Synthesis, characterization and antimicrobial evaluation of some novel (3-methyl-5-((3-phenylisoxazol-5-yl)methoxy)benzofuran-2-yl)(phenyl)methanones

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The synthesis and characterization of a novel class of benzofuran-isoxazole hybrid heterocyclic unit are described and their antimicrobial activities evaluated. The antimicrobial investigation studies reveal that the majority of the final target compounds exhibit moderate to excellent activity against tested microbes. Among the compounds screened, 7b, 7a and 7i have shown potent activity and compounds 7h, 7c, 7f show good activity compared to standard drugs Gentamicin and Nystatin.

Keywords: Benzofuran, isoxazole, hybrid-heterocyclic, antibacterial, antifungal

Benzofuran and its derivatives either natural or synthetic have been reported to exhibit diverse biological activities such as antibacterial, antimicrobial, antifungal, antitumor, anticonvulsant, anti-HIV, antitubercular, anti-inflammatory, anti-diabetic, antidepressant, and in the treatment of asthma, rheumatism and ulcers. Furthermore, certain derivatives of benzofuran present in natural products show high cytotoxicity.

On other hand isoxazoles either natural or synthetic have been drawing significant interest from medicinal and organic chemists owing to their notable biological activities. Isoxazoles are playing important role in heterocyclic chemistry as pharmacophores and extensively used as synthons in the field of organic chemistry. Isoxazole forms the basis for several drugs such as leflunomide, valdecoxib and zonisamide.

In continuation to our previous work in discovering potential antimicrobial benzofurans and isoxazoles, this work directed towards the synthesis of a diverse series of novel benzofuran-isoxazole derivatives of biological interest.

Results and Discussion

Chemistry

As shown in Scheme I, benzofuran based 3,5-disubstituted isoxazoles (7a-j) were synthesized by a 4-step protocol. Compound 2 was obtained by the reaction of 1-(2,5-dihydroxyphenyl)ethanone (1) with 3-bromoprop-1-yne in the presence of K2CO3 and acetone. The key intermediate compound 4 was obtained in 96% yield by the reaction of acetylinic ortho hydroxyl acetophenone (2) with phenacylbromide (3). This was confirmed by 1H-NMR spectra which showed characteristic peaks at δ 4.74 (d, J = 2.4 Hz, 2H) and at δ 2.53 (d, J = 2.27 Hz, 1H) indicates presence of acetylenic benzofuran. This was further confirmed by MASS spectra which gave base peak at m/z 291 [M+H]+ corresponding to the molecular weight of compound 4. Different substituted aldoximes (6a-j) were prepared by reacting aldehydes (5a-j) with hydroxylamine hydrochloride. The in situ generated nitrile oxides from oxime (6a-j) underwent a 1,3-dipolar cycloaddition with alkyne (4) to give the corresponding isoxazoles (7a-j). In 1H-NMR spectra peak between δ 4.74 to δ 7.01 indicates the formation of isoxazole ring. This was also confirmed by 13C-NMR spectra which showed characteristic peak of isoxazole between δ 97.2 to δ 97.4. The structures of the newly synthesized target molecules were characterized by IR, NMR and mass spectral data.

Biological activities

Antibacterial activity

The targeted novel benzofuran-isoxazoles (7a-j) were tested for their antibacterial activity against four
Gram positive bacteria vize *Micrococcus luteus*, Methicillin-resistant *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* & four gram negative bacteria vize *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus vulgaris* using Gentamicin sulphate as standard drug. All the tested compounds displayed excellent to moderate activity against tested bacterial strains (Table I). Among the compounds 7b, 7a, 7i, 7h and 7c were shown potent active. It was attracting that compounds with strong electron withdrawing group like NO2 of phenyl ring attached to isoxazole may increasing the antibacterial activity than other aryl substituted isoxazole. The compounds 7d, 7e, 7f, 7g and 7j were exhibited mild antibacterial activity against tested bacterial strains.

