IJBPAS, June, 2024, 13(6): 2929-2938

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ENHANCING POTENTIALITY OF FLAVONOIDS BY PREPARING SEMI SYNTHETIC DERIVATIVES

USHARANI U[1*] AND SREEDEVI A[2]

1: Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Carmelaram, Bangalore, India

2: Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Viswa Vidyalayam, Tirupati, Andhra Pradesh, India

***Corresponding Author: Ms. Ummarasetty Usharani E Mail: uusharani.pharmacy@gmail.com**

Received 15th April 2023; Revised 8th June 2023; Accepted 23rd Sept. 2023; Available online 1st June 2024

https://doi.org/10.31032/IJBPAS/2024/13.6.8115

ABSTRACT

In drug designing, molecular docking is used for understanding drug-receptor interaction. In the present study Schiffs bases of chrysin were synthesized by using different amines. Structures of the newly synthesized compounds were characterized by spectral studies. Compounds were screened for their antibacterial activity and anthelmentic activity. Compound 1A was found to be potent antibacterial against Staphylococcus aureus at 10µg/mL compared to standard drug Ofloxacin and also 1a was found to be potential when compared to standard drug Albendazole. Present study showed that by increasing water solubility of flavonoids the biological activity may increases. All the compounds were subjected to molecular docking studies for the inhibition of the enzyme DNA Gyrase (EC 5.99.1.3). The *in silico* molecular docking study results showed that, all the synthesized compounds having minimum binding energy and have good affinity toward the active pocket, thus, they may be considered as good inhibitor of DNA Gyrase.

Keywords: Molecular docking, flavonoids, antibacterial activity, chrysin, Drug design

1. INTRODUCTION

Flavonoids belong to a large group of natural poly phenol compounds and have a broad spectrum of pharmacological activity **[1]**. Many natural flavonoids have extreme low solubility in aqueous media and body fluids. This leads to certain difficulties in

creating highly effective medicines, while the solubility is one of the major biopharmaceutical characteristics that largely determine the drug bioequivalence **[2-6]**. Although flavonoids have poor bioavailability but many of these compounds appear to be effective at preventing various disorders. Flavonoids are secondary metabolites of plants, and therefore occur in the human diet. Numerous epidemiological studies show an inverse correlation between dietary flavanoid consumption and chronic degenerative diseases. Flavonoids are found ubiquitously in fruits, vegetables, nuts and plant –derived beverages, such as tea and wine. These compounds have been reported to possess a wide range of activities in the prevention of common diseases, including coronary heart disease, cancer, neurodegenerative diseases, gastrointestinal disorders and others. Their beneficial effects appear to be related to the various biological/pharmacological activities of flavonoids. Chrysin is one of the flavonoid reported for many activities like Anti-bacteria **[7-9]** Anti-oxidant **[10]**, Antiiflammatory **[11-13]**, Anti-tubercular **[14-16]** and Anti-cancer activities **[17]**. The present work is concerned with the synthesis of semi synthetic derivatives of chrysin by amine substitution. Chrysin is a hydroxylated flavon derivative, also called as 5,7 dihydroxy flavone found in propolis, passion flowers, passiflora incarnata, honey etc. The objective of this study was to improve the water solubility there by increasing the bioavailability of drug. Because of the emergency of multidrug resistance in common pathogens there is a need to develop potent antibacterial drugs with improved properties and reduced toxicity. So, the present study was designed to synthesize Schiff's bases to improve the antibacterial activity by increasing the biological activity of flavonoids by synthesizing their semisynthetic derivatives.

2. EXPERIMENTAL

2.1 Materials chemicals used in this work are procured from sigma aldrich

2.2 Method of preparation of semi synthetic derivatives

2.2.1 Procedure for the preparation of 4-Hydroxyamino-2-phenyl-4-Hchromene-5-7-diol:

0.069gms of hydroxyl amine hydrochloride in 3ml of ethanol and 0.254gms of chrysin which is dissolved in 5ml of ethanol the solution is refluxed for around 2hours. Then the product is filtered and collected to obtain highest purity the product is recrystallized with ethanol.

2.2.2 Procedure for the preparation of N-(5, 7-dihydroxy-2-phenyl-4H-chromen-4-yl) hydrazinecarbothioamide

0.111g of thiosemicarbazide and 0.254 chrysin and dissolved it in 5ml of ethanol for solubility and reflex it for 30mins the product is filtered and collected to obtained

highest purity the product is recrystallized with ethanol.

