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Synthesis, antiproliferative activities and telomerase inhibition evaluation of novel asymmetrical 1,2-disubstituted amidoanthraquinone derivatives

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ABSTRACT

A series of diversely asymmetrical mono- or disubstituted 1,2-diamidoanthraquinone derivatives were synthesized and evaluated for drug-induced cytotoxicity by SRB assay, telomerase inhibitory activity by TRAP assay, and *hTERT* expression by SEAP assay. Interestingly, compounds **4**, **11**, **21**, **32** and **36** exhibited selective potent antiproliferative activities by NCI with IC_{50} values in the micromolar range. Of these, only compound **8** showed an IC_{50} value of 0.95 μM against PC-3 cell lines (human prostate cancer) by SRB assay. All the synthesized compounds exhibited a poor or modest telomerase inhibitory activity by TRAP assay suggesting another mode of action for these compounds. Compound **11** showed broad inhibition against different types of cancer cell lines in the micromolar and submicromolar range.

1. Introduction

Anthraquinone-containing extracts from different plant sources such as senna, cascara, aloe, frangula, and rhubarb have been found to have wide variety of pharmacological activities such as antiinflammatory, wound healing, analgesic, antipyretic, antimicrobial, and antitumor activities [1,2]. The main bioactive constituents of rhubarb are anthraquinone derivatives, including emodin, aloe-emodin, rhein, chrysophanol, physcion, and danthron [3]. Anthraquinone-based drugs have been shown to process strong antiperliferative properties, such as mitoxantrone, ametantrone, and anthracycline antibiotics (daunorubicin and doxorubicin), are used for treatment of varied oncology (Scheme 1). However, these clinically used drugs also suffered from frequent cardiotoxicity that limited their applicability [4]. Doxorubicin and its analogs function as topoisomerase poisons through stabilization of the cleavable complex that forms as part of the catalytic cycle of the enzyme [5]. The detailed mechanism of how these compounds, which contain an anthraquinone core that forms between the DNA base pairs, the drug, and the enzyme in the minor groove has remained elusive.

Anthracyclines and their anthraquinone derivatives remain 'evergreen' drugs with broad clinical indications but have an improvable pharmacological index [6,7]. In an attempt to identify new targets for anticancer drugs, our attention has been focused on cytotoxicity and telomerase. Nevertheless, because of their broad bioactivities, anthraquinone-based analogs and their coplanar structural motif still attract attentions for rational design anticancer drugs. In addition, DNA appears to be the major cellular target for most anthraquinone-based structures which is generally accepted by DNA-interactive drugs [8]. It has been reported that daunorubicin and its analogs are DNA-intercalating agent, and their cytotoxicity is due to stabilization of the topoisomerase II–DNA cleavage complexes [9,10]. Furthermore, the bi-functionalized side-chains of regioisomeric amidoanthraquinones may simultaneously occupy both DNA major and minor grooves, and mono- or disubstituted of

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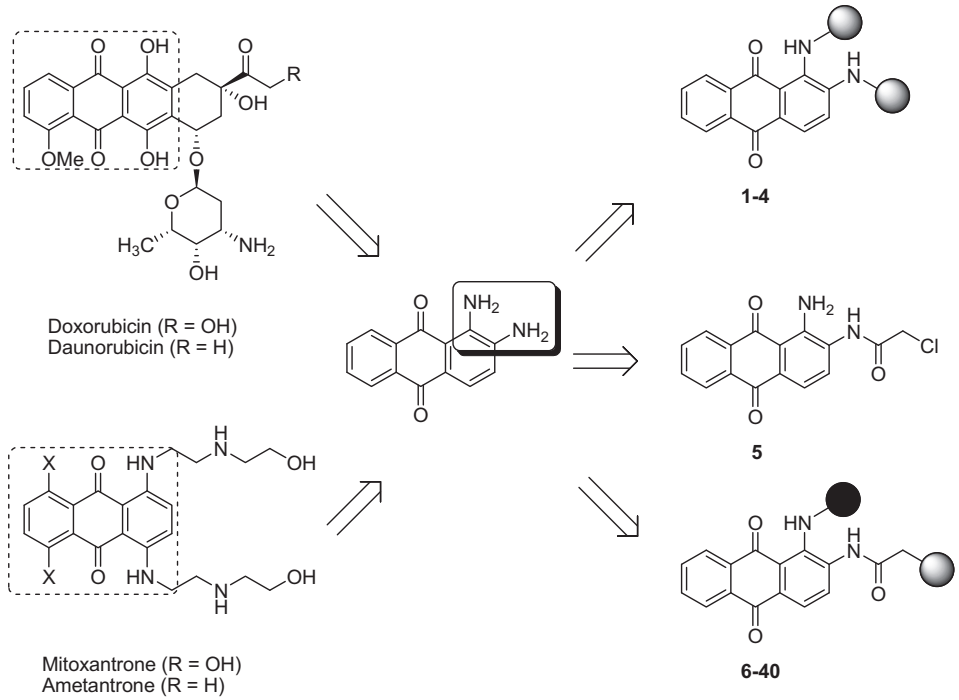


Scheme 1. Scaffold-based of bioactive pharmacophoric anthraquinones for drug design.

anthraquinone derivatives can potentially increase DNA binding affinity [11].

Telomerase is a ribonucleoprotein for telomere maintenance which utilizes its RNA component as the template to extend telomeric DNA length [12]. The activity of telomerase could be detected in about 85–90% of tumor cells, whereas it is low or not present in most somatic cells [13]. Thus, the maintenance of telomere length is considered as a biological marker for determining the proliferation of cancer cells. In previous studies [2,7,14–18], our groups have reported a series of amidoanthraquinones at 1,4-, 1,5-, 2,6- and 2,7-position that showed diversely *in vitro* antitumor activity and telomerase inhibitory activities. Here we report the design and synthesis of a series of novel asymmetrical mono- or disubstituted 1,2-diamidoanthraquinone derivatives. These compounds were evaluated for cytotoxicity and telomerase inhibitory activity using SRB assay [19–22], and repressing *hTERT*-expression, respectively. Among these derivatives, compounds **1**, **5**, **6**, **7**, **8**, **23**, **24**, **30** and **37** showed the highest potency against PC-3 (prostate cancer) with IC_{50} from 0.95 μM to 2.64 μM (Table 1). Compounds **4** (NSC747246), **11** (NSC749232), **21** (NSC749233), **32** (NSC749757), and **36** (NSC749670) were selected by the NCI for one dose screening program (Table 2) and further studies on **11** (NSC749232) and **36** (NSC749670) (Table 3) where the curves cross these lines represent the interpolated values to cause 50% growth inhibition (GI_{50}), total growth inhibition (TGI) and 50% cell killing (LC_{50}), respectively.

