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Structure Property Relationships of Carboxylic Acid Isosteres

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ABSTRACT: The replacement of a carboxylic acid with a surrogate structure, or (bio)-isostere, is a classical strategy in medicinal chemistry. The general underlying principle is that by maintaining the features of the carboxylic acid critical for biological activity, but appropriately modifying the physicochemical properties, improved analogs may result. In this context, a systematic assessment of the physicochemical properties of carboxylic acid isosteres would be desirable to enable more informed decisions of potential replacements to be used for analog design. Herein we report the structure-property relationships (SPR) of 35 phenylpropionic acid derivatives, in which the carboxylic acid moiety is replaced with a series of known isosteres. The dataset generated provides an assessment of the relative impact on the physicochemical properties that these replacements may have compared to the carboxylic

acid analog. As such, this study presents a framework for how to rationally apply isosteric replacements of the carboxylic acid functional group.

KEYWORDS: Carboxylic acid isostere; bioisostere; isosteric replacement; physicochemical properties; structure property relationship; tetrazole.

Introduction. The replacement of an atom, or group of atoms, or even an entire scaffold of a biologically active compound with a surrogate structure that exhibits broadly similar biological properties is a fundamental strategy of medicinal chemistry, known as isosteric or bio-isosteric replacement.[1-3] In general, for this approach to be successful, similarities must exist between at least some of the properties of the isostere (we use the term ”isostere” in the broadest sense to include both classical and non-classical isosteres, as well as bio-isosteres) and those of the fragment being replaced, such that the new analogs retain the biological activities of the parent compound. At the same time, however, the isosteric replacement must produce changes in the physicochemical properties or susceptibility to metabolism compared to the parent compound in order to lead to improved derivatives. The success of any isosteric replacement is invariably context dependent, however, and depends on the particular molecular environment of the biological target (*e.g.,* the ability of the target to accommodate surrogate structures), and whether those surrogate structures will also provide the desired improvements in properties. For this reason, a screening of a series of alternative structures is almost always necessary.[4] In this situation, the availability of experimental data detailing the structure-property relationship (SPR) of the existing palette of isosteres is most desirable. However, when these data are not available, the selection/prioritization of potential replacements is typically based on a variety of factors, such as the historical success rate of specific isosteres, calculated physicochemical properties, chemical/medicinal chemistry intuition, as well as synthetic accessibility.

The importance of the carboxylic acid functional group in drug design is illustrated by the fact that >450 marketed drugs are carboxylic acid containing molecules.[5] However, the presence of this functional group in a drug or a drug candidate can be responsible for undesired consequences, such as limited permeability across biological membranes, metabolic instability, and potential idiosyncratic toxicities. To circumvent one or more of these shortcomings, medicinal chemists typically resort to prodrug (*e.g.,* ester prodrugs) or isosteric replacement strategies. As part of our continued interest in the area of isosteric replacements of the carboxylic acid functional group,[4, 6-9] we set out to define the SPR of a number of acidic moieties that are frequently used as replacements of the carboxylic acid in drug design. In this particular context, the most important physicochemical parameters are arguably the acidity and lipophilicity, as well as the effect that the isosteric replacements may have on compound permeability. Since these three parameters are inter-related, even partial/incomplete information [*e.g.,* just pK_a or distribution coefficient ($logD_{7.4}$) value] can be valuable in anticipating the physiochemical outcome of an isosteric replacement. However, a systematic SPR study enabling an accurate ranking of specific properties of carboxylic acid isosteres would provide a rational basis on which design decisions could be made. Somewhat surprisingly, thus far, such a study has never been reported. Although comparison of experimentally determined physicochemical properties of a number of compounds bearing carboxylic acid isosteres to the parent acid are available in the literature,[4] these data are often incomplete or fragmented. Furthermore, these data sets are not readily comparable given that different isosteres are frequently found in completely unrelated molecules and often different methods are used to define the experimental values. Thus, to enable a more rigorous side-by-side comparison, we have assembled and characterized a library of 35 model compounds that are derivatives of phenylpropionic acid. Each compound was then evaluated experimentally for water solubility, acidity, lipophilicity, and passive permeability in a Parallel Artificial Membrane Permeability Assay (PAMPA). The effect of each of the carboxylic acid replacements on plasma protein binding was also evaluated. The data generated in these studies permit an assessment of the SPR of the most commonly incorporated carboxylic acid isosteres, which may be useful in the selection and prioritization of potential replacements of the carboxylic acid moiety to be used in analog design.

Results.

*Library Design***.** The design of the library scaffold took multiple factors into consideration. To ensure that the physicochemical properties of each member of the compound library were an accurate representation as possible of the properties of the corresponding carboxylic acid isostere, it was desirable that each model compound be only minimally functionalized. At the same time, given that the assays employed to determine the physicochemical properties would rely upon either UV- or mass spectrometry-based detection methods, the model compounds should both contain a chromophore and exhibit sufficient molecular weight (*i.e.*, MW ≥ 150 Da). Finally, a short aliphatic spacer separating the chromophore from the acidic moiety was viewed as desirable to avoid possible interference caused by conjugation that might affect the intrinsic physicochemical properties of the carboxylic acid surrogate. Based on these considerations, phenylpropionic acid (**1**, Table 1) was selected as the reference compound and each of the compounds in the library was designed as an analog thereof. In addition to the technical reasons mentioned above, the phenylpropionic acid is also an attractive template as this substructure is found in a wide range of biologically active compounds, thus making the compound library directly relevant to medicinal chemistry.

The library of model compounds (36 entries) shown in Table 1 include examples from 24 different classes of carboxylic acid isosteres for which there is evidence of successful applications in drug design.[4] These examples were chosen to represent as wide a range of carboxylic acid surrogates as possible with respect to structure and properties. They include non-carbon acyclic acids such as phosphonic and sulfonic acids and sulfonamides, modified carbon-based acids such as hydroxamic acid and acylurea, heterocycles like tetrazole and thiazolidinedione and carbacycles such as phenols and squaric acid derivatives. For some of the classes of carboxylic acid isosteres that present multiple, non-equivalent points of attachment or derivatization (*e.g.*, sulfonamides, acyl sulfonamides), a minimal set of representative congeners was prepared recognizing, however, that the physicochemical properties of these particular classes of isosteres could be effectively modulated by varying the nature of the substituents. Compounds **1**, **6**, **8**, and **10** were commercially available, while all other analogs were synthesized based on literature precedent (see Experimental section). In addition to the structures being established by standard methods (^{1}H- and ^{13}C-NMR, IR, HRMS), X-ray structures of compounds **1–3**, **5**, **6**, **8**, **10–20**, **22**, **26**, **28**, **32**, **35**, **36** were also obtained (see Supporting Information).

Determination of Physicochemical Properties. The compound library was initially evaluated for kinetic solubility in aqueous buffer (pH 7.4). These results, summarized in Table 1, reveal that the vast majority of compounds exhibit aqueous solubility that is either comparable or greater than that of the parent acid (**1**). The only exceptions were compounds **21** (which is significantly less soluble than **1**) and **34**, for which a determination of aqueous solubility was not possible due to the fact that this compound was not detected after the 24 h of incubation in aqueous buffer. With the knowledge that solubility would not limit the subsequent property evaluation of the remaining members, each test compound was evaluated for: (*a*) lipophilicity, by determining the distribution coefficient between *n*-octanol and aqueous buffer at pH 7.4 (*i.e.*, $logD_{7.4}$) via shake-flask method; (*b*) acidity, by determining the pK_a values via capillary electrophoresis; (*c*) permeability, using a PAMPA assay; and (*d*) plasma protein binding, by determining the fraction unbound (*fu*) via equilibrium dialysis. Calculated $logD_{7.4}$, and pK_a values were also obtained for comparison employing ChemAxon.[10] Table 1 assembles the accumulated data from these studies.

To highlight how the properties of each carboxylic acid isostere change in comparison to acid **1**, Figures 1 and 2 illustrate the distribution of each of the measured values relative to **1**. As seen from Figure 1, the replacement of the carboxylic acid with these surrogate structures can lead to a considerable variation of physicochemical properties relative to the reference compound **1**: pK_a values span over approximately 10 pK_a units (2–12), with most isosteres being either equally acidic or less acidic than the parent acid. Only a handful of acidic moieties (*e.g.*, non-carbon acids **6–9**, as well as squaric acid derivatives **30** and **31**) had measured pK_a values below 4. The modulation of the pK_a of a target molecule is often an important aspect in compound optimization. Influence of pK_a on compound permeability (*vide infra*) as well as binding to the pharmacological target can be profound, therefore a ranking of carboxylic acid isosteres based on their intrinsic acidity provides a starting point for prioritizing which moieties are most likely to impart pK_a values within the desired range. In addition, these data could be helpful in determining whether neighboring group substitution may be required to modulate the pK_a values of specific isosteres of interest if the intrinsic acidity falls outside of the optimal range.

In contrast to the wide range of pK_a values, lipophilicity (*i.e.*, $logD_{7.4}$) and permeability coefficient (P_{app}) values in the PAMPA assay appear to be more narrowly distributed within approximately ~3 log units, with a near equal number of isosteres leading to a relative increase and a decrease in lipophilicity and permeability. Examples of isosteres with $logD_{7.4}$ most close to the acid include tetrazole **16**, oxazolidinedione **18**, oxadiazol-5(4*H*)-thione **22**, tetramic acid **24** and cyclopentane-1,3-diones **25–27.** Permeability coefficients (P_{app}) were obtained for 33 of the 36 test compounds. The data for the oxathiadiazole **21**, and the fluorophenols derivatives **32** and **33** were considered unreliable due to high non-specific binding (**32**) or compound precipitation (**21** and **33**) on the donor plate during the assay (Table 1). Carboxylic acid isosteres with relatively high membrane permeability in the PAMPA assay (*i.e.,* log $P_{app} > -5.8$) included acylurea **15**, sulfonamide **11**, thiazolidinedione **17**, thiadiazol-5(4*H*)-one **20**, cyclopentane-1,2-diones **28** and **29** and substituted phenol **35**.

Similar to the results of pK_a determination, the effect of isosteric replacements on plasma protein binding was pronounced (Figure 2). Some derivatives displayed very high fraction unbound (*fu*) values including the acylurea **15** (77%), hydroxamic esters **4** and **5** (68%, and 64%, respectively) and sulfonamide **10** (61%), while others [oxadiazol-5(4*H*)-thione **22**, isoxazole **23**, and substituted phenols **32** & **33**] were virtually completely bound (*i.e., fu* < 1%).

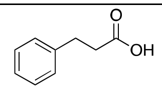
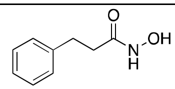
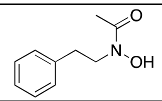
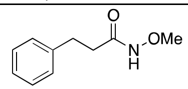
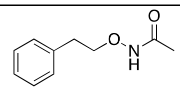
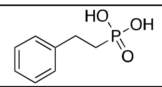
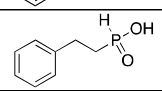
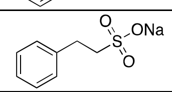
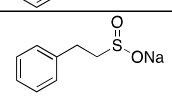
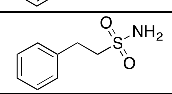
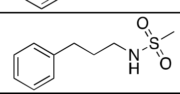
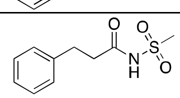
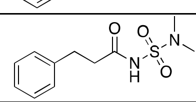
Since many medicinal chemistry optimization projects rely on computational chemistry programs to predict specific properties to prioritize compounds for synthesis, we employed programs in ChemAxon to calculate $logD_{7.4}$ and pK_a values for all analogs. From the data depicted in Table 1, a comparison of experimental and calculated $logD_{7.4}$ or pK_a values reveals that the calculated values for the carboxylic acid and most isosteres appear to correlate well with experimental results; however, there are notable exceptions in which the discrepancy can be as large as 2 or 3 log unit (see Figure 4A/B and Figure 5A/B). This variance is especially notable for the oxadiazol-5(4*H*)-thione (**22**) and conjugated 1,3-dicarbonyl systems giving rise to vinylogous acids (*e.g.*, **24–27**, **30** and **31**). As such, caution should be exercised when employing computational chemistry programs to predict properties of these structural types.

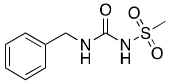
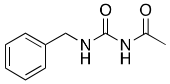
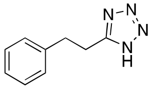
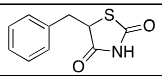
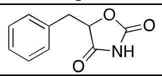
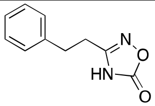
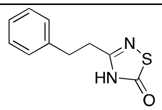
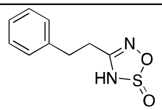
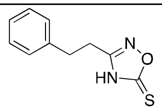
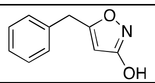
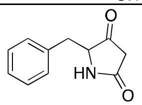
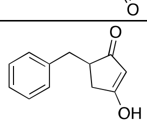
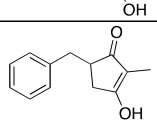
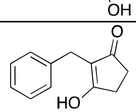
While the specific value for each of the individual properties is useful, more helpful are the correlations one can draw within the dataset. As stated above, most of the measured properties are inter-related and one property certainly influences the other. Although no overt correlation was evident between the *fu* and $logD_{7.4}$ values (Figure 2), overall there appears to be a relationship linking the ionization state of the acidic moiety with the *fu*. Thus, in most cases, the least acidic isosteres (*i.e.*, pK_a > 8), such as the hydroxamic acids (*i.e.,* **2** and **3**) and esters (*i.e.,* **4** and **5**), sulfonamides (*i.e.,* **10** and **11**), and acyl-urea (*i.e.,* **15**) were found to lead to higher *fu* values (> 29%) relative to the carboxylic acid and most other isosteres that are predominantly negatively charged at pH 7.4 (Table 1).

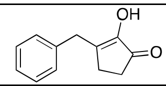
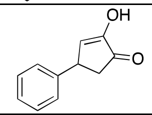
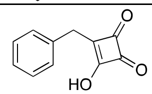
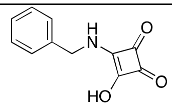
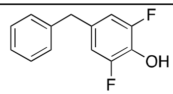
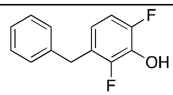
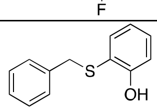
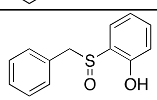
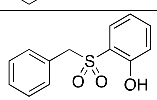
Evaluation of the data also revealed a reasonably good correlation between the lipophilicity and permeability data ($r^2=0.828$; Figure 1). However, of note, some compounds having very similar acidity and lipophilicity were found to exhibit significantly different permeability coefficients in the PAMPA assay. For example, comparison of the amino squaric acid (**31**) with the phosphinic acid (**7**) derivative suggests that the latter may be significantly less permeable than the former, in spite of comparable acidity and lipophilicity (Table 1 and Figure 1). Relatively large differences in permeability coefficients between compounds that are essentially isometric with respect to lipophilicity and acidity are also observed when comparing carboxylic acid **1** with other isosteres, such as the cyclopentane-1,3-dione (**25–27**), or the oxadiazol-5(4*H*)-thione (**20**), or the tetrazole (**16**). In particular, the difference in permeability between **1** and **16** was confirmed in three independent experiments ($p < 0.01$).

This observation indicated that, in addition to acidity and lipophilicity, other factors can influence compound permeability in the PAMPA assay. One possibility is that the de-solvation energy may be significantly different with different acidic moieties, even in those cases where acidity and lipophilicity are closely comparable. Since the solvation/de-solvation energy is determined in large part by hydrogen-bond (HB) interactions, we conducted further studies to compare specifically the HB capacity of tetrazole **16** and carboxylic acid **1**. For this endeavor, we employed a colorimetric assay[11] in which the H-bonding between a fluorescent pyrazinone sensor (HB acceptor) and the analyte HB donor (*i.e.,* **1** or **16**) was monitored by following the characteristic blue-shift of the maximum wavelength (λ_{max}) of the sensor in the UV spectrum (Figure 3). Given that the change in λ_{max} of the sensor depends on the strength of HB interaction with the analyte, stronger HB donors will cause comparatively larger shifts than weaker HB donors. Interestingly, these titration experiments revealed that the tetrazole derivative engages in significantly stronger HB interactions than the corresponding carboxylic acid (Figure 3). These results suggest that if similar differences exist in the interaction with water, then a tighter solvation and correspondingly higher de-solvation energy would be expected for **16** compared to **1**, leading to less permeability in a PAMPA assay. Further evaluation of this possibility will require, however, additional studies to compare **16** and **1** in the deprotonated forms (*i.e.*, tetrazolate and carboxylate) as HB acceptors.

Table 1.

