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Research Article

Synthesis of Novel Deoxyvasicinone Analogs and their Anti-Bacterial Studies

GVR. Sharma[1*], S. Laxman[1], YLN. Murthy[2], K. Aruna Lakshmi[3] G. Jeson Babu[3] and M. Tark Ramji[3]

[1]Department of Chemistry, GIT, GITAM University, Rushikonda, Visakhapatnam, Andhra Pradesh, India.

[2]Department of Organic Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, India.

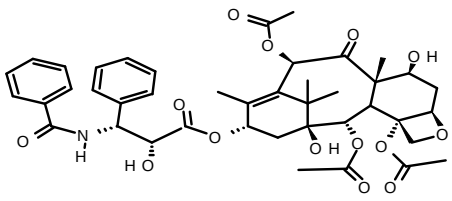
[3]Department of Biotechnology, GIT, GITAM University, Visakhapatnam, Andhra Pradesh, India.

ABSTRACT

Several synthetic analogs of natural product deoxyvasicinone, an alkaloid containing quinazolinone moiety are being reported in this communication. Novel synthetic method for the synthesis of deoxy vasicinone analogs are reported. Antibacterial activity of selected analogs are reported.

INTRODUCTION

Natural products obtained from medicinally active plants and marine sources play a significant role in improving human health. The approach to new drug discovery research based on the natural products was found to be one of the most powerful strategies in the recent times[1,2]. Natural products with the history of their medicinal importance could provide a platform to prepare new compounds with the help of either conventional synthetic methods or modern methods like combinatorial chemistry, microwave chemistry etc. We are interested to use natural products with historical medical use as standard platform, prepare their analogs and study their biological properties. In addition, Natural products and their analogs are likely to be more bioavailable due to their origin from nature, and likely to show potential biological activity. It has been observed sometimes due to the presence of chiral centres, only one of the isomers may show better biological activity which may be attributed to its favorable interactions at the active site. Frequently followed Lipinski's rules need not be applied for the design of new molecules based on natural products in drug discovery. Due to these reasons natural products play a vital role in the development of molecules of interest in the area of pharmaceutical research. There have been several reviews and articles published on natural products and their biological significance. A few of the very important drugs which are currently in use in various thereupetic areas are shown below.



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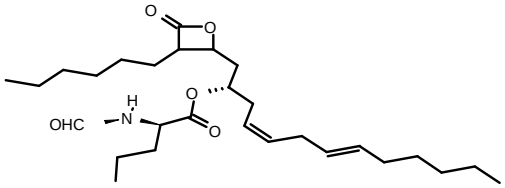

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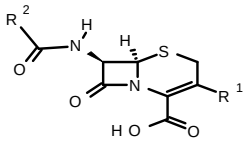

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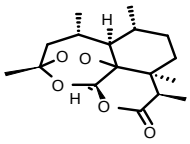

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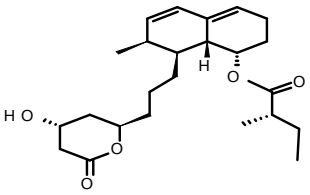
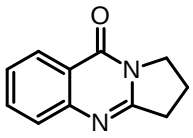


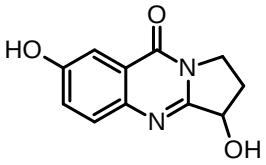
mevastatin

4(3H)-Quinazolinone moeity form a part of several medicinally active naturally occurring alkaloids isolated from plant kingdom, from animals, and from microorganisms. These have been extensively investigated due to their interesting biological activities. Also, the 4(3H) quinazolinone ring is considered as an important building block in synthesis.[1] as this moiety is capable of binding at multiple sites with high affinity and facilitate rapid pharmacological action and hence helps in the rapid discovery of more medicinally active molecules around this building block.

