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Kinetics and Thermodynamics of Reversible Thiol Additions to Mono- and Diactivated Michael Acceptors: Implications for the Design of Drugs That Bind Covalently to Cysteines

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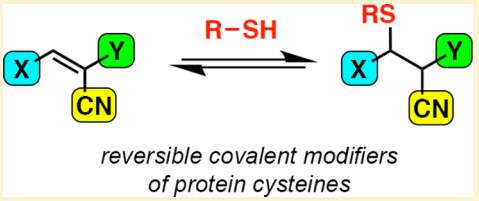
Supporting Information

ABSTRACT: Additions of cysteine thiols to Michael acceptors underpin the mechanism of action of several covalent drugs (e.g., afatinib, osimertinib, ibrutinib, neratinib, and CC-292). Reversible Michael acceptors have been reported in which an additional electron-withdrawing group was added at the α-carbon of a Michael acceptor. We have performed density functional theory calculations to determine why thiol additions to these Michael acceptors are reversible. The α-EWG group stabilizes the anionic transition state and intermediate of the Michael addition, but less intuitively, it destabilizes the neutral adduct. This makes the reverse reaction (elimination) both faster and more thermodynamically favorable. For thiol addition to be reversible, the Michael acceptor must also contain a suitable substituent on the β-carbon, such as an aryl or branched alkyl group. Computations explain how these structural elements contribute to reversibility and the ability to tune the binding affinities and the residence times of covalent inhibitors.



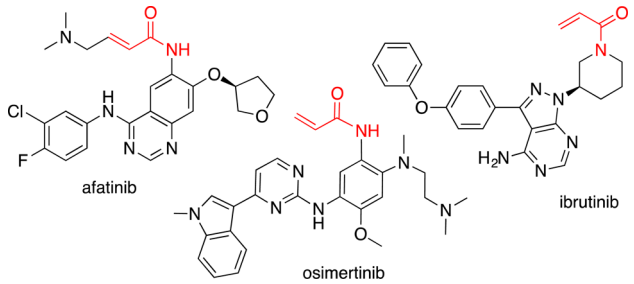
INTRODUCTION

There has been a growing interest in the rational design of drugs that covalently modify their biological targets.[1] Traditionally, structure-based drug design programs have avoided covalent modifiers, due to safety concerns. The propensity of chemically reactive inhibitors to engage in off-target interactions has been associated with elevated risks of toxicity and immunological responses. However, covalent modifiers allow access to higher binding affinities[2] and longer residence times[3] than can be achieved with traditional noncovalent inhibitors and these properties potentially translate advantageously into lower dosages, dose frequencies, and systemic exposure to the drug. A number of pharmaceutical companies have thus initiated drug discovery programs directed toward covalent inhibitors of various enzymes.

One strategy, which has already produced several FDA-approved drugs, is "targeted covalent inhibition".[1] In this strategy, a covalent warhead is tethered to a ligand that recognizes the target protein's binding site through noncovalent interactions. Once the ligand has docked into the binding site, the warhead forms a covalent bond with a nearby amino acid residue. Target selectivity is maximized by designing the inhibitor such that covalent bond formation involves a poorly conserved, noncatalytic residue.

The addition of a Michael acceptor to a cysteine thiol group is a common modality of targeted covalent inhibitor design. The cysteine-targeting acrylamides afatinib[4] and osimertinib[5] have received FDA approval for treatment of non-small-cell lung cancer (NSCLC), while ibrutinib[6] has been approved for treatment of chronic lymphocytic leukemia and mantle cell lymphoma (Scheme 1). Other cysteine-targeting Michael acceptors currently in advanced clinical trials include the EGFR/ERBB inhibitors dacomitinib,[7] CO-1686[8] (NSCLC), neratinib[9] (breast cancer), and Bruton's tyrosine kinase (BTK) inhibitor[10] CC-292[11] (blood cancers). All of these inhibitors irreversibly modify their target proteins.