Class	*Cpd #*	*Structure*	*Aq. Solub.*[a] *(μM)*	*$logD_{7.c}$*[c]	*$logD_{7.4}$ calc*[b]	*PAMPA*			*pK_a*[g]	*pK_a calc.*[b]	*PPB (% fu)*[h]
						Pe (cm/s)[d]	*% retention*[e]	*log_{Papp}*[f]			
Carboxylic acid	1[*]		110.69 ± 3.04	–0.49 ± 0.19	–0.56	1.66E-06 ± 3.48E-7	–6.8 ± 11	–5.79 ± 0.10	4.64	4.73	9.5 ± 0.4
Hydroxamic acids	2[*]		≥ 200	0.71	1.23	4.97E-06	0.03	–5.30	8.18	8.90	29 ± 2
	3[*]		≥ 200	1.52	1.16	4.53E-06	1.1	–5.34	8.83	8.37	37 ± 10
Hydroxamic esters	4		≥ 200	1.16	1.59	7.28E-06	5.3	–5.14	9.47	8.45	68 ± 3
	5[*]		199.80 ± 0.49	1.18	1.35	4.60E-06	–2.9	–5.34	9.58	8.88	64 ± 3
Phosphonic acid	6[*]		152.36 ± 1.18	–1.14	–1.54	9.40E-08	–2.7	–7.03	2.34 (8.49)	1.81	31 ± 5
Phosphinic acid	7		127.73 ± 1.96	–1.44	–1.36	1.70E-08	–2.1	–7.77	1.98	2.24	8.90 ± 0.06
Sulfonic acid	8[*]		≥ 200	–1.45	–1.17	3.84E-08	–6.1	–7.42	<2.0	–0.81	0.31 ± 0.08
Sulfinic acid	9		≥ 200	–1.30	–0.84	ND[†]	–6.8	ND[†]	2.1	2.00	5.0 ± 0.7
Sulfonamides	10[*]		≥ 200	0.96	0.63	2.13E-06	4.2	–5.67	10.04	11.38	61 ± 0.04
	11[*]		≥ 200	1.42	1.15	1.05E-05	8.1	–4.98	>12	12.06	37 ± 0.06
Acyl-sulfonamides	12[*]		199.70 ± 0.30	–1.02	–0.21	3.45E-07	1.4	–6.46	4.94	4.08	12.8 ± 0.2
	13[*]		199.03 ± 1.24	0.17	–0.22	1.53E-06	2.2	–5.81	5.86	4.12	8.1 ± 0.2

Sulfonylureas	14[*]		197.76 ± 2.24	–1.23 ± 0.06	–0.87	2.61E-07 ± 1.01E-7	–3.0 ± 5.39	–6.61 ± 0.20	5.04	4.14	31 ± 2
Acylurea	15[*]		≥ 200	1.42	0.57	1.63E-05	–3.3	–4.79	>12	11.77	77 ± 1
Tetrazole	16[*]		≥ 200	–0.25 ± 0.10	0.10	4.83E-07 ± 1.48E-7	4.7 ± 2.8	–6.33 ± 0.15	5.09	5.08	1.1 ± 0.12
Thiazolidine diones	17[*]		200.41 ± 0.41	1.07 ± 0.03	1.12	8.76E-06 ± 1.06E-6	5.5 ± 1.7	–5.06 ± 0.06	6.19	6.61	3.4 ± 0.11
Oxazolidine diones	18[*]		≥ 200	–0.16	0.70	2.46E-06	–0.6	–5.61	5.86	6.63	14 ± 1
Oxadiazol-5(4*H*)-one	19[*]		≥ 200	0.32	1.26	1.22E-06	–4.0	–5.91	5.73	6.04	1.1 ± 0.12
Thiadiazol-5(4*H*)-one	20[*]		200.47 ± 0.47	1.66	2.18	1.14E-05	–0.7	–4.94	6.50	7.19	1.2 ± 0.45
Oxathiadiazole-2-oxide	21		7.13 ± 0.74	ND	0.95	1.10E-07[‡]	–1432	–6.96[‡]	5.23	6.41	ND
Oxadiazol-5(4*H*)-thione	22[*]		≥ 200	–0.25	2.84	3.27E-07	–3.4	–6.49	3.58	7.77	0.65 ± 0.16
Isoxazole	23		≥ 200	0.46	1.34	4.65E-06	–11	–5.33	5.36	6.21	0.10 ± 0.10
Tetramic acid	24		≥ 200	–0.35	1.34	2.50E-06	1.3	–5.60	6.08	10.54	ND
Cyclopentane 1,3-diones	25		194.93 ±1.01	–0.70	2.32	2.12E-07	–3.0	–6.67	4.01	8.82	8.00 ± 0.35
	26[*]		≥ 200	–0.33	2.71	2.60E-07	–5.1	–6.58	4.47	8.72	11 ± 0.14
	27		199.04 ± 0.76	–0.60	2.16	1.54E-07	–8.8	–6.81	4.44	8.65	14 ± 0.6

Cyclopentane 1,2-diones	28*		195.03 ± 1.75	1.85	2.34	1.14E-05	1.5	–4.94	8.88	9.24	16 ± 4
Cyclopentane 1,2-diones	29		192.44 ± 4.06	1.71	1.94	1.70E-05	–8.6	–4.77	8.28	9.37	ND
Squaric acid derivatives	30		165.79 ± 0.06	–0.84	1.36	ND†	–13	ND†	<2.0	6.56	8.20 ± 0.18
	31		≥ 200	–1.40	1.18	2.24E-07	–6.6	–6.65	<2.0	7.97	6.4 ± 0.34
Substituted phenols	32*		109.17 ± 1.35	3.34	3.88	7.05E-06	49#	–5.15	7.19	7.74	0.26 ± 0.08
	33		148.70 ± 3.91	3.56	3.85	4.71E-06	–32	–5.33‡	7.05	7.62	0.52 ± 0.21
	34		ND§	>3.78	3.91	ND	ND	ND	9.06	9.18	ND
	35*		≥ 200	2.16	2.55	1.23E-05	1.1	–4.91	7.70	7.25	12 ± 3
	36*		197.11 ± 0.29	1.76	2.31	1.57E-05	7.2	–4.80	7.12	6.74	1.60 ± 0.03

[a] Kinetic solubility in aqueous phosphate buffer (pH 7.4) determined by LC/MS after 24 h of incubation (experiment run by Analyza); [b] Calculated values using ChemAxon[10]; [c] Distribution coefficient between *n*-octanol and aqueous buffer (pH 7.4) determined by LC/MS (experiment run by WuXi AppTech); [d] Effective permeability (PAMPA assay run by Analyza); [e] Membrane retention; [f] Log of the apparent permeability coefficient; [g] Pk_a values determined by capillary electrophoresis, for diprotic compounds the second equivalence point is indicated in brackets (experiment run by Analyza); [h] Plasma protein binding, fraction unbound (*fu*) determined by equilibrium dialysis; * X-ray crystal structure revealing H-bond pattern is shown in the Supporting Information; † Permeability value could not be calculated as the concentration of test compound in the acceptor plate was below limit of quantitation (<LOQ); ‡ Compound precipitated in donor plate; # Compound appeared to exhibit high non-specific binding; § Compound appeared to be unstable during the 24h incubation time of the assay; ND = not determined.

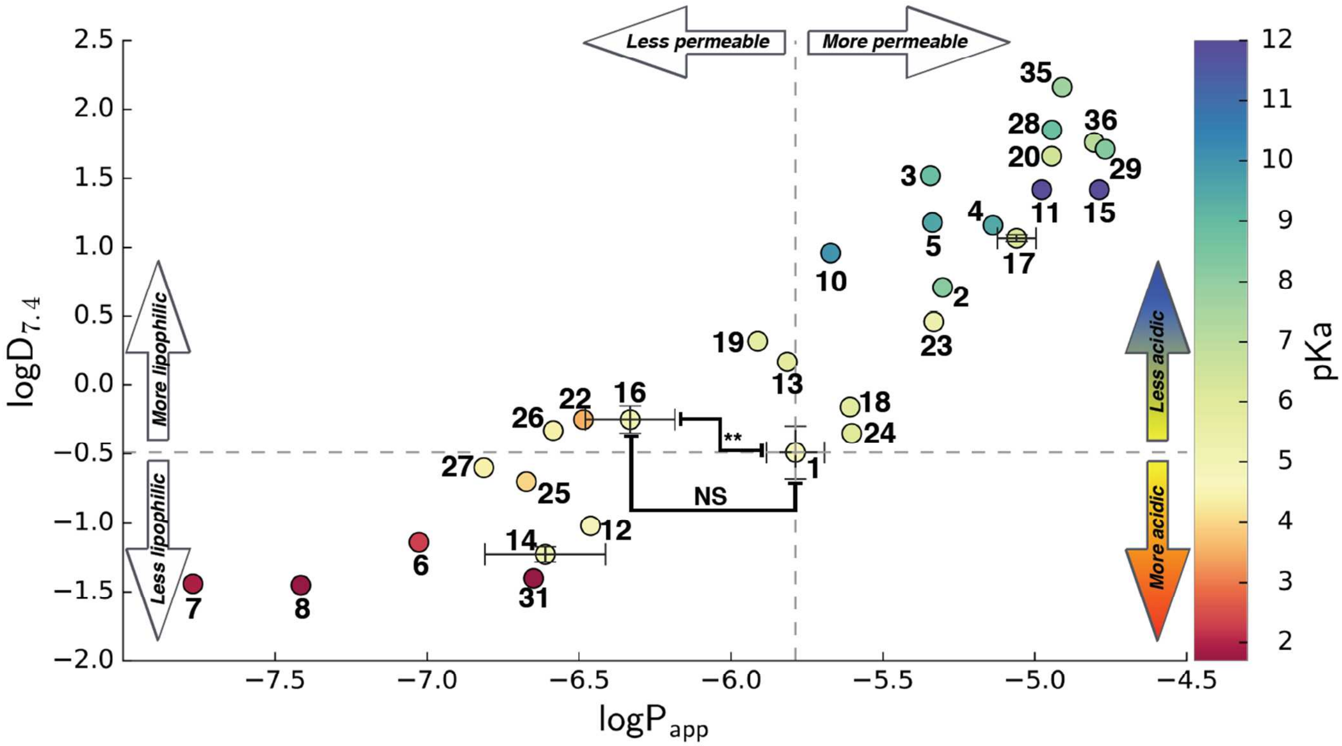


Figure 1. Plot showing lipophilicity (*i.e.,* log$D_{7.4}$), acidity (*i.e.*, pK_a), and permeability (*i.e.*, logP_{app}) of test compounds, relative to the carboxylic acid compound **1**; log$D_{7.4}$ and logP_{app} values for compounds **1**, **16**, **14** and **17** are the averages obtained from three independent experiments; (**) $p < 0.01$ by two-tailed t-test confirming statistical significant difference in membrane permeability between **16** and **1**; NS indicates that the difference in log$D_{7.4}$ values between **16** and **1** does not reach statistical significance.

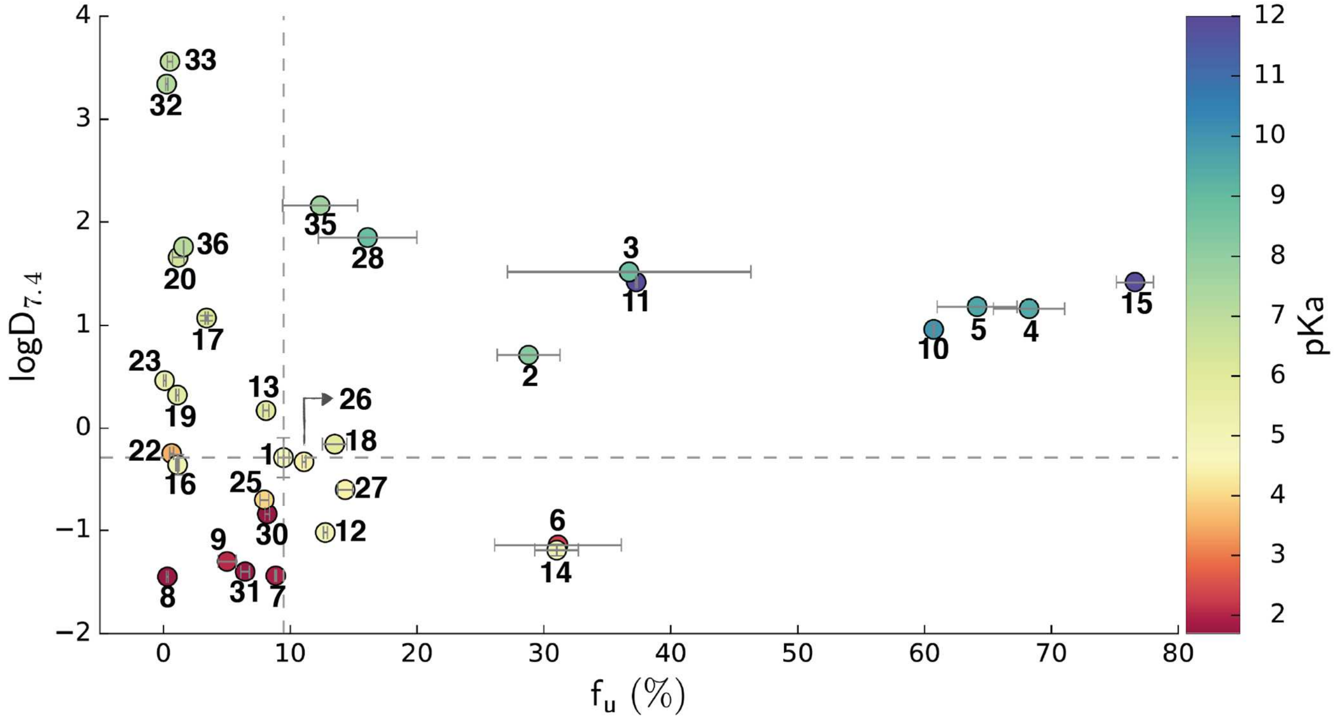


Figure 2. A plot of compound lipophilicity (*i.e.*, $\log D_{7.4}$), plasma protein binding (*i.e.*, *fu*), and acidity (*i.e.*, pK_a) relative to the carboxylic acid compound **1**; *fu* values are the averages obtained from three independent experiments; $\log D_{7.4}$ values for compounds **1**, **16**, **14** and **17** are the averages obtained from three independent experiments.

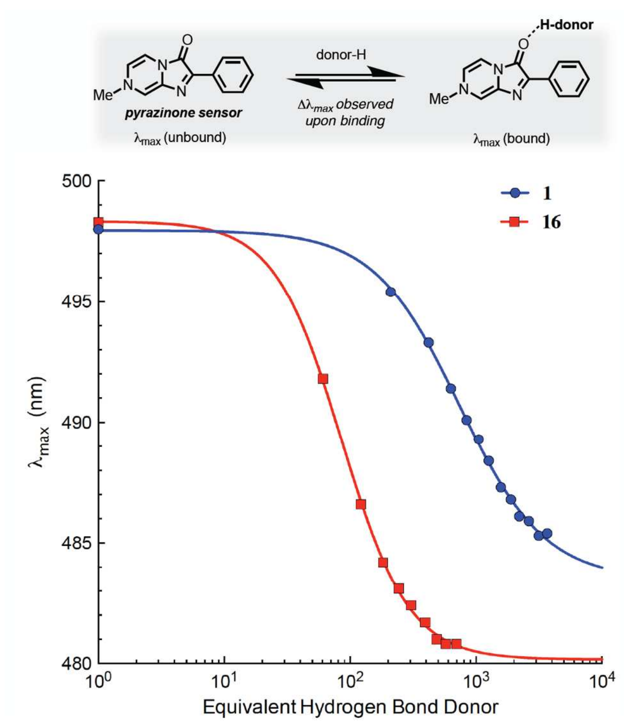


Figure 3. Titration curves from a colorimetric assay that monitors the blue-shift in λ_{max} of the pyrazinone sensor upon complexation with the HBD analyte (**1** or **16**).

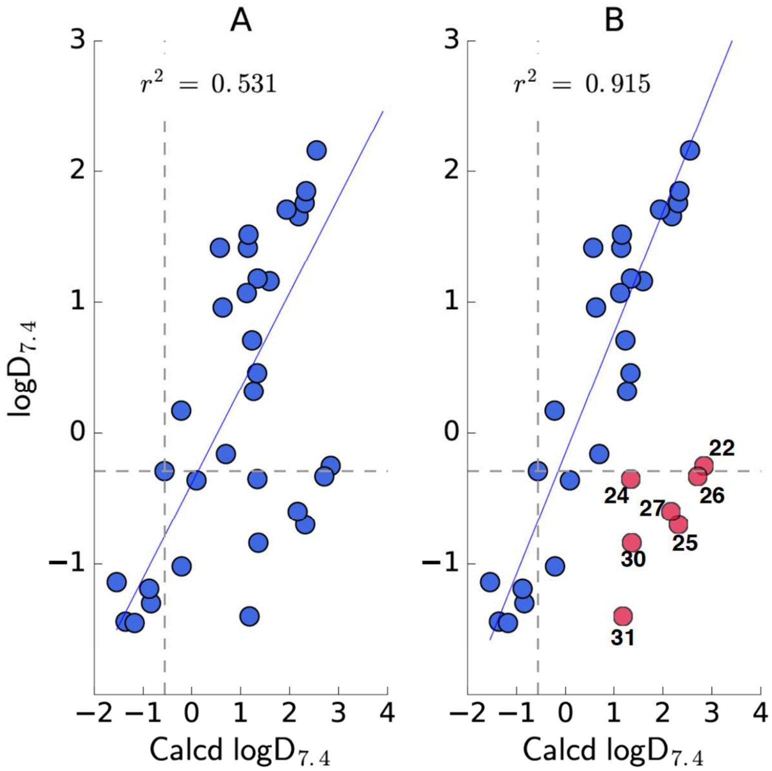


Figure 4. Comparison between experimental and calculated logD$_{7.4}$ values; (*A*) the linear regression for the entire data set; (*B*) the linear regression when major outliers (*i.e.*, discrepancy > 1 log unit, shown in red) are excluded.

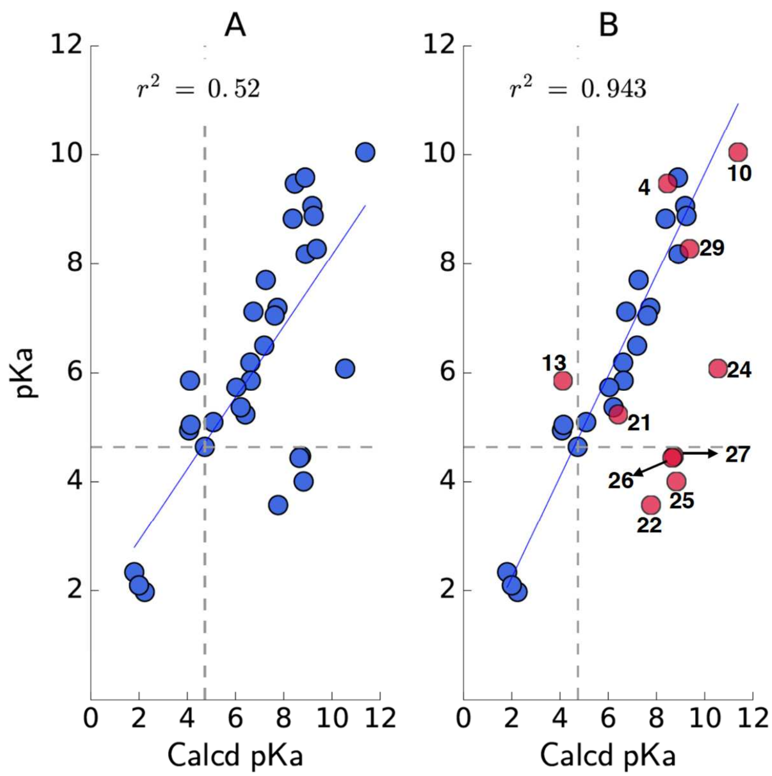


Figure 5. Comparison between experimental and calculated pK_a values; (*A*) the linear regression for the entire data set; (*B*) the linear regression when major outliers (*i.e.*, discrepancy > 1 log unit, shown in red) are excluded.

