

## How Chemical Environment Activates Anthralin and Molecular Oxygen for Direct Reaction

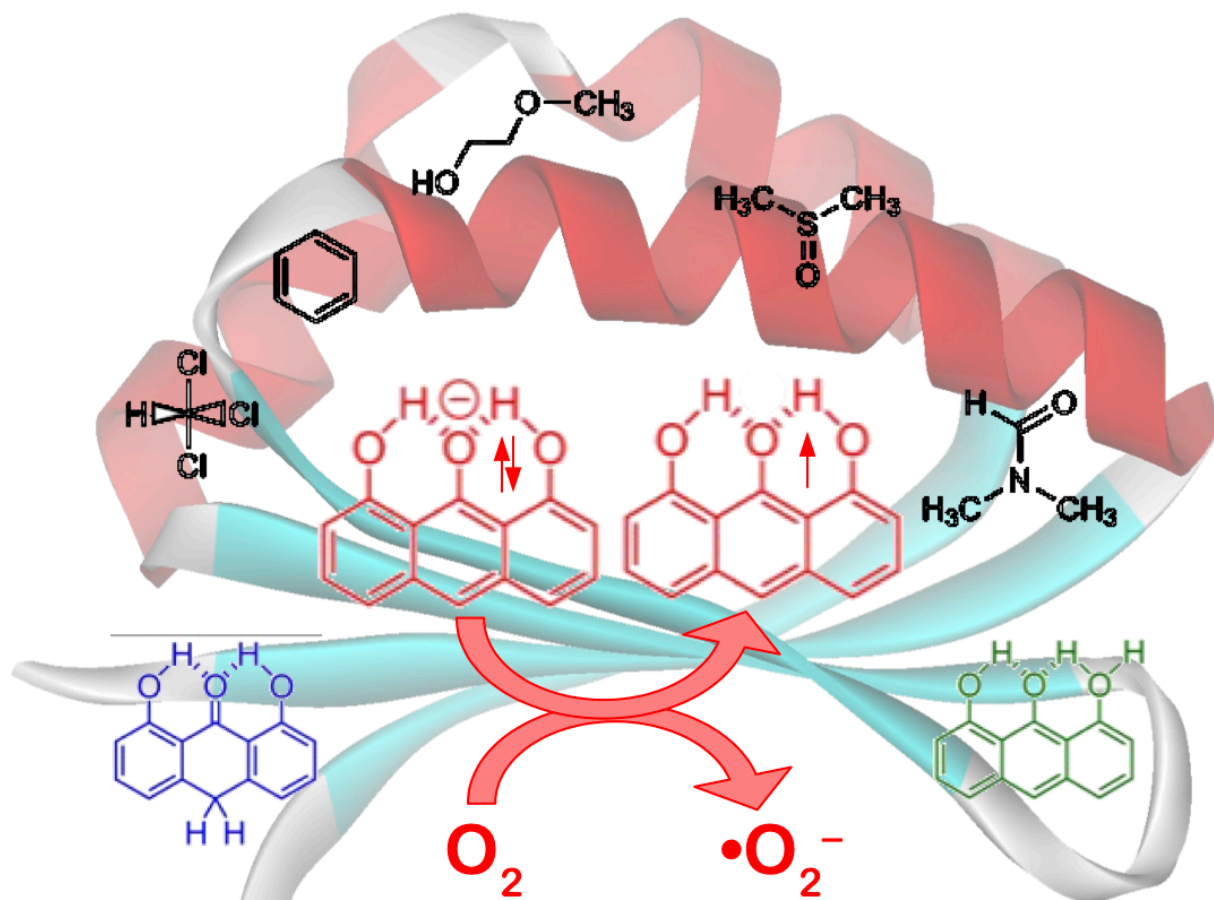
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### ABSTRACT:

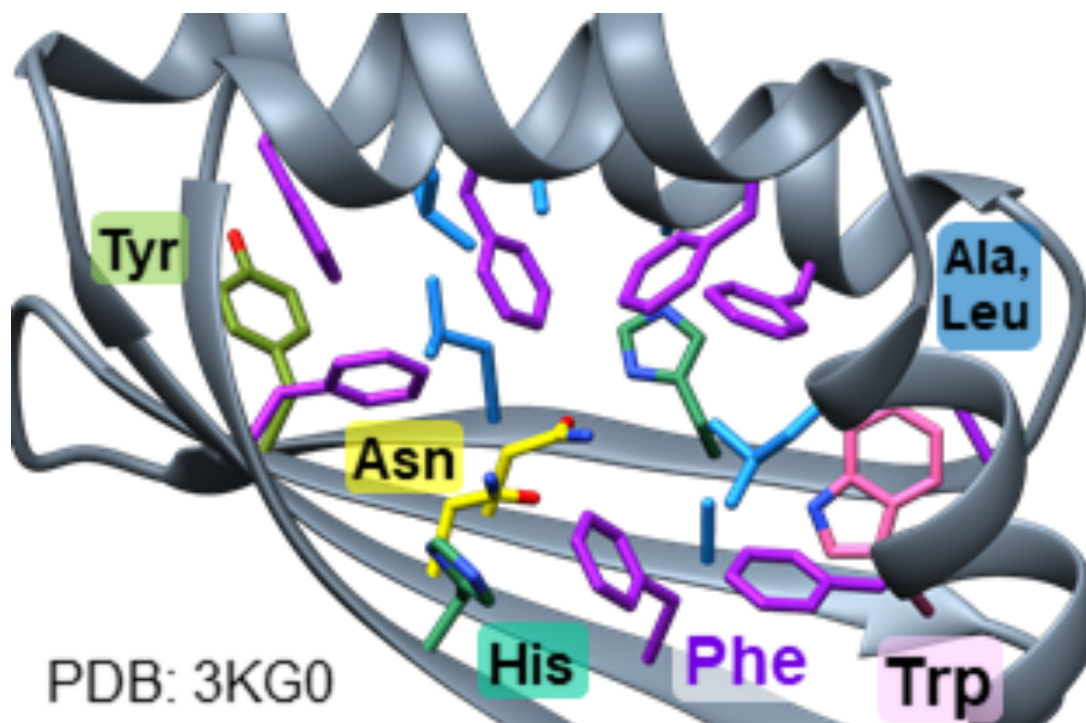
The role of the chemical environment in promoting anthralin/ $O_2$  reactions was discovered, using neat solvents to model the amino acids of a cofactor-independent oxygenase. Experimental and computational results highlight the importance of the substrate-enolate, which is accessed via energetically small, escalating steps in which the ground state keto-isomer is tautomerized to an enol and then ionized by solvent. The resulting ion-pair is poised for spontaneous electron transfer to  $O_2$ . Similar activation may be exploited in biological/non-biological oxidations involving  $O_2$ .

### TABLE OF CONTENTS/GRAPHICAL ABSTRACT FIGURE:



Enzyme active sites use particular arrangements of amino acids to catalyze many reactions with efficiencies and specificities not yet achieved by synthetic catalysts. Reactions between organic molecules and dioxygen ( $O_2$ ) are often thermodynamically favorable, but the triplet ground state of  $O_2$  imposes a significant kinetic barrier.<sup>1</sup> Nearly all enzymes that use this desirable “green” oxidant<sup>2</sup> need metabolically expensive flavin or redox-active transition metal cofactors to reductively activate  $O_2$ .<sup>1,3</sup> Cofactor-Independent Oxidases (CIOs) catalyze oxidations of organic substrates by  $O_2$  using

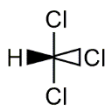
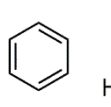
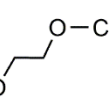
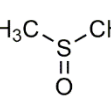
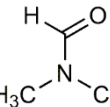






only intramolecular interactions supplied by the protein environment (Figure 1).<sup>4</sup> The exact mechanisms of substrate binding and catalysis are unknown, but previous work suggests protein/substrate interactions play a key role by tuning the substrate's O<sub>2</sub>-activating efficiency.<sup>4-7</sup> The oxygenation of dithranol (also known as anthralin or 1,8-dihydroxy-9,10-dihydroanthracen-9-one, CAS# 1143-38-0) to dithranone (Scheme 1A) is catalyzed by a CIO involved in anthracycline biosynthesis called nogalamycin monooxygenase (NMO, Figure 1).<sup>8-10</sup> This reaction also occurs in an uncatalyzed manner when dithranol is used topically to treat psoriasis. The mechanism of action is not well understood but is surmised to be related to the generation of reactive oxygen species by dithranol, analogous to the initial step shown in Scheme 1A.<sup>11</sup>



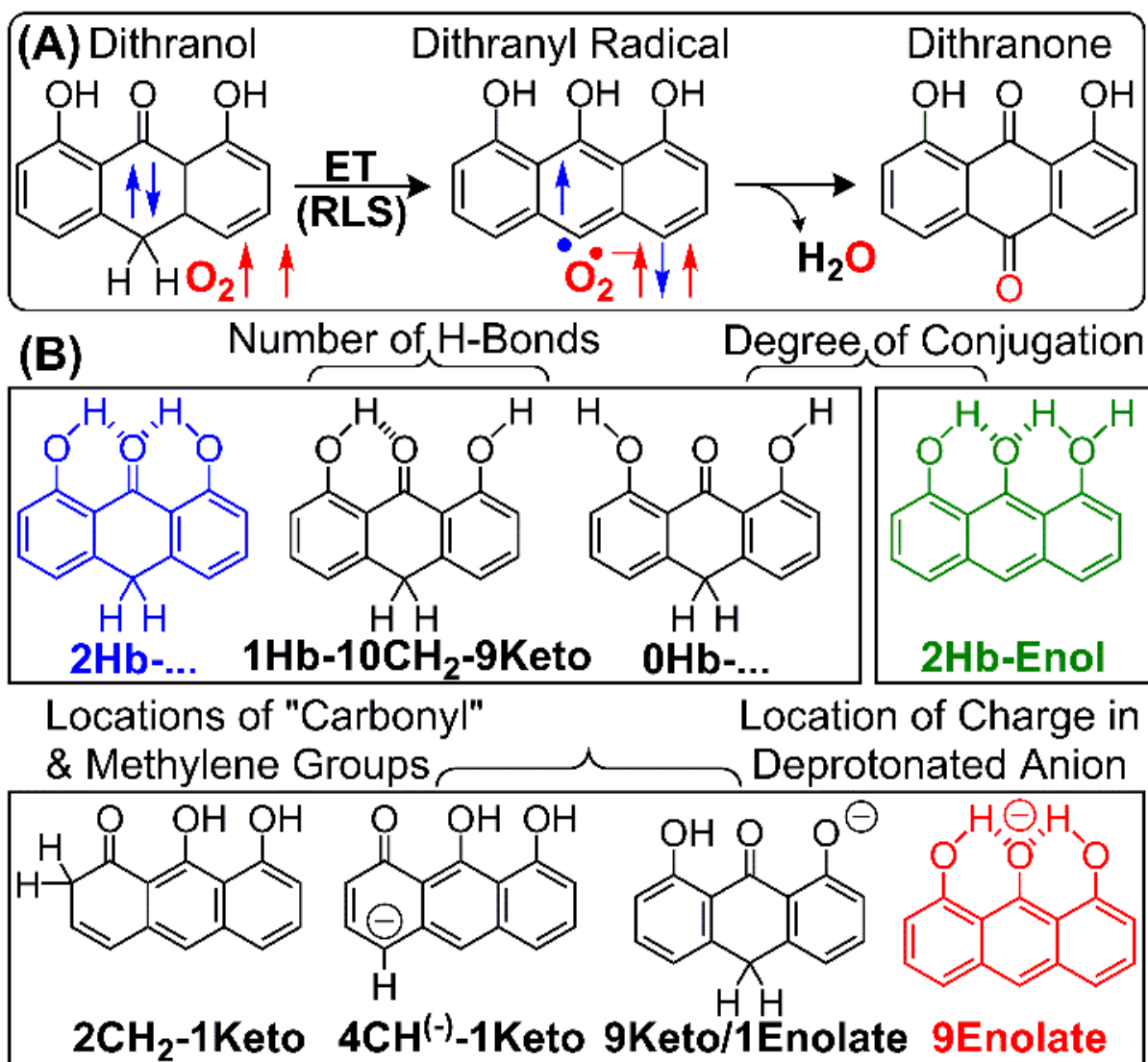
**Figure 1.** Crystal structure of cofactor-free nogalamycin monooxygenase, showing amino acid side chains lining the active site pocket. Table S1 shows proposed solvent/amino acid side chain correlations and solvent physical properties.

Our approach is to use neat solvents to deconvolute the network of supramolecular interactions between substrate, O<sub>2</sub>, and the substrate-binding pocket of a CIO (Table S1) by probing how solute/solvent interactions influence the reactivity of dithranol. It is relevant to structure/function considerations that molecules of this class can assume a remarkably large variety of tautomeric structures and be strongly influenced by modest variations in chemical environment. Three complementary spectroscopic techniques and a validated level of computational theory (Tables S2-S4) were employed to explore how each solvent environment, acting as a model for a type of substrate/amino acid interaction,<sup>12,13</sup> influences dithranol structure and relative isomer energies to activate the dithranol/O<sub>2</sub> pair toward spontaneous electron transfer.

