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Arylanthranilodinitriles: A new biaryl class of antileishmanial agents☆

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Abstract—A series of anthranilodinitrile-based biaryls were synthesized and evaluated in vitro against extracellular promastigotes and intracellular amastigotes of *Leishmania donovani*. Among various screened compounds, a biaryl with trifluoromethyl group **5f** showed 83% inhibition against promastigotes and 70% inhibition against amastigotes of *L. donovani* at 8 and 20 μg/mL concentrations, respectively.

Leishmaniases are parasitic diseases caused due to invasion of reticulo-endothelial system of vertebrate host by different species of the hemoflagellates of the genus *Leishmania*. The disease is transmitted in vertebrate after inoculation of promastigotes by the bite of the infected female phlebotomine sandfly.[1] Among various kinds of clinical manifestations, visceral leishmaniasis known as 'Kala-azar' is more fatal in untreated cases, which causes fever, cough, abdominal pain, diarrhea, hepatomegaly, splenomegaly, and pancytopenia. *Leishmania donovani* is the prime accused of visceral leishmaniasis, which is more prevalent in countries of tropical region.[2]

The current scenario for the chemotherapy of leishmaniasis is highly alarming, which is limited to handful of drugs with undesirable side effects and patients are bound to compromise with the existing arsenal of drugs.[3–5] Over the last few decades, pentavalent antimony has played an important role in the treatment of visceral leishmaniasis but the emergence of drug resistance and serious side effects (pancreatitis and hepatotoxicity) have compelled researchers to search for new potential compounds.[6] Pentamidine is a drug of choice in resistant cases of antimony class of compounds, while amphotericin B is useful in unresponsive and relapse cases of visceral leishmaniasis.[7] However, the treatments with these drugs are associated with severe toxic side effects. In a major breakthrough in antileishmanial chemotherapy, miltefosine,[8] a hexadecyl-phosphocholine, has recently found to be very effective for the treatment of human visceral *Leishmania* infections including antimony-resistant cases. Although the development of miltefosine for oral administration in patients with solid tumors was prevented by its dose-limiting gastrointestinal toxicity,[9] the long-term side effects of the drug and the emergence of resistant parasites in patients with visceral leishmaniasis are yet to come. Recent advancements[10] in the identification and understanding of genome sequence of *Leishmania major* parasite have provided new impetus to find new ways of treating this neglected disease and have generated potential hope to discover new antileishmanial agents in near future. In such enthusiastic moments, search for effective and safer chemotherapeutic agents for the treatment of leishmaniasis is highly desirable. This paper deals with synthesis and antileishmanial activity of a new class of anthranilodinitrile-based biaryls, which possess good inhibitory activity in in vitro models.

4-Arylanthranilodinitriles may be considered as biaryls in which one of the two-phenyl rings is functionalized with an amino group flanked between two nitrile substituents. This class of compound possesses interesting biological activities.[11,12] These anthranilodinitrile-based biaryl compounds have been either synthesized by the

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reaction of α,β-unsaturated carbonyl compounds with malononitrile[13] or by the reaction of α-methylene ketones and enaminoketones with malononitrile[14] separately, but they suffer from low yield of the desired product. Recently, we have developed a new procedure for the synthesis of this class of compounds through carbanion-induced ring transformation of 2*H*-pyran-2-ones.[15] A key intermediate ketene dithioacetal **1** was prepared from easily accessible precursors ethyl cyanoacetate, carbon disulfide, and methyl iodide as described earlier.[16] The ketene dithioacetal **1** on Michael addition–cyclization reaction with various commercially available substituted propiophenones **2a–c** or phenyl acetones **2d–h** under alkaline conditions furnished 2*H*-pyran-2-ones **3a–h** in high yields (Scheme 1). It is noteworthy that cyclic dienones **3a–h** generated from ketene dithioacetal **1** have promising topology as useful substrates for ring transformation reactions, flexible substitution pattern, and the presence of a good leaving alkylsulfanyl group for generating molecular diversity. Our synthetic approach to prepare functionalized biaryls **5a–h** is based on Michael–Ziegler–Thorpe–retro–Diels–Alder type reaction of 2*H*-pyran-2-ones **3a–h** with malononitrile **4** under mild reaction conditions. Hence, stirring an equimolar mixture of 2*H*-pyran-2-ones **3a–h**, malononitrile, and powdered KOH in DMF for 12–15 h at room temperature afforded functionalized biaryls in excellent yields (Scheme 1).

