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Synthesis of marine alkaloid: 8,9-Dihydrocoscinamide B and its analogues as Novel class of antileishmanial agents

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Abstract—A series of marine alkaloid 8,9-dihydrocoscinamide B, its analogues and indolylglyoxylamide derivatives have been synthesized and screened for their in vitro antileishmanial activity profile in promastigote and amastigote models. Compounds **7** and **10** have shown 99–100% inhibition against promastigotes and 97–98% inhibition against amastigotes at a concentration of 10 μg/ml.

Leishmaniasis is a growing health problem in many parts of the world, with about 350 million people living in areas of disease endemicity and about 2 million new cases each year.[1–3] Leishmaniasis is caused by different species belonging to the genus *Leishmania*, a protozoan which is transmitted to humans by the bite of an insect vector, phlebotomine sandfly. Infection by various strains of *Leishmania* causes a wide spectrum of disease in humans, with many different clinical presentations. The severity of the disease is largely dictated by the immunological status of the infected individual and by the species of *Leishmania* involved. *Leishmania* spp. exists in two morphologically distinct forms: a motile flagellated form (promastigotes) and an intracellular non-flagellated form (amastigotes). The promastigotes are ingested by mononuclear phagocytes of the host, where they transform into immotile amastigotes, multiply, rupture the host cell and then invade other cells.[4]

The visceral form of Leishmaniasis, commonly known as Kala-azar, is caused by the parasite *Leishmania donovani* and is often fatal. The drugs for leishmaniasis's treatment of all their clinical forms are sodium stilbogluconate (Pentostam) and meglumine antimonate (Glucantime), despite the fact that they exhibit renal and cardiac toxicity.[5] Alternative drugs, such as pentamidine, amphotericin B, and some azo-derivatives, are also very toxic with serious side effects.[6] Miltefosine, a phosphocholine analogue originally developed as an anticancer agent, has been found to be highly effective against leishmaniasis in vitro and in vivo. Now, this compound is the only oral agent against both cutaneous[7] and visceral[8] leishmaniasis, although presenting severe gastrointestinal problems.[9] Since the chemotherapy against leishmaniasis is still inefficient, there is an urgent need for the development of new, efficient and safe drugs for the treatment of this disease.[10]

Natural products have played a significant role in the drug discovery process throughout the last century. Plant derived natural products were used in traditional medicine as therapies of malaria and cutaneous leishmaniasis. Although most active drugs against infectious agents are derived from natural products,[11–13] medicinal scientific evaluation of the medicinal properties of marine sources remains grossly understudied because of the presence of active principle in very minute quantity. A variety of marine sources including sponges, tunicates, red alga, acorn worms and symbiotic bacteria have been shown to generate indole alkaloids, which represent the largest number and most complicated of the marine alkaloids.[14] The indole skeleton often appears in the natural products with a variety of biological activities.[15–17] It has been reported that indolylglyoxylamide derivatives have anticancer activity and bisindolic enamides,

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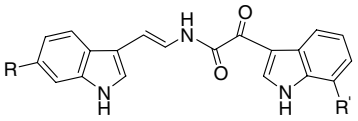
Coscinamide A–C (**1**–**3**); isolated from a marine sponge, a *Coscinoderma* sp., give cycloprotection against HIV in the NCI assay.[18,19] This class of secondary metabolites draws attention to the tremendous unexplored potential and can be used as the basis for further development as drug candidates.



