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SUPPLEMENTARY MATERIAL

Immunomodulatory and immunostimulatory effects of some bisbenzoxazole derivatives on lipopolysaccharide stimulated mammalian macrophages

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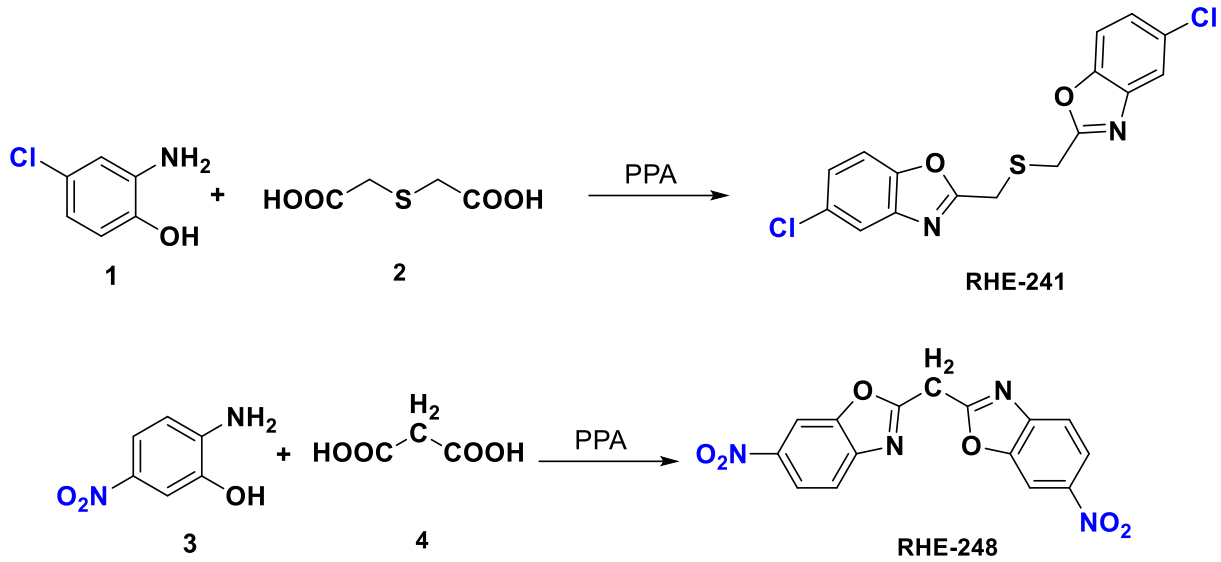
Detailed experimental procedures, characterization data and copies of ^{1}H and ^{13}C NMR for all compounds

Chemistry experimental procedure:

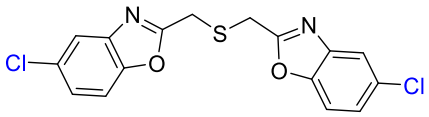
Material and Methods: *General.* All reagents used were used were commercially available unless otherwise specified and all solvents were distilled before use. Melting points were measured with *Mettler Toledo MP90* melting point device. IR Spectra: *Perkin Elmer Spectrum One* FT-IR spectrometer. ^{1}H-NMR and ^{13}C-NMR Spectra: *Bruker 400* spectrometer.

General Procedure for preparation of Compounds: General Procedure for preparation of Compounds: 5 mmol of the 4-chloro-2-aminophenol (**1**) or 5-nitro-2-aminophenol (**3**) and 2.5 mmol of the corresponding dicarboxylic acid derivatives (**2,** and **4**) are refluxed in a reflux condenser with a magnetic stirrer for a period of 13-15 hours after being dissolved in PPA heated in an oil-bath at 180 ˚C. The reaction was followed by thin layer chromatography (TLC). UV (ultraviolet) light was used in the determination of stains in the works of TLC (Kieselgel 60 F254, ready-to-use aluminum plate coated with 0.2 mm thickness) which was made by using ready-made plates. After cooling, the reaction mixture was poured onto ice water and neutralized by mixing with 5N NaOH till slightly basic pH (8–9) to get the precipitate. The resulting precipitate was filtered off and washed with cold water. Crystallized with a suitable solvate (ethanol:water = 1:1). The resulting crystalline compounds were filtered and the vacuumed product was dried.

