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Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF N[1] AND N[4] SUBSTITUTED 3, 5-DINITROSULFANILAMIDES DERIVATIVES AS ANTI-LEISHMANIA AGENTS

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ABSTRACT

Leishmaniasis is a disease caused by a protozoan parasites belonging to the genus leishmania. The neglected tropical diseases (NTDs) affect more than one-sixth of the world's population. Current drugs are toxic, expensive, and require longterm treatment. Thus, identification and development of novel, efficient and safe anti-leishmanial compounds as drug candidates are important. Quantitative structure activity relationship analysis (QSAR) was carried out to identify the ideal physicochemical characteristics of potential nitrosulfanilamide analogs. QSAR results show that some derivatives studied would be useful as prototype molecules for the planning of new derivatives with profile of anti-leishmanial drugs.In our efforts to identify novel chemical scaffolds for the development of antileishmanial agents, a series of nitro sulfanilamide compounds was synthesized and tested against the leishmaniasis. Among the synthesized derivatives, compound 1b showed significant antileishmanial efficacy against *L.donavani*. The results show that the reported compounds are promising leads for potential anti-leishmanial drug development.

Keywords: Anti-leishmanial activity, *Lesihmaniadonavani*, Nitrosulfanilamide

1. INTRODUCTION

Leishmaniasis is a parasitic disease transmitted by the bite of some species of sand flies. It is an obligate intracellular protozoan. Leishmaniasis is caused by a number of protozoa in the genus leishmania. The protozoa may be harbored in diseased rodents, canines and various other mammals. Transmitted from the infected mammal to man by bites from female sand flies of the genus phlehotomus, and then appearing in one of four major clinical syndromes: visceral leishmaniasis (internal organ), cutaneous leishmaniasis (skin), mucocutabeousleishmaniasis, or diffuse cutaneous leishmaniasis. The sand fly, the vector involved in spreading the disease, breeds in warm, humid climates and thus the disease is more common in the tropics. As many as 12 million individuals, worldwide are infected by this organism. The incubation period for cutaneous leishmaniasis ranges from a few weeks to several months. The slow-healing lesions may be seen on the skin in various regions of the body depending on the specific strain of organism. Usually these conditions exhibit spontaneous healing, but this may also occur over an extended period of time (1-2 years) [1]. Chemotherapy is the current basis for the treatment of leishmaniasis, and effective anti-leishmanial vaccines are still under study and development [2]. Pentavalent antimonial drugs are the first choice treatment for leishmaniasis, and resistance arise is frequently a problem in endemic areas. Amphotericin B and pentamidine are the second choice drugs, and have their therapeutic effectiveness limited by high toxicity and difficulties in administration [2]. In this context, and due to the restricted therapeutic alternatives and high toxicity of the current medicines, the search in biodiversity for new active compounds and extracts against leishmania is a current field in medicinal chemistry [3]. In leishmania, nitroimidazoles are activated by a novel nitro reductase that results in the generation of reactive nitrogen species, including nitric oxides (NO) that aremajor effectors of these compounds. Because the des-nitroforms of these compounds were found to be inactive against *L. donovani*, this suggests that the nitro group plays a key role in the anti leishmanial activity of this series [4]. Nifurtimox and benznidazole have been the first

effective drugs for treating acute-phase human chagas and leishmanias is infection; with the first being no longer available on the market because of undesirable side effects [5]. They are feature hetero aromatic nitro moieties that are pivotal for the antiprotozoa mechanism of action. Parasite resistance arisen with benznidazole drove the development of alternative therapies. Indeed, combined treatment of benznidazole with drugs with different mechanisms ofaction such as azoles, nitric oxide, or clomipramine could be astrategy to improve the pharmacotherapy efficacy [6]. Noteworthy, a new chemo type able to afford for leishmaniasis and carbonic anhydrase (CAs) inhibition was reported in 2016, namely, N-nitrosulfonamides [7].Considering the above and based on the QSAR study [8], in the present study a set of N- nitro sulfonamides are synthesized and screened biological activity against a pathogen *L. donovani*.

