

Synthesis, Biological Evaluation, and Structure–Activity Relationships of *N*-Benzoyl-2-hydroxybenzamides as Agents Active against *P. falciparum* (K1 strain), *Trypanosomes*, and *Leishmania*

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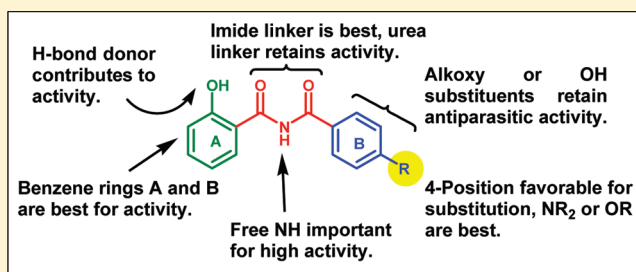
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ABSTRACT: In our efforts to identify novel chemical scaffolds for the development of new antiprotozoal drugs, a compound library was screened against *Toxoplasma gondii* tachyzoites with activity discovered for *N*-(4-ethylbenzoyl)-2-hydroxybenzamide **1a** against *T. gondii* as described elsewhere. Synthesis of a compound set was guided by *T. gondii* SAR with **1r** found to be superior for *T. gondii*, also active against Thai and Sierra Leone strains of *Plasmodium falciparum*, and with superior ADMET properties as described elsewhere. Herein, synthesis methods and details of the chemical analysis of the compounds in this series are described. Further, this series of *N*-benzoyl-2-hydroxybenzamides was repurposed for testing against four other protozoan parasites: *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, and *P. falciparum* (K1 isolate). Structure–activity analyses led to the identification of compounds in this set with excellent antileishmanial activity (compound **1d**). Overall, compound **1r** was the best and had activity 21-fold superior to that of the standard antimalarial drug chloroquine against the K1 *P. falciparum* isolate.



INTRODUCTION

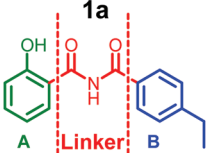
Protozoan parasites cause diseases such as malaria, amoebiasis, giardiasis, toxoplasmosis, leishmaniasis, and trypanosomiasis and are among the most serious public health problems in developing countries. Some of these parasites also cause major health problems in the U.S., Europe, and Japan.¹ According to the World Health Organization (WHO), parasitic protozoan diseases plague billions of people worldwide and kill millions annually.² Nevertheless, few medicines are available to treat those serious and sometimes lethal infections. Human African trypanosomiasis (HAT, sleeping sickness), caused by *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) and *T. b. gambiense*, threatens about 50 million people living in sub-Saharan Africa and causes an estimated 25000 deaths per year.³ There are an estimated 10 million people suffering from American trypanosomiasis (Chagas disease), which is caused by *Trypanosoma cruzi* (*T. cruzi*).⁴ Visceral leishmaniasis is caused by *Leishmania donovani* (*L. donovani*) and currently threatens 350 million persons around the world.⁴ *Plasmodium falciparum* (*P. falciparum*) is one of the causative agents of malaria and

infects about 225 million people and causes nearly 800000 deaths, mostly in children, annually.⁵ Overall, in the absence of vaccines and with few chemotherapeutics available, some with reduced efficacy and adverse effects, diseases caused by protozoan parasites still contribute significantly to morbidity and mortality. Thus, discovery and development of effective, safe and affordable antiprotozoal agents remains an urgent need.

In our efforts to identify novel chemical scaffolds as potent antiprotozoal drugs, a small compound library (ASDI) was screened initially against *Toxoplasma gondii* tachyzoites as described elsewhere.¹ Moderate activity was found for *N*-(4-ethylbenzoyl)-2-hydroxybenzamide **1a** (Figure 1) against the *T. gondii* parasites.¹ Interestingly, some salicylimides, among other compounds, have been reported to possess antimalarial activity ascribed to their targeting the hemozoin detoxification protein (HDP), a protein known to be vital for *P. falciparum*.⁶ It is also

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		IC ₅₀ (μg/mL), [μM]	
Standard		1a	
<i>T. b. rhod.</i>	melarsoprol	0.005, [0.013]	81, [301]
<i>T. cruzi</i>	benznidazole	0.48, [1.8]	4.8, [18]
<i>L. don. axe.</i>	miltefosine	0.19, [0.47]	7.4, [27]
<i>P. falc. K1</i>	chloroquine	0.11, [0.34]	5.6, [21]
L6	podophyllotoxin	0.004, [0.01]	70, [260]

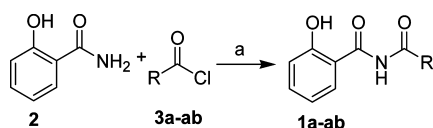
Figure 1. Hit compound 1a.

known that certain salicylamide derivatives, such as salicylhydroxamic acid, nitazoxanide, and other thiazolidines, display antiprotozoal activity.^{7–11} Herein, we report the details of the synthesis, modification, and biological evaluation of this set of *N*-benzoyl-2-hydroxybenzamides and related compounds, as potent, and in some cases selective, agents against the K1 isolate of *P. falciparum* and *L. donovani*. We furthermore studied their effects on *T. b. rhodiense* and *T. cruzi*.

CHEMISTRY

The desired *N*-benzoyl-2-hydroxybenzamides (**1a–ab**) were generated by the reaction of salicylamide (**2**) with various acid chlorides (**3a–ab**) in refluxing pyridine (Scheme 1). The crude

Scheme 1. Preparation of *N*-Benzoyl-2-hydroxybenzamides^a



^aReagents and conditions: (a) pyridine, reflux, 4 h.

products (mostly solids) were subsequently purified by crystallization or preparative HPLC to give the pure compounds in yields ranging from modest to very good (Table 1).

The hydroxybenzamides **1k** and **1ab** were further elaborated as shown in Scheme 2. Catalytic hydrogenation over palladium-on-carbon of compounds **1k** and **1ab** provided corresponding aniline **4a** and phenol **4b** derivatives in good yields (Scheme 2). Reaction of compound **4b** with methyl 3-(chlorocarbonyl)propanoate provided compound **5** in modest yield (Scheme 2). The hydroxamic acid **8** was prepared in a three-step procedure starting with protection of the hydroxyl group in **4a** as its silyl ether.¹² Subsequent reaction with 8-[(benzyloxy)amino]-8-oxooctanoyl chloride¹³ gave the intermediate **7**, which was deprotected to provide compound **8** in low yield (Scheme 2).

To better understand the importance of the imide linker for biological activity, modification of this moiety was investigated. Syntheses are shown in Scheme 3. The *N*-methyl imide **10** was prepared in high yield from 4-ethylbenzoyl chloride (**3a**) and *N*-methylsalicylamide (**9**)¹⁴ by following the standard procedure described above. In the same manner, the acylated salicylhydrazide **12** was prepared from 4-ethylbenzoyl chloride (**3a**) and salicylhydrazide (**11**) (Scheme 3). Reaction of the original hit compound **1a** with Lawesson's reagent in refluxing chlorobenzene provided the thioimide derivative **13** (Scheme 3).

Further modifications of the linker resulted in synthesis of *N*-[(4-ethylphenyl)carbamoyl]-2-hydroxybenzamide (**16**) and 1-(4-ethylphenyl)-3-(2-hydroxyphenyl)urea (**19**) (Scheme 4). The intermediate **15** was readily obtained from salicylamide following a known procedure.¹⁵ Standard hydrogenolysis of **15** gave the compound **16** in 69% yield (Scheme 4). The compound **19** bearing a urea linker was prepared by the reaction of isocyanate **17** with 4-ethylaniline and subsequent deprotection (Scheme 4).¹⁶

Several compounds **21a–c** bearing linkers that are chemically more stable than an imide function were analyzed. The compounds were prepared from the corresponding imide/amine precursors (**20a–c**) as shown in Scheme 5 by following the standard procedure described above. The oxadiazole **23** was prepared from **1a** by cyclodehydration in the presence of hydroxylamine hydrochloride and sodium acetate (Scheme 5).¹⁷ Reaction of 2-(benzyloxy)aniline (**24**) with ethyl 3-chloro-3-oxopropanoate gave the intermediate **25**, which was readily hydrolyzed to the carboxylic acid **26**.¹³ This intermediate was converted to the final bis-amide **28** through amide bond formation¹³ with 4-ethylaniline and subsequent deprotection (Scheme 5).¹⁸

RESULTS AND DISCUSSION

In search of compounds with improved activity and ADMET properties,¹ we modified the hit **1a** at three sites: the phenol ring (A), 4-ethylphenyl ring (B), and the imide linker (Figure 1).¹ ADMET studies, described in detail elsewhere,¹ revealed metabolic instability to be the main concern for **1a**.¹ Briefly, the compound was found to be quickly metabolized in human liver microsomes (HLM), and only 0.5% of the compound remained after incubation for 60 min in the presence of NADPH.¹ However, in the absence of NADPH, 53% of the compound was left unchanged after the incubation.¹ The potassium ion channel coded by *hERG* was not inhibited by **1a** at compound concentration as high as 300 μM.¹ Among the recombinant cytochrome P450 (CYP450) isozymes, 3A4, 2D6, and 2C9, only the latter was inhibited to the extent of 71% at a compound concentration of 10 μM.¹ The aqueous solubility in phosphate-based buffer of compound **1a** was 2.76 μM at pH 4.0 and 50.8 μM at pH 7.4, respectively (for comparison, the control compound diclofenac exhibits the following solubility: 9.10 μM at pH 4.0 and 303 μM at pH 7.4).¹ The tested compound presented a similar permeability as the control drugs testosterone and methotrexate.¹ However, the protein binding in human plasma of **1a** was high (99.9%) in comparison with the control drug, fluconazole (23.7%), and both its intrinsic clearance and its metabolism rate in HLM (282 μL/min/mg proteins) were faster than that of the control verapamil (38.8 μL/min/mg proteins).¹ Nonetheless, these results encouraged us to modify the hit **1a** to generate a lead compound, with the improvement of both antiprotozoal potency and metabolic stability as the primary goal.¹

All of the compounds **1a–ab**, **4a**, **4b**, **5**, **8**, **10**, **12**, **13**, **16**, **19**, **21a–c**, **23**, and **28** were evaluated for in vitro activity against four pathogenic protozoa: *T. b. rhodiense* (trypomastigote stage, STIB 900 strain), *T. cruzi* (intracellular amastigote stage, Tulahuen C4 strain), *L. donovani* (axenic amastigote stage, MHOM-ET-67/L82), and *P. falciparum* (erythrocytic stage, K1 strain, which is a chloroquine and pyrimethamine resistant strain). The toxicity of the designed compounds was assessed against a mammalian primary cell line derived from rat skeletal myoblasts (L6). The following drugs were used as the reference

Table 1. Variation of the Ring B in *N*-Benzoyl-2-hydroxybenzamides (1)

Compound	a	b	c	d	e	f	g
R							
Yield (%) ^a	51	85	22	95	55	17	29
Compound	h	i	j	k	l	m	n
R							
Yield (%) ^a	38	91	14	42	33	37	77
Compound	o	p	q	r	s	t	u
R							
Yield (%) ^a	62	39	31	8	25	29	28
Compound	v	w	x	y	z	aa	ab
R							
Yield (%) ^a	24	41	41	33	48	83	30

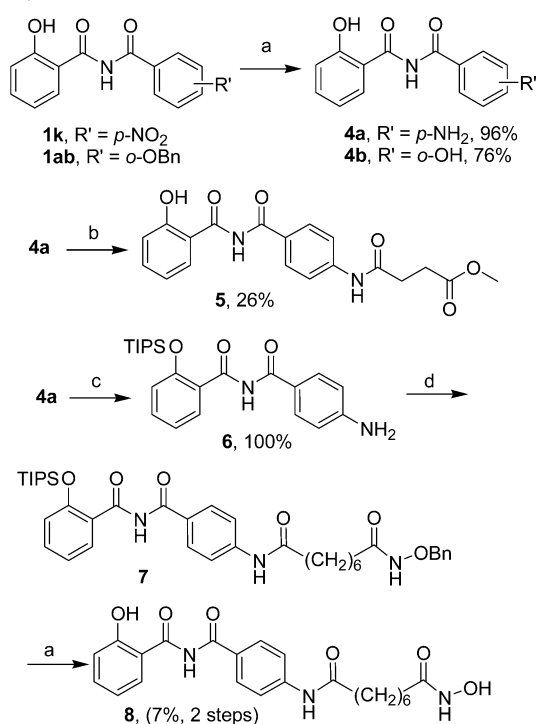
^aIsolated yield of product exhibiting >95% HPLC purity.

compounds: melarsoprol (*T. b. rhodesiense*), benznidazole (*T. cruzi*), miltefosine (*L. donovani*), chloroquine (*P. falciparum*), and podophyllotoxin (L6). The IC₅₀ values presented below indicate the concentration of the tested compound in μg/mL that is necessary to inhibit 50% of the parasite growth. The displayed numbers are the average values collected from two replicate experiments. The selectivity index is the ratio between the IC₅₀ for rat skeletal myoblasts (L6) and the IC₅₀ for the respective protozoan parasite. The screening data are presented in three tables. Table 2 shows compounds bearing various *para*-substituents on the aromatic B ring (1a–s, 4a, 5, and 8). Table 3 presents compounds with other modification of the B ring (1t–aa, 4b), and Table 4 provides results for the structures with modifications in the linker and the A ring (10–28).

