SYNTHESIS, CHARACTERIZATION AND BIOEVALUATION OF 3-SUBSTITUTED PHENYL-5-(2"',2"'-DIMETHYL,7"'-HYDROXYCHROMAN) ISOXAZOLE AND 3-(4'-CHLOROPHENYL)-5-(3"',4"',9"',10"'-TETRAHYDRO-2"', 2"',8"',8"'-TETRAMETHYL-2"'H,8"'H-DIPYRANYLBENZO [1,2-B:3,4-B'] ISOXAZOLE BESIDES MOLECULAR DOCKING STUDIES.
Synthesis, Characterization and Bioevaluation of 3-substituted phenyl-5-(2",2"-dimethyl,7"-hydroxychroman)isoxazole and 3-(4’-chlorophenyl)-5-(3",4",9",10"-tetrahydro-2",2",8",8"-tetramethyl-2"”H,8”H-dipyranylbenzo [1,2-b:3,4-b’]isoxazole besides Molecular Docking Studies.

PART: A

Introduction

Isoxazole: Azoles possess an oxygen and nitrogen next to each other at the 1, 2 positions, whereas oxazoles possess nitrogens at the 1, 3 positions. Isoxazole 1 is a π – excessive five-member ring containing two different heteroatoms, oxygen and nitrogen in adjacent positions.

![Isoxazole structure](image)

The naturally occurring antibiotic cycloserine¹ 2, the monamine oxidase inhibitor isocarboxazide 3, isoxazole steroids, ibotenic acid 4, muscimol 5 isolated from Amanita muscaria² and isoxazoline-5-ones, isolated from Legume seeds³, are potential isoxazole derivatives.
In the ultraviolet region, the unsubstituted isoxazoles ring absorb at 215 nm\(^4\) and the alkyl groups have a small effect (10-15 nm) either bathochromic or hypsochromic depending on the position of substitution\(^5\). 3-phenyl isoxazole absorbs at 239nm, whereas 5-phenylisoxazole absorbs near 260nm\(^6\).

In the IR region, ring-stretching vibrations of isoxazole ring occur in the region 1300-1600cm\(^{-1}\). The C\(_4\)-H stretching occurs in 1085-1215 cm\(^{-1}\) and C\(_5\)-H stretchings\(^7\) occur near 960cm\(^{-1}\). Strong electron acceptors at the 4-position and strong electron donors at 3– or 5- positions cause a band of high intensity\(^8\). Characteristic bands occur at 1000-1300 cm\(^{-1}\) region for 3, 4- and 3, 5-disubstituted isoxazoles, while bands below 1000cm\(^{-1}\) represent general substitution pattern\(^9,10\).

In the \(^1\)H NMR spectra of the unsubstituted isoxazoles, the C\(_3\)-H appears at δ 8.2-8.3; the C\(_4\)-H appears between δ6.3-6.5 while C\(_5\)-H appears at δ 8.4-8.6 \(^{11}\). In 3, 5-disubstituted isoxazoles, the C\(_4\)-H resonates at δ 6.77 for 3-aryl-5-phenyl isoxazole and around δ6.7-7.05 for 3-phenyl-5-aryl isoxazoles depending on the substitution. Appreciable variation of chemical shifts of C\(_4\)-H is observed when the substituted phenyl group is at 5-position\(^{12}\).

In the \(^{13}\)C NMR, the C\(_3\)-carbon resonates at δ149.1, C\(_4\)-carbon at δ103.7 and C\(_5\)-carbon at 157.9 for the unsubstituted isoxazole\(^{13}\), whilst in 3, 5-diaryl isoxazole the C\(_3\)-carbon resonates at δ161.8, C\(_4\)-carbon at δ98.0, the C\(_5\)-carbon appears at δ168.9 \(^{14}\).
The mass spectra of all diphenyl isoxazoles contain rearranged peaks at m/z 165 \((C_{13}H_{19})^+\). In addition, the spectra of 3,5-diphenyl isoxazoles contain peaks 180 \((C_{13}H_{10}N)^+\), which are produced by specific phenyl migrations\(^{15}\). Further, the fragmentation patterns of 3-aryl-5-phenyl and 3-phenyl-5-aryl-isoxazole are significantly different\(^{16}\).

The microwave spectra\(^{17}\) of an isoxazole-CO complex and four of its isotopomers have been measured between 7 and 18 GHz with a pulsed nozzle Fourier transform microwave spectrometer. Rotational constants, centrifugal distortion constants, and N-14 quadruple coupling constants have been fitted to the measured transition frequencies of each isotopomer. The permanent electric dipole moment has been determined from stark effect splitting. The permanent electric dipole moment has been determined from stark effect splitting. A unique structure has been found for the complex by taking both the moments of inertia of the isotopomers and the quadrupole splittings into account. The complex is planar, with CO lying approximately radially away from nitrogen in the isoxazole ring.

The \(^{13}\)C chemical shifts of 572 isoxazoles are reported by Martins et al\(^{18}\), some of them in several solvents. Simple models were used to rationalize, the substituent effects on the C\(_3\), C\(_4\) and C\(_5\) of the isoxazole ring. In addition, the O\(^{17}\) chemical shifts of 10 isoxazoles and N\(^{15}\) chemical shifts of 7 isoxazoles are also reported. The X-ray crystal structure of 4-phenyl-1, 5-(2, 3, 4-trimethoxy phenyl)-isoxazole, \(C_{18}H_{17}NO_4\) has been determined by Sanmantin. R. et al\(^{19}\), revealing an interesting lack of coplanarity for the aromatic rings system.
General methods of synthesis of isoxazoles:

The synthetic routes to isoxazoles may be classified according to the number of isoxazole ring atoms in each component synthon (eg. 3+2), the type of ring atom in each component (eg. C-C-C + N-O) and the chemical class to which each component belongs (eg. 1, 3-dicarboxyl compound + hydroxylamine).

Of all the synthetic routes, the (3+2) routes are of prime importance, in which there are two types of synthons, viz., CCC + NO and CNO + CC; both are 1, 3-dipolar additions.

The CCC + NO routes contain the 1, 3-dicarbonyl system, usually a 1, 3-diketone or a ketoaldehyde as CCC unit and the NO unit represented by the hydroxylamine or its N-substituted derivatives.20

3-Alkyl, 5-aryl isoxazoles 6 were also prepared from aryl cycolpropanes21 with NaNO2 in CF3COOH (Scheme I).

![Scheme I](image)

Novel preparation of 3-alkyl-5-hydroxy-5-per (poly) fluoroalkyl-4, 5-dihydro isoxazoles has been synthesized by a 1, 3-dipolar-cyclo addition of trimethylsilylnitronates to 1-bromo-1-per (poly) fluoro alkyl ethane via one pot
(or) two-step reaction\textsuperscript{22}. The formation of the isoxazole derivative takes place by cyclisation of oximes in the presence of iodine and potassium iodide\textsuperscript{23}.

Nitro compounds, N-oxides and oximes have provided the CNO sequence in the (CNO + CC) routes, by far the most important CNO component are chloroximes and nitrile oxides. This CNO + CC route i.e, 1, 3-dipolar cycloaddition reactions of nitrile oxides with C = C and C≡C dipolarophiles are the most important and many were reported. The chemistry of nitrile oxides has been well reviewed\textsuperscript{24}.

Intramolecular nitrile oxide cycloaddition is a powerful method for constructing complex ring systems\textsuperscript{25}. Thus phthalaldehyde could be mono protected as benzylidene derivative and carried through several steps including intramolecular silylnitronate or nitrile oxide cycloaddition to furnish substituted indenoisoxazole derivative 7 and 8 with high diastereo selectivity (Scheme-II).
Reagents: (a). BuPh₃P+Br⁻, 10% aq.NaOH, CH₂Cl₂ - Water 0-25⁰C/hr

(b). CH₃NO₂, NH₄OAc, AcOH, reflux, 8 hr:

(c). NaBH₄, EtOH-dioxane (reverse addition), 0-25⁰C, 3 hr;

(d). TMSCl, Et₃N, 0-25⁰C, CH₂Cl₂, 18 hr

(e). PhNCO, Et₃N, benzene, 0-25⁰C, 20 hr
Addition of nitronate anions to 3-bromochromone and 6-bromofurfurochromone results in the efficient generation of 3-hydroxy-2-(1-(hydroxyimino)alkyl, aryl or carbo alkoxy) substituted chromones and the corresponding 6, 7-disubstituted furochromones. These compounds are efficiently converted to furo(3, 2 : 6,7) benzopyrano(2,3-d)isoxazoles and chromano(2,3-d) isoxazoles respectively with N,N-dimethyl formamide and dimethyl acetal (DMF-DMA) (scheme-III).

Treating 4-methoxy benzaldehyde oxime with NCS in DMF followed by reaction of the resulting 4-methoxy benzaldehyde chloroxime with propargyl
alcohol in the presence of Et₃N in Et₂O afforded 57% of substituted (3-(4-methoxyphenyl)Isoxazol-5-yl)methanol²⁷ of the type 10.

![Chemical Structure](image)

(3-(4-methoxyphenyl)Isoxazol-5-yl)methanol 10

A novel synthesis of isomeric isoxazoles 11 & 12 was reported by Thakare, et al²⁸ via region-selective cyclo condensation of β-(2¹-furyl) acrylophenone dibromides, 2-OH-3-R-5-Cl-C₆H₂CO (CHBr)₂R₁ with hydroxyl amine hydrochloride (Scheme-IV).

