Bioorganic & Medicinal Chemistry Letters 22 (2012) 6559–6562

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Chemotherapy of leishmaniasis. Part XI: Synthesis and bioevaluation of novel isoxazole containing heteroretinoid and its amide derivatives ☆

S. N. Suryawanshi [a,*], Avinash Tiwari [a], Naveen Chandra [a], Ramesh [b], Suman Gupta [b]

[a] Division of Medicinal Chemistry, CSIR, Central Drug Research Institute, Lucknow 226001, India
[b] Division of Parasitology, CSIR, Central Drug Research Institute, Lucknow 226001, India

ARTICLE INFO

Article history:
Received 24 July 2012
Revised 3 September 2012
Accepted 6 September 2012
Available online 15 September 2012

Keywords:
Heteroretinoid
Isoxazole
Leishmania donovani
In vivo trial
Hamster

ABSTRACT

Novel isoxazole containing heteroretinoid **(4)** and its amide derivatives **(5a–j)** have been synthesized and evaluated for their in vivo antileishmanial activity against *Leishmania donovani* in hamsters. Compounds **3**, **5a**, **5d**, **5k** and **5l** inhibited **70–76%** parasite growth at 50 mg $kg^{-1} \times 5$ days. The present study has helped us in identifying a new lead that could be exploited as a potential antileishmanial agent.

Leishmaniasis is a family of parasitic diseases that affect about 12 million people in tropical and subtropical areas in the form of three clinical expressions: visceral leishmaniasis, which is fatal in the absence of treatment; muco-cutaneous leishmaniasis and cutaneous leishmaniasis, which is often self curing. Classical drugs such as antimonials (Pentostam and Glucantime) are toxic and drug resistance is increasing dangerously in the field.[1] A liposomal amphotericin B formulation (AmBisome) less toxic than amphotericin B deoxycholate is gradually becoming the first-line therapy, especially in immunocompromised patients, but this drug must be administered by a parenteral route.[2] Miltefosine (Impavido) was the first drug registered against visceral leishmaniasis in the last decade; however, its toxicity and the appearance of drug resistance justify the search for new chemical series in order to find an orally safe and active drug.[3] Currently, efforts are being made to search for new molecules from the natural sources and in this endeavor diarylheptanoids,[4a–c] oxygenated abietanes,[5] diterpene quinones[6,7] are showing promise as new lead molecules. Rationally designed heterocyclic ionone like molecules[8] and some novel terpenyl 2,4-diamino pyrimidines[9] are prominent lead molecules and are under investigation. In recent years retinoids,[10] retinoic acid analogs,[11] heteroretinoids[12] are under investigation as antiproliferative agents. Oral miltefosine, originally developed as an anticancer drug, has been used for treatment of visceral leishmaniasis in India.[13] Prompted by this and in continuation of our efforts towards design and synthesis of terpene based compounds as antileishmanial agents,[14a–c] we designed and synthesized isoxazole containing heteroretinoid and its amide derivatives.

The structure of retinoid can be divided into three parts, that is, a hydrophobic part, a linking part, and a carboxylate part. Incorporation of the structural features of naturally occurring ATRA afforded our designed synthetic heteroretinoid **(4)** shown in Figure 1. Synthetic heteroretinoid and its amide derivatives were synthesized and evaluated for antileishmanial activity against *Leishmania donovani* in hamsters and the results are reported in this communication

The synthetic procedures adopted to obtain the target compounds are depicted in Scheme 1. The reaction of α ionone with sodium hydride and diethyl oxalate in toluene was carried out at reflux temperature to furnish **2** in good yield (60%). The compound **2** on treatment with hydroxylamine hydrochloride in ethanol under refluxing conditions afforded compound **3** in 68% yield. Compound **3** was subjected to base catalyzed hydrolysis to give compound **4** in 90% yield**.** Compound **4** was reacted with oxalyl chloride to furnish acid chloride which was next coupled with a set of different aliphatic and aromatic amines to obtain compounds **5a–h**. These aliphatic and aromatic amines were selected on the basis of their easy availability, low cost and their presence as substituents in some novel antileishmanial agents.[15a,b] Compounds **(5a–h)** were prepared in good to excellent yield (68%–96%) as shown in Table 1. Compounds **5i** and **5j** were synthesized directly from compound **3** using hydrazine hydrate and hydroxylamine

