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Synthon Disconnection Strategy for the Synthesis Design of "Coelenterazine"- A Bioluminescent Marine Natural Product used in Bioassays

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ABSTRACT

Synthon disconnection strategy is a problem solving technique in the planning of organic syntheses. This strategy, developed by Prof. E.J.Corey of Harvard University, plays vital role in the synthesis design of many bioactive natural products used in analytical biochemistry, molecular biology and drug discovery process. Keeping an overview on the published works in both journals and patent literatures, a good number of synthesis schemes have been proposed based on this strategy for a bioluminescent natural product 'Coelenterazine' isolated from marine organism jellyfish Aequorea victoria *that finds extensive applications in bioassays. The proposed synthesis planning being a theoretical investigation, the actual laboratory execution requires the cross examination of a considerable number of factors such as reactions, reagents and order of events. In actual practice, generally that route is most feasible which is cost-effective, safe, and easy to carry out and gives maximum yield in a short reaction time.*

Keywords: Anti-oxidant, Bioassays, Bioluminescence, Coelenterazine, Synthon disconnection strategy, Retrosynthetic analysis.

INTRODUCTION

Organic synthesis is one of the major activities of organic chemists and the planning of synthesis is an intellectual task where chemist's imagination, art, creativity and knowledge need to be explored. The simplest synthesis of a molecule is one in which the target molecule can be obtained by submitting a readily available starting material to a single reaction that converts it to the desired target molecule. However, in most cases the synthesis is not straightforward; in order to convert a chosen starting material to the target molecule, numerous steps that add, change, or remove functional groups and steps that build up the carbon atom framework of the target molecule may need to be done. A systematic approach for designing synthetic routes to a molecule is promulgated as a result of Prof. E.J.Corey's developments of 'synthon disconnection approach'/'retrosynthetic analysis'[1-3]. Retrosynthetic analysis is a protocol of stepwise breaking of target molecule to starting materials by disconnection of bonds and functional group interchange to a sequence of progressively simpler structures along a pathway just reverse of actual synthesis. Every disconnected part is an idealized fragment, called 'synthon'. The synthons when joined by known or conceivable synthetic operations result in the formation of target molecule (TM). Retrosynthetic analysis of a target molecule usually results in more than one possible synthetic route. It is therefore necessary to critically assess each derived route in order to choose the single route that is most feasible. In actual practice, generally that route is selected which is cost-effective, safe (the toxicity and reactivity hazards associated with the reactions involved), and

easy to carry out and produces maximum yield in a short reaction time, when assessing alternative synthetic routes to a molecule.

Organic compounds from marine organisms serve as compounds of interest both in their natural form and as templates for design and discovery of bioactive compounds that play important role in analytical biochemistry, molecular biology and drug discovery process. [4-9]. Bioluminescence is the production and emission of light by a living organism as the result of a chemical reaction during which chemical energy is converted to light energy. This phenomenon is exhibited by many marine organisms such as the sea pansy *Renilla reniformis*, the deep-sea shrimp *Oplophorus gracilirostris*, jellyfish *Aequorea victoria*, the squid *Watasenia* and hydroid *Obelia* [10-13]. and has been used extensively in different formats for life science research and drug discovery owing to its extremely high sensitivity and nonhazardous nature. The luminescent system of the jellyfish *Aequorea victoria* consists of the photoprotein aequorin, which contains the molecule "Coelenterazine" fig.1 as a prosthetic group and shows considerable potential in this area. The bioluminescence reaction of coelenterazine involves an oxidative process with molecular oxygen to produce the emission of light. In bioluminescent applications, coelenterazine has been used with the photoprotein aequorin and its recombinant analogues to monitor Ca^{2+} concentrations in mammalian cells[14].Analogues of coelenterazine are also used with aequorin to study ion channels and tyrosine kinase receptors[15] Furthermore, fluorescence resonance energy transfer (FRET) assays that use coelenterazine to detect protein-protein interactions have been developed[16]. Aside from being a luminescent compound, Coelenterazine has also been studied for its anti-oxidant properties for protection against the oxygen toxicity in the environment [17]. Coelenterazine is distributed in a very small amount in its natural sources due to which the quantitative extraction and purification is a very difficult task. Therefore, synthetic methodologies for rapid access of this molecule and its templates are highly essential in synthetic organic chemistry/medicinal chemistry, for a better understanding of its chemiluminescent properties and applications in analytical biochemistry.

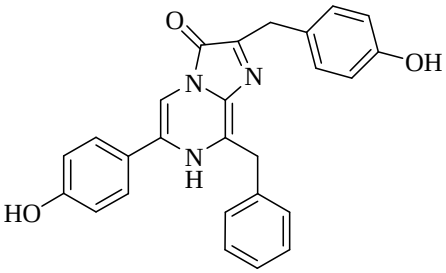


Figure I: Coelenterazine

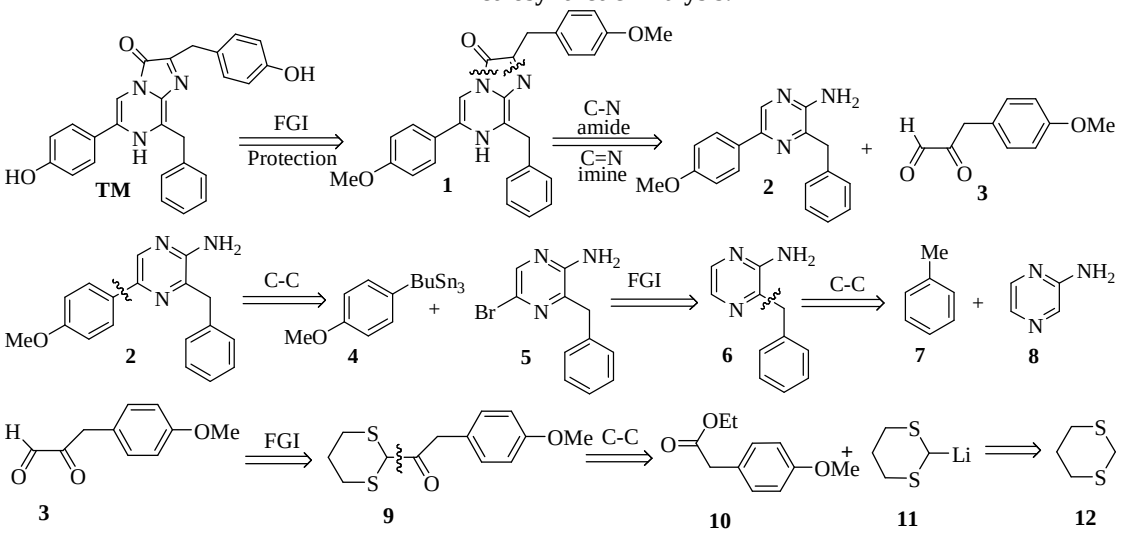
Although a few methods of synthesis of Coelenterazine are well documented in literature [18], some alternative synthetic routs are still required for its commercial success. Keeping an overview on the published works both in journals and patent literatures, an effort has been made to propose a good number of synthesis schemes for Coelenterazine based on synthon disconnection approach /retrosynthetic analysis. To the best of our knowledge, this type of work has not been reported earlier. The choice of this molecule for synthesis planning is obvious as bioassays are routinely works in life science research related to drug discovery. In this profit oriented world, the chemical/ pharmaceutical industries are also vibrant today in search of cost effective scalable synthesis. Moreover with the availability new reagents, chemical reactions, sophisticated new methods of laboratory execution and the application of synthon approach to analyse the target molecules leading to several routes have made it possible to rethink their synthesis for the improvement in existing processes to satisfy the commercial need.

