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Synthesis of 1-benzyl-3-(5-hydroxymethyl-2-furyl)selenolo[3,2-*c*]pyrazole derivatives as new anticancer agents

Li-Chen Chou [a], Li-Jiau Huang [a], Mei-Hua Hsu [a], Mei-Chi Fang [a], Jai-Sing Yang [b], Shi-Hong Zhuang [a], Hui-Yi Lin [a], Fang-Yu Lee [c], Che-Ming Teng [d], Sheng-Chu Kuo [a,*]

[a] *Graduate Institute of Pharmaceutical Chemistry, China Medical University, 91 Shueh Shih Road, North Section, Taichung 40402, Taiwan*
[b] *Departments of Pharmacology, China Medical University, 91 Shueh Shih Road, North Section, Taichung 40402, Taiwan*
[c] *Yung-Shin Pharmaceutical Industry Co., Ltd. No. 1191, Sec. 1, Chung Shan Rd., Tachia, Taichung, Taiwan*
[d] *Pharmacological Institute, College of Medicine, National Taiwan University, No. 1, Jen-Ai Road, Sec. 1, Taipei, Taiwan*

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ABSTRACT

As part of our continuing search for potential anticancer drug candidates among YC-1 analogs, 1, 3-disubstituted selenolo[3,2-*c*]pyrazole derivatives were synthesized and evaluated for their cytotoxicity against NCI-H226 non-small cell lung cancer and A-498 renal cancer cell lines. Significant cytotoxicity was observed in 3-(5-hydroxymethyl-2-furyl) derivatives (**2**, **33**, **36** and **37**). Among them, compound **2** was found to have the most potent activity. The mode of action of compound **2** seems to differ from those of the 175 anticancer agents listed in NCI's standard database and resembles that of YC-1. Thus, we recommend that compound **2** should be developed further as new drug candidate for treatment of non-small cell lung cancer and renal cancer.

1. Introduction

1-Benzyl-3-(5-hydroxymethyl-2-furyl)indazole (YC-1)(Fig. 1) is an unigure activator of soluble quanyl cyclase (sGC) that not only activates sGC in NO-independent manner, but also potentiates the NO- and CO-dependent activation of sSC [1–3]. Through sGC/cGMP pathway, YC-1 has demonstrated its multiple pharmacological actions including antiplatelet, vascular relaxation, neuroprotection, and so on [4]. Besides its role in these cGMP related pharmacological actions, YC-1 has also being studied for its high potential as a new anticancer drug candidate. According to the literatures [5–9], the anticancer effect of YC-1 may be attributable to its multiple action including anti-angiogenesis, anti-inflammation, apoptosis-induction and inhibition of matrix metalloproteinases (MMPs). Results from animal studies revealed that the oral dosing of YC-1 exhibited excellent anti-tumor activity against non-small cell lung cancer, hepatoma, prostate cancer, renal and breast cancers.

During our recent study, we found that the furo[3,2-*c*]pyrazole isostere of YC-1 (**1**)(Fig. 1) exhibited greater cytotoxicity than YC-1 against HL-60 cells [10]. However, its action mechanism seemed to differ from that of YC-1. This intrigued us into investigating the anticancer activity of various YC-1 isosteres. Meanwhile, from the chemical structure point of view, the core structure of YC-1 is an indazole, and its furo[3,2-*c*]pyrazole isostere raised its cytotoxicity considerably by introducing an oxygen heteroatom into it. Therefore, we expected that the systematic introduction of other heteroatom from the same periodical group, namely the sulfur and the selenium atoms, which yield thieno[3,2-*c*]pyrazole and selenolo[3,2-*c*]pyrazole isosteres of YC-1, might also result in differ physiochemical properties and biological activities. Since, the synthesis and biological activities of both the furo[3,2-*c*]pyrazole [10] and thieno[3,2-*c*]pyrazole analogs [11] of YC-1 were already reported by us, this study focused on the selenolo[3,2-*c*]pyrazole analogs of YC-1 having new heterocyclic core structure. At first, 1-benzyl-3-(5-hydroxymethyl-2-furyl)selenolo[3,2-*c*]pyrazole (**2**)(Fig. 1), an isostere of YC-1, was prepared. And, during its evaluation for cytotoxicity against a panel of 60 NCI human cancer cell lines, compound **2** was found to demonstrate selective inhibition toward NCI-H226 non-small cell lung cancer and A-498 renal cancer

* Corresponding author. Tel.: +86 886 4 22053366x5608; fax: +86 886 4 22030760.
E-mail address: sckuo@mail.cmu.edu.tw (S.-C. Kuo).

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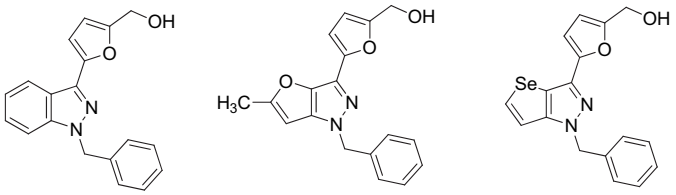


Fig. 1. Structures of YC-1, 1-benzyl-3-(5-hydroxymethyl-2-furyl)-5-methylfuro[3,2-*c*]pyrazole (**1**) and 1-benzyl-3-(5-hydroxymethyl-2-furyl)selenolo[3,2-*c*] pyrazole (**2**).

cell lines. Therefore, we synthesized a series of its analogs and evaluated for their cytotoxicty against NCI-H226 and A-498 cancer cell lines in this study.

2. Chemistry

The synthetic methods for target compounds **2** and **27**–**41** were illustrated in Scheme 1. For the preparation of intermediates **10** and **11**, the starting compounds **3** and **4** were prepared by published methods [12] and were then treated with $SOCl_2$ in CH_2Cl_2, to yield the corresponding acid chlorides (**5**, **6**) that were subjected to Friedel–Craft's reaction with 5-methyl furoate (**8**) to give the corresponding ketones (**10**, **11**). Since the method for structural confirmation of products **10** and **11** was similar, only that of the latter was described here in detail. First, the molecular formula of compound **11** was determined to be $C_{12}H_{10}O_4Se$ based on elemental analysis and the detection of a molecular ion peak in mass spectrum at *m*/*z* 298. Furthermore, its ^1H NMR spectrum displayed two signals at δ 3.92 and 2.60 which correlated with the group of OCH_3 and CH_3, respectively. Two mutually coupled signals at δ 7.22 ($J = 3.6$ Hz) and δ 7.30 ($J = 3.6$ Hz) were assigned to the H-3 and H-4 of the furan ring, and another signal at δ 7.06 ($J = 4.0$, 1.0 Hz) indicated the coupling between H-3 and C-5–CH_3, whereas the signal at δ 8.38 ($J = 4.0$ Hz) were attributed to the H-3 of the selenophene. Overall, the spectral and elemental analysis together confirmed the structure of compound **11**. Similary, compound **12** was prepared by Friedel–Crafts acylation of starting compound **7** with 4-methoxycarbonyl benzoyl choride (**9**).

