Supplementary data for article:

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SUPPLEMENTARY DATA

Selenotriapine – an isostere of the most studied thiosemicarbazone with pronounced pro-apoptotic activity, low toxicity and ability to challenge phenotype reprogramming of 3-D mammary adenocarcinoma tumors

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Content

Experimental

HSA binding experiments. A ligand (quencher) can absorb energy at both the HSA excitation (280 nm) and emission (340 nm) wavelengths. In order to overcome the inner-filter effect, the absorbance values of the ligand used were measured and corresponding corrections were made during calculation of binding parameters according to the eq. (S1)[S1]:

$$F_c = F_u \times 10^{(A_{ex} \times d_{ex} + A_{em} \times d_{em})/2} \qquad \text{(eq. S1)}$$

where F_u is the measured emission fluorescence intensity, F_c is the corrected fluorescence intensity that would be measured in the absence of any inner-filter effects, d_{ex} and d_{em} are the cell path lengths in the excitation and emission direction (1 cm), A_{ex} and A_{em} are the absorbance values of the quencher measured at the excitation and peak emission wavelength.

Fluorescence quenching data were processed using the Stern–Volmer eq. (S2):

$$F_0/F = 1 + K_{sv}[Q] = 1 + k_q\tau_0[Q \qquad \text{(eq. S2)}$$

where F_0 and F are the HSA fluorescence intensities at 340 nm before and after addition of the quencher (Se-3-AP), K_{sv} is the Stern-Volmer quenching constant, k_q stands for the fluorescence quenching rate constant, τ_0 is the average fluorescence lifetime of the fluorophore (7.09 ns for HSA)[S2] and [Q] is the concentration of the quencher[S3]. The quenching process was additionally analyzed using a modified Stern–Volmer eq. (S3)[S3]:

$$F_0/F_0 - F = 1/f_a K_a [Q] + 1/f_a \qquad \text{(eq. S3)}$$

where F_0 and F are the HSA fluorescence intensities before and after addition of the quencher at concentration [Q]. K_a represents the effective quenching constant for the accessible fluorophores, and f_a is the fraction of accessible fluorophore.

Thermodynamic parameters of binding, the enthalpy (ΔH) and entropy change (ΔS), during the binding of Se-3-AP to HSA were determined by measuring the binding constants at three temperatures, and following the Van't Hoff eq. (S4):

$$\ln K_a = -\Delta H/RT + \Delta S/R \qquad \text{(eq. S4)}$$

where R is the universal gas constant, T is the temperature (in K), and K_a is the effective quenching constant at the corresponding temperature.

The estimations of association (binding) constants (K_b) and number of binding sites (n) of HSA and Se-3-AP were done using eq. (S5)[S4]:

$$\log(F_0 - F)/F = -n\log(1/([Q] - [P] \times (F_0 - F)/F_0) + n\log K_b \qquad \text{(eq. S5)}$$

where [Q] and [P] are total concentrations of ligand (Se-3-AP) and protein (HSA), respectively.

