicity, but are also less efficient.

Turning to the more substituted compounds, the lowest singlet excitation lies in the same range as for Cou, Ps and Ang; between 3.4 and 4.1 eV. The lowest excitation energy is found for 3-CPs, 3.43 eV. Most of the UV-A active excitations (<4 eV, or >305 nm) have very low probability, whereas the photodegrading second set of excitations, in the energy region 4.0–4.3 eV (305–285 nm), all are of intermediate strength. A band of strong transitions also occurs at 4.9–5.2 eV (255–240 nm). Overall, there is a very good agreement between theory and experimentally measured excitations in aqueous solution.

The main effects of the substitutions compared to the parent compounds is a lowering of the various excitation energies by ∼0.1 eV and (in most cases) increased transition probabilities. We note, however, that there is a significant difference in energies needed for excitations of the free psoralens in aqueous solution, compared with the energetics required for adduct formation to DNA (350–400 nm, or even visible light). The rationale for this difference lies in the surrounding—when intercalated between the base stacks the π-cloud of the psoralen interacts with the π-systems

Table 3
The three lowest lying triplet energies $^3E_{0-0}$ (eV) in eV (nm) of the systems investigated in the present study, computed at the PCM/B3LYP/6-31+G(d,p) level[a]

Compound	T_1 Exp [46][b]	T_1	T_2	T_3
Fu		3.80 (326)	5.23 (237)	5.83 (213)
Pyr		2.66 (466)	4.26 (291)	4.38 (283)
BF		3.30 (376)	4.14 (300)	4.54 (273)
Cou	2.70[w]	2.78 (446)	3.53 (351)	4.04 (307)
Ps	2.72[w]	2.79 (444)	2.99 (415)	3.62 (342)
Ang		2.80 (443)	3.29 (377)	3.48 (357)
DMC	2.60[e]	2.69 (460)	3.63 (341)	4.05 (306)
5-MOP	2.63[e]	2.76 (450)	2.98 (416)	3.55 (349)
8-MOP	2.72[e]	2.75 (450)	2.90 (428)	3.48 (356)
3-CPs	2.46[w]	2.65 (468)	2.80 (443)	3.50 (354)
TMP	2.78[w]	2.84 (437)	2.92 (424)	3.58 (347)
AMT		2.85 (435)	2.93 (423)	3.57 (347)
Flavin		2.07 (600)	2.80 (443)	2.81 (441)

[a] These transitions are spin-forbidden; all extinction coefficients are therefore zero.
[b] Exp: experimental data [46] obtained in water (w) or ethanol (e) [46].

of the neighbouring nucleotides, which reduces the excitation energies further. Interestingly, the flavin molecule is significantly easier to excite than the other heterocycles investigated. The first singlet excitation energy is only 3.0 eV (413 nm), and has intermediate transition probability. The second band with possible singlet excitations lies at 3.8 eV.

The lowest lying triplet excitation energies of the entire set of furocoumarins and related are listed in Table 3. The gap between the first excited singlet and first excited triplet is reduced with increasing size of the compound. For the monocyclic compounds the S_1–T_1 gap is approximately 2 eV, for the bicyclic compounds it is closer to 1.5 eV, and for the larger and more substituted systems it is approximately 1 eV. From the present data, we find that all three lowest triplets considered here lie lower in energy than the first excited singlet. For the furan and benzofuran compounds the T_1 state lies more than 3 eV above the ground state, whereas the remaining furocoumarins have T_1 excitation energies between 2.65 and 2.85 eV. The lowest lying T_1 state is (again) found for flavin, at 2.1 eV. Comparing with experimental data obtained in either water or ethanol, we again note a very good agreement with the computed data. As for the singlet excitations, the calculated values lie 0.05–0.15 eV too high, and the trends between compounds are with one or two exceptions excellently reproduced.

3.2. Oxygen-dependent type I reactions

Residing in the long-lived first excited triplet states, the furocoumarins may undergo a series of phototoxic reactions with molecular oxygen. In type I reactions (Scheme 1), the triplet states are initially reduced by an electron donor in the near vicinity; e.g. a DNA base. In Table 4 we list the computed ground state vertical electron affinities in aqueous solution of the furocomarins under study, as well as the energy gain when reducing the corresponding T_1 states.

