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ORIGINAL ARTICLE

# 5-Nitroisatin-derived thiosemicarbazones: potential antileishmanial agents

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**Abstract**

A series of 29 previously reported $N^4$-substituted 5-nitroisatin-3-thiosemicarbazones **2**–**30** has been screened for leishmanicidal potential. Compounds **2**–**4**, **7**, **8**, **10**–**13**, **15**–**19**, **21**, **23**, **24**, **26**, **28** and **30** exhibited good to excellent antileishmanial activities with $IC_{50}$ values ranging from $0.44 \pm 0.02$ to $32.38 \pm 0.66$ μg/mL. Of these, **5**, **7**, **19** and **28** proved to be the most active antileishmanial agents, displaying activities with $IC_{50}$ values $1.78 \pm 0.35$, $0.44 \pm 0.02$, $1.91 \pm 0.04$ and $4.28 \pm 0.75$ μg/mL, respectively, which were even better than the standard drug, pentamidine ($IC_{50} = 5.09 \pm 0.04$ μg/mL). This study presents the first example of exhibition of leishmanicidal potential by isatin-thiosemicarbazones and as such furnishes a solid basis for further research on these compounds to develop more potent antileishmanial agents.

**Keywords**

Isatin-thiosemicarbazones, leishmanicidal activity, *Leishmania major*

**History**

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## Introduction

Leishmaniasis is a tropical disease caused by protozoa of the genus *Leishmania*. There are four different forms of the disease with a broad range of chemical manifestations. These include cutaneous leishmaniasis (CL), diffused cutaneous leishmaniasis (DCL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL). Of these, the last, being the most severe, causes almost 100% mortality, if untreated[1,2].

The available chemotherapy for leishmaniasis relies upon pentavalent antimonial ($Sb^v$) compounds, i.e. sodium stibogluconate (pentostam) or meglumine antimoniate (glucantime), which even though are still the first-line drugs, but are not totally safe and/or efficacious[3]. For the treatment of unresponsive cases, amphotericin B and pentamidine can be used, though they are not fully effective either and exhibit adverse side effects[4]. Miltefosine or hexadecylphosphocholine, an alkylphospholipid, originally developed as an anticancer agent, is the only oral antileishmanial drug found to be effective against both the CL[5] and VL[6], although causing serious gastrointestinal problems[7]. Furthermore, drug-resistant strains of *Leishmania* spp. have appeared, which necessitate the search for new, more effective and non- or least toxic chemotherapeutic agents.

Thiosemicarbazones are an important class of organic compounds, which possesses a number of medicinal properties, including antibacterial, antifungal, antiprotozoal, antitumoral, antiviral and more particularly, the antileishmanial activities[8–21]. Isatin-derived thiosemicarbazones have also been found to possess a variety of biological activities, such as antimicrobial, antineoplastic, antiplasmodial and antiviral[22–34]. However, antileishmanial potential of this group of thiosemicarbazones has not been explored. Very recently, during random screening of the organic compounds prepared in our laboratory, we found that certain $N^4$-substituted isatin-thiosemicarbazones bearing strong inductively electron-attracting substituents at position-5 of the isatin scaffold exhibited antileishmanial activity. This observation stimulated us to study the leishmanicidal properties of a series of 29 previously reported $N^4$-alkyl/alkenyl/aryl substituted 5-nitroisatin-3-thiosemicarbazones[35,36], prepared by the condensation of 5-nitroisatin with appropriate thiosemicarbazides (Scheme 1). This paper, therefore, describes the *in vitro* leishmanicidal potential of these compounds. It also describes the effects of the nature of substituents attached to $N^4$ of the thiosemicarbazone moiety on this very potential of the compounds. Further studies on such compounds with certain structural modifications are in progress.

## Materials and methods

All reagents and solvents were used as obtained from the suppliers or recrystallized/redistilled as necessary. The 5-nitroisatin-3-thiosemicarbazones were prepared by treating 5-nitroisatin with appropriate thiosemicarbazides. The details of the reactions along with physical, analytical and spectral data of the compounds were reported elsewhere[35,36]. *In vitro* leishmanicidal screening of the prepared compounds was made at Dr. Panjwani Center for Molecular Medicine and Drug Research, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan.