EXPERIMENTAL SECTION

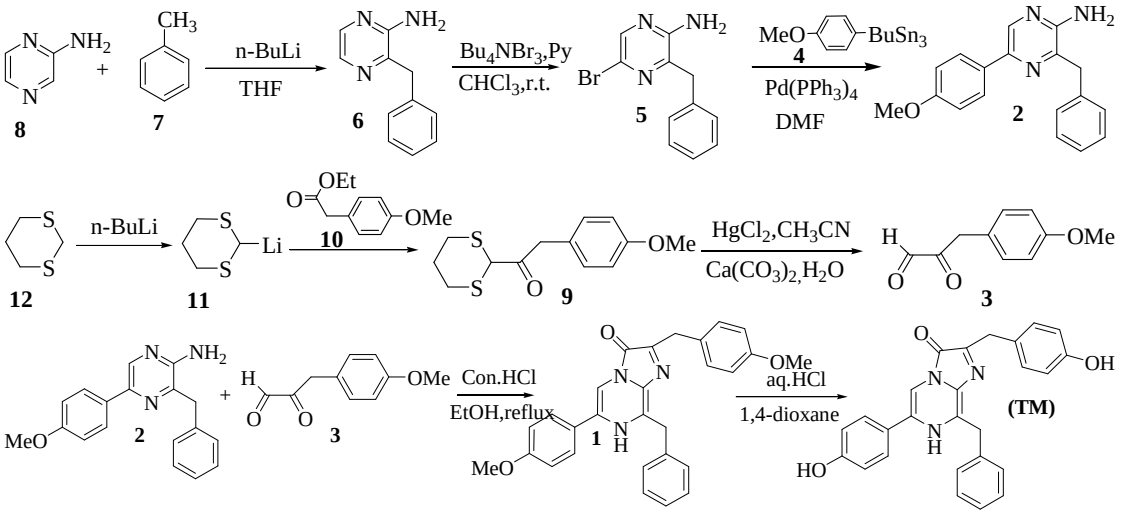
The structure and information about Coelenterazine as bioluminescent candid has been collected from different books [1, 2]. The proposed synthesis plannings are then exploited in a novel way from the result of its retrosynthetic analysis using the basic principle outlined in the pioneering works of Prof. E.J. Corey. The symbols and abbreviations are synonymous to that represented in book [3].The analysis–synthesis schemes being theoretical propositions; obviously the syntheses have not been executed in the laboratory. The actual laboratory execution requires the cross examination of a considerable number of factors such as reagents, reactions, order of events, economical viability, environmental benign, saftyness, short time and scalable synthesis.

