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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.

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 0.2–0.3 million cases of VL appeared every year worldwide and an estimated 20,000–40,000 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 is 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

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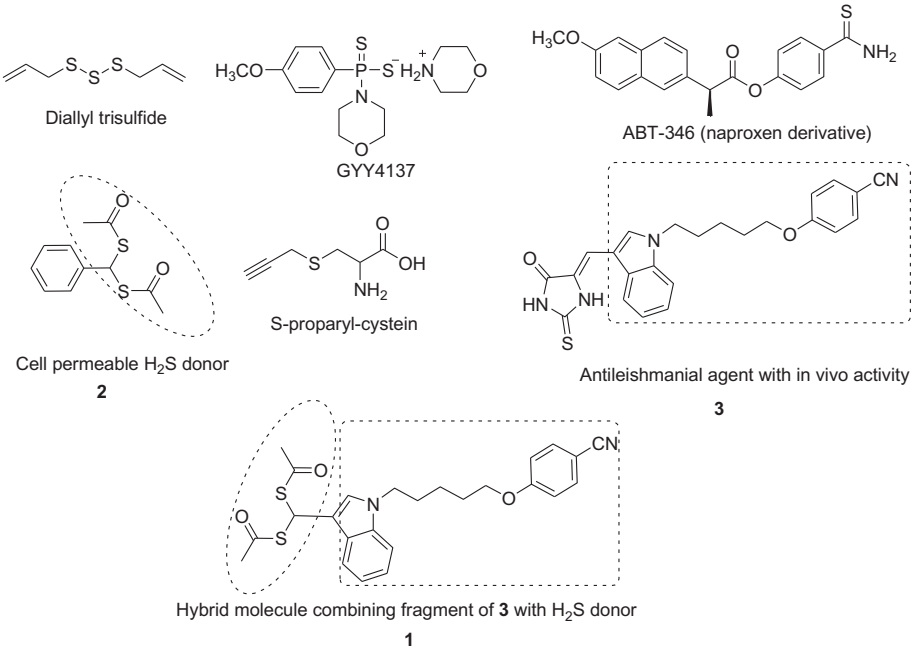


Fig. 1. Structures of various H_2S donors and basis of structure of hybrid molecule **1**.

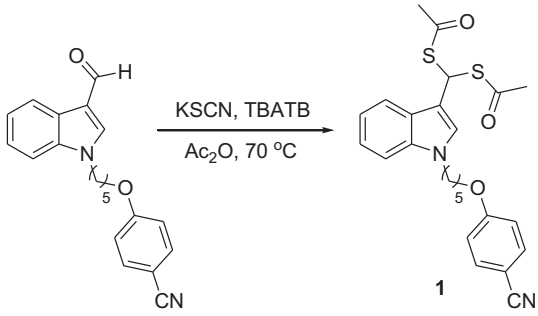
antileishmanial agent, we tried to find out the mechanism of unusual reactivity of 1-alkylated indole-3-carbaldehyde to increase the yield of product. It was found that the role of glycine in multicomponent synthesis of *gem*-dithioacetylated indole derivative was just to create acidic condition during 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).

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.

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.

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, C). 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



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

the drug treated parasites with that of the untreated control (Supplementary data).

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**)