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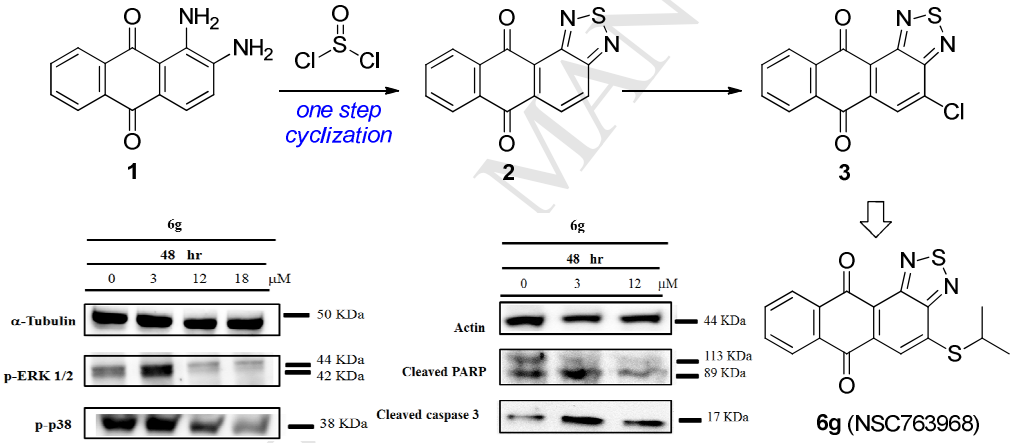

Abstract:

A series of sulfur-substituted anthra[1,2-*c*][1,2,5]thiadiazole-6,11-diones were synthesized and evaluated for cell proliferations, apoptosis, signaling pathways, and NCI-60 cell panel assay, respectively. Compounds **2**, **3**, **4a**, **4d**, **4f**, **4i**, **4k**, **5b**, **5c**, **5d**, **5f**, **5g**, **6b**, **6c**, **6d**, **6e**, **6g**, **7a** and **7g** were selected by NCI and **4d**, **4f**, **5f**, **6g** and **7a** represent the GI_{50}, TGI and LC_{50}, respectively. Among them, **6g** appeared to be the most active compound of this series that not only induced apoptosis in DU-145 cancer cells but also attenuated the ERK1/2 and p-38 signaling pathways. All test compounds exhibited diverse cytostatic and cytotoxic activities for further developing potential anticancer agents.

Keywords: Thiadiazoles, anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione, SRB assay, NCI 60-cell panel assay, apoptosis.

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Ring fusion strategy for synthesis and lead optimization of sulfur-substituted anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione derivatives as promising scaffold of antitumor agents

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Abstract:

A series of sulfur-substituted anthra[1,2-*c*][1,2,5]thiadiazole-6,11-diones were synthesized and evaluated using the cell proliferations, apoptosisand NCI-60 cell panel assays. Also, the signaling pathways that account for their activities were investigated. Compounds **2**, **3**, **4a**, **4d**, **4f**, **4i**, **4k**, **5b**, **5c**, **5d**, **5f**, **5g**, **6b**, **6c**, **6d**, **6e**, **6g**, **7a** and **7g** were selected by NCI. Among the tested compounds, **6g** appeared to be the most active compound of this series that not only induced apoptosis in DU-145 cancer cells but also attenuated the ERK1/2 and p-38 signaling pathways. All test compounds exhibited diverse cytostatic and cytotoxic activities that warrant further development as potential anticancer agents.

Keywords: Thiadiazoles, anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione, SRB assay, NCI 60-cell panel assay, apoptosis.

1. Introduction

Thiadiazoles are among the privileged pharmacological scaffold fragments because of their unique chemical properties for diverse biological and clinical applications [1], such as anticancer [2-5], anti-inflammatory [6], antihypertensive [7], antidepressant [8,9], anticonvulsant [10], antileishmanial [11,12], and antimicrobial activities [13-16]. Anthraquinone-containing extracts from different plant sources (e.g. senna, cascara, aloe, frangula, and rhubarb etc.) have been found to have wide variety of pharmacological activities such as anti-inflammatory, wound healing, analgesic, antipyretic, anti-microbial, and antitumor activities [17,18]. To date, anthracycline antibiotics (e.g. daunorubicin, doxorubicin) and the selective anthraquinone derivatives (e.g. mitoxantrone, emodin) are important anthraquinone-containing drugs, showing strong antiproliferative (or cytostatic) activities and diverse applications in clinical practice [19]. In view of the above facts and the concept of bio-isosterism we developed a ring fusion strategy that fused the thiadiazole and anthraquinone structures to alter their pharmacological and physiochemical properties for varied bioactivities. The scaffold hopping strategy aimed for the discovery of novel anticancer drugs has been generally accepted as a means to transform pharmacophoric templates, and subsequently match these with complementary features embedded into small-molecule topologies [20] (Fig. 1).

Based on the similar chemical properties and drugs comparison studies, our group illustrated that related various tricyclic and tetracyclic anthraquinone-derived analogs exhibited anticancer activities by acting as potent telomerase and/or TOP1 inhibitors [18,21-24]. Thorough analysis of these scaffold according to the associated biological activity showed that thiadiazole renders anthraquinone moieties unprecedented broad-spectrum bioactivities [22,23,25-29]. In addition, the five-membered thiadiazole ring connected to the plannar tricyclic anthraquinone

moiety gives a compact and planar structure that exhibits an extremely low aromaticity as a result of its high polarity and electron-withdrawing tendency [1]. Although a number of thiadiazole-containing drugs have advantages over other commonly discovered therapeutic scaffolds, the most fully investigated of these broad biological relevance in clinical currently on the market [30,31]. In this study we were interested in developing the widely reported drugs containing 1,2,5-thiadiazole, timolol, vedaclidine, sulfametrole, tazomeline and xanomeline taking into consideration that anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione was evaluated as bioisostere of core. Based on our previous studies, anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione was shown to exhibit significant antitumor potency against several cancers *in vitro* and *in vivo* [26-28]. Moreover, the tricyclic and tetracyclic systems, heterocyclic anthraquinones and substituents of these heterocycles with thiadiazole-containing compounds typically lead to analogues with improved activities because the sulfur atom imparts substantially increased binding to their intracellular targets as well as increased liposolubility [31].

We have previously synthesized various tricyclic and tetracyclic derivatives having different substituents at different positions which showed remarkable potent anticancer activities. In the course of our continuing search for new antitumor agents from the thiadiazole-containing anthraquinone compounds, we hypothesized that the fused system containing thiadiazole ring will have advantage over benzene and thiazole. Compared with other heterocycles, thiadiazole (benzene bioisostere) with unparalleled properties display striking differential cytotoxicity profiles. The anticancer activities of our new compounds were evaluated using a panel of 60 human tumor cell lines primary screen provided by the NCI (USA). Toward supporting the aforementioned hypothesis and exploration of SARs, we found the backbone of anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione derivatives is associated with

DNA-intercalating or topoisomerase inhibitory properties. The cytotoxicity of our compounds against two classical human prostate cancer cell lines (including PC-3 and DU-145) where evaluated by the SRB and MTT assays (Table 1). Moreover, **2** (NSC745885), **3** (NSC757963), **4a** (NSC757964), **4d** (NSC761882), **4f** (NSC761884), **4i** (NSC757966), **4k** (NSC761890), **5b** (NSC761881), **5c** (NSC761891), **5d** (NSC763955), **5f** (NSC761885), **5g** (NSC761883), **6b** (NSC761889), **6c** (NSC761886), **6d** (NSC761892), **6e** (NSC761887), **6g** (NSC763968), **7a** (NSC757965), and **7c** (NSC763965) were selected by the NCI for one dose screening program (Table 2). Considering their structure and side chains, **4d** (NSC761882), **4f** (NSC761884), **5f** (NSC761885), **6g** (NSC763968), and **7a** (NSC757965) showed potent growth inhibition activities, so they were evaluated at 5 dose concentration levels (Table 3). For the most potent compound **6g**, the effects on the cell cycle and induction of apoptosis were also tested in the human prostate cancer cell line (DU-145). In this study, it is generally accepted that we provide insight into the correlation between the cytotoxicity and inhibition activities against ERK 1/2 and p-38 phosphorylation of some of selective synthetic compounds for their anticancer activities.

2. Chemistry

According to our previous series of studies, the structural complexity of anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione, arising from the presence of tetracyclic ring has restricted most of the SAR studies by derivatization of the parent lead structure rather than by *de novo* chemical synthesis [22,23,26-29]. There has been a continuous interest in the synthesis of sulfur-substituted anthra[1,2-*c*][1,2,5]thiadiazole-6,11-diones and their systematic dissection largely on account of their complementary activities. Our primary starting material anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione (**2**) was synthesized from 1,2-diaminoanthraquinone by

thionyl chloride and trimethylamine as catalyst. Subsequently, **3** was prepared by potassium chlorate in hydrochloric acid in the presence of acetic acid and quenched by ice water. Such approach, as shown in Scheme 1, reacting **2** with the appropriate thiols and thiophenols by using ferric chloride as catalyst in DMF for several hours or reacting **3** with the appropriate thiols and thiophenols in dry THF and *N*,*N*-diisoproprylethylamine (DIPEA) afforded the sulfur-substituted anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione derivatives in good yields (overall 35-88%). It was found that the two above-mentioned methods led directly to the formation of the desired final products. In the series of designed analogues, we maintained the anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione as a core structure, and the lead optimization focused on varying the electron-withdrawing or electron-releasing substituents at the thio-position of anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione skeleton.

3. Results and discussion

3.1 In vitro antiproliferative activities

In an initial attempt to investigate the cytotoxic activities of anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione, the IC_{50} values of each target compound were measured by SRB and MTT assays against PC-3 and DU-145 cells. As illustrated in Table 1, the values of IC_{50} for nine compounds (**2**, **4d**, **4f**, **4q**, **5d**, **6f**, **6g**, **7b** and **7d**) were less than 10 μM against PC-3 and/or DU-145 cells, respectively. Through structural variation as shown in Table 1, to explore the effect of substitution in the linker, a few electron donating substituents (CH_3, OCH_3, OC_2H_5) of terminal phenyl group were predicted to have less efficacy in our assays. In addition to examining different linkers and diverse heteroaromatic substitutions on the terminal phenyl, the activity of electron withdrawing group on meta-position substitution was superior to *para*-position and *ortho*-position. These data also indicated that a suitable linker were important for enzymatic inhibition and anticancer cell proliferation. More

importantly, compound **6f** and **6g** were chosen for further optimization based on evaluating the SAR in greater detail. Among them, **6g** is the most active derivative with IC_{50} values of 3.69 μM and 4.53 μM against PC-3 and DU-145, respectively. So, compound **6g** was selected for further evaluation for the morphological changes and apoptosis in DU-145 cells, as well as inhibition activity against the ERK 1/2 and p38 phosphorylation. Understanding the cell death pathways for selective killing against cancer cells would lead to more effective strategies. It seemed from theresults for **6g** that the preclinical studies still have a long way to go because of its target and mechanism.

3.2 NCI60 human cell lines anticancer drug screening

The anticancer activity and toxicity of all the test compounds were evaluated using the National Cancer Institute (NCI) Drug Screen Program of 60 human tumor cell lines. Details of the methodology are described in our previous reports [21, 24, 25, 32-36]. There are two stages of the screening process that starts with the evaluation of the test compounds against the 60 human tumor cell lines at a single dose of 10 μM. In this initial screening, **2**, **3**, **4a**, **4d**, **4f**, **4i**, **4k**, **5b**, **5c**, **5d**, **5f**, **5g**, **6b**, **6c**, **6d**, **6e**, **6g**, **7a**, and **7c** were chosen by NCI and tested against a panel of 60 human tumor cell lines. The growth inhibition percentages obtained from the single dose experiments are shown in Table 2. Compounds **4d**, **4f**, **5f**, **6g** and **7a** showed potent and significant growth inhibition so they were evaluated further for their cytostatic and cytotoxic activities against the 60 cell lines panel using the five dose studies (0.01, 0.1, 1, 10 and 100 μM). The dose-response curve is originated from the log 10 of the corresponding drug concentration against each cell line from the subpanel groups. The percentage growth at each concentration with GI_{50}, TGI and LC_{50} of **4d**, **4f**, **5f**, **6g** and **7a** are shown in Table 3. The *in vitro* results showed that compounds **4f** and **6g** have potent broad spectrum anticancer activities against almost all of the human cancer cell

lines with GI_{50} in nano molar to micro molar range. As shown in Table 3, **4f** exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} values between 0.31 to 20.1 μM. With regard to the sensitivity against some individual cell lines, **4f** showed high activity against Ovarian cancer (OVCAR-4) and Breast cancer (MDA-MB-231/ATCC) cell lines with GI_{50} values of 0.31 and 0.37 μM, respectively. Sensitive cell lines to **4f**, exhibited an LC_{50} with as little as 6.71 μM and TGI with as little as 0.94 μM for Ovarian cancer (OVCAR-4). Results of our above study extended the initial *in vitro* observation reported in the data above and confirmed the importance of anticancer activity.

More interestingly, **6g** had the prominently potent activity against most of the tested cell lines with GI_{50} values ranging from 0.18 to 13.3 μM. With regard to the sensitivity against individual cell lines, **6g** showed high activity against Leukemia (0.18-0.31 μM); Non-small lung cancer (0.32-2.85 μM); Colon Cancer (0.29-13.3 μM); CNS Cancer (0.52-3.32 μM); Melanoma (0.20-2.72 μM); Ovarian Cancer (0.25-2.90μM); Renal Cancer (0.56-5.68 μM); Prostate Cancer (0.25-1.45 μM); Breast Cancer (0.29-2.19 μM). It is worth mentioning that **6g** had the highest selectivity against the following cell lines with GI_{50}: Leukemia (0.18-0.31 μM), Non-small lung cancer (HOP-92: 0.32 μM; NCI-H23: 0.56 μM), Colon Cancer (HCT-116: 0.29 μM; SW-620: 0.43 μM), CNS Cancer (SNB-75: 0.52 μM); Melanoma (LOX IMVI: 0.34 μM; MDA-MB-435: 0.20 μM; SK-MEL-2: 0.99 μM; UACC-257: 0.57 μM); Ovarian Cancer (IGROV1: 0.96 μM; OVCAR-3: 0.25 μM; OVCAR-4: 0.36 μM; OVCAR-8: 0.49 μM; NCI/ADR-RES: 0.52 μM); Renal Cancer (A489: 0.56 μM; CAKI-1: 0.59μM; SN12C: 0.66 μM); Prostate Cancer (PC-3: 0.25 μM); Breast Cancer (MDA-MB-231/ATCC: 0.29 μM; T-47D: 0.38 μM; MDA-MB-468: 0.37 μM). The five dose graphs and dose response curves of 6g for all tested cell lines are illustrated in Fig. 2.

3.3 The in vitro selectivity of the full NCI60 cell panel assay

All the NCI60 cell panel assays, representing nine tumor subpanels, were incubated at five different concentrations of the test compounds. The sensitivity of compounds against some individual cell lines depends on the ratio obtained by dividing the full panel MID and particular subpanel MID [37]. Ratios between 3 and 6 refer to moderate selectivity; ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria are rated non-selective [38]. All of these parameters provide insight into the selectivity and potency of the antitumor activity. Based on these parameters, **4d**, **4f**, **5f**, **6g** and **7a** were found to be moderately selective for growth inhibition toward leukemia cancer subpanel where **6g** and **7a** showed selectivity ratios of 6.44 and 3.66, respectively, whereas they were found to be less selective against the remaining cell panels (Table 4). It seemed from these results that only **6g** exhibited the unique selectivity against leukemia cancer.