2. Chemistry

There has been continuing interest in the synthesis of anthraquinone-based derivatives and their systematic dissection largely on account of their biological activities [16]. Herein, we reported on the discovery of an efficiently method for synthesis of asymmetrical mono- or disubstituted skeleton of amidoanthraquinones that involved simply synthetic route with appropriate yields. The synthesis of target compounds is illustrated and outlined in schemes. Our synthetic strategies involved both the sequential introduction of the 1,2-diamidoanthraquinone moiety and the substituted aromatic or bicyclic aromatic system. The acylated compound **5** with various acyl chlorides afforded the asymmetrical compounds **6**–**28**. The nucleophilic substitution between the compound **11** and the appropriated amines under catalysis conditions in the presence of DIPEA as well as in dry THF resulted in the formation of asymmetrical compounds **29**–**40**. Based upon our desired structures obtained asymmetrical side-chains, if we changed the order of synthetic route form acylation

Table 1
Drug screened for cytotoxicity of compounds **1**–**40** against the growth of human prostate cancer cell lines (PC-3) by SRB assay.

Cell type/PC-3 (inhibition μM)[a]			
Compound	IC_{50}	Compound	IC_{50}
1	1.19	**22**	>20
2	>20	**23**	2.44
3	3.94	**24**	2.64
4	3.16	**25**	3.78
5	1.65	**26**	4.31
6	1.23	**27**	>20
7	1.12	**28**	5.39
8	0.95	**29**	3.92
9	3.67	**30**	1.46
10	3.79	**31**	6.68
11	3.87	**32**	17.56
12	>20	**33**	>20
13	3.48	**34**	>20
14	3.14	**35**	19.06
15	3.09	**36**	3.46
16	3.51	**37**	2.07
17	>20	**38**	>20
18	3.42	**39**	>20
19	5.68	**40**	>20
20	6.67	mitoxantrone	0.29
21	>20	doxorubicin	0.25

[a] IC_{50} is the concentraion of drug (μM) required to inhibit cell growth by 50%; all experiments were independently performed at least three times ($n = 3$).

Table 2
Cytotoxicity of selected compounds in the NCI *in vitro* 60-cell drug screen program.

Panel/Cell lines	Compound/Growth percent[a]				
	4	**11**	**21**	**32**	**36**
	NSC747246	NSC749232	NSC749233	NSC749757	NSC749670
Leukemia					
CCRF-CEM	79.02	–	33.97	39.61	61.06
HL-60(TB)	73.11	−46.88	41.36	96.11	−8.54
MOLT-4	89.63	−23.17	11.40	48.20	26.04
SR	87.69	−19.84	−31.25	–	−43.70
K562	107.54	–	–	40.86	−4.52
RPMI-8226	101.77	–	–	–	–
Non-small cell lung cancer					
EKVX	–	88.22	102.08	65.93	78.07
HOP-62	105.23	−28.62	115.67	113.43	75.21
HOP-92	91.21	−68.77	73.63	59.33	90.68
NCI-H226	91.14	−25.30	80.49	90.22	93.87
NCI-H23	90.57	−85.89	64.75	73.29	89.52
NCI-H322M	97.74	95.05	104.38	82.50	72.60
NCI-H460	88.62	95.65	104.50	61.05	37.47
NCI-H522	72.05	57.88	43.37	103.02	88.21
A549/ATCC	90.46	−81.71	–	84.88	83.89
Colon cancer					
COLO 205	108.57	−53.43	–	65.60	2.29
HCC-2998	84.68	–	94.74	84.60	28.97
HCT-116	78.55	−54.27	67.55	58.46	8.37
HCT-15	102.44	−50.22	68.55	73.22	27.47
HT29	99.39	3.37	88.84	31.34	3.69
KM12	94.21	−19.53	80.59	51.05	44.05
SW-620	93.79	−43.27	83.41	82.29	39.17
CNS cancer					
SF-268	98.91	14.63	80.14	86.17	59.11
SF-295	110.81	79.70	98.48	68.97	89.86
SF-539	97.26	−31.42	107.09	81.29	62.68
SNB-19	103.91	88.40	124.27	108.22	68.75
SNB-75	102.26	6.31	109.14	66.03	82.17
U251	91.73	−44.02	66.17	82.17	22.38
Melanoma					
LOX IMVI	84.41	−57.05	36.29	80.62	1.24
MALME-3M	127.16	−22.41	47.07	82.24	10.84
M14	91.57	−22.68	87.10	88.01	−60.04
MDA-MB-435	–	4.82	80.40	79.14	11.15
SK-MEL-2	100.65	52.00	−6.49	102.17	123.64
SK-MEL-28	99.46	−31.94	88.68	92.18	−57.56
SK-MEL-5	94.04	−98.72	51.32	61.74	41.30
UACC-62	87.50	−67.80	59.67	76.82	−20.65
UACC-257	98.46	–	–	92.83	−50.95
Ovarian cancer					
IGROV1	78.71	51.41	41.03	106.07	112.20
OVCAR-3	97.90	−92.86	86.38	77.82	−6.15
OVCAR-4	104.32	34.52	80.40	47.06	57.11
OVCAR-5	110.18	109.55	119.04	102.31	106.66
OVCAR-8	85.43	8.72	–	65.02	46.43
NCI/ADR-RES	–	72.17	86.87	50.86	66.74
SK-OV-3	109.62	106.24	–	82.13	108.91
Renal cancer					
786−0	116.17	11.30	97.26	65.15	11.90
A489	124.08	123.78	91.79	68.12	67.36
ACHN	112.43	21.92	79.64	82.23	49.87
SN12C	98.59	−81.02	79.93	74.82	63.31
TK-10	124.91	130.52	120.81	86.85	101.93
UO-31	66.54	42.91	58.19	69.24	70.66
CAKI-1	94.89	–	–	55.64	69.76
RXF 393	88.25	–	–	63.80	68.84
Prostate cancer					
PC-3	82.91	6.81	–	75.00	84.75
DU145	95.93	−97.56	85.83	91.52	82.12
Breast cancer					
MCF7	71.71	−79.28	51.00	70.79	31.79
MDA-MB-231/ATCC	93.29	24.67	82.04	73.34	37.00
HS 578-T	105.96	–	66.65	69.39	87.29
BT-549	95.39	28.19	79.78	81.58	91.88
T-47D	100.00	−49.46	–	44.33	52.62
MDA-MB-468	104.17	−83.42	51.02	69.27	7.43
Mean	96.19	−1.96	73.78	74.53	46.71
Delta	29.65	96.76	105.03	43.19	106.75
Range	76.37	229.24	155.52	82.09	183.68

[a] Data obtained from NCI *in vitro* 60-cell lines drug screen program at 10 μM.

