Supplementary Material

Synthesis of new heterocycle-based selenoamides as potent cytotoxic agents

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Table of Contents

1. Materials and instruments ............................................................................................................................................. S2
2. Synthesis of carbene complexes 1(a-h) .......................................................................................................................... S2
3. NMR and HR-M spectra of 1c .......................................................................................................................................... S3
4. Synthesis of aminocarbene complexes 2(a-h) .................................................................................................................. S5
5. NMR and HR-M spectra of 2(c-g) .................................................................................................................................... S7
6. Synthesis of selenoamides 3(a-h) .................................................................................................................................... S16
7. NMR and HR-M spectra of 3(a-g) ................................................................................................................................... S19
8. X-Ray diffraction analyses of 3f ...................................................................................................................................... S31
9. Biological Studies .............................................................................................................................................................. S32
10. References ........................................................................................................................................................................ S33
1. - Materials and instruments

THF and diethyl ether were distilled from sodium/benzophenone under a nitrogen atmosphere. All reagents and solvents were obtained from commercial suppliers and used without further purification. All compounds were characterized by IR spectra, recorded on a Bruker Tensor 27 spectrophotometer, by KBr or film techniques, and all data are expressed in wave numbers (cm$^{-1}$). Melting points were obtained on a Melt-Temp II apparatus and are uncorrected. NMR spectra were measured with a JEOL Eclipse +300 and Bruker Avance III 300, using CDCl$_3$ and CD$_3$CN as solvents. Chemical shifts are in ppm (δ), relative to TMS. The MS-EI were obtained on a JEOL JMS-AX505 HA using 70 eV as ionization energy and for MS-FAB a JEOL JMS-SX 102A using nitrobenzyl alcohol and polyethylene glycol as matrix. All tested compounds synthesized are more than 95 % pure, analyzed using HPLC HP 1100 with diode-array detector.

2. - Synthesis of Fischer ethoxyarylcarbene chromium 0 complexes 1(a-h)

The preparation of Fischer-type carbene complexes was carried out using a slightly modification of the methodology previously described elsewhere.$^{1}$ To a solution of the corresponding aryl substrate (8 mmol) in 10 mL of anhydrous THF under argon atmosphere was added at 0°C a solution of nBuLi (8.2 mmol). The reaction mixture was stirred at room temperature for 20 to 60 min. and then transferred by canula to a suspension of Cr(CO)$_6$ (1.74 g, 8 mmol) in THF (20 mL). The mixture was then stirred for the time specified in Table 1 of main document, at room temperature. The solvent was removed under vacuum, and then triethyloxonium tetrafluoroborate (2 g, 10 mmol) on ice/water was added. The organic phase was washed with saturated solution of NaHCO$_3$ and then with brine. The organic phase was dried with anhydrous sodium sulfate and the solvent was evaporated under vacuum. The mixture was purified by chromatography on silica gel or alumina using hexane as eluent. Fischer carbene complexes 1a, 1b, 1d, 1e, 1f, 1g and 1h are already known and their spectroscopic data match well with literature.$^{1-3}$

$$[\text{ethoxy}(\text{benzo}[d][1,3]\text{dioxol-5-yl})\text{methylidene}]\text{pentacarbonyl chromium (0) (1c):}$$ Yield: 60 %; mp 66 – 70 (d) °C; $^1$H NMR (300 MHz, CDCl$_3$, TMS): δ = 7.36 (d, J = 7.2 Hz, 1 H; C$_6$H$_3$), 7.03 (s, 1 H; C$_6$H$_3$), 6.86 (d, J = 7.8 Hz, 1 H; C$_6$H$_3$), 6.05 (s, 2 H; OCH$_2$O), 5.12 (d, 2 H; OCH$_2$), 1.70 (s, 3H, CH$_3$) ppm. $^{13}$C NMR (75 MHz, CDCl$_3$, TMS): δ = 339.8 (C=Cr), 223.8 (CrCO$_{ax}$), 216.7 (CrCO$_{eq}$), 150.9 (C, C$_6$H$_3$), 147.9 (C, C$_6$H$_3$), 147.6 (C, C$_6$H$_3$), 124.5 (CH, C$_6$H$_3$), 107.6 (CH, C$_6$H$_3$), 105.2 (CH, C$_6$H$_3$), 101.9 (OCH$_2$O), 76.6 (OCH$_2$), 15.2 (CH$_3$) ppm. IR (KBr) (cm$^{-1}$): 2057, 1905 (CrCO). MS (EI, 70 eV) m/z (%): 370 (8) [M$^+$], 342 (27) [M$^+$-CO], 314 (47) [M$^+$-2CO], 286 (38) [M$^+$-3CO], 258 (92) [M$^+$-4CO], 230 (68) [M$^+$-5CO]. HRMS (FAB$^+$): m/z: calcd. for C$_{15}$H$_{10}$CrO$_8$: 369.9781 [M$^+$]; found: 369.9768.
3. - NMR spectra of 1c

