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Efficacy ranking of triterpenoids as inducers of a cytoprotective enzyme and as inhibitors of a cellular inflammatory response *via* their electron affinity and their electrophilicity index

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Abstract

Electron affinity (*EA*) and electrophilicity index (ω) of 16 synthetic triterpenoids (TP), previously identified as inducers of cytoprotective enzymes and as inhibitors of cellular inflammatory responses, have been calculated by the molecular orbital method. Linear correlations were obtained by plotting the values of *EA*, as well as those of ω *versus* (i) the potencies of induction of NAD(P)H quinone reductase (NQO1, EC 1.6.99.2), a cytoprotective enzyme, expressed *via* the concentration of TP required to double the specific activity of NQO1 (CD value) and (ii) the values of their anti-inflammatory activity expressed *via* the IC-50 of TP for suppression of upregulation of inducible nitric oxide synthase (iNOS, EC 1.14.13.39), both previously experimentally determined. The observed correlations demonstrate quantitatively for a series of triterpenoids that their electrophilicity is a major factor determining their potency as inducers of the cytoprotective phase 2 response and as inhibitors of inflammatory processes.

Keywords

electron affinity; inflammation; NAD(P)H-quinone oxidoreductase 1; quantum chemical calculations; structure-activity relationships

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Conflict of interest

The authors declare that there are no conflicts of interest.

1. Introduction

Recent observations have contributed to a new understanding of the 19th century Rudolf Virchow's hypothesis of a link between inflammatory processes induced by chronic injury and a predisposition to certain cancers [1]. Indeed, a chronic injury can promote a cascade of events leading to mutations and carcinogenesis *via* (i) release of leukocytes, (ii) production by leukocytes of reactive oxygen and nitrogen species (ROS, RNS), which can lead to oxidative damage and mutations, (iii) induction of cell responses involving wound healing, angiogenesis and tissue remodeling [2,3]. A linear correlation has been observed between the anti-inflammatory potencies of a series of synthetic triterpenoid (TP) analogues of oleanolic acid and their ability to induce the phase 2 enzyme NAD(P)H-quinone reductase (NQO1, EC 1.6.99.2) [4]. It has also been shown that this correlation extends to many other inducers that belong to structurally distinct chemical classes [5]. NQO1 is an obligatory two electron-reducing enzyme. Its gene expression is regulated coordinately with a battery of >100 cytoprotective enzymes via the Keap1/Nrf2/ARE pathway that protects against damaging electrophilic species, including endogenous ROS and RNS arising from aerobic metabolism, toxic electrophilic exogenous compounds, and electrophilic metabolites. The induction of NQO1 by test agents is a reliable biomarker of the ability of these agents to protect against tumor development in many animal models [6,7]. In our previous study [4] two bioassays were used: the first expressed the induction of the phase 2 enzyme NQO1 *via* the concentration of TP (called CD value) required to double the specific activity of NQO1 in the murine hepatoma cell line Hepa1c1c7, the second quantified the inhibition of a cellular inflammatory process *via* the IC-50 value of TP for suppression of induction of nitric oxide synthase (iNOS, EC 1.14.13.39) by interferon-gamma (IFN- γ) in primary mouse macrophages. This study demonstrated that (i) a Michael reaction acceptor functionality, i.e., olefinic function conjugated to electron-withdrawing group(s) in the TP, is necessary for inducer activity of NQO1 and for blocking inflammation, (ii) induction of phase 2 enzymes by TP is selective and independent of phase 1 enzymes, (iii) induction requires the presence of both Keap1, the protein sensor for inducers, and transcription factor Nrf2, (iv) the most potent TP (TP-225) reacts with thiol groups of the Keap1 sensor; such reaction renders Keap1 unable to target transcription factor Nrf2 for ubiquitination and proteasomal degradation, which allows Nrf2 to bind to the antioxidant response element (ARE), and activate the transcription of NQO1 and other ARE-regulated genes. These studies also suggested that both abilities of TP to induce NQO1 and to inhibit inflammatory processes, which stimulate the inducible form of nitric oxide (NO) synthase (iNOS), could be contributing to the potent chemoprotective effects of TP, recently observed in several animal models [8-10]. Increased levels of NO can react with superoxide anions and produce peroxynitrite anion (ONOO⁻), an oxidant and a nitrating species, which can damage DNA and increase cancer risk [11].