Scheme 1. Covalent Drugs That Bind Irreversibly to Specific Protein Cysteine Thiol Groups



A recent development is the concept of reversible covalent inhibition—i.e., where the covalent bond can be readily broken to release the inhibitor.[3,12,13] Reversible bond formation introduces the prospect of developing inhibitors that form long-lasting, but not necessarily permanent, interactions with

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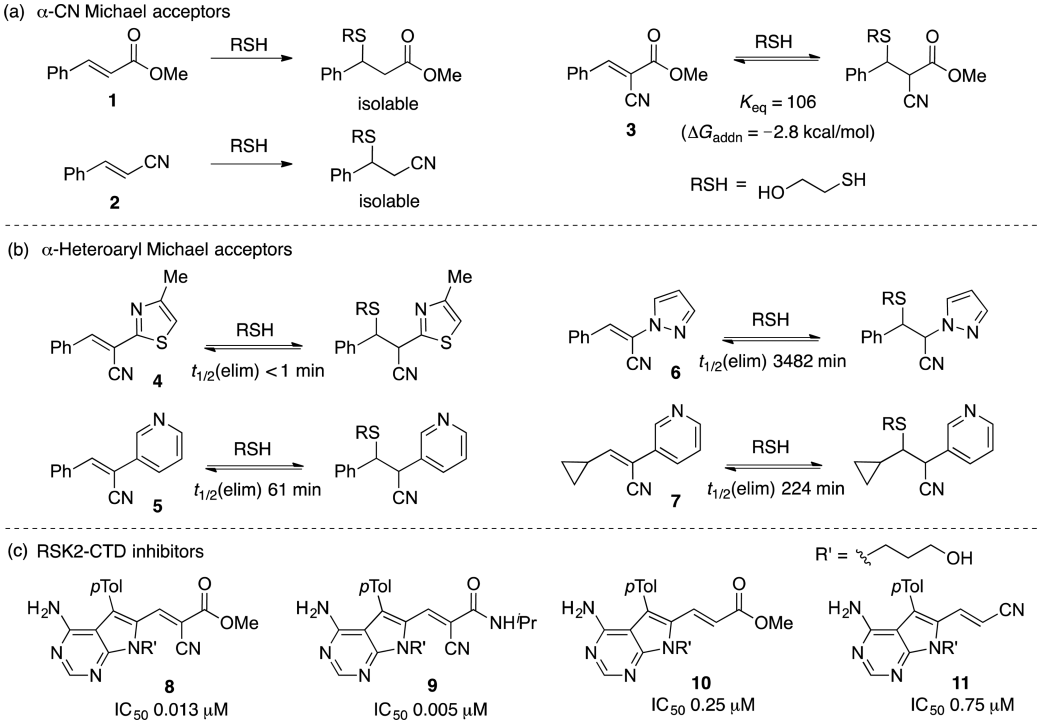
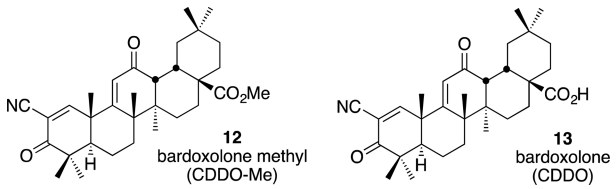
Scheme 2. (a) Effect of an α Electron-Withdrawing Group on the Reversibility of Thiol Additions to Michael Acceptors,[12] (b) α-Heteroaryl Michael Acceptors and the Half-Lives for Thiol Elimination from the Corresponding β-Mercaptoethanol Adducts,[16] and (c) Reversible and Irreversible Inhibitors of the Kinase RSK2-CTD[12]



the target protein. If the binding site does not have the correct three-dimensional structure (e.g., if the protein is unfolded, as occurs during natural protein turnover), or if the inhibitor binds to an off-target thiol, then the association will be short-lived and less likely to be harmful. Potential advantages of reversible covalent inhibitors include improved safety margins and applicability to chronic diseases.

Taunton and co-workers pioneered the design of activated Michael acceptors as reversible, cysteine-targeting inhibitors (Scheme 2).[12,14,15] Whereas simple Michael acceptors such as **1** and **2** reacted irreversibly with thiols to give isolable adducts, the thiol adducts of analogues containing an electron-withdrawing group (EWG) at the α-position, such as cyano-substituted **3**, could not be isolated and reverted to the reactants upon dilution. Similarly, acceptors **4**−**7**, in which the carboxylate group of **3** has been replaced by a heteroaromatic ring, were also shown to undergo reversible thiol additions.[16] These α-EWGs have been used to design reversible inhibitors of several kinases.[3,12,16−18] For example, α-cyanoacrylate **8** and α-cyanoacrylamide **9** displayed up to 150-fold more potent inhibition of the C-terminal kinase domain of the p90 ribosomal protein S6 kinase RSK2 (RSK2-CTD) than the irreversible inhibitors **10** and **11**, which lack an α-EWG.

