FINAL PROGRESS REPORT
OF
MINOR RESEARCH PROJECT

Title

SYNTHESIS OF NEW OXADIAZOLE INTEGRATED BENZOTHIAZEPINE ANALOGUES AS ANTI-INFLAMMATORY AGENTS

Ref: MRP(S)-0551/13-14/KAMY013/UGC-SWRO

Submitted by

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Submitted to

University Grants Commission

SOUTH WESTERN REGIONAL OFFICE

P.K Block, Palace Road, Gandhinagar

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Annexure -VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH
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NEW DELHI – 110 002

Final Report of the work done on the Minor Research Project.

1. Project report No: **Final Report**

2. UGC Reference No: **MRP(S)-0551/13-14/KAMY013/UGC-SWRO**

3. Period of report: from: **2014 to 2016**

4. Title of research project: **SYNTHESIS OF NEW OXADIAZOLE INTEGRATED BENZOTHIAZEPINE ANALOGUES AS ANTI-INFLAMMATORY AGENTS**

5. (a) Name of the Principal Investigator: **NAVEEN.P**

(b) Deptt. and University/College where work has progressed **PG**

Department of Chemistry, JSS College of Arts Commerce &

Science Ooty Road, Mysore-25

6. Effective date of starting of the project **28-03-14**

7. Grant approved and expenditure incurred during the period of the report:

a. Total amount approved Rs. **1,95,000/-**

b. Total expenditure Rs. **1,97,007/-**

c. Report of the work done:

i. Brief objective of the project

- To Promote the development of research in the synthesis of benzothiazepine analoges in the field of reducing inflammation in India with a view to manufacture and encourage such products in developing countries at affordable costs.

- To cultivate and promote standardized practices and research in the field of inflammatory diseases in India.
- To foster studies on the basic mechanisms of anti-inflammatory activity of heterocyclic compounds.
- To contribute and mutually exchange knowledge and appreciation amongst members of the society.
- To promote continuing education in the field of inflammation, organizing, special lectures and publishing journals on academic matters pertaining to the society
- To do every act and anti-inflammatory activity to achieve the above objectives and carry out all actions necessary for harmonious and cogent execution of the above objectives.

ii Work done so far

Substituted chalcones were obtained by Claisen-Schmidt condensation of aromatic aldehydes in the presence of 40% sodium hydroxide with 5-acetyl-2-hydroxybenzoicacid which was achieved by the Fries rearrangement of acetyl Salicylic acid using anhydrous aluminum chloride at 120 ^{o}C , chalcones so obtained was refluxed with 2-amionthiophenol in the presence of acetic acid in ethanol benzothiazepine was obtained

The semicarbazone were achieved by treating semicarbazide with different substituted aldehydes in the presence of sodium acetate, substituted semicarbazone were refluxed with equimolar mixture of chloramine-T in ethanol afforded aminooxadiazole, aminooxadiazole and benzothiazepine were coupled by using coupling agent TBTU in the presence of mild base lutidin, to furnish integrated benzothiazepine oxadiazole analogues.

The title compounds oxadiazole integrated benzothiazepine analogues so synthesized was submitted for characterization.

The Synthesis and inflammatory angiogenesis activity of the synthesized compound is been performed the manuscript is in preparation stage.

A detail report is enclosed

iii. Has the progress been according to original plan of work and towards achieving the objective: **yes**

iv. Please indicate the difficulties, if any, experienced in implementing the project: **No**

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet: **Completed**

vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission: **Enclosed**

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

(a) The financial assistance was helpful in training four final year chemistry post graduate students in synthesizing heterocyclic compounds.

(b) The PI is grateful to UGC for providing the financial assistance which is useful to carryout research (Ph.D), the same will be acknowledged in the Ph.D thesis.