*3.4 The cytotoxicity of **6g** to SV-HUC-1 cells (human normal urothelial cells), (WMPY-1) normal human prostatic stromal myo-fibroblasts and (RWPE-1) normal prostate epithelial cells.*

In this study, we compared the cytotoxic effect of SV-HUC-1 cells (human normal urothelial cells), WMPY-1 (normal human prostatic stromal myo-fibroblasts) and RWPE-1 (normal prostate epithelial cells) induced by **6g**, and also compared this cytotoxicity to doxorubicin (DXR). Based on our result, compound **6g** had less cytotoxic effect than DXR. As shown in Fig. 3a, **6g** and doxorubicin were compared regarding cytotoxicity in the normal cell line (SV-HUC-1 cells), 80% survival rate of the cells was observed with the highest concentration of **6g**. As shown in Fig. 3b and 3c, 50% and 60% survival rate was observed with the highest concentration of **6g** of WMPY-1 (normal human prostatic stromal myo-fibroblasts) and RWPE-1 (normal

prostate epithelial cells), respectively. These data are of interest, as it suggests that DXR are more toxic to WMPY-1 (normal human prostatic stromal myo-fibroblasts. The response of the well-known anticancer drug, DXR, was more toxic at the same concentration.

3.5. Morphological changes and apoptosis in DU-145 cells

In the present study, we assessed the effect of **6g** on human prostate cancer DU-145 cells *in vitro*, and elucidated its possible mechanisms. DU-145 cells were treated with different concentrations of **6g** and cell proliferation was assessed by MTT assay, cell apoptosis by flow cytometry, and protein levels of Bcl-2 by Western blotting. The results showed that **6g** inhibited the proliferation of DU-145 cells in a dose-dependent manner. DU-145 cells treated with different concentrations of **6g** displayed obvious morphological changes indicating apoptosis under the fluorescence microscope. In addition, **6g** increased the proportion of early apoptotic DU-145 cells, down-regulated the protein levels of Bcl-2. Morphology analysis confirmed the cytotoxic effects of **6g**. As shown in Fig. 4, the apoptotic morphological changes included cell rounding and shrinkage after 24 hour incubation with 5 μM of **6g**. Following above observation, we concluded that **6g** caused apoptosis in DU-145 cells. To investigate whether apoptosis induced by **6g** was involved in activating caspase cascades, we exposed DU-145 cells to various concentrations of the compound for 48 h. The activity of caspase-3 was then determined using western blotting analysis, which revealed the activation of caspase-3 within 48 h of **6g** treatment. The PARP, a 113 kDa nuclear enzyme, is cleaved in fragments of 89 during apoptosis. Exposure to **6g** also decreased the total PARP, increased the levels of cleaved PARP (Fig. 5) and decreased the levels of cyclinD1 and Bcl-2 (Fig. 6a, 6b). Interesting, there are more cleaved PARP and cleaved caspase 3 at concentration 3 uM than at 12 uM of **6g** might be apoptosis and necrosis.

*3.6 Inhibition activity of **6g** against ERK1/2 and p38 phosphorylation*

In view of the previous rationale and in continuation of our ongoing program, ERK1/2 is an important mitogen-activated protein kinase which control cellular activities and physiological processes [39]. Western blot analysis demonstrated that compound **6g** had significant inhibition activities against ERK 1/2 and p-38 phosphorylation in DU-145 cells. As shown in Fig. 7, compound **6g** inhibited p-38 phosphorylation in DU-145 cells at 3, 12, 18 μM with obvious concentration dependency. Interestingly, compound **6g** might induce ERK ½ phosphorylation at low concentrations but against ERK ½ phosphorylation at high concentrations. Thus, these compounds can inhibit the proliferation of DU-145 cells through the mediated signaling pathway of ERK1/2 and p-38.

4. Conclusion

In this investigation, we continue to pay attention to the role of our synthesized tetracyclic anthraquinone moiety pharmacophore and to understand the pharmacological effects of thiadiazole-containing anthraquinone derivatives. Thus, a novel structure of substituted anthra [1,2-*c*][1,2,5] thiadiazole-6,11-dione derivatives were design and synthesized. The NCI60 cancer panel, leukemia (0.18-0.31 μM), non-small cell lung cancer (0.32-2.85 μM), colon cancer (0.29-13.3 μM), CNS cancer (0.52-3.32 μM), melanoma (0.20-2.72 μM), ovarian cancer (0.25-2.90 μM), renal cancer (0.56-5.68 μM), prostate cancer (PC-3: 0.25 μM; DU-145: 1.45 μM), breast cancer (0.29-2.19 μM) were sensitive to **6g**. Also, the leukemia (0.56-0.64 μM), non-small cell lung cancer (1.00-18.6 μM), colon cancer (0.44-20.1 μM), melanoma (0.54-2.34 μM), ovarian cancer (0.31-3.97 μM), breast cancer (0.37-3.27 μM) were sensitive to **4f** whereas the CNS cancer, renal cancer and prostate cancer panels were less sensitive. The average GI_{50} values of **6g** and **4f** were 1.61 and 3.68 μM, respectively. The data showed that the compounds bearing sulfur alkyl side chains

displayed potent cytotoxicity in prostate cancer cells. Furthermore, response of the well-known anticancer drug, DXR, showed more toxicity than **6g** at high concentrations in the normal human urothelial cells. Given the striking correlations between the proliferative capacity and topoisomerase activity in tumor cells (data not shown), we expected that analysis of the anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione as a chromophore with various thiol groups side chain substitutions might produce further insight into designing better lead compounds for anti-tumor agents. Morphology analysis confirmed that the cytotoxic effects of **6g** not only induced apoptosis but also activated the caspase cascade and PARP as well as decreasing the levels of cyclinD1 and Bcl-2. Additionally, the inhibition activities of **6g** on the ERK1/2 and p38 protein phosphorylation were also tested by Western blotting. Results showed that such inhibition was concentration dependent. Thus, compound **6g** can inhibit the proliferation of DU-145 cells through the mediated signaling of ERK1/2 and p38 pathways. Collectively, the extensive SARs studies discussed here have helped us to design a further novel series of potent anticancer drugs to enhance the bioavailability and *in vivo* activity. Comparison of our experimental results lead to the conclusion that the *in vitro* anticancer activity is dependent on the length of linker between phenyl and sulfur substituents, with one and two carbons of linker typically giving rise to compounds with higher activity. Compounds containing 1,2,5-thiadiazole ring was observed to have high potency, and some of them displayed excellent activities against a range of tumor cells. The ability of thiadiazole-containing anthraquinone compounds to target DNA could explain their broad activity in unique ways which could potentially be used for chemical intervention at the gene level. Sulfur containing substituents in various heterocyclic derivatives might have a promising effect on the biological activity, so our above-mentioned results may contribute to substantial progress in this field.

5. Materials and methods

5.1 Chemistry

Melting points were determined by melting point apparatus (Büchi® 545). All reactions were monitored by TLC (Silica Gel 60 F_{254}). ^{1}H NMR spectra were recorded with GEMINI-300 MHz (Varian®) and AM-500 MHz (Bruker®). Chemical shift (δ) values were in ppm relative to TMS as an internal standard. Fourier-transform IR spectra (KBr): Perkin-Elmer 983G spectrometer. Mass spectra were obtained on Finnigan MAT 95 XL HRMS and Finnigan/Thermo Quest MAT HRMS. Reagents and solvents were purchased from Merck and Aldrich used without further purification. Typical experiments illustrating the general procedures for the preparation of the anthraquinones are described below.

5.2.General synthetic methods A：Preparation of compound 2

1.19g (5 mmole) of 1,2-diaminoanthraquinone (compound 1) was dissolved in THF (30ml). The thionyl chloride ($SOCl_2$) (0.5mL) and triethylamine (TEA) (3 mL) were added and the reaction mixture was stirred at room temperature for 24 hours. The mixture was quenched by ice water (100ml) and then collected by filtering. The crystals were recrystallized from hot EtOH to get a hot compound **NSC745885 (**anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione**)**.

General synthetic methods B：Preparation of compound 3

A solution of 0.50 g (2 mmole) of compound **NSC745885** in 15 mL of acetic acid at 90℃ was treated with 5 ml of concentrated hydrochloric acid. A solution of 2.0 g of potassium chlorate was added to the boiling suspension during a 45 minutes time interval. The mixture was then boiled for another 4 hours and the precipitate was separated in ice bath. Finally the reaction product **NSC757963** was crystallized from acetic acid.

General synthetic methods C: preparation of compounds 4a~ 4r, 5a~ 5h, 6a~ 6g

and 7a~ 7e

These compounds were synthesized from 1,2-diaminoanthraquinone. All the chemicals and organic solvents required for synthesis were purchased from either Sigma Aldrich Chemical Company or Merck Chemical Company. A mixture of compound **3** (0.3 g, 1 mmole) in dry THF and *N*,*N*-Diisoproprylethylamine (DIPEA) (0.5 mL) were heated to 65℃, and then series organosulfur compounds (2 mmole) in same solvent were added. The reaction mixtures were refluxed for 2~4 hours at 65℃. Then the crude products were purified by ethanol to afford desired compounds.

5.2.1. 4-(Phenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4a)

The pure compound was obtained as an orange red powder (yield 76%). Mp: 224-225 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1519.8 (CO), 1589.2 (CO), 1672.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): 7.58-7.62 (m, 3H), 7.71-7.74 (m, 2H, m), 7.76-7.86 (m, 3H), 8.16 (dd, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H), 8.35 (dd, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 119.93, 121.40, 127.04, 127.44, 128.32, 130.67, 130.87, 132.30, 133.97, 134.03, 134.88, 135.73, 135.90, 143.57, 151.32, 155.18, 181.68, 183.52. HRMS (EI) *m/z*: calcd for $C_{20}H_{10}N_2O_2S_2^+$ $[M]^+$; 374.0184, found, 374.0184.

5.2.2. 4-(2-Fluorophenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4b)

The pure compound was obtained as a yellow orange powder (yield 88%). Mp: 272-273 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1541.0 (CO), 1591.2 (CO), 1674.1 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.32-7.40 (m, 2H), 7.61-7.67 (m, 1H), 7.68-7.69 (m, 1H), 7.71-7.75 (m, 1H), 7.76-7.86 (m, 2H), 8.17 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.34 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz , 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 117.21, 117.51, 119.84, 121.67, 126.04, 126.09, 127.08, 127.48, 127.85, 132.20, 133.61, 133.72, 133.85, 134.13, 134.95, 135.67, 137.77, 151.28, 155.14, 161.71, 181.74, 183.47. HRMS (EI) *m/z*: calcd $[M]^+$, 392.0089

($C_{20}H_9FN_2O_2S_2^+$); found, 392.0087.

5.2.3. 4-(2-Chlorophenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4c)

The pure compound was obtained as a yellow powder (yield 81%). Mp: 263-264 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1542.9 (CO), 1670.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.47 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.57 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.63 (s, 1H), 7.70 (dd, *J*= 8.4 Hz, *J*= 1.5 Hz, 1H), 7.75-7.87 (m, 3H), 8.16-8.19 (m, 1H), 8.34-8.37 (m, 1H). ^{13}C NMR(75 MHz, $CDCl_3$): δ ppm 120.24, 121.78, 127.05, 127.44, 127.76, 128.59, 131.45, 132.26, 132.41, 133.93, 134.05, 134.90, 135.67, 137.98, 140.04, 140.75, 151.37, 155.24, 181.64, 183.41. HRMS(EI) *m/z*: calcd $[M]^+$, 407.9794 ($C_{20}H_9ClN_2O_2S_2^+$); found, 407.9796.

5.2.4. 4-(2-Bromophenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4d)

The pure compound was obtained as an orange powder (yield 85%). Mp: 210-211 ℃ (EtOH). FT-IR(KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1539.1 (CO), 1593.1 (CO), 1670.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.46-7.53 (m, 2H), 7.62 (s, 1H), 7.74-7.82 (m, 4H), 8.16 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.33 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 120.24, 121.74, 127.09, 127.48, 129.30, 129.88, 130.85, 132.26,132.45, 133.92, 134.11, 134.96, 135.68, 138.00, 140.91, 151.39, 155.19, 181.74, 183.47. HRMS (EI) *m/z*: calcd $[M]^+$, 451.9289 ($C_{19}H_9BrN_2O_2S_2^+$); found, 451.9295.

5.2.5. 4-(3-Fluorophenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4e)

The pure compound was obtained as a yellow powder (yield 78%). Mp: 254-255 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1575.7 (CO), 1670.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.28-7.34 (m, 1H), 7.41-7.45 (m, 1H), 7.49-59 (m, 2H), 7.78 (s, 1H), 7.80-7.88 (m, 2H), 8.18 (dd, *J*= 7.2 Hz, *J*= 1.5 Hz, 1H), 8.33-8.36 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 117.83, 118.03, 119.93, 122.24, 122.46, 126.87, 127.24, 131.26, 131.29 131.70, 131.79, 131.83, 133.48,

133.96, 134.79, 135.33, 141.98, 150.90, 154.72, 161.71, 164.48, 181.45, 183.09. HRMS (EI) m/z: calcd $[M]^+$, 392.0089 ($C_{20}H_9FN_2O_2S_2^+$); found, 392.0091.

5.2.6. 4-(3-Chlorophenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4f)

The pure compound was obtained as an orange powder (yield 58%). Mp: 252-253 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1500.5 (aromatic C=C), 1539.1 (CO), 1668.3 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δ ppm 7.50-7.63 (m, 3H), 7.71 (t, *J*= 1.5 Hz, 1H), 7.79 (s, 1H), 7.81-7.87 (m, 2H), 8.19 (dd, *J*= 7.8 Hz, *J*= 1.8 Hz, 1H), 8.36 (dd, *J*= 7.5 Hz, *J*= 1.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 120.61, 121.87, 127.15, 127.50, 130.43, 131.05, 131.58, 132.26, 133.78, 133.91, 134.18, 135.00, 135.33, 135.71, 136.28, 142.03, 151.31, 155.22, 181.73, 183.41. HRMS (EI) *m/z*: calcd $[M]^+$, 407.9794 ($C_{20}H_9ClN_2O_2S_2^+$); found, 407.9794.

5.2.7. 4-(3-Bromophenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4g)

The pure compound was obtained as an orange powder (yield 74%). Mp: 197-198 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1558.4 (C=O), 1668.3 (C=N). ^1H NMR(400 MHz, $CDCl_3$): δ ppm 7.40 (t, *J*= 7.6 Hz, 1H), 7.66 (d, *J*= 7.6 Hz, 1H), 7.74 (d, *J*= 7.6 Hz, 1H), 7.78-7.81 (m, 2H), 7.83-7.87 (m, 2H), 8.19 (d, *J*= 7.6 Hz, 1H), 8.36 (d, *J*= 7.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 120.04, 121.39, 123.87, 126.91, 127.27, 130.01, 131.65, 131.86, 133.50, 133.79, 134.01, 134.12, 134.82, 135.36, 137.95, 141.91, 150.93, 154.73, 181.50, 183.12. HRMS (EI) *m/z*: calcd $[M]^+$, 451.9289 ($C_{20}H_9BrN_2O_2S^+$); found, 451.9295.

