

# Synthesis and characterization of novel 6-fluoro-4-piperidinyl-1,2-benzisoxazole amides and 6-fluoro-chroman-2-carboxamides: antimicrobial studies

B. S. Priya, Basappa, S. Nanjunda Swamy and Kanchugarakoppal S. Rangappa\*

Department of Studies in Chemistry, University of Mysore, Manasagangothri, Mysore 570006, India

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**Abstract**—Novel derivatives of 6-fluoro-4-piperidinyl-1,2-benzisoxazole amides **4(I–VI)** were obtained by the condensation of different acid chlorides with 6-fluoro-3-piperidin-4yl-benzo[d]isoxazole. Also, 6-fluoro-chroman-2-carboxamides **6(I–III)** were synthesized by using nebolic acid chloride with different amines in presence of triethylamine as acid scavenger and dichloroethane as solvent. The synthesized compounds were characterized by IR, <sup>1</sup>H NMR, and CHN analysis. These molecules were evaluated for their efficacy as antimicrobials in vitro by disc diffusion and microdilution method against pathogenic strains such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Xanthomonas campestris pvs*, *X. oryzae*, *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Trichoderma* species, *F. monaliforme*, and *Penicillium* species. Compounds **4I**, **4IV**, **4V**, **6I**, **6II** and **6III** showed better inhibitory activity than compared to standard drugs. Among these compounds, **4IV** and **6III** showed potent inhibitory activity against all the strains and found to be nonstrain dependent. The title compounds represent a novel class of potent antimicrobial agents.

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## 1. Introduction

The development of antimicrobial drugs represent one of the most important advances in therapeutics, which has improved the quality of life and advances in many other areas of medicine, for example, cancer chemotherapy, organ transplantation, and major surgery etc.<sup>1</sup> Many reports ascribe interesting biological activities of 1,2-benzisoxazoles and their derivatives. The chemistry of substituted 1,2-benzisoxazole amides occupies an extremely important role in the field of pharmaceuticals and in medicinal fields. A red dye, prontosil was synthesized in German by Klarer and Mietzsch in 1932 and tested by the usual (in vitro) screening method against bacterial culture but found to be ineffective in these tests. But in 1935, Domagk reported in vivo and it was strikingly active against haemolytic streptococci and other infections, in spite of the advent of the antibiotic drugs. Sulfonamides are among the most widely used antibacterial agents used so far today.<sup>2</sup> Sulfonamides

can produce a wide variety of outward effects due partly to allergy, direct toxicity, allergic nephritis, anaemia.<sup>3</sup>

Compounds containing amide bond, benzisoxazoles, chromans and fluorine atom substitution can alter the chemical properties, disposition, and biological activities of drugs.<sup>4</sup> Many fluorinated compounds, 1,2-benzisoxazole derivatives and various amides are currently used in the treatment of diseases. These include, antidepressants, anti-inflammatory agents, antimalarial drugs, antipsychotics, antiviral agents, steroids, and general anaesthetics.<sup>5</sup> The fluorine substitution can also have a profound effect on drug disposition, in terms of distribution, drug clearance, route(s), and extent of drug metabolism.<sup>6</sup> Medicinal chemists improve both the safety and the efficacy of a drug change constructively. Therefore, the purpose of this study is to apply our continuous efforts in two-fold. First, to outline the chemical basis of changes in drug disposition that can be achieved by the introduction of bioactive groups or key functional groups of natural products or fluorine. Secondly, to consider the pharmacological and toxicological implications of such changes with respect to drug response.

Our previous studies on the synthesis of heterocycles like isoxazoles, novel isoxazolidines showed a wide

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\* Corresponding author. Tel.: +91 821 2412191; fax: +91 821 2416133/2421263; e-mail: [rangappaks@yahoo.com](mailto:rangappaks@yahoo.com)

spectrum of antimicrobial activities.<sup>7–12</sup> So we continued our efforts to synthesize a series of compounds, which have multifunctional pharmacologically active groups like 1,2-benzisoxazole, 4-piperidiny-amides, chromans, fluorine substituted amides, which can exhibit antimicrobial activities. In connection with our efforts, to identify a variety of biological targets, we report the synthesis of novel molecules and their in vitro antimicrobial activities by disc diffusion and microdilution method against eleven pathogenic strains, that is, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Xanthomonas campestris pvs*, *X. oryzae*, *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Trichoderma* species, *F. monaliforme*, and *Penicillium* species.

