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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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Abstract

In our efforts to identify novel chemical scaffolds for the development of new antiprotozoal drugs, a compound library was screened against *T. gondii* tachyzoites with activity discovered for *N*-(4-ethylbenzoyl)-2-hydroxybenzamide **1a** against *T. gondii* as described elsewhere.¹ 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 *P. falciparum*, and with superior ADMET properties as described elsewhere.¹ 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 re-purposed for testing against four other protozoan parasites: *T. b. rhodesiense*, *T. cruzi*, *L. donovani*, and *P. falciparum* (K1 isolate). Structure-activity analyses led to the identification of compounds in this set with excellent anti-leishmanial activity (compound **1d**). Overall, compound **1r** was the best and had activity 21-fold superior to that of the standard anti-malarial drug chloroquine against the K1 *P. falciparum* isolate.

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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 and also some of these parasites 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 25,000 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 800,000 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 anti-protozoal agents remains an urgent need.

In our efforts to identify novel chemical scaffolds as potent anti-protozoal drugs, a small compound library (ASDI) was screened initially against *T. 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 anti-malarial activity ascribed to their targeting the Hemozoin Detoxification Protein (HDP), a protein known to be vital for *P. falciparum*.⁶ It is also known that certain salicylamide derivatives, such as salicylhydroxamic acid, nitazoxanide and other thiazolides, display anti-protozoal 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* and 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 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 3-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, designed modification of this moiety was analyzed. Syntheses are shown in Scheme 3. The *N*-methyl imide **10** was prepared in high yield from the corresponding 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-ethylamine 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-ethylamine 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 $\mu\text{L}/\text{min}/\text{mg}$ proteins) were faster than that of the control verapamil (38.8 $\mu\text{L}/\text{min}/\text{mg}$ proteins).¹ Nonetheless, these results encouraged us to modify the hit **1a** to generate a lead compound, with the improvement of both anti-protozoal 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. rhodesiense* (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 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 analogs 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 anti-parasitic 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 (within micromolar range) against *T. cruzi*, *L. donovani*, and *P. falciparum* (K1 strain). Compounds with methyl (**1b**), fluorine (**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 methyl ester analogue **1l** gave similar results. In contrast to these results, compound **5** containing a succinic acid methyl ester side chain was inactive against all species. On the other hand, the analog **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*, though 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 anti-leishmanial and *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. Anti-plasmodial 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 analog **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 analogs 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). Based on 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 L-6 cell line (selectivity index, SI = 1640) make this compound a promising lead for the further development of anti-malarial drugs. This compound has been evaluated for its ADMET properties elsewhere¹, which 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 analog **1d**, the trifluoromethyl analog **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 analogs 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 anti-parasitic 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 anti-parasitic activity for some of the synthesized compounds (Table 3). The 2-naphthyl analog **1t**, the 6-methoxy-2-naphthyl analog **1u**, and the 2,3,4-trimethoxyphenyl analog **1y** were the most active against *P. falciparum* (K1 strain). Compound **1t** showed activity at low concentration (only 2-fold less 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 analog **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 analogs presented in Table 3 showed moderate (corresponding to micromolar) activity against *L. donovani*, and the 3-chloromethyl analog **1v** even had activity slightly better than the anti-leishmanial drug miltefosine. However, the selectivity index of compound **1v** was only 11, which makes this compound less viable for further development. Analog **1z** with a cyclohexyl B ring and **1aa** with a 2-methyl-3-furyl B ring turned out to be moderately active only 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 anti-leishmanial 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 anti-leishmanial activity. Free phenol functionality (compound **4b**) is tolerated but alkoxy substituents (compounds **1x, y**) provide a better contribution to the compounds' anti-malarial activity. Although significantly cytotoxic, the (chloromethyl)phenyl analog **1v** provided excellent potency against *L. donovani*, whereas 2,4-dichlorophenyl (compound **1w**) gave a completely inactive analog. 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 analogs with modified linker and A ring moieties. A series of 10 compounds was prepared and screened for anti-leishmania, trypanosome and plasmodium (K1) activity (Table 4). We found that most of the modifications caused a decrease in anti-*P. falciparum* (K1 strain) activity. Only the sulfur analog **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 anti-parasitic 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 anti-leishmanial 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 anti-malarial 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 anti-leishmanial 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* (K1 strain) and *L. donovani* were identified. Among the tested parasites *P. falciparum* (K1 strain) was the most sensitive to these *N*-benzoyl-2-hydroxybenzamides. 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 analog. With its excellent anti-plasmodial potency, this highly selective agent may be used as a possible lead for the further development of anti-malarial agents. Although analog **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 anti-leishmanial 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 IC₉₀s 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 L-6 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 non-aqueous 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 Bruker spectrometer at 400 MHz 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 Q-TOF-2TM (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 × 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)/1.0M 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. Non-commercial 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 SOCl_2 and heated at $80\text{ }^\circ\text{C}$ 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 H_2 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); ^{13}C 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 $\text{C}_{16}\text{H}_{16}\text{NO}_3$ 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); ^{13}C 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 $\text{C}_{15}\text{H}_{14}\text{NO}_3$ 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 ($\text{CD}_3\text{OD}/\text{CDCl}_3$) 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); ^{13}C NMR ($\text{CD}_3\text{OD}/\text{CDCl}_3$) 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); ¹³C 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 C₁₄H₁₁FNO₃ 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); ¹³C 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 C₁₅H₁₁F₃NO₃ 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); ¹³C 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); ¹³C 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 C₁₅H₉N₂O₃ 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-(methylsulphonyl)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); ¹³C 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); ¹³C 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 C₁₅H₁₃ClNO₃ 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); ¹³C 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 C₁₄H₈Cl₂NO₃ 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); ¹³C 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); ¹³C 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 C₁₇H₁₈NO₆ 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); ¹³C 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); ¹³C 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 C₁₃H₁₂NO₄ 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 mmolar 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); ¹³C 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 **1i** 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); ¹³C 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); ¹³C 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]amino]-4-oxobutanoate (5)

