

Design and Synthesis of Isoxazole Equipped Pyrazole Conjugated Benzimidazole Derivatives

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Abstract

Isoxazole-equipped pyrazole-conjugated Benzimidazole derivatives indicates a class of heterocyclic compounds synthesized through multi-step process involving cyclocondensation reactions, often starting from Benzimidazole precursors like 4-(1H-benzimidazol-2-yl)oxazol-2-amines, followed by incorporation of pyrazole and isoxazole moieties via Vilsmeier-Hack formylation. These derivatives are typically prepared by condensing arylhydrazines with aryl ketones to form pyrazole intermediates.

Key words: Pharmacophore, Benzimidazole frame works, Vilsmeier-Hack formylation, antifungal activity, Thin Layer Chromatography.

1. Introduction:

Benzimidazole is an important pharmacophore and privileged structure in therapeutic chemistry. Over the years of active research, it has evolved as an important heterocyclic system due to its presence in a wide spectrum of bioactive compounds like antifungal, anti-inflammatory agents, proton pump inhibitors and anticoagulants¹, anticonvulsants², analgesics³, antiulcers⁴, antiviral⁵, anticancer, antiparasitics⁶, antihistaminics⁷.

On the other hand, pyrazole nitrogen consisting heterocyclic scaffolds have always played an essential role in modern agrochemical and medicine fields because of their owing biological activity, which has emanated in different applications such as, antimicrobial,⁸⁻⁹ antimalarial,¹⁰⁻¹¹ anti-inflammatory,¹²⁻¹³ antiviral,¹⁴⁻¹⁵ antiproliferative,¹⁶⁻¹⁷ anticancer¹⁸ antileishmanial¹⁹⁻²¹ activities. Pyrazole based noticeable drugs are available in the market for instance, Celecoxib, Lonazolac, Pyrazofurin, Crizotinb and Encorafenib, etc. Some of pyrazole based biological active agents²²⁻²⁵ are depicted in figure 5.1. The isoxazole scaffolds are leading structural fragments and found to have a wide range of applications in synthetic, medicinal and biological activities such as, antimycobacterial,²⁶⁻²⁸ antimicrobial,²⁹⁻³¹ antiviral,³² anticancer,³³⁻³⁴ anticonvulsant,³⁵⁻³⁷ antidepressant,³⁸ anti-inflammatory,

antioxidant, antituberculosis. Some of the isoxazole based biological active compounds³⁰⁻³⁶ are depicted in Figure 1.

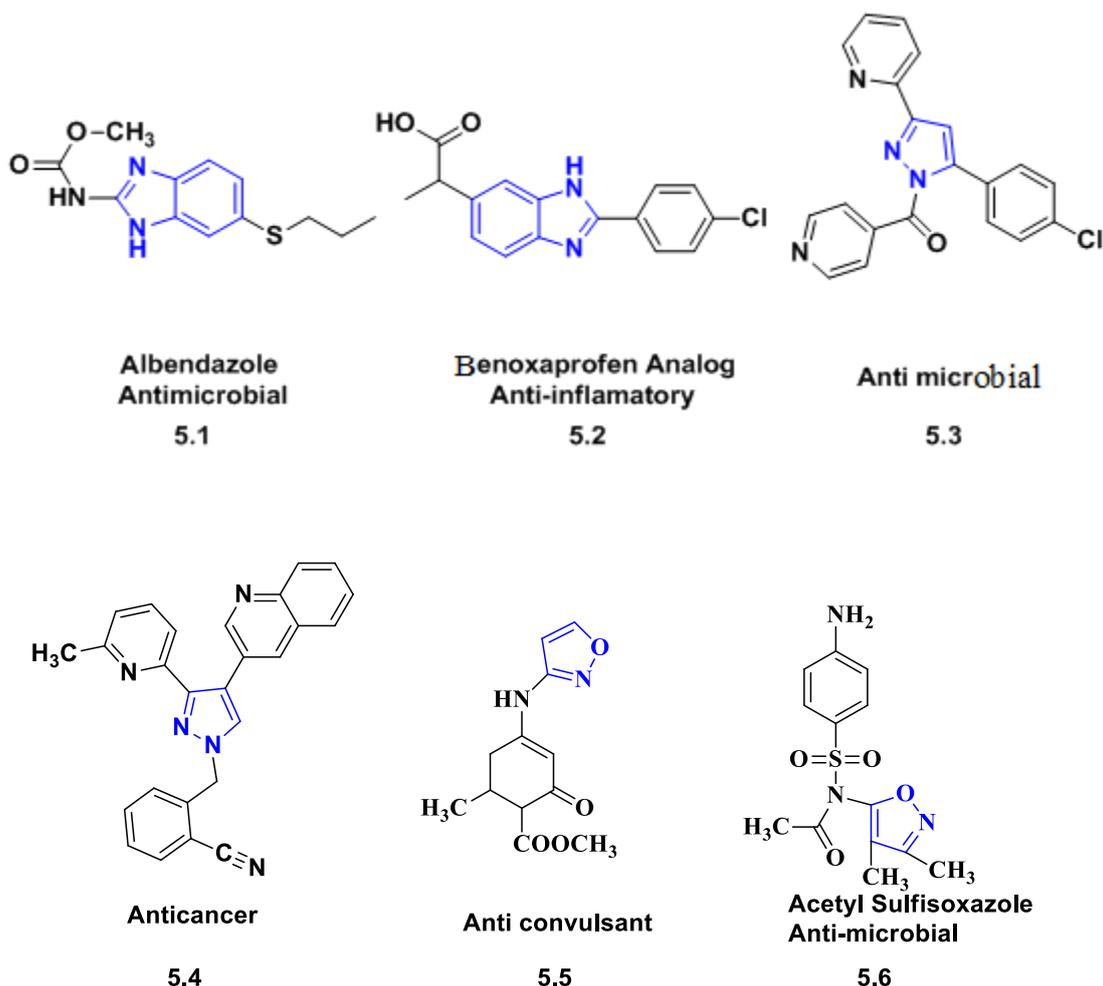


Figure 1: Benzimidazole (5.1, 5.2), pyrazole (5.3, 5.4) and Isoxazole (5.5, 5.6) containing biologically active drugs