We were pleased to find that many of the synthesized analogues were more potent against the tested parasites than the hit 1a, showing that various substituents in the *para*-position of the B ring improved the desired antiparasitic activity (Table 2). Compounds 1n, 1o, and 1r, bearing ethoxy, *n*-butoxy, and *N,N*-diethylamino substituents, respectively, showed reasonable activity against *T. cruzi*, good activity against *L. donovani*, and very good activity against *P. falciparum*

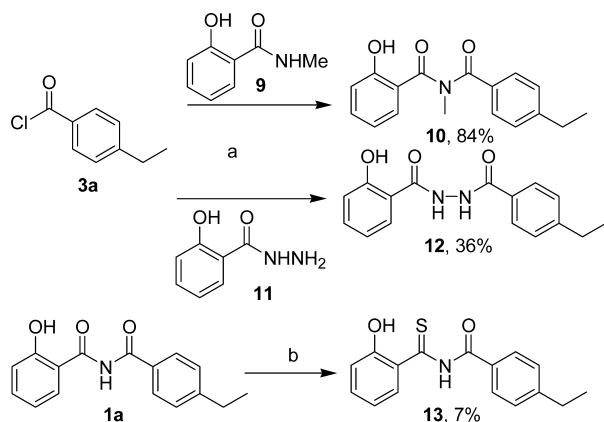
(K1 strain). Compound 1r exhibited the best IC₅₀ value against *P. falciparum* (K1 strain) among all the tested compounds. The simple replacement of ethyl in 1a by *tert*-butyl in 1d led to good and very good activity against *L. donovani* and *P. falciparum* (K1 strain), respectively. The potency of compound 1d against *L. donovani* was comparable to that of miltefosine. Compounds bearing other alkyl substituents (ethyl 1a, isopropyl 1c) or aromatic groups (phenyl 1e, benzyl 1p) displayed similar potency against *T. cruzi*, *L. donovani*, and *P. falciparum* (K1 strain). Compounds with methyl (1b), fluoro (1f), methoxy (1m), and amino (4a) groups also had similar activity as compounds 1a,c,e,p, however, only against *L. donovani* and *P. falciparum* (K1 strain). Compound 1q possessing a *para*-*N,N*-dimethylamino substituent turned out to be not only potent but very selective against *P. falciparum* (K1 strain). It was unexpected for us to see such dramatic change in the selectivity for compounds 1q,r and 4a by introducing such minor structural modifications. The morpholinyl-substituted compound 1s showed reasonable activity and selectivity against malaria parasites. To our surprise, compound 8 bearing the octanedioic acid amide hydroxyamide side chain was modestly active only against *P. falciparum* (K1 strain). The benzoic acid

Scheme 2. Elaboration of Selected *N*-Benzoyl-2-hydroxybenzamides^a



^aReagents and conditions: (a) Pd/C, H₂, MeOH, room temp, 18 h; (b) methyl 3-(chlorocarbonyl)propanoate, pyridine, CH₂Cl₂, room temp, 1 h; (c) TIPSCl, Et₃N, DMAP, DMF, room temp, 3.5 h; (d) 8-[(benzyloxy)amino]-8-oxooctanoyl chloride, pyridine, CH₂Cl₂, room temp, 2 h.

Scheme 3. Modifications of the Linker in *N*-Benzoyl-2-hydroxybenzamides^a

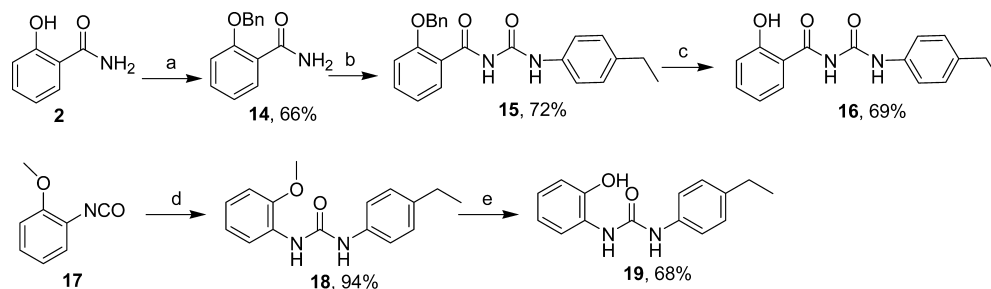


^aReagents and conditions: (a) pyridine, reflux, 4 h; (b) Lawesson's reagent, chlorobenzene, 135 °C, 3 h.

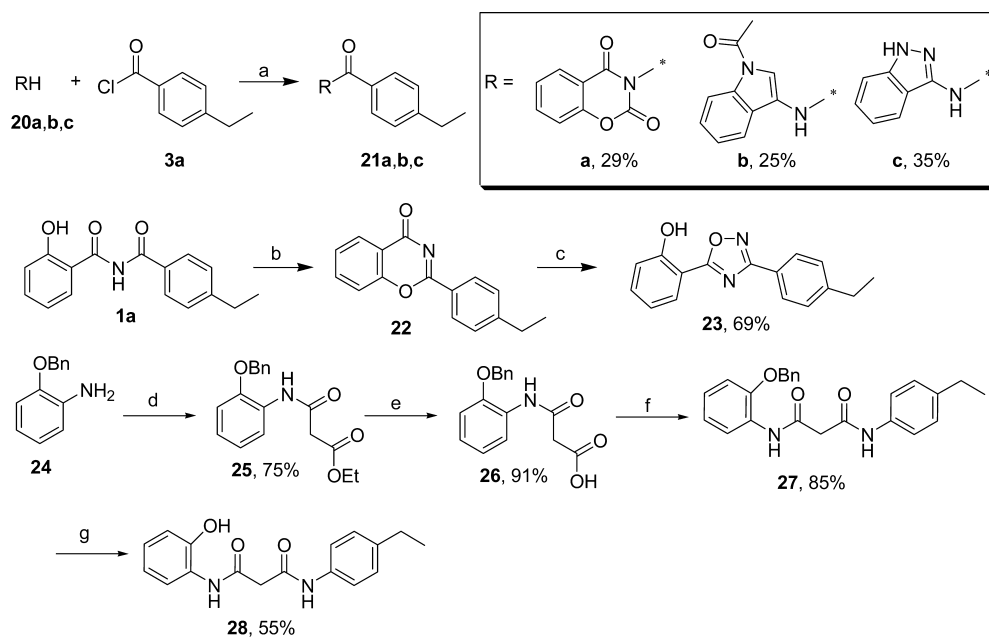
methyl ester analogue **11** gave similar results. In contrast to these results, compound **5** containing a succinamic acid methyl ester side chain was inactive against all species. On the other hand, the analogue **1g** containing a trifluoromethyl group is the only compound in this series that is potent and at the same time reasonably selective against *L. donovani*. Compound **1h** bearing a trifluoromethoxy group also appeared to be selective against *L. donovani* although significantly less potent (around 10-fold) than compound **1g**. The methanesulfonyl group in compound **1j** caused complete loss of inhibitory activity against

all tested parasites. Against *T. cruzi*, modest inhibitory activity was displayed by several compounds already mentioned above (**1a,c-e,n,p,r**). Unfortunately, none of the compounds presented in Table 2 were active against *T. b. rhodesiense*.

Summarizing the SAR data shown in Table 2, we observe a clear relationship between structural modifications at the *para* position of the B ring and antileishmanial and anti-*P. falciparum* (K1 strain) activity. Some of the most active compounds against *P. falciparum* (K1 strain) are those with bulky alkyl substituents such as *tert*-butyl (**1d**). Reduction of the substituent size to ethyl (**1a**), methyl (**1b**), or even isopropyl (**1c**) resulted in decreased potency. This finding would suggest the presence of a large hydrophobic space in the binding pocket able to accommodate branched substituents in the area contiguous to the *para* position of the B ring. In comparison with the preferred *tert*-butyl group, a phenyl substituent (compound **1e**) causes reduction in activity, therefore suggesting the preference for an alkyl group in the binding site. Antiplasmodial potency is enhanced by introduction of bulky and strongly electron-donating *N* or *O* substituents. Compound **1r** with *N,N*-diethylamino substitution turns out to be the most active and selective in this series against malaria parasites. Its *N,N*-dimethylamino analogue **1q** and the morpholinyl-substituted compound **1s** both exhibit good potency and moderate selectivity against the same parasite. The high potency of these compounds not only reiterates the significance of hydrophobic interactions involving lipophilic partial structures of the 4-substituents but also points to potential dipolar interactions or H-bonding between the ligand and the binding pocket. The aniline **4a**, which lacks the ability to engage in hydrophobic interactions via its 4-substituent, is far less active against *P. falciparum* (K1 strain) than compounds bearing a dialkylated nitrogen atom (**1q,r,s**). Potency enhancement by large, aliphatic, heteroatom-containing groups is also apparent among alkoxy-substituted compounds. High potency is observed for compounds **1n** and **1o** with ethoxy and *n*-butoxy substituents, respectively. However, the relatively small trifluoromethoxy (compound **1h**) and methoxy (compound **1m**) groups along with the large, aromatic benzyloxy substituent (compound **1p**) are less potent in comparison with **1n,o**. Enhanced potency is also observed for analogues bearing small electron-withdrawing groups such as fluoro (compound **1f**) and trifluoromethyl (compound **1g**). Other electron-withdrawing groups such as cyano (compound **1i**), methanesulfonyl (compound **1j**), and nitro (compound **1k**) lead to loss of potency; as an exception, the methoxycarbonyl-substituted compound **1l** maintains fair potency against *P. falciparum* (K1 strain). The modest activity of compound **8** bearing the octanedioic acid amide hydroxyamide moiety in comparison with the inactive compound **5** that contains a succinamic acid methyl ester side chain would suggest an alternative mode of action for the former compound against *P. falciparum* (K1 strain). On the basis of these results, we can characterize the binding site for *N*-benzoyl-2-hydroxybenzamides *para*-substituted in their B ring as a fairly large hydrophobic site with preference for branched alkyl substituents as well as strongly electron-donating groups, such as dialkylamino and alkoxy, that are able to accept H-bonds. All in all, the excellent potency of compound **1r** along with its negligible cytotoxicity against the L6 cell line (selectivity index, SI = 1640) make this compound a promising lead for the further development of antimalarial drugs. This compound has been evaluated for its ADMET properties elsewhere,¹ which

Scheme 4. Synthesis of the Carbamoylsalicylamide **16** and the Urea **19**^a

^aReagents and conditions: (a) BnBr, K₂CO₃, acetone, reflux, 16 h; (b) (1) (COCl)₂, CH₂Cl₂, room temp to 50 °C, 3 h, (2) 4-ethylaniline, MeCN, room temp, 18 h; (c) 10% Pd/C, H₂, MeOH, room temp, 24 h; (d) 4-ethylaniline, CH₂Cl₂, room temp, 19 h; (e) BBr₃, CH₂Cl₂, -78 to 0 °C.

