Pure Appl. Chem., Vol. 80, No. 8, pp. 1783–1790, 2008.
doi:10.1351/pac200880081783

Discovery of leishmanicidal agents from medicinal plants*

Atta-ur-Rahman, Samreen, Atia-tul-Wahab, and M. Iqbal Choudhary‡

H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan

Abstract: Antileishmanial activity of several classes of natural compounds was evaluated by using an in vitro parasitic assay model. This has led to the discovery of new antileishmanial agents with potential to be useful in the treatment of visceral and cutaneous leishmaniasis.

Keywords: leishmaniasis; antileishmanial agents; physalins; steroidal alkaloids; steroidal saponins.

Leishmaniasis is a group of prevalent diseases caused by protozoan parasites belonging to the genus *Leishmania*. This affects over 12 million people in 88 countries with 2–3 million new cases each year. It is estimated that about 350 million people are at risk for this infection [1]. Leishmaniasis is transmitted by the *Phlebotamine* sand fly belonging to the genera *Lutzomia* (New World) and *Phlebotamous* (Old World). Leishmaniasis is classified on the basis of symptomatology like cutaneous leishmaniasis (Oriental sore) caused by *L. major*, mucocutaneous leishmaniasis (MCL or Espundia) caused by *L. mexicana*, and *L. tropica* and visceral leishmaniasis (Kala azar) caused by *L. donovani* [2].

Cutaneous leishmaniasis (CL), the most common form, is a complex disease with a wide spectrum of clinical manifestations. It is prevalent in tropical and subtropical countries including Iran, Afghanistan, Brazil, Peru, Saudi Arabia, Pakistan, and Syria. *L. donovani* is the major causative agent of visceral leishmaniasis. It exists in the form of obligatory intracellular amastigotes found in the phagolysosomal compartment of mammalian macrophages. Visceral leishmaniasis has a high mortality rate and is characterized by irregular fever, weight loss, hepatosplenomegaly, and anemia. Some 500 000 new cases of visceral leishmaniasis are diagnosed annually in Bangladesh, Brazil, India, Nepal, and Sudan. MCL produces lesions which can lead to extensive destruction of the mucous membranes of the nose, mouth, and throat cavities. About 90 % of cases of MCL occur in Bolivia, Brazil, and Peru. Due to the limited availability of effective pharmaceuticals, most of the people in endemic areas rely on traditional medicines for the treatment of leishmaniasis [3].

The current treatment for leishmaniasis includes pentavalent antimonials such as sodium stibogluconate (pentostam) and meglumine antimonate (glucatime). The long use of antimonials leads to the accumulation of the drug in liver and spleen and causes side effects such as myalgia, pancreatitis, cardiac arrhythmia, and hepatitis, leading to the reduction or cessation of treatment. Pentamidine is among the second line of drugs for the treatment of visceral leishmaniasis in patients who failed to respond to antimonial therapy. Although pentamidine is less toxic than antimonials, it is still not free from

*Paper based on a presentation at CHEM-BIO-TECH-2007, a joint meeting of the IUPAC 1st Symposium on Chemical Biotechnology (ISCB-1) and the 8th Symposium on Bioorganic Chemistry (ISBOC-8), 8–11 August 2007, Turin, Italy. Other presentations are published in this issue, pp. 1773–1882.

‡Corresponding author: Fax: +92-21-4819018-9; E-mail: hej@cyber.net.pk

side effects such as hypoglycemia, diabetes, nephrotoxicity, and pain at the site of infection. The antibiotic amphotericin B is another drug used for the treatment of visceral leishmaniasis. It shows better results in cases resistant to antimonials and diamidines, but it leads to nephrotoxicity and cardiotoxicity. The emergence of drug resistance in *Leishmania* parasites is a major obstacle to their control. About 5–70 % of the patients in some endemic areas sometimes do not respond to standard antiparasitic drugs. Therefore, the search for novel, effective, and safe drugs for the treatment of the diseases has been a priority for health researchers [1,4].