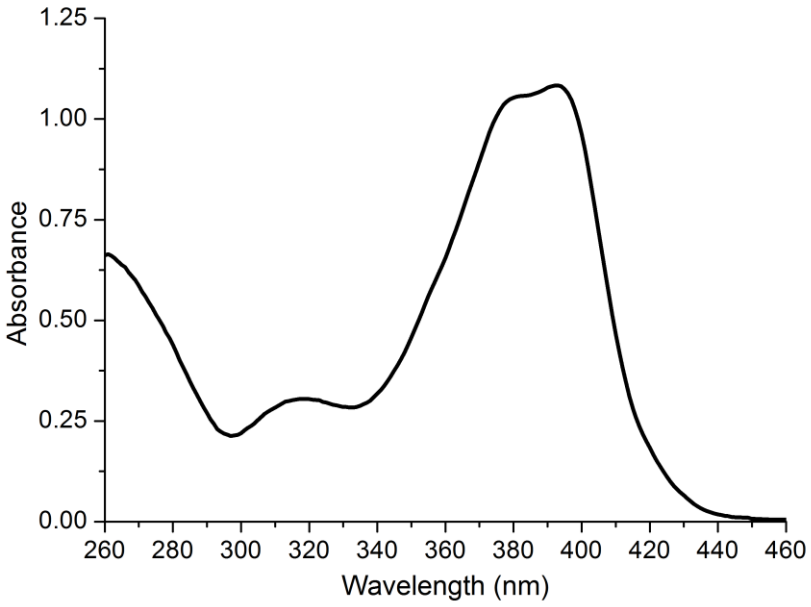

Figure S1. UV/vis spectrum of Se-3-AP (51 μM) in DMSO.

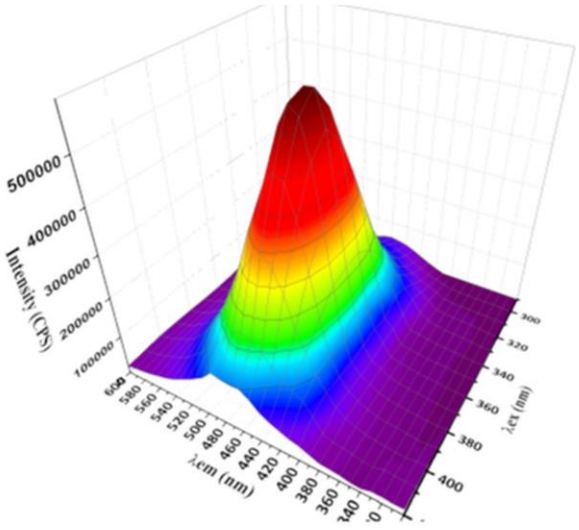

Figure S2. Fluorescence spectrum of Se-3-AP (10 mM, λ_{ex} = 360 nm) in DMSO.

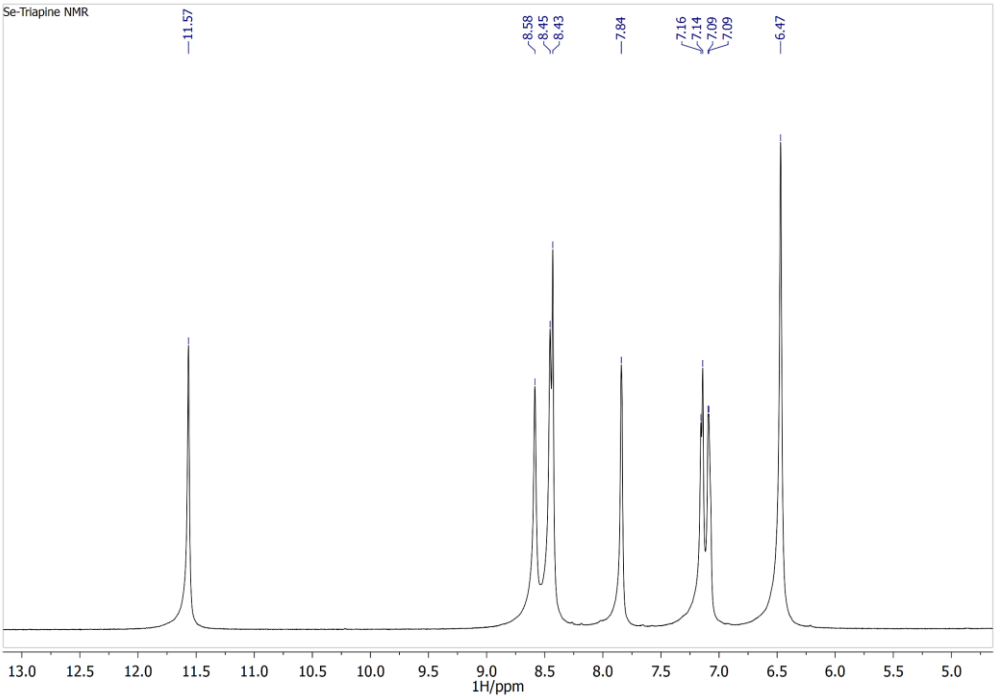

Figure S3. ^{1}H-NMR spectrum of Se-3-AP in DMSO-d_6.