5.2.8. 4-(4-Fluorophenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4h)

The pure compound was obtained as an orange red powder (yield 79%). Mp: 224-225 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1539.1 (CO), 1519.2 (CO), 1674.1 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δ ppm 7.31(d, *J*= 8.7 Hz, 2H), 7.67 (s, 1H), 7.69-7.74 (m, 2H), 7.75-7.89 (m, 2H), 8.17 (d, *J*= 7.5 Hz, 1H), 8.34 (d, *J*= 7.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 117.90, 118.19, 119.74, 121.52,

123.47, 127.10, 127.48, 132.28, 133.94, 134.12, 134.97, 135.70, 138.15, 138.26, 143.33, 151.32, 155.82, 162.95, 181.69, 183.50. HRMS (EI) *m/z*: calcd $[M]^+$, 392.0089 ($C_{20}H_9FN_2O_2S_2^+$); found, 392.0087.

5.2.9. 4-(4-Chlorophenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4i)

The pure compound was obtained as a brown powder (yield 63%). Mp: 260-261 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1519.4 (CO), 1582.2 (CO), 1664.4 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.56 (d, *J*= 8.4 Hz, 2H), 7.65 (d, *J*= 8.7 Hz, 2H), 7.76 (s, 1H), 7.78-7.85 (m, 2H), 8.19 (dd, *J*= 7.2 Hz, *J*= 1.2 Hz, 1H), 8.35 (dd, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR(75 MHz, $CDCl_3$): δ ppm 120.20, 121.76, 126.95, 127.13, 127.50, 130.97, 132.30, 133.97, 134.15, 135.00, 135.73, 137.02, 137.50, 142.54, 151.35, 155.22, 181.68, 183.48. HRMS (EI) *m/z* calcd $[M]^+$, 407.9794 ($C_{20}H_9ClN_2O_2S_2^+$); found, 407.9791.

5.2.10. 4-(4-Bromophenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4j)

The pure compound obtained as an orange powder (yield 68%). Mp: 246-247 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1522.4 (CO), 1591.2 (CO), 1668.3 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.13 (dd, *J*= 6.6 Hz, *J*= 1.8 Hz, 2H), 7.51 (dd, *J*= 6.6 Hz, *J*= 1.8 Hz, 2H), 7.75 (s, 1H), 7.77-7.86 (m, 2H), 8.19 (dd, *J*= 7.2 Hz, *J*= 1.2 Hz, 1H), 8.34 (dd, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 120.27, 121.78, 125.64, 127.14, 127.50, 127.60, 127.83, 132.29, 133.95, 134.17, 135.00, 135.72, 137.16, 142.32, 151.33, 155.22, 181.69, 183.48. HRMS (EI) *m/z*: calcd $[M]^+$, 451.9289 ($C_{20}H_9BrN_2O_2S_2^+$); found, 451.9296.

5.2.11. 4-(o-Tolylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4k)

The pure compound was obtained as a dark red powder (yield 78%). Mp: 251-252 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1502.4 (aromatic C=C), 1541.0 (CO), 1593.1 (CO), 1670.2(C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.47 (s, 3H), 7.37-7.42 (m, 1H), 7.50 (s, 1H), 7.52-7.55 (m, 2H), 7.69 (d, *J*= 7.8 Hz, 1H), 7.76 (td, *J*= 7.2 Hz, *J*= 1.2

Hz, 1H), 7,83 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.15 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.35 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 20.62, 119.17, 121.22, 127.03, 127.25, 127.43, 128.11, 131.54, 131.99, 132.32, 134.01, 134.48, 134.88, 135.79, 137.22, 142.92, 143.51, 151.43, 155.29, 181.71, 183.56. HRMS (EI) *m/z*: calcd $[M]^+$, 388.0340 ($C_{21}H_{12}N_2O_2S_2^+$); found, 388.0343.

5.2.12. 4-(m-Tolylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4l)

The pure compound was obtained as an orange red powder (yield 76%). Mp: 261-262 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1541.0 (CO), 1593.1 (CO), 1670.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.46 (s, 3H), 7.42 (t, *J*= 8.4 Hz, 1H), 7.47-7.53 (m, 3H), 7.73 (s, 1H), 7.76 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.83 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.15 (dd, *J* = 7.5 Hz, *J* = 1.5 Hz, 1H), 8.33 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 21.38, 119.93, 121.29, 127.02, 127.43, 127.90, 130.44, 131.71, 132.32, 132.88, 134.00, 134.87, 135.75, 136.32, 140.75, 143.86, 151.31, 155.18, 181.70, 183.58. HRMS (EI) *m/z*: calcd $[M]^+$, 388.0340 ($C_{21}H_{12}N_2O_2S_2^+$); found, 388.0344.

5.2.13. 4-(p-Tolylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4m)

The pure compound was obtained as a light orange powder (yield 80%). Mp: 294-295 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1502.4 (aromatic C=C), 1591.2 (CO), 1666.4 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.50 (s, 3H), 7.40 (d, *J*= 7.8 Hz, 2H), 7.73 (d, *J*= 8.1 Hz, 2H), 7.71 (s, 1H), 7.76 (td, *J*= 1.5 Hz, *J*= 7.2 Hz, 1H), 7.83 (td, *J*= 1.5 Hz, *J*= 7.5 Hz, 1H), 8.16 (dd, *J*= 7.2 Hz, *J*= 1.2 Hz, 1H), 8.35 (dd, *J*= 7.2 Hz, *J*= 1.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 21.52, 119.67, 121.26, 124.55, 127.03, 127.45, 131.50, 132.35, 134.01, 134.89, 135.78, 135.88, 141.38, 144.25, 151.35, 155.18, 181.73, 183.68. HRMS (EI) *m/z*: calcd $[M]^+$, 388.0340 ($C_{21}H_{12}N_2O_2S_2^+$); found, 388.0341.

5.2.14. 4-(2-Isopropylphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4n)

The pure compound was obtained as a light brown powder (yield 68%). Mp: 266-267 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1672.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.23 (d, *J* = 6.9 Hz, 6H), 3.47-3.52 (m, 1H), 7.35-7.41 (m, 1H), 7.54 (s, 1H), 7.58-7.62 (m, 2H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.75 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.82 (td, *J*= 7.5 Hz, *J*= 1.2 Hz, 1H), 8.13-8.16 (m, 1H), 8.32-8.35 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 23.99, 31.48, 119.52, 121.15, 126.05, 127.02, 127.41, 127.69, 127.95, 131.98, 132.30, 133.98, 134.86, 134.87, 135.73, 137.41, 144.01, 151.36, 153.90, 155.14, 181.70, 183.53. HRMS (EI) *m/z*: calcd $[M]^+$, 416.0653 ($C_{23}H_{16}N_2O_2S_2^+$); found, 416.0651.

5.2.15. 4-(4-Isopropylphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4o)

The pure compound was obtained as a brown powder (yield 57%). Mp: 232-233 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1539.1 (CO), 1593.1 (CO), 1666.4 (C=N). ^{1}H NMR(300 MHz, $CDCl_3$): δ ppm 1.36 (d, *J*= 7.2 Hz, 6H), 3.03-3.07 (m, 1H), 7.45 (d, *J*= 7.8 Hz, 2H), 7.63 (d, *J*= 7.8 Hz, 2H), 7.74 (s, 1H), 7.77 (d, *J*= 7.5 Hz, 1H), 7.81 (d, *J*= 7.2 Hz, 1H), 8.15 (d, *J*= 7.5 Hz , 1H), 8.33 (d, *J*= 7.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 23.86, 34.21, 119.73, 121.27, 124.84, 127.04, 127.45, 128.87, 132.37, 134.00, 134.87, 135.81, 135.93, 144.17, 151.35, 152.20, 155.21, 181.75, 183.65. HRMS (EI) *m/z*: calcd $[M]^+$, 416.0653 ($C_{23}H_{16}N_2O_2S_2^+$); found, 416.0657.

5.2.16. 4-(2-Ethylphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4p)

The pure compound was obtained as a brown powder (yield 57%). Mp: 232-233℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1542.9 (CO), 1593.1 (CO), 1668.3 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.21 (t, *J* = 7.5 Hz, 3H), 2.84 (q, *J*= 12.9 Hz, *J*= 7.5 Hz, 2H), 7.39 (td, *J*= 1.8 Hz, *J*= 7.4 Hz, 1H), 7.51 (s, 1H), 7.54 (d, *J*= 5.1 Hz, 1H), 7.58 (td, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H), 7.67 (dd, *J*= 7.8 Hz, *J*= 1.2 Hz, 1H), 7.75 (td, *J*= 7.5 Hz, *J*= 1.8 Hz, 1H), 7.81 (td, *J*= 7.5 Hz, *J*= 1.2 Hz, 1H), 8.13 (dd,

J= 7.5 Hz, *J*= 1.2 Hz, 1H), 8.32 (dd, *J*= 7.5 Hz, *J*= 1.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.54, 27.47, 119.28, 121.07, 126.44, 126.99, 127.40, 128.12, 130.49, 131.78, 132.20, 133.87, 134.02, 134.89, 135.68, 137.49, 143.74, 149.35, 151.29, 155.12, 181.73, 183.55. HRMS (EI) *m/z*: calcd $[M]^+$, 402.0497 ($C_{22}H_{14}N_2O_2S_2^+$); found, 402.0499.

5.2.17. 4-(4-Ethylphenylthio)anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4q)

The pure compound was obtained as an orange powder (yield 80%). Mp: 232-233 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1502.4 (aromatic C=C), 1591.2 (CO), 1668.3 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.35 (t, *J*= 7.5 Hz, 3H), 2.80 (q, *J*= 7.5 Hz, 2H), 7.42 (d, *J*= 8.1 Hz, 2H), 7.62 (d, *J*= 8.1 Hz, 2H), 7.71 (s, 1H), 7.80 (t, *J*= 7.8 Hz, 1H), 7.79-7.84 (m, 1H), 8.15 (dd, *J*= 7.5 Hz, *J*= 1.2 Hz, 1H), 8.33 (d, *J*= 7.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 15.20, 28.85, 119.56, 121.32, 124.55, 127.01, 127.43, 130.27, 132.25, 133.91, 134.02, 134.89, 135.71, 135.95, 144.22, 147.58, 151.27, 155.07, 181.74, 183.47. HRMS (EI) *m/z*: calcd $[M]^+$, 402.0497 ($C_{22}H_{14}N_2O_2S_2^+$); found, 402.0500.

5.2.18. 4-(4-Tert-butylphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (4r)

The pure compound was obtained as a yellow powder (yield 77%). Mp: 298-299℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1670.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.43 (s, 9H), 7.58-7.65 (m, 4H), 7.75 (s, 1H), 7.76 (td, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H), 7.83 (td, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H), 8.15 (dd, *J*= 7.5 Hz, *J*= 1.2 Hz, 1H), 8.32 (dd, *J*= 7.8 Hz, *J*= 1.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 31.34, 35.13, 119.74, 121.25, 124.60, 127.01, 127.42, 127.73, 127.74, 132.33, 133.98, 134.84, 135.59, 135.79, 144.04, 151.32, 154.52, 160.69, 181.71, 183.61. HRMS (EI) *m/z*: calcd $[M]^+$, 430.0810 ($C_{24}H_{18}N_2O_2S_2^+$); found, 430.0813.

5.2.19. 4-(2,5-Dimethylphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (5a)

The pure compound was obtained as a dark brown powder (yield 47%). Mp: 243-244

℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1500.5 (aromatic C=C), 1522.8 (CO), 1591.2 (CO), 1674.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.40 (6H, s, -CH_3), 7.33 (d, *J*= 7.8 Hz, 1H), 7.39 (d, *J*= 7.5 Hz, 1H), 7.51-7.53 (m, 2H), 7.76 (t, *J*= 7.5 Hz, 1H), 7.83 (t, *J*= 7.8 Hz, 1H), 8.15 (d, *J*= 7.5 Hz, 1H), 8.34 (d, *J*= 7.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 20.08, 20.82, 119.24, 121.23, 125.36, 126.79, 127.02, 127.45, 131.79, 132.41, 134.00, 134.01, 134.87, 135.84, 137.54, 137.93, 140.25, 143.17, 151.45, 155.30, 181.74, 183.68. HRMS (EI) *m/z*: calcd $[M]^+$, 402.0497 ($C_{22}H_{14}N_2O_2S_2^+$); found, 402.0506.

5.2.20. 4-(2,4-Dimethylphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (5b)

The pure compound was obtained as an orange powder (yield 39%). Mp: 250-251 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1502.4 (aromatic C=C), 1522.4 (CO), 1591.2 (CO), 1668.3 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.42 (s, 3H), 2.46 (s, 3H), 7.20 (d, *J*= 7.8 Hz, 1H), 7.32 (s, 1H), 7.52 (s, 1H), 7.56 (d, *J*= 8.1 Hz, 1H), 7.76 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.83 (td, *J*= 7.2 Hz, *J*= 1.5 Hz, 1H), 8.16 (dd, *J*= 7.8 Hz, *J*= 1.2 Hz, 1H), 8.35 (dd, *J*= 7.5 Hz, *J*= 1.2 Hz, 1H). ^{13}C NMR(75 MHz, $CDCl_3$): δ ppm 20.51, 21.43, 119.03, 121.12, 123.55, 126.98, 127.42, 128.96, 132.32, 132.84, 133.97, 134.87, 135.78, 137.12, 141.96, 143.26, 143.53, 151.45, 155.31, 181.75, 183.76. HRMS (EI) *m/z*: calcd $[M]^+$, 402.0497 ($C_{22}H_{14}N_2O_2S_2^+$); found, 402.0497.

5.2.21. 4-(3,5-Dimethylphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (5c)

The pure compound was obtained as an orange red powder (yield 41%). Mp: 283-284 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1502.4(aromatic C=C), 1539.1(CO), 1595.0(CO), 1672.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.41 (s, 6H), 7.22 (s, 1H), 7.32 (s, 2H), 7.73 (s, 1H), 7.78 (td, *J*= 7.5 Hz, *J*= 1.2 Hz, 1H), 7.85 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.16 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.35 (dd, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR(75 MHz, $CDCl_3$): δ ppm 21.29, 119.87, 121.13, 127.00, 127.42, 132.29, 132.72,

133.35, 133.94, 134.00, 134.87, 135.75, 140.47, 144.53, 151.27, 155.13, 181.73, 183.66. HRMS (EI) *m/z*: calcd $[M]^+$, 402.0497 ($C_{22}H_{14}N_2O_2S_2^+$); found, 402.0491.

5.2.22. 4-(2,6-Dimethylphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (5d)

The pure compound was obtained as a dark brown powder (yield 35%). Mp: 213-214 ℃ (EtOH). FT-IR(KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1668.3 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δ ppm 2.48 (s, 6H), 7.30 (d, *J*= 7.8 Hz, 2H), 7.40 (s, 1H), 7.41-7.46 (m, 1H), 7.76 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.82 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.12 (dd, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H), 8.34 (dd, *J*= 7.5 Hz, *J*= 1.2 Hz, 1H). ^{13}C NMR(75 MHz, $CDCl_3$): δ ppm 21.54, 29.77, 118.29, 121.22, 126.65, 127.01, 127.43, 128.30, 129.38, 129.54, 131.22, 132.35, 134.00, 135.88, 142.34, 143.75, 144.31, 151.54, 158.87, 181.73, 183.66. HRMS (EI) *m/z*: calcd $[M]^+$, 402.0497 ($C_{22}H_{14}N_2O_2S_2^+$); found, 402.0496.

