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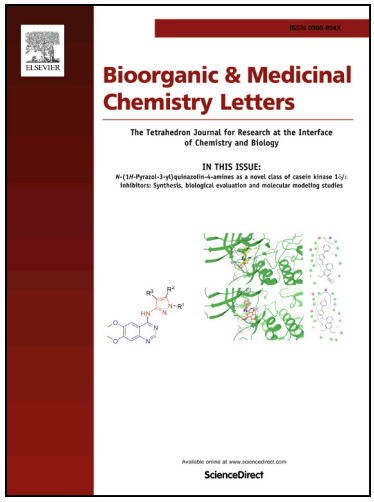

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gem-Dithioacetylated Indole Derivatives as Novel Antileishmanial Agents.

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Abstract:
In this communication we report a serendipitously discovered hybrid molecule **1**, combining fragment of **3** (an *in vivo* active antileishmanial molecule) with H_2S donor moiety (known for bimodal behavior of cytoprotection and apoptosis), as antileishmanial agent. Compound **1** suppresses 99.82% parasitemia of *L. donovani* infected macrophages at 12.5 μg/ml without even deforming them (CC_{50} > 100 μg/ml). This compound appears cytotoxic for intracellular amastigotes while cytoprotective to host macrophages. The concept can be utilized to develop high therapeutic index NCE (New Chemical Entities) for other macrophage mediated diseases like tuberculosis and cancer.

Key words: Hybrid, H_2S donor, antileishmanial, cyctoprotection.

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Leishmaniasis is a vector born disease caused by protozoa of genus *Leishmania* which is transmitted by female *phlebotomine* sand fly to human host. Depending on causative protozoan species it is manifested in four clinical forms: visceral leishmaniasis, cutaneous leishmaniasis, mucocutaneous leishmaniasis and post kala azar dermal leishmaniasis (PKDL). Among these four clinical forms of leishmaniasis, visceral leishmaniasis (VL, also known as kala azar) is fatal if left untreated. There are 0.2 to 0.3 million cases of VL appeared every year worldwide and an estimated 20000 to 40000 people die annually due to VL.[1]

Treatment of VL relied on set of four drugs; sodium stibogluconate (antimonial therapy), liposomal amphotericin B (polyene antibiotic), paromomycin (aminoglycoside antibiotic) and miltefosin (phospholipid).[2] All the four drugs have well documented toxicity and suffer from problem of resistance. The antimonial therapy induces cardiac arrhythmia and pancreatitis especially in malnourished and immunocompromised patients.[3] Liposomal amphotericin B, though a highly effective option for treatment of VL, however this drug formulation is very expensive and suffered from life threatening side effects such as hypokalemia (low K^+ level in the blood), nephrotoxicity and first dose anaphylaxis.[3] The third option miltefosin is contraindicated in pregnant woman due to teratogenicity and cause mild to severe gastrointestinal side effects.[4] Similarly, paromomycin is suffered from high tone ototoxicity and increased hepatic transaminases.[5] In spite of toxicity of prevalent treatments, the wide spread treatment failure are observed with antimonials [3] and the other three drugs are also prone to development of resistance.[6] Climate change, mass movement of population and the emergence of PKDL have further increased the risk of spread of leishmaniasis.[1] The development of new safe and efficacious drugs are thus required for VL therapy.

H_2S is recently recognized as endogenous gasotransmitter like NO and CO. It has multiple roles in normal physiology and pathogenesis of diseases. At low concentration, H_2S activates many intracellular signaling pathway promoting vasodilation and cyctoprotection, while at high concentration it becomes detrimental to viability of cell through various mechanisms (mitochondrial inhibition, intracellular acidification etc.).[7] To exploit the role of H_2S in cellular biology, slow H_2S donors have emerged as important chemotype to treat various pathological conditions.[16] The slow H_2S donors; diallyl trisulfide, GYY4137 and S-propargyl-cystein were found active in animal models of cancer (Fig. 1).[7] Furthermore, conjugation of H_2S donating moiety with known drug (naproxen) was found to reduce the toxicity of parent drug.[8]

Previously, our group has developed novel antileishmanial agents combining natural product scaffold with drug fragments or other privileged structures.[9] In one of our report we combined pentamidine fragment with 2-thio analogue of aplysinopsin to create novel antileishmanial chemotype **3**.[9a] Herein, we report that the fragment of above chemotype **3** when conjugated to H_2S donor moiety, can provide an antileishmanial agent with better activity profile (Fig. 1).