![Chemical Reaction](image)

Scheme IV
The application of microwaves in promoting the cyclo addition reactions of allylic alcohols with nitrile oxides using a domestic microwave oven and a focused monomode microwave reactor demonstrated that not only was the reaction time substantially reduced, but also the reaction yields were significantly improved over the conventional stirred reactions. Microwave irradiation alters the region-selectivity of the cycloaddition reaction, which favours the non-hydrogen bond directed cycloadducts \(^{29}\), Isoxazolines 13.

\[
\begin{align*}
R_1 &= \text{H, Me} \\
R_2 &= \text{H, Me, Et, Pr, Ph} \\
R_3 &= \text{H, Me, Et.}
\end{align*}
\]

Naik, et al \(^{30}\) synthesized some 2\(^{1}\)-hydroxy-3\(^{1}\)-bromo-5\(^{1}\)-ethyl chalcones and 3-(2\(^{1}\)-hydroxy-3\(^{1}\)-bromo-5\(^{1}\)-ethyl phenyl)-5-substituted phenyl-2-isoxazoles by condensation of \(\alpha,\beta\)-dibromo chalcones with hydroxylamine hydrochloride in EtOH in presence of KOH, which showed medium antibacterial activity.

All reactions leading to isoxazoles must involve cyclization via, a cyclic transition state, which contains all five atoms of the isoxazole ring. In some cases the acyclic intermediates are stable and capable of isolation, in which the acyclic precursor is either the starting material or an isolable intermediate \(^{31}\), which constitute the (5+0) routes for the synthesis of isoxazoles; in these reactions, the substituents in the products are unambiguously located (Scheme-V).
Synthesis of isoxazoles from other heterocyclic compounds has been reported. Ring contraction of condensed 4-pyrones continues to play a useful role in the synthesis of steroidal isoxazoles. The formation of the isoxazolone
from the 1, 3, 5-dioxazolidone $14$ and diethylaminopropyne, exemplifies the principle and the compound $14$ represents a cyclic NOC synthon (Scheme-VI).

![Scheme-VI](image)

Other transformations giving isoxazoles and / or their derivatives are (i) Heterocyclization reaction with ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate$^{34}$; (ii) An efficient region-selective cleavage of pyrano[3,4-C] isoxazoles with boron trihalide $^{35}$; (iii) Intramolecular cyclization of 3-aryl-2-nitro acrylates promoted by titanium tetrachloride$^{36}$; (iv) Thermolysis of 2-(4-nitro-1H-imidazol-5-yl) acetate and malonate derivatives$^{37}$; (v) 1,3-dipolar cyclo additions of pyrazole nitrile oxides for the synthesis of novel C-60-fused isoxazolines$^{38}$; (vi) Heterocyclic synthesis via enaminones$^{39}$; (vii) Intramolecular cyclo addition of 3-O-alkynyl carbohydrate nitrile oxides$^{40}$; (viii) Intramolecular oxime olefin cycloadditions$^{41}$; (ix) Improved microwave induced condensation of 1,3-dihydro-3-(2-phenyl-2-oxoethylidene)-2H-indole-2-ones with hydroxylamine hydrochloride$^{42}$; (x) 1,3-dipolar cyclo addition reaction between nitrile oxides and stannyl alkynes which proceeds in a region-specific manner$^{43}$; (xi) Synthesis of
isoxazoles using poly(ethylene glycol) as support\textsuperscript{44}; (xii) Formation of acetyl and 3-benzoylisoxazole derivatives via nitrile oxide using CAN\textsuperscript{45}.

Murthy et al\textsuperscript{46} had synthesized and characterized a few isoxazoles and examined their pharmacological activities. In continuation of the above investigations on isoxazoles; In this Chapter we describe the synthesis and characterization of chromanoisoxazoles, \textbf{26-30}; (\textbf{Section A & B}) and biological activity studies (\textbf{Section-C}). The compounds synthesized are as follows:

1) 3-(3\textsuperscript{1}, 4\textsuperscript{1}-dimethoxy phenyl)-5-(2\textsuperscript{“},2\textsuperscript{“}-dimethyl, 7\textsuperscript{”}-hydroxy chroman) isoxazole \textbf{26}.

2) 3-(4\textsuperscript{‘}-N, N\textsuperscript{’}-dimethylamino phenyl)-5-(2\textsuperscript{“}, 2\textsuperscript{”}-dimethyl, 7\textsuperscript{”}-hydroxy chroman) isoxazole \textbf{27}.

3) 3-(4\textsuperscript{”}-chloro phenyl)-5-(2\textsuperscript{“}, 2\textsuperscript{”}-dimethyl, 7\textsuperscript{”}-hydroxy chroman) isoxazole \textbf{28}.

4) 3-(4\textsuperscript{‘}-methoxy phenyl)-5-(2\textsuperscript{“}, 2\textsuperscript{”}-dimethyl, 7\textsuperscript{”}-hydroxy chroman) isoxazole \textbf{29}.

5) 3-(4\textsuperscript{‘}-cyano phenyl)-5-(2\textsuperscript{“},2\textsuperscript{”}-dimethyl, 7\textsuperscript{”}-hydroxy chroman) isoxazole \textbf{30}. 


PART: B

Present work

Section-A: Synthesis, Characterization and Bioevaluation of 3-substituted phenyl-5-(2"",2""-dimethyl,7""-hydroxychroman)isoxazole.

In this chapter the synthesis and characterization of some new isoxazoles is presented. A brief review given about highlights their spectral characteristics and methods of synthesis.

The method employed for the synthesis of isoxazoles has been the common (3+2) route. These isoxazoles were prepared from chalcones, which are important intermediate products, as they possess varied biological and pharmacological activities. They can be obtained by the acid or base catalyzed aldol condensation of hydroxyl acetophenones with benzaldehydes.\(^{47-49}\)

For example, 2-hydroxy acetophenone and benzaldehyde react in the presence of 0.1M NaOH to give the chalcone \(16\) (Scheme-VII)\(^ {50}\).

![Scheme-VII](image)

Cinnamic acid condenses with resorcinol in chloroform in the presence of boron trifluoride to yield the chalcone \(17\) (Scheme-VIII)\(^ {51}\).

![Scheme-VIII](image)
The synthesis and characterization of five new chalcones have been reported below adopting the following synthetic procedure (Scheme-IX).

Resorcinol 18 was acylated using acetic acid in the presence of fused ZnCl$_2$ at 140-150$^\circ$C for 15 min with stirring. The reaction mixture was left for 1 hr and then 100 ml 1:1 HCl was added to break the zinc chloride complex and within 5
minutes precipitation commenced. The precipitate was washed with very dilute HCl and water. A red precipitate was obtained, which was crystallized from 20% HCl to give resacetophenone needles and characterized by comparing its spectral data (IR, NMR) with those reported in literature.\(^{52}\)

The resacetophenone 19 on nuclear prenylation\(^{53}\) with isoprene in the presence of PPA/Xylene, at room temperature gave a mixture of three products (20A, 20B and 20C) in the ratio of 2:3:1. They were separated by column chromatography on silica gel and crystallized. The slowest moving compound 20C gave a negative ferric chloride reaction but a positive DNP test and its elemental analysis showed the incorporation of two isoprene units which was further confirmed by the NMR data. Both the compounds 20A and 20B gave positive ferric chloride reaction and their elemental analysis showed the incorporation of one isoprene unit. On the basis of \(^1\)H and \(^{13}\)CNMR data, the compounds 20A and 20B were characterized as 3,4-dihydro-5-hydroxy-2,2-dimethyl-6-acetyl-2H-1-benzopyran and 3,4-dihydro-7-hydroxy-2,2-dimethyl-6-acetyl-2H-1-benzopyran respectively. Compound 20C was characterized as 3, 4, 9, 10-tetrahydro-2, 2, 8, 8-tetramethyl-6-acetyl-2H, 8H-benzo [1, 2-b: 3,4b’] dipyran, and its structure was confirmed by spectral data and elemental analysis. \(^1\)H NMR and IR spectra of compound 20B are presented in figure-1 & figure-2 respectively. Figures-3 & 4, depict the \(^1\)H NMR and IR spectra of compound 20C. The spectral values of compounds 20A, 20B and 20C are:
Fig 1: $^1$H spectrum of compound 20B.
Fig 2: IR spectrum of compound 20B.
Compound 20A:

$^1$H NMR (CDCl$_3$): δ1.46 (s, 6H); δ1.91 (t, 2H); δ2.72 (t, 2H); δ2.5 (s, 3H); δ6.35 (d, 1H); δ7.4 (d, 1H); δ13.1 (s, 1H, OH).

IR ($v_{\text{max}}$ cm$^{-1}$, Nujol): 3350 cm$^{-1}$(OH, str); 1680 cm$^{-1}$(C=O, str).

Compound 20B:

$^1$H NMR (CDCl$_3$): δ1.3 (s, 6H); δ1.8 (t, 2H); δ2.7 (t, 2H); δ2.5 (s, 3H); δ6.3 (s, 1H); δ7.5 (s, 1H); δ12.2 (s, 1H, OH). (Fig 1)

IR ($v_{\text{max}}$ cm$^{-1}$, Nujol): 3343.3 cm$^{-1}$(OH, str); 1660.18 cm$^{-1}$(C=O, str). (Fig 2)

Compound 20C:

$^1$H NMR (CDCl$_3$): δ1.33 (s, 6H); δ1.36 (s, 6H); δ1.6 (2H); δ1.7 (t, 3H); δ2.61 (t, 2H); δ2.71 (t, 2H); δ2.59 (s, 3H, COCH$_3$); δ7.49 (s, 1H). (Fig 3)

IR ($v_{\text{max}}$ cm$^{-1}$, Nujol): 1685.68 cm$^{-1}$(C=O, str). (Fig 4)

The chroman 20B on condensation with different substituted benzaldehyde in the presence of 30% alcoholic alkali at room temperature resulted in the formation of the corresponding chalcone derivatives 21-25 (yield, 85%) (Scheme-IX).
Fig 3: $^1$H NMR spectrum of compound 20C.
Fig 4: IR spectrum of compound 20C.
The thin layer chromatography of these chalcones showed characteristic colour spots with methanol-sulphuric acid (9:1) as a spraying reagent. They also exhibited the characteristic colour test with antimony trichloride \(^{54}\). With the above procedure, the following five compounds \(21,22,23,24\) and \(25\) were synthesized.

