## Synthesis, characterization of hetero-atom substituted quinoxaline heterocyclic derivatives and their antimicrobial activity

S Mahaboob Basha,<sup>A,B</sup> S Syed Shafi,<sup>A\*</sup> Chamarthi Naga Raju,<sup>B</sup> Palaa Krishna,<sup>B</sup> Shaik Thaslim Basha<sup>b</sup>

<sup>\*a</sup>Department of Chemistry, Thiruvallur University, Serkkadu, Vellore-632115, Tamil Nadu, India.
<sup>b</sup>Department of Chemistry, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.
Corresponding Author: S Mahaboob Basha

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## I. Introduction

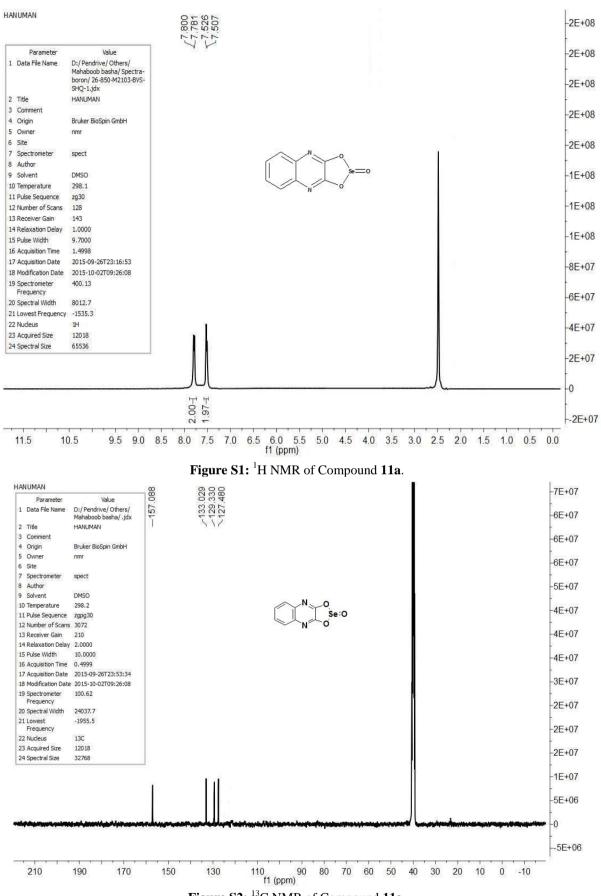
## Antibacterial activity

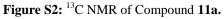
Antibacterial activity was assayed for the title compounds against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Klebsiella pneumoniae* by agar well diffusion method.<sup>29</sup> 5  $\mu$ g/mL of the test compounds were dissolved in 1 mL of DMSO solvent. Centrifuged pellets of bacteria from 24 h old culture containing approximately 10<sup>4</sup>-10<sup>6</sup> colony forming unit (CFU) per mL was spread on the surface of Muller Hinton Agar (MHA) plates. Nutrient agar medium was prepared by suspended nutrient agar 28 g in 1 liter of distilled water, autoclaved and cooled to 45 °C, and then it was seeded with 15 mL of prepared inocula to have 10<sup>6</sup> CFU/mL. Petri dishes were prepared by pouring 10 mL of seeded nutrient agar. Wells were created in medium with the help of a sterile metallic borer and test solution was added. Experimental plates were incubated for 24 h at 37 °C and antibacterial activity was defined as the diameter (mm) of the clear inhibition zone formed around the well. Ciprofloxacin was used as standard drug for antibacterial activity. For each treatment, three replicates were carried out and the mean of the diameter of the inhibition zone values were calculated and presented in **Table 2**.