(c) Publication of results

1. **The research finding of the intermediate step is published in European Journal of Medicinal Chemistry 114 (2016) 153-161 with an Impact factor of 3.902 a copy of the same is enclosed.**
2. **The crystal study of the intermediate is 5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-amine IUCrData (2016). 1-3.**
3. **The crystal study of the intermediate is Methyl 2-(benzoyloxy)benzoate IUCrData (2016). 1-3.**
4. **The plane of research was presented in a UGC sponsored two days national conference on " Application of modern analytical techniques to fundamental research in chemistry" (AMAT-FRC 2016) Organized by JSS College of Arts Commerce and Science, BN Road Mysore.**

PRINCIPAL INVESTIGATOR **SIGNATURE OF THE PRINCIPAL**

SUMMARY OF THE FINDINGS

Chemistry

The 4-Hydroxy-3-(-3-phenylacryloyl) benzoic acid analogues (**5)** were obtained as represented in Scheme **1**. 3-Acetyl-4-hydroxybenzoic acid **(3)** was obtained by Fries rearrangement of 4-acetoxybenzoic acid **(2)** in the presence of anhydrous aluminum chloride, wherein the compound **2** was achieved by acetylating 4-hydroxybenzoic acid **(1**) in the presence of triethylamine and dichloromethane. Compound **3** on condensing with benzaldehyde aldehydes (**4**) in the presence of strong base furnished 4-hydroxy-3-(-3-phenylacryloyl) benzoic acid derivatives (**5)** in moderate to excellent yield. Compound 4-hydroxy-3-(-3-phenylacryloyl) benzoic acid was refluxed with 2-amionthiophenol in the presence of acetic acid in ethanol to obtain benzothiazepine. The 5-phenyl-1,3,4-oxadiazol-2-amine analogues (**9a-j)** were synthesized by reported method [14]**.** The title compounds **10a-j** were obtained**,** by acid amine coupling of compounds **5**, with compounds (**9a-j**) in the presence of HATU and TEA [15].

All the synthesized compounds were characterized by IR, NMR and mass spectral studies, In the IR spectrum of compound **2** the presence of OH absorption at 3409-3490 and C=O was observed at 1670 cm^{-1} and C-O at 1213 cm^{-1} and in ^{1}H NMR spectrum the signal at 2.3 ppm as a singlet for methyl group and the signal between 7.1-8.1 ppm as a multiple for aromatic protons and a singlet at 11.8 of COOH indicates the formation of compound 2-acetoxybenzoic acid (**2**). Further, in the IR spectrum appearance of a broad band between 3480-3580 cm^{-1} of phenolic OH, and a peak at 1722-1732 for $COCH_3$ and also in NMR spectra the appearance of a broad singlet around 9.06 ppm, and decrease in the number of aromatic proton clearly indicate, the

formation of 3-acetyl-4-hydroxybenzoic acid **(3)**. In the IR spectrum of compound **5a** absorption of C=C band at 1645 cm^{-1} and increase in the number of aromatic protons compared to compound (**3**) with appearance of two doublet at the range of 7.4 ppm and 7.9 ppm in ^{1}HNMR indicates the formation 4-Hydroxy-3-(-3-phenylacryloyl)benzoic acid **(5),** Further the increase in the number of aromatic signal clearly indicates the formation on compound **6.**

Similarly, among compounds **10a-j,** compound **10a** has been taken as a representative example to discuss spectral characterization. In the IR spectrum of compound **10a,** the disappearance of O-H of COOH band at 3403 -3495 cm^{-1} and also the disappearance of the COOH in ^{1}H NMR signal at 11.1 and the appearance of a N-H as single at the range 6.1 and the increase in number of aromatic protons indicate the coupling of compound **5a** with Phenyl-1,3,4-oxadiazol-2-amine to form compound **10a**.

Biology

*Structure Activity Relationship (SAR) and selection of **10j** as potent inflammatory angiogenesis agents*

Present study is concerted to evaluate the biological significance of synthesized benzothiazepine embedded oxadiazole analogues **10a-j** among the series of compounds **10a-j** the compound **10j** has exhibited a potent inflammatory angiogenesis activity. From the study it is interesting to note that the presence of hydroxyl substitution, particularly at the ortho position of the phenyl ring attached to 4^{th} carbon atom of the oxadiazole ring exhibited a good anti-inflammatory angiogenesis activity. Among the series of compound **10e,** also comprising of the hydroxy group at para positions of the phenyl ring attached to 4^{th} carbon atom of the oxadiazole ring has not shown remarkable activity.

Compound 10j emerged as lead molecule.