Related to these observations is the anti-inflammatory agent bardoxolone methyl (CDDO-Me, **12**),[19] a semisynthetic oleanolic acid derivative that reached phase III clinical trials for chronic kidney disease in diabetic patients.[1h,20] Spectroscopic measurements on the parent bardoxolone (**13**) showed that thiols add reversibly to the α-cyanoenone group.[19a] Reversible modification of specific protein cysteine residues by the α-cyanoenone moiety of bardoxolone methyl is believed to trigger activation of the Keap1-Nrf2 pathway and inhibition of NF-κB, leading to the observed anti-inflammatory activity.



It is not obvious why an α-EWG should make the addition of a thiol to a Michael acceptor reversible. Taunton noted that the α-EWG makes the thiol adduct more acidic, stabilizing the conjugate base (enolate or related anion), which is an intermediate in the base-catalyzed elimination.[12,16] However, an α-EWG also activates the Michael acceptor toward reaction with thiolate anion, implying that C−S bond formation would be more favorable. We have investigated the influence of these effects on rates and equilibria of variously substituted Michael acceptors.

We report here a theoretical study showing how the substituents on a Michael acceptor influence the kinetics and thermodynamics of thiol additions, leading to reversibility. Density functional theory calculations yield the unexpected conclusion that an α-EWG not only lowers the barrier for thiol addition or elimination but also makes addition less thermodynamically favorable. In addition to the α-EWG, the substituent on the β-carbon is also crucial to the overall energetics and is an equally important element of reversible inhibitor design.

RESULTS AND DISCUSSION

Density functional theory (DFT) calculations were performed on the additions of thiols to a range of Michael acceptors (Table 1).[21] Initially, we evaluated the performance of several DFT methods by comparing the calculated and experimental[12]

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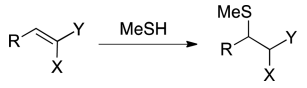
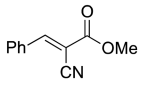
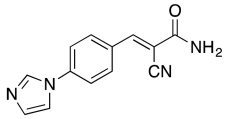
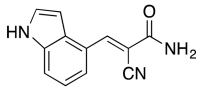
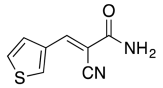
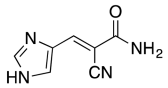
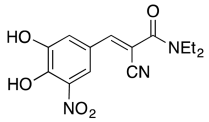
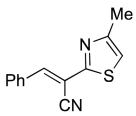
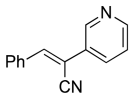
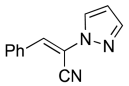
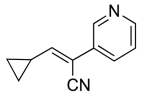
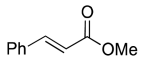
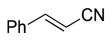
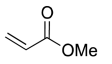
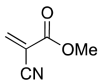
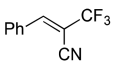
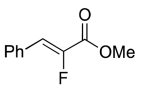
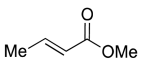
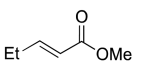
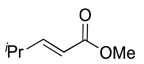
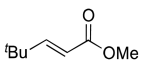
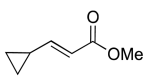
Table 1. Computed Thermodynamics of Thiol Additions to Michael Acceptors in Water[a]



A. α-Cyano Michael acceptors		Calc ΔH	Calc (Expt[b]) ΔG
	**3**	−13.7	−2.9 (−2.8)
	**14**	−14.6	−3.8 (−3.9)
	**15**	−13.8	−2.6 (−3.0)
	**16**	−12.9	−2.8 (−2.6)
	**17**	−12.1	−1.3 (−2.0)
	**18**[c]	−16.5	−5.7 (−3.2)

B. α-Heteroaryl Michael acceptors		Calc ΔH	Calc ΔG
	**4**	−14.5	−3.6
	**5**	−15.4	−4.7
	**6**	−17.4	−6.6
	**7**	−14.7	−3.4

C. Michael acceptors		Calc ΔH	Calc ΔG
	**1**	−16.7	−6.4
	**2**	−16.9	−5.8
	**19**	−20.0	−10.1
	**20**	−20.4	−10.3
	**21**	−18.0	−7.7
	**22**	−16.2	−4.6
	**23**	−17.5	−6.0
	**24**	−17.8	−7.6
	**25**	−17.8	−7.3
	**26**	−17.7	−6.8
	**27**	−17.0	−6.1
	**28**	−16.6	−5.7