Medicinal plants have been used for centuries for the treatment of various ailments. Plants are an important source for drug candidates, particularly against parasites because of their long association with parasites. Over 100 plants have been reported to be active against various forms of leishmanial parasites [5].

During the current study, we focused our efforts on discovering effective antileishmanial agents to control visceral and cutaneous leishmaniasis. Our main strategy was to screen a large number of natural products that researchers have obtained from medicinal plants and other living sources. For this purpose, a high-throughput in vitro assay was employed. *L. major* promastigotes were cultured in the logarithmic phase, and the final concentration of parasites was adjusted to 2×106 parasites/mL. The test compounds were dissolved in dimethyl sulfoxide (DMSO), and their volume was made up with RPMI-1640. Test compounds were added in serial dilution. The parasite suspension was added into each well of the 96-well plates, and incubated at 21–22 °C for 72 h, in the presence and absence of amphotericin B (pos. control). The experiments were carried out in duplicate, and the number of surviving parasites were counted. The 50 % inhibitory concentration (IC_{50}) was determined. The details of the assay are reported in references herein. As a result, the following classes were identified as antileishmanial agents.

PHYSALINS

Several physalins, **1–4** and **7–10**, and a withanolide **11** were isolated from *Physalis minima* Linn. (Solanaceae) [6–8]. Compounds **5**, **6**, and **9** were obtained by biotransformation of **3** by *Rhizopus stolonifer* and *Cunninghamella elegans* (Fig. 1). Physalins are steroidal lactone constituents of *Physalis* and other closely related genera. All these compounds showed potent leishmanicidal activity against the promastigotes of *L. major* (Table 1). Structure analysis of physalins showed that the presence of hydroxyl groups at C-5, C-6, and C-11 appears to play a major role in the activity against leishmaniasis. Compound **1** showed IC_{50} = 0.92 μg/mL as compared to the standard drug amphotericin B, IC_{50} = 0.12 μg/mL, while the absence of OH groups significantly decreased the activity as shown by the IC_{50} value of compound **2** (IC_{50} = 5.00 μg/mL).

Compound **3** also showed substantial antileishmanial activity (IC_{50} = 3.39 μg/mL). The biotransformed products **5–6** and **9** showed a decrease in activity probably due to the absence of the C-6 hydroxyl group from compound **3**. Withanolide **11** did not show any activity (IC_{50} = 38.9 μg/mL).