Discussion. The carboxylic acid is one of the most frequent functional groups in small molecules that bind to protein targets, and is considered a privileged sub-structure.[12] The acidity, combined with the ability to establish relatively strong electrostatic interactions and hydrogen-bonds, as well as the ability to participate in interactions with dipoles make this functional group highly versatile, and in turn able to engage in molecular interactions with a wide array of complementary functional groups. These properties also imply that the carboxylic acid moiety can impart relatively high water solubility, which is an important attribute for a drug-like molecule. On occasion, these same properties can also contribute to some of the deficits of carboxylic acids as drug candidates, including, for example, a relatively poor permeability across biological membranes. In addition, other potential liabilities that have been associated with the carboxylic acid functional group in drugs or drug candidates include a relatively rapid metabolism mediated by uridine 5'-diphospho-glucuronosyl-transferase (UGTs).[13] This phase 2 metabolic process can be responsible for limited compound half-life and for the formation of reactive acyl-glucuronides that can cause covalent modifications of proteins and potentially serious adverse side effects.[14] The replacement of the carboxylic acid with appropriate isosteres can be especially useful to circumvent these possible drawbacks while maintaining the positive aspects of the carboxylic acid functional group. Furthermore, in addition to these specific applications, carboxylic acid isosteres can be employed more broadly in analog design to explore the effects of diversification of structure and physicochemical properties of compounds of interest. The utility of this strategy is evident from the fact that several carboxylic acid isosteres, such as tetrazole, thiazolidinedione, sulfonamides, and acyl sulfonamides have ultimately led to the clinical development of entire classes of important therapeutic agents (*e.g.*, see Figure 6).

Based on the importance of acid isosteres to the field of medicinal chemistry and the critical role that these fragments can play in tuning the properties of acidic, biologically active compounds, we assembled a set of 35 carboxylic acid isosteres on the same scaffold and measured a focused set of key physicochemical properties, such as acidity, lipophilicity, and permeability. Furthermore, given that molecules possessing the carboxylic acid functionality are often found to exhibit high degree of plasma protein binding,[15, 16] the effect of these isosteric replacements on the fraction unbound was also investigated. Finally, using computational chemistry programs, we calculated pK_a and $logD_{7.4}$ values to compare to the experimental values. The dataset generated in this study provides an assessment of the effect that such isosteres can have on the physicochemical properties of the corresponding carboxylic acid. In addition, our results highlight some correlations between properties that can be used during the selection/prioritization of isosteres for analog design.

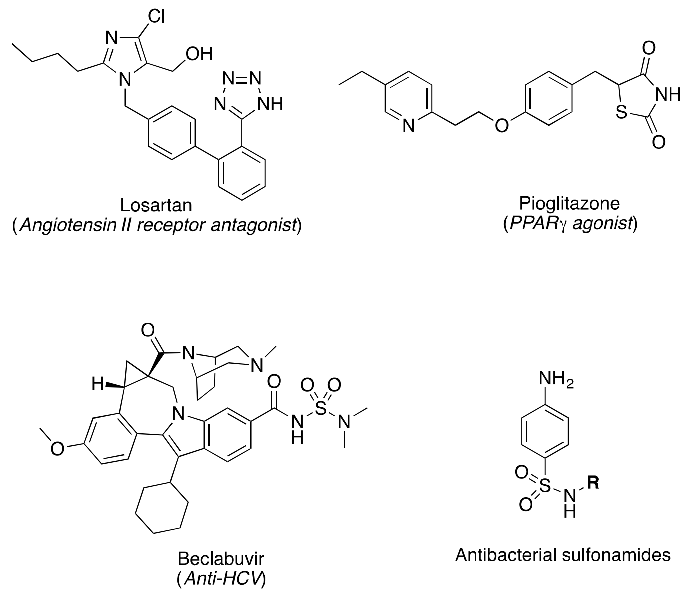


Figure 6. Representative examples of drugs/drug candidates containing carboxylic acid bioisosteres.

In agreement with previous studies,[17] our results indicate that overall there is a clear correlation between the experimental $\log D_{7.4}$ values and the apparent permeability coefficients in the PAMPA assay (Figure 1), such that, generally, more lipophilic, less acidic compounds exhibit higher rates of passive diffusion. However, in selected cases the impact that isosteric replacements produce on the compound permeability appears to deviate significantly from this relationship. These deviations are most effectively illustrated by the comparison between tetrazole **16** and carboxylic acid **1**. The tetrazole, which among the carboxylic acid isosteres is one that has been the focus of intense studies,[18, 19] is generally considered to be more lipophilic[20] and thus potentially more permeable than the carboxylic acid.[21] However, our comparative studies with model compounds reveal that whereas the two acidic moieties exhibit very similar pK_a and $\log D_{7.4}$ values, the tetrazole is in fact significantly less permeable in the PAMPA assay. Interestingly, previous Caco2 bi-directional permeability studies with a series of carboxylic acids and corresponding 1*H*-tetrazoles derivatives found that several tetrazoles were less permeable than the carboxylic acids due to active transport mechanisms.[22, 23] However, in our studies, given the artificial nature of the PAMPA assay, active transport systems, metabolism, and protein binding are excluded from consideration. Therefore, any differences in the rate of transport should ultimately result from specific differences in physicochemical properties between the acidic moieties. Our studies found that the model compound bearing the tetrazole moiety can establish considerably stronger HB interactions than the corresponding compound bearing the carboxylic acid. This result is consistent with previous reports in which other 1*H*-tetrazoles were found to be relatively strong HB donors,[24] and suggests that in water the tetrazole derivative may be more tightly solvated than the carboxylic acid leading to correspondingly higher de-solvation energies. As the de-solvation energy is known to affect compound permeability across biological membranes,[25, 26] the different rates of diffusion that have been observed for **16** and **1** in the PAMPA assay may be related, at least in part, to the difference in HB strength between the tetrazole and the carboxylic acid. In this regard, it should be noted that the lack of equivalence between the hydrogen-bonding and the acidity scale is not unexpected, as this phenomenon has been documented.[24, 27]

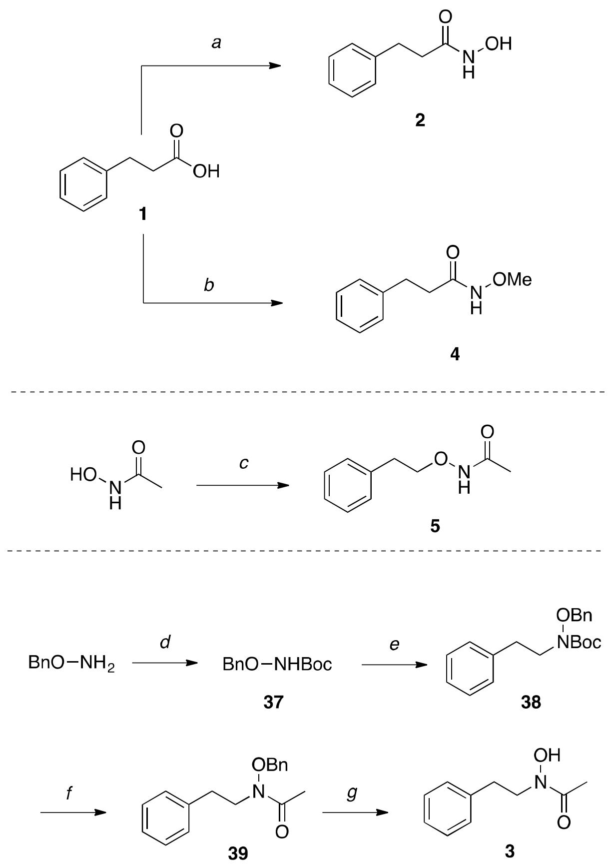
In addition to the differences in the permeability rates in the PAMPA assay, **16** and **1** were also found to exhibit significant differences in the plasma protein-binding assay with the tetrazole derivative being more tightly bound than the corresponding carboxylic acid compound. A similar observation was reported when comparing the plasma protein-binding of other match paired tetrazoles and carboxylic acid analogs.[28] Also consistent with previous reports,[15, 16] which illustrated the importance of the ionization state of molecules on plasma protein binding, a broader examination of the plasma protein-binding data from all carboxylic acid isosteres evaluated in this study suggests that derivatives that are predominantly neutral at pH 7.4 generally exhibit larger *fu* values. However, there seems to be no clear correlation between the plasma protein binding and the lipophilicity of test compounds. Although this outcome appears to be in disagreement with previous studies that reported a relatively good correlation between the calculated $\log D_{7.4}$ values and plasma protein binding of a series of carboxylic acid molecules,[29] it is possible that such a correlation can be established only within specific families of closely related compounds and not between different classes of carboxylic acid isosteres. Indeed when we compared the *fu* values of **1**, **25–27** with the corresponding, but more lipophilic congeners that were brominated in the *para* position, we found that within each of the matching pairs the *fu* was considerably lower for the brominated derivative (see Supporting Information).

Finally, the discrepancies between the calculated and the experimental $\log D_{7.4}$ and pK_a values that are evident for all vinylogous acids tested likely arise from these or similar vinylogous systems being absent or underrepresented in the training set used by the software. As such, the predictive ability of the software could be readily improved by simply updating the training set with the data provided in this study. Our results in general underscore the importance of experimental values and suggest that caution should be exercised when calculated physicochemical properties are used to prioritize carboxylic acid isosteres for analog design.

Conclusions. Taken together, the data generated in this study permit an assessment of the relative differences in physicochemical properties of carboxylic acid isosteres, compared to the corresponding carboxylic acid. This data set comprises a benchmark that may be useful to compare the physicochemical properties of carboxylic acid isosteres and ultimately permit more informed and effective prioritizations of isosteric replacements in medicinal chemistry for analog design.

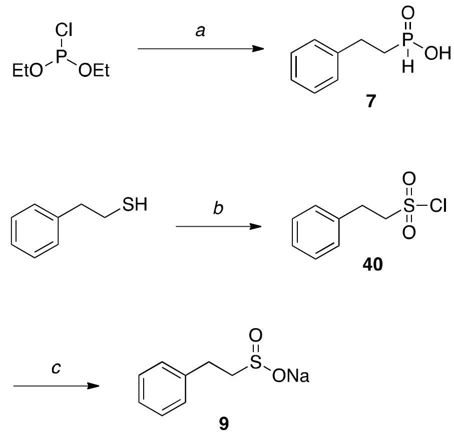
Experimental Section

The synthesis of test compounds is highlighted in Schemes 1–11. The hydroxamic acids **2**[30] and **3**[31] (Scheme 1) were prepared starting respectively from carboxylic acid **1** and *O*-benzylhydroxylamine.[32] Hydroxamic ester derivative **4**[33] was obtained via 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) mediated coupling of **1** with *O*-methyl hydroxylamine, whereas reverse hydroxamic ester **5**[34] was obtained by *O*-alkylating acetohydroxamic acid, following procedures described by Wrigglesworth and co-workers.[35]



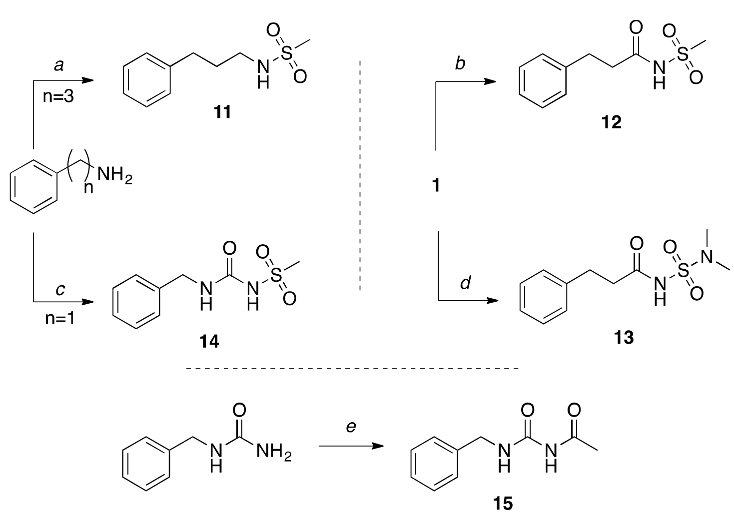
Scheme 1. Synthesis of hydroxamic acid and ester derivatives. *Reagents and Reaction conditions:* (a) CDI, hydroxylamine, CH_3CN, r.t., 14 h, (70%); (b) *N*-methylmorpholine, EDCI•HCl, *O*-methyl hydroxylamine, r.t., 16 h, (83%); (c) *(i)* K_2CO_3, Na_2CO_3, MeOH/H_2O, 50 ˚C, 10 min; *(ii)* (2-bromoethyl)benzene, 50 ˚C, 16 h, (32%); (d) Boc_2O, THF/H_2O, NEt_3, r.t., 2 h, (45%); (e) *(i)* NaH, DMF, r.t., 30 min; *(ii)* (2-bromoethyl)benzene, r.t., 16 h, (53%); (f) *(i)* TFA/CH_2Cl_2, r.t., 18 h, (85%); *(ii)* Ac_2O, DMAP, pyridine, CH_2Cl_2, r.t., 2 h, (79%); (g) H_2 (1 atm), Pd/C, MeOH, r.t., 3 h, (77%).

With respect to phosphinic (**7**[36]) and sulfinic acid (**9**[37]) derivatives (Scheme 2), the former was obtained by reacting triethylphosphite with phenethylmagnesium bromide, while the latter was prepared in two steps starting from 2-phenylethanethiol, via an oxidation-reduction sequence.



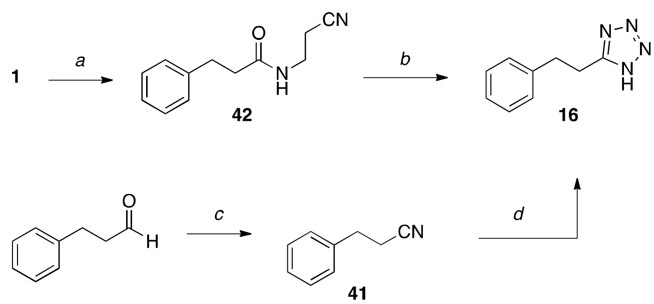
Scheme 2. Synthesis of phosphinic and sulfinic acid derivatives. *Reagents and Reaction conditions:* (a) Phenethylmagnesium bromide, Et_2O, 5 ˚C to r.t., 16 h, (32%); (b) NCS, CH_2Cl_2/H_2O, r.t., 3.5 h, (90%); (c) Na_2SO_3, $NaHCO_3$, CH_3CN/H_2O, r.t., 24 h, (75%).

The model compounds bearing the sulfonamide (**11**[38]) and acylurea (**15**[39]) functional groups were synthesized starting respectively from 3-phenylpropan-1-amine and benzyl urea as previously described (Scheme 3). The acylsulfonamide derivatives **12** and **13** (Scheme 3) were obtained by coupling **1** with the appropriate sulfonamides, whereas sulfonylurea **14** was obtained by reacting methylsulfonamide with benzyl-carbamoylimidazole, which can be readily prepared from benzylamine hydrochloride and 1,1'-carbonyldiimidazole (CDI).[40]



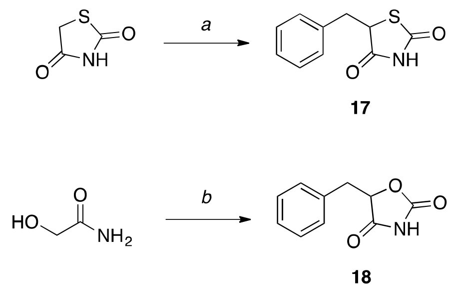
Scheme 3. Synthesis of sulfonamide, acylsulfonamide, sulfonylurea, and acylurea derivatives. *Reagents and Reaction conditions:* (a) MsCl, NEt_3, CH_2Cl_2, 4 ˚C, 24 h, (88%); (b) methanesulfonamide, EDCI•HCl, DMAP, CH_2Cl_2, 45 ˚C, 8 h, then r.t., 48 h, (70%); (c) *(i)* 4 M HCl in dioxane, r.t., 5 min; *(ii)* CDI, DMF, CH_3CN, r.t., 2 h; *(iii)* NaH, methanesulfonamide, DMF, r.t., 16 h, (11%); (d) *(i)* TBTU, DIEA, CH_2Cl_2, r.t., 25 min; *(ii) N*,*N*-dimethylsulfamide, r.t., 14 h, (81%); (e) Ac_2O, CH_2Cl_2, 50˚C, 24 h, then r.t., 48 h, (75%).

The tetrazole derivative **16**[41] (Scheme 4) was prepared from nitrile **41** and sodium azide,[42] or alternatively, from the cyanoalkylamide **42** and TMS-azide, under Mitsunobu conditions, followed by elimination of the acrylonitrile.[43]



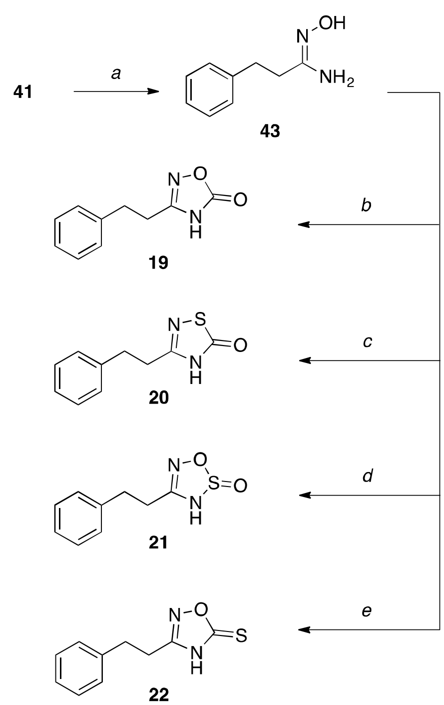
Scheme 4. Synthesis of tetrazole derivative. *Reagents and Reaction conditions:* (a) EDCI•HCl, HOBt, DMF, DIEA, 3-aminopropionitrile, r.t., 15 h, (95%); (b) *(i)* PPh_3, DIAD, $TMSN_3$, 0 ˚C, 30 min, then r.t., 2 h, then 50 ˚C, 17 h; *(ii)* DBU, CH_2Cl_2, r.t., 6 h, (63%); (c) NH_2OH•HCl, DMSO, 90 ˚C, 3.5 h, (61%); (d) NaN_3, $ZnBr_2$, H_2O, 150 ˚C, 24 h, (20%).

In the case of thiazolidine-2,4-dione (**17**[44]) and oxazolidine-2,4-dione (**18**) derivatives, these model compounds were obtained by reacting the appropriately lithiated heterocycle with benzyl bromide (Scheme 5).



