Anti-Leishmanial Activities of Synthetic Biscoumarins

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(Received on 30th November 2015, accepted in revised form 13th July 2016)

Summary: Synthetic biscoumarins **1-19** were screened to check their anti-leishmanial activity. Out of nineteen, analogs **2** (IC_{50} = 46.95 ± 1.23 μM), **4** (IC_{50} = 63.03 ± 0.20 μM), **5** (IC_{50} = 41.35 ± 0.51 μM), **6** (IC_{50} = 35.62 ± 0.15 μM), **7** (IC_{50} = 75.35 ± 0.35 μM), **10** (IC_{50} = 82.36 ± 1.93 μM), **11** (IC_{50} = 76.45 ± 0.13 μM), **16** (IC_{50} = 59.21 ± 1.00 μM), **18** (IC_{50} = 75.73 ± 1.54 μM), and **19** (IC_{50} = 76.43 ± 0.04 μM) exhibited moderate to weak anti-leishmanial potential, if compared with the standard pentamidine (IC_{50} = 5.09 ± 0.04 μM), whilst rest of the analogs were found to be inactive.

Keywords: Synthesis, Biscoumarins, *In vitro*, Anti-leishmanial activities, Pentamidine

Introduction

Leishmaniasis is infectious disorder affected by parasite of *Leishmania* genus from family *trypanosomatidae*. This disorder expresses itself on three types that are mucocutaneous, visceral and cutaneous leishmaniasis [1, 2]. With different spectrum of clinical expression cutaneous leishmaniasis is common group of disease that ranges from minor cutaneous nodules to large mucosal tissue damage [3]. Visceral leishmaniasis is the most dangerous type in which parasite is drifted to vital tissues. It is unbearable, severe disorder having symptom like sustained fever, hyper γ-globulinemia, splenomegaly and pancytopenia. Patient usually dies nearly, if untreated due to illness over a period of months [4]. Leishmaniasis disease is transfer by protozoan affected female phlebotomine sandflies bite. Then it is stewed in bone marrow, liver and spleen through macrophages parasite [5-7]. Each year new cases about 500,000 of visceral disease and 1500,000 of cutaneous disease occur. Worldwide more than in 70 countries, cutaneous leishmaniasis is common and about 90% cases are reported from Brazil, Algeria, Afghanistan, Peru, Pakistan, Syria and Saudia Arabia [8-12]. In 65 countries, visceral leishmaniasis disease occurs in agricultural area and in peripheral poor five countries *e.g.* India, Brazil, Bangladesh and Sudan [1, 13]. Internationally numbers of cases are growing by a shocking rate [14].

In past few years the versatile medical and biological properties of biscoumarin such as antiinflammatory, antioxidant, anticancer and antibacterial activities have been discovered [15-17]. Strong inhibition of tubulin aggregation of biscoumarin derivatives played a great role against cancer and through cell cycle cancer cells were able to prevent progression [18-20]. Biscoumarins derivatives act as anticoagulant and are also identified as hemorrhagic agents in the spoiled clover disease of cattle's [21].

In this study, we have evaluated for anti-leishmanial properties of synthetic biscoumarins **1-19**.

Results and Discussion

Chemistry

Biscoumarins **1-19** were synthesized by addition of catalytic tetraethyl ammonium bromide (TEAB) to a stirred mixture of different substituted aldehydes (2 mmol) and coumarin derivatives (4 mmol) in water. Tetraammonium ethyl bromide was used as catalyst which is highly water soluble compound. It interacts with the aldehyde and coumarin and make the reaction possible in water. The mixture was refluxed for 1-2 hours at 60 °C. Reaction completion was checked by thin layer chromatography. On completion, reaction mixture was washed with water to afford desired products. Column chromatographic technique was used in some case for separation of products and acetone and *n*-hexane (3:7) were used as eluent. ^{1}H NMR and EI-MS spectroscopic techniques were used for the structural determination of biscoumarins **1-19** [22].