**Antifungal activity**

The target benzofuran-isoxazoles (7a-j) were also screened for antifungal activity studies against three dermatophytes vize, *Microsporum canis*, *Microsporum gypseum* and *Epidermophyton floccosum*. According to the results obtained (Table II) among all screened analogues, 7b, 7a, 7i, 7h, 7c and 7f have shown more active against tested fungal strains compared to standard drug Nystatin. Compounds 7d and 7e exhibited moderate active.

**Experimental Section**

The melting points were measured in open capillaries and are uncorrected. The IR spectra were recorded on a Perkin–Elmer 337 grating IR spectrophotometer for solid samples pelleted in KBr. The NMR spectra were obtained on Bruker AV-400 and AV-300 NMR spectrometers for CDCl3 solutions. The chemical shifts were measured in ppm against internal TMS. Electron Spray Ionization (ESI) mass spectra were recorded on a QSTARXL hybrid MS system (Applied Bio Systems) under electro spray ionization. Thin layer chromatography was carried out on Merck TLC silica gel 60 F254 plates. The spots were visualized in UV light at 254 nm. Column chromatography was performed on a Merck silica gel 60A (100–200 mesh).

**Synthesis of compound 2**

Compound 1 (2 g, 0.0131 mol) was dissolved in dry acetone and was added K₂CO₃ (1.81 g, 0.0131 mol) and 3-bromoprop-1-yne (1.56 g, 0.0131 mol). This reaction mixture was heated at reflux temperature for 8 hours and monitored by TLC. After consumption of starting materials the reaction mixture was allowed to room temperature then excess acetone was removed. The crude was diluted with water and extracted with ethyl acetate. The combined organic layer was subjected to reduced pressure to remove
excess solvent. The crude obtained was purified by column chromatography to offer 1-(2-hydroxy-5-(prop-2-yn-1-yloxy)phenyl)ethanone 2.

IR (KBr, \(\nu_{\text{max}}\) cm\(^{-1}\)): 3408, 2345, 1642. White solid, Yield 84%, mp 184-186°C. 1H NMR (300 MHz, CDCl\(_3\)): 12.4 (s, 1H), 7.62 (dd, \(J = 7.07, 2.3\) Hz, 1H), 6.40–6.40 (m, 2H), 4.73 (d, \(J = 2.3\) Hz, 2H), 2.54 (s, 3H), 2.53 (d, \(J = 2.28\) Hz, 1H). MS: \(m/z\) 191 [M+H]+.

Synthesis of (3-methyl-5-(prop-2-yn-1-yloxy)benzofuran-2-yl) (phenyl)methanone, 4

In dry acetone compound 2 (2.5 g, 0.013 mol) was dissolved followed by K\(_2\)CO\(_3\) (2.72 g, 0.019 mol), 2-bromo-1-phenylethanone (2.61 g, 0.013 mol) was added and was stirred at room temperature for 20 hours. After completion of reaction as indicated by TLC, excess acetone was removed under reduced pressure then diluted with water and extracted with ethyl acetate. The organic layer was concentrated to get crude product which was subjected to column chromatography which offered pure compound 4 in 96% yield. IR spectrum, \(\nu\), cm\(^{-1}\): 1664. White solid, mp 199-201°C. \(^1\)H NMR (300 MHz, CDCl\(_3\)): 7.6-7.2 (m, 6H), 6.4–6.4 (m, 2H), 4.7 (d, \(J = 2.4\) Hz, 2H), 2.56 (s, 3H), 2.53 (d, \(J = 2.27\) Hz, 1H). MS: \(m/z\) 291 [M+H]+.

Procedure for the preparation of aldoximes, 6a-j

To a solution of aldehyde 5a-j (1 eq) in methanol was added hydroxylamine hydrochloride (1 eq)
followed by sodium acetate (1.5 eq). The resulting reaction mixture was stirred at room temperature for 3 hours. After completion of the reaction, the reaction mixture was quenched by adding crushed ice and the precipitate formed was isolated by filtration, washed with pet ether, and dried to afford substituted aldoximes 6a-j.

