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Published in:
Bioorganic & Medicinal Chemistry Letters

DOI:
[10.1016/j.bmcl.2016.07.028](https://doi.org/10.1016/j.bmcl.2016.07.028)

Publication date:
2016

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Document Version
Version created as part of publication process; publisher's layout; not normally made publicly available

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Bensasson, R. V., Dinkova-Kostova, A. T., Zheng, S., Saito, A., Li, W., Zoete, V., & Honda, T. (2016). Electron affinity of tricyclic, bicyclic, and monocyclic compounds containing cyanoenones correlates with their potency as inducers of a cytoprotective enzyme. *Bioorganic & Medicinal Chemistry Letters*, 26(17), 4345-4349. <https://doi.org/10.1016/j.bmcl.2016.07.028>

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Accepted Manuscript

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PII: S0960-894X(16)30741-7
DOI: <http://dx.doi.org/10.1016/j.bmcl.2016.07.028>
Reference: BMCL 24072

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 10 June 2016
Revised Date: 11 July 2016
Accepted Date: 12 July 2016

Please cite this article as: Bensasson, R.V., Dinkova-Kostova, A.T., Zheng, S., Saito, A., Li, W., Zoete, V., Honda, T., Electron affinity of tricyclic, bicyclic, and monocyclic compounds containing cyanoenones correlates with their potency as inducers of a cytoprotective enzyme, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: <http://dx.doi.org/10.1016/j.bmcl.2016.07.028>

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Electron affinity of tricyclic, bicyclic, and monocyclic compounds containing cyanoenones correlates with their potency as inducers of a cytoprotective enzyme

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ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

Electron affinity

Energy of the lowest unoccupied molecular orbital

Keap1/Nrf2/ARE pathway

Nrf2 activator

NAD(P)H:quinone oxidoreductase 1 (NQO1) inducer

QSAR

ABSTRACT

Tricyclic, bicyclic, and monocyclic compounds containing cyanoenones induce various anti-inflammatory and cytoprotective enzymes through activation of the Keap1/Nrf2/ARE (antioxidant response element) pathway. The potency of these compounds as Nrf2 activators was determined using a prototypic cytoprotective enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) in Hepa1c1c7 murine hepatoma cells. The electron affinity (EA) of the compounds, expressed as the energy of their lowest unoccupied molecular orbital [E (LUMO)], was evaluated using two types of quantum mechanical calculations: the semiempirical (AM1) and the density functional theory (DFT) methods. We observed striking linear correlations [$r = 0.897$ (AM1) and 0.936 (DFT)] between NQO1 inducer potency of these compounds and their E (LUMO) regardless of the molecule size. Importantly and interestingly, this finding demonstrates that the EA is the essentially important factor that determines the reactivity of the cyanoenones with Keap1.

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Oxidative stress and inflammation are closely involved in the pathogenesis of ageing and chronic degenerative diseases, including cardiovascular and neurodegenerative diseases as well as cancer.^{1,2} Therefore, a strategy has been proposed with the aim of reducing oxidative stress and inflammation in order to inhibit or retard aging and carcinogenesis. The redox-sensitive signaling system known as the Keap1/Nrf2/ARE (Kelch-like ECH-associated protein 1/NF-E2 p45-related factor 2/antioxidant response element) pathway plays a key role in maintenance of cellular homeostasis under stress, inflammatory, carcinogenic, and proapoptotic conditions. Electrophilic agents oxidize highly reactive cysteine residues of the protein sensor for inducers, Keap1, leading to a conformation change that allows the transcription factor Nrf2 to accumulate and undergo nuclear translocation and binding (as a heterodimer with a small Maf protein) to the AREs, specific DNA sequences present in the promoter/enhancer regions of cytoprotective genes.³⁻⁵ These processes enhance transcription of ARE-regulated anti-inflammatory and cytoprotective enzymes. Thus, activators of the Keap1/Nrf2/ARE pathway (briefly, Nrf2 activators) are

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considered to be potential cytoprotective and anti-inflammatory agents.