## 2. Chemistry

The synthesis of 1,2-benzisoxazole series began with the synthesis of 4-[(2,4-difluoro-phenyl)-methoxy-methyl]-piperidin-1-carbaldehyde via Friedel–Crafts acylation method by using *n*-formyl-isonipecotic acid and 1,3-difluorobenzene. The amine, 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole was obtained in one step from the above intermediate, which involves the hydroxylamine

sulfate/powdered potassium hydroxide mediated oxime formation and its subsequent internal cyclization followed by alkaline hydrolysis of the protected piperidiny group simultaneously, which is reported for the first time from our laboratory.<sup>13</sup>

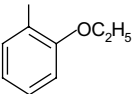
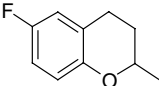
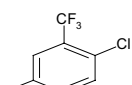
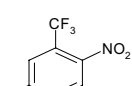
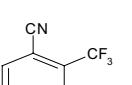
Further, the condensation of the previously synthesized different acid chlorides with 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole in presence of acid scavenger, triethylamine in dichloroethane as solvent and DMF as catalyst. The newly synthesized molecules are tabulated in Table 1 and schematic diagram is as shown in Scheme 1. Similarly the nebolic acid amides are synthesized by the condensation reaction of the above acid chloride with the different amines, which is shown in Scheme 2 and Table 1.

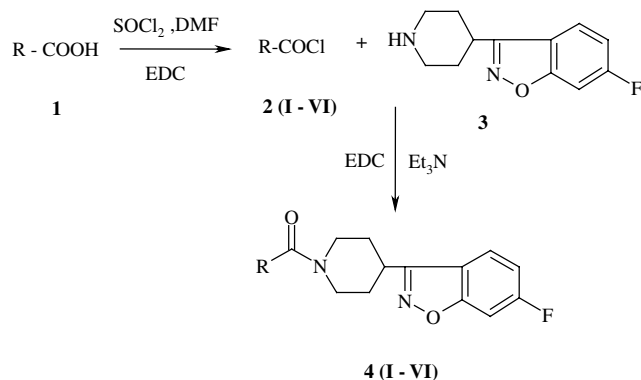
## 3. Results and discussion

### 3.1. Chemistry

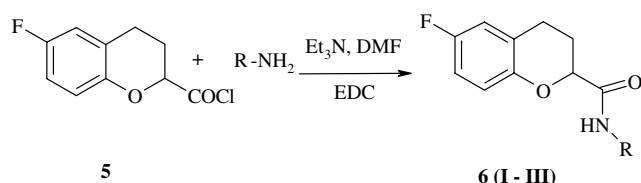
The condensation reaction of different acid chlorides with the amines like 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole and chroman derivatives gave a good yield

**Table 1.** Reaction condition and physical data of 1,2-benzisoxazole and chroman amides (**4I–VI** and **6I–III**)

| Amides      | R                                                                                   | <i>R<sub>f</sub></i> value | Eluent                     | Yield (%) | Mp (°C) |
|-------------|-------------------------------------------------------------------------------------|----------------------------|----------------------------|-----------|---------|
| <b>4I</b>   |  | 0.50                       | Chloroform–methanol, 9:1   | 90        | 150–152 |
| <b>4II</b>  |  | 0.70                       | Chloroform–methanol, 9:1   | 80        | 120–125 |
| <b>4III</b> |  | 0.55                       | Chloroform–methanol, 9:1   | 75        | 110–115 |
| <b>4IV</b>  |  | 0.6                        | Chloroform–methanol, 9:1   | 95        | Oily    |
| <b>4V</b>   |  | 0.45                       | Chloroform–methanol, 9:1   | 75        | Oily    |
| <b>4VI</b>  |  | 0.50                       | Chloroform–methanol, 9:1   | 65        | 130–132 |
| <b>6I</b>   |  | 0.80                       | Benzene–ethyl acetate, 9:1 | 70        | Oily    |
| <b>6II</b>  |  | 0.64                       | Benzene–ethyl acetate, 9:1 | 78        | 140–145 |
| <b>6III</b> |  | 0.52                       | Benzene–ethyl acetate, 9:1 | 82        | 172–175 |



**Scheme 1.** Where R = 2-ethoxy-phenyl, **4I**; R = 6-fluoro-chroman-2-yl-, **4II**; R = 3-pyridinyl, **4III**; R = 2-pyridinyl, **4IV**; R = 2-chloro-phenyl, **4V**; R = 2,3-dichloro-phenyl **4VI**.