2.2.3 Procedure for the preparation of 5,7-dihydroxy-4-hydroxyl-chromen-4-yl)amino}acetic acid

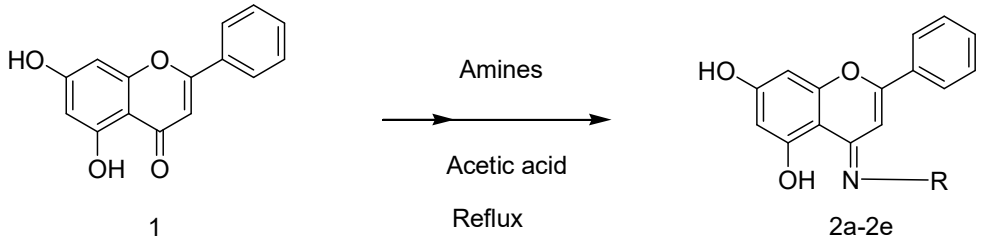
0.075g of chrysin dissolved in 3ml of DMF and 0.0254g of chrysin in 5ml of ethanol. This solution is mixed and reflex one hour the obtained product is filtered and collected and for highest purity product is again recrystallized with ethanol.

2.2.4 Procedure for the preparation of 4E)-4-[(2-hydroxyethyl)imino]-2-phenyl-3,4-dihydro-2H-chromene-5,7-diol

0.075gms of semi carbazide and 0.54g of chrysin dissolved in 5ml of ethanol/ and reflex for 2hours. Obtained product is filtered and collected and for highest purity product is again recrystallized with ethanol.

2.2.5 Procedure for the preparation of (2E)-2-(5,7-dihydroxy-2-phenyl-2,3-dihydro-4H-chromen-4-ylidene)hydrazinecarboxamide

0.254gms of chrysin in 2ml of ethanol add 3ml of DEA and mix it well, reflex for 1hour obtained product is filtered and collected and for highest purity product is again recrystallized with ethanol.



R:

a: -OH
b: -CS-NH-NH2
c: -CH2-COOH
d: -CH2-CH2-OH
e: -NH-CO-NH2

Scheme: Synthetic root for the compounds from 2a to 2e

2.2.1 Characterisation of synthesized compounds

2.2.1.1 1a 4-Hydroxyamino-2-phenyl-4-Hchromene-5-7-diol:

IR (KBr, v_{max} cm^{-1}) 3570 (O-H str), 1694 (C=N str), 1505 (C=C Ar str), ^{1}H NMR (400 MHZ, DMSO): δ 6.22-8.05 (8H, Ar-H), 8.07-10.92 (2H, Ar-OH), 12.88 (IH, OH); MS (ESI) m/z : 269.25 [M]+. Elemental composition C15 H_{11} NO_4 C, 66.91%; H, 4.12%; N, 5.20%; O, 23.77%

2.2.1.2 1b N-(5, 7-dihydroxy-2-phenyl-4H-chromen-4-yl) hydrazinecarbothioamide:

IR (KBr, v_{max} cm^{-1}) 3451 (O-H str), 3030 (C-H, Ar-H), 1648 (C=N str), ^{1}H NMR (400 MHZ, DMSO): δ 6.22-8.06 (9H, Ar-H), 10.92-12.88 (2H, N-H); MS (ESI) m/z:

327.35 [M]+. Elemental composition $C_{16}H_{13}N_3O_3S$ C, 58.7%, H, 4.0%, N, 12.84%, O, 14.66%, S, 9.80%

2.2.1.3 **1c.Preparation of {(5,7-dihydroxy)-4-hydroxyl-chromen-4-yl)amino}acetic acid**

IR (KBr, v_{max} cm^{-1}) 3630 (O-Hstr), 3030 (C-H Ar-H), 1500 (C=N str), ^{1}H NMR (400 MHZ, DMSO): δ 6.23-8.07 (9H, Ar-H), 10.92-12.88 (2H, OH), MS (ESI) m/z: 359.41 [M]+. Elemental composition $C_{16}H_{25}NO_5$ C, 66.83%, H, 7.0%, N, 3.90%, O, 22.26%