Compound **1** was serendipitously formed during one pot muticomponent synthesis of **3**.[10] After discovering **1** as potential antileishmanial agent, we tried to find out the mechanism of unusual reactivity of 1-alkylated indole-3-carbaldehyde to increase the yield of product. It was found that the role of glycine in multicomponent synthesis of *gem*-dithioacetylated indole derivative was just to create acidic condition during reaction, instead of generating acetylthiocyanate (CH_3CO-SCN) species (through intermediate 1-acetylthiohydantoin) as we previously proposed.[10] Since tetrabutylammonium tribromide (TBATB) was used as acid

catalyst in *gem*-diacetyl protection of aldehyde and ketone,[11] we also used the same catalyst for synthesis of *gem*-dithiolacetylated indole derivatives. Use of TBATB was found slightly more effective in comparison to glycine in above KSCN and acetic anhydride mediated *gem*-dithiolacylation of indole-3-carbaldehyde (Scheme 1).

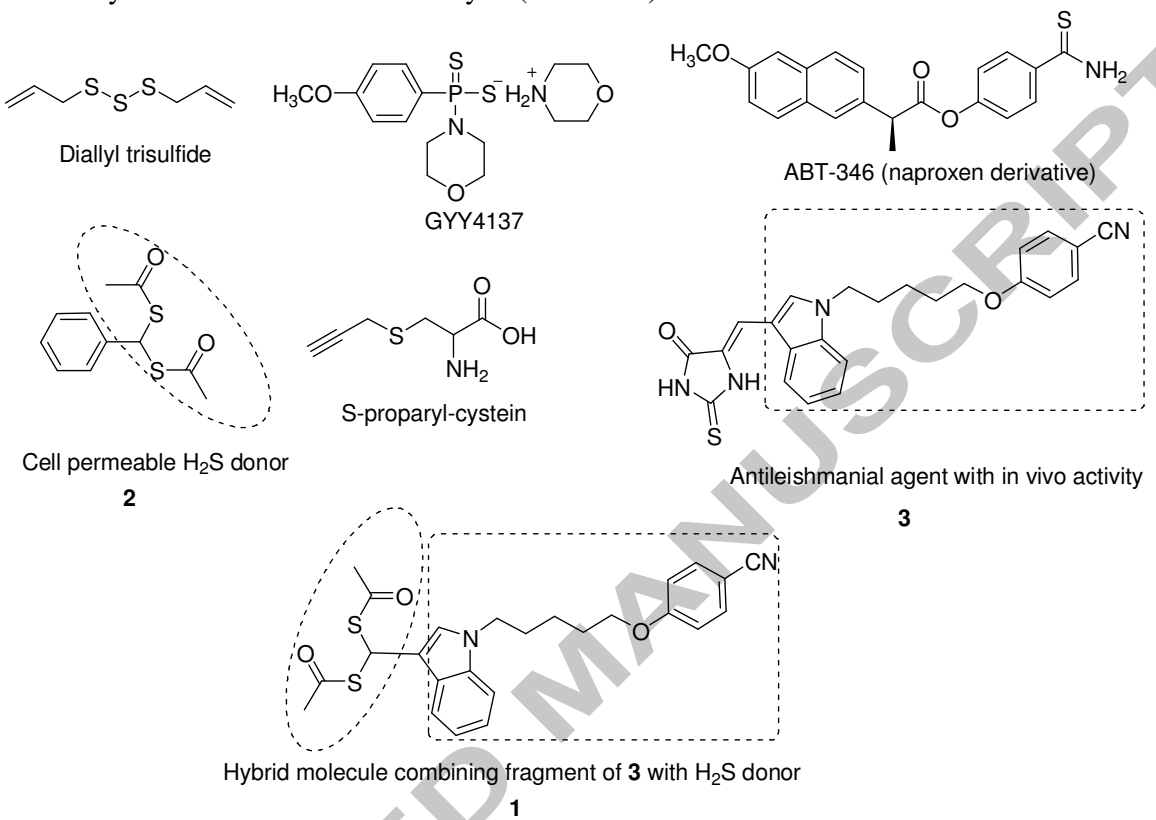
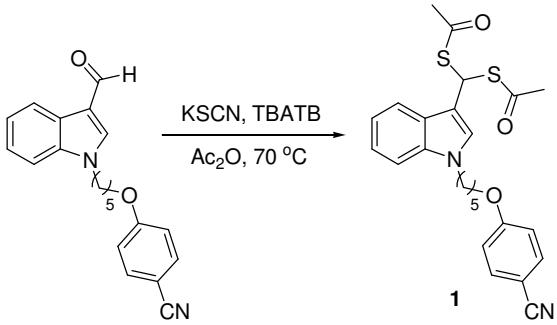