RESULTS AND DISCUSSION

Retrosynthetic Analysis: 1



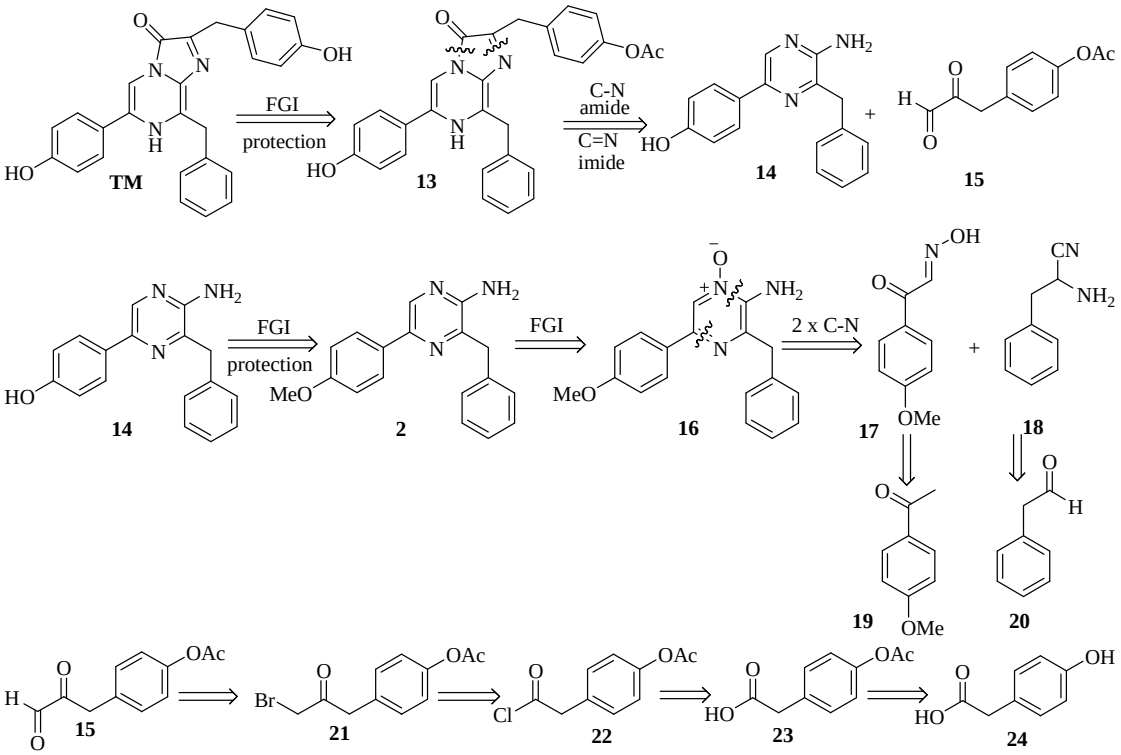
Synthesis: 1



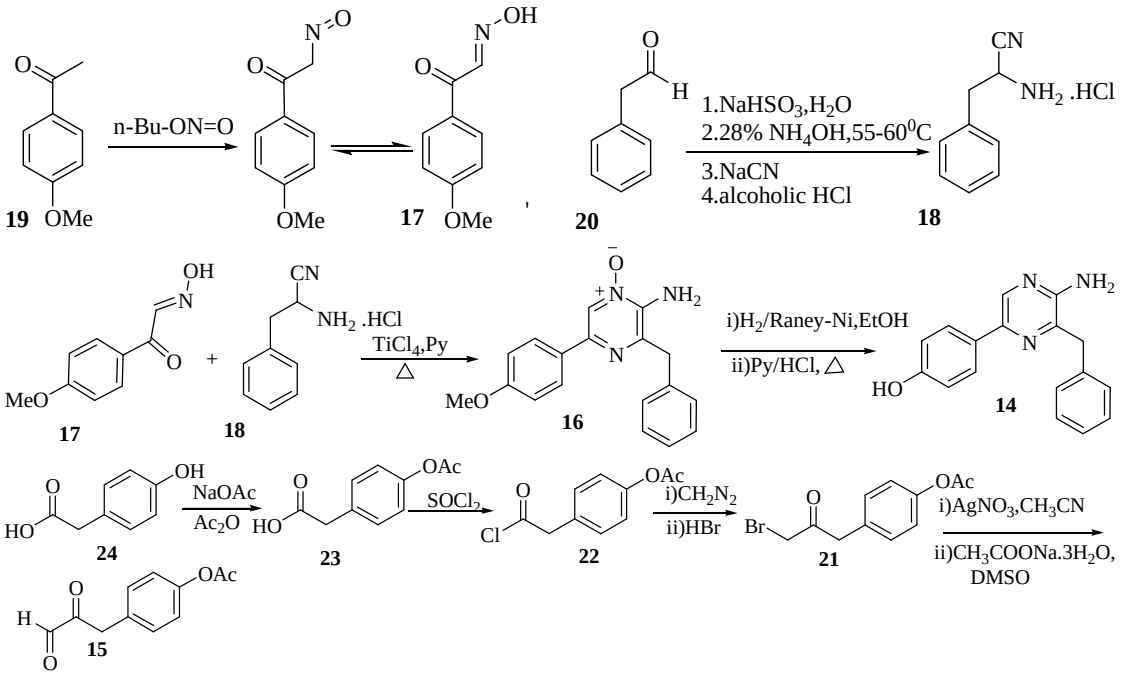
Scheme: 1

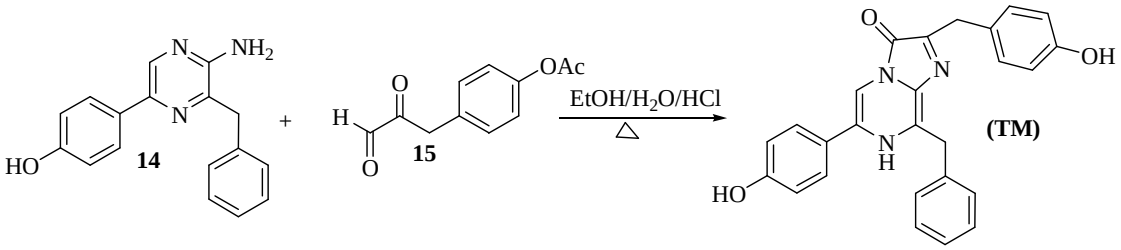
Regioselective alkylation of 2-amino pyrazin **8** with benzyllithium prepared from toluene **6** & n-butyl lithium in THF, forms 2-amino-3-benzylpyrazine **6.**Selective bromination of **6** using tetra-n-butylammonium tribromide(Sato's method)[19] produces 3-benzyl-5-bromo-2 aminopyrazine **5.**Treatment of **5** with organostannane reagent **4** in presence of $Pd(PPh_3)_4$ in DMF undergoes Stille coupling to form **2**. *p*-methoxyphenylglyoxal **3** is prepared from ethyl *p*-methoxyphenyl acetate **10** by exploring dithiane based-route. Coupling of **2** with glyoxal **3** under acidic condition forms **1**.Deprotection of methyl ether group of **1** under mild acidic condition forms the target molecule (**TM**).

