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ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · OCTOBER 2013

Impact Factor: 5.45 · DOI: 10.1021/jm4006127 · Source: PubMed

Journal of Medicinal Chemistry

Article

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In Vitro and in Vivo Antileishmanial and Trypanocidal Studies of New *N*-Benzene- and *N*-Naphthalenesulfonamide Derivatives

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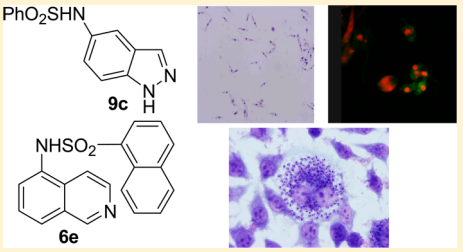
ABSTRACT: We report in vivo and in vitro antileishmanial and trypanocidal activities of a new series of *N*-substituted benzene and naphthalenesulfonamides **1**−**15**. Compounds **1**−**15** were screened in vitro against *Leishmania infantum*, *Leishmania braziliensis*, *Leishmania guyanensis*, *Leishmania amazonensis*, and *Trypanosoma cruzi*. Sulfonamides **6e**, **10b**, and **10d** displayed remarkable activity and selectivity toward *T. cruzi* epimastigotes and amastigotes. **6e** showed significant trypanocidal activity on parasitemia in a murine model of acute Chagas disease. Moreover, **6e**, **8c**, **9c**, **12c**, and **14d** displayed interesting IC_{50} values against *Leishmania* spp promastigotes as well as *L. amazonensis* and *L. infantum* amastigotes. **9c** showed excellent in vivo activity (up to 97% inhibition of the parasite growth) in a short-term treatment murine model for acute infection by *L. infantum*. In addition, the effect of compounds **9c** and **14d** on tubulin as potential target was assessed by confocal microscopy analysis applied to *L. infantum* promastigotes.



INTRODUCTION

Protozoa of the order Kinetoplastida are the causative agents of a number of human and animal diseases including Chagas disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania* spp.).[1] According to the World Health Organization, leishmaniasis is an uncontrolled tropical disease with high morbidity and mortality rates in Africa, Asia, and the Americas.[2] Current chemotherapy is based on pentavalent antimonials, such as sodium stibogluconate and meglumine antimoniate. Both drugs only exist in their parenteral forms and need to be used under medical supervision. Now, second-line compounds are being used, including pentamidine and amphotericin B. A new antileishmanial, miltefosine, has been used in India and currently is undergoing clinical trials in other countries. However, current drugs for leishmaniasis are toxic, expensive, and cause several adverse effects.[3] Moreover, the development of drug resistance, especially in HIV−*Leishmania* coinfected patients, has also worsened the problem.[4]

Human American trypanosomiasis or Chagas disease, mostly found in the American continent, is also an important cause of mortality and morbidity in the region.[5] At present, the only therapeutic agents of value against Chagas disease are benznidazole and nifurtimox. Nevertheless, both drugs suppress parasitemia and are efficacious exclusively during the early stages of infection. Moreover, limitations such as the long treatment period (30, 60, or 90 days), toxicity, extreme side effects, and regional variations in efficacy due to naturally resistant *T. cruzi* strains cause a high rate of patient noncompliance.[6]

In spite of the social and economic importance of leishmaniasis and Chagas disease, efforts directed toward the discovery of new drugs against them remain undeveloped.[7] Therefore, there is an urgent need for the discovery of new therapeutics displaying antitrypanosomal and leishmanicidal activities.

Sulfonamide pharmacophore is an important structural core in medicinal chemistry. In the literature, sulfonamides with different pharmacological profiles, such as antimicrobial,[8] diuretic,[9] hypoglycaemic,[10] antithyroid,[11] antitumoral,[12] and antiviral[13] activities, are described. In addition, the antiparasitic efficacy of several benzenesulfonamides has also been reported.[14] Thus, in vitro antileishmanial and trypanocidal effects of compounds containing the sulfonamide moiety have been shown.[15] However, a limited number have been tested in murine animal models, and neither of them displayed significant in vivo activity. In this context, we have previously shown the in vivo activity of *p*-nitrobenzenesulfonamides against *Leishmania infantum*.[16] This result prompted us to synthesize and in vitro evaluate novel sulfonamides for

Received: April 24, 2013
Published: October 23, 2013

dx.doi.org/10.1021/jm4006127 | *J. Med. Chem.* 2013, 56, 8984−8998

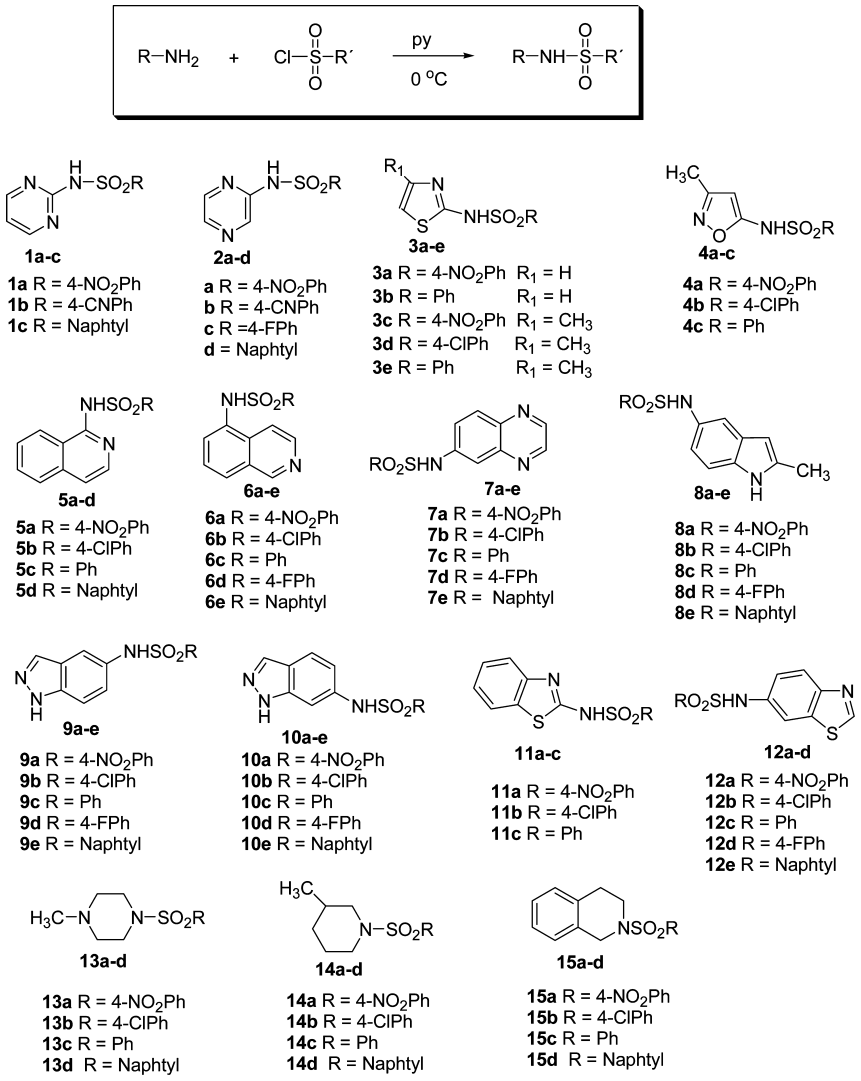


Figure 1. Synthetic reaction and structures of sulfonamide derivatives **1**–**15**.

antiprotozoal activity against *Leishmania guayanensis*, *Leishmania amazonensis*, *Leishmania braziliensis*, the etiological agents of most cases of cutaneous and mucocutaneous leishmaniasis, and against *T. cruzi*, the causative agent of Chagas disease. Encouraged by our recent results,[17] we report here in vivo and in vitro trypanocidal and leishmanicidal studies on more than 60 aromatic sulfonamide derivatives (three of them previously described). In order to investigate a potential mechanism of action, the antitubulin effect of the most promising sulfonamides was assessed by using immunofluorescence confocal microscopy. In addition, a structure–activity relationship (SAR) analysis will be discussed.

RESULTS AND DISCUSSION

Chemistry. Previous research from our group showed that *N-p*-nitrobenzenesulfonamides of 2-amine-substituted pyrimidine and pyrazine rings, sulfonamides **1a** and **2a**, displayed an interesting in vivo activity against *L. infantum*, as their administration (5 mg/kg/day for 10 days, injected ip route) led to a clear-cut parasite burden reduction (nearly 99%).[16] To develop alternative structures to parent compounds **1a** and **2a**, our design approach was based on the combination of diverse monocyclic and bicyclic amines with different naphthalene and benzenesulfonyl chlorides, to afford sulfonamides **1**–**15** (Figure 1). Compounds **1**–**15** were prepared in moderate to high yields (40–99%) by the reaction of the appropriate amines with the corresponding arylsulfonyl chlorides in anhydrous pyridine at 0 °C. The synthesis of the target compounds followed the procedure described by us for this type of derivative.[16] NMR and HRMS spectral data were used to provide complete structural characterization of sulfonamides **1**–**15**.

BIOLOGICAL ACTIVITY

In Vitro Activity against *Leishmania* spp. and *T. cruzi*. Sulfonamides **1**–**15** were tested for antiprotozoal activity against four different *Leishmania* species (*L. infantum*, *L. braziliensis*, *L. guyanensis*, and *L. amazonensis*) and *T. cruzi*, as well as for cytotoxicity against J774 macrophages and NCTC929 fibroblasts, following established procedures (see Experimental Section). Table 1 presents sulfonamide derivatives shown to be active in vitro against *Leishmania* spp. and *T. cruzi* extracellular forms (promastigotes and epimastigotes, respectively). As can be seen in Table 1, compounds **2a**, **6e**, **10b**, and **10d** displayed promising IC_{50} values against *T. cruzi* epimastigotes with a moderate–good selectivity index (SI = CC_{50}/IC_{50}) on fibroblasts of >4.7, >1.9, >9.9, and >196.9,

dx.doi.org/10.1021/jm4006127 | J. Med. Chem. 2013, 56, 8984–8998

Table 1. Leishmanicidal and Trypanocidal Activities of Sulfonamides 1−15 on Extracellular Forms (*Leishmania* promastigotes and *T. cruzi* epimastigotes) and Cytotoxicity on J774 Macrophages and NCTC929 Fibroblasts

	parasites IC_{50}[b] (μM)					cell lines CC_{50}[b] (μM)	
	L. infantum	*L. braziliens.*	*L. guyanen.*	*L. amazon.*	*T. cruzi*	macrophages J774	fibroblasts NCTC929
1a[f]	76.4	48.8	46.4	68.6	156.1	NC[e]	ND[d]
2a[f]	66.2	61.5	84.2	NA[a]	76.1	NC	317
5a	39.5	42.5	24.6	27	136.3	41.3	ND
5b	51.4	37	4.3	82.8	NA	NC	ND
5c	NA	162.1	93.5	93.5	ND	NC	ND
6b	21	29.8	22.6	8.4	ND	60.8	ND
6e	20.3	23	15.8	24.5	97.6	NC	187
7a	81.7	60.8	101.4	197.7	NA	NC	ND
7b	51.5	80.9	25.9	46.2	ND	23.8	ND
7d	33.4	13.4	29.8	14.2	ND	43.9	ND
8a	35.2	16.8	19.6	15.8	ND	132	ND
8b	12.4	23	10.4	18.2	ND	194.8	ND
8c	23.4	29	20.9	15.4	NA	NC	ND
8d	34.5	42.7	116.6	12.5	ND	40.4	ND
8e	11.9	11.6	13.6	7.4	ND	24.6	ND
9a[f]	61.3	80.8	139.6	66.7	ND	132.9	ND
9c	37.3	40.3	47.9	38.7	NA	NC	ND
9d	65.4	86.6	101.9	115.7	NA	NC	ND
9e	37.3	39.7	52.8	37.7	ND	30	ND
10a	43.3	21.7	48.1	60.3	NA	NC	ND
10b	43.5	35.6	46.1	59.1	25.9	NC	211
10d	94.8	88.8	96.3	107.9	1.3	NC	223
12a	85.5	31	53.7	89.6	NA	NC	ND
12c	18.4	30.6	23.7	82.6	NA	NC	ND
14c	112.4	115.3	54.3	40.9	NA	NC	ND
14d	6.7	9.9	7.2	24.6	NA	NC	ND
15d	NA	NA	NA	NA	113.7	NC	>310
M[c]	17.6	7.6	44.6	34.1		135.9	ND
B[c]					54.7	NC	>194

[a]NA, nonactive at tested concentrations. [b]IC_{50}, concentration of the compound that produced a 50% reduction in parasites; CC_{50}, concentration of the compound that produced a 50% reduction of cell viability in treated culture cells with respect to untreated ones. [c]**M**, miltefosine; **B**, benznidazole. [d]ND, not determined. [e]NC (noncytotoxic), CC ≥ 300 μM. [f]Data published in ref 17.

respectively. Moreover, compounds **10b** and **10d** exhibited higher activity than the reference drug benznidazole: **10b** (IC_{50} = 25.9 μM), **10d** (IC_{50} = 1.3 μM), and benznidazole (IC_{50} = 54.7 μM) ($p < 0.05$ and 0.01, respectively).

As outlined in Table 1, from the antiparasitic screening against cultured promastigotes of *Leishmania* spp., 12 compounds (**1a**, **2a**, **5b**, **6e**, **8c**, **9c**, **9d**, **10a**, **10b**, **12a**, **12c**, and **14d**) displayed activity at the micromolar level, without cytotoxicity on J774 macrophage cells (CC_{50} ≥ 300 μM). Whereas sulfonamides **1a**, **2a**, **5b**, **9d**, and **12a** showed lower activity compared to the standard drug miltefosine, used as reference ($p < 0.05$), compounds **6e**, **8c**, **9c**, **10a**, **10b**, **12c**, and **14d** showed IC_{50} values close to that of miltefosine ($p = 1.00$), the sulfonamides **6e**, **8c**, **9c**, **12c** and **14d** being the most active compounds. Although their potency against some *Leishmania* species was lower when compared to that of miltefosine, overall our compounds showed better selectivity index (SI = CC_{50}/IC_{50}), resulting in promising therapeutic utility.

It is interesting to note that sulfonamide **2a** was active against all *Leishmania* species except for *L. amazonensis*, whereas compound **5b** exhibited a higher activity against *L. guyanensis* promastigotes (IC_{50} = 4.3 μM) in comparison to those of *L. infantum* (IC_{50} = 51.4 μM), *L. braziliensis* (IC_{50} = 37 μM), and *L. amazonensis* (IC_{50} = 82.8 μM). These results pointed toward different susceptibility among *Leishmania* species to these compounds.

Sulfonamides **6e**, **8c**, **9c**, **12c**, and **14d**, the most potent compounds in the antipromastigote assay (selectivity index ranging from >10.4 to >16.2 for **6a**, from >8.8 to >16.6 for **8c**, from >5.3 to >6.9 for **9c**, from >3.1 to >13.9 for **12c**, and from >10.4 to >38.2 for **14d**, among the species), could be good candidates for subsequent investigations against the clinically relevant *Leishmania* amastigote forms. Consequently, these sulfonamides were tested against *L. amazonensis* and *L. infantum* amastigotes. The antileishmanial activity of these derivatives decreased against the intracellular forms, in comparison to extracellular forms, except for **9c**, **12c**, and **14d** on *L. amazonensis*, where a higher profile against amastigotes than promastigotes was shown. Likewise, compounds **2a**, **6e**, **10b**, and **10d**, with activity against *T. cruzi* epimastigotes, were assayed in an in vitro model of intracellular *T. cruzi* amastigotes. Of the five compounds tested, sulfonamides **6e**, **10b**, and **10d** showed higher activity than the reference compound benznidazole (Table 2).