The aim of the present investigation is to search for quantitative structure-activity relationships between the physico-chemical properties of TP and their potencies as inducers of NQO1 and as inhibitors of inflammation. Since these TP are electrophiles and interact, *via* their Michael acceptor functionalities, with sulfhydryl groups of Keap1, the property involved in this interaction should be the reduction potential E (TP/TP^{•-}). A quantum mechanical calculation of the energy of the lowest unoccupied molecular orbital E (LUMO) represents a satisfactory measure of the electron-acceptor properties of the molecule in question [12]. This E (LUMO) is linearly correlated with the reduction potential E (TP/TP^{•-}) in solution and with the electron affinity (EA) of TP in the gas phase as already demonstrated for a series of aromatic hydrocarbons [13].

In the present work, three possible theoretical approaches for estimating the electron affinity (EA) of our triterpenoid compounds are tested. One uses directly the E (LUMO) value of the

neutral species TP, the second and the third consider a system where an electron is removed from the open-shell structure of the $TP^{\bullet-}$ anion, using either the energy of the highest occupied molecular orbital, E (HOMO), or the energy of the singly occupied molecular orbital, E (SOMO), located between E (HOMO) and E (LUMO) on the axis of the orbital energies of $TP^{\bullet-}$. For ranking the efficacy of our 16 TP, another descriptor has been used, the electrophilicity index ω . Further comments concerning the determinations and usefulness of these two descriptors, electron affinity (EA) and electrophilicity index (ω), will be given in the section below dealing with computational methods.

In search of correlations, the three different values of EA, as well as those of ω of the triterpenoid compounds were plotted versus: (i) the induction potencies of NQO1 expressed *via* the concentration of TP (CD) required to double the specific activity of NQO1, and (ii) their anti-inflammatory activity expressed *via* the half maximal inhibitory concentration, IC-50, of TP for suppression of induction of nitric oxide synthase (iNOS), both previously experimentally determined [4,14,15].

In addition to the purely electronic factor, another physico-chemical property will be considered, the hydrophobic character of the TP expressed by $\log P$, P representing the relative distribution in an octanol/water mixture obtained from the ratio of the concentration of a compound in octanol over its concentration in aqueous solution.

2. Computational background and calculation methods

2.1 Electron affinity (EA)

It is customary to justify the theoretical procedures of electron affinity (EA) determination by referring to the so-called Koopmans theorem, specific of self-consistent field (SCF) treatments for closed-shell systems [16]. Formal relationships between E (HOMO), or the E (LUMO) energy levels of a closed-shell system and their energy change when an electron is respectively removed from the highest occupied molecular orbital (HOMO) or attached to the lowest unoccupied molecular orbital (LUMO) are given in classical textbooks of Quantum Chemistry [17]. Linear correlations between (i) E (HOMO) of aromatic hydrocarbons and their ionization potentials in the gas phase as well as their electrochemical oxidation potentials in solution, and (ii) E (LUMO) of aromatic hydrocarbons and their electron affinities (EA) in the gas phase and their electrochemical reduction potentials in solution are reported by Pullman and Pullman [18]. Moreover, the Koopmans theorem, valid for closed-shell structures, without unpaired electrons, is extended to radicalic open-shell systems, using E (SOMO) [19]. Some overlooked details of this Koopmans theorem have been clearly stated by Angeli [20].

Given the structural complexity of the TP molecules, self-consistent field (SCF) calculations by the AM1 quantum mechanical procedure have been carried out with the Hyperchem 7.51 program. The restricted Hartree-Fock (RHF) formalism, using standard AM1 basis sets of atomic orbitals was retained for computing the orbital energy levels of TP and $TP^{\bullet-}$ systems. A set of filled and empty molecular orbitals is generated for each of them, from which were selected the relevant HOMO, LUMO and SOMO levels linearly correlated with electron affinities EA of (TP) and reduction potentials E ($TP/TP^{\bullet-}$). Let us present now some details on the validity of our approach from the theoretical point of view.