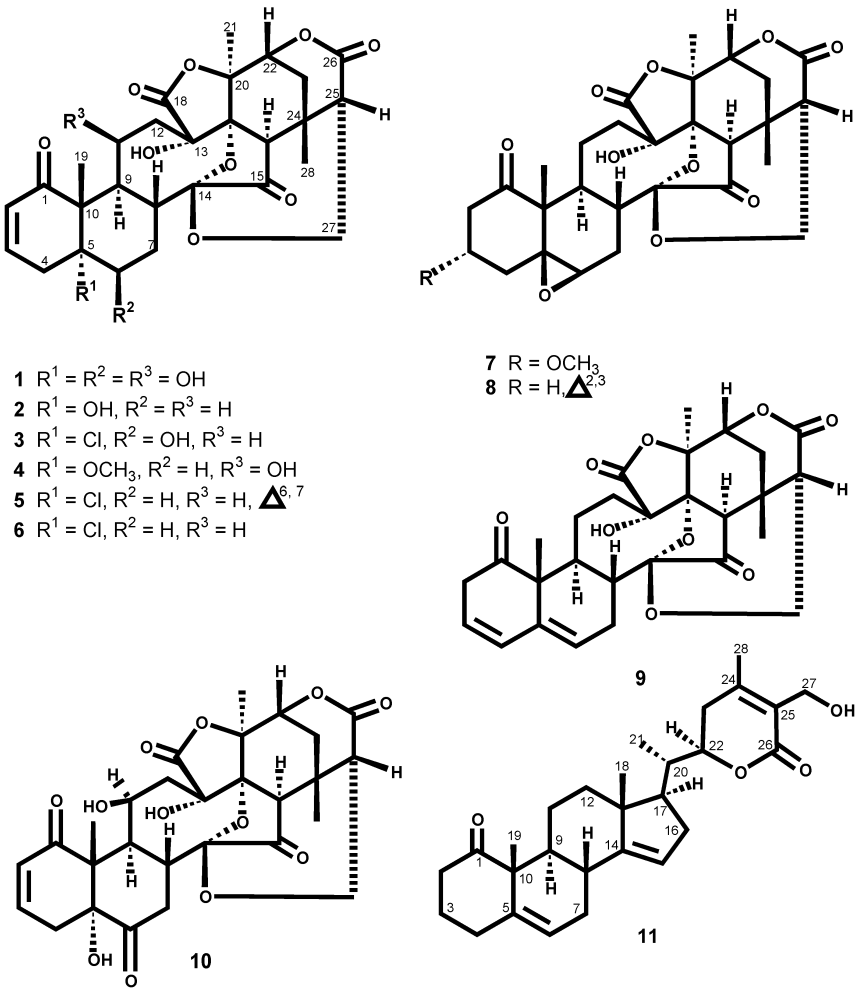


Fig. 1 Structures of physalins **1–4** and **7–10** and a withanolide **11** isolated from *P. minima* and their biotransformed products **5**, **6**, and **9**.

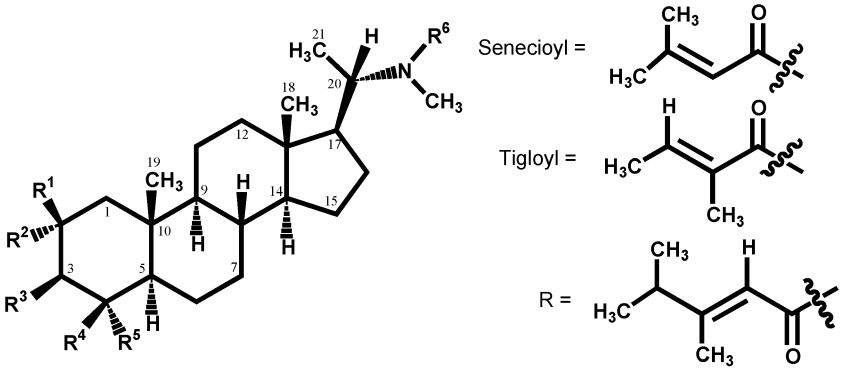
Table 1 In vitro antileishmanial activity of compounds **1–11** and standard drug amphotericin B.

Compound	IC_{50} (μg/mL) ± SD[a]	Compound	IC_{50} (μg/mL) ± SD[a]
1	0.92 ± 0.001	**7**	19.4 ± 0.180
2	5.00 ± 0.010	**8**	3.39 ± 0.005
3	3.39 ± 0.005	**9**	7.05 ± 0.050
4	4.86 ± 0.056	**10**	3.65 ± 0.070
5	4.21 ± 0.015	**11**	38.9 ± 0.105
6	3.46 ± 0.030	**Amphotericin B**[b]	0.12 ± 0.105

[a]Standard deviation.
[b]Standard drug for leishmaniasis.

STEROIDAL ALKALOIDS

Several steroidal alkaloids **12–28** were isolated from *Sarcococca hookeriana* (Baill.) Hook. f. (Buxaceae) (Fig. 2). Steroidal alkaloids are commonly called "steroidal alkamines" and bear a cyclopentanophenanthrene skeleton. These compounds and their derivatives were found to have potent-to-mild antileishmanial activity [9].