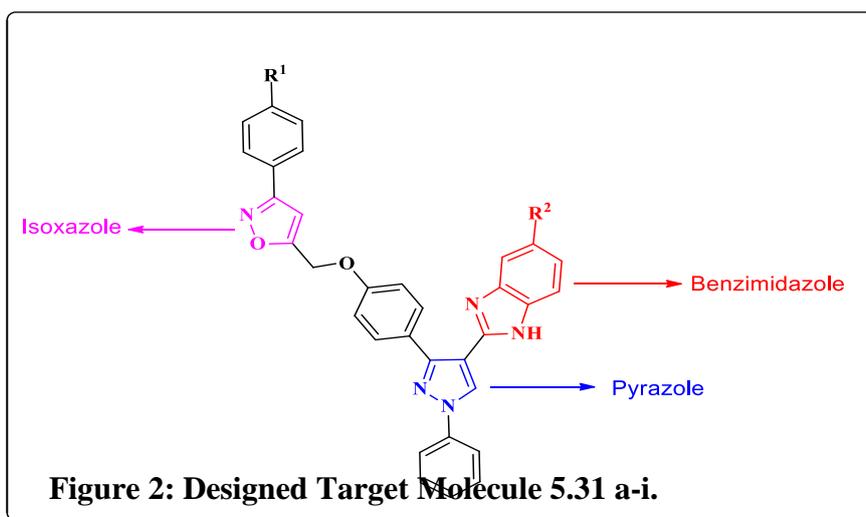
Based on the literature search results, it is evident that synthesis of biological active compounds containing isoxazole, pyrazole and benzimidazole scaffolds have been attracted tremendous interest among researchers individually due to their potential applications as cited above. However, heterocyclic building blocks consisting three moieties such as isoxazole, pyrazole and benzimidazoles have not been reported till now. Because of the superior biological activity and inspiration from the above cited findings we have been directed towards synthesis of single molecule containing three vital pharmacophores, isoxazole, pyrazole and benzimidazole, which could be anticipated to exhibit better biological activity.

In this context, in continuation of “our research on the synthesis of biologically active” moieties we have designed a new synthetic strategy to prepare a single molecule having three moieties isoxazole, pyrazole and benzimidazole and their derivatives. Some of the previous approaches used for the synthesis of benzimidazole, pyrazole and isoxazole are discussed below.

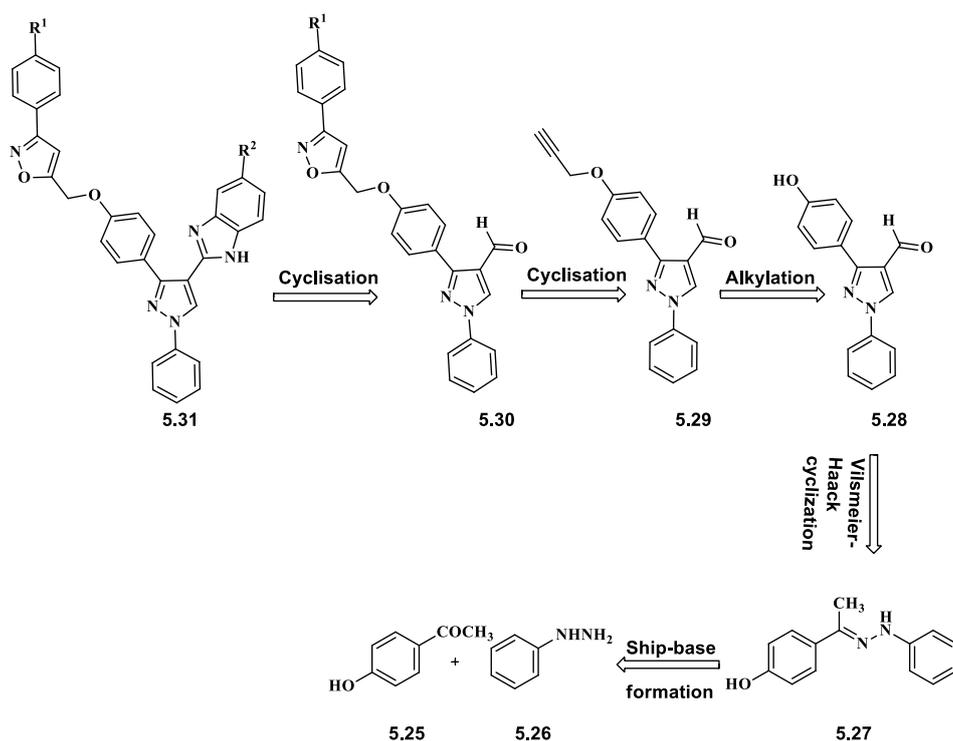
Present work:

In view of the above analysis and the wide range of biological applications of isoxazole, pyrazole aldehyde conjugated benzimidazole frameworks, we have developed synthesis of hybrid molecules containing isoxazole, pyrazole aldehyde and benzimidazole frameworks.

In continuation to our work on the synthesis of biological active moieties, we have synthesized a series of novel, “5-((4-(4-(1H-benzo[d]imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)”-3-phenylisoxazole derivatives (**5.31 a-i**) **Fig 2** and evaluated their antimicrobial and antifungal activities.