In the survey of Mulliken on molecular orbital theory [21], it was pointed out that the success of predictions based on Koopmans theorem for ionization potentials comes from a balance between two sources of error in these calculations, namely lack of self-consistency of the electronic and geometrical structure assigned to the final cation and neglect of correlation energies in the picture of both initial and final states. It can be shown without any

calculation that these two terms have opposite signs, hence the Mulliken conclusion. Extending the argument of Mulliken to non-closed shell configurations is not immediate, but Koopmans theorem is probably valid, although to a minor extent, for the SOMO orbital of radicals as discussed by Dodds and McWeeny [22]. It is not possible to argue in the same way about the method using E (LUMO) of neutral TP for estimating electron affinities *EA*, because there is no reason why the two sources of error mentioned above should have opposite signs in this case, in contrast with the use of E (HOMO) or E (SOMO) for the estimation of ionization potentials. Thus, *EA* evaluations from orbital energies only lie finally in a comparison *a posteriori* between the calculation results and the experimental data. A good linear relationship observed between them in a series of chemically related molecules means that there is probably a quantity transferable from a compound to another, compensating all the deficiencies of the treatment. Let us add finally that the evaluation of electron affinities of molecules *M* via a calculation of the ionization energies of the charged species *M*^{•-} obtained by putting an extra electron in a SOMO or a LUMO level, would be a valuable alternative [23]. However, the application of Koopmans theorem to the resulting anion TP^{•-} implies that a SCF treatment of the charged species has been made effectively, in addition to the treatment carried out from the neutral TP, as it has been done in the present work by the AM1 method. The electron affinities (*EA*) of our TP reflect the capability of these agents to accept one electron from a donor.

2.2 Electrophilicity index (ω)

In the frame of the molecular orbital method, it is possible to describe the efficacy of TP compounds by means of another reactivity descriptor expressed in terms of E (HOMO) and E (LUMO) orbital energies of the neutral TP, namely the electrophilicity index (ω)

$$\omega = \frac{\chi^2}{2\eta}$$

where (χ) is the electronegativity and (η) the chemical hardness of the molecule. Use of ω was suggested empirically by Maynard *et al.* in their study of the reaction between the human immunodeficiency virus type 1 (HIV-1) nucleocapsid p7 (NCp7) zinc finger thiolates and different electrophilic ligands [24]. This was justified later by Parr *et al.* in the frame of the density functional theory (DFT) [25]. The alternative of using standard DFT calculations would be possible if theoretical values in agreement with experimental ionization potentials could be obtained in a simple way. Now the E (HOMO) DFT values are generated by the so-called non-interacting electron model and therefore are lower than the exact ones by a deviation ΔE , the evaluation of which would necessitate an intricate linear response procedure [26]. Moreover, the determination of realistic E (LUMO) values *via* DFT with respect to experimental *EA* is still debated [27]. Let us add that, in connection with the anti-inflammatory properties of some non-steroidal drugs, we have recently pointed out the fact that correlation coefficients between E (HOMO) and biological efficacy computed by our AM1 calculations are better than those computed by corresponding DFT or even *ab initio* methods [28]. Equations for the calculations of χ and η are :

$$\chi = \frac{I+EA}{2} \quad \text{and} \quad \eta = I - EA$$

where *I* and *EA* are respectively the ionization potential and electron affinity. In terms of orbital energies, these equations can be written as:

$$\chi = -\frac{E(\text{HOMO}) + E(\text{LUMO})}{2} \quad \text{and as} \quad \eta = E(\text{LUMO}) - E(\text{HOMO}).$$

Thus, the electrophilicity index becomes

$$\omega = \frac{[E(\text{HOMO}) + E(\text{LUMO})]^2}{8[E(\text{LUMO}) - E(\text{HOMO})]}$$

This concept of electrophilicity index, its usefulness as well as the electrophilicity scales based on different physico-chemical properties have been reviewed by Chattaraj *et al.* [29]. In the section “Results”, we will briefly compare the electrophilicity scale, ω observed for our TP with the ranking based on their electron affinities *EA*.

2.3 Hydrophobic character

The calculation of $\log P$ was carried out using the XlogP program, which is based on the summation of atomic contributions and includes correction factors for some intramolecular interactions [30].

Statistical calculations and graph plotting were performed using the Kaleidagraph software (version 3.6). This software calculates the linear curve fits, using the least-squared error method.