### *In vitro* antileishmanial activity

*Leishmania major* were grown in bulk in modified Novy-MacNeal-Nicolle (NNN) biphasic medium by using normal

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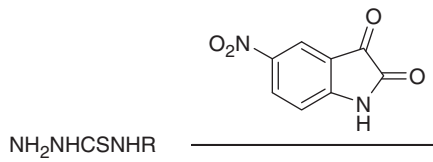
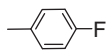
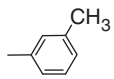
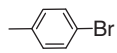
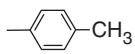
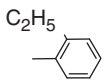
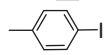
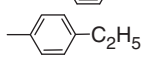
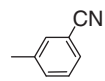
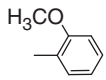
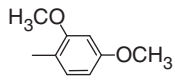
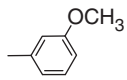
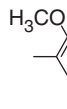
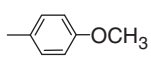
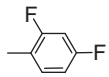
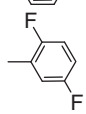
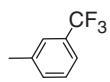
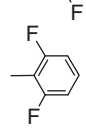
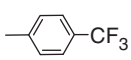
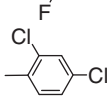
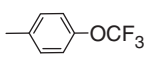



**1** **2-30**

| Compounds | R | Compounds | R |
|---|---|---|---|
| **2** |  | **17** |  |
| **3** | —$CH_2$ $(CH_2)_4$ $CH_3$ | **18** |  |
| **4** | —$CH_2$ CH=$CH_2$ | **19** |  |
| **5** |  | **20** |  |
| **6** |  | **21** |  |
| **7** |  | **22** |  |
| **8** |  | **23** |  |
| **9** |  | **24** |  |
| **10** |  | **25** |  |
| **11** |  | **26** |  |
| **12** |  | **27** |  |
| **13** |  | **28** |  |
| **14** |  | **29** |  |
| **15** |  | **30** |  |
| **16** |  | – | – |

Scheme 1. Synthesis of title compounds **2**–**30**.

physiological saline. *Leishmania* promastigotes were cultured with Rosewell Park Memorial Institute (RPMI) 1640 medium, supplemented with 10% heat inactivated foetal bovine serum. Parasites at log phase were centrifuged at 2000 rpm for 10 min and washed three times with saline at the same speed and time. Parasites were diluted with fresh culture medium to a final density of $10^6$ cells/mL.

In a 96-well microtiter plate, 180 µL of medium was added in first row and 100 µL of medium was added in other wells. A 20 µL of the experimental compound was added in medium and serially diluted. A 100 µL of parasite culture was in all wells and two rows were left for negative and positive controls. Negative controls received only medium, whereas the positive control contained varying concentrations of standard leishmanicidal compound, pentamidine. The plate was incubated at 21–22 °C for 72 h. The culture was examined microscopically on an improved Neubauer counting chamber and $IC_{50}$ values of compounds were calculated by Software Ezfit 5.03 (Perella Scientific, Amherst, NH). All assays were performed in triplicate.

## Results and discussion

This study describes the *in vitro* determination of the leishmanicidal effects of 29 $N^4$-substituted 5-nitroisatin-3-

Table 1. *In vitro* antileishmanial activity of compounds **2–30**.

| Compounds | $IC_{50} \pm SEM^*$ (μg/mL) | Compounds | $IC_{50} \pm SEM^*$ (μg/mL) |
|---|---|---|---|
| **2** | 74.31 ± 0.69 | **17** | 12.94 ± 0.02 |
| **3** | 25.84 ± 0.205 | **18** | 13.00 ± 0.08 |
| **4** | 27.14 ± 0.06 | **19** | 1.91 ± 0.04 |
| **5** | 1.78 ± 0.35 | **20** | 51.20 ± 0.27 |
| **6** | 52.66 ± 0.28 | **21** | 26.95 ± 0.08 |
| **7** | 0.44 ± 0.02 | **22** | 63.89 ± 1.39 |
| **8** | 29.11 ± 0.70 | **23** | 25.93 ± 0.03 |
| **9** | 71.45 ± 0.61 | **24** | 25.86 ± 0.18 |
| **10** | 27.37 ± 1.05 | **25** | 53.65 ± 0.51 |
| **11** | 32.38 ± 0.66 | **26** | 26.79 ± 0.90 |
| **12** | 31.43 ± 0.18 | **27** | 82.12 ± 0.86 |
| **13** | 12.98 ± 0.145 | **28** | 4.28 ± 0.075 |
| **14** | 65.53 ± 1.14 | **29** | >100 |
| **15** | 20.75 ± 0.01 | **30** | 15.58 ± 0.11 |
| **16** | 5.74 ± 0.01 | | |
| **Pentamidine**† | 5.09 ± 0.04 | | |

*Standard error of the mean.
†Standard drug for leishmanicidal activity.

thiosemicarbazones **2–30**, the synthesis of which has been reported elsewhere[35,36].