The above synthesized intermediates **10**, **11** and **12** were then subjected to condensation reaction with phenylhydrazine (**13**) or benzylhydrazine (**14**), in toluene, in the presence of acetic acid, to afford the corresponding hydrazones (**15**–**20**). As expected from our previous experience [12], both *E*- and *Z*-form isomers were produced. However, they led to the same rarget compounds in subsequent reactions and were not isolated. The above prepared hydrazones (**15**–**20**) were then treated with $Pb(OAc)_4$, in CH_2Cl_2, to form intermediates **21**–**26** that were subsequently cyclized by treatment with $BF_3{\cdot}Et_2O$ to yield the corresponding selenolo[3,2-*c*]pyrazoles (**27**–**32**). The method for structural determination of these products was similar, and was exemplified by that of product **32**. First, its molecular formula was determined to be $C_{19}H_{16}N_2O_3Se$ based on elemental analysis and the detection of its molecular ion peak in mass spectrum (*m*/*z* 400, M^+). On its ^1H NMR spectrum, the signals at δ 2.56, 3.89 and 5.46 were correlated with the CH_3, OCH_3 and –CH_2– groups, respectively. And H-6 signal of the selenolo[3,2-*c*]pyrazole ring was located at δ 6.62 ($J = 1.2$ Hz), whereas the H-3 signal of the furan ring was seen at δ 7.76 ($J = 3.6$ Hz). Signals for the other 6 protons were located between δ 7.18 and 7.32. Based on the above spectral and elemental analysis, its structure was confirmed as the expected 1-benzyl-3-(5-methoxycarbonyl-2-furyl)-5-methylselenolo[3,2-*c*]pyrazole (**32**).

Subsequently, compounds **27**–**32** were reduced with $Ca(BH_4)_2$ into their corresponding carbinols (**33**, **2**, **34**–**37**)either, or hydrolyzed with NaOH into their corresponding carboxylic acid derivatives (**38**–**41**).

3. Results and discussion

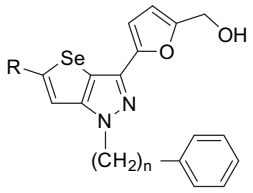
3.1. Cytotoxicity of compound 2 against human cancer cell line panel

Compound **2** was evaluated for cytotoxicity with a panel of NCI human cancer cell lines [13,14]. As shown by the data in Fig. 2, its mean graph midpoint (MID) values for log GI_{50}, log TGI, log LC_{50} were −4.68, −4.12 and −4.06, respectively, indicating its poor cytotoxicity toward human cancer cell lines. Nevertheless, compound **2** demonstrated selective inhibition against NCI-H226 and A-498 human cancer cell lines. As comparison, its log GI_{50}, log TGI and log LC_{50} values against NCI-H226 non-small cell lung cancer cells were −6.60, −6.01 and −5.19, respectively, indicating about 20–100 times more potent than the MID values. Similarly, its log GI_{50}, log TGI, log LC_{50} values against A-498 renal cancer cells were −6.79, −6.49 and −6.20 respectively, that were more than 100 times the values at the MID. We further utilized NCI's COMPARE program to compare the cancer-type-specific Fingerprint (log GI_{50}) of compound **2** with NCI's standard agent database which is consisted of 175 drugs with cancer treatment New Drug Application (NDA) or Investigational New Drug Application (IND) as well as compounds that had reached a particular high stage of interest at the NCI in recent years for the list. The result in Table 1, indicated that the correlation coefficient is lower than 0.5 suggesting that the mode of action of compound **2** might differ significantly from most of the 175 drugs in the NCI database. On the other hand, the fingerprint of compound **2** established in US NCI seemed to have great degree of similarity with the fingerprint of YC-1 established in the Japanese Foundation for Cancer Research (JFCR) [9]. Although the panel of cancer cell line used in JFCR was somewhat different.

3.2. Cytotoxicity of 1, 3-disubstituted selenolo[3,2-c]pyrazoles (2, 27–41)

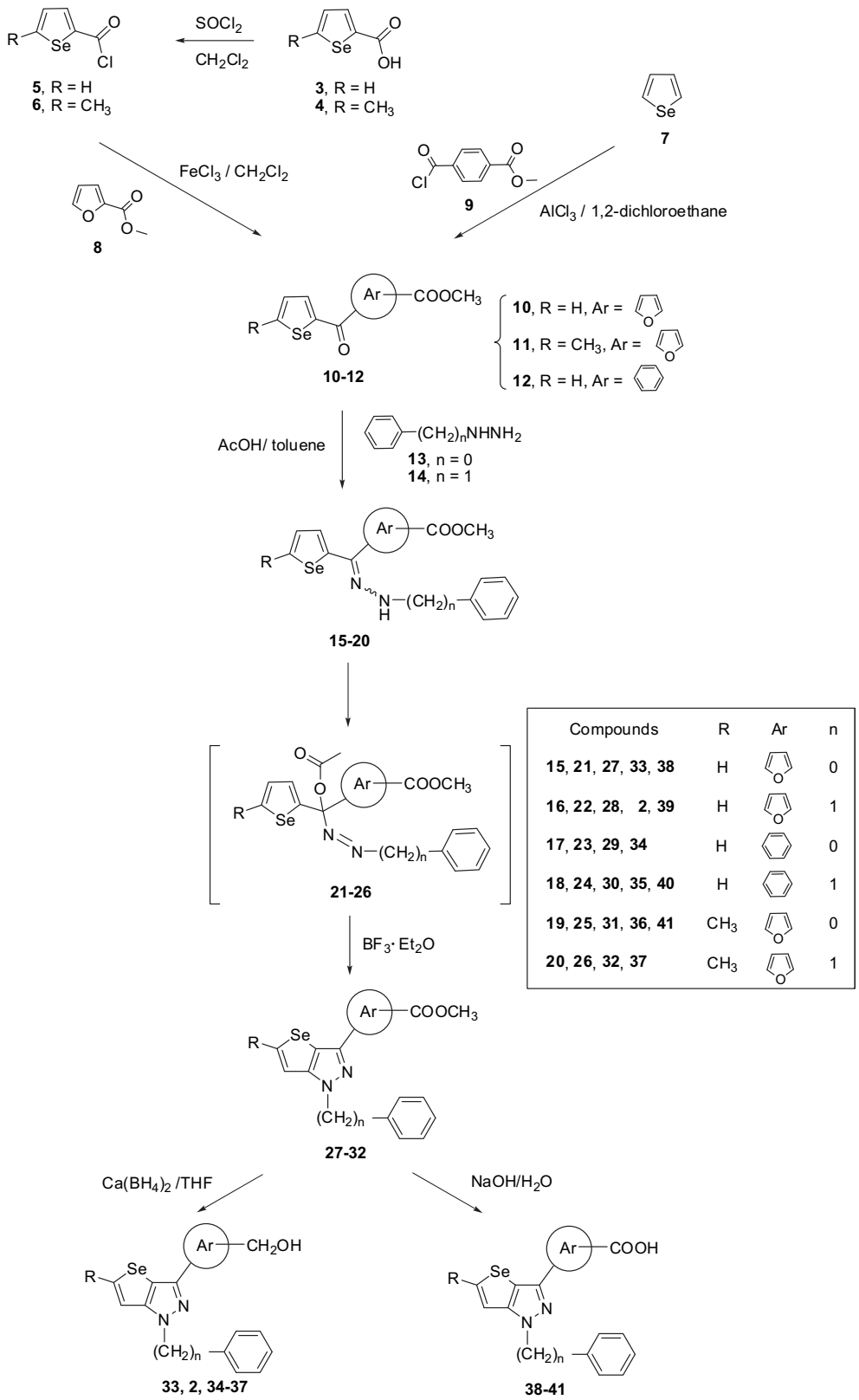
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of target compounds (**2**, **27**–**41**) against NCI-H226 and A-498 cell lines, and the result was summarized in Table 2.