Compounds	R^1	R^2	R^3	R^4	R^5	R^6	Unsat^n.
12	OH	H	NH-Senecioyl	OH	H	CH_3	$\Delta^{16, 17}$
12a	OAc	H	NH-Senecioyl	OAc	H	CH_3	$\Delta^{16, 17}$
13	H	OH	NH-Senecioyl	OAc	H	CH_3	-
13a	H	OAc	NH-Senecioyl	OAc	H	CH_3	-
14	OAc	H	NH-Senecioyl	H	H	CH_3	-
15	H	H	NMe-R	H	H	CHO	-
16	OAc	H	NH-Senecioyl	H	H	CH_3	$\Delta^{14, 15}$
17	H	-	NH-Tigloyl	O	H	H	$\Delta^{2, 3}$
18	H	H	NMeCOPh	OAc	H	CH_3	-
19	H	-	NH-Tigloyl	O	H	H	-
20	H	H	NHMe	H	H	CH_3	$\Delta^{14, 15}$
21	H	H	OH	H	OH	CH_3	-
21a	H	H	OAc	H	OAc	CH_3	-
21b	H	H	$O(CH_2)_4CH_3$	H	O-C_5H_{11}	CH_3	-
21c	H	H	O	H	O	CH_3	-
22	H	H	NH_2	H	H	CH_3	-
23	H	H	NMe_2	H	H	CH_3	-
24	H	H	NHMe	H	H	CH_3	-
25	H	H	NH-R	H	H	CH_3	-
26	H	-	NH-Tigloyl	O	-	CH_3	$\Delta^{16, 17/ 2, 3}$
27	H	-	NH-Tigloyl	O	-	CH_3	-
28	H	H	NMeCOPh	H	H	CH_3	-

Fig. 2 Structures of steroidal alkaloids, isolated from *S. hookeriana* and their derivatives.

The study indicated that the functionalities present on the steroidal skeleton play an important role in the leishmanicidal activity (Table 2). Compound **13** with senecioylamino, α-hydroxy, and β-acetoxy groups at C-3, C-2, and C-4 positions, respectively, showed the most potent antileishmanial activity (IC_{50} = 0.20 μg/mL), comparable to the standard drug, amphotericin B (IC_{50} = 0.12 μg/mL). Compound **16** with a β-acetoxy group at C-2 and a double bond between C-14/C-15 showed mild activity (IC_{50} = 3.36 μg/mL). Compound **14**, which lacks C-14/C-15 double bond, showed comparatively less activity (IC_{50} = 3.78 μg/mL). The acetylated derivative of **12** was relatively more active (IC_{50} = 6.67 μg/mL) than the derivative **13a** (IC_{50} = 12.74 mg/mL) with the C-3 β-acetoxy configuration and with no C-16/C-17 double bond. Compound **12** with β-hydroxyl groups at C-2 and C-4 positions was less active (IC_{50} = 52.74 μg/mL). These observations indicated that a combination of C-4 β-acetoxy, C-2 α-hydroxyl, and olefinic functionalities at the five-membered ring play an important role in the activity of compounds with a C-3 seneciolyamino group.

Table 2 In vitro antileishmanial activity of compounds **12–28** and the standard drug, amphotericin B.

Compound	IC_{50} (μg/mL) ± SD[a]	Compound	IC_{50} (μg/mL) ± SD[a]
12	52.74 ± 0.80	**21a**	2.12 ± 0.23
12a	6.67 ± 0.47	**21b**	1.12 ± 0.27
13	0.20 ± 0.09	**21c**	3.30 ± 0.67
13a	12.74 ± 0.85	**22**	3.41 ± 0.54
14	3.78 ± 0.23	**23**	13.00 ± 0.25
15	6.49 ± 0.32	**24**	3.44 ± 0.76
16	3.36 ± 0.40	**25**	0.46 ± 0.06
17	2.29 ± 0.73	**26**	3.41 ± 0.27
18	61.44 ± 0.55	**27**	1.28 ± 0.43
19	3.27 ± 0.37	**28**	26.82 ± 0.67
20	2.00 ± 0.50	**Amphotericin B**	0.12 ± 0.10
21	55.33 ± 0.80		

Compounds with a tigloylamino moiety at C-3 and an α,β-unsaturated carbonyl functionality at ring A and/or at ring D were also studied. Compound **27** showed a higher activity (IC_{50} = 1.28 μg/mL) than compounds with an unsaturated five-membered ring and a monomethylamino group at C-20 position (compounds **17**, **19**, and **26**).