**Synthesis of 7-hydroxy-6-(4′-chloro) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2H benzo(1,2b) pyran (23):**

2,2-Dimethyl-6-acetyl-7-hydroxy chroman \(20\) on condensation with chlorobenzaldehyde in the presence of 30% alcoholic alkali at room temperature resulted in the formation of chalcone \(23\) [yield:75%]; crystallized from methanol as yellow needles. Mol. For: \(C_{20}H_{19}O_3Cl\); m.p.210\(^{0}\)C. The purity of the compound \(23\) was checked by TLC and HPLC. The \(^1\)H NMR spectrum was recorded and data it is presented below.

\(^1\)H NMR spectrum/CDCl\(_3\) (TMS): \(\delta 1.35(s, \text{ CH}_3)\) and \(1.4(s, \text{ CH}_3)\): gem dimethyls, \(\delta 1.8(t, 2H, \text{ C}_3\text{-methylene protons})\) and \(\delta 2.8 (t, 2H, \text{ C}_4\text{-methylene protons})\). \(\Delta7.3-7.5 \text{ (m,4H, aromatic protons), } \delta 7.63(d,1H) \& \delta 8.05 (d,1H)\) CH=CH double bond protons, \(\delta 7.3 \text{ (s, 1H, C}_5\text{-H) and } \delta 6.45(s, 1H, C}_5\text{-H) and } \delta 6.45(s, 1H, C}_8\text{-H)\)

Further in its \(^{13}\)C NMR spectrum, the \(\alpha-\beta\) unsaturated carbonyl carbon was observed at \(\delta 189.8\), confirming the chalcone \(23\).
Fig 5: $^1$H NMR spectrum of compound 23.
Fig 6: IR spectrum of compound 23.
Adopting the above procedure, the chroman 20B was condensed with veratraldehyde, 4-N, N’-dimethylamino benzaldehyde, 4-methoxy benzaldehyde, and 4-Cyano benaldehyde and the corresponding chalcones 21, 22, 24, 25 were obtained in good yields and purified using column chromatography. The structures of chalcones (21-25) were confirmed by elemental analysis and various spectroscopic techniques. The ¹H NMR spectral characteristics of these five chalcones are given in Table-1. The figures 5 & 6, show ¹H NMR and IR spectra of compound 23, 7-hydroxy-6-(4’-chboro) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2H benzo (1,2b) pyran.
Table 1

$^1$H NMR data of Pyrano chalcones (21-25)*

<table>
<thead>
<tr>
<th>Proton No.</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>1.32 (s, 3H, CH$_3$)</td>
<td>1.33 (s, 3H, CH$_3$)</td>
<td>1.35 (s, 3H, CH$_3$)</td>
<td>1.3 (s, 3H, CH$_3$)</td>
<td>1.31 (s, 3H, CH$_3$)</td>
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<tr>
<td>2b</td>
<td>1.41 (s, 3H, CH$_3$)</td>
<td>1.43 (s, 3H, CH$_3$)</td>
<td>1.4 (s, 3H, CH$_3$)</td>
<td>1.4 (s, 3H, CH$_3$)</td>
<td>1.45 (s, 3H, CH$_3$)</td>
</tr>
<tr>
<td>3</td>
<td>1.83 (t, 2H,3-CH$_2$)</td>
<td>1.8 (t, 2H,3-CH$_2$)</td>
<td>1.7 (t, 2H,3-CH$_2$)</td>
<td>1.71 (t, 2H,3-CH$_2$)</td>
<td>1.85 (t, 2H,3-CH$_2$)</td>
</tr>
<tr>
<td>4</td>
<td>2.57 (t, 2H,4-CH$_2$)</td>
<td>2.7 (t, 2H,4-CH$_2$)</td>
<td>2.8 (t, 2H,4-CH$_2$)</td>
<td>2.6 (t, 2H,4-CH$_2$)</td>
<td>2.7 (t, 2H,4-CH$_2$)</td>
</tr>
<tr>
<td>5</td>
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<td>7.5 (s, 1H)</td>
<td>7.3 (s, 1H)</td>
<td>7.45 (s, 1H)</td>
<td>7.38 (s, 1H)</td>
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<td>8</td>
<td>6.27 (s, 1H)</td>
<td>7.0 (s, 1H)</td>
<td>6.45 (s, 1H)</td>
<td>6.7 (s, 1H)</td>
<td>6.37 (s, 1H)</td>
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<tr>
<td>CH=CH</td>
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<td>7.89 (d, 1H)</td>
<td>7.63 (d, 1H)</td>
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<td>7.56 (d, 1H)</td>
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<td>OCH$_3$</td>
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<td>-</td>
<td>-</td>
<td>3.9 (s)</td>
<td>-</td>
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<tr>
<td>N-Me$_2$</td>
<td>-</td>
<td>2.85 (-N(CH$_3$)$_2$)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

*CDCl$_3$ / TMS
The chalcones synthesized are:

1. 7-Hydroxy-6-(3’, 4’-dimethoxy) cinnamoyl, 3,4-dihydro-2,2-dimethyl-2H benzo(1,2b) pyran 21.

2. 7-Hydroxy-6-(4’-N,N-dimethyl amino) cinnamoyl, 3,4-dihydro-2,2-dimethyl-2H benzo (1,2b) pyran 22.

3. 7-Hydroxy-6-(4’-chloro) cinnamoyl, 3,4-dihydro-2,2-dimethyl-2H benzo (1,2b) pyran 23.

4. 7-Hydroxy-6-(4’-methoxy) cinnamoyl, 3,4-dihydro-2,2-dimethyl-2H benzo (1,2b) pyran 24.

5. 7-Hydroxy-6-(4’-cyano) cinnamoyl, 3, 4-dihydro-2,2-dimethyl-2H benzo (1,2b) pyran 25.

These chalcones, forming the C₃ framework, on condensation with hydroxylamine hydrochloride, gave 3-substituted phenyl-5 (2”, 2”-dimethyl, 7”-hydroxy chroman) isoxazoles.

Synthesis of 3-substituted phenyl-5-(2”’,2”’-dimethyl, 7”’-hydroxy chroman) isoxazoles (26-30):

A large number of 3, 5-diaryl isoxazoles have been prepared in literature adopting the (3+2) route (in several cases the carbon units being chalcone derivatives). These isoxazoles were also reported to possess pharmacological activity. This prompted the present work in preparing some new 3-substituted phenyl-5-(2”, 2”-dimethyl-7”-
hydroxy chroman) isoxazoles. For this purpose, above synthesized chalcones (21-25) were condensed with hydroxylamine hydrochloride.

![Scheme X](image)

**Scheme X**

21. $R_1, R_4, R_5 = H$ & $R_2, R_3 = OCH_3$.  
22. $R_1, R_2, R_4, R_5 = H$, $R_3 = N(CH_3)_2$.  
23. $R_1, R_2, R_4, R_5 = H$, $R_3 = Cl$.  
24. $R_1, R_2, R_4, R_5 = H$, $R_3 = OCH_3$.  
25. $R_1, R_2, R_4, R_5 = H$, $R_3 = CN$.  
26. $R_1, R_4, R_5 = H$ & $R_2, R_3 = OCH_3$.  
27. $R_1, R_2, R_4, R_5 = H$, $R_3 = N(CH_3)_2$.  
28. $R_1, R_2, R_4, R_5 = H$, $R_3 = Cl$.  
29. $R_1, R_2, R_4, R_5 = H$, $R_3 = OCH_3$.  
30. $R_1, R_2, R_4, R_5 = H$, $R_3 = CN$.

The above chalcones were condensed with hydroxylamine hydrochloride in presence of KOH/ethanol; after usual work up and purification by column chromatography the major compound was isolated and crystallized from methanol which furnished crystalline products (Scheme-X).

The elemental analysis for the above compounds were determined and found satisfactory. In IR spectra the absence of chalcone carbonyl was noticed and the
characteristic ring stretching vibrations of isoxazoles between 1600-1300 cm$^{-1}$ and 1300 - 1200 cm$^{-1}$ was observed, confirming the formation of isoxazole ring (7, 8).

When the aryl rings are differently substituted it is possible to get a mixture of isomeric 3, 5-diaryl isoxazoles. The condensation of different aryl substituted
chalones with hydroxyl amine hydrochloride can give rise to the two isomeric 3,5-
diaryl isoxazoles, depending upon the site of the initial attack by the nucleophilic
amino nitrogen on the carbonyl carbon (or) the β-carbon of the α,β-unsaturated
carbonyl system. In the former case the reaction goes through the initial formation of
the oxime which subsequently cyclizes to give isoxazoline derivatives and subsequent
oxidation to give isoxazoles of type I. In the later case the hydroxylamine
hydrochloride undergoes Michael addition at the β-carbon to give adducts; they
cyclize to give isoxazolines by elimination of a molecule of water. The isoxazolines
further get oxidized to give isoxazoles of Type II (Scheme-XI).