[a] ΔH and ΔG in kcal/mol computed at the M06-2X/6-311+G(d,p) level of theory in CPCM water. [b] ΔG for Michael additions of β-mercaptoethanol in PBS containing 1−2% DMSO; data from ref 12. [c] NEt_2 was modeled as NMe_2.

values of ΔG for additions of β-mercaptoethanol to α-cyano substituted Michael acceptors **3** and **14**−**18** (Table 1A). In the calculations, β-mercaptoethanol was modeled as MeSH.[22] We evaluated a variety of functionals (B3LYP, M06-2X, and ωB97X-D), basis sets, methods of computing entropy, and solvent models. Full details are given in the Supporting Information. Among the methods examined, the closest agreement with experiment was obtained from computations with M06-2X/6-311+G(d,p) in conjunction with the CPCM continuum solvent model. The discussion below will refer to the results obtained with this theoretical method. Results of computations at other levels of theory are discussed in the Supporting Information.

The agreement between the calculated and experimental ΔG values is very good, within 0.1−0.7 kcal/mol in most cases. Entacapone (**18**) is an exception; the predicted ΔG value is 2.5 kcal/mol more negative than the experimental value. This may be due to partial ionization of the nitrocatechol group of **18** under the experimental conditions (PBS, 1−2% DMSO), which is not modeled by the calculations. Overall, the calculations correctly predict both the absolute magnitudes of ΔG and the general trends in relative reactivities of the α-cyano-substituted Michael acceptors.

Calculations on the additions of MeSH to a range of other Michael acceptors are shown in Table 1B,C. Table 1B contains a selection of α-heteroaryl acrylonitriles. The half-lives for elimination of β-mercaptoethanol from the adducts of these acceptors were reported by Taunton[16] and are shown in Scheme 2b. Table 1C contains a series of model Michael

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acceptors which were computed to enable analysis of substituent effects.

Two observations are clear from Table 1. First, MeSH additions to α-cyanoacrylates (e.g., **3** and **21**) are about 3 kcal/mol less thermodynamically favorable than additions to the corresponding parent acrylates (**1** and **19**) or acrylonitriles (**2** and **20**). The lower thermodynamic driving force for addition to the α-cyano derivatives is unexpected.[23] It reveals that reversibility is determined by more than simply the acidity of the adduct. Indeed, the smaller thermodynamic driving force is the fundamental reason why the thiol adduct of **3** undergoes elimination upon dilution,[12] while additions to α-unsubstituted analogues **1** and **2** are effectively "irreversible".

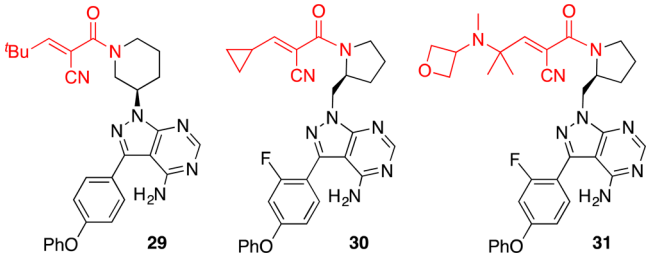
Thiol addition to **1** or **2** has an equilibrium constant of about $K = 10^4$ ($\Delta G = -6$ kcal/mol), whereas addition to **3** has a constant of $K = 10^2$ ($\Delta G = -3$ kcal/mol). The position of the equilibrium in the reaction of **3** is more sensitive to concentration than it is in reactions involving **1** or **2**. For example, in a solution initially containing 100 mM thiol and 100 μM **1**, 100% of **1** will be converted to the thiol adduct at equilibrium. If the equilibrium mixture is then diluted 10-fold, only 0.2% of the adduct will revert to **1** and free thiol. In contrast, the reaction of **3** with a thiol at the same concentrations will give 93% conversion to the adduct, and after a 10-fold dilution, 39% of the adduct will revert to **3** and free thiol. For the reversibility of thiol addition to **3** to be experimentally detectable, the kinetics of addition and elimination must be fast. However, if the cyano group did not affect ΔG, then the adduct of **3** would undergo minimal thiol elimination, even if the barrier were low.