Scheme 5. Preparation of Compounds with Modified Linkers^a

^aReagents and conditions: (a) pyridine, reflux, 4 h; (b) 200 °C, 1 h; (c) NH₂OH·HCl, NaOAc, absolute EtOH, 16 h; (d) pyridine, CH₂Cl₂, ethyl 3-chloro-3-oxopropanoate, room temp, 18 h; (e) THF, LiOH, room temp, overnight; (f) DIPEA, BOP-Cl, 4-ethylaniline, room temp, 22 h; (g) Pd(OH)₂/C (25 wt %), EtOH–cyclohexene (2:1), reflux, 2.5 h.

turned out to be more favorable than those of the hit **1a**.¹ Additionally, compounds **1q** and **1s** have reasonable selectivity index values (110 and 272, respectively) and can thus also be regarded as attractive leads.

Similar trends in the SAR were noticed for *L. donovani*, suggesting that this protozoan possesses a homologous molecular target for this series of compounds. The most active compounds were the *tert*-butyl analogue **1d**, the trifluoromethyl analogue **1g**, the ethoxy and *n*-butoxy compounds **1n** and **1o**, as well as the *N,N*-dimethylamino compound **1r**. Decreased steric bulk of the substituents resulted in a decrease (compounds **1a,b,c,h,m**) or complete loss (compound **1q**) of potency. Reduced activity was also observed for compounds **1e,p** containing aromatic substituents and for the morpholinyl derivative **1s**. This would suggest a strict cutoff for the size of the *para* substituent at the *L. donovani* binding site. Complete loss of activity was observed for the compounds bearing large and strongly electron-withdrawing groups (**1i,j,k,l**), or bearing long polar chains (**5** and **8**). The *L. donovani* binding site tolerates small electron-withdrawing groups (compounds **1f,g**) as well as or better than that of *P. falciparum* (K1 strain). This

result may assist in designing further structural modifications aimed at providing potent and selective compounds against *L. donovani*.

In analogy to the SAR for *P. falciparum* (K1 strain) and *L. donovani*, the most active compounds against *T. cruzi* were analogues with branched and electron-donating substituents (**1a,c,d,n**, and **r**). However, all of them were less potent against this protozoan than against *P. falciparum* (K1 strain) and *L. donovani*. On the other hand, compounds bearing electron-withdrawing groups resulted in the complete loss of antiparasitic activity (**1f,g,i–l**). Surprisingly, the aromatic substituents in **1e** and **1p** retained moderate activity against *T. cruzi*, indicating possible π – π interaction in the binding pocket. None of the preceding compounds turned out to be active against *T. b. rhodesiense*.

In the final series of compounds, we probed the influence of other modifications of the B ring. Encouragingly, we observed an improvement in the antiparasitic activity for some of the synthesized compounds (Table 3). The 2-naphthyl analogue **1t**, the 6-methoxy-2-naphthyl analogue **1u**, and the 2,3,4-trimethoxyphenyl analogue **1y** were the most active against *P.*

Table 2. Effect of Variation in the *para*-Substituent of the B Ring on Biological Activity

compd	<i>T. b. rhodesiense</i>		<i>T. cruzi</i>		<i>L. donovani</i> axe.		<i>P. falciparum</i> (K1)		L6
	IC ₅₀ ^a	SI ^b	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀
1a	81	0.86	4.8	15	7.4	9.3	5.6	12	70
1b	>100	<0.46	38	1.2	3.2	14	1.4	33	46
1c	65	0.29	2.0	9.5	1.3	14	1.50	12	19
1d	61	0.038	6.0	0.38	0.14	17	0.029	80	2.3
1e	87	>1.2	7.7	>13	7.4	>14	3.9	>26	>100
1f	64	0.60	51	0.75	1.6	23	1.5	25	38
1g	70	0.19	50	0.26	0.57	23	3.8	3.4	13
1h	65	1.3	72	1.2	5.3	16	22	3.8	84
1i	>100	<0.55	100	0.55	12	4.7	14	3.9	55
1j	>100	1.0	51	>2.0	>100	1.0	38	2.7	>100
1k	>100	<0.53	56	0.94	11	4.9	22	2.4	53
1l	>50	<0.83	20	2.1	>50	<0.83	2.9	14	42
1m	50	0.78	31	1.3	1.7	23	1.6	24	39
1n	60	0.28	4.1	4.1	0.45	37	1.0	17	17
1o	55	0.23	11	1.2	0.94	14	0.26	50	13
1p	62	1.0	4.4	15	1.5	42	4.1	16	64
1q	81	0.66	37	1.4	18	3.0	0.49	110	54
1r	>50	<0.16	2.8	2.9	0.91	9.1	0.005	1640	8.2
1s	>50	1.0	>50	1.0	16	>3.1	0.18	>272	>50
4a	46	0.84	40	0.98	7.8	5.0	3.9	10	39
5	>50	1.0	>50	1.0	22	>2.3	21	>2.4	>50
8	65	>1.5	49	>2.1	>100	1.0	3.5	>29	>100
standard	0.005 ^c		0.48 ^d		0.19 ^e		0.11 ^f		0.004 ^g

^aValues indicate the inhibitory concentration of a compound or standard in $\mu\text{g}/\text{mL}$ that is necessary to achieve 50% growth inhibition (IC₅₀). Data shown in the table represent mean values from two independent determinations. ^bSelectivity index = (IC₅₀ for L6)/(IC₅₀ for the respective protozoan parasite). ^cStandard compound: melarsoprol. ^dStandard compound: benznidazole. ^eStandard compound: miltefosine. ^fStandard compound: chloroquine. ^gStandard compound: podophyllotoxin.

Table 3. Effect of Other Modifications of the B Ring in *N*-Benzoyl-2-hydroxybenzamides on Biological Activity

compd	<i>T. b. rhodesiense</i>		<i>T. cruzi</i>		<i>L. donovani</i> axe.		<i>P. falciparum</i> (K1)		L6
	IC ₅₀ ^a	SI ^b	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀
1a	81	0.86	4.8	15	7.4	9.3	5.6	12	70
1t	26	0.78	8.5	2.4	1.0	20	0.22	94	21
1u	42	1.3	5.1	11	1.4	40	0.98	57	56
1v	20	0.087	17	0.10	0.16	11	4.4	0.40	1.8
1w	>100	<0.62	58	1.1	11	5.5	14	4.4	62
1x	>50	<0.47	27	0.86	>50	<0.47	4.2	5.6	24
1y	79	0.26	40	0.51	3.2	6.5	0.78	26	21
1z	>100	<0.55	16	3.4	6.0	9.2	13	4.4	55
1aa	48	0.33	36	0.44	2.0	7.8	4.7	3.4	16
4b	62	0.63	14	2.8	1.9	21	1.1	36	39
standard	0.005 ^c		0.48 ^d		0.19 ^e		0.11 ^f		0.004 ^g

^aValues indicate the inhibitory concentration of a compound or standard in $\mu\text{g}/\text{mL}$ that is necessary to achieve 50% growth inhibition (IC₅₀). Data shown in the table represent mean values from two independent determinations. ^bSelectivity index = (IC₅₀ for L6)/(IC₅₀ for the respective protozoan parasite). ^cStandard compound: melarsoprol. ^dStandard compound: benznidazole. ^eStandard compound: miltefosine. ^fStandard compound: chloroquine. ^gStandard compound: podophyllotoxin.

falciparum (K1 strain). Compound **1t** showed activity at low concentration (only 2-fold less potent than the standard) against malaria parasites. Also compounds **1u** and **1y** displayed potency within the same range as **1t** against the same protozoan. We also observed modest potency of these compounds (**1t,u,y**) against *L. donovani*. Compound **1aa** with 2-methyl-3-furyl as the B ring, and the symmetric analogue **4a** showed dual activity against malaria and leishmania parasites. However, both were less active than **1t,u,y**. The piperonyl-substituted compound **1x** turned out to be weakly active only against *P. falciparum* (K1 strain) and thus is somewhat selective

against this protozoan. Compounds with 2,4-dichlorophenyl (**1w**) or cyclohexyl (**1z**) as their B rings were inactive against *P. falciparum* (K1 strain).

Many of the analogues presented in Table 3 showed moderate (corresponding to micromolar) activity against *L. donovani*, and the 3-chloromethyl analogue **1v** even had activity slightly better than the antileishmanial drug miltefosine. However, the selectivity index of compound **1v** was only 11, which makes this compound less viable for further development. Analogues **1z** with a cyclohexyl B ring and **1aa** with a 2-methyl-3-furyl B ring turned out to be moderately active only

Table 4. Effect of Variations in the Linker and A Ring on Biological Activity

compd	<i>T. b. rhodesiense</i>		<i>T. cruzi</i>		<i>L. donovani</i> axe.		<i>P. falciparum</i> (K1)		L6
	IC ₅₀ ^a	SI ^b	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀
1a	81	0.86	4.8	15	7.4	9.3	5.6	12	70
10	>100	<0.094	29	0.33	9.1	1.0	28	0.33	9.4
12	17	0.28	2.2	2.2	6.2	0.77	27	0.18	4.8
13	61	0.28	10	1.7	5.3	3.3	4.2	4.1	17
16	36	2.0	74	0.95	100	0.70	26	2.8	70
19	18	0.54	7.0	1.4	3.7	2.6	2.1	4.6	9.6
21a	83	0.31	8.8	3.0	1.9	13	6.9	3.7	26
21b	>50	<0.46	28	<0.81	>50	<0.46	23	1.0	23
21c	8.2	1.0	8.9	0.93	3.0	2.8	8.5	0.96	8.2
23	61	>1.6	39	>2.6	>100	>1.0	27	>3.7	>100
28	35	0.44	20	0.76	13	1.2	12	1.3	16
standard	0.005 ^c		0.48 ^d		0.19 ^e		0.11 ^f		0.004 ^g

^aValues indicate the inhibitory concentration of a compound or standard in $\mu\text{g}/\text{mL}$ that is necessary to achieve 50% growth inhibition (IC₅₀). Data shown in the table represent mean values from two independent determinations. ^bSelectivity index = (IC₅₀ for L6)/(IC₅₀ for the respective protozoan parasite). ^cStandard compound: melarsoprol. ^dStandard compound: benznidazole. ^eStandard compound: miltefosine. ^fStandard compound: chloroquine. ^gStandard compound: podophyllotoxin.

against *L. donovani*. Compounds **1w** and **1x** were completely inactive against leishmania parasites. The fact that compounds with B rings other than benzene still retained moderate activity against *L. donovani* when compared to other protozoa provides valuable guidance for the design of further potential antileishmanial agents. Besides the hit compound **1a**, the naphthyl derivatives **1t** and **1u** were the only compounds moderately active against *T. cruzi*. In accord with the previous observations, none of these compounds were active against *T. b. rhodesiense* parasites.

Concluding the SAR for 2-hydroxybenzamides presented in Table 3, we can state that the identity of the B ring as benzene seems to be important for the anti-*P. falciparum* (K1 strain) activity as presented by **1t,u,v,y**. However, cyclohexane (compound **1z**) and furan (compound **1aa**) substitutes retain moderate antileishmanial activity. Free phenol functionality (compound **4b**) is tolerated, but alkoxy substituents (compounds **1x,y**) provide a better contribution to the compounds' antimalarial activity. Although significantly cytotoxic, the (chloromethyl)phenyl analogue **1v** provided excellent potency against *L. donovani*, whereas 2,4-dichlorophenyl (compound **1w**) gave a completely inactive analogue. These conclusions are in good agreement with the initial SAR described for the *para* position of *N*-benzoyl-2-hydroxybenzamides (Table 2) and confirm the importance of the aromatic nature of the ligand's B ring, preferably bearing electron-donating groups attached through a heteroatom.

