Electronic Supplementary Material (ESI) for RSC Advances.


**Electronic Supplementary Information**

# Group VI transition metal carbonyl hydrosulfides Na[M(CO)$_5$(SH)] (M = Cr, Mo, W) as water soluble hydrogen sulfide releasing agents.

Hwa Tiong Poh, Ashfaq A. Bengali and Wai Yip Fan*

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543

*Corresponding author

E-mail: chmfanwy@nus.edu.sg

Tel: +65-6516-6823

Fax: +65-6779-1691

## Materials

Chromium hexacarbonyl ($Cr(CO)_6$, 99%), molybdenum hexacarbonyl ($Mo(CO)_6$, 98%) and tungsten hexacarbonyl ($W(CO)_6$, 99%) were purchased from Strem Chemicals. Sodium hydrosulfide (NaSH, 90%) and tetrafluoroboric acid was purchased from Sigma-Aldrich. Methanol ($CH_3OH$, 99%) and acetone ($C_7H_6O$, 99.5%) were purchased from Merck. All solvents and reagents were used without further purification.

## Instrumentation

Photochemical experiments were conducted with Legrand Broadband lamp (200-800 nm, 11 W) and Nd-YAG pulsed laser system (Quantel Brillant b, 355 nm). All infrared (IR) spectra were obtained from Shimadzu IR Prestige-21 spectrometer, using a 0.1 mm path length $CaF_2$ cell for liquid samples and a 15 cm path length $CaF_2$ gas cell for gas samples. UV-visible spectroscopy was done using Shimadzu UV-1600 Series spectrometer with 1 cm path length in quartz cell. Electrospray Ionization (ESI) mass spectrometry was conducted using Finnigan MAT LCQ spectrometer.

## Preparation of $Na[M(CO)_5SH]$ (M = Cr, Mo, W)

All reactions were carried out under vacuum using standard vacuum line and Schlenk techniques unless otherwise stated. NaSH (25.4 mg, 0.452 mmol) was added to a solution of $Cr(CO)_6$ (10.0 mg, 45.7 μmol) in 2 mL acetone and subjected to UV irradiation using a Legrand Broadband lamp (200 – 800 nm, 11 W) for 2 hours. The crude product was purified by precipitating the excess NaSH using a solvent mixture of methanol and diethyl ether (1:5 ratio). The volume was subsequently reduced and hexane was added to induce precipitation of the yellow $Na[Cr(CO)_5(SH)]$ in 76% yield. IR (acetone): 2046, 1939, 1887 $cm^{-1}$. ESI MS (acetone): 224.7 ($M^-$) and 196.8 ($M^- - CO$). UV (methanol): $\lambda_{max}$: 388 nm. Anal. calcd. for NaCrC5HO5S: C 26.68, S 14.24; found: C 26.59, S 14.21. The same procedure was applied to $W(CO)_6$ using the same ratio of starting materials and the orange $Na[W(CO)_5(SH)]$ was obtained in 85% yield. IR (acetone): 2076, 1932, 1885 $cm^{-1}$. ESI MS (acetone): 356.7 ($M^-$) and 328.9 ($M^- - CO$). UV (methanol): $\lambda_{max}$: 463 nm. Anal. calcd. for NaWC5HO5S: C 16.82, S 8.98; found: C 16.72, S 8.92. For the case of $Mo(CO)_6$, instead of UV irradiation, a 355 nm pulsed laser was used for the synthesis. Irradiation of the starting materials in acetone for 2 hours formed the brown product in 79% yield. IR (acetone): 2054, 1943, 1872 $cm^{-1}$. ESI MS (acetone): 270.6 ($M^-$) and 242.8 ($M^- - CO$). UV (methanol): $\lambda_{max}$: 439 nm. Anal. calcd. for

NaMoC5HO5S: C 22.32, S 11.92; found: C 22.47, S 11.97. ESI spectra for the three metal hydrosulfides are shown in Fig. S1 to Fig. S3.

**Hydrogen Sulfide Release**

A 5mM methanolic solution of the respective metal hydrosulfide was prepared by the addition of the appropriate mass into 10 ml of methanol. This solution was then subjected to hydrogen sulfide release by adding in either 10 ml of phosphate buffer at pH 6.5 or 4% aqueous $HBF_4$. $HBF_4$ was chosen because the anion is non-coordinating. The release of hydrogen sulfide was then monitored directly or indirectly by three methods.