3. Results

3.1 Potencies of synthetic TP analogues of oleanolic acid as inducers of NQO1 versus their electron affinity (EA)

The molecular structures of the TP under study are shown in Fig.1

Induction potencies are assessed by using the CD value, the Concentration of each TP required to Double the NQO1 specific activity in Hepa 1c1c7 murine hepatoma cells. These CD values have been previously experimentally determined by us [4]. For convenience, the induction potencies are now reported as pCD values (Table 1), where pCD is the logarithm of the reciprocal of CD (expressed in M units). Notably, these vary by four orders of magnitude from 9.55 for the most active TP-225 to 5.41 for the least active TP-46.

In Table 1 are also reported the energy values in eV of the E (LUMO) of neutral TP, the E (HOMO) of TP^{•-} anions, the E (SOMO) of TP^{•-} anions, the E (HOMO) of neutral TP, the CD values, and the pCD values of the TP. The E (LUMO) of TP, and E (HOMO) and E (SOMO) of the anion TP^{•-} reported in Table 1, have a sign which is the inverse of those of the corresponding reduction potentials E (TP/TP^{•-}). Thus, the most electrophilic TP is 225 and the least electrophilic is 46. Of note, TP-46, as well as TP-69 and TP-156 are the least potent inducers and have one Michael acceptor function (α,β -unsaturated carbonyl group), in contrast with the other 13 TP, which have two Michael acceptor functions. In addition, inactive oleanolic acid has no Michael acceptor function (Fig.1).

An absence of correlation of the biological activities of these TP with their electron-donating property is observed when plotting the pCD values *versus* the E (HOMO) of the TP, which represents the ease of a TP to donate an electron and is linearly correlated with their oxidation potential E (TP^{•+}/TP). This absence of correlation is consistent with the fact that the TP act as electron-acceptors *via* their electrophilic Michael acceptor groups in their direct interaction with highly reactive thiols of the sensor protein Keap1 [4]. Plots of the

pCD *versus* E (LUMO), *versus* E (HOMO) of the anion TP^{•-} and *versus* the energy of the singly occupied molecular orbital E (SOMO) for the 16 active TP (Fig.1) give respectively linear correlations expressed by equations (1), (2) and (3) with correlation coefficients r:

$$pCD = 5.53 - 3.71 E \text{ (LUMO)} \quad (1), r=0.75$$

$$pCD = 0.53 - 2.57 E \text{ (HOMO) of TP}^{\bullet-} \text{ anion} \quad (2), r=0.72$$

$$pCD = 11.23 - 2.65 E \text{ (SOMO)} \quad (3), r=0.80$$

The plots of correlations (1), (2), and (3) are rather similar to one another with the same main outliers TP-233 and 191.

The linear plot pCD *versus* E (SOMO) corresponding to equation (3) has the best correlation coefficient r and is shown in Fig.2.

Outlier TP-233 is dissimilar to the other compounds in the series by its large hydrophobic substituent -CO₂(CH₂)₇CH₃ in position 17 (Fig.1), which is the cause of its high log*P* value (8.65) relatively to the log*P* values of the other TP (Table 1). In contrast with TP-233, outlier TP-191 is dissimilar by the presence of two hydrophilic substituents -CO₂H in positions 2 and 17, cause of the lowest log*P* value (1) in the TP series. These special structures can be considered as the cause of the presence of TP-233 and TP-191 relatively far from the linear fit of the correlations (1), (2), and (3) (Fig.2), and (5), (6), and (7) (Fig. 3). If the TP-233 and TP-191 are taken out, the correlation coefficients r are significantly improved:

$$pCD = 5.50 - 4.15 E \text{ (LUMO)} \quad (1'), r=0.85$$

$$pCD = 0.19 - 2.92 E \text{ (HOMO) of the TP anion} \quad (2'), r=0.83$$

$$pCD = 11.75 - 2.89 E \text{ (SOMO)} \quad (3'), r=0.88$$

The value of the correlation coefficients might still be improved by taking into account other physico-chemical properties such as the steric factors of the TP approach to their binding site, which are more difficult to quantify. The present correlations demonstrate an unquestionable general quantitative tendency: the higher is the electron affinity of a TP [i.e: the higher is its reduction potential E (TP/TP^{•-})], the greater its potency as NQO1 inducer. The importance of the electron affinity (EA) of the TP for their biological efficacy is consistent with the observations that the introduction of a second Michael acceptor functionality increases their potency and that a reaction of the TP occurs with the thiol groups of the Keap 1 sensor [4]. Reactions of Michael acceptor-containing TP and their tricyclic derivatives with nucleophile thiols have been reported previously [31-33].