Scheme 5. Synthesis of thiazolidine-2,4-dione and oxazolidine-2,4-dione derivatives. *Reagents and Reaction conditions:* (a) *(i) n*-BuLi, THF, –78 ˚C, 10 min *(ii)* benzyl bromide, –78 ˚C to r.t, 1.5 h, (53%); (b) *(i) t*-BuOK, diethyl carbonate, MeOH, 70 ˚C (microwave irradiation), 19 h, then r.t., 24 h; *(ii) n*-BuLi, THF, –78 °C to 0 ˚C, 30 min; *(iii)* benzyl bromide, –78 ˚C to r.t., 2 h, (5%).

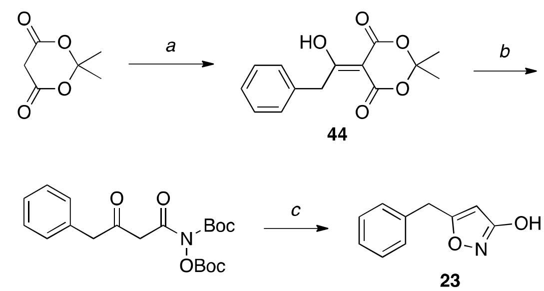
For the synthesis of the oxadiazole derivatives (**19** and **22**) as well as related thiadiazole (**20**) and oxathiadiazole (**21**) compounds (Scheme 6), common precursor *N'*-hydroxy-3-phenylpropanimidamide (**43**) was reacted with the appropriate acylating agent according to conditions reported by Kohara and co-workers.[45]



Scheme 6. Synthesis of 5-oxo-1,2,4-oxadiazole and related thiadiazole derivatives. *Reagents and Reaction conditions:* (a) NH_2OH, H_2O, EtOH, 75 ˚C, 5.5 h, (92%); (b) *(i)* isobutyl chloroformate,

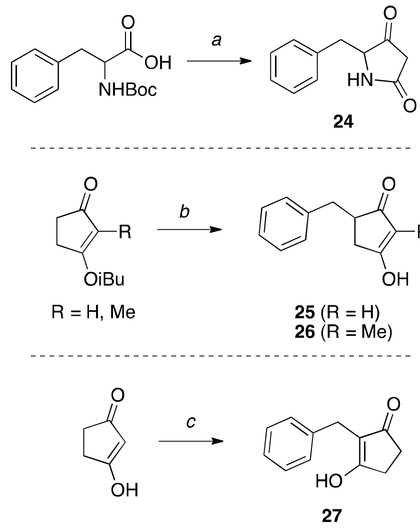
pyridine, DMF, 0 ˚C to r.t., 16 h; *(ii)* toluene, 120 ˚C, 2 h, then 140 ˚C, 24 h, (59%); (c) *(i)* 1,1'-thiocarbonyldiimidazole, THF, r.t., 40 min; *(ii)* BF_3•OEt_2, THF, r.t., 3 h, (28%); (d) $SOCl_2$, THF, CH_2Cl_2, 0 ˚C, 1 h, (<10%); (e) DBU, 1,1'-thiocarbonyldiimidazole, CH_3CN, r.t., 24 h, (43%).

The synthesis of known 3-hydroxyisoxazol **23**[46] was conducted starting from Meldrum's acid as highlighted in Scheme 7.



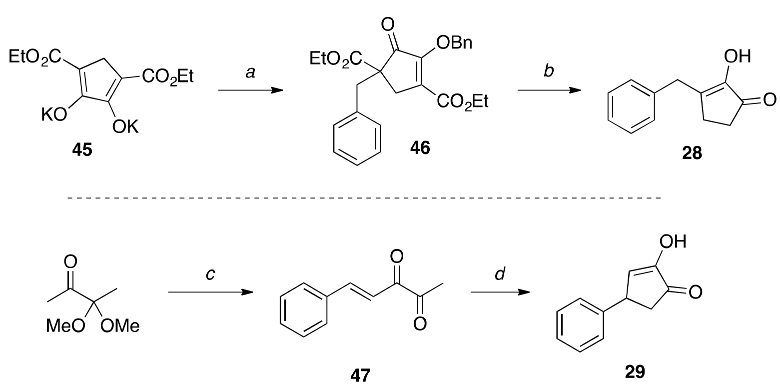
Scheme 7. Synthesis of isoxazol-3-ol derivative. *Reagents and Reaction conditions:* (a) *(i)* pyridine, CH_2Cl_2, 0 ˚C, 10 min; *(ii)* phenylacetyl chloride, 0 ˚C, 4 h, (91%); (b) *N,O*-di-Boc-hydroxylamine, toluene, 65 ˚C, 16 h, (quant.); (c) 4 M HCl, MeOH, r.t., 16 h, (10%).

The synthesis of a tetramic acid derivative (**24**[47]) was accomplished according to known procedures in three steps, starting from *N*-Boc protected phenylalanine and Meldrum's acid, while structurally related cyclopentane-1,3-diones (**25** and **26**) were obtained via chemoselective benzylation of the appropriate isobutyl-protected cyclopentane-1,3-dione with benzyl bromide under conditions reported by Koreeda and co-workers,[48] followed by hydrolysis of the vinylogous ester. The 1,3-dione derivative **27** was prepared in one step from cyclopentane-1,3-dione and benzaldehyde under reductive alkylation conditions.[49]



Scheme 8. Synthesis of tetramic acid and cyclopentane-1,3-dione derivatives. *Reagents and Reaction conditions:* (a) *(i)* Meldrum's acid, DMAP, CH_2Cl_2, EDCI•HCl, 0 ˚C to r.t., 15 h; *(ii)* EtOAc, reflux, 30 min; *(iii)* TFA/CH_2Cl_2, r.t., 15 min, (91%); (b) *(i)* LDA, THF, –78 ˚C, 20 min; *(ii)* benzyl bromide, –78 ˚C to r.t., 3 h, (27–45%); *(iii)* acetone, HCl (2 M), r.t., 16 h, (42–67%); (c) Hanztsch ester, *L*-proline, benzaldehyde, CH_2Cl_2, r.t., 16 h, (60%).

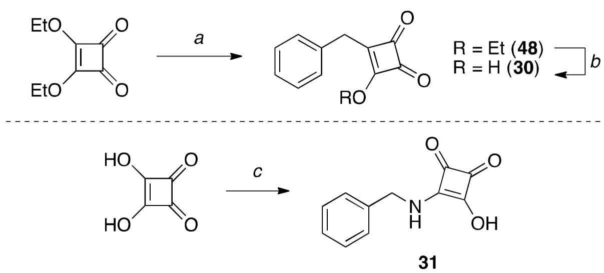
The synthesis of cyclopentane-1,2-dione derivative **28**[50] (Scheme 9) was accomplished in two steps by reacting known di-potassium salt **45**[51] with benzyl bromide to give intermediate **46**, followed by a de-benzylation/de-carboxylation sequence of the latter to obtain the desired 1,2-dione. The 1,2-dione **29**[52] was synthesized via magnesium methoxide induced cyclization of *α*,*β*-unsaturated dione **47**.



Scheme 9. Synthesis of cyclopentane-1,2-dione derivatives. *Reagents and Reaction conditions:* (a) benzyl bromide, DMF, 120 ˚C, 2 h, (47%); (b) AcOH, HCl (37%), 130 ˚C, 3 h, (71%); (c) *(i)*

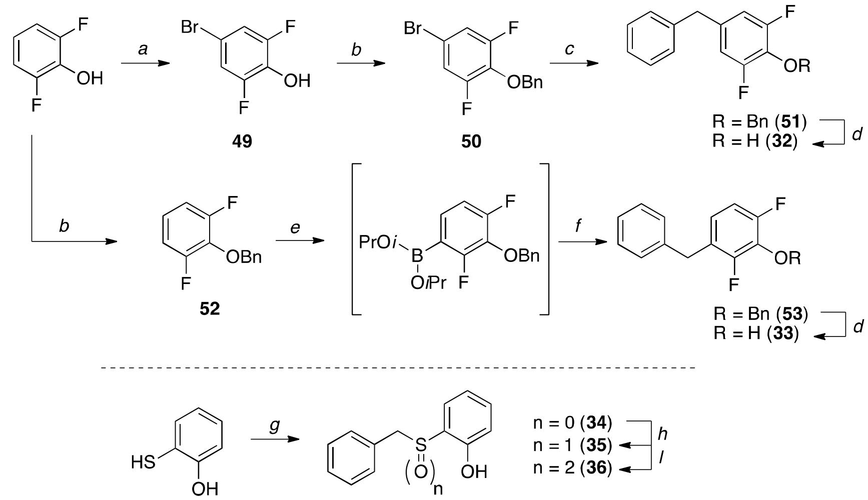
benzaldehyde, NaOH, MeOH, r.t., 24 h; *(ii)* TsOH•H_2O, acetone, r.t., 38 h, (73%); (d) $Mg(OMe)_2$, MeOH, reflux, 90 min, (53%).

The cyclobutane-1,2,3-trione derivative **30** (Scheme 10) was obtained by reacting diethyl squarate with benzylmagnesium bromide, followed by acid-mediated hydrolysis. Squaric acid monoamide derivative **31**[53] (Scheme 10) was prepared by direct condensation of benzylamine with squaric acid under microwave assisted conditions.



Scheme 10. Synthesis of squaric acid derivatives. *Reagents and Reaction conditions:* (a) benzylmagnesium bromide, THF, 0 ˚C to r.t., 40 min, (82%); (b) 3 M HCl, acetone, r.t., 3 h, (66%); (c) benzylamine, 200 ˚C (microwave irradiation), 20 min, (22%).

The synthesis of fluorophenol derivative **32** (Scheme 11) was completed in 4 steps starting from 2,6-difluorophenol. Thus, after bromination and protection of the phenol as benzyl-ether, Negishi cross-coupling conditions were employed to obtain **51**, which was finally deprotected by hydrogenolysis to give model compound **32**. The corresponding *meta*-benzylated isomer **33** was obtained via selective *ortho*-borylation of protected phenol **52**, followed by a Suzuki-Miyaura cross-coupling reaction with benzyl bromide,[54] and final deprotection.[55] Finally, the synthesis of substituted phenols **34–36** was accomplished starting from 2-hydroxythiophenol via benzylation reaction (**34**), followed by *S*-oxidation to the corresponding sulfoxide (**35**) and sulfone (**36**).



Scheme 11. Synthesis of substituted phenol derivatives. *Reagents and Reaction conditions:* (a) NBS, DMF, 0 ˚C to r.t., 40 h, (86%); (b) *(i)* K_2CO_3, acetone, 70 ˚C, 1 h; *(ii)* benzyl bromide, 70 ˚C, 3 h then r.t., 14 h, (99%); (c) $ZnBr_2$, benzylmagnesium chloride, PEPPSI-IPr®, THF, r.t., 16 h, (74%); (d) H_2 (1 atm), Pd/C, MeOH or EtOH, r.t., 1 h, (97–99%); (e) *(i) n*-BuLi, THF, –50 ˚C, 20 min; *(ii)* B(O-*i*-Pr)$_3$, –50 ˚C to r.t., 1 h; (f) $(PPh_3)_2$Pd(Br)(Succ), benzyl bromide, Na_2CO_3, THF/H_2O, 60 ˚C, 14 h, (42%); (g) *(i)* $KHCO_3$, DMF, r.t., 5 min; *(ii)* benzyl bromide, r.t., 16 h, (93%); (h) AcOH, UHP, r.t., 20 h, (98%); (l) *N*-methylmorpholine *N*-oxide, $K_2OsO_4{\bullet}2H_2O$, CH_2Cl_2, r.t., 20 h, (95%).

Materials and methods. All solvents were reagent grade. All reagents were purchased from Aldrich or Fisher Scientific and used as received. Thin layer chromatography (TLC) was performed with 0.25 mm E. Merck pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040–0.062 mm) supplied by Silicycle and Sorbent Technologies. TLC spots were detected by viewing under a UV light, or using $KMnO_4$ or ceric ammonium molybdate stains. Melting points (mp) were acquired on a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Jasco Model FT/IR-480 Plus spectrometer. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker AMX-500 spectrometer. Chemical shifts were reported relative to the residual solvent's peak. High-resolution mass spectra were measured at the University of Pennsylvania Mass Spectrometry Center on either a VG Micromass 70/70H or VG ZAB-E spectrometer. Singlecrystal X-ray structure determinations were performed at the University of Pennsylvania with an Enraf Nonius CAD-4 automated diffractometer. Analytical reverse-phased (Sunfire™ C18; 4.6x50 mm, 5 mL) high-performance liquid chromatography (HPLC) was performed with a Waters binary gradient module 2525 equipped with Waters 2996 PDA and Waters micromass ZQ. All samples were analyzed employing a linear gradient from 10% to 90% of CH_3CN in H_2O over 8 min and flow rate of 1 mL/min, and unless otherwise stated, the purity level was >95%. Preparative reverse-phase HPLC purifications were performed on a Gilson instrument (*i.e.*, Gilson 333 pumps, a 215 liquid handler, 845Z injection module, and PDA detector) employing Waters SunFire™ preparative C_{18} OBD™ columns (5 µm 19x50 or 19x100 mm). Unless otherwise stated, HPLC purifications were carried out employing a linear gradient from 10% to 90% of CH_3CN in H_2O for 15 min with a flow rate of 20 mL/min. Unless otherwise stated, all final compounds were found to be >95% as determined by HPLC/MS and NMR.

3-phenylpropionic acid (1): Commercially available. X-Ray quality crystals were obtained by slow evaporation from a CH_2Cl_2 solution (see Supporting Information): mp (CH_2Cl_2) 49–51 °C.

***N*-(Hydroxy)-3-phenylpropanamide (2):** To a stirred solution of **1** (1.00 g, 6.66 mmol, 1.00 equiv) in anhydrous CH_3CN (22 mL) at r.t. under N_2, CDI (1.30 g, 7.99 mmol, 1.20 equiv) was added in one portion, and the resulting solution was stirred for 35 min. A solution of hydroxylamine (50% wt in H_2O, 0.450 mL, 7.32 mmol, 1.10 equiv) was then added dropwise, and the solution was stirred for 14 h at r.t.. The reaction was quenched by careful addition of 1 M $KHSO_4$ (until pH 1–2), then extracted with EtOAc (×3). The combined organic extracts were washed with phosphate buffer (pH 7), H_2O, brine, then dried over Na_2SO_4, filtered, and concentrated in vacuo. Recrystallization from EtOAc/hexanes provided the title product as a colorless to off-white solid (0.774 g, 4.69 mmol, 70%). X-Ray quality crystals were obtained by layer diffusion of cyclohexane into a CH_2Cl_2 solution (see Supporting Information): mp (CH_2Cl_2/cyclohexane) 73–74 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.08 (br s, 2H), 7.27–7.14 (m, 5H), 2.90 (t, J = 7.8 Hz, 2H), 2.40 (t, J = 7.8 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ

171.3, 140.3, 128.8, 128.5, 126.6, 34.9, 31.5 ppm; IR (KBr) ν 3204, 3059, 3025, 2925, 2861, 1642, 1496, 1452, 1382 cm^{-1}; HRMS (ES^+) calculated for $C_9H_{11}NNaO_2$ $[M+Na]^+$ 188.0687, found 188.0688.

***N*-Hydroxy-*N*-phenethylacetamide (3):** To a solution of **39** (0.183 g, 0.679 mmol, 1.00 equiv) in MeOH (3.40 mL), Pd/C (10% wt (wet), 0.018 g) was added. The suspension was stirred under an H_2 atmosphere (balloon) for 3 h. The reaction mixture was then filtered through a pad of Celite, washed with EtOAc, and concentrated in vacuo. Purification by a short silica gel column chromatography (20% EtOAc in hexanes) afforded the title compound (0.083 g, 0.46 mmol, 68%). X-Ray quality crystals were obtained by slow evaporation from a CH_2Cl_2/hexanes solution (see Supporting Information): mp (CH_2Cl_2/hexanes) 49–51 °C; ^{1}H NMR (500 MHz, $CDCl_3$) mixture of *E/Z* isomers δ 7.44–7.08 (m, 3H), 3.97–3.71 (m, 2H), 2.98 (dt, J = 32.7, 6.7 Hz, 1H), 2.10 (s, 1H), 1.61 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) mixture of *E/Z* isomers δ 172.3, 165.2, 138.7, 138.0, 129.1, 128.9, 128.6, 127.1, 126.5, 51.6, 49.7, 33.5, 33.1, 20.5, 18.4 ppm; IR (KBr) ν 3172, 2922, 2848, 1614 cm^{-1}; HRMS (ES^+) calculated for $C_{10}H_{14}NO_2$ $[M+H]^+$ 180.1025, found 180.1020.

***N*-Methoxy-3-phenylpropanamide (4):** To a solution of **1** (0.210 g, 1.40 mmol, 1.00 equiv) in CH_2Cl_2 at r.t. under N_2, *N*-methylmorpholine (0.190 mL, 1.70 mmol, 1.20 equiv) was added dropwise, and the reaction mixture was stirred for 15 min. EDCI•HCl (0.328 g, 1.70 mmol, 1.20 equiv) was added, and the suspension was stirred until a clear solution was obtained. *O*-methylhydroxylamine hydrochloride (0.146 g, 1.70 mmol, 1.20 equiv) was then added and the solution was stirred for 16 h at r.t.. The reaction was quenched with satd. aq. NH_4Cl, and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with H_2O, brine, then dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (2–10% MeOH in CH_2Cl_2) afforded the title compound (0.210 g, 1.17 mmol, 83%) as a clear oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.05 (s, 1H), 7.28–7.21 (m, 5H), 3.66 (s, 3H), 2.98 (t, J = 7.8 Hz, 2H), 2.45 (app t, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 140.5, 128.6, 128.4, 126.4, 64.0, 34.8, 31.5 ppm; IR (KBr) ν 3182, 3000, 2974, 2936, 1659, 1497, 1453 cm^{-1}; HRMS (ES^+) calculated for $C_{10}H_{13}NNaO_2$ $[M+Na]^+$ 202.0844, found 202.0838.