(a) infrared spectroscopy

(b) UV-visible spectroscopy

(c) pH measurements

**Infrared Measurements**

A 0.01M solution of NaSH in deionised water was prepared for calibration purposes. The round bottom flask was then attached to a 15cm long gas cell connected to the headspace above the solution. 15 mL of 1M hydrochloric acid was then added and the release of H2S was then monitored until there were no further changes to the infrared absorbance obtained. A calibration curve was then plotted and subsequently used to determine the total gas concentration in the hydrolysis of the metal hydrosulfide. A separate solution containing 0.5mM of the respective metal hydrosulfide was then added to an equivalent volume of pH 6.5 phosphate buffer. The reaction flask was equipped with the same 15 cm long gas cell connected to the headspace above the aqueous solution. The FTIR spectrum of the fundamental band of $H_2S$ was obtained every 5 minutes for a total duration of 40 minutes. The concentration every 5 minutes were then calculated from the calibration curve and plotted against time.

**UV-Visible Measurements**

To a solution containing 0.5mM of metal hydrosulfide, an initial UV spectrum was taken at time $t$ = 0. 0.2 ml of 4% aqueous $HBF_4$ was then added to the solution of metal hydrosulfide in a quartz cuvette and an UV spectrum was obtained every 5 minutes over a period of 1 hour. The absorbance of the metal hydrosulfides starting material were used as an indicator of hydrogen sulfide release as the final product bands were too broad to be of any use.

**pH Measurements**

To a 0.01M solution containing the metal hydrosulfide, the pH of the initial solution was measured using a pH meter. The pH was monitored every 5 minutes for a total duration of 1 hour.

**Cytotoxicity Testing**

Experimental cultures of the mammary epithelial cells MCF-10A were obtained from the American Type Culture Collection (ATCC) and cultured in tissue culture dishes (Nunc Inc., Naperville, IL, USA). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS), 1% Lglutamate (GIBCO Laboratories), and 1% penicillin/streptomycin (GIBCO Laboratories) at 37 °C under a 5% CO2 atmosphere. Cell cultures were maintained in an antibiotic-free condition during cell growth and experiments. Phosphate buffered saline (PBS) was obtained from 1st Base. 40μM of Na[Mo(CO)5SH] in DMSO was prepared and serially diluted to obtain concentrations of 20μM, 10μM, 5μM and 1μM. 100μL of each concentrations were then added to the cells in triplicates and incubated at 37°C and 5% CO2 for 24 hours. A similar procedure was applied to the MDA-MB-231 cell line.

For the proliferation assay, approximately 10000 cells per well were placed in a 96-well plate and allowed to adhere for approximately 24 h. To each well was added 20% of Cell Titer 96 Aqueous One Cell Proliferation Assay (Promega), and then the mixtures were left to incubate in a 37 °C incubator with 5% CO2 over 2 h. Each sample was done in triplicate. The absorbance intensities at 490 nm were then measured and cell viabilities relative to the control (DMSO) were calculated.