In Vivo Activity against *L. infantum*. Supported by the in vitro leishmanicidal efficacy, sulfonamides **6e**, **8c**, **9c**, **12c**, and **14d** were also evaluated in vivo in a murine model of acute infection by *L. infantum*. The compounds were assayed at concentrations of 5 mg/kg, administered daily by the

dx.doi.org/10.1021/jm4006127 | J. Med. Chem. 2013, 56, 8984−8998

Table 2. In Vitro Activity of Sulfonamides on *L. amazonensis*, *L. infantum*, and *T. cruzi* Intracellular Forms (amastigotes)

	intracellular amastigotes IC_{50}[a] (μM)		
compd	*L. infantum*	*L. amazonensis*	*T. cruzi*
2a	NA[c]	NA	223.7
6e	23.0	42.9	141.15
8c	47.3	64.1	NA
9c	83.2	30.8	NA
10b	NA	NA	12.35
10d	NA	NA	28.5
12c	39.2	77.5	NA
14d	7.1	18.0	NA
M[b]	23.7	20.9	
B[b]			192.1

[a]IC_{50}, concentration of the compound that produced a 50% reduction in parasites. [b]M: miltefosine, B: benznidazole. [c]NA, nonactive at tested concentrations

intraperitoneal route up to a total of five doses, using a method previously described.[16] In a subsequent study, an additional dose of 10 mg/kg/day of compound **9c** was also tested. The results are summarized in Table 3.

Table 3. In Vivo Antileishmanial Effect of Sulfonamides 6e, 8c, 9c, 12c, and 14d against *L. infantum*

	percentage reduction (mean ± SD)[a]	
compd	spleen	liver
6e	NS[b]	NS
8c	NS	NS
9c (5 mg/kg dose)	55.50 ± 18.53*	78.90 ± 37.10*
9c (10 mg/kg dose)	96.34 ± 3.83**	97.57 ± 2.55**
12c	53.51 ± 67.09	NS
14d	80.17 ± 18.53**	NS

[a]Reduction of parasite burden in spleens and livers of treated mice in relation to those in the control (untreated) groups expressed as percentage. The standard deviation (SD) was calculated by comparing individual data for each treated animal with the mean value for the control group. [b]NS: No suppression of parasite burden. *$p < 0.05$; **$p < 0.01$.

Two out of the five compounds tested (**6e**, **8c**) did not show any *L. infantum* growth inhibition, suggesting a poor bioavailability. Compound **12c** was moderately active against spleen parasites but not against those in the liver, probably due to a rapid biotransformation of the compound in the latter organ. Compound **14d** was totally inactive against liver parasites as well; however, it exhibited high leishmanicidal activity against spleen forms (80.17 ± 18.53% reduction regarding controls). Unfortunately, the administration of **14d** was followed by the appearance of clear signs of hyperactivity together with local skin irritation. The sulfonamide **9c** was the only compound that displayed good activity against both spleen and liver parasite forms with marked increase at 10 mg/kg dose (96.34 ± 3.83% and 97.57 ± 2.55% reduction, respectively, regarding untreated controls). No evident signs of toxicity, such as gross weight loss or hair loss, were observed in any of the animals at the end of the assay, strongly suggesting that **9c** is well-tolerated by the infected mice. Therefore, sulfonamide **9c** can be seen as a promising prototype compound for treating leishmaniasis.

In Vivo Anti-*T. cruzi* Activity. Taking into consideration the in vitro anti-*T. cruzi* activity of compounds **2a**, **6e**, **10b**, and **10d**, in vivo assays in a murine model of acute infection by *T. cruzi* were performed, as previously described.[18−20] Sulfonamides **2a**, **6e**, **10b**, and **10d** were administered intraperitoneally (10 mg/kg/day) for 5 days. A group of untreated mice was also included as control.

The in vivo results are shown in Table 4. Overall, the isoquinolyl derivative **6e** exhibited the highest antiparasitic activity with 72% and 60% reduction of parasitemia, after the administration of three and five doses, respectively. The *p*-nitrobenzene pyrazin-2-yl derivative **2a** was less active than **6e**, with 14% and 57% reduction in parasitemia. The remaining compounds (sulfonamides **10b** and **10d**) were not active, and even an increase in parasitemia was observed after three doses. Nevertheless, when five doses of both compounds were given a reduction of 14% and 44% was recorded.

Table 4. Percentage Reduction of *T. cruzi* Parasitemia in BALB/c Mice Treated after Three and Five Doses of Compounds 2a, 6e, 10b, and 10d

	percentage reduction (mean ± SD)	
compd	8 dpi[a] (three doses[b])	10 dpi[a] (five doses[b])
2a	14 ± 7	57 ± 24
6e	72 ± 20	60 ± 9
10b	−10 ± 25	14 ± 15
10d	−50 ± 20	44 ± 19

[a]dpi: days postinfection. [b]10 mg/kg/day ip.

As outlined in Figure 2, the sulfonamide **6e** showed the lowest parasitemia levels after three and five doses (Figure 2A). Moreover, the percentage of survival in treated mice with this compound was higher than for untreated controls (Figure 2B). In the case of sulfonamide **2a**, the survival rate in treated mice remained always lower than that recorded for the controls. Unfortunately, compounds **10b** and **10d**, which had displayed the best in vitro IC_{50} values (Tables 1 and 2), showed a poor in vivo activity as assessed in reduction of parasitemia (Figure 2A) as well as and survival rates (Figure 2B).

These findings encourage supplementary investigations on sulfonamide **6e** as a potential new trypanocidal drug. Assays with higher dosing in frequency and/or drug quantity should be advisable.

■ CONFOCAL MICROSCOPY

In the literature, it has been reported that sulfonamides possess selective antimicrotubule activity against kinetoplastids.[21,22] Consequently, immunofluorescence studies with confocal microscopy, using specific antibodies against β-tubulin, were carried out to evaluate the effect of sulfonamides **9c** and **14d** on the tubulin distribution profile in *L. infantum* promastigotes.

Confocal microscopic analysis clearly showed the effects of sulfonamide derivatives **9c** and **14d** on *L. infantum* promastigotes after in vitro incubation for 24 and 48 h, in comparison to untreated controls. As shown in untreated promastigotes (Figure 3, A1−A6), antitubulin antibody is homogenously bound alongside cell cytoskelet. This pattern is visibly altered after 24 h incubation with compound **14d**, where an irregular binding pattern of the antibody onto parasite cytoskelet was observed (Figure 3, B1−B3). This disorganizing effect was even more dramatic after 48 h, involving also the

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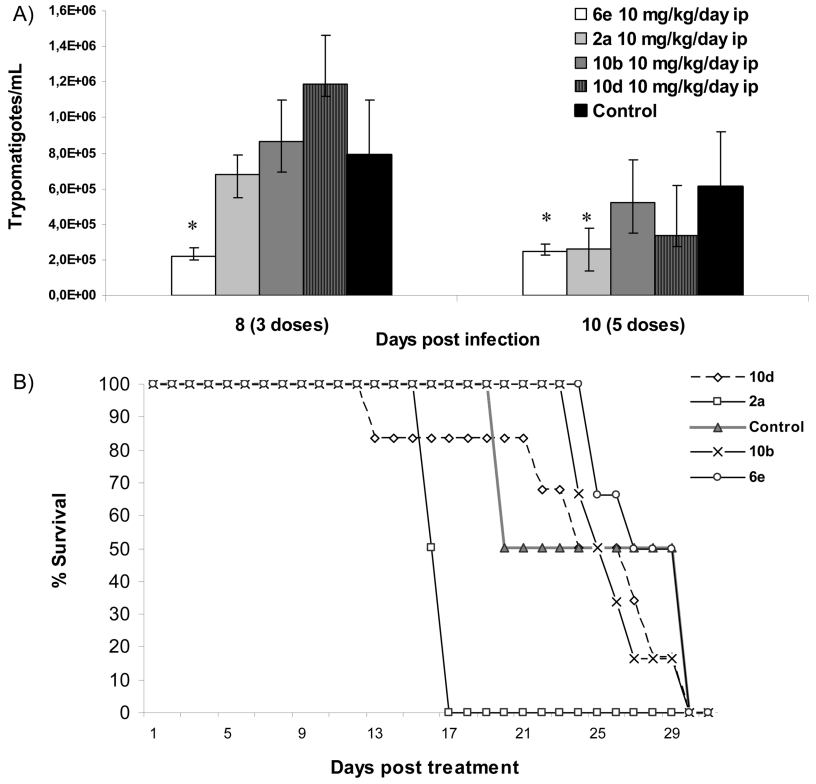

Figure 2. Survival of *T. cruzi* infected mice. (A) Parasitemia levels during the acute infection period in BALB/c mice infected with 10^4 bloodstream trypomastigotes of *T. cruzi*. Mice were treated with **2a**, **6e**, **10b**, and **10d** for 5 days consecutively (5−10 postinfection). Parasitemia was determined by counting the number of trypomastigotes in 5 μL of fresh blood collected from the tail (means ± SEMs). *$p < 0.05$. Bar (left to right): **6e**, **2a**, **10b**, **10d**, and control. (B) Percentage of mouse survival in the control and sulfonamides **2a**, **6e**, **10b**, and **10d** treatment.

DNA (Figure 3, B4−B6). As shown with propidium iodide staining, the kinetoplast DNA (kDNA) is missing and the nuclei appear to be undergoing apoptosis (Figure 3, B5). Immunofluorescence images of *Leishmania* promastigotes treated with sulfonamide **9c** show a marked disorganizing effect on microtubules after 48 h of contact (Figure 3, section C1 and C4 as compared to A1 and A4). However, in contrast to compound **14d**, no evident changes on nuclear and kinetoplastid DNA were evidenced (Figure 3, C5−C6). Therefore, cytoskelet β-tubulin appears to be exclusively targeted by compound **9c**, whereas neither nuclear nor kinetoplastid DNA seem to be altered for this compound.

Microtubules are vital for cell shape, form, motility, growth differentiation, and survival/infectivity of Kinetoplastid parasites.[23,24] In this context, recent research has shown the antitubulin activity of sulfonamide-containing compounds,[25,26] analogously to our results.

Structure−Activity Relationship Studies and in Silico Pharmacokinetic Evaluation. A structure−activity relationship (SAR) analysis was carried out to establish structural features for the antiprotozoal efficacy. Regarding to the relationships between the structure of the *N*-substituent on the sulfonamide moiety and the detected antiparasitic properties, it seems that the nature of the heterocyclic scaffold is important for the activity. None of the amino monocyclic aromatic derivatives (compounds **1b**, **1c**, **2b−d**, **3a−e**, and **4a−c**) showed an improvement in potency over the hit compounds **1a** and **2a** (data not shown). However, analogues where the monocyclic rings of compounds **1−4** were replaced by bicyclic heteroaromatic rings (compounds **5−12**) showed overall an increase in potency, suggesting the requirement of a benzo-fused aromatic substructure for optimal effect. Thus, among the benzo-fused heteroaromatic series, the best in vitro activities against *Leishmania* spp. and *T. cruzi*, without toxicity on mammalian cells, were achieved by an isoquinoline (**6e**), an indole (**8c**), indazoles (**9c**, **10b**, **10d**), and a benzothiazole (**12c**). On the other hand, when ring systems containing saturated heterocycles, such as compounds **13−15**, were considered, only the naphthyl-substituted derivative 3-methylpiperidine **14d** exhibited excellent in vitro leishmanicidal efficacy. Therefore, the presence of the naphthyl substituent on the sulfonamide group seems useful for enhancing the antiprotozoal activity.

In addition, we also aim to test the influence of the substituents in the *para*-position of the benzenesulfonyl moiety. In relation to the leishmanicidal efficacy, the most active compounds against *Leishmania* amastigotes without cytotoxicity were the nonsubstituted sulfonamides **6e**, **8c**, **9c**, **12c**, and **14d**, whereas the addition of an electron-withdrawing substituent did not increase the antileishmanial properties. On the other hand, the in vitro trypanocidal screening revealed the halogenated *p*-chloro- and *p*-fluorobenzenesulfonamides **10b** and **10d** as potent anti-*T. cruzi* compounds, with IC_{50} values lower than for the reference drug benznidazol.

Surprisingly, the presence of the nitro group on the benzenesulfonamide moiety did not confer good antiprotozoal activities, as might be expected taking into account our previous results.[16] This fact indicates different mechanisms of action for the studied compounds, suggesting a different target. Probably, a combination of the electronic distribution, the size, and the lipophilicity of the compounds could explain the differences in the antiparasitic activity.

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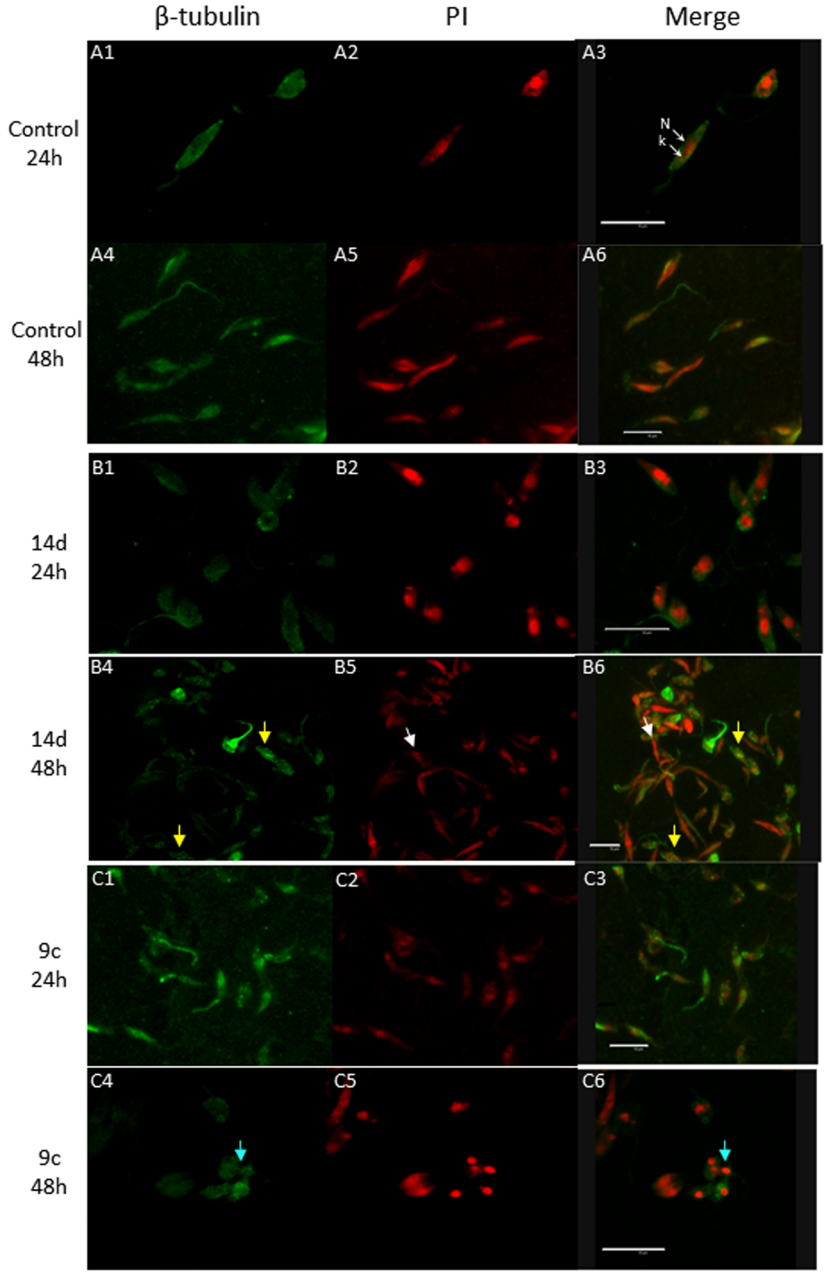

Figure 3. The effect of compounds **9c** and **14d** on tubulin in log-phase promastigotes: Section A shows untreated promastigotes after 24 h (A1−A3) and 48 h (A4−A6) incubation, section B are promastigotes after treatment with **14d** for 24 h (B1−B3) and 48 h (B4−B6), and section C shows promastigotes treated with **9c** for 24 h (C1−C3) and 48 h (C4−C6). Panels A1, A4, B1, B4, C1, and C4 show anti-β-tubulin green fluorescence; panels A2, A5, B2, B5, C2, and C5 show propidium iodide staining; and panels A3, A6, B3, B6, C3, and C6 are the merged images. Scale bar represents 10 μm. In Figure A3, K indicates kinetoplastid and N, the nucleus. Yellow arrows indicate irregular tubulin accumulation in parasite cytoskelet, white arrows mark nuclear and kinetoplastid DNA alterations, and blue arrows show a marked disorganizing effect on microtubules.