To a suspension of **4a** (244 mg, 0.95 mmol) and pyridine (0.085 mL, 1.05 mmol) in CH₂Cl₂ (2 mL) was added a solution of methyl 3-(chlorocarbonyl)propanoate (150 mg, 1.0 mmol) in CH₂Cl₂ (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 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); ¹³C 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); ¹³C 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 (CDCl₃) 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); ¹³C NMR (CDCl₃) 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); ¹³C 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 (CDCl₃) 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); ¹³C NMR (CDCl₃) 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 mmolar 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); ¹³C 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); ¹³C 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 CH₂Cl₂ (4 mL) was added dropwise 4-ethylalanine (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 CH₂Cl₂ (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); ¹³C 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 CH₂Cl₂ (25 mL) was added dropwise BBr₃ (4.0 mL of a 1.0M solution in CH₂Cl₂, 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 sat. NaHCO₃ (2 × 50 mL), and H₂O (2 × 50 mL). The organic phase was dried over Na₂SO₄, 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); ¹³C 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); ¹³C 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-1H-indol-3-yl)-4-ethylbenzamide (**21b**)

To a suspension of 1-(1H-indol-3-yl)ethanone (796 mg, 5 mmol) in 95% ethanol (6 mL) was added NH₂OH·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 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 using hexane–EtOAc (2:1) as the eluent to give (*E*)-1-(1H-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. H₂SO₄ (several drops). The mixture was refluxed for 4 h, concentrated in vacuo, and then diluted with H₂O (40 mL) and washed with CHCl₃ (3 × 20 mL). The aqueous layer was basified with 2N NaOH to pH 9 and extracted with EtOAc (3 × 40 mL). The combined organic layers were washed with brine (20 mL), dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using CHCl₃/MeOH (10:1) as the eluent to give 1-(3-amino-1H-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 $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ (0.78 mL, 25 mmol). After refluxing for 4 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 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 (CDCl_3) 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 (CDCl_3) 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 $\text{NH}_2\text{OH}\cdot\text{HCl}$ (8.4 mg, 0.12 mmol) were added. The mixture was stirred for 16 h, diluted with EtOAc (50 mL), washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4 , 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 (CDCl_3) 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 (CDCl_3) 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 (CDCl_3) 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 (CDCl_3) 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 ($\text{DMSO}-d_6$) 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 ($\text{DMSO}-d_6$) 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 ($\text{DMSO}-d_6$) 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 ($\text{DMSO}-d_6$) 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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Abbreviations