1 Coscinamide A R=Br, R'=H
2 Coscinamide B R=R'=H
3 Coscinamide C R=Br, R'=OH

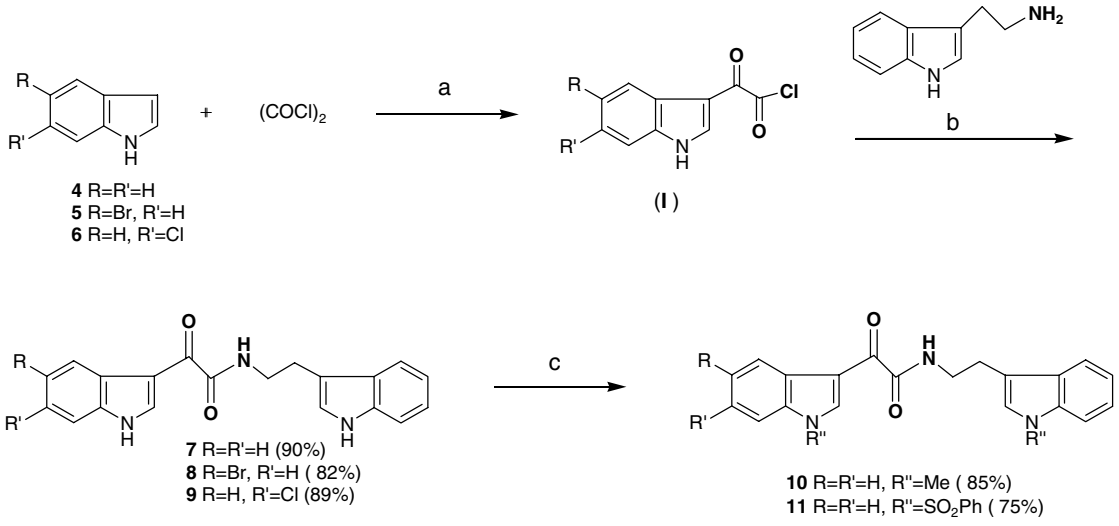
Apart from the synthesis of bioactive heterocycles,[20] we undertook the synthesis of bioactive marine natural products and under this ongoing programme, we investigated the antileishmanial in vitro activity of 8,9-dihydrocoscinamide B, its analogues and indolylglyoxylamide derivatives. In this communication, we have reported in vitro antileishmanial activity of these synthesized compounds.

8,9-Dihydrocoscinamide B (**7**) and its analogues (**8**–**11**) were synthesized in high yield by reacting indole oxalyl chloride (**I**) with tryptamine in dry THF at 0 °C to ambient temperature for 24 h. (Scheme 1). Same set of conditions was applied for the synthesis of various indolylglyoxylamide derivatives (**12**–**22**) by using different amines instead of tryptamine (Scheme 2) in quantitative yield. All the synthesized compounds are well characterized by spectroscopic method such as IR, mass, NMR and elemental analysis.[23]

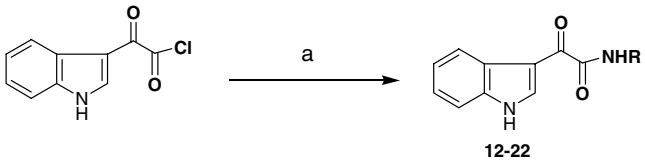
Luciferase transfected *L. donovani* promastigotes, which are more stable under the influence of G 418, were maintained at 25 ± 1 °C in medium 199 supplemented with 10% Fetal Calf Serum (GIBCO). The effect of compounds on the growth of promastigotes was assessed by monitoring the luciferase activity of viable cells after treatment. The transgenic promastigotes of late log phase were seeded at 5×10^5/100 μl medium 199/well in 96-well flat-bottomed microtitre (MT) plates (CELLSTAR) for 72 h in medium alone or in presence serial dilutions of drugs (250 ng/ml to 10 μg/ml) in DMSO.[21] Parallel dilutions of DMSO were used as controls. After incubation, an aliquot (50 μl) of promastigote suspension was aspirated from each well of 96-well plate and mixed with equal volume of Steady Glo® reagent (Promega) and luminescence was measured in luminometer. The values were expressed as RLU (relative luminescence unit).

For assessing the activity of compounds against amastigote stage of the parasite, mouse macrophage cell line (J-774A.1) infected with promastigotes expressing luciferase firefly reporter gene was used. Cells were seeded in a 96-well plate (5×10^4 cells/100 μl/well) in RPMI-1640 containing 10% fetal calf serum and the plates were incubated at 37 °C in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary-phase promastigotes (2.5×10^5/100 μl/well). Promastigotes invade the macrophage and are transformed into amastigotes. The test material in appropriate concentration (50 and 10 μg/ml) in complete medium was added after replacing the previous medium and the plates were incubated at 37 °C in a CO_2 incubator for 24 h or more. After incubation, the drug-containing medium was decanted and 50 μl PBS was added to each well and mixed with an equal volume of the steady Glo reagent. After gentle shaking for 1–2 min, the reading was taken in a luminometer.