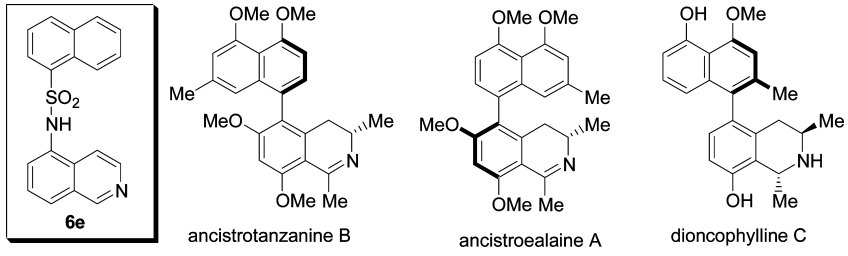

Figure 4. Naphthylisoquinoline alkaloids ancistrotanzanine B, ancistroealaine A, and dioncophylline C and the structurally related analog sulfonamide **6e**.

dx.doi.org/10.1021/jm4006127 | J. Med. Chem. 2013, 56, 8984−8998

Remarkably, when sulfonamides were screened for in vivo activity against *T. cruzi*, quite good activity was found for compound **6e**, a simplified analog of dioncophyline C, ancistrotanzanine B, and its atropisomer ancistroealaine A, natural naphthylisoquinoline alkaloids with known activities against various tropical diseases, such as malaria, leishmaniasis, and Chagas disease.[27] In compound **6e**, instead of the naphthyl group at the 5-position bearing three substituents, as found in the natural naphthylisoquinoline alkaloids dioncophyline C, ancistrotanzanine B, and ancistroealaine A, a naphthyl moiety without substituents is coupled with the isoquinoline ring, through an additional sulfonamide bridge between the naphthalene and the isoquinoline portions. Moreover, the isoquinoline portion is significantly simplified by the fact that it is now devoid of the two methyl groups at C-1 and C-3, thus avoiding the existence of any of the stereogenic centers present in the natural alkaloids (Figure 4).

Finally, compounds **6e** and **9c**, the most in vivo antiprotozoal active sulfonamides, were also submitted to an in silico pharmacokinetic properties evaluation.[28]

Since good absorption is necessary for oral administration, we analyzed the number of free rotatable bonds (n-ROTB) and Lipinski's "rule of five" for both derivatives. Lipinski descriptors describe the molecular properties for drug pharmacokinetics in the human body, especially for oral absorption. The rule states that the most "druglike" molecules present clogP $\leq$ 5, molecular weight $\leq$ 500, number of hydrogen bond acceptors $\leq$ 10, and hydrogen bond donors $\leq$ 5. Compounds **6e** and **9c**, analogously to other antileishmanial and antimalarial sulfonamides,[15c] showed excellent n-ROTB values ($\leq$10) and fulfilled the Lipinski rule of five, an important characteristic for future drug development. In addition, ADMET (absorption, distribution, metabolism, excretion and toxicity) properties were calculated using admetSAR (http://www.admetexp.org/), a freely accessible Web-based application.[28b] The predicted data for BBB (blood–brain barrier) penetration, HIA (human intestinal absorption), and Caco-2 cell permeability are positive for **6e**. In the case of compound **9c**, although permeability is negative, it has a moderate probability value. In the case of metabolism, various cytochrome P450 (CYP) were evaluated. In terms of toxicity, it was found that both compounds may not show mutagenic toxicity with respect to the AMES test and carcinogen effect. Lipinski data and the predicted data of some ADMET properties of compounds **6e** and **9c** are summarized in Table 5.

Table 5. Oral Bioavailability, Molecular Properties, and Predicted ADMET Properties of Compounds 6e and 9c[a]

	6e		9c	
	result	probability (%)	result	probability (%)
		Absorption		
BBB	+	96.80	+	97.33
HIA	+	99.55	+	100.00
Caco-2	+	70.12	−	51.36
		Metabolism		
CYP450 2C9 substrate	NS	75.39	NS	76.15
CYP450 2D6 substrate	NS	81.45	NS	80.95
CYP450 3A4 substrate	NS	66.10	NS	65.93
CYP450 1A2 inhibitor	I	90.43	I	89.57
CYP450 2C9 inhibitor	I	61.63	I	54.59
CYP450 2D6 inhibitor	I	55.60	NI	60.41
CYP450 2C19 inhibitor	I	77.96	I	78.52
CYP450 3A4 inhibitor	NI	53.44	NI	58.57
		Toxicity		
AMES toxicity	−	85.79	−	73.95
carcinogens	−	88.98	−	82.38
n-ROTB ($\leq$10)	2		2	
		Lipinski Molecular Descriptors		
HBA ($\leq$10)	4		5	
HBD ($\leq$5)	1		2	
clogP ($\leq$5)	3.40 ± 0.76		1.97 ± 0.78	
MW ($\leq$500)	334.39		273.31	

[a]BBB, blood–brain barrier; HIA, human intestinal absorption; I, inhibitor; NI, noninhibitor; NS, nonsubstrate; n-ROTB, number of rotatable bonds; HBA, number of hydrogen bond acceptors; HBD, number of hydrogen bond donors; clogP, logarithm of compound partition coefficient between *n*-octanol and water; MW, molecular weight.

CONCLUSION

In summary, we have reported the design, synthesis, and antiparasitic activity of a small library of readily available new *N*-substituted benzene and naphthalenesulfonamide derivatives **1**–**15**.

Compounds **6e**, **8c**, **9c**, **12c**, and **14d** demonstrated potent inhibition on the promastigote form of four *Leishmania* species (*L. infantum*, *L. braziliensis*, *L. guyanensis*, and *L. amazonensis*). They were also active on the amastigote form of *L. amazonensis* and *L. infantum*. Finally, in vivo antileishmanial screening and computational studies revealed the indazolyl derivative **9c** as a good candidate for preformulation studies and clinical development.

Compounds **2a**, **6e**, **10b**, and **10d** displayed remarkable activity toward *T. cruzi* epimastigotes. Studies in vivo on these sulfonamides indicated that **6e** showed a substantial reduction in parasitemia levels in a murine model of acute Chagas disease. Moreover, **6e** showed excellent in silico Lipinski and n-ROTB values. Therefore, **6e** is a promising anti-*T. cruzi* candidate, and further clinical investigation could be useful in the development of new antichagasic drugs.

In this work, we demonstrate the antinuclear and/or antitubulin effects on *L. infantum* promastigotes of compounds **9c** and **14d**, by confocal microscopy analysis. Thus, our previous findings on the mode of action of leishmanicidal sulfonamides have been extended by using alternative molecular tools and, at the same time, opening new windows toward the precise characterization of the mechanism of action of these compounds.

In conclusion, the *N*-isoquinolin-5-yl-1-naphthalenesulfonamide (**6e**) and *N*-(1*H*-indazol-5-yl)benzenesulfonamide (**9c**) represent an important class of simple and readily obtainable compounds with promising in vivo activity against *Leishmania* and *Trypanosoma* parasites and interesting in silico ADME properties. Both sulfonamides might be useful lead scaffolds in the development of new antiprotozoals, and therefore, they merit further pharmacological exploration.

dx.doi.org/10.1021/jm4006127 | J. Med. Chem. 2013, 56, 8984–8998

EXPERIMENTAL SECTION

Chemistry. *General.* All reagents were purchased from Aldrich and used without purification. All experiments were made under nitrogen atmosphere. Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Column chromatography was performed using silica gel (Merck 60, 70−230 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-300 instrument. Chemical shifts (δ values) and coupling constants (J values) are given in parts per million and hertz, respectively. HRMS were obtained using a VG Autospec TRIO 1000 instrument. The ionization mode used in mass spectra was electron impact (EI), fast atom bombardment (FAB), or time-of-flight mass spectrometry (TOFMS). Elemental analysis were performed by the Servicio de Espectroscopía Atómica, Molecular y Óptica, Universitat de València-SCSIE (Servei Central de Suport a la Investigació Experimental), València, Spain. The purity of the compounds (≥95%) and molecular mass were confirmed by elemental microanalysis and HRMS. The analytical results for C, H, and N were within ±0.4 of the theoretical values. Compounds **1a**, **2a**, **3a**, **3c**, and **9a** were synthesized as previously described.[16]

General Procedure for the Synthesis of Sulfonamides 1−15. To an ice-cooled solution of the amine (20 mmol) in pyridine (8 mL) was slowly added the corresponding sulfonyl chloride (30 mmol) in pyridine (6 mL). The mixture was stirred at 0 °C for 2 h and allowed to reach room temperature. Water was added (100 mL) and the solid was collected and recrystallized from MeOH:CH_2Cl_2.

*4-Cyano-N-pyrimidin-2-ylbenzenesulfonamide (**1b**).* Yield 40%; mp 132−133 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.05 (t, J = 5, 1H), 8.06 (d, J = 8.7, 2H), 8.11 (d, J = 8.7, 2H), 8.50 (d, J = 5, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 115.4 (CH), 118.1 (C), 126.8 (CN), 128.5 (CH), 133.4 (CH), 145.4 (C), 156.8 (C), 158.6 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{11}H_9N_4O_2S$ 261.0446, found 261.0450.

*N-Pyrimidin-2-yl-1-naphthalenesulfonamide (**1c**).* Yield 40%; mp 199−202 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.93 (t, J = 5, 1H), 7.67 (m, 3H), 8.05 (d, J = 7.5, 1H), 8.22 (d, J = 8, 1H), 8.40 (m, 3H), 8.77 (d, J = 8, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 115.7 (CH), 124.6 (CH), 124.7 (CH), 127.1 (CH), 127.8 (C), 128.4 (CH), 129.3 (CH), 131.4 (CH), 133.9 (C), 134.6 (CH), 135.3 (C), 157.2 (C), 158.6 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{14}H_{12}N_3O_2S$ 286.0650, found 286.0647.

*4-Cyano-N-pyrazin-2-ylbenzenesulfonamide (**2b**).* Yield 20%; mp 107−110 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.08 (d, J = 8.3, 2H), 8.10 (d, J = 8.3, 2H), 8.15 (d, J = 2.7, 1H), 8.21 (d, J = 2.7, 1H), 8.35 (s, 1H); HRMS (ES$^+$) m/z [M + H] calcd for $C_{11}H_9N_4O_2S$ 261.0446, found 261.0446.

*4-Fluoro-N-pyrazin-2-ylbenzenesulfonamide (**2c**).* Yield 52%; mp 212−215 °C (lit.[29] mp 180−181 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.42 (t, J = 9, 2H), 8.03 (dd, J = 5.3, J = 9, 2H), 8.22 (dd, J = 1.3, J = 2.6, 1H), 8.23 (d, J = 2.6, 1H), 8.36 (d, J = 1.3, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 116.6 (d, J_{C-F} = 18, CH), 130.7 (d, J_{C-F} = 6, CH), 135.3 (CH), 136.6 (d, J_{C-F} = 18, C), 139.3 (CH), 142.4 (CH), 148.3 (C), 165.0 (d, J_{C-F} = 250, C).

*N-Pyrazin-2-yl-1-naphthalenesulfonamide (**2d**).* Yield 99%; mp 207−210 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.70 (m, 3H), 8.09 (m, 3H), 8.25 (d, J = 8.2, 1H), 8.33 (d, J = 1.3, 1H), 8.4 (dd, J = 1.1, J = 7.4, 1H), 8.7 (d, J = 8.2, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 124.3 (CH), 124.9 (CH), 127.3 (CH), 127.6 (C), 128.7 (CH), 129.5 (CH), 131.0 (CH), 134.0 (C), 134.7 (CH), 134.8 (C), 135.1 (CH), 138.8 (CH), 142.2 (CH), 148.4 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{14}H_{12}N_3O_2S$ 286.0650, found 286.0648.

*N-Thiazol-2-ylbenzenesulfonamide (**3b**).* Yield 57%; mp 167−170 °C (lit.[30] mp 171.5−172.5 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.83 (s, 1H), 7.26 (s, 1H), 7.52 (m, 3H), 7.80 (m, 2H), 12.76 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 108.6 (CH), 124.8 (CH), 126.1 (CH), 129.3 (CH), 132.4 (CH), 142.7 (C), 169.3 (C).

*4-Chloro-N-(4-methylthiazol-2-yl)benzenesulfonamide (**3d**).* Yield 50%; mp 106−108 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.06 (s, 3H), 6.33 (s, 1H), 7.58 (d, J = 8.7, 2H), 7.76 (d, J = 8.7, 2H); HRMS (EI$^+$) m/z calcd for $C_{10}H_9ClN_2O_2S_2$ 287.9793, found 287.9794.

*N-(4-Methylthiazol-2-yl)-benzenesulfonamide (**3e**).* Yield 30%; mp 152−153 °C (lit.[31] mp 161−162 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.15 (s, 3H), 6.46 (s, 1H), 7.61 (m, 3H), 7.86 (dd, J = 1.7, J = 8.3, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 13.7 (CH_3), 102.6 (CH), 116.4 (C), 126.1 (CH), 129.2 (CH), 132.2 (CH), 135.0 (C), 168.0 (C).

*N-(3-Methylisoxazol-5-yl)-4-nitrobenzenesulfonamide (**4a**).* Yield 54%; mp 148−153 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.03 (s, 3H), 5.49 (s, 1H), 8.04 (d, J = 9.1, 2H), 8.35 (d, J = 9.1, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 11.8 (CH_3), 86.9 (CH), 124.8 (CH), 128.3 (CH), 147.5 (C), 149.7 (C), 160.8 (C), 165.2 (C); HRMS (EI$^+$) m/z calcd for $C_{10}H_9N_3O_5S$ 283.0262, found 283.0264.

*4-Chloro-N-(3-methylisoxazol-5-yl)benzenesulfonamide (**4b**).* Yield 45%; mp 158−160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.29 (s, 3H), 6.14 (s, 1H), 7.69 (d, J = 8.8, 2H), 7.86 (d, J = 8.8, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 12.4 (CH_3), 95.8 (CH), 129.0 (CH), 129.9 (CH), 138.6 (C), 138.7 (C), 157.6 (C), 170.9 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{10}H_{10}ClN_2O_3S$ 273.0101, found 273.0105.

*N-(3-Methylisoxazol-5-yl)benzenesulfonamide (**4c**).* Yield 80%; mp 146−152 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.95 (s, 3H), 5.74 (s, 1H), 7.60 (t, J = 7.6), 7.70 (d, J = 7.6, 1H), 7.86 (d, J = 7.6, 2H).

*N-Isoquinolin-1-yl-4-nitrobenzenesulfonamide (**5a**).* Yield 54%; mp 195−200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.24 (d, J = 6.2, 1H), 7.65 (d, J = 6.8, 1H), 7.80 (m, 1H), 7.87 (d, J = 8, 2H), 7.95 (m, 2H), 8.20 (d, J = 8, 2H), 8.54 (d, J = 8.3, 1H), 8.95 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 111.7 (CH), 117.9 (C), 123.7 (CH), 124.8 (CH), 125.8 (CH), 127.2 (CH), 128.0 (CH), 129.0 (CH), 135.1 (CH), 137.3 (C), 147.7 (C), 154.3 (C), 154.4 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{15}H_{12}N_3O_4S$ 330.0549, found 330.0547. Anal. Calcd for $C_{15}H_{11}N_3O_4S(H_2O)_{0.6}$: C, 52.97; H, 3.61; N, 12.35; S, 9.43. Found: C, 52.85; H, 3.89; N, 12.37; S, 9.11.