Reversible covalent drugs, which bind to their protein targets but not permanently, combine the advantages and circumvent the disadvantages of irreversible covalent and reversible non-covalent drugs. These compounds demonstrate unique biological features by selectively targeting protein cysteine residues in a reversible covalent fashion,^{6,7} avoiding off-target drug interactions. Owing to the reversible nature of the reaction, toxicity issues commonly associated with covalent protein modifications can be minimized. However, reversible covalent drugs have been largely ignored because of the lack of reactive compounds to produce the reversible covalent adducts with protein targets. Based on previously published evidence,^{8,9} the nonenolizable cyanoenone functionality is considered to be one of the best fragments that can be used for exploring reversible covalent drugs which are targeting the Keap1/Nrf2/ARE pathway. Although the reversible Michael adducts involved in equilibrium mixtures have been observed in solution by NMR, UV, and mass spectrometry, they have never been isolated and structurally characterized because of the rapid equilibrium that favors the reverse reaction.⁸⁻¹⁰ Most recently, a 3D structure of a reversible thiol Michael adduct was observed in situ in a crystalline sponge.¹¹

Presently, a Nrf2 activator, bardoxolone methyl (Figure 1), which is a semisynthetic triterpenoid with cyanoenone functionality in ring A and a reversible covalent drug,⁸ has been evaluated in Phase II clinical trials for the treatment of PAH (pulmonary arterial hypertension) in the USA¹² and diabetic nephropathy in Japan¹³. During the development of bardoxolone methyl and its analogues, tri-, bi-, and monocyclic compounds with cyanoenones have been designed and explored.^{9,10,14} They are also highly potent Nrf2 activators and reversible covalent drugs. Amongst them, tricyclic compound **1** (TBE-31, Figure 1 and Table 1) is the most potent Nrf2 activator.¹⁴ Although **1** was initially designed based on bardoxolone methyl, **1** is not a simple mimic since **1** is not a triterpenoid but a tricyclic compound, with two cyanoenones in rings A and C, and an ethynyl group at C10a. To understand such high biological potency of **1** in a series of tri-, bi-, and monocyclic compounds with cyanoenones, we have focused on the electron affinity of these compounds expressed as the energy of their lowest unoccupied molecular orbital [E (LUMO)] because it has been previously reported that electron affinity is a major molecular property for determining the potency of bardoxolone methyl analogues, semisynthetic triterpenoids, as Nrf2 activators.¹⁵ We have found that **1** has extremely low E (LUMO) among them. Also, notably, we have observed striking linear correlations between the inducer potency of the compounds and their E (LUMO) regardless of the molecule size. We now report these interesting quantitative structure–activity relationships (QSAR) in this communication.

Figure 1. Structures of bardoxolone methyl and TBE-31

The potency of tri-, bi-, and monocyclic compounds containing cyanoenones as Nrf2 activators was evaluated using a prototypic cytoprotective enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) in Hepa1c1c7 murine hepatoma cells by CD values (the concentration required to double the specific enzyme activity of NQO1).^{9,10,14} For this study, the pCD values (logarithm of the reciprocal of CD values expressed in M (mol/L) units) are employed for inducer potency. NQO1 is the prototypical Nrf2 target gene, and the NQO1 bioassay is widely recognized as a highly quantitative readout for Nrf2 activity. To our knowledge, the cyanoenones are not direct substrates for NQO1. We have previously shown that cyanoenones react with cysteine residues in Keap1, the main negative regulator of Nrf2.^{6,7,14} In a variety of cell lines and animal tissues, we have demonstrated that Nrf2 activation by cyanoenones leads to the coordinate transcriptional upregulation of NQO1 together with other Nrf2-target genes, such as multiple isoforms of glutathione *S*-transferase (GST), heme oxygenase 1, γ -glutamyl cysteine ligase catalytic (GCLC) subunit, as well as an ARE-luciferase reporter, a direct readout of Nrf2-mediated transcription.^{6,7,14} Furthermore, Nrf2 is required for the NQO1 inducer activity of cyanoenones as NQO1 induction is not observed in Nrf2-deficient cells.^{6,7}