**Table S1.** Summary of solvent/amino acid side chain correspondence and solvent parameters used from Universal Solvation Model Based on Solute Electron Density (SMD model<sup>20,21</sup>).<sup>‡</sup>

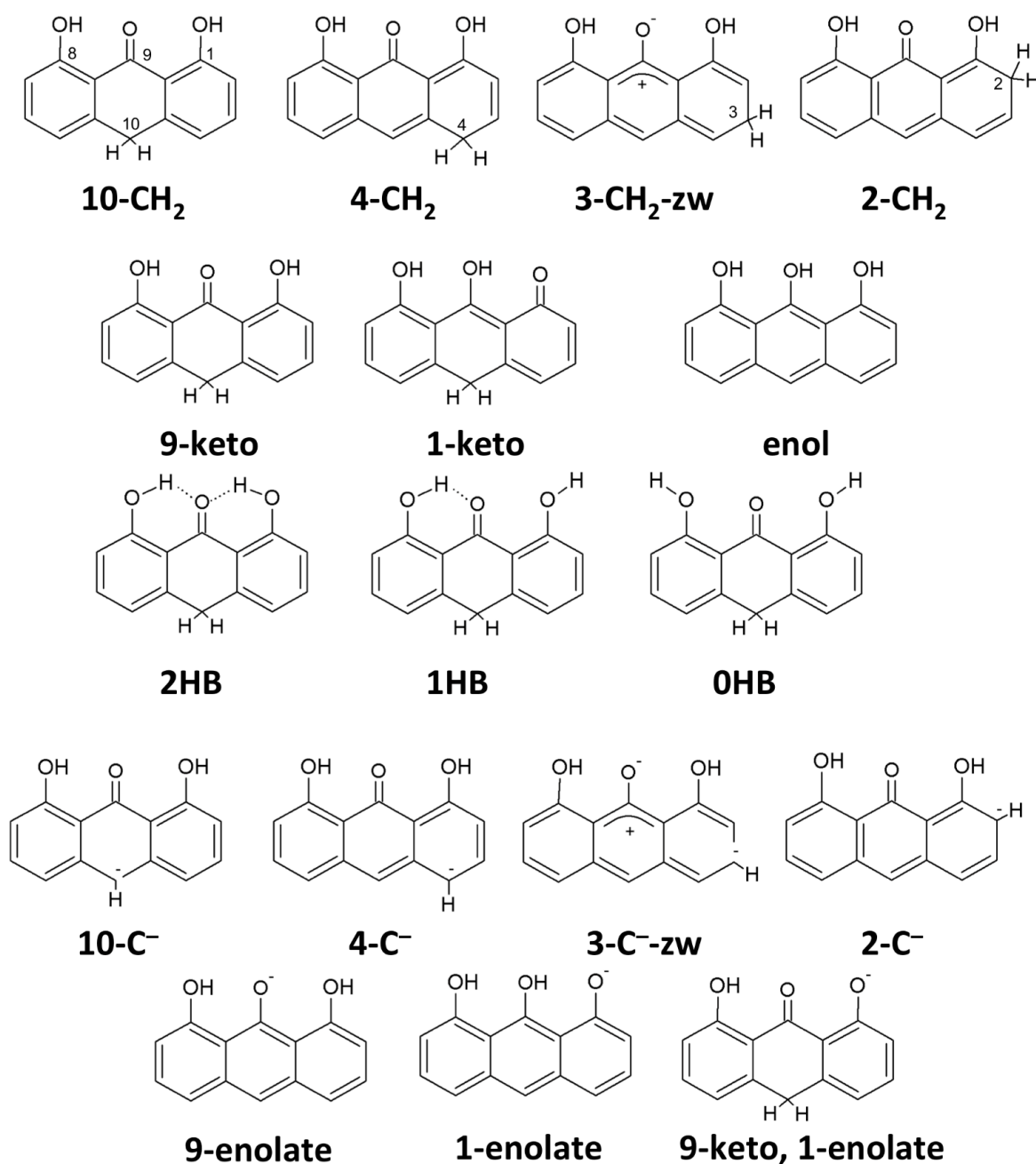
		H <sub>2</sub> O	CHCl <sub>3</sub>	Benzene	2ME	DMSO	DMF
	Structure						
	Amino Acid Side-chain Modeled						
H-bond Acidity	( $\alpha$ )	0.82	0.15	0.00	0.30	0.00	0.00
H-bond Basicity	( $\beta$ )	0.35	0.02	0.14	0.84	0.88	0.74
Bulk Dielectric Constant	( $\epsilon$ )	78.3553	4.7113	2.244	17.2	46.826	37.219
Dielectric Constant at infinity	( $\epsilon_\infty$ )	1.7785	2.0906	2.247	1.9973	2.0079	2.0392
Mole Fraction Solubility of O <sub>2</sub> <sup>*</sup>	( $X_{O_2} \times 10^4$ )	0.2301 <sup>a</sup>	7.30 <sup>a</sup>	8.10 <sup>a</sup>	[ 7.5 ] <sup>b</sup>	1.09 <sup>c</sup>	3.89 <sup>d</sup>
Solvent Radius	( $\text{\AA}$ )	1.385	2.480	2.63	3.16	2.455	2.89
Molecular Volume	( $\text{\AA}^3$ )	18.07	80.70	88.91	101.6	70.94	101.1
Density	( $\text{g/cm}^3$ )	0.997	1.4832	0.876	0.9647	1.0955	0.944
Numeral Density	( $1/\text{cm}^3$ )	3.348	0.7482	0.6773	0.7661	0.8490	0.7370
Thermal Expansion Coefficient	( $10^{-3}/\text{K}$ )	0.257	1.255	1.380	0.956	98.2	0.9744
Index of Refraction	( $n_{20}$ )	1.3328	1.4459	1.5011	1.4024	1.4170	1.4305
Surface Tension at Liquid-Air Interface	( $\gamma$ )	103.60	38.39	40.62	44.39	61.78	49.56
Carbon Aromaticity	( $\phi$ )	0	0.00	1.00	0	0	0
Electronegative Halogenicity	( $\psi$ )	0	0.5	0.00	0	0	0

<sup>‡</sup> Side chain/solvent groups may form intermolecular interactions with the substrate. Solvents used here to capture the essential chemical properties of diverse amino acid side chains include: CHCl<sub>3</sub> (similar to alanine and comparable with a gas phase model), benzene (phenylalanine), DMSO (histidine), DMF (asparagine, glutamine), and 2ME (serine). Neat H<sub>2</sub>O was used as a control for the no-enzyme condition; <sup>\*</sup> Mole fraction ( $X_{O_2}$ ) of dissolved oxygen at T = 298K, partial pressure (O<sub>2</sub>) = 1 atm; <sup>a</sup> Miyamoto, H.; Yampolski, Y.; Young, C. L. IUPAC-NIST Solubility Data Series. 103. Oxygen and Ozone in Water, Aqueous Solutions, and Organic Liquids (Supplement to Solubility Data Series Volume 7). *J. Phys. Chem. Ref. Data* **2014**, 43 (3), 033102. <https://doi.org/10.1063/1.4883876>. <sup>b</sup> Estimate from average of values for diethylether (14.32) and ethylene glycol (0.723); <sup>c</sup> *Oxygen and Ozone: Solubility Data Series*, Vol 7.; Battino, R., Ed.; Pergamon Press: New York, 1981. <sup>d</sup> Sato, T.; Hamada, Y.; Sumikawa, M.; Araki, S.; Yamamoto, H. Solubility of Oxygen in Organic Solvents and Calculation of the Hansen Solubility Parameters of Oxygen. *Ind. Eng. Chem. Res.* **2014**, 53 (49), 19331–19337. <https://doi.org/10.1021/ie502386t>.



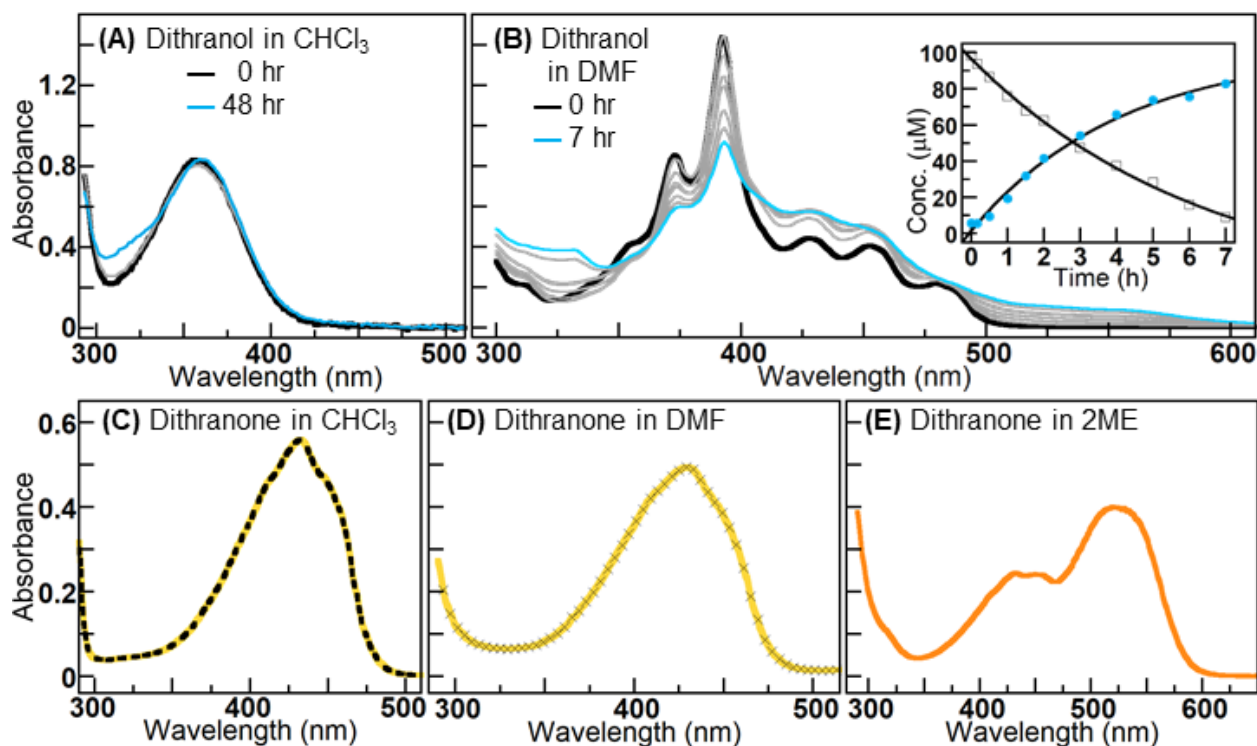
**Scheme 1.** (A) NMO-catalyzed oxygenation of dithranol with (B) a representative set of potentially accessible tautomers. Lowest-energy isomers of the three main forms of dithranol are colored: blue = keto; green = enol; red = anion (enolate). For full set of isomers see Scheme S1.

Our results indicate that ion-pairing between an enolate isomer and protonated solvent molecules is critical for stabilizing the reactive substrate anion along the way to form an enol/superoxide radical pair (Scheme 1A). This result is unexpected because the structure of the reactive dithranolate anion is often predicted to be a C10 carbanion due to the weak C-H bond.<sup>14</sup> Additionally, prior theoretical investigations<sup>13,14</sup> strongly favor dominance of the keto isomer in neat solvents, contrary to our experimental observations. The mechanism invoked here was initially inspired by how flavin-dependent oxidases prime the reduced flavin to activate O<sub>2</sub>.<sup>3</sup> In subsequent experimental work under semi-aqueous conditions (2:1 Tris/NaCl buffer:2-methoxyethanol [2ME], pH 8), only the anionic form of dithranol reacted appreciably with O<sub>2</sub>,<sup>6</sup> suggesting that the enolate is activated to form the experimentally detectable dithranyl/superoxide (O<sub>2</sub>•<sup>-</sup>) radical pair in a rate-determining step. Following intersystem crossing, the radical pair generates the oxygenation product (dithranone) and water (Scheme 1A).<sup>6,7</sup>



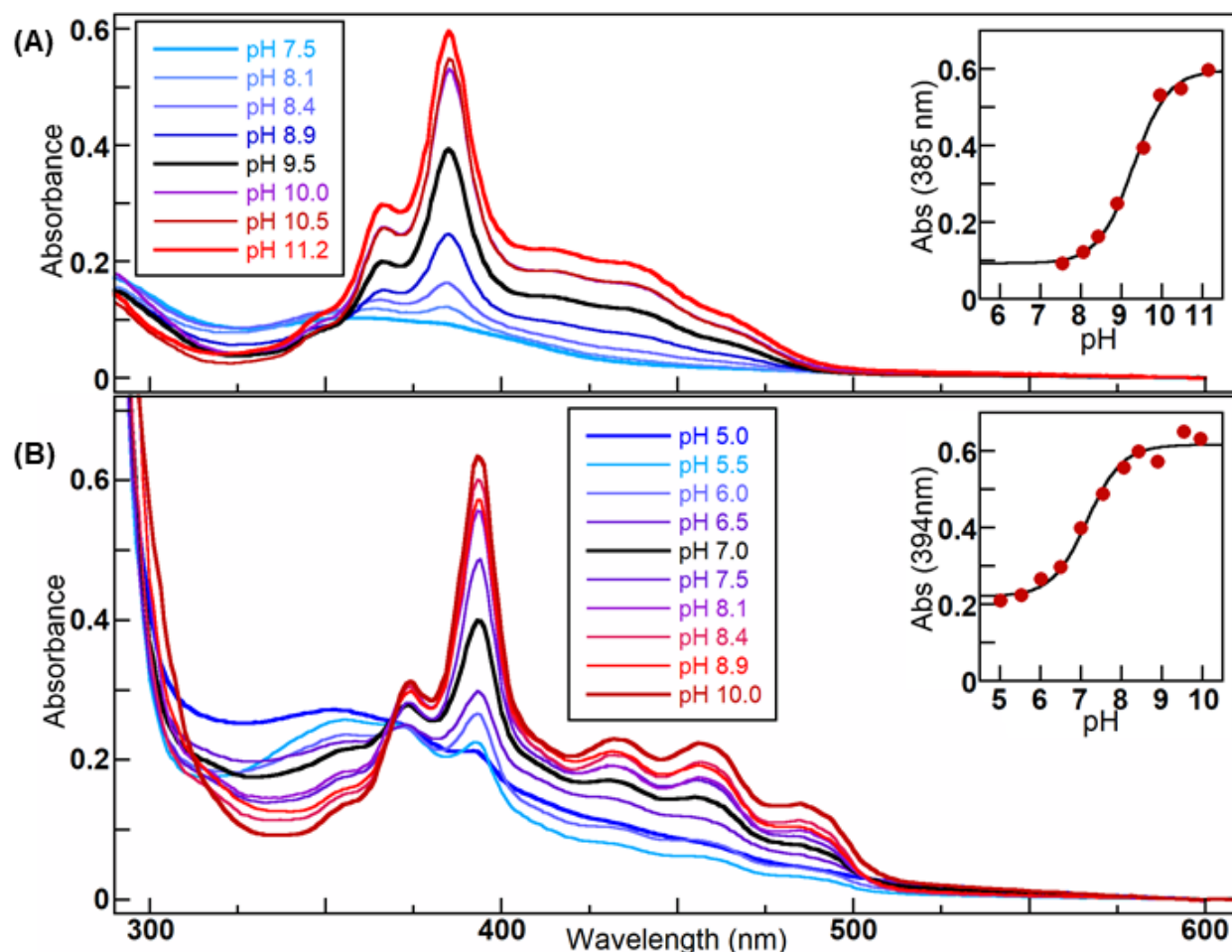
**Scheme S1.** Overview of dithranol tautomers with key C-H and O-H positional variations and representative hydrogen bonding patterns.

The presence of enolate likewise correlated with rapid reaction between dithranol/O<sub>2</sub> in neat solvents. For example, dithranol in CHCl<sub>3</sub> (100% ketone, Figure 2) did not react with O<sub>2</sub> within 48h. By contrast, the half-life for the same reaction in DMF (100% enolate, Figure 2) was 2.9h (Figure S1). Oxygen is more soluble in CHCl<sub>3</sub> than DMF (Table S1), suggesting that the discrepancy can be ascribed to the intrinsic reactivity of the dithranol/O<sub>2</sub> pair,<sup>15</sup> which in turn correlates with the presence of the enolate. The pK<sub>a</sub> of 9.28±0.07 measured for the dithranol ⇌ enolate + H<sup>+</sup> equilibrium in water decreased by two units (7.15±0.09) for NMO-bound dithranol, indicating that the protein stabilizes the enolate (Figure S2).