The isoxazoles obtained in the reaction might be a mixture of these two, unless the
reaction is regio-specific giving one or the other exclusively. Although the products in
the reaction were found to be single on TLC, the possibility of a mixture cannot be
excluded in view of closeness in structure. If they form Type I, isomers the isoxazoles
formed with a 5-aryl substitution change, while 3-aryl substitution is not changed.
Alternatively, if they form Type II isomers, 5-aryl substitution is not changed, while
the 3-aryl substitution changes. In the absence of regio-specificity, simultaneous attack
of the reagent at both the sides give mixtures and consequently the isomer ratio might
depend probably more on the reaction conditions and to a less extent on the aryl
substitution pattern. It is rather difficult to decide between these two structures by the
chemical shift of the lone olefinic proton of the isoxazole system for, the chemical
shift of that proton in either system might be very close. However, a report in
literature (12) on the $^1$H NMR spectral study of several 3, 5-disubstituted isoxazoles and it is observed that the chemical shifts of the C₄-H were essentially unaffected by the presence of para or meta substitution in the phenyl group at position 3 and appreciable variation (6.7-7.05) in chemical shift of C₄-H with changes in substitution in the phenyl group at position 5. Similar conclusions were drawn from the $^{13}$C chemical shifts (14) of 3,5-diaryl isoxazoles when substitution pattern in 3-aryl group has little effect on the carbon of isoxazole ring while the effect of substituents in the 5-aryl series was more pronounced on the chemical shifts of isoxazole ring atoms.

In the light of the earlier studies by Murthy et al 46, 55 and above observations, it was concluded that the isoxazoles formed are of type II.

The isoxazoles thus synthesized are:

1. 3-(3’,4’-dimethoxy phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole 26.
2. 3-(4’-N,N’-dimethyl amino phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole 27.
3. 3-(4’-chloro phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole 28.
4. 3-(4’-methoxy phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole 29.
5. 3-(4’-cyano phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole 30.

Synthesis of 3-(3’,4’-dimethoxy phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole 26:
7-hydroxy-6-(3’, 4’-dimethoxy) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl 2H benzo (1,2b) pyran 21 and hydroxylamine hydrochloride in presence of KOH/absolute ethanol was refluxed on a water bath for 4 hrs. Then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water, which resulted in the formation of a brown precipitate, which was purified using column chromatography. The pure compound 26 was crystallized from methanol as pale yellow needles in 60% yield. The purity of the compound was checked by HPLC (Figure-7).

Column : Shim-pack CLC ODS column

Flow rate : 1 ml/min

Injected quantity : 5µl

% Purity : 95.7959 %

Retention time (R_t) 4.33 min

Compound 26 analyzed for C_{22}H_{23}O_{5}N, m.p.178^{0}C. IR spectrum was recorded and the following values were observed IR (ν_{max}): 3435, 2974, 2927, 1615, 1524, 1387, 1277, 1196, 1040 cm^{-1}. 
Fig 7: HPLC chromatogram of compound 26
Fig 8: $^1$H NMR chromatogram of compound 26
The $^1$H NMR spectrum (Figure-8) of compound 26 showed the characteristic $C_4$-H at $\delta 6.9$ as singlet. Two methyl groups at $\delta 1.38$ (s, 6H); two methylene groups at $\delta 1.7$-1.9 (br, t, $3''$-CH$_2$) and $\delta 2.8$ (t, $4''$-CH$_2$), 3.9 (s, OCH$_3$), $C_5$-H and $C_8$-H at 7.55 (s,1H) and $\delta 6.35$ (s, 1H), aromatic protons – 6.8(s, 1H, $C_2$-H), 7.8(d, 1H, $C_6$-H), 7.6 (d, 1H, $C_5''$-H).

The $^{13}$C NMR spectrum further confirmed the structure 26. The characteristic $C_3$-carbon resonates at $\delta 151.4$, $C_4$-carbon at $\delta 100.3$ and $C_5$-carbon appears at $\delta 156.8$. Based on the above spectral studies compound 26 was confirmed as 3-(3’, 4’-dimethoxy phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole.

**Synthesis of 3-(4’-N, N-dimethyl amino phenyl)-5-(2”, 2”-dimethyl, 7”-hydroxy chroman) isoxazole (27):**

7-Hydroxy-6-(4’-N, N-dimethyl amino) cinnamoyl, 3, 4-dihydro-2,2-dimethyl 2H benzo (1,2b) pyran 22 and hydroxylamine hydrochloride in absolute ethanol in presence of KOH was refluxed on a water bath for 5 hrs. The reaction mixture after neutralization with acetic acid and usual work up, the product slowly separated out as brown precipitate, which slowly separated out. The precipitate was filtered and was chromatographed over silica gel eluting with hexane and EtOAc. The pure compound 27 crystallized from methanol as colour less needles in 62% yield. The purity of the compound was checked by HPLC.

Compound 27 was analyzed for $C_{22}H_{24}O_3N_2$; m.p.206$^0$C.
The $^1$H NMR spectrum (Figure-9) of compound 27 showed the characteristic C$_4$-H at δ7.44 as singlet. Two methyl groups at δ1.34(s) and δ1.38(s) and two methylene groups at δ1.78(t, 3″-CH$_2$) and δ2.6(t, 4″-CH$_2$), 3.01(N-methyis), 7.55(s, 1H, C$_5$″), δ7.51(s, 1H, C$_8$″), 6.65(d,2H, C$_3$″ and C5″), 7.49(d,2H, C$_2$″ and C$_6$″) supporting the structure as 27.

IR spectrum (Figure-10) showed the characteristic ring stretching vibrations (O-N) at 1638 cm$^{-1}$.

IR($\nu_{max}$): 3436,3103,2970,2946,2926,1638,1600,1475,1366,1140,1015 cm$^{-1}$.

The $^{13}$C NMR spectrum further confirmed the structure 27. The characteristic C$_3$-carbon resonates at δ150.8, C$_4$ carbon at δ99.0 and C$_5$ carbon appears at 157.2.

Based on the above spectral studies, the compound 27 is confirmed as 3-(4’-N,N-dimethyl amino phenyl)-5-(2″,2″-dimethyl, 7″-hydroxy chroman) isoxazole.

Adopting the above synthetic procedure the isoxazoles 28, 29, 30 were synthesized. The physical and spectral characteristics of five new isoxazoles were tabulated and presented in Tables-2, 3 and 4. The purity of the above compounds also was checked by HPLC and HPLC chromatograms for the compounds 29 and 30 are shown in figures-11 and 12 respectively. Similarly, 1H NMR and IR spectra recorded for the compound 30 are shown in figures-13 and 14.
Fig 9: $^1$H NMR chromatogram of compound 27
Fig 10: IR chromatogram of compound 27.
Fig 11: HPLC chromatogram of compound 29.
Fig 12: HPLC chromatogram of compound 30.
Fig 13: $^1$H NMR spectrum of compound 30
Fig 14: IR spectrum of compound 30.
### Table-2

Physical and analytical data of 3-substituted phenyl-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazole (26-30).

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Molecular formula</th>
<th>Yield (%)</th>
<th>$R_f$ Values</th>
<th>m.p ($^\circ$C)</th>
<th>HPLC Purity* (%)</th>
<th>$R_t$ (min)</th>
<th>Elemental analysis Calc. (Found)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$C$</td>
</tr>
<tr>
<td>26</td>
<td>C$<em>{22}$H$</em>{23}$O$_5$N</td>
<td>60</td>
<td>0.4</td>
<td>178</td>
<td>95.79</td>
<td>4.322</td>
<td>69.29 (69.01)</td>
</tr>
<tr>
<td>27</td>
<td>C$<em>{22}$H$</em>{24}$O$_3$N$_2$</td>
<td>62</td>
<td>0.6</td>
<td>206</td>
<td>95.62</td>
<td>3.931</td>
<td>72.52 (71.99)</td>
</tr>
<tr>
<td>28</td>
<td>C$<em>{20}$H$</em>{18}$O$_3$NCl</td>
<td>56</td>
<td>0.5</td>
<td>176</td>
<td>98.54</td>
<td>4.245</td>
<td>67.60 (67.41)</td>
</tr>
<tr>
<td>29</td>
<td>C$<em>{21}$H$</em>{21}$O$_4$N</td>
<td>64</td>
<td>0.4</td>
<td>185</td>
<td>100</td>
<td>4.390</td>
<td>71.79 (71.43)</td>
</tr>
<tr>
<td>30</td>
<td>C$<em>{21}$H$</em>{18}$O$_3$N$_2$</td>
<td>52</td>
<td>0.7</td>
<td>146</td>
<td>100</td>
<td>3.914</td>
<td>75.90 (75.47)</td>
</tr>
</tbody>
</table>

@ Solvent system – Hexane: EtOAc (9:1)

*H.P.L.C Experimental Conditions: Mobile Phase - Acetonitrile.

Column – Silica gel

Injected quality- 10µl

Flow rate – 1.0ml/min

Detector: UV detector; $\lambda_{max}$: 254nm.