2. EXPERIMENTAL

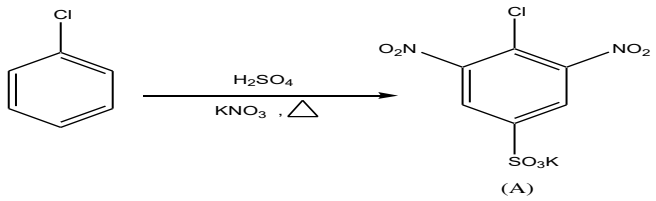
2.1. Material and methods

All the chemicals used in the synthesis of compounds were of synthetic grade, and they were procured from Sigma - Aldrich, SD-fine and Hi media. All the melting points were taken in open capillaries tube and are uncorrected. The purity of compounds was checked routinely by thin layer chromatography (TLC) (0.2-mm thickness) using silica gel plates (Merck), and spots were visualized by exposing the dry plates in iodine vapors and UV detector (long and short wavelength).Theλ_{max} of the synthesized compounds in ethanol was determined on Shimadzu 1700 UV double beam spectrophotometer by scanning between 400-200 nm. Infrared (IR) spectra (λ_{max} in cm^{-1}) were recorded on a Perkin Elmer FT-IR using KBr technique. 1H-nuclear magnetic resonance (NMR) spectra were observed on a BRUKER DRX-300 (300 MHz) NMR instrument using Deuterated chloroform ($CDCl_3$)- as solvent and TMS as internal reference (chemical shifts in d ppm). The peak and abbreviations were used to indicate multiplicity: s (singlet), d (doublet), dd (double doublet), ddd (double doublet, doublet), t (triplet), and m (multiplet). The chemical shifts were reported in δ units and the coupling constants (J) were reported in Hertz. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 Mass spectrometer/Data system using Argon/Xenon (6kv, 10 mA) as the FAB gas using fast atom bombardment (FAB) technique.

2.2. Chemistry

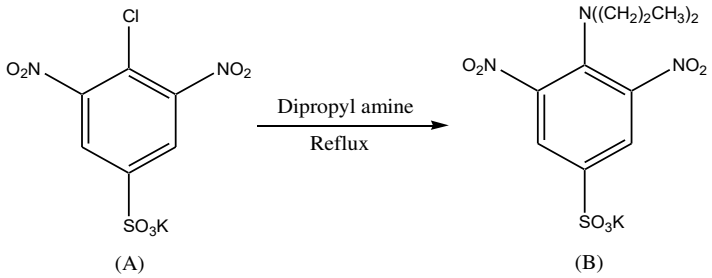
2.2.1. General Procedure for synthesis of N^1 and N^4 Substituted 3, 5-Dinitro Sulfanilamides Derivatives

The different synthetic routes were used for synthesis of the compounds in this study shown in the following scheme I and scheme II as reported by Harry P. Schultz [9] and Gautam Bhattacharya *et al* [10] respectively.



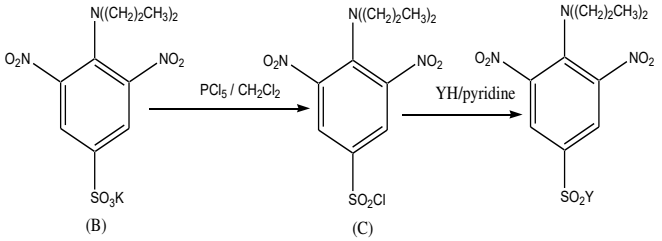
Scheme I: General procedure for Synthesis of Potassium, 4-chloro-3, 5-dinitrobenzenesulfonate (A)

In a round-bottom flask fitted with a mechanical stirrer placed 50 ml (55.4 g, 0.49 mole) of chlorobenzene, 300 ml of concentrated sulfuric acid (Sp. Gr. 1.84), and 50 ml of fuming sulfuric acid (containing approximately 20% free sulfur trioxide). The mixture was stirred and heated on a steam bath for two hours and then cooled to room temperature. The stirrer was removed from the reaction flask and replaced with a thermometer. To the clear solution, 170 g (1.68 moles) of potassium nitrate in 4 portions was added. The temperature of the mixture during this time was held at 40-60^{0}C by cooling the flask and its contents in ice water. After the mixture was swirled briefly in the reaction flask to dissolve most of the potassium nitrate, it was heated to 110-115^0 and held at that temperature for 24 hours. The hot contents of the flask were poured onto 2 kg of cracked ice. After the ice was melted, the precipitate was filtered with suction and pressed as dry as possible. Without further drying, the potassium, 4-chloro-3,5-dinitrobenzenesulfonate was recrystallized from 600 ml. of boiling water.