Figure S1. $^1$H NMR spectrum of 1c

Figure S2. $^{13}$C NMR spectrum of 1c
Figure S3. HR-MS of compound 1c
4. - Synthesis of Fischer Aminocarbenes chromium 0 complexes 2(a-h) (Route A)

To a solution of Fischer ethoxycarbene complex (1) (2.3 mmol) in 20 mL of anhydrous diethyl ether under nitrogen atmosphere was added 4.9 mmol of ethanolamine. The reaction mixture was stirred at room temperature for 5 to 20 min. and then diluted with 20 mL of water. The organic phase was separated and dried with anhydrous Na₂SO₄, and the solvent was evaporated in vacuum. The crude product was purified by flash column chromatography using alumina and Hexane-AcOEt mixture (95:5) as eluent. Compounds 2a, 2b and 2h are known and their spectroscopic data match well with already described in the literature.¹⁻³

[(benzo[d][1,3]dioxo-5-yl)(2-hydroxyethylamino)methylidene]pentacarbonyl chromium (0) (2c) (Unstable in solution): Yield: 81%；Yellow oil; ¹H NMR (300 MHz, CDCl₃, TMS): δ = 9.51 (s, 1 H; NH₆), 9.06 (s, 1 H; NH₂), 6.83 (s, 1 H; C₆H₃), 6.34 – 6.26 (m, 2H; C₆H₅), 5.99 (s, 2H; OCH₂O), 3.76 (s, 2 H; OCH₂), 3.39 (s, 2 H, NCH₂), 1.25 (s, 1 H; OH) ppm. IR (Film), cm⁻¹: 3410 (OH), 3352 (NH), 2054, 1974, 1911 (CrCO). MS (FAB⁺) m/z (%): 385 (12) [M⁺], 357 (16) [M⁺-CO], 329 (15) [M⁺-2CO], 301 (21) [M⁺-3CO], 273 (28) [M⁺-4CO], 245 (72) [M⁺-5CO]; HRMS (FAB⁺) m/z: calcd. for C₁₅H₁₁CrNO₈: 384.9890 [M⁺]; found: 384.9888.

[(N-methylpyrrol-2-yl)(2-hydroxyethylamino)methylidene]pentacarbonyl chromium (0) (2d): Yield: 90%; Yellow oil; ¹H NMR (300 MHz, CDCl₃, TMS): δ = 9.40 (s, 1 H; NH₆), 8.62 (s, 1 H; NH₂), 6.61 and 6.55 (s, 1 H; C₄H₅N), 6.17 (s, H; C₄H₅N), 5.97 (s, H; C₄H₅N), 3.54-3.38 (m, 7 H; OCH₂, NCH₂, NCH₃) 1.25 (s, 1 H; OH) ppm. ¹³C NMR (75 MHz, CDCl₃, TMS): δ = 277.2 and 265.4 (CCr, Isomers E and Z), 223.5 (CO₉, Isomer E), 217.7 (COeq Isomers Z), 217.2 (COeq Isomer E), 211.5 (COeq Isomer Z), 163.3 (C, C₄H₅N), 145.1 (CH, C₄H₅N), 123.2 (CH, C₄H₅N), 109.0 and 106.1 (CH, C₄H₅N, Isomers E and Z), 62.9 and 60.9 (OCH₂, Isomers E and Z), 54.4 and 52.6 (NCH₂, Isomers E and Z), 52.6 ppm (NCH₃). IR (Film) cm⁻¹: 3609 (OH), 3373 (NH), 1998, 1870 (CrCO). MS (FAB⁺) m/z (%): 345 (80) [M⁺+1], 317 (12) [M⁺+1-CO], 289 (65) [M⁺+1-2CO], 261 (100) [M⁺+1-3CO], 233 (57) [M⁺+1-4CO], 204 (14) [M⁺+1-5CO]; HRMS (FAB⁺) m/z: calcd. for C₁₃H₁₂CrN₂O₆: 344.0100 [M⁺]; found: 344.0110.