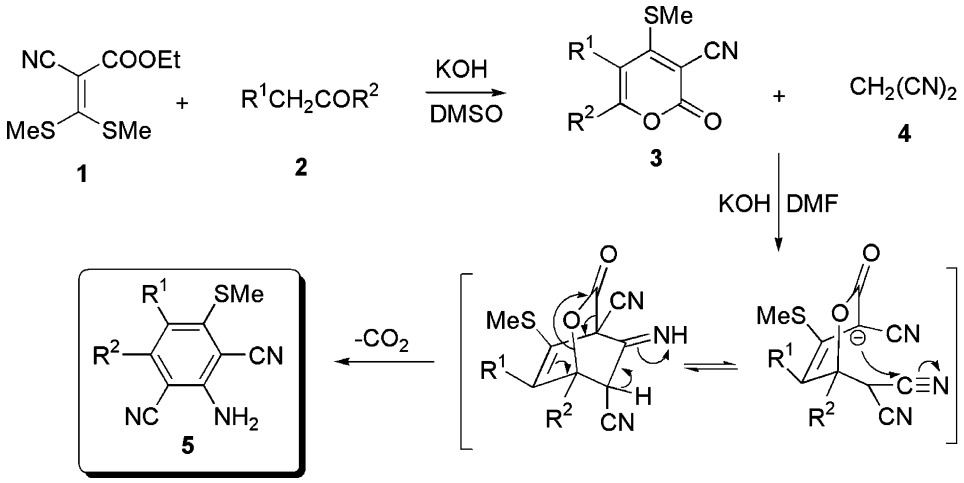
A plausible mechanism, depicted in Scheme 1, suggests that the reaction is initiated by the Michael addition of an anion, generated from a molecule of active methylene ketone **2**, to the ketene dithioacetal **1** followed by intra-molecular cyclization to form a 2*H*-pyran-2-one intermediate **3**. The lactone **3** has three electrophilic centers; C2, C4, and C6 in which C6 is likely to be highly electrophilic due to extended conjugation, and the presence of an electron-withdrawing nitrile group at position 3. The 2*H*-pyran-2-one is attacked by carbanion generated from malononitrile, followed by Thorpe cyclization involving one of the nitrile functionalities of malononitrile and C-3 of the pyranone ring to form a bicyclic intermediate and further by decarboxylation to furnish biaryl **5** in excellent yield. All the synthesized compounds were characterized by spectroscopic analysis.[17,18]

In the sandfly vector, *Leishmania* parasites exist as extracellular promastigotes, while in the mammalian hosts they exist primarily as intracellular amastigotes within phagolysosomes of macrophages. Various in vitro models have been developed to access in vitro antileishmanial activity of drug candidates. Our biological test system involves microscopic counting of live promastigotes in control and treated culture. Taking pentamidine as a control, we have evaluated antileishmanial activity of functionalized biaryls against extracellular promastigotes of *L. donovani* and intracellular amastigotes residing in murine macrophages at various concentrations. The activity profile of the compounds is summarized in Table 1.

The WHO reference strain of *L. donovani* promastigotes (MHOM/IN/80/Dd-8) originally obtained from Imperial College, London, has been maintained since then in this laboratory in NNN (Nacy, McNeal, and Nicoll) medium and as amastigotes in golden hamster.

To assess the inhibition of promastigote growth, 1×10^6 promastigotes/mL were allowed to multiply for 4 days in medium alone or in the presence of serial dilution of drug ranging from 4 μg to 8 μg/mL.The protozoan counts were taken using hemocytometer. Pentamidine was used as positive control.

For assessing activity of compound against amastigote stage of the parasite, mouse macrophage cell line J-774A was used. Cells were seeded in 96-well plate at 5×10^4 cells/100μL/well and the plates were incubated at 37° in CO_2 (5%) incubator. After 24 h, the medium was replaced with fresh medium containing stationary phase promastigotes (2.5×10^5 promastigotes/100 μL/well). Promastigotes invade the macrophages and transformed into amastigotes. The test compounds in appropriate concentration (20 μg/mL in complete medium) were added after replacing the previous medium. Plate was incubated at 37 °C in CO_2 (5%) incubator for 48 h



Scheme 1.

Table 1. In vitro antileishmanial activity for the compounds **5a**–**h**

Compound	R^1	R^2	Concentration (μg/mL)	Promastigotes[a] (% inhibition)	Concentration (μg/mL)	Amastigotes[a] (% inhibition)
5a	CH_3	C_6H_5	4	25	20	23
			8	48		
5b	CH_3	4-ClC_6H_4	4	46	20	10
			8	50		
5c	CH_3	4-$CH_3OC_6H_4$	4	31	20	25
			8	53		
5d	2-FC_6H_4	CH_3	4	37	20	**61**
			8	**60**		
5e	4-FC_6H_4	CH_3	4	43	20	**62**
			8	**65**		
5f	4-$CF_3C_6H_4$	CH_3	4	67	20	**70**
			8	**83**		
5g	2,4-$(OCH_3)_2C_6H_3$	CH_3	4	36	20	**56**
			8	**59**		
5h	3,4-$(OCH_3)_2C_6H_3$	CH_3	4	60	20	**53**
			8	**69**		
Pentamidine			2	71	20	48

[a] Values are the average of percentage inhibition after day 1, 2, 3, and 4.

more. Pentamidine was used as positive control. After incubation, the drug containing medium was decanted and the cell monolayers were stained with Giemsa for 45 min and at least 100 infected macrophages per sample were counted under optical microscope. Efficacy was expressed as the percent inhibition of amastigote multiplication using the following formula:

$$PI = 100 - \{(AT \times 100)/AC\},$$

where PI is percent inhibition of amastigote multiplication, AT is actual number of amastigotes cells in treated groups, and AC is actual number of amastigotes per 100 macrophage cells in untreated control group.