☆ CSIR-CDRI Communication NO.: 208/2011/SNS

* Corresponding author. Tel.: +91 2212411 18; fax: +91 0522 2223405/938/504.
E-mail address: shivajins@yahoo.co.in (S.N. Suryawanshi).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.09.024

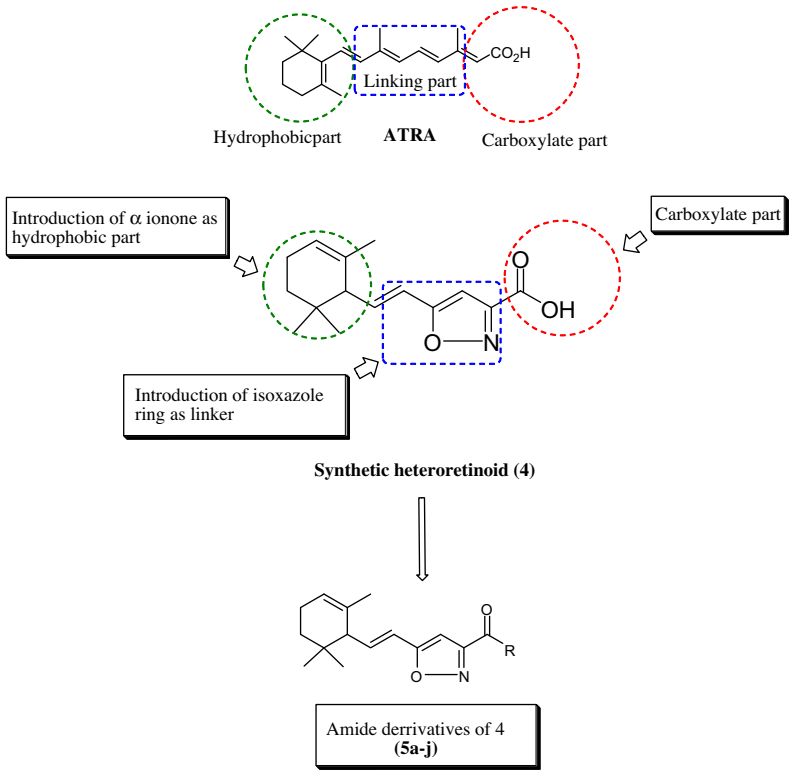


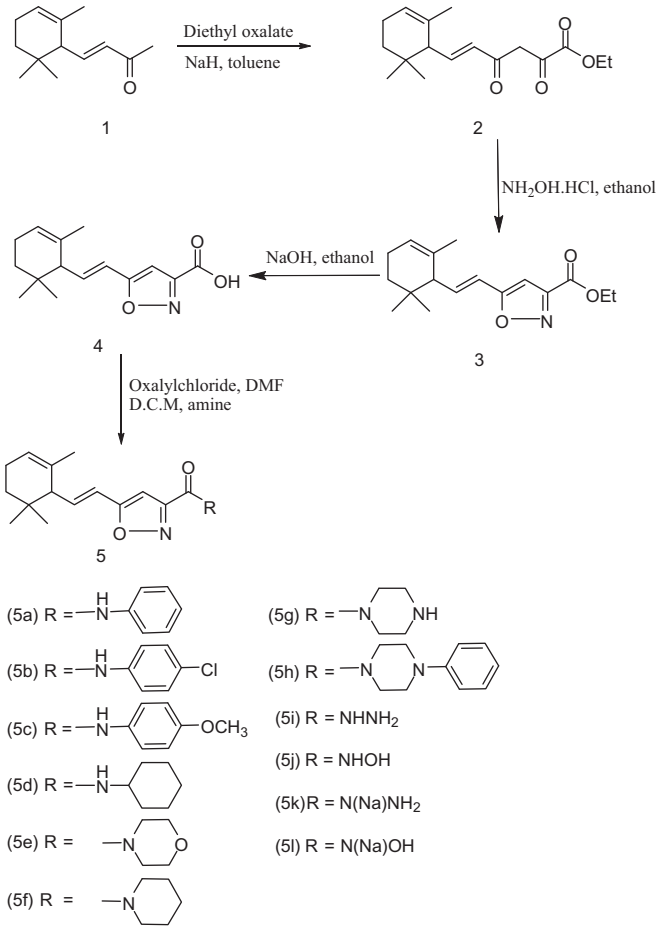
Figure 1. Designing of synthetic heteroretinoid **(4)**.