Scheme II: General procedure for Synthesis of 3, 5-Dinitro-N^4, N^4-di-n-propylsulfonate (B)

A suspension of potassium, 4-chloro-3,5-dinitrobenzenesulfonate (2.82 g, 8.8 mmol) in dipropylamine (50 ml) was refluxed for 3 hrs. By that time almost all the solid had dissolved. After 3 hrs, the solvent was evaporated, and water was added. The resulting yellow precipitated was filtered and collected.



Scheme III: General procedure for the synthesis of N^1-Aryl 3,5-Dinitro-N^4,N^4-di-n-propylsulfanilamide derivatives

To a suspension of 3,5-Dinitro-N^4, N^4-di-n-propylsulfonate (**B**, 347 mg, 0.90 mmol) in dichloromethane (10ml), was added PCl_5 (624 mg, 3 mmol), and the reaction mixture was stirred for 2 hrs. The dichloromethane layer was extracted after washing with water and drying with Na_2SO_4. The product 3,5-Dinitro-N^4, N^4-di-n-propylsulfonyl chloride (**C**) was used without further purification. Pyridine was added, the temperature was adjusted to 40 °C, 2 to 6 equivalent mole of the aniline derivative was added, and the reaction mixture was stirred for 3 hrs at 40 ^{0}C. The progress of the reaction was monitored through TLC. Pyridine was then evaporated in vacuum; the dark residue was washed with water and extracted with ethyl acetate. The organic layers were combined, dried over anhydrous sodium suphate, filtered and evaporated under reduced pressure. The resulting crude residue was purified by silica gel to get the corresponding dinitro sulfanilamide derivatives.

2.3. Biology

2.3.1. In vitro anti-leishmanial assay

The anti-leishmanial activity of the compounds was tested in vitro against a culture of *L. donovani* promastigotes.

2.3.2. Drugs

The stock solutions of the various nitro sulfanilamide synthesized derivatives (50 μM) were prepared in dimethyl sulfoxide (DMSO). DMSO has no effect on the proliferation or morphology of parasites when its concentration does not exceed 1% v/v in cell culture.

2.3.3. Leishmania culture

Promastigote infective forms of *L. donovani* were maintained by weekly transfers in brain heart infusion medium (BHI) supplemented with 10% fetal bovine serum (FBS) at 26°C. The infectivity of the parasites was maintained by performing a periodic inoculation into hamster footpads.

2.3.4. Antileishmanial evaluation

Promastigote forms of *L. donovani* [3 × 106 cell/ml] were incubated (24 h) in BHI supplemented with 10% FBS in the absence or presence of the 5-(4,5-dihydro-1*H*-imidazol-2-yl)-4-(arylamino)thieno[2,3-*b*]pyridine derivatives (6.25 to 50μM). Amphotericin B was used as the positive control and in previous pilot, 200μM led to 73.9% inhibitory effect. Untreated controls were performed with or without DMSO. The growth inhibitory effect was quantitatively monitored by direct counting of parasites in a Neubauer chamber using optical microscopy (Olympus B × 41). Each experiment was performed in triplicate, and control groups (non-treated parasites) were performed in presence of DMSO 1% as it showed no effect on the assays. Serial dilutions of nitro sulphanilamide derivatives was performed promastigotes forms of *L. donovani* [3×106 cell/ml] were incubated with the derivative (6.25, 12.5, 25 and 50μM) for 24 h. Cell density was determined by direct counting in a Neubauer chamber. For the most active compounds, we determined the effective concentration that is able to inhibit 50% of the *L. donovani* growth after 24 h (IC_{50}). IC_{50} and graphs were determined using the Microcal Origin program. The results were analyzed using Student's t test, and significant differences were determined at $P < 0.05$.