**Scheme 2.** R = 3-trifluoromethyl-4-chloro-phenyl-**6I**; R = 3-trifluoromethyl-4-nitro-phenyl-**6II**; R = 4-cyano-3-trifluoromethyl-phenyl-**6III**.

in the ratio of 65% to 75% with a purity 90–95%. In case of chromane derivative, N–H stretching broad peak in the range of  $3300\text{--}3400\text{ cm}^{-1}$  and carbonyl bond stretching in the range of  $1620\text{--}1700\text{ cm}^{-1}$  were observed. But in case of 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole derivative, we observed only carbonyl group bond stretching in the region of  $1620\text{--}1660\text{ cm}^{-1}$ . The products were obtained in good yield and greater than purity of 95%. The reaction condition and physical data of these amides are given in Table 1. All the synthesized compounds were characterized by IR,  $^1\text{H}$  NMR, and CHN elemental analysis.

### 3.2. Biology: in vitro evaluation of antimicrobial activity

In approach of synthesizing new antimicrobial compounds, we have synthesized, 6-fluoro-4-piperidinyl-

1,2-benzisoxazole amides **4(I–VI)** and new 6-fluoro-chroman-2-carboxamides **6(I–III)** and evaluated for their efficacy as antimicrobials in vitro by disc diffusion and microdilution method against the various pathogenic strains. Nystatin was used as standard drug against fungi, streptomycin and tetracycline were tested against bacteria. In all determinations, tests were performed in duplicate and the results were reported as mean of atleast three determinations. Our results showed that, from the 6-fluoro-4-piperidinyl-1,2-benzisoxazole amide series, compounds **4I**, **4IV** and **4V** showed significant inhibition in the order **4IV** > **4V** > **4I**. The significant inhibition shown by the compounds **4I** and **4V** might be due to the presence of chloro and ethoxy groups in the second position of the benzene ring. Compound **4IV** showed potent inhibition against all the strains tested and was not strain dependant, this result reveal that the compound **4IV** bearing 2-pyridine ring plays a pivotal role for the inhibitory activity when compared to **4III**, which is substituted at third position, in which no significant inhibition was observed. Among the other series of compounds tested 6-fluoro-chroman-2-carboxamides **6(I–III)**, the inhibition was in the order **6III** > **6I** > **6II**. Both the key functional moieties have fluorine atom at the sixth position of 1,2-benzisoxazole and chroman groups. The inhibition shown may be possibly due to the presence of trifluoromethyl group, a most lipophilic group, which can exert effect compared to a phenyl ring or even a *tert*-butyl function.<sup>14</sup> Also the inhibitory activity may be due to the presence of nitrile, chloro and nitro groups in **6III**, **6I** and **6II** respectively. Compounds **4II**, **4III** and **4VI** were not effective against any of the strains tested (Tables 2–5).

### 4. Conclusion

In conclusion, we report the antimicrobial studies of new 6-fluoro-chroman-2-carboxamides and 6-fluoro-4-piperidinyl-1,2-benzisoxazole amides. Compounds **4IV** and **6III** showed potent inhibition against all the strains tested and found to be nonstrain dependant. Modifications to improve the potency of this series by diversification of the position and type of amides are currently under progress in our lab.