**Retro Synthetic Analysis:**

The essential target molecule (**5.31a-i**) was expected by the combination of isoxazole linked pyrazole aldehyde with substituted ortho di amino phenyl. The isoxazole linked pyrazole aldehyde was in turn synthesized by the cyclisation of 1-phenyl-3-(4-(prop-2-yn-1-yloxy)-phenyl)-1H-pyrazole-4-carbaldehyde with aryl oximes. The aldehyde was obtained by the propargylation of “1-phenyl-3-(4hydroxy)-phenyl)-1H-pyrazole-4-carbaldehyde”. The compound 3-(3-hydroxyphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde could be obtained from substituted acetophenone and phenyl hydrazine via hydrazones followed by Vilsmeier-Haack cyclization.

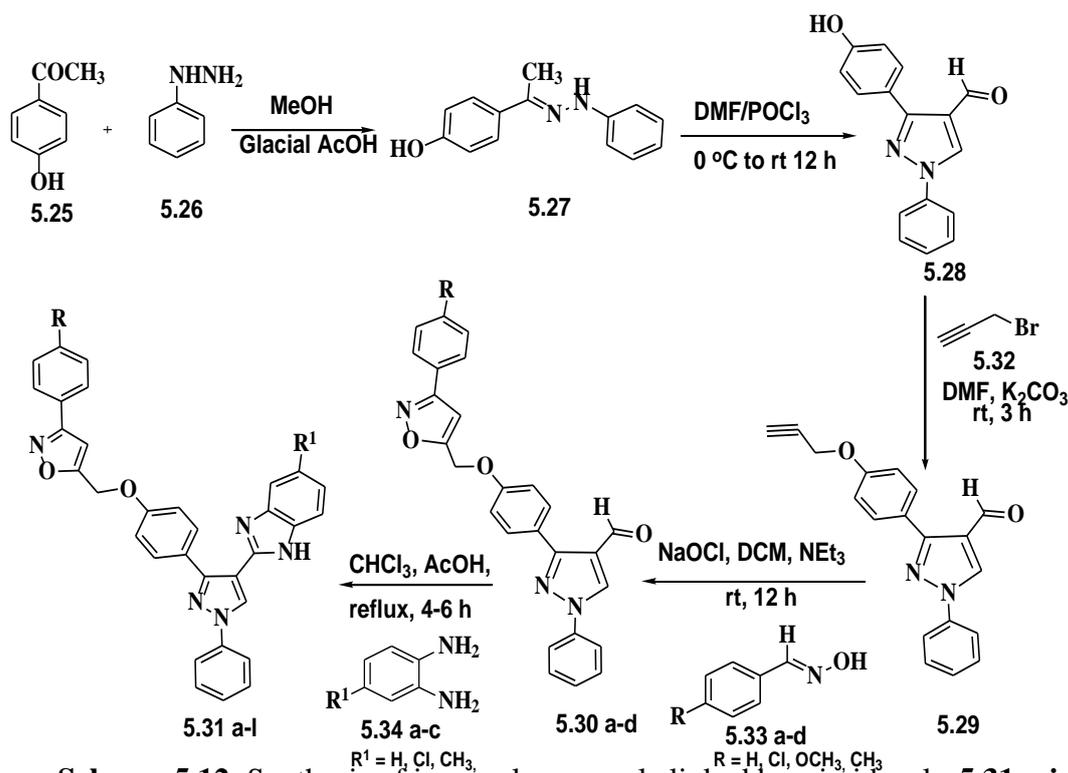


Scheme: Retro Synthesis of the Target Molecule **5.31**.

Synthetic Scheme:

In this section we have described the synthesis a series of isoxazole, pyrazole linked benzimidazole derivatives in five steps as shown in **Scheme-5-12**. In the first step Schiff's base, hydrazone was formed by the condensation of 4-hydroxy-acetophenone with phenyl hydrazine. It was further cyclized with hydroxonium salt in the presence of DMF/ POCl_3 to give pyrazole aldehyde⁵⁴; it is called as Vilsmeier-Haack cyclization.

Substituted 4-hydroxy pyrazole aldehyde **5.28** was treated with propargyl bromide **5.32** in presence of DMF/ K_2CO_3 to afforded propargylated pyrazole aldehyde which was cyclized with aryl oximes to form isoxazole conjugated pyrazole aldehyde **5.30 a-d**. Thus, the substituted pyrazole aldehydes **5.30 a-d** were treated with substituted orthophenyl di amine **5.34 a-c** in chloroform as a solvent, few drops of acetic acid under reflux conditions for 4-6 hours to afford substituted "5-((4-(4-(1H-benzo[d]imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)) phenoxy)methyl)-3-phenylisoxazole derivative **5.31 a-l**. These final isoxazole, pyrazole linked benzimidazoles **5.31 a-l** were confirmed by their spectral data.



Scheme-5.12: Synthesis of isoxazole, pyrazole linked benzimidazoles **5.31 a-i**

Synthesis of isoxazole, pyrazole linked benzimidazoles involves five steps

Step-I) Synthesis of (E)-4-(1-(2-phenylhydrazono)-ethyl) phenol (**5.27**)