*4-Chloro-N-isoquinolin-1-ylbenzenesulfonamide (**5b**).* Yield 60%; mp 183−188 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.1 (d, J = 7, 1H), 7.60 (m, 4H), 7.80 (m, 2H), 7.98 (d, J = 8.6, 2H), 8.26 (d, J = 8.3, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 110.9 (CH), 124.2 (C), 127.1 (CH), 127.2 (CH), 128.1 (CH), 128.5 (CH), 128.7 (CH), 129.5 (CH), 134.2 (CH), 137.2 (C), 142.4 (C), 148.0 (C), 152.8 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{15}H_{12}ClN_2O_2S$ 319.0308, found 319.0313.

*N-Isoquinolin-1-ylbenzenesulfonamide (**5c**).* Yield 51%; mp 174−176 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.07 (d, J = 7, 1H), 7.57 (m, 5H), 7.80 (m, 2H), 7.98 (dd, J = 1.6, J = 8.1, 2H), 8.38 (d, J = 8.3, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 110.5 (CH), 124.3 (C), 126.1 (CH), 127.1 (CH), 127.2 (CH), 128.4 (CH), 129.4 (CH × 2), 132.4 (CH), 134.1 (CH), 137.1 (C), 148.0 (C), 152.8 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{15}H_{13}N_2O_2S$ 285.0698, found 285.0693. Anal. Calcd for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85; S, 11.28. Found: C, 63.15; H, 4.13; N, 9.72; S, 11.08.

*N-Isoquinolin-1-yl-1-naphthalenesulfonamide (**5d**).* Yield 87%; mp 255−258 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.23 (d, J = 7, 1H), 7.44−7.50 (m, 3H), 7.67 (d, J = 7, 1H), 7.76 (m, 1H), 7.89 (d, J = 8.3, 2H), 7.97 (m, 3H), 8.54 (d, J = 8.3, 1H), 8.85 (d, J = 8.5, 1H), 8.99 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 111.7 (CH), 117.9 (C), 124.7 (CH), 124.8 (CH), 125.8 (CH), 126.0 (CH), 126.1 (CH), 127.9 (CH), 128.0 (CH), 128.1 (CH), 128.2 (CH), 129.0 (CH), 129.3 (C), 129.9 (CH), 133.9 (C), 135.1 (CH), 137.3 (C), 144.0 (C), 154.2 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{19}H_{15}N_2O_2S$ 335.0854, found 335.0848

*N-Isoquinolin-5-yl-4-nitrobenzenesulfonamide (**6a**).* Yield 36%; mp 265−268 °C (lit.[32] mp 270 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.35 (dd, J = 1.2, J = 7.5, 1H), 7.78 (m, 2H), 8.26 (d, J = 8.9, 2H), 8.59 (d, J = 8.9, 2H), 8.62 (d, J = 6.7, 1H), 8.65 (d, J = 6.7, 1H), 9.76 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 116.9 (CH), 117.4 (CH), 120.7 (CH), 123.7 (CH), 126.7 (C), 127.7 (CH), 128.4 (C), 129.4 (C), 132.2 (CH), 145.1 (C), 147.4 (CH), 150.0 (C), 154.6 (CH).

dx.doi.org/10.1021/jm4006127 | J. Med. Chem. 2013, 56, 8984−8998

*4-Chloro-N-isoquinolin-5-ylbenzenesulfonamide (**6b**).* Yield 84%; mp 205−207 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.58 (m, 3H), 7.66 (m, 3H), 7.99 (d, J = 6, 1H), 8.12 (d, J = 8.2, 1H), 8.51 (d, J = 6, 1H), 9.46 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 117.4 (CH), 127.7 (CH), 128.6 (CH), 128.9 (C), 129.0 (CH), 129.1 (CH), 129.8 (CH), 132.0 (C), 133.0 (C), 138.3 (C), 138.5 (C), 140.4 (CH), 151.6 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{15}H_{12}ClN_2O_2S$ 319.0308, found 319.0301. Anal. Calcd for $C_{15}H_{11}ClN_2O_2S(H_2O)_{0.3}$: C, 55.57; H, 3.61; N, 8.64; S, 9.89. Found: C, 55.24; H, 3.48; N, 8.79; S, 10.27.

*N-Isoquinolin-5-ylbenzenesulfonamide (**6c**).* Yield 40%; mp 198−200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.42 (m, 2H), 7.60 (m, 2H), 7.68 (m, 2H), 7.80 (dd, J = 3, J = 8.8, 2H), 7.97 (d, J = 8, 1H), 8.39 (d, J = 6, 1H), 9.26 (s, 1H), 10.44 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 116.0 (CH), 127.0 (CH), 127.6 (CH), 128.7 (CH), 129.2 (C), 129.6 (CH), 130.1 (CH), 131.9 (C), 133.3 (CH), 135.5 (C), 140.0 (C), 143.2 (CH), 152.8 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{15}H_{13}N_2O_2S$ 285.0698, found 285.0697.

*4-Fluoro-N-isoquinolin-5-ylbenzenesulfonamide (**6d**).* Yield 95%; mp 233−237 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.35 (t, J = 8.7, 2H), 7.46 (d, J = 8, 1H), 7.61 (t, J = 8, 1H), 7.72 (dd, J = 5.2, J = 8.7, 2H), 7.80 (d, J = 6, 1H), 8.00 (d, J = 8, 1H), 8.43 (d, J = 6, 1H), 9.28 (s, 1H), 10.49 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 115.9 (CH), 116.8 (d, J_{C-F} = 22.5, CH), 126.8 (CH), 127.6 (CH), 127.7 (CH), 129.2 (C), 130.1 (d, J_{C-F} = 9.75, CH), 131.8 (C), 132.0 (C), 136.2 (d, J_{C-F} = 3, C), 143.3 (CH), 152.8 (CH), 164.7 (d, J_{C-F} = 250.5, C); HRMS (TOF$^+$) m/z [M + H] calcd for $C_{15}H_{12}FN_2O_2S$ 303.0598, found 303.0605.

*N-Isoquinolin-5-yl-1-naphthalenesulfonamide (**6e**).* Yield 81%; mp 196−200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.43 (dd, J = 1.1, J = 7.5, 1H), 7.52 (m, 2H), 7.62 (m, 2H), 7.72 (d, J = 6.5, 1H), 7.90 (d, J = 8, 1H), 8.02 (d, J = 6.5, 1H), 8.04 (d, J = 8, 1H), 8.20 (d, J = 8.3, 1H), 8.24 (dd, J = 1.1, J = 7.5, 1H), 8.78 (d, J = 8.3, 1H), 9.20 (s, 1H), 10.79 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 124.8 (CH), 126.3 (CH), 126.5 (CH), 127.3 (CH), 127.6 (CH), 128.0 (C), 128.4 (CH), 128.8 (CH), 129.4 (CH), 129.8 (CH), 129.9 (CH), 131.6 (C), 131.9 (C), 132.7 (C), 134.1 (C), 134.8 (CH), 134.9 (C), 143.0 (CH), 152.7 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{19}H_{15}N_2O_2S$:335.0854, found 335.0862. Anal. Calcd for $C_{19}H_{14}N_2O_2S$: C, 68.24; H, 4.22; N, 8.38; S, 9.59. Found: C, 68.54; H, 4.00; N, 8.72; S, 9.34.

*4-Nitro-N-quinoxalin-6-ylbenzenesulfonamide (**7a**).* Yield 98%; mp 251−254 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.64 (dd, J = 2.4, J = 9, 1H); 7.72 (d, J = 2.4, 1H); 8.01 (d, J = 9, 1H); 8.11 (d, J = 9, 2H); 8.37 (d, J = 9, 2H); 8.58 (bs, 1H, NH); 8.81 (d, J = 1.9, 1H); 8.85 (d, J = 1.9, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 116.2 (CH), 124.0 (CH), 125.2 (CH), 128.7 (CH), 131.0 (CH), 138.9 (C), 139.8 (C), 143.0 (C), 144.8 (C), 145.1 (CH), 146.7 (CH), 150.4 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{14}H_{11}N_4O_4S$ 331.0501, found 331.0506. Anal. Calcd for $C_{14}H_{10}N_4O_4S$: C, 50.90; H, 3.05; N, 16.96; S, 9.71. Found: C, 51.02; H, 3.31; N, 17.31; S, 9.53.

*4-Chloro-N-quinoxalin-6-ylbenzenesulfonamide (**7b**).* Yield 99%; mp 190−192 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.66 (m, 4H), 7.85 (d, J = 9, 2H), 8.01 (d, J = 9, 1H), 8.81 (s, 1H), 8.84 (s, 1H), 11.2 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 115.7 (CH), 123.9 (CH), 129.0 (CH), 130.1 (CH), 130.9 (CH), 138.3 (C), 138.6 (C), 139.4 (C), 139.0 (C), 143.0 (C), 144.9 (CH), 146.6 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{14}H_{11}ClN_3O_2S$ 320.0261, found 320.0264. Anal. Calcd for $C_{14}H_{10}ClN_3O_2S$: C, 52.58; H, 3.15; N, 13.14; S, 10.03. Found: C, 52.63; H, 3.27; N, 13.10; S, 10.04.

*N-Quinoxalin-6-ylbenzenesulfonamide (**7c**).* Yield 46%; mp 195−200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.57 (m, 3H), 7.62 (dd, J = 2.4, J = 9, 1H), 7.68 (d, J = 2.4, 1H), 7.87 (dd, J = 1.7, J = 7.5, 2H), 7.94 (d, J = 9, 1H), 8.6 (bs, 1H, NH); 8.78 (d, J = 1.8, 1H), 8.83 (d, J = 1.8, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 115.3 (CH), 123.8 (CH), 127.1 (CH), 129.9 (CH), 130.7 (CH), 133.7 (CH), 139.5 (C), 139.7 (C), 143.0 (C), 144.7 (CH), 146.6 (CH), 150.0 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{14}H_{12}N_3O_2S$ 286.0650, found 286.0647.

*4-Fluoro-N-quinoxalin-6-ylbenzenesulfonamide (**7d**).* Yield 78%; mp 186−190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.41 (t, J = 8.8, 2H), 7.63 (dd, J = 2.3, J = 9, 1H), 7.70 (d, J = 2.3, 1H), 7.94 (dd, J = 5.1, J = 8.8, 2H), 8.01 (d, J = 9, 1H), 8.80 (d, J = 1.7, 1H), 8.84 (d, J = 1.7, 1H), 11.1 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 115.6 (CH), 117.1 (d, J_{C-F} = 23.25, CH), 123.9 (CH), 130.2 (d, J_{C-F} = 9.75, CH), 130.8 (CH), 135.8 (C), 139.5 (C), 139.6 (C), 143.0 (C), 144.8 (CH), 146.6 (CH), 164.8 (d, J_{C-F} = 251.25, C); HRMS (TOF$^+$) m/z [M + H] calcd for $C_{14}H_{11}FN_3O_2S$ 304.0551, found 304.0661. Anal. Calcd for $C_{14}H_{10}FN_3O_2S$: C, 55.44; H, 3.32; N, 13.85; S, 10.57. Found: C, 55.58; H, 3.69; N, 13.96; S, 10.22.

*N-Quinoxalin-6-yl-1-napthalenesulfonamide (**7e**).* Yield 99%; mp 115−119 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.38 (m, 1H), 7.65 (m, 4H), 7.78 (td, J = 1.5, J = 7, 1H), 7.92 (d, J = 9, 1H), 8.06 (d, J = 8.3, 1H), 8.21 (d, J = 8.3, 1H), 8.40 (dd, J = 1.3, J = 7.5; 1H), 8.58 (bs, 1H, NH), 8.73 (d, J = 2, 1H), 8.78 (d, J = 2, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 114.1 (CH), 123.0 (CH), 124.3 (CH), 124.9 (CH), 127.5 (CH), 127.6 (C), 128.8 (CH), 129.6 (CH), 130.7 (CH), 134.0 (C), 134.1 (C), 135.3 (CH), 139.2 (C), 139.4 (C), 143.0 (C), 144.5 (CH), 146.5 (CH), 149.9 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{18}H_{14}N_3O_2S$ 336.0807, found 336.0805.

*N-(2-Methyl-1H-indol-5-yl)-4-nitrobenzenesulfonamide (**8a**).* Yield 94%; mp 184−186 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.31 (s, 3H), 6.02 (s, 1H), 6.69 (dd, J = 2, J = 8.5, 1H), 7.08 (m, 2H), 7.88 (d, J = 9, 2H), 8.32 (d, J = 9, 2H), 10.08 (bs, 1H, NH), 10.91 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 13.7 (CH_3), 99.6 (CH), 111.1 (CH), 114.0 (CH), 116.7 (CH), 124.7 (CH), 128.0 (C), 128.7 (CH), 129.0 (C), 134.4 (C), 137.3 (C), 145.5 (C), 149.9 (C); HRMS (EI$^+$) m/z calcd for $C_{15}H_{13}N_3O_4S$: 331.0626, found 331.0623. Anal. Calcd for $C_{15}H_{13}N_3O_4S$: C, 54.37; H, 3.95; N, 12.68; S, 9.68. Found: C, 54.52; H, 4.21; N, 12.35; S, 9.47.

*4-Chloro-N-(2-methyl-1H-indol-5-yl)benzenesulfonamide (**8b**).* Yield 87%; mp 188−190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.39 (s, 3H), 6.20 (s, 1H), 6.95 (dd, J = 2, J = 8.7, 1H), 7.06 (d, J = 2, 1H), 7.15 (d, J = 8.7, 1H), 7.55 (d, J = 8.8, 2H), 7.60 (d, J = 8.8, 2H), 9.8 (s, 1H, NH), 10.90 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ =13.7 (CH_3), 99.5 (CH), 111.0 (CH), 113.7 (CH), 116.5 (CH), 128.6 (C), 129.0 (CH), 129.5 (CH), 134.3 (2C), 137.1 (C), 137.7 (C), 138.9 (C). HRMS (TOF$^+$) m/z [M + H] calcd for $C_{15}H_{14}ClN_2O_2S$ 321.0459, found 321.0459. Anal. Calcd for $C_{15}H_{13}ClN_2O_2S(H_2O)_{0.4}$: C, 54.93; H, 4.24; N, 8.54; S, 9.78. Found: C, 54.70; H, 3.94; N, 8.78; S, 9.51.

*N-(2-Methyl-1H-indol-5-yl)benzenesulfonamide (**8c**).* Yield 84%; mp: 182−184 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.31 (s, 3H), 6.00 (s, 1H), 6.71 (dd, J = 2, J = 8.5, 1H), 7.06 (d, J = 8.5, 1H), 7.08 (s, 1H), 7.47 (m, 3H), 7.65 (d, J = 7.2, 2H), 9.72 (s, 1H, NH), 10.85 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 13.7 (CH_3), 99.5 (CH), 110.9 (CH), 113.4 (CH), 116.4 (CH), 124.3 (C), 127.0 (CH), 128.9 (C), 129.3 (CH), 132.7 (CH), 134.2 (C), 137.0 (C), 140.1 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{15}H_{15}N_2O_2S$ 287.0854, found 287.0844. Anal. Calcd for $C_{15}H_{14}N_2O_2S$: C, 61.94; H, 5.02; N, 9.63; S, 11.02. Found: C, 62.22; H, 4.89; N, 9.23; S, 10.66.

*4-Fluoro-N-(2-methyl-1H-indol-5-yl)benzenesulfonamide (**8d**).* Yield 92%; mp 155−157 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.50 (s, 3H), 6.01 (s, 1H), 6.67 (dd, J = 2, J = 8.7, 1H), 7.06 (d, J = 2, 1H), 7.09 (d, J = 8.7, 1H), 7.33 (t, J = 8.8, 2H), 7.68 (dd, J = 5.1, J = 8.8, 2H), 9.74 (s, 1H, NH), 10.86 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 13.7 (CH_3), 99.5 (CH), 111.0 (CH), 113.7 (CH), 116.3 (CH), 116.6 (CH), 124.5 (C), 128.7 (C), 130.1 (CH), 134.3 (C), 136.3 (C), 137.1 (C), 162 (d, J_{C-F} = 260, C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{15}H_{14}FN_2O_2S$ 305.0760, found 305.0766. Anal. Calcd for $C_{15}H_{13}FN_2O_2S(H_2O)_{0.3}$: C, 58.16; H, 4.42; N, 9.04; S, 10.35. Found: C, 58.10; H, 4.34; N, 9.16; S, 10.22.