**Table 2.** Minimal inhibitory concentration (MIC) in  $\mu\text{g/mL}$  of synthesized compounds against tested bacterial strains by microdilution method

| Compounds    | Minimal inhibitory concentration (MIC) in $\mu\text{g/mL}$ |                |                       |                           |                  |
|--------------|------------------------------------------------------------|----------------|-----------------------|---------------------------|------------------|
|              | <i>B. subtilis</i>                                         | <i>E. coli</i> | <i>P. fluorescens</i> | <i>X. campestris pvs.</i> | <i>X. oryzae</i> |
| <b>4I</b>    | 14                                                         | 11             | 9                     | 8                         | 10               |
| <b>4II</b>   | 25                                                         | 19             | 22                    | 19                        | 24               |
| <b>4III</b>  | 28                                                         | 24             | 29                    | 19                        | 24               |
| <b>4IV</b>   | 9                                                          | 7              | 5                     | 3                         | 5                |
| <b>4V</b>    | 11                                                         | 9              | 6                     | 6                         | 8                |
| <b>4VI</b>   | 27                                                         | 24             | 19                    | 25                        | 30               |
| <b>6I</b>    | 10                                                         | 10             | 7                     | 8                         | 9                |
| <b>6II</b>   | 15                                                         | 12             | 11                    | 10                        | 12               |
| <b>6III</b>  | 8                                                          | 6              | 6                     | 2                         | 4                |
| Streptomycin | 20                                                         | 14             | 12                    | —                         | —                |
| Tetracycline | —                                                          | —              | —                     | 9                         | 14               |

**Table 3.** Minimal inhibitory concentration (MIC) in  $\mu\text{g/mL}$  of synthesized compounds against tested fungal strains by microdilution method

| Compounds   | Minimal inhibitory concentration (MIC) in $\mu\text{g/mL}$ |                  |                     |                            |                       |                            |
|-------------|------------------------------------------------------------|------------------|---------------------|----------------------------|-----------------------|----------------------------|
|             | <i>A. niger</i>                                            | <i>A. flavus</i> | <i>F. oxysporum</i> | <i>Trichoderma</i> species | <i>F. moniliforme</i> | <i>Penicillium</i> species |
| <b>4I</b>   | 11                                                         | 12               | 10                  | 9                          | 7                     | 9                          |
| <b>4II</b>  | 22                                                         | 20               | 26                  | 23                         | 20                    | 24                         |
| <b>4III</b> | 19                                                         | 22               | 29                  | 27                         | 23                    | 20                         |
| <b>4IV</b>  | 8                                                          | 6                | 5                   | 6                          | 3                     | 5                          |
| <b>4V</b>   | 10                                                         | 9                | 7                   | 8                          | 6                     | 8                          |
| <b>4VI</b>  | 12                                                         | 10               | 5                   | 10                         | 4                     | 8                          |
| <b>6I</b>   | 11                                                         | 10               | 8                   | 9                          | 7                     | 8                          |
| <b>6II</b>  | 13                                                         | 11               | 9                   | 10                         | 8                     | 9                          |
| <b>6III</b> | 7                                                          | 7                | 6                   | 5                          | 4                     | 6                          |
| Nystatin    | 16                                                         | 14               | 12                  | 12                         | 8                     | 10                         |

**Table 4.** Inhibitory zone (diameter) mm of synthesized compounds against tested bacterial strains by disc diffusion method

| Compounds    | Inhibitory zone (diameter) mm |                |                       |                           |                  |
|--------------|-------------------------------|----------------|-----------------------|---------------------------|------------------|
|              | <i>B. subtilis</i>            | <i>E. coli</i> | <i>P. fluorescens</i> | <i>X. campestris pvs.</i> | <i>X. oryzae</i> |
| <b>4I</b>    | 14                            | 17             | 21                    | 15                        | 14               |
| <b>4II</b>   | 9                             | 8              | 6                     | 8                         | 7                |
| <b>4III</b>  | 9                             | 7              | 10                    | 8                         | 5                |
| <b>4IV</b>   | 18                            | 22             | 25                    | 19                        | 20               |
| <b>4V</b>    | 16                            | 18             | 21                    | 16                        | 15               |
| <b>4VI</b>   | 6                             | 5              | 8                     | 4                         | 3                |
| <b>6I</b>    | 14                            | 17             | 20                    | 15                        | 13               |
| <b>6II</b>   | 13                            | 16             | 21                    | 14                        | 12               |
| <b>6III</b>  | 19                            | 20             | 24                    | 20                        | 19               |
| Streptomycin | 12                            | 14             | 18                    | —                         | —                |
| Tetracycline | —                             | —              | —                     | 12                        | 11               |

Streptomycin sulfate (25  $\mu\text{g/disc}$ ); tetracycline (25  $\mu\text{g/disc}$ ) were used as positive reference standard antibiotic discs, synthesized compounds (25  $\mu\text{g/disc}$ ).

