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RESEARCH PAPER

Predicting Drug Substances Autoxidation

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ABSTRACT

Purpose Chemical degradation and stability in formulation is a recurrent issue in pharmaceutical development of drugs. The objective of the present study was to develop an in silico risk assessment of active pharmaceutical ingredients (APIs) stability with respect to autoxidation.

Methods The chemical degradation by autoxidation of a diverse series of APIs has been investigated with molecular modelling tools. A set of 45 organic compounds was used to test and validate the various computational settings. Aiming to devise a methodology that could reliably perform a risk assessment for potential sensibility to autoxidation, different types of APIs, known for their autoxidation history were inspected. To define the level of approximation needed, various density functional theory (DFT) functionals and settings were employed and their accuracy and speed were compared.

Results The Local Density Approximation (LDA) gave the fastest results but with a substantial deviation (systematic over-estimation) to known experimental values. The Perdew-Burke-Ernzerhof (PBE) settings appeared to be a good compromise between speed and accuracy.

Conclusions The present methodology can now be confidently deployed in pharmaceutical development for systematic risk assessment of drug stability.

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KEY WORDS Degradation · Autoxidation · Computational chemistry · Pharmaceutical · DFT

INTRODUCTION

The pharmaceutical properties of drug candidates are routinely characterised during pharmaceutical development to optimize drug manufacturability and in vivo performance as these depend, in part at least, upon their physico-chemical properties [1]. Solubility and drug bio-availability are probably the main concerns in early development [2]. Another vital component of drug early development is the study of stability of drug candidates, [3]. Drugs are often subject to chemical decomposition in the active substance and even more once formulated in either liquid or solid dosage forms [[4]CBV. Degradation can lead to an increased level of impurity in the solid state during storage and/or within the formulation; it can originate from intrinsic chemical weakness or from interaction with formulation excipients [5]. There is a clear benefit to screening drug substance candidates with respect to their stability [6].

Typically, drugs have many different chemical functions and thus are subject to degradation via multiple mechanisms. The two main modes of drug degradation commonly reported in literature are hydrolysis and oxidation by molecular oxygen, so called autoxidation. Stress testing is an essential part of stability testing according to ICH Q1A (R2) stability testing guidelines [7]. It is judicious to perform it at the early development phases in order to support formulation and the development of stability-indicating analytical procedures.

Valuable recommendations for formulation design with regards to chemical compatibility issues could be informed by in silico studies. These recommendations would allow focussing the development efforts on the "excipient domain" with an improved probability of success and to anticipate impurity problems to avoid genotoxic impurity formation.

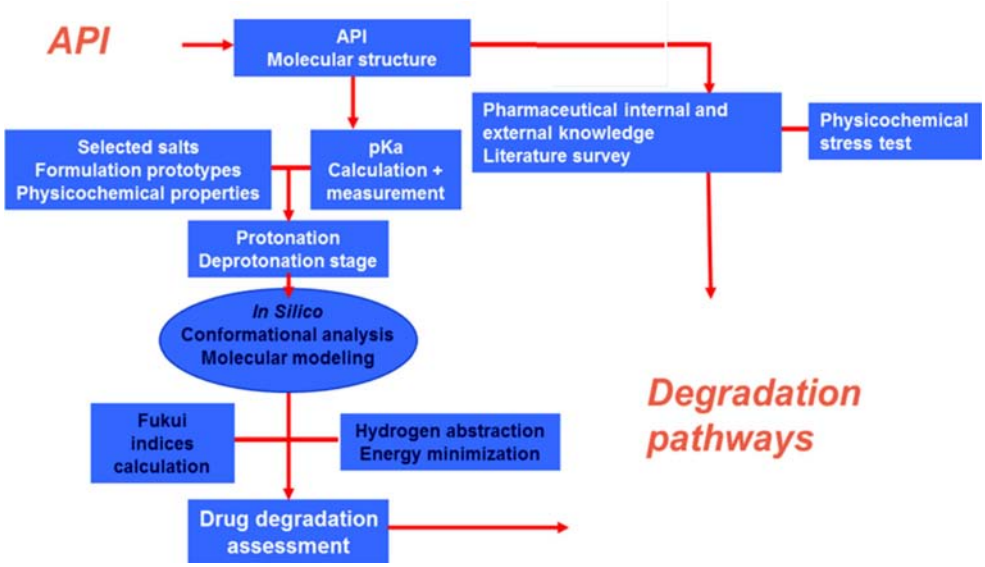


Fig. 1 Schematic overall logic flow of drug degradation anticipation

Moreover, impurities suspected of being exceptionally active or that may produce toxic effects should be avoided. Calculations based on molecular modeling techniques such as the density Functional Theory (DFT) or molecular dynamics (MD) are widely used in the pharmaceutical industry and belong to an integrated part of the degradation prediction workflow, as shown in Fig. 1. A previous study on drug substances stability using molecular modeling techniques suggested that, in particular, DFT could be reliably used to predict the most stable hydrogen-abstracted radicals [8].