### Table 3
\(^1\)H NMR of data of 3-substituted phenyl-5-(2”, 2”-dimethyl, 7”-hydroxy chroman) isoxazole (26-30).

<table>
<thead>
<tr>
<th>Proton No</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 a</td>
<td>1.38(s,6H,CH(_3))</td>
<td>1.34(s,3H,CH(_3))</td>
<td>1.3 (s,3H,CH(_3))</td>
<td>1.33 (s,3H,CH(_3))</td>
<td>1.3 (s,3H,CH(_3))</td>
</tr>
<tr>
<td>2 b</td>
<td>1.38 (s,3H,CH(_3))</td>
<td>1.4 (s,3H,CH(_3))</td>
<td>1.4 (s,3H,CH(_3))</td>
<td>1.45(s,3H,CH(_3))</td>
<td></td>
</tr>
<tr>
<td>3’</td>
<td>1.7-1.9(br t,2H,3”-CH(_2))</td>
<td>1.78(t,2H,3”-CH(_2))</td>
<td>1.83(t,2H,3”-CH(_2))</td>
<td>1.82(t,2H,3”-CH(_2))</td>
<td>1.85(t,2H,3”-CH(_2))</td>
</tr>
<tr>
<td>4’</td>
<td>2.9(t,2H,4”-CH(_2))</td>
<td>2.6(t,2H,4”-CH(_2))</td>
<td>2.7(t,2H,4”-CH(_2))</td>
<td>2.7(t,2H,4”-CH(_2))</td>
<td>2.8(t,2H,4”-CH(_2))</td>
</tr>
<tr>
<td>5’</td>
<td>7.55(s,1H)</td>
<td>7.55(s,1H)</td>
<td>7.1 (s,1H)</td>
<td>7.5(s,1H)</td>
<td>7.6(s,1H)</td>
</tr>
<tr>
<td>8’</td>
<td>6.35(s,1H)</td>
<td>7.51(s,1H)</td>
<td>6.36(s,1H)</td>
<td>6.3(s,1H)</td>
<td>6.3(s,1H)</td>
</tr>
<tr>
<td>4</td>
<td>6.9(s,1H)</td>
<td>7.44(s,1H)</td>
<td>7.32(s,1H)</td>
<td>7.31(s,1H)</td>
<td>7.3(s,1H)</td>
</tr>
<tr>
<td>Aromatic protons</td>
<td>6.8(s,1H,C(_2))</td>
<td>6.69(d,2H,C(_3), C(_5))</td>
<td>7.37(d,2H,C(_3), C(_5))</td>
<td>6.83(d,2H,C(_3), C(_5))</td>
<td>7.7(d,2H,C(_3), C(_5))</td>
</tr>
<tr>
<td></td>
<td>7.6(d,1H,C(_3))</td>
<td>7.49(d,2H,C(_2), C(_6))</td>
<td>7.48(d,2H,C(_2), C(_6))</td>
<td>7.77(d,2H,C(_2), C(_6))</td>
<td>6.9(d,2H,C(_2), C(_6))</td>
</tr>
<tr>
<td>OCH(_3)</td>
<td>3.9(s, OCH(_3))</td>
<td>-</td>
<td>-</td>
<td>3.9(s, OCH(_3))</td>
<td>-</td>
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<tr>
<td>N-Methyl</td>
<td>-</td>
<td>3.65</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\*CDCl\(_3\)/TMS
Table-4

$^{13}$C NMR Data of 3-(4’-chlorophenyl-5-(2'', 2''-dimethyl, 7''-hydroxy chroman) isoxazole (28)

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>Chemical shift</th>
<th>Carbon No.</th>
<th>Chemical shift</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>151.4</td>
<td>7”</td>
<td>152.84</td>
</tr>
<tr>
<td>4</td>
<td>100.3</td>
<td>8”</td>
<td>109.67</td>
</tr>
<tr>
<td>5</td>
<td>156.8</td>
<td>1’</td>
<td>127.5</td>
</tr>
<tr>
<td>2”a,b</td>
<td>26.76</td>
<td>2’</td>
<td>127.5</td>
</tr>
<tr>
<td>2”</td>
<td>75.62</td>
<td>3’</td>
<td>128.2</td>
</tr>
<tr>
<td>3”</td>
<td>47.2</td>
<td>4’</td>
<td>133.4</td>
</tr>
<tr>
<td>4”</td>
<td>30.63</td>
<td>5’</td>
<td>128.2</td>
</tr>
<tr>
<td>5”</td>
<td>130.25</td>
<td>6’</td>
<td>127.5</td>
</tr>
<tr>
<td>6”</td>
<td>114.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The five new 3-substituted phenyl 5-(2”, 2”-dimethyl, 7”-hydroxy chroman) isoxazoles thus synthesized were:

1. 3-(3’, 4’-dimethoxy-phenyl)-5-(2”, 2”-dimethyl, 7”-hydroxy chroman) isoxazole 26.

2. 3-(4’-N, N-dimethyl amino phenyl)-5-(2”, 2”-dimethyl, 7”-hydroxy chroman) isoxazole 27.

3. 3-(3’, 4’-chloro-phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole 28.

4. 3-(4’-methoxy-phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole 29.

5. 3-(4’-cyano phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole 30.
Section-B:

Synthesis and characterization of New chromanoisoxazoles: 3-(4’-chlorophenyl)-5-(3”,4”,9”,10”-tetrahydro-2”,2””-8”,8””-tetramethyl-2”H,8”H-dipyranylbenzo [1,2-b:3,4-b’]) isoxazole (32)

Double chroman, compound 20C was condensed with 4-chlorobenzaldehyde in the presence of alcoholic KOH and stirred for 72 hr. at room temperature. This after usual work up and purification by column chromatography furnished the pure chalcone 31 (Scheme XII). Purity of the chalcone obtained was checked by HPLC, and found to be above 99%.

![Scheme XII]
The chalcone 31 thus obtained was condensed with hydroxylamine hydrochloride in the presence of KOH/C$_2$H$_5$OH. After usual work up and purification by column chromatography, compound 32 was isolated. It was further crystallized from methanol, which furnished white crystalline product. The compound showed purity of 100% as evidenced by HPLC data Figure-15. The IUPAC nomenclature of the compound 32 is 3-(4’-chlorophenyl)-5-(3’”,4’”,9””,10””-tetrahydro-2””,2””,8””,8””-tetramethyl-2””H,8””H-dipyranyl benzo [1,2-b:3,4-b’] isoxazole. The compound was characterized using $^1$H NMR & $^{13}$C NMR spectra and the data is presented tables-5 & 6. The$^1$H NMR spectrum is presented in figure-16.

**TLC:** R$_f$ value: 0.9

**HPLC purity:** 100%; (Hexane: Ethyl acetate, 9:1) (fig-15).

**m.p:** 235°C

**Molecular formula:** C$_{25}$H$_{26}$O$_3$NCl;

**Elemental analysis:**

**Found (%):** C 70.08; H 6.06; N 3.24; Cl 8.26%.

**Required (%):** C 70.76; H 6.13; N 3.30; Cl 8.37%.

$^1$H NMR(CDCl$_3$/TMS): δ1.35(s, 2a”, 8a”, 6H, CH$_3$), 1.42(s, 2b”, 8b”, 6H, CH$_3$), 1.8(t, 3””,9””4H, CH$_2$), 2.6(t,10””,2H, CH$_2$), 2.6(t, 10”,2H,CH$_2$),2.7(t,4””,2H,CH$_2$), 6.9(s, 1H, C-4), aromatic protons 7.45(d,2H,C’3& c’$_5$), 7.81(d,2H,C’3& C’$_6$).

**IR($v_{max}$):** 3247,2970,1618,1582,1335,1290,1185,1043,740cm$^{-1}$. 
The compound 32, showed UV absorption maximum at 324.4 nm (CH$_3$CN).

Similarly the fluorescence emission maximum was found to be 404 nm in CH$_3$CN, and $^{13}$C NMR spectrum is presented in Figure-17.
Fig 15: HPLC chromatogram of compound 32.
Fig 16: $^1$H spectrum of compound 32.
Fig 17: 13C spectrum of compound 32.
Table 5: $^1$H NMR data of Chromanoisoxazole 32

<table>
<thead>
<tr>
<th>Proton</th>
<th>Compound 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>2”a, 8”a,</td>
<td>$\delta$1.35(s, 6H, CH$_3$)</td>
</tr>
<tr>
<td>2”b, 8”b</td>
<td>$\delta$ 1.42(s, 6H, CH$_3$)</td>
</tr>
<tr>
<td>3”, 9”</td>
<td>$\delta$ 1.8(t,4H,CH$_2$)</td>
</tr>
<tr>
<td>4”</td>
<td>$\delta$2.7(t, 2H, CH$_2$)</td>
</tr>
<tr>
<td>10”</td>
<td>$\delta$2.6(t, 2H, CH$_2$)</td>
</tr>
<tr>
<td>12”</td>
<td>$\delta$ 7.5(s, 1H)</td>
</tr>
<tr>
<td>4</td>
<td>$\delta$6.9(s, 1H)</td>
</tr>
<tr>
<td>Aromatic Protons</td>
<td>$\delta$ 7.45(d,2H,C’3 &amp; C’5),</td>
</tr>
<tr>
<td></td>
<td>$\delta$ 7.81(d,2H,C’2&amp; C’6).</td>
</tr>
</tbody>
</table>

Table 6: $^{13}$C NMR data of Chromanoisoxazole 32

<table>
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<tr>
<th>Carbon No.</th>
<th>Chemical shift</th>
<th>Carbon No.</th>
<th>Chemical shift</th>
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<td>159.84</td>
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<td>4</td>
<td>99.5</td>
<td>9”</td>
<td>32.5</td>
</tr>
<tr>
<td>5</td>
<td>165.0</td>
<td>10”</td>
<td>22.4</td>
</tr>
<tr>
<td>2”a,b</td>
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<td>1’</td>
<td>134.46</td>
</tr>
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<td>8”a,b</td>
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</tr>
<tr>
<td>2”, 8”</td>
<td>74.61</td>
<td>3’</td>
<td>129.57</td>
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<tr>
<td>3”</td>
<td>34.5</td>
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<td>4”</td>
<td>17.4</td>
<td>5’</td>
<td>129.57</td>
</tr>
<tr>
<td>5”</td>
<td>110.25</td>
<td>6’</td>
<td>128.15</td>
</tr>
</tbody>
</table>
PART C

Biological activity studies of the newly synthesized Chromanoisoxazoles (26-30):

Literature survey revealed that isoxazoles possess a wide variety of biological activities. In continuation of our studies, we have investigated the antimicrobial activity studies of chromanoisoxazoles

Antimicrobial activity studies of chromanoisoxazoles

Preparation of media: In case of antibacterial activity studies, 37 gms of nutrient agar medium (Himedia) was dissolved in 1000 ml of distilled water and the pH was adjusted to 7.0. Where as in case of antifungal activity studies, potato dextrose agar (Himedia), 39 gm was dissolved in 1000 ml distilled water and the pH was adjusted to 5.6. Each 20ml portion of media was distributed to test tubes and these test tubes were plugged with non-adsorbent cotton and kept in autoclave (121.1°C) for sterilization for an hour.