The electron affinity of these new compounds can be calculated *via* the energy of their lowest unoccupied molecular orbital, E (LUMO) in eV, and was quantified at two quantum mechanical levels: the semiempirical AM1,¹⁶ and the density functional theory (DFT)¹⁷ (see Table 1). Briefly, no solvent

Table 1.

Compd# ^a	Structure	pCD ^b	E (LUMO) AM1 ^c [eV]	E (LUMO) DFT ^d [eV]
1 (TBE-31)		9.04	-1.2576	-3.0814
2		8.12	-1.2017	-2.9450
3		7.74	-1.1735	-2.9566
4		7.74	-1.1540	-2.9429
5		7.70	-1.0204	-2.9314
6		7.67	-1.0076	-2.8759
7		7.52	-1.1794	-2.9096
8		7.48	-0.9497	-2.7263
9		7.38	-0.9845	-2.8550
10		7.25	-0.9257	-2.7208
11		7.00	-0.7791	-2.7309
12		7.00	-0.9026	-2.7056
13		6.82	-0.9307	-2.7589
14		6.82	-0.9143	-2.6855
15		6.80	-0.8224	-2.6710
16		6.74	-1.0863	-3.0365
17		5.72	-0.6147	-2.4689

^aThese compounds are racemic or optically inactive (meso compounds).

^bLogarithm of the reciprocal of CD values expressed in M units and reported in references (5) and (6).

^{c,d}E (LUMO) in eV calculated using the AM1¹⁶ or the DFT¹⁷ approaches, with the Gaussian 09 program.³⁰

model was used for the calculation of the LUMO energies. Since the molecules are neutral and mainly non polar, we do not expect any significant change in the relative LUMO energies upon the application of an implicit solvent model. All molecules were fully energy-optimized before LUMO energy calculations, both at the DFT and AM1 levels, using the "Opt= Tight" keyword of the Gaussian 09 program. Since the molecules have few conformational degrees of freedom, we consider that this energy minimization is sufficient.

Recent examples demonstrate the continuing growth of predictive QSAR modeling using quantum-chemical methods for the analysis of physico-chemical properties of congeneric series of chemicals that govern their biological and medicinal properties.^{18,19} In addition to pursuit for the QSAR between electron affinity and biological potency of the tri-, bi-, and monocyclic compounds, the present study has another aim, that is, to check if the AM1 quantum mechanical procedure is as relevant as DFT quantum mechanical method for this type of application. This question is still controversial, since fast AM1 calculations are frequently underrated, due to the domination of heavy DFT calculations; as stated in a Nature article: "The DFT method is the most heavily cited concept in physical sciences. It can be used to describe all chemistry, biochemistry, biology, nanosystems and materials."²⁰

Since tri-, bi-, and monocyclic compounds under study interact *via* their Michael acceptors with sulfhydryl groups of the protein Keap1, the critical feature involved in this interaction is their electron-acceptor property, which can be expressed by their reduction potential $E(T/T^{\cdot-})$, T = a compound of these tri-, bi-, and monocyclic compounds). The relative electron-acceptor properties of a compound (T) can be represented by its electron affinity (EA) in the gas phase and its electrochemical reduction potential in solution $E(T/T^{\cdot-})$. The EA of a compound (T) represents the energy liberated when an electron adds to T in the gas phase to form $T^{\cdot-}$. Linear correlations between the EA within a series of related compounds, called congeners, and the energy of their lowest unoccupied molecular orbital $E(LUMO)$ are presented in classical textbooks of Quantum Chemistry.^{21,22} A quantum mechanical calculation of $E(LUMO)$ of T represents a satisfactory measure of the electron-acceptor properties of the molecule in question.²³ This $E(LUMO)$ is linearly correlated with the reduction potential $E(T/T^{\cdot-})$ in solution and with the EA of T in the gas phase as already demonstrated for a series of aromatic hydrocarbons.²⁴ The fact that $E(LUMO)$ can be considered as representative of the EA is initially based on Koopmans' theorem,²⁵ and on its analysis by Angeli,²⁶ Mulliken,²⁷ Dodds and McWeeny,²⁸ and Heinrich *et al.*²⁹