***N*-Phenethoxyacetamide (5):** Acetohydroxamic acid (0.276 g, 3.67 mmol, 1.00 equiv) and K_2CO_3 (0.508 g, 3.67 mmol, 1.00 equiv) were dissolved in MeOH/H_2O (1:1, 17 mL) and the resulting solution was stirred at r.t. for 10 min. Na_2CO_3 (0.428 g, 4.04 mmol, 1.10 equiv) was added in one portion at r.t., then the reaction was stirred at 50 °C for 10 min. Phenethyl bromide (0.500 mL, 3.67 mmol, 1.00 equiv) was added dropwise, and the reaction mixture was vigorously stirred for 16 h at the same temperature. After cooling to r.t., the reaction mixture was concentrated in vacuo, then diluted with 1 M HCl (until pH 2) and extracted with CH_2Cl_2 (×4). The combined organic extracts were washed with brine, then dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (40–100% EtOAc in hexanes) provided the title compound (0.208 g, 1.16 mmol, 32%) as a colorless solid. X-Ray quality crystals were obtained by slow evaporation from an Et_2O/toluene solution (see Supporting Information): mp (Et_2O/toluene) 87–87.5 °C; Lit.[34] (benzene) 91–93 °C; ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.28–7.17 (m, 5H), 4.89 (s, 1H), 4.02 (t, J = 7.1 Hz, 2H), 2.94 (t, J = 7.1 Hz, 2H), 1.85 (s, 3H) ppm; ^{13}C NMR (125 MHz, MeOH-d_4) δ 168.7, 137.9, 128.5, 128.1, 126.0, 100.2, 76.6, 34.0, 18.0 ppm.

Phenethylphosphonic acid (6): Commercially available. X-Ray quality crystals were obtained by slow diffusion of ligroin into an *i*-PrOH solution (see Supporting Information): mp (*i*-PrOH/ligroin) 135–136 °C.

Phenethylphosphinic acid (7): Diethylchlorophosphite (1.50 g, 9.58 mmol, 1.00 equiv) in anhydrous Et_2O (10 mL) was cooled at 5 °C under N_2. Phenethylmagnesium chloride (1.0 M in THF, 10.0 mL, 10.0 mmol, 1.04 equiv) was added dropwise while maintaining the internal temperature between 0–10 ˚C. The reaction mixture was stirred at r.t. for 16 h, then filtered under N_2. The filtrate was concentrated in vacuo to give a clear, colorless liquid. The liquid was dissolved in H_2O (1.5 mL) then concentrated HCl (37% wt, 0.050 mL) was added. The resulting mixture was stirred for 15 min at r.t., and then extracted with EtOAc (×3). The combined organic extract were washed with brine, then dried over $MgSO_4$, filtered and concentrated in vacuo. The clear liquid was diluted in 2 M aq. NaOH (4.0 mL) at r.t. and the solution was stirred for 1 h, then washed once with Et_2O. The aqueous layer was acidified with concentrated HCl (37% wt, until pH 1) and extracted with EtOAc (×3). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford the title compound (0.520 g, 3.06 mmol, 32%) as a pale yellow oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.98–1.88 (m, 2H), 2.83–2.73 (m, 2H), 6.98 (d, J = 522.9 Hz, 1H), 7.22–7.15 (m, 1H), 7.32–7.22 (m, 4H), 9.77 (s, 1H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.3, 31.9 (d, J = 90.0 Hz), 126.7, 128.7, 129.0, 141.7 (d, J = 16.3 Hz) ppm; ^{31}P NMR (146 MHz, $CDCl_3$) δ 29.0 ppm; IR (KBr) ν 3027, 2920, 2861, 2367, 1497, 1455 cm^{-1}; HRMS (ES$^-$) calculated for $C_8H_{10}O_2P$ [M–H]$^-$ 169.0418, found 169.0448.

Sodium 2-phenylethane-1-sulfonate (8): Commercially available. X-Ray quality crystals were obtained by slow vapor diffusion of Et_2O into a MeOH solution (see Supporting Information): mp (Et_2O/MeOH) > 150 °C.

Sodium 2-phenylethane-1-sulfinate (9): A solution of **40** (0.205 g, 1.00 mmol, 1.00 equiv) in CH_3CN (1.2 mL) was added dropwise to a stirred solution of Na_2SO_3 (0.189 g, 1.50 mmol, 1.50 equiv) and $NaHCO_3$ (0.252 g, 3.00 mmol, 3.00 equiv) in H_2O (1.4 mL) at r.t., and the resulting solution was vigorously stirred for 24 h. The solvents were removed in vacuo, and the colorless solid residue was suspended in hot EtOH, then filtered through a short pad of Celite® (rinsed three times with hot EtOH). Evaporation of the solvents in vacuo provided the title compound (0.144 g, 0.749 mmol, 75%) as a colorless solid. An analytically pure sample was obtained by recrystallization from MeOH/Et_2O: mp (Et_2O/MeOH) > 150 °C; ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.27–7.22 (m, 4H), 7.17–7.13 (m, 1H), 2.91–2.88 (m, 2H), 2.54–2.51 (m, 2H) ppm; ^{13}C NMR (125 MHz, MeOH-d_4) δ 129.4, 126.9, 64.6, 29.7 ppm; IR (KBr) ν 3443, 3085, 3061, 3026, 2961, 2927, 2854, 1602, 1214, 1180 cm^{-1}.

2-Phenylethane-1-sulfonamide (10): Commercially available. X-Ray quality crystals were obtained by slow evaporation from EtOAc (see Supporting Information): mp (EtOAc) 121–122 °C.

***N*-(3-Phenylpropyl)methanesulfonamide (11):** To a solution of 3-phenylpropylamine (1.00 mL, 7.03 mmol, 1.00 equiv) in CH_2Cl_2 (20 mL) at 0 °C under N_2, NEt_3 (3.00 mL, 21.5 mmol, 3.06 equiv) was added. The solution was stirred at the same temperature for 5 min, then methanesulfonyl chloride (0.550 mL, 7.06 mmol, 1.00 equiv) was added dropwise, then the flask was placed in a fridge (approx.

4 °C) for 24 h. The solvents were removed in vacuo, and the residue was diluted with satd. aq. $NaHCO_3$ and extracted with EtOAc (×3). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (10–90% EtOAc in hexanes) afforded the title compound (1.32 g, 6.19 mmol, 88%) as a colorless solid. An analytically pure sample was obtained by recrystallization from cold Et_2O. X-Ray quality crystals were obtained from a mixture of CH_3CN/H_2O (see Supporting Information): mp (Et_2O) 45–46 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (t, *J* = 7.4 Hz, 2H), 7.24–7.15 (m, 3H), 4.86 (t, *J* = 5.8 Hz, 1H), 3.13 (t, *J* = 6.8 Hz, 2H), 2.93 (s, 3H), 2.75–2.60 (m, 2H), 1.90 (t, *J* = 7.2 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.9, 128.6, 128.4, 126.2, 42.7, 40.1, 32.8, 31.6 ppm; IR (KBr) ν 3458, 2923, 1326, 1161 cm^{-1}; HRMS (ES^+) calculated for $C_{10}H_{15}NNaO_2S$ $[M+Na]^+$ 236.0721, found 236.0711.

***N*-(Methylsulfonyl)-3-phenylpropanamide (12):** A solution of **1** (0.546 g, 3.63 mmol, 1.00 equiv), methanesulfonamide (0.346 g, 3.63 mmol, 1.00 equiv), EDCI•HCl (0.836 g, 4.36 mmol, 1.20 equiv), and DMAP (0.533 g, 4.36 mmol, 1.20 equiv) in CH_2Cl_2 (20 mL) was stirred at 45 °C for 8 h, then at r.t. for 48 h. The reaction was quenched with H_2O, then extracted with CH_2Cl_2 (×3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by reverse phase HPLC afforded the title compound (0.574 g, 2.52 mmol, 70%) as a colorless solid. X-ray quality crystals were obtained from a mixture of CH_3CN/H_2O (see Supporting Information): mp (CH_3CN/H_2O) 105.5–106.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.30 (t, *J* = 7.4 Hz, 2H), 7.21 (dd, *J* = 18.3, 7.3 Hz, 3H), 3.21 (s, 3H), 2.97 (t, *J* = 7.6 Hz, 2H), 2.63 (t, *J* = 7.6 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 139.6, 128.9, 128.5, 126.8, 41.6, 38.3, 30.5 ppm; IR (KBr) ν 3439, 2919, 1339, 1144 cm^{-1}; HRMS (ES^+) calculated for $C_{10}H_{13}NNaO_3S$ $[M+Na]^+$ 250.0514, found 250.0511.

***N*-(*N*,*N*-Dimethylsulfamoyl)-3-phenylpropanamide (13):** To a suspension of **1** (0.052 g, 0.244 mmol, 1.00 equiv) and TBTU (0.133 g, 0.413 mmol, 1.00 equiv) in anhydrous CH_2Cl_2 (2.3 mL) at r.t. under N_2, DIEA (0.300 mL, 1.72 mmol, 5.00 equiv) was added dropwise, and the resulting suspension was stirred at r.t. for 25 min. *N*,*N*-dimethylsulfamide (0.051 g, 0.293 mmol, 1.20 equiv) was then added in one portion, and the resulting solution was stirred at r.t. for 14 h. The reaction was quenched by addition of 1 M aq. $KHSO_4$, then extracted with EtOAc (×3). The combined organic extracts were washed with brine, then dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (7–40% EtOAc in hexanes) provided the title compound (0.071 g, 0.277 mmol, 81%) as a colorless crystalline solid. X-ray quality crystals were obtained by slow evaporation from EtOAc/hexanes (see Supporting Information): mp (EtOAc/hexanes) 103–104 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.30–7.26 (m, 2H), 7.20 (m, 3H), 2.96 (t, J = 7.5 Hz, 2H), 2.83 (s, 6H), 2.60 (t, J = 7.6 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 139.8, 128.7, 128.5, 126.6, 38.2, 37.6, 30.6 ppm; IR (KBr) ν 3439, 2919 cm^{-1}; HRMS (ES^{+}) calculated for $C_{11}H_{16}N_2O_3NaS$ $[M+Na]^+$ 279.0779, found 279.0773.

***N*-(Benzylcarbamoyl)methanesulfonamide (14):** To a stirred solution of benzylamine (0.107 g, 1.00 mmol, 1.00 equiv) in CH_2Cl_2 (1.0 mL) was added 4 M HCl in dioxane (0.250 mL, 1.00 mmol, 1.00 equiv) and the solution was stirred at r.t. for 5 min under N_2. The solvents were removed in a stream of air, then the residue was dried under high vacuum for 3 h. The residue was dissolved in anhydrous CH_3CN (0.60 mL), then a solution of CDI (0.178 g, 1.1 mmol, 1.10 equiv) dissolved in DMF (0.30 mL) was added. The solution was stirred at r.t. for 2 h. The solvents were removed in a stream of air. Purification by silica gel column chromatography (5% MeOH in CH_2Cl_2) provided the benzylcarbamoylimidazole intermediate (0.050 g, 2.48 mmol, 25%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (d, J = 5.9 Hz, 1H), 7.98 (s, 1H), 7.39 (s, 1H), 7.32–7.12 (m, 5H), 6.76 (s, 1H), 4.45 (d, J = 5.5 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.3, 137.4, 136.0, 129.6, 128.9, 128.0, 116.8, 45.0 ppm.

To a stirred solution of methylsulfonamide (0.028 g, 0.298 mmol, 1.20 equiv) in DMF (1.0 mL) was added NaH (60% wt in oil, 0.007 g, 0.298 mmol, 1.20 equiv). The solution was stirred for 15 min at r.t.. The benzylcarbamoylimidazole intermediate (0.050 g, 0.248 mmol) was added, and the resulting solution was stirred at r.t. for 16 h. The reaction was diluted with EtOAc, then extracted with H_2O. The combined aqueous layers were acidified with 1 M HCl (until pH 2–3), then extracted with EtOAc (×3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by reverse phase HPLC provided the title compound (0.025 g, 0.110 mmol, 44%). X-ray quality crystals were obtained by slow evaporation from EtOAc (see Supporting Information): mp (EtOAc) 163–164 °C; ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.47–7.19 (m, 5H), 4.41 (s, 2H), 3.29 (d, J = 1.3 Hz, 3H) ppm; ^{13}C NMR (125 MHz, MeOH-d_4) δ 153.3, 138.6, 128.4, 127.1, 127.1, 47.5, 47.3, 43.3, 40.6 ppm; IR (KBr) ν 3338, 2920, 1648, 1562 cm^{-1}.

***N*-(Benzylcarbamoyl)acetamide (15):** To a suspension of *N*-benzylurea (0.415 g, 2.75 mmol, 1.00 equiv) in CH_2Cl_2 (20 mL), Ac_2O (2.09 mL, 22.1 mmol, 8.00 equiv) was added dropwise, and the resulting suspension was stirred at 50 °C for 24 h, then at r.t. for 48 h. The solvents were removed in vacuo. Purification by reverse phase HPLC provided the title compound (0.396 g, 2.06 mmol, 75%) as a colorless solid. X-ray quality crystals were obtained by slow evaporation from CH_2Cl_2 (see Supporting Information): mp (CH_2Cl_2) 118–119 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.54 (s, 1H), 8.94 (t, J = 5.3 Hz, 1H), 7.39–7.20 (m, 5H), 4.49 (d, J = 6.0 Hz, 2H), 2.09 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 155.3, 138.2, 128.7, 127.5, 127.5, 43.6, 23.9 ppm; IR (KBr) ν 3447, 3356, 2922, 1689, 1630 cm^{-1}; HRMS (ES$^+$) calculated for $C_{10}H_{12}N_2NaO_2$ [M+Na]$^+$ 215.0796, found 215.0798.

5-Phenethyl-1*H*-tetrazole (16): *Method A*. 3-phenylpropanenitrile **41** (0.100 g, 0.762 mmol, 1.00 equiv), 1 M aq. NaN_3 (0.840 mL, 0.840 mmol, 1.10 equiv) and 1 M aq. $ZnBr_2$ (0.760 mL, 0.760 mmol, 1.00 equiv) were added to a microwave vial. The vial was sealed, warmed to 150 °C and vigorously stirred for 24 h. The reaction was quenched with 3 M HCl (1.5 mL) and extracted with EtOAc (×3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by reverse phase HPLC provided the title compound (0.026 g, 0.149 mmol, 20%) as a colorless solid. *Method B*. A suspension of amide **42** (0.105 g, 0.519 mmol, 1.00 equiv) and PPh_3 (0.340 g, 1.30 mmol, 2.50 equiv) in anhydrous CH_3CN (5 mL) under N_2, was cooled to 0 °C and stirred for 10 min. DIAD (0.250 mL, 1.30 mmol, 2.50 equiv) was added dropwise (discoloration after each drop) and the suspension was stirred at 0 °C for 5 min. $TMSN_3$ (0.210 mL, 1.56 mmol, 3.00 mmol) was then added dropwise in 5 min, and the reaction was stirred at 0 °C for 30 min, then at r.t. for 2 h, and finally at 50 °C for 15 h. The reaction was cooled to 0 °C, quenched by addition of 3 M aq. $NaNO_2$ (0.520 mL, 1.56 mmol, 1.00 equiv). After 20 min stirring at 0 °C, 0.5 M aq. CAN (1.45 mL, 0.726 mmol, 1.40

equiv) was added and the stirring was continued at 0 °C for 30 min. The reaction was then diluted with H_2O and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (50–70% EtOAc in hexanes) afforded a mixture of 3-(5-phenethyl-1*H*-tetrazol-1-yl)propanenitrile, PPh_3 and EtOAc (0.106 g, 90% wt NMR purity), which was used directly in the next step: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.24 (m, 3H), 7.10–7.08 (m, 2H), 4.12 (t, J = 6.9 Hz, 2H), 3.22–3.14 (m, 4H), 2.68 (t, J = 6.9 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.9, 139.3, 129.1, 128.5, 127.1, 115.9, 42.1, 33.9, 25.5, 18.3 ppm.

To a stirred solution of 3-(5-phenethyl-1*H*-tetrazol-1-yl)propanenitrile (0.106 g, 0.418 mmol, 1.00 equiv) in CH_2Cl_2 (3.8 mL) under N_2, freshly distilled DBU (0.440 mL, 2.93 mmol, 7.00 equiv) was added dropwise and the resulting clear yellow solution was stirred at r.t. for 6 h. The reaction was diluted with CH_2Cl_2 and washed with 1 M HCl (×2), brine, then dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (12–100% EtOAc in hexanes) provided the title compound (0.046 mg, 0.264 mmol, 63% over 2 steps) as a colorless solid. X-ray quality crystals were obtained by slow evaporation from CH_2Cl_2 (see Supporting Information): mp (CH_2Cl_2) 97–99 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.97 (s, 1H), 7.23 (t, J = 7.2 Hz, 2H), 7.20–7.13 (m, 3H), 3.40 (t, J = 7.8 Hz, 2H), 3.16 (t, J = 7.8 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.2, 139.2, 128.9, 128.5, 127.0, 33.8, 25.5 ppm; IR (KBr) ν 3354, 3130, 2919, 2722, 1565, 1495 cm^{-1}; HRMS (ES^+) calculated for $C_9H_{11}N_4$ $[M+H]^+$ 175.0984, found 175.0989.

5-Benzylthiazolidine-2,4-dione (17): Thiazolidine-2,4-dione (0.586 g, 5.00 mmol, 1.00 equiv) was weighed in a flame-dried round-bottom flask. The flask was evacuated and backfilled with N_2 (×5), then anhydrous THF (20 mL) was added. The resulting clear solution was cooled to –78 °C and stirred for 10 min. *n*-BuLi (2.34 M in hexanes, 4.30 mL, 10 mmol, 2.00 equiv) was added dropwise over 5 min under vigorous stirring (a precipitate formed during the addition). The resulting deep yellow solution was stirred at the same temperature for 15 min, then warmed to 0 °C and stirred for 20 min. The reaction was cooled to –78 °C, and benzyl bromide (0.600 mL, 5.00 mmol, 1.00 equiv) was added dropwise. The solution was stirred for 20 min at the same temperature, then warmed to r.t. and stirred for 1.5 h. The reaction was quenched with satd. aq. NH_4Cl, then extracted with EtOAc (×3). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (5–40% EtOAc in hexanes) provided the title compound (0.549 g, 2.65 mmol, 53%) as a colorless solid. X-ray quality crystals were obtained by slow evaporation from an Et_2O/hexanes solution (see Supporting Information): mp (Et_2O/hexanes) 74–75 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.35–7.23 (m, 5H), 4.54 (dd, *J* = 9.9, 3.8 Hz, 1H), 3.56 (dd, *J* = 14.1, 3.8 Hz, 1H), 3.13 (dd, *J* = 14.0, 10.0 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.7, 170.9, 135.9, 129.3, 129.0, 127.8, 53.6, 38.8 ppm; IR (KBr) ν 3320, 2943, 1748 cm^{-1}; HRMS (ES^-) calculated for $C_{10}H_8NO_2S$ $[M–H]^-$ 206.0281, found 206.0289.