[(N-methylindol-2-yl)(2-hydroxyethylamino)methylidene]pentacarbonyl chromium (0) (2e): Yield: 92%; Yellow oil; ¹H NMR (300 MHz, CDCl₃, TMS): δ = 9.92 (s, 1 H; NH), 7.59 (d, J= 7.5 Hz, 1 H; C₈H₅N), 7.33 – 7.12 (m, 3 H; C₈H₅N), 6.18 (s, 1 H; C₈H₅N), 3.72 - 360 (m, 7 H; OCH₂, NCH₂, NCH₃), 3.34 (s, 1 H; OH) ppm. ¹³C NMR (75 MHz, CDCl₃, TMS): δ = 279.0 (CCr), 223.1 (CrCO₉), 216.8 (CrCOeq), 145.0 (C, C₈H₅N), 137.0 (C, C₈H₅N), 128.1 (C, C₈H₅N), 122.1 (CH, C₈H₅N), 121.0 (CH, C₈H₅N), 120.4 (CH, C₈H₅N), 109.4 (CH, C₈H₅N), 96.0 (CH, C₈H₅N), 60.6 (OCH₂), 50.3 (NCH₂), 30.8 (NCH₃) ppm. IR (Film) cm⁻¹: 3349 (OH) and (NH), 2054, 1976, 1912 (CrCO). MS (FAB⁺) m/z (%): 395
(11) [M′'-1], 366 (62) [M′'-CO], 338 (18) [M′'-2CO], 310 (11) [M′'-3CO], 282 (100) [M′'-4CO], 254 (84) [M′'-5CO].

HRMS (FAB+) m/z: calcd. for C_{17}H_{14}CrN_{2}O_{6}: 394.0257 [M′']; found: 394.0260.

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[[\text{thien}-2-\text{yl})(2-\text{hydroxyethylamino)methyliden}]{p}pentacarbonyl \text{chromium (0) (2f)}: \text{Yield: 95%; m.p. 105 -107°C (d);} \\
^1H \text{NMR (300 MHz, CDCl}_3\text{, TMS): } \delta = 9.45 (s, 1 \text{ H}; \text{NH}_2), 9.13 (s, 1 \text{ H}; \text{NH}_2), 7.47 \text{ and } 7.41 (m, \text{ 2 H}; \text{C}_4\text{H}_3\text{S, Isomers E and Z}), 7.11 \text{ and } 7.06 (m, \text{ 2 H}; \text{C}_4\text{H}_3\text{S, Isomers E and Z}), 6.84 (s, \text{ 1H}; \text{C}_4\text{H}_3\text{S}) 4.27 \text{ and } 4.09 (d, \text{ 4 H}; \text{OCH}_2, \text{Isomers E and Z}), 3.86 \text{ and } 3.66 (d, \text{ 4 H}; \text{OCH}_2, \text{Isomers E and Z}), 1.99 (s, \text{ 1 H}; \text{OH}) \text{ ppm.} \\
^{13}C \text{NMR (75 MHz, CDCl}_3\text{, TMS): } \delta = 272.9 \text{ and } 261.2 (\text{CCr, Isomers E and Z}), 223.2 (\text{CO}_ax, \text{Isomers E and Z}), 217.5 \text{ and } 217.1 (\text{CO}_eq, \text{Isomers E and Z}), 155.7 \text{ and } 149.01(\text{C, C}_4\text{H}_3\text{S, Isomers E and Z}), 129.3 \text{ and } 128.2 (\text{CH, C}_4\text{H}_3\text{S, Isomers E and Z}), 127.6 \text{ and } 127.3 (\text{CH, C}_4\text{H}_3\text{S, Isomers E and Z}), 126.7 \text{ and } 122.6 (\text{CH, C}_4\text{H}_3\text{S, Isomers E and Z}), 61.0 \text{ and } 60.9 (\text{OCH}_2), 54.6 \text{ and } 52.3 (\text{NCH}_2, \text{Isomers E and Z}) \text{ ppm.} \\
\text{IR (KBr) cm}^{-1}: 3581 (\text{OH}), 3308 (\text{NH}), 2053, 1869 (\text{Cr- CO}). \text{MS (EI, 70 eV) m/z (%): } 347 (14.96) [M′'], 319 (7.04) [M′'-CO], 291 (8.16) [M′'-2CO], 263 (20.41) [M′'-3CO], 235 (36.73) [M′'-4CO], 207 (13.6) [M′'-5CO]. \text{HRMS (FAB+) m/z: calcd for C}_{12}\text{H}_9\text{CrNO}_7: 346.9556 [M′']; found: 346.9560.\\