The $E(LUMO)$ of the electrophilic tri-, bi-, and monocyclic compounds under study was calculated by two quantum mechanical techniques: the semiempirical AM1,¹⁶ and the density functional theory (DFT- B3LYP/6-31+G**) methods,¹⁷ using the Gaussian 09 program revision A.02 (Gaussian Inc., Wallingford CT, 2009).³⁰

The software Kaleidagraph (version 3.6.4) was used for plotting our data and for the determination of the correlation coefficients r of the linear correlations observed.

Plotting the potencies for induction of NQO1 expressed by pCD values of these compounds 1–17 excluding compound 16 versus their $E(LUMO)$ calculated by the AM1 and DFT methods leads to linear correlations spanning more than three orders of magnitude, and displayed in Figures 2 and 3.

The linear correlation shown in Figure 2 is expressed by equation (1)

$$pCD = 3.6268 - 3.763E(LUMO, AM1) \text{ with a correlation coefficient } (r) = 0.8969 \quad (1)$$

The linear correlation shown in Figure 3 is expressed by equation (2)

$$pCD = -5.2486 - 4.4834E(LUMO, DFT) \text{ with a correlation coefficient } (r) = 0.9356 \quad (2)$$

Figure 2. Plot of the pCD of the monocyclic, bicyclic, and tricyclic compounds (logarithm of the reciprocal of CD, expressed in M (mol/L) units, CD being the concentration of inducer required to double the specific enzyme activity NQO1) versus their electron affinity expressed as their $E(LUMO)$ in eV, calculated via the semiempirical AM1 method.

Linear correlation and correlation coefficient $r = 0.89687$ corresponding to equation 1.

Figure 3. Same as Figure 1, with $E(LUMO)$ calculated at the DFT level.

Linear correlation and correlation coefficient $r = 0.9356$ corresponding to equation 2.

Notably and importantly, striking linear correlations excluding 16 are observed regardless of the molecule size. If the pCD of 16 followed the linear correlations observed for other compounds, its pCD values should be respectively pCD = 7.72 and 8.36 [the value is as low as pCD (9.04) of 1 (TBE-31)] from equations (1) and (2). We speculate that the reasons why the biological potency (6.74) of 16 is lower than expected are as follows. Since it has been previously reported that 16 has the

highest reactivity with a sulfhydryl group and a chloride anion in this series of compounds,¹⁰ a large portion of **16** could be “quenched” by abundant cellular thiols, such as the cysteine residue of glutathione, which is present at millimolar concentrations, and/or **16** could be inactivated by chloride anion in the cell culture medium used for biological testing.³¹

The two linear correlations (Figures 2 and 3) show that both methods of E (LUMO) calculations lead to rather similar correlation coefficients. The similarities between the correlation coefficients *r* observed at the AM1 and DFT levels for the linear correlations displayed in Figures 2 and 3 demonstrate the relevance of the semiempirical AM1 method for this particular application in front of the heavier yet more frequently used DFT method, a question which was still controversial.