The vertical electron affinities in aqueous solution are all, with the exception of the smaller monocycles, in the energy range 2.1–2.9 eV. If the furocoumarin is instead first excited into the T_1 state, the electron affinities increase to between 4.2 and 5.5 eV. The largest VEA(T_1) is noted for the substituted 3-CPs; most of the compounds have VEA(T_1) energies close to or just above 5 eV. The larger the energy gain for reduction of the T_1 state, the easier it will be for the compound to extract an electron from the surrounding (Scheme 1, step 1). A possible set of electron donors are the DNA nucleobases; at the IEF-PCM/B3LYP/6-31+G(2df,p)//B3LYP/6 - 31 + G(d,p) level the vertical ionisation potentials are A: 5.94 eV, G: 5.49 eV, T: 6.45 eV and C: 6.47 eV [36]. Hence, at the present level it is only 3-CPs (T_1) that will gain sufficient energy to overcome the IP of guanine; $\Delta_r E_{0K}$ = VIP(G)–VEA(T_1). Several of the other compounds are however within 0.5 eV of the VIP of guanine; the local surrounding of the base stack will most certainly reduce the VIPs of the nucleobases further, plus that explicit interactions between the psoralens and water or DNA bases not considered here should stabilise the anions even further.

Once the reduced form of the psoralen compound is formed ($Ps^{\bullet -}$), this may undergo electron transfer to molecular oxygen, forming the reactive superoxide radical anion and regenerating the ground state psoralen (Scheme 1, step 2). The adiabatic electron affinity of ground state molecular oxygen in bulk water is calculated to be 3.91 eV. This should be compared with the ground state electron affinities of the psoralens (Table 4), all being <2.9 eV. Hence, once the reduced drug has been formed, our calculations suggest that the extra electron readily could be transferred to molecular oxygen with a net energy release of at least 1 eV ($\Delta_r E_{0K}$ = VEA(Ps) − VEA($^3O_2$)). Since the activation energies of ET are usually very low, the ET step could well be the driving force in this mechanism.

The superoxide anion undergoes fast bimolecular decay to yield oxidising species as H_2O_2, O_2, and $^{\bullet}OH$ in near-neutral and acid solutions. In addition, $O^{\bullet}{}_2{}^-$ in itself may even function as a reducing agent for other triplet psoralens (VEA(T_1) data, Table 4). In this case, it thus seems that the oxygen molecules will be able to scavenge the generated triplet psoralens, which may be one rationale for the fact that psoralen-induced photosensitivity (phototoxicity) in general is believed to be non-oxygen dependent [47]. The VEA of the DNA bases in aqueous solution are only in the range of 1.3–2.0 eV [36] and, hence, not strong enough oxidising agents to withdraw the electron from the superoxide anion (back electron transfer).

3.3. Formation of singlet molecular oxygen

A second type of reactions with molecular oxygen is the transfer of excitation energy from the T_1 state; the so-called electron-exchange or type II photosensitisation mechanism (cf. Scheme 1). In this case, the de-excitation energy of

Table 4
Vertical electron affinities (VEA), and vertical and adiabatic ionisation potentials (VIP, VEA) in eV of the furocoumarin and related compounds S_0 and T_1 states in vacuum (vac) or bulk water (aq). 1 eV = 23.06 kcal/mol.

Compound	VEA_{aq}	$VEA(T_1)_{aq}$ [a]	VIP_{vac}	AIP_{vac}	VIP_{aq}	AIP_{aq}	$VIP(T_1)_{aq}$ [b]
Fu	0.46	4.26	8.68	8.51	6.25	5.87	2.45
Pyr	2.15	4.81	8.83	8.68	6.59	6.18	3.93
BF	1.16	4.46	8.04	7.87	6.03	5.72	2.73
Cou	2.34	5.12	8.41	8.30	6.45	6.34	3.67
Ps	2.30	5.09	7.93	7.74	6.12	5.89	3.33
Ang	2.23	5.03	7.96	7.81	6.08	5.90	3.28
DMC	2.16	4.85	7.45	7.43	5.86	5.76	3.17
4′,5-DMA	2.11	–	7.65	7.47	5.92	5.65	–
5-MOP	2.34	5.10	7.83	7.35	5.98	5.51	3.22
8-MOP	2.32	5.07	7.71	7.32	5.87	5.59	3.12
3-CPs	2.82	5.47	7.97	7.76	6.14	5.99	3.49
TMP	2.15	4.99	7.43	7.23	5.76	5.43	2.92
AMT	2.11	4.96	7.25	7.00	5.64	5.41	2.79
Flavin	2.36	4.42	7.83	7.72	6.06	6.00	3.99

[a] $VEA(T_1)_{aq} = VEA(S_0)_{aq} + {}^3E_{0-0}$.
[b] $VIP(T_1)_{aq} = VIP(S_0)_{aq} - {}^3E_{0-0}$.

the psoralen T_1 states should thus be compared with the energy needed to bring molecular oxygen to the reactive $^1\Delta_g$ state; an energy of approximately 1.0 eV ($\Delta_r E_{0K} = {}^3E_{0-0}(^3O_2) - {}^1E_{0-0}(Ps)$).

