**Antimicrobial evaluation of spirooxindolopyrrolidine engrafted indoles against multidrug resistant ESKAPE clinical pathogens**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

General methods

All reagents and solvents were purchased from commercial suppliers (Merck chemicals) and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on silica gel. 1H, and 13C NMR spectra were recorded on a JEOL 400 MHz instrument in CDCl3 using Tetramethylsilane (TMS) as internal standard. Standard Bruker software was used throughout. Chemical shifts are given in parts per million (δ-scale) and the coupling constants are given in Hertz. Elemental analyses were performed on a Perkin-Elmer 2400 series II elemental CHNS analyzer. Mass spectra were recorded on JEOL-DX303 HF mass spectrometer.

Spirocompound **4c**: Pale yellow solid; 1H NMR (CDCl3, 500 MHz): *δ*/ppm 2.14 (t, *J* = 13.2 Hz, 1H), 2.82 (d, *J* = 13.2 Hz, 1H), 3.26 (d, *J* = 13.2 Hz, 1H), 3.42 (s, 3H), 3.71 (d, *J* = 13.2 Hz, 1H), 4.80-4.82 (m, 1H), 6.77-6.95 (m, 4H, ArH), 7.07 (t, *J* = 9.0 Hz, 1H), 7.10 (s, 1H), 7.18-7.32 (m, 4H, ArH), 7.50 (s, 1H), 8.00 (d, *J* = 9.0 Hz, 1H), 10.28 (s, 1H), 10.32 (s, 1H), 10.70 (s, 1H); 13C NMR (CDCl3, 100 MHz) *δ*/ppm: 29.7, 41.6, 51.5, 62.2, 64.7, 64.9, 74.9, 109.3, 110.8, 110.9, 113.5, 117.4, 119.5, 120.7, 121.2. 123.4, 124.8, 125.8, 127.7, 128.3, 129.2, 131.2, 136.4, 142.8, 143.0, 172.5, 177.7, 182.6; LC/MS(ESI): *m/z* = 600 (M+); Anal. Calcd for C30H25BrN4O5: C, 59.91; H, 4.19; N, 9.32; Found C, 60.2; H, 4.30; N, 9.44.

 Spirocompound **4d**: Pale yellow solid; 1H NMR (CDCl3, 500 MHz): *δ*/ppm 2.13-2.19 (t, *J* = 13.2 Hz, 1H), 2.91 (d, *J* = 14.0 Hz, 1H), 3.25 (d, *J* = 13.2 Hz, 1H), 3.40 (s, 3H), 3.64 (d, *J* = 12.4 Hz, 1H), 4.84-4.88 (m, 1H), 6.25 (s, 1H), 6.76-6.82 (m, 3H, ArH), 6.92-7.09 (m, 3H), 7.15-7.21 (m, 3H), 7.28 (d, *J* = 8.0 Hz, 1H, ArH), 7.49 (d, *J* = 7.2 Hz, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 10.26 (s, 1H), 10.34 (s, 1H), 10.68 (s, 1H); 13C NMR (CDCl3, 100 MHz) *δ*/ppm: 38.9, 41.2, 51.4, 62.3, 64.7, 65.0, 75.2, 109.2, 109.3, 110.9, 113.1, 117.9, 119.5, 120.6, 121.2, 121.9, 123.2, 124.3, 124.5, 127.5, 128.7, 129.3, 131.3, 132.7, 136.1, 141.9, 142.8, 172.3, 178.0, 182.6; LC/MS(ESI): *m/z* = 522 (M+); Anal. Calcd for C30H26N4O5: C, 68.95; H, 5.02; N, 10.72; Found C, 69.05; H, 5.13; N, 10.83.

*Antibiotic sensitivity test*

The antibiotics resistant profile of several standard antibiotics was determined by disk diffusion methods (Bauer et al., 1996). Targeted ESKAPE pathogens were evaluated against eight standard antibiotics available in our lab namely; Ampicillin (AMP-25 µg); Chloramphenicol (C-30 µg); Ciprofloxacin (CIP-5 µg); Gentamycin (GEN-10 µg); Penicillin-G (P-10 units); Streptomycin (S-10 µg); Tetracycline (TE-30 µg); Vancomycin (VA-30 µg). The following bacterial pathogens *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* sp., were used to determined antibiotics sensitivities test.

**Table S1**: Antibiotic sensitivity profile of ESKAPE pathogens

|  |  |  |
| --- | --- | --- |
| ESKAPE pathogens | No of resistant antibiotics | Resistant antibiotics\* |
| *Enterococcus faecium* | 5 | AMP, C, CIP, P, S,  |
| *Staphylococcus aureus* | 6 | AMP, C, GEN, P, TE, VA  |
| *Klebsiella pneumoniae*  | 5 | AMP, CIP, GEN, P, TE |
| *Acinetobacter baumannii* | 7 | AMP, C, CIP, GEN, P, TE, VA |
| *Pseudomonas aeruginosa*  | 6 | AMP, C, GEN, P, TE, VA |
| *Enterobacter* species | 4 | AMP, P, S, VA |

\*Ampicillin (AMP-25µg); Chloramphenicol (C-30µg); Ciprofloxacin (CIP-5µg); Gentamycin (GEN-10µg); Penicillin-G (P-10units); Streptomycin (S-10µg); Tetracycline (TE-30µg); Vancomycin (VA-30µg).