3.2 Potencies of synthetic TP for suppression of iNOS induction *versus* their electron affinity (EA)

Table 2 reports the IC-50 and the pIC-50 values, cologarithm of IC-50, determined for the suppression of induction of nitric oxide synthase (iNOS) (M) in mouse macrophages, expressing the anti-inflammatory activities of our series of TP.

The plot of pIC-50 *versus* the E (LUMO), *versus* E (HOMO) of the anion TP^{•-} and *versus* the energy of the singly occupied molecular orbital E (SOMO) for the 16 active TP of Fig.1, give respectively linear correlations expressed by equations (4), (5) and (6) with correlation coefficients r :

$$\text{pIC-50} = 5.527 - 5.49 \text{ E (LUMO)} \quad (4) \quad r=0.77$$

$$\text{pIC-50} = -1.96 - 3.75 \text{ E (HOMO) of TP anion} \quad (5) \quad r=0.73$$

$$\text{pIC-50} = 13.77 - 3.97 \text{ E (SOMO)} \quad (6) \quad r=0.83$$

The plot pIC-50 *versus* E (SOMO) corresponding to equation (6) has the best correlation coefficient r, and is shown in Fig. 3.

Again, TP-233 and TP-191 are outliers for reasons related to their structure. If they are taken out of the plot, the correlation coefficients r are improved:

$$\text{pIC-50} = 5.23 - 6.14 \text{ E (LUMO)} \quad (4') \quad r=0.88$$

$$\text{pIC-50} = -3.05 - 4.27 \text{ E (HOMO) of TP anion} \quad (5') \quad r=0.84$$

$$\text{pIC-50} = 14.52 - 4.30 \text{ E (SOMO)} \quad (6') \quad r=0.92$$

New equations expressing the correlations for all compounds as a function of both parameters EA and logP are reported below. These new expressions lead to two remarks: (i) the EA parameters are by far the major factor controlling the biological properties, (ii) the new correlation coefficients are identical to those expressing pCD and pIC-50 as an exclusive function of the EA parameters (see equations 1, 2, 3, 4, 5, 6)

$$\begin{aligned} \text{pCD} &= 5.67 (\pm 0.72) - 3.79 (\pm 0.91) \text{ E (LUMO)} - 0.041 (\pm 0.15) \log P, R=0.75 \\ \text{pCD} &= 0.51 (\pm 1.82) - 2.56 (\pm 0.68) \text{ E (HOMO) of TP anion} + 0.017 (\pm 0.15) \log P, R=0.72 \\ \text{pCD} &= 11.59 (\pm 1.14) - 2.73 (\pm 0.56) \text{ E (SOMO)} - 0.059 (\pm 0.14) \log P, R=0.80 \end{aligned}$$

$$\begin{aligned} \text{pIC}_{50} &= 5.36 (\pm 1.01) - 5.60 (\pm 1.28) \text{ E (LUMO)} - 0.029 (\pm 0.21) \log P, R=0.77 \\ \text{pIC}_{50} &= -2.10 (\pm 2.63) - 3.72 (\pm 0.98) \text{ E (HOMO) of TP anion} + 0.059 (\pm 0.22) \log P, R=0.73 \\ \text{pIC}_{50} &= 14.20 (\pm 1.55) - 4.09 (\pm 0.77) \text{ E (SOMO)} - 0.059 (\pm 0.19) \log P, R=0.83 \end{aligned}$$

The two remarks above are also valid for the equations 1', 2', 3', 4', 5', 6', where the two outliers TP-233 and TP-191 have been taken out.