The ΔG values for MeSH additions to α-heteroaryl-substituted acrylonitriles **4**–**7** are between −3.4 and −6.6 kcal/mol. These values are intermediate between the ΔG value for addition to cyanoacrylate **3** (−3 kcal/mol) and those for additions to **1** or **2** (−6 kcal/mol), with the exception of pyrazolyl derivative **6**, which has $\Delta G = -6.6$ kcal/mol. Experimentally, thiol additions to **4**–**7** are reversible, with elimination half-lives of seconds (**4**), minutes (**5**), hours (**7**), or days (**6**).[16] Alternatively, a σ-acceptor group (CF_3) at the α-position is also predicted to make addition less favorable, as shown by **22**, in which the α-CF_3 group raises ΔG by an amount comparable to that for the 3-pyridyl group of **5** ($\Delta G = -4.6$ kcal/mol). A fluoro substituent at C-α (**23**) has a smaller effect, raising ΔG by 0.4 kcal/mol in comparison to **1**. α-CF_3- and α-F-substituted acrylamides have been explored as constructs for designing reversible covalent inhibitors of drug-resistant EGFR mutants.[24,25]

The second important observation from Table 1 is that an α-EWG is not, by itself, sufficient to make thiol addition reversible. The substituent on the β-carbon of the Michael acceptor plays an equally important role. The ΔG values for MeSH additions to Michael acceptors without a β-substituent (**19**–**21**) are 4–5 kcal/mol more negative than those for additions to the corresponding β-phenyl-substituted acceptors (**1**–**3**) and predict that additions to the unsubstituted Michael acceptors would be effectively irreversible.[26,27] Thus, the ability to chemically tune a Michael acceptor, so that it reacts reversibly with thiols, depends not only on the α-EWG but even more so on the presence of a suitable substituent at C-β.

The earliest reversible cysteine-targeting inhibitors (e.g., **8** and **9**, Scheme 2) contained a heteroaryl substituent at the β position, but more recent work[3,16] has employed branched-chain alkyl groups at this position. For example, a thiol adduct of **7**, containing a cyclopropyl substituent at C-β, underwent elimination over a period of several hours (Scheme 2), while acrylamides **29**–**31** (Scheme 3) reversibly inhibited BTK with

Scheme 3. Reversible Covalent BTK Inhibitors with Branched-Chain Alkyl β-Substituents[3]



residence times ranging from 1 day to 1 week. Computations predict that the ΔG value for MeSH addition to **7** (−3.4 kcal/mol) is about 1 kcal/mol less negative than the ΔG value for addition to the β-phenyl analogue **5**. The computed ΔG values for MeSH additions to **1** and **28** differ by a similar amount. In the series of acyclic alkyl-substituted acrylates **24**–**27**, thiol addition becomes increasingly less favorable as the degree of branching of the β-substituent increases. An ^{i}Pr or ^{t}Bu group (**26** and **27**) has an effect on the energetics of thiol addition similar to that of a Ph group (**1**).

We modeled the addition of a thiol to bardoxolone methyl (Figure 1). In this case, geometry optimizations were

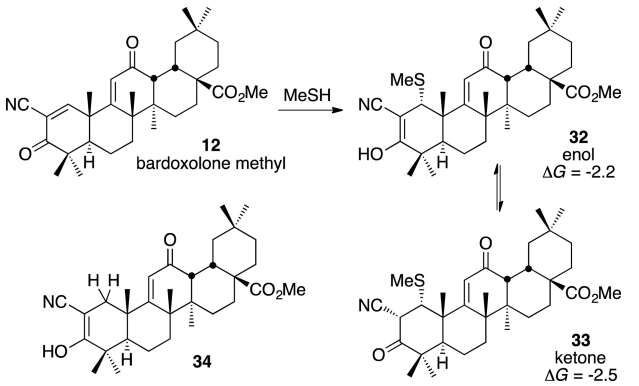


Figure 1. Addition of MeSH to bardoxolone methyl, computed with M06-2X/6-311+G(d,p)//M06-2X/6-31+G(d) in CPCM water (ΔG in kcal/mol).

conducted using the 6-31+G(d) basis set and single-point energies were subsequently calculated with the 6-311+G(d,p) basis set. Overall, the conjugate addition of MeSH to **12**, giving **33**, is predicted to have $\Delta G = -2.5$ kcal/mol. This small negative value of ΔG agrees with experiments, which showed that additions of thiols to bardoxolone are reversible.[19a] Interestingly, enol **32** is predicted to have almost the same energy as ketone **33** (−2.2 kcal/mol). This result is also consistent with the original experimental report, in which the structure of a thiol adduct of bardoxolone in aqueous solution or DMSO was assigned as the enol, rather than as the ketone, on the basis of spectroscopic similarity with the known enol **34**.[19a,28] The relatively high stability of enol **32** is not a general property of additions to α-cyano Michael acceptors: for comparison, the MeSH adduct of **3** is 15 kcal/mol less stable as the enol than as the ketone.[29]