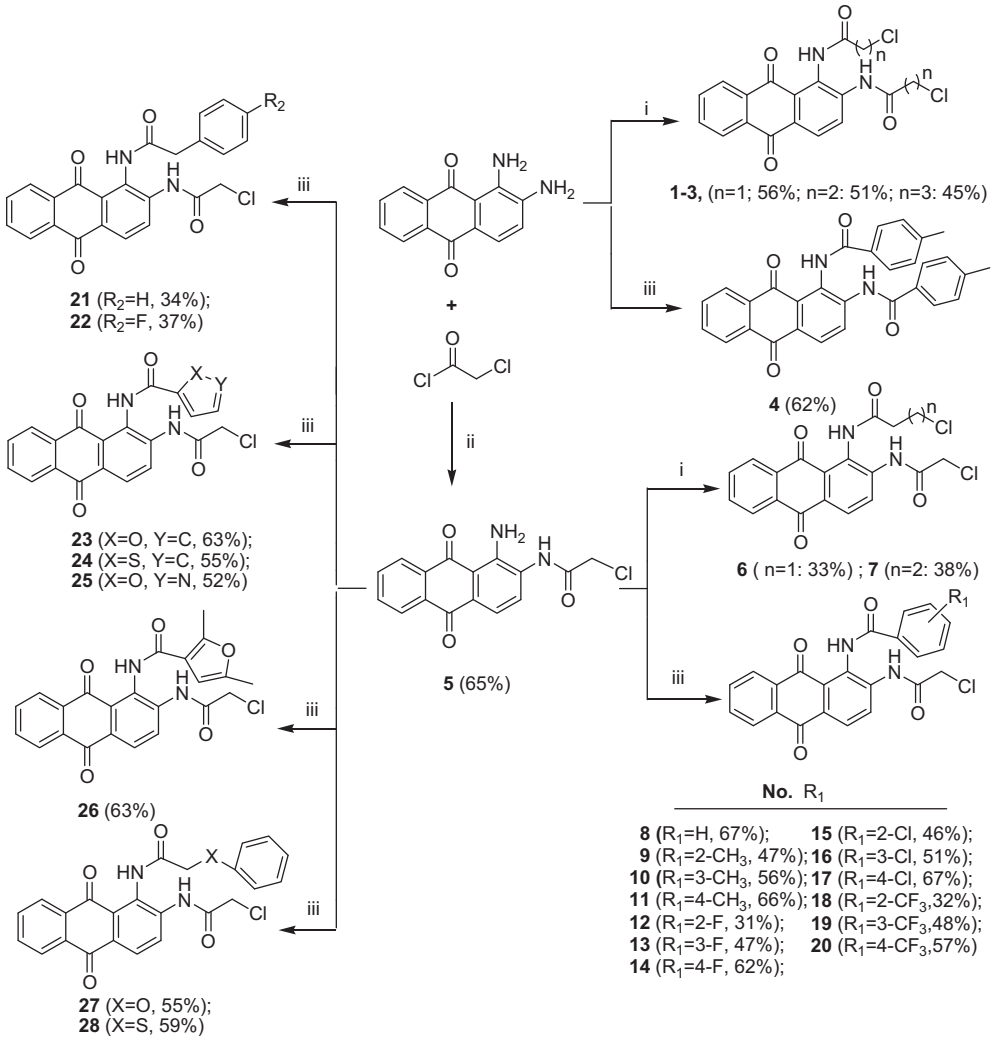
Table 3
In vitro anticancer activity of compounds **11** (NSC749232) and **36** (NSC749670) in the NCI's 60 human cancer cell lines.

Panel/Cell lines (μM)	**11** (NSC749232)			**36** (NSC749670)		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Leukemia						
CCRF-CEM	2.54	>50	>50	–	–	–
HL-60(TB)	6.14	24.6	>50	–	–	–
MOLT-4	2.29	2.95	>50	–	–	–
SR	1.59	>50	>50	–	4.65	>100
K562	1.87	4.04	>50	2.09	–	–
RPMI-8226	1.35	>50	>50	3.25	>100	>100
Non-small cell lung cancer						
EKVX	1.98	14.9	>50	–	>100	>100
HOP-62	2.97	9.49	24.4	>100	>100	>100
HOP-92	9.73	31.7	>50	0.87	9.19	>100
NCI-H226	8.81	20.8	49.1	>100	>100	>100
NCI-H23	1.78	8.28	31.4	>100	>100	>100
NCI-H322M	3.76	13.7	45.4	>100	>100	>100
NCI-H460	5.81	14.1	34.1	3.36	>100	>100
NCI-H522	0.88	2.03	–	–	>100	>100
A549/ATCC	12.3	29.2	>50	–	>100	>100
Colon cancer						
COLO 205	2.95	9.65	23.7	2.22	4.08	>100
HCC-2998	8.61	1.59	29.4	3.00	>100	>100
HCT-116	1.05	2.46	9.32	3.08	>100	>100
HCT-15	6.28	1.34	28.4	3.36	>100	>100
HT29	3.36	2.41	>50	3.09	>100	>100
KM12	5.56	12.6	28.3	–	>100	>100
SW-620	1.87	9.31	>50	–	>100	>100
CNS cancer						
SF-268	5.74	2.12	>50	–	>100	>100
SF-295	5.46	1.52	42.2	>100	>100	>100
SF-539	2.92	1.01	25.1	–	–	–
SNB-19	8.38	1.52	27.6	–	–	>100
SNB-75	7.66	1.53	30.5	>100	>100	>100
U251	1.80	6.83	30.2	3.64	>100	>100
Melanoma						
LOX IMVI	0.78	1.84	4.29	–	>100	>100
MALME-3M	5.89	15.6	41.1	–	–	>100
M14	4.72	12.4	31.4	2.22	4.65	–
MDA-MB-435	5.73	19.5	>50	3.13	>100	>100
SK-MEL-2	1.34	4.40	>50	>100	>100	>100
SK-MEL-28	7.63	15.2	30.1	1.87	–	–
SK-MEL-5	0.83	1.53	2.83	1.94	–	>100
UACC-257	9.92	17.9	32.2	2.52	–	>100
UACC-62	1.61	6.27	24.3	2.23	5.81	>100
Ovarian cancer						
IGROV1	5.60	17.0	>50	>100	>100	>100
OVCAR-3	1.35	3.54	15.2	2.43	–	>100
OVCAR-4	5.52	12.0	25.9	–	>100	>100
OVCAR-5	11.3	19.2	32.7	>100	>100	>100
OVCAR-8	3.32	28.5	>50	>100	>100	>100
NCI/ADR-RES	9.46	30.7	>50	–	>100	>100
SK-OV-3	13.2	35.9	>50	>100	>100	>100
Renal cancer						
786–0	9.48	17.2	31.2	5.61	>100	>100
A489	9.87	22.5	>50	>100	>100	>100
ACHN	7.18	14.3	28.6	–	>100	>100
CAKI-1	6.87	15.2	33.7	–	>100	>100
RXF 393	7.84	16.5	34.6	–	>100	>100
SN12C	1.22	3.66	>50	–	>100	>100
TK-10	2.34	4.94	29.2	>100	>100	>100
UO-31	7.21	1.73	41.3	–	>100	>100
Prostate cancer						
PC-3	6.54	15.4	36.5	–	>100	>100
DU145	2.69	9.07	23.0	>100	>100	>100
Breast cancer						
MCF7	1.12	4.95	>50	3.13	>100	>100
MDA-MB-231/ATCC	6.51	>50	>50	3.30	>100	>100
HS 578-T	1.64	9.12	>50	> 100	>100	>100
BT-549	2.58	16.3	>50	> 100	>100	>100
T-47D	1.19	4.04	46.3	2.62	–	>100
MDA-MB-468	0.83	1.82	3.98	2.58	>100	>100

