COUMARIN ANALOGUES AS A POTENTIAL INHIBITOR OF LEISHMANIASIS: A MULTI-TARGETING PROTEIN INHIBITION APPROACH BY MOLECULAR DOCKING

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ABSTRACT
Objective: Leishmaniasis is one of the most dreadful diseases as a leading cause of death in most of the developed countries. Objective of the current work was to identify more potent and highly effective novel compound for the treatment of leishmaniasis.

Methods: In the given study molecular docking study was performed on the library of coumarin analogues as anti-leishmaniasis agents. Total 300 coumarins analogues were taken from Pubmed and were studied using a molecular docking study on trypanothione reductase from Leishmania infantum (PDB code: 2JK6 and 2P18) and Leishmania mexicana (PDB code: 3PP7).

Results: Molecular docking result revealed that most active compound COU-130 and COU-220 bind to the active site of the protein with amino acids present in the various proteins. In PDB 2JK6 the active compound binds to the amino acid thr-51 and ser-144 were binding to the active site, and in PDB 3PP7 the active compound binds amino acid thr-26 and in PDB 2P18 the active compound binds to the amino acid phe-219 and try-212.

Conclusion: Further in vitro and in vivo study of selected coumarin analogues can be studied for their therapeutic potential in treating leishmaniasis.

Keywords: Coumarins, leishmaniasis, molecular docking.

INTRODUCTION
Objective of the current work was to identify more potent and highly effective novel compound for the treatment of leishmaniasis, which could be further used as a therapeutic agent in treating leishmaniasis. Leishmaniasis is one of the most dreadful diseases and is a leading cause of deaths in developing countries. Leishmaniasis is a complex disease mostly found in the Indian sub-Continent caused by Leishmania spp. and carried by sand fly. Clinical classification of the disease comprises visceral and cutaneous Leishmaniasis, but the infection remains asymptomatic in many cases. Compared to chemical synthesis, plant derived natural products represents an attractive source of biologically active agents since they are natural and are economic to afford. Leishmania has an intricate life cycle and one of the most developed forms, the amastigote which is present in the immunological cell of the host organism, which makes the targeting of the drug more challenging. Compared to chemical synthesis, plant derived natural products represents an attractive source of biologically active agents since they are natural and are economic to afford.

Objective of the given work is to identify more potent and highly effective novel compound for the treatment of leishmaniasis, which could be further used as a therapeutic agent in treating leishmaniasis. Excessive use of antimonials as a primary drugs in treatment of the disease, their therapeutic window is short and they posses heavy metal toxicity as well. However they are being regularly used as a major drug in the third world countries.

MATERIALS AND METHODS
Molecular Docking: Molecular docking is an important tool in drug discovery and CADD; the importance of ligand-protein docking is that it predicts a predominant binding mode between the three dimensional protein structures and the ligand. Use of docking in virtual-screening has become very important because, it helps in the screening of large libraries. Using different scoring functions helps in...
The coumarins are of great interest due to their pharmacological properties. In particular, their physiological, bacteriostatic and anti-tumor activity makes these compounds attractive backbone derivatisation and screening as novel therapeutic agents. Coumarins are naturally occurring benzopyrones. It consists of benzene ring with a pyrone ring. The coumarins consist of umbelliferone, esculetin and scopoletin.