Synthesis of benzofuran based 3,5-disubstituted isoxazoles, 7a-j

To a solution of benzaldehyde oximes 6a-j (100 mg, 1 eq) in DMF was added N-chloro succinimide (1 eq) for chlorination, the resulting mixture was stirred at room temperature for 30 minutes. The reaction mixture was cooled to 0 °C and slowly added catalytic amount of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.2eq) followed by compound 4 (1 eq). This resulting reaction mixture was stirred for 4 hours at room temperature. After consumption of the starting materials, as indicated by TLC, ice-cold water was added to the reaction mixture, precipitate so obtained was filtered, washed with water and cold MeOH to afford the pure substituted benzofuran based 3,5-disubstituted isoxazoles 7a-j in excellent yields (Table III).

### Table III — Physical data of compounds 7a-j

<table>
<thead>
<tr>
<th>Compd</th>
<th>Physical State</th>
<th>Yield (%)</th>
<th>m.p. (°C)</th>
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<tbody>
<tr>
<td>7a</td>
<td>Light yellow solid</td>
<td>84</td>
<td>147-149</td>
</tr>
<tr>
<td>7b</td>
<td>Light yellow solid</td>
<td>75</td>
<td>157-159</td>
</tr>
<tr>
<td>7c</td>
<td>White solid</td>
<td>88</td>
<td>86-88</td>
</tr>
<tr>
<td>7d</td>
<td>White solid</td>
<td>90</td>
<td>77-79</td>
</tr>
<tr>
<td>7e</td>
<td>White solid</td>
<td>80</td>
<td>72-73</td>
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<tr>
<td>7f</td>
<td>Light yellow solid</td>
<td>95</td>
<td>104-106</td>
</tr>
<tr>
<td>7g</td>
<td>White solid</td>
<td>93</td>
<td>112-114</td>
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<tr>
<td>7h</td>
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<td>90</td>
<td>95-97</td>
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<tr>
<td>7i</td>
<td>White solid</td>
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</tr>
<tr>
<td>7j</td>
<td>White solid</td>
<td>86</td>
<td>126-128</td>
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IR spectrum, ν, cm⁻¹: 1653 (C=O). ¹H NMR (400 MHz, CDCl₃): 8.18-7.99 (m, 3H), 7.64-7.42 (m, 4H), 7.22-7.11 (m, 2H), 6.86 (s, 1H), 5.37 (s, 2H), 2.61 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): 185.7, 167.7, 159.1, 157.9, 155.8, 147.9, 145.6, 138.5, 138.0, 132.3, 129.8 (2C), 128.5 (2C), 127.5, 126.7, 125.8, 123.8, 123.3, 122.2, 120.7, 115.2, 114.2, 97.3, 61.4, 10.1. MS: m/z 444 [M+H]⁺.

(5-((3-(4-Chlorophenyl)isoxazol-5-yl)methoxy)-3-methylbenzofuran-2-yl)(phenyl)methanone, 7c: IR (KBr, νmax cm⁻¹): 1647 (C=O). ¹H NMR (400 MHz, CDCl₃): 8.18-7.99 (m, 3H), 7.63-7.38 (m, 7H), 7.15 (s, 1H), 7.03 (d, J = 8.28 Hz, 1H), 6.86 (s, 1H), 5.36 (s, 2H), 2.61 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): 185.6, 167.8, 159.0, 158.4, 155.7, 148.0, 145.5, 137.7, 138.4, 132.3, 129.9 (2C), 128.6 (2C), 128.2 (2C), 127.4, 126.6, 123.6, 122.2, 121.6 (2C), 114.2, 97.2, 61.6, 10.1. MS: m/z 444 [M+H]⁺.

(5-((3-(3-Chlorophenyl)isoxazol-5-yl)methoxy)-3-methylbenzofuran-2-yl)(phenyl)methanone, 7d: IR (KBr, νmax cm⁻¹): 1654 (C=O). ¹H NMR (400 MHz, CDCl₃): 8.18-7.99 (m, 3H), 7.63-7.38 (m, 7H), 7.15 (s, 1H), 7.03 (d, J = 8.28 Hz, 1H), 6.86 (s, 1H), 5.36 (s, 2H), 2.61 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): 185.6, 167.8, 159.0, 158.4, 155.7, 148.0, 145.5, 137.7, 138.4, 132.3, 129.9 (2C), 128.6 (2C), 128.2 (2C), 127.4, 126.6, 123.6, 122.2, 121.6 (2C), 114.2, 97.2, 61.6, 10.1. MS: m/z 444 [M+H]⁺.
159.3, 158.4, 155.4, 148.6, 145.9, 145.1, 138.3, 132.6, 132.1 (2C), 129.8 (2C), 128.5 (2C), 127.4, 124.3, 123.7, 122.3, 121.6 (2C), 114.1, 97.3, 61.7, 10.4. MS: m/z 488 [M+H]^+.