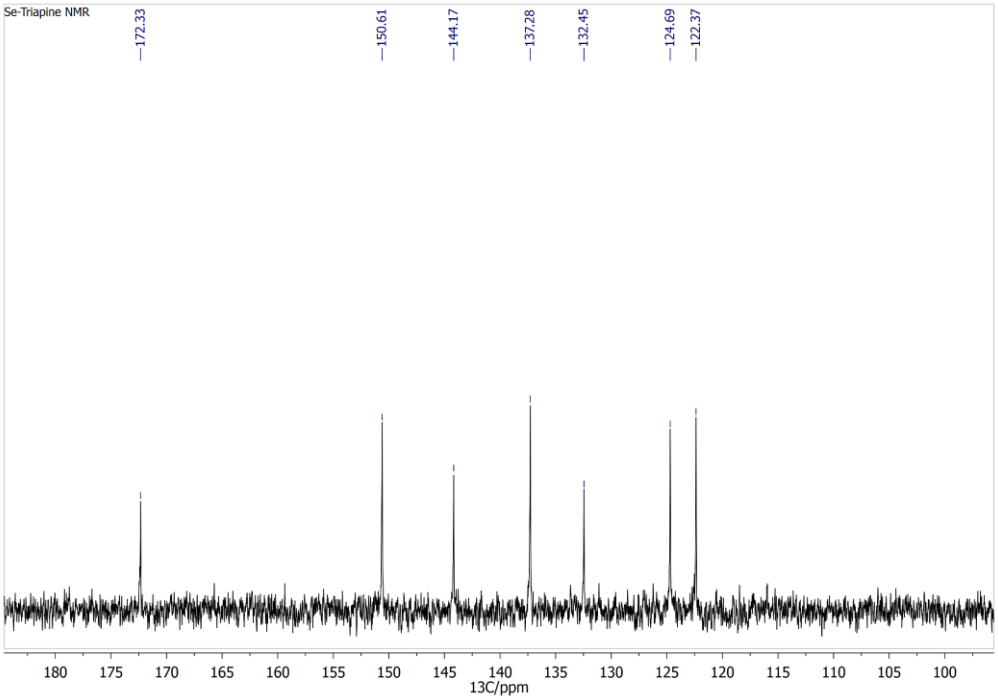

Figure S4. ^{13}C-NMR spectrum of Se-3-AP in DMSO-d_6.

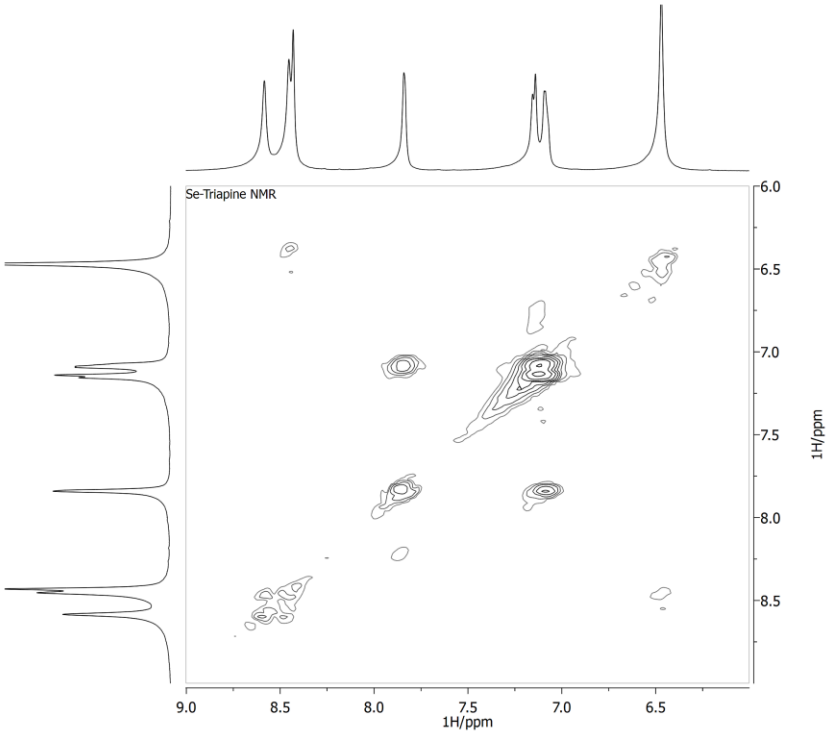

Figure S5. COSY spectrum of Se-3-AP in DMSO-d_6.