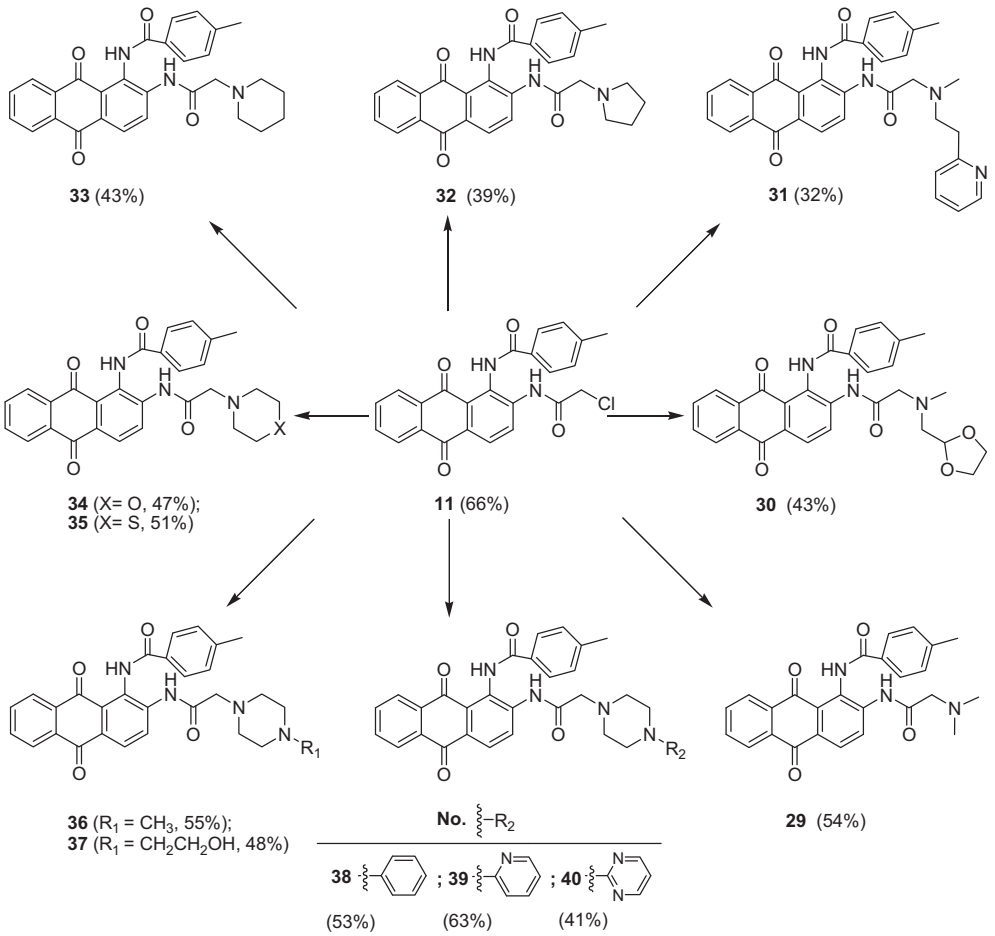
to amination, it should be noted for initially beginning amination of compound **5**. The reaction was due to the mono-substituted side chain of intermediate **5** bearing an electrophilic carbon atom would be possibly cyclized to imidazole group by heating in miniclave at 60 °C [7] and some undesired byproducts before further subjected to nucleophilic displacement with chlorine atom to a series of amines. Notably, there was an interesting reaction to mentioned that the starting material 1,2-diaminoanthraquinone, which was treated with three kind of conditions but different equivalents of chloroacetyl chloride and corresponding of catalyst in the presence of pyridine, as well as control of reaction temperature and time that could generate at least three kind of products and byproducts. Consequently, the reactivity of compounds **1** and **5** has been explored in a simply nucleophilic substitution *via* the regioselective of 1,2-diaminoanthraquinone. The symmetrically side-chains of compound **1** needed longer reaction time than compound **5**. This resulted in the intramolecular hydrogen bonding interaction between first-positional aromatic amine (hydrogen bond donors) and the neighboring quinone carbonyl group (hydrogen bond acceptors) of the l,2-diaminoanthraquinone [23]. By contrast with compound **5**, the course of compound **1** through the intermediate of intramolecular hydrogen bond existing could probably affect the difference of both reactive orientation (slight steric hindrance) and rate. According to the ratio of the reaction mixture indicated two spots (compounds **1** and **5**) on TLC which allowed us to isolate them as being either major or minor. Besides, it was not only compounds **1** and **5**, but also all the final products of these reactions were monitored by TLC and the quantities of the byproducts were separated from their physical chemistry properties and purification by tedious recrystallization and chromatography. The chemical structures of the compounds were established by FT-IR, high resolution mass (HRMS), ^{1}H NMR and ^{13}C NMR spectra and the results are presented in the experimental part Schemes 2 and 3.

3. Biological activity

All synthesized compounds (**1**–**40**) were tested against human prostate cancer cell lines (PC-3) using SRB assay. Moreover, five of these compounds (**4**, **11**, **21**, **32**, and **36**) were chosen by NCI and tested against a panel of 60 human tumor cell lines. In addition, compounds **11** and **36** were selected for further cell growth inhibition analyses to determine the values of GI_{50}, TGI, and LC_{50}. We also evaluated the effects of compounds (**1**–**40**) on telomerase inhibition by PCR-based telomeric repeat amplification protocol (TRAP) assay [2,24]. Finally, their effects on the transcriptional activity of human telomerase reverse transcriptase (*hTERT*) was



Scheme 2. Synthesis of disubstituted anthraquinone derivatives **1**–**28**. Reagents and conditions: (i) acyl chloride, anhydrous DMF, pyridine, rt, 24 h; (ii) anhydrous DMF, rt, 2 h; (iii) acyl chloride, pyridine, anhydrous THF, miniclave, 3–4 h.



Scheme 3. Synthesis of disubstituted anthraquinone derivatives **29**–**40**. Reagents and conditions: secondary amine, DIPEA, anhydrous THF, reflux, 14–16 h.

evaluated using the secreted alkaline phosphatase (SEAP) as the reporter system [25].