Retrosynthetic Analysis: 2



Synthesis: 2

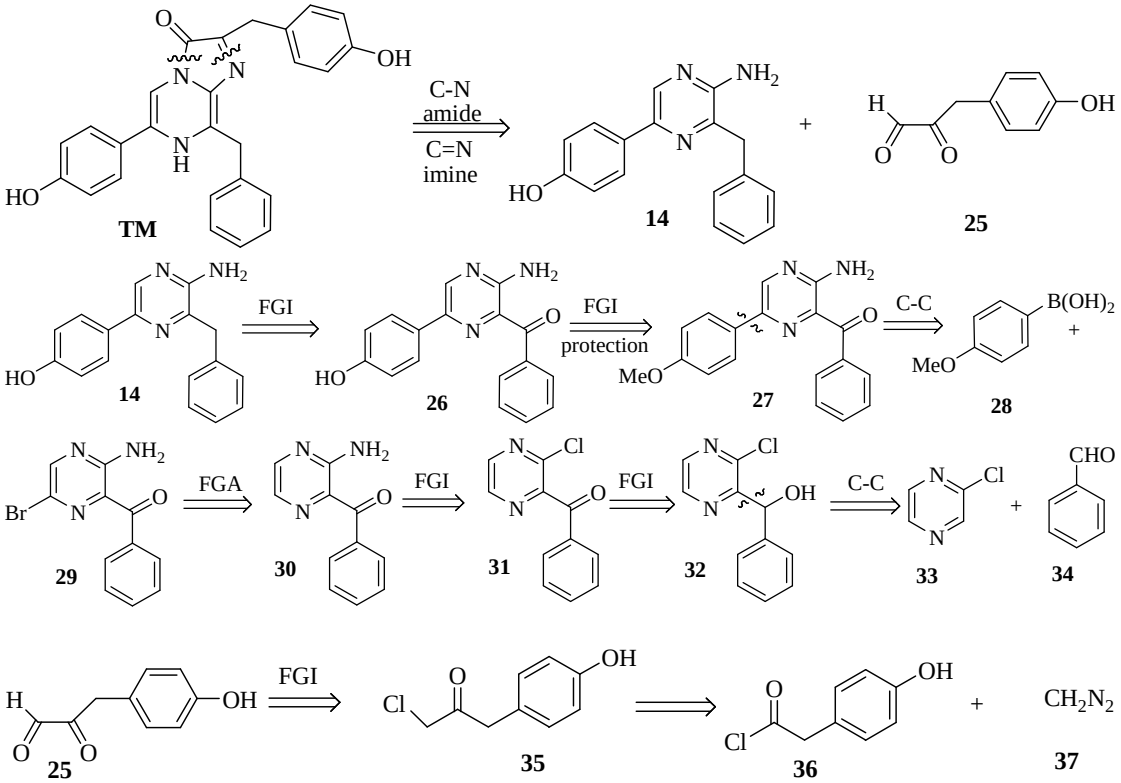




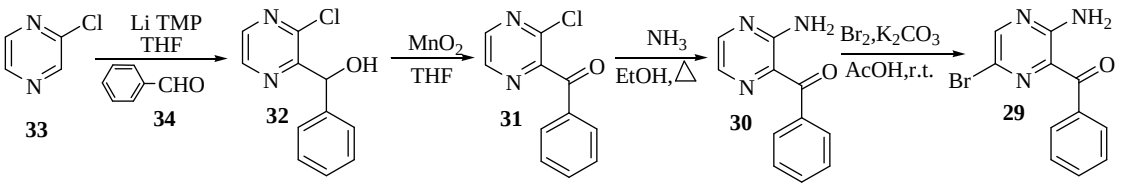
Scheme: 2

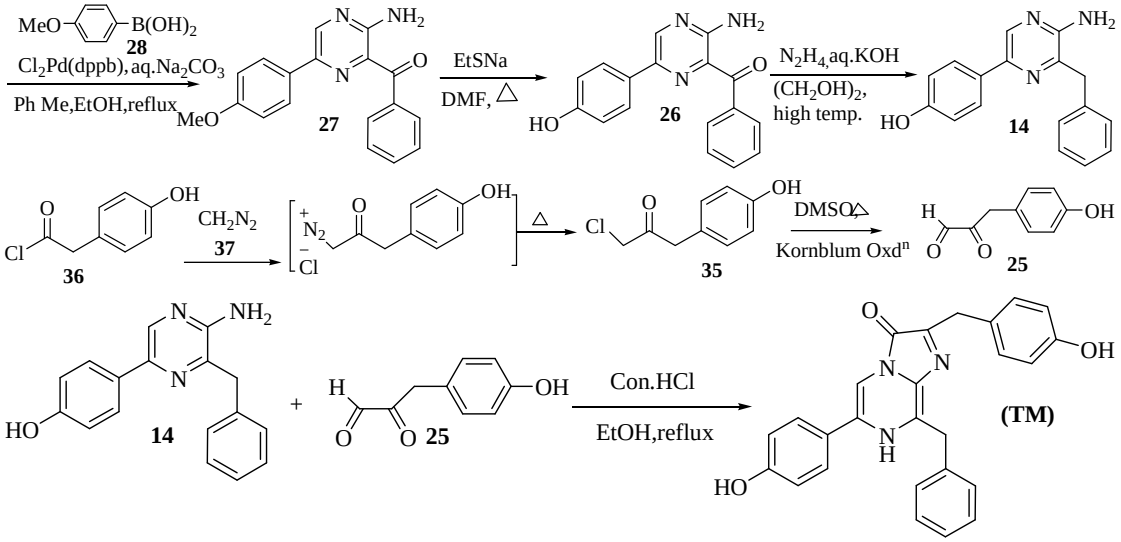
p-methoxyphenylglyoxal aldoxime **17** is formed from *p*-methoxyphenyl ethanone **19** on treatment with n-Butyl nitrite and subsequent tautomerisation.The α-amino nitrile **18** is prepared from 2-phenyl acetaldehyde **20** by following Freifelder and Hasbrouck method[20]. Nucleophilic addition of **18** to the carbonyl group of **17** in presence of $TiCl_4$/Py forms pyrazine *N*-oxide which is reduced and deprotected to give aminopyrazine **14**.The phenyl acetic acid **23** prepared from **24** is then transformed to α-bromoketone **21** and then benzylglyoxal **15**.Cyclization of **14** with **15** in an acidic medium forms the target molecule (**TM**).