5-Benzyloxazolidine-2,4-dione (18): 2-Hydroxyacetamide (0.300 g, 4.00 mmol, 1.00 equiv) was weighed in a flame dried microwave vial, then dissolved in anhydrous MeOH (4.2 mL). Potassium *tert*-butoxide (0.450 g, 4.00 mmol, 1.00 equiv) was added, followed by diethyl carbonate (0.508 mL, 4.80 mmol, 1.20 equiv). The vial was sealed and stirred at 70 °C for 19 h, then at r.t. for 24 h. The solvents were removed in vacuo, the residue was taken up in H_2O, then acidified to pH 2 with 3 M HCl and extracted with EtOAc (×3). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford oxazolidine-2,4-dione, which was directly used in the next step without further purification. To a solution of crude oxazolidine-2,4-dione (0.105 g, 1.00 mmol, 1.00 equiv) in anhydrous THF (3.6 mL) at −78 °C, *n*-BuLi (2.65 M in hexanes, 0.780 mL, 2.10 mmol, 2.10 equiv) was added dropwise and the mixture was stirred at −78 °C for 15 min, then at 0 °C for 30 min. The solution was then cooled to −78 °C and benzyl bromide (0.120 mL, 1.00 mmol, 1.00 equiv) was added dropwise. The resulting solution was warmed to r.t. and stirred for 2 h. The reaction was quenched with satd. aq. NH_4Cl, then extracted with EtOAc (×3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by reverse phase HPLC provided the title compound (0.009 g, 0.05 mmol, 5%) as a colorless solid. X-ray quality crystals were obtained by slow evaporation from $CHCl_3$ (see Supporting Information): mp ($CHCl_3$) 85–87 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.30 (ddd, *J* = 9.6, 6.2, 3.2 Hz, 3H), 7.26–7.20 (m, 2H), 5.08 (dd, *J* = 5.7, 4.3 Hz, 1H), 3.32 (dd, *J* = 14.8, 4.2 Hz, 1H), 3.16 (dd, *J* = 14.8, 5.7 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 154.1, 133.0, 129.7, 128.9, 127.9, 81.4, 36.6 ppm; IR (KBr) ν 3355, 2916, 2848, 1823, 1743 cm^{-1}; HRMS (ES^-) calculated for $C_{10}H_8NO_3$ $[M-H]^-$ 190.0504, found 190.0503.

3-Phenethyl-1,2,4-oxadiazol-5(4*H*)-one (19): To a solution of amidoxime **43** (0.120 g, 0.700 mmol, 1.00 equiv) and pyridine (0.061 mL, 0.760 mmol, 1.10 equiv) in DMF (1.20 mL) at 0 °C, isobutyl chloroformate (0.091 mL, 0.700 mmol, 1.00 equiv) was added dropwise, and the solution was stirred for 16 h while slowly warming to r.t.. The reaction was diluted with H_2O and extracted with EtOAc (×3). The combined organic extracts were washed with H_2O, brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was suspended in toluene (1.50 mL) in a microwave vial and stirred at 120 °C for 2 h, then at 140 °C for 24 h. After cooling to r.t., the mixture was concentrated in vacuo. Purification by reverse phase HPLC provided the title compound (0.080 g, 0.448 mmol, 59%) as a colorless solid. X-ray quality crystals were obtained by slow evaporation from Et_2O (see Supporting Information): mp (Et_2O) 98–100 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 10.53 (s, 1H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.23 (dd, *J* = 7.6, 6.4 Hz, 3H), 3.02 (t, *J* = 7.8 Hz, 2H), 2.91 (t, *J* = 7.7 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.6, 158.9, 138.7, 128.9, 128.4, 127.1, 31.7, 27.0 ppm; IR (KBr) ν 3189, 2956, 1768, 1606, 1454 cm^{-1}; HRMS (ES^-) calculated for $C_{10}H_9N_2O_2$ $[M-H]^-$ 189.0664, found 189.0675.

3-Phenethyl-1,2,4-thiadiazol-5(4*H*)-one (20): To a solution of amidoxime **43** (0.100 g, 0.610 mmol, 1.00 equiv) in anhydrous THF (1.00 mL) at r.t. under Ar, 1,1'-thiocarbonyldiimidazole (0.160 g, 0.910 mmol, 1.50 equiv) was added, and the solution was stirred for 40 min. The reaction was quenched with H_2O, then extracted with CH_2Cl_2 (×3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was dissolved in anhydrous THF (1.0 mL), then BF_3•OEt_2 (48%, 0.230 mL, 1.80 mmol, 3.00 equiv) was added dropwise, and the resulting mixture was stirred at r.t. for 3 h. The reaction was diluted with H_2O and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with 1 M HCl, dried over Na_2SO_4, filtered, and concentrated in vacuo.

Purification by reverse phase HPLC afforded the title compound (0.035 g, 0.170 mmol, 28%) as a colorless solid. X-ray quality crystals were obtained by slow evaporation from CH_2Cl_2 (see Supporting Information): mp (CH_2Cl_2) 119–122 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.43 (br s, 1H), 7.32–7.20 (m, 5H), 3.06 (t, J = 7.8 Hz, 2H), 2.92 (t, J = 7.7 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.9, 139.5, 128.9, 128.4, 126.8, 33.2, 32.4 ppm; IR (KBr) ν 3437, 2918, 1666, 1564, 1450 cm^{-1}; HRMS (ES$^-$) calculated for $C_{10}H_9N_2OS$ [M–H]$^-$ 205.0436, found 205.0427.

4-Phenethyl-3*H*-1,2,3,5-oxathiadiazole 2-oxide (21): To a solution of amidoxime **43** (0.100 g, 0.610 mmol, 1.00 equiv) in THF (18 mL), pyridine (0.130 mL, 1.60 mmol, 2.60 equiv) in CH_2Cl_2 (3.5 mL) was added, and the solution was cooled to 0 °C. Thionyl chloride (0.058 mL, 0.800 mmol, 1.30 equiv) was then added dropwise, and the reaction was stirred at 0 °C for 1 h, during which a colorless precipitate was formed. The solvents were removed in vacuo, and the residue was diluted with H_2O and extracted with $CHCl_3$ (×3). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by reverse phase HPLC afforded the title compound (0.012 g, 0.060 mmol, 10%) as a colorless solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (br s, 1H), 7.35–7.31 (m, 2H), 7.28–7.25 (m, 1H), 7.23–7.21 (m, 2H), 3.00 (t, J = 7.60 Hz, 2H), 2.94–2.85 (m, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.2, 139.2, 129.2, 128.6, 127.3, 32.7, 25.7 ppm; IR (KBr) ν 3223, 2925, 1606, 1404, 1176 cm^{-1}; HRMS (ES$^-$) calculated for $C_9H_9N_2O_2S$ [M–H]$^-$ 209.0390, found 209.0385.

3-Phenethyl-1,2,4-oxadiazole-5(4*H*)-thione (22): To a solution of amidoxime **43** (0.100 g, 0.610 mmol, 1.00 equiv) in CH_3CN (5.5 mL), 1,1'-thiocarbonyldiimidazole (0.120 g, 0.670 mmol, 1.10 equiv) and DBU (0.360 mL, 2.40 mmol, 3.9 equiv) were added and the reaction was stirred at r.t. for 24 h. The solvents were removed in vacuo, and the residue was diluted with H_2O. The pH was adjusted to pH 4 with 1 M HCl, and the mixture was extracted with EtOAc (×3). The combined organic extracts were concentrated in vacuo, and the residue was dissolved in 1 M NaOH and washed with Et_2O. The pH was again adjusted to pH 4 with 1 M HCl, and extracted with EtOAc (×3). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, filtered and concentrated in vacuo. Purification by reverse phase HPLC afforded the title compound (0.056 g, 0.271 mmol, 43%) as a colorless solid. X-ray quality crystals were obtained by slow evaporation from CH_2Cl_2 (see Supporting Information): mp (CH_2Cl_2) 96–100 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.37 (br s, 1H), 7.35 (t, J = 7.3 Hz, 2H), 7.29 (d, J = 7.3 Hz, 1H), 7.23–7.18 (m, 2H), 3.05 (dd, J = 8.3, 5.0 Hz, 2H), 3.03–2.96 (m, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.2, 159.3, 138.3, 129.1, 128.4, 127.3, 32.2, 25.8 ppm; IR (KBr) ν 3420, 2920, 1603, 1472 cm^{-1}; HRMS (ES$^-$) calculated for $C_{10}H_9N_2OS$ [M–H]$^-$ 205.0436, found 205.0441.

5-Benzylisoxazol-3-ol (23): To a stirred solution of **44** (0.571 g, 2.18 mmol, 1.00 equiv) in toluene (20 mL), *N,O*-di-Boc-hydroxylamine (0.508 g, 2.18 mmol, 1.00 equiv) was added. The solution was stirred at 65 °C for 16 h. After cooling to r.t., the solvents were removed in vacuo to provide the β-keto hydroxamic acid intermediate (0.962 g, 100%), which was used directly in the next step without further purification. Crude β-keto hydroxamic acid intermediate (0.268 g, 0.681 mmol, 1.00 equiv) was dissolved in MeOH (6.0 mL) and 4 M HCl (9.0 mL), and the resulting solution was stirred at r.t. for 16 h. The mixture was concentrated in vacuo, diluted with H_2O, and extracted with EtOAc (×3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (10% EtOAc in hexanes, buffered with 1% AcOH) provided the title compound (0.013 g, 0.074 mmol, 10%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 –7.22 (m, 5H), 5.65 (s, 1H), 3.98 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 171.3, 135.4, 129.0, 128.9, 127.4, 94.4, 33.9 ppm; IR (KBr) ν 3399, 2919, 2848, 1620, 1524 cm^{-1}; HRMS (ES$^+$) calculated for $C_{10}H_{10}NO$ [M+H]$^+$ 176.0712, found 176.0714.

5-Benzylpyrrolidine-2,4-dione (24): To a solution of *N*-Boc-(*DL*)-phenylalanine (2.00 g, 7.54 mmol, 1.00 equiv), DMAP (1.29 g, 10.6 mmol, 1.40 equiv) and freshly recrystallized Meldrum's acid (1.20 g, 8.29 mmol, 1.10 equiv) in CH_2Cl_2 (50 mL) at 0 °C, EDCI•HCl (1.73 g, 9.05 mmol, 1.20 equiv) was added in one portion. The reaction mixture was stirred at 0 °C for 5 min, then at r.t. for 15 h. The solvents were removed in vacuo, then the residue was diluted in EtOAc, washed with 1 M $KHSO_4$, 5% wt aq. citric acid, brine (×3), then dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting colorless solid was dissolved in EtOAc (100 mL) and stirred at reflux for 30 min. The solvents were removed in vacuo to yield a colorless foam. The residue was dissolved in CH_2Cl_2 under N_2 at r.t., then TFA (10 mL) was added in a steady stream and the reaction was stirred at r.t. for 15 min. The solvents were removed in vacuo, using toluene (2 portions) to azeotrope the residual TFA. The crude solid was dissolved in a minimum amount of Et_2O, then cooled to –78 °C and precipitated with hexanes. Filtration afforded the title compound (1.30 g, 6.88 mmol, 91%) as a colorless to pale yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (t, *J* = 7.2 Hz, 2H), 7.28 (d, *J* = 7.2 Hz, 1H), 7.16 (d, *J* = 7.0 Hz, 2H), 6.54 (br s, 1H), 4.23 (dd, *J* = 7.7, 3.3 Hz, 1H), 3.16 (dd, *J* = 13.9, 3.7 Hz, 1H), 2.93 (d, *J* = 22.2 Hz, 1H), 2.84 (dd, *J* = 13.9, 8.3 Hz, 1H), 2.71 (d, *J* = 22.2 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 170.8, 135.3, 129.5, 129.2, 127.6, 65.3, 40.9, 38.4 ppm; IR (KBr) ν 3174, 2944, 1770, 1695 cm^{-1}; HRMS (ES$^+$) calculated for $C_{11}H_{12}NO_2$ $[M+H]^+$ 190.0868, found 190.0865.

5-Benzyl-3-hydroxycyclopent-2-en-1-one (25): To a solution of 3-isobutoxycyclopent-2-en-1-one (0.130 g, 0.844 mmol, 1.00 equiv) in anhydrous THF (2.00 mL) at –78 ˚C under N_2, a freshly prepared solution of LDA (1 M in THF, 1 mL, 1 mmol, 1.18 equiv) was added dropwise. The resulting solution was stirred at –78 °C for 45 min, then a solution of benzyl bromide (0.100 mL, 0.844 mmol, 1.00 equiv) in THF (3 mL) was added dropwise. The solution was stirred for 1 h while slowly warming to r.t.. The reaction was quenched with satd. aq. NH_4Cl and extracted with EtOAc (×2). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (3% MeOH in CH_2Cl_2) provided the 5-benzyl-3-isobutoxycyclopent-2-en-1-one intermediate (0.055 g, 0.225 mmol, 27%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.25 (m, 2H), 7.21 (t, *J* = 6.8 Hz, 3H), 5.26 (s, 1H), 3.71 (d, *J* = 6.6 Hz, 2H), 3.27 (dd, *J* = 14.0, 4.1 Hz, 1H), 2.88–2.74 (m, 1H), 2.67–2.51 (m, 2H), 2.43–2.30 (m, 1H), 2.04 (hept, *J* = 6.6 Hz, 1H), 0.97 (d, *J* = 6.7 Hz, 6H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.3, 189.4, 139.6, 129.0, 128.6, 126.4, 103.9, 78.1, 46.8, 37.3, 34.3, 28.0, 19.1, 19.1 ppm. To a mixture of 5-benzyl-3-isobutoxycyclopent-2-en-1-one (0.035 g, 0.143 mmol, 1.00 equiv) in acetone (2.0 mL) at r.t., 3 M HCl (1.0 mL) was added and the mixture was stirred for 16 h. The reaction was then concentrated in vacuo. Purification by reverse phase HPLC provided the title compound (0.018 g, 0.096 mmol, 67%) as a colorless solid: ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.27 (t, *J* = 7.6 Hz, 2H), 7.23–7.15 (m, 3H) 3.15 (dd, *J* = 13.8, 4.2 Hz, 1H), 3.01–2.92 (m, 1H), 2.64 (dd, *J* = 13.8, 9.4 Hz, 1H), 2.51 (dd, *J* = 18.1, 6.9 Hz, 1H), 2.24 (dd, *J* = 18.1, 2.4 Hz, 1H) ppm; ^{13}C NMR (125 MHz, MeOH-d_4) δ 139.0, 128.9, 128.2, 126.2, 44.8, 36.9, 36.1 ppm; IR (KBr) ν 3026, 2920, 2680, 2565, 1644, 1553 cm^{-1}; HRMS (ES$^+$) calculated for $C_{12}H_{13}O_2$ $[M+H]^+$ 189.0916, found 189.0913.

5-Benzyl-3-hydroxy-2-methylcyclopent-2-en-1-one (26): Prepared as **25** from 3-isobutoxy-2-methylcyclopent-2-en-1-one (0.130 g, 0.773 mmol, 1.00 equiv) and benzyl bromide (0.092 mL, 0.773 mmol, 1.00 equiv). Purification by silica gel column chromatography (3% MeOH in CH_2Cl_2) provided the 5-benzyl-3-isobutoxy-2-methylcyclopent-2-en-1-one intermediate (0.090 g, 0.348 mmol, 45%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (dd, *J* = 8.7, 6.7 Hz, 2H), 7.23–7.17 (m, 3H), 3.81 (d, *J* = 6.6 Hz, 2H), 2.82–2.73 (m, 1H), 3.31 (dd, *J* = 14.1, 4.1 Hz, 1H), 2.66–2.56 (m, 1H), 2.50 (dd, *J* = 14.0, 10.7 Hz, 1H), 2.28 (dt, *J* = 17.5, 2.0 Hz, 1H), 2.01–1.90 (m, 1H), 1.64 (d, *J* = 1.8 Hz, 3H), 0.95 (dd, *J* = 6.7, 1.6 Hz, 5H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.6, 183.4, 139.8, 129.0, 128.6, 126.4, 115.3, 75.6, 46.2, 37.5, 31.2, 28.8, 19.0, 6.2 ppm. After hydrolysis of the 5-benzyl-3-isobutoxy-2-methylcyclopent-2-en-1-one intermediate (as for **25**), purification by reverse phase HPLC provided the title compound (0.030 g, 0.148 mmol, 43%) as a colorless solid: ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.27 (dd, *J* = 8.3, 7.1 Hz, 2H), 3.32 (dq, *J* = 3.0, 1.5 Hz, 1H), 4.94 (s, 2H), 3.17 (dd, *J* = 13.7, 4.1 Hz, 1H), 2.88 (dq, *J* = 10.5, 3.7, 3.2 Hz, 1H), 2.58 (dd, *J* = 13.7, 9.6 Hz, 1H), 7.23–7.16 (m, 3H), 2.47 (ddt, *J* = 17.8, 6.7, 1.1 Hz, 1H), 1.57 (s, 3H), 2.20 (ddt, *J* = 17.8, 2.3, 1.1 Hz, 1H) ppm; ^{13}C NMR (125 MHz, MeOH-d_4) δ 139.1, 128.8, 128.1, 126.1, 112.5, 48.3, 48.1, 47.9, 47.8, 47.6, 47.4, 47.2, 44.0, 37.1, 34.7, 4.3 ppm; IR (KBr) ν 3410, 3028, 2927, 1780, 1714 cm^{-1}; HRMS (ES$^-$) calculated for $C_{13}H_{13}O_2$ $[M-H]^-$ 201.0916, found 201.0926.

2-Benzyl-3-hydroxycyclopent-2-en-1-one (27): To a solution of cyclopentane-1,3-dione (0.300 g, 3.06 mmol, 1.03 equiv), diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (0.775 g, 2.98 mmol, 1.00 equiv) and (*L*)-proline (0.017 g, 0.148 mmol, 5 mol%) in CH_2Cl_2 (10 mL) was added benzaldehyde (0.915 mL, 9.00 mmol, 3.00 equiv), and the resulting mixture was allowed to stir at r.t. for 30 min. The reaction mixture was concentrated in vacuo. Purification by silica gel column chromatography (30–100% EtOAc in hexanes) provided the title compound (0.350 g, 1.86 mmol, 61%) as a colorless solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.75 (s, 1H), 7.21 (t, J = 7.5 Hz, 2H), 7.18–7.14 (m, 2H), 7.14–7.08 (m, 1H), 3.33 (s, 2H), 2.40 (s, 4H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 141.1, 128.7, 128.6, 126.1, 116.1, 27.1 ppm; IR (KBr) ν 3365, 2920, 2852, 1667, 1567 cm^{-1}; HRMS (ES^{-}) calculated for $C_{12}H_{11}O_2$ [M–H]$^{-}$ 187.0759, found 187.0761.