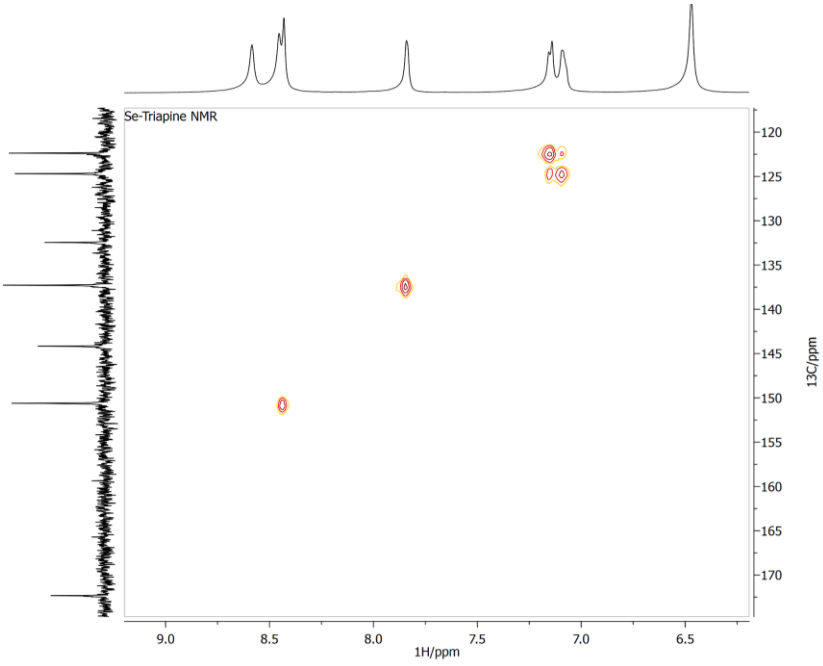

Figure S6. ^{1}H-^{13}C HSQC spectrum of Se-3-AP in DMSO-d_6.

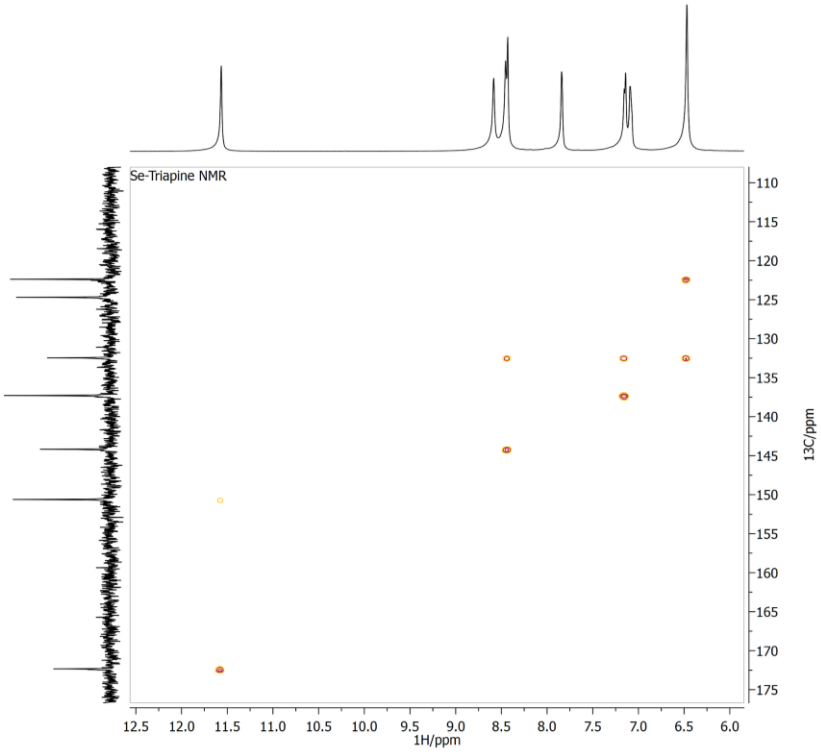

Figure S7. ^{1}H-^{13}C HMBC spectrum of Se-3-AP in DMSO-d_6.