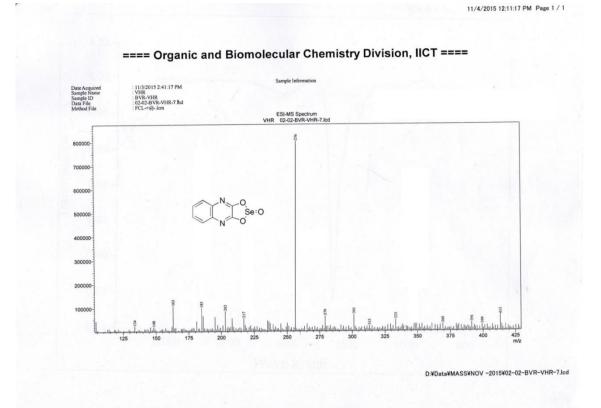
## Antifungal activity

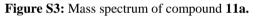
Antifungal activity of newly synthesized compounds were screened against the fungal strains like *Aspergillus flavus, Aspergillus niger, Aspergillus funigatus* and *Candida albicans* by the poison plate technique.<sup>30</sup> All the tested compounds were dissolved in DMSO before mixing with potato dextrose agar (PDA). The final concentration of the compounds in the medium was fixed at 50  $\mu$ g/mL. The fungi were incubated in PDA at 25±1 °C for 5 days to get new mycelium for antifungal activity, and then a mycelia disc of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. The inoculated plates were incubated at 25±1 °C for 5 days. Amphotericin-B was used as a standard drug for antifungal activity. The radial growth of the fungal colonies was measured on the sixth day. For each treatment, three replicates were carried out and the mean of the diameter of the inhibition zones were calculated and presented in **Table 3**.