The in vitro biological activity of 8,9-dihydrocoscinamide B (**7**), its analogues (**8**–**11**) and indolylglyoxylamide derivatives (**12**–**22**) has shown encouraging results against *L. donovani*. The percentage inhibition of these compounds against promastigote has been



Scheme 1. Reagents and conditions: (a) THF, 0 °C; (b) tryptamine, THF, 0 °C–rt; (c) R″X (X = I/Cl), DCM, 0 °C.



12-22

Comp. no	12	13	14	15	16	17	18	19	20	21	22
Yield (%)	85	70	70	75	82	78	70	85	70	72	75

Scheme 2. Reagents and conditions: (a) different amines, THF, 0 °C–ambient temp.

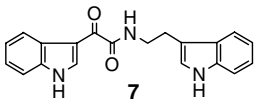
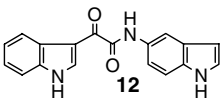
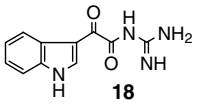
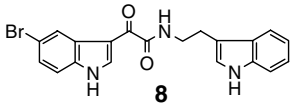
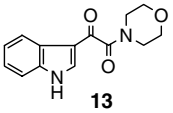
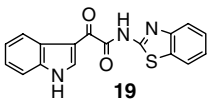
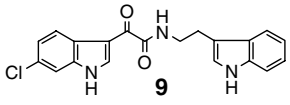
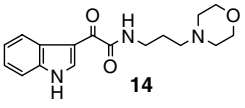
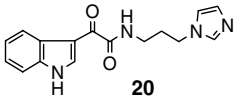
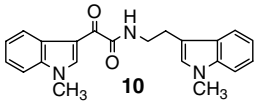
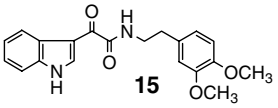
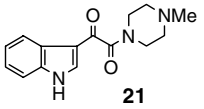
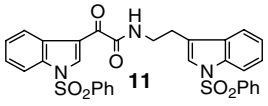
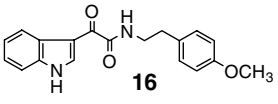
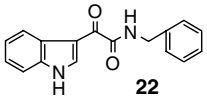
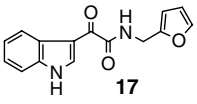
shown in Table 1, while compounds having percentage inhibition against promastigote more than 80% were further screened for amastigote model (Table 2). Five compounds (**7**–**10**) and **17** showed 99–100% inhibition against promastigotes, whereas two compounds **7** and **10** exhibited 98% inhibition in amastigotes at a concentration of 10 μg/ml. 8,9-dihydrocoscinamide B (**7**) showed 100% inhibition against promastigotes and ~98% inhibition against amastigotes. 5-bromo-8,9-dihydrocoscinamide B (**8**) showed 99% and 75% inhibition in promastigote and amastigote, respectively.

While 6-chloro-8,9-dihydrocoscinamide B (**9**) showed 99% against promastigotes and 59% inhibition in amastigotes. 5-Bromo-8,9-dihydrocoscinamide B (**8**) is more active than 6-chloro-8,9-dihydrocoscinamide (**9**) is in agreement with the results reported in the literature that bromo alkaloids are more active as compared to chloro alkaloids.[22] We observed that indeed 6-chloro-8,9-dihydrocoscinamide (**9**) acts as inhibitors, while 5-bromo-8,9-dihydrocoscinamide (**8**) enhances antileishmanial activity.

Substitution at N in 8,9-dihydrocoscinamide B is also very important and responsible for activity. Compound (**10**) showed 99% inhibition in promastigotes and ~98% inhibition in amastigotes, whereas compound (**11**) showed only 49% inhibition in promastigotes. These data reveal that presence of electron donating groups enhances the percentage inhibition, whereas electron withdrawing groups retard the percentage inhibition.

