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Evaluation of Antimicrobial Activity of 3-(4-1H-Indol-3-yl)-(2,3-dihydro-1H-benzo[b]diazepin-2-yl)- 2H-chromen-2-one

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Abstract— 1,5- Benzodiazepines and its derivatives constitute an important class of heterocyclic compounds which possess wide range of therapeutic and pharmacological properties as anticonvulsant, analgesic, sedative, antidepressive, and hypnotic agents. In the last decade, the area of biological interest of 1,5-Benzodiazepines has been extended to several diseases such as cancer, viral infection and cardiovascular disorders. With this background, we had synthesized RVB-01 (3-(4-1H-Indol-3-yl)-2,3-dihydro-1H-benzo[b][1,5]diazepin-2-yl)-2H-Chromen-2-one) . Structure of this compound have been elucidated by using physical and extensive spectral data. The antimicrobial activity of RVB-01 against *B subtilis , P aeruginosa, E coli, M luteus, S aureus* was determined by agar diffusion method at 10, 50, 500 and 1000 mcg/ml using ciprofloxacin as a reference. MIC value of RVB-01 against *B subtilis* was determined by tube dilution method. RVB-01 showed remarkable activity against all microorganisms except *E coli* and having an MIC value of 250-300 mcg/ml against *B subtilis.* The results indicated that 2,3-Dihydro-1H-1,5-Benzodiazepines could be the potential candidate eliciting antibacterial activity, and further studies can be conducted using molecular modeling tools for designing 1,5-benzodiazepines having better activity.

Keywords : 1,5- Benzodiazepine, Antimicrobials, Kirby bauer disc diffusion method.

INTRODUCTION

Benzodiazepines are important organic molecules with a wide range of array of biological activities and therapeutic functions. Particularly 1,5-benzodiazepines are useful precursor for the synthesis of some fused rings benzodiazepine derivatives, such as triazolo-, oxadiazolo-, oxazino- or furano-benzodiazepines[1-3].

Many members of this family are widely used as antimicrobial[4], anticonvulsant, antianxiety, analgesic, sedative, antidepressive, hypnotic and anti-inflammatory agents[5-11]. Although the first benzodiazepine was introduced as a drug nearly 30 year ago, the research in this area is still very active and is directed towards the synthesis of compounds of enhanced pharmacological activity. Despite their wide range of pharmacological activity, industrial and synthetic applications, the synthesis of 1,5-benzodiazepines has received little attention. Although we are having traditional method of the synthesis of 1,5-benzodiazepines using α,β- unsaturated compounds as precursor, but all the previously used method have problems like drastic reaction conditions and also severe side reactions[12-13].

Surface-mediated solid phase reactions are of growing interest because of their ease of execution and work up, mild reaction conditions, rate of reaction, high yields, lack of solvent and low cost in comparison with their homogenous counterparts [13-20] due to the rising concern over environmental issues and the need for more efficient catalysts. Literature data revealed the significance of tetranitrile-silver complex[21], cage type mesoporous aluminosilicate[22], boric acid[11], phosphorous oxychloride[13], Clay (KSF and K10)-supported heteropoly acids[2], zirconium (IV) chloride[23], heteropoly acids including $H_{14}[NaP_5W_{30}O_{110}]$, $H_5[PMo_{10}V_2O_{40}]$, $H_6[P_2W_{18}O_{62}]$[24], 2,4,6-trichloro-1,3,5-triazine(TCT)[25], Molecular iodine[26], $CdCl_2$[27], InC_3[28], camphor sulphonic acid[29], piperidine[30], potassium iodide[31], versatile superacid catalyst "sulfated zirconia"[32], polymer supported ytterbium perfluoroctane sulfonate($Yb(OPf)_3$)[33], MgO,$Yb(OTf_3)$, $Sc(OTf_3)$, Al2O3, BF3-etherate, acetic acid and SiO2[34].

$KAl(SO_4)_2$. 12 H_2O has been widely used as a non-toxic, reusable, inexpensive and easily available catalyst for the processing of pechmann condensation[35]. The current work is to use this catalyst for the synthesis of 1,5-benzodiazepine and evaluate the molecule for its anti microbial property.