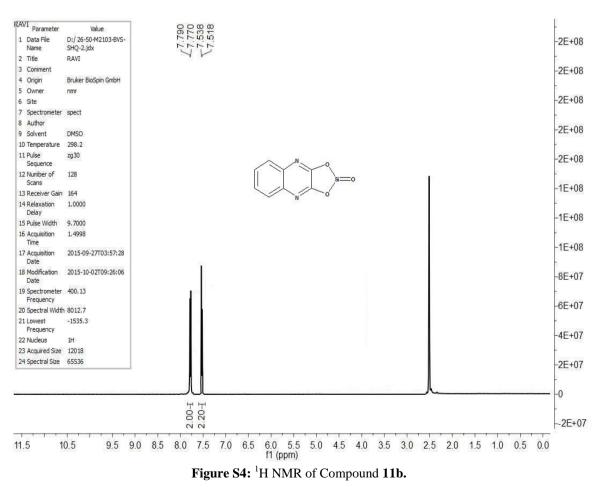
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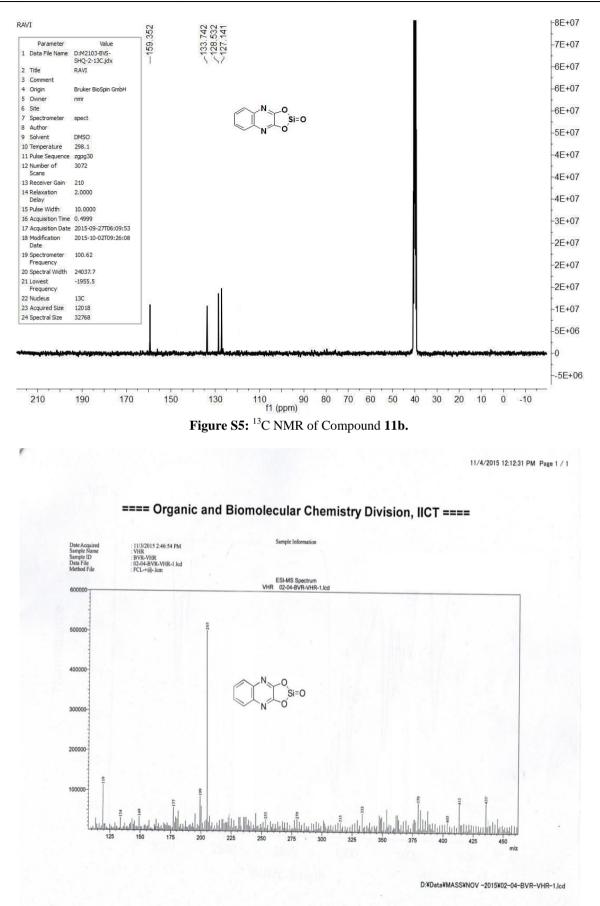
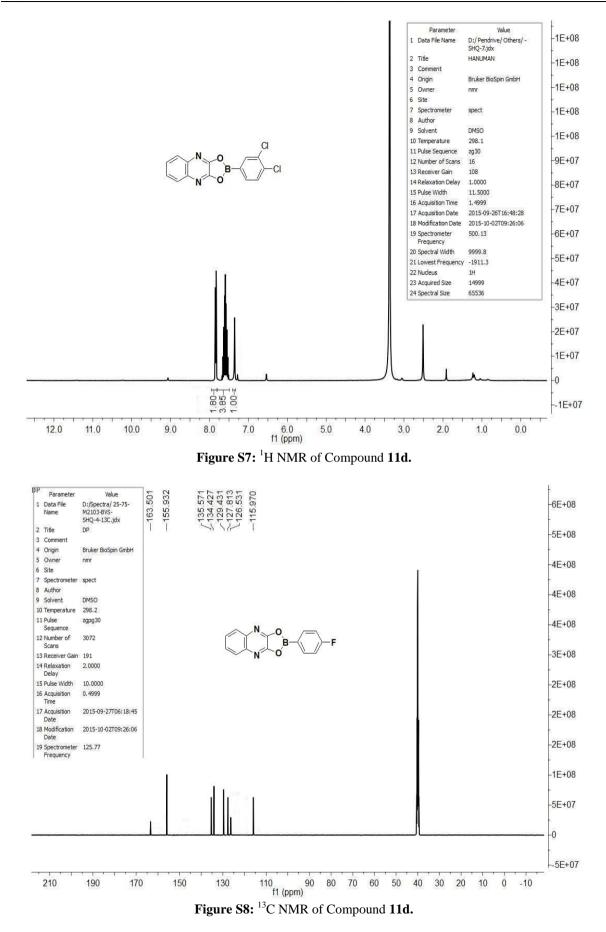
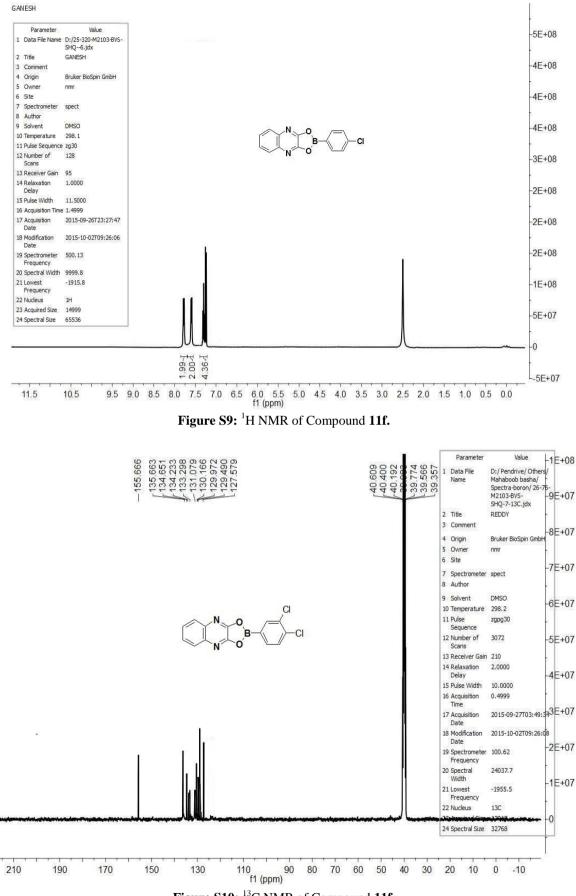