The data suggest that several arylanthranilodinitriles represent interesting leads as antileishmanial agents. The structure–activity relationship of the screened biaryls revealed that antileishmanial activities are significantly dependent on the point of attachment of aryl ring (R^1 or R^2 = aryl) on the basic anthranilodinitrile ring. In general, aryl ring *para* to amino group (**5d**–**h**, R^1 = aryl, R^2 = Me) of anthranilodinitrile ring possessed good inhibitory activity against extracellular promastigotes (59–83% at 8 μg/mL concentration) and intracellular amastigotes (53-70% at 20 μg/mL concentration) of *L. donovani*, while aryl ring *meta* to amino functionality (**5a**–**c**) showed moderate inhibition. It is evident from the activity profile of **5d**–**h** that the compounds have weak inhibitory effect on the *L. donovani* promastigotes, comparing with standard drug pentamidine, whereas they exhibited better inhibition against intracellular amastigotes of the *L. donovani*. Among various screened compounds, 4-amino-2-methyl-6-methylsulfanyl-4′-trifluoromethyl-biphenyl-3,5-dicarbonitrile (**5f**) was found to be the most active, which showed 83% inhibition against promastigotes and 70% inhibition against amastigotes of *L. donovani* at 8 and 20 μg/mL concentrations, respectively. Rest of the compounds (**5d**, **e**, **g**, **h**) possessed slightly better inhibition against amastigotes comparing with standard drug.

In conclusion, we have identified a new series of anthranilodinitrile-based biaryls as inhibitors of *L. donovani*. The preliminary investigations revealed that the anthranilodinitrile class of compounds has potential as antileishmanial agents and has opened a new avenue for further exploration.

Acknowledgments

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17. General procedure for the synthesis of 2*H*-pyran-2-ones **3a**–**h**. A mixture of ethyl 2-cyano-3,3-di(methylsulfanyl)acrylate **1** (10 mmol), propiophenone or aryl acetone (11 mmol), and powdered KOH (12 mmol) in dry DMSO (50 mL) was stirred at room temperature for 10 h. After completion, the reaction mixture was poured into ice water with constant stirring. The precipitate thus obtained was filtered and purified on a silica gel column using chloroform as eluent; **3a**: Yellow solid; mp 180–182 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.17 (s, 3H, CH_3), 3.02 (s, 3H, SCH_3), 7.48–7.54 (m, 5H, ArH); IR (KBr) 1707 (CO), 2216 cm^{-1} (CN); MS (FAB) 258 (M^++1).
18. General procedure for the synthesis of **5**. A mixture of 2*H*-pyran-2-ones **3a**–**h** (1 mmol), malononitrile (**4**, 1.2 mmol), and powdered KOH (1.2 mmol) in dry DMF (5 mL) was stirred at room temperature for 12–15 h. At the end the reaction mixture was poured into ice water with vigorous stirring and finally neutralized with dilute HCl. The solid thus obtained was filtered and purified on a silica gel column using chloroform–hexane (1:2) as eluent. Compound **5a**: white solid; yield 94%; mp 220–222 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.16 (s, 3H, CH_3), 2.59 (s, 3H, SCH_3), 5.12 (br s, 2H, NH_2), 7.20–7.25 (m, 2H, ArH), 7.47–7.50 (m, 3H, ArH); IR (KBr) 2221 (CN), 3348 (NH), 3407 cm^{-1}(NH); MS (FAB) 280 (M^++1). Compound **5b**: white solid; yield 91%; mp 200–202 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.16 (s, 3H, CH_3), 2.59 (s, 3H, SCH_3), 5.15 (br s, 2H, NH_2), 7.18 (d, J = 8.2 Hz, 2H, ArH), 7.48 (d, J = 8.2 Hz, 2H, ArH); IR (KBr) 2221 (CN) 3350 (NH), 3413 (NH), cm^{-1}; MS (FAB) 314 (M^++1). Compound **5c**: white solid; yield 89%; mp 188–190 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.19 (s, 3H, CH_3), 2.57 (s, 3H, SCH_3), 3.87 (s, 3H, OCH_3), 5.13 (br s, 2H, NH_2), 7.00 (d, J = 8.6 Hz, 2H, ArH), 7.17 (d, J = 8.6 Hz, 2H, ArH); IR (KBr) 2228 (CN) 3364 (NH), 3457 (NH) cm^{-1}; MS (FAB) 310 (M^++1).