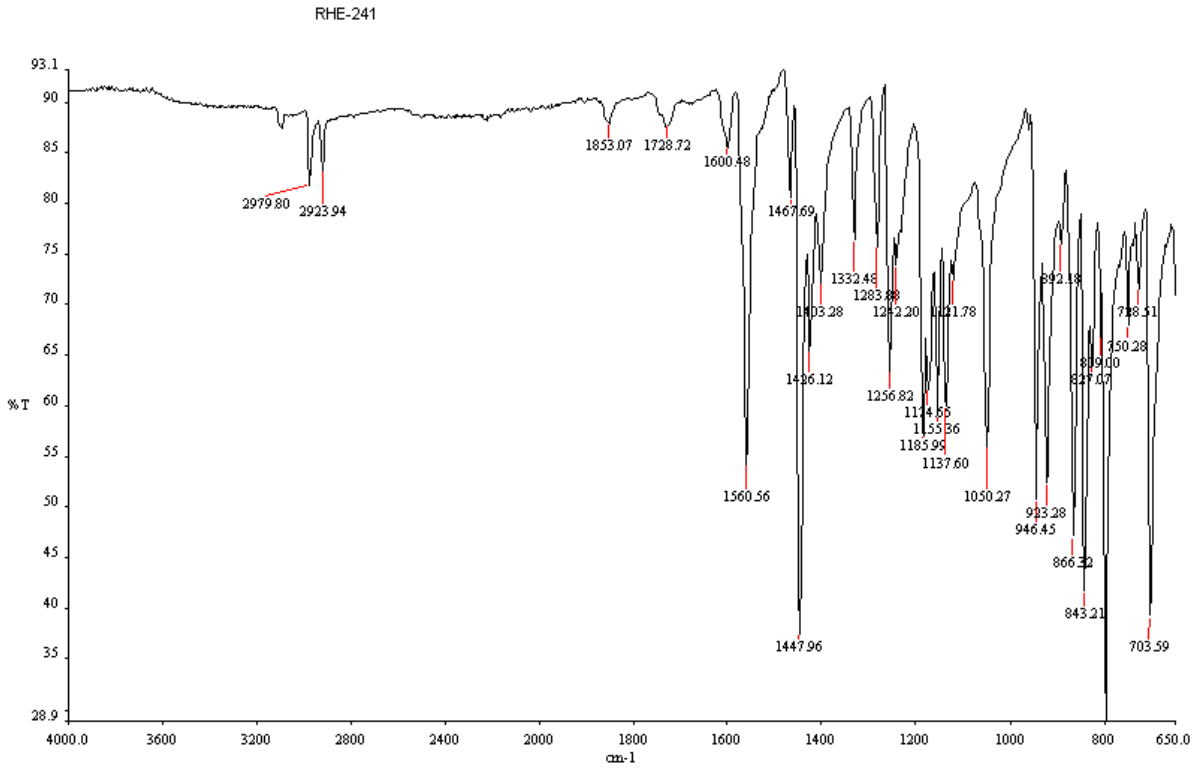


Scheme 1: Synthesis method of Compounds.

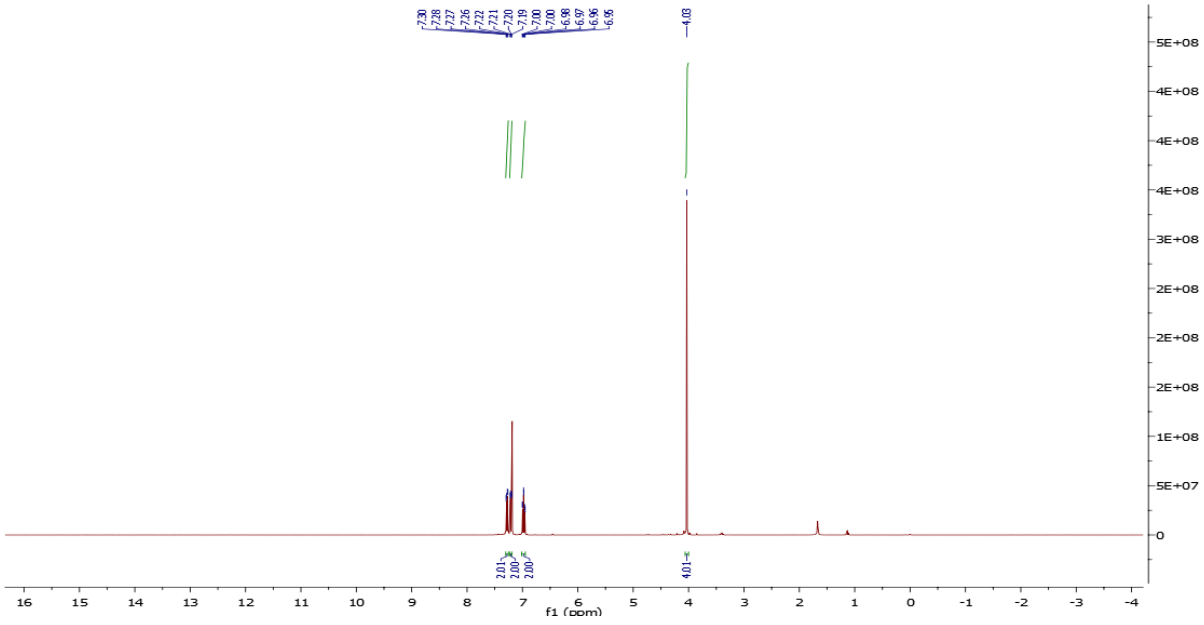
Bis((5-chlorobenzo[d]oxazol-2-yl)methyl)sulfane (RHE-241):