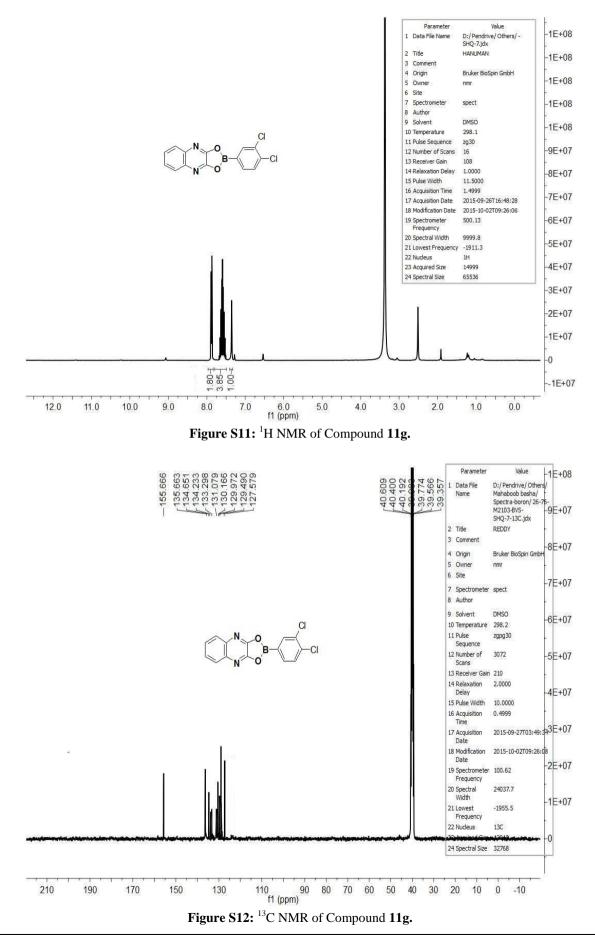
Figure S6: Mass spectrum of Compound 11b.

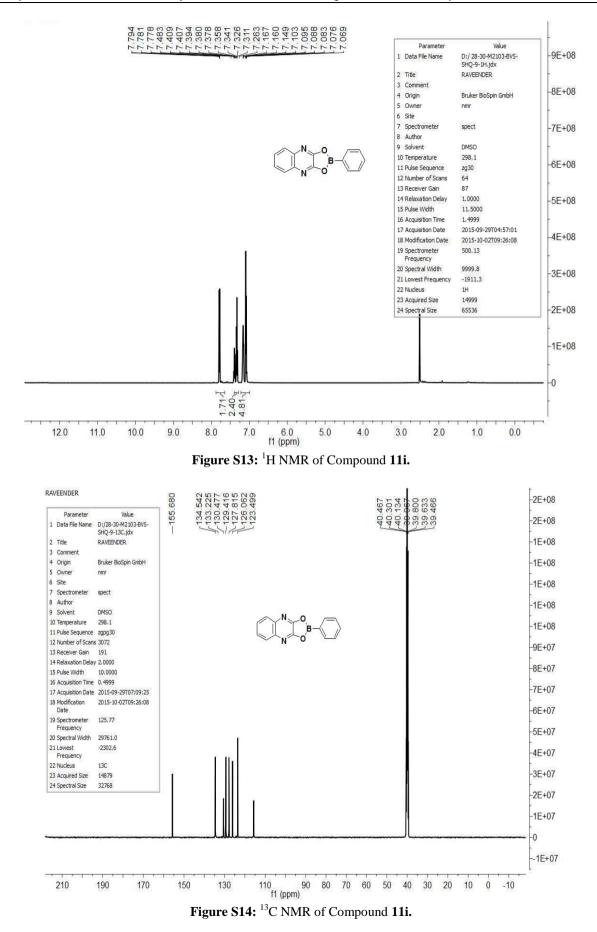


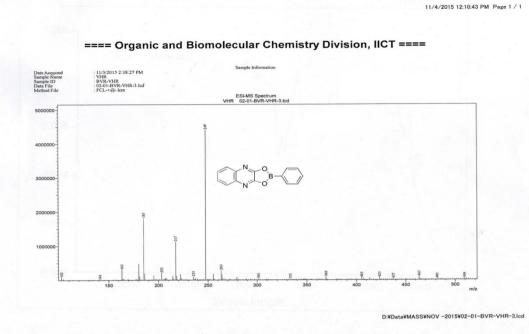
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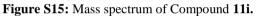


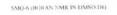












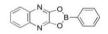


Figure S16: <sup>11</sup>B NMR of Compound 11i.

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