Compound (**12**), a bisindole alkaloid, showed ~94% inhibition in promastigotes, while showing total cell loss in amastigote. Indolylglyoxylamide derivatives showed lesser degree of inhibition as compared to 8,9-dihydrocoscinamide B derivatives. Compound (**13**), the only indolylglyoxylamide derivative, which showed 83% inhibition in promastigote, also showed 78% inhibition in

Table 1. Antileishmanial in vitro activity against luciferase–promastigote system

Structures of 8,9-dihydrocoscinamide B and its analogues	% inhibition (at 10 μg/ml) promastigote	Structures of indolylglyoxylamides	% inhibition (at 10 μg/ml) promastigote	Structures of indolylglyoxylamides	% inhibition (at 10 μg/ml) promastigote
 **7**	100	 **12**	93.65	 **18**	55
 **8**	99.2	 **13**	83.2	 **19**	18
 **9**	99.13	 **14**	73	 **20**	55.5
 **10**	99.0	 **15**	78.21	 **21**	35.9
 **11**	49	 **16**	68.5	 **22**	53.9
—	—	 **17**	99.2	—	—
		SSG®—a, b Pentamidine®—c, d, e			

a: SSG (sodium stilbogluconate) shows 40–50% inhibition in promastigotes at 500 μg/ml, b: SSG (sodium stilbogluconate) shows 21% inhibition in amastigotes at 50 μg/ml, c: pentamidine shows 85–90% inhibition in promastigotes at 0.5 μg/ml, d: pentamidine shows 90–99% inhibition in amastigotes at 50 μg/ml, e: pentamidine shows 30% inhibition in amastigotes at 10 μg/ml.

Table 2. Antileishmanial in vitro activity against luciferase–amastigote system

Compound	% inhibition (at 10 μg/ml)
7	97.72
8	75.06
9	59.06
10	97.78
12	TCL
13	78.06
17	NI

TCL, total cell loss; NI, no inhibition.

amastigote. Whereas compound (**17**) showed ~99% inhibition in promastigotes but no inhibition against amastigote. Compounds (**15**) and (**16**) showed 78% and ~69% inhibition against promastigotes. It again strengthens the fact that electron donating group present either in indole ring or phenyl ring enhances the activity. Compound (**15**) having two methoxy groups present in the phenyl ring showed higher percentage of inhibition as compared to (**16**), where only one methoxy group attached to the phenyl ring. Rest of the compounds (**18**–**22**) exhibit low percentage of inhibition in promastigotes at a concentration of 10 μg/ml. The results prove that 8,9-dihydrocoscinamide B (**7**) and its analogues (**8**–**11**) exhibited a better correlation of activity to indolylglyoxylamide derivatives (**12**–**22**). Comparatively inhibited activity of indolylglyoxylamide derivatives may be attributed to the presence of one indole ring.

Leishmania occurs in third world countries. Prescribed treatments are antimonials and benzamidines, known for high toxicity. This necessitates development of safer and more chemotherapeutic agents for treatment. 8,9-dihydrocoscinamide B and its analogues have shown promising in vitro activity, needing the concentrated attention and perseverance of the scientific community so that this lead compound may ultimately be developed as very useful drug for the future benefits of mankind.