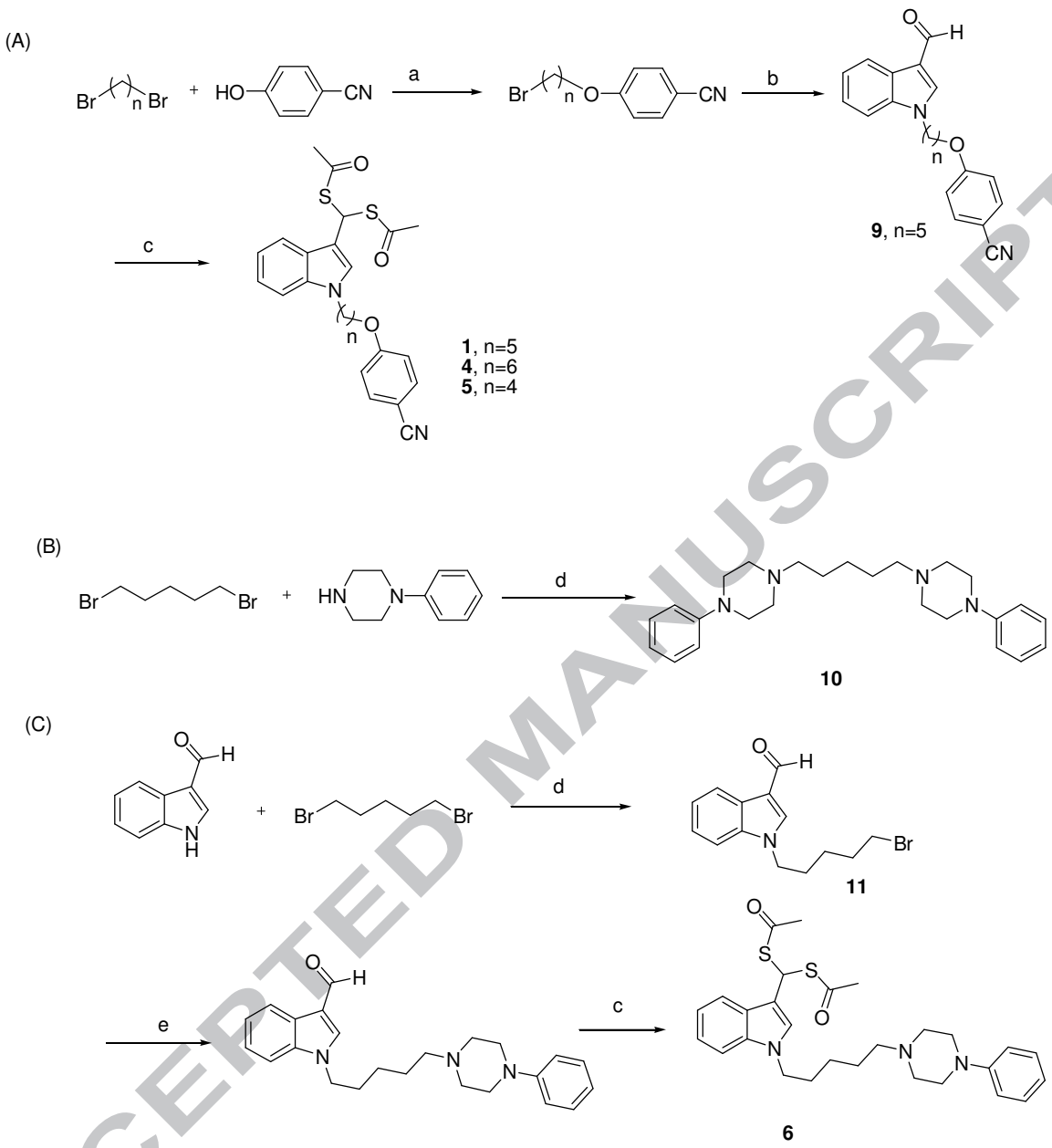
Fig 1. Structures of various H_2S donors and basis of structure of hybride molecule **1**.

