Electronic Supplementary Data

Co(II) complex of 2-amino-6-methylbenzothiazole: Synthesis, structure and biological evaluation

S Jone Kirubavathy\textsuperscript{a}, R Velmurugan\textsuperscript{b}, R Karvemb\textsuperscript{c}, N S P Bhuvanesh\textsuperscript{d}, I V M V Enoch\textsuperscript{e}, P Mosae Selvakumare & S Chitra\textsuperscript{a} *

\textsuperscript{a}Department of Chemistry, PSGR Krishnammal College for Women, Coimbatore 641 004, India
Email: chitrapsgrkc@gmail.com

\textsuperscript{b}Department of Chemistry, Kongunadu Arts and Science College, Coimbatore 641029, India

\textsuperscript{c}Department of Chemistry, National Institute of Technology, Tiruchirappalli 620015, India

\textsuperscript{d}Department of Chemistry, Texas A&M University, College Station, TX 77842, USA

\textsuperscript{e}Department of Chemistry, Karunya University, Coimbatore 641114, India

No. Contents Pg No.
1 Fig. S1–Electronic spectrum of CoCl\textsubscript{2} with the title complex. 2
2 Fig. S2–Electronic spectrum of the title complex. 2
3 Fig. S3–Magnetization versus applied field plot for Co(II) complex. 3
4 Standard procedures for biological studies. 3
5 Table S1–Antimicrobial activity of the ligand and complex. 5
Fig. S1–Electronic spectrum of CoCl$_2$ with the title complex

Fig. S2–Electronic spectrum of the title complex
Fig. S3–Magnetization versus applied field plot for Co(II) complex

**Standard procedures for biological studies**

*In vitro* antimicrobial activity

The test organisms were grown on nutrient agar and potato dextrose for antibacterial and antifungal studies respectively, in petri plates. The plates were incubated for 24 h for bacteria and 72 h for fungi. Then, the test solutions were diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration of the samples was noted. DMF is used as the negative control, and *amikacin* and *ciprofloxacin* were used as positive standards for antibacterial study and *nystatin* for antifungal activity. The minimum inhibitory concentration was determined by serial dilution technique.

*In vitro* anticancer activity–Cell treatment procedure and MTT assay

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1 \times 10^5$ cells/mL. Cell suspensions were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37 °C. After 24 h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in DMSO and diluted to twice the desired final maximum
test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µL of these different sample dilutions were added to the appropriate wells already containing 100 µL of medium, resulted the required final sample concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 ºC. The medium without samples was served as control and triplicate was maintained for all concentrations.

3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15 µL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37 ºC for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µL of DMSO and then measured the absorbance at 570 nm using micro plate reader.

**Antituberculosis activity**

The antimycobacterial activity of the compounds was assessed against *M. tuberculosis* using microplate alamar blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96-wells plate received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this time, 25 µL of freshly prepared 1:1 mixture of alamar blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.
<table>
<thead>
<tr>
<th>Name of organism</th>
<th>Zone of inhibition(mm)</th>
<th>2-amino-6-methyl benzothiazole</th>
<th>Co(II) complex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 20% 40% 60% 80%</td>
<td>C 20% 40% 60% 80%</td>
<td></td>
</tr>
<tr>
<td>1 Pseudomonas aeruginosa</td>
<td></td>
<td>- - 06 08 10</td>
<td>- - 12 14 16</td>
</tr>
<tr>
<td>2 Aeromonas hydrophila</td>
<td></td>
<td>- - - 09 12</td>
<td>- - - 10 14</td>
</tr>
<tr>
<td>3 Thiobacillus thidurance</td>
<td></td>
<td>- - - 11 14</td>
<td>- - - 15 18</td>
</tr>
<tr>
<td>4 Serratia marcescens</td>
<td></td>
<td>- - - 15 18</td>
<td>- - - 16 22</td>
</tr>
<tr>
<td>5 Acinetobacter baumanii</td>
<td></td>
<td>- - 06 07 10</td>
<td>- - 12 14 16</td>
</tr>
<tr>
<td>6 Aspergillus niger</td>
<td></td>
<td>- - 08 10 12</td>
<td>- 16 17 20 24</td>
</tr>
<tr>
<td>7 Candida tropicalis</td>
<td></td>
<td>- - - - 10</td>
<td>- - - - 18</td>
</tr>
</tbody>
</table>