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[[\text{furan}-2-\text{yl)(2- hydroxyethylamino)methyliden}]{p}pentacarbonyl \text{ chromium (0) (2g): Yield: 96%; m.p. 85 -86 °C;} \\
^1H \text{NMR (300 MHz, CDCl}_3\text{, TMS): } \delta = 9.73 (s, 1 \text{ H}; \text{NH}_E), 8.89 (s, 1 \text{ H}; \text{NH}_Z), 7.67 \text{ and } 6.48 (s, \text{ 2H; C}_4\text{H}_3\text{O, Isomers E and Z}), 7.19 (s, \text{ 1 H}; \text{C}_4\text{H}_3\text{O}), 6.61 (s, \text{ 2 H}; \text{C}_4\text{H}_3\text{O}, \text{ Isomers E and Z}), 4.26 \text{ - 3.92 (m, 8 H; OCH}_2\text{, NH}_2, \text{ Isomers E and Z}), 1.88 (s, \text{ 1 H; OH} ) \text{ ppm.} \\
^{13}C \text{NMR (75 MHz, CDCl}_3\text{, TMS): } \delta = 244.2 (\text{CCr}), 222.7 (\text{CO}_ax), 218.0 \text{ and } 217.8 (\text{CO}_eq \text{Isomers E and Z}), 145.8 \text{ and } 144.2 (\text{C, C}_4\text{H}_3\text{O, Isomers E and Z}), 125.1 \text{ and } 123.4 (\text{CH, C}_4\text{H}_3\text{O, Isomers E and Z}), 113.4 \text{ and } 112.9 (\text{CH, C}_4\text{H}_3\text{O, Isomers E and Z}), 61.0 \text{ and } 60.5 (\text{OCH}_2, \text{Isomers E and Z}), 54.1 (\text{NCH}_2) \text{ ppm.} \\
\text{IR (KBr) cm}^{-1}: 3581 (\text{OH}), 3308 (\text{NH}), 2053, 1869 (\text{Cr- CO}). \text{MS (EI, 70 eV) m/z (%): } 331 (15) [M′'], 303 (7) [M′'-CO], 275 (8) [M′'-2CO], 247 (20) [M′'-3CO], 219 (37) [M′'-4CO], 191 (14) [M′'-5CO]. \text{HRMS (FAB+) m/z: calcd for C}_{12}\text{H}_9\text{CrNO}_7: 330.9784 [M′']; found: 330.9792.\\

5. - NMR and HR-MS spectra of 2c-g

Figure S4. $^1$H NMR spectrum of 2c (E/Z ratio 72:28)

Figure S5. HR-MS of compound 2c
Figure S6. $^1$H NMR spectrum of 2d (E/Z ratio 61:39)

Figure S7. $^{13}$C NMR spectrum of 2d
Figure S8. HR-MS of compound 2d

| Observed m/z | Int% | Estimated m/z | Error [ppm] | U.S. C | H | O | N | Cr |
|--------------|------|---------------|-------------|--------|---|---|---|----|
| 344.0110     | 75.1 | 344.0100      | 2.9         | 11     | 13| 12| 6 | 2  | 1  |
Figure S9. $^1$H NMR spectrum of 2e

Figure S10. $^{13}$C NMR spectrum of 2e
[ Elemental Composition ]

Data : Dr-Jose-G-Lopez093  Date: 30-Jan-2013  18:31
Sample: 186 MeIndCrNMeOH
Note : Luis-Velasco
Inlet : Direct  Ion Mode : FAB+
RT : 0.96 min  Scan#: (4,8)
Elements : C 30/0, H 49/0, O 9/0, N 4/1, Cr 2/0
Mass Tolerance : 100ppm, 2mmu if m/z >2

| Observed m/z | Int%  | Estimated m/z | Error [ppm] | U.S. C | H  | O  | N  | Cr |
|--------------|-------|---------------|-------------|-------|----|----|----|----|
| 394.0260     | 58.2  | 394.0257      | -0.7        | 14    | 17 | 14 | 6  | 2  | 1  |

Figure S11. HR-MS of compound 2e
Figure S12. $^1$H NMR spectrum of 2f (E/Z ratio 56:44)

Figure S13. $^{13}$C NMR spectrum of 2f
**Figure S14.** HR-MS of compound 2f

| Observed m/z       | Int%  | Estimated m/z | Error [ppm] | U.S. | C | H | O | N | S | Cr |
|--------------------|-------|---------------|-------------|------|---|---|---|---|---|----|
| 346.9560           | 22.5  | 346.9556      | 1.1         | 11   | 12| 9 | 6 | 1 | 1 | 1  |
Figure S15. $^1$H NMR spectrum of 2g (E/Z ratio 55:45)

Figure S16. $^{13}$C NMR spectrum of 2g
Figure S17. HR-MS of compound 2g
### 6. - Synthesis of selenoamides 3(a-f) (Route A)

#### Table S1. Synthesis of arylselenoamides 3(a-f)

| Entry | Ar         | Compound | Time (min) | Demetalation step | Route A | Route B |
|-------|------------|----------|------------|-------------------|---------|---------|
|       |            |          |            |                   | Yield (%)<sup>a</sup> | Global Yield (%)<sup>a</sup> | Yield (%)<sup>b</sup> | Global Yield (%)<sup>b</sup> |
| 1     |            | 3a       | 25         |                   | 94      | 70      | 93      | 74      |
| 2     |            | 3b       | 20         |                   | 96      | 78      | 95      | 80      |
| 3     |            | 3c       | 35         |                   | 91      | 44      | 90      | 64      |
| 4     |            | 3d       | 45         |                   | 83      | 46      | 90      | 64      |
| 5     |            | 3e       | 45         |                   | 72      | 63      | 80      | 75      |
| 6     |            | 3f       | 35         |                   | 90      | 60      | 91      | 76      |
| 7     |            | 3g       | 45         |                   | 90      | 69      | 92      | 74      |
| 8     | Fc         | 3h       | 15         |                   | 91      | 77      | 95      | 81      |

For <sup>a</sup>Route A and <sup>b</sup>Route B, the global yield was determined from the corresponding aryl substrate used as starting material.