2.2.1.4 1d. Preparation of(4E)-4-[(2-hydroxyethyl)imino]-2-phenyl-3,4-dihydro-2H-chromene-5,7-diol

IR (KBr, v_{max} cm^{-1}) 3451 (O-H str), 3030 (C-H,Ar-H), 1677 (C=Nstr), ^{1}H NMR (400 MHZ, DMSO): δ 6.23-8.07 (9H, Ar-H), 10.92-12.88 (2H,OH), MS (ESI) m/z: 299.3 [M]+.Elemental composition $C_{17}H_{17}NO_4$ C, 66.21%, H, 5.72%, N, 4.68%, O, 21.38%

2.2.1.5 1e. Preparation of(2E)-2-(5,7-dihydroxy-2-phenyl-2,3-dihydro-4H-chromen-4-ylidene)hydrazinecarboxamide

IR (KBr, v_{max} cm^{-1}) 3430 (O-H str), 2980 (C-H, Ar-H), 1677 (C=Nstr), 823 (N-H rocking) ^{1}H NMR (400 MHZ, DMSO): δ 6.23-8.07 (9H, Ar-H), MS (ESI) m/z: 311.2 [M]+. Elemental composition $C_{16}H_{13}N_3O_4$ C, 61.73%, H, 4.21%, N, 13.50%, O, 20.56%

2.3. ANTIBACTERIAL STUDIES

Antibacterial activity of newly synthesized compounds was determined by Spread plate method. The petri dishes were washed thoroughly and sterilized in hot air woven at 160^0c for 1 hr. 20 ml of sterile nutrient agar medium was poured into sterile petridishes and allow to solidify. The petridishes were incubated at 37^0c for 24 hrs to check for sterility. The medium was seeded with the organism by spread plate method using sterile cotton swabs. Bores were made on the medium using sterile borer and 0.1 ml of the ofloxacin at a concentration of 10 microgram per ml was taken as standard reference. A control having only ethanol in the petri dishes was maintained in each plate. The petridishes were kept in refrigerator for 48 hrs and zone of inhibition were observed and measured using a scale. Antibacterial activity of all the compounds was carried out against the microorganisms. The zone inhibition is as shown in table1. The media was used for both sub culturing and also for estimating antibacterial activity **[18-19]**. Antibacterial results were compared with standard drug Ofloxacin and summarised in table1. Aactivity of active compounds showed in **Figure 1**.

Table 1: Results showing antibacterial activity of synthesised compounds against *Escherichia coli*

Compound	Zone of inhibition			
	5µg/ml	10 µg/ml	15 µg/ml	20 µg/ml
Std ofloxacin	----	26	----	-----
Chrysin	14	16	21	26
1a	15	20	25	29
1b	11	19	23	28
1c	16	23	25	30
1d	12	22	24	31
1e	10	17	20	25

1a 1b 1c

Figure 1: showing results antibaterial activity of active compounds

DISCUSSION

Flavonoids were reported for various activities. Among them we have selected chrysin, reported for various activities but their major drawback is their high lipophillicity and low bioavailability. So present scheme was designed to synthesise their semisynthetic derivatives by amine substitution to enhance the activity. Initially chrysin Schiff's bases were prepared by using hydrazine, semi carbazide, glycine, and diethyl amine. Chrysin Schiff's bases were synthesized. And all these derivatives were characterized using TLC, NMR and IR spectroscopy. And these compounds were evaluated for antibacterial activity and compared with standard ofloxacin. Compound **1a**, showed good activity which contain hydroxyl amine in their structure. Compounds **1b**, and **1c** showed moderate activity which contains thiosemicarbazide and glycine respectively.

2.4 ANTHELMENTHIC ACTIVITY

Helmenthiasis

- **Collection**: earthworm Pheretima posthuma (Annelida)

Average size of earthworm being 7-10 cm

- **Assay**: Earthworm (Pheretima posthuma) = intestinal roundworm of human beings
- **Procedure**: Each petridish was placed with 1 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously, the time death of worm was recorded after

ascertaining that worms neither moved when shaken nor when given

- external stimuli. The test results were compared with reference compound Albendazole (1, 10 mg/ml) treated samples. Pheretima posthuma worms are easily available and used as a suitable model for screening f anthelmintic drug.

Table 2: Results showing Anthelmenthic activity of synthesized compounds (10mg/ml).