As shown in Table 2, among the selenolo[3,2-*c*]pyrazoles (**2**, **27**–**41**) tested, only compounds **2**, **33**, **36** and **37** demonstrated significant cytotoxicity. These four compounds have the same common structure (**A**) shown in the following.



(A)

Among them, compounds **2** and **37** with –CH_2– link ($n = 1$) exhibited relatively greater cytotoxicity than those (**33**, **36**) without –CH_2– link ($n = 0$). Between two compounds with $n = 1$, the one with R═H showed greater cytotoxicity than the other with R═CH_3 (**37**). Among these four, the cytotoxicity of compound **2** is the greatest, which is comparable with the YC-1 control, and is much greater than that of its furo[3,2-*c*]pyrazole (**1**) [10] and thieno[3,2-*c*]pyrazole (**42**) [11] counterpart. Although the cytotoxicity of compounds **33**, **36** and **37** is relatively weaker than compound **2**,



Compounds	R	Ar	n
15, **21**, **27**, **33**, **38**	H		0
16, **22**, **28**, **2**, **39**	H		1
17, **23**, **29**, **34**	H		0
18, **24**, **30**, **35**, **40**	H		1
19, **25**, **31**, **36**, **41**	CH_3		0
20, **26**, **32**, **37**	CH_3		1

Scheme 1.