hydrochloride respectively. We also synthesized the sodium salts of compounds **5i** and **5j** using an aqueous solution of NaOH to make them water soluble and to check the effect of this increased water solubility on antileishmanial activity of these compounds. All the products were characterized by the various spectroscopic (^{1}H and ^{13}C NMR) and spectrometric data (Mass).

The in vivo leishmanicidal activity was determined in golden hamsters (*Mesocricetus auratus*) infected with MHOM/IN/80/Dd8 strain of *Leishmania donovani* obtained through the courtesy of P.C.C. Garnham, Imperial College, London (UK). The method of Beveridge et al.[16] as modified by Bhatnagar et al.[17] and Gupta et al.[18] was used for in vivo evaluation. Golden hamsters (Inbred strain) of either sex weighing 40–45 g were infected intracardially with 1×10^7 amastigotes per animal. The infection is well adapted to the hamster model and establishes itself in 15–20 days. Meanwhile, hamsters gain weight (85–95 g) and can be subjected to repeated spleen biopsies. Pre-treatment spleen biopsy was carried out in all the animals to assess the degree of infection. The animals with +1 infection (5–15 amastigotes/100 spleen cell nuclei) were included in the chemotherapeutic trials. The infected animals were randomized into several groups on the basis of their parasitic burdens. Five to six animals were used for each test sample. Drug treatment by intraperitoneal (i.p.) route was initiated after 2 days of biopsy and continued for 5 consecutive days. Post-treatment biopsies were done on day 7 of the last drug administration and amastigote counts are assessed by Giemsa staining. Intensity of infection in both, treated and untreated animals, and also the initial count in treated animals were compared and the efficacy was expressed in terms of percentage inhibition (PI) using the following formula:-

$$PI = 100 - [ANAT \times 100/(INAT \times TIUC)]$$

Where PI is Percent Inhibition of amastigotes multiplication, ANAT is Actual Number of Amastigotes in Treated animals, INAT is Initial Number of Amastigotes in Treated animals and TIUC is Time Increase of parasites in Untreated Control animals.



Scheme 1. Synthesis of isoxazole containing heteroretinoid and its amide derivatives.

Table 1
Reaction conditions and percentage yield of different amide derivatives

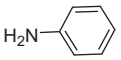
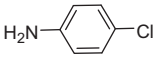
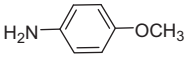
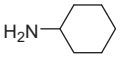
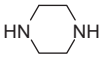
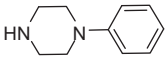
Substrate	Amine	Reaction conditions	Compd, %
4		Oxalyl chloride/DMF/DCM, rt, 2 h	**5a**, 78
4		Oxalyl chloride/DMF/DCM, rt, 2 h	**5b**, 90
4		Oxalyl chloride/DMF/DCM, rt, 2 h	**5c**, 83
4		Oxalyl chloride/DMF/DCM, rt, 2 h	**5d**, 70
4		Oxalyl chloride/DMF/DCM, rt, 2 h	**5e**, 65
4		Oxalyl chloride/DMF/DCM, rt, 2 h	**5f**, 96
4		Oxalyl chloride/DMF/DCM, rt, 2 h	**5g**, 90
4		Oxalyl chloride/DMF/DCM, rt, 2 h	**5h**, 93
3	NH_2NH_2	Ethanol, reflux, 3 h	**5i**, 78
3	NH_2OH	THF, rt, 17 h	**5j**, 84

Table 2
Antileishmanial activity of compounds against *Leishmania donovani* in hamsters

Compd	Dose (mg/kg) × 5 days	% Inhibition on day-7 P.T.[a]
2	50	Inactive
3	50	76
4	50	45
5a	50	74
5b	50	63
5c	50	58
5d	50	70
5e	50	Inactive
5f	50	ND[b]
5g	50	ND
5h	50	Inactive
5i	50	50
5j	50	65
5k	50	70
5l	50	71
SSG[c]	50	89.0 ± 8.31
Paromomycin	50	46.7 ± 9.82

[a] P.T: post treatment.
[b] ND: not done.
[c] SSG: reference drug (Sodium stibogluconate).