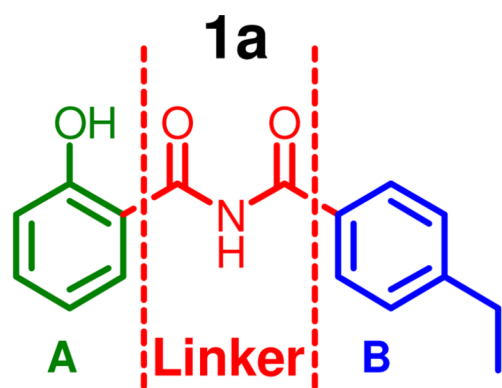
ADMET	absorption, distribution, metabolism, excretion, toxicity
CYP450	cytochrome P450
DMSO-<i>d</i>₆	deuterated dimethyl sulfoxide
<i>hERG</i>	the human <i>Ether-à-go-go</i> -Related Gene
HLM	human liver microsomes
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
HTS	high-throughput screening
<i>L. donovani</i>	<i>Leishmania donovani</i>
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
<i>T. b. rhodesiense</i>	<i>Trypanosoma brucei rhodesiense</i>
<i>T. cruzi</i>	<i>Trypanosoma cruzi</i>
<i>T. gondii</i>	<i>Toxoplasma gondii</i>
TLC	thin layer chromatography
TMS	tetramethylsilane
<i>P. falciparum</i>	<i>Plasmodium falciparum</i>