*2-Methyl-1H-indol-5-yl-1-naphthalenesulfonamide (**8e**).* Yield 44%; mp 198−190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.27 (s, 3H), 5.92 (s, 1H), 6.59 (dd, J = 2, J = 8.7, 1H), 6.99 (m, 2H), 7.50 (t, J = 8.1, 1H), 7.66 (td, J = 1.3, J = 7, 1H), 7.71 (td, J = 1.5, J = 7, 1H), 8.04 (m, 2H), 8.13 (d, J = 8.1, 1H), 8.80 (d, J = 8.7, 1H), 10.10 (s, 1H, NH), 10.78 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 13.7 (CH_3), 99.4 (CH), 110.9 (CH), 112.8 (CH), 115.9 (CH), 124.7

dx.doi.org/10.1021/jm4006127 | J. Med. Chem. 2013, 56, 8984−8998

(CH), 125.0 (CH), 127.1 (CH), 128.0 (C), 128.2 (CH), 128.7 (C), 128.9 (C), 129.3 (CH), 130.0 (CH), 134.0 (C), 134.2 (CH), 135.2 (C), 137.0 (C), 145.7 (C); HRMS (ES^+) m/z [M + H] calcd for $C_{19}H_{17}N_2O_2S$ 337.1011, found 337.1013. Anal. Calcd for $C_{19}H_{16}N_2O_2S$: C, 67.83; H, 4.79; N, 8.33; S, 9.53. Found: C, 67.96; H, 5.12; N, 8.15; S, 9.32.

*4-Chloro-N-(1H-indazol-5-yl)benzenesulfonamide (**9b**).*[33] Yield 20%; mp 171−173 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.79 (m, 4H), 7.25 (dd, J = 0.9, J = 8.8, 2H), 7.73 (s, 2H), 12.57 (bs, 1H, NH), 13.06 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 100.9 (CH), 110.6 (CH), 118.5 (CH), 124.2 (C), 129.0 (CH), 129.6 (CH), 131.7 (CH), 135.0 (C), 137.9 (C), 138.6 (C), 142.4 (C).

*N-(1H-Indazol-5-yl)benzenesulfonamide (**9c**).* Yield 78%; mp 166−168 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.10 (dd, J = 2, J = 9, 1H), 7.41 (m, 2H), 7.51 (m, 3H), 7.69 (dd, J = 1.7, J = 7, 2H), 7.99 (s, 1H), 10.07 (s, 1H, NH), 13.02 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 111.0 (CH), 113.2 (CH), 122.6 (CH), 123.1 (C), 127.0 (CH), 129.4 (CH), 130.5 (C), 133.0 (CH), 133.8 (CH), 138.0 (C), 139.7 (C); HRMS (ES^+) m/z [M + H] calcd for $C_{13}H_{12}N_3O_2S$ 274.0650, found 274.0645. Anal. Calcd for $C_{13}H_{11}N_3O_2S$: C, 57.13; H, 4.06; N, 15.37; S, 11.73. Found: C, 57.28; H, 4.33; N, 15.09; S, 11.65.

*4-Fluoro-N-(1H-indazol-5-yl)benzenesulfonamide (**9d**).* Yield 89%; mp 214−218 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.06 (dd, J = 2, J = 9, 1H), 7.32 (td, J = 2, J = 8.8, 2H), 7.41 (d, J = 2, 1H), 7.42 (d, J = 9, 1H), 7.73 (dd, J = 5.3, J = 8.8, 2H), 7.99 (s, 1H), 10.0 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 111.1 (CH), 113.6 (CH), 116.5 (d, J_{C-F} = 18, CH), 122.8 (CH), 123.2 (C), 130.0 (d, J_{C-F} = 6, CH), 130.3 (C), 133.8 (CH), 136.0 (d, J_{C-F} = 3, C), 138.1 (C), 164.5 (d, J_{C-F} = 250, C); HRMS (ES^+) m/z [M + H] calcd for $C_{13}H_{11}FN_3O_2S$ 292.0556, found 292.0559. Anal. Calcd for $C_{13}H_{10}FN_3O_2S$: C, 53.60; H, 3.46; N, 14.42; S, 11.01. Found: C, 53.61; H, 3.30; N, 14.49; S, 10.63.

*N-(1H-Indazol-5-yl)naphthalene-1-sulfonamide (**9e**).* Yield 91%; mp 196−199 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.98 (dd, J = 2, J = 9, 1H), 7.32 (m, 2H), 7.53 (t, J = 7.8, 1H), 7.70 (m, 2H), 7.91 (s, 1H), 8.05 (d, J = 7.8, 1H), 8.10 (d, J = 7.3, 1H), 8.13 (d, J = 8.4, 1H), 8.77 (d, J = 8.4, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 111.1 (CH), 112.3 (CH), 121.9 (CH), 123.1 (C), 124.7 (CH), 124.8 (CH), 127.3 (CH), 127.9 (C), 128.4 (CH), 129.4 (CH), 130.1 (CH), 130.3 (C), 133.7 (CH), 134.0 (C), 134.5 (CH), 134.8 (C), 137.8 (C); HRMS (ES^+) m/z [M + H] calcd for $C_{17}H_{14}N_3O_2S$ 324.0801, found 324.0808. Anal. Calcd for $C_{17}H_{13}N_3O_2S$: C, 63.14; H, 4.05; N, 12.99; S, 9.92. Found: C, 63.30; H, 4.39; N, 13.20; S, 9.53.

*N-(1H-Indazol-6-yl)-4-nitrobenzenesulfonamide (**10a**).* Yield 65%; mp 185−188 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.9 (dd, J = 1.7, J = 8.7, 1H), 7.28 (s, 1H), 7.63 (d, J = 8.7, 1H), 7.95 (s, 1H), 8.0 (d, J = 8.8, 2H), 8.35 (d, J = 8.8, 2H), 10.72 (bs, 1H, NH), 12.91 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 101.3 (CH), 115.5 (CH), 120.6 (C), 121.9 (CH), 125.0 (CH), 128.6 (CH), 133.8 (CH), 135.5 (C), 140.4 (C), 145.2 (C), 150.2 (C); HRMS (TOF^+) m/z [M + H] calcd for $C_{13}H_{11}N_4O_4S$ 319.0496, found 319.0486

*4-Chloro-N-(1H-indazol-6-yl)benzenesulfonamide (**10b**).* Yield 95%; mp 187−191 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.89 (dd, J = 1.7, J = 8.7, 1H), 7.25 (s, 1H), 7.61 (d, J = 8.7, 2H), 7.62 (d, J = 8.7, 1H), 7.73 (d, J = 8.7, 2H), 7.95 (s, 1H), 10.48 (bs, 1H, NH), 12.90 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 100.9 (CH), 115.4 (CH), 120.4 (C), 121.7 (CH), 128.9 (CH), 129.8 (CH), 133.8 (CH), 136.0 (C), 138.2 (C), 138.6 (C), 140.4 (C); HRMS (TOF^+) m/z [M + H] calcd for $C_{13}H_{11}ClN_3O_2S$ 308.0255, found 308.0251. Anal. Calcd for $C_{13}H_{10}ClN_3O_2S$: C, 50.73; H, 3.27; N, 13.65; S, 10.42. Found: C, 50.49; H, 3.01; N, 13.31; S, 10.10.

*N-(1H-Indazol-6-yl)benzenesulfonamide (**10c**).* Yield 93%; mp 180−184 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.91 (dd, J = 2, J = 8.6, 1H), 7.28 (s, 1H), 7.53 (m, 3H), 7.60 (d, J = 8.6, 1H), 7.78 (dd, J = 8.1, J = 1.5, 2H), 7.94 (s, 1H), 10.43 (bs, 1H, NH), 12.87 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 100.4 (CH), 115.2 (CH), 120.2 (C), 121.6 (CH), 127.0 (CH), 129.6 (CH), 133.3 (CH), 133.8 (CH), 136.4 (C), 139.8 (C), 140.5 (C); HRMS (TOF^+) m/z [M + H] calcd for $C_{13}H_{12}N_3O_2S$ 274.0645, found 274.0645. Anal. Calcd for $C_{13}H_{11}N_3O_2S$: C, 56.02; H, 4.19; N, 15.08; S, 11.50. Found: C, 56.01; H, 4.46; N, 14.80; S, 11.52.

*4-Fluoro-N-1H-indazol-6-ylbenzenesulfonamide (**10d**).* Yield 96%; mp 159−162 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.88 (dd, J = 1.8, J = 8.7, 1H), 7.26 (s, 1H), 7.37 (t, J = 8.8, 2H), 7.61 (d, J = 8.7, 1H), 7.81 (dd, J = 5.2, J = 8.8, 2H), 7.95 (s, 1H), 10.44 (s, NH), 12.89 (s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 100.7 (CH), 115.3 (CH), 116.8 (d, J_{C-F} = 22.5, CH), 120.3 (C), 121.7 (CH), 130.0 (d, J_{C-F} = 7.5, CH), 130.3 (C), 133.9 (CH), 136.0 (d, J_{C-F} = 3, C), 140.4 (C), 164.5 (d, J_{C-F} = 247.5, C); HRMS (TOF^+) m/z [M + H] calcd for $C_{13}H_{11}FN_3O_2S$ 292.0551, found 292.0549. Anal. Calcd for $C_{13}H_{10}FN_3O_2S(H_2O)_{0.4}$: C, 52.31; H, 3.65; N, 14.08; S, 10.74. Found: C, 52.11; H, 3.24; N, 13.72; S, 11.00.

*N-(1H-Indazol-6-yl)naphthalene-1-sulfonamide (**10e**).* Yield 75%; mp 207−210 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 6.88 (dd, J = 1.7, J = 8.7, 1H), 7.17 (s, 1H), 7.52 (d, J = 8.7, 1H), 7.59 (t, J = 7.8, 1H), 7.65 (dd, J = 7.2, J = 7.7, 1H), 7.75 (ddd, J = 1.3, J = 6.8, J = 8.5, 1H), 7.88 (s, 1H), 8.04 (d, J = 8.1, 1H), 8.17 (d, J = 8.5, 1H), 8.21 (d, J = 7.6, 1H), 8.78 (d, J = 8.7, 1H), 10.80 (s, 1H, NH), 12.77 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 99.3 (CH), 114.5 (CH), 120.3 (C), 121.6 (CH), 124.6 (CH), 124.8 (CH), 127.4 (CH), 127.8 (C), 128.6 (CH), 129.5 (CH), 130.2 (CH), 133.7 (CH), 134.1 (C), 134.6 (C), 134.8 (CH), 136.1 (C), 140.4 (C); HRMS (TOF^+) m/z [M + H] calcd for $C_{17}H_{14}N_3O_2S$ 324.0801, found 324.0798.

*N-Benzothiazol-2-yl-4-nitrobenzenesulfonamide (**11a**).*[34] Yield 67%; mp 244−247 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.32 (td, J = 2, J = 8.1, 1H), 7.47 (m, 2H), 7.84 (d, J = 9, 2H), 7.89 (d, J = 8.1, 1H), 8.20 (d, J = 9, 2H), 9.50 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 114.8 (CH), 123.3 (CH), 123.7 (CH), 124.5 (CH), 124.7 (C), 127.3 (CH), 127.8 (CH), 140.1 (C), 147.6 (C), 154.5 (C), 169.4 (C).

*N-Benzothiazol-2-yl-4-chlorobenzenesulfonamide (**11b**).*[35] Yield 47%; mp 212−216 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.28 (td, J = 1.5, J = 7.9, 1H), 7.38 (d, J = 8.7, 2H), 7.46 (m, 2H), 7.60 (d, J = 8.7, 2H), 7.88 (dd, J = 0.4, J = 7.9, 1H), 9.38 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 115.1 (CH), 123.2 (CH), 124.2 (CH), 125.2 (C), 127.6 (CH), 127.8 (CH), 128.0 (CH), 128.1 (C), 133.3 (C), 140.1 (C); 147.6 (C), 169.2 (C).

*N-Benzothiazol-2-ylbenzenesulfonamide (**11c**).* Yield 87%; mp 262−267 °C (lit.[36] mp 288−289); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.20 (td, J = 1.5, J = 7.9, 1H), 7.35 (d, J = 8.7, 2H), 7.6 (m, 3H), 7.75 (d, J = 8.7, 2H), 7.88 (dd, J = 0.4, J = 7.9, 1H);^{13}C NMR (75 MHz, DMSO-d_6): δ = 113.2 (CH), 123.1 (CH), 124.1 (CH), 125.1 (C), 126.1 (CH), 127.6 (CH), 129.5 (CH), 132.8 (CH), 136.5 (C), 142.3 (C), 167.4 (C).

*N-Benzothiazol-6-yl-4-nitrobenzenesulfonamide (**12a**).* Yield 99%; mp 242−247 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.26 (dd, J = 2, J = 8.7, 1H), 7.91 (d, J = 2, 1H), 7.97 (d, J = 8.7, 1H), 8.02 (d, J = 8.8, 2H), 8.35 (d, J = 8.8, 2H), 9.28 (s, 1H), 10.86 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 114.5 (CH), 120.7 (CH), 124.0 (CH), 125.1 (CH), 128.7 (CH), 134.9 (C), 136.5 (C), 145.0 (C), 150.2 (C), 150.7 (C), 156.4 (CH); HRMS (ES^+) m/z [M + H] calcd for $C_{13}H_{10}N_3O_4S_2$ 336.0113, found 336.0110. Anal. Calcd for $C_{13}H_9N_3O_4S_2$: C, 46.56; H, 2.70; N, 12.53; S, 19.12. Found: C, 46.81; H, 2.75; N, 12.82; S, 18.76.

*N-Benzothiazol-6-yl-4-chlorobenzenesulfonamide (**12b**).* Yield 88%; mp 221−223 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.24 (dd, J = 2, J = 8.8, 1H), 7.61 (d, J = 6.8, 2H), 7.77 (d, J = 6.8, 2H), 7.88 (d, J = 2, 1H), 7.96 (d, J = 8.8, 1H), 9.27 (s, 1H), 10.60 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 114.0 (CH), 120.4 (CH), 123.9 (CH), 129.0 (CH), 129.8 (CH), 134.9 (C), 135.3 (C), 138.3 (C), 138.4 (C), 150.5 (C), 156.1 (CH); HRMS (ES^+) m/z [M + H] calcd for $C_{13}H_9ClN_2O_2S_2$ 324.9872, found 324.9879.

*N-Benzothiazol-6-ylbenzenesulfonamide (**12c**).* Yield 96%; mp 204−208 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.26 (dd, J = 2, J = 8.8, 1H), 7.56 (m, 3H), 7.79 (d, J = 7, 2H), 7.87 (d, J = 2, 1H), 7.94 (d, J = 8.8, 1H), 9.25 (s, 1H), 10.55 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 113.5 (CH), 120.1 (CH), 123.8 (CH), 127.0 (CH), 129.7 (CH), 133.4 (CH), 134.9 (C), 135.7 (C), 139.6 (C), 150.3 (C), 155.9 (CH); HRMS (ES^+) m/z [M + H] calcd for

$C_{13}H_{11}N_2O_2S_2$ 291.0262, found 291.0260. Anal. Calcd for $C_{13}H_{10}N_2O_2S_2$: C, 53.77; H, 3.47; N, 9.65; S, 22.09. Found: C, 53.77; H, 2.96; N, 9.49; S, 22.45.

N-Benzothiazol-6-yl-4-fluorobenzenesulfonamide (**12d**). Yield 97%; mp 231−233 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.25 (dd, J = 2.1, J = 8.8, 1H), 7.37 (t, J = 8.8, 2H), 7.84 (dd, J = 5.3, J = 8.8, 2H), 7.88 (d, J = 2.1, 1H), 7.95 (d, J = 8.8, 1H), 9.27 (s, 1H), 10.60 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 113.8 (CH), 116.8 (d, J_{C-F} = 22.5, CH), 120.4 (CH), 123.8 (CH), 130.1 (d, J_{C-F} = 9.75, CH,), 134.9 (C), 135.6 (C), 136.0 (d, J_{C-F} = 3, C), 150.4 (C), 156.0 (CH), 164.7 (d, J_{C-F} = 255, C).