Isoxazole containing heteroretinoid **(4)** and its amide derivatives **(5a–j)** were synthesized (Scheme 1). All the synthesized compounds were screened for their leishmanicidal activity against *L. donovani* in hamsters at 50 mg kg^{-1} × 5 days dose and results have been presented in Table 2. Among all fifteen tested compounds, five compounds **(3**, **5a**, **5d**, **5k** and **5l)** have shown 70–76% inhibition of parasite growth. The efficacy of these compounds was more or less similar to sodiumstibogluconate (89% inhibition of parasite growth) and superior to paromomycin (46% parasite inhibition). Four compounds **(4**, **5b**, **5c** and **5j)** exhibited 45–65% inhibition of parasite multiplication. Compounds **5e** and **5h** were found to be inactive. Interestingly, primary amine based amides **(5a–d**, **5i** and **5j)** have shown better activity as compared to amides **(5e** and **5h)** synthesized using secondary amines. The presence of N–H bond in secondary amides seems to play an important role in the mode of action of the agent, since going from secondary amide **(5a–d**, **5i** and **5j)** to tertiary amide **(5e** and **5h)** leads to a complete disappearance of antileishmanial effect. It is likely that N–H bond present in secondary amide may form hydrogen bond with the macromolecular target in the parasite. Sodium salt formation of compounds **5i** and **5j** increased the parasite inhibitory activity of these compounds because of increased water solubility (Table 2, entry-14, 15).

Table 3
Molinspiration calculation of molecular properties for the lipinski rule

Compd	nViol	MW	miLog P	nON	nOHNH	natoms	nrotb
Acceptable range	⩽1	<500	⩽5	<10	<5	–	–
2	0	292.375	2.431	4	0	21	7
3	0	289.375	4.89	4	0	21	5
4	0	261.321	4.254	4	1	19	3
5a	1	336.435	5.573	4	1	25	4
5b	1	370.88	6.251	4	1	26	4
5c	1	366.461	5.629	5	1	27	5
5d	1	342.483	5.781	4	1	25	4
5e	0	330.428	3.966	5	0	24	3
5f	1	328.456	5.028	4	0	24	3
5g	0	329.444	3.416	5	1	24	3
5h	1	405.542	5.709	5	0	30	4
5i	0	275.352	3.065	5	3	20	3
5j	0	276.336	3.558	5	2	20	3

nViol, no. of violations; MW, molecular weight; miLog P, molinspiration predicted Log P; nON, no. of hydrogen bond acceptors; nOHNH, no. of hydrogen bond donors; natoms, no. of atoms; nrotb, no. of rotatable bond.

The synthesized compounds were also checked for compliance to the Lipinski rule of five, and the results are summarized in Table 3. The rule states that a molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: log*P* (octanol–water partition coefficient) $\leqslant$5, molecular weight $\leqslant$500, number of hydrogen bond acceptors $\leqslant$10 and number of hydrogen bond donors $\leqslant$5. Molecular properties of synthesized compounds were calculated by www.molinspiration.com software, and it was found that majority of the synthesized compounds followed the above criteria (Table 3). Therefore, these compounds have a good potential for eventual development as oral agents and can be potentially active drug candidate.

In conclusion, we have identified a promising new hit for the treatment of leishmaniasis. The potent activity and simple synthesis of these heteroretinoids suggest that they can be a possible lead for the development of novel drug against leishmania.

Acknowledgements

The authors are thankful to the division of Sophisticated Analytical Instrument Facility (SAIF), CDRI for providing spectral and elemental analysis data. Financial support from U.G.C. is gratefully acknowledged. Technical assistance by Mrs. Manju is gratefully acknowledged.