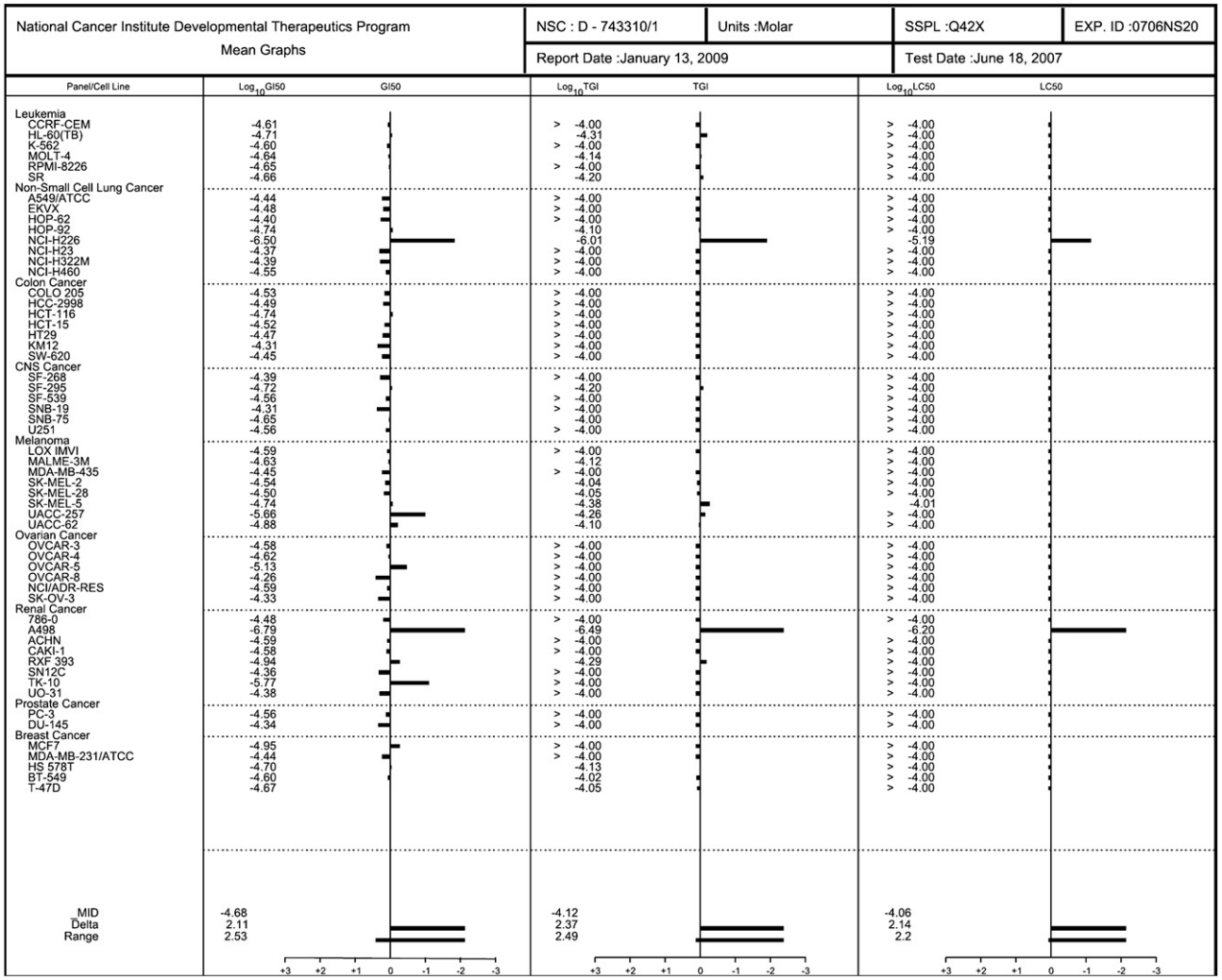


Fig. 2. Differential activity patterns for compound **2** against 60 human cancer cell lines. MG-MID: mean of log *X* values (X = GI_{50}, TGI, and LC_{50}). Delta: logarithm of the difference between the MG-MID and the log *X* of the most sensitive cell line. Range: logarithm of the difference between the log *X* of the most resistant cell line and the log *X* of the most sensitive cell line.

their exhibition of selective cytotoxicity toward A-498 cells deserves further investigation.

4. Conclusion

A series of 1, 3-disubstituted selenolo[3,2-*c*]pyrazoles (**2**, **27**–**41**) were synthesized and evaluated for cytotoxicity against NCI-H226 and A-498 cancer cell lines. Four compounds (**2**, **33**, **36** and **37**) that demonstrated significant cytotoxicity were found to have the similar 5-substituted 1-benzyl or 1-phenyl-3-(5-hydroxymethyl-2-furyl) selenolo[3,2-*c*]pyrazole structure (**A**). The mode of action of compound **2**, which demonstrated the greatest cytotoxicity, seems to differ from that of the 175 anticancer agents listed in NCI's standard database, and resembles that of YC-1. Details of its action mechanism is currently under investigation and will be published when available. Our preliminary data suggest that cyclophlin A is an important target of compound **2** cytotoxicity on A-498 cells. We thereby recommend that compound **2** be further developed as the YC-1-derived, new drug candidate for treatment of lung and renal cancers. On the other hand, the structure–activity relationship established in the present study will serve as guideline for future study.

Table 1
COMPARE correlation at GI_{50} level for compound **2**.

Rank	Compounds (NCI number)	r^a
1	O6-methylguanine (S37364)	0.354
2	Bruceantin (S165563)	0.296
3	Pyrrolizine dicarbamate (S278214)	0.254
4	Cytembena (S104801)	0.246
5	Hydroxyurea (S32065)	0.221
6	Dihydro-5-azacytidine (S264880)	0.218
7	Morpholino-ADR (S354646)	0.215

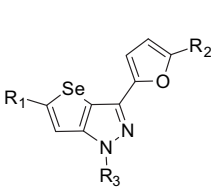
[a] *r*: correlation coefficient.

5. Experimental

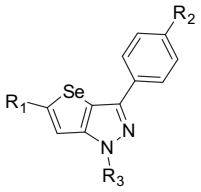
5.1. Chemistry

All of the solvents and reagents were obtained commercially and used without further purification. The progress of all reactions was monitored by TLC on 2 × 6 cm pre-coated silica gel 60 F_{254} plates of thickness 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm. The following adsorbent was

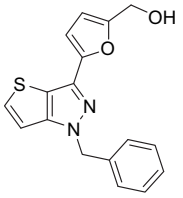
Table 2
Cytotoxicity of selenolo[3,2-*c*]pyrazoles.



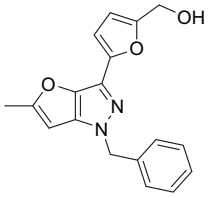
27, 28, 31, 32, 33, 2, 36, 37, 38, 39, 41



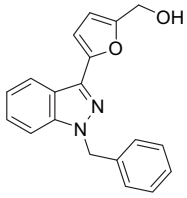
29, 30, 34, 35,40



42



1



YC-1

Comp'd	R_1	R_2	R_3	IC_{50}[a](μM)	
				NCI-H226[b]	A498[b]
27	H	$COOCH_3$	phenyl	>50	>50
28	H	$COOCH_3$	benzyl	>100	>100
29	H	$COOCH_3$	phenyl	>100	>100
30	H	$COOCH_3$	benzyl	>100	>100
31	CH_3	$COOCH_3$	phenyl	>100	>100
32	CH_3	$COOCH_3$	benzyl	>100	>100
33	H	CH_2OH	phenyl	29.5	2.2
2	H	CH_2OH	benzyl	1.4	0.4
34	H	CH_2OH	phenyl	>100	>100
35	H	CH_2OH	benzyl	>100	>100
36	CH_3	CH_2OH	phenyl	25	1.9
37	CH_3	CH_2OH	benzyl	8.7	0.9
38	H	COOH	phenyl	>100	>100
39	H	COOH	benzyl	>100	>100
40	H	COOH	benzyl	>100	>100
41	CH_3	COOH	phenyl	>100	>100
42 [11]				8.0	0.6
1 [10]				2.0	0.4
YC-1 [12]				1.9	0.3

Human tumor cells were treated with different concentrations of samples for 48 h.
[a] Data was presented as IC_{50} (μM, the concentration of 50% proliferation-inhibitory effect).
[b] Human lung cancer cell (NCI-H226), human renal cancer cell (A-498).

used for column chromatography: silica gel 60 (Merck, particle size 0.063–0.200 mm). Melting points were determined with a Yanaco MP-500D melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu IR-Prestige-21 spectrophotometers as KBr pellets. NMR spectra were obtained on a Bruker Avance DPX-200 FT-NMR spectrometer in $CDCl_3$ or DMSO. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; and m, multiplet. MS spectra were measured with an HP 5995 GC-MS instrument. Elemental analyses (C, H, and N) were carried out at the instruments center of National Chung Hsing University, Taichung, Taiwan and performed on a Perkin–Elmer 2400 Series II CHNS/O analyzer or Elementar vario EL III Heraeus CHNOS Rapid F002 and the results were within ±0.4% of the calculated values.