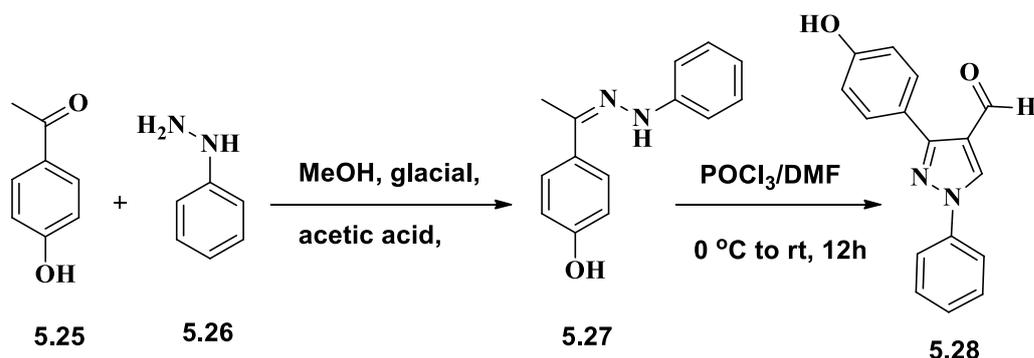
Step-II) “Synthesis of substituted 3-(3-hydroxyphenyl)-1-phenyl-1H-pyrazole-4”-carbaldehyde (**5.28**)

Step-III) Synthesis of 1-phenyl-3-(4-(prop-2-yn-1-yloxy)-phenyl)-1H-pyrazole-4-carbaldehyde (**5.29**)

Step-IV) Synthesis of 1-phenyl-3-(4-((3-phenylisoxazol-5-yl)-methoxy)-phenyl)-1H-pyrazole-4-carbaldehyde derivatives (**5.30 a-c**)

Step-V) Synthesis of substituted “5-((4-(4-(1H-benzo-[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl) phenoxy) methyl)-3-phenylisoxazole (**5.31 a-i**)”

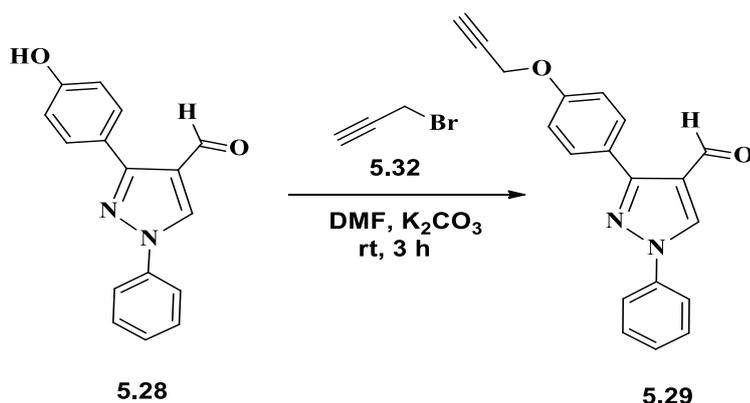
The desired starting material, **5.28** was synthesized by following previous reported methods⁵⁴⁻⁵⁵. The 4-Hydroxy acetophenone (**5.25**) was treated with phenyl hydrazine (**5.26**) to yield corresponding hydrazone (**5.27**) which was followed by subjected to Vilsmeier-Haack cyclization reaction in the presence of DMF/ POCl_3 which gave desired compound (**5.28**) in good yield.



Scheme 5.11:“Synthesis of 3-(3-hydroxyphenyl)-1-phenyl-1H-pyrazole-4-carbal-dehyde”(5.28)

3) Synthesis of 1-phenyl-3-(4-(prop-2-yn-1-yloxy)-phenyl)-1H-pyrazole-4-carbaldehyde (5.29)

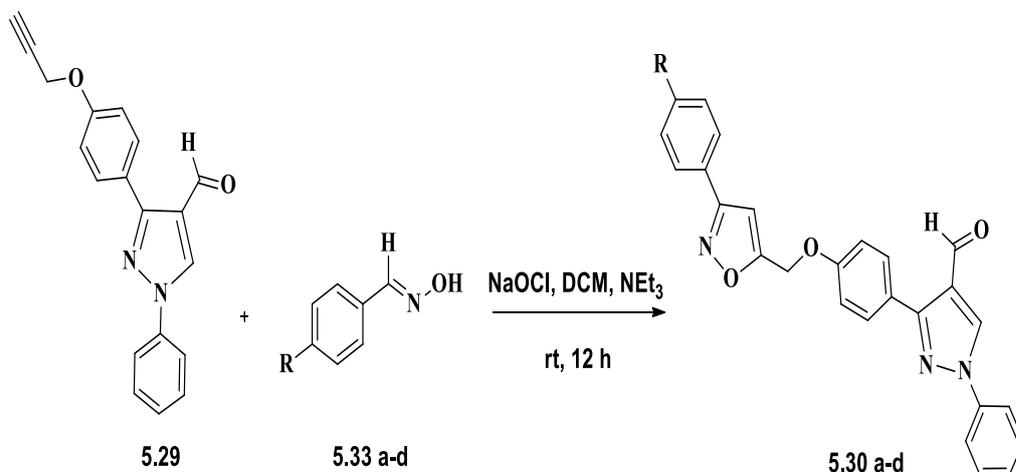
In the third step, anhydrous K_2CO_3 was added to 4-hydroxy pyrazole aldehyde (5.28) dissolved in DMF. To this was added Propargyl bromide (5.32) 80% in toluene “the reaction mixture was stirred at room temperature for 3” hours to afford 1-phenyl-3-(4-(prop-2-yn-1-yloxy)-phenyl)-1H-pyrazole-4-carbaldehyde(5.29) in good yields.



Scheme 5.12: Synthesis of 1H-pyrazole-4-carbal-dehyde (4.30).

4) Synthesis of various substituted 1-phenyl-3-(4-((3-phenylisoxazol-5-yl)-methoxy)-phenyl)-1H-pyrazole-4-carbaldehyde derivatives (5.30 a-c)

In fourth step, achieved the synthesis of substituted 1-phenyl-3-(4-((3-phenylisoxazol-5-yl)-methoxy) phenyl)-1H-pyrazole-4-carbaldehyde (5.30 a-d) where the compound 5.29 was reacted with substituted (E)-benzaldehyde oximes (5.33 a-d) in DCM solvent, in presence of NaOCl, Et_3N , at room temperature.