### Table 1: Coumarin Analogues used in the study

| Coumarin Analogue                        | Structure                                      |
|------------------------------------------|------------------------------------------------|
| 1H-2-Benzopyran-1-one                    | 5-formyl-6-hydroxy coumarin                   |
| 2H-Chromen-2-one                         | 2-oxo-2h-1-benzopyran-7-carboxylic acid       |
| 8-aza-coumarin                           | 7-Hydroxy-3,4,8-trimethylcoumarin             |
| 3,4-dihydrocoumarin                      | 2-Oxo-2H-chromene-6-carboxylic acid           |
| 5,6,7,8-tetradecaertochromen-2-one       | [1,3]Dioxolo[4,5-g]chromen-6-one              |
| 3,4,5,6,7,8-hexadecaertochromen-2-one    | 2-Oxo-2H-chromene-4-carboxylic acid           |
| 2H-1-Benzopyran-2-one                    | Coumarin-3-carboxylic acid                    |
| Octahydrocoumarin                        | 2H-1-Benzopyran-2-one                         |
| Octahydro-2H-Chromen-2-one               | 4-Hydroxy-5,7-dimethyl-2H-1-benzopyran-2-one |
| epoxy coumarin                           | 4-Methoxy-3-methyl-2H-chromen-2-one           |
| 5-Methylcoumarin                         | 2H-1-Benzopyran-2-one                         |
| 7-Methylcoumarin                         | 7-methoxy-8-methyl-2H-chromen-2-one           |
| 3-Methylcoumarin                         | 5-hydroxy-4,7-dimethyl-2H-chromen-2-one       |
| 8-Methylcoumarin                         | 7-Methoxy-4-methylcoumarin                    |
| 4-Methylcoumarin                         | 7-Ethoxy-4-methylcoumarin                     |
| 6-Methylcoumarin                         | 7-hydrazinyl-4-methyl-2h-chromen-2-one        |
| coumarin hydrzone                        | 4-Methylenamo-3-aminocoumarin                 |
| 4-Amino-chromen-2-one                    | 3,4-dihydro-4,5,7-trimethyl                   |
| 3-Aminocoumarin                          | 2H-1-Benzopyran-2-one                         |
| 6-Aminocoumarin                          | 7-Nitrocoumar                                  |
| coumarin-6-one                           | 7-amino-3-hydroxy-4-methyl-coumarin           |
| 4-Hydroxycoumarin                        | Amino methoxy coumarin                        |
| Chroman-2,3-dione                        | 4-methyl-1-aminoxy-coumarin                   |
| 5-Hydroxycoumarin                        | 7-amino-4-methoxy-coumarin                    |
| 7-hydroxycoumarin                        | 7-hydroxy-4-(amino methyl)coumarin            |
| Coumarin 3,4-epoxide                     | 5-amino-6-hydroxy-4-methyl-coumarin           |
| 8-Hydroxycoumarin                        | 8-amino-7-hydroxy-4-methyl-2H-chromen-2-one   |
| 6-Hydroxycoumarin                        | 7-dihydroxy-4-methyl coumarin                 |
| 3-Hydroxycoumarin                        | 4-methyl-7-hydroxy-coumarin                   |
| 2-Thiocoumarin                           | methoxy-8-hydroxy-coumarin                    |
| 8-amino-3,4-dihydro-coumarin             | 4-Hydroxy-7-methoxy-coumarin                  |
| coumarin water                           | 4-Methylaphthetin                              |
| 4-Methyl(5,6,7,8-2H4)Coumarin             | 5,7-dihydroxy-4-methylcoumarin                |
| 7-Hydroxy Coumarin-13C3                  | 4-Methyleculetin                               |
| 7-hydroxycoumarin                        | 6-Methyleculetin                               |
| 7-Hydroxy Coumarin-13C6                  | coumarin ethanol                              |
| 6-Methylotachydrocoumarin                | 6-hydroxy-4,4-dimethyl-3,4-dihydro-2H-1-benzopyran-2-one |
| 7-Ethynylcoumarin                        | 7-Mercapto-4-methyl-2H-chromen-2-one          |
| ethynyl coumarin                         | 4-hydroxy-3-(hydroxyl aminocoumarin)          |
| 3-Cyanocoumarin                          | 3-Amino-4,7-dihydroxycoumarin                 |
| 8-formyl coumarin                        | 7-amino-4-fluoromethyl coumarin               |
| 2-Oxo-2H-chromene-7-carbaldehyde         | 4,6,7-trihydroxycoumar                        |