Molecular modeling studies of active ingredients can help estimate their chemical stability. Fukui indices calculations [9] help identify reactive fragments in molecules thus allowing anticipation of the main degradation pathways such as hydrolysis. With regard to the second main degradation mechanism, oxidation, Fukui index for radical susceptibility only predicts oxidation proceeding through electron transfer. The most common oxidation mechanism through radical abstraction by radical initiators; for this latter case bond dissociation energy (BDE) evaluation is more appropriate [8].

The radical oxidation mechanism caused by molecular oxygen (referred to as *Autoxidation*) is described in Fig. 2.

Initiators usually come from trace impurities, often peroxides. Therefore, autoxidation only leads to significant degradation if the reaction cycle depicted in Fig. 2 runs several times for each radical produced before termination by radical destruction. Taking this in mind, oxidation products come from the reaction of the intact C-H bond with the peroxide radical $ROO^{\cdot}$. The main reaction is therefore:

$$RH + ROO^{\cdot} \rightarrow ROOH + R^{\cdot}$$

that can thermodynamically be interpreted as the sum of two reactions:

$$RH \rightarrow R^{\cdot} + H^{\cdot}$$
$$ROO^{\cdot} + H^{\cdot} \rightarrow ROOH$$
$$RH + ROO^{\cdot} \rightarrow ROOH + R^{\cdot}$$

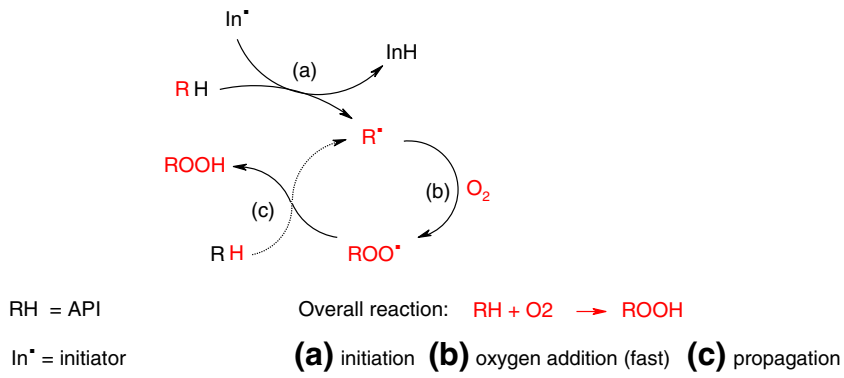


Fig. 2 Simplified autoxidation degradation pathway

where it is evident that the energetic balance is the difference between the BDE of the intact substrate RH (first reaction enthalpy) and the BDE of the hydroperoxide ROOH (second reaction enthalpy). It is worth noting that, taking into account that in the mechanism depicted in Fig. 2, initiation is the rate-determining step. Molecular oxygen O_2 operates