At the same time, we also prepared analogues with modified linker and A ring moieties. A series of 10 compounds was prepared and screened for antileishmanial, -trypanosomal, and -plasmodium (K1 strain) activity (Table 4). We found that most of the modifications caused a decrease in anti-*P. falciparum* (K1 strain) activity. Only the sulfur analogue **13** and urea-linked compound **19** were slightly more potent against malaria parasites than the hit compound **1a**.¹ On the other hand, the benzoxazinanedione **21a** and indazolylbenzamide **21c** were slightly less active against the same parasites than the hit **1a**. Although anti-*P. falciparum* (K1 strain) activity was retained by compounds **13**, **19**, and **21a,c**, their small selectivity indices suggest safety problems. The above observations support the notion that the H-bond donor-acceptor pattern in the imide linker is crucial for antiparasitic

activity. This was additionally supported by methylation of the nitrogen atom in the imide linker, which gave the inactive compound **10**.

Pleasingly, the present set of structural modifications provided some compounds with improved potency against *L. donovani*. Good antileishmanial activity was shown by compounds which had already proven activity against *P. falciparum* (K1 strain) (**13**, **19**, and **21a,c**). In addition to these compounds, the *N*-methylated compound **10** and the acylated salicylhydrazide **12** showed modest potency against *L. donovani*. As in the case of compounds active against malaria parasites, all the compounds active against leishmania parasites suffered from lack of selectivity (SI < 5). Four compounds, the acylated salicylhydrazide **12**, the urea-linked compound **19**, the benzoxazinanedione **21a**, and the indazolylbenzamide **21c**, were only moderately active against *T. cruzi*. The only compound, which inhibited at moderate concentration the growth of *T. b. rhodesiense* was **21c**. Despite its moderate activity, this compound was 10-fold more potent than the parent compound **1a**. Contrary to this, compounds which had chemically stable linkers, i.e., the acylated urea **16**, indolylbenzamide **21b**, diphenyloxadiazole **23**, and bis-amide **28**, turned out to be inactive across all four parasites. Summarizing the SAR for this series of compounds, we can state that the imide linker with an unsubstituted *N* atom is crucial for antimalarial activity. However, we were pleased to see that compounds bearing other linker/A ring combinations, such as the urea **19**, the benzoxazinanedione **21a**, and the indazolylbenzamide **21c**, retained moderate antileishmanial activity.

CONCLUSIONS

This research provides solid ground for the discovery of new and effective agents to treat protozoan infections that cause malaria and leishmaniasis. The hit compound **1a** was discovered by initial HTS screening against *T. gondii*,¹ and the preliminary ADMET results, while encouraging, revealed this agent to be metabolically unstable in HLM.¹ The metabolic instability is ascribed to the imide linker, and therefore extensive modifications of this moiety as well as the B-ring were pursued.¹ Several promising lead compounds active against the K1 strain of *P. falciparum* and *L. donovani* were identified. Among the tested parasites *P. falciparum* (K1 strain)

was the most sensitive to these *N*-benzoyl-2-hydroxybenzamide. Compound **1r** is 21-fold more potent against the K1 isolate of malaria than the standard drug, chloroquine. Moreover, it exhibits an SI value of 1640, suggesting both high selectivity and low toxicity for this compound. The SAR for *T. gondii* reported elsewhere is similar to that described for *P. falciparum* K1 isolate.¹ Efficacy of compound **1a** was found to be much lower for other malaria isolates, and **1r** was also found less effective in different types of assays¹ for other *P. falciparum* or other plasmodial isolates.

Additionally, the electron-donating diethylamino group in compound **1r** stabilizes the imide linker, thus improving the chemical and metabolic stability of this analogue. With its excellent antiplasmodial potency, this highly selective agent may be used as a possible lead for the further development of antimalarial agents. Although analogue **1g** is 4 times less potent than **1d**, it shows better selectivity against *L. donovani* parasites than malaria, therefore both compounds are attractive leads for further research including assessment of activity in infected macrophages. Compound **1v**, with a 3-chloromethyl substituent on the B ring, might also be regarded as an interesting compound for the development of antileishmanial agents. Moreover, the fact that the *L. donovani* binding site better tolerates small electron-withdrawing groups provides a good starting point for the design of more potent and selective agents to combat this protozoan. Compounds **1c** and **12** are the most potent members of this series against *T. cruzi*. Because of its hydrolytic stability, the latter compound can be considered an attractive scaffold for further work. *T. b. rhodesiense* was generally resistant to the compounds used in this study, and compound **21c** was the only one showing weak activity against this parasite. Studies to determine IC₅₀s and whether the compounds are cidal will be important in the future. While the data generated in this research may be useful in the design and synthesis of novel agents with improved potencies and safety margins to treat infections caused by *L. donovani* and *P. falciparum* (K1 strain), we call attention to the fact that it will be essential to study the potential teratogenicity of these imides, as they do bear some structural relationship to teratogen thalidomide.

EXPERIMENTAL SECTION

Antiprotozoal Assays. The *in vitro* activities against *T. b. rhodesiense*, *T. cruzi*, *L. donovani*, and *P. falciparum* (K1 strain) were determined as described earlier.¹⁹ The following strains and parasite forms were used: *T. b. rhodesiense*, STIB900, trypomastigote form; *T. cruzi*, Tulahuen C2C4, amastigote form in L6 rat myoblasts; *L. donovani*, MHOM/ET/67/L82, axenic amastigote form; *P. falciparum*, K1 (chloroquine and pyrimethamine resistant) erythrocytic stage.

Chemistry. All chemicals and solvents were purchased from Sigma-Aldrich and/or Fisher Scientific and were used without further purification. Anhydrous THF and CH₂Cl₂ were obtained by distillation over sodium wire or CaH₂, respectively. All nonaqueous reactions were carried out under argon atmosphere with exclusion of moisture from reagents, and all reaction vessels were oven-dried. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker spectrometer at 400 and 100 MHz, respectively, and were referenced to the residual peaks of CHCl₃ at 7.26 ppm or DMSO-*d*₆ at 2.50 ppm (¹H NMR) and CDCl₃ at 77.23 ppm or DMSO-*d*₆ at 39.51 ppm (¹³C NMR). Chemical shifts were reported in parts per million downfield of TMS and the following abbreviations used to denote coupling patterns: s = singlet; d = doublet; t = triplet; q = quartet; br = broad; fs = fine splitting. Mass spectra were measured in the ESI mode at an ionization potential of 70 eV with an LC-MS MSD (Hewlett-Packard). HRMS experiments were performed on a Q-TOF-2TM instrument

(Micromass). TLC was performed on Merck 60 F₂₅₄ silica gel plates. Column chromatography was performed using Merck silica gel (40–60 mesh). Preparative HPLC was carried out on an ACE 5 AQ column (150 mm × 20 mm), with detection at 254 and 280 nm on a Shimadzu SPD-10A VP detector; flow rate = 17 mL/min; gradient from 8% to 100% methanol in water (both solvents containing 0.05 vol% of CF₃COOH). Purities of final compounds were established by analytical HPLC, which was carried out on an Agilent 1100 HPLC system with a Synergi 4 μ Hydro-RP 80A column, with detection at 254 nm on a variable wavelength detector G1314A; flow rate = 1.4 mL/min; gradient from 10% to 100% methanol in water (both solvents containing 0.05 vol% of CF₃COOH) within 20 min. All the final compounds were of purity ≥95% as determined by the method described above.

General Procedure for the Synthesis of Imide Compounds.

To a solution of amide (5 mmol) in pyridine (6 mL) was added an acyl chloride (5 mmol). After refluxing for 4 h, the resulting mixture was diluted with Et₂O (30 mL)/H₂O (20 mL) or H₂O (20 mL)/1.0 M HCl (5 mL). The resulting precipitate was collected by filtration, washed with water, and dried, or the resulting mixture was diluted with EtOAc, washed with H₂O (20 mL) and brine (20 mL), dried over Na₂SO₄, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel or recrystallization from MeOH. Noncommercial acyl chlorides were obtained from the corresponding carboxylic acid by the following procedure: to a solution of acid (5 mmol) in anhydrous CH₂Cl₂ (6 mL) was added (COCl)₂ (6 mmol) and 1 drop of DMF at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred for 2 h. After evaporation of the solvent, the residue was used without purification in the next step. Alternatively, the acid was dissolved in SOCl₂ and heated at 80 °C for 1.5–3 h before cooling to room temperature and evaporating the volatiles *in vacuo*. The residue was used in the next step without further purification.

Hydrogenation reactions were carried out with 10% Pd/C (10–15 wt %) and a H₂ balloon in MeOH as the solvent for 18 h at room temperature.

***N*-(4-Ethylbenzoyl)-2-hydroxybenzamide (1a).** The general procedure was used with salicylamide (**2**) and 4-ethylbenzoyl chloride (**3a**) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (51%). ¹H NMR (DMSO-*d*₆) δ 11.70 (s, 1H), 11.60 (s, 1H), 7.72–7.88 (m, 3H), 7.28–7.42 (m, 3H), 6.88–6.98 (m, 2H), 2.62 (q, *J* = 8.0 Hz, 2H), 1.13 (t, *J* = 8.0 Hz, 3H). ¹³C NMR (DMSO-*d*₆) δ 164.8, 164.2, 156.4, 149.4, 134.3, 131.3, 131.1, 128.4, 127.9, 120.0, 119.1, 117.1, 28.8, 15.3. HPLC purity 99.4%. MS (ESI) *m/z* 270 (M + H)⁺. HRMS (ESI) calcd for C₁₆H₁₆NO₃ 270.1125 (M + H)⁺, found 270.1131.

2-Hydroxy-*N*-(4-methylbenzoyl)benzamide (1b). The general procedure was used with salicylamide (**2**) and 4-methylbenzoyl chloride (**3b**) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (85%). ¹H NMR (DMSO-*d*₆) δ 11.88 (s, 1H), 11.77 (s, 1H), 7.88 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.46 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 2H), 6.96–7.08 (m, 2H), 2.36 (s, 3H). ¹³C NMR (DMSO-*d*₆) δ 164.7, 164.1, 156.4, 143.3, 134.3, 131.1, 131.0, 129.5, 127.8, 120.0, 119.1, 117.1, 99.6, 21.1. HPLC purity 97.6%. HRMS (ESI) calcd for C₁₅H₁₄NO₃ 256.0968 (M + H)⁺, found 256.0978.

2-Hydroxy-*N*-(4-isopropylbenzoyl)benzamide (1c). The general procedure was used with salicylamide (**2**) and 4-isopropylbenzoyl chloride (**3c**) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (22% over two steps). ¹H NMR (CD₃OD/CDCl₃) δ 8.09 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 2H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.00 (m, 2H), 3.00 (q, *J* = 6.8 Hz, 1H), 1.28 (d, *J* = 6.8 Hz, 6H). ¹³C NMR (CD₃OD/CDCl₃) δ 167.1, 166.2, 158.0, 156.1, 136.0, 132.8, 132.3, 128.9, 128.1, 121.6, 119.3, 117.9, 35.4, 24.0. HPLC purity 97.6%. MS (ESI) *m/z* 284 (M + H)⁺.

***N*-(4-*tert*-Butylbenzoyl)-2-hydroxybenzamide (1d).** The general procedure was used with salicylamide (**2**) and 4-*tert*-butylbenzoyl chloride (**3d**) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (95%). ¹H

NMR (DMSO- d_6) δ 11.94 (s, 1H), 11.91 (s, 1H), 7.84–7.92 (m, 3H), 7.57 (d, J = 8.0 Hz, 2H), 7.43 (dt, J = 8.0, 2.0 Hz, 1H), 6.94–7.05 (m, 2H), 1.31 (s, 9H). ^{13}C NMR (DMSO- d_6) δ 164.8, 164.2, 156.4, 156.0, 134.3, 131.1, 127.7, 125.8, 120.0, 119.1, 117.1, 34.9, 30.9; HPLC purity 98.0%. HRMS calcd for $\text{C}_{18}\text{H}_{19}\text{NO}_3$ 297.1365 (M) $^+$, found 297.1369.