N-(Benzothiazol-6-yl)naphthalene-1-sulfonamide (**12e**). Yield 98.5%; mp 201−203 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.19 (dd, J = 2, J = 8, 1H), 7.73 (m, 5 H), 8.06 (d, J = 7, 1H), 8.20 (d, J = 8, 1H), 8.28 (dd, J = 1.2, J = 7, 1H), 8.78 (d, J = 8, 1H), 9.20 (s, 1H), 10.96 (bs, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 112.3 (CH), 119.2 (CH), 123.7 (CH), 124.6 (CH), 124.8 (CH), 127.4 (CH), 127.8 (C), 128.6 (CH), 129.5 (CH), 130.4 (CH), 134.1 (C), 134.5 (C), 134.8 (C), 134.9 (CH), 135.6 (C), 149.9 (C), 155.6 (CH); HRMS (TOF$^+$) m/z [M + H] calcd for $C_{17}H_{13}N_2O_2S_2$ 341.0413, found 341.0422.

1-Methyl-4-(4-nitrobenzenesulfonyl)piperazine (**13a**). Yield 42%; mp 159−160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.14 (s, 3H), 2.36 (t, J = 4.7, 4H), 2.97 (t, J = 4.7, 4H), 8.00 (d, J = 9, 2H), 8.45 (d, J = 9, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 45.6 (CH_3), 46.0 (CH_2), 53.8 (CH_2), 125.1 (CH), 129.4 (CH), 141.1 (C), 150.4 (C).

4-Chloro-4-methylpiperazin-1-ylbenzenesulfonamide (**13b**). Yield 49%; mp 109−100 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.26 (s, 3H), 2.53 (m, 4H), 2.95 (m, 4H), 7.75 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 45.0 (CH_3), 45.6 (2 × CH_2), 53.5 (2 × CH_2), 129.8 (CH), 130 (CH), 134.1 (C), 138.7 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{11}H_{16}ClN_2O_2S$ 275.0621, found 275.0621.

4-Methylpiperazin-1-ylbenzenesulfonamide (**13c**). Yield 60%; mp 130−134 °C (lit.[37] mp 129−130 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.14 (s, 3H), 2.36 (t, J = 5, 4H), 2.88 (t, J = 5, 4H), 7.70 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 45.5 (CH_3), 46.0 (2 × CH_2), 53.8 (2 × CH_2), 127.9 (CH), 129.7 (CH), 133.6 (CH), 135.1 (C).

Methyl-4-(naphthalene-1-sulfonyl)piperazine (**13d**). Yield 99%; mp 121−124 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.06 (s, 3H), 2.26 (m, 4H), 3.06 (m, 4H), 7.69 (m, 3H), 8.13 (dd, J = 1.5, J = 8.1, 1H), 8.15 (dd, J = 1.3, J = 7.3, 1H), 8.29 (d, J = 8.3, 1H), 8.66 (d, J = 8.5, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 45.5 (2 × CH_2), 45.6 (CH_3), 54.1 (2 × CH_2), 124.9 (CH), 125.0 (CH), 127.3 (CH), 128.5 (CH), 129.4 (CH), 130.6 (CH), 132.3 (C), 134.3 (C), 135.0 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{15}H_{19}N_2O_2S$ 291.1167, found 291.1169.

3-Methyl-1-(4-nitrobenzenesulfonyl)piperidine (**14a**). Yield 81%; mp 147−150 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.83 (d, J = 6.4, 3H), 0.87 (m, 1H), 1.46 (m, 1H), 1.65 (m, 3H), 1.99 (t, J = 11, 1H), 2.30 (td, J = 2.4, J = 11.5, 1H), 3.53 (t, J = 10.5, 2H), 8.0 (d, J = 9, 2H), 8.44 (d, J = 9, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 19.0 (CH_3), 24.5 (CH_2), 30.6 (CH), 31.5 (CH_2), 46.4 (CH_2), 52.8 (CH_2), 125.0 (CH), 129.3 (CH), 141.8 (C), 150.3 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{12}H_{17}N_2O_4S$: 285.0909, found 285.0902.

1-(4-Chlorobenzenesulfonyl)-3-methylpiperidine (**14b**). Yield 43.5%; mp 111−113 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.83 (d, J = 6.4, 3H), 0.85 (m, 1H), 1.44 (m, 1H), 1.62 (m, 3H), 1.91 (t, J = 10.5, 1H), 2.21 (td, J = 3, J = 11.5, 1H), 3.47 (m, 2H), 7.72 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 19.0 (CH_3), 24.4 (CH_2), 30.5 (CH), 31.5 (CH_2), 46.4 (CH_2), 52.9 (CH_2), 129.7 (CH), 129.9 (CH), 134.8 (C), 138.3 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{12}H_{17}ClNO_2S$ 274.0669, found 274.0663.

1-Benzenesulfonyl-3-methylpiperidine (**14c**). Yield 74%; mp 98−100 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.82 (d, J = 6.4, 3H + m, 1H), 1.45 (m, 1H), 1.62 (m, 3H), 1.85 (t, J = 11, 1H), 2.17 (td, J = 3, J = 11.5, 1H), 3.5 (m, 2H), 7.71 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 19.1 (CH_3), 24.5 (CH_2), 30.5 (CH), 31.6 (CH_2), 46.5 (CH_2), 53.0 (CH_2), 127.7 (CH), 129.7 (CH), 133.6 (CH), 135.9 (C); HRMS (ES$^+$) m/z [M + H] calcd for $C_{12}H_{18}NO_2S$ 240.1058, found 240.1061. Anal. Calcd for $C_{12}H_{17}NO_2S$: C, 60.22; H, 7.16; N, 5.85; S, 13.40. Found: C, 60.60; H, 7.51; N, 5.66; S, 13.45.

3-Methyl-1-(naphthalene-1-sulfonyl)piperidine (**14d**). Yield 84%; mp 65−69 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.78 (d, J = 6.4, 3H), 0.9 (m, 1H), 1.37 (m, 1H), 1.62 (m, 3H), 2.2 (t, J = 10.2, 1H), 2.56 (m, 1H), 3.59 (m, 2H), 7.71 (m, 3H), 8.10 (dd, J = 1.5, J = 8.7, 1H), 8.15 (dd, J = 1.1, J = 7.5, 1H), 8.27 (d, J = 8.1, 1H), 8.67 (d, J = 8.7, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 18.9 (CH_3), 24.7 (CH_2), 30.7 (CH), 31.7 (CH_2), 45.9 (CH_2), 52.4 (CH_2), 125.0 (CH), 125.0 (CH), 127.3 (CH), 128.4 (CH), 128.5 (C), 129.4 (CH), 130.2 (CH), 133.3 (C), 134.3 (C), 134.7 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{16}H_{20}NO_2S$ 290.1215, found 290.1212. Anal. Calcd for $C_{16}H_{19}NO_2S(H_2O)_{0.4}$: C, 64.79; H, 6.73; N, 4.72; S, 10.81. Found: C, 65.06; H, 7.02; N, 5.07; S, 10.65.

2-(4-Nitrobenzenesulfonyl)-1,2,3,4-tetrahydroisoquinoline (**15a**). Yield 45%; mp 171 °C (lit.[38] mp 161 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.86 (t, J = 6, 2H), 3.40 (t, J = 6, 2H), 4.30 (s, 2H), 7.15 (m, 4H), 8.10 (d, J = 9, 2H), 8.41 (d, J = 9, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 28.2 (CH_2), 43.8 (CH_2), 47.4 (CH_2), 125.0 (CH), 126.5 (CH), 126.7 (CH), 127.1 (CH), 129.1 (CH), 129.3 (CH), 131.7 (C), 133.2 (C), 142.2 (C), 150.4 (C).

2-(4-Chlorobenzenesulfonyl)-1,2,3,4-tetrahydroisoquinoline (**15b**). Yield 99%; mp 154−156 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.85 (t, J = 6, 2H), 3.35 (t, J = 6, 2H), 4.22 (s, 2H), 7.14 (m, 4H), 7.70 (d, J = 8.7, 2H), 7.84 (d, J = 8.7, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 28.3 (CH_2), 43.8 (CH_2), 47.5 (CH_2), 126.5 (CH), 126.8 (CH), 127.0 (CH), 129.0 (CH), 129.7 (CH), 129.9 (CH), 131.8 (C), 133.3 (C), 135.3 (C), 138.5 (C); HRMS (TOF$^+$) m/z [M + H] calcd for $C_{15}H_{15}ClNO_2S$ 308.0507, found 308.0504.

2-Benzenesulfonyl-1,2,3,4-tetrahydroisoquinoline (**15c**). Yield 97%; mp 156−158 °C (lit.[39] mp 158−159 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.84 (t, J = 5.7, 2H), 3.22 (t, J = 5.7, 2H), 4.20 (s, 2H), 7.13 (m, 4H), 7.64 (t, J = 7, 2H), 7.69 (d, J = 7, 1H), 7.82 (d, J = 7, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 28.3 (CH_2), 43.9 (CH_2), 47.6 (CH_2), 126.5 (CH), 126.8 (CH), 127.0 (CH), 127.8 (CH), 129.0 (CH), 129.8 (CH), 131.9 (C), 133.3 (C), 133.6 (CH), 136.3 (C).

2-(Naphthalen-1-sulfonyl)-1,2,3,4-tetrahydroisoquinoline (**15d**). Yield 94%; mp 135−137 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.81 (t, J = 6, 2H), 3.52 (t, J = 6, 2H), 4.40 (s, 2H), 7.12 (m, 4H), 7.7 (m, 3H), 8.1 (dd, J = 1.3, J = 8, 1H), 8.2 (dd, J = 1.3, J = 7.3; 1H), 8.28 (d, J = 8.3, 1H), 8.65 (d, J = 8.3, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 28.5 (CH_2), 43.2 (CH_2), 46.8 (CH_2), 121 (C), 124.8 (CH), 125.0 (CH), 126.4 (CH), 126.7 (CH), 127.0 (CH), 127.3 (CH), 128.3 (C), 128.6 (CH), 129.1 (CH), 129.5 (CH), 130.2 (CH), 132.2 (C), 133.4 (C), 134.3 (C), 134.9 (CH); HRMS (ES$^+$) m/z [M + H] calcd for $C_{19}H_{18}NNaO_2S$ 346.0872, found 346.0890. Anal. Calcd for $C_{19}H_{17}NO_2S$: C, 70.56; H, 5.30; N, 4.33; S, 9.91. Found: C, 70.43; H, 5.45; N, 4.34; S, 9.90.

Biological Activity. *Drugs and Reagents.* Resazurin sodium salt was obtained from Sigma-Aldrich (St. Louis, MO) and stored at 4 °C protected from light. The solution of resazurin was prepared at 2.5 mM in phosphate-buffered saline solution (PBS), pH 7.4, and filtered through 0.22 μm prior use. Chlorophenol red−β-D-galactopyranoside (CPRG; Roche, Indianapolis, IN) was dissolved in 0.9% Triton X-100 (pH 7.4). Reference drugs miltefosine and benznidazole were purchased from Sigma-Aldrich. The stock solution of benznidazole was prepared in sterile distilled water with 3% Tween 80 and before use it was diluted in sterile distilled water for oral administration.

Leishmanicidal Assays. *Parasites and Culture Procedure.* The following species of *Leishmania* were used: an autochthonous isolate of *L. infantum* (MCAN/ES/96/BCN150) obtained from an asymptomatic dog from the Priorat region (Catalunya, Spain), kindly given by Prof. Montserrat Portús (Universidad de Barcelona); *L. braziliensis* 2903, *L. amazonensis* (MHOM/Br/79/Maria), and *L. guyanensis* 141/93 were kindly provided by Prof. Alfredo Toraño (Instituto de Salud Carlos III, Madrid). Promastigotes were cultured in Schneider's insect medium (Sigma) at 26 °C supplemented with 20% heat-inactivated fetal bovine serum (FBS) (Sigma) and 100 U/mL of penicillin plus 100 μg/mL of streptomycin (Sigma) in 25 mL culture flasks.

In Vitro Promastigote Susceptibility Assay. The assay was performed following a method previously described.[16] Briefly, log-phase promastigotes (2.5×10^5parasites/well) were cultured in 96-well plastic plates. Compounds were dissolved in dimethyl sulfoxide (DMSO) and were added at 2-fold serial dilutions up to 200 μL final volume. The final solvent (DMSO) concentrations never exceeded 0.2% (v/v). After 48 h at 26 °C, 20 μL of 2.5 mM resazurin solution was added and the fluorescence intensity (535 nm excitation wavelength and 590 nm emission wavelength) was determined with a fluorometer Infinite 200 (Tecan i-Control) to calculate growth inhibition (%). All tests were carried out in triplicate. Miltefosine was used as reference drug. The efficacy of each compound was estimated by calculating the IC_{50} (concentration of the compound that produced a 50% reduction in parasites) and GI% (growth inhibitory percentage).

In Vitro Intracellular Amastigote Susceptibility Assay. The assay was carried out as described by Bilbao-Ramos et al.[40] Briefly, 5×10^4 J774 macrophages and stationary promastigotes in a 1:10 rate were seeded in each well of a microtiter plate, suspended in 200 μL of culture medium and incubated for 24 h at 33 °C, 5% CO_2 in a humidity chamber. After this first incubation, the temperature was increased up to 37 °C for another 24 h. Thereafter, cells were washed several times in culture medium by centrifugation at 1500*g* for 5 min in order to remove free noninfective promastigotes. Finally, the supernatant was replaced by 200 μL/well of culture medium containing 2-fold serial dilutions of the test compounds in a triplicate assay. Following incubation for 48 h at 37 °C, 5% CO_2 in a humidity chamber, the culture medium was replaced by 200 μL/well of the lysis solution (RPMI-1640 with 0.048% HEPES and 0.006% SDS) and incubated at room temperature for 20 min. Thereafter, the plates were centrifuged at 1500*g* for 5 min and the lysis solution was replaced by 200 μL/well of Schneider's insect medium. The culture plates were then incubated at 26 °C for other 3 days to allow transformation of viable amastigotes into promastigotes and proliferation. Afterward, 20 μL/well of 2.5 mM resazurin was added, and the plates were left for another 3 h incubation. Finally, fluorescence emission was measured as described above.

In Vivo Leishmanicidal Assay. The in vivo assay was performed in BALB/c mice infected with the virulent *L. infantum* strain MCAN/ES/96/BCN150. The infection was carried out under the same conditions as previously described.[16] Briefly, each mouse was infected with 10^7 promastigotes at stationary phase, given by the intracardiac (ic) route following anesthesia with sodium pentobarbital. The splenic and hepatic parasite burdens were estimated by the limiting dilution assay described by Titus et al.,[41] adapted to the conditions of our laboratory.

The therapeutic protocols were carried out as follows: Mice were randomly sorted into eight groups. One group was kept as untreated control. Treatment started on day 21 postinfection and lasted continuously for 5 days. Animals were dosed once daily and the compounds were administered ip at 5 mg/kg in a 0.1 mL final volume of propylene glycol solution. Seven days later, the mice were sacrificed, and the parasitic burden was evaluated. In a subsequent study, a dose of 10 mg/kg/day of compound **9c** was tested according to the previous schedule.

All animal experiments and procedures were approved by the institution's committee on the ethical handling and protection of laboratory animals used for experimental and other scientific purposes.

Trypanocidal Assays. *Parasites and Culture Procedure.* For in vitro studies, the clone CL-B5 of *T. cruzi* was used. The parasites, stably transfected with the *Escherichia coli* β-galactosidase gene (lacZ), were kindly provided by Dr. F. Buckner through the Universidad Complutense de Madrid (Spain). The epimastigotes were grown at 28 °C in liver infusion tryptose broth (LIT) complemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin and harvested during the exponential growth phase.