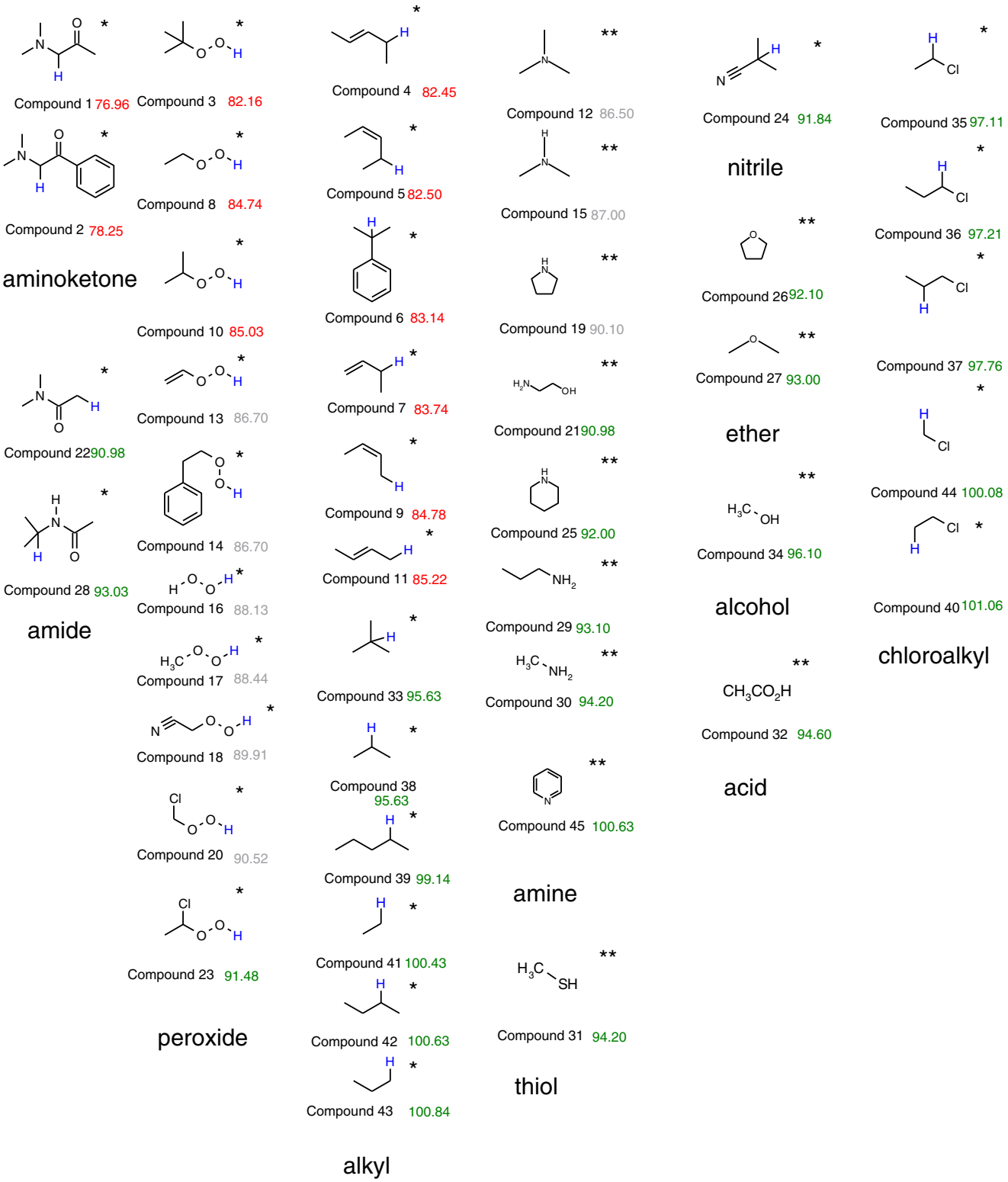


Fig. 3 Compound testing set. *Experimental C-H BDE results described by* M. Coote et al. [16]* and Lewin/Cramer (2004) [32]**, color code green: unlikely, grey: equivocal, red: likely. (kcal mol^{-1})

after this step and therefore does not appear in the kinetic equation of autoxidation; i.e., radical oxidation is zero-order with respect to oxygen. The consequence is that the only way to accelerate and to control this reaction in stress studies is the addition of compounds leading to a controlled production of peroxide radicals. This experimentally confirms that the above reaction is the crucial point in describing the oxidation susceptibility to autoxidation. Moreover, it is clear that considering all the C-H bonds of the molecule, only those with a BDE lower than that of the peroxide radical are likely be dissociated [10, 11].

Experimental bond dissociation energies can be found in the literature though they are not easy to measure. Three different techniques can be utilised: (i) radical kinetics study (ii) photoionization mass spectrometry and (iii) the acidity/electron affinity cycle [12]. Electronic structure methods have also been used reliably to predict bond dissociation energies. Recently, Sharp T.R. [13] reported a method using semi-empirical and DFT models where the carbon-hydrogen BDEs of a set of APIs were computed and used to estimate drug stability. In particular, semi-empirical models such as RM1 or PM6 [14, 15] could be used at low computational cost whilst more accurate higher level of theory require much higher computational expense. Autoxidation also had been investigated for polymer systems by Coote M. et al. [16]. The authors used quantum mechanical methods to demonstrate that the bond dissociation energies of the R-H bond of the polymer backbone were significantly higher than that of the R-OOH bond (BDE of ~377 kJ/mol [17]) thus revisiting a known reaction scheme describing the propagation step of the autoxidation by Bolland and Gee [18].

The ultimate goal of such analysis would be a calculation of reaction rates of all possible oxidation reactions and an estimation of thermodynamic concentrations of oxidation products, especially of the potentially harmful molecules. However, given a huge number of possible reactions, such a head-on approach is not feasible. Alternatively, one can identify parts of the drug molecule most susceptible to a specific degradation channel and introduce descriptors characterizing the stability of the weakest part as a measure of overall stability. In the context of autoxidation two main assumptions are usually made:

A. Radical initiation is a rate determining step in the auto oxidation process;
B. Radical initiation primarily occurs via abstraction of the weakest carbon hydrogen bond.

It follows from these assumptions that the propensity toward autoxidation is characterized by the bonding strength of the weakest hydrogen. So, hydrogen abstraction energy may be a reliable descriptor of degradation susceptibility. The energy required for homolytic bond ($R^{-}H$) cleavage is

Table I Experimentally determined BDE energies (see Fig. 3) and their calculated values. (kcal mol^{-1})