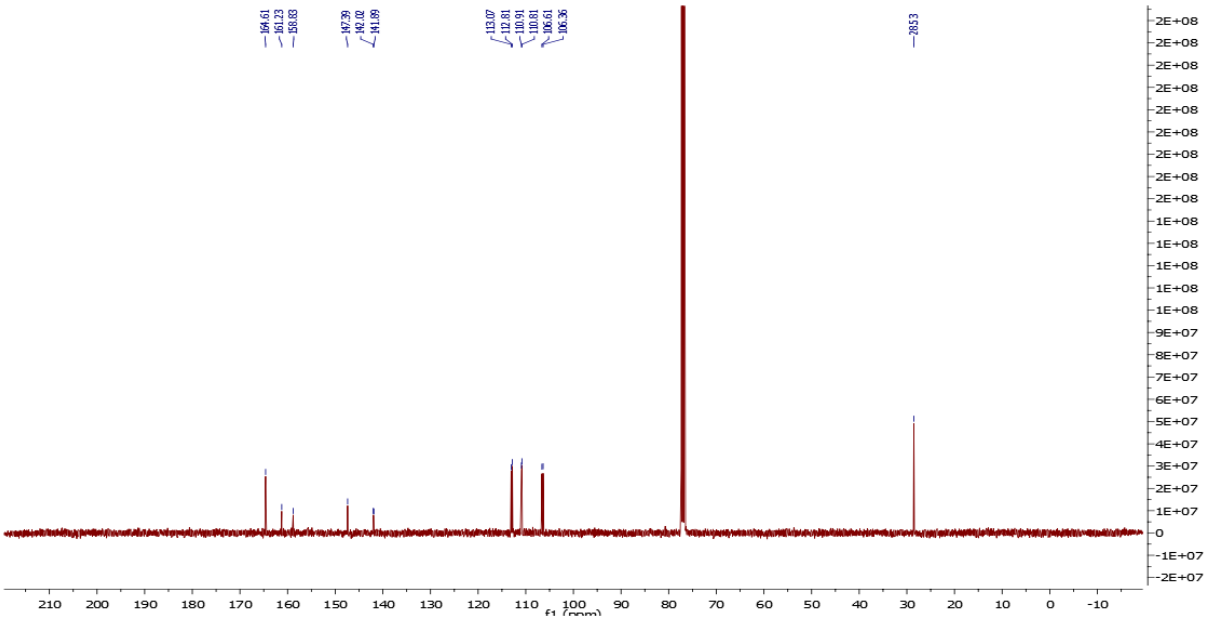
The above procedure was followed with **1** and **2** to yield **RHE-241** as a crystalline solid (45% yield). The crystallization solvent is ethanol-water (1:1). **Rf** (Hexan:Ethylacetate 1:1)= 0,64 ; **mp**= 197 °C; **IR** (KBr, cm^{-1}) V_{max} 2979, 2923, 1560, 1447, 1332, 812, 703; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ = 7.30-7.26 (m, 2H, Ar-H), 7.21 (dd, J=2.58 Hz, J=8.69 Hz, 2H, Ar-H), 6.98 (td, J=2.58 Hz, J=8.69 Hz, 2H, Ar-H), 4.03 (s, 4H, $-CH_2$). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ=164.6, 161.2, 158.8, 147.4, 142.0, 141.9, 113.1, 112.8, 110.9, 110.8, 106.6, 106.4, 28.5.