MATERIALS AND METHODS

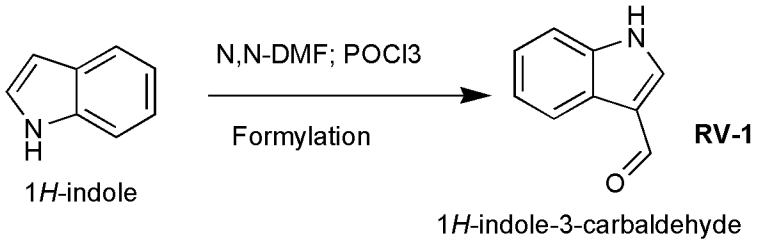
Drugs & Chemicals

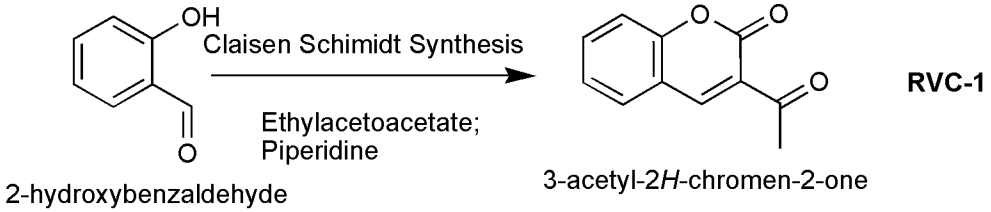
Reactants for the synthesis of molecule RVB-01 were procured from Sigma-aldrich ltd. Media and other materials for antimicrobial activity were procured from Hi-media ltd. All the drugs and chemicals except the test compound (RVB-01) were dissolved or diluted in distilled water and used for the experimentation purpose. **RVB-01** was dissolved in 100% DMSO and dilutions were made with distilled water so that the final concentration of DMSO did not exceed (0.1 % v/v).

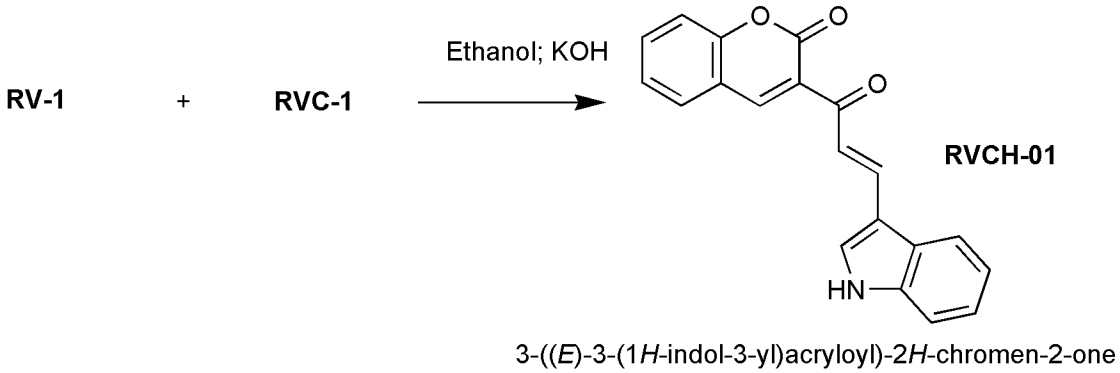
Experimental

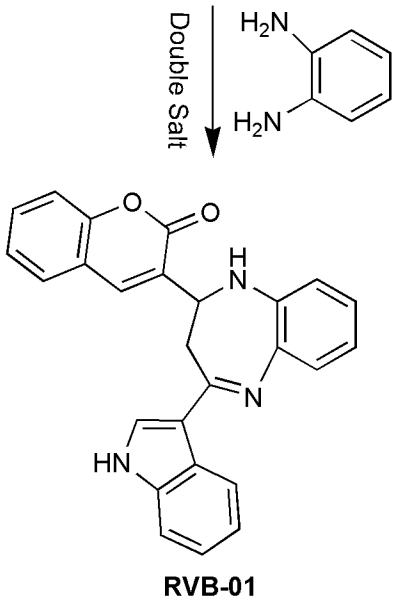
Melting points were determined using open capillary tube in Toshniwal Melting point apparatus and are presented without any corrections. The UV spectra were recorded on Shimadzu UV 1650. The infrared (IR) spectra were recorded on a FTIR-8310 Shimadzu spectrometer using potassium bromide pellets. The proton nuclear magnetic resonance (1H-NMR) spectra were recorded on AMX 400 at 200 MHz using tetramethylsilane (TMS) as the internal standard and DMSO as solvent, collected from IISC, Bangalore, India. The Mass spectral study was taken using Shimadzu LC-ESI- MS at Manipal Accunova, Manipal. Cell counting was done using haemocytometer of Rohem instrument pvt. Ltd. Rota evaporator from servewell instruments pvt ltd., Bangalore. UV chamber from Servewell instruments pvt ltd. All reagents were of the highest purity commercially available. The chemical shifts are expressed in part per million (ppm) downfield from the internal standard; the coupling constants are in Hz, and signals are quoted as *s* (singlet), *d*