In vitro anti-inflammatory activity (HRBC membrane stabilization) of compounds **10a-j** were treated with increasing concentration of the compounds (50, 100, 150, 200, 250 µg/ml) were evaluated and compound **10j** possess significant membrane stabilizing activity compared with the control group and selected as lead compound. Inhibitory concentration (IC 50%) values of 160.076 µg/ml with values for indomethacin (121.68µg/ml), (**Table 1**) Based on the IC_{50} values, compound **10j** was chosen as lead compound.

*Angiopreventive activity of compound **10j**.*

Angioprevention effect was assessed by the CAM assay which is one of the most reliable model for angiogenesis studies [16]. In the present study we have employed rVEGF165 induced neovascularisation in *in-vivo* CAM model to study the efficacy of the compound **10j**. A clear formation of vascular zone around the implanted is a clear evident for the regression of neovessels in the developing embryos with compound **10j** indicates the inhibition of angiogenesis in CAM.

Acute toxicity studies

The animals which received 5000 mg/Kg body weight of compound **10j** were immediately died on the other hand animals which received 4000 mg/Kg body weight of compound **10j** died after 24h. Abnormal aggressive behavior was observed in the animals which received 3000 mg/Kg body weight and died after 24h. Besides the animals with 2000 mg/Kg body weight of compound **10j** died after 24h and the animals which received AEE and LIE with same concentration showed abnormal behavior with less food and water consumption. Moreover animals with 250 mg/Kg body weight of compound **10j** was found to be normal and hence $1/10^{th}$ of this dose i.e. 25mg body weight were selected as therapeutic dose for further analysis.

Inhibition of inflammatory corneal neovascularization by compound **10j**.

Inflammatory corneal angiogenesis assay is one of the most reliable models to study the effect of drug on inflammatory angiogenesis. Three days after the corneal alkali burn, corneal neovascularization (CNV) occurred in both the control and treated groups (**Figure 1**). Compared to the corneas of the control group, corneas in the compound **10j** treated group showed less CNV. The amount of CNV was quantified by measuring the area of neovascularization. It was found that the area of CNV was significantly smaller in the compound **10j** treated group than in the control group, which indicates an inhibitory effect of compound **10j** on CNV.

Reduction of carrageenan-induced paw edema by Compound **10j**.

Injection of mice with carrageenan caused a significant increase in the paw weight percentage was measured after 5 h compared to the control values. Pretreatment of carrageenan injected mice with indomethacin (10 mg/kg) significantly decreased paw weight % measured after 5 h compared to carrageenan injected mice whereas compound **10j** (25 mg/kg/body weight) treated animals showed significantly decreased paw weight % measured after 5 h (75%) compared to that of carrageenan injected mice.

MPO activity of compound **10j**.

The neutrophil migration towards carrageenan induced inflammation in mouse paws was indirectly determined based on MPO activity in the target tissue. Treatment with compound **10j** significantly prevented the increase in MPO activity induced by carrageenan.

Conclusion

The present has focused on the synthesis of a series of benzothiazepine integrated oxadiazole (**10a-j**) with different substitutions, among the series of compounds compound **10j** exhibit potential inflammatory angiogenesis. The activity of synthesized compounds showed that benzothiazepine appended oxadiazole play a major role in enhancing the activity. From the study it is interesting to note that the presence hydroxyl substitution, particularly at the ortho position

of the phenyl ring attached to 4th carbon atom of the oxadiazole ring exhibited a good anti-inflammatory angiogenesis activity. Among the series of compound **10e,** also comprising of the hydroxy group at para positions of the phenyl ring attached to 4th carbon atom of the oxadiazole ring has not shown remarkable activity.

