IN VITRO LEISHMANICIDAL ACTIVITY OF 1,3-DISUBSTITUTED UREA

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Abstract

Protozoan parasites are among the most common pathogens. Leishmania affects large populations of the world especially in the third world countries including Pakistan. We attempted to assess synthesized 1,3-disubstituted ureas for leishmanicidal activity. Compounds, *N*-(2,6-dimethylphenyl)-*N*'-(2-nitrophenyl) urea and *N*-butyl-*N*'-(4-nitrophenyl)urea showed pronounced inhibition with $IC_{50} \pm$ S.D (μg/mL) of 29.16 $\pm$ 1.04 and 40.91 $\pm$ 0.12, respectively.

Introduction

Leishmaniasis is a syndrome caused by a number of species of *Dimorphic protozoa* Leishmania acting on reticuloendolethial system of the host (Pearson *et al*., 1989; Ram and Nath, 1996). The syndrome is classified as cutaneous, visceral and mucosal leishmaniasis and infected sand flies are a chief source to spread it from animals to humans (Evans, 1993; Lerner *et al*., 1991; Lerner and Shoemaker, 1992; Peter, 1963). It is one of the major health problems of tropical, subtropical and mediterranean regions. Pentostam, sterols and purines analogues were suggested as chemotherapeutic agents of leishmaniasis in 1980s (Berman, 1988). The WHO report indicate that about 53 million people all over the world are in danger of suffering from leishmaniasis and it has been also noted that each year 12 million new cases of leishmaniasis arise. The causative agent of this disease commonly famous as leishmania (Ram and Nath, 1996). The leishmaniases causes zoonotic infections whose degree of virulence depends on the host immunity and tropism of the parasite. The disease symptoms includes lesions, weight loss, fever, discomfort, change in hair colour, disorder in function of liver, bone marrow and spleen, ulceration, nasal blockage, swelling of lips and nose and thickening in plaques (Magill, 1995). A safe and sound vaccine is not yet available. Some currently used antimonial drug such as tartar emetic, sodium stibogluconate (pentostam), (Berman, 1988) urea stibamine, (Khan *et al*., 2003) and meglumine antimoniate (Steck, 1972) have severe adverse side effects. Various pentamidines and amphotericine B are also known for the treatment of leishmaniasis especially for visceral type. A number of forms of leishmaniasis are resistant to conventional drug therapy, especially in HIV-1 leishmania co-infected patients. The development of new effective drug is therefore, an urgent task (Cappuccino and Stauber, 1959; Bernier *et al*., 1995; Davidson *et al*., 1994). More recently Pitzer *et al*. (1998) synthesised 4-chloro-3,5-dinitrobenzotri fluoride analogues and its biological evaluation as antileishmanial agents is determined.

Sulfonamide and urea derivatives of quinacrine were tested for inhibition of protozoa and urea analogues of quinacrine have admirable activity against Leishmania and other parasitic protoza (Chibale *et al*., 2001). In this paper 1,3-disubstituted ureas (1-6) are investigated for their leishmanicidal activity

Materials and Methods

In order to search new and improved pharmaceuticals of low toxicity and high availability six compounds of the urea derivatives (1-6) were synthesized, characterized by spectroscopic techniques and finally evaluated for leishmanicidal activity.

***N*-(2,6-dimethylphenyl)-*N*'-(2-nitrophenyl)urea (1)**: 0.30 mL (2.44 mmol) of 2,6-dimethylaniline was dissolved in approximately 5-10 mL of 1,4-dioxane and than 0.20 gm (1.22 mmol) of *o*-nitrophenyl isocyanate was added to afford light yellow crystals of title compound (Perveen *et al*., 2012).

***N*-(4-chlorophenyl)-*N*'-(2-nitrophenyl)urea (2):** 0.31 gm (2.44 mmol) of 4-chloroaniline was dissolved in approximately 5-10 mL of 1,4-dioxane and than 0.20 gm (1.22 mmol) of *o*-nitrophenyl isocyanate was added to afford yellow crystals of title compound (Perveen *et al*., 2012).

***N*-butyl-*N*'-(3-nitrophenyl)urea (3):** 0.24 mL (2.44 mmol) of butyl amine was dissolved in approximately 5-10 mL of 1,4-dioxane and than 0.20 gm (1.22 mmol) of *m*-nitrophenyl isocyanate was added to afford off white crystals of title compound (Perveen *et al*., 2012).

***N*-(4-chlorophenyl)-*N*'-(3-nitrophenyl)urea (4):** 0.31 mL (2.44 mmol) of 4-chloroaniline was dissolved in approximately 5-10 mL of 1,4-dioxane and than 0.20 gm (1.22 mmol) of *m*-nitrophenyl isocyanate was added to afford yellow crystals of title compound (Perveen *et al*., 2012).