For in vivo assays, bloodstream trypomastigotes of the Y strain were used throughout and were harvested by heart puncture from *T. cruzi*-infected BALB/c mice on the day of peak parasitemia, as described.[18]

Epimastigote Susceptibility Assay. The screening assay was performed in 96-well microplates (SARSTEDT) with cultures that had not reached the stationary phase, as described.[42] Briefly, epimastigotes were seeded at 1×10^5 per milliliter in 200 μL. The plates were then incubated with the drugs at 28 °C for 72 h, at which time 50 μL of the substrate solution CPRG was added to give a final concentration of 200 μM. The plates were incubated at 37 °C for an additional 6 h and were then read at 595 nm. Benznidazole was used as reference drug. Each concentration was tested in triplicate. Each experiment was performed twice separately. The efficacy of each compound was estimated by calculating the IC_{50}.

In Vitro Intracellular Amastigote Susceptibility Assay. The activity was evaluated by colorimetric method using CPRG.[43] NCTC-929 fibroblasts were established in 24-well tissue culture plates at a previously determined optimal concentration of 2.5×10^3 cells/well. NCTC-929-derived trypomastigotes were added to the monolayers at a parasite:cell ratio of 5:1 and incubated for 24 h at 33 °C with 5% CO_2. The infected cells were then washed twice with PBS to remove the extracellular trypomastigotes. The drugs were added in triplicate to give a final volume of 900 μL/well. The plates were incubated for 7 days at 33 °C. At this time, 100 μL of CPRG solution (final concentration 400 μM) in 0.3% Triton X-100 was added. Following 4 h of incubation at 37 °C, the colorimetric reaction was quantified by measuring optical densities (OD) at 595 nm wavelength. The results were expressed as percentages of antiamastigote activity (%AA) relative to control wells, as follows: %AA = 100 − (OD experimental wells/OD control wells) × 100. Background controls (only NCTC-929 cells) were subtracted from all values.

In Vivo Trypanocidal Assay. Mice Infection. BALB/c mice (20−24 g) were obtained from the Animal Facility of the Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción (UNA, Paraguay). Mice were housed at a maximum of six per cage and kept in a specific pathogen free (SPF) room at 20−24 °C under a 12/12 h light/dark cycle and provided with sterilized water and chow ad libitum. The animals were allowed to acclimate for 7 days before starting the experiments. Infection was performed by ip injection of 10^4 trypomastigotes.

All animal experiments and procedures were approved by the institution's committee on the ethical handling and protection of laboratory animals used for experimental and other scientific purposes.

Treatment. The in vivo protocol[19] allows the analyses of the effect of drugs on the parasite load using BALB/c female mice infected with 10^4 bloodstream trypomastigotes from the Y strain. Compounds **2a**, **6e**, **10b**, and **10d** were dissolved in DMSO and then freshly diluted with propylene glycol. Animals are treated intraperitoneally (ip) with the compounds at 10 mg/kg/day for 5 days consecutively, with treatment beginning 5 days postinfection (dpi). Only those animals that are positive for parasitemia were used. The following parameters are followed: (i) parasitemia is evaluated microscopically by the Pizzi−Brener method[20] at 5, 8, and 10 dpi and (ii) survival is defined as an animal living 30 days after the end of the treatment. Results from each compound tested according to these parameters will be compared to the results achieved following the reference protocol dosing with 100 mg/kg/day benznidazole. The mortality rates were checked daily until 30 dpi and expressed as survival percentage (%S).

Cytotoxicity Assays. *Cells.* J774 murine macrophages were grown in RPMI 1640 medium (Sigma) supplemented with 10% heat-inactivated FBS (30 min at 56 °C), penicillin G (100 U/mL), and streptomycin (100 μg/mL). For the experiments, cells in the preconfluence phase were harvested with trypsin. Cell cultures were maintained at 37 °C in a humidified environment with 5% CO_2.

Fibroblast NCTC929 were grown in Dulbecco's modified Eagle medium (DMEM; Sigma) supplemented with 10% FBS, 2 mM L-glutamine, and antibiotics (50 units/mL penicillin and 50 g/mL streptomycin).

Cytotoxicity Assays on Macrophages and Fibroblasts. The procedure for cell viability measurement was evaluated with resazurin by a colorimetric method described previously.[43]

For macrophages, J774 cell lines were seeded (5×10^4 cells/well) in 96-well flat-bottom microplates with 100 μL of RPMI 1640 medium. The cells were allowed to attach for 24 h at 37 °C, 5% CO_2, and the medium was replaced by different concentrations of the drugs in 200 μL of medium and exposed for another 24 h. Growth controls were

also included. Afterward, a volume of 20 μL the 2.5 mM resazurin solution was added, and plates were returned to the incubator for another 3 h to evaluate cell viability. The reduction of resazurin was determined by the fluorescence intensity (535 nm excitation wavelength and 590 nm emission wavelength) as in the promastigotes assay. Each concentration was assayed three times. Medium and drug controls were used in each test as blanks.

For fibroblasts, NCTC clone 929 cells were plated in 96-microtiter plates at 3×10^4 cells per well in 100 μL of growth medium. The cells were grown overnight at 37 °C, 5% CO_2. Thereafter the medium was removed, and the compounds were added in 200 μL of medium for 24 h. After incubation, 20 μL of 2 mM resazurin solution was added to each well. The plates were incubated for 3 h to allow optimal oxidation−reduction. The plates were read at 570 and 595 nm on a microplate reader.

Cytotoxicity effect of compounds was defined as the 50% reduction of cell viability of treated culture cells with respect to untreated culture (CC_{50}).

Confocal Microscopy. *Reagents.* Mouse polyclonal anti-β-tubulin antibody was purchased from NeoMarkers (Thermo Scientific), goat anti-rabbit IgG (H+L)−Alexa Fluor 488 dye (Life Technologies) and Fluoroshield with propidium iodide (PI) were from Sigma.

Procedure. Promastigote forms of *L. infantum* in log-phase growth were treated with compounds **9c** and **14d** at concentrations corresponding to the IC_{50} for 24 and 48 h. After incubation, the parasites were washed with PBS containing 0.25 mM $MgCl_2$ and 0.35 mM $CaCl_2$, fixed with 4% paraformaldehyde and then permeabilized with 0.2% saponin in blocking solution for 20 min (1% bovine serum albumin in PBS containing Mg^{2+} and Ca^{2+}). Thereafter, promastigotes were incubated overnight in a humid chamber with anti-β-tubulin as described by Chavan et al.[24] Then, the parasites were washed with PBS containing Ca^{2+} and Mg^{2+} and were stained with the secondary reagent, goat anti-rabbit IgG (H+L)−dye in blocking solution for 1 h at 37 °C. Then, promastigotes were mounted in Fluoroshield with PI. Slides were then observed under a Leica DM IRE2 confocal laser scanning microscope and visualized with a Leica SP2 (Leica Microsystems Heidelberg GmbH).

Statistics. Data are reported as means of three repeated experiments. The efficacy against parasite (IC_{50}) and the cytotoxicity effect (CC_{50}) of compounds were determined by probit multilineal analysis curves. For in vitro test all data were analyzed by Tukey's HSD-test posthoc, and in vivo assays were analyzed by Mann−Whitney *U* test. Statistical significance was considered at $p \leq 0.05$ using SPSS v20.0 and Microsoft Excel 2007 software.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank the Conselleria de Sanitat-Generalitat Valenciana (Projects AP158/10 and AP173/11), the Universidad Cardenal Herrera CEU (Projects PRCEU-UCH 19/10 and PRCEU-UCH 36/11), Ministerio de Asuntos Exteriores y Cooperación (Project MAEC-AP/038991/11), and the Ministerio de Ciencia e Innovación (CONSOLIDER CSD2010-00065) for financial support. P.B.-R. is a MAEC-AECID predoctoral fellow at the Complutense University of Madrid. C.G.-R. is indebted to the Generalitat Valenciana for a contract. C.V. and M.R. are grateful to PRONII/CONACYT (Programa Nacional de Incentivo a Investigadores/Consejo Nacional de Ciencia y Tecnología), Paraguay.

ABBREVIATIONS USED

AA, antiamastigote activity; B, benznidazole; CC_{50}, 50% reduction of cell viability of treated culture cells with respect to untreated culture; CPRG, chlorophenol red−β-D-galactopyranoside; DMEM, Dulbecco's modified Eagle medium; dpi, days postinfection; FBS, fetal bovine serum; GI%, growth inhibitory percentage; HIA, human intestinal absorption; I, inhibitor; LIT, liver infusion tryptose; M, miltefosine; kDNA, kinetoplastic DNA; n-ROTB, number of rotatable bonds; NI, noninhibitor; NS, nonsubstrate; PI, propidium iodide; SI, selectivity index; SPF, specific pathogen free.

REFERENCES

(1) (a) Nussbaum, K.; Honek, J.; Cadmus, C. M. C. v. C.; Efferth, T. Trypanosomatid Parasites Causing Neglected Diseases. *Curr. Med. Chem.* **2010**, *17*, 1594−1617. (b) Graebin, C. S.; Uchoa, F. D.; Bernardes, L. S. C.; Campo, V. L.; Carvalho, I.; Eifler-Lima, V. L. Antiprotozoal Agents: An Overview. *Anti-Infect. Agents Med. Chem.* **2009**, *8*, 345−366.

(2) http://www.who.int/leishmaniasis/en/ (last accessed April 9, 2013)

(3) (a) http://www.dndi.org/diseases/vl/current-treatment.html (last accessed April 9, 2013). (b) Monzote, L. Current Treatment of Leishmaniasis: A Review. *Open Antimicrob. Agents J.* **2009**, *1*, 9−19. (c) Cruz, A. K.; Simões de Toledo, J.; Falade, M.; Terrão, M. C.; Kamchonwongpaisan, S.; Kyle, D. E.; Uthaipibull, C. Current Treatment and Drug Discovery Against *Leishmania* spp. and *Plasmodium* spp.: A Review. *Curr. Drug Targets* **2009**, *10*, 178−192. (d) Croft, S. L. Kinetoplastida New Therapeutic Strategies. *Parasite* **2008**, *15*, 522−527. (e) Kedzierski, L.; Sakthianandeswaren, A.; Curtis, J. M.; Andrews, P. C.; Junk, P. C.; Kedzierska, K. Leishmaniasis: Current Treatment and Prospects for New Drugs and Vaccines. *Curr. Med. Chem.* **2009**, *16*, 599−614.

(4) Croft, S. L.; Sundar, S.; Fairlamb, A. H. Drug Resistance in Leishmaniasis. *Clin. Microbiol. Rev.* **2006**, *19*, 111−126.

(5) (a) http://www.who.int/mediacentre/factsheets/fs340/en/index.html (last accessed April 9, 2013). (b) Rassi, A., Jr.; Rassi, A.; Marin-Neto, J. A. Chagas Disease. *Lancet* **2010**, *375*, 1388−1402.

(6) (a) Castillo, E.; Dea-Ayuela, M. A.; Bolás-Fernández, F.; Rangel, M.; González-Rosende, M. E. The Kinetoplastid Chemotherapy Revisited: Current Drugs, Recent Advances and Future Perspectives. *Curr Med. Chem.* **2010**, *17*, 4027−4051. (b) Filardi, L. S.; Brener, Z. Susceptibily and Natural Resistance of *Trypanosoma cruzi* Strains to Drugs Used Clinically in Chagas Disease. *Trans. R. Soc. Trop. Hyg.* **1987**, *81*, 755−759.

(7) (a) Richard, J. V.; Werbovetz, K. A. New Antileishmanial Candidates and Lead Compounds. *Curr. Opin. Chem. Biolog.* **2010**, *14*, 447−455. (b) Cerecetto, H.; González, M. Synthetic Medicinal Chemistry in Chagas' Disease: Compounds at The Final Stage of "Hit-To-Lead" Phase. *Pharmaceuticals* **2010**, *3*, 810−838.

(8) (a) Argyropoulou, I.; Geronikaki, A.; Vicini, P.; Zani, F. Synthesis and Biological Evaluation of Sulfonamide Thiazole and Benzothiazole Derivatives As Antimicrobial Agents. *Arkivoc* **2009**, *6*, 89−102. (b) Mitscher, L. A.; Lemke, T. L.; Gentry, E. J. Antibiotics and Antimicrobial Agents. In *Foye's Principles of Medicinal Chemistry*; Lemke, T. L., Williams, D. A., Eds.; Lippincott, Williams & Wilkins: Philadelphia, 2008; pp 1028−1083.

(9) (a) Vullo, D.; Innocenti, A.; Supuran, C. T. Diuretics with Carbonic Anhydrase Inhibitory Activity: Toward Novel Applications for Sulfonamide Drugs. In *Drug Design of Zinc−Enzyme Inhibitors*; Supuran, C. T., Winum, J.-Y., Eds.; Wiley & Sons, Inc.: New York, 2009; pp 155−170. (b) Supuran, C. T.; Scozzafava, A. Carbonic Anhydrase Inhibitors and Their Therapeutic Potential. *Exp. Opin. Ther. Patents* **2000**, *10*, 575−600.

(10) (a) Navarrete-Vazquez, G.; Paoli, P.; León-Rivera, I.; Villalobos-Molina, R.; Medina-Franco, J. L.; Ortiz-Andrade, R.; Estrada-Soto, S.; Camici, G.; Diaz-Coutiño, D.; Gallardo-Ortiz, I.; Martinez-Mayorga,

K.; Moreno-Díaz, H. Synthesis, in Vitro and Computational Studies of Protein Tyrosine Phosphatase 1B Inhibition of a Small Library of 2-Arylsulfonylaminobenzothiazoles with Antihyperglycemic Activity. *Bioorg. Med. Chem.* **2009**, *17*, 3332−3341. (b) Selam, J. L. Pharmacokinetics of Hypoglycemic Sulfonamides: Ozidia, a New Concept. *Diabetes Metab.* **1997**, *23*, 39−43.

(11) Thornber, C. W. Isosterism and Molecular Modification in Drug Design. *Chem. Soc. Rev.* **1979**, *8*, 563−580.

(12) (a) Shah, S. S. A.; Rivera, G.; Ashfaq, M. Recent Advances in Medicinal Chemistry of Sulfonamides. Rational Design as Anti-Tumoral, Anti-Bacterial and Anti-Inflammatory Agents. *Mini-Rev. Med. Chem.* **2013**, *13*, 70−86. (b) Abbassi, N.; Chicha, H.; Rakib, E. M.; Hannioui, A.; Alaoui, M.; Hajjaji, A.; Geffken, D.; Aiello, C.; Gangemi, R.; Rosano, C.; Viale, M. Synthesis, Antiproliferative and Apoptotic Activities of *N*-(6(4)-Indazolyl)-benzenesulfonamide Derivatives as Potential Anticancer Agents. *Eur. J. Med. Chem.* **2012**, *57*, 240−249. (c) Ghorab, M.; Fatma, M.; Ragab, A.; Hamed, M. M. Design, Synthesis and Anticancer Evaluation of Novel Tetrahydroquinoline Derivatives Containing Sulfonamide Moiety. *Eur. J. Med. Chem.* **2009**, *44*, 4211−4217. (d) Hu, L.; Li, Z.; Jiang, J.; Boykin, D. W. Novel Diaryl or Heterocyclic Sulfonamides As Antimitotic Agents. *Anti-Cancer Agents Med. Chem.* **2008**, *8*, 739−745.

(13) (a) Wolfe, C.; Hicks, C. Profile of Darunavir in the Management of Treatment-Experienced HIV Patients. *HIV/AIDS* **2009**, *1*, 13−21. (b) Stranix, B. R.; Lavallée, J. F.; Sevigny, G.; Yelle, J.; Perron, V.; LeBerre, N.; Herbart, D.; Wu, J. J. Lysine Sulfonamides as Novel HIV-Protease Inhibitors: *Nε*-Acyl Aromatic *α*-Amino Acids. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3459−3462. (c) Supuran, C. T.; Innocenti, A.; Mastrolorenzo, A.; Scozzafava, A. Antiviral Sulfonamide Derivatives. *Mini-Rev. Med. Chem.* **2004**, *4*, 189−200.