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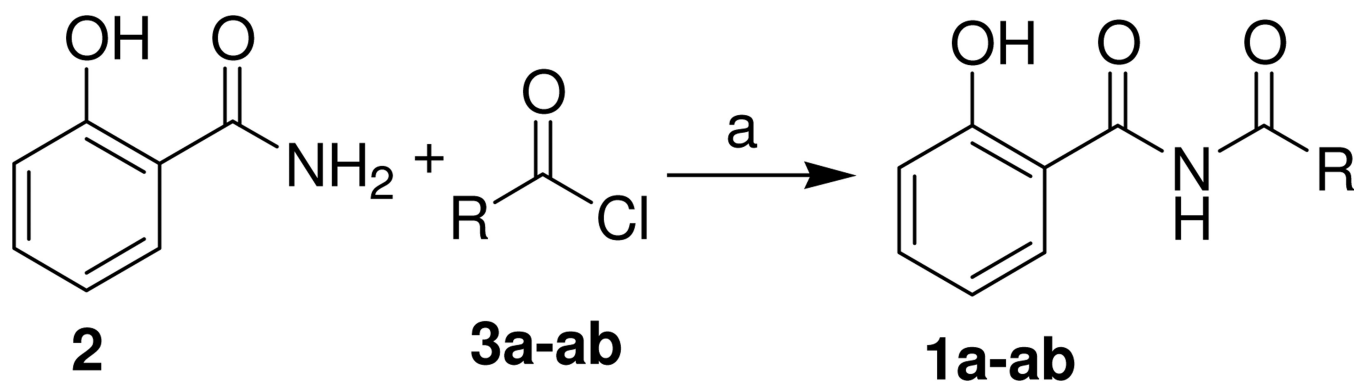
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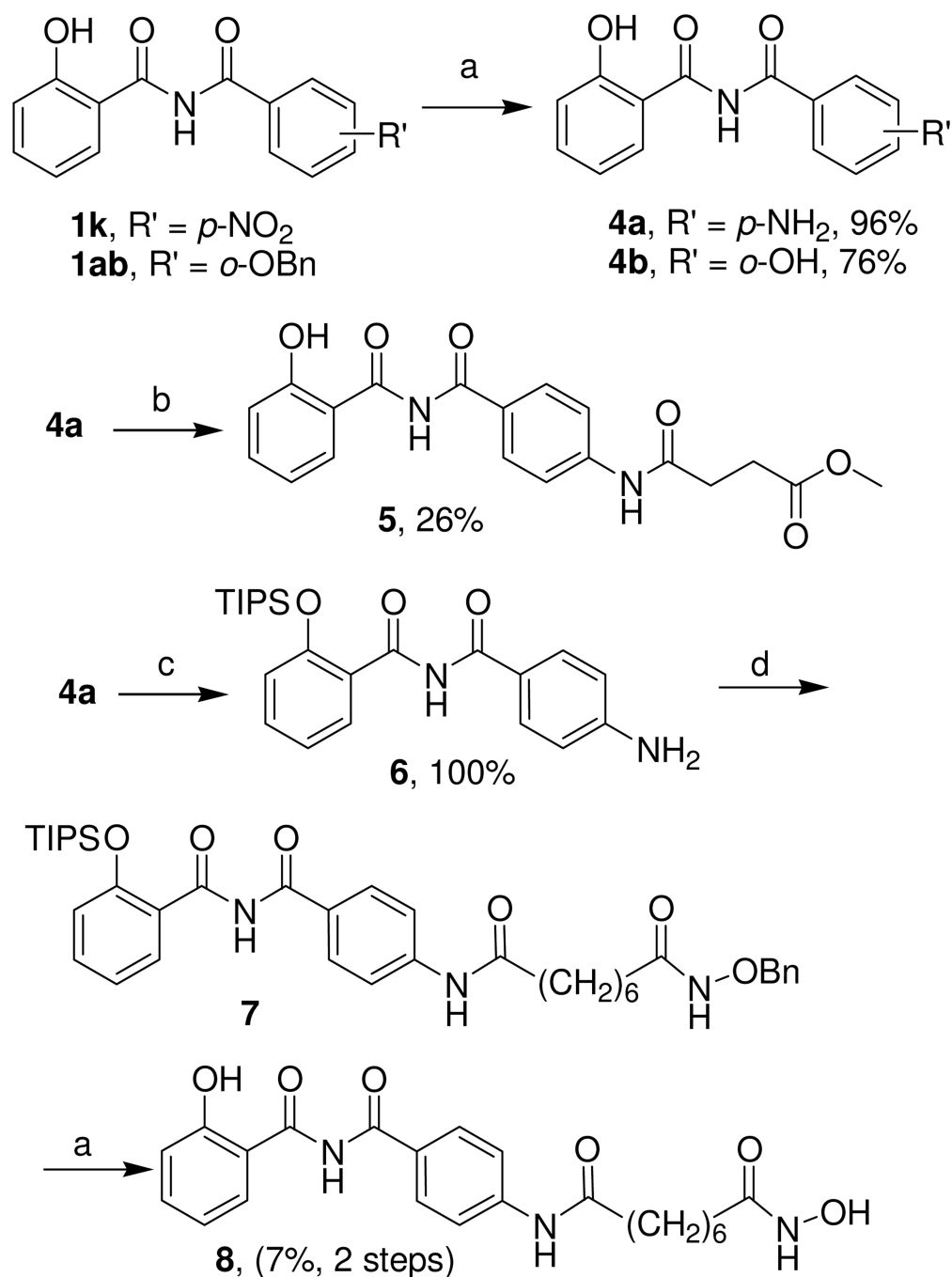


		IC₅₀ (μg/mL), [μM]	
Standard		1a	
<i>T. b. rhod.</i>	melarsoprol	0.005, [0.013]	81, [301]
<i>T. cruzi</i>	benznidazole	0.48, [1.8]	4.8, [18]
<i>L. don. axe.</i>	miltefosine	0.19, [0.47]	7.4, [27]
<i>P. falc. K1</i>	chloroquine	0.11, [0.34]	5.6, [21]
L6	podophyllotoxin	0.004, [0.0]1	70, [260]