Entry	EXP	LDA	PBE
1*	77.0	81.1	72.9
2*	78.2	80.3	72.2
3*	82.2	93.1	83.2
4*	82.4	97.5	86.3
5*	82.5	96.0	85.2
6*	83.1	96.0	87.1
7*	83.7	98.1	86.7
8*	84.7	94.5	84.8
9*	85.0	101.1	89.6
10*	85.0	93.8	84.1
11*	85.2	101.4	89.9
12**	86.5	107.2	95.8
13*	86.7	94.8	84.7
14*	86.7	94.4	84.6
15**	87.0	106.9	95.8
16*	88.1	96.7	87.1
17*	88.4	94.9	85.3
18*	89.8	96.8	86.9
19**	90.1	105.4	93.8
20*	90.5	96.2	86.6
21**	90.7	101.7	91.7
22**	91.0	113.0	101.4
23**	91.5	102.8	91.5
24*	91.8	99.1	89.2
25**	92.0	104.8	94.0
26**	92.1	106.5	95.8
27**	93.0	119.1	100.2
28*	93.0	101.9	92.1
29**	93.1	116.1	93.9
30**	94.2	115.8	96.3
31**	94.2	98.9	91.0
32**	94.6	120.3	107.6
33*	95.6	109.7	98.3
34**	96.1	111.3	100.0
35*	97.1	110.4	100.1
36*	97.2	110.7	100.3
37*	97.8	111.8	99.4
38*	98.0	113.7	101.8
39*	99.1	113.8	102.0
40*	100.1	118.0	105.2
41*	100.4	118.7	106.2
42*	100.6	114.0	102.0
43*	100.8	119.9	106.8
44*	101.1	114.1	103.3
45*	105.0	119.2	109.2

Experimental C-H BDE results described by M. Coote et al. [16]* and Lewin/Cramer (2004) [31]**

Additional similar calculations were performed with the B3LYP functional. However, it was found that the quality of results was not improved significantly; moreover, such level of theory needs a dramatic increase in the duration of the calculations. In general the B3LYP hybrid results gave lower BDE values than that of LDA, however ranking of compounds BDE were similar in both cases

referred to as the hydrogen Bond Dissociation Energy (BDE) or the hydrogen abstraction energy. It is the aim of this paper to test this hypothesis and to establish correlation between weakest hydrogen BDE and oxidation resistance of the test molecules.

Relative bond dissociation energies (or bond dissociation enthalpies - BDEs) of the R-H bond are indicators of the primary site(s) of auto-oxidation. Isodesmic methods combined with DFT calculations have been successfully employed [19] with errors typically less than ± 2.5 kcal.mol^{-1} [20]. Leopoldini et al. [21] also reported BDEs of phenolic compounds estimated by DFT calculations using the B3LYP hybrid functional [30] in order to study their auto-oxidative properties. Paracetamol metabolic oxidation has been studied by Alves et al. [22] also using the B3LYP hybrid DFT functional. Similarly to calculations presented in this paper, BDEs were used to estimate the hydrogen abstraction energies. Their results showed that initial hydrogen abstraction from the phenolic hydroxyl group is favored by ~29.87 kcal.mol^{-1} over an initial hydrogen abstraction from the acetylamine nitrogen atom. This study demonstrates the capability of DFT to predict the most likely site for auto-oxidation.

In the present study, a methodology to predict the risk of autoxidation of pharmaceuticals using quantum-mechanical methods is presented. We develop the workflow for automated calculation and ranking of hydrogen abstraction energies for an arbitrary molecule. The method is applied to a selection of APIs. Based on these calculations, an energy threshold could be established to estimate autoxidation resistance of new molecular entities and to identify potentially unstable molecules at an early stage of development.

The paper is organized as follows. The next section presents methodology details. In the following part the results of all computations are presented. The compound test set results are presented first, followed by the computations on both APIs with known sensitivity to autoxidation and APIs without an autoxidation history. In the subsequent part, results are discussed in terms of accuracy versus experimental data, when available. Results obtained with different computational settings are compared in order to devise the best compromise between accuracy and speed.

METHODOLOGY

In this part the computational tools employed to estimate the hydrogen abstraction energies are described. In particular, the Density Functional Theory [23, 24] (DFT), a recent popular *ab initio* tool to perform electronic structure calculation, is briefly introduced as well as its implementation and particularities inside the DMol^3 module [25, 26] of Materials Studio® [27]. Due to the numerous calculations performed on the radicals, the Pipeline Pilot [27] tool was employed to generate all required structures and to perform all calculations systematically and automatically distributing them efficiently on multiple CPUs. An output summary report at the end of the execution of the protocol created was generated.

Computational Strategies

In this paper we adopt the following strategy: for any studied molecule we calculate the bond dissociation energy for all symmetrically non-equivalent hydrogen atoms. This is achieved via the following steps:

1. The geometry of the initial molecule is optimized and its ground state energy, E(RH), calculated.
2. The initial structure of every single possible radical is generated one at a time by systematic removal of one hydrogen atom from the initial molecule.