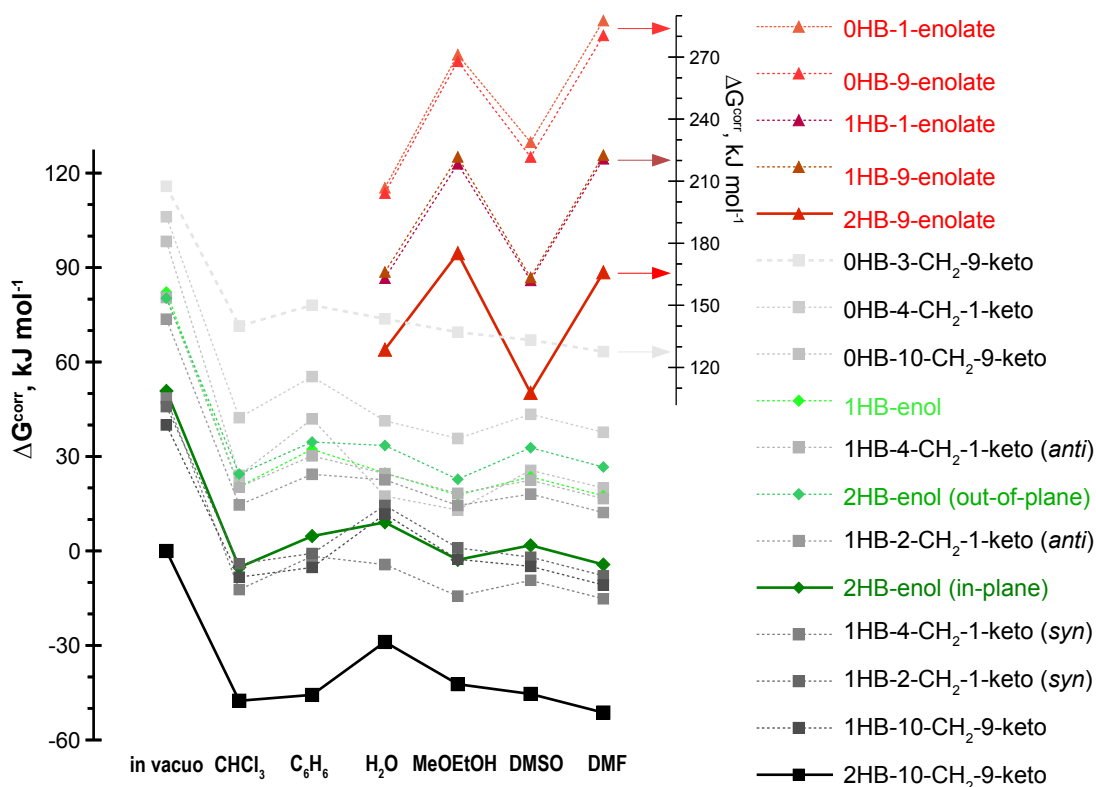
**Figure S1.** The rate of dithranol oxidation in air varies with solvent, according to the initial fraction of enolate present, as illustrated by time resolved UV/vis spectroscopy and HPLC. Reactions of dithranol in air (room temperature, 100 rpm shaking, air-saturation) were monitored over time by UV/vis absorbance in **(A)**  $\text{CHCl}_3$  (124  $\mu\text{M}$  dithranol) and **(B)** DMF (110  $\mu\text{M}$  dithranol). **(B, inset)** Discontinuous HPLC was used to monitor the disappearance of dithranol (white squares) and appearance of dithranone (blue circles) over time in DMF. The data were fit to single exponential curves yielding  $k = 0.15 \text{ h}$  (dithranol disappearance) or  $k = 0.24 \text{ h}$  (dithranone appearance). Dithranol oxidation can occur by  $\text{O}_2$ -dependent pathways that lead either to the dimerization product (bisanthrone) or monooxygenation (dithranone). The least error in HPLC determination of progress of reaction was previously shown to occur by monitoring dithranone production, which has  $k = 0.24 \text{ h}$  or  $t_{1/2} = 2.9 \text{ h}$ . **(C-E)** UV/vis absorbance spectra of the oxygenation product, dithranone (50  $\mu\text{M}$ ), in **(C)**  $\text{CHCl}_3$ , **(D)** DMF, and **(E)** 2ME.





**Figure S2.** NMO lowers the  $pK_a$  of dithranol by greater than 2 pH units. Spectrophotometric pH titrations of **(A)** 46  $\mu\text{M}$  dithranol and **(B)** 39  $\mu\text{M}$  dithranol mixed with 80  $\mu\text{M}$  NMO (NMO-dithranol complex) were performed discontinuously using a series of aqueous buffers containing 100 mM buffer and 300 mM NaCl pre-adjusted to pH 5.02, 5.53, 5.97, 6.50, 7.00, 7.57, 8.06, 8.43, 8.90, 9.54, 9.95, 10.47, 11.15 (see Solvents and Reagents section of Experimental). An approximate isosbestic point at 345 nm is observed in panel A. An isosbestic point at 368 nm is observed in panel B. Upon binding to the enzyme, the absorbance maxima in dithranol's high-pH spectrum are observed to red shift by approximately 8 nm. The individual peaks, especially above 400 nm, are more well-resolved. **(Insets)**  $pK_a$  values were determined by plotting the absorbance at 385 nm (dithranol) or 394 nm (NMO-dithranol complex) as a function of pH and fitting the data to a sigmoidal curve. The active site pocket of NMO lowers the  $pK_a$  of dithranol from  $9.28 \pm 0.07$  to  $7.15 \pm 0.09$  ( $\Delta pK_a = 2.13 \pm 0.11$ ).

To understand how, we first considered the structure of the substrate dithranol (Scheme S1) in light of its array of C-H/O-H bond tautomers, methylene positional isomers, ionization isomers, and varied intramolecular H-bonding patterns. While in the gas phase these were calculated to span a ca. 200  $\text{kJ}\cdot\text{mol}^{-1}$  relative energy range (Figure S3) using validated density functional theory, the energetic order may be readily altered by the protein environment toward promoting deprotonation and reaction with  $\text{O}_2$ . The level of theory (MN15<sup>16</sup>/6-311++G\*\*<sup>17-19</sup>/SMD<sup>20,21</sup>) was validated using CBS-Q3 results<sup>14</sup> from the literature (Table S2).



**Figure S3.** Gibbs free energy landscape (corrected for translational entropy in 100  $\mu\text{M}$  solution at MN15/6-311++G\*\*/SMD level of theory) of dihydranol tautomers as defined in Scheme S1. All energy values are given relative to the lowest energy keto isomer in gas phase. Isomers are ordered according to their relative energies in gas phase as shown on the left-hand side of the figure. Enolate structures were mapped considering protonated solvents at infinite separation and both embedded in polarizable continuum model SMD.

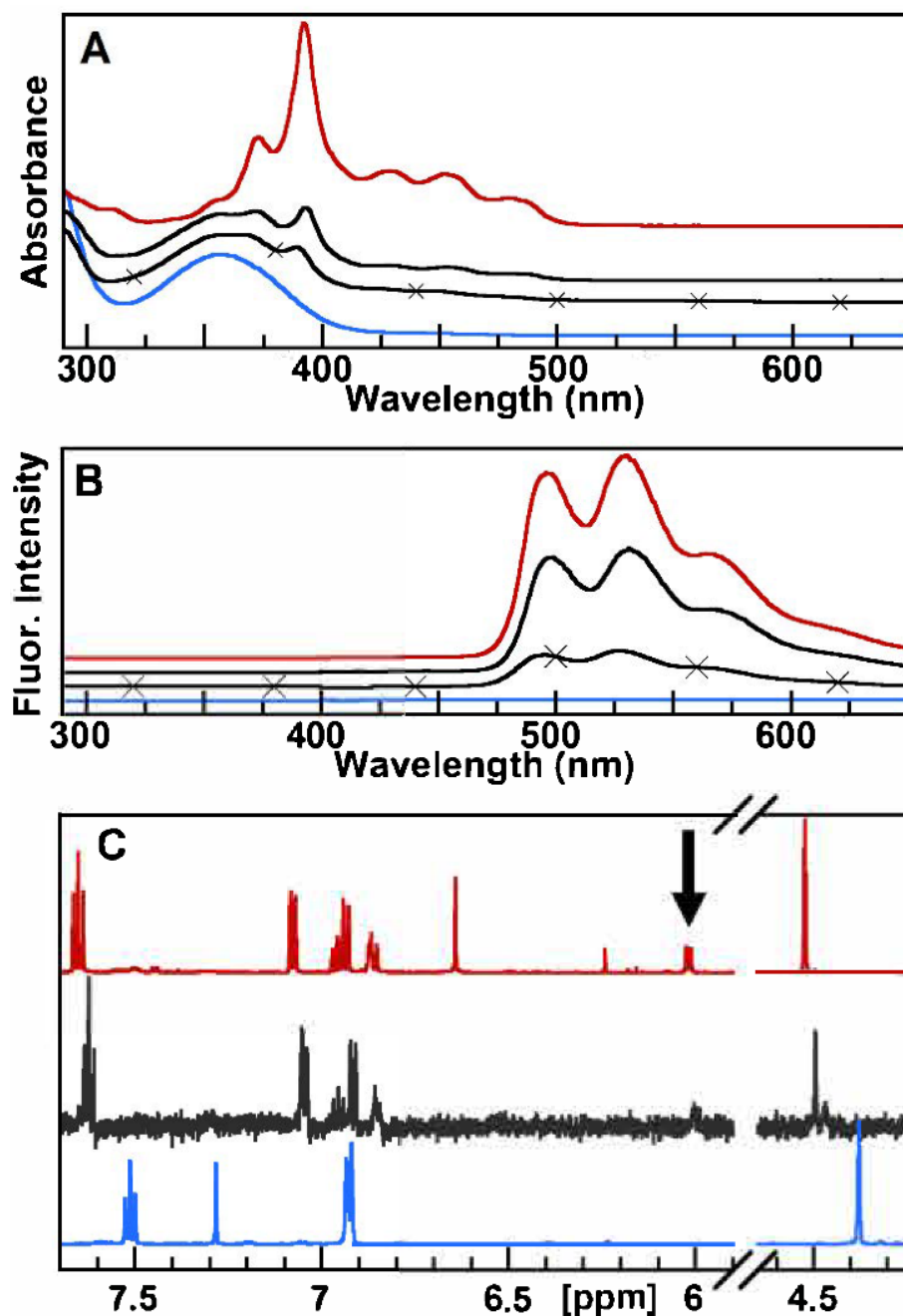
**Table S2.** Errors in tautomerisation enthalpies ( $\Delta\Delta^{\ddagger}\text{H}$ ,  $\text{kJ mol}^{-1}$ ) calculated using various density functionals relative to the corresponding CBS-QB3 reference values<sup>2</sup> for equilibrium structures of gas phase isomers for 1,8-dihydroxyanthrone (keto) and 1,8,9-trihydroxy-anthracene (enol) molecules.<sup>‡</sup>

		1HB-keto	2HB-enol	1HB-enol	0HB-keto	RMS
Rung 2	BP86	+8	+13	+14	+21	+15.0
	BLYP	+5	+10	+10	+13	+ 9.8
	PBE	+7	+13	+13	+20	+14.2
Rung 3	TPSS	+7	+16	+17	+19	+15.5
	revTPSS	+6	+20	+20	+16	+16.3
	M06L	+4	+16	+13	+11	+11.6
Rung 4	B3LYP	+5	+6	+9	+11	+ 8.2
	cam-B3LYP	+7	+13	+13	+20	+14.2
	cam-B3LYP+GB3J	+6	+14	+17	+13	+13.1
	$\omega$ B97XD	+4	+19	+20	+7	+14.3
	MN15	+3	+1	+2	+7	+ 4.0

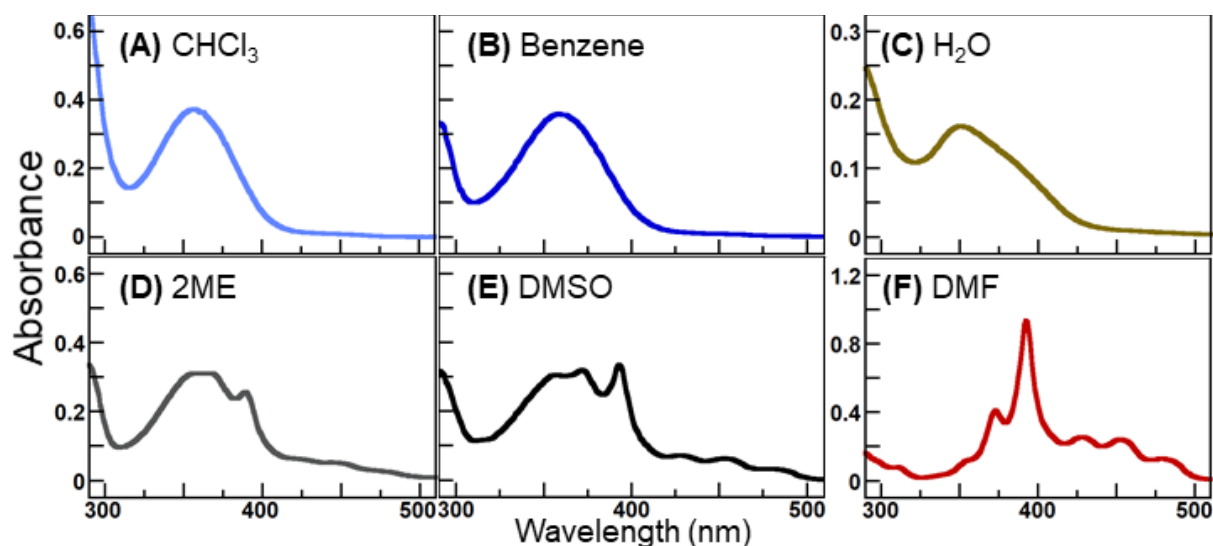
<sup>‡</sup>The CBS-QB3 tautomerisation enthalpies ( $\Delta^{\ddagger}\text{H}$ ) relative to **2HB-keto** form are taken from Ref. 2 as **1HB-keto** +47  $\text{kJ mol}^{-1}$ ; **2HB-enol** +52  $\text{kJ mol}^{-1}$ ; **1HB-enol** +84  $\text{kJ mol}^{-1}$ ; **0HB-keto** +47  $\text{kJ mol}^{-1}$ . All calculations find the 2HB-10-CH<sub>2</sub>-9-keto form (Scheme S1) to be the most stable isomer. The lowest root-mean-square (RMS) deviation was used to select the density functional with the highest chemical accuracy. The 6-311++G\*\* basis set was used in all calculations.