Retrosynthetic Analysis: 3



Synthesis: 3

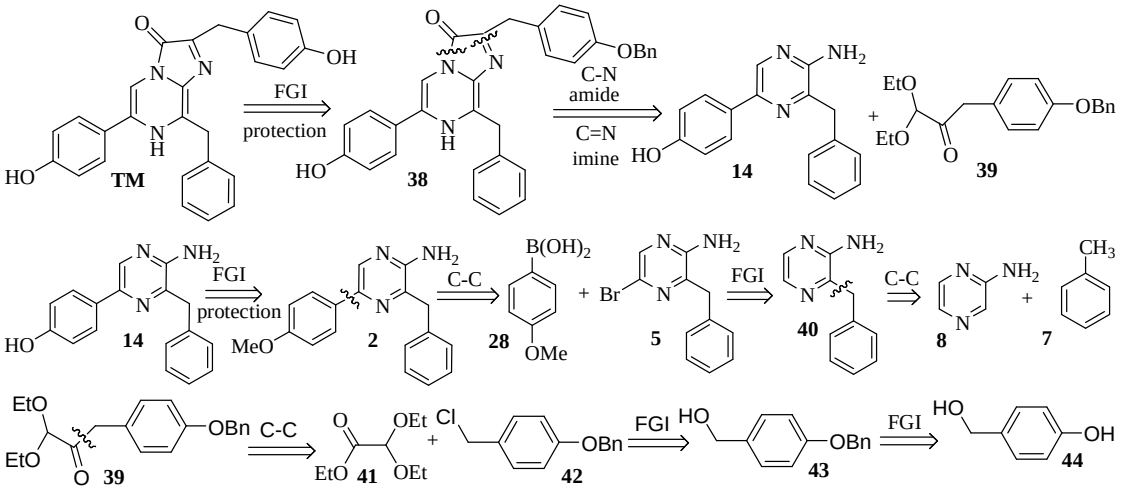




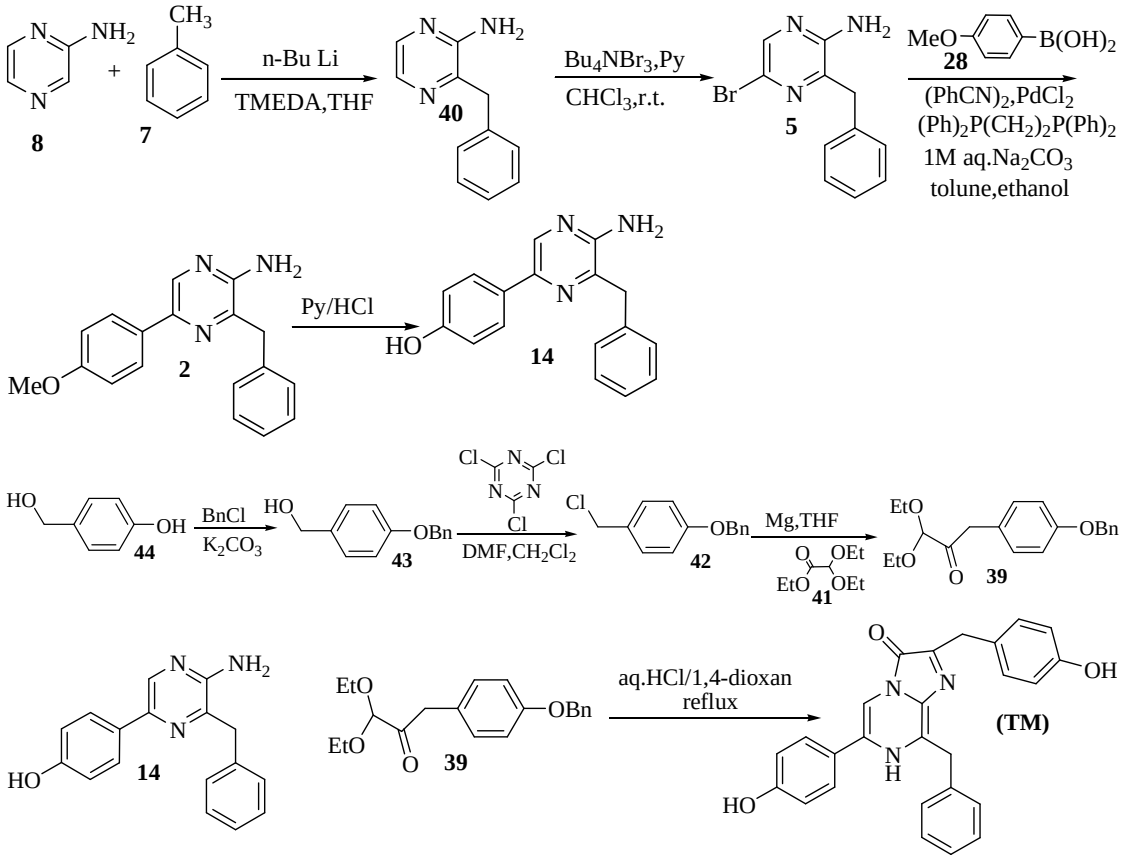
Scheme: 3

Condensation of 2-chloropirazine **33** with benzaldehyde **34** in presence of a strong base 2,2,6,6-tetramethylpiperidyl lithium (LiTMP) forms the chloropyrazinoalcohal **32**.Oxidation of **32** by MnO_2 forms the ketopyrazine **31**.The chlorine substitution is then achieved using NH_3 in EtOH furnishing the aminopyrazine **30**.Bromination of aminopyrazine by Br_2/K_2CO_3 affords the bromopyrazine **29**.The bromopyrazine then undergoes Suzuki coupling with p-methoxyphenylboronic acid **28** to form the aminopyrazine **27.**Deprotection of methyl aryl ether group of **27** using ethanethiolate in DMF to form **26**.Wolf-Kishner reduction of **26** affords the aminopyrazine **14**. Diazomethylation and subsequent decomposition of *p*-hydroxyphenyl acetyl chloride **36** forms corresponding α-chloro ketone **35**.Kornblum oxidation of **35** affords *p*-hydroxybenzylglyoxal **25** Coupling of **14** with glyoxal **25** under acidic condition forms the target molecule (**TM**).