*5.1.1. General procedure for the synthesis of compounds **10**–**12***

*5.1.1.1. 5-Methoxycarbonyl-2-furyl 2-selenophenyl ketone (**10**).* A mixture of selenophene-2-carboxylic acid (**3**) (7.0 g, 0.04 mol) in 50 mL of dry CH_2Cl_2 and $SOCl_2$ (9.52 g, 0.08 mol) was heated under refluxing for 12 h. After evaporation, the crude product selenophene-2-carbonyl chloride (**5**) was given. Into the crude product **5** was added dry CH_2Cl_2 (50 mL), then methyl furan-2-carboxylate (**8**) (5.04 g, 0.04 mol) and anhydrous ferric chloride (6.50 g, 0.04 mol) were added. The reaction mixture was then heated under refluxing for 4 h, cooled and quenched with ice water. The CH_2Cl_2 layer was sequentially washed with water, 5% $NaHCO_3$ solution, and water, till neutral, then dried over $MgSO_4$ and filtered. The solvent of the filtrate was evaporated under reduced pressure, the residue was recrystallized from *n*-hexane to afford compound **10** (7.0 g, 0.025 mol). Yield: 62%; yellow crystals; mp 116–118 °C; MS (EI, 70 eV): *m*/*z* 284 (M^+); IR (KBr): 1618, 1717 (C═O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.84 (s, 3H), 7.43 (d, 1H, $J = 3.8$ Hz), 7.52–7.57 (m, 2H), 8.41 (d, 1H, $J = 3.8$ Hz); 8.76 (d, 1H, $J = 5.5$ Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.92, 119.58, 119.91, 132.12, 137.73, 143.86, 146.45, 147.74, 152.69, 158.40, 174.33; Anal. Calcd for $C_{11}H_8O_4Se$: C, 46.66; H, 2.85, Found: C, 46.76; H, 2.81.

*5.1.1.2. 5-Methoxycarbonyl-2-furyl 5-methyl-2-selenophenyl ketone (**11**).* 5-Methylselenophene-2-carboxylic acid (**4**) (7.6 g, 0.04 mol) and methyl furan-2-carboxylate (**8**) (5.04 g, 0.04 mol) were allowed

to react as in the preparation of compound **10** to afford **11** (5.94 g, 0.02 mol). Yield 50%; yellow crystals; mp 101–104 °C; MS (EI, 70 eV): *m/z* 298 (M^+); IR (KBr): 1600, 1736 (C═O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.60 (s, 3H), 3.92 (s, 3H), 7.06 (dd, 1H, *J* = 4.0, 1.0 Hz); 7.22 (d, 1H, *J* = 3.6 Hz), 7.30 (d, 1H, *J* = 3.6 Hz); 8.38 (d, 1H, *J* = 4.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.92, 52.41, 118.18, 118.69, 130.40, 138.33, 145.47, 146.01, 153.88, 158.61, 159.41, 173.87; Anal. Calcd for $C_{12}H_{10}O_4Se$: C, 48.50; H, 3.39, Found: C, 48.45; H, 3.42.

*5.1.1.3. p-Methoxycarbonylphenyl 2-selenophenyl ketone (**12**).* Anhydrous $AlCl_3$ (10.19 g, 0.076 mol) and, 4-methoxycarbonyl benzoyl chloride (**9**) (8.34 g, 0.042 mol) were dissolved in 1,2-dichloroethane (100 mL), then selenophene (**7**) (5.00 g, 0.038 mol) was added dropwise. The reaction mixture was then heated under refluxing for 30 min, cooled, and quenched with ice water. The organic layer was sequentially washed with water, 5% $NaHCO_3$ solution, and water, till neutral, then dried over $MgSO_4$ and filtered. The solvent of the filtrate was evaporated under reduced pressure, and the residue was recrystallized from *n*-hexane to afford compound **12** (4.78 g, 0.016 mol). Yield 43%; yellow crystals; mp 124–127 °C; MS (EI, 70 eV): *m/z* 294 (M^+); IR (KBr): 1618, 1715 (C═O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 3.94 (s, 3H), 7.41 (dd, 1H, *J* = 4.0, 5.4 Hz), 7.79 (d, 1H, *J* = 4.0 Hz); 7.85 (d, 2H, *J* = 8.6 Hz), 8.13 (d, 2H, *J* = 8.6 Hz); 8.47 (d, 1H, *J* = 5.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 52.46, 128.95, 129.63, 130.89, 133.06, 137.77, 141.23, 141.51, 150.01, 166.29, 188.77; Anal. Calcd for $C_{13}H_{10}O_3Se$: C, 53.26; H, 3.44. Found: C, 53.46; H, 3.41.

*5.1.2. General procedure for the synthesis of compounds **27–32***

*5.1.2.1. 1-Phenyl-3-(5-methoxycarbonyl-2-furyl)selenolo[3,2-c]pyrazole (**27**).* Into the solution of compound **10** (5.66 g, 0.02 mol) in toluene (100 mL) was added phenylhydrazine (**13**) (4.36 g, 0.04 mol) and acetic acid (1.5 mL). The mixture was heated under refluxing for 3 h. After cooling, the solvent was evaporated and the residue was purified by column chromatography (silica gel, toluene) to give 5-methoxycarbonyl-2-furyl 2-selenophenyl phenylhydrazone (**15**). The crude product **15** was dissolved in CH_2Cl_2 (50 mL), then $Pb(OAc)_4$ (26.60 g, 0.06 mol), followed with boron trifluoride diethyl etherate (98% in ether, 100 mL). After shaking for 10 min, the reaction mixture was poured into ice water (200 mL) and allowed to stand until two layers formed. The organic layer was washed with water, then 10% NaOH solution till neutral, then dried over $MgSO_4$ and filtered. The solvent of the filtrate was evaporated and the residue was purified by column chromatography (silica gel, CH_2Cl_2) to afford compound **27** (2.74 g, 7.4 mmol). Yield 37%; white solid; mp 145–146 °C; MS (EI, 70 eV): *m/z* 372 (M^+); IR (KBr): 1724 (C═O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.82 (s, 3H), 7.04 (d, 1H, *J* = 3.4 Hz), 7.21–7.58 (m, 5H), 7.75–8.85 (m, 2H), 8.38 (d, 1H, *J* = 5.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.42, 109.36, 113.99, 117.59, 120.81, 128.94, 129.54, 131.05, 131.40, 137.11, 137.94, 139.51, 143.71, 150.98, 152.05, 158.64; Anal. Calcd for $C_{17}H_{12}N_2O_3Se$: C, 55.00; H, 3.26; N, 7.55. Found: C, 55.04; H, 3.23; N, 7.59.