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| Compound                                      | Formula                                      | Description                                           |
|-----------------------------------------------|----------------------------------------------|-------------------------------------------------------|
| 2-oxo-2H-chromene-4-carbaldehyde             | 4,5,7-Trihydroxycoumarin                    | 5-Methyl-4-(methylthio)coumarin                       |
| Coumarin-6-carboxaldehyde                    | 2H-1-Benzopyran-2-one                        | 4-Hydroxy-3-nitrocoumarin                            |
| 3,6-Dimethyl-2H-1-benzopyran-2-one           | 3-Methyl-6-chlorocoumarin                    | 7-Hydroxy-8-(hydroxyaminomethyl)coumarin             |
| 4,7-dimethylchromen-2-one                    | 6-chloro-7-hydroxy-2H-chromen-2-one          | 7-Hydroxy-8-(aminoxy) methylcoumarin                 |
| 3-Ethyl-2H-1-benzopyran-2-one                | methyl coumarin hydrochloride               | 3-Amino-4,7-dihydroxy-8-methylcoumarin               |
| 6-aminoethylcoumarin                         | 6-Aminoocoumarinhydrochloride               | 8-fluoro-3-carboxy-coumarin                          |
| 6-Amino-4-methyl-2H-chromen-2-one            | 4-Methylumbelliferone sodium                 | 4,7,8-trihydroxy-3-methyl                            |
| 3-(Aminomethyl)-2H-chromen-2-one             | 6,7-Dihydroxycoumarin sodium                | 7,8-Dihydroxy-6-methoxy-coumarin                     |
| 7-Amino-4-methylcoumarin                     | propynloxy coumarin                         | 7-ethoxy-4-fluoro-coumarina                          |
| 4-Hydroxy-3-methyl-2H-chromen-2-one          | 4-hydroxy-3-(prop-2-ynyl)-2H-coumarin       | 7-(2-fluoroethyl)oxy-coumarin                        |
| 4-Hydroxy-6-methylcoumarin                   | 6-(2-propynyl-ox)coumarin                   | Coumarin-3-carboxylic acid chloride                 |
| Hydroxymethyl coumarin                       | 2H-1-Benzopyran-2-one                       | chloromethyl amino coumarin                          |
| 6-(hydroxymethyl)-2H-chromen-2-one           | 2H-1-Benzopyran-2-one                       | 3-Chloro-7-hydroxy-4-methyl-2H-chromen-2-one        |
| 5-methoxy-2H-chromen-2-one                   | 7-(Propargyloxy) coumarin                   | 4-(chloromethyl)-6-hydroxy-2H-chromen-2-one          |
| 7-hydroxy-8-methylcoumarin                   | 4-propargylthio-coumarin                    | hydroxybenzo coumarin                                |
| 5-methylumbelliferone                        | Monosodium esculentin                       | 3-furyl coumarin                                     |
| 4-methylumbelliferone                        | cyanomethoxy coumarin                      | 3-furanyl coumarin                                   |
| 4-Methoxycoumarin                            | 6-cyano-7-methoxy-coumarin                  | 6-(3-pyrazolyl)coumarin                              |
| 8-methoxycoumarin                            | 3-azidomethyl coumarin                      | Benzoyl[d,E]-3-H-coumarin                            |
| 6-Hydroxy-4-methylcoumarin                   | 4-(allylamo)coumarin                       | 6-(isoxazol-5-yl)coumar                               |
| 7-Methoxycoumarin                            | coumarin-6,8-dicarbaldehyde                | 3-(1,3,4-triazol-2-yl)coumar                         |
| 3,4-Diaminocoumarin                          | dihydrofurfo-[3,2-g]coumarin-6-one          | 7-Dimethylamino-4-ethylcoumar                        |
| 2H-1-Benzopyran-2-one                        | 3-Glyoxyloycoumarin                        | 3-(1,3,4-oxadiazol-2-yl)coumar                      |
| 3-methyl-thia-coumarin                       | 3-allyl-4-hydroxycoumarin                   | 4-(trifluoromethyl)coumar                            |
| hydroxymino-coumarin                         | 7-glycidylcoumarin                          | 3-(trifluoromethyl)chromen-2-one                     |
| aminohydroxy-coumarin                        | 3-acetyl-5-methyl coumarin                  | 4-oxadiazolyl coumarin                               |
| 4,7-Dihydroxycoumarin                        | 4-allyl-3-hydroxy-coumarin                  | 3-(1,3,4-oxadiazol-2-yl)coumar                      |
| 5,7-Dihydroxy-2H-chromen-2-one               | 6-methyl-3-acetyl coumarin                  | 6-(2-butynol)oxy-coumar                              |
| 6,7-Dihydroxycoumarin                        | 6-(Allyloxy)coumarin                       | 4-Methyl-7-(3-hydroxy-1-propynyl)coumar              |
| 7,8-Dihydroxycoumarin                        | 4-allyloxy-coumarin                         | 7-(2-Butynol)oxy-coumar                              |
| fluoromethyl coumarin                        | 7-Allyloxy-coumarin                         | 3-(2,5-Dihydrofuran-2-yl)coumar                     |
| 8-fluoro-4-hydroxy-2H-chromen-2-one          | 3-acetyl-7-methyl-2H-chromen-2-one          | 7-(1-Methylpropargyloxy)coumar                       |
| 3-Chlorocoumarin                             | coumarin KOH                                | 4-(4-Hydroxy-1-butynyl)coumar                        |
| 4-chloro-2H-chromen-2-one                    | 3-Butylcoumar                               | Giparpene                                            |
| 6-Chlorocoumarin                             | 3-azido-7-hydroxycoumarin                  | 6-prenyl-coumar                                      |
| coumarin hydrochloride                       | 3-Acetamidocoumarin                         | dimethyl-allyl-coumarin                              |
| 2H-1-Benzopyran-2-one                        | 6-Acetamidocoumarin                         | isopentenyl coumarin                                 |
| 6-Methyl-2-oxo-2H-chromene-3-carbonitride    | coumarin isothiocyanate                    |                                                    |
| 3-Cyano-4-methylcoumarin                     | dimethylaminomethyl coumarin               | 3-(4-Pentenyl)coumarin                               |
| Angelicin                                    | 4-(propylamino)chromen-2-one               | 3-(1',1'-dimethylallyl)coumar                        |
| 7H-Furo[3,2-g]chromen-7-one                  | 7-(Ethylamino)-4-methylcoumarin            | 4,4-dichloro-2H-chromen-2-one                        |
| cyclopropyl coumarin                         | 4,6-Dimethyl-7-methylaminocoumarin         | N-Coumarin-3-ylacrylamide                           |
| isopropenyl coumarin                         | 7-Dimethylamino-4-methylcoumarin           | 4-azido-3-ethyl-coumar                               |
| coumarin isocyanate                          | 5-Fluorangelic                             | 5-Allyl-6-(methyl aminocoumarin)                     |
| 7-(2-oxoethyl)coumarin                       | acetylhydroxy-coumarin                    | 4-Methyl-6,7,8,9-tetrahydro-2H-pyran[3,2-g]quolin-2- one; |
The coumarins are of great interest due to their pharmacological properties. In particular, their physiological, bacteriostatic and anti-tumor activity makes these compounds attractive backbone derivatisation and screening as novel therapeutic agents\(^\text{11}\).