IR spectrum of bis((5-chlorobenzo[d]oxazol-2-yl)methyl)sulfane **(RHE-241).**

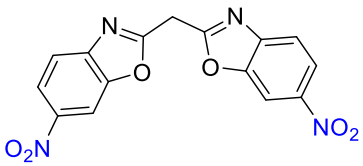


^{1}H-NMR spectrum of bis((5-chlorobenzo[d]oxazol-2-yl)methyl)sulfane **(RHE-241).**

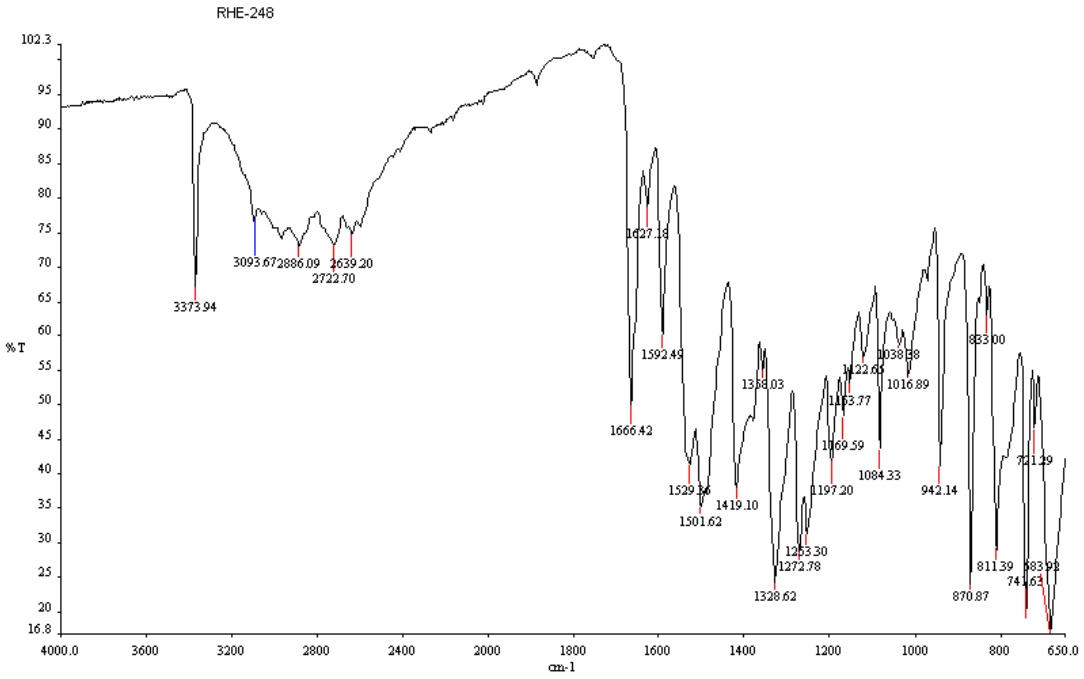


^{13}C-NMR spectrum of bis((5-chlorobenzo[d]oxazol-2-yl)methyl)sulfane **(RHE-241).**

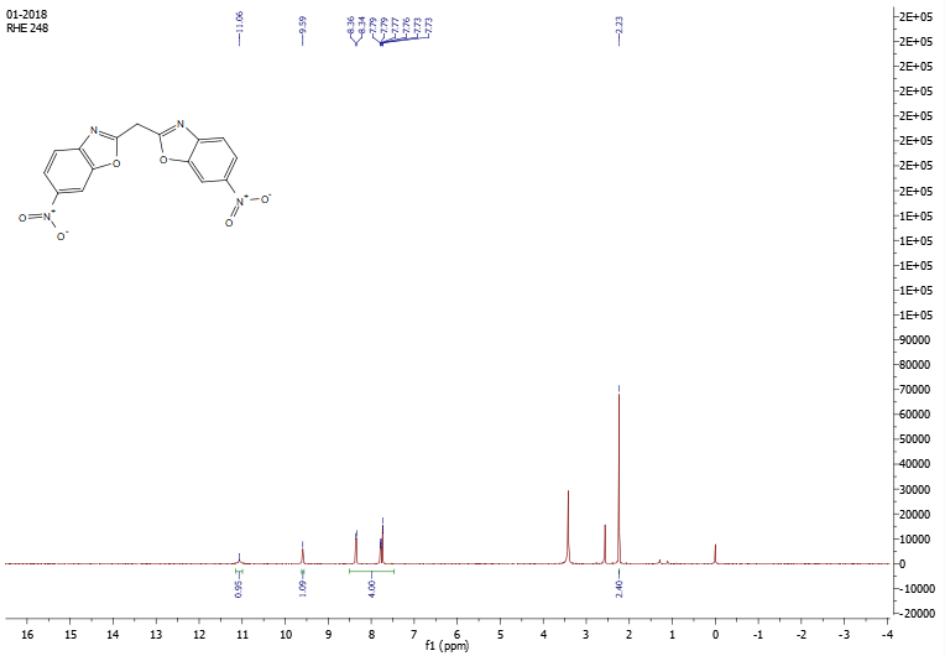
Bis(6-nitrobenzo[d]oxazol-2-yl)methane (RHE-248):