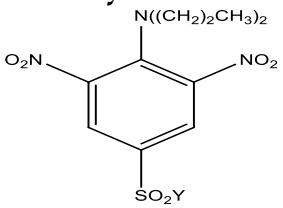
3. RESULTS AND DISCUSSION

Considering that there is no vaccine available to prevent leishmaniasis, discovery of new effective and low toxic drugs for the treatment this neglected disease is an urgent need.In last years some works from literature have showed that some of nitro sulfanilamide derivatives could be interesting for the development of new anti-protozoan agents. Based on the QSAR study, few analogs were designed for further study. Thus the structures of the synthesized compounds were in confirmation of the designed analogs. It would have

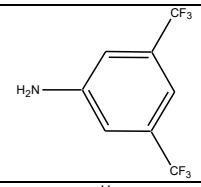
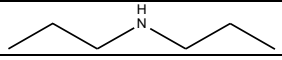
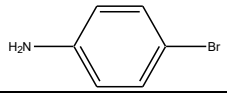
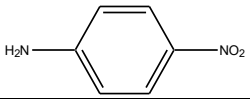
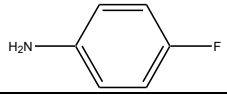
been very interesting to determine the anti-leishmaniasis activity of these compounds which would have confirm the success of the QSAR analysis by comparing theoretical and experimental values in the study. However due to practical problems and time constraints research upto this level was attempted.

In the present study, nitro sulfanilamides were synthesized employing four steps based on the reported methods, using as chlorobenzene starting materials and substituted aniline. In first step, 4-chloro-3,5-dinitrobenzenesulfonate synthesized from chloro-benzene and then propyl sulfonates B prepared from A reaction with dipropyl amine in second step, nitro sulfanilamides are prepared from B, reaction with phosphorus pentachloride and DCM and then react with substituted aniline in presence of pyridine. The desired compound could be easily synthesized in good yield (more than 70%) in two to four steps, as either crystalline or solid compound. The structure, physical properties and biological activity of the synthesized compounds were shown in Table 1.

Table1: Structure and Physical Properties of the Synthesized Compounds



1a - 1e

Comp. no	Y	Molecular formula	Melting Point (˚C)	Mol. wt	Solubility	% yield	L. donvani IC_{50} (µg/mL)
1a		$C_{20}H_{20}F_6N_4O_6S$	115-120	558	Chloroform	80%	10.54
1b		$C_{16}H_{30}N_4O_6S$	128-133	402	Chloroform	46%	8.84
1c		$C_{18}H_{21}BrN_4O_6S$	180-185	501	Chloroform	66%	20.43
1d		$C_{18}H_{21}N_5O_8S$	100-105	467	Chloroform	50%	21.10
1e		$C_{18}H_{21}FN_4O_6S$	190-195	440	DMF	48%	12.49
Standard	Pentamidine	-	-		-	-	4.70
Standard	Amphotericin B	-	-		-	-	0.44

For evaluation of anti-leishmanial properties of target compounds, the *in-vitro* activity was assessed against promastigote forms of *L. donovani*. The inhibitory effects of compounds against promastigotes forms of *L. major* were measured after 72 h. following established procedures, at concentrations ranging from 25 to 100 µm. The IC50 values registered after 24 h. of exposure are represented in Table 1 including Pentamidine and Amphoteracin B as the reference drug.

In the present work, all of the synthesized compounds (1a-e) were more effective against promastigote forms of *L.donovani*. The activity is increased in the following order: 1d>1c>1e>1a>1b. Compound 1b was found to be the most active compound in this series (IC50 = 8.84 µm). Screening results indicate that the substitutions in phenyl ring increase the efficacy and synthesized compounds shows significant inhibitory activity against leishmaniasis.

3.1. Synthesis of N-(3,5-Bis-trifluoromethyl-phenyl)-4-dipropylamino-3,5-dinitro-benzenesulfonamide (1a)

Pale yellow crystalline solid, λ_{Max} (nm) in Ethanol 288;R_f Value: 0.88; IR Spectra (KBr) $\upsilon_{max}(cm^{-1})$: 1156.6 (Sulfonamides), 1505.9 & 1362.3 (C-NO_2), 1315.8 (C-N), 1010.1 (C-F); ^{1}H NMR ($CDCl_3$, 300 MHz):δ 0.863-0.887 (m, 6H,-$(CH_2CH_2CH_3)_2$), 1.538 - 1.567 (m, 4H, -$(CH_2CH_2CH_3)_2$, 2.976 (t,4H, N-$(CH_2CH_2CH_3)_2$), 7.108 (d, 2H, Aromatic), 7.565-7.592 (m, 1H, aromatic), 8.193 (s, 2H, aromatic); Mass Spectra: FAB-MS m/z 559 [M^+].