*5.1.2.2. 1-Benzyl-3-(5-methoxycarbonyl-2-furyl)selenolo[3,2-c]pyrazole (**28**).* Compound **10** (5.66 g, 0.02 mol), benzylhydrazine (**14**) (4.88 g, 0.04 mol) and acetic acid (1.5 mL) were allowed to react as in the preparation of compound **27** to afford **28** (1.54 g, 4.0 mmol). Yield: 20.1%; white solid; mp 130–131 °C; MS (EI, 70 eV): *m/z* 386 (M^+); IR (KBr): 1724 (C═O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.80 (s, 3H), 5.59 (s, 2H), 6.88 (d, 1H, *J* = 3.8 Hz), 7.23–7.34 (m, 5H), 7.38 (d, 1H, *J* = 3.8 Hz), 7.53 (d, 1H, *J* = 5.7 Hz), 8.19 (d, 1H, *J* = 5.7 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.33, 54.73, 108.32, 113.54, 116.64, 120.85, 128.07, 128.31, 129.14, 136.02, 137.26, 137.90, 143.20, 151.60, 151.69, 158.68; Anal. Calcd for $C_{18}H_{14}N_2O_3Se$: C, 56.11; H, 3.66; N, 7.27. Found: C, 56.18; H, 3.62; N, 7.29.

*5.1.2.3. 1-Phenyl-3-(p-methoxycarbonylphenyl)selenolo[3,2-c]pyrazole (**29**).* Compound **12** (5.86 g, 0.02 mol), phenylhydrazine (**13**) (4.36 g, 0.04 mol) and acetic acid (1.5 mL) were allowed to react as in the preparation of compound **27** to afford **29** (1.93 g, 5.06 mmol). Yield: 25.3%; white solid; mp 165–166 °C; MS (EI, 70 eV): *m/z* 382 (M^+); IR (KBr): 1713 (C═O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.85 (d, 3H), 7.37 (t, 1H, *J* = 7.2 Hz), 7.56 (t, 2H, *J* = 8.2 Hz), 7.81–7.89 (m, 3H), 7.95–8.11 (m, 4H), 8.40 (d, 1H, *J* = 5.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.67, 114.64, 119.47, 120.84, 125.99, 127.27, 129.45, 130.23, 130.57, 136.57, 139.25, 140.13, 144.47, 149.93, 166.36; Anal. Calcd for $C_{19}H_{14}N_2O_2Se$: C, 59.85; H, 3.70; N, 7.35. Found: C, 59.72; H, 3.64; N, 7.42.

*5.1.2.4. 1-Benzyl-3-(p-methoxycarbonylphenyl)selenolo[3,2-c]pyrazole (**30**).* Compound **12** (5.86 g, 0.02 mol), benzylhydrazine (**14**) (4.88 g, 0.04 mol) and acetic acid (1.5 mL) were allowed to react as in the preparation of compound **27** to afford **30** (2.49 g, 6.3 mmol). Yield: 31.4%; white solid; mp 150–153 °C; MS (EI, 70 eV): *m/z* 396 (M^+); IR (KBr): 1717 (C═O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.91 (s, 3H), 5.53 (s, 2H), 7.00 (d, 1H, *J* = 5.8 Hz), 7.21–7.38 (m, 5H), 7.85–7.95 (m, 3H), 8.10 (d, 2H, *J* = 8.6 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.12, 55.48, 112.51, 117.20, 125.41, 127.52, 128.13, 128.86, 130.16, 134.83, 136.12, 137.11, 143.55, 150.91, 166.95; Anal. Calcd for $C_{20}H_{16}N_2O_2Se$: C, 60.77; H, 4.08; N, 7.09. Found: C, 60.79; H, 4.06; N, 7.11.

*5.1.2.5. 1-Phenyl-3-(5-methoxycarbonyl-2-furyl)-5-methylselenolo[3,2-c]pyrazole (**31**).* Compound **11** (5.94 g, 0.02 mol), phenylhydrazine (**13**) (4.36 g, 0.04 mol) and acetic acid (1.5 mL) were allowed to react as in the preparation of compound **27** to afford **31** (2.86 g, 7.4 mmol). Yield: 37.2%; white solid; mp 156–158 °C; MS (EI, 70 eV): *m/z* 386 (M^+); IR (KBr): 1732 (C═O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.64 (s, 3H), 3.82 (s, 3H), 7.00 (d, 1H, *J* = 3.8 Hz), 7.36–7.45 (m, 2H), 7.50–7.58 (m, 3H), 7.79 (d, 2H, *J* = 8.2 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 19.79, 52.36, 109.17, 112.72, 117.30, 120.77, 127.26, 130.12, 137.50, 139.95, 143.62, 148.19, 151.05, 154.29, 158.61; Anal. Calcd for $C_{18}H_{14}N_2O_3Se$: C, 56.11; H, 3.66; N, 7.27. Found: C, 56.15; H, 3.86; N, 7.32.

*5.1.2.6. 1-Benzyl-3-(5-methoxycarbonyl-2-furyl)-5-methylselenolo[3,2-c]pyrazole (**32**).* Compound **11** (5.94 g, 0.02 mol), benzylhydrazine (**14**) (4.88 g, 0.04 mol) and acetic acid (1.5 mL) were allowed to react as in the preparation of compound **27** to afford **32** (1.56 g, 3.9 mmol). Yield: 19.5%; white solid; mp 142–145 °C; MS (EI, 70 eV): *m/z* 400 (M^+); IR (KBr): 1730 (C═O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.56 (s, 3H), 3.89 (s, 3H), 5.46 (s, 2H), 6.62 (d, 1H, *J* = 1.2 Hz), 7.76 (d, 1H, *J* = 3.6 Hz), 7.18–7.32 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 19.68, 51.84, 55.34, 107.46, 110.09, 115.72, 120.02, 127.43, 128.10, 128.84, 136.01, 136.46, 143.45, 149.57, 151.75, 152.52, 159.12; Anal. Calcd for $C_{19}H_{16}N_2O_3Se$: C, 57.15; H, 4.04; N, 7.02. Found: C, 57.18; H, 4.07; N, 7.12.