Retrosynthetic Analysis: 4



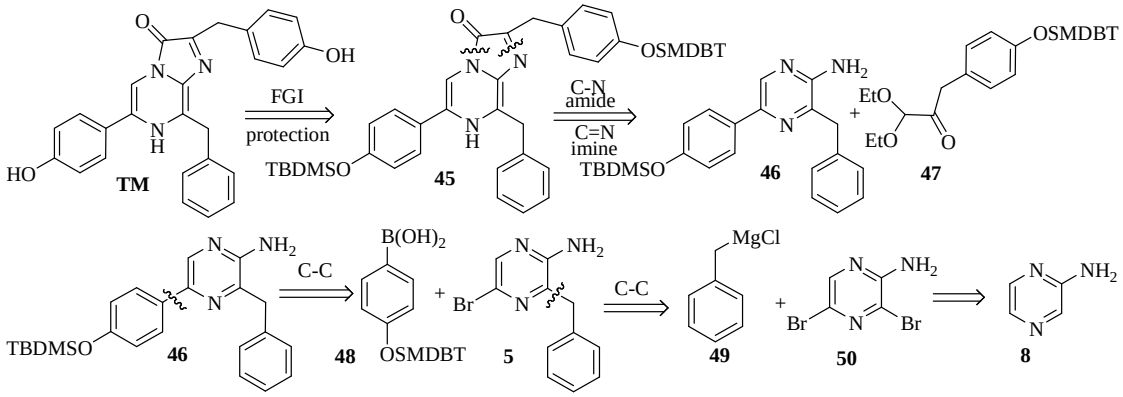
Synthesis: 4

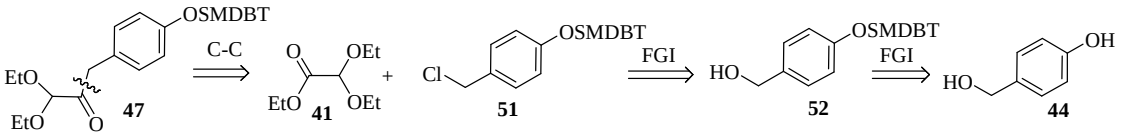


Scheme: 4

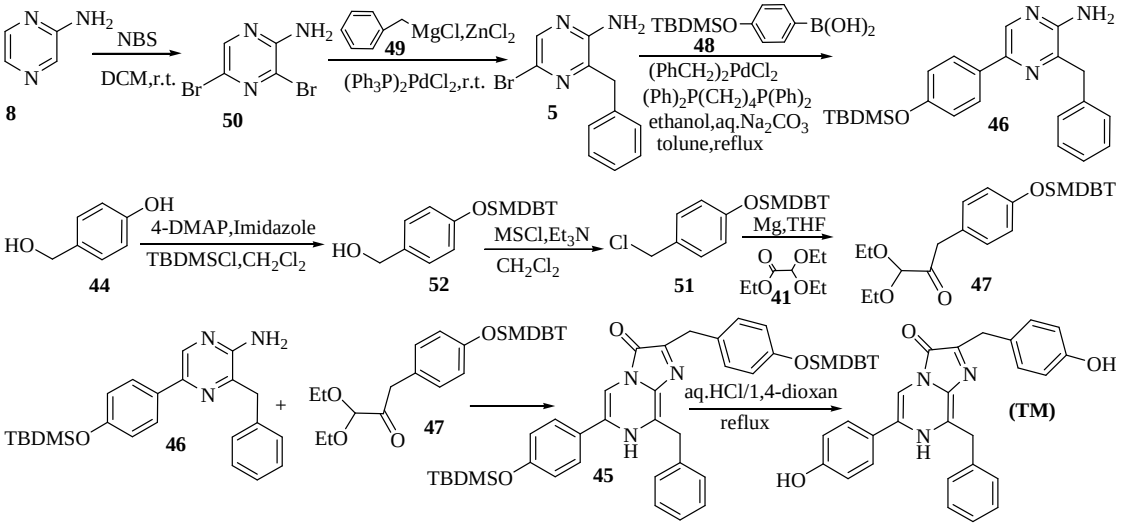
3-benzyl-5-bromo-2 aminopyrazine **5** is produced from 2-aminopyrazine **8** as discussed in scheme**:**1**.** Treatment of **5** with commercially available p-methoxyphenylboronic acid **28** in presence of 1,4-*bis* (diphenylphosphino)butane and *bis* (benzonitrile)dichloropalladium II gives 3,5 disubstituted aminopyrzine **2**.The methoxy group of **2** is cleaved using pyridine hydrochloride to afford the aminopyrazine **14**. Benzylprotected *p*-hydroxybenyl alcohol **43** obtained from *p*-hydroxybenzylalcohol **44** is chlorinated by using cyanuric chloride in DMF to form *p*-benzoyloxy benzyl chloride **42**.The chloride is then converted in to its corresponig Grignard reagent and then subjected to a reaction with the commercially available ethyl dietoxyacetate **41** to form highly protected dicarbonyl compound **39**.Coupling of **14** with glyoxal **39** under acidic condition forms the target molecule (**TM**).