3.3 Potencies of synthetic TP analogues of oleanolic acid as inducers of NQO1 and as inhibitors of iNOS induction *versus* their electrophilicity index (ω)

As mentioned above, the electrophilicity index, ω , of every TP, reported in Table 3 is calculated in terms of E (HOMO) and E (LUMO) orbital energies of the neutral TP by

means of the formula: $\omega = \frac{\chi^2}{2\eta}$

Plotting this electrophilicity index, ω , versus E(SOMO) gives a linear correlation expressed by:

$\omega = 1.90 - 0.30 E(\text{SOMO})$ with a correlation coefficient $r = 0.92$ This indicates that both scales, based on distinct physico-chemical parameters, are rather similar. Compared with the plots of pCD and pIC50 *versus* E(SOMO) expressed by equations (3), (3'), (6), and (6'), plots of pCD and pIC50 as a function of the electrophilicity index, ω , showed rather similar correlation coefficients expressed by the following equations: for all our 16 TP

$$\text{pCD} = -4.02 + 7.8\omega \quad (7) \quad r = 0.77$$

$$\text{pIC50} = -8.36 + 11.23\omega \quad (8) \quad r = 0.76$$

and without the outliers TP 233 and 191

$$\text{pCD} = -5.67 + 9.1\omega \quad (7') \quad r = 0.89$$

$$\text{pIC50} = -10.74 + 13.06\omega \quad (8') \quad r = 0.89$$

The efficacy ranking observed by using the electrophilicity index, ω , is in good agreement with that obtained by means of *EA* expressed by the E (SOMO) for our 16 TP.

Our approach could be used to analyze the efficacy ranking of other electrophilic ligands reacting with the highly reactive thiolates of Keap1 as well as those of other proteins.

4. Discussion

More than 20 years ago, Talalay *et al.* provided the first insight into the structural and electronic requirements for phase 2 inducer activity by demonstrating that many inducers are Michael reaction acceptors [34]. Our calculations on triterpenoid electrophiles, acting as Michael acceptors *via* their α,β -unsaturated ketone functionalities, rationalize these observations. The present findings complement earlier studies on other classes of NQO1 inducers, considered as “anti-oxidants”, including diphenols [35], phenylpropenoids [36], and flavonoids [37] for which linear correlations between their NQO1 induction potencies and their E (HOMO) are observed. These linear correlations are due to the fact that these classes of electron-donating molecules (diphenols, phenylpropenoids, and flavonoids) act *via* a two-step mechanism involving (i) oxidation of these inducers to their quinone or

semiquinone derivatives, which are Michael acceptors and (ii) oxidation or alkylation of highly reactive thiols of Keap1 by these electrophilic quinones or semiquinones. The transformation of these diphenols, phenylpropenoids, and flavonoids into oxidant species can occur by various routes, including their oxidation by reactive oxygen and nitrogen species (ROS, RNS), by autooxidation or by non-enzymatic (e.g., metal-catalyzed) as well as enzymatic mechanisms [35]. This necessary transformation of the diphenols, phenylpropenoids, and flavonoids into oxidant species is consistent with an important observation of Prochaska *et al.* [38,39] that among diphenol inducers, only those with oxidizable phenolic hydroxyl groups (i.e., *ortho*- or *para*-, but not *meta*- diphenols) are inducers. Very recently, we have obtained experimental evidence that under aerobic conditions, Cu⁺⁺ catalyzes the oxidation of *ortho*- and *para*-hydroquinones to their corresponding quinones, which subsequently react with cysteine residues of Keap1, resulting in activation of Nrf2, and that such oxidation to quinones is both necessary and sufficient for activation of the Keap1/Nrf2 pathway by oxidizable diphenols [40].

The correlations between the electron affinity (EA), and their electrophilicity index, ω , of the TP and their potencies as inducers of the cytoprotective phase 2 response and as inhibitors of inflammatory processes provide insights into the mechanism(s) by which the TP is recognized by the intracellular sensor molecules. In the case of the Keap1/Nrf2/ARE-dependent upregulation of the cytoprotective phase 2 response, it is now widely accepted that highly reactive cysteine residues of Keap1 are the sensors for inducers. Similarly, cysteine 179 of I κ B kinase β (IKK β) has been implicated as the intracellular target of TP which triggers events that ultimately lead to inhibition of the IFN γ -mediated upregulation of iNOS [41,42]. Up to date, adducts of TP and cysteine residues of either Keap1 or IKK β have not been identified despite of the strong spectroscopic (UV and NMR) evidence that the Michael addition reaction does occur when TP are incubated with thiols [4,43], suggesting that such adducts do form, but are unstable, and reversible. Indeed, NMR spectroscopic evaluation has demonstrated the nucleophilic addition of dithiothreitol to TP-151 and the decomposition of the resulting adduct to regenerate the TP [43].