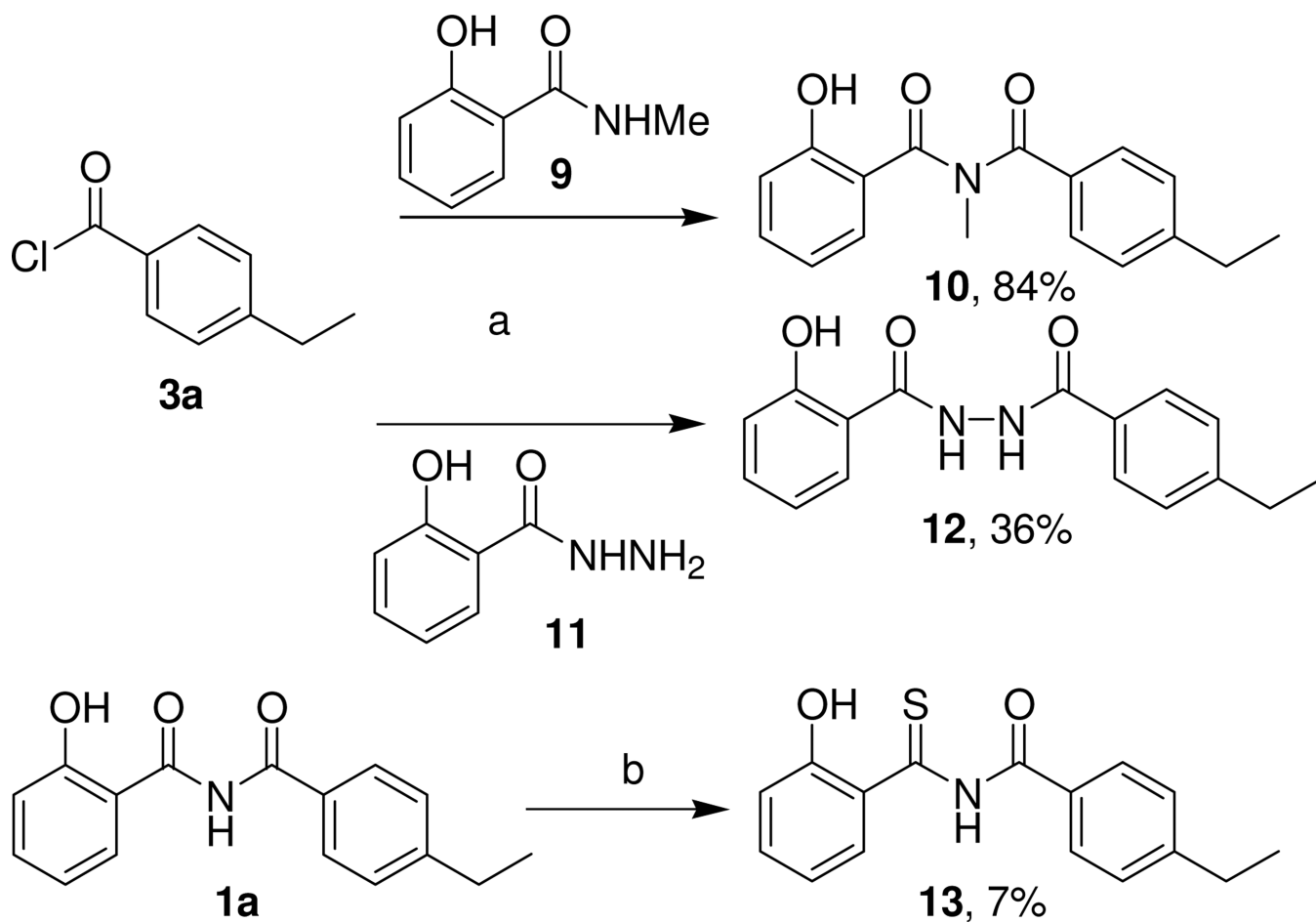
Figure 1.
Hit compound **1a**



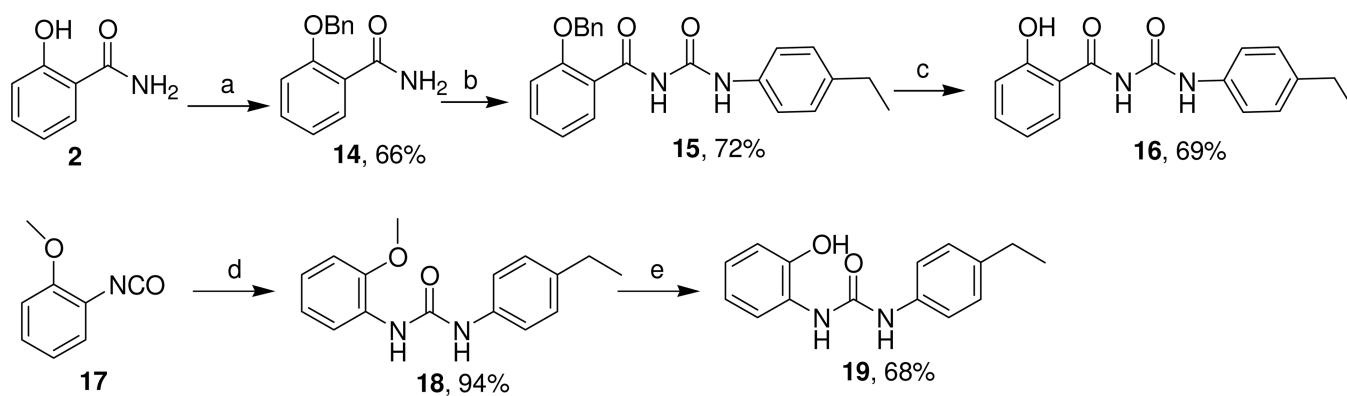
Scheme 1.
Preparation of *N*-benzoyl-2-hydroxybenzamides
Reagents and conditions: (a) pyridine, reflux, 4 h.