4. Results and discussion

4.1. Chemistry

Based upon the ^{1}H NMR spectrum, there were obviously two major differences between compounds **1** and **5**: (i) two singlet peaks at δ 4.43 and δ 4.45 ppm were present in compound **1** and two protons of alkyl group were each for symmetrical moiety (−C$\underline{H}_2$− and −C$\underline{H}_2$−) of double side chain than only one singlet peak in the presence of δ 4.38 ppm for alkyl group (−C$\underline{H}_2$−), respectively; (ii) compound **5** emerged from the broad peak containing two protons where the first-positional amine of aromatic ring (Ar−N$\underline{H}_2$) was located at δ 7.96 ppm, and one singlet at δ 9.80 ppm for second-positional amido group (Ar−NH−CO) of aromatic ring. Compound **1** appeared as two siglets at δ 9.83 and 10.29 ppm due to the double amine of amido group. There were two multiplets at δ 7.90–7.93 ppm and δ 8.12–8.18 ppm and a triple-doublet appeared at δ 7.88 ppm. In addition to ^{1}H NMR data, the ^{13}C NMR showed a couple of peaks at δ 165.61 and 166.02 ppm for the symmetrically double-side chains of amido carbon (−N$\underline{C}$O). Moreover, both peaks at δ 37.71 and 43.25 ppm were also elucidated on the two carbon of alkyl group (−$\underline{C}H_2$−) than only one peak at 165.72 ppm. Compared with **5**, compound **1** was making the condition more basic by addition of pyridine into the reaction mixture, and accelerated the reactivity of acylation in S_N2 reaction.

4.2. In vitro anti-proliferative activities

The SRB assay provides a rapid, sensitive method for measuring the cell growth inhibition in 96 well microtiter plates [21]. The results were summarized in Table 1 and expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}). As a result of SAR studies showed that the amide-side chains obtained one carbon atom as well as bearing chlorine atom (**1** and **5**–**28**) were much potential antitumor activities then followed by amination of amido-side chains (compounds **29**–**40**). However, the amination compounds **36** (IC_{50} = 3.46 μM) and **37** (IC_{50} = 2.07 μM) were 10-folds more active than others. Both symmetrical and asymmetrical side-chains results indicated that the length of carbon side chain between amido group and chlorine atom at secondary position of 1,2-diaminoanthraquinone scaffold played an important role in enhancing their cytotoxic activies of synthesized compounds. Compounds **4**, **11**, **21**, **32**, and **36** were choosed and studied for their antiproliferative activity against human cancer cell lines in NCI Drug Screen Program. As shown in Table 2, compounds **11** and **36** showed strong growth-inhibitory effects that several cancer cell lines showed <10% growth upon 10 μM compound-treatments. Compounds **4**, **21**, and **32** did not show significant antiproliferative activities for tumor-cell lines. Compounds **11** and **36** were further analyzed by NCI to determine

the values of GI_{50}, TGI, and LC_{50}. As shown in Table 3, both compounds **11** (NSC749232) and **36** (NSC749670) showed potent activity with GI_{50} ranging from 0.78 to 13.2 μM (mean value = 3.63 μM) and 2.09 to > 100 μM (mean value = 12.88 μM), respectively. It is also apparent that compound **11** exhibited stronger cancer-inhibitory effects than compound **36** with LC_{50} and TGI as low as 2.83 and 1.01 μM in tested cell lines, respectively. It exhibited the best activity against melanoma cell lines with GI_{50} ranging from 0.78 to 9.92 μM. The breast cancer subpanel was ranked second sensitive with GI_{50} ranging from 0.83 to 6.51 μM. It is also interesting to note that both compounds **11** and **36** displayed relatively differential cytotoxic activity profile against NCI 60-cell lines. Although the mechanism of how these compounds showed differential growth-inhibitory effects toward cancer cells, asymmetrical side chain within compound **11** might have a role on the activity Fig. 1.

4.3. Telomerase activities

Anthraquinone analogs are one of the main structures that targets telomere and affects telomerase activity [26–28]. Since telomerase is an important component of cancers, we are interested in examining the effects of our synthesized compounds on telomerase activities in the cell free extracts prepared from H1299 cells. However, none of the synthesized compounds showed telomerase inhibitory effects at 10 μM concentration by TRAP assay. We next analyzeds the *hTERT* repressing activity of these compounds. The expression of SEAP in H1299 cells harboring P_{hTERT}-SEAP was used as the criteria to evaluate that anthraquinone derivatives inhibited the expression of *hTERT* in cancer cells [29]. Results of all tested compounds which showed MTT and SEAP inhibition activity at 1, 10 and 100 μM were summarized in Table 4. We found all of the synthesized compounds showed parallel inhibitory effects toward