Table II Calculated BDE Energies Values on Autoxidation Historic Drug. (kcal mol^{-1})

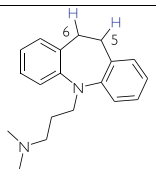
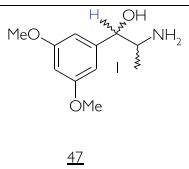
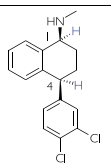
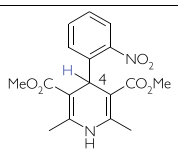
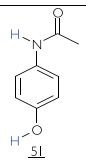
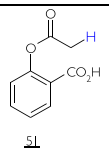
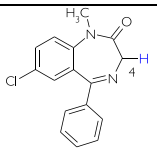
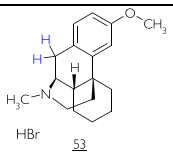
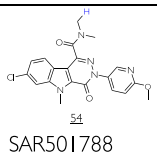
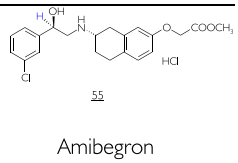
Drug	 46 Imipramine	 47 Methoxamine	 48 Sertraline	 49 Nifedipine	 51 Paracetamol
LDA ΔE kcal/mol	(H5) 97.9 (H6) 97.8	86.3	(H1) 90.2 (H4) 86.6	84.5	(O-H) 95.6 (NH) 106.9
PBE ΔE kcal/mol	(H5) 88.4 (H6) 88.4	86.1	(H1) 79.6 (H4) 83.0	76.6	(O-H) 86.1 (N-H) 96.3

Table III Calculated BDE Energies Values on Non-autoxidation Historic Drug. (kcal mol^{-1})

Drug	 51 Aspirin	 52 Diazepam	 HBr 53 Dextro--methorphan	 54 SAR501788	 55 Amibegron
LDA ΔE kcal/mol	114.9	90.2	99.2 101.6 (protonated)	106.5	98.1 (protonated)
PBE ΔE kcal/mol	103.4	82.7	88.2 89.3 (protonated)	110.5	89.9 (protonated)

3. All structures of radicals are optimized and their ground state energy, E(R), are computed.
4. The energy of the isolated H atom E(H) is also computed.
5. The hydrogen BDE is calculated for each radical as

$$BDE(R) = E(R) + E(H) - E(RH)$$

6. Hydrogen BDE's are ranked for analysis.

All steps of the workflow including jobs submission, execution on a remote cluster, and analysis are fully automated using Accelrys Pipeline Pilot © software platform [27]. These calculations are conducted via secure web interface allowing for streamlined analysis, reporting and sharing the results.

Total energy and geometry optimization calculations of steps 1,3, and 4 of the workflow were carried out using $DMol^3$ code distributed as part of Accelrys Materials Studio © version 6.0 and Accelrys Pipeline Pilot ©. $DMol^3$ implements density functional theory (DFT) for molecules and solids using all electron approach and efficient numerical local orbital basis sets [25–29]. In order to assess the accuracy, efficiency, and robustness of the BDE calculations, we have undertaken extensive tests using local density approximation (LDA) functional of Vosko, Wilk and Nusair (VWN) [29] , the Generalised Gradient Corrected Approximation (GGA) functional of Perdew, Burke and Ernzerhof (PBE) [30], and non-local hybrid functional B3LYP [31]. All calculations were performed using spin-unrestricted DFT. For all density functional, the BDE energies were found to be saturated for DNP (double zeta with polarization) basis sets [31]. Production

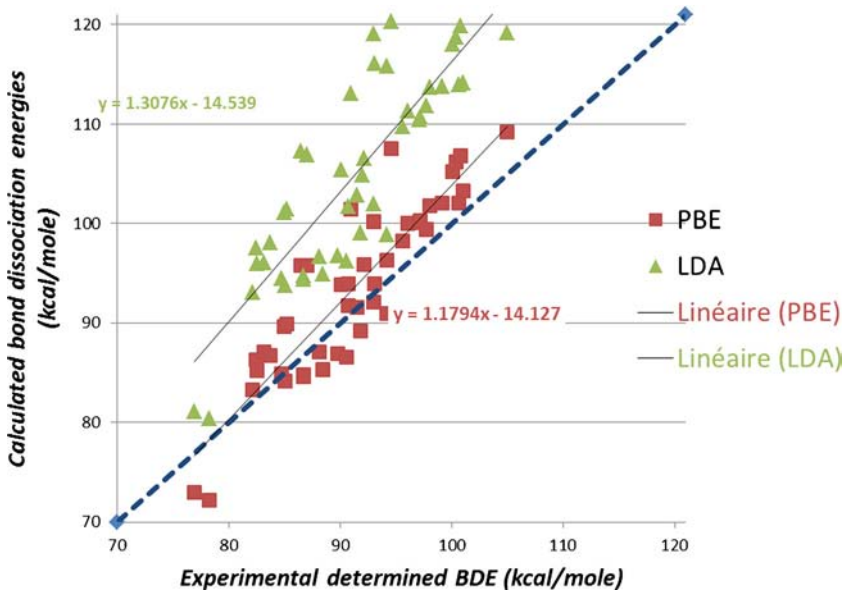


Fig. 4 Correlation calculated BDE vs experimental on compound testing set (kcal mol^{-1})

Table IV Regression statistics for computer *vs.* experimentally determined BDE energies of the compound testing set

	linear fit	Root mean square deviation (kcal mol^{-1})
LDA	$y = 1.3076x - 14.539$	14.6
PBE	$y = 1.1794x - 14.127$	4.5

calculations were performed with this basis and the orbital cutoff of 3.1 Å. The self-consistent energy was converged to within $2*10^{-5}$ Hartree and atomic forces to less than $4*10^{-3}$ Hartree/Å. All calculations of radicals also included the spin density and HOMO and LUMO charge density maps for further analysis. Applicability of various functional for the BDE calculations is discussed in the next section.