NAME OF THE COMPOUND	At 10mg/ml	
	Paralysis time(sec)	Death time(sec)
1a	12	32.34
1b	16	33
1c	16.1	34
1d	18	36
1e	19	35
Albendazole	11.05	32.3

Normal warm

Paralysed warm

Death warm

Figure 2: Showing the acttion of synthesized compound against warm

2.5 INSILICO MOLECULAR DOCKING STUDIES

Molecular docking is an important component of drug development tool box. In academic communities the usage of this is increasing day by day because of the simplicity. Molecular docking is also referred to as small molecular docking. Molecular docking is a study of how two or more molecular structures, for instance, drug

and catalyst or macromolecule receptor, match along to be a perfect fit. (Gane & Dean, 2000) Molecular docking helps in the identification of target sites of the ligand and the receptor molecule. Docking also helps in understanding of different enzymes and their mechanism of action.The "scoring" feature in docking helps in selecting the best fit or the best drug from an array of options.(r) Molecular docking is a method which predicts the preferred relative orientation of one molecule (key) when bound in an active site of another molecule (lock) to form a stable complex such that free energy of the overall system is minimized. It exploits the concept of molecular shape and physicochemical complementarity. The structures interact like a hand in a glove, where both shape and physicochemical properties contribute to the fit(R).

To gain insight into the mechanism of synthesised semisynthetic flavonoid derivatives they were subjected to molecular docking studies against bacterial enzyme DNA gyrase. DNA gyrase is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. Gyrase belongs to a class of enzymes known as topoisomerases that are involved in the control of topological transitions of DNA. Bacterial DNA gyrase (topoisomerase II) and topoisomerase IV are required for DNA synthesis. Inhibition of DNA gyrase blocks relaxation of supercoiled DNA, relaxation being a requirement for transcription and replication.

DOCKING STUDIES OF FLAVONOID DERIVATIVES

Software used: 1-Click Docking

Protein used for docking **[20]**: The details of protein used for docking are mentioned in **Table 3**.

The ligands were drawn in chem Draw Ultra 8.0 (Chem Office package) all compounds are docked against DNA gyrase. Docking scores of compounds and docking poses of the compounds showed in **Table 4 and Table 5** respectively.

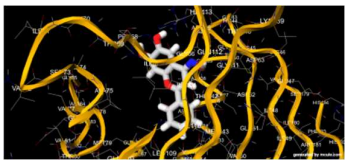
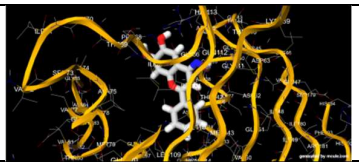
Table 3: Details of the protein used for docking studies

S. No.	Name of the protein	Source	PDB ID	Name of organism
1	DNA Gyrase subunitB	Sc-PDB	1aj6	Escherichia coli

Table 4.: Docking scores of compounds against bacteria and logP values

S. No.	Product	Docking score	logP values
1	Chrysin	-8.0	2.87
2	1a	-8.9	2.80
3	1b	-7.6	3.25
4	1c	-7.2	2.70
5	1d	-7.0	2.40
6	1e	-8.4	3.25
7	Standard ofloxacin	-7.7	1.54

Table 5: Docking poses of active compounds

S. No.	Product	Docking score	Docking pose
1	1a	-8.9	
2	1b	-7.6	

3. RESULTS AND DISCUSSION

Schiffs bases of flavonoid chrysin were synthesized and they were characterized using TLC, IR, NMR and Mass spectroscopy. These compounds were subjected to molecular docking studies. These synthesised compounds were evaluated for antibacterial activity by comparing with standard drug Ofloxacin and aslo anthelminthic activity by comparing with Albendazole. Compound 1a, showed good antibacterial and almenthic acitivity which contain hydroxyl amine in their structure. Compounds 1a, and 1c showed moderate activity which contains thiosemicarbazide and glycine respectively. Compound 1e showing least activity when compared to the standard drugs.The derivatives of chrysin are showing better activity than the chrysin.