### *In vitro* antileishmanial activity

The synthetic thiosemicarbazones **2–30** were screened for their antileishmanial activity according to the literature protocol[37]. Compound **2**, i.e. 1-(5-nitro-2-oxindolin-3-ylidene)-4-phenylthiosemicarbazide severed as a reference point to evaluate the effects of different substituents (alkyl, alkenyl and aryl bearing one or two functions about the phenyl ring) attached to $N^4$ of the thiosemicarbazone moiety on the leishmanicidal potential of these compounds. All the compounds except **29** were found to be active against *Leishmania major*, showing varied leishmanicidal activity ($IC_{50} = 0.44 \pm 0.02$–$82.12 \pm 0.86$ μg/mL) (Table 1). Compounds **3** and **4** having *n*-hexyl and allyl substituents at $N^4$ of the thiosemicarbazone moiety were found to exhibit good antileishmanial activity ($IC_{50} = 25.84 \pm 0.205$ and $27.14 \pm 0.06$ μg/mL, respectively), whereas **2** bearing phenyl group at the same position displayed weak activity ($IC_{50} = 74.31 \pm 0.69$ μg/mL). Amongst the aryl-substituted compounds **5–7** possessing methyl substituents at different positions of the phenyl ring, the *ortho*-substituted compound **5** exhibited significant activity with an $IC_{50}$ value of $1.78 \pm 0.35$ μg/mL, while the *para*-substituted compound **7** displayed excellent leishmanicidal effect ($IC_{50} = 0.44 \pm 0.02$ μg/mL); however, the *meta*-substituted isomer **6** showed moderate activity ($IC_{50} = 52.66 \pm 0.28$ μg/mL). To the contrary, amongst the ethyl-substituted compounds **8** and **9**, the *para*-substituted compound **9** was found to demonstrate weak leishmanicidal activity ($IC_{50} = 71.45 \pm 0.61$ μg/mL), when compared with the *ortho*-substituted compound **8**, which displayed good activity ($IC_{50} = 29.11 \pm 0.70$ μg/mL). However, all the three methoxy-substituted compounds **10–12**, regardless of the positions of substituents about the phenyl ring, exhibited good activity with $IC_{50}$ values $27.37 \pm 1.05$, $32.38 \pm 0.66$ and $31.43 \pm 0.18$ μg/mL, respectively. In the cases of trifluoromethyl-substituted compounds **13–15**, the *ortho*- and *para*-substituted compounds **13** and **15** were found to show significant activity ($IC_{50} = 12.98 \pm 0.145$ and $20.75 \pm 0.01$ μg/mL, respectively), when compared with the *meta*-substituted compound **14**, which exhibited weak activity ($IC_{50} = 65.53 \pm 1.14$ μg/mL). On the contrary, all the three monofluoro-substituted compounds **17–19** demonstrated significant activity with $IC_{50}$ values ranging from $1.91 \pm 0.04$ to $13.00 \pm 0.08$ μg/mL. Of these, the *para*-substituted compound **19** showed much pronounced activity with an $IC_{50}$ value of $1.91 \pm 0.04$ μg/mL. It is pertinent to mention that compound **16**, the only trifluoromethoxy derivative (*para*-substituted) tested in the assay, showed leishmanicidal activity comparable with that of the standard drug, pentamidine ($IC_{50} = 5.74 \pm 0.01$ versus $5.09 \pm 0.04$ μg/mL). Amongst the two bromo-substituted compounds **20** and **21**, the latter having a *para* substituent displayed good antileishmanial activity with an $IC_{50}$ value of $26.95 \pm 0.08$ μg/mL, whereas the former with an *ortho* substituent exhibited moderate activity ($IC_{50} = 51.20 \pm 0.27$ μg/mL). In contrast, amongst the two iodo-substituted compounds **22** and **23**, compound **23** possessing a *para* substituent showed significant leishmanicidal effect ($IC_{50} = 25.93 \pm 0.03$ μg/mL), while the ortho-substituted compound **22** demonstrated weak activity ($IC_{50} = 63.89 \pm 1.39$ μg/mL). Interestingly, compound **24**, the only cyano derivative (*meta*-substituted) tested in the assay, was found to exhibit significant activity ($IC_{50} = 25.86 \pm 0.18$ μg/mL), when compared with the corresponding trifluoromethyl derivative **14**, which demonstrated weak leishmanicidal influence ($IC_{50} = 65.53 \pm 1.14$ μg/mL). Of the remainder active $N^4$-aryl-substituted compounds **25–28** and **30**, compound **25** having methoxy substituents at *ortho*, *para* (2,4) positions of the phenyl ring displayed moderate antileishmanial activity ($IC_{50} = 53.65 \pm 0.51$ μg/mL), whereas **26** with the same functions at *ortho*, *meta* (2,5) positions exhibited good activity ($IC_{50} = 26.79 \pm 0.90$ μg/mL). This shows that in the case of **25**, substitution of additional methoxy substituent at *para* position of the phenyl ring caused significant reduction in activity, as the respective *ortho*-substituted compound **10** gave an $IC_{50}$ value of $27.37 \pm 1.05$ μg/mL in this assay. However, such an additional substitution at *meta* position of the phenyl ring was not found to cause any considerable change in the activity of compound **26** ($IC_{50} = 27.37 \pm 1.05$ versus $26.79 \pm 0.90$ μg/mL). More interestingly, in compound **28**, substitution of additional fluoro function at *meta* position of the phenyl ring caused pronounced enhancement in the leishmanicidal activity ($IC_{50} = 4.28 \pm 0.075$ μg/mL), as the respective *ortho*-substituted compound **17** gave an $IC_{50}$ value of $12.94 \pm 0.02$ μg/mL in the present assay. Furthermore, as in compound **25**, such an additional substitution of fluoro group at *para* position of the phenyl ring of compound **27** resulted into a decrement in its activity but to a level of much higher degree ($IC_{50}$ $12.94 \pm 0.02$ μg/mL → $82.12 \pm 0.86$ μg/mL). Compound **27** thus proved to be the least active derivative in the present series. Compound **30**, the only dichloro derivative (*ortho*, *para*-substituted) tested in this assay, demonstrated significant activity ($IC_{50} = 15.58 \pm 0.11$ μg/mL). These structure–activity relationships (SARs) may serve as a basis for structural modifications aimed at the development of certain leishmanicidal agents of medicinal interest/importance.