(5-(3-(Bromophenyl)isoxazol-5-yl)methoxy)-3-methylbenzofuran-2-yl)(phenyl)methanone, 7g: IR (KBr, ν_max cm⁻¹): 1655 (C=O). ¹H NMR (400 MHz, CDCl₃): 8.17-8.05 (m, 2H), 7.69-7.58 (m, 5H), 7.58-7.55 (m, 2H), 7.50-7.43 (m, 1H), 7.27-7.24 (m, 1H), 7.22-7.19 (m, 1H), 7.01 (s, 1H), 5.39 (s, 2H), 2.62 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): 185.6, 167.8, 159.2, 158.4, 155.6, 148.5, 145.5, 138.4, 137.5, 132.9, 132.8, 132.5, 132.3, 129.9 (2C), 128.6 (2C), 127.4, 123.6, 122.1, 121.9, 121.8, 114.2, 97.2, 61.6, 10.3. MS: m/z 488 [M+H]^+.

(5-(3-(Methoxyphenyl)isoxazol-5-yl)methoxy)-3-methylbenzofuran-2-yl)(phenyl)methanone, 7h: IR (KBr, ν_max cm⁻¹): 1654 (C=O). ¹H NMR (400 MHz, CDCl₃): 8.10-8.06 (m, 2H), 7.80 (s, 1H), 7.70 (s, 1H), 7.61 (t, J = 7.48 Hz, 1H), 7.58-7.47 (m, 4H), 7.44-7.40 (m, 1H), 7.21 (d, J = 2.4 Hz, 1H), 7.13 (dd, J = 8.6, 2.3 Hz, 1H), 6.92 (s, 1H), 5.39 (s, 2H), 3.86 (s, 3H), 2.61 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): 185.5, 167.4, 158.8, 158.1, 156.8, 147.8, 146.0, 138.7, 138.1, 132.3, 130.2 (2C), 128.6 (2C), 128.1 (2C), 127.3, 126.5, 123.4, 122.1, 121.5 (2C), 114.3, 97.3, 61.6, 56.5, 10.2. MS: m/z 440 [M+H]^+.

(5-(3-(4-Methoxyphenyl)isoxazol-5-yl)methoxy)-3-methylbenzofuran-2-yl)(phenyl)methanone, 7i: IR (KBr, ν_max cm⁻¹): 1653 (C=O). ¹H NMR (400 MHz, CDCl₃): 8.07-7.98 (m, 2H), 7.75 (s, 1H), 7.70 (s, 1H), 7.58 (t, J = 7.3 Hz, 1H), 7.56-7.47 (m, 4H), 7.39-7.36 (m, 1H), 7.20 (d, J = 2.4 Hz, 1H), 7.16 (dd, J = 8.6, 2.4 Hz, 1H), 6.95 (s, 1H), 5.36 (s, 2H), 3.88 (s, 3H), 2.61 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): 185.7, 167.8, 159.1, 157.2, 155.1, 148.1, 149.9, 137.8, 132.5, 131.9 (2C), 129.8 (2C), 128.4 (2C), 127.7, 124.3, 123.6, 122.1, 121.5 (2C), 114.3, 97.4, 61.7, 56.7, 10.4. MS: m/z 440 [M+H]^+.

Conclusions

A new series of benzofuran-isoxazole heterocyclics 7a-j were synthesized in high yields and characterized by different spectroscopic techniques. These synthesized molecules were screened for antimicrobial activity and demonstrated excellent activity against tested bacterial and fungal strains. The highest activities were found for compounds 7b, 7a, 7i, 7h, 7c and 7f. These results positively encouraged us for further developing novel bioactive agents.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

References