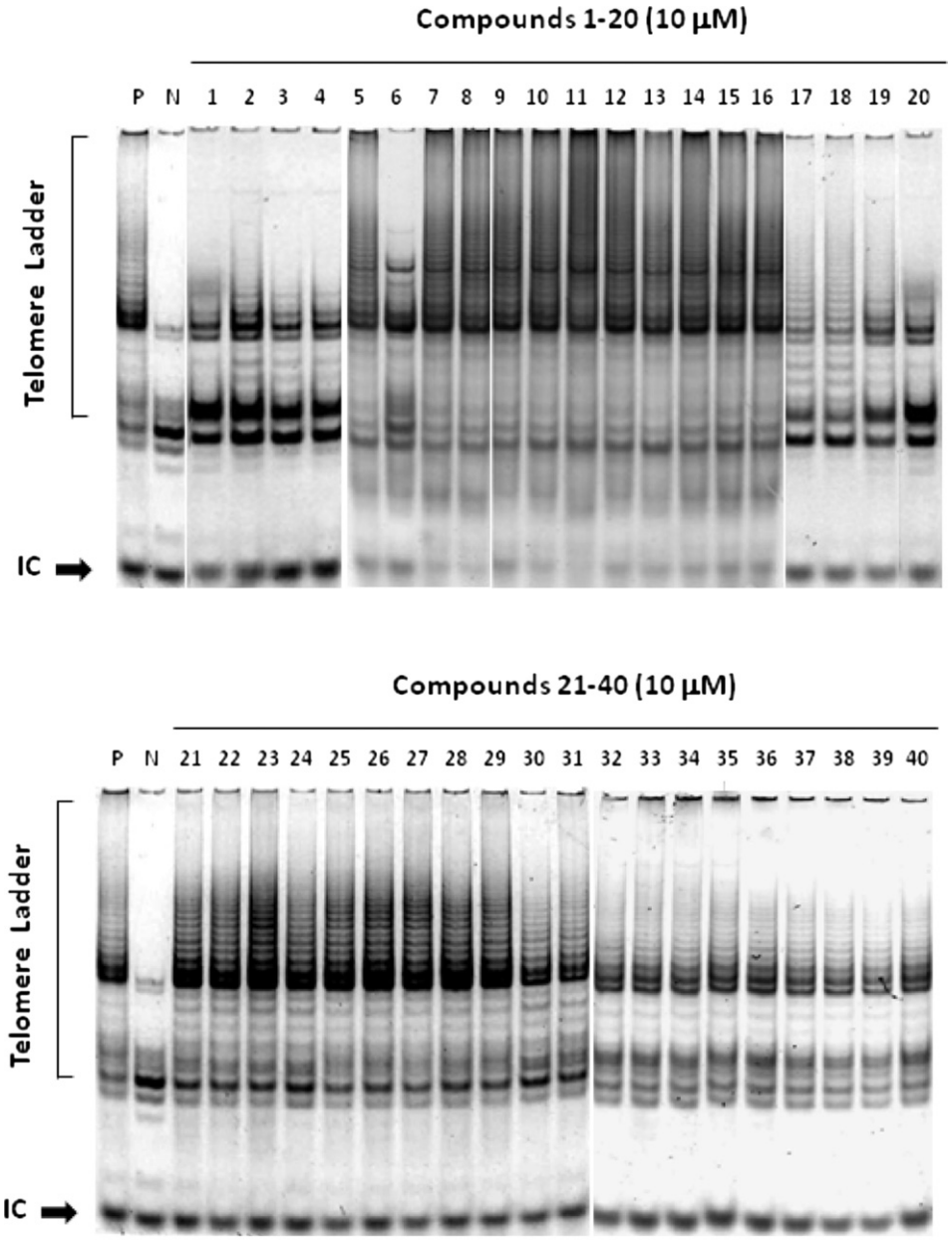


Fig. 1. Compounds **1**–**40** were tested the inhibition of telomerase activity by TRAP assay under concentration of 10 μM. [P: positive control (no inhibitor); N: negative control (RNase A-treated cell extract, no inhibitor); IC: internal control].

Table 4
Effects of compounds **1**–**40** on activating or repressing *hTERT* expression and telomerase activity.

Cell type	H1299 (inhibition μM ± SD)[a]					
Compound	MTT			SEAP		
	100	10	1	100	10	1
1	0 ± 4	0 ± 3	8 ± 3	13 ± 1	13 ± 1	15 ± 1
2	5 ± 1	83 ± 6	101 ± 4	15 ± 1	94 ± 5	96 ± 3
3	19 ± 5	79 ± 6	103 ± 7	15 ± 1	92 ± 6	113 ± 6
4	62 ± 6	116 ± 4	100 ± 4	30 ± 2	97 ± 4	108 ± 9
5	0 ± 4	0 ± 3	8 ± 3	13 ± 1	13 ± 1	15 ± 1
6	19 ± 5	79 ± 6	103 ± 7	15 ± 1	92 ± 6	113 ± 6
7	62 ± 5	116 ± 4	100 ± 4	30 ± 2	97 ± 4	108 ± 9
8	5 ± 1	83 ± 6	101 ± 4	15 ± 1	94 ± 5	96 ± 3
9	0 ± 2	0 ± 3	73 ± 7	0 ± 2	0 ± 2	98 ± 7
10	0 ± 1	0 ± 1	72 ± 3	0 ± 0	0 ± 0	116 ± 1
11	0 ± 1	2 ± 0	103 ± 7	0 ± 0	0 ± 0	109 ± 2
12	0 ± 1	2 ± 0	78 ± 7	2 ± 0	5 ± 0	105 ± 1
13	1 ± 0	8 ± 1	18 ± 1	0 ± 0	1 ± 0	26 ± 1
14	0 ± 0	4 ± 0	10 ± 2	0 ± 2	2 ± 3	11 ± 9
15	0 ± 0	6 ± 1	101 ± 4	1 ± 2	5 ± 2	81 ± 5
16	0 ± 0	5 ± 0	89 ± 2	3 ± 0	6 ± 0	80 ± 3
17	4 ± 0	5 ± 1	92 ± 7	4 ± 0	6 ± 0	98 ± 5
18	0 ± 0	3 ± 1	93 ± 2	2 ± 0	4 ± 1	93 ± 6
19	3 ± 1	5 ± 1	94 ± 3	0 ± 1	4 ± 0	99 ± 4
20	0 ± 0	6 ± 0	86 ± 2	6 ± 0	8 ± 0	85 ± 8
21	3 ± 1	5 ± 1	81 ± 1	7 ± 0	9 ± 0	84 ± 3
22	6 ± 0	7 ± 0	88 ± 5	1 ± 0	8 ± 0	93 ± 5
23	2 ± 0	0 ± 0	80 ± 3	2 ± 0	2 ± 0	84 ± 2
24	0 ± 0	2 ± 0	0 ± 0	2 ± 0	3 ± 0	5 ± 2
25	0 ± 1	3 ± 0	105 ± 7	1 ± 0	4 ± 0	92 ± 0
26	0 ± 0	2 ± 0	96 ± 2	0 ± 0	2 ± 0	81 ± 1
27	4 ± 0	66 ± 1	101 ± 4	4 ± 0	72 ± 2	82 ± 1
28	0 ± 0	5 ± 0	88 ± 2	0 ± 0	3 ± 0	80 ± 4
29	0 ± 0	88 ± 5	106 ± 2	9 ± 1	78 ± 3	99 ± 3
30	3 ± 0	88 ± 7	104 ± 2	10 ± 0	76 ± 7	100 ± 3
31	1 ± 1	46 ± 1	90 ± 1	22 ± 2	11 ± 2	94 ± 6
32	5 ± 1	69 ± 3	88 ± 4	3 ± 0	59 ± 2	81 ± 4
33	10 ± 2	70 ± 2	86 ± 6	8 ± 0	70 ± 3	85 ± 7
34	15 ± 3	56 ± 5	81 ± 6	3 ± 0	54 ± 3	98 ± 0
35	20 ± 2	69 ± 3	85 ± 7	12 ± 1	72 ± 3	88 ± 1
36	3 ± 3	13 ± 4	77 ± 1	8 ± 4	29 ± 7	78 ± 3
37	0 ± 1	44 ± 4	71 ± 5	0 ± 0	8 ± 3	76 ± 9
38	23 ± 3	76 ± 7	80 ± 6	15 ± 1	88 ± 9	93 ± 4
39	6 ± 9	100 ± 7	105 ± 3	9 ± 2	64 ± 6	97 ± 7
40	59 ± 13	91 ± 5	102 ± 5	19 ± 5	79 ± 3	87 ± 7

[a] SD, standard derivation, all experiments were independently performed at least three times ($n = 3$).

both MTT and SEAP assays, indicating that none of these compounds showed specific *hTERT* repression activity.