**Preparation of selenating agent:** To a solution of 0.01 mol of NaBH₄ in 10 mL of ethanol was added 0.01 mol of powdered selenium, and the mixture was vigorously stirred at room temperature for 30 min under nitrogen atmosphere. The selenating agent was then added to a solution (0.001 mol) of the corresponding aminocarbene complex in 5 mL of ethanol, under nitrogen atmosphere; the reaction was monitored by TLC on silica-gel. After
the reaction was completed, the solvent was evaporated under vacuum, the residual mixture was dissolved in distilled water and the product was extracted with CH₂Cl₂ and then dried with anhydrous Na₂SO₄. After the evaporation of the solvent, the resultant mixture was purified by silica-gel column using a mixture Hexane: CH₂Cl₂ (1:1) as eluent. Selenoamides 3a and 3f are known and their spectroscopic data match well with literature.³

**N-(2-hydroxyethyl)-4-methoxybenzenecarboselenoamide (3b).** Yield: 97%; m.p. 68 - 73°C; ¹H NMR (300 MHz, CDCl₃, TMS): δ = 8.51 (s, 1 H; NH), 7.79 (d, J = 8.4 Hz, 2 H; C₆H₄), 6.86 (d, J = 8.4 Hz, 2 H; C₆H₄), 4.07 – 4.00 (m, 4 H; OCH₂, NCH₂), 3.83 (s, 3 H; OCH₃), 2.17 (s, 1 H; OH) ppm. ¹³C NMR (75 MHz, CDCl₃, TMS): δ = 203.0 (C=Se), 162.3 (C, C₆H₄), 137.0 (C, C₆H₄), 128.7 (2 CH, C₆H₄), 113.7 (2 CH, C₆H₄), 58.9 (OCH₂), 55.0 (OCH₃), 49.7 (NCH₂) ppm. IR (KBr) cm⁻¹: 3377 (NH), 1607 (C=Se); MS (EI, 70 eV) m/z (%): 259 (7) [M⁺], 177 (97) [M⁺-H₂Se], 134 (15) [ArCNH⁺]; HRMS (FAB⁺): m/z: calcd for C₁₀H₁₃NO₂Se: 259.0112 [M⁺]; found: 259.0100.

**N-(2-hydroxyethyl)benzo[d][1,3]dioxole-5-carboselenoamide (3c).** Yield: 90%; m.p. 254 - 256°C; ¹H NMR (300 MHz, CD₃CN, TMS): δ = 9.05 (s, 1 H; NH), 7.33 – 7.31 (m, 2 H; C₆H₃), 6.80 (d, 1 H; C₆H₃), 6.00 (s, 2 H; OCH₂O), 3.88-3.82 (m, 4 H; OCH₂, NCH₂), 3.05 (s, 1 H; OH) ppm. ¹³C NMR (75 MHz, CD₃CN, TMS): δ = 202.0 (C=Se), 149.8 (C, C₆H₃), 147.3 (C, C₆H₃), 138.6 (C, C₆H₃), 121.2 (CH, C₆H₃), 107.6 (CH, C₆H₃), 107.0 (CH, C₆H₃), 101.9 (CH₂, OCH₂O) 58.4 (OCH₂), 52.0 (NCH₂) ppm. IR (KBr) cm⁻¹: 3357 (NH), 1607 (C=Se). MS (EI, 70 eV) m/z (%): 273 (40) [M⁺], 191 (14) [M⁺-H₂Se], 148 (100) [ArCNH⁺]; HRMS (FAB⁺): m/z: calcd for C₁₀H₁₂NO₃Se: 273.9982 [M⁺+1]; found: 273.9977.

**N-(2-hydroxyethyl)-1-methyl-1H-pyrrole-2-carboselenoamide (3d).** Yield: 93%; m.p. 79 - 80°C; ¹H NMR (300 MHz, CDCl₃, TMS): δ = 7.86 (s, 1 H; NH), 6.78 (s, 1 H; C₆H₅N), 6.48 – 6.47 (m, 1 H; C₆H₅N), 6.08 – 6.06 (m, 1 H; C₆H₅N), 3.99 (s, 3 H; NCH₃), 3.93 – 3.90 (m, 4 H; NCH₂CH₂), 2.45 (s, 1 H; OH) ppm. ¹³C NMR (75 MHz, CDCl₃, TMS): δ = 190.4 (C=Se), 137.2 (C, C₆H₅N), 130.5 (CH, C₆H₅N), 110.2 (CH, C₆H₅N), 107.7 (CH, C₆H₅N), 60.3 (OCH₂), 50.2 (NCH₂), 37.3 (NCH₃) ppm. IR (KBr) cm⁻¹: 3351 (NH), 1652 (C=Se). MS (EI, 70 eV) m/z (%): 232 (8) [M⁺], 149 (100) [M⁺-H₂Se], 107 (24) [M⁺-ArCNH⁺]; HRMS (FAB⁺): m/z: calcd for C₈H₁₂N₂OSe: 232.0115 [M⁺]; found: 232.0122.