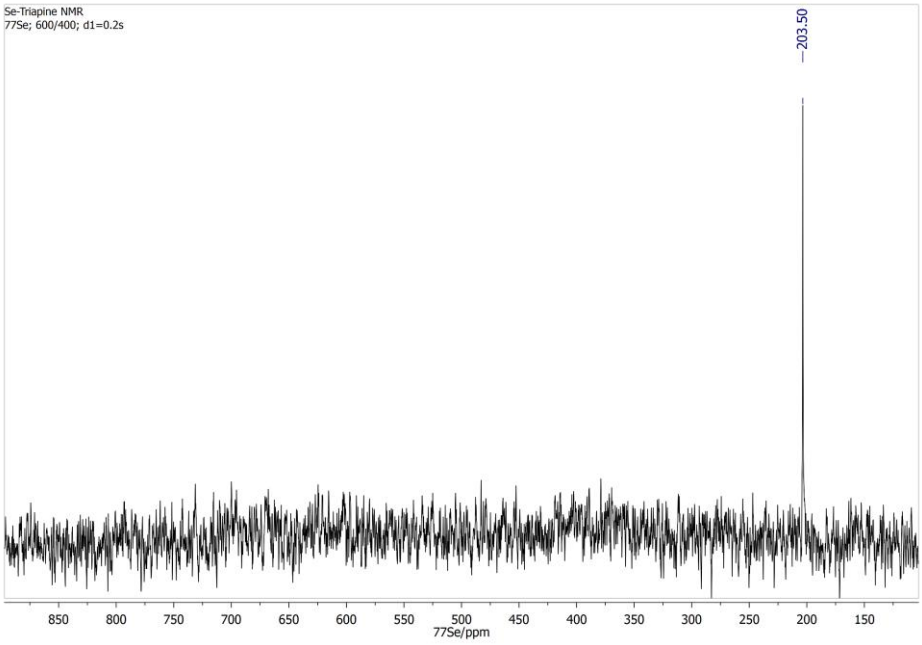

Figure S8. ^{77}Se-NMR spectrum of Se-3-AP in DMSO-d_6.

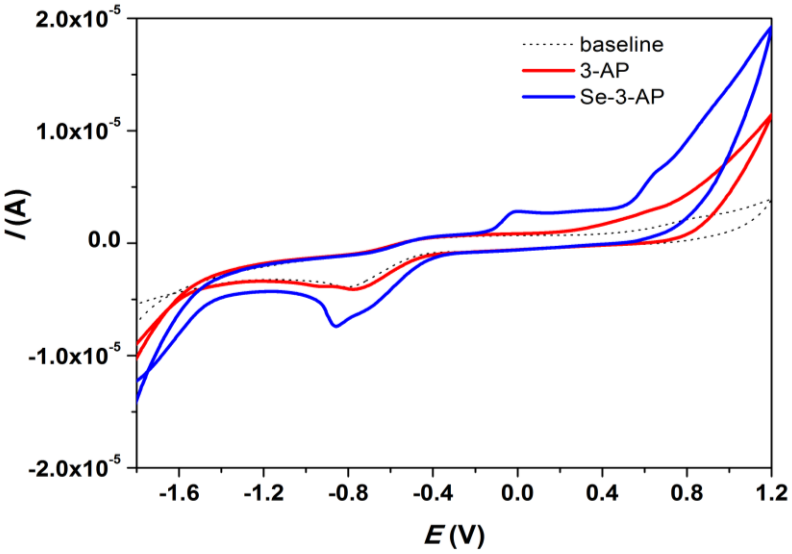

Figure S9. Cyclic voltammograms of Se-3-AP and 3-APin 0.10 M [*n*-Bu_4N]PF_6/DMSO, GC electrode.