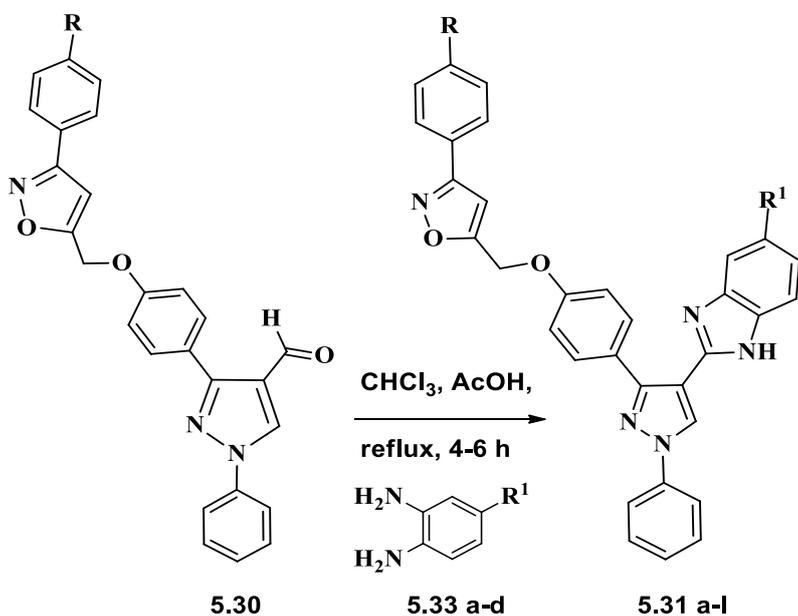


S.NO.	R
5.30a	H
5.30b	Cl
5.30c	OCH ₃
5.30d	CH ₃

Scheme 5.13: Substituted 1-phenyl-3-(4-((3-phenylisoxazol-5-yl)-methoxy)-phenyl)-1H-pyrazole-4-carbaldehyde (**5.30 a-d**)

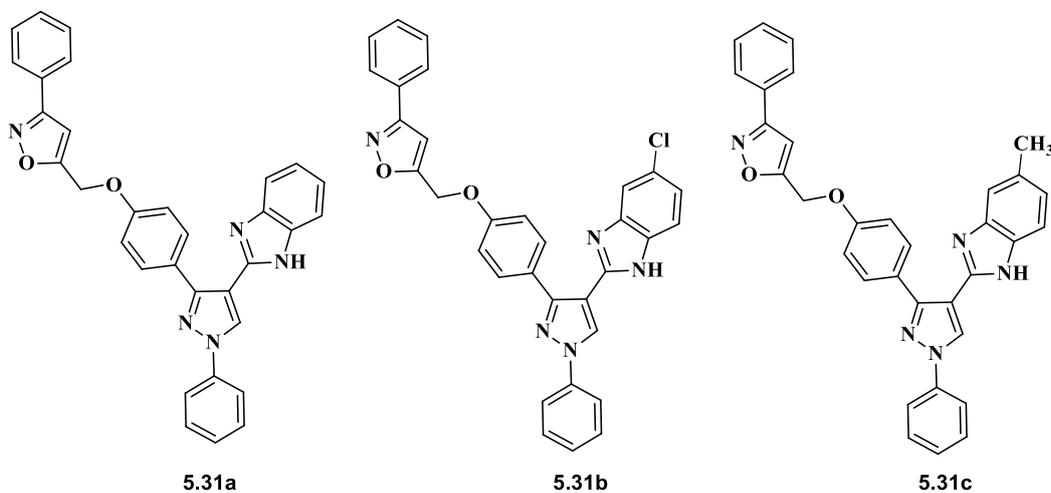
5) Synthesis of various substituted 5-((4-(4-“(1H-benzo[d]imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)”phenoxy)methyl)-3-phenylisoxazole (**5.31 a-i**)

A mixture of substituted 1-phenyl-3-(4-((3-phenylisoxazol-5-yl)-methoxy)-phenyl)-1H-pyrazole-4-carbaldehyde (**5.30**) and ortho phenyl di amine (**5.33 a-c**) were dissolved in CHCl₃ solvent, in presence of acetic acid (few drops) under reflux condition yielded substituted 5-((4-(4-“(1H-benzo [d] imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)”phenoxy)methyl)-3-phenylisoxazole (**5.31 a-i**).



Scheme 5.14: Substituted 5-((4-(4-“(1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)” phenoxy)methyl)-3-phenylisoxazole(5.31 a-l)

Newly Synthesized benzimidazole derivatives (5.31 a-l):