**SAR prediction**

On the basis of energy map generated from the following PDB, structures were selected on the basis of molecular weight. The energy map predicts the presence of different energies in the protein, which helps in the prediction of structures. On the basis of energy map it was determined that presence of an electron donating and with drawing group will give an efficient binding. The SAR prediction was done on Molegro Virtual Docker 6.0.

**Docking Protocol**

1. **Protein preparation**

Various proteins were downloaded from the Protein data bank PDB for standard bioinformatics (RSCB) that contains various X-ray crystal structures for proteins and other macromolecules. Then it was corrected by addition of missing hydrogen, atoms and incorrect bonding types and the charges were balanced.

2. **Ligand preparation**

Ligands were downloaded from the small molecules site ‘PubChem’, in SDF format.

3. **Docking**

Molecular docking was performed on the respective proteins retrieved from the protein data bank in Molegro Virtual Docker ver. 6.0.

4. **Validation**

Each and every docking run needs to be validated before the run. It’s carried out by re-docking the co-crystallized ligand that is present in the protein, with the same protein. The re-docked ligand is then compared with the original one by superimposition\(^\text{12}\).

**RESULTS AND DISCUSSION**

Molecular docking results revealed that most active compound COU-130 and COU-220 binds to the active site of the protein [PDB code: 2JK6, 2P18 and 3PP7]. In PDB 2JK6 the active compound binds to the amino acid thr-51 and ser-14 were binding to the active site Figure 2a, and in PDB 3PP7 the active compound binds amino acid thr-26 Figure 2b and in PDB 2P18 the active compound binds to the amino acid phe-219 and try-212 Figure 2c.

**Table 2: Code with resolution**

| Code | Name | Resolution |
|------|------|------------|
| 2JK6 | Structure of Trypanothione Reductase from *Leishmania infantum* | 2.95 Å |
| 3PP7 | Crystal structure of *Leishmania mexicana* pyruvate kinase in complex with the drug suramin, an inhibitor of glycolysis. | 2.35 Å |
| 2P18 | Crystal structure of the *Leishmania infantum* glyoxalase II | 1.8 Å |

Molecular docking helps in understanding the binding of the compound on the active site of the protein, this study helps in determining the binding of coumarin analogues which can be used in designing in effective and less toxic compounds against the treatment of Leishmaniasis.