5.2.23. 4-(3,4-Dimethylphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (5e)

The pure compound was obtained as an orange powder (yield 67%). Mp: 221-222 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1541.0 (CO), 1668.3 (C=N). ^1H NMR(300 MHz, $CDCl_3$): δ ppm 2.35 (s, 3H), 2.40 (s, 3H), 7.33 (d, *J*= 7.8 Hz, 1H), 7.42-7.46 (m, 2H), 7.73 (s, 1H), 7.76 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.83 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.15 (dd, *J*= 7.2 Hz, *J*= 1.5 Hz, 1H), 8.35 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR(75 MHz, $CDCl_3$): δ ppm 19.85, 19.88, 119.22, 120.68, 123.84, 126.76, 127.21, 131.69, 131.91, 133.20, 133.56, 133.84, 134.71, 135.41, 136.54, 139.16, 139.90, 144.27, 150.93, 154.67, 181.52, 183.44. HRMS (EI) *m/z*: calcd $[M]^+$, 402.0497 ($C_{22}H_{14}N_2O_2S_2^+$); found, 402.0489.

5.2.24. 4-(2,4-Difluorophenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (5f)

The pure compound was obtained as a brown powder (yield 78%). Mp: 217-218 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1510.2 (aromatic C=C), 1589.2 (CO), 1666.4 (C=N). ^1H NMR (400 MHz, $CDCl_3$): δ ppm 7.09-7.15 (m, 2H), 7.65 (s, 1H), 7.71-7.74 (m,

1H), 7.75-7.79 (m, 1H), 7.81-7.85 (m, 1H), 8.16 (d, *J*= 7.6 Hz, 1H), 8.33 (d, *J*= 7.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 105.63, 105.89, 106.15, 110.39, 110.43, 110.58 113.40, 113.44, 113.62, 113.66, 119.20, 119.262, 121.38, 126.82, 127.22, 127.29, 131.77, 133.42, 133.95, 134.79, 134.83, 135.25, 138.69, 138.79, 140.42, 150.89, 154.62, 162.23, 163.81, 163.92, 164.75, 164.88, 166.35, 166.47, 181.36, 183.40. HRMS (EI) *m/z*: calcd $[M]^+$, 409.9995 ($C_{20}H_8F_2N_2O_2S_2^+$); found, 409.9992.

5.2.25. 4-(2,5-Dichlorophenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (5g)

The pure compound was obtained as a yellow powder (yield 77%). M.p: 248-249 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1539.1 (CO), 1670.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.52 (dd, *J*= 8.4 Hz, *J*= 2.7 Hz, 1H), 7.62 (d, *J*= 7.2 Hz, 1H), 7.72 (s, 1H), 7.77 (d, *J*= 2.7 Hz, 1H), 7.81 (td, *J*= 7.5 Hz, *J*=1.5 Hz, 1H), 7.85 (td, *J*= 7.5 Hz, *J*= 1.8 Hz, 1H), 8.20 (dd, *J*= 7.2 Hz, *J*= 1.8 Hz, 1H), 8.36 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 121.16, 122.33, 127.16, 127.52, 129.91, 132.18, 132.27, 133.92, 134.10, 134.19, 135.02, 135.66, 136.94, 138.02, 139.12, 151.40, 155.35, 181.66, 183.31. HRMS (EI) *m/z*: calcd $[M]^+$, 441.9404 ($C_{20}H_8Cl_2N_2O_2S_2^+$); found, 441.9399.

5.2.26. 4-(2,4,5-Trichlorophenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (5h)

The pure compound was obtained as a yellow powder (yield 75%). Mp: 261-262 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1542.9 (CO), 1593.1 (CO), 1670.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.76 (s, 1H), 7.79 (s, 1H), 7.81 (d, *J*= 7.5 Hz, 1H), 7.83 (t, *J*= 1.5 Hz, 1H), 7.86 (s, 1H), 8.20-8.23 (m, 1H), 8.35-8.38 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 120.61, 122.02, 126.95, 127.31, 127.44, 131.81, 132.20, 132.48, 133.46, 134.10, 134.91, 135.27, 136.20, 137.87, 138.18, 138.35, 151.00, 154.81, 181.43, 183.02. HRMS (EI) *m/z*: calcd $[M]^+$, 475.9015 ($C_{20}H_7Cl_3N_2O_2S_2^+$); found, 475.9013.

5.2.27. 4-(2-Methoxyphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (6a)

The pure compound was obtained as a yellow powder (yield 83%). Mp: 227-228 ℃ (EtOH). FT-IR(KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1670.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 3.85 (s, 3H), 7.15-4.17 (m, 2H), 7.60-7.63 (m, 2H), 7.62 (s, 1H), 7.76 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.80 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.14 (dd, *J*= 7.2 Hz, *J*= 1.2 Hz, 1H), 8.32 (d, *J*= 7.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 56.25, 112.61, 115.84, 119.61, 121.15, 122.30, 126.97, 127.42, 132.38, 133.14, 133.91, 134.06, 134.82, 135.72, 137.69, 142.52, 151.39, 155.35, 160.68, 181.71, 183.76. HRMS (EI) *m/z*: calcd $[M]^+$, 404.0289 ($C_{21}H_{12}N_2O_3S_2^+$); found, 404.0295.

5.2.28. 4-(3-Methoxyphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (6b)

The pure compound was obtained as a yellowish powder (yield 81%). Mp: 237-238 ℃ (EtOH). FT-IR(KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1544.9 (CO), 1589.2 (CO), 1668.3 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 3.87 (s, 3H), 7.14 (dd, *J*= 8.4 Hz, *J*= 2.7 Hz, 1H), 7.25 (d, *J*= 7.8 Hz, 1H), 7.30 (d, *J*= 7.8 Hz, 1H), 7.51 (t, *J*= 7.8 Hz, 1H), 7.74-7.86 (m, 3H), 8.15 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.35 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 55.71, 116.94, 120.14, 120.90, 121.47, 127.08, 127.47, 127.91, 129.25, 131.43, 132.37, 134.04, 134.91, 135.81, 143.40, 151.35, 155.22, 161.31, 181.72, 183.56. HRMS (EI) *m/z*: calcd $[M]^+$, 404.0289 ($C_{21}H_{12}N_2O_3S_2^+$); found, 404.0281.

5.2.29. 4-(4-Methoxyphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (6c)

The pure compound was obtained as an orange powder (yield 85%). Mp: 293-294 ℃ (EtOH). FT-IR(KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1593.1 (CO), 1664.5 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 3.94 (s, 3H), 7.11 (d, *J*= 8.4 Hz, 2H), 7.63 (d, *J*= 8.7 Hz, 2H), 7.69 (s, 1H), 7.77-7.83 (m, 2H), 8.17 (d, *J*= 7.2 Hz, 1H), 8.35 (d, *J*= 7.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 55.50, 116.07, 117.66, 119.30, 126.80, 127.21, 129.35, 131.94, 133.59, 133.84, 134.71, 135.41, 137.47, 144.60, 150.98,

154.62, 161.65, 181.53, 183.41. HRMS (EI) *m/z*: calcd $[M]^+$, 404.0289 ($C_{21}H_{12}N_2O_3S_2^+$); found, 404.0283.

5.2.30. 4-(3-Ethoxyphenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (6d)

The pure compound was obtained as an orange powder (yield 84%). Mp: 249-250 ℃ (EtOH). FT-IR(KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1589.2 (CO), 1670.2 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δ ppm 1.44 (t, *J*= 6.9 Hz, 3H), 4.09 (q, *J*= 6.9 Hz, 2H), 7.10-7.14 (m, 1H), 7.23 (t, *J*= 2.4 Hz, 1H), 7.27-7.30 (m, 1H), 7.49 (t, *J*= 8.0 Hz, 1H), 7.77 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.79 (s, 1H), 7.83 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.17 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.35 (dd, *J*= 7.8 Hz, *J*= 1.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 14.75, 64.11, 117.54, 120.12, 121.47, 127.08, 127.65, 127.75, 129.12, 131.40, 132.37, 134.04, 134.90, 135.83, 136.80, 143.53, 151.35, 155.22, 160.66, 181.74, 183.58. HRMS (EI) *m/z*: calcd $[M]^+$, 418.0446 ($C_{22}H_{14}N_2O_3S_2^+$); found, 418.0445.

5.2.31. 4-(4-(Methylthio)phenylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (6e)

The pure compound was obtained as a dark brown powder (yield 70%). Mp: 214-215 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1558.4 (CO), 1668.3 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δ ppm 2.58 (s, 3H), 7.41 (d, *J*= 8.4 Hz, 2H), 7.60 (d, *J*= 8.4 Hz, 2H), 7.76 (s, 1H), 7.80 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 7.83 (td, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.17 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H), 8.35 (dd, *J*= 7.5 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR(75 MHz, $CDCl_3$): 15.27, 119.69, 123.43, 127.07, 127.47, 127.75, 132.27, 133.92, 134.10, 134.95, 135.72, 136.13, 143.21, 143.77, 151.31, 153.79, 181.76, 183.63. HRMS (EI) *m/z*: calcd $[M]^+$, 420.0061 ($C_{21}H_{12}N_2O_2S_3^+$); found, 420.0069.

5.2.32. 4-(Propylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (6f)

The pure compound was obtained as a dark brown powder (yield 85%). Mp: 194-195 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1591.2 (CO), 1668.3

(C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.20 (t, *J*= 7.2 Hz, 3H), 1.94 (s, *J*= 7.2 Hz, 2H), 3.30 (t, *J*= 7.2 Hz, 2H), 7.80-7.88 (m, 2H), 8.14 (s, 1H), 8.28 (dd, *J*= 7.2 Hz, *J* =1.2 Hz, 1H), 8.36 (dd, *J*= 7.2 Hz, *J*= 1.2 Hz, 1H). ^{13}C NMR(75 MHz, $CDCl_3$): δ ppm 13.63, 21.78, 33.56, 118.47, 121.02, 127.16, 127.49, 127.60, 132.37, 134.01, 134.98, 135.64, 143.04, 151.22, 155.94, 181.73, 183.96. HRMS (EI) *m/z*: calcd $[M]^+$, 340.0340 ($C_{17}H_{12}N_2O_2S_2^+$); found, 340.0338.

5.2.33. 4-(Isopropylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (6g)

The pure compound was obtained as a yellow powder (yield 88%). Mp: 193-194 ℃ (EtOH). FT-IR(KBr, ν_{max} cm^{-1}): 1502.4 (aromatic C=C), 1539.1 (CO), 1591.2 (CO), 1668.3 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.58 (d, *J*= 6.6 Hz, 6H), 4.05 (m, 1H), 7.81-7.89 (m, 2H), 8.23 (s, 1H), 8.27-8.30 (m, 1H), 8.36-8.39 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 22.81, 36.08, 119.67, 121.17, 127.17, 127.51, 127.82, 132.40, 134.03, 134.98, 135.64, 142.33, 151.36, 156.24, 181.77, 183.95. HRMS (EI) *m/z*: calcd $[M]^+$, 340.0340 ($C_{17}H_{12}N_2O_2S_2^+$); found, 340.0345.

5.2.34. 4-(Benzylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (7a)

The pure compound was obtained as a pale brown powder (yield 88%). Mp: 243-244 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1498.6 (aromatic C=C), 1585.4 (C=O), 1666.4 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 4.56 (s, 2H), 7.31 (t, *J*= 7.2 Hz, 1H), 7.38 (t, *J*= 7.2 Hz, 2H), 7.55 (d, *J*= 7.2 Hz, 2H), 7.81 (td, *J*= 7.2 Hz, *J*= 1.6 Hz, 1H), 7.86 (dd, *J*= 7.2 Hz, *J*= 1.6 Hz, 1H), 8.26-8.28 (m, 2H), 8.36 (dd, *J*= 7.2 Hz, *J*= 1.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 36.24, 118.76, 120.01, 120.90, 126.94, 127.25, 128.02, 128.93, 129.07, 131.90, 133.57, 133.88, 134.67, 134.81, 135.24, 141.72, 150.79, 181.53, 183.48. HRMS (EI) m/z: calcd [M]+, 388.0340 ($C_{21}H_{12}N_2O_2S_2^+$); found, 388.0340.

5.2.35. 4-(2-Chlorobenzylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (7b)

The pure compound was obtained as a dark yellowish orange powder (yield 81%).

Mp: 254-255 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1508.2 (aromatic C=C), 1542.9 (CO), 1674.1 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 4.67 (s, 2H), 7.25-7.28 (m, 2H), 7.44-7.47 (m, 1H), 7.56-7.59 (m, 1H), 7.80-7.86 (m, 2H), 8.26 (dd, *J*= 7.35 Hz, *J*= 1.5 Hz, 1H), 8.27 (s, 1H), 8.35 (dd, *J*= 7.35 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 33.92, 118.59, 121.00, 126.92, 127.21, 127.26, 129.52, 130.05, 130.90, 131.85, 132.49, 133.52, 133.86, 134.55, 134.77, 135.23, 141.42, 150.75, 155.28, 181.741, 183.34. HRMS (EI) *m/z*: calcd $[M]^+$, 421.9950 ($C_{21}H_{11}ClN_2O_2S_2^+$); found, 421.9954.

5.2.36. 4-(4-Chlorobenzylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dion (7c)

The pure compound was obtained as a light yellow powder (yield 82%). Mp: 261-262 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1510.2 (aromatic C=C), 1670.2 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 4.52 (s, 2H), 7.34 (d, *J*= 8.0 Hz , 2H), 7.48 (d, *J*= 7.6 Hz, 2H), 7.80-7.88 (m, 2H), 8.24 (s, 1H), 8.27 (d, *J*= 7.6 Hz, 1H), 8.36 (d, *J*= 7.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 35.53, 119.00, 126.97, 127.28, 129.12, 130.36, 131.88, 133.35, 133.58, 133.93, 134.85, 135.19, 140.99, 150.82, 155.33, 181.48, 183.40. HRMS (EI) *m/z*: calcd $[M]^+$, 421.9950 ($C_{21}H_{11}ClN_2O_2S_2^+$); found, 421.9942.

5.2.37. 4-(4-Tert-butylbenzylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dion (7d)

The pure compound was obtained as an orange yellow powder (yield 62%). Mp: 256-257 ℃ (EtOH). FT-IR(KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1544.9 (CO), 1670.2 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.31 (s, 9H), 4.53 (s, 2H), 7.39 (d, *J*= 8.4 Hz, 2H), 7.35 (d, *J*= 8.4 Hz, 2H), 7.83 (td, *J*= 1.8 Hz, *J*= 7.5 Hz, 2H), 8.27-8.30 (m, 2H), 8.36 (d, *J*= 7.2 Hz, 1H). ^{13}C NMR(75 MHz, $CDCl_3$): δ ppm 31.29, 34.60, 35.89, 35.89, 118.61, 120.84, 125.89, 126.96, 127.27, 128.83, 131.52, 131.94, 133.62, 133.88, 134.81, 135.28, 142.09, 151.09, 181.56, 183.55. HRMS (EI) *m/z*; calcd $[M]^+$, 444.0966 ($C_{25}H_{20}N_2O_2S_2^+$); found, 444.0970.