Deoxyvasicinone (2,3-dihydropyrrolo[2,1-*b*]quinazolin-9(1*H*)-one) **2** is an alkaloid isolated from the aerial parts of *Adhatoda vasica* (from the family Acanthacea, Sankrit-Vasaka), an evergreen sub-herbaceous bush, used extensively in indigenous medicine for cold, cough, bronchitis, and asthma.[11] Deoxyvasicinone possesses antimicrobial, anti-inflammatory and antidepressant activities.[12] In addition, deoxyvasicinone is very important key intermediate for the synthesis of various natural products such as vasicinone,[13] isaindigotone,[14] and luotonin A.[14]



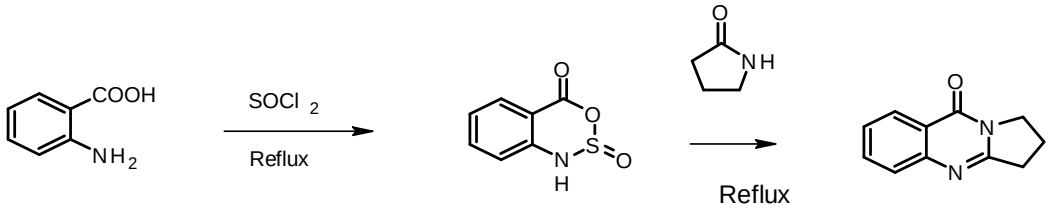
Deoxyvasicinone
Isaindigotone



Vasicinone
Luotonin A

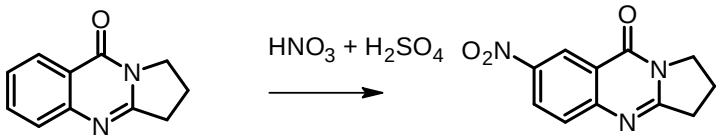
RESULTS AND DISCUSSION

Several methods are reported in the literature for the preparation of quinazolinones from anthranilic acid acid, anthranilamide etc. We chose to start with anthranilic acid which when refluxed with thionyl chloride underwent in situ cyclization to form a cyclic intermediate. Without isolating the cyclic intermediate, we proceeded to react with 2-pyrrolidone under refluxing conditions to provide deoxyvasicinone.



1

Deoxyvasicinone was characterized by 1HNMR, IR, m.p. The deoxyvasicinone was treated with a nitration mixture (Nitric acid and Sulfuric acid) to obtain nitrodeoxyvasicinone. The nitrodeoxyvasicinone was reduced with tin and hydrochloric acid under refluxing conditions to obtain aminodeoxyvasicinone. Both nitro and aminodeoxyvasicinones were characterized by 1HNMR, IR, Mass.



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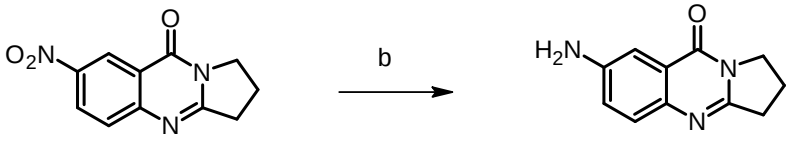
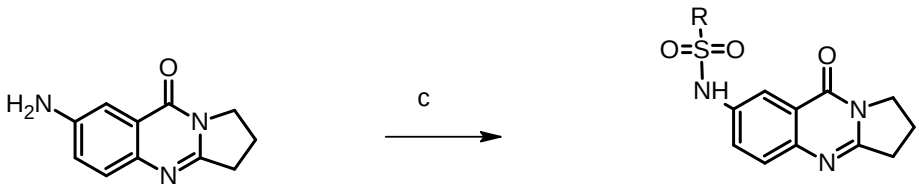


3

The amino deoxyvasicinone was treated with various sulfonyl chlorides in pyridine at room temperature to moderately high temperatures (50°C) to obtain the corresponding sulfonamides which are characterized by 1HNMR, IR, Mass.