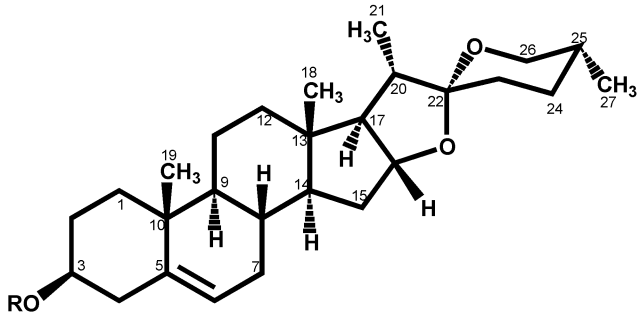
Compounds with a 3',4'-dimethyl-2'-pentenamido functionality at C-3 position such as **25** displayed potent activity (IC_{50} = 0.46 μg/mL), while compound **15** showed only mild (IC_{50} = 6.49 μg/mL) antileishmanial activity. This indicated that the presence of the *N*,*N*-formyl(methyl)amino group at C-20 decreases the activity.

In compounds **18** and **28** with a C-3 *N*-methylamino benzoyl moiety at C-3, compound **18** was found to be less active (IC_{50} = 61.44 mg/mL) than compound **28** which lacks an acetoxy group (IC_{50} = 26.82 μg/mL).

Compound **20** with a C-14/C-15 double bond and a C-3 amino methyl group was found to be more active (IC_{50} = 2.00 μg/mL) than other compounds without such a double bond (e.g., compound **24**), whereas the C-3 amino compound **22** was more active than the C-3 *N*,*N*-dimethylamino-containing compound **23** (IC_{50} = 13.00 μg/mL). The antileishmanial activities of compound **21** and its synthetic derivatives were also studied. The three derivatives were found to be far more active than the parent compound **21**.

Steroidal saponins

Saponins **29–31**, isolated from *Paris polyphylla* Smith. (Trilliaceae) (Fig. 3), showed mild-to-moderate antileishmanial activities. Steroidal saponins are glycosylated derivatives of steroids. Compound **29** exhibited significant activity with IC_{50} = 1.59 μg/mL as compared to the standard drug amphotericin B (IC_{50} = 0.12 μg/mL). Compounds **30**, **31**, and **31a** did not show any significant antileishmanial activity (Table 3) [10].



Compounds	R
29	α-L-Rhamnopyranosyl-(1⟶3)-β-D-glucopyranoside
30	[α-L-Rhamnopyranosyl-($1_{Rha}\rightarrow 2_{Glc}$)-α-L-arabino-furanosyl-($1_{Ara}\rightarrow 4_{Glc}$)]-β-D-glucopyranoside
31	[α-L-Rhamnopyranosyl-($1_{Rha}\rightarrow 2_{Glc}$)-α-L-rhamno-pyranosyl-($1_{Rha}\rightarrow 4_{Glc}$)]-β-D-glucopyranoside
31a	H

Fig. 3 Structure of saponins isolated from *P. polyphylla* and their derivatives.

Table 3 In vitro antileishmanial activity of compounds **29–31** and the standard drug amphotericin B.

Compound	IC_{50} (μg/mL) ± SD[a]
29	1.59 ± 0.08
30	81.67 ± 0.85
31	83.72 ± 0.25
31a	58.75 ± 0.30
Amphotericin B	0.12 ± 0.10

Species of seaweeds

The crude ethanolic extract of various seaweed were also tested for their in vitro antileishmanial activity. These weeds showed mild-to-weak inhibitory activity against the parasites (Table 4) [11–12].

Table 4 In vitro antileishmanial activity of species of seaweeds.