5.2.38. 4-(Phenethylthio) anthra[1,2-c][1,2,5]thiadiazole-6,11-dione (7e)

The pure compound was obtained as a yellow powder (yield 62%). Mp: 119-120 ℃ (EtOH). FT-IR (KBr, ν_{max} cm^{-1}): 1506.3 (aromatic C=C), 1542.9 (C=O), 1668.3 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 3.19 (t, *J*= 7.5 Hz, 2H), 3.58 (t, *J*= 7.5 Hz, 2H), 7.23-7.30 (m, 1H), 7.33-7.37 (m, 4H), 7.79-7.88 (m, 2H), 8.22 (s, 1H), 8.29 (dd, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H), 8.37 (dd, *J*= 7.8 Hz, *J*= 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 33.07, 34.85, 118.73, 121.16, 125.79, 127.17, 127.20, 127.41, 127.49, 127.68, 128.88, 129.07, 132.32, 134.03, 134.48, 134.98, 135.19, 135.58, 139.43, 142.28, 151.21, 155.92, 181.67, 183.80. HRMS (EI) *m/z*: calcd $[M]^+$, 402.0497 ($C_{22}H_{14}N_2O_2S_2^+$); found, 402.0491.

5.3. Cell Cultures and Sulforhodamine B (SRB) assay

Human hormone-refractory prostate cancer cell lines (PC-3) were from American Type Culture Collection (Rockville, MD). The cells were cultured in RPMI1640 medium with 10% FBS (v/v) and penicillin (100 units/ml)/streptomycin (100 μg/ml). Cultures were maintained in a humidified incubator at 37 ^{o}C in 5% CO_2. Cells were seeded in 96-well plates in medium with 5% FBS. After 24 h, cells were fixed with 10% trichloroacetic acid (TCA) to represent cell population at the time of compound addition (T_0). After additional incubation of vehicle (0.1% DMSO) or the indicated compound for 48 h, cells were fixed with 10% TCA and SRB at 0.4% (w/v) in 1% acetic acid was added to stain cells. Unbound SRB was washed out by 1% acetic acid and SRB bound cells were solubilized with 10 mM Trizma base. The absorbance was read at a wavelength of 515 nm. Using the following absorbance measurements, such as time zero (T_0), control growth (C), and cell growth in the presence of compound (T_X), the percentage growth was calculated at each of the compound concentrations levels. Percentage growth inhibition was calculated as: $100 - [(T_X-T_0)/(C-T_0)] \times 100$ for concentrations for which $T_X \geq T_0$. Growth inhibition of 50% (IC_{50}) was determined at the drug concentration which resulted in 50% reduction of total protein increased in

control cells during the compound incubation.

5.4. Cell Cultures and MTT assay for cell viability

The Prostate cancer cell line (DU-145 cells) and human urothelial cell line (SV-HUC-1) were used in this study. DU-145 was maintained in RPMI-1640 culture medium and SV-HUC-1 was maintained in Ham's F-12k culture medium containing 5% heat inactivated fetal calf serum in an atmosphere of 5% CO_2 in a humidified incubator at 37℃.The tetrazolium reagent (MTT; 3-(4,5-di-methylthiazol)-2,5-diphenyltetrazolium bromide, USB) was designed to yield a colored formazan upon metabolic reduction by viable cells. Approximately 2×10^3 cells were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37℃ for 24 h. To assess the *in vitro* cytotoxicity, each compound was dissolved in DMSO and prepared immediately before the experiments and was diluted into the complete medium before addition to cell cultures. Test compounds or vehicle (0.1% DMSO) were then added to the culture medium for designated various concentrations. After 72 h, an amount of 100 μL of MTT solution was added to each well, and the samples were incubated at 37℃ for 4 h. A 100 μL solution of DMSO was added to each well and incubated at 37℃ for another 3 h. The absorbency at 570 nm was measured using an ELISA reader.

5.5. Cell morphology

The DU-145 cells were plated at density 5×10^5 cells per well in a 10 cm dish and then with various concentration of **6g** for 24 hour. Cells were directly examined and photographed under a phase contrast microscope.

5.6. Western blot assay

Treated cells were collected and washed with PBS. After centrifugation, cell were lysed in a lysis buffer. The lysates were incubated on ice for 30 min and centrifuged at

120000g for 15 min. Supernatants were collected, and protein concentrations were then determined using Bradford assay. The protein samples were electrophoresed on 12% SDS-polyacrylamide gels and transferred to a PVDF membrane. Immuno-reactivity was detected using western blot chemiluminescence regent system. β-actin was used as a loading control.

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Table 1.

No.	SRB assay	MTT assay	No.	SRB assay	MTT assay
	PC-3 (μM)	DU-145 (μM)		PC-3 (μM)	DU-145 (μM)
2	2.5	7.41	**5a**	7.44	11.97
3	13.5	6.68	**5b**	>15	15.89
4a	>15	>15	**5c**	>15	>15
4b	>15	7.64	**5d**	6.74	7.88
4c	>15	>15	**5e**	>15	14.09
4d	5.76	9.58	**5f**	14.8	9.12
4e	8.82	12.68	**5g**	>15	>15
4f	5.64	1.75	**5h**	>15	>15
4g	12.4	10.68	**6a**	>15	12.91
4h	6.7	11.12	**6b**	>15	>15
4i	>15	>15	**6c**	>15	8.26
4j	>15	13.28	**6d**	>15	>15
4k	>15	>15	**6e**	>15	>15
4l	>15	>15	**6f**	5.39	2.55
4m	>15	>15	**6g**	3.69	4.53
4n	>15	14.60	**7a**	>15	6.35
4o	>15	13.85	**7b**	5.48	6.38
4p	>15	>15	**7c**	>15	7.51
4q	7.5	2.52	**7d**	9.5	3.66
4r	>15	8.38	**7e**	>15	9.65

[a] IC_{50} is the concentration of drug (μM) required to inhibit cell growth by 50% of the mean (n = 3).

Table 2.

Panel/Cell Line	2 NSC745885	3 NSC757963	4a NSC75796	4d NSC761882	4f NSC761884	4i NSC757966	4k NSC761890	5b NSC761881	5c NSC761891	5d NSC763955
Leukemia										
CCRF-CEM	-30.81	-41.78	15.74	N.T.	N.T.	79.34	N.T.	N.T.	N.T.	34.84
HL-60(TB)	-21.77	1.87	83.94	-43.96	N.T.	87.34	79.53	99.93	73.66	47.83
K562	49.61	2.44	52.03	29.66	N.T.	94.91	32.30	67.97	75.57	43.14
MOLT-4	-40.09	-34.12	31.08	29.67	N.T.	59.21	67.31	91.01	71.40	32.64
RPMI-8226	-27.97	27.81	78.21	9.40	N.T.	90.15	48.56	85.14	89.13	55.63
SR	4.96	-23.86	86.41	2.94	N.T.	110.89	23.00	70.26	78.44	35.52
Non-small cell lung cancer										
A549/ATCC	104.18	103.21	105.68	95.37	107.48	103.88	104.68	109.80	107.23	90.16
EKVX	103.18	82.19	97.77	77.74	43.65	94.80	104.56	108.79	100.45	82.44
HOP-62	-100.00	75.8	110.22	4.88	42.12	101.98	59.51	79.34	90.96	90.01
HOP-92	N.T.	-52.25	117.67	37.57	N.T.	94.77	89.91	97.83	93.51	N.T.
NCI-H226	97.80	100.39	101.59	92.07	78.07	104.26	95.71	01.50	87.97	78.31
NCI-H23	16.07	75.02	79.21	18.02	-26.91	100.11	53.21	89.40	90.39	67.37
NCI-H322M	125.82	108.2	100.74	N.T.	N.T.	99.52	N.T.	N.T.	N.T.	83.83
NCI-H460	77.41	100.18	100.56	58.24	N.T.	102.62	88.86	102.01	104.64	87.37
NCI-H552	N.T.	89.53	94.91	30.77	7.18	93.41	74.81	96,81	105.78	61.39
Colon cancer										
COLO 205	111.54	99.08	117.53	97.36	105.98	121.41	99.23	106.63	106.04	98.76
HCC-2998	-0.74	105.67	110.88	78.44	101.66	106.82	124.00	121.23	108.80	100.96
HCT-116	-50.00	48.15	90.08	16.62	-67.84	98.25	92.15	94.55	92.29	64.99
HCT-15	1.48	96.8	93.89	39.74	17.97	98.10	38.57	83.04	90.91	47.54
HT29	116.95	N.T.	N.T.	96.46	102.17	N.T.	104.31	101.47	104.91	97.73
KM12	98.60	95.88	99.5	57.85	N.T.	101.50	103.11	96.42	105.36	95.96
CNS cancer										
SF-268	16.29	37.03	102.24	-5.11	N.T.	104.55	74.73	90.61	108.19	79.48
SF-295	126.17	109.08	106.36	100.07	N.T.	97.26	104.92	N.T.	91.11	91.11
SF-539	-47.11	108.52	106.54	-5.66	-52.72	107.00	78.04	100.22	94.94	84.51
SNB-19	78.98	105.75	108.79	71.38	56.88	105.18	103.54	102.33	106.60	94.75
SNB-75	79.03	85.25	96.12	47.20	26.08	91.73	76.30	76.05	80.61	69.38
U251	-89.26	29.19	88.75	11.49	-69.50	103.69	62.36	104.99	107.58	53.94
Melanoma										
LOX IMVI	-50.63	10.23	42	-15.34	-59.25	83.24	30.01	90.38	90.18	44.61
MALME-3M	-71,64	97.09	106.43	35.27	-86.56	105.10	96.77	108.23	101.93	73.72
M14	74.54	102.73	106.41	59.45	-76.44	105.44	94.25	100.77	100.28	121.55
MDA-MB-435	-87.88	N.T.	N.T.	-72.95	-82.33	106.67	80.61	105.58	104.60	96.77
SK-MEL-2	-73.16	0.21	112.49	35.02	-53.17	109.49	73.29	98.52	107.90	67.54
SK-MEL-28	15.47	131.75	127.12	64.65	24.96	130.48	105.73	118.64	111.81	89.33
SK-MEL-5	8.52	72.45	85.68	46,26	19.02	N.T.	87.54	109.99	107.45	70.23
UACC-257	-83.07	76.09	92.75	4.75	-84.59	95.45	87.32	121.93	115.60	86.57
UACC-62	-82.32	72.91	86.28	39.58	-64.98	90.65	90.69	94.50	102.82	91.72
Ovarian cancer										
IGROV1	-88.34	52.47	84.4	27.94	-13.50	84.93	80.06	95.00	96.70	66.38
OVCAR-3	-15.47	49.88	96.67	-65,85	N.T.	118.64	5.30	105.38	118.49	1.00
OVCAR-4	-94.43	-75.03	57.83	N.T.	N.T.	76.82	N.T.	N.T.	N.T	4.00
OVCAR-5	-20.07	N.T.	N.T.	120.52	108.55	N.T.	126.28	106.46.	107.20	113.96
OVCAR-8	-10.07	-4.57	82.3	2.47	-22.92	88.60	76.29	104,59	92.19	58.98
NCI/ADR-RES	-2.53	N.T.	N.T.	-29,94	-15.13	102.42	59.53	97.75	97.53	59.68
SK-OV-3	100.70	109.05	105.6	66.68	95.44	108.96	93.79	110.36	97.49	N.T.
Renal cancer										
786-0	-25.98	102.14	110.04	-6.19	-2.06	108.70	100.36	105.11	98.25	115.19
A498	130.09	93.79	95.01	24.25	N.T.	93.03	85.54	84.37	93.56	N.T.
ACHN	-94.31	N.T.	N.T.	25,50	-92.53	N.T.	89.76	94.24	98.02	83.18
CAKI-1	1.97	96.51	89.04	29.67	-3.69	86.97	81.47	N.T.	91.05	61.03
RXF 393	20.11	71.92	114.31	-8.53	-27.84	117.52	95.88	105.93	112.19	76.16
SN12C	-73.27	71.92	96.58	22.56	-66.17	86.95	85.97	98.68	104.90	67.67
TK-10	96.99	134.06	143.47	76.52	53.81	139.80	122.97	127.11	131.58	119.41
UO-31	-79.30	80.15	80.12	56.15	-74.79	79.46	62.14	65.72	69.14	66.73
Prostate cancer										
PC-3	N.T.	23.54	87.32	4.23	N.T.	86.94	73.26	76.11	81.30	59.94
DU145	40.76	96.95	102.73	50.57	N.T.	117.15	101.53	109.69	122.43	106.29
Breast cancerc										
MCF7	-50.74	65.36	94.88	-6.39	-69.36	86.11	66.12	79.77	75.84	68.59
MDA-MB-231/ATCC	-17.33	-23.35	66.67	16.85	-29.08	89.05	60.72	92.27	99.22	30.57
HS 578T	7.40	72.54	95.89	54.25	N.T.	103.37	83.69	99.06	104.51	82.43
BT-549	N.T.	101.5	104.68	52.19	36.21	98.88	95.35	94.55	95.06	105.06
T-47D	-45.52	-54.59	93.21	-33.05	-42.26	85.04	51.89	75.36	81.07	66.81
MDA-MB-468	-73.17	-88,31	87.29	-79.88	-79.19	46.13	53.68	100.45	116.25	45.85
Mean	2.68	54.91	93.48	29.61	-5.75	98.08	80.61	97.26	98.32	73.30
Delta	102.68	143.22	77.74	109.49	86.78	51.95	75.31	31.54	29.18	72.30
Range	230.09	222.37	123.73	200.40	201.08	93.67	120.98	61.39	62.44	120.55