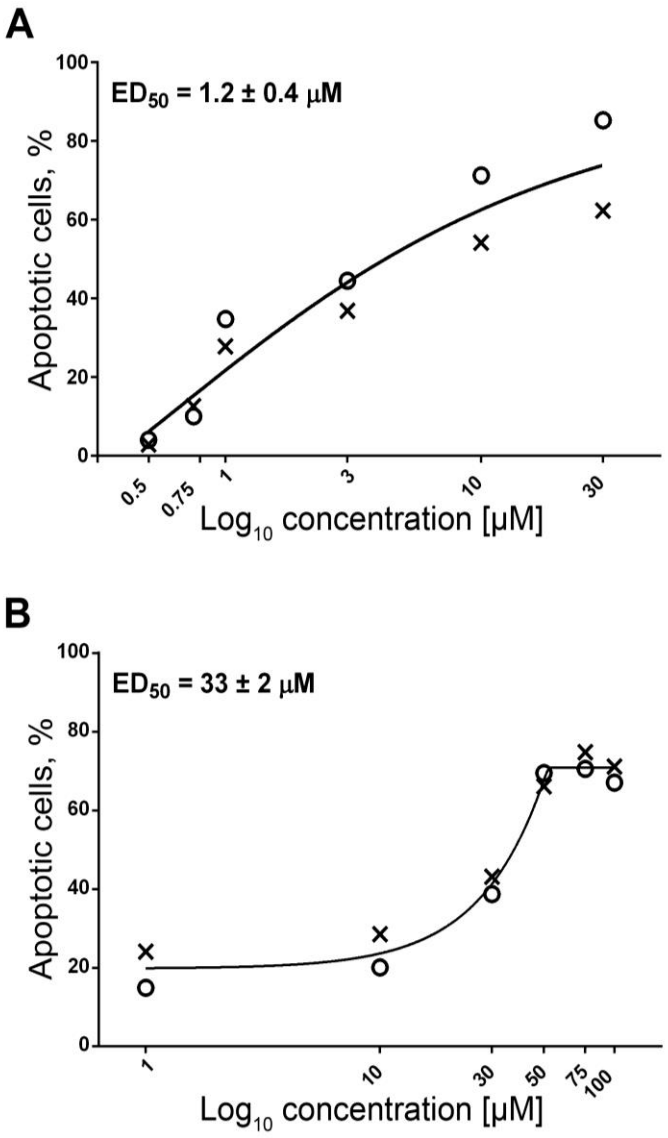

Figure S10. Concentration-response curves and ED_{50} values for Se-3-AP on THP-1 (A) and MCF-7 cells (B) after 24 h treatment. Results are presented as percentages of apoptotic cells determined by means of Annexin V/propidium iodide double staining method for two independent experiments (circles and crosses), with asymmetric five-parameter sigmoidal curve computed for both replicates in GraphPad Prism software.