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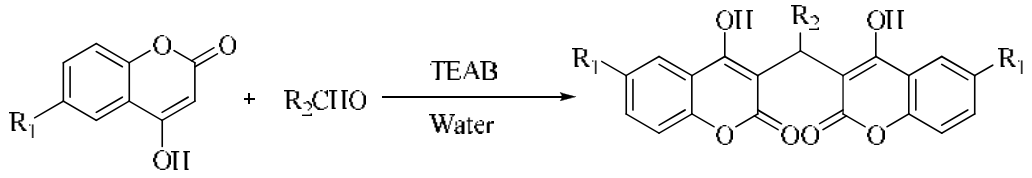


Scheme-1: Synthesis of Biscoumarin Derivatives **1-19**

Table-1: Different Substituents of Biscoumarin Derivatives **(1-19).**

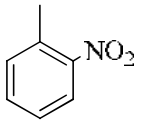
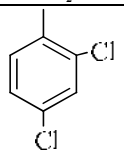
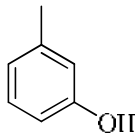
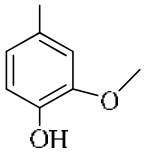
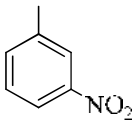
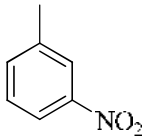
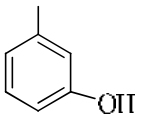
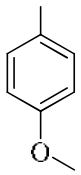
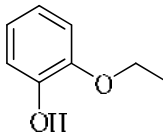
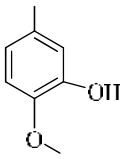
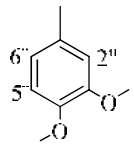
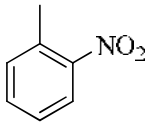
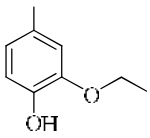
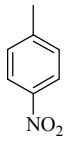
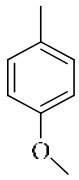
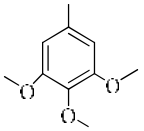
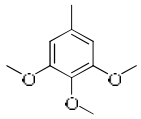
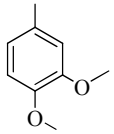
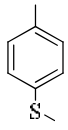
Compd No.	R_1	R_2	Compd No.	R_1	R_2	Compd No.	R_1	R_2
1	**Cl**		**8**	**Cl**		**15**	**CH_3**	
2	**Cl**		**9**	**Cl**		**16**	**CH_3**	
3	**Cl**		**10**	**Cl**		**17**	**CH_3**	
4	**Cl**		**11**	**CH_3**		**18**	**CH_3**	
5	**Cl**		**12**	**CH_3**		**19**	**CH_3**	
6	**Cl**		**13**	**CH_3**				
7	**Cl**		**14**	**CH_3**				

Table-2: Results of Leishmanicidal Potential of Biscoumarin Derivatives **(1-19).**

Compd No.	IC_{50} ± SEM[a] (μM)	Compd No.	IC_{50} ± SEM[a] (μM)	Compd No.	IC_{50} ± SEM[a] (μM)
1	**NA[b]**	**8**	**NA[b]**	**15**	**NA[b]**
2	**46.95 ± 1.23**	**9**	**NA[b]**	**16**	**59.21 ± 1.00**
3	**NA[b]**	**10**	**82.36 ± 1.93**	**17**	**NA[b]**
4	**63.03 ± 0.20**	**11**	**76.45 ± 0.13**	**18**	**75.73 ± 1.54**
5	**41.35 ± 0.51**	**12**	**NA[b]**	**19**	**76.43 ± 0.04**
6	**35.62 ± 0.15**	**13**	**NA[b]**	**Pentamidine[c]**	**5.09 ± 0.04**
7	**75.35 ± 0.35**	**14**	**NA[b]**		

SEM[a] = Standard Error of the Mean, N. A.[b] = Not Active, Standard[c] for anti-leishmanial activity.

Anti-Leishmanial Activity

All synthetic analogs **1-19** were evaluated for anti-leishmanial activity. Among the series, analogs **2**, **4**, **5**, **6**, **7**, **10**, **11**, **16**, **18**, and **19** exhibited moderate to weak anti-leishmanial activity with IC_{50} values of 46.95 ± 1.23, 63.03 ± 0.20, 41.35 ± 0.51, 35.62 ± 0.15, 75.35 ± 0.35, 82.36 ± 1.93, 76.45 ± 0.13, 59.21 ± 1.00, 75.73 ± 1.54 and 76 ± 0.04 μM, respectively, when compared with the standard pentamidine having IC_{50} value 5.09 ± 0.04 μM. The remaining analogs were found to be inactive.