Plating of media: Sterilized media was heated in a water bath thoroughly. Molten media was poured on to the Petri dish (pre-sterilized in oven for 3 hours at 110°C in order to avoid contamination). The plated Petri dishes were kept on plain surface to avoid non-uniform solidification of medium. Micro wells (6mm diameter) were made with bore-puncher at equidistance (four micro wells were made on a 4” assay-plate). All these operations were performed in “sterile room” which was equipped with a “laminar flow”.

a) **Antibacterial Activity**

The compounds 26-30 were tested for their antibacterial activity against Gram-positive bacteria, *Bacillus subtilis* & *Bacillus pumilus* and Gram-negative bacteria *Escherichia coli* & *proteus vulgaris*, at concentrations of 5, 10, 20, 50, 100 and 200µg/ml. The cultures of *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli* and *Proteus vulgaris* grown over night at 37°C, were used for testing the antibacterial activity which was checked employing agar well diffusion method. Nutrient agar medium (Himedia, India) was dissolved in water and pH was adjusted to 7.0. This was then disturbed in 20ml quantity in boiling tubes; they were then plugged tightly with non-absorbent cotton and sterilized in an autoclave. The bacterial culture (50µl) was then added aseptically to the agar medium maintained at 45°C, mixed well and poured in to petriplates. Test solutions of different concentrations of compounds 26-30 were prepared in DMSO. After hardening, cups of 6mm diameter each were cut into agar and 50µl test solutions of varying concentrations (5, 10, 20, 50, 100 & 200µg/ml) were placed in these cups. The plates were incubated at 37°C for 24 hours and the diameter of inhibition zone was measured in mm. Solvent DMSO alone was kept as control, which did not have any inhibition zone. The activity was compared with standard antibiotic Benzyl Penicillin and the antibacterial activities inhibition zones of the compounds are measured and presented in Table-7. The zone of Inhibition are photographed and presented in figures 18-22(five photographs).
Antifungal activity

Antifungal activity of the compounds was tested against *Candida albicans* using agar well diffusion method. Potato dextrose agar (Himedia, India) was dissolved in water and pH was adjusted to 5.6. This was then distributed 20ml each in boiling tubes which were plugged tightly with non-absorbent cotton and sterilized. To this 50µl of fungal spore suspension was added and thoroughly mixed with 20 ml medium aseptically and poured in to petriplates. When agar solidified, cups of 6mm diameter were made on each of the seeded plates. These cups were filled with 50µl of test samples of concentrations of 5, 10, 20, 50, 100 & 200µg/ml the petriplates were incubated at 28°C for 2 days. The inhibition zones produced by test compounds were compared with inhibition zones produced by pure ketoconazole used as standard. Inhibition zone of the compounds are presented in table 7.
### Table: 7
Zone of inhibition in mm of compounds 26-30

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>Concentration of the compound µg/ml</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>B.subtilis</td>
<td>B.pumilus</td>
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<td>5</td>
<td>9</td>
<td>9</td>
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<tr>
<td></td>
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<td>10</td>
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<tr>
<td></td>
<td>20</td>
<td>11</td>
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<tr>
<td>Ketoconazole</td>
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‘NA’ indicates no Zone,  
DMSO did not exhibit inhibition zone
Fig 18: Zone of inhibition of the compound 26 against test pathogens
Fig 19: Zone of inhibition of the compound 27 against test pathogens
Fig 20: Zone of inhibition of the compound 28 against test pathogens
Fig 21: Zone of inhibition of the compound 29 against test pathogens
Fig 22: Zone of inhibition of the compound 30 against test pathogens
Results:

The results show that all the five compounds 26-30 showed antibacterial activity against all the three organisms. The minimum inhibition concentration (MIC) was 5µg/ml against *B. subtilis* for compound 26 and 29 where as it was 20µg/ml for compound 27, 10µg/ml for compound 28 and 50µg/ml in case of compound 30. MIC was 5µg/ml against *B. pumilus* for compounds 26 and 27; 20µg/ml for compounds 28 and 29 and in case of compound 30 MIC was 100µg/ml. In case of gram-negative bacteria, *E. coli* MIC was 20µg/ml for compound 26; 50µg/ml for 27 & 28; 10µg/ml for compound 29 and 200µg/ml for compound 30. On the whole it was observed that compound 26 and 29 both having methoxy substituents in the phenyl ring showed more activity in all the organisms than that of other compounds.

In case of antifungal activity studies, MIC was 10µg/ml for compounds 26 and 20µg/ml for 27 while it was 10µg/ml for compounds 28 and 29. Whereas it was 200µg/ml for compound 30 against *C. albicans*. 
PART D

Molecular Docking Studies of newly synthesized Chromanoisoxazoles (26-30):

Drug discovery in modern times involves production numerous molecules and examine vast libraries in least time. This gave rise to computer-aided drug design (CADD). Computer-aided drug design (CADD) represents computational methods for the storage, management, analysis and modeling of compounds. Digital repositories are developed for to study chemical interaction relationships, computer programs for designing compounds of interest, and tools for assessment of potential leads prior to their synthesis and testing. Only small number of compounds are required to be synthesized when computer aided drug screening methods are used. Interaction theory between drugs and their target forms basis for these methods. Drug and the target interaction involve Steric, electrostatic and hydrophobic complementarity. In a structure-based drug design, three-dimensional structures of target proteins are known while in case of ligand-based drug design the structures are unknown. In the computer-aided structure-based drug design, the three-dimensional structure of drug-protein complex is build and binding affinity of the drug to the protein is calculated by the software programme called molecular docking

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example
scoring functions. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore docking is useful for predicting both the strength and type of signal produced.

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking \(^{58-59}\).

![Process of docking](image)

**Applications**

A binding interaction between a small molecule ligand and an enzyme protein may result in activation or inhibition of the enzyme. If the protein is a receptor, ligand
binding may result in agonism or antagonism. Docking is most commonly used in the field of drug design, most drugs are small organic molecules and docking may be applied to:

- Hit identification – docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest.

- Lead optimization – docking can be used to predict in where and in which relative orientation a ligand binds to a protein (also referred to as the binding mode or pose). This information may in turn be used to design more potent and selective analogues.

- Bioremediation – Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes.

**iGEM Dock**

iGEMDOCK is the GEMDOCK, which is a robust and well-developed tool. Using iGEMDOCK, the predicted poses generated from the GEMDOCK are able to be directly visualized by a molecular visualization tool and analyzed by post-analysis tools. iGEMDOCK provides the post-analysis tools by using k-means and hierarchical clustering methods based on the docked poses (i.e. protein-ligand interactions) and compound properties (i.e. atomic compositions). Atomic composition (AC), which is similar to the amino acid composition of a protein sequence, is a new concept for measuring compound similarity. We validated the
protein-ligand docking accuracy and screening accuracies of iGEMDOCK by using a test set with 100 protein-ligand complexes and four targets, respectively, which are thymindine kinase, estrogen receptor for antagonists and agonists, and human DHFR. Experimental results show that iGEMDOCK keeps then advantages of GEMDOCK and provided graphical-integrated environment for virtual screening and docking. We also evaluated the AC method on a test set with 76 compounds. The results indicate that the AC method performs better than the comparative methods in this set. It believes that iGEMDOCK, which integrates the structure-based virtual screening and post-screening analysis, is a useful system for drug discovery.

Molecular docking studies

Method

X-Ray crystal structure of Topoisomerase I (PDB ID 1T8I) used in docking studies was obtained from Protein Data Bank. Co-crystallized ligands and water molecules were removed from target protein using Argus lab. Ligands were prepared by using Chemoffice (Cambridge). Energy minimization was done using molecular mechanics. The minimization was executed until the root mean square gradient value reached a value smaller than 0.001kcal/mol. Such energy minimized ligands and receptors used for docking studies using GEMDOCK (Generic Evolutionary Method for molecular DOCKing) and PyMol for better visualization.
Table 8: Docking studies of compounds on topoisomerase I.

<table>
<thead>
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<th>Compound (R)</th>
<th>Binding energy (- Kcal/mol)</th>
<th>Amino acids Involved</th>
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</table>
| 26 (-OCH$_3$)$_2$ | -78.1 | VAL-226  
| | | PHE-227  
| | | PRO-229  
| | | PRO-230  
| | | LEU-334  
| | | TYR-338  
| | | ARG-376 |
| 27 -N(CH$_3$)$_2$ | -80.1 | LYS-284  
| | | GLU-285  
| | | PRO-358  
| | | LEU-373  
| | | LYS-374  
| | | ARG-375  
| | | ARG-376 |
| 28 (-Cl) | -86.8 | LYS-493  
| | | GLU-495  
| | | SER-506  
| | | ARG-508  
| | | ASP-562  
| | | ASP-563 |
| 29 (-OCH$_3$) | -84.1 | LYS-493  
| | | VAL-502  
| | | LYS-532  
| | | ASP-533  
| | | LYS-587 |
| 30 (-CN) | -73.0 | GLY-531  
| | | LYS-532  
| | | ILE-535  
| | | HIS-632  
| | | HIS-632  
| | | THR-718 |
| Camptothecin (standard) | -85.8 | ASN-631  
| | | TYR-537  
| | | ARG-488  
| | | CYS-630  
| | | ILE-535 |
Fig 23: Cl substituted compound (28) interaction with topoisomerase I (PDB ID 1T8I).
Results and Discussion

Type I topoisomerases cut one strand of double-stranded DNA, relax the strand, and reanneal the strands. As topoisomerases generate breaks in DNA, they are targets of small-molecule inhibitors that inhibit the enzyme. Type 1 topoisomerase is inhibited by irinotecan, topotecan and camptothecin which are cytotoxic drugs. Novel topoisomerases inhibitors are needed for development of cytotoxic drugs.