(14) (a) Martyn, D. C.; Cortese, J. F.; Tyndall, E.; Dick, J.; Mazitschek, R.; Munoz, B.; Clardy, J. Antiplasmodial Activity of Piperazine Sulfonamides. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 218−221. (b) Hernandez-Nunez, E.; Tlahuext, H.; Moo-Puc, R.; Torres-Gomez, H.; Reyes-Martinez, R.; Cedillo-Rivera, R.; Nava-Zuazo, C.; Navarrete-Vazquez, G. Synthesis and in Vitro Trichomonicidal, Giardicidal and Amebicidal Activity of *N*-Acetamide(sulfonamide)-2-methyl-4-nitro-1*H*-imidazoles. *Eur. J. Med. Chem.* **2009**, *44*, 2975−2984. (c) Ekoue-Kovi, K.; Yearick, K.; Iwaniuk, D. P.; Natarajan, J. K.; Alumasa, J.; de Dios, A. C.; Roepe, P. D.; Wolf, C. Synthesis and Antimalarial Activity of New 4-Amino-7-chloroquinolyl Amides, Sulfonamides, Ureas and Thioureas. *Bioorg. Med. Chem.* **2009**, *17*, 270−283. (d) Parai, M. K.; Panda, G.; Srivastava, K.; Puri, S. K. Design, Synthesis and Antimalarial Activity of Benzene and Isoquinoline Sulfonamide Derivatives. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 776−778. (e) Krungkrai, J.; Krungkrai, S. R.; Supuran, C. T. Malarial Parasite Carbonic Anhydrase and Its Inhibitors. *Curr. Top. Med. Chem.* **2007**, *7*, 909−917. (f) Norose, K.; Aosai, F.; Mun, H.-S.; Yano, A. Effects of Sulfamethoxazole on Murine Ocular Toxoplasmosis in Interferon-*γ* Knockout Mice. *Invest. Ophthalmol. Vis. Sci.* **2006**, *47*, 265−271.

(15) (a) Papadopoulou, M. V.; Bloomer, W. D.; Rosenzweig, H. S.; Chatelain, E.; Kaiser, M.; Wilkinson, S. R.; McKenzie, C.; Ioset, J.-R. Novel 3-Nitro-1*H*-1,2,4-triazole-Based Amides and Sulfonamides as Potential Antitrypanosomal Agents. *J. Med. Chem.* **2012**, *55*, 5554−5565. (b) Marra, R. K. F.; Bernardino, A. M. R.; Proux, T. A.; Charret, K. S.; Lira, M.-L. F.; Castro, H. C.; Souza, A. M. T.; Oliveira, C. D.; Borges, J. C.; Rodrigues, C. R.; Canto-Cavalheiro, M. M.; Leon, L. L.; Amaral, V. F. 4-(1*H*-Pyrazol-1-yl)benzenesulfonamide Derivatives: Identifying New Active Antileishmanial Structures for Use against a Neglected Disease. *Molecules* **2012**, *17*, 12961−12973. (c) Barea, C.; Pabón, A.; Castillo, D.; Zimic, M.; Quiliano, M.; Galiano, S.; Pérez-Silanes, S.; Monge, A.; Deharo, E.; Aldana, I. New Salicylamide and Sulfonamide Derivatives of Quinoxaline 1,4-Di-*N*-oxide with Anti-leishmanial and Antimalarial Activities. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4498−502. (d) Goodarzi, M.; da Cunha, E. F. F.; Freitas, M. P.; Ramalho, T. C. QSAR and Docking Studies of Novel Antileishmanial Diaryl Sulfides and Sulfonamides. *Eur. J. Med. Chem.* **2010**, *45*, 4879−4889. (e) Pagliero, R. J.; Lusvarghi, S.; Pierini, A. B.; Brun, R.; Mazzieri, M. R. Synthesis, Stereoelectronic Characterization and Antiparasitic Activity of New 1-Benzenesulfonyl-2-methyl-1,2,3,4-tetrahydroquinolines. *Bioorg. Med. Chem.* **2010**, *18*, 142−150. (f) da Silva, L. E.; de Sousa, P. T., Jr.; Maciel, E. N.; Nunes, R. K.; Eger, I.; Steindel, M.; Rebelo, R. A. In Vitro Antiprotozoal Evaluation of Zinc and Copper Complexes Based on Sulfonamides Containing 8-Aminoquinoline Ligands. *Lett. Drug Des. Discovery* **2010**, *7*, 679−685. (g) Souza, A. M. T.; Castro, H. C.; Brito, M. A.; Andrighetti-Frohner, C. R.; Magalhaes, U. O.; Oliveira, K. N.; Gaspar-Silva, D.; Pacheco, L. K.; Joussef, A. C.; Steindel, M.; Simoes, C. M. O.; Santos, D. O.; Albuquerque, M. G.; Rodrigues, C. R.; Nunes, R. J. *Leishmania amazonensis* Growth Inhibitors: Biological and Theoretical Features of Sulfonamide 4-Methoxychalcone Derivatives. *Curr. Microbiol.* **2009**, *59*, 374−379. (h) Khan, K. M.; Khan, M. Z.; Taha, M.; Maharvi, G. M.; Saify, Z. S.; Parveen, S.; Choudhary, M. I. Leishmanicidal Potential of *N*-Substituted Morpholine Derivatives: Synthesis and Structure−Activity Relationships. *Nat. Prod. Res.* **2009**, *23*, 479−484. (i) Altenkamper, M.; Bechem, B.; Perruchon, J.; Heinrich, S.; Madel, A.; Ortmann, R.; Dahse, H.-M.; Freunscht, E.; Wang, Y.; Rath, J.; Stich, A.; Hitzler, M.; Chiba, P.; Lanzer, M.; Schlitzer, M. Antimalarial and Antitrypanosomal Activity of a Series of Amide and Sulfonamide Derivatives of a 2,5-Diaminobenzophenone. *Bioorg. Med. Chem.* **2009**, *17*, 7690−7697. (j) Ajibade, P. A.; Kolawole, G. A.; Brien, P. O. Metal Complexes of 4-Amino-*N*-(2-pyrimidinyl)benzene Sulfonamide: Synthesis, Characterization and Antiprotozoal Studies. *Synth. React. Inorg. Met.-Org. Nano-Metal Chem.* **2007**, *37*, 653−659.

(16) Dea-Ayuela, M. A.; Castillo, E.; González-Álvarez, M.; Vega, C.; Rolon, M.; Bolas-Fernández, F.; Borras, J.; González-Rosende, M. E. In Vivo and in Vitro Anti-Leishmanial Activities of 4-Nitro-*N*-pyrimidin- and *N*-Pyrazin-2-ylbenzenesulfonamides, and N^2-(4-Nitrophenyl)-N^1-propylglycinamide. *Bioorg. Med. Chem.* **2009**, *17*, 7449−7456.

(17) Bilbao-Ramos, P.; Galiana-Roselló, C.; Dea-Ayuela, M. A.; González-Alvarez, M.; Vega, C.; Rolón, M.; Pérez-Serrano, J.; Bolás-Fernández, F.; González-Rosende, M. E. Nuclease Activity and Ultrastructural Effects of New Sulfonamides with Anti-Leishmanial and Trypanocidal Activities. *Parasitol. Int.* **2012**, *61*, 604−613.

(18) Meirelles, M. N.; de Araújo Jorge, T. C.; de Souza, W. Interaction of *Trypanosoma cruzi* with Macrophages in Vitro: Dissociation of the Attachment and Internalization Phases by Low Temperature and Cytochalasin B. *Z. Parasitenkd.* **1982**, *68*, 7−14.

(19) Romanha, A. J.; Castro, S. L.; Soeiro, M.; de, N.; Lannes-Vieira, J.; Ribeiro, I.; Talvani, A.; Bourdin, B.; Blum, B.; Olivieri, B.; Zani, C.; Spadafora, C.; Chiari, E.; Chatelain, E.; Chaves, G.; Calzada, J. E.; Bustamante, J. M.; Freitas-Junior, L. H.; Romero, L. I.; Bahia, M. T.; Lotrowska, M.; Soares, M.; Andrade, S. G.; Armstrong, T.; Degrave, W.; Andrade, Z.; de, A. In Vitro and in Vivo Experimental Models for Drug Screening and Development for Chagas Disease. *Mem. Inst. Oswaldo Cruz* **2010**, *105*, 233−238.

(20) Brener, Z. Therapeutic Activity and Criterion of Cure on Mice Experimentally Infected with *Trypanosoma cruzi*. *Rev. Inst. Med. Trop. Sao Paulo* **1962**, *4*, 386−396.

(21) Lama, R.; Sandhu, R.; Zhong, B.; Li, B.; Su, B. Identification of Selective Tubulin Inhibitors as Potential Anti-Trypanosomal Agents. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5508−5516.

(22) George, T. G.; Endeshaw, M. M.; Morgan, R. E.; Mahasenan, K. V.; Delfín, D. A.; Mukherjee, M. S.; Yakovich, A. J.; Fotie, J.; Li, C.; Werbovetz, K. A. Synthesis, Biological Evaluation, and Molecular Modeling of 3,5-Substituted-N^1-phenyl-N^4,N^4-di-*n*-butylsulfanilamides as Antikinetoplastid Antimicrotubule Agents. *Bioorg. Med. Chem.* **2007**, *15*, 6071−6079.

(23) Kohl, L.; Gull, K. Molecular Architecture of the Trypanosome Cytoskeleton. *Mol. Biochem. Parasitol.* **1998**, *93*, 1−9.

(24) Chavan, H. D.; Singh, G.; Dey, C. S. Confocal Microscopic Investigation of Tubulin Distribution and Effect of Paclitaxel on Posttranslationally Modified Tubulins in Sodium Arsenite Resistant *Leishmania donovani*. *Exp. Parasitol.* **2007**, *116*, 320−326.

(25) Chang, J. Y.; Hsieh, H. P.; Chang, C. Y.; Hsu, K. S.; Chiang, Y. F.; Chen, C. M.; Kuo, C. C.; Liou, J. P. 7-Aroyl-aminoindoline-1-sulfonamides as a Novel Class of Potent Antitubulin Agents. *J. Med. Chem.* **2006**, *49*, 6656−6659.

(26) Yoshimatsu, K.; Yamaguchi, A.; Yoshino, H.; Koyanagi, N.; Kitoh, K. Mechanism of Action of E7010, an Orally Active Sulfonamide Antitumor Agent: Inhibition of Mitosis by Binding to the Colchicine Site of Tubulin. *Cancer Res.* **1997**, *57*, 3208−3213.
(27) (a) Bringmann, G.; Zhang, G.; Ölschläger, T.; Stich, A.; Wud, J.; Chatterjee, M.; Brun, R. Highly Selective Antiplasmodial Naphthylisoquinoline Alkaloids from *Ancistrocladus tectorius*. *Phytochemistry* **2013**, *91*, 220−228. (b) Bringmann, G.; Brun, R.; Kaiser, M.; Neumann, S. Synthesis and Antiprotozoal Activities of Simplified Analogs of Naphthylisoquinoline Alkaloids. *Eur. J. Med. Chem.* **2008**, *43*, 32−42. (c) Maya, J. D.; Cassels, B. K.; Iturriaga-Vásquez, P.; Ferreira, J.; Faúndez, M.; Galanti, N.; Ferreira, A.; Morello, A. Mode of Action of Natural and Synthetic Drugs against *Trypanosoma cruzi* and Their Interaction with the Mammalian Host. *Comp. Biochem. Phys. A* **2007**, *146*, 601−620.
(28) (a) Calculated by using Advanced Chemistry Development (ACD/Labs) Software V 11.02 (copyright 1994−2012 ACD/Labs). (b) Cheng, F.; Li, W.; Zhou, Y.; Shen, J.; Wu, Z.; Liu, G.; Lee, P. W.; Tang, Y. admetSAR: A Comprehensive Source and Free Tool for Evaluating Chemical ADMET Properties. *J. Chem. Inf. Model.* **2012**, *52*, 3099−3105.
(29) Vigorita, M. G.; Grasso, S.; Zappala, M.; Ottana, R.; Monforte, M. T.; Barbera, R.; Trovato, A. Aminopyrazinyl Derivatives: Synthesis and Evaluation of Antiinflammatory and Related Activities. *Farmaco* **1994**, *49*, 271−276.
(30) Cejudo, R.; Alzuet, G.; González-Alvarez, M.; García-Giménez, J. L.; Borras, J.; Liu-González, M. DNA Cleavage Reaction Induced by Dimeric Copper(II) Complexes of *N*-Substituted Thiazole Sulfonamides. *J. Inorg. Biochem.* **2006**, *100*, 70−79.
(31) Sprague, J. M.; Kissinger, L. W. Sulfonamido Derivatives of Thiazoles. *J. Am. Chem. Soc.* **1941**, *63*, 578−580.
(32) Hill, A. J.; Hall, G. E. Some Sulfanilamide Derivatives of the Isoquinoline Series. *J. Am. Chem. Soc.* **1952**, *74*, 666−668.
(33) Li, X.; Chu, S.; Feher, V. A.; Khalili, M.; Nie, Z.; Margosiak, S.; Nikulin, V.; Levin, J.; Sprankle, K. G.; Tedder, M. E.; Almassy, R.; Appelt, K.; Yager, K. M. Structure-Based Design, Synthesis, And Antimicrobial Activity of Indazole-Derived SAH/MTA Nucleosidase Inhibitors. *J. Med. Chem.* **2003**, *46*, 5663−5673.
(34) Xu, X.; Weitzberg, M.; Keyes, R. F.; Li, Q.; Wang, R.; Wang, X.; Zhang, X.; Frevert, E. U.; Camp, H. S.; Beutel, B. A.; Sham, H. L.; Gu, Y. G. The Synthesis and Structure-Activity Relationship Studies of Selective Acetyl-CoA Carboxylase Inhibitors Containing 4-(Thiazol-5-yl)but-3-yn-2-amino Motif: Polar Region Modifications. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1803−1807.
(35) Verma, S. M.; Khani, L.; Gunakkunru, A.; Saha, A. Synthesis and Anticonvulsant Activity of Some Heterocyclic Sulfonamides. *J. Inst. Chem. (India)* **2008**, *80*, 8−13.
(36) Lacova, M.; Nga, N. T.; Halgas, J. Vilsmeier−Haack Reaction of 2-Amino-X-benzothiazoles with *N*-Phenylformamide in the Presence of Benzenesulfonyl Chloride. *Collect. Czech. Chem. Commun.* **1988**, *53*, 311−318.
(37) Jacob, R. M. 1-Alkylsulfonyl-4-alkylpiperazines. US Patent 2507408, 1950.
(38) Ito, K.; Tanaka, H. Syntheses of 1,2,3,4-Tetrahydroisoquinolines from *N*-Sulfonylphenethylamines and Aldehydes. *Chem. Pharm. Bull.* **1977**, *25*, 1732−1739.
(39) Lukanov, L.; Venkov, A.; Mollov, N. Application of the Intramolecular α-Amidoalkylation Reaction for the Synthesis of 2-(Arylsulfonyl)-1,2,3,4-tetrahydroisoquinolines. *Synthesis* **1987**, 204−206.
(40) Bilbao-Ramos, P.; Sifontes-Rodríguez, S.; Dea-Ayuela, M. A.; Bolás-Fernández, F. A Fluorometric Method for Evaluation of Pharmacological Activity against Intracellular *Leishmania* Amastigotes. *J. Microbiol. Methods* **2012**, *89*, 8−11.
(41) Titus, R. G.; Marchand, M.; Boon, T.; Louis, J. A. A Limiting Dilution Assay for Quantifying *Leishmania major* in Tissues of Infected Mice. *Parasite Immunol.* **1985**, *7*, 545−555.
(42) Vega, C.; Rolón, M.; Martínez-Fernández, A. R.; Escario, J. A.; Gómez-Barrio, A. A New Pharmacological Screening Assay with *Trypanosoma cruzi* Epimastigotes Expressing beta-Galactosidase. *Parasitol. Res.* **2005**, *95*, 296−298.
(43) Rolón, M.; Seco, E. M.; Vega, C.; Nogal, J. J.; Escario, J. A.; Gómez-Barrio, A.; Malpartida, F. Selective Activity of Polyene Macrolides Produced by Genetically Modified Streptomyces on *Trypanosoma cruzi*. *Int. J. Antimicrob. Agents* **2006**, *28*, 104−109.