**Table 5.** Inhibitory zone (diameter) mm of synthesized compounds against tested fungal strains by disc diffusion method

| Compounds   | Inhibitory zone (diameter) mm |                  |                     |                            |                       |                            |
|-------------|-------------------------------|------------------|---------------------|----------------------------|-----------------------|----------------------------|
|             | <i>A. niger</i>               | <i>A. flavus</i> | <i>F. oxysporum</i> | <i>Trichoderma</i> species | <i>F. moniliforme</i> | <i>Penicillium</i> species |
| <b>4I</b>   | 15                            | 13               | 18                  | 19                         | 15                    | 14                         |
| <b>4II</b>  | 6                             | 5                | 5                   | 6                          | 4                     | 2                          |
| <b>4III</b> | 4                             | 3                | 1                   | 4                          | 5                     | 3                          |
| <b>4IV</b>  | 20                            | 19               | 24                  | 29                         | 23                    | 21                         |
| <b>4V</b>   | 18                            | 16               | 20                  | 21                         | 19                    | 18                         |
| <b>4VI</b>  | 5                             | 4                | 2                   | 7                          | 5                     | 4                          |
| <b>6I</b>   | 19                            | 17               | 23                  | 28                         | 22                    | 23                         |
| <b>6II</b>  | 16                            | 14               | 19                  | 20                         | 16                    | 15                         |
| <b>6III</b> | 21                            | 20               | 25                  | 28                         | 25                    | 22                         |
| Nystatin    | 8                             | 10               | 14                  | 16                         | 12                    | 10                         |

Nystatin (25  $\mu\text{g/disc}$ ) was used as positive reference standard antibiotic discs, synthesized compounds (25  $\mu\text{g/disc}$ ).

## 5. Experimental

The melting points were determined on SELACO-650 hot stage apparatus and are uncorrected. IR (Nujol) spectra were measured on Shimadzu 8300 IR spectrophotometer,  $^1\text{H}$  NMR were recorded on Shimadzu AMX 400-Bruker, 400 MHz spectrometer by using  $\text{CDCl}_3$  as solvent and TMS as an internal standard (chemical shift in  $\delta$  ppm). Elemental analyses were obtained on a Vario-EL instrument. TLC was conducted on 0.25 mm silica gel plates (60F<sub>254</sub>, Merck) and Column by silica gel BDH 60–120 mesh. All extracted solvents were dried over  $\text{Na}_2\text{SO}_4$ , followed by evaporation in vacuo.

### 5.1. Synthesis of 6-fluoro-3-piperidin-4yl-benzo[d]isoxazole 3 was reported already from our laboratory<sup>13</sup>

**5.1.1. General procedure for the synthesis of acid chlorides 2(I–VI).** A solution of respective acids in dichloroethane (10 mL) was taken. Thionyl chloride (2 mL) in dichloroethane (3 mL) was added dropwise to a solution and a drop of N,N-dimethylformamide was added. The reaction mixture was refluxed for 6–7 h at 60–70 °C till the completion of the reaction, solvent was evaporated under vacuum, dichloroethane was added twice and evaporated again to dryness in order to remove the excess of thionyl chloride present in the reaction mixture. The

crude acid chlorides were taken directly to the next reactions.

**5.1.2. General procedure for the synthesis of 1,2-benzisoxazole substituted amides.** A solution of amine **3** (1 equiv) in dichloroethane was taken and cooled to 0 °C. The crude acid chloride (1.2 equiv) was added dropwise and triethylamine was added at the same temperature. The reaction mixture was stirred for another 1 h at 0 °C. The reaction mass was gradually allowed to room temperature. After completion of the reaction, the solvent was evaporated under vacuum and extracted with dichloroethane thrice. The organic layer was washed with 5% HCl solution and 10% NaHCO<sub>3</sub> solution in order to remove unreacted amines and acids, respectively. Finally water wash was given to the organic layer and dried with anhydrous sodium sulfate, evaporated the solvent. Using appropriate mixture of solvent like benzene, *n*-hexane, ethylacetate as eluent in silica gel column, pure product was separated.