3.2. Synthesis of 4-Dipropylamino-N,N-diethy l-3,5-dinitro-benzenesulfonamide (1b)

White crystalline solid, λ_{Max} (nm) in Ethanol 405;R_f Value: 0.87; IR Spectra (KBr) $\upsilon_{max}(cm^{-1})$: 1122.7 (Sulfonamides), 1505.9 & 1355.4 (C-NO_2), 1355.4 (C-N); ^{1}H NMR ($CDCl_3$, 300 MHz): δ0.834-0.883 (m, 6H, -$(CH_2CH_2CH_3)_2$), 1.581-1.686 (m, 4H, -$(CH_2CH_2CH_3)_2$, 2.969 (t, 4H, N-$(CH_2CH_2CH_3)_2$), 7.124 (d, 2H, Aromatic), 7.980-8.103 (m, 1H, aromatic), 8.243 (s, 2H, aromatic); Mass Spectra: FAB-MS m/z 403 [M^+].

3.3. Synthesis of N-(4-Bromo-phenyl)-4-dipro-pylamino-3,5-dinitro-benzenesul-fonamide (1c)

Yellow crystalline solid, λ_{Max} (nm) in Ethanol 405;R_f Value: 0.95; IR Spectra (KBr) $\upsilon_{max}(cm^{-1})$: 1178.6 (Sulfonamides), 1505.5 & 1317.9 (C-NO_2), 1317.9 (C-N), 516 (C-Br);^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.826-0.870 (m, 6H, -$(CH_2CH_2CH_3)_2$), 1.520-1.607 (m, 4H, -$(CH_2CH_2CH_3)_2$, 2.857 (t, 4H, N-$(CH_2CH_2CH_3)_2$), 7.140 (d, 2H, Aromatic), 7.410-7.439 (m, 1H, aromatic), 8.157 (s, 2H, aromatic); Mass spectra: FAB-MS m/z 501 [M^+]

3.4. Synthesis of N-(4-Nitro-phenyl)-4-dipro-pylamino-3,5-dinitro benzenesulfonamide (1d)

White crystalline solid, λ_{Max} (nm) in Ethanol: 380;R_f Value: 0.88; IR Spectra (KBr) $\upsilon_{max}(cm^{-1})$: 1140.5 (Sulfonamides), 1515 & 1321.6 (C-NO_2), 1321.6 (C-N); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.80-0.87 (m, 6H, -$(CH_2CH_2CH_3)_2$), 1.59-1.67 (m, 4H, -$(CH_2CH_2CH_3)_2$, 2.83 (t, 4H, N-$(CH_2CH_2CH_3)_2$), 6.61 (d, 2H, Aromatic), 7.43-7.50 (m, 1H, aromatic), 8.23 (s, 2H, aromatic); Mass spectra: FAB-MS m/z - 467 [M^+]

3.5. Synthesis of N-(4-Fluoro-phenyl)-4-dipro-pylamino-3,5-dinitro-benzenesulfonamide (1e)

Yellow crystalline solid, λ_{Max} (nm) in Ethanol: 406;R_f Value: 0.81; IR Spectra (KBr) $\upsilon_{max}(cm^{-1})$: 1150.4 (Sulfonamides), 1518.9 & 1321.8 (C-NO_2), 1321.8 (C-N), 1070.7 (C-F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.765-0.788 (m, 6H, -$(CH_2CH_2CH_3)_2$), 1.484-1.507 (m, 4H, -$(CH_2CH_2CH_3)_2$, 2.791 (t, 4H, N-$(CH_2CH_2CH_3)_2$), 7.152 (d, 2H, Aromatic), 7.374-7.403 (m, 1H, aromatic), 8.180 (s, 2H, aromatic); Mass spectra: FAB-MS m/z 441 [M^+].

4. CONCLUSION

In summary, a series of N^1 and N^4 substituted 3, 5-dinitro sulfanilamides were synthesized and evaluated for their *in-vitro* inhibitory activity against the *Leishmania* parasite. All of the target compounds exhibited good anti-leishmanial activity against *L. donovani*. These data encourage furthermore studies evaluating the effect on intracellular *in-vitro*, *in-vivo* efficacy and cytotoxicity of the compounds. The experimental data proposes that dinitro-sulfanilamides derivatives may be further investigated as a drug candidate for treatment of leishmaniasis. Hence the field is further open for these studies.

5. ACKNOWLEDGEMENTS

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Conflict of interest: Nil

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