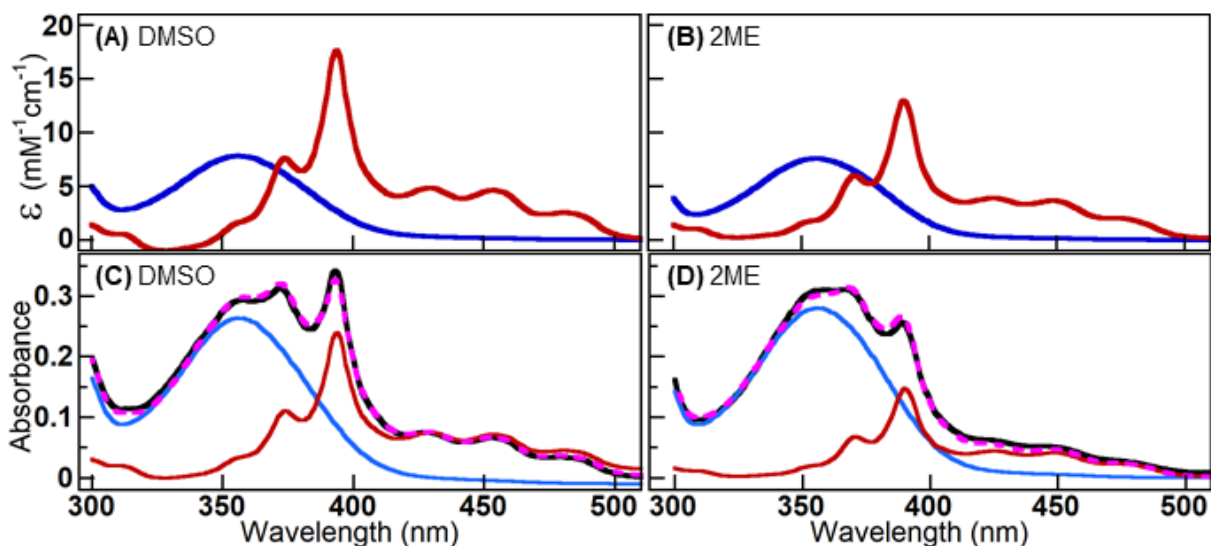
To understand the activating effects of the chemical environment, we used UV/visible absorbance (UV/vis), fluorescence, and proton nuclear magnetic resonance ( $^1\text{H}$ -NMR) spectroscopies to determine the most stable isomer(s) of dithranol in a series of solvents chosen based on the side chains in NMO (Figure 1, Table S1). The results indicated a spontaneous shift from the pure dithranol-keto isomer in  $\text{CHCl}_3$ , benzene, and  $\text{H}_2\text{O}$ , to keto:enolate mixtures in 2ME and DMSO (80:20 and 70:30), to apparently pure enolate in DMF (Figures 2, S4-S7 and Tables S5-S6). NMR characterizations were limited by the tendency of isomer mixtures to change in composition between solute concentrations typical of NMR ( $\geq 1$  mM) and concentrations used to investigate the enzyme ( $\leq 300$   $\mu\text{M}$ ). We speculate that solute-solvent intramolecular interactions predominate over solute-solute interactions only at lower solute concentrations. Additionally, the use of infrared spectroscopy was prohibited by the overlap of diagnostic carbonyl and  $\text{C}=\text{C}$  stretches for these tricyclic aromatic molecules. These challenges may have precluded observation of the enolate in prior experimental studies, in which it has long been assumed that keto isomers of anthralin exclusively constituted the ground state.



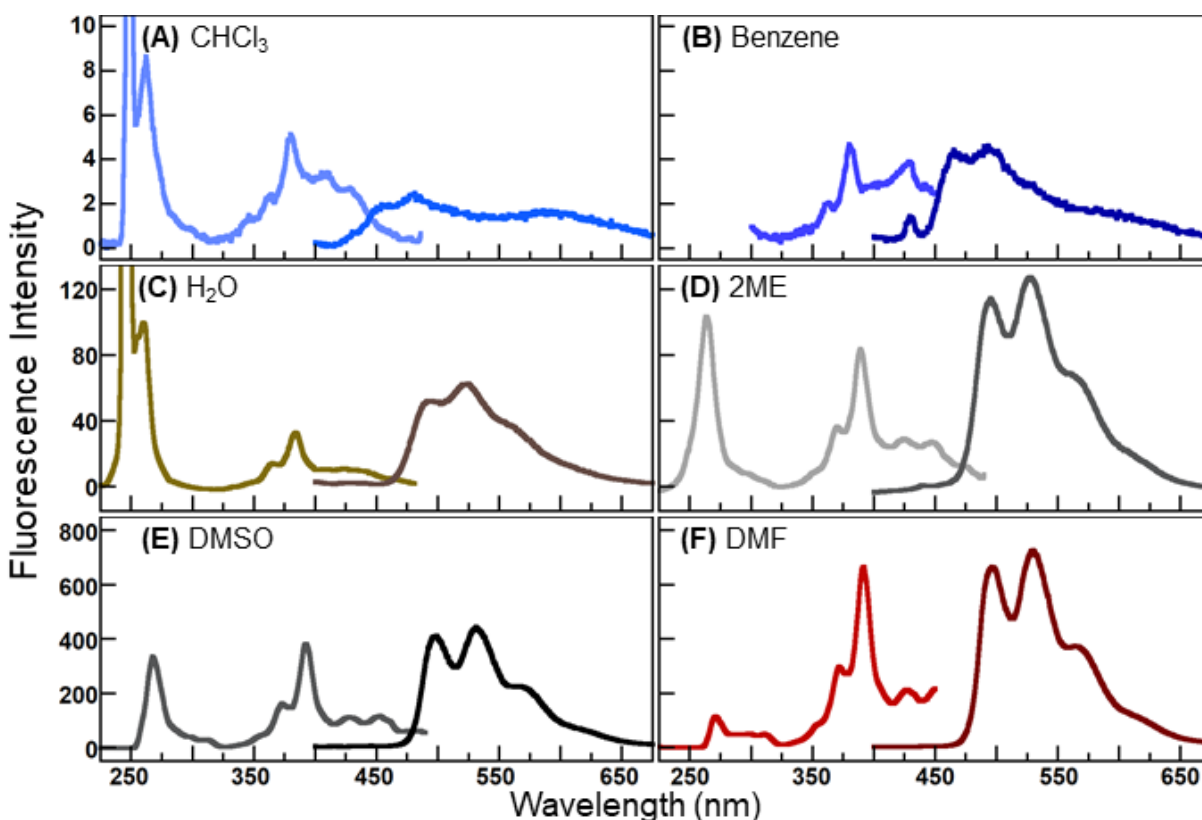
**Figure 2.** Representative spectra demonstrating solvent-dependent stabilization of dithranol isomers. (A) UV/vis spectra of 50  $\mu$ M dithranol in CHCl<sub>3</sub> (blue, keto form), DMF (red, enolate), DMSO (black solid lines, 70:30 keto:enolate mixture), and 2ME (black (x), 80:20 keto:enolate mixture (Figure S5)). (B) Fluorescence spectra of 50  $\mu$ M dithranol in CHCl<sub>3</sub> (blue,  $\lambda_{\text{exc.}}$ =380 nm), 5  $\mu$ M dithranol in DMSO (black,  $\lambda_{\text{exc.}}$ = 393 nm), 5  $\mu$ M dithranol in 2ME (black (x),  $\lambda_{\text{exc.}}$ = 393 nm) and DMF (red,  $\lambda_{\text{exc.}}$ = 391 nm). The fully conjugated, aromatic enolate isomer fluoresces strongly, while the keto does not (Figure S6). (C) <sup>1</sup>H-NMR spectra of dithranol in CDCl<sub>3</sub> (blue, 10 mM), d<sup>6</sup>-DMSO (black, 0.16 mM), and d<sup>7</sup>-DMF (red, 0.32 mM). The black arrow indicates a doublet in the region expected for aromatic protons, absent in the blue spectrum and tentatively assigned to the proton on the fully conjugated central ring of the enolate isomer. (See also Figure S7.) Dithranol yields equivalent keto spectra in all three solvents at concentrations >1 mM (not shown). Corrected concentration.



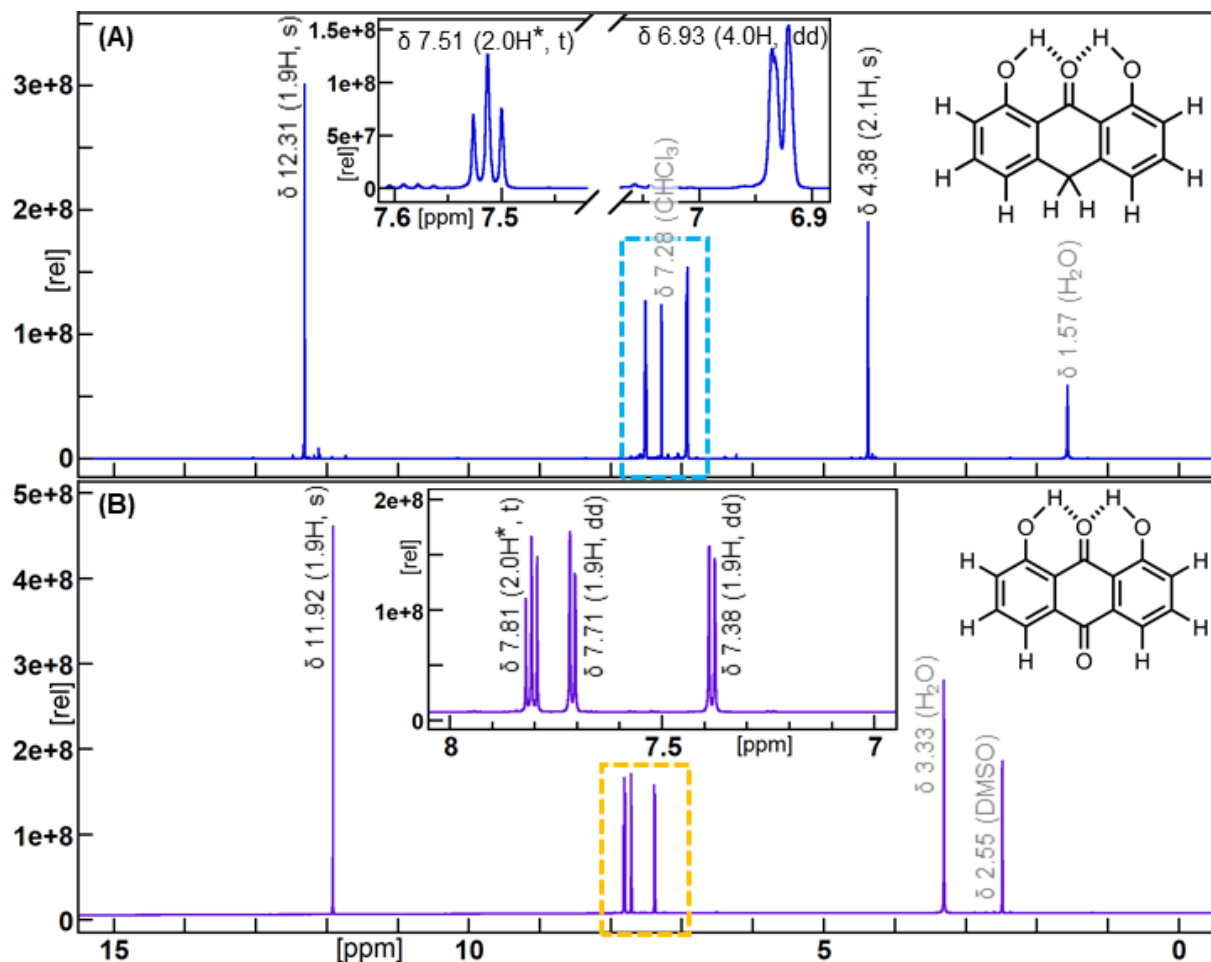
**Figure S4.** The UV/vis absorbance spectrum of dithranol is solvent-dependent. Representative spectra of 50  $\mu\text{M}$  dithranol in **(A)**  $\text{CHCl}_3$ , **(B)** benzene, **(C)**  $\text{H}_2\text{O}$  (25  $\mu\text{M}$  dithranol) **(D)** 2ME, **(E)** DMSO, and **(F)** DMF. In  $\text{CHCl}_3$  and benzene, the spectrum of dithranol exhibits a single broad peak centered at 358 and 359 nm, respectively, which is similar to that observed in aqueous buffer at low pH ( $< 1$  unit from the  $\text{pK}_a$ ). (See Figure S2A.) The spectrum in pure distilled water **(C)** resembles the spectra shown in **(A)** and **(B)**; however, the source of asymmetry in this spectrum is unclear. In DMF, dithranol's spectrum is similar to the NMO-dithranol spectrum measured in high pH ( $> 1$  unit from  $\text{pK}_a$ ) aqueous buffer: dominated by a sharp peak at 391 nm with a shoulder at 373 nm and three minor peaks at 428, 453, and 479 nm. (See Figure S2B.) The bathochromic shift and appearance of additional peaks at higher wavelengths are indicative of increased conjugation (*e.g.*, see spectra for benzene, naphthalene, and anthracene, representing increases in conjugation) consistent with the assignment of a ketone isomer to the single-peak spectrum and a more-conjugated, enol(ate) isomer to the multi-peak, red-shifted spectrum. In DMSO and 2ME, dithranol's spectrum exhibits features of both pH-extremes, suggesting that dithranol exists as a mixture of the two species in these solvents. (See Figure S5).



**Figure S5.** The absorbance spectra of dithranol in neat DMSO or 2ME can be simulated as linear combinations of spectra measured in acidic or alkaline solvent. UV/Vis absorption spectra of dithranol at varying concentrations were measured in DMSO or 2ME in the presence of excess (1 mM) HCl or NaOH. Simulated absorbance spectra were generated as linear combinations of the two component spectra (acidic and alkaline) representing the protonated ketone and deprotonated enolate forms of dithranol. Molar absorptivity is plotted as a function of wavelength for dithranol in **(A)** DMSO<sub>Acidic</sub> (blue) or DMSO<sub>Alk</sub> (red) and **(B)** 2ME<sub>Acidic</sub> (blue) or 2ME<sub>Alk</sub> (red). **(C)** The absorbance spectrum of 50  $\mu$ M dithranol in neat DMSO (black) and a simulated spectrum (pink/dashed) that was generated as the sum of the weighted component spectra shown in blue (73%) and red (27%). **(D)** Absorbance spectrum of 50  $\mu$ M dithranol in 2ME (black) and a simulated spectrum (pink/dashed) that was generated as the sum of the weighted component spectra shown in blue (76%) and red (24%).



**Figure S6.** The fluorescence emission intensity and excitation spectra of dithranol show different solvent-dependent trends. Excitation spectra were measured by illuminating dithranol over a range of wavelengths (lighter-colored lines, 250–470 nm) and monitoring emitted fluorescence at 495 nm. The emission spectra (darker lines, 400–650 nm) were then produced by exciting dithranol at the visible wavelength at which maximum fluorescence intensity was observed in the excitation spectrum ( $\lambda_{\text{Exc}}$ ). Representative spectra are shown in **(A)**  $\text{CHCl}_3$  (50  $\mu\text{M}$ ,  $\lambda_{\text{Exc}}$  = 380 nm), **(B)** benzene (50  $\mu\text{M}$ ,  $\lambda_{\text{Exc}}$  = 380 nm), **(C)**  $\text{H}_2\text{O}$  (25  $\mu\text{M}$ ,  $\lambda_{\text{Exc}}$  = 383 nm), **(D)** 2ME (5  $\mu\text{M}$ ,  $\lambda_{\text{Exc}}$  = 389 nm), **(E)** DMSO (5  $\mu\text{M}$ ,  $\lambda_{\text{Exc}}$  = 393 nm), and **(F)** DMF (5  $\mu\text{M}$ ,  $\lambda_{\text{Exc}}$  = 391 nm). Little to no fluorescence was observed in solutions of dithranol in  $\text{CHCl}_3$  and benzene. By contrast, dithranol fluoresces orders of magnitude more intensely in DMF than any other neat solvent. (Note the differences in the various y-scales.) Intermediate fluorescence intensity is observed in neat DMSO or 2ME, which can be increased by addition of NaOH (not shown). Keto forms (Scheme S1) lacking conjugation in the center ring are expected to be flexible, diminishing conjugation in the 3-ring system and consequently lowering fluorescence. The intense transitions in dithranol's fluorescence emission spectrum in DMF are consistent with a fully conjugated, planar 3-ring system. The fluorescence spectra of dithranol in DMSO and 2ME are of intermediate intensity, reflecting the mixed ketone/enol(ate) composition. The small amount of fluorescence observed in solutions of dithranol in  $\text{CHCl}_3$  and benzene could suggest that a very small fraction of dithranol exists as a planar enol in those solvents. This is supported by the shape of the fluorescence excitation spectra (200–450 nm) when emission at 495 nm is monitored. This has a maximum near 380 nm with small peaks at higher wavelengths, rather than a single broad peak near 360 nm as in the absorbance spectrum for the ketone, which would be expected if the ketone isomer itself was minimally fluorescent, e.g. while transiently planar. This blue-shift in maximum excitation wavelength is accompanied by a blue-shift in the entire emission spectrum, which suggests that it belongs to a dithranol isomer other than the keto or enolate.