N-(2-Hydroxybenzoyl)-[1,1'-biphenyl]-4-carboxamide (1e). The general procedure was used with salicylamide (2) and biphenyl-4-carbonyl chloride (3e) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (54% over two steps). ^1H NMR (DMSO- d_6) δ 11.8 (s, 1H), 11.7 (s, 1H), 8.02 (d, J = 4.0 Hz, 2H), 7.89 (t, J = 8.0 Hz, 3H), 7.77 (d, J = 8.0 Hz, 2H), 7.49 (m, 4H), 7.01 (q, J = 8.0 Hz, 2H). ^{13}C NMR (DMSO- d_6) δ 164.6, 164.3, 156.4, 144.4, 138.8, 134.2, 132.5, 131.0, 129.1, 128.4, 127.1, 127.0, 120.0, 119.1, 117.0, 99.5. HPLC purity 98.9%. MS (ESI) m/z 318 (M + H) $^+$.

N-(4-Fluorobenzoyl)-2-hydroxybenzamide (1f). The general procedure was used with salicylamide (2) and 4-fluorobenzoyl chloride (3f) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (17%). ^1H NMR (DMSO- d_6) δ 11.82 (s, 2H), 7.90–8.05 (m, 2H), 7.84 (dd, J = 7.6, 2.0 Hz, 1H), 7.36–7.48 (m, 3H), 6.94–7.02 (m, 2H). ^{13}C NMR (DMSO- d_6) δ 166.0, 164.3 (d, J = 172 Hz), 163.6, 156.4, 134.3, 131.0, 130.7 (d, J = 38 Hz), 130.3 (d, J = 12 Hz), 119.9, 119.2, 117.1, 116.1, 115.9. HPLC purity 96.0%. HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{11}\text{FNO}_3$ 260.0717 (M + H) $^+$, found 260.0720.

2-Hydroxy-N-(4-(trifluoromethyl)benzoyl)benzamide (1g). The general procedure was used with salicylamide (2) and 4-(trifluoromethyl)benzoyl chloride (3g) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (29%). ^1H NMR (DMSO- d_6) δ 11.64 (s, 1H), 11.46 (s, 1H), 8.09 (d, J = 8.0 Hz, 2H), 7.91 (d, J = 8.0 Hz, 2H), 7.84 (dd, J = 8.0, 2.0 Hz, 1H), 7.46 (dt, J = 8.0, 2.0 Hz, 1H), 6.90–7.04 (m, 2H), 3.85 (s, 3H). ^{13}C NMR (DMSO- d_6) δ 164.8, 164.6, 156.6, 137.8, 134.4, 132.4 (q, J = 32 Hz), 131.0, 129.2 (2C), 125.8 (q, J = 4 Hz), 123.5 (q, J = 270 Hz), 119.9, 119.0, 117.1. HPLC purity 98.0%. HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{11}\text{F}_3\text{NO}_3$ 310.0685 (M + H) $^+$, found 310.0689.

2-Hydroxy-N-(4-(trifluoromethoxy)benzoyl)benzamide (1h). The general procedure was used with salicylamide (2) and 4-(trifluoromethoxy)benzoyl chloride (3h) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (38%). ^1H NMR (DMSO- d_6) δ 11.7 (br s, 2H), 8.04 (d, J = 8.0 Hz, 2H), 7.85 (dd, J = 8.0, 4.0 Hz, 1H), 7.56 (d, J = 8.0 Hz, 2H), 7.46 (t, J = 8.0 Hz, 1H), 7.01 (m, 2H). ^{13}C NMR (DMSO- d_6) δ 164.7, 164.3, 156.5, 151.2 (q, J = 1.8 Hz), 134.3, 133.0, 131.0, 130.3 (2C), 121.1 (2C), 119.9 (q, J = 258 Hz), 119.8, 119.2, 117.1. HPLC purity 99.0%. MS (ESI) m/z 326 (M + H) $^+$.

N-(4-Cyanobenzoyl)-2-hydroxybenzamide (1i). The general procedure was used with salicylamide (2) and 4-cyanobenzoyl chloride (3i) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (91%). ^1H NMR (DMSO) δ 11.73 (s, 2H), 8.04 (app s, 4H), 7.84 (dd, J = 7.6, 2.0 Hz, 1H), 7.46 (dt, J = 7.6, 2.0 Hz, 1H), 6.95–7.05 (m, 2H), 2.36 (s, 3H). ^{13}C NMR (DMSO) δ 164.7, 164.4, 156.6, 137.9, 134.4, 132.6, 131.0, 128.5, 119.9, 119.0, 118.4, 117.5, 114.9. HPLC purity 96.4%. HRMS (ESI) calcd for $\text{C}_{15}\text{H}_9\text{N}_3\text{O}_3$ 265.0619 (M - H) $^+$, found 265.0629.

2-Hydroxy-N-[4-(methylsulfonyl)benzoyl]benzamide (1j). The general procedure was used with salicylamide (2) and 4-(methylsulfonyl)benzoyl chloride (3j), prepared from the corresponding carboxylic acid) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a pale-pink solid (90 mg, 14%). ^1H NMR (DMSO- d_6) δ 11.73 (br s, 1H), 11.61 (br s, 1H), 8.14–8.09 (m, 4H), 7.84 (dd, J = 7.9, 1.7 Hz, 1H), 7.47 (ddd, J = 8.5, 7.3, 1.8 Hz, 1H), 7.03–6.98 (m, 2H), 3.31 (s, 3H). ^{13}C NMR (DMSO- d_6) δ 164.8, 164.6, 156.5, 144.0, 138.4, 134.3, 130.9, 128.8, 127.4, 119.8, 119.0, 117.0, 43.2. HPLC purity 99.7%. MS (ESI) m/z 320 (M + H) $^+$.

2-Hydroxy-N-(4-nitrobenzoyl)benzamide (1k). The general procedure was used with salicylamide (2) and 4-nitrobenzoyl chloride (3k) as reactants. Purification of the crude material by preparative

HPLC gave the title compound as a white solid (42%). ^1H NMR (DMSO- d_6) δ 11.8 (s, 1H), 11.7 (br s, 1H), 8.36 (d, J = 8.0 Hz, 2H), 8.11 (d, J = 8.0 Hz, 2H), 7.87 (d, J = 8.0 Hz, 1H), 7.44 (t, J = 8.0 Hz, 1H), 6.99 (m, 2H). ^{13}C NMR (DMSO- d_6) δ 164.5, 164.1, 156.6, 149.6, 139.4, 134.3, 130.9, 129.1, 123.7, 119.7, 118.5, 117.0. HPLC purity 99.8%. MS (ESI) m/z 287 (M + H) $^+$.

Methyl 4-[(2-hydroxybenzoyl)carbamoyl]benzoate (1l). The general procedure was used with salicylamide (2) and 4-(chlorocarbonyl)benzoic acid methyl ester (3l, prepared from the corresponding carboxylic acid) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (200 mg, 33%). ^1H NMR (DMSO- d_6) δ 11.75 (br s, 2H), 8.11 (d, J = 8.3 Hz, 2H), 8.03 (d, J = 8.3 Hz, 2H), 7.86 (dd, J = 7.7, 1.1 Hz, 1H), 7.46 (td, J = 7.5, 1.4 Hz, 1H), 7.04–6.97 (m, 2H), 3.90 (s, 3H). ^{13}C NMR (DMSO- d_6) δ 165.5, 164.6, 164.5, 137.8, 134.3, 133.0, 131.0, 129.5, 128.1, 119.9, 119.0, 117.0, 52.5. HPLC purity 99.5%. MS (ESI) m/z 300 (M + H) $^+$.

2-Hydroxy-N-(4-methoxybenzoyl)benzamide (1m). The general procedure was used with salicylamide (2) and 4-methoxybenzoyl chloride (3m) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (37%). ^1H NMR (DMSO- d_6) δ 11.81 (s, 1H), 11.72 (s, 1H), 7.80–7.91 (m, 3H), 7.46 (dt, J = 8.4, 2.0 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 6.90–7.05 (m, 2H), 3.85 (s, 3H). ^{13}C NMR (DMSO- d_6) δ 164.3, 163.0, 156.4, 134.2, 131.1, 129.2, 125.9, 119.9, 119.2, 117.1, 114.2, 99.6, 55.7. HPLC purity 95.0%. HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{14}\text{NO}_4$ 272.0917 (M + H) $^+$, found 272.0927.

N-(4-Ethoxybenzoyl)-2-hydroxybenzamide (1n). The general procedure was used with salicylamide (2) and 4-ethoxybenzoyl chloride (3n) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (77%). ^1H NMR (DMSO- d_6) δ 11.87 (s, 1H), 11.56 (s, 1H), 7.90–8.05 (m, 3H), 7.46 (dt, J = 8.4, 2.0 Hz, 1H), 6.93–7.05 (m, 4H), 4.13 (d, J = 4.0 Hz, 2H), 1.30 (t, J = 4.0 Hz, 3H). ^{13}C NMR (DMSO- d_6) δ 164.2, 162.3, 156.4, 134.2, 131.1, 129.9, 125.6, 120.0, 119.2, 117.0, 114.6, 63.7, 14.6. HPLC purity 99.6%. HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{14}\text{NO}_4$ 284.0928 (M - H) $^+$, found 284.0936.

N-(4-Butoxybenzoyl)-2-hydroxybenzamide (1o). The general procedure was used with salicylamide (2) and 4-butoxybenzoyl chloride (3o) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (62%). ^1H NMR (CDCl_3) δ 8.12 (d, J = 8.4 Hz, 2H), 7.90 (m, 1H), 7.50 (m, 1H), 7.32 (m, 1H), 7.20 (d, J = 8.0 Hz, 1H), 6.97 (d, J = 8.4 Hz, 2H), 6.48 (br, 1H), 6.33 (br, 1H), 4.05 (d, J = 6.4 Hz, 2H), 1.81 (m, 2H), 1.52 (m, 2H), 0.99 (d, J = 7.2 Hz, 3H). ^{13}C NMR (CDCl_3) δ 167.6, 164.8, 164.2, 148.6, 132.6, 132.4, 130.7, 127.7, 126.4, 123.5, 120.8, 114.8, 68.3, 31.2, 19.3, 14.0. HPLC purity 99.8%. MS (ESI) m/z 314 (M + H) $^+$.

N-[4-(Benzyloxy)benzoyl]-2-hydroxybenzamide (1p). The general procedure was used with salicylamide (2) and 4-(benzyloxy)benzoyl chloride (3p) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (39%). ^1H NMR (DMSO- d_6) δ 11.7 (br s, 1H), 11.6 (br s, 1H), 7.87 (m, 3H), 7.41 (m, 6H), 7.17 (d, J = 8.0 Hz, 2H), 7.00 (m, 2H), 5.22 (s, 2H). ^{13}C NMR (DMSO- d_6) δ 164.2, 164.1, 162.0, 156.3, 136.5, 134.2, 131.0, 129.8, 128.5, 128.1, 127.8, 126.0, 119.9, 119.2, 117.0, 115.0, 69.6. HPLC purity 98.3%. MS (ESI) m/z 348 (M + H) $^+$.

N-[4-(Dimethylamino)benzoyl]-2-hydroxybenzamide (1q). The general procedure was used with salicylamide (2) and 4-(dimethylamino)benzoyl chloride (3q) as reactants. Purification of the crude material by preparative HPLC gave the title compound as yellow solid (31%). ^1H NMR (DMSO- d_6) δ 11.8 (s, 1H), 11.5 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 8.0 Hz, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.02 (m, 2H), 6.77 (d, J = 8.0 Hz, 2H), 3.03 (s, 6H). ^{13}C NMR (DMSO- d_6) δ 164.6, 164.4, 156.7, 153.6, 134.5, 131.5, 130.0, 120.3, 120.0, 119.6, 117.5, 111.5, 40.8. HPLC purity 98.3%. MS (ESI) m/z 285 (M + H) $^+$.