Retrosynthetic Analysis: 5





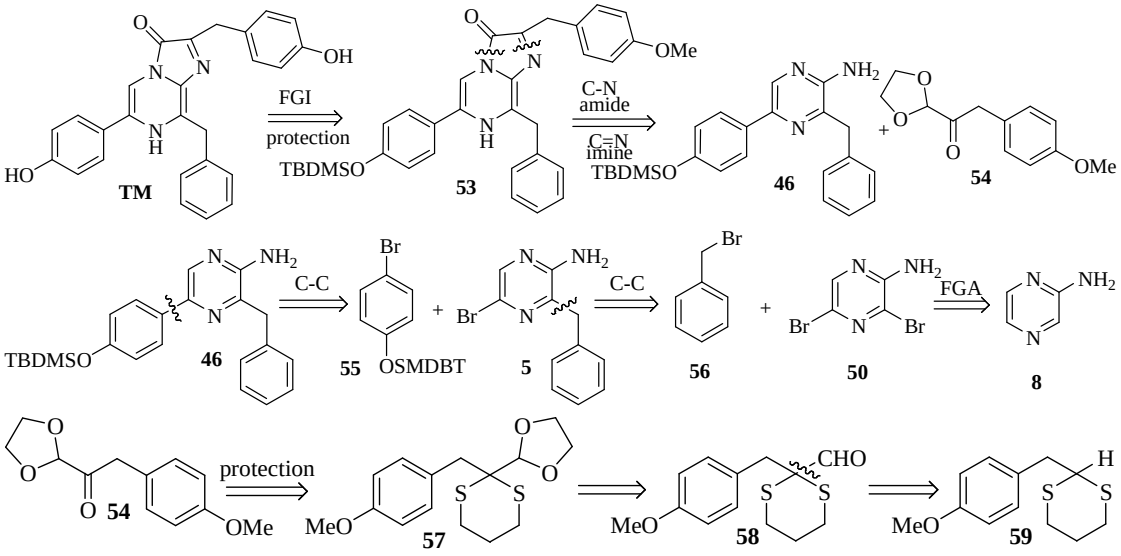
Synthesis: 5

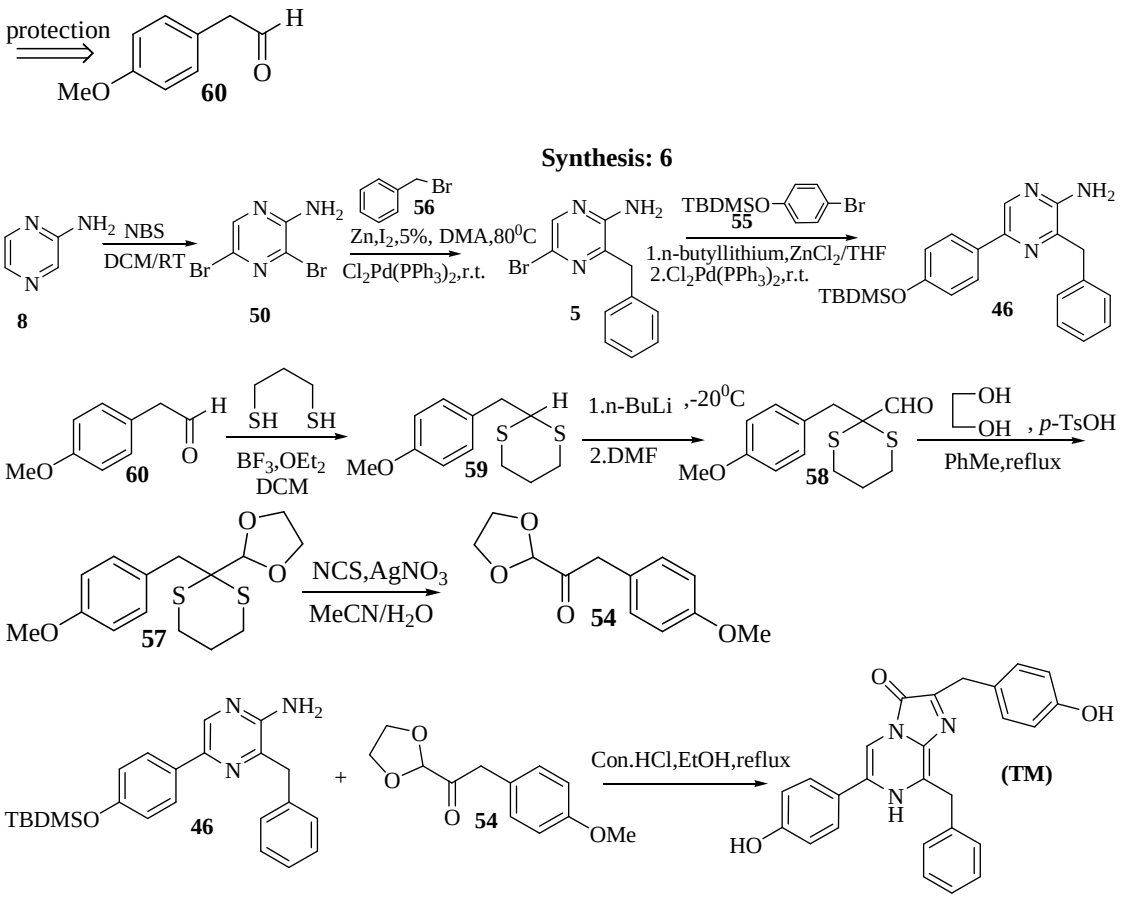


Scheme: 5

Bromination of 2-aminopyrazine **8** with N-bromosuccinamide in DCM forms 3,5-dibromo-2-aminopyrazine **50.** Palladium catalysed coupling of **50** with benzylzinc chloride reagent **49** (formed from benzylmagnesium chloride and anhydrous $ZnCl_2$) affords 3-benzyl-5-bromo-2 aminopyrazine **5.**Under Suzuki coupling condition, **5** condenses with boronic acid **48** to afford **46.** Acid catalysed addition of α-keto acetal **47 (**formed as above) to **46** forms 3,7-dihydroimidazo[1,2a]pyrazine-3-one **45**.Deprotection of silyl groups of **45** under mild acidic condition affords the target molecule (**TM**).