**N-(2-hydroxyethyl)-1-methyl-1H-indole-2-carboselenoamide (3e).** Yield: 72%; m.p.70 - 71°C; ¹H NMR (300 MHz, CDCl₃, TMS): δ = 8.63 (s, 1 H; NH), 7.63 (d, 1 H; C₆H₅N), 7.34 (s, 2 H; C₆H₅N), 7.14 (s, 1 H; C₆H₅N), 6.73 (s, 1 H; C₆H₅N), 4.02 – 4.01 (m, 7H; OCH₂, NCH₂, NCH₃) 1.65 ppm (s, 1 H; OH). ¹³C NMR (75 MHz, CDCl₃, TMS): δ = 192.5 (C=Se), 143.3 (C, C₆H₅N), 140.3 (C, C₆H₅N), 126.3, (C, C₆H₅N), 124.2 (CH, C₆H₅N), 121.9 (CH, C₆H₅N), 121.0
(CH, C₆H₅N), 110.4 (CH, C₆H₅N), 101.7 (CH, C₆H₅N), 60.4 (OCH₂), 50.6 (NCH₂), 32.4 ppm (NCH₃). IR (KBr): ν = 3421 (OH), 3150 (NH), 1644 cm⁻¹ (C=Se). MS (EI, 70 eV) m/z (%): 282 (100) [M⁺], 201 (29) [M⁺-H₂Se], 157 (79) [ArCNH⁺].

HRMS (FAB⁺) m/z: calcd. for C₁₂H₁₄N₂OSe: 282.0271 [M⁺]; found: 282.0285.

**N-(2-hydroxyethyl)thiophene-2-carboselenoamide (3f).** Yield: 75%; m.p. 118-120°C; ¹H NMR (300 MHz, CDCl₃, TMS): δ = 8.42 (s, 1 H; NH), 7.60 (d, J = 6.0 Hz, 1 H; C₄H₃S), 7.52 (d, J = 3.0 Hz, 1 H; C₄H₃S), 7.13 (d, J = 3.0 Hz, 1 H; C₄H₃S), 4.08 - 4.04 (m, 4 H; OCH₂, NCH₂), 1.87 ppm (s, 1 H; OH). ¹³C NMR (75 MHz, CDCl₃, TMS): δ = 192.2 (C=Se), 149.8 (C, C₄H₃S), 133.2 (CH, C₄H₄), 128.2 (CH, C₄H₃S), 124.4 (CH, C₄H₃S), 60.4 (OCH₂), 51.2 ppm (NCH₂). IR (KBr): ν = 3370 (NH), 1658 cm⁻¹ (C=Se). MS (EI, 70 eV) m/z (%): 235 (100) [M⁺], 154 (32) [M⁺-H₂Se], 110 (89) [ArCNH⁺]. HRMS (FAB⁺) m/z: calcd. for C₇H₉NOSSe: 234.9570 [M⁺]; found: 234.9582.

**N-(2-hydroxyethyl)furan-2-carboselenoamide (3g).** Yield: 97%; m.p. 74-76°C; ¹H NMR (300 MHz, CDCl₃, TMS): δ = 8.90 (s, 1 H; NH), 7.55 – 7.50 (m, 2 H; C₄H₃O), 6.47 (s, 1 H; C₄H₃O), 4.06 - 3.98 (m, 4 H; OCH₂, NCH₂), 2.51 ppm (s, 1 H; OH). ¹³C NMR (75 MHz, CDCl₃, TMS): δ = 185.1 (C=Se), 155.1 (C, C₄H₃O), 144.4 (CH, C₄H₃O), 120.8 (CH, C₄H₃O), 113.7 (CH, C₄H₃O), 60.4 (CH, OCH₂) 50.0 ppm (NCH₂). IR (KBr): ν = 3330 (NH), 1532 cm⁻¹ (C=Se). MS (EI, 70 eV) m/z (%): 219 (100) [M⁺], 138 (18) [M⁺-H₂Se], 94 (83) [ArCNH⁺]. HRMS (FAB⁺) m/z: calcd. for C₇H₉NO₂Se: 218.9799 [M⁺]; found: 218.9785.
7. - NMR and HR-MS spectra of 3b-g