**Figure S7.**  $^1\text{H}$ -NMR spectra of 10 mM dithranol (**A**;  $\text{CDCl}_3$ , 600 MHz) and 18 mM dithranone (**B**;  $(\text{CD}_3)_2\text{SO}$ , 600 MHz). In chloroform, the spectrum for dithranol (**A**) exhibits four sets of peaks, which integrate to a total of ten protons, consistent with the keto isomer of dithranol as shown. The presence of a sharp singlet peak at 12.3 ppm, which integrates to two protons, suggests that the two potentially exchangeable hydroxyl protons are not labile, but rather, involved in stable H-bonds<sup>4</sup> with the carbonyl oxygen on the central ring, as observed in the crystal structure (Scheme S1, **2HB-keto** structure). Two protons are represented by a triplet and four by doublet-of-doublet peaks in the region typically associated with aromaticity (6-8 ppm), highlighted in the dashed box and shown on an expanded scale in the inset. An up-field peak at 4.4 ppm represents the two protons of the methylene carbon of the central ring, and their presence is unique to the keto isomers. **Dithranol  $^1\text{H}$ -NMR summary ( $\text{CDCl}_3$ , 600 MHz):**  $\delta_{\text{H}}$  12.31 (2H, s), 7.51 (2H\*, t,  $J = 7.9$ ), 6.93 (4H, dd,  $J = 1.6, 0.5$ ), 4.38 (2H, s). In DMSO, the NMR spectrum of dithranone (**B**) exhibits four peaks, which integrate to a total of eight protons. The hydroxyl protons are represented by a sharp singlet peak at 11.9 ppm which integrates to two protons, suggesting that these are involved in stable H-bonds<sup>4</sup> with the carbonyl oxygen on the central ring, as observed in dithranol. Two protons are represented by a triplet and four by doublet-of-doublet peaks in the region typically associated with aromaticity (dashed box, shown on an expanded scale in the inset). **Dithranone  $^1\text{H}$ -NMR summary ( $(\text{CD}_3)_2\text{SO}$ , 600 MHz):**  $\delta_{\text{H}}$  11.9 (2H, s), 7.8 (2H, t), 7.7 (2H, d), 7.5 (2H, d). \* Integration normalization peak.



**Table S5.** Summary of UV/visible absorbance data for dithranol and the oxygenation product dithranone: Wavelengths (nm) of peak maxima and shoulders.

[Dithranol]	Solvent	$\lambda_1$	$\lambda_2$	$\lambda_3$	$\lambda_4$	$\lambda_5$	$\lambda_6$	$\lambda_7$	$\lambda_8$	$\lambda_9$
50 $\mu$ M	CHCl <sub>3</sub>	258 <sup>b</sup>	288	-	358 <sup>a</sup>	-	-	-	-	-
50 $\mu$ M	benzene	- <sup>d</sup>	289	-	359 <sup>a</sup>	-	-	-	-	-
50 $\mu$ M	DMSO	265 <sup>b</sup>	289	-	358	372	393 <sup>a</sup>	415	453	480
50 $\mu$ M	DMSO <sub>Acid</sub>	261 <sup>d</sup>	291	-	357	-	-	-	-	-
50 $\mu$ M	DMSO <sub>Alk</sub>	269	289 <sup>c</sup>	312	357 <sup>c</sup>	374	394 <sup>a</sup>	429	454	480
50 $\mu$ M	2ME	263 <sup>b</sup>	289	-	358	367 <sup>a</sup>	389	424	443	475
50 $\mu$ M	2ME <sub>Acid</sub>	- <sup>d</sup>	290	-	358 <sup>a</sup>	-	-	-	-	-
50 $\mu$ M	2ME <sub>Alk</sub>	- <sup>d</sup>	289	310 <sup>c</sup>	354 <sup>c</sup>	371	390 <sup>a</sup>	425	449	470
50 $\mu$ M	DMF	- <sup>d</sup>	- <sup>d</sup>	311	355 <sup>c</sup>	373	392 <sup>a</sup>	428	453	479
25 $\mu$ M	H <sub>2</sub> O	258 <sup>b</sup>	286	-	351 <sup>a</sup>	389 <sup>c</sup>	-	-	-	-

[Dithranone]	Solvent	$\lambda_1$	$\lambda_2$	$\lambda_3$	$\lambda_4$	$\lambda_5$	$\lambda_6$	$\lambda_7$	$\lambda_8$	$\lambda_9$
50 $\mu$ M	CHCl <sub>3</sub>	248 <sup>b</sup>	286	-	432 <sup>a</sup>	-	-	-	-	-
50 $\mu$ M	benzene	- <sup>d</sup>	281	-	432 <sup>a</sup>	-	-	-	-	-
50 $\mu$ M	DMSO	266 <sup>b</sup>	283 <sup>c</sup>	-	428 <sup>a</sup>	-	-	-	-	-
50 $\mu$ M	DMSO <sub>Acid</sub>	266 <sup>b</sup>	283 <sup>c</sup>	-	429 <sup>a</sup>	-	-	-	-	-
50 $\mu$ M	DMSO <sub>Alk</sub>	- <sup>d</sup>	284	332	-	565 <sup>a</sup>	-	-	-	-
50 $\mu$ M	2ME	- <sup>d</sup>	283 <sup>b</sup>	-	432	520 <sup>a</sup>	-	-	-	-
50 $\mu$ M	2ME <sub>Acid</sub>	- <sup>d</sup>	284	-	-	-	-	-	-	-
50 $\mu$ M	2ME <sub>Alk</sub>	- <sup>d</sup>	284	316 <sup>c</sup>	-	517 <sup>a</sup>	-	-	-	-
50 $\mu$ M	DMF	274	284	-	430 <sup>b</sup>	-	-	-	-	-
25 $\mu$ M	H <sub>2</sub> O	273 <sup>b</sup>	287 <sup>c</sup>	-	412 <sup>a</sup>	470 <sup>c</sup>	-	-	-	-

<sup>a</sup> Visible absorbance maximum (>300 nm); <sup>b</sup> Absorbance maximum (depends on UV-transparency of solvent);

<sup>c</sup> Shoulder; "peak" wavelength is estimated; <sup>d</sup> Not measured.

**Table S6a.** Summary of fluorescence data for dithranol: Wavelengths (nm) of peak maxima and shoulders. The upper section summarizes dithranol fluorescence emission spectra while the lower section summarizes fluorescence excitation spectra, where light emission at 495 nm was monitored. Note: The oxygenation product, dithranone, does not fluoresce under any conditions tested.

Conc.	Solvent	$\lambda_{Exc}$	$\lambda_1$	$\lambda_2$	$\lambda_3$	$\lambda_4$	$\lambda_5$
50 $\mu$ M	CHCl <sub>3</sub>	380	454	481 <sup>a</sup>	-	591	-
50 $\mu$ M	Benzene	380	465	493 <sup>a</sup>	530 <sup>c</sup>	585 <sup>c</sup>	-
5 $\mu$ M	DMSO	393	-	499	531 <sup>a</sup>	565	617 <sup>c</sup>
5 $\mu$ M	DMSO <sub>Acidic</sub>	392	-	499 <sup>a</sup>	534	566 <sup>c</sup>	617 <sup>c</sup>
5 $\mu$ M	DMSO <sub>Alk</sub>	392	-	498	531 <sup>a</sup>	565	617 <sup>c</sup>
5 $\mu$ M	2ME	389	-	495	528 <sup>a</sup>	565	613 <sup>c</sup>
5 $\mu$ M	2ME <sub>Acidic</sub>	388	459 <sup>c</sup>	491 <sup>a</sup>	524 <sup>c</sup>	570 <sup>c</sup>	-
5 $\mu$ M	2ME <sub>Alk</sub>	389	-	495	528 <sup>a</sup>	565 <sup>c</sup>	613 <sup>c</sup>
5 $\mu$ M	DMF	391	-	496	530 <sup>a</sup>	564	617 <sup>c</sup>
25 $\mu$ M	H <sub>2</sub> O	383	-	492	524 <sup>a</sup>	560 <sup>c</sup>	611 <sup>c</sup>

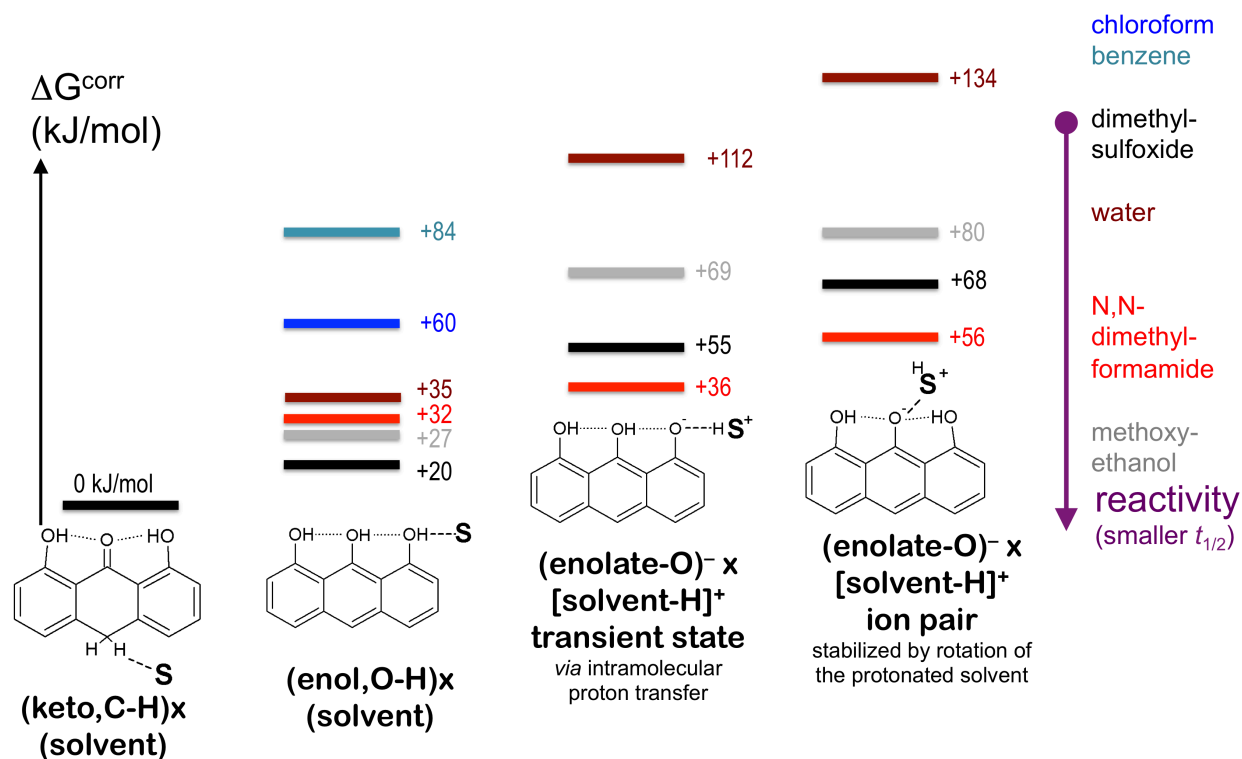
**Table S6a.** Summary of fluorescence data for dithranol: Wavelengths (nm) of peak maxima and shoulders. The upper section summarizes dithranol fluorescence emission spectra while the lower section summarizes fluorescence excitation spectra, where light emission at 495 nm was monitored. Note: The oxygenation product, dithranone, does not fluoresce under any conditions tested.

Conc.	Solvent	$\lambda_{Em}$	$\lambda_1$	$\lambda_2$	$\lambda_3$	$\lambda_4$	$\lambda_5$	$\lambda_6$	$\lambda_7$	$\lambda_8$	$\lambda_9$	$\lambda_{10}$
50 $\mu$ M	CHCl <sub>3</sub>	495	248 <sup>c</sup>	262 <sup>b</sup>	-	-	364 <sup>c</sup>	380 <sup>a</sup>	410	430	-	-
5 $\mu$ M	Benzene	495	- <sup>d</sup>	- <sup>d</sup>	-	-	363	380 <sup>a</sup>	-	429	-	-
5 $\mu$ M	DMSO	495	-	267	-	354 <sup>c</sup>	373	393 <sup>b</sup>	-	427	453	480
5 $\mu$ M	DMSO <sub>Acidic</sub>	495	-	268	-	-	380 <sup>c</sup>	392 <sup>b</sup>	-	429	451	480
5 $\mu$ M	DMSO <sub>Alk</sub>	495	-	268	313	354 <sup>c</sup>	373	392 <sup>b</sup>	417	428	453	480
5 $\mu$ M	2ME	495	-	264b	-	354 <sup>c</sup>	370	389 <sup>a</sup>	-	425	447	479 <sup>c</sup>
5 $\mu$ M	2ME <sub>Acidic</sub>	495	-	305 <sup>a</sup>	-	-	370 <sup>c</sup>	387	-	430 <sup>c</sup>	-	-
5 $\mu$ M	2ME <sub>Alk</sub>	495	-	263b	-	354 <sup>c</sup>	371	389 <sup>a</sup>	-	425	447	479
5 $\mu$ M	DMF	495	-	271	311 <sup>c</sup>	354 <sup>c</sup>	372	392 <sup>b</sup>	-	426	450	-
25 $\mu$ M	H <sub>2</sub> O	495	246 <sup>c</sup>	260b	-	354 <sup>c</sup>	365	383 <sup>a</sup>	410	426	441	-

<sup>a</sup> Emission maximum or (visible, >300 nm) excitation maximum; <sup>b</sup> Excitation maximum (depends on UV-transparency of solvent); <sup>c</sup> Shoulder; "peak" wavelength is estimated; <sup>d</sup> Not measured.