Serial no.	Plant name	IC_{50} (μg/mL) ± SD[a]
	Division: Chlorophyta	
1	*Caulerpa faridii*	34.05 ± 0.15
2	*Caulerpa racemosa*	37.50 ± 0.45
3	*Codium flabellatum*	34.00 ± 0.90
4	*Codium iyengarii*	60.13 ± 0.45
5	*Ulva fasciata*	50.00 ± 1.00
6	*Ulva reticulata*	64.75 ± 0.20
7	*Ulva rigida*	65.69 ± 0.45
	Division: Rhodophyta	
8	*Botryocladia leptopoda*	60.81 ± 0.41
9	*Centroceras clavulatum*	57.89 ± 0.75
10	*Gracilaria corticata*	37.50 ± 0.45
11	*Laurencia pinnatifida*	6.25 ± 0.35
12	*Melanothamnus afaqhusainii*	32.60 ± 0.20
13	*Scinaia hatei*	14.10 ± 0.20
14	*Scinaia indica*	59.60 ± 0.30
15	*Amphotericin B*	0.07 ± 0.19

CONCLUSIONS

This study demonstrates the potential of natural products and their derivatives in the discovery of antileishmanial agents. Several new classes of antileishmanial agents of natural origin were identified during this systematic study. This led to the identification of several scaffolds for the future synthesis of second-generation compounds with optimized pharmacological profile for the treatment of leishmaniasis.

ACKNOWLEDGMENTS

We acknowledge the enabling role of the Higher Education Commission Islamabad, Pakistan, and appreciate its financial support through "Merit Scholarship Scheme for Ph.D. studies in Science and Technology". We would also like to acknowledge the contribution of Ms. H. Sabina, Dr. R. Aliya, Ms. S. Tasneem, Ms. Kausar Yasmeen, Dr. Sammer Yousuf, Dr. Shakil Ahmed, Dr. Adnan Ali Shah, Dr. Krishna P. Devkota, Dr. Rosa Ranjit, Dr. Norbert Sewald, Dr. M. Tareq H. Khan, and Dr. A. Meli Lannang to this work.

REFERENCES

1. S. Pandey, S. N. Suryawanshi, S. Gupta, V. M. L. Srivastava. *Eur. J. Med. Chem.* **39**, 969 (2004).
2. P. Desjeux. *Trans. R. Soc. Trop. Med. Hyg.* **95**, 239 (2001).
3. R. W. Ashford. *Int. J. Parasitol.* **30**, 1269 (2000).
4. P. Minodier, P. Parola. *Travel Med. Infect. Dis.* **5**, 150 (2007).
5. L. G. Rocha, J. R. G. S. Almeida, R. O. Macêdo, J. M. Barbosa-Filho. *Phytomedicine* **12**, 514 (2005).
6. M. I. Choudhary, S. Yousuf, S. Ahmed, Samreen, K. Yasmeen, Atta-ur-Rahman. *Chem. Biodivers*. **2**, 1164 (2005).

7. M. I. Choudhary, S. Yousuf, Samreen, S. Ahmed, Atta-ur-Rahman. *Nat. Prod. Res*. **21**, 877 (2007).
8. M. I. Choudhary, S. Yousuf, Samreen, A. A. Shah, S. Ahmed, Atta-ur-Rahman. *Chem. Pharm. Bull*. **54**, 927 (2006).
9. K. P. Devkota, M. I. Choudhary, R. Ranjit, Samreen, N. Sewald. *Nat. Prod. Res*. **21**, 292 (2007).
10. K. P. Devkota, M. T. H. Khan, R. Ranjit, A. M. Lannang, Samreen, M. I. Choudhary. *Nat. Prod. Res*. **21**, 321 (2007).
11. H. Sabina, S. Tasneem, Samreen, Y. Kausar, M. I. Choudhary, R. Aliya. *Pak. J. Bot.* **37**, 163 (2005).
12. H. Sabina, Samreen, M. I. Choudhary, R. Aliya. *Int. J. Phycol. Phycochem*. **2**, 53 (2006).