**5.1.3. Synthesis of (2-ethoxy-phenyl)-[4-(6-fluoro-benzo[d]isoxazole-3-yl)-piperidin-1-yl]-methanone **4I**.** It was obtained from 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole **3** (0.2 g, 0.909 mmol), 2-ethoxybenzoyl chloride (0.201 g, 1.09 mmol), and triethylamine (0.551 g, 5.454 mmol). The product obtained was pure white solid. IR (cm<sup>-1</sup> Nujol): 1620.1, 1454.2, 1492.8, 1348.1, 1244, 1043.4. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 1.42 (t, 3H), 1.65 (q, 2H), 3.0–3.18 (m, 1H), 3.53 (q, 4H), 4.02–4.15 (t, 2H), 6.9 (d, 1H, *J* = 18 Hz, Ar-H), 6.96–7.02 (t, 2H, Ar-H), 7.4 (t, 1H, Ar-H), 7.8 (d, 1H, Ar-H), 6.85 (s, 1H, Ar-H), 7.6–7.67 (dd, 1H, *J* = 2 Hz, *J* = 13 Hz, Ar-H). Anal. Calcd CHN: 68.478, 5.706, 7.608. Found 68.602, 5.689, 7.312.

**5.1.4. Synthesis of [4-(6-fluoro-benzo[d]isoxazole-3-yl)-piperidin-1-yl]-(6-fluoro-chroman-2-yl)-methanone **4II**.** It was obtained from 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole **3** (0.2 g, 0.909 mmol), nebulic acid chloride (0.213 g, 1.0908 mmol), and triethylamine (0.551 g, 5.454 mmol). The product obtained was brownish yellow solid. IR (cm<sup>-1</sup> Nujol): 1640, 1434.9, 1515.9, 1060. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 1.8 (q, 4H), 3.4 (t, 4H), 2.5–2.6 (t, 2H, chroman-CH<sub>2</sub>), 2.6–2.8 (m, 1H), 2.2 (q, 2H), 4.4 (t, 2H), 6.78 (s, 1H, Ar-H), 6.65 (d, 1H, Ar-H), 6.9 (d, 1H, Ar-H), 7.31 (d, 1H, Ar-H), 7.12 (d, 1H, Ar-H), 7.4 (s, 1H, Ar-H). Anal. Calcd CHN: 66.331, 5.02, 7.035. Found 66.521, 4.98, 7.21.

**5.1.5. Synthesis of [4-(6-fluoro-benzo[d]isoxazole-3-yl)-piperidin-1-yl]-pyridin-3-yl-methanone **4III**.** It was obtained from 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole **3** (0.2 g, 0.909 mmol), nicotinic acid chloride (0.134 g, 1.0908 mmol), and triethylamine (0.551 g, 5.454 mmol). The product obtained was pale yellow solid. IR (cm<sup>-1</sup> Nujol): 1654, 1480, 1440, 1210. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 1.65 (q, 4H), 2.9 (m, 1H), 3.46 (t, 4H), 6.85 (d, 1H, Ar-H), 7.24–7.28 (dd, 1H, Ar-H), 6.90–6.95 (s, 1H, Ar-H), 7.34–7.48 (t, 1H, Ar-H), 7.82 (d, 1H, Ar-H), 7.99 (dd, 2H, Ar-H), 8.59–8.62 (s, 1H, Ar-H). Anal. Calcd CHN: 66.461, 4.923, 12.923. Found 66.625, 4.879, 12.982.

**5.1.6. Synthesis of [4-(6-fluoro-benzo[d]isoxazole-3-yl)-piperidin-1-yl]-pyridin-2-yl-methanone **4IV**.** It was obtained from 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole **3** (0.2 g, 0.909 mmol), picolinic acid chloride (0.134 g, 1.0908 mmol), and triethylamine (0.551 g, 5.454 mmol). The product obtained was oily. IR (cm<sup>-1</sup> Nujol): 1624.1, 1510, 1480, 1060. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 1.8–1.95 (q, 4H), 2.5 (m, 1H), 3.46 (t, 4H), 6.97 (s, 1H, Ar-H), 7.25–7.30 (dd, 1H, Ar-H), 7.34–7.38 (d, 1H, Ar-H), 7.64–7.7 (t, 1H, Ar-H), 7.81–7.85 (t, 1H, Ar-H), 8.6 (d, 1H, *J* = 10 Hz, Ar-H), 8.12 (d, 1H, Ar-H). Anal. Calcd CHN: 66.461, 4.923, 12.923. Found 66.321, 4.965, 12.894.