4

c. RSO_2Cl, Pyridine, RT-50 degC

R= Me, Ph, 4-MePh, 4-NO_2Ph, 2-NO2Ph

EXPERIMENTAL

Synthesis of Deoxyvasicinone

Anthranilic acid (g, mmols) was taken in thionyl chloride (ml) and refluxed for 3 hrs followed by distillation of the thionylchloride. The crude product was diluted with toluene and 2-pyrrolidone (gm, mmols) and heated at 80 deg C for 5 hrs. The reaction mixture was diluted with toluene, washed with water, dried over anhydrous sodium sulfate and concentrated on rotavapor followed by purification on the silicagel column. Gms (mmols) deoxyvasicinone was obtained whose melting point matched with literature value. 1HNMR (CDCl3): 1-8-2.8 (6H), 7.0 to 8.0 (aromatic 4H), Mass: 186

Synthesis of 6-Nitrodeoxyvasicinone

To a mixture of nitric acid and sulfuric acid (ml) deoxyvasicinone (gm, mmols) was added at ice cold temperature and stirred for 2 hrs followed by dilution with water and extraction with dichloromethane. The dichloromethane layer was dried over anhyd. Sodium sulfate and concentration provided 6-Nitrodeoxyvasicinone (gm, mmol).
1HNMR (CDCl3): 2-3 (m, 6H), 7-8.5 (m, 3H, Ar); Mass: 231

Synthesis of 6-Aminodeoxyvasicinone

To 6-Nitrodeoxyvasicinone (gm, mmols) in hydrochloric acid, tin (gm) was added and heated at reflux for 2 hrs. Filtration, neutralization followed by extraction with dichloromethane, drying over anhydrous sodium sulfate,

concentration provided 6-Aminodeoxyvasicinone (gm, mmol).
1HNMR (CDCl3): 2-3 (m, 6H, aliphatic), 7-8 (m, 3H, Aromatic)
Mass: 201

Synthesis of 6-Phenyl sulfonilamylamino deoxyvasicinone

To 6-aminodeoxyvasicinone (gm, mmols) in pyridine (ml) was added phenylsulfonyl chloride (gm, mmols) and heated to 50 deg C for 2 hrs followed by dilution with water and extraction with dichloromethane, drying and concentration, followed by chromatographic purification provided sulfonamide derivative (gm, mmols).
1HNMR (CDCl3): 2-3 (m, 6H, aliphatic), 7-8 (m, 8H, aromatic)
Mass: 341.

Antibacterial activity

Screening for the antibacterial activity of compounds

The bacterial cultures were grown in peptone water medium and incubated at 37^0C after 6hrs of growth, bacteria were at a concentration of 10^6 cells/ml were inoculated on the surface of Mueller-Hinton agar plates subsequently, filter paper disc(6mm in diameter) saturated with compound (50µg/ml) was placed on surface of each inoculated plate. To evaluate the efficiency of the methodology, 50µl of the extract was inserted simultaneously in a hole made in new plates. The plates were incubated at 37^0C for 24hrs and inhibition zone was observed. Cultured bacteria with halos equal to or greater than 7mm were considered susceptible to the compound. The compound dissolved in 2% DMSO. 2% DMSO served as a negative control. The compound was later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial sample.

Determination of Minimum Inhibitory Concentration

The antibacterial studies of each compound was analysed by Micro broth dilution assay. The assays were repeated at least three times

Microbroth-dilution assay

The MIC was determined as per the guidelines of Clinical and Laboratory Standards Institute[1]. All the bacteria used in this study were incubated for 24hrs at 37^oC. Bacterial suspensions were prepared by suspending 24hrs grown culture in sterile normal saline. The turbidity of the bacterial suspensions was adjusted to a McFarland standard of 0.5, which is equivalent to $1.5x10^8$ CFU/ml. The twofold serial dilutions of each compound was prepared in Muller Hinton broth, 100µl of the bacterial inoculum was added to each well of the plate, resulting in a final inoculum of $5x10^5$ CFU/ml in the well; final concentration of the compound ranged from 10mg/ml to 62.5µg/ml. The plates were incubated at 37^0C for 24hrs. The minimum concentration of the compound that showed 100% reduction of the original inoculum was recorded as the MIC.
The Minimum Bactericidal Concentration (MBC) was determined by spreading a 100-µl volume on a LB agar plate from the wells showing no visible growth. The plates were incubated at 37°C for 24 h. The minimum concentration of compound that showed ≥99.9% reduction of the original inoculum was recorded as the MBC.