N-[4-(Diethylamino)benzoyl]-2-hydroxybenzamide (1r). The general procedure was used with salicylamide (2) and 4-(diethylamino)benzoyl chloride (3r, prepared from the corresponding

carboxylic acid) as reactants. Purification of the crude material by preparative HPLC gave the title compound as yellow solid (8% over two steps). ^1H NMR (DMSO- d_6) δ 11.8 (br s, 1H), 11.5 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 8.0 Hz, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.00 (m, 2H), 6.73 (d, J = 8.0 Hz, 2H), 3.41 (q, J = 8.0 Hz, 4H), 1.12 (t, J = 8.0 Hz, 6H). ^{13}C NMR (DMSO- d_6) δ 164.6, 164.4, 156.8, 151.2, 134.5, 131.5, 130.2, 120.3, 119.6, 119.0, 117.5, 110.9, 44.3, 12.7. HPLC purity 98.5%. MS (ESI) m/z 313 (M + H) $^+$.

2-Hydroxy-*N*-(4-morpholinobenzoyl)benzamide (1s). The general procedure was used with salicylamide (2) and 4-morpholinobenzoyl chloride (3s, prepared from the corresponding carboxylic acid) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (25% over two steps). ^1H NMR (DMSO- d_6) δ 11.8 (br s, 1H), 11.5 (s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 8.0 Hz, 2H), 7.46 (t, J = 8.0 Hz, 1H), 7.00 (m, 4H), 3.75 (t, J = 4.0 Hz, 4H), 3.30 (t, J = 4.0 Hz, 4H). ^{13}C NMR (DMSO- d_6) δ 164.8, 164.5, 156.7, 154.4, 134.5, 131.5, 129.8, 122.8, 120.4, 119.6, 117.5, 113.8, 66.3, 47.3. HPLC purity 98%. MS (ESI) m/z 327 (M + H) $^+$.

***N*-(2-Hydroxybenzoyl)-2-naphthamide (1t).** The general procedure was used with salicylamide (2) and naphthalene-2-carbonyl chloride (3t, prepared from the corresponding carboxylic acid) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (170 mg, 29% over two steps). ^1H NMR (DMSO- d_6) δ 11.89 (br s, 2H), 8.58 (s, 1H), 8.10 (m, 2H), 8.04 (d, J = 7.6 Hz, 1H), 7.96 (d, J = 8.5 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.71–7.64 (m, 2H), 7.47 (t, J = 7.5 Hz, 1H), 7.06 (d, J = 8.1 Hz, 1H), 7.01 (d, J = 7.5 Hz, 1H). ^{13}C NMR (DMSO- d_6) δ 165.1, 164.4, 156.6, 134.8, 134.2, 132.1, 131.1, 131.0, 129.2, 128.7, 128.6, 128.5, 127.7, 127.2, 123.8, 119.8, 119.1, 117.1. HPLC purity 100%. MS (ESI) m/z 292 (M + H) $^+$.

***N*-(2-Hydroxybenzoyl)-6-methoxy-2-naphthamide (1u).** The general procedure was used with salicylamide (2) and 6-methoxynaphthalene-2-carbonyl chloride (3u, prepared from the corresponding carboxylic acid) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a pale-yellow solid (180 mg, 28% over two steps). ^1H NMR (DMSO- d_6) δ 11.79 (s, 1H), 11.76 (s, 1H), 8.51 (s, 1H), 8.02 (d, J = 8.8 Hz, 1H), 7.97 (d, J = 8.8 Hz, 1H), 7.92 (dd, J = 3.4, 1.8 Hz, 1H), 7.90 (m, 1H), 7.50–7.45 (m, 2H), 7.29 (dd, J = 8.9, 2.5 Hz, 1H), 7.05 (d, J = 8.1 Hz, 1H), 7.01 (t, J = 7.7 Hz, 1H), 3.93 (s, 3H). ^{13}C NMR (DMSO- d_6) δ 165.0, 164.4, 159.3, 156.4, 136.7, 134.2, 131.1, 130.9, 128.6, 127.4, 127.3, 124.4, 119.9, 119.8, 119.2, 117.1, 106.0, 55.5. HPLC purity 97.2%. MS (ESI) m/z 322 (M + H) $^+$.

***N*-[3-(Chloromethyl)benzoyl]-2-hydroxybenzamide (1v).** The general procedure was used with salicylamide (2) and 3-(chloromethyl)benzoyl chloride (3v) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (24%). ^1H NMR (DMSO- d_6) δ 11.85 (s, 2H), 7.99 (s, 1H), 7.84–7.90 (m, 2H), 7.73 (d, J = 7.6 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.46 (dt, J = 7.6, 1.6 Hz, 1H), 6.90–7.02 (m, 2H), 4.87 (s, 2H). ^{13}C NMR (DMSO- d_6) δ 164.7, 164.4, 156.5, 138.7, 134.3, 134.2, 133.2, 131.1, 129.3, 128.3, 127.5, 120.0, 119.1, 117.1, 45.5. HPLC purity 96.2%. HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{13}\text{ClNO}_3$ 290.0578 (M + H) $^+$, found 290.0584.

2,4-Dichloro-*N*-(2-hydroxybenzoyl)benzamide (1w). The general procedure was used with salicylamide (2) and 2,4-dichlorobenzoyl chloride (3w) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (41%). ^1H NMR (DMSO- d_6) δ 11.60 (s, 2H), 7.68–7.82 (m, 2H), 7.60 (d, J = 7.6 Hz, 1H), 7.54 (dd, J = 7.6, 2.0 Hz, 1H), 7.47 (dt, J = 7.6, 2.0 Hz, 1H), 6.90–7.02 (m, 2H). ^{13}C NMR (DMSO- d_6) δ 166.2, 165.0, 157.3, 135.2, 134.9, 130.7, 130.6, 129.9, 129.2, 127.6, 119.8, 117.8, 117.2. HPLC purity 98.8%. HRMS (ESI) calcd for $\text{C}_{14}\text{H}_8\text{Cl}_2\text{NO}_3$ 307.9887 (M + H) $^+$, found 307.9891.

***N*-(2-Hydroxybenzoyl)benzo[d][1,3]dioxole-5-carboxamide (1x).** The general procedure was used with salicylamide (2) and benzo[d][1,3]dioxole-5-carbonyl chloride (3x, prepared from the corresponding carboxylic acid) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid

(234 mg, 41% over two steps). ^1H NMR (DMSO- d_6) δ 11.75 (br s, 1H), 11.57 (s, 1H), 7.87 (dd, J = 7.8, 1.4 Hz, 1H), 7.52 (dd, J = 8.1, 1.5 Hz, 1H), 7.47–7.41 (m, 2H), 7.08 (d, J = 8.2 Hz, 1H), 7.03–6.97 (m, 2H), 6.16 (s, 2H). ^{13}C NMR (DMSO- d_6) δ 164.2, 163.8, 156.3, 151.2, 147.9, 134.2, 131.0, 127.7, 123.3, 119.9, 119.1, 117.0, 108.3, 107.6, 102.2. HPLC purity 98.7%. MS (ESI) m/z 286 (M + H) $^+$.

***N*-(2-Hydroxybenzoyl)-3,4,5-trimethoxybenzamide (1y).** The general procedure was used with salicylamide (2) and 3,4,5-trimethoxybenzoyl chloride (3y) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (33%). ^1H NMR (DMSO- d_6) δ 11.63 (s, 1H), 11.60 (s, 1H), 7.86 (dd, J = 7.6, 2.0 Hz, 1H), 7.23 (s, 1H), 6.96–7.10 (m, 2H). ^{13}C NMR (DMSO- d_6) δ 164.2, 164.1, 156.3, 141.4, 134.2, 131.1, 128.8, 120.0, 119.5, 117.1, 105.3, 60.2, 56.2. HPLC purity 96.9%. HRMS (ESI) calcd for $\text{C}_{17}\text{H}_{18}\text{NO}_6$ 332.1129 (M + H) $^+$, found 332.1135.

***N*-(Cyclohexanecarbonyl)-2-hydroxybenzamide (1z).** The general procedure was used with salicylamide (2) and cyclohexanecarbonyl chloride (3z) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (354 mg, 48%). ^1H NMR (DMSO- d_6) δ 11.44 (s, 1H), 10.80 (s, 1H), 7.81 (dd, J = 7.9, 1.7 Hz, 1H), 7.44 (ddd, J = 8.3, 7.2, 1.7 Hz, 1H), 6.98 (dd, J = 8.2, 0.9 Hz, 1H), 6.94 (d, J = 7.1, 1.1 Hz, 1H), 2.87 (tt, J = 11.0, 3.3 Hz, 1H), 1.89–1.86 (m, 2H), 1.75–1.72 (m, 2H), 1.66–1.63 (m, 1H), 1.39–1.13 (m, 5H). ^{13}C NMR (DMSO- d_6) δ 176.2, 164.8, 156.7, 134.2, 130.7, 119.7, 118.6, 117.0, 44.6, 28.5, 25.4, 25.1. HPLC purity 100%. MS (ESI) m/z 248 (M + H) $^+$.

***N*-(2-Hydroxybenzoyl)-2-methylfuran-3-carboxamide (1aa).** The general procedure was used with salicylamide (2) and 2-methylfuran-3-carbonyl chloride (3aa) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (83%). ^1H NMR (DMSO- d_6) δ 11.65 (s, 1H), 11.24 (s, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.68 (s, 1H), 7.45 (t, J = 7.6 Hz, 1H), 6.82–7.05 (m, 2H), 6.77 (s, 1H), 2.50 (s, 3H). ^{13}C NMR (DMSO- d_6) δ 164.1, 161.0, 158.8, 156.2, 141.6, 134.2, 131.0, 119.9, 119.2, 117.0, 115.9, 108.8, 13.5. HPLC purity 98.7%. HRMS (ESI) calcd for $\text{C}_{13}\text{H}_{12}\text{NO}_4$ 246.0761 (M + H) $^+$, found 246.0765.

2-(Benzyloxy)-*N*-[(2-hydroxyphenyl)carbonyl]benzamide (1ab). The general procedure was used with salicylamide (2) and 2-(benzyloxy)benzoyl chloride (3ab) as reactants. The reaction was carried out on 4 mmol scale and provided crude material as a brown oil (423 mg, 30%), which was pure enough to be used in the next step without further purification. Purification of a portion of the crude material by preparative HPLC gave the title compound as a white solid (60 mg). ^1H NMR (DMSO- d_6) δ 11.68 (br s, 1H), 11.39 (br s, 1H), 7.79 (dd, J = 7.9, 1.6 Hz, 1H), 7.73 (dd, J = 7.7, 1.7 Hz, 1H), 7.52 (ddd, J = 8.5, 7.5, 1.8 Hz, 1H), 7.44 (ddd, J = 8.3, 7.3, 1.8 Hz, 1H), 7.38–7.35 (m, 2H), 7.25–7.21 (m, 4H), 7.09 (td, J = 7.6, 0.5 Hz, 1H), 6.96 (dd, J = 8.3, 0.5 Hz, 1H), 6.93 (td, J = 8.0, 0.8 Hz, 1H), 5.29 (s, 2H). ^{13}C NMR (DMSO- d_6) δ 164.8, 164.4, 156.8, 155.8, 136.3, 134.4, 133.4, 130.9, 130.8, 128.4 (2C), 127.9, 127.3 (2C), 123.8, 121.1, 119.8, 118.4, 117.0, 113.6, 69.9. HPLC purity 99.2%. MS (ESI) m/z (M + H) $^+$.