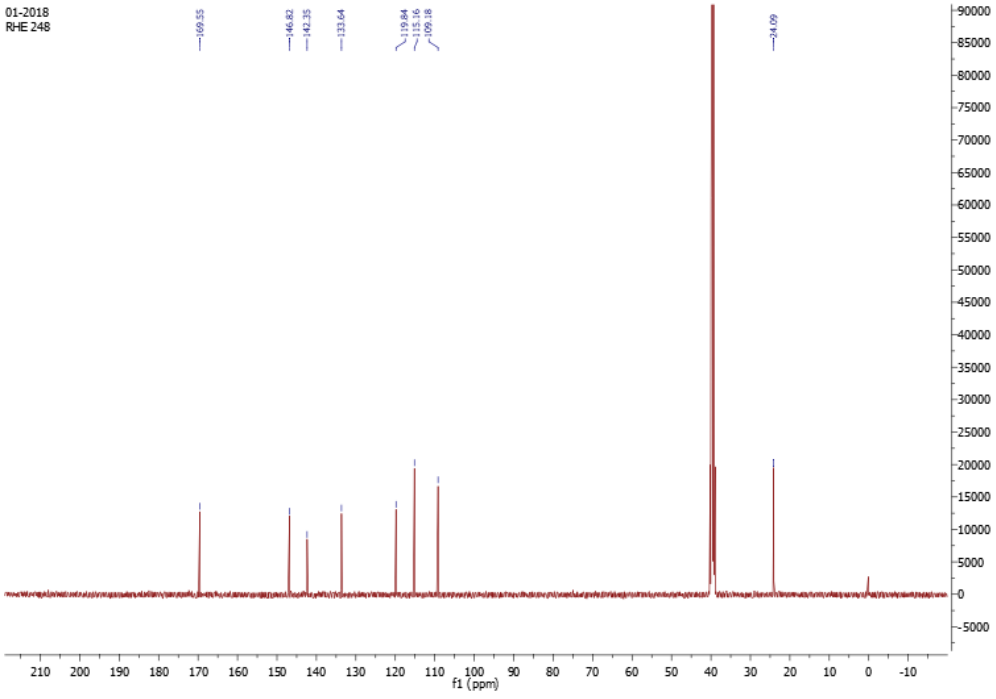
The above procedure was followed with **3** and **4** to yield **RHE-248** as a brown crystalline solid (50% yield). The crystallization solvent is ethanol-water (1:1). **Rf** ($CHCl_3$)= 0,40; **mp**= 190 °C; **IR** (KBr, cm^{-1}) V_{max} 3373, 3093, 2886, 1592, 1501, 1328, 811, 741, 683. **^{1}H-NMR** (400 MHz, d_6-DMSO) δ = 11.06 (s, 1H, Ar-H), 9.59 (s, 1H, Ar-H), 7.91 (m, 4H, Ar-H), 2.23 (s, 2H, CH_2). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ=169.5, 146.8, 142.3, 133.6, 119.8, 115.2, 109.2, 24.1.



IR spectrum of bis(6-nitrobenzo[d]oxazol-2-yl)methane **(RHE-248)**



^{1}H-NMR spectrum of bis(6-nitrobenzo[d]oxazol-2-yl)methane **(RHE-248)**



^{13}C-NMR spectrum of bis(6-nitrobenzo[d]oxazol-2-yl)methane **(RHE-248)**