RESULTS

Validation – Choice of the Functional Versus Experimental Results

The energy required to break an individual C-H bond is dependent on the structure and electronic environment. Therefore, much attention has been given to developing and selecting the most appropriate functional to improve calculation accuracies. Thus, we tested mainly LDA and PBE as functional to determine the most appropriate in terms of result compliance and computing time. Occasionally B3LYP was employed, however these settings, though giving the most reliable results, were deemed too computationally expensive for rapid treatment of APIs.

Experimental Domain

The reference set of compounds, for which high quality experimental bond dissociation energies have been measured, was assembled by Lewin and Cramer (2004) [32], and is further documented in the review by Blanksby and Ellison (2003) [33] and later on by Coote M. et al. (2011) [16]. Compounds and experimental energies are collected in Fig. 3. The abstracted hydrogen is marked in blue when known.

Benchmarking of Computer Results

Comparison of computerized values with good experimental BDE determinations is a valuable indicator of the validity of calculations. Our own evaluations of the reliability of computed results are summarized in Table I using the LDA and PBE functionals.

BDE Analysis Applied to Pharmaceutical Relevant Drug Substance

Imipramine, methoxamine, sertraline, nifedipine, and paracetamol, have been examined because they have a significant accumulated history of information on their stability and degradant identities related to autoxidation or oxidative degradation. Similarly, the following drug substances were processed: aspirin, diazepam, dextromethorphan, and Sanofi compounds SAR501788 as well as SR58611A (amibegron). These have little or no autoxidation behaviour reported so far. The ability of our approach to predict a low propensity toward autoxidation was investigated. Results are presented in Tables II and III The weakest hydrogen atom is shown in blue.

These results are discussed in the following section.

DISCUSSION

The LDA, PBE correlations with experimental values are plotted on Fig. 4

The ideal correlation in y = ax + b would be slope =1 and b =0. The solid line is a fit to the correlation for the 45 references of the test set compounds. The root mean square deviation clearly indicates the superiority of PBE functional in terms of calculation results as compared to experimental data

Table V Autoxidation risk table for calculated drugs

	Autoxidation reported					No autoxidation reported				
Drug	46	47	48	49	50	51	52	53	54	55
LDA ΔE KCal	(H5) 96.9 (H6) 98.8	86.3	(H1) 90.2 (H4) 85.6	84.5	95.6	114.9	90.2	101.6	106.5	98.1
PBE ΔE KCal	(H5) 88.4 (H6) 88.4	86.1	(H1) 79.6 (H4) 83.0	76.6	85.1	103.4	82.7	89.3	110.5	89.9

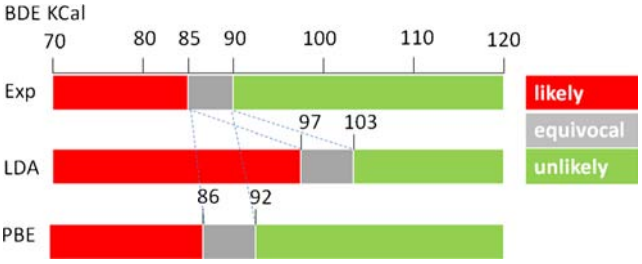


Fig. 5 Risk scale correlation calculated BDE vs experimental (kcal mol^{-1})

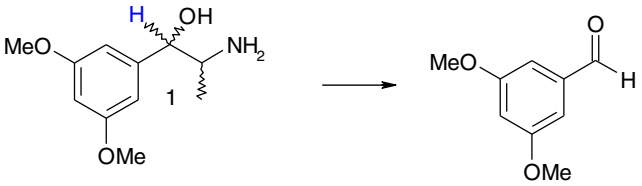


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Fig. 7 Methoxamin degradation benzylic oxidation

as seen on above Fig. 4. PBE results display a slope much closer to the bisectrix y = x. Statistical regression results are compiled in Table IV for comparison.

Our calculations demonstrated that both qualitatively and quantitatively, BDE can be predicted with our protocol. Though the compounds used in this validation study have significant lower molecular weight than common active pharmaceutical ingredients, their chemical diversity does encompass that of typical drugs. Effect of larger size compounds on radical energies, such as stabilization via resonance, might not be effectively accounted for.