5. Conclusion

In the course of our continuing search for new antitumor agents from anthraquinone moiety, we described a method of synthesizing diversely symmetrical or asymmetrical substituted 1,2-diamidoanthraquinone derivatives and comparing to their cytotoxicity and telomerase activity. In this investigation, we focused our attention on the role of our systematic synthesized tricyclic pharmacophore bearing the symmetrical or asymmetrical side chain linked to the planar anthraquinone moiety and to understand the basis of tricyclic system selectivity. Forty new compounds have been prepared among which twenty-seven displayed a broad spectrum of antitumor activity below 10 μM range. As shown in the Tables 2 and 3, the cytotoxicity elicited by the five analogs upon human-derived carcinoma cells, is largely clustered within the Leukemia, Non-small cell lung cancer, Colon cancer, CNS cancer, Melanoma, Ovarian cancer, Renal cancer, Prostate cancer and Breast cancer panels of the NCI 60 cell line screen. To understand the unique antiproliferative activity pattern of test compounds in the NCI-60 cell lines screen, we computed structure activity relationships between the five compounds **4** (NSC747246), **11** (NSC749232), **21** (NSC749233), **32** (NSC749757), and **36** (NSC749670), respectively. A variable pattern of sensitivity against some individual cell lines was observed and the most potent compound **8** revealed a high activity toward a prostate cancer cell lines ($IC_{50} = 0.95$ μM, PC-3). They also exhibited dose-dependent inhibition of proliferation in all 60 cancer cell lines. As shown in Table 3 for compound **11** (NSC 749232), the average concentration required to inhibit GI_{50} was 3.63 μM with a range of 0.78 μM (Melanoma: LOX IMVI) to 9.92 μM (Melanoma: UACC-257). As shown in Table 3 for compound **36** (NSC749670), the average concentration required to inhibit GI_{50} with a range of 0.87 μM (Non-small cell lung cancer: HOP-92) to >100 μM. All these data were summarized in Tables 2 and 3 and used for further analysis.

Although the mechanisms of how compounds **11** and **36** rendered it cytotoxic activity are still unclear, there is still no significant correlation between telomerase activity and cytotoxicity. Moreover, we anticipate that analysis of anthraquinone-based small molecules with various asymmetrical amido side chains might yield further insight into designing better lead compounds for anticancer agents. The physical, chemical and biological properties of anthraquinone-base derivatives are greatly affected by its various substituents of the planar ring system which considered as aglycon analogs of anthracycline antibiotics. We are still interested in examining the effects of these compounds on telomerase activity in the cell free extracts prepared from H1299 cells. As shown in Table 4, none of the synthesized compounds exhibited telomerase inhibitory activity on SEAP expression, a tentative explanation suggested could be a steric hindrance induced by substituents or by other interactions due to the presence of functional groups on these compounds. Although the mechanism of preferential inhibition of compounds is still unclear to us, these results suggested that the cell proliferation inhibition activity of our compounds could be attributed to another mechanism of action. Therefore, more biological experiments are underway to determine a possible mechanism, which would give some information on how to improve the efficiency of our leads by chemical structure modulation.

6. Materials and methods

6.1. 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. Mass spectra were obtained on Finnigan MAT 95 XL HRMS and Finnigan/Thermo Quest MAT HRMS. FT-IR spectra were taken on Perkin–Elmer FTIR-1615 spectrometer. The starting material of 1,2-diaminoanthraquinone was purchased form Aldrich Chemicals. Reagents and solvents were purchased from Aldrich and Merck used without further purification.

6.2. General synthetic methods

All anthraquinone-based derivatives were synthesized by using a simply acylation or two-stage reaction in miniclave (Büchi). These compounds were obtained in good yield and their purity was determined using FT-IR, ^{1}H NMR, ^{13}C NMR and high resolution mass (HRMS) spectrometry.

6.2.1. General procedure A: preparation of compound (*1*–*3*)

1,2-diaminoanthraquinone (0.92 g, 4 mmol) was dissolved in anhydrous DMF (30 mL) in ice-bath and acyl chloride (12 mmol)

and pyridine (0.5 mL) were added under nitrogen for 24 h. Ice was added to precipitate out the crude product. The resulting precipitate was collected and purified by crystallization from hot ethanol to afford desired compounds **1**–**3**.

*6.2.2. General procedure B: preparation of compound (**4**)*

1,2-diaminoanthraquinone (0.92 g, 4 mmol) was dissolved in anhydrous THF (30 mL) and 4-toluoyl chloride (1.6 mL, 12 mmol), pyridine (0.5 mL) were added dropwise under nitrogen. The reaction mixture was heated and refluxed at 70 °C in miniclave for 3–4 h. After removal of THF, the reaction mixture was washed with ethyl acetate/hexane and the crude product was extracted in ethyl acetate. The organic layer was collect and dried over anhydrous Na_2SO_4, and then the solvent was evaporated. The crude compound was washed and purified by crystallization from hot ethanol to afford desired compound **4**.

*6.2.3. General procedure C: preparation of compound (**5**)*

1,2-diaminoanthraquinone (1.19 g, 5 mmol) was dissolved in anhydrous DMF (30 mL) and chloroacetyl chloride (0.5 mL, 6.0 mmol) was added under nitrogen for 2 h. Ice was added to precipitate out the crude product. The resulting precipitate was collected and purified by crystallization from hot ethanol to afford desired compound **5**.

*6.2.4. General procedure D: preparation of compound (**6**–**7**)*

A solution of compound **5** (1.28 g, 4 mmol) was dissolved in anhydrous DMF (30 mL) and acyl chloride (12 mmol) was added under nitrogen for 1 h. Ice was added to precipitate out the crude product. The resulting precipitate was collected and purified by recrystallization from hot ethanol to afford desired compounds **6**–**7**.

*6.2.5. General procedure E: preparation of compound (**8**–**28**)*

A solution of compound **5** (1.28 g, 4 mmol) was dissolved in anhydrous THF (30 mL), and acyl chloride (12 mmol), pyridine (0.5 mL) were added dropwise under nitrogen. The reaction mixture was heated and refluxed at 70 °C in miniclave for 3–4 h. After removal of THF, the reaction mixture was washed with ethyl acetate/hexane and the crude product was extracted in ethyl acetate. The organic layer was collect and dried over anhydrous Na_2SO_4, and then the solvent was evaporated. The crude compound was washed and purified by crystallization from hot ethanol to afford desired compounds **8**–**28**.

*6.2.6. General procedure F: preparation of compound (**29**–**40**)*

A solution of compound **11** (0.83 g, 2 mmol) was dissolved in anhydrous THF (30 mL). The mixture was stirred for 10 min before the addition of appropriate amines (8 mmol) and DIPEA (1 mL, 6 mmol), after which the reaction mixture was refluxed for 16 h. After removal of THF, the reaction mixture was washed with ethyl acetate/hexane and the crude product was extracted in ethyl acetate. The organic layer was collected and dried over anhydrous Na_2SO_4, and then the solvent was evaporated. The crude compound was washed and purified by crystallization from hot ethanol to afford desired compounds (**28**–**40**).