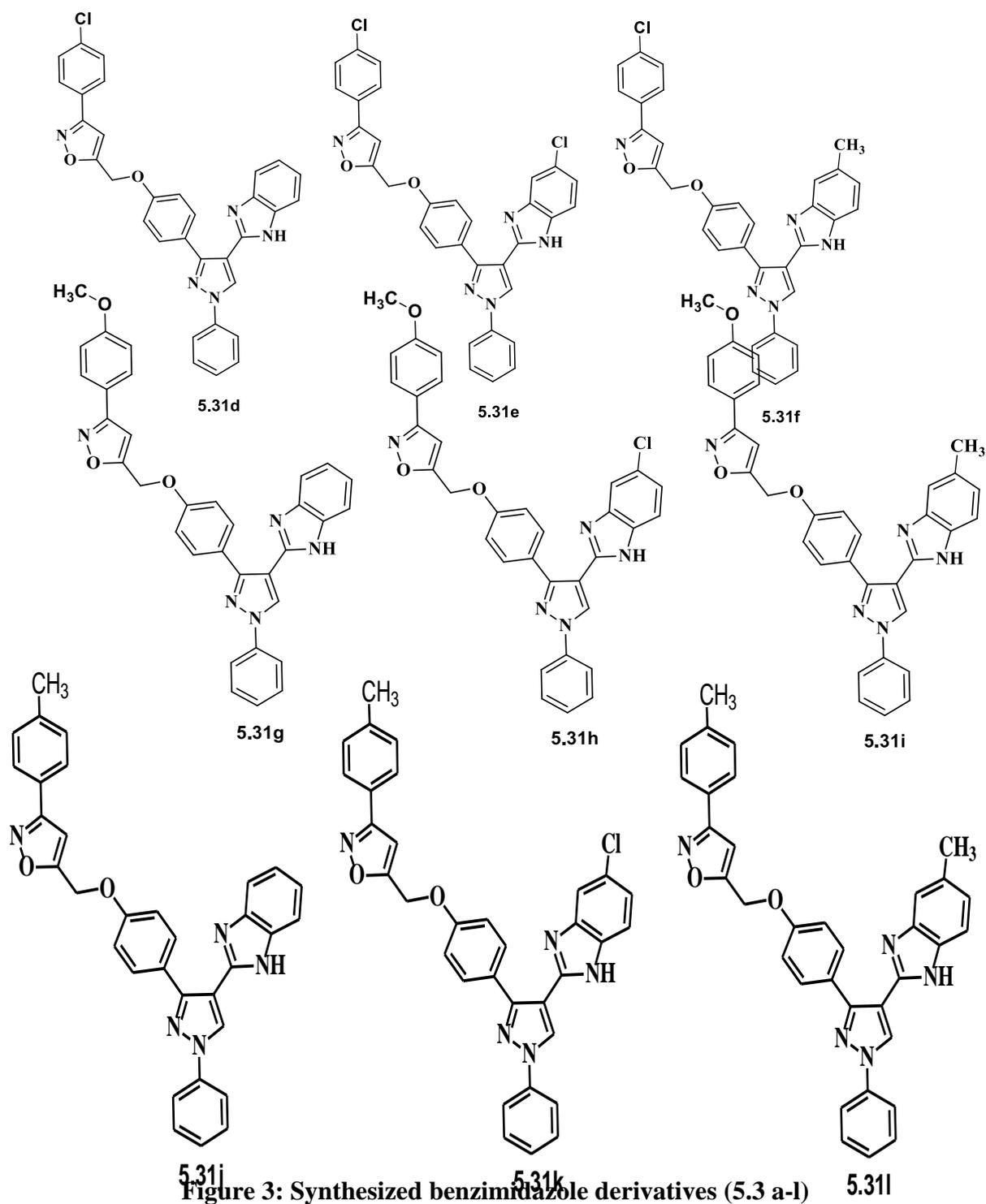


Figure 3: Synthesized benzimidazole derivatives (5.3 a-l)

Table-1. Physical data of substituted isoxazole, pyrazole conjugated benzimidazole derivatives (5.31a-l)

S No.	Compound	M.P. (°C)	M.F. (M.Wt.)	Time (h)	Yield (%)
1	“5-((4-(4-(1H-Benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-phenylisoxazole” (5.31a)	158-160	C ₃₂ H ₂₃ N ₅ O ₂ (509)	5.30	80
2	“5-((4-(4-(5-Chloro-1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-phenylisoxazole” (5.31b)	162-164	C ₃₂ H ₂₂ N ₅ O ₂ Cl (543)	5.00	86
3	“5-((4-(4-(5-Methyl-1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-phenylisoxazole” (5.31c)	154-156	C ₃₃ H ₂₅ N ₅ O ₂ (523)	6.00	84
4	“5-((4-(4-(1H-Benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-(4-chlorophenyl)isoxazole” (5.31d)	160-162	C ₃₂ H ₂₂ N ₅ O ₂ Cl (543)	5.00	85
5	“5-((4-(4-(5-Chloro-1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-(4-chlorophenyl)isoxazole” (5.31e)	170-172	C ₃₂ H ₂₁ N ₅ O ₂ Cl ₂ (577)	4.30	88
6	“5-((4-(4-(5-Methyl-1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-(4-chlorophenyl)isoxazole” (5.31f)	174-176	C ₃₃ H ₂₄ N ₅ O ₂ Cl (557)	5.30	84
7	“5-((4-(4-(1H-Benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-(4-methoxyphenyl)isoxazole” (5.31g)	164-166	C ₃₃ H ₂₅ N ₅ O ₃ (539)	6.00	81
8	“5-((4-(4-(5-Chloro-1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-(4-	176-178	C ₃₃ H ₂₄ N ₅ O ₃ Cl (573)	5.30	83