3-Benzyl-2-hydroxycyclopent-2-en-1-one (28): A mixture of **46** (0.155 g, 0.367 mmol, 1.00 equiv) in glacial AcOH (0.70 mL) and concentrated HCl (37% wt, 0.70 mL) in a sealed tube was stirred at 130 °C for 3 h. After cooling to r.t., the mixture was carefully quenched with H_2O, then diluted in aq. buffer (pH 3). The pH was adjusted to pH 3 with 20% wt aq. NaOH, then the mixture was extracted with EtOAc (×5). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (5–20% EtOAc in hexanes) provided the title compound (0.049 g, 0.260 mmol, 71%) as a colorless solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33–7.29 (m, 2H), 7.26–7.22 (m, 3H), 5.68 (s, 1H, OH), 3.74 (s, 2H), 2.39–2.38 (m, 2H), 2.36–2.34 (m, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.7, 148.9, 146.2, 137.8, 129.1, 128.8, 126.7, 35.0, 32.1, 24.9 ppm; IR (KBr) ν 3327, 3024, 2923, 1695, 1652, 1387, 1107 cm^{-1}.

2-Hydroxy-4-phenylcyclopent-2-en-1-one (29): A solution of **47** (0.027 g, 0.160 mmol, 1.00 equiv) in a 10% wt solution of $Mg(OMe)_2$ in MeOH (15 mL) was stirred at reflux for 90 min. After cooling to r.t., the reaction mixture was concentrated in vacuo. The residue was taken up in Et_2O and H_2O, then the pH was adjusted to pH 7 with 1 M HCl. The organic layer was washed with H_2O (×4), dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (50% EtOAc in hexanes) provided the title compound (0.015 g, 0.086 mmol, 53%) as a colorless solid. X-ray quality crystals were obtained by slow evaporation from CH_2Cl_2 (see Supporting Information): mp (CH_2Cl_2) 106–107 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.32 (m, 2H), 7.27–7.24 (m, 1H), 7.20–7.18 (m, 2H), 6.61 (m, 1H), 6.25 (br s, 1H), 4.04–4.03 (m, 1H), 2.99 (dd, J = 19.4, 6.4 Hz, 1H), 2.36 (d, J = 19.5 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.1, 142.7, 129.0, 127.3, 127.1, 42.4, 40.1 ppm; IR

(KBr) ν 3334, 3058, 3025, 1699, 1403 cm^{-1}; HRMS (CI^+) calculated for $C_{11}H_{10}O_2$ $[M]^+$ 174.0681, found 174.0688.

3-Benzyl-4-hydroxycyclobut-3-ene-1,2-dione (30): To a solution of **48** (0.099 g, 0.460 mmol, 1.00 equiv) in acetone (5.0 mL) at r.t., 3 M HCl (5.0 mL) was added and the solution was stirred at r.t. for 3 h. The acetone was removed in vacuo, and the aqueous layer was extracted with Et_2O (×3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by reverse phase HPLC afforded the title compound (0.057 g, 0.303 mmol, 66%) as a colorless solid: ^1H NMR (500 MHz, acetone-d_6) δ 11.59 (br s, 1H), 7.36–7.30 (m, 4H), 7.25–7.23 (m, 1H), 3.98 (s, 2H) ppm; ^{13}C NMR (125 MHz, acetone-d_6) δ 197.6, 196.6, 181.3, 136.2, 129.3, 129.2, 127.4, 30.4 ppm; IR (KBr) ν 3214, 2974, 1812, 1644 cm^{-1}; HRMS (ES^-) calculated for $C_{11}H_7O_3$ $[M–H]^-$ 187.0401, found 187.0405.

3-(Benzylamino)-4-hydroxycyclobut-3-ene-1,2-dione (31): To a suspension of squaric acid (0.114 g, 1.00 mmol, 1.00 equiv) in H_2O (3.0 mL) in a microwave vial under N_2, benzylamine (0.220 mL, 2.00 mmol, 2.00 equiv) was added dropwise, the tube was sealed and heated to 200 °C under microwave irradiation (Biotage Initiator) for 20 min. *Note: the internal pressure reached up to 17 bars.* After cooling to r.t., the reaction was diluted with 3 M HCl (40 mL), and extracted with Et_2O (×5). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford the title compound (0.045 g, 0.221 mmol, 22%) as a colorless solid: ^1H NMR (500 MHz, DMSO-d_6) δ 8.87 (t, J = 6.0 Hz, 1H), 7.36 (t, J = 7.5 Hz, 2H), 7.37–7.27 (m, 6H), 7.30–7.28 (m, 3H), 4.59 (d, J = 6.3 Hz, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 184.8, 173.7, 138.7, 128.6, 127.4, 47.0 ppm *(C=O carbons not observed due to rapid tautomeric exchange).*

4-Benzyl-2,6-difluorophenol (32): To a solution of **51** (0.310 g, 1.00 mmol, 1.00 equiv) in MeOH (10 mL) under N_2, Pd/C (10% wt (wet), 0.031 g) was added. The resulting suspension was purged with H_2 for 10 min, then stirred for 1 h under an H_2 atmosphere (balloon). The reaction mixture was then purged with N_2 for 10 min, filtered through a pad of Celite® and thoroughly washed with EtOAc. Removal of the volatiles in vacuo provided the title compound (0.220 g, 0.999 mmol, 99%) as a colorless solid. X-Ray quality crystals were obtained by slow layer diffusion of hexanes into a solution of the product in Et_2O at r.t. (see Supporting Information): mp (hexanes/Et_2O) 60.5–61.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (t, *J* = 7.4 Hz, 2H), 7.29 (t, *J* = 7.3 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 2H), 6.80–6.74 (m, 2H), 5.16 (br s, 1H), 3.91 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.7 (dd, *J* = 242.5, 5.7 Hz), 140.0, 133.4 (t, *J* = 7.6 Hz), 130.9 (t, *J* = 16.2 Hz), 128.9, 128.8, 126.6, 112.1–111.9 (m), 41.0 ppm; IR (KBr) ν 3343, 2964, 1220 cm^{-1}; HRMS (ES^-) calculated for $C_{13}H_9F_2O$ $[M–H]^-$ 219.0627, found 219.0625.

3-Benzyl-2,6-difluorophenol (33): To a solution of **53** (0.100 g, 0.322 mmol, 1.00 equiv) in EtOH (3.2 mL) under N_2, $Pd(OAc)_2$ (~0.001 g, ~1 mol%) and activated carbon (0.010 g) were added. The resulting suspension was purged with H_2 for 10 min, then stirred for 45 min under an H_2 atmosphere (balloon). The reaction mixture was then purged with N_2 for 10 min, filtered through a pad of Celite® and thoroughly washed with EtOAc. Removal of the volatiles in vacuo provided the title compound (0.069 g, 0.313 mmol, 97%) as a pale yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (t, *J* = 7.4 Hz, 2H), 7.24–7.19 (m, 3H), 6.82 (td, *J* = 9.2, 1.7 Hz, 1H), 6.62 (td, *J* = 8.3, 5.9 Hz, 1H), 5.20 (s, 1H), 3.96 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.4 (dd, *J* = 240.8, 4.4 Hz), 150.1 (dd, *J* = 241.2, 4.7 Hz), 139.5, 132.9 (t, *J* = 16.3 Hz), 128.8, 128.7, 126.5, 124.7 (dd, *J* = 14.1, 3.5 Hz), 120.3 (dd, *J* = 8.2, 5.1 Hz), 111.1 (dd, *J* = 18.0, 3.7 Hz), 34.6 (d, *J* = 2.6 Hz) ppm; IR (KBr) ν 3390, 3085, 3063, 3029, 2929, 2853, 1604, 1501, 1471 cm^{-1}; HRMS (ES^-) calculated for $C_{13}H_9OF_2$ $[M–H]^-$ 219.0621, found 219.0625.

2-(Benzylthio)phenol (34): To a stirred suspension of $KHCO_3$ (0.508 g, 5.08 mmol, 1.05 equiv) in anhydrous DMF (5.0 mL) at r.t. under N_2, 2-hydroxythiophenol (0.500 mL, 4.83 mmol, 1.00 equiv) was added dropwise. The suspension was stirred for 5 min, then benzyl bromide (0.580 mL, 4.88 mmol, 1.01 equiv) was added dropwise and the mixture was stirred for 16 h. The reaction was quenched with satd. aq. NH_4Cl, then extracted with Et_2O (×5). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (7% EtOAc in hexanes) provided the title compound (0.972 g, 4.49 mmol, 93%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31–7.26 (m, 5H), 7.13–7.10 (m, 2H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.83 (t, *J* = 7.5

Hz, 1H), 6.58 (s, 1H), 3.87 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.2, 137.7, 136.5, 131.5, 128.9, 128.6, 127.5, 120.7, 118.3, 114.9, 41.5 ppm; IR (KBr) ν 3407, 3062, 3029, 2924, 1573, 1470, 1455 cm^{-1}; HRMS (ES^-) calculated for $C_{13}H_{11}OS$ $[M–H]^-$ 215.0531, found 215.0539.

2-(Benzylsulfinyl)phenol (35): To a solution of **34** (0.100 g, 0.462 mmol, 1.00 equiv) in glacial AcOH (3.80 mL), UHP (0.049 g, 0.509 mmol, 1.10 equiv) was added in one portion at r.t., and the resulting solution was stirred at r.t. for 20 h. The reaction mixture was diluted with satd. aq. $NaHCO_3$, and then extracted with CH_2Cl_2 (×4). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (30% EtOAc in hexanes) provided the title compound (0.105 g, 0.452 mmol, 98%) as a colorless crystalline solid. X-Ray quality crystals were obtained by slow vapor diffusion of hexanes into a solution of the product in THF at r.t. (see Supporting Information): mp (THF/hexanes) 134–134.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.07 (br s, 1H), 7.33–7.26 (m, 4H), 7.09 (d, J = 7.1 Hz, 2H), 6.94–6.88 (m, 2H), 6.83 (t, J = 7.5 Hz, 1H), 4.36 (d, J = 12.7 Hz, 1H), 4.27 (d, J = 12.7 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.2, 132.9, 130.6, 129.0, 128.6, 128.6, 125.9, 122.0, 119.6, 118.3, 60.5 ppm; IR (KBr) ν 3062, 3031, 2923, 2847, 2696, 2562, 1587, 1452 cm^{-1}; HRMS (ES^-) calculated for $C_{13}H_{11}O_2S$ $[M–H]^-$ 231.0480, found 231.0487.

2-(Benzylsulfonyl)phenol (36): To a solution of **34** (0.100 g, 0.462 mmol, 1.00 equiv) in CH_2Cl_2, *N*-methylmorpholine *N*-oxide (0.135 g, 1.15 mmol, 2.50 equiv) and K_2OsO_4•$2H_2O$ (0.008 g, 0.023 mmol, 5 mol%) were added at r.t., and the resulting solution was stirred for 20 h. The solvents were removed in vacuo. Purification by silica gel column chromatography (30% EtOAc in hexanes) provided the title compound (0.109 g, 0.439 mmol, 95%) as a colorless crystalline solid. X-Ray quality crystals were obtained by slow diffusion of hexanes into a solution of the product in EtOAc at r.t. (see Supporting Information): mp (EtOAc/hexanes) 105–105.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.47 (td, J = 7.8, 1.4 Hz, 1H), 7.39 (dd, J = 7.9, 1.6 Hz, 1H), 7.35 (t, J = 7.4 Hz, 1H), 7.27 (t, J = 7.6 Hz, 3H), 7.09 (d, J = 7.4 Hz, 2H), 6.92 (td, J = 7.7, 1.1 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 4.36 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.8, 136.7, 130.9, 129.8, 129.2, 128.8, 127.2, 120.3, 119.9, 118.6, 63.4

ppm; IR (KBr) ν 3340, 2929, 2848, 1596, 1474, 1453 cm $^{-1}$; HRMS (ES$^-$) calculated for $C_{13}H_{11}O_3S$ [M–H]$^-$ 247.0429, found 247.0438.

***tert*-Butyl (benzyloxy)carbamate (37) :** To a suspension of *O*-benzylhydroxylamine hydrochloride (0.798 g, 5.00 mmol, 1.00 equiv) in THF/H_2O (1:1, 10 mL), NEt_3 (0.700 mL, 5.00 mmol, 1.00 equiv) was added dropwise. A solution of di-*tert*-butyl dicarbonate (1.09 g, 5.00 mmol, 1.00 equiv) in THF (2.5 mL) was added dropwise at r.t. in 30 min, and the resulting solution was stirred at r.t. for 1.5 h. The volatiles were removed in vacuo, and the residue was taken up in EtOAc, then washed with 0.5 M citric acid (×2), H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo to provide the title compound (0.500 g, 2.24 mmol, 45%), which was directly used in the next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.31 (m, 5H), 7.10 (s, 1H), 4.86 (s, 2H), 1.48 (s, 9H) ppm.

***tert*-Butyl (benzyloxy)(phenethyl)carbamate (38) :** To a stirred solution of **37** (0.500 g, 2.24 mmol, 1.00 equiv) in anhydrous DMF (4.5 mL) under N_2, NaH (60% wt, 0.059 g, 2.46 mmol, 1.10 equiv) was added and the resulting solution was stirred at r.t. for 30 min. Phenethyl bromide (0.340 mL, 2.46 mmol, 1.10 equiv) was added dropwise and the reaction mixture was stirred at r.t. for 16 h. The reaction was poured into H_2O and extracted with hexanes (×3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (10% EtOAc in hexanes) provided the title compound (0.388 g, 1.19 mmol, 53%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56–6.95 (m, 10H), 4.82 (s, 2H), 3.62 (t, J = 7.6 Hz, 2H), 2.89 (t, J = 7.9, 7.5 Hz, 2H), 1.46 (s, 9H) ppm.

***N*-(Benzyloxy)-*N*-phenethylacetamide (39) :** A solution of **38** (0.264 g, 0.806 mmol, 1.00 equiv) in CH_2Cl_2/TFA (3:1 v/v, 2.00 mL) was stirred at r.t. for 18 h. The reaction mixture was made alkaline by addition of 1 M $NaHCO_3$ (4.2 mL) and extracted with CH_2Cl_2 (×3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo to provide *O*-benzyl-*N*-phenethylhydroxylamine (0.162 g, 0.713 mmol, 88%), which was used directly in the next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (m, 4H), 7.43–7.35 (m, 3H), 7.34–7.27 (m, 3H), 5.60 (s, 1H), 4.83 (s, 2H), 3.28 (t, J = 7.1 Hz, 2H), 2.95 (t, J = 7.1 Hz, 2H) ppm. To a solution of crude *O*-benzyl-*N*-

phenethylhydroxylamine (0.060 g, 0.267 mmol, 1.00 equiv) in CH_2Cl_2 (7.0 mL), DMAP (0.016 g, 0.134 mmol, 0.50 equiv), pyridine (0.043 mL, 0.534 mmol, 2.00 equiv) and Ac_2O (0.050 mL, 0.534 mmol, 2.00 equiv) were added successively. The solution was stirred at r.t. for 2 h. The reaction was diluted with CH_2Cl_2 and washed with satd. aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (30% EtOAc in hexanes) provided the title compound (0.056 g, 0.208 mmol, 79%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45–7.33 (m, 5H), 7.33–7.27 (m, 2H), 7.25–7.18 (m, 3H), 4.78 (s, 2H), 3.86 (bs, 2H), 2.94 (t, J = 7.7 Hz, 2H), 2.08 (d, J = 6.2 Hz, 3H) ppm.

2-Phenylethane-1-sulfonyl chloride (40): To a suspension of NCS (2.00 g, 14.9 mmol, 4.00 equiv) in CH_2Cl_2 (15 mL) at r.t., 2-phenylethanethiol (0.500 mL, 3.73 mmol, 1.00 equiv) was added dropwise, followed by H_2O (7.5 mL). A rapid color change (from colorless to yellow) and a vigorous bubbling were observed for 5 min. The resulting colorless biphasic mixture was stirred at r.t. for 3.5 h. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (×2). The combined organic extracts were washed with satd. aq. $NaHCO_3$ (×3), H_2O, brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (5% EtOAc in hexanes) afforded the title compound (0.683 g, 3.34 mmol, 90%) as a low-melting colorless solid: mp (CH_2Cl_2) 30–30.5 °C; Lit.[56] = 32–33 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.36 (m, 2H), 7.33–7.30 (m, 1H), 7.26–7.25 (m, 2H), 3.93–3.90 (m, 2H), 3.37–3.33 (m, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.7, 129.2, 128.6, 127.7, 66.3, 30.5 ppm; IR (KBr) ν 3030, 2924, 1497, 1456 cm^{-1}.

3-Phenylpropanenitrile (41): To a suspension of hydroxylamine hydrochloride (0.570 g, 8.20 mmol, 1.10 equiv) in DMSO (3.0 mL) at r.t., 3-phenylpropanal (0.999 g, 7.44 mmol, 1.00 equiv) was added. The resulting mixture was stirred at 90 °C for 3.5 h. After cooling to r.t., the reaction was diluted with H_2O (5 mL) and extracted with EtOAc (×2). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (10–20% EtOAc in hexanes) provided the title compound (0.600 g, 4.57 mmol, 61%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.34 (m, 2H), 7.31–7.28 (m, 1H), 7.27–7.24 (m, 2H), 2.97 (t, J =

7.4 Hz, 2H), 2.63 (t, *J* = 7.4 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2, 129.1, 128.4, 127.4, 119.3, 31.7, 19.5 ppm; IR (KBr) ν 3063, 3030, 2933, 2869, 2247, 1603, 1496, 1453 cm^{-1}; HRMS (ES^+) calculated for $C_9H_{10}N$ $[M+H]^+$ 132.0813, found 132.0819.