**Table 3: The Molecular docking score**

| Compound | PDB code | Moldock score | Rerank score |
|----------|----------|---------------|--------------|
| COU-130  | 2JK6     | -172.948      | -122.454     |
| COU-130  | 3PP7     | -127.413      | -100.061     |
| COU-220  | 2P18     | -116.818      | 84.517       |

The crystal structure superposition of the structure and the final conformations suggests that the ligands were docked into the same site of binding and have a close resemblance to the pose of the ligand which was present in the crystal structure.

**CONCLUSION**

Molecular docking helped in understanding the efficacy of binding of the particular group of coumarins. The coumarins selected on the basis of the lowest binding energy. The molecules were selected on the basis of a lower molecular weight; so that it will have an efficient binding on the selected proteins. The given study is valuable, inexpensive and important for further *in vitro* and *in vivo* studies. Selected coumarins analogues can be studied for their therapeutic potential in treating Leishmaniasis.
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**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

**REFERENCES**

1. Ostyn B, Gidwani K, Khanal B, Picado A, Chappuis F, Singh SP, et al. Incidence of symptomatic and asymptomatic Leishmania donovani infections in high-endemic foci in India and Nepal: a prospective study. PLoS Negl Trop Dis 2011; 5(10):e1284. https://doi.org/10.1371/journal.pntd.0001284.

2. Andrade-Narvaez FJ, LorôÂa-Cervera EN, Sosa-Bibiano EI, Van Wynsberge NR. Asymptomatic infection with American cutaneous leishmaniasis: epidemiological and immunological studies. Mem Inst Oswaldo Cruz 2016; 111(10):1590.0074-02760160158.

3. Croft SL, Olliaro P. Leishmaniasis chemotherapy challenges and opportunities. Clin Microbiol Infect 2011; 17(10):1478-1483. https://doi.org/10.1111/j.1469-0691.2011.03630.x.

4. Mitropoulos PL, Konidas P, Durkin-Konidas M. New World cutaneous leishmaniasis: updated review of current and future diagnosis and treatment. J Am Acad Dermatol 2010; 63(2):309-322. https://doi.org/10.1016/j.jaad.2009.06.088.

5. Paola B, Gianni C. Molecular basis of antimony treatment in leishmaniasis. J Med Chem 2009; 52: 2603–2612.

6. Hugh P, Iain W. The trypanocidal drug suramin and other trypan blue mimetics are inhibitors of pyruvate kinases and bind to the adenosine site. The J Biol Chem 2011; 286(36): 31232–31240. https://doi.org/10.1074/jbc.M110.212613.

7. Marta S, Liâdia B, Catalysis and Structural Properties of Leishmania infantum Glyoxalase II: Trypanothione Specificity and Phylogeny. Biochemistry 2008; 47:195-204. https://doi.org/10.1021/bi070989m.

8. Agarwal R. Synthesis and biological screening of some novel coumarin derivatives. Biochem Pharmacol 2000; 6: 1042-1051. https://doi.org/10.1016/S0006-2952(00)00057-8.

9. Bruneton J. Immunotoxicity of epicutaneously applied anticoagulant rodenticide warfarin. Hampshire UK, Intercept Ltd. 1999; 2: 245-263. https://doi.org/10.1016/S0300-483X(03)00047-7.

10. Bosland MC. Synthesis of vanillin ethers from bromomethyl coumarins as anti-inflammatory agents. San Diego Academic Press 1991; 6: 162-177. https://doi.org/10.1016/S0223-5234(03)00016-3.

11. Kamath N, Hurley JS. A novel series of 5- (substituted)-aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines and pharmacological evaluation reported as a novel anti-inflammatory and analgesic effects. Eur J Can 1998; 14: 19-27.

12. Cooke D, Fitzpatrick B, O’ Kennedy R, McCormack T, Egan D. Coumarin biochemical profile and recent developments. John Wiley and Sons 1997; 3: 311-322. https://doi.org/10.7324/JAPS.2012.2643.

13. Kirk E. Hevener,1 Wei Zhao et al. Validation of molecular docking programs for virtual screening against dihydropteroate synthase. J Chem Inf Model 2009; 49(2): 444–460. https://doi.org/10.1021/ci800293n.