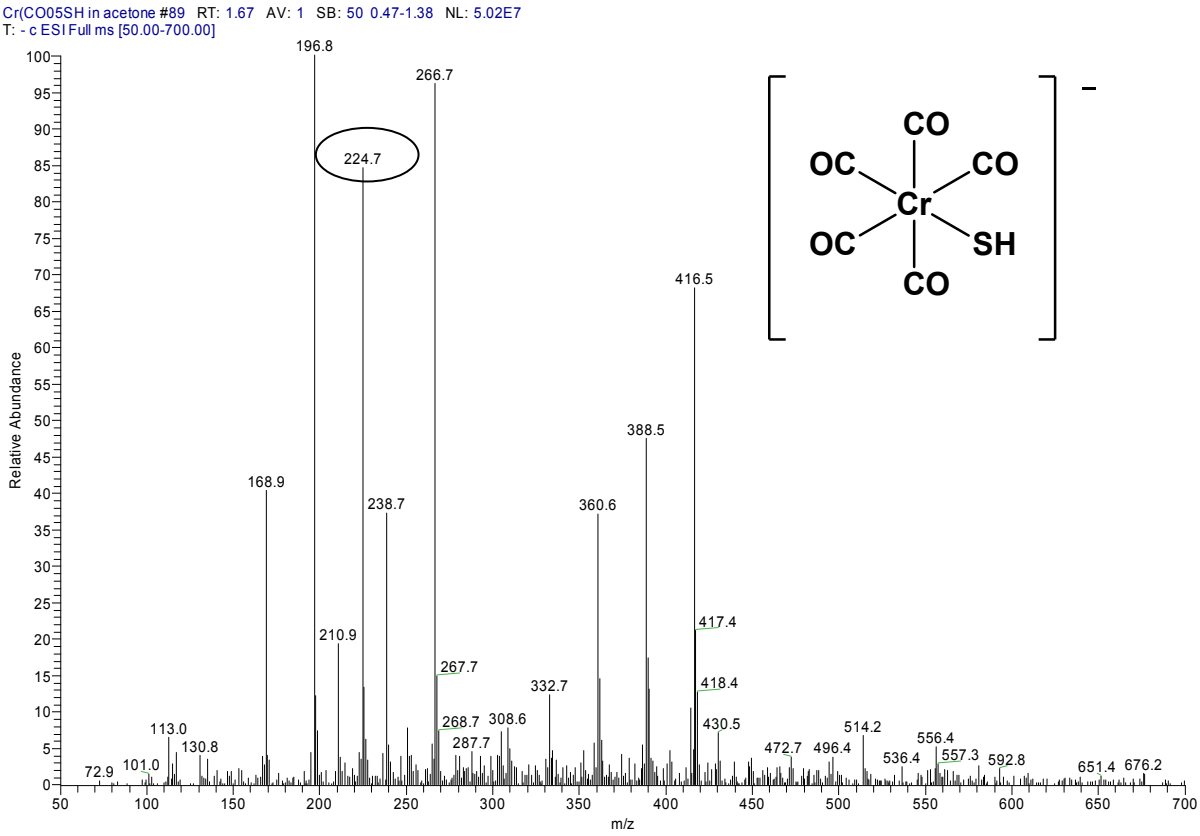


Figure S1: ESI spectrum for $Na[Cr(CO)_5(SH)]$

Assignment of major peaks :

| m/e | ion |
|---|---|
| 168.9 | $Cr(CO)_3SH^-$ |
| 196.8 | $Cr(CO)_4SH^-$ |
| 224.7 | $Cr(CO)_5SH^-$ |
| 360.6 | $Cr_2(CO)_8SH^-$ |
| 388.5 | $Cr_2(CO)_9SH^-$ |
| 416.5 | $(Cr(CO)_5)_2SH^-$ |

266.7 unknown but can be matched to $(CH_3CO)Cr(CO)_5SH^-$ ($CH_3CO$ fragment comes from acetone solvent)