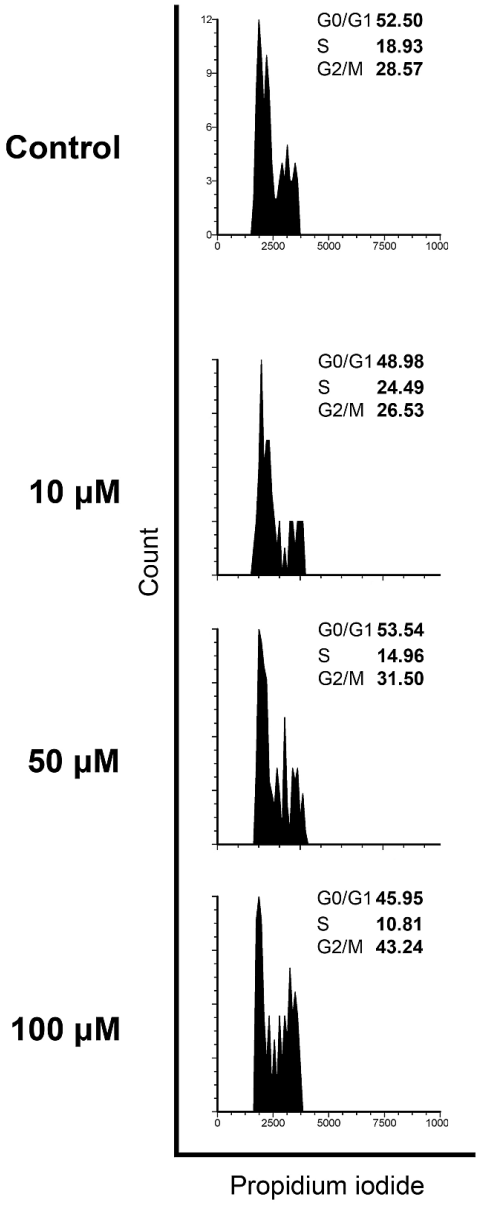

Figure S11. Changes in distribution of MCF-7 cells within phases of mitotic division induced by 24 h treatment with 3-AP. Analysis has been performed on the same cell samples represented in Figure 2A (right panel) on the remaining cells after Annexin V/PI read out. Incidences of cells found at the G0/G1, S and G2/M phases were determined according to non-treated control population. Results represent percentages of cells within phases of cell cycle obtained from a single experiment considering additional replicates have not been acquired due to 3-AP lack of activity.

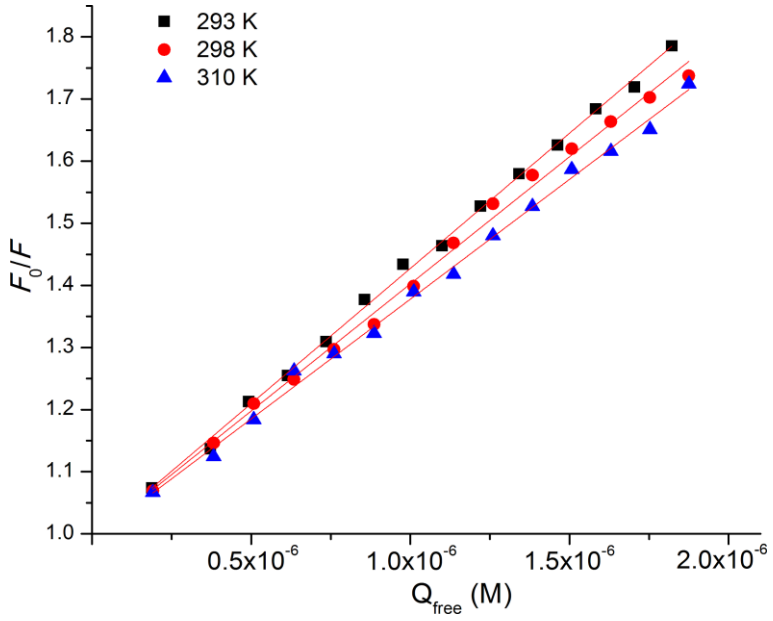

Figure S12. Stern-Volmer plot of F_0/Fvs [Q] at three different temperatures, where F_0 and F represent HSA fluorescence intensities in absence (F_0) and in the presence of the quencher (F), and [Q] is the concentration of the quencher (Se-3-AP).