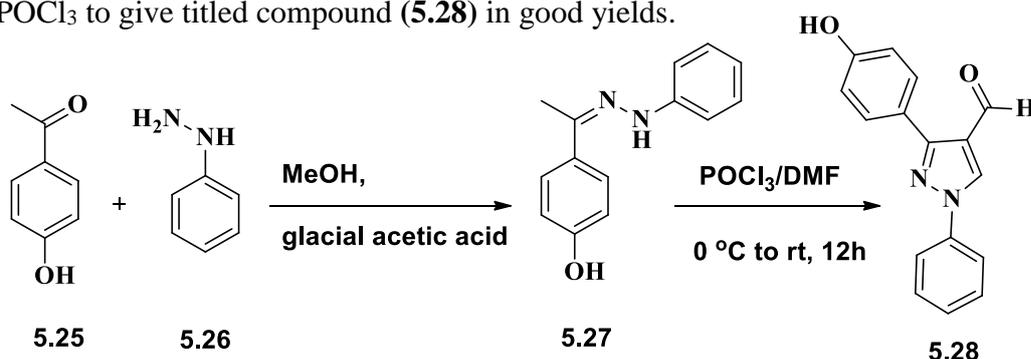
	methoxyphenyl)isoxazole” (5.31h)				
9	“5-((4-(4-(5-Methyl -1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-(4-methoxyphenyl)isoxazole” (5.31i)	168-170	C ₃₄ H ₂₇ N ₅ O ₃ (553)	6.00	80
10	“5-((4-(4-(1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-(p-tolyl)isoxazole” (5.31j)	152-154	C ₃₃ H ₂₅ N ₅ O ₂ (524)	5.30	85
11	“5-((4-(4-(5-chloro-1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-(p-tolyl)isoxazole” (5.31k)	144-146	C ₃₃ H ₂₄ N ₅ O ₂ Cl (553)	6.00	83
12	“5-((4-(4-(5-methyl-1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl)phenoxy)methyl)-3-(p-tolyl)isoxazole” (5.31l)	150-152	C ₃₄ H ₂₇ N ₅ O ₂ (538)	5.00	81

The newly synthesized compounds **5.31 a-l** were characterized by using their Proton, ¹³C-NMR, IR data and ESI-MS analysis. For example a descriptive spectral analysis of 5-“((4-(4-(1H-benzo[d]-imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl) phenoxy)methyl)-3-phenylisoxazole(**5.31a**), was discussed below.

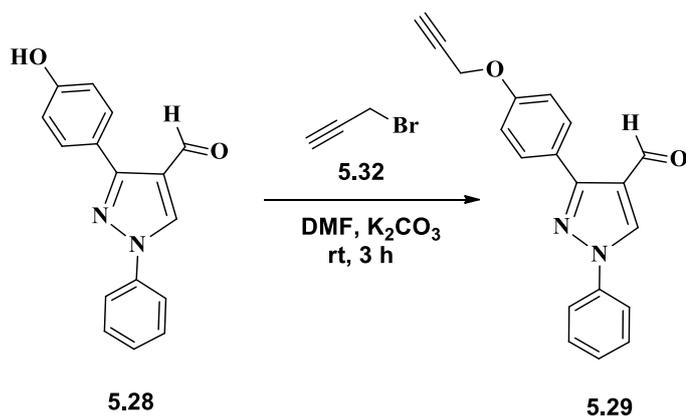
The ¹H NMR Spectra (400 MHz, CDCl₃) of compound **5.31a** (Fig-5.3) exhibited one singlet corresponding to the proton of pyrazole at δ~8.54 and one singlet corresponding to the proton of isoxazole at δ~8.07. All the aromatic protons were appeared in the range of δ ~7.82 – 6.82. The methylene two protons attached to oxygen were appeared as singlet at δ ~5.37. ¹³C-NMR spectrum (100 MHz, CDCl₃) of compound **5.31a** (Fig-5.4) revealed all the aromatic carbons in the range of δ ~155.8 – 114.8. Oxygen attached aliphatic carbon was appeared at δ ~61.5. IR spectrum (neat, Fig-5.5) of compound **5.31a** exhibited characteristic –C=N absorption band at 1608 cm⁻¹ and the ether band was observed at 1036 cm⁻¹. EI-Mass spectrum of **5.31a** (Fig-5.6) gave its base peak at m/z: 510 (M+H)⁺.

Experimental:
“Synthesis of 3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazole-4” -carbaldehyde(5.28):

The desired starting material **5.28** was synthesized by following previous reported methods.⁴⁸⁻⁵⁹ The 4-hydroxy acetophenones (**5.25**) was treated with phenyl hydrazine (**5.26**) to yield corresponding hydrazones (**5.27**), which was then subjected to Vilsmeier-Haack cyclization reaction in the presence of DMF/POCl₃ to give titled compound (**5.28**) in good yields.


Synthesis of 1-phenyl-3-(4-(prop-2-yn-1-yloxy)-phenyl)-1H-pyrazole-4-carbaldehyde (5.29):

The starting compound **5.29** was prepared by the propargylation of compound **5.28**, (1.0 eq), using propargyl bromide (**5.32**), (1.5 eq), in presence of K₂CO₃, DMF solvent (10 mL), under inert condition at room temperature for 3 hours. “After completion of the reaction, as confirmed by TLC, it was extracted using ice cold water (3 x 50 mL) and DCM. The combined organic layers was dried with anhydrous Na₂SO₄ and concentrated under vacuum. After that, the crude material was purified by silica gel column” chromatography using 20% ethyl acetate: hexane, as an eluent to afford compound **5.29** as yellow solid with 85% yield. The spectral data of isolated compound **5.29** is in good agreement with the reported spectral data.


Synthesis of substituted 1-phenyl-3-(4-((3-phenylisoxazol-5-yl)-methoxy)-phenyl)1H-pyrazole-4-carbaldehyde (5.30 a-c):

To a solution of compounds **5.29** (1.00 mmol) in DCM (1mL), compound **5.33 a-c** (1.1 mmol), tri ethyl amine (1.5 mmol), and sodium hypo chloride (9-12% in H₂O, 12mL) were added at 0 °C temperature, under inert conditions and the reaction was stirred for 12 hours. “After completion of the reaction, monitored by TLC, the resulting solution was diluted with ice cold water, extracted with ethyl acetate; the organic layer was dried over sodium sulphate, evaporated under vacuo. The

crude product was purified using silica gel column"; elution of the column with 20% ethyl acetate in hexane afforded the pure compound **5.30 a-I** in 60-70% yield, as yellow coloured solid.