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

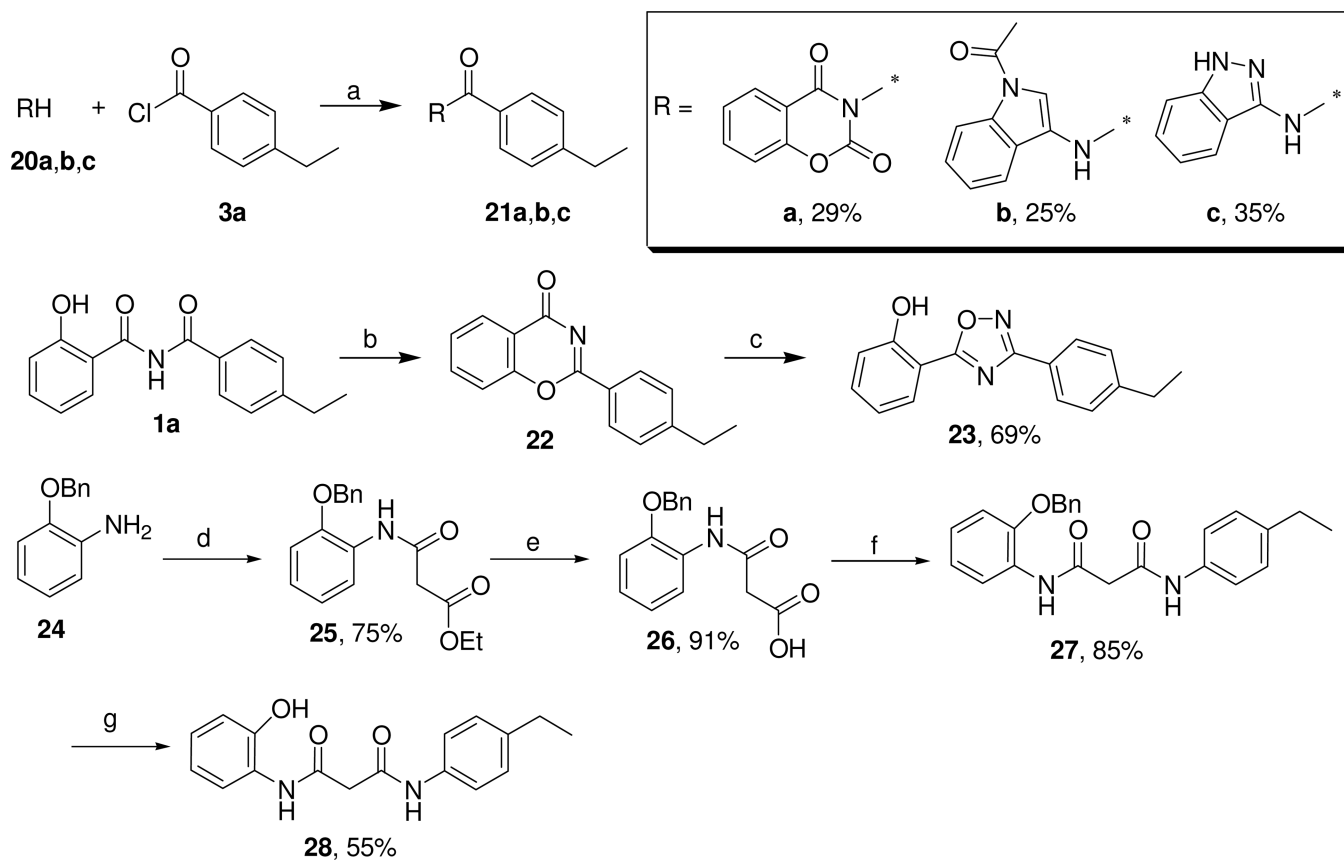
Reagents and conditions: (a) Pd/C, H₂, MeOH, room temp, 18 h; (b) methyl 3-(chlorocarbonyl)propanoate, pyridine, CH₂Cl₂, room temp, 1 h; (c) TIPSCl, Et₃N, DMAP, DMF, room temp, 3.5 h; (d) 8-[(benzyloxy)amino]-8-oxooctanoyl chloride, pyridine, CH₂Cl₂, room temp, 2 h.