The exact mechanism of antileishmanial activity displayed by the test thiosemicarbazones **2–30** is not known. However, it is generally accepted that the mode of leishmanicidal action of this class of agents involves primarily the inhibition of cysteine proteases found in different *Leishmania* species. Such an enzymatic inhibition may be attributed to nucleophilic addition of the active site cysteine thiol to the thiocarbonyl function of the thiosemicarbazone moiety, as recently proposed for cruzain, the related major parasitic cysteine protease from *Trypanosomal cruzi*[10,12,29]. The antileishmanial activity shown by our test compounds **2–30** may also be attributed to inhibition of the ribonucleoside diphosphate reductase (RDR), an iron (Fe)-dependent enzyme catalyzing the rate-limiting step of DNA synthesis in the parasite. Notably, it is the ability of these compounds to act as tridentate metal chelators that leads to their leishmanicidal activity. The chelation of Fe from intracellular Fe pools results in the inhibition of RDR. The tridentate chelating

thiosemicarbazones have been proposed to act by inhibiting RDR[13,38–40]. Such compounds have also been proposed to act by inhibiting dihydrofolate reductase[41,42].

It is only very recently that the thiosemicarbazone-metal complex, i.e. benzaldehyde thiosemicarbazone derived from limonene complexed with copper, termed as BenzCo, has been found to exhibit leishmanicidal activity against the promastigote, axenic amastigote and intracellular amastigote forms of *Leishmania amazonensis*. This leishmanicidal effect demonstrated by BenzCo was associated with the production of reactive oxygen species (ROS) leading to mitochondrial dysfunction, ultimately causing parasite death[43].

ROS may lead to oxidative damage of virtually any biomolecule. Mitochondria are particularly susceptible to such a damage caused by ROS that are continuously generated by the mitochondrial respiratory chain[44]. The generation of ROS can also be induced by certain drugs, affecting parasite mitochondrial functions[45–48]. The same principle worked for BenzCo in killing parasites. The treatment of parasites with BenzCo dose-dependently generated ROS and enhanced mitochondrial membrane lipid peroxidation, resulting in irreversible loss of mitochondrial functions such as mitochondrial respiration, oxidative phosphorylation and ion transport[44].

## Conclusions

Conclusively, we have demonstrated the potential of $N^4$-substituted 5-nitroisatin-3-thiosemicarbazones to exhibit antileishmanial activity. To the best of our knowledge, such a group of compounds has been scarcely studied previously for this activity. All the compounds of the present series except **29** were found to be active in the leishmanicidal assay. Of the active compounds, 20, i.e. **2**–**4**, **7**, **8**, **10**–**13**, **15**–**19**, **21**, **23**, **24**, **26**, **28** and **30** displayed good to excellent antileishmanial activity. Compounds **5**, **7**, **19** and **28** proved to be potent antileishmanial agents, showing leishmanicidal activity even better than the standard drug, pentamidine. These compounds may represent valid leads for further studies aimed at the development of efficacious antileishmanial compounds of medicinal interest. The SAR studies revealed that the antileishmanial potential of the trial compounds depended mainly on the electronic effects of the substituents attached to $N^4$ of the thiosemicarbazone moiety. Nevertheless, extensive studies are required to determine the mechanism by which these compounds exhibit the leishmanicidal activity.

## Declaration of interest

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