This protocol is specific only to N-alkylated indole carbaldehyde since N-alkylation increases electron density at 2-position of indole.[10] It was further confirmed when we observed no reaction with 3-formylazaindole and poor yield with 5-bromo substituted 1-alkylindole under this multicomponent protocol (Supplementary data). It indicates that TBATB catalyzed KSCN based *gem*-dithioacetylation is very sensitive to electron density at 2-position of indole.



Scheme 1: Synthesis of *gem*-dithioacetylated indole using TBATB instead glycine.

To determine role of various fragments of **1** in its antileishmanial activity we synthesized compounds **4**, **5** and **6**. Syntheses of all these compounds are shown in Scheme 2.



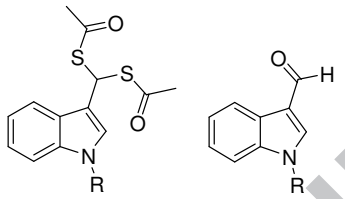
Scheme 2: Reagents and conditions: (a) Acetone, K_2CO_3, dibromoalkane (n = 4, 5, 6; 5 equiv.), *p*-cyanophenol (1.0 equiv.); (b) 3-formylindole, NaOH (aq), TBAB, toluene; (c) KSCN (2.0 equiv.), Ac_2O, TBATB, 70 ^oC; (d) 1,5-dibromopentane (5.0 equiv.), *N*-phenylpiperazine/3-formylindole (1.0 equiv., added dropwise), acetone, K_2CO_3; (e) DIPEA, AcCN, N-phenylpiperazine.

In synthesis of N-phenylpiperazine analogue **6** we have to change the reaction sequence (through intermediate **11**) since alkylation of N-phenylpiperazine with dibromopentane provided exclusively the *bis*-substituted product **10** (Scheme 2B, 2C).

Methyl and butyl substituted analogues (**7** and **8** respectively) were synthesized following reaction sequence shown in Scheme 2A.

Initially compound **7**, **8** and **1** were screened for antileishmanial activity against promastigote and intracellular amastigote stages of parasite. Firefly luciferase transfected *L. donovani* promastigotes were used for screening against promastigotes. For screening against intracellular amastigote stage of parasite, mouse macrophages were infected with firefly luciferase expressing promastigotes, which transformed to amastigotes in macrophages. The inhibition was determined by comparison of luciferase activity of the drug treated parasites with that of the untreated control (Supplementary data).

Table 1: Antileishmanial activity of *gem*-dithioacetylated indole derivatives



1-8 **9**

Comp.	R	% Inhibition promastigote stage [a, b]	% Inhibition intracellular amastigote stage [a]	Cytotoxcity CC_{50} (µg/ml)
1	*p*-cyanophenoxypentyl	96	99.82	NT[c]
4	*p*-cyanophenoxyhexyl	91.56	40.16	NT
5	*p*-cyanophenoxybutyl	90.87	97.79	NT
6	N-phenylpiperazinylpentyl	60.10	86.77	13.83
7	methyl	31	ND[d]	ND
8	butyl	74	ND	ND
9	*p*-cyanophenoxypentyl	NI[e]	NI	ND

[a] inhibition measured at 12.5 µg/ml. [b] promastigote strain: MHOM/IN/Dd8, [c] NT: not toxic i.e. CC_{50} > 100 µg/ml. [d] ND: not determined, [e] NI: no inhibition.