The most potent analog **6** (IC_{50} = 35.62 ± 0.15 μM) have three methoxy groups at ring R_2 and chloro substitution as R_1. Its structurally similar analog **7** (IC_{50} = 75.35 ± 0.35 μM) lacks one methoxy group at ring R_2 showed two-fold lower activity than compound **6**. Similarly, compound **10** (IC_{50} = 82.36 ± 1.93 μM) which has only one methoxy instead of three also found to be weak anti-leishmanial agent. Another, analog **5** (IC_{50} = 41.35 ± 0.51 μM) have 3-ethoxy-4-hydroxyl group attached to phenyl part showed moderate activity. Its structurally similar analog **2** (IC_{50} = 46.95 ± 1.23 μM) which have methoxy instead of ethoxy showed almost similar activity. Similarly, compound **17** which has distinctly similar structure as **5** but only has methyl instead of chloro, showed no activity. Limited structure activity relationship suggested that electron donating groups on R_2 and chloro group as R_1 showed better activity than other analogs.

Experimental

Materials and Methods

NMR spectroscopy was carried out on Avance Bruker AM-300 and AMX-400 MHz instruments. EI-MS measurements were carried out on Finnegan MAT-311 (Germany) instrument. Silica gel (E. Merck, type 60, 70-230 mesh) was used for column chromatography. Pre-coated silica gel aluminum plates (kieselgel 60, 254. E. Merck, Germany) was used for TLC analysis. Chromatograms were visualized with the help of UV lamp having wavelength of 254 and 365 nm.

Anti-Leishmanial Bioassay

Using normal physiological saline in modified NNN biphasic medium *Leishmania major* were grown. *Leishmania promastigotes* was cultured in RPMI 1640 medium additional with 10% heat deactivated foetal bovine serum. For 10 minutes at 2000 rpm, parasites were centrifuged and washed thrice with salty solution. To final density of 10^6 cells/ml parasites were watered in fresh cultured medium. To first row 100 μL and 180 μL of medium was added to remaining wells in 69-well micro titer plate. In medium 20 μL experimental compound were added and in sequence diluted. In all wells parasites culture of 100 μL was added. For negative and positive controls, two rows were left. Medium was used as negative and positive control has different concentration of standard anti-leishmanial compound pentamidine. Incubation of plates was carried out at 21-22 $^{\circ}$C for 72 h. All cultures were tested microscopically on improved Neubauer counting chamber. Ez-fit 5.03 software (Perella Scientific) was used for calculating the IC_{50} value of the synthetic compound. All these experiments were performed in triplicate [23].

General Procedure for Preparation of Biscoumarins **1-19**

Biscoumarins **1-19** were synthesized by addition of catalytic tetraethyl ammonium bromide (TEAB) to a stirred mixture of different substituted aldehydes (2 mmol) and coumarin derivatives (4 mmol) in water. The mixture was refluxed for 1-2 h at 60 $^{\circ}$C. Reaction completion was checked by thin layer chromatography. On completion, reaction mixture was washed with water to give desired products. Column chromatography was used in some case for separation of products and acetone and *n*-hexane (3:7) were used as eluent. ^{1}H NMR and EI-MS spectroscopic techniques were employed for the structural determination of biscoumarins **1-19** [22].

Characterization of the Representative Compound

3,3-{(2-Nitrophenyl) methylene} bis (6-chloro-4-hydroxy-2H-chromen-2-one) (**1**)

Yield: 0.21 g (80%); ^{1}H-NMR (DMSO, 300 MHz); δ 7.69 (d, 2H, J = 2.7Hz, H-5/5'), 7.5 (m, 4H, H-7/7'/3''/6''), 7.3 (m, 4H, H-8/8'/4''/5''), 6.4 (s, 1H, Ar_3CH); EI-MS m/z (rel. int. %): 526 (M+, 42), 298 (67), 283 (58), 196(27), 154 (100).

Conclusion

In conclusion synthetic biscoumarins **1-19** were evaluated for anti-leishmanial activity. Compounds **2**, **4**, **5**, **6**, **7**, **10**, **11**, **16**, **18**, and **19** displayed moderate to weak anti-leishmanial activity. Further research on these molecules may result in viable anti-leishmanial agent.

Acknowledgements

The authors are thankful to Higher Education Commission (HEC) Pakistan for financing

the Project No. 20-2073 under National Research Program for Universities.

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