***N*-(2-Cyanoethyl)-3-phenylpropanamide (42):** To a solution of **1** (0.082 g, 0.547 mmol, 1.00 equiv), EDCI•HCl (0.262 g, 1.37 mmol, 2.5 equiv), and HOBt•H_2O (0.209 g, 1.55 mmol, 2.80 equiv) in DMF (2.8 mL) under N_2 at r.t., DIEA (0.380 mL, 2.19 mmol, 4.00 equiv) was added dropwise, followed 5 min later by 3-aminopropionitrile (0.100 mL, 1.37 mmol, 2.5 equiv). The resulting solution was stirred at r.t. for 15 h. The reaction was diluted with EtOAc, washed with 1 M HCl, H_2O, satd. aq. $NaHCO_3$, brine, The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to provide the title compound (0.105 g, 0.519 mmol, 95%) as a pale yellow solid, which was used directly in the next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.22 (m, 5H), 6.52 (br s, 1H), 3.46 (q, *J* = 6.2 Hz, 2H), 3.00 (t, *J* = 7.5 Hz, 2H), 2.58–2.53 (m, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 140.6, 128.6, 128.3, 126.4, 118.3, 37.9, 35.6, 31.5, 18.3 ppm.

***N′*-Hydroxy-3-phenylpropanimidamide (43):** To a solution of **41** (0.466 g, 3.60 mmol, 1.00 equiv) in EtOH (3.5 mL) in a microwave vial, hydroxylamine (50% wt in H_2O, 0.440 mL, 7.10 mmol, 1.97 equiv) was added, the vial was sealed, and the mixture was stirred at 75 °C for 5.5 h. After cooling to r.t., the solvents were evaporated in vacuo to provide the title compound (0.548 g, 3.30 mmol, 92%), which was directly used in the next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (br s, 1H), 7.31–7.21 (m, 5H), 4.55 (br s, 2H), 2.89 (t, *J* = 8.1 Hz, 2H), 2.46 (t, *J* = 8.1 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.7, 140.9, 128.7, 128.4, 126.4, 33.1 ppm.

5-(1-Hydroxy-2-phenylethylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (44): To a solution of freshly recrystallized Meldrum's acid (1.00 g, 6.94 mmol, 1.00 equiv) in CH_2Cl_2 (2.7 mL) at 0 ˚C under N_2, anhydrous pyridine (1.36 mL, 16.8 mmol, 2.40 equiv) was added dropwise in 10 min. Then, a solution of freshly distilled phenylacetyl chloride (0.920 mL, 6.94 mmol, 1.00 equiv) in anhydrous CH_2Cl_2 (2.2 mL) was added over 2 h, and the resulting solution was stirred at 0 ˚C for 2 h. The reaction was diluted with CH_2Cl_2, acidified with 2 M HCl, and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with 2 M HCl (×2), dried over Na_2SO_4, filtered, and concentrated in vacuo to provide the title compound (1.60 g, 6.10 mmol, 91%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, *J* = 7.3 Hz, 2H), 7.36–7.27 (m, 3H), 4.43 (bs, 2H), 1.72 (s, 6H) ppm.

Diethyl 1-benzyl-4-(benzyloxy)-5-oxocyclopent-3-ene-1,3-dicarboxylate (46): To a solution of **45**[51] (0.250 g, 0.785 mmol, 1.00 equiv) in anhydrous DMF (4.6 mL) under N_2, benzyl bromide (0.280 mL, 2.36 mmol, 3.00 equiv) was added in one portion at r.t., and the solution was stirred at 120 °C for 2 h. The reaction was diluted with EtOAc, then carefully quenched with 30% aq. AcOH. The aqueous layer was extracted with EtOAc (×3). The combined organic layers were washed with brine/1 M HCl (2:1), brine (×3), dried over $MgSO_4$, filtered, and concentrated in vacuo using toluene to azeotrope the residual DMF and AcOH. Purification by silica gel column chromatography (1–20% EtOAc in hexanes) provided the title compound (0.155 g, 0.367 mmol, 47%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.28 (m, 7H), 7.23–7.19 (m, 3H), 7.09–7.07 (m, 2H), 5.46 (d, *J* = 12.1 Hz, 1H), 5.40 (d, *J* = 12.1 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.24 (s, 2H), 3.07 (d, *J* = 17.9 Hz, 1H), 2.70 (d, *J* = 17.9 Hz, 1H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.8, 169.7, 163.8, 155.1, 136.6, 135.3, 130.0, 128.6, 128.4, 128.4, 128.2, 128.2, 128.1, 127.7, 127.1, 72.3, 66.3, 62.0, 61.1, 57.4, 39.4, 33.3, 32.0, 14.1, 14.0 ppm.

(*E*)-5-Phenylpent-4-ene-2,3-dione (47): To a solution of 3,3-dimethoxybutan-2-one (2.00 mL, 14.9 mmol, 1.00 equiv) and freshly distilled benzaldehyde (1.51 mL, 14.9 mmol, 1.00 equiv) in MeOH (35 mL), a solution of NaOH (0.776 g, 19.4 mmol, 1.30 equiv) in H_2O (11.5 mL) was added dropwise at r.t., and the resulting yellow solution was stirred for 24 h. The solvents were removed in vacuo, and the residue was extracted with EtOAc (×4). The combined organic extracts were washed with satd. aq. $NaHSO_3$ (×2), brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The yellow residue was dissolved in acetone (75 mL), then TsOH•H_2O (0.567 g, 2.98 mmol, 20 mol%) was added and the resulting mixture was stirred at r.t. for 38 h. The solvents were removed in vacuo, the residue was taken up in toluene, and washed with H_2O (until pH 7), brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (5–10% EtOAc in hexanes) afforded the title compound (1.89 g, 10.9 mmol, 73% over 2 steps) as a yellow crystalline solid: mp (CH_2Cl_2) 47–48 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, *J* = 16.2 Hz, 1H), 7.63–7.60 (m, 4H), 7.44–7.37 (m, 4H), 2.43 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 198.9, 186.8, 147.8, 134.5, 131.5, 129.1, 129.0, 118.0, 24.5 ppm; IR (KBr) ν 3446, 3058, 3026, 1714, 1683, 1605, 1576, 1449 cm^{-1}; HRMS (CI^+) calculated for $C_{11}H_{10}O_2$ $[M]^+$ 174.0681, found 174.0672.

3-Benzyl-4-ethoxycyclobut-3-ene-1,2-dione (48): To a solution of diethyl squarate (0.441 g, 2.59 mmol, 1.00 equiv) in anhydrous THF (10 mL) at 0 °C, benzyl magnesium bromide (0.6 M in THF, 5.49 mL, 3.29 mmol, 1.27 equiv) was added dropwise, and the solution was stirred at 0 °C for 10 min, then at r.t. for 30 min. The reaction was quenched with 3 M HCl and extracted with Et_2O (×3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (30% EtOAc in hexanes) afforded the title compound (0.460 g, 2.13 mmol, 82%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.15 (m, 5H), 4.75 (q, *J* = 7.1 Hz, 2H), 3.91 (s, 2H), 1.45 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 198.2, 194.6, 194.1, 181.2, 134.6, 128.9, 128.8, 127.3, 70.9, 30.8, 15.6 ppm.

4-Bromo-2,6-difluorophenol (49): To a solution of 2,6-difluorophenol (1.30 g, 10.0 mmol, 1.00 equiv) in anhydrous DMF at 0 °C under N_2, recrystallized NBS (1.87 g, 10.5 mmol, 1.05 equiv) was added in one portion, and the clear solution was stirred in the dark for 40 h while warming to r.t.. The reaction was quenched with H_2O, then extracted with Et_2O (×4). The combined organic extracts were washed with 1 M Na_2SO_3, brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (10% EtOAc in hexanes) provided the title compound (1.80 g, 8.63 mmol, 86%) as a yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12–7.06 (m, 2H), 5.17 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.8 (dd, *J* = 246.8, 6.3 Hz), 132.6 (t, *J* = 15.8 Hz), 115.8–115.5 (m), 110.4 (t, *J* = 10.9 Hz) ppm; IR (KBr) ν 3214, 2974, 1812, 1644 cm^{-1}; HRMS (ES^-) calculated for $C_6H_2BrF_2O$ $[M–H]^-$ 206.9257, found 206.9250.

2-(Benzyloxy)-5-bromo-1,3-difluorobenzene (50): A mixture of **49** (0.879 g, 4.20 mmol, 1.00 equiv) and K_2CO_3 (0.697 g, 5.04 mmol, 1.2 equiv) in acetone (21 mL) in a sealed tube was stirred at

70 °C for 1 h. After cooling to approximately 40 °C, benzyl bromide (0.600 mL, 5.04 mmol, 1.20 equiv) was added in a steady stream. The tube was sealed, warmed to 70° C and stirred for 3 h, then cooled to r.t. and stirred for 14 h. The reaction was diluted with acetone, filtered through a short pad of Celite®, then concentrated in vacuo. Purification by silica gel column chromatography (0–5% EtOAc in hexanes) provided the title compound (1.24 g, 4.15 mmol, 99%) as a pale yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55–7.49 (m, 2H), 7.48–7.38 (m, 3H), 7.16–7.06 (m, 2H), 5.24 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.2 (dd, *J* = 252.5, 6.5 Hz), 134.7 (t, *J* = 14.2 Hz), 116.1 (dd, *J* = 19.7, 6.9 Hz), 114.3 (t, *J* = 11.4 Hz), 76.1 (t, *J* = 3.2 Hz) ppm; IR (KBr) ν 3214, 2974, 1812, 1644 cm^{-1}; HRMS (ES$^+$) calculated for $C_{13}H_{10}BrF_2O$ $[M+H]^+$ 298.9878, found 298.9876.

5-Benzyl-2-(benzyloxy)-1,3-difluorobenzene (51): Anhydrous $ZnBr_2$ (1.08 g, 4.80 mmol, 2.40 equiv) was weighed in a round-bottom flask and flame-dried under high vacuum, cooled to r.t., then dissolved in THF (9.4 mL, previously sparged with Ar for 15 min) under Ar. A solution of benzylmagnesium chloride (0.33 M in THF, 12.1 mL, 3.99 mmol, 2.00 equiv) was added dropwise (formation of a colorless precipitate) and the resulting suspension was vigorously stirred for 15 min at r.t.. A solution of **50** (0.623 g, 2.00 mmol, 1.00 equiv) and PEPPSI-IPr® (0.068 g, 0.100 mmol, 5 mol%) in THF (6.0 mL, previously sparged with Ar for 15 min) was then cannulated dropwise, and the resulting orange mixture was stirred at r.t. for 16 h. The reaction was quenched carefully with 1 M HCl, then diluted with Et_2O and filtered through a short pad of Celite®. The layers were separated, and the aqueous layer was extracted with Et_2O (×3). The combined organic extracts were washed with satd. aq. $NaHCO_3$ (×2), brine, dried over $MgSO_4$, decolorized with activated carbon, filtered through a short pad of Celite®, and concentrated in vacuo. Purification by silica gel column chromatography (2–40% CH_2Cl_2 in hexanes) provided the title compound (0.459 g, 1.48 mmol, 74%) as a pale yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.2 Hz, 2H), 7.40–7.32 (m, 5H), 7.26 (t, *J* = 7.3 Hz, 2H), 7.17 (d, *J* = 7.4 Hz, 2H), 6.74–6.69 (m, 2H), 5.14 (s, 2H), 3.89 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.1 (dd, *J* = 248.6, 6.1 Hz), 139.7, 137.2 (t, *J* = 8.1 Hz), 136.7, 133.3 (d, *J* = 14.3 Hz), 129.0, 128.8, 128.6, 128.5, 128.4, 126.7, 126.7, 112.5 (dd, *J* = 17.3, 5.4 Hz), 76.2 (t, *J* = 3.0 Hz), 41.3 ppm; IR (KBr) ν 3424, 1514 cm^{-1}; HRMS (ES^+) calculated for $C_{13}H_{10}BrF_2O$ $[M+H]^+$ 298.9878, found 298.9876.

2-(Benzyloxy)-1,3-difluorobenzene (52): Prepared as **50**, starting from 2,6-difluorophenol (0.911 g, 7.01 mmol, 1.00 equiv), K_2CO_3 (1.16 g, 8.41 mmol, 1.2 equiv) and benzyl bromide (1.00 mL, 8.41 mmol, 1.20 equiv). Purification by silica gel column chromatography (0–10% CH_2Cl_2 in hexanes) provided the title compound (1.51 g, 6.87 mmol, 98%) as a colorless oil: ^1H NMR (500 MHz, $CDCl_3$) δ 7.51–7.49 (m, 2H), 7.42–7.35 (m, 3H), 7.00–6.87 (m, 3H), 5.21 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.5 (dd, *J* = 248.2, 5.6 Hz), 136.6, 135.3 (t, *J* = 14.1 Hz), 128.6, 128.5, 128.4, 123.1 (t, *J* = 9.2 Hz), 112.2 (dd, *J* = 17.3, 5.4 Hz), 76.1 (t, *J* = 3.1 Hz) ppm; IR (KBr) ν 3067, 3034, 2946, 2890, 1593, 1496, 1475 cm^{-1}.

1-Benzyl-3-(benzyloxy)-2,4-difluorobenzene (53): To a solution of *n*-BuLi (2.34 M in hexanes, 1.28 mL, 3.00 mmol, 1.50 equiv) in THF (6.0 mL, previously sparged with Ar for 15 min) under N_2 at –50 °C (dry ice/acetone bath) in a flame-dried round-bottom flask, a solution of **52** (0.440 g, 2.00 mmol, 1.00 equiv) in THF (3.0 mL, previously sparged with Ar for 15 min) was cannulated dropwise over 10 min at –50 °C. The resulting bright yellow solution was stirred at the same temperature for 35 min, during which the color changed from bright yellow to bright green, then pale yellow. Triisopropyl borate (0.740 mL, 3.20 mmol, 1.60 equiv) was added dropwise at –50 °C, then the reaction was warmed to r.t. and stirred for 1 h. To this crude mixture were added successively $[(PPh_3)_2Pd(Br)(Succ)•0.5\ CH_2Cl_2]$[54] (0.085 g, 0.100 mmol, 5 mol%), benzyl bromide (0.480 mL, 4.00 mmol, 2.00 equiv) and aq. $NaHCO_3$ (3.3 M in H_2O, previously sparged with Ar for 15 min, 1.50 mL, 5.0 mmol, 2.50 equiv). The resulting mixture was vigorously stirred at 60 °C for 14 h. After cooling to r.t., the reaction mixture was filtered through a pad of Celite®, thoroughly washed with Et_2O, then concentrated in vacuo. Purification by silica gel column chromatography (3–30% CH_2Cl_2 in hexanes) provided the title compound (0.259 g, 0.835 mmol, 42%) as a colorless oil: ^1H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.1 Hz, 2H), 7.39–7.33 (m, 3H), 7.30 (t, *J* = 7.6 Hz, 2H), 7.23 (t, *J* = 7.3 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 2H), 6.82–6.74 (m, 2H), 5.18 (s, 2H), 3.95 (s, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.9 (dd, *J* = 246.7, 4.9 Hz), 154.5 (dd, *J* = 247.7, 5.4 Hz), 139.6, 136.7, 135.2 (t, *J* = 14.7 Hz), 128.8, 128.7, 128.6, 128.5, 128.4, 126.5, 125.0 (dd, *J* = 14.9, 3.6 Hz), 124.1 (dd, *J* = 8.8, 5.6 Hz), 111.6 (dd, *J* = 19.2, 3.8 Hz), 76.1 (t, *J* = 3.2 Hz), 34.6 (d, *J* = 3.0 Hz) ppm; IR (KBr) ν 3063, 3030, 2919, 1498, 1453 cm^{-1}; HRMS (CI^+) calculated for $C_{20}H_{16}F_2O$ $[M]^+$ 310.1169, found 310.1171.

Determination of plasma unbound fraction: Plasma unbound fraction was determined using rapid equilibrium dialysis units (Thermo Scientific Pierce, Rockford, IL). Compounds at 50 mM in DMSO were diluted with pooled normal human plasma (Innovative Research, Novi, MI) to a final concentration of 5 μM. In duplicate, 100 μL aliquots of plasma were placed in sample chambers and dialyzed against 300 μL of phosphate buffered saline at 37 °C with gentle shaking. A dialysis unit was included for each compound at 5 μM in buffer to confirm stability and that equilibrium was reached over the length of the experiment. After 6 h, 20 μL aliquots were removed from each side of the dialysis membrane and mixed with an equal volume of plasma or buffer so that the matrix compositions were identical. Samples were extracted with 120 μL MeOH, vortexed and centrifuged at 6000*g* for 20 min at 4 °C. Supernatants were analyzed by LC-MS using an Acquity UPLC-TQ MS (Waters Corporation, Milford, MA). A number of H_2O/CH_3CN mobile phases were used to promote electrospray ionization. Methods were optimized for each compound using 0.1% formic acid, 10 mM ammonium formate, or 10 mM ammonium hydroxide. Sample injections, 2 μL, were separated using a gradient from 5% to 95% acetonitrile over 2 min on an Acquity BEH C18 column (1.7 μm, 2.1 × 50 mm) at 6 μL/min and 35 °C. Compounds were detected using selected ion recording (SIR). In 4 cases were matrix effects interfered with compound detection, multiple reaction monitoring (MRM) of a specific collision induced ion was used. Fraction unbound (*fu*) was calculated as the buffer chamber to plasma chamber peak area ratio.

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List of Nonstandard Abbreviations: CDI = 1,1'-carbonyldiimidazole; DIAD = diisopropyl azodicarboxylate; EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt = hydroxybenzotriazole; $(PPh_3)_2Pd(Br)(Succ)$ = *trans*-Bromo(*N*-succinimidyl)bis(triphenylphosphine)-palladium(II); UGTs = uridine 5'-diphospho-glucuronosyl-transferase.

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Supporting Information Available: NMR spectra of test compounds; X-ray crystal structures of compounds **1** (CCDC 1428182), **2** (CCDC 1427573), **3** (CCDC 1427548), **5** (CCDC 1427579), **6** (CCDC 1427789), **8** (CCDC 1427589), **10** (CCDC 1427790), **11** (CCDC 1428190), **12** (CCDC 1428042), **13** (CCDC 1427584), **14** (CCDC 1427791), **15** (CCDC 1428191), **16** (CCDC 1428195), **17** (CCDC 1427583), **18** (CCDC 1427834), **19** (CCDC 1428038), **20** (CCDC 1428058), **22** (CCDC 1428049), **26** (CCDC 1427564), **28** (CCDC 1436469), **32** (CCDC 1427567), **35** (CCDC 1427585), **36** (CCDC 1427787). Authors will release the atomic coordinates and experimental data upon article publication; experimental details for the permeability assay (PAMPA), $logD_{7.4}$ and pK_a determinations, as well as UV-Vis titrations; the SMILES string structures along the full data set in tabular form (csv file format). This material is available free of charge via the Internet at http://pubs.acs.org.

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