The relevance of AM1 method versus the DFT method has also been observed in the case of other groups of chemoprotective molecules with correlation coefficients being in certain cases slightly higher for the AM1 method and in other cases lower than for the DFT method. These correlation coefficients are reported in a review dealing with biological efficacy and redox properties of diphenols, phenylpropenoids, flavonoids, triterpenoids, benzoic and salicylic acids.³² For instance, in the case of 27 anti-inflammatory molecules (12 salicylic acids and 15 phenols) the semiempirical AM1 method gives a better correlation coefficient *r* than the DFT procedure.³²

In summary, we observed striking linear correlations [*r* = 0.897 (AM1) and 0.936 (DFT)] between NQO1 inducer potency (pCD) of these tri-, bi-, and monocyclic compounds and their E (LUMO) regardless of the molecule size. Importantly and interestingly, this finding demonstrates that the electron affinity is the essentially important factor for the cyanoenones to reversibly bind with Keap1. Consequently, we could clarify that **1** (TBE-31) is the most potent inducer because the both E (LUMO, AM1) and E (LUMO, DFT) for **1** are the lowest. This QSAR encourages the design of new reversible covalent drugs containing cyanoenones as warheads.

Also, our investigations indicate (i) that the semiempirical AM1 quantum mechanical procedure is as relevant as the density functional theory (DFT) quantum mechanical method for this application dealing with cyanoenone compounds, and (ii) that simple low-cost *in silico* determination of the electron affinity of candidate molecules in a set of congeners can potentially reduce the number of bioassays and improve the forward search for novel chemoprotective drugs.

Acknowledgments

The authors remember with gratitude Gaston Berthier (*Laboratoire de Chimie Théorique de Paris*) for his invaluable inspiration. We thank Dr Ute Röhrig (*Swiss Institute of Bioinformatics*) for her help in using the Gaussian program. We thank for their precious support Professors Sylvie Rebuffat, Bernard Bodo, Thérèse Garestier and Alexandre Boutorine (*Muséum National d'Histoire Naturelle*). This investigation was supported by funds from Stony Brook Foundation, Reata Pharmaceuticals Inc., the Muséum National d'Histoire Naturelle, the Solidar-Immun Foundation and Cancer Research UK (C20953/A18644). SZ, AS, and WL are grateful to the Institute of Chemical Biology & Drug Discovery Postdoctoral Scholarships.