Scheme 3.
Modifications of the Linker in *N*-Benzoyl-2-hydroxybenzamides
Reagents and conditions: (a) pyridine, reflux, 4 h; (b) Lawesson's reagent, chlorobenzene, 135 °C, 3 h.

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

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

**Scheme 5.**

Preparation of Compounds with Modified Linkers

Reagents and conditions: (a) pyridine, reflux, 4 h; (b) 200 °C, 1 h; (c) $\text{NH}_2\text{OH}\cdot\text{HCl}$, NaOAc, absolute EtOH, 16 h; (d) pyridine, CH_2Cl_2 , 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) $\text{Pd}(\text{OH})_2/\text{C}$ (25 wt %), EtOH-cyclohexene (2:1), reflux, 2.5 h.

Table 1

Variation of the Ring B in *N*-Benzoyl-2-hydroxybenzamidides (**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

^a Isolated yield of product exhibiting >95% HPLC purity.

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

Compound	<i>T. b. rhod.</i>		<i>T. cruzi</i>		<i>L. don. axc.</i>		<i>P. falc. (K1)</i>		<i>L6</i>	
	IC ₅₀ ^a	SI ^b	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀	SI
1a	81	0.86	4.8	1.5	7.4	9.3	5.6	1.2	70	
1b	>100	<0.46	38	1.2	3.2	14	1.4	3.3	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	1.5	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	

^a Values indicate the inhibitory concentration of a compound or standard in µg/mL that is necessary to achieve 50% growth inhibition (IC₅₀). Data shown in the table represent mean values from two independent determinations.

^b Selectivity index = (IC₅₀ for L6)/(IC₅₀ for the respective values for the four protozoan organisms).

Standard compounds: ^c melarsoprol, ^d benzimidazole, ^e mitelfosine, ^f chloroquine, ^g podophyllotoxin.
Marked field, IC₅₀ < 0.1 µg/mL.

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

Compound	<i>T. b. rhod.</i>		<i>T. cruzi</i>		<i>L. don. axc.</i>		<i>P. falc. (K1)</i>		L6
	IC ₅₀ ^a	SI ^b	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀	SI	
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

^a Values indicate the inhibitory concentration of a compound or standard in µg/mL that is necessary to achieve 50% growth inhibition (IC₅₀). Data shown in the table represent mean values from two independent determinations.

^b Selectivity index = (IC₅₀ for L6)/(IC₅₀ for the respective protozoan parasite).

Standard compounds: ^c melarsoprol, ^d benznidazole, ^e miltefosine, ^f chloroquine, ^g podophyllotoxin.

Marked field, 0.1 < IC₅₀ < 1.0 µg/mL.

Table 4

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

Compound	<i>T. b. rhod.</i>		<i>T. cruzi</i>		<i>L. don. axae.</i>		<i>P. falc. (K1)</i>		<i>L6</i>	
	IC ₅₀ ^a	SI ^b	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀	SI	IC ₅₀	SI
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	

^a Values indicate the inhibitory concentration of a compound or standard in µg/mL that is necessary to achieve 50% growth inhibition (IC₅₀). Data shown in the table represent mean values from two independent determinations.

^b Selectivity index = (IC₅₀ for L6)/(IC₅₀ for the respective protozoan parasite).

Standard compounds: ^c melarsoprol, ^d benznidazole, ^e miltefosine, ^f chloroquine, ^g podophyllotoxin.
Marked field, 1.0 < IC₅₀ < 10 µg/mL.