From Table 3 we see that all psoralens have T_1 states lying more than 2.0 eV above the S_0 ground states. Thus, based on the present energy calculations, the transfer of the T_1 excitation energy from any of the furocoumarins to molecular oxygen in order to generate singlet molecular oxygen should in principle be possible.

From the two oxygen-dependent reactions we may thus conclude that in absence of reducing medium the drug may be able to transfer the excitation energy from the T_1 state to molecular oxygen, thus being a source for singlet oxygen. However, if the $Ps(T_1)$ drug is reduced, i.e. in presence of a suitable electron donor, then the reduced drug may in turn donate its excess electron to molecular oxygen. The generated superoxide anion will in turn be able to reduce new $Ps(T_1)$ molecules, which in turn become reduced by $Ps^{\bullet -}$, and so forth. Given the right conditions, one single oxygen molecule could thus be able to scavenge (bring back to ground state) a large number of excited drugs from their reactive triplet state. The present calculations hence suggest that also the furocoumarins undergo the same kind of oxygen-dependent photoxic reactions as other drugs, but that in this case the net effect thereof is eliminated due to back-reactions with the molecular oxygen.

3.4. Direct electron transfer to $^3O_2$

Another proposed mechanistic type of photochemical psoralen reactions involves ionisation (oxidation) of the drug by radiation, coupled with electron uptake by either molecular oxygen or the target compound (e.g. a nucleobase) and subsequent rearrangements, fragmentations, dimerisations and other reactions. It is thus of interest to compare the vertical and adiabatic ionisation potentials (VIP and AIP, respectively) for a set of compounds, and the effects of the surrounding on these, in particular in relation to the electron affinities of $^3O_2$ and the various nucleobases. The difference between VIP and AIP indicates the extent of relaxation of the cation, i.e. how much the structure of the compound is effected by the ionisation.

Starting with the small "building blocks" furan (Fu) and pyrone (Pyr), we see from Table 4 that furan is easier to ionise than the larger pyrone ring by between 0.2 and 0.5 eV (4–8 kcal/mol), depending on vacuum or bulk water surrounding. The ionisation potentials of the small heterocycles are however significantly larger than if we fuse the system with a benzene ring to form benzofuran (BF) and coumarin (Cou), respectively. The effect of the increased aromaticity is smaller in aqueous solution. The full parent compounds psoralen (Ps) and angelicin (Ang) lie approximately 0.5 eV lower in ionisation energies than coumarin, but are still higher than the benzofuran system. We also note that for all systems investigated, the inclusion of the solvent reduces the IPs in the order of 40 kcal/mol (1.7 eV).

Several modifications to the basic parent compounds have been suggested, primarily by methyl or methoxy substituents (cf. Fig. 1). Two of the most active and widely used furocoumarins are 8-methoxy psoralen (8-MOP) and TMP. With the exception of 3-CPs, the substituents lower the ionisation energies by 0.3–0.6 eV compared to their unsubstituted parent compounds (Table 4). In bulk water the effects are somewhat smaller. The largest effects are observed for TMP and AMT. The IPs of flavin are very similar to those of the unsubstituted psoralen.

The relaxation effects of the cation are throughout rather small, only a few tenths of an eV. The reason for this is that most aromatic compounds retain their planarity also upon ionisation, and thus the structural reorganisations are small. Similar observations have been made for, e.g. the various

nucleobases [35]. The largest effects are again noted for the more substituted compounds 5-MOP, 8-MOP, TMP and AMT.

The adiabatic electron affinity of molecular oxygen in its $^3\sum_g^-$ ground state amounts to 0.6 eV (experimental value 0.45 eV) in vacuum, and 3.91 eV in bulk water solvent. From this data, it is clear that once sufficient energy is provided to eject an electron from the furocoumarin, this may readily be captured by the molecular oxygen. In addition, the ionisation potential of $^3O_2$ in aqueous solution (>12 eV in vacuum and 8.93 eV in bulk water) far exceeds the IP of the furocoumarins in the corresponding medium. It is thus unlikely that molecular oxygen would be ionised in the process, followed by electron uptake by the drug.

For the DNA bases, the computed VEA in aqueous solution lie in the range 1.3–2.0 eV (IEF-PCM/B3LYP/6-31+G(2df,p)//B3LYP/6-31+G(d,p) level [36]). Provided that the furcoumarin is ionised by radiation, it is reasonable to assume that the electron will primarily be captured by molecular oxygen, if present. In addition, the VEA of the DNA bases all lie below those of the entire furocoumarin family (cf. Table 4), and it thus appears more likely that an electron ejected from the drug will be captured by another drug molecule, than by any of the nucleobases.