Retrosynthetic Analysis: 6

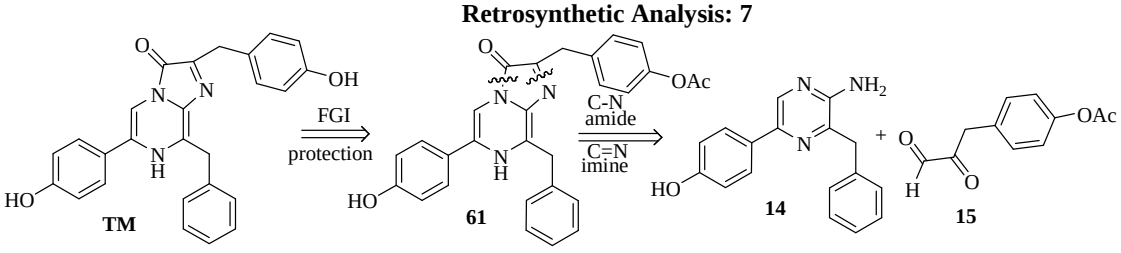


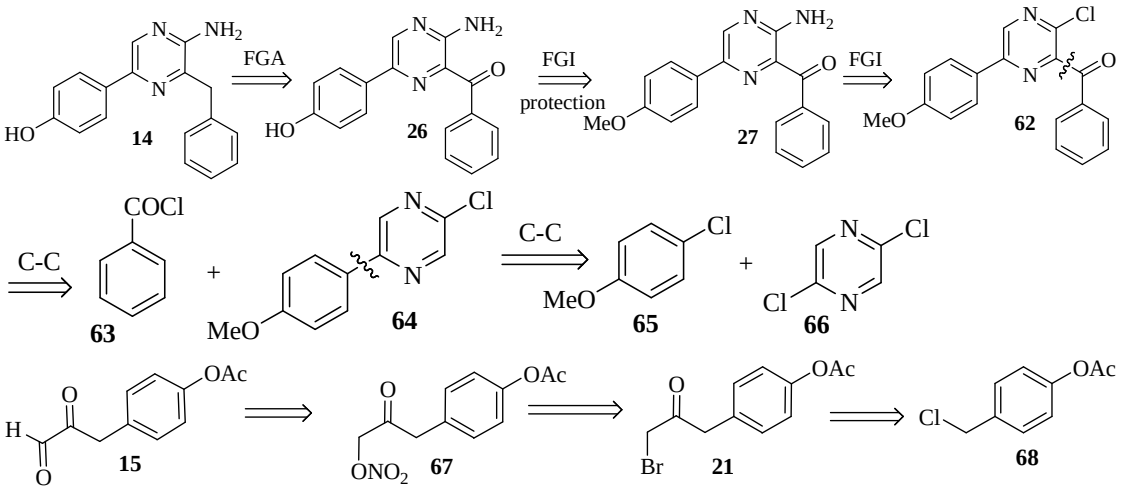


Scheme: 6

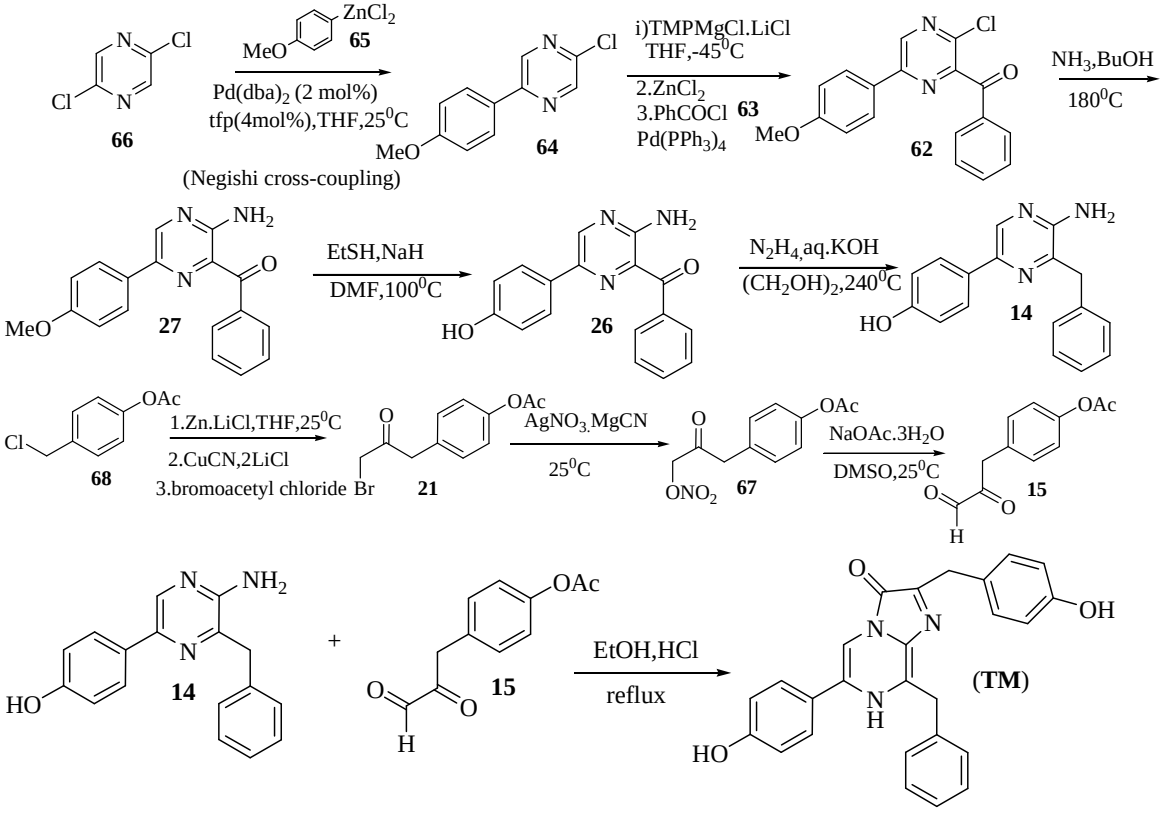
Bromination of 2-aminopyrazine **8** with NBS forms 3,5-dibromo-2-aminopyrazine **50**.Negishi cross-coupling reaction of **50** with benzyl zincbromide formed from benzyl bromide **56** by Huo's method [21] to form 3-benzyl-5-bromo-2 aminopyrazine **5.** The in situ generated phenyl lithium organometallic from *tetra*-butyldimethylsilyl (TBDMS) protected bromophenol **55** with n-butyl lithium is then treated with anhydrous $ZnCl_2$ to create the phenyl zinc chloride (Buchwald's and Miline's approach to zincation)[22] which undergoes cross coupling with the aryl bromide **5** using Pd (II) catalyst to form **46** . The aldehyde group of phenylacetaldehyde **60** is protected by dithiane and then lithiated by n-BuLi.The lithiated derivative on treatment with DMF forms another aldehyde **58**. This aldehyde is then protected to the corresponding acetal to form thioketal acetal moiety **57**.Selective hydrolysis of the thioketal acetal by N-chlorosuccinamide(NCS) and $AgNO_3$ /MeCN provides α-keto acetal **54**.Condensation of **54** with **46** in acidic condition affords the target molecule (**TM**).

Retrosynthetic Analysis: 7





Synthesis: 7



Scheme: 7

Negishi cross-coupling of 2, 5-dichloropyrazine **66** with organozinc compound **65** forms the aryl pyrazine **64.**Magnesiation of **64** with TMPMgCl.LiCl gives after transmetalation with $ZnCl_2$ and acylation with benzoyl chloride **63**, the ketopyrazine **62**.Aminopyrazine **27** is then formed from **62** on treatment with NH_3in BuOH.Cleavage of the methyl ether with sod.thioethanoate in DMF yields the corresponding alcohol **26**.Wolf-Kishner reduction of alcohol affords the coelenteramine **14**. Addition of Zn-dust to 4-(chloromethyl) phenyl acetate **68** and subsequent trapping with bromoacetyl chloride after transmetalation with CuCN.2LiCl, furnish the acetyl derivative **21**.Nitration of **21** with $AgNO_3$ forms the nitrate ester **67** which upon reaction with NaOAc in DMSO

affords the corresponding acetoxy α- keto aldehyde **15**.Condensation of **14** with α- keto aldehyde **15** in 1:1 HCl/EtOH provides the target molecule (**TM)**.

CONCLUSION

The planning of a multistep synthesis via synthon disconnection approach/ retrosynthetic analysis is a challenging task that requires not only a thorough knowledge of synthetic reactions, but also a logical approach for disconnecting a complex target molecule into simpler or commercially available starting materials for a chemical synthesis. It is a paper exercise; a full analysis of this type will provide many routes for synthesizing the target molecule. Taking the privilege of this approach, we have proposed a good number of synthesis schemes for bioluminescent natural product "Coelenterazine". Being a theoretical proposition, the synthesis works have not been executed in the laboratory. Strategic application of this approach can determine different routes to synthesize the target molecule even if the target molecule has never been synthesized earlier. Scalable synthetic routes for newly discovered natural products, drug molecules and useful compounds not available in adequate quantities from natural resources can be best provide by this approach. With the advancement and development of new reactions and reagents, the synthesis of best selling drugs can be rethinking for the improvements in existing process through this approach.

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