M. Coote et al. [16] reported that thermodynamic favourability C-H bond dissociation probability is strongly favoured for ΔE <85 kcal mol^{-1} , disfavoured ΔE >90 kcal mol^{-1} and questionable between 85 and 90 kcal mol^{-1}, which is consistent with BDE values reported in Table II for the ROO-H bond that is responsible of the chain propagation.

Thermodynamic favourability of hydrogen abstraction reaction is reflected by the following colour code: strongly favoured; red – strongly disfavoured; green – equivocal; grey)

Indeed, the risk scale appears to be fit sharply with compounds 46 through 55 propensities to autoxidation. Results for each individual compound are discussed below in Table V (Fig. 5).

BDE Calculation Applied to Imipramine

We applied the BDE calculations to imipramine, compound 46, which undergoes oxidation at the benzylic site to form the corresponding benzyl hydroxyl compound (Fig. 6) [34]. Subsequent elimination of the hydroxyl occurs to give an extended conjugation in the molecule or another pathway is observed by ring rearrangement. Both degradation paths experienced by formulation developers are expected to be coming from the weakly bonded benzylic hydrogen represented in blue.

BDE calculations, listed in Table II, with LDA and PBE functional results clearly stated that the weakest hydrogens are adjacent to a benzyl ring systems, in position 5 and 6 in structure 46. The calculated energy is lower than the threshold of the level likely or equivocal, as described in risk Table V.

BDE Calculation Applied to Methoxamine

In the case of methoxamine 47, which contains a benzyl hydroxyl moiety, decomposition was observed in aqueous solution to the primary degradant, corresponding benzaldehyde, presumably via a benzylic radical autoxidation according to Florey (1991)[34] (Fig. 7).

BDE calculations with LDA and BDE functional, listed in Table II, confirmed both mechanistic aspects of this radical initiated degradation on benzylic position H1. The calculated

Fig. 6 Imipramine degradation and benzylic oxidation

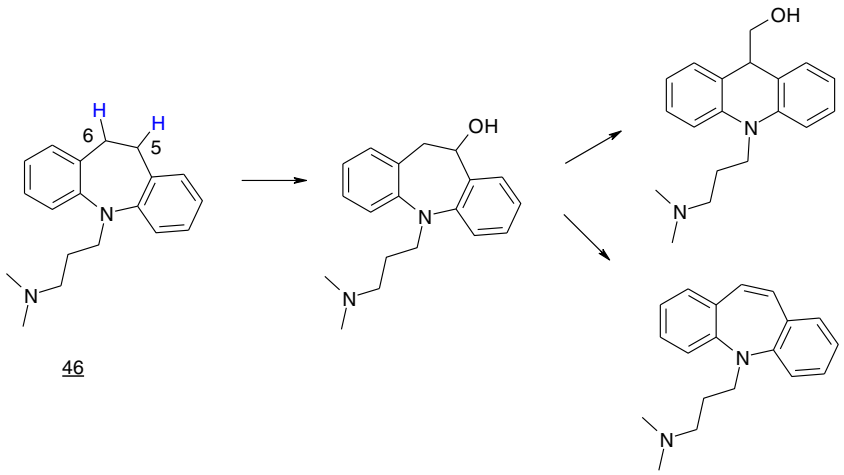

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Table VI Autoxidation risk table for calculated drugs

	ΔE kcal mol^{-1}	
	LDA	PBE
Protonated sertraline H1	102.1	92.3
Protonated sertraline H4	88.6	81.9

energy was below the likely threshold, as reported in risk Table V.

BDE Calculation Applied to Sertraline

In the case of sertraline 48, our calculation efforts focused on a molecule with two known weak benzylic C-H bonds. The molecule is reported to be oxygen sensitive and to give only degradation products from radical oxidation of position 4.

Sertraline is manufactured as the hydrochloride salt [13], therefore we calculated BDE for both free and protonated forms. Our calculations clearly show that C(4)-H is sensitive to oxidation according to both DFT methods, and is definitely the weakest of the two considered bonds (Table VI).

Moreover, epimerization reaction occurs under photochemical conditions to give the trans sertraline product as described in Fig. 8 [35].

A photochemical reaction does not start with a hydrogen abstraction, but with electronic excitation, so the calculated BDE cannot be used to predict reactivity. Nevertheless, calculated weakness of the C(4)-H bond is in agreement with this preferential involvement in epimerization.

BDE Calculation Applied to Nifedipine

For nifedipine 49, our calculation efforts focused on a molecule with a highly functionalized structure. Indeed, certain nitro-aromatic groups are susceptible to photochemical reactivity. The most well-known example is nifedipine, where the nitro moiety triggers the aromatization to a substituted pyridine[36, 37]. Calculations with both LDA and PBE functionals are in agreement with the fact that C(4)-H is the weakest CH bond in the molecule.

BDE Calculation Applied to Paracetamol

Paracetamol metabolic oxidation has been studied by Alves et al. [22]. Their calculation stated that the initial more labile hydrogen is from the phenolic hydroxyl group, with BDE 75.4 kcal mol^{-1}, while hydrogen removal from the acetylamine nitrogen atom is 87.4 kcal mol^{-1}.