4.CONCLUSION

The Research work was done by synthesizing chrysin Schiff's bases. These compounds were screened for antibacterial activity .All these synthesized compounds showed good antibacterial activity and antheminthic activity. Schiff's bases were reported for wide variety of activities. In the present work semisynthetic derivatives were synthesized by amine substitution to enhance the activity. So this will be the new scaffold for the future research work

REFERENCES

[1] Chebil. L, Humaey. C, Anthoni. J. Solubility of Flavonoids in Organic Solvents; Journal of Chemical & Engineering Data 2007;14: 1552-1556.

[2] Olga Ferreira and Siamo pinho.P. Solubility of Flavonoids in Pure Solvents; Industrial & Engineering Chemistry and Research 2012; 51: 6586-6590.

[3] Feizi. S, Jabbari. M, Farajtabar. A. A systemic study on solubility and solvation of bio active compound chrysin in some water and co solvent

mixture; Journal of Molecular Liquids 2016; 220: 478-483.

[4] Kumar and Pandey. Chemistry and biological activities of flavonoids; Scientific world journal 2013

[5] Shashank Kumar, Abhay K Pandey. Chemistry and biological activities of flavonoids; The scientific world journal 2013.

[6] Shouqin. Z, Chang. W. High hydrostatic pressure extraction of flavonoids from propolis. Journal of chemical Technology & Biotechnology; International Research in process, Environmental & clean Technology 2005; 80:50-54.

[7] Zearah.SA and Kananay-AL. Anti - bacterial activity of flavonoid compound isolated from inula greaveonens 1; Plant on selected pathogenic bacteria. Basrah journal of veterinary research, 2014. Vol (13;1-10)

[8] Suresh Babu. K, Hari Babu. T, Srinivas. PV. Synthesis and biological evaluation of novel C (7) modified chrysin analogues as antibacterial agents. Bioorganic & Medicinal Chemistry Letters 2005, 16: 1; 221-224

[9] Tim Cushnie, Andrew J. Antimicrobial activity of flavonoids. International journal of antimicrobial agents 2005 (26: 5; 343-356)

[10] Sreedevi A. Usha. U, Bharati.K, Effect of chrysin isolated from oroxylum indicum against cisplatin induces nephrotoxicity; Recent Res Modern Med, 2011; 302-307.

[11] Dao TT, chi YS, kim HP. Synthesis and inhibitory activity against COX-2 catalyzed prostaglandin production of chrysin derivatives. Bioorg Med Chem. Lett 2004; 8: 1165-1167

[12] Heeyeong Cho, Cheol-won Yun, Woo-Kyu Park, Jae-Yang Kong. Modulation of the activity of pro-inflammatory enzymes, COX-2 and Inos ;Pharmacological research 2004;49:37-43.

[13] Tran Thanh Dao, Yeon Sook Chi, Jeongsoo Kim, Hyun Pyo Kim, Sanghee Kim, Haeil Park. Synthesis and inhibitory activity against COX-2 catalysed prostaglandin production of Chrysin derivatives. Bioorganic & medicinal chemistry letters 2004; 14: 1165-1167.

[14] Akilesh k yadav, jayaprakash Thakur, OM prakash. Screening of flavonoids for antitubercular activity and their structure- activity relationships.; Medicinal chemistry research 2013; 22: 2706-2716.

[15] Saeed Samarghandian, TaherehFakhondeh .Protective effects of chrysin against drugs and toxic agents ;Dose-Response 2017;15:2:1559-1562.

[16] Havsteen. B. H. The biochemistry and medical significance of flavonoids; Pharmacology and therapeutics 2002; 96: 67-202

[17] Xing Zheng, Wei-dong meng, Yang-Yanyu, Jian-Guo Cao, Feng –Ling Qing. Synthesis and anticancer effects of Chrysin derivatives; Bioorganic and medicinal chemistry letters 2003; 13: 881-884.

[18] Zhao, X. B. Mei, Zuo. M. F, Bai. W. J, Dai. H. F, Antibacterial activity of flavonoids from Dalbergia Odorifera on Ralstonia Solanaceareum.; Molecules 2011; 16.

[19] Geetha M, Usha rani U. Raghuveer P, Raveendra Reddy J, New quinoxalinyl chalcone derivatives: search for potent antimicrobial agents; Journal of antimicrobial agents 2018; 3.

[20] Worldwide Protein Data Bank [www.pdb.org]. The Protein Data Bank archive (PDB) the single repository of information about the 3D structures of proteins, nucleic acids, and complex assemblies, Since 1971, Available from: https://www.wwpdb.org/.