(doublet), *t* (triplet), *q* (quartet), or *m* (multiplet). The purity of the compounds was checked by Thin Layer Chromatography using Merck Pre-coated silica gel GF aluminium plates and Ethyl acetate : Chloroform (15:85) as solvent system.









Synthesis of RVB-01(Refer Scheme 1)

Synthesis of Potassium Aluminium Sulfate Dodecahydrate:

Add 25ml of 3M KOH in a 250 ml Beaker containing Aluminium Pieces. Proceed the reaction in fuming hood and filter it while hot to remove undissolved carbon particles. Cool the reaction mixture and acidify it with continuous stirring using 3M H2SO4. Concentrate the mixture and allow it to stand for overnight to crystallize Potassium aluminium sulfate dodecahydrate, a catalyst(Double Salt).

*Synthesis of Indole -3-aldehyde (**RV-1**)*

Took 5 gm of indole & dissolved in 10 ml N,N-Dimethyl Formamide. In separate conical flask, Took 16 ml of N,N-DMF, kept it in ice bath & maintain temp 10-12 ^{o}C, then add 4 ml dropwise phosphorous chloride with continuous stirring for 30 min, bring the reaction mixture to R.T. The first solution of indole in DMF was added dropwise to this reaction mixture with continuous stirring over an additional 1.5 Hrs. Reaction mixture was poured into chipped ice which produces a clear red solution. Basify it with 5N NaOH till the precipitation occurs. Allow it to settle down. Dilute it with 250 ml Hot water. Cool it again followed by filtration of product using rota evaporator. Washed the product with cold water, dry it under vaccum conditions. Yield 78%.

*Synthesis of 3-acetyl-2H-chromen-2-one (**RVC-1**)*

Took 0.05 mole of Salicylaldehyde & 0.05 mole of ethylacetoacetate in a conical flask. Mixture was subjected to cool in an ice bath, followed by addition on 1 ml of piperidine with continuous stirring. The reaction mixture was kept at freezing pt. temp for 3 hrs, followed by addition of cold ethanol to break the lumps, filter the product and wash the product using cold ethanol, Dried it in the vacuum condition. Yield 64.15 %.

*Synthesis of 3-((E) -3-(1H-indol-3-yl)acryloyl)-2H-Chromen-2-one (**RVCH-01**)*

Equimolar concentration of RV-1 & RVC-1 was added in 50 ml ethanol with continuous stirring at 32 ^{o}C for 30 min, followed by addition of 10% KOH. The reaction mixture was continued to stir for next 4 Hrs. The container was kept overnight at room temperature, following by pouring of the reaction mixture into ice cold water, acidified using dilute HCL. The product was filtered out and dried in vacuum conditions. Yield 73.61 %

*Synthesis of 3-(4-1H-Indol-3-yl)-2,3-dihydro-1H-benzo[b][1,5]diazepin-2-yl)-2H-Chromen-2-one (**RVB-01**)*

0.05 moles of RVCH-01 & 0.06 moles of 0-phenylenediamine were added in 40 ml of ethanol containing 500 mg of double salt with continuous stirring for 2 hrs, followed by dilution with water. Extract the benzodiazepine analogue using ethylacetate successively for 3 times. Decant it and passed it through sodium sulfate. Kept it under dessicator for drying. RVB-01 was successfully formed with 75.81 % yield. Mol. Formula $C_{26}H_{19}N_3O_2$, Mol. Wt. 405.45, Log P(Calculated): 3.98$\pm$1.10, Melting point(^{o}C) 118-120, Solubility in DMF, DMSO, Acetonitrile, Acetone & Chloroform; UV : 275.4 nm ;LC-ESI-MS : 404.8 m/z$(M)^{+}$, 426.7 $(M\text{-}1+Na)^{+}$;IR(KBr): 3329.25 (NH), 3055.35,2922.25(Ar-H), 1716.70 (C=O), 1602.90 (C=C), 1226(C-O-C); 1H-NMR (CD_3OD): 3.3(s, 1H, 3^{o} - C<u>H</u>), 3.9 (s, 1H, Diazepin-N<u>H</u>), 1,225, 2.2161(d, 2H, 2^{o}- C<u>H</u>), 6.7-8.5 (m, 14H, Ar-<u>H</u>), 10.7(s, 1H, indolyl-N<u>H</u>) elucidated the synthesis of **RVB-01.**