Results, listed in Table II, clearly support that the phenol moiety is the weakest with respect to a radical attack during degradation and metabolism. These results are consistent with those of Alves's [22]. The phenol BDE is below our likely threshold for both LDA and PBE functionals.

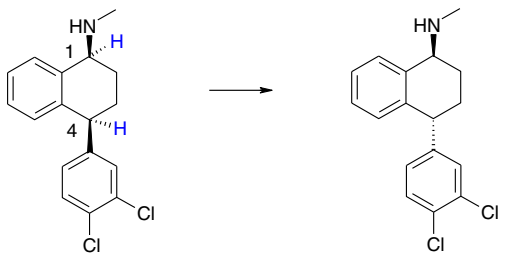
BDE Calculations Applied to Autoxidation for Drugs with Unknown Historical Autoxidation

For compound 51 and 54, no moiety is expected to bring any autoxidation susceptibility; therefore it is quite logical that our BDE calculation results, listed on Table IV, are above the threshold unlikely for both LDA and PBE functionals.

For compounds 53 and 55, no autoxidation is yet reported, however our calculations indicate that the probability is equivocal.

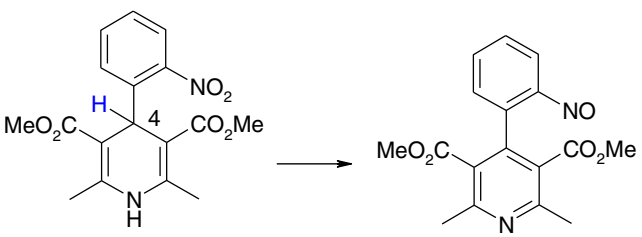
Dextromethorphan, a widely used antitussive drug, as hydrobromide, is a well-known stable compound. Nevertheless, susceptibility of the benzylic methylene to photooxidation has been reported, even if under particular conditions involving $FeCl_3$ catalysis [38]. Moreover, European Pharmacopeia reports the ketone from benzylic oxidation as qualified impurity, confirming that degradation is possible, although very weak. Equivocal BDE values from Table IV are in agreement with reported data.

Compound 55, amibegron, is a potent β-3 agonist clinically tested against severe depression, but discontinued in 2010. Its API is very stable and the substance is also rather stable in oral solid formulations; the only degradation pathway detected in stress studies and in stability studies is the hydrolysis of the



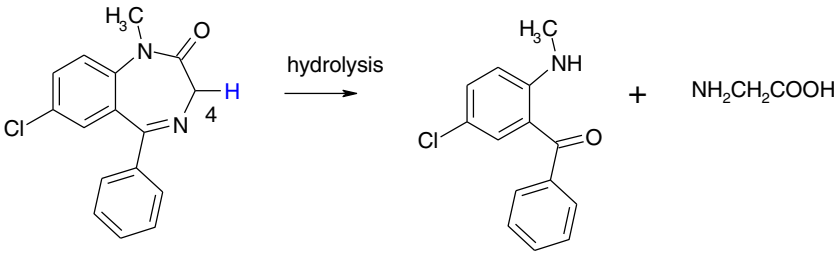
48

Fig. 8 Sertraline photo-epimerization



49

Fig. 9 Nifedipine aromatization



52

Fig. 10 Diazepam degradation pathway hydrolysis

ethyl ester [39]. Equivocal computed BDE values thus confirm known experimental data.

Special case: BDE Calculations Applied to Diazepam

BDE values have been calculated for diazepam 52, results are listed in Table IV. Hydrogen H4 shows the lowest BDE, being adjacent to nitrogen heteroatom and carbonyl of amide moiety. However oxidation degradation for this molecule has not been reported as a degradation pathway competing with the primary hydrolysis pathway of the imine and amide [40] removing the weakness on H4 from its structure (Figs. 9 and 10).

Our study revealed that the molecule is prone to oxidative degradation that hasn't been revealed due to competition with hydrolysis.

CONCLUSION

The primary focus of this work was to develop a computational method to estimate the autoxidation potential of a compound prior to experimental work. A method based on two functional, LDA and PBE, was outlined and the results were compared to a diverse set of chemical entities. This set of reference compounds allowed comparing the accuracy of the calculated BDE with experimental values in order to achieve reliable prediction. In this perspective, PBE functional provided, within reasonable calculation time, useful predictions both in qualitative and quantitative terms. Indeed, the correct weakest C-H bond was identified in each of the chemical moieties tested. Moreover a risk scale could be set up to quantify the level of likelihood for an autoxidation event to occur. An in depth chemical "understanding" of the reacting moiety of the molecule is still necessary to achieve reliable degradation prediction when several degradation pathways are competing.

BDE calculations may be taken as a complementary source of information with the experimental stress testing [7] for early compound stability profiling. Additionally, there is no need to be a highly skilled computational expert to routinely use our pipeline pilot protocol.

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