One may then speculate that the ultimate result of such a reversible addition could be a change in the oxidation state of the sensor cysteine(s), e.g. formation of disulfide bond(s) within Keap1 and IKK β . Indeed, we have previously detected intermolecular disulfide-linked dimers of Keap1 in extracts of cells that were treated with inducers of three different chemical types, i.e., sulforaphane, 1,2-dithiole-3-thione, and bis(2-hydroxybenzylidene)acetone, and their facile conversion to reduced monomers by treatment with dithiothreitol [44]. Likewise, treatment with either TP-155 or TP-151 resulted in formation of a species, corresponding to a dimer of IKK β based on its molecular mass, that reacts with anti-IKK β antibody in cells ectopically expressing wild-type-, but not C179A mutant IKK β [42]. These findings imply that following chemical modification of specific cysteine thiols, the modifying agents are displaced by intermolecular sulfhydryl disulfide interchange to lead to a covalent disulfide-linked dimer of Keap1 or IKK β as shown in Scheme 1.

Notably, there is now experimental evidence for the formation of both intra- and intermolecular disulfide bridges in Keap1. Thus, very recently, Fourquet *et al.* [45] reported that exposure to reactive oxygen (ROS) and nitrogen (RNS) species leads to formation of an intramolecular disulfide linking Cys226 and Cys613, as well as an intermolecular disulfide bridging two Keap1 molecules through Cys151 of each monomer, and that even in untreated cells a fraction of Keap1 carries the intramolecular disulfide. In the case of a triterpenoid reacting with Keap1, such speculated outcome as shown in Scheme 1 will allow for the possibility of regeneration of the sensor proteins upon reduction of the disulfide bridge(s),

unlike an irreversible inactivation by a stable adduct formation that may result in toxicity and will require *de novo* protein synthesis for regain of function.

5. Conclusion

In our present calculations, the TP outer energy levels computed by the molecular orbital method are correlated to (i) their electron affinity (*EA*) in the gas phase, and (ii) to their reduction potential E (TP/TP^{•-}) in solution. Our quantum chemical study establishes quantitative structure-activity relationships between *EA* and biological efficacies. The electrophilicity index ω of the TP calculated using electronegativity and chemical hardness parameters, gives correlations and efficacy ranking analogous to those obtained by the efficacy scaling *via EA*, expressed by E (SOMO) calculations. These correlations demonstrate quantitatively, for this series of triterpenoids, that their electron-acceptor property is by far the major factor that determines their potency as inducers of the cytoprotective phase 2 response and as inhibitors of inflammatory processes. Our results illustrate the Szent-Györgyi concept of “submolecular electronic processes”, in particular those involving electron-donor or electron-acceptor properties of exogenous substances in biological systems [46]. More specifically, in the present study, the efficacy of the Michael addition of a thiol nucleophile to an electron deficient carbon of a triterpenoid has been interpreted using the electron affinity of the acceptor as a global reactivity descriptor. Moreover, the energy change predicted from orbital energies gives information on the most favored attack position of the electrophilic compound considered, as pointed out by A. Pullman [47].

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Figure 1.
Structures of the triterpenoids under study

Figure 2. Plot of pCD (logarithm of the reciprocal of CD, expressed in M units) of the 16 triterpenoid inducers of NQO1 *versus* their E (SOMO), corresponding to equation (3)

Figure 3.

Plot of pIC₅₀ (logarithm of the reciprocal of IC₅₀, expressed in M units) of the 16 triterpenoid inhibitors of induction of iNOS expressing the anti-inflammatory activities *versus* their E (SOMO), corresponding to equation (6)

Scheme 1.

A nucleophilic attack on the electrophilic carbon (C1) on ring A of a Michael-acceptor bearing triterpenoid by a cysteine thiolate ion of a protein sensor leads initially to adduct formation that in turn is rapidly attacked by a second cysteine thiolate to form an intermolecular (**A**) or intramolecular (**B**) protein disulfide. Only partial structures (i.e., ring A) are shown.