**5.1.7. Synthesis of (2-chloro-phenyl)-[4-(6-fluoro-benzo[d]isoxazole-3-yl)-piperidin-1-yl]-methanone **4V**.** It was obtained from 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole **3** (0.2 g, 0.909 mmol), 2-chloro benzoyl chloride (0.170 g, 1.0908 mmol) and triethylamine (0.551 g, 5.454 mmol). The product obtained was oily. IR (cm<sup>-1</sup> Nujol): 1670, 1492, 1450, 1244. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 1.65 (q, 4H), 3.12–3.2 (m, 1H), 3.72 (t, 4H), 7.39 (t, 1H, Ar-H), 7.74 (d, 1H, *J* = 12 Hz, Ar-H), 6.9 (s, 1H, Ar-H), 6.96–7.02 (d, 1H, Ar-H), 7.05–7.11 (dd, 1H, Ar-H), 7.6–7.67 (t, 1H, Ar-H), 7.15–7.2 (d, 1H, Ar-H). Anal. Calcd CHN: 63.598, 4.463, 7.810. Found 63.556, 4.461, 7.825.

**5.1.8. Synthesis of (2,3-dichloro-phenyl)-[4-(6-fluoro-benzo[d]isoxazole-3-yl)-piperidin-1-yl]-methanone **4VI**.** It was obtained from 6-fluoro-3-piperidin-4-yl-benzo[d]isoxazole **3** (0.2 g, 0.909 mmol), 2,3-dichloro benzoyl chloride (0.209 g, 1.0908 mmol), and triethylamine (0.551 g, 5.454 mmol). The product obtained was yellow solid. IR (cm<sup>-1</sup> Nujol): 1656.9, 1480, 1440, 1228.6. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 1.98 (q, 4H), 3.14 (t, 4H), 4.13–4.29 (m, 1H), 6.75 (s, 1H, Ar-H), 7.32 (t, 1H, Ar-H), 7.35 (d, 1H, Ar-H), 7.45 (dd, 1H, Ar-H), 7.52 (t, 1H, Ar-H), 7.24 (d, 1H, Ar-H). Anal. Calcd CHN: 58.015, 3.817, 7.12. Found 58.142, 3.792, 7.20.

**5.1.9. Synthesis of 6-fluoro-chroman-2-carboxylic acid (4-chloro-3-trifluoromethyl-phenyl)-amide **6I**.** It was obtained from 5-amino-2-chloro-benzotrifluoride (0.2 g, 1.022 mmol), nebulic acid chloride (0.24 g, 1.227 mmol), and triethyl amine (0.62 g, 6.132 mmol). The product obtained was oily. IR (cm<sup>-1</sup> Nujol): 3360, 1640, 1458. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 2.42 (t, 2H), 2.12–2.2 (q, 2H), 4.1 (t, 2H), 3.12 (s, 1H, -NH), 7.39 (d, 1H, Ar-H), 7.74 (s, 1H, Ar-H), 6.9 (d, 1H, Ar-H), 6.96–7.02 (dd, 1H, *J* = 8 Hz, Ar-H), 7.05–7.11 (t, 1H, Ar-H), 7.5 (dd, 1H, Ar-H). Anal. Calcd CHN: 54.618, 3.212, 3.748. Found 54.653, 3.199, 3.792.

**5.1.10. Synthesis of 6-fluoro-chroman-2-carboxylic acid (4-nitro-3-trifluoromethyl-phenyl)-amide **6II**.** It was obtained from 5-amino-2-nitro-benzotrifluoride (0.2 g, 0.970 mmol), nebulic acid chloride (0.228 g, 1.1643 mmol), and triethyl amine (0.589 g, 5.82 mmol). The product obtained was white crystalline solid. IR (cm<sup>-1</sup> Nujol): 3320, 1660, 1425, 1594. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 2.1 (q, 2H), 2.9 (t, 2H), 3.46 (m, 1H), 4.1 (s, 1H, -NH), 6.65–6.70 (d, 1H, Ar-H),



6.95–7.1 (dd, 1H, Ar–H), 7.82 (d, 1H, Ar–H), 7.99 (dd, 2H, Ar–H), 8.05 (s, 1H, Ar–H). 7.25 (s, 1H, Ar–H). Anal. Calcd CHN: 53.125, 3.125, 7.291. Found 53.202, 3.110, 7.296.