Minimum inhibitory concentrations (MIC)

Organism	De-oxy vasicinone mg/ml	Nitro-deoxyvasicinone mg/ml	Amino-deoxyvasicinone mg/ml	Sulphonyl amino de-oxy vasicinone mg/ml	Methyl sulphonyl amino de-oxivasicinone mg/ml
E.coli	5.0	2.5	5.0	10.0	2.5
P.aeruginosa	10.0	5.0	10.0	5.0	2.5
K.pneumonia	5.0	2.5	5.0	5.0	2.5
K.oxytoca	5.0	2.5	5.0	5.0	2.5
P.vulgaris	5.0	2.5	5.0	5.0	2.5
P.mirabilis	5.0	2.5	5.0	5.0	2.5
S.dysentriae	5.0	2.5	5.0	5.0	2.5
S.typhi	5.0	2.5	5.0	5.0	2.5
S.aureus	2.5	1.25	2.5	2.5	1.25
S.pyogenes	2.5	1.25	2.5	2.5	1.25

Organism	De-oxy vasicinone mg/ml	Nitro-deoxyvasicinone mg/ml	Amino-deoxyvasicinone mg/ml	Sulphonyl amino de-oxy vasicinone mg/ml	Methyl sulphonyl amino de-oxivasicinone mg/ml
E.coli	10.0	5.0	10.0	>10.0	5.0
P.aeruginosa	>10.0	10.0	>10.0	10.0	5.0
K.pneumonia	10..0	5.0	10.0	10.0	5.0
K.oxytoca	10.0	5.0	10.0	10.0	5.0
P.vulgaris	10.0	5.0	10.0	10.0	5.0
P.mirabilis	10.0	5.0	10.0	10.0	5.0
S.dysentriae	10.0	5.0	10.0	10.0	5.0
S.typhi	10.0	5.0	10.0	10.0	5.0
S.aureus	5.0	2.5	5.0	5.0	2.5
S.pyogenes	5.0	2.5	5.0	5.0	2.5

Minimum Bactericidal concentrations

Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytoca, Klebsiella pneumonia, Proteus vulgaris, Proteus mirabilis, Shigell dysentriae, Salmonella typhi, Staphylococcus aureus, Streptococcus pyogenes.

MIC of organic compounds towards fungus

Organism	De-oxy vasicinone mg/ml	Nitro-deoxyvasicinone mg/ml	Amino-deoxyvasicinone mg/ml	Sulphonyl amino de-oxy vasicinone mg/ml	Methyl sulphonyl amino de-oxivasicinone mg/ml
A.flavus	10.0	5.0	10.0	5.0	10.0
A.fumigatus	10.0	5.0	10.0	5.0	10.0
A.niger	10.0	5.0	10.0	5.0	10.0
F.oxysporum	10.0	5.0	10.0	5.0	10.0
P.notatum	10.0	5.0	10.0	5.0	10.0
Candida albicans	5.0	10.0	5.0	2.5	5.0

Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Fusarium oxysporum, Pencillium notatum

Organism	De-oxy vasicinone mg/ml	Nitro-deoxyvasicinone mg/ml	Amino-deoxyvasicinone mg/ml	Sulphonyl amino de-oxy vasicinone mg/ml	Methyl sulphonyl amino de-oxivasicinone mg/ml
A.flavus	>10.0	10.0	>10.0	10.0	>10.0
A.fumigatus	>10.0	10.0	>10.0	10.0	>10.0
A.niger	>10.0	10.0	>10.0	10.0	>10.0
F.oxysporum	>10.0	10.0	>10.0	10.0	>10.0
P.notatum	>10.0	10.0	>10.0	10.0	>10.0
Candida albicans	>5.0	10.0	10.0	5.0	10.0

CONCLUSION

We have reported in this communication, a novel series of natural product analogs and their antibacterial studies. Further work is in progress and the results will be communicated in an appropriate journal.

ACKNOWLEDGEMENTS

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