Figure S18. $^1$H NMR spectrum of 3b

Figure S19. $^{13}$C NMR spectrum of 3b
Figure S20. HR-MS of compound 3b

| Observed m/z | Int%  | Estimated m/z | Error [ppm] | U.S. C | H | O | N | Se |
|--------------|-------|---------------|-------------|--------|---|---|---|----|
| 259.0100     | 70.1  | 259.0112      | -4.6        | 5      | 10| 13| 2 | 1  |
Figure S21. $^1$H NMR spectrum of 3c

Figure S22. $^{13}$C NMR spectrum of 3c
Figure S23. HR-MS of compound 3c

| Observed m/z | Int%  | Estimated m/z | Error [ppm] | U.S. C | H | O | N | Se |
|--------------|-------|---------------|-------------|--------|---|---|---|----|
| 273.9977     | 100.0 | 273.9982      | -2.1        | 6.5    | 10| 12| 3 | 1  | 1  |

[ Elemental Composition ]
Data: Dr-Jose-G-Lopes015 Date: 04-Mar-2013 17:48
Sample: 577 OOSeneEtOH
Note: Luis-Velasco
Inlet: Direct Ion Mode: FAB+
RT: 4.47 min Scan#: (11,13)
Elements: C 40/0, H 49/0, O 3/0, N 3/0, Se 1/0
Mass Tolerance: 100 ppm, 2 mmu if m/z > 2
Figure S24. $^1$H NMR spectrum of 3d

Figure S25. $^{13}$C NMR spectrum of 3d.
Figure S26. HR-MS of compound 3d

| Observed m/z | Int%  | Estimated m/z | Error [ppm] | U.S. C | H | O | N | Se |
|--------------|-------|---------------|-------------|-------|---|---|---|----|
| 232.0122     | 7.1   | 232.0115      | 3.0         | 4     | 8 | 12| 1 | 2  | 1  |
Figure S27. $^1$H NMR spectrum of 3e

Figure S28. $^{13}$C NMR spectrum of 3e
Figure S29. HR-MS of compound 3e

| Observed m/z | Int%   | Estimated m/z | Error [ppm] | U.S. C | H | O | N | Se |
|--------------|--------|---------------|-------------|--------|---|---|---|----|
| 282.0285     | 100.0  | 282.0271      | 4.9         | 7      | 12 | 14| 1 | 2  |
|              |        |               |             |        |    |   |   |    |

[ Elemental Composition ]

Data : Dr-Jose-G-Lopes016   Date :06-Nov-2012  19:24
Sample: 2772  Caro2
Note : Luis-Velasco
Inlet : Direct          Ion Mode :  FAB+
RT : 0.31 min            Scan# : (6,10)
Elements : C 40/0, H 49/0, O 3/0, N 3/0, Se 1/0
Mass Tolerance : 100ppm, 2mmu if m/z >2
Figure S30. $^1$H NMR spectrum of 3f

Figure S31. $^{13}$C NMR spectrum of 3f
Figure S32. HR-MS of compound 3f
Figure S33. $^1$H NMR spectrum of 3g

Figure S34. $^{13}$C NMR spectrum of 3g
Figure S35. HR-MS of compound 3g
8. X-Ray diffraction analyses

Suitable X-ray quality crystals of 3f were grown by slow evaporation of chloroform at room temperature. A crystal of 3f was mounted on a glass fiber at room temperature, then placed on a Bruker Smart Apex CCD diffractometer, equipped with Mo KR radiation; decay was negligible in both cases. Details of crystallographic data collected for compound 3f are provided in Table S2. Systematic absences and intensity statistics were used in space group determination. The structure was solved using direct methods. Anisotropic structure refinements were achieved using full matrix, least-squares technique on all non-hydrogen atoms. All hydrogen atoms were placed in idealized positions, based on hybridization, with isotropic thermal parameters fixed at 1.2 times the value of the attached atom. Structure solutions and refinements were performed using SHELXTL V6.10. CCDC-1015044 (3f) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.ac.uk/data_request/cif.

Table S2. Crystal data and structure refinement for 3f.

| Property                                      | 3f                  |
|-----------------------------------------------|---------------------|
| Empirical Formula                             | C7H9NOSSe           |
| Formula Weight (g mol⁻¹)                      | 234.17              |
| Crystal size (mm)                             | 0.28 x 0.22 x 0.07  |
| Color                                         | Orange              |
| Crystal system                                | Monoclinic          |
| Space Group                                   | P2₁/c               |
| a (Å)                                        | 8.595(10)           |
| b (Å)                                        | 13.301(15)          |
| c (Å)                                        | 8.139(9)            |
| α (°)                                        | 90                  |
| β (°)                                        | 107.864(4)          |
| γ (°)                                        | 90                  |
| V (Å³)                                       | 885.7(17)           |
| Z                                            | 4                   |
| Dcalc (g cm⁻³)                                | 1.756               |
| Number of collected reflections               | 5879                |
| Number of independent reflections (Rint)      | 2026, Rint = 0.0202 |
| Absorption correction method                  | Semi-empirical from equivalents |
| Maximum and minimum transmission              | 0.7456 and 0.5658   |
| Data/restraints/parameters                    | 2026/2/1065         |
| Final R indices [I>2σ(I)]                    | R = 0.0292, wR2 = 0.0703 |
| R indices (all data)                          | R = 0.0367, wR2 = 0.0741 |
| Goodness-of-fit on F²                         | 1.057               |
9. Biological studies.