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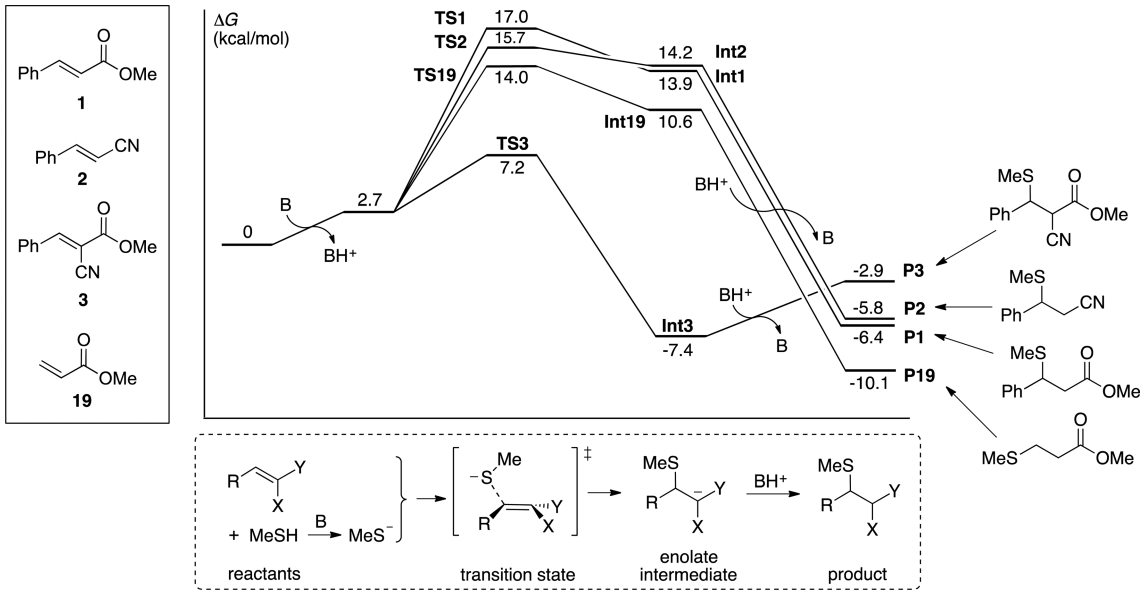

Figure 2. Free energy profiles for additions of MeSH to Michael acceptors **1**–**3** and **19** in water, giving products **P1**–**P3** and **P19**, respectively, computed at the M06-2X/6-311+G(d,p) level of theory in CPCM water. DBU was used as a model base catalyst (B).

In order to determine how α-EWG and β-Ph groups influence the kinetics of thiol additions, we computed free energy profiles for the base-catalyzed additions of MeSH to Michael acceptors **1**–**3** and **19** (Figure 2). The first step of the Michael addition is the deprotonation of the thiol to give the thiolate anion. Addition of the thiolate to the Michael acceptor, leading to the enolate (or related anion) intermediate, is rate-determining.[30] Numerous general or specific base catalysts may deprotonate the thiol in a buffered solution or within a cell; we used DBU as a model base because this allows the ionic and neutral reactions to be compared over a convenient energy scale. In aqueous solution, the choice of base catalyst affects the stability of the thiolate anion and the enolate (or enolate-like) intermediate, due to the role of the pK_a of the base on the relevant proton transfer equilibria: that is, the relative energies of [BH^+][MeS^-] and of [BH^+][**Int**] on the plot.[31] However, the base has no effect on the overall thermodynamics of thiol addition or on the barrier for addition of the free thiolate anion to the Michael acceptor in aqueous solution.