Table 2 *(continued)*

Panel/Cell Line	5f NSC761885	5g NSC761883	6b NSC761889	6c NSC761886	6d NSC761892	6e NSC761887	6g NSC763968	7a NSC757965	7c NSC763956
Leukemia									
CCRF-CEM	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	-45.05	0.65	N.T.
HL-60(TB)	-37.71	72.72	84.53	102.73	N.T.	105.25	-41.24	70.32	57.42
K562	-21.06	47.80	38.52	91.98	N.T.	67.50	-26.17	10.51	67.53
MOLT-4	-41.57	1.27	35.83	82.89	N.T.	59.93	-54.56	-20.97	22.72
RPMI-8226	6.29	77.16	52.03	101.16	N.T.	88.58	-38.03	43.23	86.78
SR	0.20	41.15	36.14	101.71	N.T.	96.31	-47.97	1.25	63.73
Non-small cell lung cancer									
A549/ATCC	111.54	102.75	109.52	103.32	111.48	104.69	-8.14	101.64	100.62
EKVX	98.12	109.03	104.94	148.76	102.26	113.82	-6,58	94.98	N.T.
HOP-62	58.46	63.18	61.81	95.20	91.94	102.09	13.18	83.65	89.15
HOP-92	-18.39	65.12	100.52	91.38	N.T.	04.31	N.T.	-14.36	N.T.
NCI-H226	88.85	112.00	95.26	79.51	90.47	79.69	10.21	101.09	82.14
NCI-H23	9.53	74.59	76.42	89.13	94.07	81.49	-42.19	55.30	82.13
NCI-H322M	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	-0.80	98.44	94.10
NCI-H460	83.60	100.98	100.27	107.16	N.T.	106.20	4.92	92.01	104.55
NCI-H552	61.06	91.29	82.41	95.69	98.19	95.39	-59.54	78.90	82.61
Colon cancer									
COLO 205	104.04	109.65	109.57	112.25	106.49	117.30	102.47	102.93	99.94
HCC-2998	107.02	109.37	104.94	106.85	105.49	111.01	21.00	115.02	102.54
HCT-116	-63.06	81.94	85.66	94.64	100.93	101.97	-36.70	53.06	114.73
HCT-15	45.84	92.85	71.89	109.35	103.08	111.56	15.31	54.72	97.91
HT29	102.45	106.27	105.25	101.30	106.40	102.26	48.08	N.T.	96.41
KM12	51.79	101.18	97.06	101.28	N.T.	100.42	19.33	103.02	105.46
CNS cancer									
SF-268	8.47	82.95	89.75	94.81	N.T.	89.67	7.06	56.01	107.19
SF-295	68.77	78.93	N.T.	N.T.	N.T.	98.897	-16.57	91.56	N.T.
SF-539	58.79	88.74	107.05	93.53	93.72	96.48	-47,01	68.26	97.95
SNB-19	87.35	84.39	105.92	113.97	103.22	101.61	21.85	94.26	104.30
SNB-75	42.86	47.00	81.80	79.05	77.25	75.08	9.12	39.58	63.35
U251	34.47	63.57	100.67	102.73	109.69	103.89	-23.10	30.51	94.44
Melanoma									
LOX IMVI	-42.03	73.05	70.96	90.24	84,11	68.98	-21.59	7.49	86.69
MALME-3M	39.46	94.30	113.06	89.52	108.70	104.58	-86.04	80.81	86.38
M14	53.77	98.95	94.23	92.89	103.80	107.55	-31.54	86.54	126.40
MDA-MB-435	-85.27	105.64	72.50	118.61	106.07	118.40	-79.28	-48.29	N.T.
SK-MEL-2	51.79	101.38	82.62	99.24	102.98	89.27	-55.62	-3.95	109.63
SK-MEL-28	79.45	122.69	101.77	115.63	112.20	119.66	-8.32	95.00	95.35
SK-MEL-5	48.53	108.82	100.11	106.14	106.82	101.45	-99.20	22.53	89.57
UACC-257	43.93	119.38	104/27	118.11	126.28	109.51	-91.69	58.86	78.70
UACC-62	53.27	94.09	87.72	108.99	108.85	110.99	-57.09	43.08	101.52
Ovarian cancer									
IGROV1	975	78.13	99.91	103.17	94.67	95.67	-32.91	43.88	84.25
OVCAR-3	-52.32	99.41	85,83	109.57	N.T.	102.89	-60.32	25.27	113.46
OVCAR-4	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	-100.00	-68.15	84.19
OVCAR-5	110.21	108.82	122.58	103.00	116.60	108.31	-27.85	N.T.	104.48
OVCAR-8	0.32	85.13	97.32	99.50	112.41	97.37	-22.64	6.01	94.19
NCI/ADR-RES	-26.24	83.13	92.67	97.60	101.92	91.79	-24.93	62.34	95.73
SK-OV-3	92.58	84.09	110.30	106.18	99.36	102.46	N.T.	80.29	N.T.
Renal cancer									
786-0	3.74	67.49	102.97	94.93	100.25	109.83	12.37	92.60	117.98
A498	50.80	87.70	83.74	84.77	N.T.	56.04	N.T.	88.61	N.T.
ACHN	34.93	88.50	98.70	102.26	103.33	103.64	0.80	N.T.	94.35
CAKI-1	24.02	108.19	N.T.	108.29	84.23	99.44	-5.68	73.77	N.T.
RXF 393	36,69	91.47	124.15	109.28	118.35	106.98	13.64	68.12	104.79
SN12C	9.12	96.65	95.93	106.54	104.76	98.04	-28.62	66.11	93.61
TK-10	111.93	111.76	130.80	135.79	125.26	128.10	30.33	129.90	121.91
UO-31	70.30	91.54	75.49	68.31	76.69	67.56	-28.43	73.75	68.47
Prostate cancer									
PC-3	-9.72	80.59	75.99	82.81	N.T.	78.37	-13.43	38.44	82.91
DU145	41.32	102.44	98.87	112.48	N.T.	101.05	6.66	82.23	115.38
Breast cancerc									
MCF7	6.58	85.16	72.77	93.78	78.81	82.87	-68.82	56.44	82.97
MDA-MB-231/ATCC	-9.73	82.44	87.08	87.58	100.81	78.80	-35.94	-13.36	95.23
HS 578T	50.08	72.51	93.84	96.71	N.T.	96.47	-9.16	59.96	88.59
BT-549	66.40-	79.66	94.91	92.92	103.88	107.82	10.84	96.19	115.07
T-47D	-28.61	63.44	69.09	89.66	86.51	86.56	-24.37	-46.11	83.77
MDA-MB-468	-86.96	90,73	93.53	112.95	108.00	82.25	-78.76	-85.29	83.39
Mean	32.54	87.13	91.30	100.77	101.69	96.80	-21.64	51.63	92.68
Delta	119.50	85.86	55.47	32.46	25.00	40.76	78.36	136.92	69.96
Range	198.89	121.42	94.97	80.45	49.59	72.06	202.47	215.19	103.68

[a] Data obtained from NCI *in vitro* 60-cell drug screen program at 10^{-5} molar concentration.

[b] N.T. = No test.

Table 3.

Panel/Cell Line (μM)	**4d** (NSC761882)			**4f** (NSC761884)			**5f** (NSC761885)			**6g** (NSC763968)			**7a** (NSC757965)		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Leukemia															
CCRF-CEM	4.88	>50	>50	0.56	6.26	>100	0.70	6.35	57.8	0.21	0.58	>100	1.30	5.06	>50.0
HL-60(TB)	17.8	>50	>50	2.51	6.71	>100	2.68	7.70	>100	0.23	0.617	40.8	1.63	>50	>50.0
K-562	18.5	>50	>50	2.41	7.27	>100	3.32	11.8	68.4	0.26	1.35	14.3	1.47	6.50	>50.0
MOLT-4	7.21	41.2	>50	0.64	3.27	>100	1.11	5.46	>100	0.18	0.49	>100	1.20	3.62	>50.0
RPMI-8226	11.3	>50	>50	1.42	6.32	>100	2.20	8.08	63.9	0.29	1.78	>100	8.45	33.4	>50.0
SR	17.0	>50	>50	2.00	8.09	>100	2.97	9.06	52.7	0.31	1.19	7.46	1.99	13.1	>50.0
Non-small cell lung cancer															
A549/ATCC	>50	>50	>50	6.66	24.4	73.6	14.9	42.4	>100	2.43	7.94	51.4	>50	> 50.0	> 50.0
EKVX	>50	>50	>50	16.0	29.7	55.0	20.8	66.5	>100	-	-	-	14.2	26.3	> 50.0
HOP-62	13.3	30.4	>50	1.85	4.04	8.81	1.82	3.30	6.01	2.10	6.53	24.2	10.7	13.6	30.4
HOP-92	18.4	>50	>50	2.30	5.23	15.8	2.03	5.01	16.3	0.32	2.18	14.5	6.05	> 50.0	> 50.0
NCI-H226	19.4	>50	>50	2.39	6.06	57.5	3.09	11.1	59.1	1.83	4.64	14.6	>50.0	35.2	> 50.0
NCI-H23	13.5	>50	>50	1.00	3.58	22.8	1.88	6.99	43.1	0.56	2.58	8.48	8.25	> 50.0	> 50.0
NCU-H322M	>50	>50	>50	18.6	33.9	61.9	96.5	>100	>100	2.85	11.0	38.7	>50.0	> 50.0	> 50.0
NCI-H46-	36.1	>50	>50	4.29	18.9	75.8	25.9	>100	>100	2.02	8.33	40.5	>50.0	16.8	32.8
NCI-H522	14.9	>50	>50	1.84	4.26	9.91	3.76	12.4	37.1	2.38	4.87	9.96	8.64	> 50.0	> 50.0
Colon Cancer															
COLON 205	>50	>50	>50	20.0	35.0	61.3	13.7	27.7	56.2	13.3	20.6	51.0	35.5	>50.0	>50.0
HCC2998	>50	>50	>50	16.2	31.7	62.1	11.3	25.3	56.4	3.46	11.1	34.9	>50.0	>50.0	>50.0
HCT-116	7.87	19.9	>50	0.44	2.66	16.3	1.55	3.22	6.69	0.29	1.01	4.22	7.29	14.2	27.5
HCT-15	12.3	>50	>50	2.71	11.3	47.1	2.84	12.9	50.0	1.69	6.91	40.4	8.27	17.1	35.3
HT-29	>50	>50	>50	20.1	66.7	>100	8.72	36.3	>100	12.2	33.6	92.7	>50.0	>50.0	>50.0
KM12	41.9	>50	>50	12.0	28.0	65.7	9.91	77.3	>100	3.19	11.3	41.1	19.5	>50.0	>50.0
SW-620	23.6	>50	>50	2.29	5.21	28.3	3.99	16.3	69.5	0.43	2.21	11.7	9.99	18.7	35.1
CNS Cancer															
SF-295	40.6	>50	>50	7.84	22.9	57.9	9.0	44.2	>100	1.88	4.30	9.83	>50.0	>50.0	>50.0
SF-539	10.3	20.5	40.5	2.19	4.07	7.55	2.08	4.24	8.63	1.73	3.51	7.14	10.5	20.2	38.9
SNB-19	32.2	>50	>50	8.64	21.2	47.1	13.9	30.9	68.6	3.32	13.1	36.3	12.7	38.8	>50.0
SNB-75	14.9	>50	>50	1.99	6.23	23.7	1.57	4.22	13.1	0.52	3.95	23.9	8.26	16.0	31.0
U251	10.9	31.7	>50	1.70	4.00	9.39	1.90	4.35	9.94	1.48	3.16	6.75	6.46	14.0	30.3
Melanoma															
LOX IMVI	12.6	>50	>50	0.54	2.08	5.73	1.21	2.64	5.77	0.34	1.31	4.73	5.68	12.8	29.1
MALME-3M	16.8	40.2	>50	1.98	3.68	6.85	3.26	8.65	41.7	1.12	2.38	5.03	11.0	22.2	44.6
M14	17.4	>50	>50	1.66	3.39	6.94	3.72	15.8	43.0	1.54	3.18	6.53	26.0	>50.0	>50.0
MDA-MB-435	9.23	17.4	32.7	1.53	3.33	7.24	1.94	3.70	7.07	0.20	0.39	0.74	9.19	18.5	37.3
SK-MEL-2	13.8	34.1	>50	2.34	4.81	9.87	2.34	4.69	9.41	0.99	2.42	5.87	8.58	18.6	33.7
SK-MEL-28	20.9	>50	>50	1.93	4.19	9.09	1.87	3.81	7.75	2.72	5.99	18.8	10.3	17.5	42.9
SK-MEL-5	5.08	11.1	24.3	0.99	2.21	4.87	1.41	2.84	5.72	1.29	2.60	5.23	7.17	21.2	49.8
UACC-257	8.56	20.9	>50	1.52	3.15	6.54	1.59	4.64	18.0	0.57	1.88	4.47	9.05	17.1	35.4
UACC-62	12.0	40.8	>50	1.60	3.26	6.54	1.73	4.37	12.9	1.35	2.67	5.26	8.22	18.6	33.7
Ovarian Cancer															
IGROV1	19.1	>501	>50	2.07	5.37	26.7	2.93	10.2	46.6	0.96	3.01	9.18	6.06	15.4	38.9
OVCAR-3	10.2	22.8	>50	0.45	1.74	6.5	1.82	3.44	6.52	0.25	0.638	2.98	8.16	15.1	27.9
OVCAR-4	2.78	10.1	30.1	0.31	0.94	6.71	0.34	1.38	4.18	0.36	1.34	3.67	6.05	13.2	29.0
OVCAR-5	3.44	>50	>50	2.74	6.08	68.2	8.18	24.0	63.3	2.07	4.05	7.94	-	-	-
OVCAR-8	11.1	>50	>50	0.59	2.26	6.81	1.58	3.63	8.34	0.49	2.37	9.85	3.50	25.8	>50.0
NCI/ADR-RES	10.4	36.9	>50	0.50	4.59	>100	1.74	4.59	>100	0.52	3.20	>100	12.9	>50.0	>50.0
SK-OV-3	21.0	>50	>50	3.97	12.1	34.8	2.34	5.08	13.0	2.90	12.6	36.9	13.7	18.5	>50.0
Renal Cancer															
786-0	11.2	29.7	>50	2.91	8.97	31.1	1.85	3.53	6.75	2.75	8.14	34.9	12.3	25.4	>50.0
A489	10.7	>50	>50	13.0	29.2	6.54	2.26	58.5	>100	0.56	4.28	23.6	>50.0	>50.0	>50.0
ACHN	12.8	32.5	>50	1.62	3.27	6.63	1.85	3.63	7.14	1.25	3.26	8.50	-	-	-
CAKI-1	17.2	>50	>50	2.67	9.15	>100	2.51	7.04	>100	0.59	2.59	9.08	9.68	26.2	>50.0
RXF 393	9.14	19.6	42.2	2.15	4.70	10.9	2.02	3.69	6.67	1.77	4.83	16.9	7.20	14.0	27.4
SN12C	10.5	39.7	>50	1.05	2.58	6.35	1.64	4.96	19.4	0.66	2.41	7.09	7.16	14.5	29.3
TK-10	29.7	>50	>50	5.31	14.8	39.9	5.07	14.1	38.6	5.68	18.0	87.6	32.0	>50.0	>50.0
UO-31	14.9	>50	>50	1.78	4.16	9.73	3.03	15.0	42.6	1.16	3.56	11.9	7.83	18.5	43.5
Prostate Cancer															
PC-3	14.8	>50	>50	2.27	11.8	69.9	1.96	6.41	85.7	0.25	1.38	75.4	10.8	43.4	>50.1
DU-145	21.5	>50	>50	2.27	5.09	14.3	6.30	18.7	45.0	1.45	6.64	26.4	10.2	19.9	38.8
Breast Cancer															
MCF7	7.28	16.6	37.8	1.30	3.37	8.78	1.31	3.11	7.39	1.71	3.80	8.42	8.33	17.0	34.7
MDA-MB-231/ATCC	7.52	37.3	>50	0.37	4.10	31.6	1.20	3.61	12.9	0.29	1.41	4.90	5.14	13.4	34.8
HS 578-T	17.8	>50	>50	1.97	6.55	>100	2.81	>100	>100	1.62	6.27	>100	17.3	>50.0	>50.0
BT-549	20.5	>50	>50	3.27	15.1	40.3	3.99	17.2	43.0	2.19	8.38	29.8	15.2	28.4	>50.0
T-47D	3.70	18.3	>50	1.29	3.35	8.72	0.86	2.57	7.02	0.38	2.15	9.11	1.16	5.29	42.9
MDA-MB-468	0.95	2.00	4.20	0.95	2.23	5.10	0.45	1.52	4.19	0.37	1.42	4.39	0.89	1.93	4.21

Concentration required for 50% inhibition of cell growth (GI_{50}), total growth inhibition (TGI) and 50% lethal concentration (LC_{50}).

Table 4.