In present study, Compounds were docked into empty binding site of experimentally known crystal structure of human topoisomerase I (70 kDa) in complex with the drug molecule Camptothecin and covalent complex with A 22 Base pair DNA Duplex (PDB ID 1T8I). Human topo I C-terminal domain is a crucial for the catalytic activity. The phosphate of tyrosine and DNA phosphodiester bond makes close interaction with the guanidinium groups of Arg 488 and Arg 590 are implicated in the reaction. Lys532, Thr 718 and Tyr 723 are other important DNA catalytic amino acids of topo I. In molecular docking studies, Compounds binding energy found to be between the ranges from -73.0 to -86.8 Kcal/mol and commonly interacted with Lysine, Arginine of topo I (Table. 23). Binding energy inversely proportional to binding affinity. Cl substituted compound 28 showed highest binding energy (-83.8 Kcal/mol) among other docked compounds. Compounds showed comparable binding affinity with Camptothecin (Cytotoxic drug).
Experimental

Preparation Resacetophenone:

Freshly fused ZnCl$_2$(33g) was dissolved in glacial acetic acid (32ml) while heating and when all the ZnCl$_2$ is almost dissolved resorcinol (22 g) was added and heated to 140-150$^\circ$C for 15 min with stirring. After that this is left for 1 hr and then 1:1 HCl (100ml) was added to break the zinc chloride complex. Within 5 minutes precipitate obtained was crystallized from HCl 20% to give resacetophenone needles,

$R_f$: 0.4 (Hexane: EtOAc, 8:2)

m.p: 145$^\circ$C.

Molecular formula: C$_8$H$_8$O$_3$

Elemental analysis:

Found (%): C, 63.06; H, 5.23%

Required (%): C, 63.15; H, 5.26%.

$^1$H NMR (CDCl$_3$/TMS): $\delta$2.65(s, 3H, COCH$_3$), $\delta$6.35(s, 1H), $\delta$6.5(d, 1H), $\delta$7.62(d, 1H), $\delta$11.55(s, OH).

IR ($v_{max}$): 3450cm$^{-1}$(-OH str.); 1685cm$^{-1}$(C=O str).

Nuclear prenylation of resacetophenone:

A solution of isoprene (1.5 ml, 0.015 mol) in xylene (5ml) was added drop wise for 8 hr to a mixture of resacetophenone (1.41g, 0.0072 mol) and PPA (2ml) in xylene (3ml) with constant stirring at 30-35$^\circ$C stirring was continued for further
14hrs. The reaction mixture was taken in chloroform (100 ml) and the chloroform solution was washed with NaHCO$_3$ (5%, 3x60ml) water; dried over MgSO$_4$ and removed under reduced pressure to give gummy material which had three spots on TLC. This on separation using column chromatography over silica gel yielded three compounds 20A, 20B and 20C in the ratio 2:3:1, on elution with hexane EtOAc.

**Compound 20A:**

- **m.p:** 148-149°C
- **Molecular formula:** C$_{13}$H$_{16}$O$_3$
- **Elemental analysis:**
  - **Found (%):** C, 70.85; H, 7.3%
  - **Required (%):** C, 70.90; H, 7.27%
- **$^1$H NMR (CDCl$_3$/TMS):** δ1.46 (s, 6H), δ1.91(t, 2H), δ2.72(t, 2H), δ6.35(d, 1H), δ7.4(d, 1H); δ2.5(s,3H,COCH$_3$); δ13.1(s,1H).
- **IR ($v_{max}$):** 3350cm$^{-1}$(-OH str.); 1680cm$^{-1}$(C=O str).

**Compound 20B:**

- **m.p:** 92-93°C
- **Molecular formula:** C$_{13}$H$_{16}$O$_3$
- **Elemental analysis:**
  - **Found (%):** C, 70.85; H, 7.3%
  - **Required (%):** C, 70.90; H, 7.27%
\(^1\text{H NMR (CDCl}_3/\text{TMS):}\) \(\delta 1.3\) (s, 6H), \(\delta 1.8\) (t, 2H), \(\delta 2.7\) (t, 2H), \(\delta 6.3\) (d, 1H), \(\delta 7.5\) (d, 1H); \(\delta 2.5\) (s, 3H, COCH\(_3\)); \(\delta 12.2\) (s, 1H).

\(\text{IR (v}\_\text{max})\): 3350 cm\(^{-1}\) (–OH str.); 1680 cm\(^{-1}\) (C=O str).

**Compound 20C:**

\(m.p: 107-108^0\) C

**Molecular formula:** C\(_{18}\)H\(_{24}\)O\(_3\)

**Elemental analysis:**

**Found (%):** C, 74.85; H, 8.29%

**Required (%):** C, 75.0; H, 8.33%.

\(^1\text{H NMR (CDCl}_3/\text{TMS):}\) \(\delta 1.33\) (s, 6H), \(\delta 1.36\) (s, 6H), \(\delta 1.6\) (s, 2H), \(\delta 1.7\) (t, 2H), \(\delta 2.61\) (t, 2H); \(\delta 2.71\) (t, 2H); \(\delta 7.49\) (s, 1H);

\(\delta 2.59\) (s, 3H, COCH\(_3\)).

**IR (v\_max):** 1685.68 cm\(^{-1}\) (C=O str).

**Synthesis of chalcones – General procedure:**

**Condensation of 2,2-dimethyl-6-acetyl-7-hydroxy chroman 20B with substituted benzaldehydes:**

A mixture of 2,2-dimethyl-6-acetyl-7-hydroxy chroman 20B (0.01 mol), substituted benzaldehydes (0.01 mol) in ethanol (30 ml) and aqueous potassium hydroxide (15 g in 15 ml of water) was kept at room temperature for 24 hours. On acidification with 1:1 hydrochloric acid, yellow (or) orange red chalcone
derivatives were obtained in 80-85% yield. It was filtered and crystallized from appropriate solvent and characterized using spectral data and elemental analysis.

2. 2-dimethyl-6-acetyl-7-hydroxy chroman 20B was condensed with various benzaldehydes, veratraldehyde, 4-N, N-dimethylamino benzaldehyde, 4-chlorobenzaldehyde, 4-methoxy benzaldehyde and 4-cyano benzaldehyde to furnish the respective chalcones viz.,

1. 7-Hydroxy-6-(3,’4’-dimethoxy) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2H benzo(1,2b)pyran 21:

   Molecular formula: C_{22}H_{24}O_{5}

   Elemental analysis:

   Found (%): C, 74.85; H, 8.29%

   Required (%): C, 71.72 H, 6.57%

2. 7-Hydroxy-6-(4’-N, N-dimethyl amino) cinnamoyl, 3, 4-dihydro-2,2-dimethyl-2H benzo(1,2b)pyran 22.

   Molecular formula: C_{22}H_{25}O_{3}N

   Elemental analysis:

   Found (%): C, 75.08; H, 7.11; N, 3.85%

   Required (%): C, 75.19 H, 7.17; N, 3.99%

3. 7-Hydroxy-6-(4’-chloro) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2Hbenzo (1,2b) pyran 23.

   Molecular formula: C_{20}H_{19}O_{3}Cl

   Elemental analysis:

   Found (%): C, 70.01; H, 5.47; Cl, 10.28%
Required (%): C, 70.07; H, 5.59; Cl, 10.30%

4. 7-Hydroxy-6-(4’-methoxy) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2Hbenzo (1,2b) pyran 24.

Molecular formula: C$_{21}$H$_{22}$O$_4$

Elemental analysis:

Found (%): C, 74.47; H, 6.52%;

Required (%): C, 74.54; H, 6.55%.

5. 7-Hydroxy-6-(4’-cyano) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2H benzo(1,2b) pyran 25.

Molecular formula: C$_{21}$H$_{19}$O$_3$N

Elemental analysis:

Found (%): C, 75.59; H, 5.73% N, 4.15%;

Required (%): C, 75.66; H, 5.74; N, 4.20%

Synthesis of 3-(3,’4’-dimethoxy phenyl)-5-(2”, 2”-dimethyl, 7”-hydroxy chroman) isoxazole (26):

7-Hydroxy-6-(3,’4’-dimethoxy) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2H benzo (1,2b) pyran 21 (324 mg, 1 mmol), hydroxylamine hydrochloride (600 mg), KOH (500 mg) in ethanol was refluxed on a water bath for 4 hrs. Then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water (30ml). Where upon a light brown precipitate slowly separated out. The precipitate was collected and chromatographed over silica gel and crystallized from methanol as pale yellow needles in 60% yield.
m.p: 178°C

Elemental analysis:

**Found (%):** C, 69.01; H, 6.01; N, 3.45%;

**Required (%):** C, 69.29; H, 6.04; N, 3.67%

$^1$H NMR (CDCl$_3$/TMS): δ1.38(s, 6H, CH$_3$), δ1.7-1.9 (br t, 2H, 3”-CH$_2$), δ2.8 (t, 2H, 4”-CH$_2$), δ6.9 (s, 1H, C$_4$-H), δ7.55 (s, 1H, C”$_5$-H), δ6.35 (s, 1H, C”$_8$-H), δ6.8(s, 1H, C$_2$’-H), δ7.8(d, 1H, C$_5$’-H), δ7.8(d, 1H, C’$_6$-H), δ7.6 (d, 1H, C$_5$’-H), δ3.9(s, OCH$_3$).