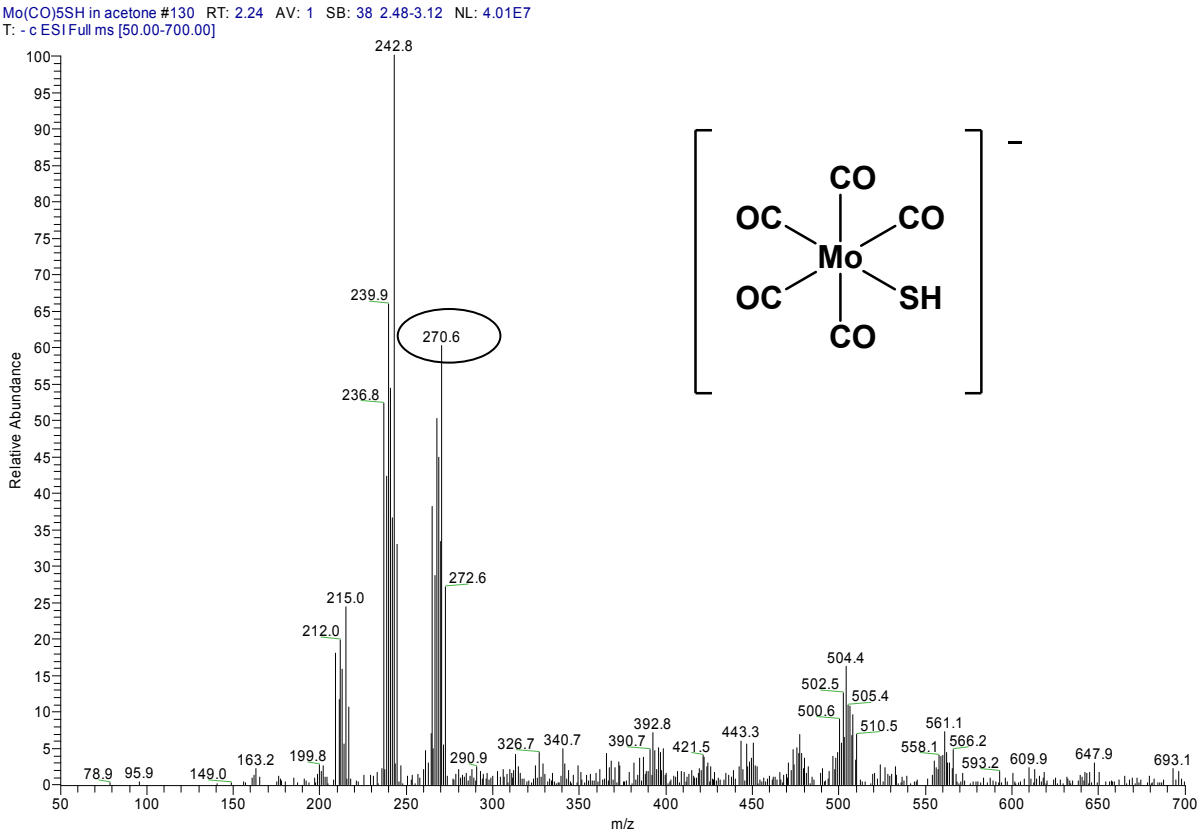


Figure S2: ESI spectrum for $Na[Mo(CO)_5(SH)]$

Assignment of major peaks :

| m/e | ion |
|---|---|
| 215.0 | $Mo(CO)_3SH^-$ |
| 242.8 | $Mo(CO)_4SH^-$ |
| 270.6 | $Mo(CO)_5SH^-$ |

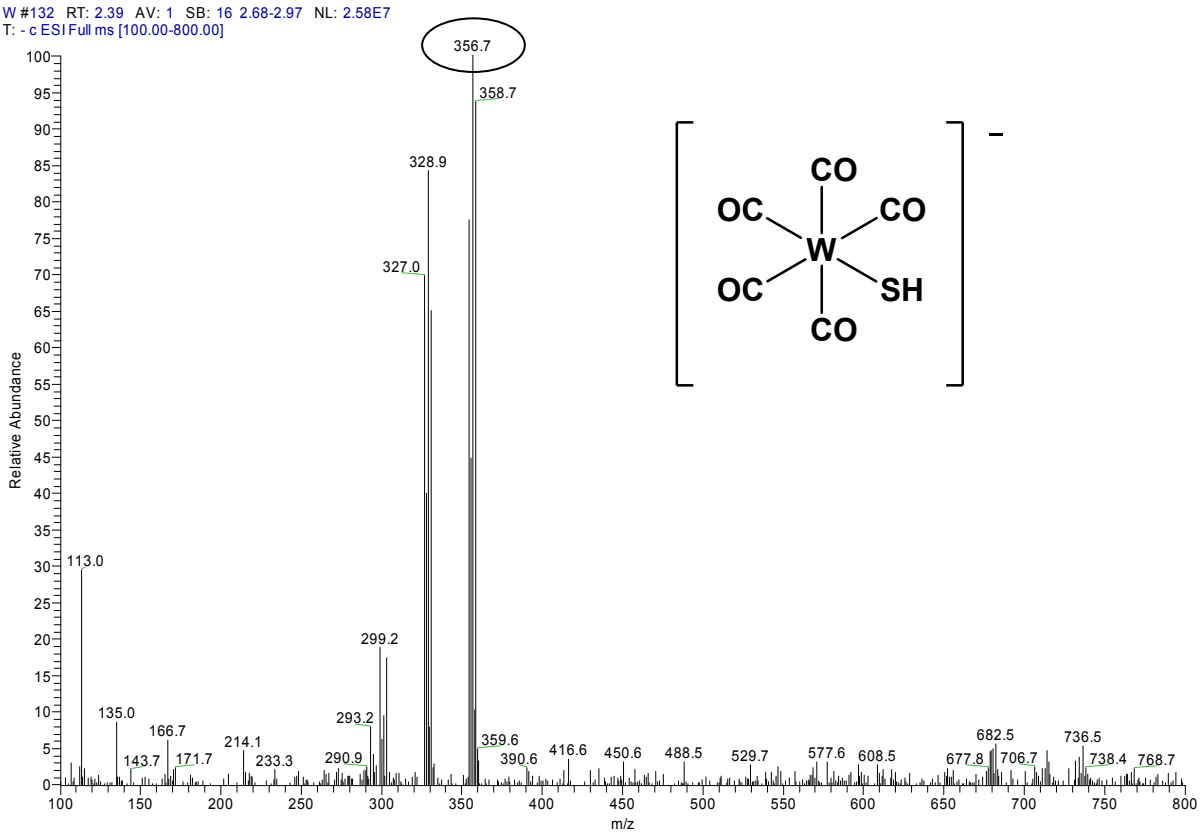


Figure S3: ESI spectrum for $Na[W(CO)_5(SH)]$

Assignment of major peaks :

| m/e | ion |
|---|---|
| 299.2 | $W(CO)_3SH^-$ |
| 328.9 | $W(CO)_4SH^-$ |
| 356.7 | $W(CO)_5SH^-$ |