As shown in Table 1 methyl and butyl substituted compounds **7** and **8** have shown only moderate activity against promastigote stage of parasite. The compound **1** with *p*-cyanophenoxypentyl chain was found active against both stages of parasites. Compound **1** suppressed 99.82% parasitemia of *L. donovani* infected macrophages at 12.5 µg/ml concentration, while similar compound **3** with same *p*-cyanophenoxypentyl indole fragment (Fig. 1) had suppressed only 62.0% parasitaemia.[9a] Surprisingly, there was no visible deformation of host macrophages treated with compound **1** (CC_{50} > 100 µg/ml). Encouraged by good activity profile of **1**, additional compounds **4**, **5** (variation of linker) and **6** (variation of *p*-cyanophenoxy group) were synthesized (Scheme 2) to understand SAR of this novel *gem*-dithioacetylated indole scaffold.

The SAR study (Table 1) shows that removal of *gem*-dithioacetyl group from **1** (the compound **9**) resulted in almost no activity. It highlights the importance of H_2S donor functionality in compound **1** for suppression of parasitemia. Also, the moderate activities of **7** and **8** show the importance of *p*-cyanophenoxy moiety for anti parasitic activity. The importance of linker chain joining the above two fragments was shown by **4** and **5**. Changing the linker from pentyl chain to butyl (compound **5**) produce slight drop in bioactivity in comparison to **1**, while making this chain longer (compound **4**) resulted in substantial reduction in bioactivity. The pentyl chain is most suitable linker for bioactivity.

We also replaced cyanophenoxy moiety of **1** with N-phenylpiperazine, an important fragment of many bioactive compounds. The combination of N-phenylpiperazine with *gem*-dithioacetylated indole (compound **6**) though retained antiparasitic activity, but it resulted in toxic molecule. Similar replacement of *p*-cyanophenoxy moiety in **3** by N-phenylpiperazine (which is more polar than *p*-cyanophenol) had resulted in an inactive molecule towards intracellular amastigotes. [9a] In our previous manuscript we reported the involvement of lipophilicity in enhancement of antiparasitic activity of compound **3**, since N-phenylpiperazine is a polar fragment so it abolishes bioactivity of **3**.[9a] However for compound **1**, it looks its lipophilicity is sufficient that replacement of *p*-cyanophenoxy fragment by more polar N-phenylpiperazine is easily tolerated.

Following mechanisms may explain the efficient killing of intracellular amastigotes within macrophages. As par the nature of H_2S donors, which are cytotoxic at high concentration and cytoprotective at low concentration[7], compound **1** show almost complete inhibition of parasite at 12.5 μg/ml while no inhibition at 6.5μg/ml.[12] This H_2S generation may occur either inside or outside of amastigote in a macrophage. The generation of H_2S inside amastigote would be possible by selective accumulation of **1** inside intracellular amastigotes, where it may be triggered to release H_2S most probably by thiols of trypanothione,[13] which is a key molecule involved in parasite defense against oxidative stress.[14] At high concentration of H_2S inside amastigotes the parasite survival could be hampered probably due to inhibition of mitochondrion bioenergetics, acidification of intracellular environment, activation of caspase 9 and apoptosis.[15] However, small amount of H_2S that may have been generated in host macrophages could have provided the cyctoprotective effect for host macrophages by enhancing cellular bioenergetics and reducing reactive oxidative species ROS.[16] This bimodal pharmacological effect of H_2S, [7] may be the probable cause of selective suppression of parasitemia without deforming the host macrophages.

However, if **1** does not accumulate inside amastigote and H_2S is generated outside amastigote, then it may trigger generation NO in macrophages[17] to kill intracellular amastigotes. In addition to that, the pharmacological actions of some slow H_2S donors[7] extend beyond the biological effect of H_2S as these were found to act on other targets such as HDAC. It is likely that **1** might also act as inhibitor of HDAC. A detailed study is needed to determine whether **1** donates H_2S inside or outside intracellular amastigote (and at what rate) or it acts via inhibition of other targets that would be reported in our future communication.

In conclusion we have developed a hybrid molecule conjugating H_2S donor moiety with fragment of *in vivo* active antileishmanial compound. This hybrid shows

bimodal pharmacological character of H_2S, killing intracellular amastigotes while protecting host macrophages. The concept can be utilized in developing therapeutics for other macrophage associated diseases like TB and cancers by designing the H_2S donor conjugated hybrid molecules that selectively accumulate in *mycobacterium* or cancer cells while protecting or stimulating host macrophages.[18] Efforts are underway to incorporate other hydrogen sulfide donor moieties [19] with above bioactive fragment to obtain antileismanial candidate with good *in vivo* antileishmanial profile.