The tendency to shift from predominantly keto to the reactive/charged enolate does not correlate straightforwardly with solvent (**S**) polarizability as expressed by the dielectric constant (Table S1). Water, for example, with the highest dielectric constant, overwhelmingly favors the keto form. Instead, enolate stabilization correlates with a combination of parameters including H-bond acceptance ( $\beta$ ), zwitterionic character, and  $\pi/\pi$ -stacking, in addition to the dielectric constant (Table S1). The solvents with the greatest enolate content (DMF > DMSO = 2ME) share high  $\beta$ -values (0.7-0.9, Table S1). Each can adopt a zwitterionic resonance form stabilized by accepting a proton at an oxygen (DMSO, 2ME) or nitrogen (DMF) lone pair, forming a protonated solvent cation (**SH**<sup>+</sup>). The zwitterionic form of these solvents draws a parallel to the imidazole side chain of histidine, which accepts a proton to form cationic imidazolium<sup>5</sup> that can in turn interact with a substrate anion.<sup>22</sup> Solvents with a  $\pi$ -electron cloud (benzene, DMF, DMSO) could form energetically significant  $\pi/\pi$ -stacking interactions that are further elaborated by the stacking of dithranol molecules in its crystal structure.<sup>23</sup>

In parallel to experiments, we calculated Gibbs free energy diagrams for the lowest energy dithranol isomers and their ionized forms as solutes in order to establish an atomic-scale description of how solvents may activate dithranol solute. Figure 3 and Table S3 present the condensed-phase translational entropy-corrected<sup>24</sup> (Table S4) Gibbs free energy ( $\Delta G^{corr}$ ) diagram for models including both explicit solvent molecules and polarizable continuum environments.<sup>20,21</sup> For clarity, we only show the lowest energy/predominant keto and enol tautomers, a transient state with an inverted H-bonding pattern of deprotonated dithranol and protonated solvent (when structurally available), and the ionized form (Figure S3, red lines). In the latter, we evaluated supramolecular assemblies where the protonated solvent rests near the anionic solute molecule, forming stable (enolate<sup>-</sup>):(**SH**<sup>+</sup>) ion-pairs.



**Figure 3.** The condensed-phase translational entropy corrected Gibbs free energy ( $\Delta G^{\text{corr}}$ ) diagram is shown for the lowest energy keto, enol, and transient and stationary enolate isomers of dithranol. Explicit solvent and protonated solvent molecules are embedded in a polarizable solvent environment. Transient state geometry was fixed by keeping the distances between the ionized dithranolate- $\text{O}^-$  and protonated solvent fixed with an imaginary normal mode along the  $(\text{C}-\text{O}^-) \cdots \text{HS}^+$  interaction path.

**Table S3.** Summary of nuclear repulsion ( $E_{\text{nuc}}$ , a.u.), total electronic energy ( $E_{\text{SCF}}$ , a.u.), enthalpy (H, a.u.), uncorrected (G, a.u.) and corrected ( $G^{\text{corr}}$ , a.u.) Gibbs free energy, condensed phase translational entropy ( $\text{J mol}^{-1} \text{K}^{-1}$ , at 100  $\mu\text{M}$  concentration,  $T = 298 \text{ K}$ ) calculated at MN15/6-311++G\*\*/SMD(solvent) level of theory.

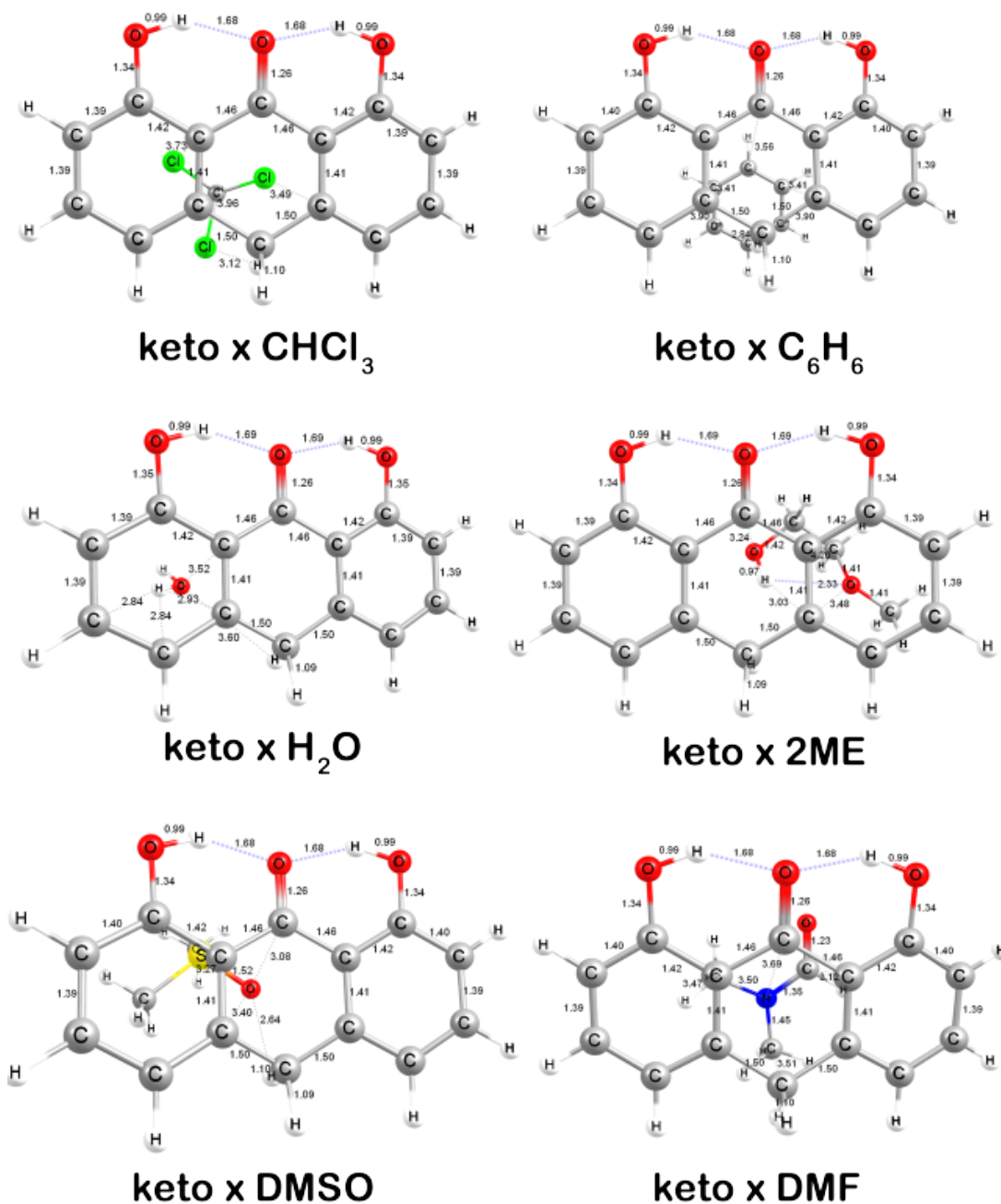
<i>phase/isomer</i> <sup>a</sup>	$E_{\text{nuc}}$	$E_{\text{SCF}}$	H	G	$S^{\text{trans}}$	$G^{\text{cor}}$
<i>gas phase</i>						
keto	1142.01524	-764.53700	-764.31532	-764.36776	176.2	-764.3678
enol	1140.63011	-764.51652	-764.29519	-764.34839	176.2	-764.3484
enolate <sup>-</sup>	1126.15900	-764.01145	-763.80452	-763.85538	176.1	-763.8554
<i>chloroform (CHCl<sub>3</sub>)</i>						
keto × S	2155.55557	-2183.61862	-2183.37015	-2183.44084	190.9	-2183.4398
enol × S	2056.54026	-2183.60034	-2183.35193	-2183.42503	190.9	-2183.4240
<i>benzene (C<sub>6</sub>H<sub>6</sub>)</i>						
keto × S	1884.93015	-996.57625	-996.24713	-996.31587	188.5	-996.3149
enol × S	1759.79442	-996.55467	-996.22536	-996.29705	188.5	-996.2829
<i>Water (H<sub>2</sub>O)</i>						
keto × S	1286.15937	-840.94298	-840.69559	-840.75983	193.1	-840.7580
enol × S	1263.76578	-840.93290	-840.68562	-840.74653	193.1	-840.7447
[enolate <sup>-</sup> × SH <sup>+</sup> ] <sup>*</sup>	1265.04536	-840.90436	-840.65720	-840.71700	193.1	-840.7152
enolate <sup>-</sup> × SH <sup>+</sup>	1257.43611	-840.89258	-840.64470	-840.70529	193.0	-840.7035
<i>2-methoxyethanol (2ME)</i>						
keto × S	1879.21619	-1033.90527	-1033.56108	-1033.63275	189.4	-1033.6317
enol × S	1754.07878	-1033.88844	-1033.54468	-1033.61890	189.4	-1033.6178
[enolate <sup>-</sup> × SH <sup>+</sup> ] <sup>*</sup>	1768.82969	-1033.86105	-1033.51973	-1033.58759	189.4	-1033.5865
enolate <sup>-</sup> × SH <sup>+</sup>	1894.72000	-1033.43953	-1033.10918	-1033.17729	189.3	-1033.1762
<i>dimethylsulfoxide (DMSO)</i>						
keto × S	1888.67638	-1317.54228	-1317.23278	-1317.30249	190.2	-1317.3013
enol × S	1714.39712	-1317.53408	-1317.22581	-1317.29472	190.2	-1317.2935
[enolate <sup>-</sup> × SH <sup>+</sup> ] <sup>*</sup>	1728.52540	-1317.52080	-1317.21524	-1317.28163	190.2	-1317.2805
enolate <sup>-</sup> × SH <sup>+</sup>	1883.98253	-1317.51733	-1317.20859	-1317.27656	190.1	-1317.2754
<i>N,N-dimethylformamide (DMF)</i>						
keto × S	1898.73921	-1012.86871	-1012.53578	-1012.60525	189.5	-1012.6041
enol × S	1704.15983	-1012.85236	-1012.51945	-1012.59302	189.5	-1012.5919
[enolate <sup>-</sup> × SH <sup>+</sup> ] <sup>*</sup>	1738.61714	-1012.83713	-1012.52618	-1012.50715	189.5	-1012.5710
enolate <sup>-</sup> × SH <sup>+</sup>	1840.07851	-1012.39642	-1012.07764	-1012.14720	189.4	-1012.1461

<sup>a</sup> [ ]<sup>\*</sup> transient state w/frozen O<sup>-</sup>...H<sup>+</sup>-S distances.

**Table S4.** Worksheet for correcting gas phase translational entropy (according to Whitesides *et al.*<sup>3)</sup> to consider condensed phase free volume of in 100  $\mu\text{M}$  solution of dithranol and its isomers in the evaluated solvents.

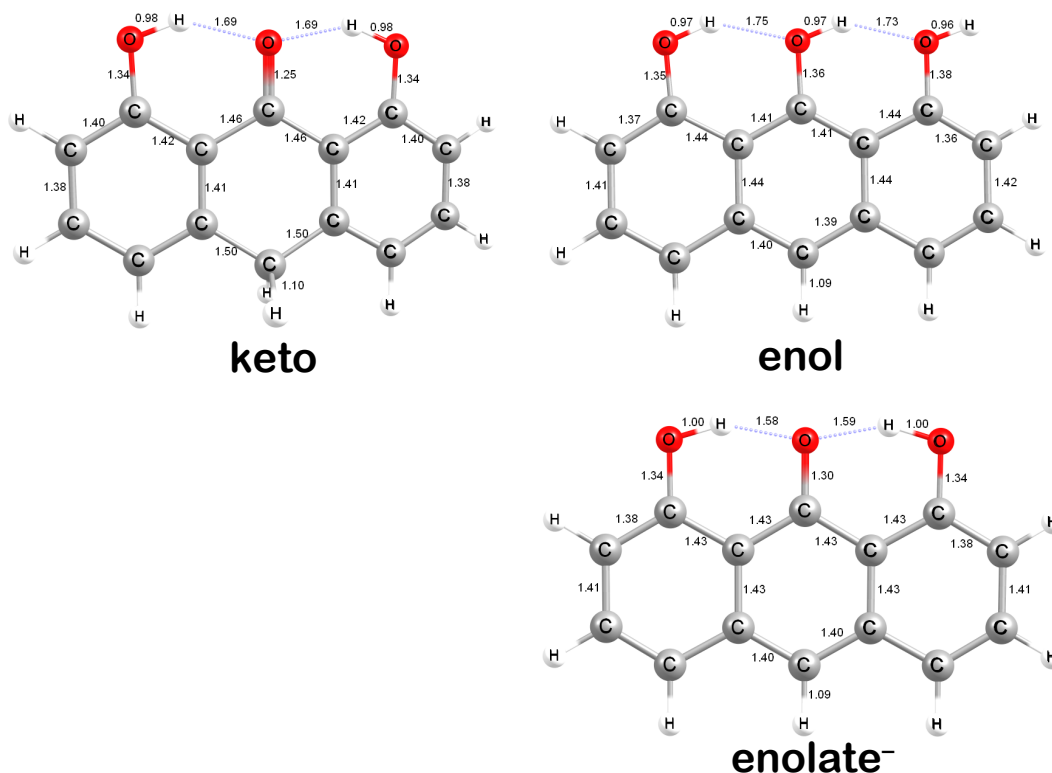
JOC 63 3821 worked out example	CHCl <sub>3</sub>	C <sub>6</sub> H <sub>6</sub>	2ME	DMF	DMSO	H <sub>2</sub> O	
<b>density, g cm<sup>-3</sup></b>	<b>1.490</b>	<b>1.492</b>	<b>0.876</b>	<b>0.965</b>	<b>0.944</b>	<b>1.100</b>	<b>1.000</b>
[solvent], mol dm <sup>-3</sup>	12.5	12.5	11.2	12.7	12.9	14.1	55.5
numeral density rel. to ideal gas	2.806E-06	2.801E-06	2.514E-06	2.842E-06	2.895E-06	3.156E-06	1.244E-05
V <sup>free,macro</sup> 1 L occupies, cm <sup>3</sup>	7.5	7.5	6.8	7.6	7.8	8.5	33.4
V available for a solvent, Å <sup>3</sup>	132.6	132.9	148.1	131.0	128.6	117.9	29.9
Inter Solvent Distance, Å	5.10	5.10	5.29	5.08	5.05	4.90	3.10
<b>V of a molecule, Å<sup>3</sup></b>	<b>97</b>	<b>104</b>	<b>107</b>	<b>102</b>	<b>101</b>	<b>96</b>	<b>25</b>
solvent radius, Å		2.920	2.945	2.895	2.889	2.840	1.814
MOPAC PM7 COSMO V, Å <sup>3</sup>		104	107	101	101	96	25
MOPAC PM7 COSMO A, Å <sup>2</sup>		116	119	117	115	110	42
G16PCM cavity V, Å <sup>3</sup>		141	134	111	125	111	19
G16PCM cavity A, Å <sup>2</sup>		145	143	124	138	124	36
SMD Rsolv, Å		2.48	2.63	2.46		2.46	1.39
SMD Vmol, Å <sup>3</sup>		80.70	88.91	70.94		70.94	18.07
R(solvent molecule), Å	4.59	4.71	4.75	4.67	4.66	4.58	2.92
V(non-excluded void), Å <sup>3</sup>	36	29	41	29	28	22	5
R(free), Å	1.01	0.73	1.09	0.82	0.72	0.60	0.33
V <sup>free,nano</sup> for single, Å <sup>3</sup>	1.03	0.39	1.28	0.56	0.37	0.22	0.04
shape: 8=cube; 6.3 sphere	8	6.3	8	8	6.3	6.3	6.3
<b>M<sub>w</sub> (solvent), g mol<sup>-1</sup></b>	<b>119.0</b>	<b>119.4</b>	<b>78.1</b>	<b>76.1</b>	<b>73.1</b>	<b>78.1</b>	<b>18.0</b>
<b>gas phase</b>							
			36.9+12.5lnM+12.5lnT				
standard state, [X] mol dm <sup>-3</sup>	0.0446	0.0446	0.0446	0.0446	0.0446	0.0446	0.0446
approx. S <sub>trans</sub> <sup>‡</sup> , J mol <sup>-1</sup> K <sup>-1</sup>	167.9	168.0	162.7	162.3	161.8	162.7	144.3
part 1, Eq.2 in JOC 63 3821	-233.2	-233.2	-233.2	-233.2	-233.2	-233.2	-233.2
part 2, Eq.2 in JOC 63 3821	253.3	253.3	252.7	252.7	252.6	252.7	250.5
exact S <sub>trans</sub> <sup>‡</sup> , J mol <sup>-1</sup> K <sup>-1</sup>	167.6	167.7	162.4	162.0	161.5	162.4	144.1
exact S <sub>trans</sub> <sup>‡</sup> , cal mol <sup>-1</sup> K <sup>-1</sup>	40.1	40.1	38.8	38.8	38.6	38.8	34.5
<b>solutions</b>							
		solute trans. S <sub>trans</sub> <sup>‡</sup> has to be dominated by SOLVENT molecules					
[analyte], 10 <sup>-3</sup> mol dm <sup>-3</sup>	1000	0.1	0.1	0.1	0.1	0.1	0.1
M <sub>w</sub> (analyte), g mol <sup>-1</sup>	32.0	33.0	33.0	33.0	33.0	33.0	33.0
[analyte] <sup>eff.</sup> , mol dm <sup>-3</sup>	132.620	0.013	0.015	0.013	0.013	0.012	0.003
<b>simple model</b>							
			11.1+12.5lnM+12.5lnT				
approx. S <sub>trans</sub> <sup>‡</sup> , J mol <sup>-1</sup> K <sup>-1</sup>	126	203	203	203	203	203	203
using [analyte] <sup>eff.</sup> or V <sup>free</sup>		11.1+12.5lnM+12.5lnT-8.1ln[analyte, effective]					
approx. S <sub>trans</sub> <sup>‡</sup> , J mol <sup>-1</sup> K <sup>-1</sup>	85	162	161	162	162	163	174
change	68%	80%	79%	80%	80%	80%	86%
approx. S <sub>trans</sub> <sup>‡</sup> , J mol <sup>-1</sup> K <sup>-1</sup>	85.0	162.0	161.1	162.1	162.2	162.9	174.4
part 1, Eq.2 in JOC 63 3821	-241.2	-232.0	-232.1	-232.0	-231.9	-231.8	-230.5
part 2, Eq.2 in JOC 63 3821	251.4	251.4	251.4	251.4	251.4	251.4	251.4
exact S <sub>trans</sub> <sup>‡</sup> , J mol <sup>-1</sup> K <sup>-1</sup>	84.8	161.7	160.8	161.8	162.0	162.7	174.1
exact S <sub>trans</sub> <sup>‡</sup> , cal mol <sup>-1</sup> K <sup>-1</sup>	20.3	38.7	38.5	38.7	38.7	38.9	41.6
Free energy correction kJ mol <sup>-1</sup>	-12.1	-12.1	-12.4	-12.1	-12.0	-11.8	-8.4