Compounds / Subpanel Cell lines	**4d** (NSC761882)		**4f** (NSC761884)		**5f** (NSC761885)		**6g** (NSC763968)		**7a** (NSC757965)	
	Subpanel MID[b]	Selectivity ratio	Subpanel MID[b]	Selectivity ratio	Subpanel MID[b]	Selectivity ratio	Subpanel MID[b]	Selectivity ratio	Subpanel MID[b]	Selectivity ratio
Leukemia	12.78	1.23	1.59	2.31	2.16	2.51	0.25	6.44	2.67	3.66
Non-small cell lung cancer	19.27	0.82	5.84	0.63	18.96	0.28	1.81	0.89	9.57	1.02
Colon cancer	21.42	0.73	10.53	0.35	7.43	0.71	4.94	0.33	16.11	0.61
CNS cancer	21.78	0.72	4.47	0.82	5.69	0.93	1.79	0.90	9.48	1.03
Melanoma	12.93	1.22	1.56	2.36	2.12	2.49	1.12	1.44	10.58	0.92
Ovarian cancer	11.15	1.41	1.52	2.42	2.70	1.96	1.08	1.49	8.40	1.16
Renal cancer	14.52	1.08	3.81	0.97	2.53	2.09	1.58	1.02	12.70	0.77
Prostate Cancer	18.15	0.87	2.27	1.62	4.13	1.28	0.85	1.89	10.50	0.93
Breast cancer	9.62	1.64	1.52	2.42	1.77	2.98	1.09	1.52	8.00	1.22
MID[a]	15.74		3.68		5.28		1.61		9.78	

MID[a] = Average sensitivity of all cell line in μM.

MID[b] = Average sensitivity of all cell line of a particular subpanel in μM.

Selectivity ratio= MID[a] : MID[b]

Scheme 1.

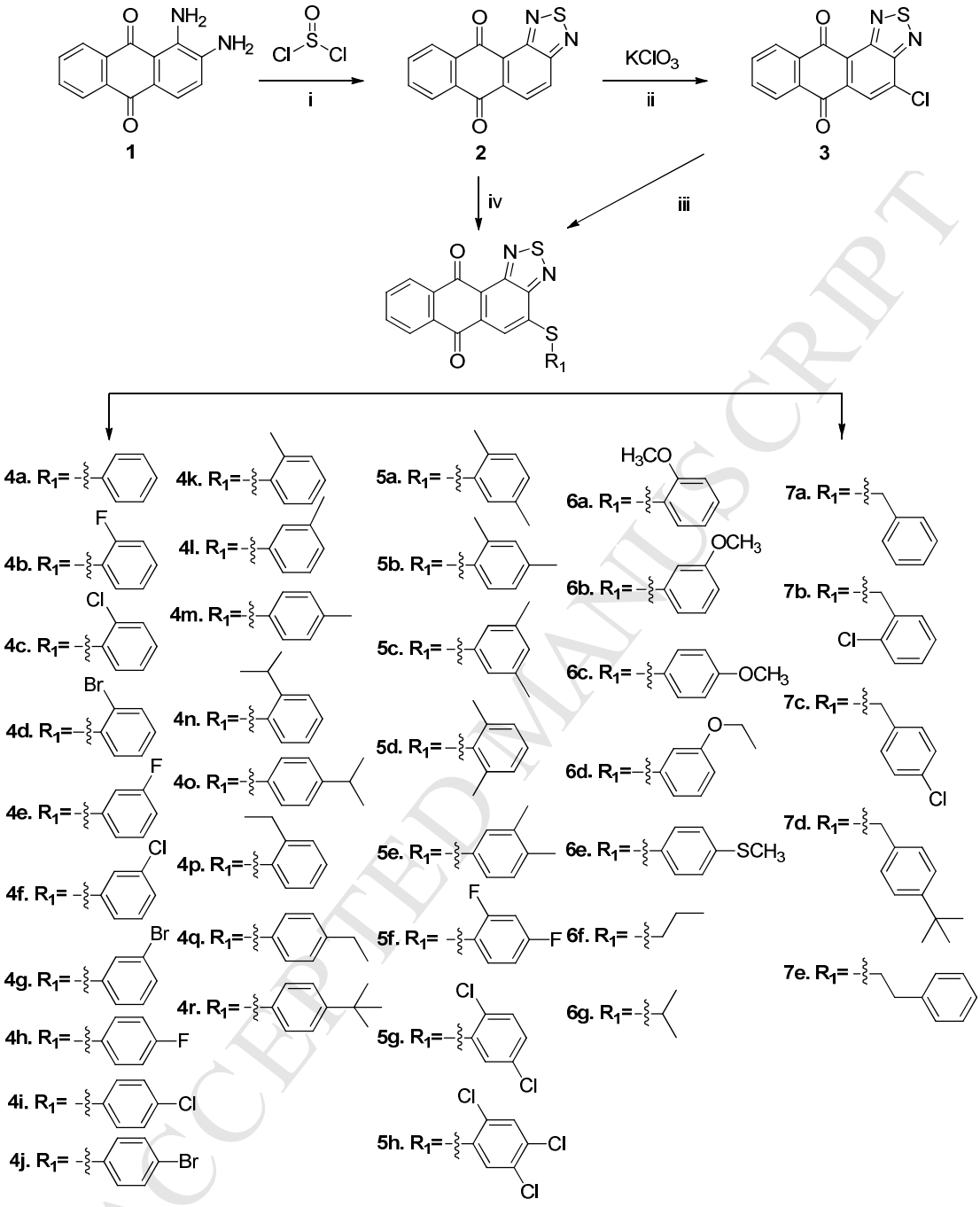


Figure 1:

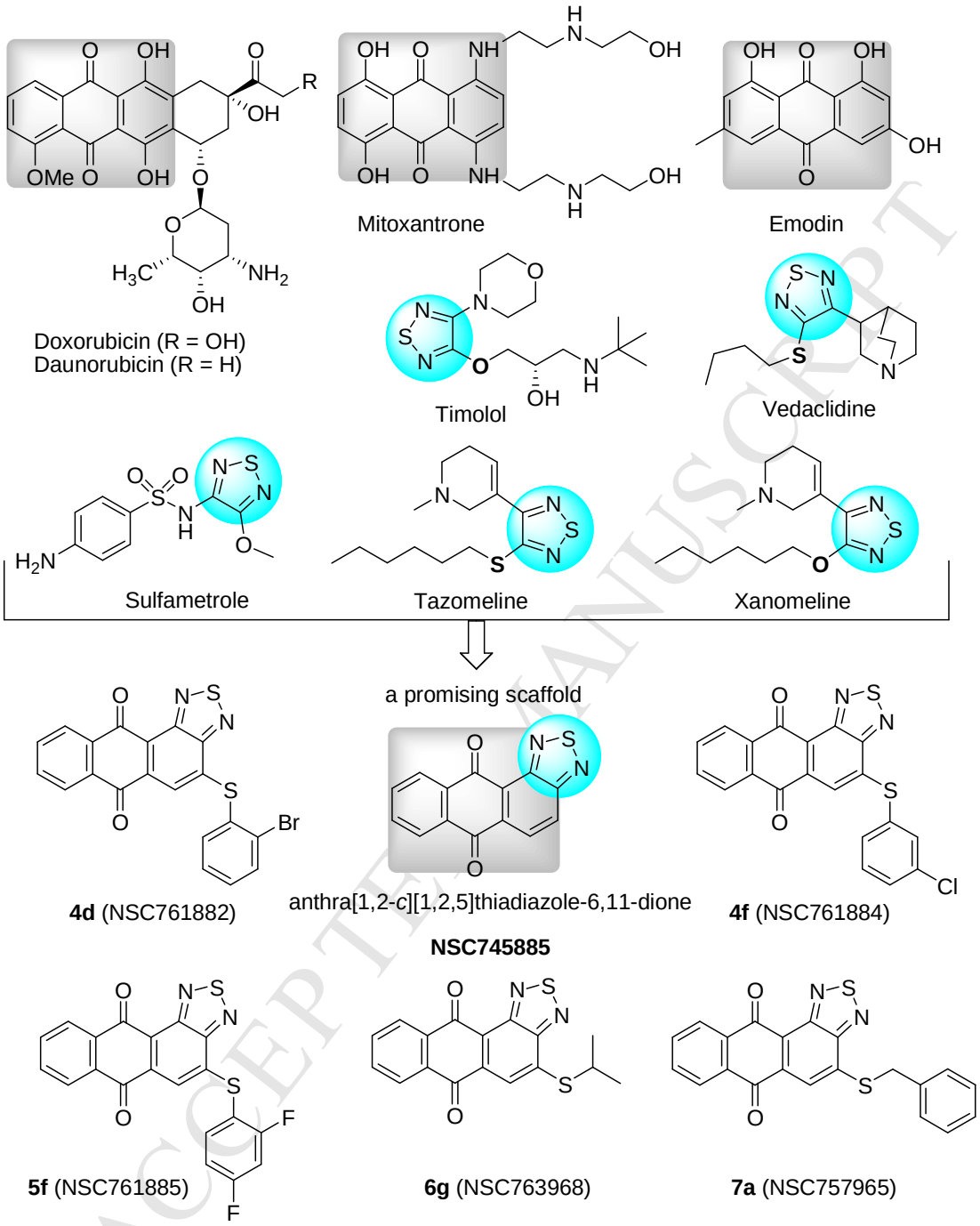


Figure 2.

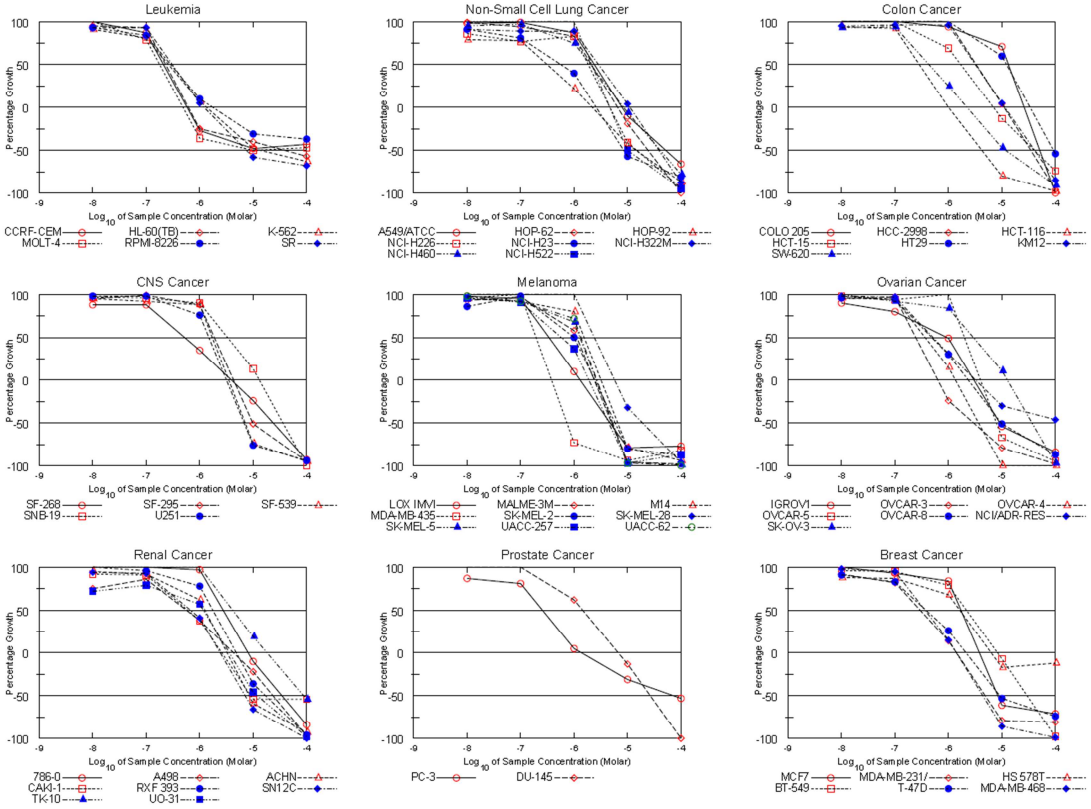
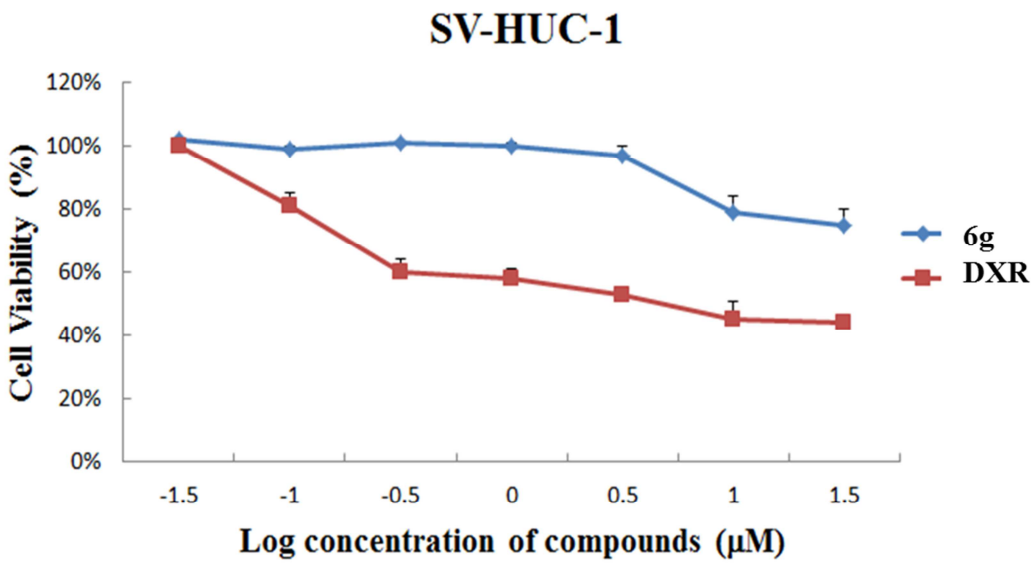
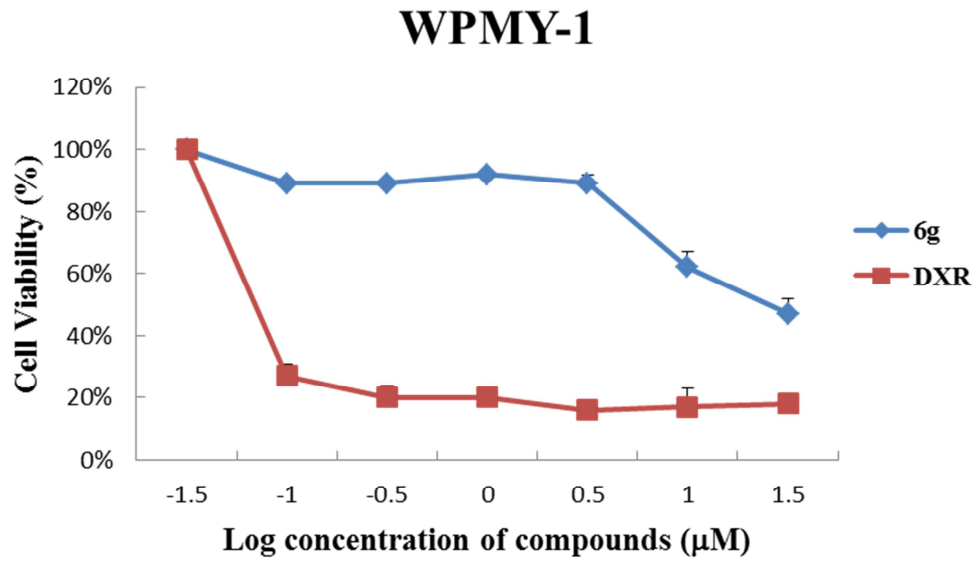


Figure 3.