*5.1.3. General procedure for the synthesis of compounds **33**, **2**, **34–37***

*5.1.3.1. 1-Phenyl-3-(5-hydroxymethyl-2-furyl)selenolo[3,2-c]pyrazole (**33**).* Compound **27** (744 mg, 2.0 mmol) was dissolved in a homogenous solution of THF (50 mL) dispersed with $Ca(BH_4)_2$ (1.26 g, 0.018 mol). The mixture was heated under refluxing for 6 h and then filtered. The solvent was evaporated and the residue was recrystallized from *n*-hexane and then purified by column chromatography (silica gel, *n*-hexane: ethyl acetate = 1:1) to afford compound **33** (515 mg, 1.5 mmol). Yield 75.1%; white solid; mp 54–55 °C; MS (EI, 70 eV): *m/z* 344 (M^+); IR (KBr): 3200–3500 (OH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 4.46 (d, 2H, *J* = 5.2 Hz), 5.35 (t, 1H, *J* = 5.2 Hz), 6.46 (d, 1H, *J* = 3.2 Hz), 6.79 (d, 1H, *J* = 3.2 Hz), 7.33–7.37 (m, 1H), 7.53 (t, 2H, *J* = 8.2 Hz), 7.77–7.83 (m, 3H), 8.36 (d,

1H, J = 5.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 56.18, 108.48, 109.72, 114.60, 118.63, 120.46, 126.88, 130.18, 139.10, 140.18, 146.62, 149.11, 151.99, 156.45; Anal. Calcd for $C_{16}H_{12}N_2O_2Se$: C, 59.89; H, 3.79; N, 7.91. Found: C, 59.87; H, 3.81; N, 7.88.

*5.1.3.2. 1-Benzyl-3-(5-hydroxymethyl-2-furyl)selenolo[3,2-c]pyrazole (**2**).* Following the same synthetic procedure as for compound **33**, compound **2** (530 mg, 1.48 mmol) was prepared by reacting compound **28** (772 mg, 2.0 mmol). Yield: 74.2%; white solid; mp 126–129 °C; MS (EI, 70 eV): m/z 358 (M^+); IR (KBr): 3200–3400 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.41 (d, 2H, J = 5.6 Hz), 5.29 (t, 1H, J = 5.6 Hz), 5.53 (s, 2H), 6.38 (d, 1H, J = 3.0 Hz), 6.59 (d, 1H, J = 3.0 Hz), 7.25–7.33 (m, 5H), 7.48 (d, 1H, J = 5.8 Hz), 8.11 (d, 1H, J = 5.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 54.46, 56.11, 107.11, 109.49, 113.53, 115.37, 127.99, 128.19, 129.09, 137.22, 137.62, 147.28, 151.24, 155.68; Anal. Calcd for $C_{17}H_{14}N_2O_2Se$: C, 57.15; H, 3.93; N, 7.80. Found: C, 56.25; H, 3.90; N, 7.86.

*5.1.3.3. 1-Phenyl-3-(p-hydroxymethylphenyl)selenolo[3,2-c]pyrazole (**34**).* Following the same synthetic procedure as for compound **33**, compound **34** (585 mg, 1.66 mmol) was prepared by reacting compound **29** (764 mg, 2.0 mmol). Yield: 82.9%; white solid; mp 133–134 °C; MS (EI, 70 eV): m/z 354 (M^+); IR (KBr): 3100–3500 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.53 (d, 2H, J = 5.8 Hz), 5.28 (t, 1H, J = 5.8 Hz), 7.34 (t, 1H, J = 7.0 Hz), 7.45 (d, 2H, J = 8.0 Hz), 7.55 (t, 2H, J = 8.0 Hz), 7.79–7.86 (m, 5H), 8.37 (d, 1H, J = 5.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 63.13, 114.70, 118.89, 120.53, 125.70, 126.84, 127.61, 130.17, 130.65, 138.72, 140.31, 143.34, 145.59, 149.55; Anal. Calcd for $C_{18}H_{14}N_2OSe$: C, 61.20; H, 3.99; N, 7.93. Found: C, 61.18; H, 3.96; N, 7.88.

*5.1.3.4. 1-Benzyl-3-(p-hydroxymethylphenyl)selenolo[3,2-c]pyrazole (**35**).* Following the same synthetic procedure as for compound **33**, compound **35** (564 mg, 1.54 mmol) was prepared by reacting compound **30** (792 mg, 2.0 mmol). Yield: 76.8%; white solid; mp 158–160 °C; MS (EI, 70 eV): m/z 368 (M^+); IR (KBr): 3100–3500 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.49 (d, 2H, J = 5.2 Hz), 5.21 (t, 1H, J = 5.2 Hz), 5.56 (s, 2H), 7.20–7.33 (m, 5H), 7.39 (d, 2H, J = 8.0 Hz), 7.51 (d, 1H, J = 5.8 Hz), 7.68 (d, 2H, J = 8.0 Hz), 8.16 (d, 1H, J = 5.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 54.49, 63.12, 113.68, 115.57, 125.24, 127.49, 128.05, 128.16, 129.08, 131.27, 136.85, 137.70, 142.57, 143.91, 151.63; Anal. Calcd for $C_{19}H_{16}N_2OSe$: C, 62.13; H, 4.39; N, 7.63. Found: C, 62.13; H, 4.27; N, 7.66.

*5.1.3.5. 1-Phenyl-3-(5-hydroxymethyl-2-furyl)-5-methylselenolo[3,2-c]pyrazole (**36**).* Following the same synthetic procedure as for compound **33**, compound **36** (613 mg, 1.7 mmol) was prepared by reacting compound **31** (772 mg, 2.0 mmol). Yield: 85.9%; white solid; mp 67–68 °C; MS (EI, 70 eV): m/z 358 (M^+); IR (KBr): 3100–3500 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.62 (s, 3H), 4.45 (d, 2H, J = 5.8 Hz), 5.33 (t, 1H, J = 5.8 Hz), 6.44 (d, 1H, J = 3.2 Hz), 6.73 (d, 1H, J = 3.2 Hz), 7.28–7.36 (m, 1H), 7.47–7.56 (m, 3H), 7.76 (d, 2H, J = 8.4 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 19.87, 56.16, 108.40, 109.66, 112.92, 116.41, 120.43, 126.81, 130.13, 138.77, 140.18, 146.62, 147.83, 153.80, 156.36; Anal. Calcd for $C_{17}H_{14}N_2O_2Se$: C, 60.22; H, 3.93; N, 7.80. Found: C, 60.18; H, 3.93; N, 7.78.