"Irreversible" additions of MeSH to **1** and **2** display similar free energy profiles, with ΔG = −6.4 and −5.8 kcal/mol, respectively (**P1** and **P2**) and $\Delta G^{\ddagger}$ = 17.0 and 15.7 kcal/mol, respectively (**TS1** and **TS2**). The intermediates (**Int1** and **Int2**) lie 1–3 kcal/mol below the rate-determining transition states. In comparison, the energy surface for MeSH addition to α-cyanoacrylate **3** is qualitatively different. The transition state (**TS3**) is about 9 kcal/mol lower in energy than **TS1** or **TS2** ($\Delta G^{\ddagger}$ = 7.2 kcal/mol), and the enolate (**Int3**) is about 21 kcal/mol more stable than **Int1** or **Int2** (−7.4 kcal/mol). In the disubstituted enolate (**Int3**), each EWG has nearly the same capacity for delocalization of negative charge as it does in a monosubstituted analogue. In **Int3**, the Mulliken partial charges on the CO_2Me group (−0.60 e) and CN group (−1.15 e) are similar to the charge on the CO_2Me group in **Int1** (−0.63 e) and the CN group in **Int2** (−1.37 e). This corresponds to a nearly additive capacity of the two EWGs for delocalization of negative charge, which explains the strong stabilization of **Int3**. The calculations verify Taunton et al.'s proposal that the α-EWG induces a marked increase in the acidity of the adduct.[12] However, the increased acidity results from both the stabilization of the enolate and, to a lesser extent, the destabilization of the adduct. That is, while enolate **Int3** is 21 kcal/mol more stable than **Int1** or **Int2**, adduct **P3** is about 3 kcal/mol less stable than **P1** or **P2**. The effect of the α-EWG on product stability is different from its effects on the stabilities of the reactant, TS, and intermediate.

While the substantial stabilization of the transition state and intermediate by the α-EWG is responsible for the observed acceleration of the rates of additions and eliminations, the fact that reversibility (i.e., elimination) is observed at all for thiol additions to α-EWG-containing substrates stems from both the faster reaction rates and the smaller overall ΔG, the latter of which is unrelated to kinetics.

Figure 2 also shows how a β-aryl group influences the kinetics and thermodynamics of thiol addition. Comparison of β-Ph-substituted acrylate **1** with the unsubstituted analogue **19** reveals that the C-β phenyl group raises the barrier for thiol addition by 3 kcal/mol and makes the addition overall 4 kcal/mol less favorable. Hence, the barrier for the reverse reaction, thiol elimination, is lower for **P1** than for **P19**, corresponding to faster elimination from the Ph-substituted derivative. Computations on MeS^- additions to β-alkyl-substituted Michael acceptors **24**–**28** (see the Supporting Information) indicate that the barriers for additions to the Me, Et, and ^{i}Pr derivatives are similar to those for Ph-substituted **1**, while the overall ΔG values are more negative; hence, thiol additions to these acceptors would have rates similar to that of **1**, but thiol eliminations from the adducts would be slower. A ^{t}Bu or cyclopropyl group raises the barrier by 1–2 kcal/mol; this would decrease the rate of addition but would have less effect on the rate of elimination.

Why are additions to diactivated Michael acceptors less thermodynamically favorable? The isodesmic reactions in eqs 1–6 (Figure 3) provide quantitative measurements of the effects of the α-CN and β-Ph groups on the stabilities of unsaturated and saturated species. In these equations we have used high-accuracy CBS-QB3 calculations to compute values of ΔH in the gas phase, to allow analysis of the intrinsic effects of

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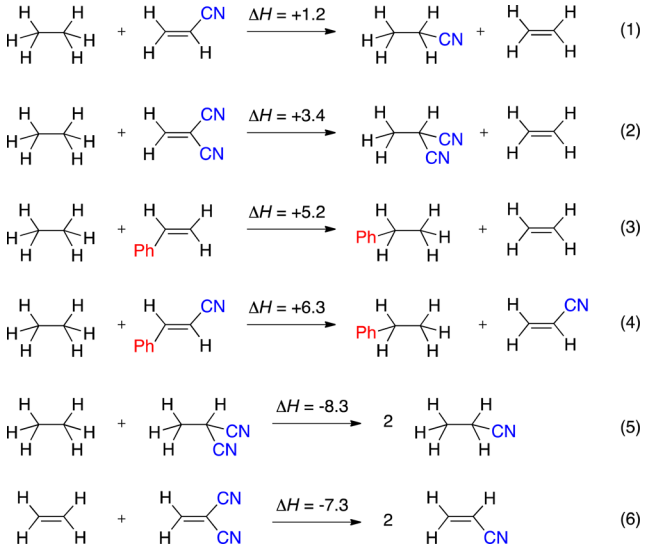