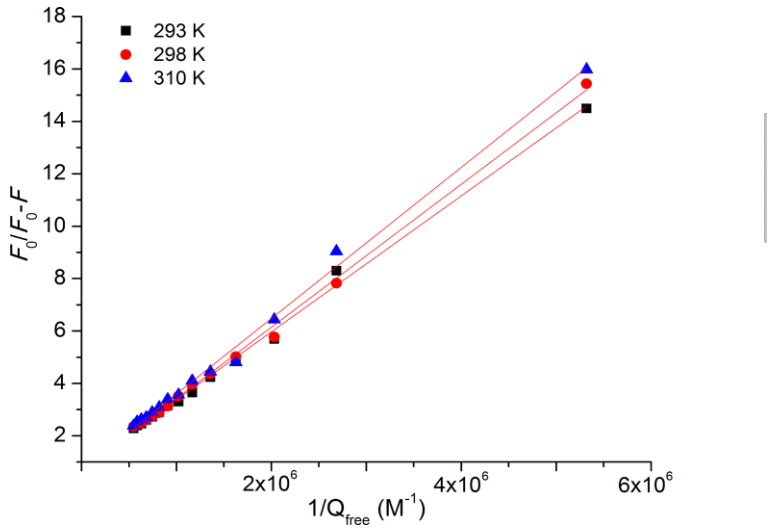

Figure S13. Modified Stern-Volmer plot for binding of Se-3-AP to HSA at three temperatures.

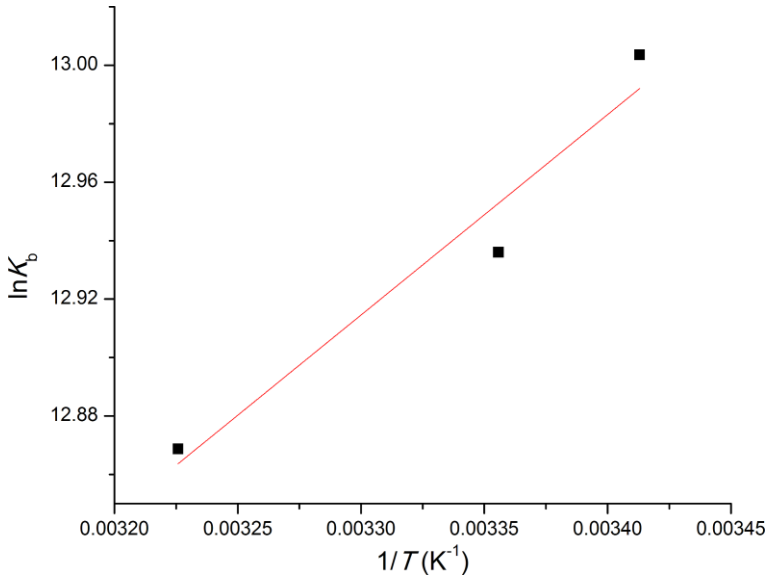

Figure S14. The plot of lnK_b *vs* 1/T for the interaction of Se-3-AP with HSA. K_b is given in M^{-1}.

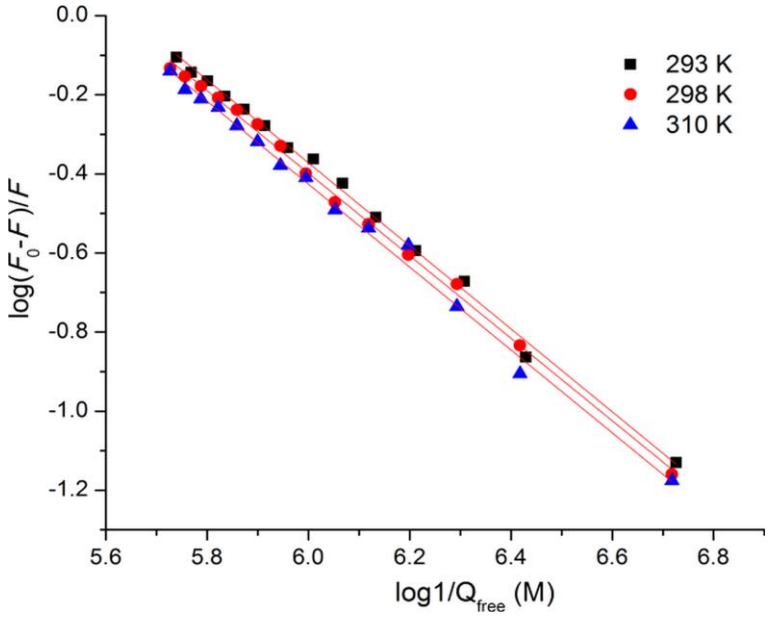

Figure S15. Double-log plot for determination of binding constants K_b, and the number of binding sites n at three temperatures; concentration of the quencher [Q] is given in M.

References

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