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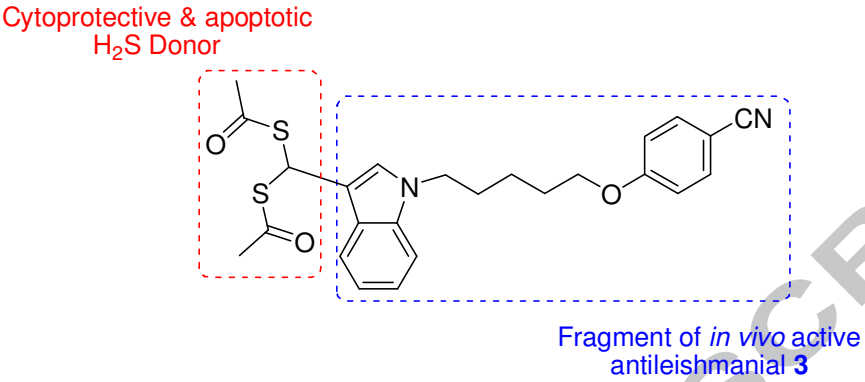
CDRI Communication No: 9557

Supplementary data
Synthesis procedures, analytical data of synthesized compounds and screening protocols can be found in the online version.

[1] WHO fact sheet http://www.who.int/mediacentre/factsheets/fs375/en/ (Accessed on Apr, 2016).
[2] Gupta, S.; Martin, D.; Puri, S. K. *J. Antimicrob. Chemother.* **2015**, *70*, 518.
[3] Chappuis, F.; Sunder, S. *Nat. Rev. Microbiology* **2007**, *5*, 873.
[4] Berman, J. *Expert Opin. Pharmacother.* **2005**, *6*, 1381
[5] Sundar, S. *N. Engl. J. Med.* **2007**, *356*, 2571.
[6] Freitas-Junior, L. H. *International Journal for Parasitology: Drugs and Drug Resistance* **2012**, *2*, 12.
[7] Szabo, C. *Nat. Rev. Drug Disc.* **2016**, *15*, 185.
[8] Gemici, B.; Wallace, J. L. *Nitric Oxide* **2015**, *46*, 25.
[9] (a) Porwal, S.; Chauhan, P. M. S. *J. Med. Chem.* **2009**, *52*, 5793. (b) Sharma, M.; Chauhan, P. M. S. *J. Med. Chem.* **2013**, *56*, 4374. (c) Chauhan, K.; Chauhan, P. M. S. *ACS Med. Chem. Lett.* **2013**, *4*, 1108. (d) Pandey, S.; Chauhan, P. M. S. *Eur. J. Med. Chem.* **2016**, *110*, 237. (e) Chauhan, S. S.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6191.
[10] Porwal, S.; Chauhan, P. M. S. *Tetrahedron Lett.* **2006**, *47*, 5863.
[11] Kavala, V.; Patel, B. K. *Eur. J. Org. Chem.* **2005**, 441.
[12] This class of compounds did not behave in dose dependent manner towards activity against intracellular amastigotes, which made it difficult to determine IC_{50} values see; Sebaugh, J. E. *Pharmaceut. Statist.* **2011**, *10*, 128.
[13] Zhao, Y.; Xian, M. *Org. Lett.* **2014**, *16*, 4536.
[14] Parveen, S.; Douglas, K. T. *J. Med. Chem.* **2005**, *48,* 8087.
[15] (a) Han, Y. *Cell. Physiol. Biochem.* **2013**, *31*, 795. (b) Lee, Z. W. *Br. J. Pharmacol.* **2014**, *171*, 4322.
[16] Wallace, J. L.; Wang, R. *Nat. Rev. Drug Disc.* **2015**, *14*, 329.
[17] Whiteman, M.; Li, L.; Rose, P.; Tan, C. H.; Parkinson, D. B.; Moore, P. K. *Antioxidants & Redox Signaling*, **2010**, *12*, 1147-1154.
[18] Altaany, W.; Yang, G.; Wang, R. *J. Cell. Mol. Med.* **2013**, *17*, 879.
[19] Zhao, Y.; Xian, M. *J. Am. Chem. Soc.* **2011**, *133*, 15.

Graphical Abstract



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