IR (ν$_{max}$): 3435, 2974, 2927, 1615, 1524, 1387, 1277, 1196, 1040 cm$^{-1}$.

**Synthesis of 3-(4’-N, N-dimethyl amino phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole (27):**

7-Hydroxy-6-(4’-N,N-dimethylamino)cinnamoyl,3,4-dihydro-2,2-dimethyl-2H benzo (1,2b) pyran 22 (364 mg, 1mmol), hydroxylamine hydrochloride (600mg), KOH (500 mg) in ethanol was refluxed on a water bath for 5 hrs. then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water (30 ml), where upon a light brown precipitate slowly separated out. The precipitate was filtered and purified by column chromatography and crystallized from methanol as light brown colour needles in 62% yield.

m.p: 206°C.

Elemental analysis:

**Found (%):** C, 72.51; H, 6.27; N, 7.43%;
Required (%): C, 72.52; H, 6.59; N, 7.69%

$^1$H NMR (CDCl$_3$/TMS): \( \delta 1.34(s, 3H, CH_3) \), \( \delta 1.38(s, 3H, CH_3) \), \( \delta 1.78(t, 2H, 3'' -CH_2) \), \( \delta 2.6(t, 2H, 4'' -CH_2) \), \( \delta 7.52(s, 1H, C''_8-H) \), \( \delta 7.55(s, 1H, C''_5-H) \), \( \delta 7.49(d, 2H, C'_2'-H \) and \( C'_6'-H) \), \( \delta 6.69(d, 2H, C'_3'-H \) and \( C'_5'-H) \), \( \delta 7.44(s, 1H, C'_4'-H) \), \( \delta 3.02 (N-Me_2) \).

IR (\( \nu_{max} \)): 3436, 3103, 2970, 2946, 1638, 1600, 1448, 1366, 1181, 1073, 980 cm$^{-1}$.

Synthesis of 3-(4’-chloro phenyl)-5-(2”,2”-dimethyl, 7”-hydroxy chroman) isoxazole (28):

7-Hydroxy-6-(4’-chloro) cinnamoyl, 3,4-dihydro-2,2-dimethyl-2H benzo (1,2b) pyran 23 (366 mg, 1 mmol), hydroxylamine hydrochloride (600 mg), KOH (500 mg) in ethanol was refluxed on a water bath for 5 hrs. Then the reaction mixture was neutralized with AcOH and the whole contents were poured in ice-cold water (30ml), where upon a light brown precipitate slowly separated out. The precipitate was collected and chromatographed over silica gel and crystallized from MeOH as pale yellow crystals in 56% yield.

m.p: 176$^0$C

Elemental analysis:

Found (%): C, 67.41; H, 5.01; N, 3.83, Cl, 9.71%;

Required (%): C, 67.60; H, 5.07; N, 3.94; Cl, 9.85%

$^1$H NMR (CDCl$_3$/TMS): \( \delta 1.3(s, 3H, CH_3) \), \( \delta 1.4(s, 3H, CH_3) \), \( \delta 1.8(t, 2H, 3'' -CH_2) \), \( \delta 2.7(t, 2H, 4'' -CH_2) \), \( \delta 6.35(s, 1H, \)
C”s-H), δ7.1(s, 1H, C”5-H), δ7.32(s, 1H, C4’-H) and δ 7.48(d, 2H, C’2-H, C’6-H), δ7.37(d, 2H, C’3-H and C5’-H),

IR (νmax): 3247, 2975, 2620, 2582, 1342, 192, 1185, 1043, 745cm⁻¹.

Synthesis of 3-(4′-methoxy phenyl)-5-(2″,2″-dimethyl, 7″-hydroxy chroman) isoxazole (29): 7-Hydroxy-6-(4′-methoxy)cinnamoyl,3,4-dihydro-2,2-dimethyl-2Hbenzo(1,2b)pyran 24 (338 mg, 1mmol), hydroxylamine hydrochloride (600mg), KOH (500 mg) in EtOH was refluxed on a water bath for 4 hrs. then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water (30 ml), where upon a light brown precipitate slowly separated out. The precipitate was collected and purified b column chromatography and crystallized from MeOH as pale yellow colour needles (64% yield).

m.p: 185⁰C.

Elemental analysis:

Found (%): C, 71.43; H, 5.82; N, 3.72%;

Required (%): C, 71.79; H, 5.98; N, 3.98; %

¹H NMR (CDCl₃/TMS): δ1.33(s, 3H, CH₃), δ1.4 (s, 3H, CH₃), δ1.8 (t, 2H, 3”-CH₂), δ2.8 (t, 2H, 4”-CH₂), δ6.3 (s,1H, C”s-H), δ7.5 (s, 1H, C”5-H), δ7.31(s, 1H, C4’-H) and δ 7.7(d,2H, C’2-H, C’6-H), δ6.83(d, 2H, C’3-H and C5’-H), δ3.9(s, OCH₃).

IR (νmax): 3299, 3210, 1605, 1518, 1465, 1440, 1255, 1030cm⁻¹.
Synthesis of 3-(4’-cyano phenyl)-5-(2”-2”-dimethyl, 7”-hydroxy chroman) isoxazole (30):

7-Hydroxy-6-(3’-cyano) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2H benzo (1,2b) pyran 25 (336 mg, 1 mmol), hydroxylamine hydrochloride (600 mg), KOH (500 mg) in EtOH was refluxed on a water bath for 5 hrs. then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water (30 ml), where upon a light brown precipitate slowly separated out. The precipitate was collected and chromatographed over silica gel and crystallized from methanol as colourless crystals in 52% yield.

**m.p:** 146⁰C

**Elemental analysis:**

**Found (%):** C, 75.47; H, 5.31; N, 8.22%;

**Required (%):** C, 75.90; H, 5.42; N, 8.43; %

**¹H NMR (CDCl₃/TMS):** δ1.3(s, 3H, CH₃), δ1.45 (s, 3H, CH₃), δ1.85 (t, 2H, 3”–CH₂), δ2.8 (t, 2H, 4”–CH₂), δ6.3 (s, 1H, C’₈-H), δ7.6(s, 1H, C’₅-H), δ7.3(s, 1H,C₄’-H )and δ6.9(d,2H, C’₂-H, C’₆-H), δ7.7 (d, 2H, C’₃-H and C₅’-H), δ11.1(s, OH).

**IR (νmax):** 3263, 2973, 2931, 2360, 1608, 1587, 1456, 1338, 1250, 1153, 1082, 875cm⁻¹.
Synthesis of New chromanisoxazole, 3-(4’-chlorophenyl)-5-(3”,4”,9”,10”-tetrahydro-2”,2”,8”,8”-tetramethyl-2”H,8”H-dipyranyl benzo [1,2-b:3,4-b’]) isoxazole (32):

Double chroman, compound 20C (0.01 mol) was condensed with 4-chlorobenzaldehyde (0.01 mol) in ethanol (30 ml) and KOH (15g in 15 ml water) and stirred for 72 hr at room temperature. This after usual work up and purification by column chromatography furnished the pure chalcone 31.

The chalcone 31 (1 mmol) thus obtained was condensed with hydroxylamine hydrochloride (600 mg) in the presence of KOH (500 mg) and EtOH (30 ml). After usual work up and purification by column chromatography, compound 32 was isolated. It was further crystallized from methanol, which furnished white crystalline product. Yield 50%.

m.p: 235°C,

Molecular formula: C_{25}H_{26}O_{3}NCl;

TLC R_{f} value: 0.9 (Hexane:Ethyl acetate 9:1),

HPLC purity: 100%;

Elemental analysis:

Found (%): C, 70.08; H, 6.06; N, 3.24; Cl 8.26%;

Required (%): C, 70.76; H, 6.13; N, 3.30; Cl 8.37 %

^1H NMR (CDCl_{3}/TMS): δ1.3(s, 2”a, 8”a, 6H), δ1.42 (s, 2b”, 8b”, 6H), δ1.8 (t, 3”,9” 4H) δ2.6 (t, 10”,2H), δ2.7 (t, 4”,2H), δ7.5(s, 12”, 1H), δ 6.9(s, 1H, C-4), aromatic
protons δ7.45 (d, 2H, C’3 & C’5), δ7.81 (d, 2H, C’2 & C’6).

**IR (νmax):** 3247, 2970, 1618, 1582, 1335, 1290, 1185, 1043, 740 cm⁻¹.
REFERENCES


   b) Grundmann. C. *Synthesis*. 7; 344: **1970**.

25. Mita Dhar and Anup Bhattacharya. *Indian J.Chemistry*. 40; 1140: **2001**.
_Tetrahedron Letters._ 33(8); 993: **1992.**

Zofia (Instytut Przemyslu Organicz nego, Pol) Pol.PL 177, 171 (Cl.CO7 D 261 06) (29 Oct. **1999**).


32. Vander Plas. H.C, Ring transformations of Heterocycles, Vol. 1 and 2,  
_Academic Press,_ New York, **1973.**


34. Wardakhan. W.W, Phosphorus Sulfur Silicon and the related elements, 162;  
275: **2000.**


   b) Lowenbein. A. *Ber*. 57; 1515: **1924**.

50. Reichel. L and Muller. K. *Ber*. 74; 1741: **1941**.