Reference

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PRINCIPAL INVESTIGATOR **SIGNATURE OF THE PRINCIPAL**

Annexure – VII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

1. TITLE OF THE PROJECT **SYNTHESIS OF NEW OXADIAZOLE INTEGRATED BENZOTHIAZEPINE ANALOGUES AS ANTI-INFLAMMATORY AGENTS**
2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR **NAVEEN.P**
3. NAME AND ADDRESS OF THE INSTITUTION **JSS College of Arts Commerce & Science Ooty Road, Mysore-25**
4. UGC APPROVAL LETTER NO. AND DATE **MRP(S)-0551/13-14/KAMY013/UGC-SWRO**
5. DATE OF IMPLEMENTATION: **28-03-14**
6. TENURE OF THE PROJECT: **18 months**
7. TOTAL GRANT ALLOCATED: **1,95,000.00/-**
8. TOTAL GRANT RECEIVED: **1,37,500.00**/-
9. FINAL EXPENDITURE: **1,97,007.00**/-
10. TITLE OF THE PROJECT **SYNTHESIS OF NEW OXADIAZOLE INTEGRATED BENZOTHIAZEPINE ANALOGUES AS ANTI-INFLAMMATORY AGENTS**
11. OBJECTIVES OF THE PROJECT

    - To Promote the development of research in the synthesis of benzothiazepine analoges in the field of reducing inflammation in India with a view to manufacture and encourage such products in developing countries at affordable costs.

- To cultivate and promote standardized practices and research in the field of inflammatory diseases in India.
- To foster studies on the basic mechanisms of anti-inflammatory activity of heterocyclic compounds.
- To contribute and mutually exchange knowledge and appreciation amongst members of the society.
- To promote continuing education in the field of inflammation, organizing, special lectures and publishing journals on academic matters pertaining to the society
- To do every act and anti-inflammatory activity to achieve the above objectives and carry out all actions necessary for harmonious and cogent execution of the above objectives.

12. WHETHER OBJECTIVES WERE ACHIEVED: **Yes**

13. ACHIEVEMENTS FROM THE PROJECT:

14. SUMMARY OF THE FINDINGS

Benzothiazepine integrated oxadiazole moiety (**10a-j**) were synthesized and characterized by IR, ^{1}H, ^{13}C NMR, elemental and mass spectral analyses and their inflammatory angiogenesis activity were evaluated. The anti-inflammatory activity of compounds **10a-j** was investigated on that human red blood cells (HRBC) *in-vitro*. The results implicate that among the series of **10a-j,** compound **10j** possess significant membrane stabilizing activity in comparison with the control. *In -vivo* CAM and rat corneal anti angiogenesis assays were performed to assess the effect of compound **10j** on endothelial cell migration which inferred that **10j** also inhibits the proliferation of endothelial cells. From the study it is interesting to note that the presence of hydroxyl substitution, particularly at the ortho position of the phenyl ring attached to 4^{th} carbon atom of

the oxadiazole ring exhibited a good anti-inflammatory angiogenesis activity. Among the series of compound **10e,** also comprising of the hydroxy group at para positions of the phenyl ring attached to 4^{th} carbon atom of the oxadiazole ring has not shown remarkable activity. The study reports the inflammatory and anti angiogenic activity of compound **10j** which could be translated into new drug in future.

15. CONTRIBUTION TO THE SOCIETY

The study reveals the significance of compound **10j** has the inflammatory and anti angiogenic agent **10j** This study could be benchmark for the trans.lation of compound **10j** into new drug in future.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT

The Principal Investigator has registered for Ph.D in Mysore University with the ORDER NO. AC.6/Ph.D/PR/65/2011, he his grateful to UGC for providing the financial assistance which is useful to carryout research (Ph.D), the same will be acknowledged in the Ph.D thesis**.**

17. NO. OF PUBLICATIONS OUT OF THE PROJECT **Three Publication**

- **The research finding of the intermediate step is published in European Journal of Medicinal Chemistry 114 (2016) 153-161 with an Impact factor of 3.902 a copy of the same is enclosed.**
- **The crystal study of the intermediate is 5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-amine IUCrData (2016). 1-3.**
- **The crystal study of the intermediate is Methyl 2-(benzoyloxy)benzoate IUCrData (2016). 1-3.**

PRINCIPAL INVESTIGATOR PRINCIPAL

(Seal)

JSS MAHAVIDYAPEETHA

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CERTIFICATE

This is to certify that the final report of the work done in Minor Research Project (MRP(S)-0551/13-14/KAMY013/UGC-SWRO entitled "Synthesis of New Oxadiazole Integrated Benzothiazepine Analogues as Anti-Inflamatory Agents" carried out by Mr Naveen P, Assistant Professor, Department of Chemistry has been kept in the library of the college and the executive summary of the study has been posted on the website of the college.

Principal