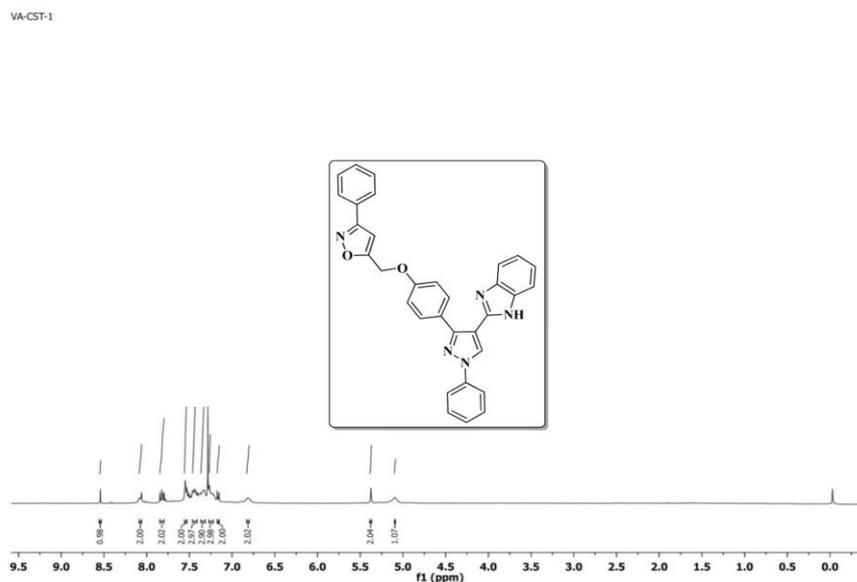


Fig: 5.4 ¹H NMR Spectrum of compound 5.30a (400 MHz, CDCl₃)

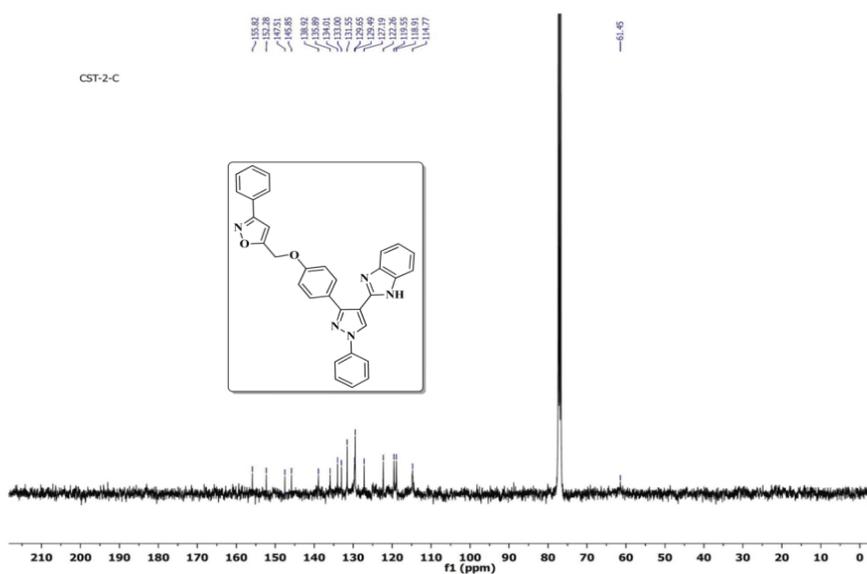


Fig: 5.5 ¹³C NMR Spectrum of compound 5.30a (100 MHz, CDCl₃)

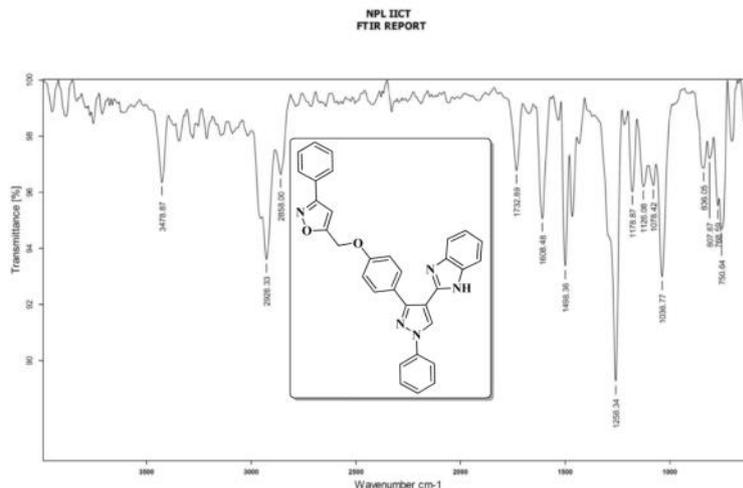


Fig: 56 FT-IR Spectrum of compound 5.30a

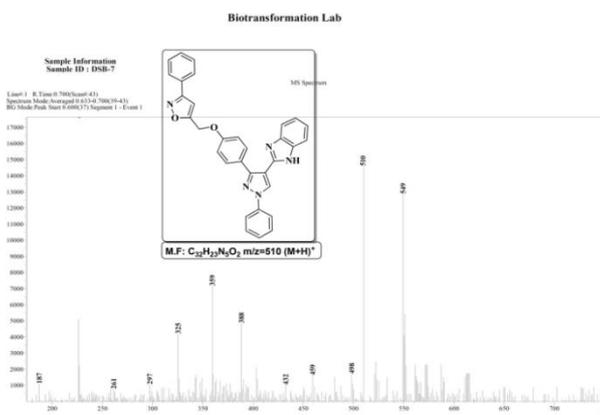


Fig: 5.7ESI- MASS Spectrum of compound 5.30a

Conclusion:

In summary, we have developed an efficient protocol for the synthesis of new family of isoxazole pyrazole conjugated benzimidazole with moderate to good yields. All the synthesized compounds will be submitted for other antimicrobial and antifungal activities.

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