Evaluation of Antimicrobial Activity

Kirby Baur Agar diffusion Method

RVB-01 was tested for antibacterial activity against the variety of test organisms Bacillus Subtilis, M. luteus, *Escherichia coli*, *Pseudomonas aeruginosa* and Coagulase positive *Staphylococcus aureus (COPS)* by the punch well and Disc diffusion methods on the Muller Hinton agar medium using Ciprofloxacin(5 µg) as the standard drug. The antibacterial screening was carried out with four different concentration of the synthesized novel molecule RVB-01 i.e. 10µg, 50µg, 500µg, 1000µg using DMSO as solvent.[36]

Tube Dilution Method

The minimum inhibitory concentration for RVB-01 against the same *Bacillus subtilis* organism used in the preliminary screening was carried out using microdilution susceptibility method. Ciprofloxacin was used as standard drugs[37, 38].

RESULTS & DISCUSSION

We have synthesized 3-(4-1H-Indol-3-yl)-2,3-dihydro-1H-benzo[b][1,5]diazepin-2-yl)-2H-Chromen-2-one and its structure was established using physical and spectral data like UV, FT-IT, 1H-NMR and Mass values. In the IR spectrum of RVB-01, prominent peaks are visible for C=O and NH functional groups at 1716.70 cm-1 and 3329.25 cm-1 respectively. This shows that these two functional groups are still existing in non-bonded stage in the compound. The peak of C-O-C appear at 1226 cm-1 in the spectra of compound and doesn't show major shifting. Similarly C=C and Ar-H peaks are appearing at 1602.90 and 3055.35 cm-1 and are showing slight shifting due to reduction in resonance characteristic of compound. In the proton NMR spectra of RVB-01, a peak at 3.3 appears due to tertiary CH group. A doublet is visible at 1.2, 2.2 which is due to splitting caused by secondary CH group. NH group shows its singlet at 10.7 and in the bonded form as diazepine NH, its peak is visible at 3.9. The multiplet in the range 6.7 – 8.5 is due to protons in the aromatic system. As far as mass spectrum data is concerned, a molecular ion peak at 404.8 can be assigned to the molecule of RVB-01 whereas after the addition of sodium ion most probably at amino group of indole ring, a peak appears at 426.7 due to $(M-1+Na)^+$. The results of preliminary antibacterial testing of compound RVB-01 are shown in **Table 1**.

ANTI MICROBIAL ACTIVITY OF RVB-01(Zone of Inhibition in mm)				
Name of Organism	***10 µg***	***50 µg***	***500 µg***	***1000 µg***
B. subtilis	*13*	*15*	*21*	*23*
P. aeruginosa	*15*	*17*	-	-
E. coli	-	-	*24*	*30*
M. luteus	*11*	*17*	*16*	*23*
S. aureus	*11*	*14*	*13*	*15*

Table 1 Results of Anti microbial screening of RVB-01.

The results of preliminary antimicrobial screening reveals that RVB-01 has significant effect on *B. subtilis, P. aeruginosa* even at 10 µg concentration, though the best results comes only at 500 µg & 50 µg respectively. In case of *E coli*, RVB-01 have no effect till 50 µg but have a significant bactericidal property at 500 and 1000 µg. In *M. luteus & S aureus*, RVB-01 have excellent antibacterial activity only at 1000 µg. The preliminary data put further the scope of RVB-01 against *B subtilis*. MIC value of RVB-01 against *B. subtilis* was found out to be 250-*300* µg/ml.

CONCLUSION

This study led us to conclude that $KAl(SO_4)_2$. $12H_2O$ can be better catalyst for the synthesis of 1,5- benzodiazepines. Derivative of 1,5-benzodiazepine RVB-01 have significant antimicrobial activity.

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