It is important to highlight that, independent of the level of theory or the solvation model, the crystallographically characterized keto form<sup>23</sup> was the most stable isomer. Depending on their H-bond acceptor and  $\pi/\pi$ -stacking properties, explicit solvent molecules either stack with dithranol or form significant (10-20 kJ/mol) C-H $\cdots$ Cl/N/O interactions with the solute. All considered keto models (Figure S8) were constructed with the energetically most favorable methylene group/solvent interactions. The most stable form of the neutral enol (still higher in energy than the keto forms) had two intramolecular H-bonds between a peripheral and the central phenol groups, while the third, dangling peripheral O-H bond provided a H-bond donor to solvent (Figures S8-S9).



**Figure S8.** Solution phase optimized equilibrium structures (no imaginary normal modes) of keto isomers of dithranol with explicit solvents calculated at MN15/6-311++G\*\*/SMD level of theory.



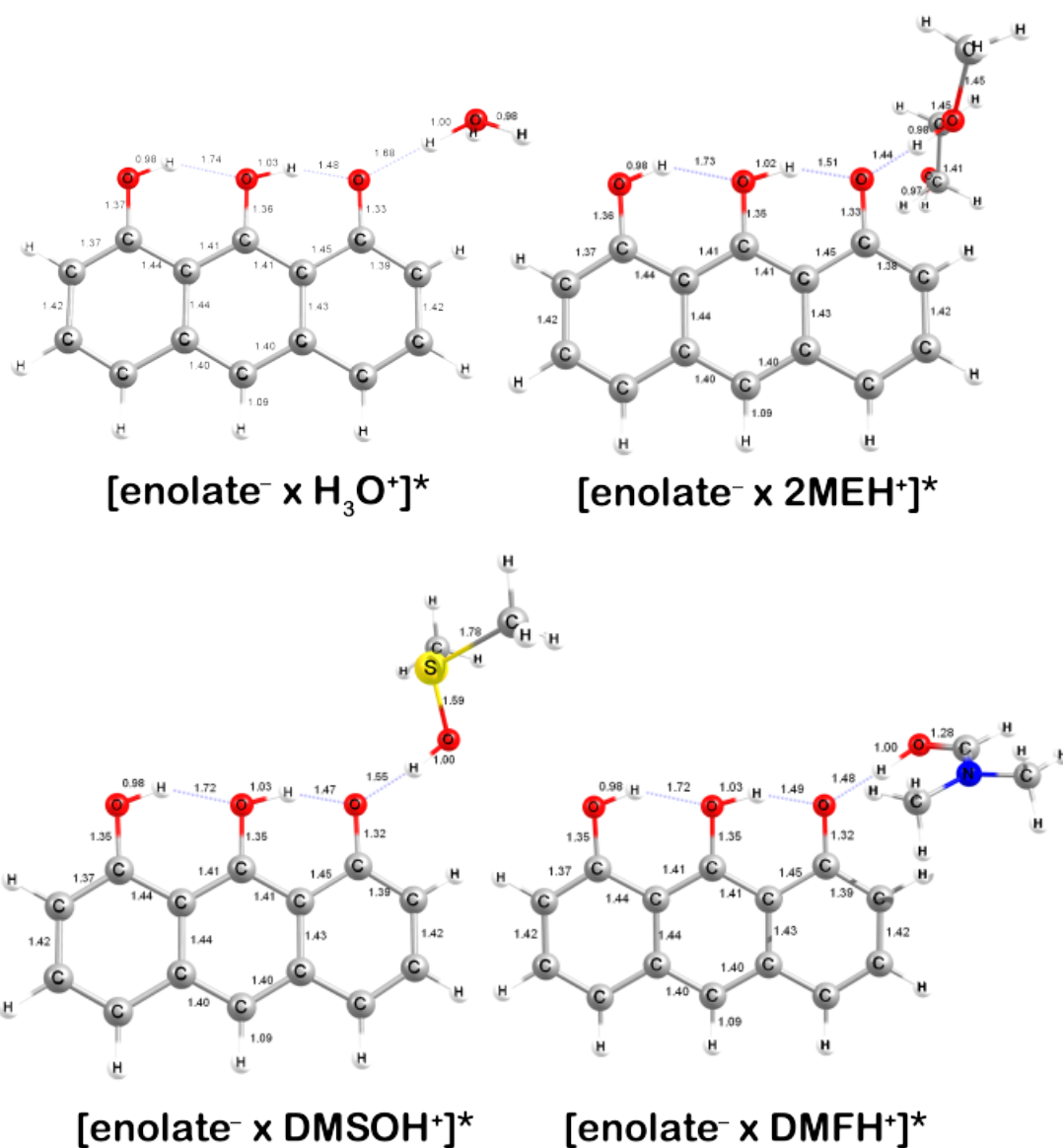


**Figure S9.** Gas phase optimized equilibrium structures calculated at MN15/6-311++G\*\* level of theory.

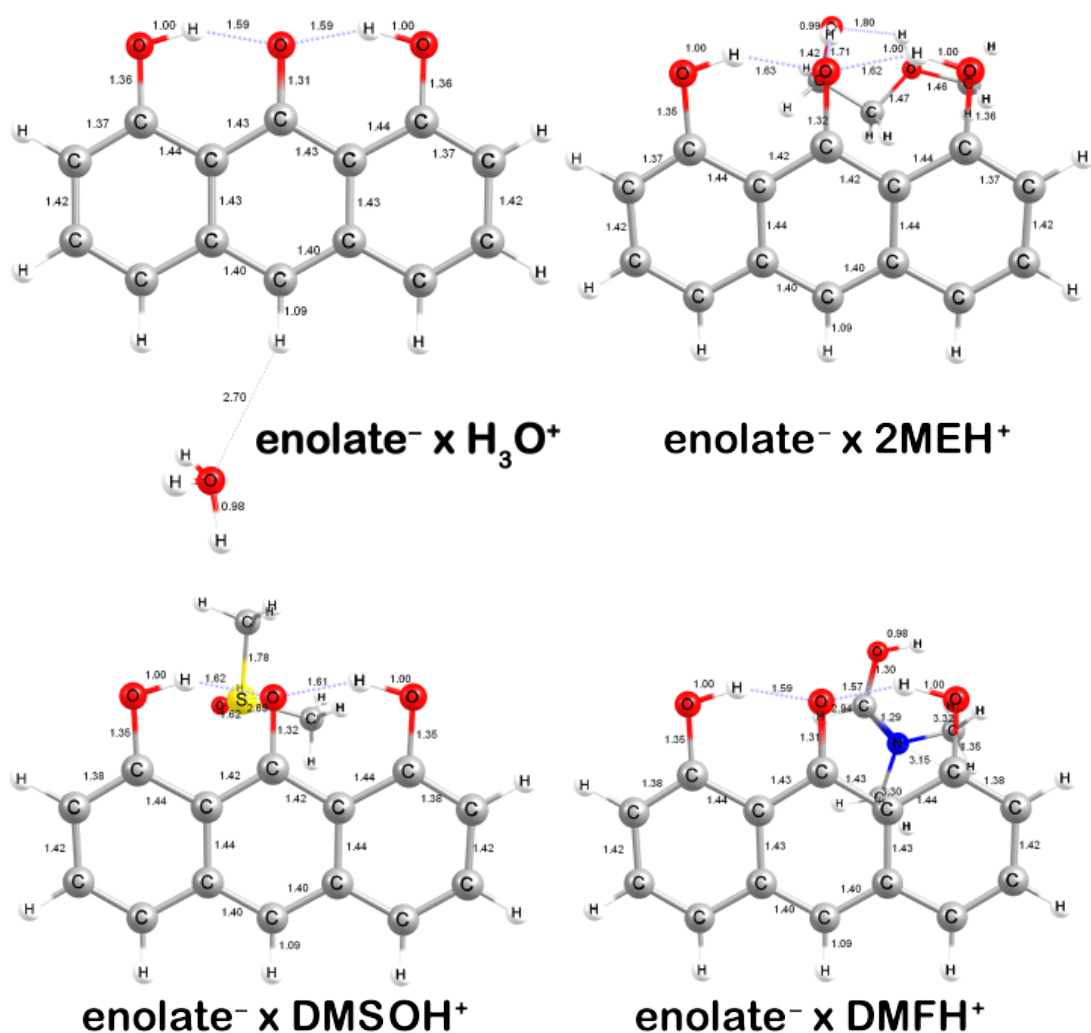
Solvents (**S**) with nucleophilic O/N (DMSO, DMF, H<sub>2</sub>O, 2ME) showed less than 35 kJ·mol<sup>-1</sup> endothermicity in forming the solvent/enol isomer supramolecular assembly. The lower energy block of (enol-O-H)×(**S**) tautomers (Figure S3) was followed by the chloroform adduct due to a dithranol-O(H)⋯H-CCl<sub>3</sub> interaction. Benzene formed an O-H⋯π-cloud interaction at the peripheral phenol group (Figure S10). The modest energy gap between the keto×(**S**) and enol×(**S**) tautomers thus defines a thermodynamically accessible stepping-stone toward ionization of the dithranol substrate, leading to the key dithranyl•/O<sub>2</sub>•<sup>-</sup> radical-pair intermediate (Scheme 1A).

A transiently ionized dithranol state was generated by inverting the H-bonding pattern (enol-O<sup>δ-</sup>-H⋯S<sup>δ+</sup>) between the solvent molecule's nucleophilic center and the peripheral phenol group (Figures S10-S11). This is another critical stepping-stone toward the enolate-O<sup>-</sup>⋯H-S<sup>+</sup> ion-pair, where the relative Gibbs free energies for each state correlate with the thermodynamic stability of the protonated solvent molecule. The two lowest energy structures were calculated for the DMFH<sup>+</sup> and DMSOH<sup>+</sup> (Figure S12) supra-molecular assemblies with the enolate. These solvents also form the most stable zwitterions, through which they are primed to accept H<sup>+</sup>.





**Figure S11.** Solution phase optimized stationary structures of transient enolate isomers with protonated solvent calculated at MN15/6-311++G\*\*/SMD level of theory. The frozen O<sup>-</sup>⋯H(solvent) distance resulted in a single imaginary normal mode with displacements along the O<sup>-</sup>⋯H<sup>+</sup>-S vector.



**Figure S12.** Solution phase optimized equilibrium structures (no imaginary normal modes) of enolate form of dithranol with protonated solvent calculated at MN15/6-311++G\*\*/SMD level of theory.

**SH<sup>+</sup>** was rotated above the dithranol/enolate plane to prevent spontaneous back-transfer of the proton from **SH<sup>+</sup>** to the phenolate group. The fully ionized, deprotonated enolates with equilibrium structures (Figure S12) adopt a highly similar H-bonding pattern to the neutral keto form (Figures S11-S12). The ion-pair is stabilized by a short-range Coulomb interaction. The relative Gibbs free energy values shift upward in energy by only ca. 20 kJ·mol<sup>-1</sup> (Figure 3). Most of the ion-pairs remain within the energy range that can be considered reasonable (~100 kJ·mol<sup>-1</sup>) for observing spontaneous reactivity under ambient conditions.