Figure 3. Isodesmic equations quantifying the effects of α-CN and β-Ph substituents on the stabilities of alkanes and alkenes. ΔH values were calculated with CBS-QB3 (kcal/mol).

substituents in the absence of entropic or solvation effects. Equation 1 shows that a cyano group prefers to be attached to an sp^2 alkene carbon, rather than to an sp^3 alkane carbon. Equation 2 shows that a geminal dicyano group also prefers an sp^2 environment, but the preference is greater than that of a single cyano group. In eq 3, the preference of a Ph group to be attached to an sp^2 alkene carbon is shown to be greater still, about 4 times that of a single CN group. This preference is magnified by a further 1 kcal/mol when the alkene bears an α-CN group (eq 4).

The preference of a single CN or Ph group to be attached to an sp^2 carbon rather than an sp^3 carbon is due to stabilization of the alkene by conjugation. For di-CN substitution, conjugation in the alkene plays a role, but the preference for an sp^2 environment mainly reflects destabilization of the saturated product. This is quantified by eqs 5 and 6 in Figure 3, which show that, while geminal disubstitution of either a saturated (eq 5) or unsaturated (eq 6) carbon by two cyano groups is destabilizing (compared to the reference monosubstituted species), the destabilization incurred by two cyano groups is 1 kcal/mol greater for a saturated carbon. The first EWG makes the saturated carbon more positive, and the addition of a second EWG is unfavorable. In an alkene, the destabilization is slightly smaller because the additional positive charge introduced by the first cyano group is stabilized to a degree by the polarization of the π bond (this is further enhanced by a β-aryl group). Taken together, the isodesmic reactions in Figure 3 indicate that the reversibility of thiol additions to Michael acceptors bearing an α-EWG and a β-aryl group stem from a combination of reactant stabilization and product destabilization and that the greatest individual substituent effect is that of the β-aryl group.

CONCLUSION

Theoretical calculations show that there is no general relationship between reactivity and thermodynamics in the Michael additions of thiols. The degree of reversibility of thiol adduct formation depends on both of these factors. An α-EWG on a Michael acceptor lowers the barrier for thiol addition but also makes the addition thermodynamically less favorable. The α-EWG, in conjunction with a suitable C-β substituent (e.g., aryl or branched-chain alkyl), gives rise to a rapidly reversible, weakly exergonic thiol addition. The acidities of the adduct, the thiol, and the bases that deprotonate these two species along the pathway for addition or elimination do not directly affect the thermodynamics but influence the reaction rates that ultimately determine to what extent thiol elimination will take place over a chemically or biologically relevant time scale.

In chemical experiments, the degree of reversibility of thiol addition is defined by the values of ΔG and $\Delta G^{\ddagger}$ for addition and elimination. In covalent drug design, these properties are one piece of a much more complex picture; inhibitor binding affinities and on/off rates also depend on local pH and ionic strength, the pK_as of the cysteine residue and catalytic bases, the conformation of the thiol adduct, the accessibility of the α-proton, concentration fluxes, and importantly, the noncovalent interactions between the inhibitor and the target binding site.[3,12] Computations on reactions in solution are not expected to correlate perfectly with inhibitor potencies or residence times, but they do reveal important fundamental features of warhead reactivities that contribute to reversibility and demonstrate that reversible thiol reactivity can be predicted by in silico methods. Our calculations have considered a range of reversible covalent warhead classes which show promise for the design of novel inhibitors against numerous therapeutic targets.

THEORETICAL CALCULATIONS

Density functional theory calculations were performed in Gaussian 09.[32] Calculations were conducted with M06-2X[33]/6-311+G(d,p), using the CPCM[34] implicit solvent model to simulate aqueous solution. The ultrafine integration grid was used. Harmonic vibrational frequency calculations indicated whether stationary points were minima or transition states and also provided unscaled zero-point energy and thermal corrections. Gibbs free energies are reported at a standard state of 1 mol/L and 25 °C. The choice of M06-2X/6-311+G(d,p) to model the thiol additions was based on validation studies in which we assessed a variety of other functionals (B3LYP,[35] M06-2X, and ωB97X-D[36]), basis sets, methods of computing entropy, and solvent models. Details of these calculations are given in the Supporting Information. For the isodesmic reactions in Figure 3, computations were performed with the high-accuracy CBS-QB3 method.[37]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02188.

Computational data and details of computations performed with different DFT methods (PDF)

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The authors declare no competing financial interest.

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