**5.1.11. Synthesis of 6-fluoro-chroman-2-carboxylic acid (4-cyano-3-trifluoromethyl-phenyl)-amide 6III.** It was obtained from 4-amino-2-trifluoromethyl-benzonitrile (0.2 g, 1.074 mmol), nebolic acid chloride (0.252 g, 1.289 mmol), and triethyl amine (0.652 g, 6.444 mmol). The product obtained was pale yellow solid. IR (cm<sup>-1</sup> Nujol): 3325, 2220, 1655, 1480. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$ : 2.65 (t, 2H), 2.3 (q, 2H), 3.92 (t, 2H), 3.56 (s, 1H, –NH), 7.24 (d, 1H, Ar–H), 7.68 (s, 1H, Ar–H), 6.76 (d, 1H, Ar–H), 7.16–7.23 (dd, 1H, *J* = 12 Hz, Ar–H), 7.35–7.4 (t, 1H, Ar–H), 7.56 (dd, 1H, Ar–H). Anal. Calcd CHN: 59.340, 3.296, 7.692. Found 59.362, 3.298, 7.565.

## 5.2. Biology

**5.2.1. Materials and methods.** Bacteria and fungal species used were obtained from microbiology department, university of Mysore, India. Namely, *Bacillus subtilis*, *E. coli*, *P. fluorescens*, *X. campestris pvs*, *X. oryzae*, *A. niger*, *A. flavus*, *F. oxysporum*, *Trichoderma* species, *F. monaliforme*, and *Penicillium* species. The bacterial strains were maintained on LB agar medium and the filamentous fungi were maintained on Potato dextrose agar (PDA) medium at 28 °C. The disc diffusion method<sup>15</sup> was used to determine antimicrobial activity of synthesized compounds. Paper discs with only DMSO were used as negative controls (Table 3).

The bacteria inoculum was prepared by suspending in 9 mL of sterile water for colonies from 24 h culture on LB agar medium. For the filamentous fungi, the inoculum was prepared with the spores derived from 5 to 15 days culture on PDA medium. The mycelia were covered with 10 mL of distilled water and the conidia were scraped using sterile pipette. The spores were recovered after filtration on sterile absorbent cotton and were resuspended in sterile distilled water (Table 4).

The cell density of each inoculum was adjusted with hemocytometer in order to obtain a final concentration of approximately 10<sup>4</sup> CFU/mL and 10<sup>6</sup> CFU/mL for the bacteria and filamentous fungi, respectively.

Nystatin (Himedia) was used as positive control for fungi and streptomycin and tetracycline for bacteria. Each disc contained 25  $\mu$ g of standard drugs and synthesized compounds. Plates were first kept at 4 °C for at least 2 h to allow the diffusion of chemicals, and then incubated at 28 °C. Inhibition zones were measured after 24 h of incubation for bacteria and after 48 h of incubation for fungi (Table 5).

The microdilution method<sup>16</sup> was followed to determine the minimum inhibitory concentration (MIC) of all the synthesized compounds. The Nutrient liquid medium and Potato dextrose liquid medium were used as test

media. Tests were performed in 96-well round bottom sterile culture plates. The suspensions of yeast and filamentous fungi were adjusted in sterile water to match the density of a 0.5 McFarland Standard. The wells of a microdilution plate were inoculated with 180  $\mu$ L of the culture medium containing a final inoculum of 0.5–2.5  $\times$  10<sup>3</sup> CFU/mL. All the compounds previously solubilized in DMSO were serially diluted to two folds in the liquid medium and gave a range of concentration from 640 to 0.1  $\mu$ g/mL. 20  $\mu$ L of each concentration were added to each wells containing culture suspension except the growth control well. The final concentration ranged from 64 to 0.01  $\mu$ g/mL. Plates were incubated at 35 °C for 48 h. Fungal growth was assessed at 494 nm by measuring the optical density in each well using an enzyme immunoassay multiwell reader (Sigma diagnostic).

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