The experimentally observed reactivity patterns (Figure S1)<sup>6</sup> can be explained in part using the computational models. Lack of reactivity in chloroform and benzene suggests that neither the keto tautomer nor the explicitly solvated enol, which is energetically accessible from the keto, is likely to be sufficiently activated for dioxygen reactivity.<sup>1</sup> Aqueous/2ME<sup>6,7</sup> and DMF are optimal due to reasonable O<sub>2</sub> solubility (Table S1) and strong stabilization of the enolate and **SH<sup>+</sup>**. Despite the favorable energetics for the enolate/**SH<sup>+</sup>** ion-pair, low O<sub>2</sub> solubility hinders reactivity in DMSO. The low O<sub>2</sub> solubility and energetically unfavorable zwitterionic form of water further poses a challenge for achieving aqueous reactivity.

An activated enolate/ $\text{SH}^+$  ion-pair is generated through a series of endothermic yet energetically attainable steps under ambient conditions, beginning with the conversion of the keto to an enol tautomer. Conversion of the enol to the ion-pair via an appropriate solvent/sidechain yields an energized intermediate, which may serve the role of a transition state from which activation of  $\text{O}_2$  to  $\text{O}_2^{\bullet-}$  by the substrate anion may occur. We envision similar steps in the NMO active site that may increase the rate of substrate oxidation over 2,000-fold relative to the non-catalytic process.<sup>6</sup> These results also suggest a mechanism whereby anthralin derivatives, delivered topically in organic matrices as psoriasis drugs or systemically as chemotherapeutics, may react with  $\text{O}_2$ . The principle of cooperation between substrate/solvent to form an ion-pair that converts to the reactive radical pair could be exploited in some organic transformations in which  $\text{O}_2$  is the desired oxidant.<sup>25</sup>

## EXPERIMENTAL

**Solvents and Reagents.** All reagents and solvents were purchased commercially and used without further purification. Acidic DMSO ( $\text{DMSO}_{\text{Acid}}$ ) was prepared by adding concentrated HCl (12.18 M, 37 w/w%) to DMSO to a final concentration of 1 mM. Alkaline DMSO ( $\text{DMSO}_{\text{Alk}}$ ) was prepared by adding NaOH from a concentrated solution (12.18 M, 39 w/w%) in deionized water to DMSO to a final concentration of 1 mM. Acidic and alkaline 2ME ( $\text{2ME}_{\text{Acid}}$  and  $\text{2ME}_{\text{Alk}}$ ) were prepared similarly. Aqueous buffers containing 100 mM buffer and 300 mM NaCl were adjusted to the following pHs with 5 M HCl or NaOH: citric acid/trisodium citrate (pH 5.02, 5.53, 5.97), *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) (pH 6.50, 7.00, 7.54), tris(hydroxymethyl)-aminomethane (tris/tris-HCl) (pH 8.06, 8.43), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) (pH 8.90, 9.54), and *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS) (pH 9.95, 10.47, 11.15). Solvents and aqueous buffer solutions used for anaerobic experiments were degassed by repeatedly freezing and thawing under vacuum with argon purge, except for DMSO and 2ME, which were degassed by repeated vacuum/Ar purge cycles, and DMF, deuterated  $\text{CHCl}_3$ , and deuterated DMSO, which were oxygen-free as purchased and stored in a dry anaerobic chamber (MBraun). Dithranol stock solutions were prepared in degassed, HPLC-grade DMSO and transferred via gas-tight syringe to crimp sealed amber vials. Stock concentrations were measured by UV/vis in  $\text{DMSO}_{\text{Acid}}$ :  $\epsilon_{356\text{nm}} = 7.83 \pm 0.03 \text{ mM}^{-1} \text{ cm}^{-1}$ . Stocks of the product dithranone (1,8-dihydroxy-9,10-anthracenone) were prepared in DMSO.<sup>26</sup>

**Expression and Purification of NMO.** The gene encoding the N-terminally His<sub>6</sub>-tagged NMO (pBad vector) was received as a kind gift from the Schneider laboratory, University of Turku, Finland.<sup>10</sup> The NMO was expressed and purified as described previously.<sup>6</sup> For anaerobic experiments, NMO was purged of  $\text{O}_2$  by gently stirring on ice in an anaerobic chamber for 30-40 min.

**UV/Visible (UV/vis) Absorbance Spectroscopy.** UV/Vis spectra were measured using a Varian Cary50 spectrophotometer under ambient conditions and plotted/analyzed using Kaleidagraph 4.0 (Synergy Software, Reading, PA). Samples of dithranol were prepared by adding dithranol from a concentrated stock to solvent in an unsealed cuvette because, with the exception of aqueous buffers and alkaline solvents, no oxidation products were detected on the timescale of the measurements. Samples of dithranol in alkaline solvents and aqueous buffers were prepared by sealing degassed solvent into a cuvette in an anaerobic chamber (Coy) and adding dithranol to the sealed cuvette via gas tight syringe. Quartz or glass cuvettes were used for all measurements for broader solvent compatibility.

**Fluorescence Spectroscopy.** Fluorescence emission spectra were measured in quartz cuvettes using an Agilent Cary Eclipse spectrophotometer. Solutions of dithranol in various solvents were prepared as described for UV/vis spectra measurements. Excitation wavelengths were chosen based on the wavelengths of maximum absorbance in the fluorescence excitation spectrum when emission at 495 nm is monitored (CHCl<sub>3</sub> and benzene, 380 nm; H<sub>2</sub>O, 383 nm; 2ME, 389 nm; DMF, 391 nm; DMSO, 393 nm).

**Nuclear Magnetic Resonance (NMR) Spectroscopy.** NMR spectra were obtained using a Bruker 600 MHz Avance III spectrometer. Solutions of dithranol or dithranone were prepared gravimetrically by dissolving the solid into deuterated solvent (CDCl<sub>3</sub>, (CD<sub>3</sub>)<sub>2</sub>SO, or (CD<sub>3</sub>)<sub>2</sub>NCDO) in a dry anaerobic chamber (MBraun) and sealing the tube with a plastic cap. Low-concentration samples were prepared by serial dilution with deuterated solvent in a dry anaerobic chamber.

**Aqueous Spectrophotometric pH Titrations.** The UV/vis spectra of free dithranol and the dithranol:NMO complex as a function of pH were measured as described above. All pH titrations were performed by a discontinuous method using a different aqueous buffer for each pH (see above) due to instability of the enzyme when concentrated acid or base is added. To determine a pK<sub>a</sub> for dithranol in the absence of enzyme a 46 μM dithranol solution in aqueous buffer was prepared anaerobically at each pH >7.5, and its absorbance spectrum measured. Because of dithranol's poor solubility in neutral and acidic aqueous solution, the pK<sub>a</sub> for the dithranol:NMO complex was determined by first adding a stoichiometric excess of NMO (80 μM) anaerobically to each solution, before adding 39 μM dithranol. The solutions were incubated for 5 min before the absorbance was measured.

Values for pK<sub>a</sub> were determined by plotting the absorbance at 385 nm ( $\lambda_{\text{max}}$  for dithranolate anion) or 393 nm ( $\lambda_{\text{max}}$  for dithranolate:NMO complex) as a function of pH and fitting the data to a curve (Kaleidagraph 4.0) describing a single pK<sub>a</sub> transition  $(B \times 10^{-\text{pH}} + A \times 10^{-\text{pK}_a}) / (10^{-\text{pH}} + 10^{-\text{pK}_a})$ , where *A* and *B* are the highest and lowest absorbance values, respectively, extrapolated by the fit.

**Estimation of Autoionization in DMSO and 2ME.** In both DMSO and 2ME, addition of sufficient concentrated HCl or NaOH fully converts the absorbance spectrum of dithranol to that observed in low- or high-pH aqueous buffer, respectively. UV/vis absorptivity constants for the fully protonated and deprotonated forms of dithranol ( $\epsilon_{\text{DH}_2}$  and  $\epsilon_{\text{DH}(-)}$ ) were obtained by measuring the absorbance spectra of each at varying concentrations in DMSO or 2ME in the presence of a large excess (1 mM) of concentrated HCl or NaOH. The data were used to generate standard curves ( $\text{Abs}_\lambda = \epsilon_\lambda[\text{Dithranol}]$ ) at 1 nm increments over  $\lambda = 300\text{--}500$  nm. These were in turn used to simulate the UV/vis spectrum measured in neat solvent as a linear combination of  $\epsilon_{\text{DH}_2}$  and  $\epsilon_{\text{DH}(-)}$  according to  $\text{Abs} = (A \cdot \epsilon_{\text{DH}_2} + B \cdot \epsilon_{\text{DH}(-)}) \cdot [\text{Dithranol}]$ , where *A* and *B* represent the mole fraction of protonated and deprotonated dithranol, respectively, and  $A + B = 1$  (*assuming no other speciation is present*). The fit parameters *A* and *B* were calculated by minimizing the sum-square of the residuals ( $\sum (\text{Abs}_{\text{calc}} - \text{Abs}_{\text{meas}})^2$ ) over the range of 300–500 nm at 1 nm intervals, using Excel's Solver tool to vary  $A + B = 1$  (GRG non-linear, default parameters with non-negative *A*, *B*).

**Reagent Quantification by High-Performance Liquid Chromatography (HPLC).** Quantification of dithranol and its oxygenation product dithranone was performed using an Agilent 1100 LC system (Agilent Technologies, Santa Clara, CA) equipped with a G1315B diode array detector. Each sample or standard was injected at a volume of 20 μL onto a Symmetry C<sub>18</sub> 5 μm, 4.6 × 150 mm column (Waters) maintained at 50 °C. Solvents used to separate the analytes of interest were (A) water +0.1% (v/v) TFA and (B) acetonitrile +0.1% (v/v) TFA. The separation was carried out with a



gradient: 0-3 min (60→90 %B, 1.5→2.5 mL/min), 3-4 min (90→60 %B, 2.5→1.5 mL/min), 4-4.1 min (60 %B, 1.5 mL/min). Analytes were monitored at 354 nm (dithranol) and 430 nm (dithranone). Other oxidation products are not reliably quantified by HPLC. Integrated intensities of the dithranol and dithranone peaks were compared against standard curves to determine concentration.

**Uncatalyzed Oxidation of Dithranol in Air.** Consumption of dithranol and formation of oxidation products were monitored by UV/vis and discontinuous HPLC as described above. In an anaerobic chamber, 10 mL solutions of 100  $\mu$ M dithranol in each solvent were prepared in 50 mL round bottom flasks covered with foil to exclude light. Zero-time samples were measured for HPLC and UV/vis analysis before the reaction mixtures were removed from the chamber. The reaction flasks were continuously shaken at room temperature at 100 rpm to establish and maintain air-saturation. Minimal evaporation was observed in most solvents, but reactions in highly volatile solvents such as chloroform were sealed with an ungreaed ground-glass stopper between measurements to reduce evaporation. Samples (50  $\mu$ L) were removed for HPLC and UV/vis analysis at regular time intervals and discarded.

**Computational Methods.** The X-ray crystal structure of dithranol<sup>23</sup> (REFCODE: ANTHLN) was obtained from the Cambridge Crystallographic Database.<sup>27</sup> The crystal packing informed us of a network of weak interactions that solute and solvent molecules could be engaged in. The  $\pi/\pi$ -stacking and the intramolecular H-bonding interactions among stacks of dithranol molecules in the crystalline state were utilized to create structures for various solute/solvent pairing.

The level of theory in our study was chosen by considering the results of high-level, *ab initio* wave function calculations carried out by others for and extensive set of anthrone and related hydroxyarene molecules using the CBS-QB3 level of theory.<sup>28,29</sup> Table S2 summarizes the calculated deviations in relative isomer energies between the reference *ab initio* wave function level and representative pure GGA (BP86,<sup>30,31</sup> BLYP,<sup>31,32</sup> and PBE,<sup>33,34</sup> Rung 2), metaGGA (TPSS,<sup>35</sup> revised TPSS,<sup>36,37</sup> and M06L,<sup>38</sup> Rung 3), and hyperGGA (B3LYP,<sup>32,39</sup> cam-B3LYP+GB3J,<sup>40,41</sup> and  $\omega$ B97xD,<sup>42,43</sup> and global-hybrid MN15:<sup>16</sup> Rung 4) density functionals. While qualitatively all functionals predicted the correct energetic order of dithranol isomers, we found a significant improvements in the absolute relative energy values (within +4.0 kJ/mol error) when using MN15<sup>16</sup> functional with triple- $\zeta$  basis set supplemented with both polarization and diffuse functions for all heavy and light atoms (6-311++G\*\*).<sup>17-19</sup> Moreover, we also evaluated the performance of three implicit solvation models (PCM,<sup>44</sup> COSMO,<sup>45</sup> and SMD<sup>20</sup>) using solvent parameters reported in Table S1. We found that only the SMD polarizable continuum model with electrostatic solvent/solute and non-electrostatic solvent/solvent interactions for the CHCl<sub>3</sub> and DMSO solutions provide negative solvation energy of the solvent molecule itself. Thus, our validated level of theory employed implicitly and explicitly solvated model calculations is the MN15<sup>16</sup>/6-311++G\*\*<sup>17-19</sup>/PCM(SMD<sup>20</sup>). This particular combination of level of theory and solvation model is notable given that they were developed by the same team of scientists, which ensures a degree of self-consistency.

All reported optimized geometries were confirmed to correspond to equilibrium structures by vibrational analysis showing no imaginary normal modes with the exception of transient state structure with constrained intermolecular distances. These frequency calculations also provided us with the predicted IR vibrational spectra that clearly showed the significant overlap of the C=O and aromatic C=C stretching modes. This prevents employing FTIR as a diagnostic technique for differentiating among various tautomers. Given that our thesis is the critical influence of solvents on relative energies of substrate isomers, we supplemented the standard thermochemical calculations with translational entropy correction for a solute at 100  $\mu$ M concentration in various solvents, as

proposed by Whitesides.<sup>24</sup> In brief, we determined the ‘effective solute concentration’ in the space of the solution that is available for the solute and not excluded by the solvent molecules. The overestimation of translational entropy using the ideal gas phase model can be as much as 30%. The translational entropy of a solute was then calculated using the equation  $S^{\text{trans,corr}} = 11.1 + 12.5 \ln(M_w^{\text{solute}}) + 12.5 \ln(T) - 8.1 \ln([\text{analyte}]^{\text{eff}})$ . The numerical details of the translational entropy corrections are summarized in Table S4.

## ASSOCIATED CONTENT

**Supporting Information.** Computational models, validations of the level of theory, UV/vis and fluorescence spectral data and fits, and UV/vis and HPLC data monitoring dithranol reactions are included in the supporting information. This material is available free of charge via the Internet at <http://pubs.acs.org>. Additional electronic supporting information is available at the free, publically accessible Zenodo dataset depository, DOI: 10.5281/zenodo.3554508. This includes downloadable files containing the atomic positions of each tautomer considered, validation of several density functionals, and detailed worksheet for correcting calculated free energy with condensed phase translation entropy.

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**Acknowledgement.** We thank Dr. Brian Tripet of the Montana State University NMR facility for his assistance in measuring, plotting, and interpreting NMR spectral data. The NMR facility was funded by the NSF-Major Research Instrumentation program (NSF-MRI: DBI-1532078), the Murdock Charitable Trust Foundation (015066:MNL), and support from the MSU Vice-President for Research and Economic Development office. We thank Garrett Moraski for helpful discussions. Financial support for this work is from the US National Science Foundation grant MCB1715176. No competing financial interests have been declared.

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