***N*-(4-Aminobenzoyl)-2-hydroxybenzamide (4a).** Compound 4a was prepared by standard hydrogenolysis of compound 1a as a pale-yellow solid (2.468 g, 96%). ^1H NMR (DMSO- d_6) δ 11.77 (br s, 1H), 11.44 (s, 1H), 7.90 (d, J = 7.4 Hz, 1H), 7.64 (d, J = 8.2 Hz, 2H), 7.45 (t, J = 7.3 Hz, 1H), 7.03 (d, J = 8.1 Hz, 1H), 6.99 (t, J = 7.4 Hz, 1H), 6.62 (d, J = 8.2 Hz, 2H), 6.05 (br, 2H). ^{13}C NMR (DMSO- d_6) δ 164.1, 163.9, 156.3, 153.5, 134.0, 131.0, 129.7, 119.9, 119.5, 119.1, 117.0, 112.8. HPLC purity 97.7%. MS (ESI) m/z 257 (M + H) $^+$.

2-Hydroxy-*N*-(2-hydroxybenzoyl)benzamide (4b). Compound 4b was prepared by standard hydrogenolysis of compound 1ab as a white solid (76%). ^1H NMR (DMSO- d_6) δ 11.54 (br s, 3H), 7.82 (dd, J = 7.8, 1.5 Hz, 2H), 7.45 (td, J = 8.5, 1.6 Hz, 2H), 7.02–6.96 (m, 4H). ^{13}C NMR (DMSO- d_6) δ 164.6, 156.9, 134.3, 130.7, 119.7, 119.0, 117.1. HPLC purity 98.5%. MS (ESI) m/z 258 (M + H) $^+$.

Methyl 4-[[4-[(2-Hydroxybenzoyl)carbamoyl]phenyl]aminol]-4-oxobutanoate (5). To a suspension of 4a (244 mg, 0.95 mmol) and pyridine (0.085 mL, 1.05 mmol) in CH_2Cl_2 (2 mL)

was added a solution of methyl 3-(chlorocarbonyl)propanoate (150 mg, 1.0 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was stirred in the cold bath for 30 min and then at room temperature for 1 h before quenching with HCl (0.1M) to give a pale-green precipitate, which was filtered off, washed with water, and dried. The crude material was recrystallized from MeOH and purified further by HPLC to give the title compound as a white solid (90 mg, 26%). ^1H NMR (DMSO- d_6) δ 11.72 (s, 1H), 11.61 (s, 1H), 10.38 (s, 1H), 7.88 (d, J = 8.5 Hz, 3H), 7.75 (d, J = 8.7 Hz, 2H), 7.46 (td, J = 7.5, 1.8 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 7.00 (t, J = 8.2 Hz, 1H), 3.60 (s, 3H), 2.69–2.60 (m, 4H). ^{13}C NMR (DMSO- d_6) δ 172.8, 170.5, 164.2, 156.4, 143.3, 134.2, 131.0, 128.9, 127.6, 119.9, 119.1, 118.4, 117.0, 51.4, 31.0, 28.3. HPLC purity 97.7%. MS (ESI) m/z 371 (M + H) $^+$.

N-Hydroxy-*N'*-[4-[[2-(hydroxyphenyl)carbonyl]carbamoyl]phenyl]octanediamide (8). Compound 8 was prepared by standard hydrogenolysis of 7 as a pale-yellow solid (15 mg, 7% over two steps). Compound 7 was obtained from 6 by following the same methodology as employed for the preparation of 5 from 4a, with the exception of using 8-[(benzyloxy)amino]-8-oxooctanoyl chloride as the acylating agent and extending the reaction time to 2 h. ^1H NMR (DMSO- d_6) δ 11.73 (br s, 1H), 11.62 (br s, 1H), 10.33 (br s, 1H), 10.25 (br s, 1H), 8.65 (br s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.88 (d, J = 8.7 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H), 7.46 (td, J = 7.5, 1.6 Hz, 1H), 7.04–6.98 (m, 2H), 2.35 (t, J = 7.5 Hz, 2H), 1.94 (t, J = 7.3 Hz, 2H), 1.59 (t, J = 7.5 Hz, 2H), 1.49 (t, J = 7.0 Hz, 2H), 1.29 (br, 4H). ^{13}C NMR (DMSO- d_6) δ 171.9, 169.1, 164.2, 156.3, 143.5, 134.2, 131.0, 128.8, 127.6, 119.9, 119.1, 118.5, 117.0, 36.4, 32.2, 28.4, 25.0, 24.8. HPLC purity 96.0%. MS (ESI) m/z 428 (M + H) $^+$.

N-(4-Ethylbenzoyl)-2-hydroxy-*N*-methylbenzamide (10). The general procedure was used with 2-hydroxy-*N*-methylbenzamide (9) 14 and 4-ethylbenzoyl chloride (3a) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (84% over two steps). ^1H NMR (CDCl_3) δ 8.08 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 7.6 Hz, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 2H), 7.27 (t, J = 7.6 Hz, 1H), 7.18 (t, J = 8.0 Hz, 1H), 6.58 (br s, 1H), 2.79 (d, J = 4.8 Hz, 3H), 2.73 (q, J = 7.6 Hz, 2H), 1.25 (t, J = 7.6 Hz, 3H). ^{13}C NMR (CDCl_3) δ 166.4, 165.1, 151.1, 148.2, 131.6, 130.4, 129.9, 128.9, 128.4, 126.4, 126.2, 123.2, 29.1, 26.7, 15.2. HPLC purity 98.7%. MS (ESI) m/z 284 (M + H) $^+$.

***N'*-(4-Ethylbenzoyl)-2-hydroxybenzhydrazide (12).** The general procedure was used with salicylhydrazide (11) and 4-ethylbenzoyl chloride (3a) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (36%). ^1H NMR (DMSO- d_6) δ 12.0 (br s, 1H), 10.7 (br s, 1H), 10.6 (s, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 2H), 7.46 (t, J = 8.0 Hz, 1H), 7.36 (d, J = 8.0 Hz, 2H), 6.97 (m, 2H), 2.68 (q, J = 8.0 Hz, 2H), 1.21 (t, J = 8.0 Hz, 3H). ^{13}C NMR (DMSO- d_6) δ 168.2, 165.9, 159.8, 148.6, 134.6, 130.2, 128.7, 128.3, 128.1, 119.5, 117.8, 115.0, 28.5, 15.7. HPLC purity 97.5%. MS (ESI) m/z 285 (M + H) $^+$.

4-Ethyl-*N*-(2-hydroxythiobenzoyl)benzamide (13). To a suspension of compound 1a (231 mg, 0.86 mmol) in chlorobenzene (3 mL) was added Lawesson's reagent (522 mg, 1.29 mmol) under argon. The resulting mixture was refluxed for 3 h to produce a clear dark-red solution. After evaporation of the bulk of solvent, the crude product was directly purified by column chromatography on silica gel using hexane–EtOAc (2:1) as the eluent to give 13 as a yellow solid (16.5 mg, 7%). ^1H NMR (CDCl_3) δ 8.10 (d, J = 8.0 Hz, 2H), 7.86 (dd, J = 8.0, 1.6 Hz, 1H), 7.64 (br, 1H), 7.48 (t, J = 8.0 Hz, 1H), 7.41 (m, 4H), 7.16 (d, J = 8.0 Hz, 1H), 2.75 (q, J = 8.0 Hz, 2H), 1.28 (t, J = 8.0 Hz, 3H). ^{13}C NMR (CDCl_3) δ 200.6, 166.0, 151.4, 145.6, 134.6, 131.6, 130.7, 130.6, 128.4, 126.4, 126.1, 123.1, 29.1, 15.2. HPLC purity 97.8%. MS (ESI) m/z 286 (M + H) $^+$.

***N*-[[4-Ethylphenyl]carbamoyl]-2-hydroxybenzamide (16).** The intermediate 14 was prepared on 20 mmol scale to give the crude product as a white solid. The crude material was recrystallized from acetone to provide the product as a white crystals (3.0 g, 66%), which was subsequently converted into compound 15. 15 The latter was obtained as a white solid (540 mg, 72%) containing an unidentified impurity (17% by HPLC). NMR data for the product 15: ^1H NMR (DMSO- d_6) δ 10.59 (s, 1H), 10.52 (s, 1H), 7.77 (dd, J =

7.5, 1.6 Hz, 1H), 7.60–7.53 (m, 3H), 7.46 (d, J = 8.3 Hz, 2H), 7.42–7.31 (m, 4H), 7.22–7.11 (m, 3H), 5.29 (s, 2H), 2.57 (q, J = 7.5 Hz, 2H), (t, J = 7.6 Hz, 3H); ^{13}C NMR (DMSO- d_6) δ 167.5, 156.2, 150.3, 139.2, 136.1, 135.2, 133.8, 130.3, 128.5, 128.2, 128.1, 127.6, 122.0, 121.0, 119.8, 113.7, 70.3, 27.5, 15.6; MS (ESI) m/z 375 (M + H) $^+$. Standard hydrogenolysis of the intermediate 15 afforded the final compound 16 as a clean product (254 mg of a white powdery solid, 69%). ^1H NMR (DMSO- d_6) δ 11.83 (br s, 1H), 10.69 (s, 1H), 10.57 (s, 1H), 7.96 (dd, J = 7.9, 1.4 Hz, 1H), 7.50 (dd, J = 8.0, 1.4 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 8.3 Hz, 2H), 7.06 (d, J = 8.2 Hz, 1H), 7.01 (t, J = 7.6 Hz, 1H), 2.57 (q, J = 7.5 Hz, 2H), 1.16 (t, J = 7.6 Hz, 3H). ^{13}C NMR (DMSO- d_6) δ 166.7, 156.8, 150.4, 139.3, 135.2, 135.0, 130.8, 128.2, 120.0, 119.9, 117.2, 117.0, 27.6, 15.7. HPLC purity 99.8%. MS (ESI) m/z 285 (M + H) $^+$.

1-(4-Ethylphenyl)-3-(2-hydroxyphenyl)urea (19). To a solution of 17 (0.27 mL, 2.0 mmol) in CH_2Cl_2 (4 mL) was added dropwise 4-ethylaniline (0.25 mL, 2.0 mmol). The resulting mixture was stirred for 19 h at room temperature, and the resulting precipitate was then filtered off and washed with CH_2Cl_2 (25 mL) to give the desired crude 18 as a pale-yellow solid (507 mg, 94%). ^1H NMR (DMSO- d_6) δ 9.20 (s, 1H), 8.17 (s, 1H), 8.12 (dd, J = 7.8, 1.7 Hz, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.1 (d, J = 8.4 Hz, 2H), 7.01 (dd, J = 7.9, 1.4 Hz, 1H), 6.93 (td, J = 7.7, 1.8 Hz, 1H), 6.88 (td, J = 7.7, 1.5 Hz, 1H), 3.87 (s, 3H), 2.54 (q, J = 7.6 Hz, 2H), 1.15 (t, J = 7.6 Hz, 3H). ^{13}C NMR (DMSO- d_6) δ 152.4, 147.6, 137.5, 137.1, 128.8, 128.0, 121.6, 120.5, 118.2, 118.1, 110.7, 55.8, 27.5, 15.8. HPLC purity 99.7%. MS (ESI) m/z 271 (M + H) $^+$. To a solution of 18 (270 mg, 1.0 mmol) in CH_2Cl_2 (25 mL) was added dropwise BBr_3 (4.0 mL of a 1.0 M solution in CH_2Cl_2 , 4.0 mmol) at –78 °C. The resulting mixture was allowed to warm to 0 °C within 2 h. After stirring for further 2 h at 0 °C, the reaction mixture was quenched with MeOH (25 mL) and washed with satd NaHCO_3 (2 \times 50 mL) and H_2O (2 \times 50 mL). The organic phase was dried over Na_2SO_4 , filtered, and concentrated to give the crude product which was dissolved in MeOH to yield 19 as pale-yellow crystals (175 mg, 68%). ^1H NMR (DMSO- d_6) δ 9.89 (s, 1H), 9.19 (s, 1H), 8.11 (s, 1H), 8.03 (dd, J = 7.6, 1.3 Hz, 1H), 7.35 (d, J = 8.3 Hz, 2H), 7.10 (d, J = 8.3 Hz, 2H), 6.83 (dd, J = 7.6, 1.4 Hz, 1H), 6.78 (td, J = 7.2, 1.5 Hz, 1H), 6.73 (td, J = 7.3, 1.4 Hz, 1H), 2.54 (q, J = 7.6 Hz, 2H), 1.15 (t, J = 7.5 Hz, 3H). ^{13}C NMR (DMSO- d_6) δ 152.6, 145.6, 137.6, 137.0, 128.0, 127.9, 121.6, 119.1, 118.5, 118.0, 114.4, 27.5, 15.8. HPLC purity 100%. MS (ESI) m/z 257 (M + H) $^+$.