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23. Spectroscopic data for **7**. MS: 332 (M+1); mp 198–200 °C; IR (KBr) 3410, 3350, 3260, 2930, 1700, 1660, 1500, 1435, 1230, 740 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 12.20 (br s, 1H, NH), 10.81 (br s, 1H, NH), 8.39 (t, 1H, J = 5.4 Hz, NHCO), 7.64 (d, 2H, J = 7.6 Hz), 7.36–7.25 (m, 6H), 7.12 (s, 1H), 6.94 (s, 1H), 3.69 (t, 2H, J = 6.3 Hz), 3.08 (t, 2H, J = 6.7 Hz); ^{13}C ($CDCl_3$, 50 MHz): 182.6, 163.8, 138.9, 136.6, 127.6, 126.6, 123.8, 123.1, 122.9, 121.7, 118.7, 118.5, 112.9, 112.5, 111.9, 111.7, 39.8, 25.3. Anal. Calcd for $C_{20}H_{17}N_3O_2$: C, 72.49; H, 5.17; N, 12.68. Found: C, 71.58; H, 4.26; N, 11.74. Spectroscopic data for **8**. MS: 411 (M+1); mp 200–202 °C; IR (KBr) 3421, 3261, 2930, 1723, 662, 1505, 1435, 1223, 793, 742 cm^{-1};^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 12.0 (br s, 1H, NH), 10.99 (br s, 1H, NH), 8.99 (t, 1H, J = 6.2 Hz, NHCO), 8.51 (s, 1H), 7.78–7.66 (m, 6H), 7.58 (s, 1H), 7.03 (s, 1H), 3.12 (t, 2H, J = 7.22 Hz), 2.38 (t, 2H, J = 7.5 Hz); ^{13}C ($CDCl_3$, 50 MHz): 182.5, 163.4, 139.9, 136.6, 135.5, 128.4, 127.6, 126.4, 125.3, 123.8, 123.0, 121.3, 118.7, 115.7, 115.0, 112.1, 111.9, 111.7, 30.8, 25.2. Anal. Calcd for $C_{20}H_{16}BrN_3O_2$: C, 58.55; H, 3.93; N, 10.24. Found: C, 59.28; H, 2.96; N, 9.27. Spectroscopic data for **9**. MS: 367 (M+1); mp 199–202 °C; IR (KBr) 3423, 3321, 2919, 1730, 1662, 1500, 1436, 1240, 816, 747 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 12.21 (br s, 1H, NH), 10.81 (br s, 1H, NH), 8.65 (t, 1H, J = 5.0 Hz, NHCO), 7.99 (s, 1H), 7.90–7.85 (m, 6H), 7.21 (s, 1H), 6.80 (s, 1H), 3.55 (t, 2H, J = 7.5 Hz), 3.0 (t, 2H, J = 8.0 Hz); ^{13}C ($CDCl_3$, 50 MHz): 183.0, 162.5, 140.0, 136.6, 135.1, 128.2, 127.6, 126.4, 125.3, 125.1, 123.4, 121.4, 119.2, 115.0, 112.2, 112.1, 111.9, 111.1, 30.8, 25.0. Anal. Calcd for $C_{20}H_{16}ClN_3O_2$: C, 65.67; H, 4.41; N, 11.49. Found: C, 6.75; H, 3.38; N, 10.56. Spectroscopic data for **10**. MS: 360 (M+1); mp 196–198 °C; IR (KBr) 3270, 2929, 2850, 1721, 1665, 1500, 1443, 1181, 738 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.78 (t, 1H, J = 5.5 Hz, NHCO), 7.50 (d, 2H, J = 7.5 Hz), 7.49–7.36 (m, 6H), 7.0 (s, 1H), 6.8 (s, 1H), 3.86 (s, 3H), 3.76 (s, 3H), 3.55 (t, 2H, J = 6.0 Hz), 3.02 (t, 2H, J = 6.5 Hz); ^{13}C ($CDCl_3$, 50 MHz): 184.9, 162.2,139.9, 136.4, 127.6, 126.4, 123.8, 123.0, 122.9, 121.3, 118.6, 113.4, 112.4, 111.9, 111.8, 40.0, 39.1, 25.8. Anal. Calcd for $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69. Found: C, 72.55; H, 4.88; N, 1.17. Spectroscopic data for **17**. MS: 269 (M+1); mp 160–162 °C; IR (KBr) 3390, 3261, 2930, 1725, 1665, 1508, 1430, 740 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 12.0 (br s, 1H, NH), 8.5 (t, 1H, J = 5.5 Hz, NHCO), 7.56–7.32 (m, 4H), 7.25 (d, 1H, J = 9.4 Hz), 7.03 (s, 1H), 5.95 (d, 1H, J = 6.5 Hz), 6.12–6.09 (m. 1H), 4.7 (s, 2H); ^{13}C ($CDCl_3$, 50 MHz): 185.0, 161.5, 149.9, 140.2, 135.0, 129.9, 120.8, 120.0, 121.6, 115.9, 109.4, 108.82, 106.0, 99.9, 40.4. Anal. Calcd for $C_{15}H_{12}N_2O_3$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.20; H, 4.06; N, 9.45.