*5.1.3.6. 1-Benzyl-3-(5-hydroxymethyl-2-furyl)-5-methylselenolo[3,2-c]pyrazole (**37**).* Following the same synthetic procedure as for compound **33**, compound **37** (612 mg, 1.65 mmol) was prepared by reacting compound **32** (800 mg, 2.0 mmol). Yield: 82.5%; white solid; mp 146–148 °C; MS (EI, 70 eV): m/z 372 (M^+); IR (KBr): 3200–3400 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.54 (s, 3H), 4.39 (d, 2H, J = 3.6 Hz), 5.27 (t, 1H, J = 3.6 Hz), 5.46 (s, 2H), 6.36 (d, 1H, J = 3.0 Hz), 6.54 (d, 1H, J = 3.0 Hz), 7.19–7.33 (m, 6H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 19.78, 54.36, 56.09, 107.05, 109.45, 111.56, 113.32, 127.90, 128.16, 129.09, 137.14, 137.66, 147.28, 149.90, 151.90, 155.59; Anal. Calcd for $C_{18}H_{16}N_2O_2Se$: C, 58.23; H, 4.34; N, 7.54. Found: C, 58.18; H, 4.45; N, 7.53.

*5.1.4. General procedure for the synthesis of compounds **38**–**41***

*5.1.4.1. 1-Phenyl-3-(5-hydroxycarbonyl-2-furyl)selenolo[3,2-c]pyrazole (**38**).* Compound **27** (744 mg, 2.0 mmol) in 20 mL of 10% NaOH solution was heated under refluxing for 2 h, cooled and acidified with dilute HCl. The precipitates were collected, then recrystallized from ethanol to afford compound **38** (557 mg, 1.56 mmol). Yield: 78.0%; white solid; mp 236–237 °C; MS (EI, 70 eV): m/z 358 (M^+); IR (KBr): 1697 (C═O), 2400–3300 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.01 (d, 1H, J = 4.0 Hz), 7.32–7.40 (m, 2H), 7.54 (t, 2H, J = 7.8 Hz), 7.76–7.83 (m, 3H), 8.37 (d, 2H, J = 5.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 109.20, 114.52, 119.39, 120.21, 120.84, 127.35, 130.21, 137.87, 139.60, 139.98, 144.96, 149.50, 150.59, 159.61; Anal. Calcd for $C_{16}H_{10}N_2O_3Se$: C, 53.80; H, 2.82; N, 7.84. Found: C, 53.82; H, 2.85; N, 7.81.

*5.1.4.2. 1-Benzyl-3-(5-hydroxycarbonyl-2-furyl)selenolo[3,2-c]pyrazole (**39**).* Following the same synthetic procedure as for compound **38**, compound **39** (595 mg, 1.60 mmol) was prepared by reacting compound **28** (772 mg, 2.0 mmol). Yield: 80.2%; white solid; mp 243–245 °C; MS (EI, 70 eV): m/z 372 (M^+); IR (KBr): 1697 (C═O), 2500–3400 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 5.58 (s, 2H), 6.82 (d, 1H, J = 3.6 Hz), 7.18–7.35 (m, 6H), 7.52 (d, 1H, J = 5.6 Hz), 8.18 (d, 1H, J = 5.6 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 54.69, 108.03, 113.52, 116.44, 119.55, 128.07, 128.29, 129.13, 136.37, 137.33, 137.75, 145.30, 150.86, 151.51, 160.03; Anal. Calcd for $C_{17}H_{12}N_2O_3Se$: C, 55.00; H, 3.26; N, 7.55. Found: C, 54.96; H, 3.24; N, 7.57.

*5.1.4.3. 1-Benzyl-3-(p-hydroxycarbonylphenyl)selenolo[3,2-c]pyrazole (**40**).* Following the same synthetic procedure as for compound **38**, compound **40** (649 mg, 1.70 mmol) was prepared by reacting compound **30** (792 mg, 2.0 mmol). Yield: 85.2%; white solid; mp 288–290 °C; MS (EI, 70 eV): m/z 382 (M^+); IR (KBr): 1684 (C═O), 2400–3200 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 5.60 (s, 2H), 7.20–7.38 (m, 5H), 7.55 (d, 1H, J = 5.8 Hz), 7.82 (d, 2H, J = 8.2 Hz), 8.01 (d, 2H, J = 8.2 Hz), 8.20 (d, 1H, J = 5.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 54.66, 113.65, 116.42, 125.36, 128.08, 128.26, 129.13, 130.21, 130.64, 136.72, 137.38, 143.01, 151.91, 167.53; Anal. Calcd for $C_{19}H_{14}N_2O_2Se$: C, 59.85; H, 3.70; N, 7.35. Found: C, 59.81; H, 3.68; N, 7.36.

*5.1.4.4. 1-Phenyl-3-(5-hydroxycarbonyl-2-furyl)-5-methylselenolo[3,2-c]pyrazole (**41**).* Following the same synthetic procedure as for compound **38**, compound **41** (647 mg, 1.74 mmol) was prepared by reacting compound **31** (772 mg, 2.0 mmol). Yield: 87.2%; white solid; mp 238–239 °C; MS (EI, 70 eV): m/z 372 (M^+); IR (KBr): 1663 (C═O), 2200–3300 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.63 (s, 3H), 6.96 (d, 1H, J = 3.6 Hz), 7.31–7.42 (m, 2H), 7.45–7.57 (m, 3H), 7.77 (d, 2H, J = 8.0 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 19.85, 109.14, 112.78, 117.20, 120.21, 120.80, 127.32, 130.19, 137.75, 139.96, 144.88, 148.22, 150.57, 154.40, 159.62; Anal. Calcd for $C_{17}H_{12}N_2O_3Se$: C, 55.00; H, 3.26; N, 7.55. Found: C, 55.03; H, 3.24; N, 7.51.

5.2. Biological evaluation

5.2.1. Cell culture and treatment

The human cancer cell line NCI-H226 and A498 were purchased from the ATCC (Manassas, VA). In this study, the NCI-H226 and A498 cells were cultured in RPMI-1640 and DMEM medium (GIBCO) respectively, supplemented with 10% FBS, penicillin (100 unit/mL)/streptomycin (100 μg/mL) and 1% L- glutamine. All cells were grown in a humidified atmosphere containing 5% CO_2 at 37 °C.

5.2.2. Cytotoxicity assay

The cytotoxicity was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [15]. NCI-H226 and A498 cells were seeded at 1×10^4 cells/well into 96-well plates. After 24 h incubation to allow for cell attachment, cells were treated with test compounds for 48 h. After treatment, cells were washed once with PBS and incubated with 1 mg/ml MTT (Sigma, St. Louis, MO, USA) for 2 h. Then the formazan precipitate was dissolved in 150 μL DMSO and the absorbance was measured on an ELISA reader at a best wavelength of 570 nm.

5.2.3. Statistic evaluation

Values are expression as the mean ± S.D. of three independent experiments. Student's *t* tests were used to assess the statistical significance of the differences, with "*P*" values of less than 0.05 being considered statistically significant.

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