***N*-(4-nitrophenyl)-*N*'-(1-phenylethyl)urea (5):** 0.31 mL (2.44 mmol) of 1-phenylethyl amine was dissolved in approximately 5-10 mL of 1,4-dioxane and than 0.20 gm (1.22 mmol) of *p*-nitrophenyl isocyanate was added to afford light yellow crystals of title compound (Perveen *et al*., 2012).

***N*-butyl-*N*'-(4-nitrophenyl)urea (6)**: 0.24 mL (2.44 mmol) of butyl amine was dissolved in approximately 5-10 mL of 1,4-dioxane and than 0.20 gm (1.22 mmol) of *p*-nitrophenyl isocyanate was added to afford white crystals of title compound (Perveen *et al*., 2012).

Melting points were recorded on Gallenkamp melting point apparatus. R_f (retention factor) values were calculated on Kieselgel 60 F_{254}, E. Merk, Germany precoated silica gel plates. Reagent grade solvents and chemicals were used for synthesis and bioscreening of compounds. Bioscreening were done in laboratories of the PCMD, H. E. J Research Institute of Chemistry, International Center for Chemical and Biological Science, University of Karachi, Pakistan.

Leishmanicidal Bioassay: (*In Vitro*): The cultivated parasites were aseptically sedimented down at 3000 rpm and counted by neubaver chamber under the microscope. The parasites were serially diluted to a final concentration of 2.0 x 10^6 parasites/mL.

The test compounds (urea derivatives) were serially diluted to a final concentration of 1.0 mg/mL of PBS (Phosphate buffer saline of pH = 7.4, 0.5 % methanol and 0.5 % DMSO) was added as negative control. In the same concentration the amphotericin B (IC_{50} ± S.D = 0.50 ± 0.02 μM) and pentamidine (IC_{50} ± S.D = 2.56 ± 0.09 μM) were prepared separately as positive control. The plate was incubated at 22 °C and place in dark for 4 days. After incubation, the control organisms multiplied about 3-6 times. The culture was monitored under microscope on a neubaver chamber and IC_{50} of urea derivatives were calculated (Evans, 1989; Ben Salah *et al*., 1995).

Results and Discussion

A number of pharmaceutical products have been discovered and are available in markets for the treatment of leishmaniasis but they are associated with a number of complications including undesirable secondary effects.

These synthesized urea derivates have been studied previously for their antidepressant drugs *in vivo* (Perveen *et al*., 2012). *In vitro* studies of these synthesized ureas (Table 1) gave encouraging results. Compound 1 [*N*-(2,6-dimethylphenyl)-*N*'-(2-nitrophenyl)urea] found most significant among the series with IC_{50} = 29.16 ± 1.04 μg/mL. Compound 6 *para* analogue of nitrophenyl urea with *n*-butyl showed good activity (IC_{50} = 40.91 ± 0.12 μg/mL), while its *meta* analogue compound 3 [*N*-butyl-*N*'-(3-nitrophenyl)urea] had low activity. In the same way when compound 4, a *meta* analogue with 4-chlorophenyl moiety demonstrated moderate activity (IC_{50} = 59.01 ± 1.87 μg/mL) while its *ortho* analogue was inactive in the assay with IC_{50} value >100 μg/mL. The variation of activity of these compounds may be due to the position of NO_2 group on phenyl ring. Compound 5 [*N*-(4-nitrophenyl)-*N*'-(1-phenylethyl)urea] also exhibited low leishmanicidal activity as compared to the standard drugs.

Table 1. Synthesis and *In vitro* leishmanicidal screening of disubstituted ureas 1-6.

Compound No.	IUPAC Name	% Yield	*R_f	M.P °C	IC_{50} ± S.D (μg/mL)
1	*N*-(2,6-dimethylphenyl)-*N*'-(2-nitrophenyl)urea	100	0.59	208-209	29.16 ± 1.04
2	*N*-(4-chlorophenyl)-*N*'-(2-nitrophenyl)urea	69	0.70	240-241	>100
3	*N*-butyl-*N*'-(3-nitrophenyl)urea	85	0.73	139-140	84.71 ± 1.78
4	*N*-(4-chlorophenyl)-*N*'-(3-nitrophenyl)urea	80	0.89	240-241	59.01 ± 1.87
5	*N*-(4-nitrophenyl)-*N*'-(1-phenylethyl)urea	68	0.76	185-186	84.22 ± 2.73
6	*N*-butyl-*N*'-(4-nitrophenyl)urea	100	0.70	136-137	40.91 ± 0.12

Standard Drugs: Amphotericin B (IC_{50} ± S.D) = 0.50 ± 0.02 (μg/mL) & Pentamidine IC_{50} ± S.D = 2.56 ± 0.09 (μg/mL) *R_f (Dichloromethane: Hexane) = Retention factor & M.P = Melting Point

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