3a)



3b)



3c)

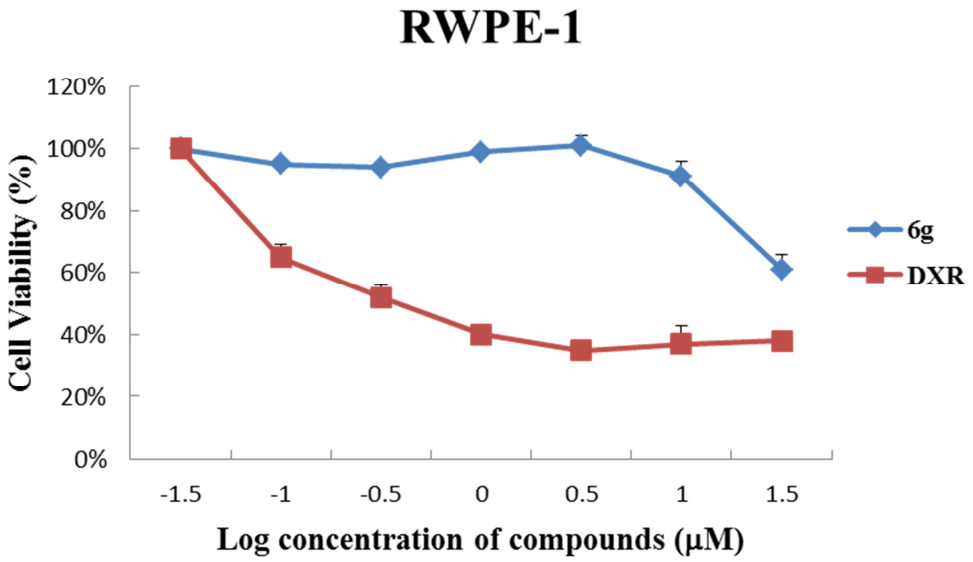


Figure 4.

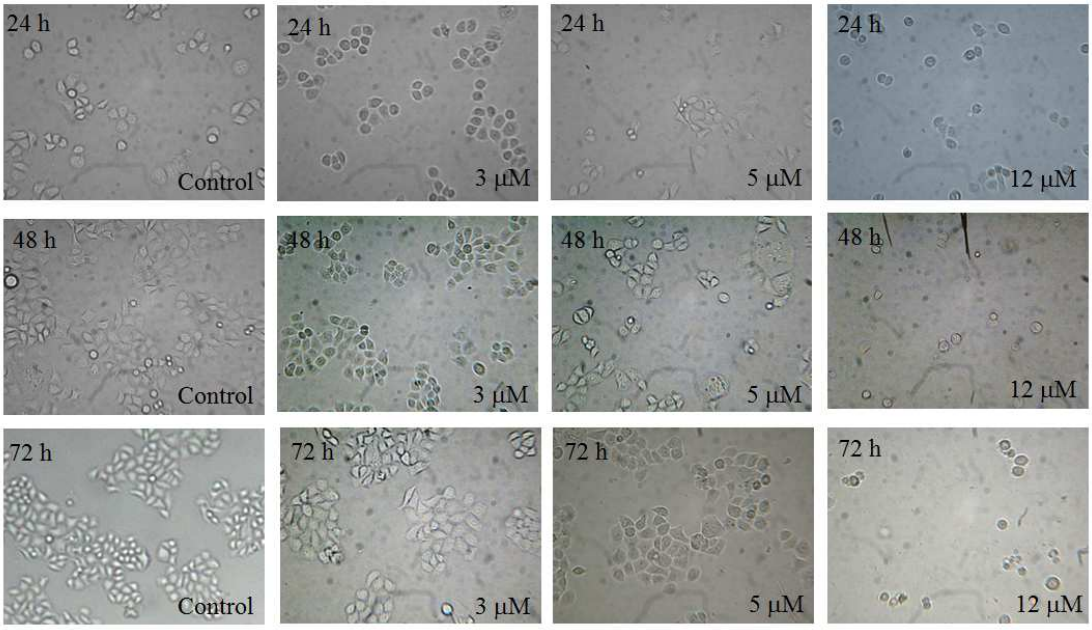


Figure 5.

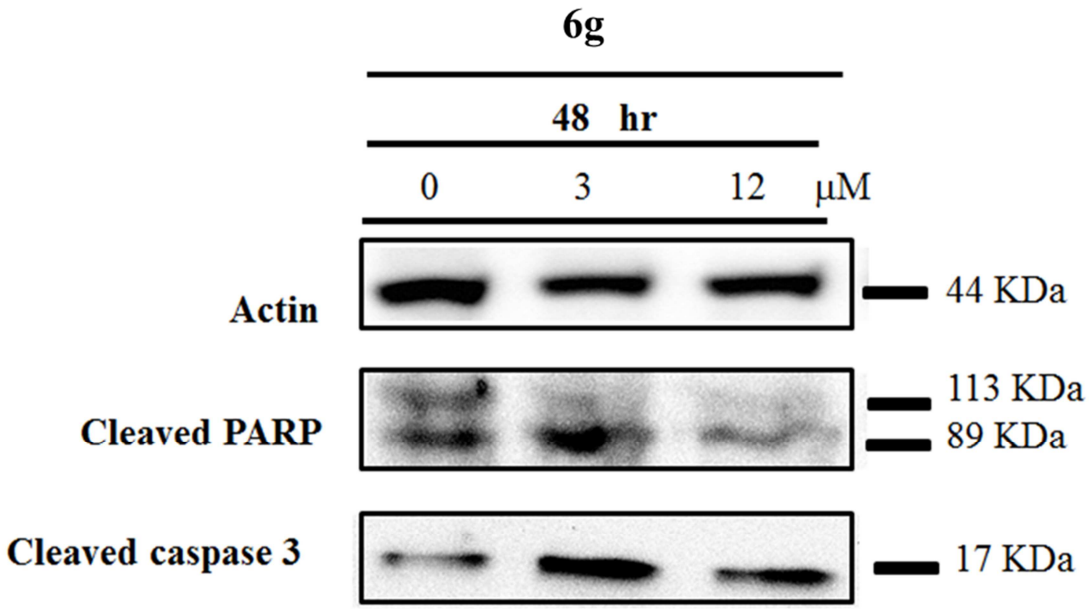
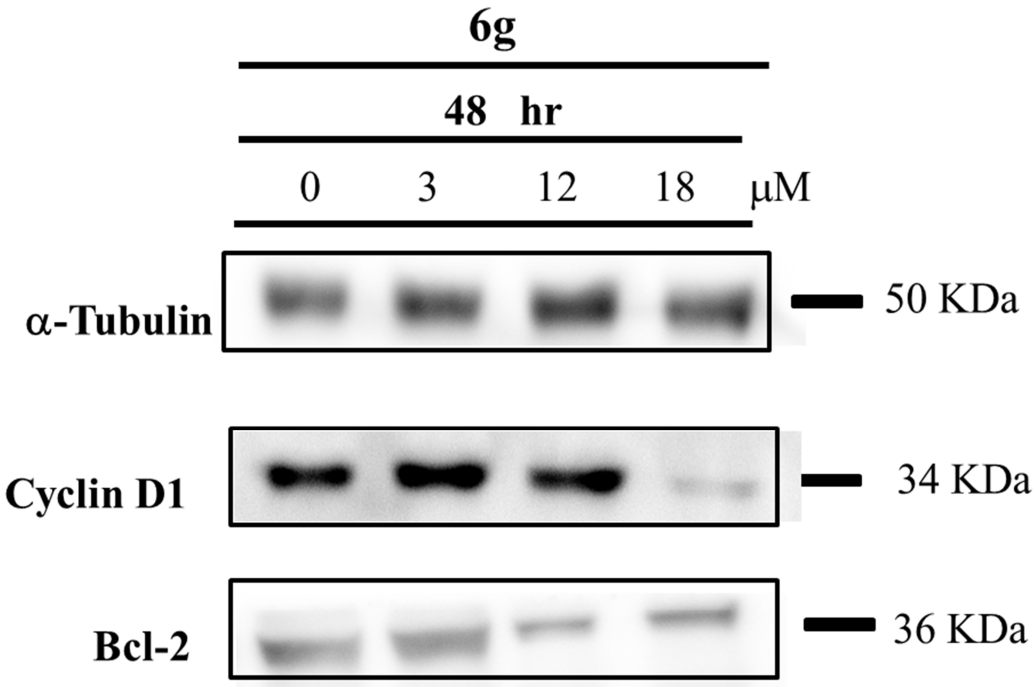


Figure 6.

6a)



6b)

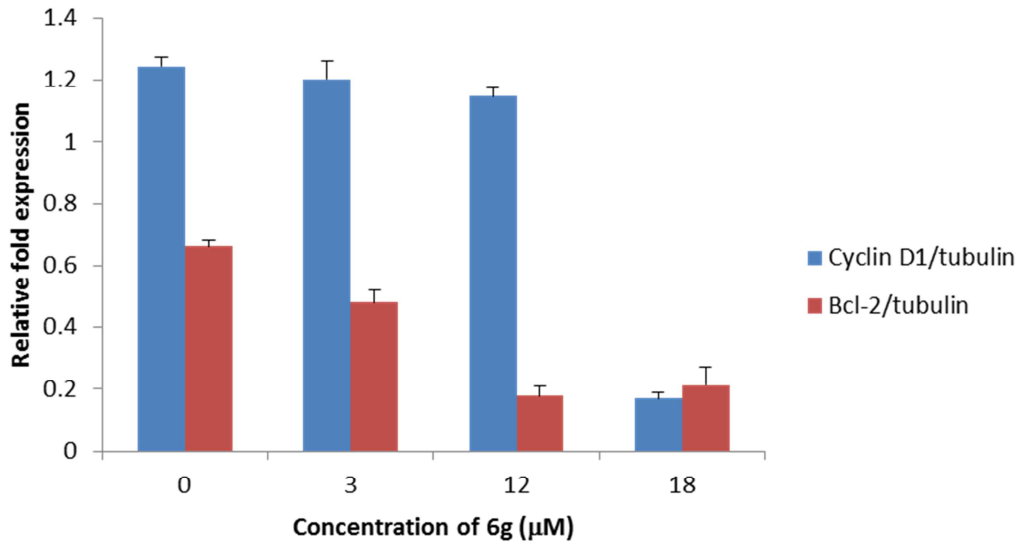
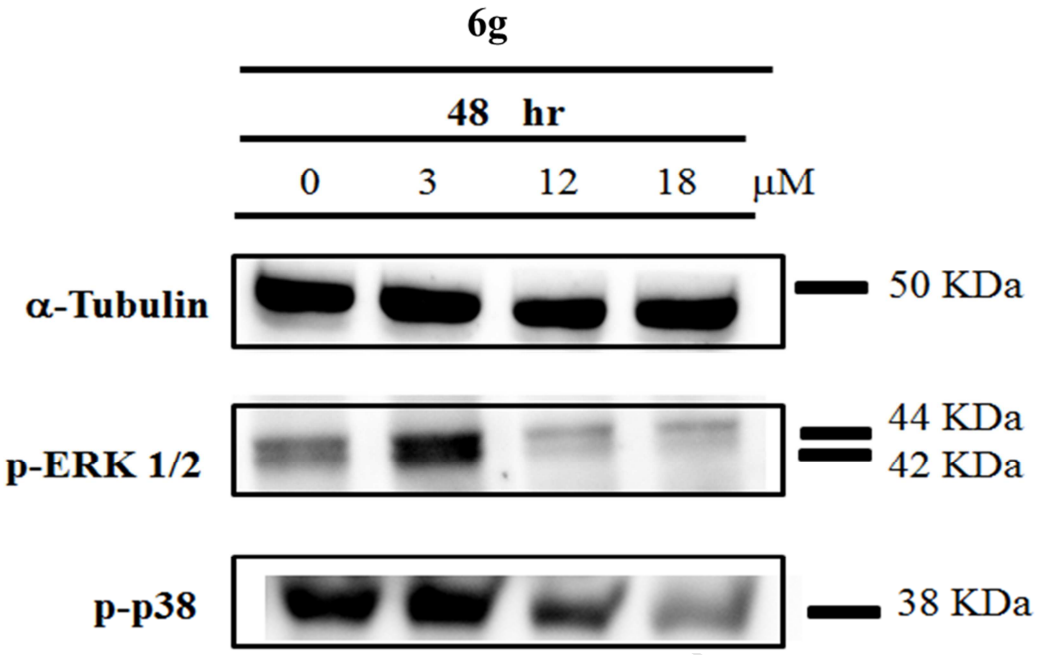


Figure 7.



Legends

Scheme 1 . Synthesis of sulfur-substituted anthra[1,2-*c*][1,2,5]thiadiazole-6,11-dione derivatives. Reagents and conditions: (i). $SOCl_2$, THF, r.t., 24 h; (ii). $KClO_3$, acetic acid, HCl, 90℃, 45 min + 4 h; (iii). various organosulfur derivatives, DIPEA, dry THF, 65℃, 2 h~4 h; (vi). various organosulfur derivatives, ferric chloride, DMF, 90℃, 3 h~4 h.

Table 1. Effects of sulfur-substituted anthra[1,2-*c*][1,2,5]thiadiazole-6,11-diones on cytotoxicity by SRB assay in PC-3 cell line and MTT assay in DU-145 cell line.

Table 2. Growth percentage of selected compounds in the NCI Developmental Therapeutics Program's *in vitro* 60 cell line screen program.

Table 3. *In vitro* antitumor activity (GI_{50}, μM), toxicity (LC_{50}, μM) and TGI data of **4d (**NSC761882**)**, **4f** (NSC761884), **5f** (NSC761885), **6g** (NSC763968), and **7a** (NSC757965).

Table 4. Median growth inhibitory concentration (GI_{50}, μM) and GI_{50} selectivity ratios of **4d**, **4f**, **5f**, **6g**, and **7a** in the NCI *in vitro* 60-cell Drug Screen.

Figure 1. Some examples of core structure of 1,2,5-thiadiazole-containing drugs and representative investigational structural sets used in the pharmacophore studies.

Figure 2. Durg response curve from five dose study against compound **6g**.

Figure 3. 3a) The cytotoxicity of **6g** and DXR, to SV-HUC-1 cells (human normal urothelial cells). 3b) to WMPY-1 (normal human prostatic stromal myo-fibroblasts). 3c) to RWPE-1 (normal prostate epithelial cells).

Figure 4. Morphological changes and apoptosis induced by **6g** in DU-145 cells.

Figure 5. Treatment with **6g** induced apoptotic pathways in DU-145 cells. Protein samples were separated using SDS-PAGE and subjected to immunoblotting with antibodies specific to cleaved PARP, cleaved caspase 3 and β-actin (n= 3 independent experiments). β-actin was used as a loading control.

Figure 6. 6a) Dose effect of compound **6g** on their inhibition activities against Cyclin D1 and Bcl-2 in DU-145. α-tubulin was used as a loading control (n= 3 independent experiments). 6b) Relative protein expression changes of compound **6g** in PC-3 cells.

Figure 7. Dose effect of compound **6g** on their inhibition activities against ERK 1/2 and p38 phosphorylation in DU-145.

Highlights

▲ sulfur-substituted anthra[1,2-*c*][1,2,5]thiadiazole-6,11-diones were synthesized

▲19 compounds were selected and tested by NCI for their anticancer potency

▲**6g** induced apoptosis and attenuated ERK1/2 and p-38 signaling pathways