References and notes

1. Statdman, E. R.; Berlett, B. S. *Chem. Res. Toxicol.* **1997**, *10*, 485.
2. Coussens, L. M.; Werb, Z. *Nature*, **2002**, *420*, 860.
3. Dinkova-Kostova, A. T.; Holtzclaw, W. D.; Cole, R. N.; Itoh, K.; Wakabayashi, N.; Katoh, Y.; Yamamoto, M.; Talalay, P. *Proc. Natl. Acad. Sci. USA*. **2002**, *99*, 11908.
4. Kobayashi, M.; Li, L.; Iwamoto, N.; Nakajima-Takagi, Y.; Kaneko, H.; Nakayama, Y.; Eguchi, M.; Wada, Y.; Kumagai, Y.; Yamamoto, M. *Mol. Cell. Biol.* **2009**, *29*, 493.
5. Hayes, J. D.; Dinkova-Kostova, A. T. *Trends Biochem. Sci.* **2014**, *39*, 199.
6. Dinkova-Kostova, A.T.; Liby, K. T.; Stephenson, K. K.; Holtzclaw, D. W.; Gao, X.; Suh, N.; Williams, C.; Risingsong, R.; Honda, T.; Gribble, G. W.; Sporn, M. B.; Talalay, P. *Proc. Natl. Acad. Sci.* **2005**, *102*, 4584.
7. Dinkova-Kostova, A. T.; Talalay, P.; Sharkey, J.; Zhang, Y.; Holtzclaw, W. D.; Wang, X. J.; David, E.; Schiavoni, K. H.; Finlayson, S.; Mierke, D. F.; Honda, T. *J. Biol. Chem.* **2010**, *285*, 33747.
8. Couch, R. D.; Browning, R. G.; Honda, T.; Gribble, G. W.; Wright, D. L.; Sporn, M. B.; Anderson, A. C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2215.
9. Zheng, S.; Laxmi, Y. R. S.; David, E.; Dinkova-Kostova, A. T.; Shrivani, K. H.; Ren, Y.; Zheng, Y.; Trevino, I.; Bumeister, R.; Ojima, I.; Wigley, W. C.; Bliska, J. B.; Mierke, D. F.; Honda, T. *J. Med. Chem.* **2012**, *55*, 4837.
10. Li, W.; Zheng, S.; Higgins, M.; Morra Jr., R. P.; Mendis A., Chien, C.-W.; Ojima, I.; Mierke, D. F.; Dinkova-Kostova, A. T.; Honda T. *J. Med. Chem.* **2015**, *58*, 4738.
11. Duplan, V.; Hoshino, M.; Li, W.; Honda, T.; Fujita, M. *Angew. Chem. Int. Ed.* **2016**, *55*, 4919–4923.
12. NCT02036970, ClinicalTrials.gov.
13. NCT02316821, ClinicalTrials.gov.
14. Honda T.; Yoshizawa, H.; Sundarajan, C.; David, E.; Lajoie, M. J.; Favalaro Jr., F. G.; Janosik, T.; Su, X.; Honda, Y.; Roebuck, B. D.; Gribble, G. W. *J. Med. Chem.* **2011**, *54*, 1762.
15. Bensasson, R. V.; Zoete, V.; Berthier, G.; Talalay P.; Dinkova-Kostova, A. T. *Chem.-Biol. Interact.* **2010**, *186*, 118.
16. Dewar, M.J.S.; Zoebisch, E.G.; Healy, E.F.; Stewart, J.J.P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
17. Parr, R. G.; Yang W. In *Density-Functional Theory of Atoms and Molecules*; Oxford University Press: Oxford, 1989.
18. Cherkasov, A.; Muratov, E. N.; Fourches, D.; Varnek, A.; Baskin, I. I.; Cronin, M.; Dearden, J.; Gramatica, P.; Martin, Y. C.; Todeschini, R.; Consonni, V.; Kuz'min, V.E.; Cramer, R.; Benigni, R.; Yang, C.; Rathman, J.; Terfloth, L.; Gasteiger, J.; Richard, A.; Tropsha, A. *J. Med. Chem.* **2014**, *57*, 4977.
19. Matta, C. J. *Comput. Chem.* **2014**, *35*, 1165.
20. Van Norden, R.; Maher, B.; Nuzzo, R. *Nature* **2014**, *514*, 550.
21. Daudel, R.; Lefebvre, R.; Moser, C. In *Quantum Chemistry, Methods and Applications*; Interscience Publishers: New York & London, 1959.
22. Pullman, B.; Pullman, A. In *Quantum Biochemistry*; Interscience Publishers: New York & London, 1960; pp. 132.
23. Maccoll, A. *Nature* **1949**, *163*, 178.
24. Matsen, F. A. *J. Chem. Phys.* **1956**, *305*, 178.
25. Koopmans, T. *Physica* **1933**, *1*, 104.
26. Angeli, C. *J. Chem. Educ.* **1998**, *75*, 1494.
27. Mulliken, R.S. *Phys. Rev.* **1948**, *74*, 736.
28. Dodds, J. L.; McWeeny, R. *Chem. Phys. Lett.* **1972**, *13*, 9.
29. Heinrich, N.; Koch, W.; Frenking, G. *Chem. Phys. Lett.* **1986**, *124*, 20.
30. Gaussian 09, revision A.02 Gaussian Inc., Wallingford CT, 2009. http://www.gaussian.com/g_tech/g_ur/m_citation.htm.
31. Although it is quite interesting issue to clarify why the second ethynyl group of **16** increases the reactivity, the clarification is out of this project.
32. Bensasson, R.V.; Sowlati-Hashjin, S.; Zoete, V.; Dauzonne D.; Matta C. *Int. Rev. Phys. Chem.* **2013**, *32*, 393.