Supporting Information to
High Antiproliferative Activity of
Hydroxythiopyridones Over Hydroxypyridones and
their Organoruthenium Complexes

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Additional XRD, NMR spectroscopic and cell biological data
Table S1. X-ray diffraction analysis measurement parameters.

|                         | 1b-MeOH       | 1d             |
|-------------------------|---------------|----------------|
| CCDC                    | 2049245       | 2049246        |
| Empirical formula       | C_{15}H_{19}NO_3 | C_{13}H_{13}NOS |
| Formula weight / g mol^{-1} | 261.31        | 231.32         |
| Temperature / K         | 100           | 100            |
| Crystal system          | triclinic     | triclinic      |
| Space group             | P-1           | P-1            |
| a / Å                   | 7.5960(3)     | 6.9685(3)      |
| b / Å                   | 10.0395(3)    | 8.2820(3)      |
| c / Å                   | 10.4920(4)    | 11.1698(4)     |
| α / °                   | 112.378(2)    | 110.175(2)     |
| β / °                   | 107.967(2)    | 93.567(2)      |
| γ / °                   | 94.975(2)     | 108.548(2)     |
| Volume / Å^3            | 684.56(4)     | 562.85(4)      |
| Z                       | 2             | 2              |
| ρ calc / g cm^{-3}      | 1.268         | 1.365          |
| μ / mm^{-1}             | 0.088         | 0.264          |
| F(000)                  | 280.0         | 244.0          |
| Crystal size / mm^3     | 0.28 × 0.14 × 0.12 | 0.4 × 0.28 × 0.15 |
| 2Θ range for data collection / ° | 5.796 to 50.498 | 5.49 to 50.5 |
| Index ranges            | -9 ≤ h ≤ 9    | -8 ≤ h ≤ 8     |
|                         | -12 ≤ k ≤ 12  | -9 ≤ k ≤ 9     |
|                         | -12 ≤ l ≤ 12  | -13 ≤ l ≤ 13   |
| Reflections collected   | 12123         | 10428          |
| Independent reflections | 2472 [R_{int} = 0.0537, R_{sigma} = 0.0411] | 2028 [R_{int} = 0.0428, R_{sigma} = 0.0300] |
| Data/restraints/parameters | 2472/0/179   | 2028/0/147    |
| Goodness-of-fit on F^2  | 1.030         | 1.088          |
| Final R indexes [I>2σ(I)] | R_I = 0.0384, wR_2 = 0.0900 | R_I = 0.0320, wR_2 = 0.0877 |
| Final R indexes [all data] | R_I = 0.0559, wR_2 = 0.0996 | R_I = 0.0337, wR_2 = 0.0896 |
| Largest diff. peak/hole / e Å^{-3} | 0.26/-0.18 | 0.28/-0.25 |

Table S2. Selected bond lengths [Å] and angles [°] for 1b and 1d.

| Bond lengths Å / angles ° | 1b              | 1d              |
|---------------------------|-----------------|-----------------|
| C4–O2/S                   | 1.2802(18)      | 1.7188(15)      |
| C3–O1                     | 1.3640(17)      | 1.3582(17)      |
| C3–C4                     | 1.428(2)        | 1.421(2)        |
| C2–C3                     | 1.373(2)        | 1.378(2)        |
| O2/S–C4–C3–O2             | 1.93            | 0.57            |
**Figure S1.** (a) π-stacking interaction found in the molecular structure of 1b with the shortest distance at 3.312 Å indicated as dashed, red lines; (b) Inter- and intramolecular H bond formation between two molecules of 1b and co-crystallized methanol indicated as a dashed, blue lines.

**Figure S2.** Stacking of four molecules of 1d and π-stacking interaction found between two molecules of 1d with the shortest distance at 3.523 Å indicated as a dashed, red line.
Table S3. Selectivity index (SI) of potent hydroxypyridone derivatives (1d and 1e) in different human cancer cell lines. SI values were calculated considering human prostate epithelial PNT1A cell line as normal cells.

| Compound | EC50 (µM) PNT1A | Selectivity Index |
|----------|-----------------|-------------------|
|          | A549            | NCI-H522          | MDA-MB-231 | MDA-MB-468 | PC3 |
| 1d       | 1.29 ± 0.06     | 3.58              | 4.61       | 0.46       | 0.75 | 3.91 |
| 1e       | 1.12 ± 0.02     | 3.50              | 4.86       | 0.42       | 0.33 | 0.77 |

Figure S3. Cell cycle analysis in A549 and NCI-H522 cells exposed to 1d and 1e. A549 (1 × 10⁶ cells per dish) cells were seeded in 10 cm cell culture dishes and NCI-H522 (3.0 × 10⁵ cells per well) cells were seeded in 6-well plates and left to attach for 24 h at 37 °C. (a) A549 cells were treated with 0.72 µM of 1d and 0.64 µM of 1e while (b) NCI-H522 cells were treated with 0.56 µM of 1d and 0.46 µM of 1e, both for 6 and 12 h. Vehicle control cells were incubated with DMSO (0.5%). Bars indicate the mean proportion of cells in the different cell cycle phases (% of total) ± SEM (n = 3). Data were analyzed with a two-way ANOVA coupled with a Bonferroni post-hoc test. No statistical significances were observed (p < 0.01).
Figure S4. Effect of 1d and 1e on (a) acetyl-H3, and cyclin D1 and (b) B1 expression in A549 cells.
Figure S5. Effect of 1d and 1e on (a) acetyl-H3, and cyclin D1 and (b) B1 expression in NCI-H522 cells.
Figure S6. Number of live, apoptotic and necrotic NCI-H522 cells following treatment with 1d and 1e. NCI-H522 (3.0 × 10^5 cells per well) cells were seeded in 6-well plates. Representative flow cytometry image of live (Q1), apoptotic (early apoptotic: Q2; late apoptotic: Q3) and necrotic (Q4) NCI-H522 cells were treated with 2× the EC50 of 1d and 1e for 12 h (a) and 24 h (b). Vehicle control cells were treated with DMSO (0.5%). PI: Propidium iodide.
NMR spectra

Figure S7. $^1$H NMR spectrum of 1d in $d_6$-DMSO.

Figure S8. $^1$H NMR spectrum of 1e in $d_6$-DMSO.
Figure S9. $^1$H NMR spectrum of 1f in $d_4$-MeOD.
Figure S10. $^1$H NMR spectrum of 2a in $d_4$-MeOD.

Figure S11. $^{13}$C{$^1$H} NMR spectrum of 2a in $d_4$-MeOD.
Figure S12. $^1$H NMR spectrum of 2b in CDCl$_3$.

Figure S13. $^{13}$C\{$^1$H\} NMR spectrum of 2b in CDCl$_3$. 
Figure S14. $^1$H NMR spectrum of 2c in $d_4$-MeOD.

Figure S15. $^{13}$C {$^1$H} NMR spectrum of 2c in $d_4$-MeOD.
**Figure S16.** $^1$H NMR spectrum of 2d in CDCl$_3$.

**Figure S17.** $^{13}$C{$^1$H} NMR spectrum of 2d in CDCl$_3$. 
Figure S18. $^1$H NMR spectrum of 2e in CDCl$_3$.

Figure S19. $^{13}$C {$^1$H} NMR spectrum of 2e in CDCl$_3$. 
Figure S20. $^1$H NMR spectrum of 2f in CDCl₃.

Figure S21. $^{13}$C{$^1$H} NMR spectrum of 2f in CDCl₃.