Table 1

Energy values in eV of E (LUMO) of TP, E (HOMO) of TP^{-•}, E (SOMO) of TP^{-•}, E (HOMO) of TP^{-•}, E (HOMO) of TP^{-•}, CD values in μM unit, the corresponding pCD values and log P values of the TP

TP[a]	E(LUMO)	E(HOMO) of TP anion	E (SOMO) of TP anion	E(HOMO)	CD[b]	pCD	log P
Ol. acid	1.1517		3.1530	-9.2948	-----	-----	3.56
46	0.0847	-1.8999	2.3050	-9.4198	3.9	5.41	3.53
69	-0.0326	-2.0420	2.2300	-10.1753	1.8	5.74	2.83
82	-0.1660	-2.1102	1.6210	-10.1038	0.28	6.55	3.61
191	-0.6704	-2.9702	1.2870	-10.2660	0.22	6.66	1.00
222	-0.6506	-2.9673	1.3050	-10.2308	0.022	7.66	3.78
190	-0.6541	-2.9647	1.3020	-10.2397	0.018	7.74	3.36
151	-0.7185	-2.8980	1.1370	-10.3069	0.0023	8.64	3.17
155	-0.7051	-2.8866	1.1690	-10.2788	0.001	9.00	5.54
223	-0.6721	-2.9424	1.2110	-10.2028	0.0013	8.89	4.48
224	-0.6705	-2.9451	1.2090	-10.0514	0.001	9.00	4.97
226	-0.6656	-2.9289	1.2130	-9.6932	0.0068	8.17	5.11
235	-0.7697	-3.0019	0.9075	-9.6187	0.0033	8.48	4.68
233	-0.7059	-2.8957	1.1420	-10.2794	0.22	6.66	8.65
225	-0.7670	-2.9271	1.0530	-10.4076	0.00028	9.55	5.35
162	-0.4550	-2.6002	1.2770	-10.2278	0.1	7.00	4.75
156	-0.5381	-2.6670	1.7280	-9.5244	1.5	5.82	5.46

[a] In this column the numbers represent the different triterpenoids as reported in Fig. 1. The oleanic acid (Ol. acid) is inactive because it has no electrophile function.

[b] Taken from reference [4]

Table 2

IC-50 values taken from references (14,15) and pIC-50 values for inhibition of iNOS activation by IFN-gamma by TP in primary mouse macrophages

TP ^[a]	IC-50 μ M	pIC-50
46	5.6	5.25
69	3.3	5.48
82	0.20	6.7
191	0.20	6.7
222	0.008	8.1
190	8.0 E-04	9.1
151	2.0 E-04	9.7
155	1.0E-04	10.0
223	9.8 E-05	10.008
224	0.00058	9.24
226	0.00155	8.81
235	1.4 E-05	10.85
233	0.0604	7.22
225	3.5 E-06	11.45
162	0.02	7.7
156	0.7	6.15

^[a] In this column the numbers represent the different triterpenoids as reported in Fig. 1.

Table 3Electronegativity, χ , chemical hardness, η , and electrophilicity index, ω , of our triterpenoids

TP ^[a]	$2\chi = E(\text{HOMO}) \text{ plus } E(\text{LUMO})$ ^[b]	$\eta = E(\text{LUMO}) \text{ minus } E(\text{HOMO})$ ^[b]	χ^2	Electrophilicity index $\omega = \chi^2/2\eta$
Ol. acid				
46	-9.3351	9.5045	21.7860	1.1460
69	-10.2079	10.1427	26.0503	1.2841
82	-10.2698	9.9378	26.3671	1.3266
191	-10.9364	9.5956	29.9012	1.5580
222	-10.8814	9.5802	29.6012	1.5449
190	-10.8938	9.5856	29.6687	1.5475
151	-11.0254	9.5884	30.3898	1.5847
155	-10.9839	9.5737	30.1615	1.5752
223	-10.8749	9.5307	29.5659	1.5511
224	-10.7219	9.3809	28.7398	1.5318
226	-10.3588	9.0276	26.8262	1.4857
235	-10.3884	8.849	26.9797	1.524
233	-10.9853	9.5735	30.1692	1.5756
225	-11.1746	9.6406	31.2179	1.6200
162	-10.6828	9.7728	28.5305	1.4596
156	-10.0625	8.9863	25.3134	1.4084

^[a] In this column the numbers represent the different triterpenoids as reported in Fig. 1.

^[b] The energy values E(HOMO) and E(LUMO) are reported in Table 1