References and notes

1. Frézard, F.; Demicheli, C.; Ribeiro, R. R. *Molecules* **2009**, *14*, 2317.
2. Moore, E. M.; Lockwood, D. N. *J. Global Infect. Dis.* **2010**, *2*, 151.
3. Maltezou, H. C. *Biomed. Biotechnol.* **2010**, *2010*, 617521.
4. (a) Alves, L. V.; Do Canto-Cavalheiro, M. M.; Cysne-Finkelstein, L.; Leon, L. *Biol. Pharm. Bull.* **2003**, *26*, 453; (b) Koide, T.; Nose, M.; Ogihara, Y.; Yabu, Y.; Ohta, N. *Biol. Pharm. Bull.* **2002**, *25*, 131; (c) Gomes, D. C. F.; Alegrio, L. V.; Lima, M. E. F.; Leon, L. L.; Aranjo, C. A. C. *Arzneim-Farsch Drug Res.* **2002**, *52*, 120.
5. Tan, N.; Kaloga, M.; Radtke, O.; Kiderlen, A. F.; Oksuz, S.; Ulubelen, A.; Kolodziej, H. *Phytochemistry* **2002**, *61*, 881.
6. Sairafianpour, M.; Christensen, J.; Staerk, D.; Budnik, B. A.; Kharazmi, A.; Bagherzadeh, K.; Jaroszewski, J. W. *J. Nat. Prod.* **2001**, *64*, 1398.
7. Valderrama, A. J.; Benites, J.; Cortes, M.; Pessoa-Mahana, H.; Prina, E.; Fournet, A. *Bioorg. Med. Chem.* **2003**, *11*, 4713.
8. Anzaldi, M.; Sottofattori, E.; Rizzetto, R.; Granello di Casaleto, B.; Balbi, A. *Eur. J. Med. Chem.* **1999**, *34*, 837.
9. Rosowsky, A.; Papoulis, A. T.; Queener, S. F. *J. Heterocyclic Chem.* **1999**, *36*, 723.
10. Aggarwal, B. B.; Sundaram, C.; Prasad, S.; Kannappan, R. *Biochem. Pharmacology* **2010**, *80*, 1613.
11. Sadikoglou, E.; Magoulas, G.; Theodoropoulou, C.; Athanassopoulos, C. M.; Giannopoulou, E.; Theodorakopoulou, O.; Drainas, D.; Papaioannou, D.; Papadimitriou, E. *Eur. J. Med Chem.* **2009**, *44*, 3175.
12. Simoni, D.; Invidiata, F. P.; Rondanin, R.; Grimaudo, S.; Cannizzo, G.; Barbusca, E.; Porretto, F.; D'Alessandro, N.; Tolomeo, M. *J. Med. Chem.* **1999**, *42*, 4961.
13. Richard, J. V.; Werbovetz, K. A. *Curr. Opin. Chem. Biol.* **2010**, *14*, 447.
14. (a) Pandey, S.; Suryawanshi, S. N.; Gupta, S.; Srivastava, V. M. L. *Eur. J. Med. Chem.* **2004**, *39*, 969; (b) Chandra, N.; Ramesh; Ashutosh; Goyal, N.; Suryawanshi, S. N.; Gupta, S. *Eur. J. Med. Chem.* **2005**, *40*, 552; (c) Chandra, N.; Pandey, S.; Ramesh; Suryawanshi, S. N.; Gupta, S. *Eur. J. Med. Chem.* **2006**, *41*, 779.
15. (a) Sunduru, N.; Agarwal, A.; Katiyar, S. B.; Nishi; Goyal, N.; Gupta, S.; Chauhan, P. M. S. *Bioorg. Med. Chem.* **2006**, *14*, 7706; (b) Sunduru, N.; Nishi; Palne, S.; Chauhan, P. M. S.; Gupta, S. *Eur. J. Med. Chem.* **2009**, *44*, 2473.
16. Beveridge, S., Schnitzer, R. J., Hanoking, F. I., Eds.*Experimental Chemotherapy* **1963**, *1*, 257.
17. Bhatnagar, S.; Guru, P. Y.; Katiyar, J. C.; Srivastava, R.; Mukherjee, A.; Akhtar, M. S.; Seth, M.; Bhaduri, A. P. *J. Indian Med. Res.* **1989**, *89*, 439.
18. Gupta, S.; Tiwari, S.; Bhaduri, A. P.; Jain, G. K. *Acta Trop.* **2002**, *84*, 165.