3-(4-Ethylbenzoyl)-2H-benz[e][1,3]oxazine-2,4(3H)-dione (21a). The general procedure was used with 2H-benz[e][1,3]oxazine-2,4(3H)-dione (20a) and 4-ethylbenzoyl chloride (3a) as reactants. Purification of the crude material by preparative HPLC gave the title compound as a white solid (29%). ^1H NMR (DMSO- d_6) δ 8.17 (d, J = 8.4 Hz, 2H), 8.00 (d, J = 6.8 Hz, 1H), 7.91 (t, J = 7.6 Hz, 1H), 7.52 (m, 2H), 7.44 (d, J = 8.0 Hz, 2H), 2.72 (q, J = 7.6 Hz, 2H), 1.21 (t, J = 7.6 Hz, 3H). ^{13}C NMR (DMSO- d_6) δ 167.5, 160.4, 153.3, 153.0, 145.8, 137.0, 131.3, 128.9, 128.4, 127.1, 125.7, 116.8, 114.3, 28.4, 15.0. HPLC purity 97.4%. MS (ESI) m/z 296 (M + H) $^+$.

***N*-(1-Acetyl-1*H*-indol-3-yl)-4-ethylbenzamide (21b).** To a suspension of 1-(1*H*-indol-3-yl)ethanone (796 mg, 5 mmol) in 95% ethanol (6 mL) was added $\text{NH}_2\text{OH}\cdot\text{HCl}$ (417 mg, 6 mmol) and NaOAc (492 mg, 6 mmol). After refluxing for 2 h, the mixture was cooled, diluted with EtOAc (50 mL), washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4 , and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using hexane–EtOAc (2:1) as the eluent to give (*E*)-1-(1*H*-indol-3-yl)ethanone oxime as a white solid (797 mg, 92%). 20 The oxime was dissolved in ethanol (6 mL) followed by addition of conc H_2SO_4 (several drops). The mixture was refluxed for 4 h, concentrated in vacuo, and then diluted with H_2O (40 mL) and washed with CHCl_3 (3 \times 20 mL). The aqueous layer was basified with 2N NaOH to pH 9 and extracted with EtOAc (3 \times 40 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4 , and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using CHCl_3 /MeOH (10:1) as the eluent to give 1-(3-amino-1*H*-indol-1-yl)ethanone (20b) 20 as a dark-green solid (310 mg, 51%). Then the final compound 21b was

obtained as a dark-green solid (380 mg, 25% over three steps) following the general procedure. ¹H NMR (DMSO-*d*₆) δ 11.7 (s, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 4.0 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 2H), 7.45 (m, 3H), 7.20 (m, 2H), 2.72 (q, *J* = 8.0 Hz, 2H), 2.52 (s, 3H), 1.23 (t, *J* = 8.0 Hz, 3H). ¹³C NMR (DMSO-*d*₆) δ 163.6, 161.1, 150.3, 137.5, 130.9, 129.7, 128.8, 127.1, 124.5, 123.4, 123.1, 121.2, 112.3, 111.1, 28.7, 15.7, 14.5. HPLC purity 98.8%. MS (ESI) *m/z* 307 (M + H)⁺.

4-Ethyl-*N*-(1*H*-indazol-3-yl)benzamide (21c). To a solution of 2-fluorobenzonitrile (0.54 mL, 5 mmol) in *n*-BuOH (6 mL) was added NH₂NH₂·H₂O (0.78 mL, 25 mmol). After refluxing for 4 h, the mixture was cooled, diluted with EtOAc (50 mL), washed with H₂O (20 mL) and brine (20 mL), dried over Na₂SO₄, and concentrated in vacuo. The resulting crude 1*H*-indazol-3-amine (20c)²¹ was converted without purification following the general procedure to compound 21c, which was obtained as a white solid (465 mg, 35% over two steps). ¹H NMR (CDCl₃) δ 9.76 (s, 1H), 8.73 (s, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.39 (m, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.19 (m, 1H), 2.73 (q, *J* = 7.6 Hz, 2H), 1.28 (t, *J* = 7.6 Hz, 3H). ¹³C NMR (CDCl₃) δ 165.5, 149.1, 141.8, 141.6, 131.2, 128.3, 127.7, 123.4, 120.8, 116.2, 109.6, 28.9, 15.3. HPLC purity 98.1%. MS (ESI) *m/z* 266 (M + H)⁺.

2-[3-(4-Ethylphenyl)-[1,2,4]oxadiazol-5-yl]phenol (23). Neat compound 1a (22 mg, 0.08 mmol) was heated to its melting point (~200 °C) for 1 h. The resulting product 22 was cooled to room temperature and suspended in absolute ethanol (2 mL). Solid NaOAc (9 mg, 0.12 mmol) and NH₂OH·HCl (8.4 mg, 0.12 mmol) were added. The mixture was stirred for 16 h, diluted with EtOAc (50 mL), washed with H₂O (20 mL) and brine (20 mL), dried over Na₂SO₄, and concentrated in vacuo.¹⁷ The crude product was purified directly by column chromatography on silica gel using hexane–EtOAc (10:1) as the eluent to give 7 as a white solid (15 mg, 69% over two steps). ¹H NMR (CDCl₃) δ 10.5 (s, 1H), 8.06 (d, *J* = 8.0 Hz, 2H), 8.03 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.56 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 2.76 (q, *J* = 8.0 Hz, 2H), 1.31 (t, *J* = 8.0 Hz, 3H). ¹³C NMR (CDCl₃) δ 174.1, 167.1, 158.1, 148.4, 135.1, 128.5, 127.8, 127.6, 123.1, 120.1, 117.7, 108.2, 28.9, 15.3. HPLC purity 99.8%. MS (ESI) *m/z* 267 (M + H)⁺.

***N*-(4-Ethylphenyl)-*N'*-(2-hydroxyphenyl)propanediamide (28).** Compound 25 was obtained from 24 by following the same methodology as employed for the preparation of 5 from 4a, with the exception of using ethyl 3-chloro-3-oxopropanoate as the acylating agent and extending the reaction time to 18 h. The crude material was subsequently purified by column chromatography on silica gel with hexane–EtOAc (gradient from 0 to 30% EtOAc) as the eluent to provide pure compound 25 as a pale-yellow oil (497 mg, 75%). ¹H NMR (CDCl₃) δ 9.53 (br s, 1H), 8.38 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.50 (d, *J* = 7.0 Hz, 2H), 7.42–7.32 (m, 3H), 7.04 (td, *J* = 7.7, 1.5 Hz, 1H), 6.99–6.95 (m, 2H), 5.16 (s, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.47 (s, 2H), 1.26 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CDCl₃) δ 169.1, 162.8, 147.5, 136.4, 128.5 (2C), 128.1, 127.7, 127.4 (2C), 124.1, 121.3, 120.3, 111.7, 70.7, 61.6, 42.5, 14.0. HPLC purity 94%. MS (ESI) *m/z* 314 (M + H)⁺.

Hydrolysis¹³ of the ester 25 gave the desired acid 26 as orange crystals (260 mg, 91% on a 1.0 mmolar scale). The crude product was pure enough to be used in the next step without further purification. ¹H NMR (DMSO-*d*₆) δ 9.63 (br s, 1H), 8.00 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.51 (d, *J* = 7.0 Hz, 2H), 7.40–7.29 (m, 3H), 7.09 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.03 (td, *J* = 7.8, 1.5 Hz, 1H), 6.90 (td, *J* = 7.9, 1.3 Hz, 1H), 5.22 (s, 2H), 3.50 (s, 2H). ¹³C NMR (DMSO-*d*₆) δ 170.1, 164.6, 148.2, 137.0, 128.4 (2C), 127.8, 127.6, 127.3 (2C), 124.4, 121.7, 120.6, 112.9, 69.6, 43.1. HPLC purity 97.8%. MS (ESI) *m/z* 284 (M + H)⁺.

Compound 27 was obtained as a yellow oil (300 mg, 85%) by following the literature procedure.¹³ The crude product was of adequate purity to be used in the next step without further purification. ¹H NMR (DMSO-*d*₆) δ 10.21 (br s, 1H), 9.99 (br s, 1H), 8.12 (d, *J* = 7.5 Hz, 1H), 7.58 (d, *J* = 6.9 Hz, 2H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.38–7.30 (m, 3H), 7.18–7.13 (m, 3H), 7.04 (t, *J* = 7.4 Hz, 1H), 6.92 (t, *J* = 7.6 Hz, 1H), 5.22 (s, 2H), 3.59 (s, 2H), 2.57 (q, *J* = 7.5 Hz, 2H), 1.16 (t, *J* = 7.5 Hz, 3H). ¹³C NMR (DMSO-*d*₆) δ 166.4, 165.0, 147.8,

139.2, 136.9, 136.4, 128.4 (2C), 128.0 (2C), 127.8, 127.7, 127.4 (2C), 124.2, 120.9, 120.8, 119.4 (2C), 112.7, 69.7, 44.6, 27.7, 15.8. HPLC purity >95%. MS (ESI) *m/z* 411 (M + Na)⁺.

The final compound 28 was obtained as a yellow solid by following a literature procedure.¹⁸ Purification by preparative HPLC provided the desired compound as a white solid (110 mg, 55% on a 0.67 mmolar scale). ¹H NMR (DMSO-*d*₆) δ 9.68 (br s, 1H), 9.43 (br s, 1H), 9.20 (br s, 1H), 9.48 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.03 (d, *J* = 8.3 Hz, 2H), 6.69 (d, *J* = 8.3 Hz, 2H), 6.46 (td, *J* = 8.0, 1.3 Hz, 1H), 6.41 (dd, *J* = 8.0, 1.3 Hz, 1H), 6.30 (td, *J* = 8.0, 1.3 Hz, 1H), 3.10 (s, 2H), 2.09 (q, *J* = 7.6 Hz, 2H), 0.69 (t, *J* = 7.5 Hz, 3H). ¹³C NMR (DMSO-*d*₆) δ 166.0, 165.3, 147.2, 139.1, 136.4, 128.0 (2C), 126.4, 124.3, 121.1, 119.4 (2C), 119.0, 115.2, 45.0, 27.6, 15.7. HPLC purity 97.7%. MS (ESI) *m/z* 299 (M + H)⁺.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ADMET, absorption, distribution, metabolism, excretion, toxicity; CYP450, cytochrome P450; DMSO-*d*₆, deuterated dimethyl sulfoxide; *hERG*, human ether-à-go-go-related gene; HLM, human liver microsomes; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; HTS, high-throughput screening; *L. donovani*, *Leishmania donovani*; L6, toxicity of the tested compounds assessed against a mammalian primary cell line derived from rat skeletal myoblasts; NADPH, nicotinamide adenine dinucleotide phosphate; SAR, structure–activity relationships; SI, selectivity index; *T. b. rhodesiense*, *Trypanosoma brucei rhodesiense*; *T. cruzi*, *Trypanosoma cruzi*; *T. gondii*, *Toxoplasma gondii*; TLC, thin layer chromatography; TMS, tetramethylsilane; *P. falciparum*, *Plasmodium falciparum*

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