Cells lines culture and culture medium.

The compounds 3(a-h) were screened in vitro against six tumor cell lines: HCT-15 (human colorectal adenocarcinoma), U251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), MCF-7 (human mammary adenocarcinoma), K562 (human chronic myelogenous leukemia) and SKLU-1 (human lung adenocarcinoma), and the healthy cell line MT2 (Human T-lymphocyte). These cell lines were supplied by National Cancer Institute (USA). The human tumor cytotoxicity was determined using the protein-binding dye Sulforhodamine B (SRB) in microculture assay to measure cell growth, as described in the protocols established by the NCI. The cell lines were cultured in RPMI-1640 medium (Sigma Chemical Co., Ltd., St. Louis, MO, U.S.A.) supplemented with 10% fetal bovine serum which was purchased from Invitrogen Corporation, 2 mM L-glutamine, 10,000 units/mL penicillin G sodium, 10,000 µg/mL streptomycin sulfate and 25 µg/mL amphotericin B (Gibco) and 1% non-essential amino acids (Gibco). They were maintained at 37°C, in a 5% CO₂ atmosphere with 95% humidity. The viability of the cells used in the experiments exceeds 95%, which was determined with trypan blue.

Cytotoxicity assays

The cells were removed from the tissue culture flasks by treatment with trypsin, and then diluted with fresh media. Of this cell suspension, 100 µL containing 5000-10,000 cell per well were pipetted into 96 well microtiter plates (Costar) and the material was incubated for 24 h, at 37 °C in a 5% CO₂ atmosphere. A 20 mM stock solution of each compound was prepared using ethanol as solvent and the solutions of lower concentrations (50 to 1 µM) were prepared by accurate dilution. To determine the inhibition of the growth (%) of human tumor cell lines for 3(a-h) at 50 µM in EtOH, 100 µL of an ethanolic solution 50 µM of each compound were added to each well (Table S2). The final percentage of ethanol in each well was 0.05%. The cultures were exposed for 48 h. After the incubation period, cells were fixed in situ by the addition of 50 mL of cold 50 % (w/v) trichloroacetic acid. The plates were incubated for 1 h, at 4 °C. The supernatant was discarded, and the plates were washed three times with water and air-dried. Cultures fixed with trichloroacetic-acid were stained for 30 min with 100 µL of 0.4% SRB solution.

Protein-bound dye was extracted with 10 mM unbuffered tris base and the optical densities were read on a Microplate Reader Synergy HT (Elx 808, BIOTEK Instruments, Inc., U.S.A.), with a test wavelength of 515 nm. Results were expressed as IC₅₀ values, they were calculated according to the protocol of Monks, were a dose-response curve was plotted for each compound, and the concentration giving 50% inhibition (IC₅₀) was estimated from non-linear regression equations.

A similar procedure was followed for obtaining the IC₅₀ of each compound, which was conducted using the three human cancer cell lines that exhibited the best results. For comparative purposes, the MT2 cell line described above was also used. The solutions of test compounds were prepared at concentrations ranging from 1.0 to 50 µM.
**Table S3.** Inhibition of the growth (%) of human tumor cell lines for 3(a-h) at 50 μM in EtOH

| Entry | Compound | HCT-15 | U251 | PC-3 | MCF-7 | K562 | SKLU-1 |
|-------|----------|--------|------|------|-------|------|--------|
| 1     | 3a       | 62.6   | 39.9 | 88.3 | 59.5  | 25.4 | 41.8   |
| 2     | 3b       | 68.7   | 87.48| 100  | 97.36 | 46.88| 54.3   |
| 3     | 3c       | 67.16  | 71.96| 84.14| 62.25 | 90.74| 35.66  |
| 4     | 3d       | 83.92  | 56.78| 74.84| 72.15 | 35.57| 21.73  |
| 5     | 3e       | >100   | 91.0 | >100 | >100  | 99.0 | 86.1   |
| 6     | 3f       | >100   | 97.3 | >100 | 78.9  | 62.15| 48.3   |
| 7     | 3g       | 62.65  | 60.49| 64.19| 51.91 | 44.62| 37.54  |
| 8     | 3h (Lead)| 98.51  | 98.27| 88.64| >100  | 62.68| 95.7   |

10. - References

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