3.5. Auto-ionisation of furocoumarins

The final reaction type considered in the present work is that of auto-ionisation of the psoralens. That is, once a psoralen molecule is lifted to the first excited triplet state, this may be reduced by a neighbouring psoralen molecule. The energy gain in reducing psoralens in their T_1 states range between 4.2 and 5.5 eV (Table 4). The VIP in aqueous solution, on the other hand, amounts to 5.6–6.6 eV. It is hence unlikely that a triplet psoralen can be reduced in this fashion. For two psoralens in the T_1 state, however, the energy gain for reduction, VEA(T_1), is 4.2–5.5 eV, whereas the corresponding vertical ionisation potentials, VIP(T_1) (Table 4) only lie between 2.4 and 4.0 eV. Hence, it will be possible for two neighbouring drug molecules to transfer an electron and autoionise, once excited.

4. Concluding remarks

In the present work, the phototoxic one-electron properties of a family of furocoumarins, proposed for use in, e.g. photochemical treatment of various skin disorders, have been explored by the hybrid Hartee-Fock—density functional theory (HF-DFT) approach B3LYP and polarised split valence basis sets. Besides the furocoumarins, or psoralens, we have also investigated the properties of their mono and bicyclic building blocks, and flavin. Comparison is made with the corresponding properties of molecular oxygen, in order to explore the possibility for electron and excitation energy transfer from the furocoumarin.

The oxygen-dependent photochemical reactions involve electronic excitation to the first excited singlet state, intersystem crossing to the first excited triplet state, and finally reduction and electron transfer (type I), or direct transfer of the triplet excitation energy (type II), to molecular oxygen. The six lowest singlet excitations and the three lowest triplet excitations of the entire set of compounds were computed using time-dependent DFT (TD-DFT), the B3LYP functional and a basis set augmented with a single set of diffuse functions (6-31+G(d,p)). The computed data agree to within ca. 0.2 eV with experimental data, and reveal a band of low-lying excitations with weak to intermediate transition probability at around 4 eV, and a band of stronger transitions around 5 eV. For flavin, the corresponding bands lie approximately 1 eV lower in energy. The first excited triplet states are found at 2.6–2.9 eV, and again very well reproduce known experimental data. For flavin, the lowest triplet lies 2.1 eV above the ground state.Type I reactions involve reduction of the T_1 state of the drug followed by electron transfer to molecular oxygen. Based on the computed excitation energies and electron affinities of the psoralens, and the electron affinity of molecular oxygen, we conclude that in the presence of an electron donor that will reduce the T_1 state of the drug (which requires a VIP less than 4.2–5.5 eV in aqueous solution, depending on drug molecule), the electron can be readily transfered to molecular oxygen (adiabatic electron affinity 3.9 eV in water). It is however unclear whether nucleobases are sufficiently strong reducing agents to provide the reducing electron to the T_1 states of the drugs. For flavin, the computed VEA$(T_1)_{aq}$ is among the lowest of the entire set, 4.42 eV. Compared to the other drugs considered here, flavin is hence among those least likely to undergo reduction by the nucleobases—contrary to the mechanism proposed in PET.

We have furthermore observed that the IP of the superoxide anion is lower than the reduction energy of the T_1 states of all the compounds currently explored. It is proposed that once a drug is reduced and has transferred its excess electron to molecular oxygen, the latter can initiate a chain of scavenging reaction events of drug molecules in their T_1 states. This would explain the oxygen-independent nature of psoralen-induced photosensitivity.

In oxygen-dependent type II reactions, the excitation energy of the T_1 state is transferred to molecular oxygen, generating ground state psoralens and molecular oxygen in the reactive $^1\Delta_g$ state. The transition energy back to the ground state is for all systems explored well above the excitation energy of molecular oxygen (the latter being 1–1.6 eV), and it is concluded that once the drug is situated in the T_1 state, this may generate singlet molecular oxygen unless it undergoes rapid reduction (see Section 4).

The final two aspects considered in this work involved direct electron transfer between the drug and molecular oxygen, and the question of auto-ionisation. In the direct electron transfer, radiation will ionise the drug, forming psoralen radical cations, whereas the ejected electron may be captured by an oxygen molecule. In aqueous solution,

the vertical ionisation potentials of the systems explored lie in the range 5.6–6.6 eV. For the T_1 states, however, the VIPs are all below 4 eV. Hence, once the drug is in the first excited triplet, this may be even be oxidised by molecular oxygen. The only exceptions to this are Pyr and Flavin. In the case of auto-ionisation, i.e. spontaneous electron transfer from one drug molecule to another, we conclude that such a process is possible only if the two part-taking systems reside in the T_1 state.

The current work represents a first study of photoactive compounds; subsequent work involving specific reactions between psoralens and DNA or lipid bilayers, as well as the phototoxicity of a wide range of suggested photosensitisers are currently under way.

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