*6.2.7. 1, 2-Bis-(chloroacetamido)-anthraquinone (**1**)*

The pure compound was obtained as a yellowish brown powder (yield 56%). Mp: 254–255 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1718 (CO), 3273 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 4.43 (s, 2H), 4.45 (s, 2H), 7.90–7.93 (m, 2H), 8.12–8.18 (m, 2H), 8.22 (d, J = 8.1 Hz, 1H), 8.38 (d, J = 9.0 Hz, 1H), 9.83 (s, 1H), 10.29 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 37.71, 43.25, 126.20, 126.93, 127.90, 128.04, 128.14, 130.33, 132.28, 134.25, 134.35, 134.42, 134.63, 139.77, 165.61, 166.02, 181.60, 183.25. HRMS (EI) m/z: calcd $[M]^+$, 390.0174 ($C_{18}H_{12}Cl_2N_2O_4^+$); found, 390.0170.

*6.2.8. 1, 2-Bis-(3-chloropropionamido)-anthraquinone (**2**)*

The pure compound was obtained as a yellow powder (yield 51%). Mp: 179–180 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1704 (CO), 3301 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 2.94–3.03 (m, 4H), 3.86–3.94 (m, 4H), 7.88–7.90 (m, 2H), 8.10–8.15 (m, 2H), 8.08 (d, J = 8.1 Hz, 1H), 8.42 (d, J = 8.7 Hz, 1H), 9.50 (s, 1H), 10.02 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 39.58, 40.20, 40.26, 43.33, 125.94, 126.29, 126.87, 127.61, 127.79, 128.33, 129.79, 132.27, 134.26, 134.36, 134.52, 140.28, 168.86, 169.43, 181.63, 183.22. HRMS (EI) m/z: calcd $[M]^+$, 418.0487 ($C_{20}H_{16}Cl_2N_2O_4^+$); found, 418.0494.

*6.2.9. 1, 2-Bis-(4-chlorobutyramido)-anthraquinone (**3**)*

The pure compound was obtained as a yellow powder (yield 45%). Mp: 154–155 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1666 (CO), 3313 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 2.04–2.12 (m, 4H), 2.57–2.66 (m, 4H), 3.71 (t, J = 6.6 Hz, 2H), 3.78 (t, J = 6.6 Hz, 2H), 7.88–7.91 (m, 2H), 8.08–8.16 (m, 3H), 8.36 (d, J = 8.7 Hz, 1H), 9.52 (s, 1H), 9.82 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 27.68, 27.76, 33.11, 33.37, 44.75, 44.99, 125.55, 126.28, 126.85, 127.88, 128.21 128.40, 129.57, 132.28, 134.23, 134.47, 134.53, 140.45, 171.12, 171.60, 181.71, 183.46. HRMS (EI) m/z: calcd $[M]^+$, 446.0800 ($C_{22}H_{20}Cl_2N_2O_4^+$); found, 446.0793.

*6.2.10. 1, 2-Bis-(4-methylbenzamido)-anthraquinone (**4**)*

The pure compound was obtained as a brown powder (yield 62%). Mp: 227–229 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1673 (CO), 3330 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 2.36 (s, 3H), 2.42 (s, 3H), 7.33 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 8.1 Hz, 2H), 7.90–7.93 (m, 2H), 8.01 (d, J = 8.1 Hz, 2H), 8.13–8.16 (m, 1H), 8.18–8.20 (m, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.42 (d, J = 8.7 Hz, 1H), 10.06 (s, 1H), 10.90 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.37, 20.48, 125.02, 125.90, 126.61, 126.90, 127.45, 128.88, 128.92, 129.28, 129.70, 129.99, 130.39, 130.57, 131.79, 133.85, 134.00, 134.14, 138.94, 139.10, 142.12, 142.20, 164.50, 165.85, 181.21, 183.84. HRMS (EI) m/z: calcd $[M]^+$, 474.1580 ($C_{30}H_{22}N_2O_4^+$); found, 474.1572.

*6.2.11. 1-(Amino)-2-(chloroacetamido)-anthraquinone (**5**)*

The pure compound was obtained as a red powder (yield 65%). Mp: 193–194 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1678 (CO), 3237 (NH), 3294, 3416 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 4.38 (s, 2H), 7.48 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.88 (td, J = 12 Hz, J = 1.8 Hz, 2H), 7.96 (br, 2H), 8.14 (dd, J = 7.2 Hz, 1.8 Hz, 1H), 8.22 (dd, J = 7.8 Hz, 1.5 Hz, 1H), 9.80 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.25, 112.98, 115.62, 126.69, 126.55, 128.88, 128.92, 130.25, 131.01, 132.64, 133.65, 134.37, 146.11, 165.72, 182.28, 184.38. HRMS (EI) m/z: calcd $[M]^+$, 314.0458 ($C_{16}H_{11}N_2O_3^+$); found, 314.0455.

*6.2.12. 1-[(3-Chloropropanyl)amido]-2-(chloroacetamido)-anthraquinone (**6**)*

The pure compound was obtained as a dark yellow powder (yield 33%). Mp: 182–183 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1670 (CO), 3271 (NH). ^1H NMR (300 MHz, $CDCl_3$): δ ppm 3.13 (t, J = 6.3 Hz, 2H), 3.96 (t, J = 6.6 Hz, 2H), 4.21 (s, 2H), 7.81–7.84 (m, 2H), 8.25–8.34 (m, 4H), 9.73 (s, 1H), 11.51 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ ppm 40.12, 40.36, 43.33, 125.92, 126.28, 126.82, 127.90, 128.00, 128.92, 130.28, 132.18, 134.19, 134.28, 134.52, 139.56, 164.50, 165.85, 181.45, 183.09. HRMS (EI) m/z: calcd $[M]^+$, 404.0331 ($C_{19}H_{14}C_{l2}N_2O_4^+$); found, 404.0334.

*6.2.13. 1-[(4-Chlorobutanyl)amido]-2-(chloroacetamido)-anthraquinone (**7**)*

The pure compound was obtained as a yellow brown powder (yield 38%). Mp: 163–164 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1673 (CO), 3290 (NH). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.28–2.34 (m, 2H), 2.88 (t, J = 7.2 Hz, 2H), 3.73 (t, J = 6.3 Hz, 2H), 4.22 (s, 2H), 7.81–7.83 (m, 2H), 8.26–8.34 (m, 4H), 9.84 (s, 1H), 11.46 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ ppm 27.76, 33.05, 39.92, 43.34, 44.99, 125.67, 126.26, 126.79, 127.91, 128.01, 128.73, 130.21, 132.16, 134.23, 134.26, 134.52, 165.34, 171.81, 181.49, 183.21. HRMS (EI) m/z: calcd $[M]^+$, 418.0487 ($C_{20}H_{16}Cl_2N_2O_4^+$); found, 418.0494.

*6.2.14. 1-(Benzamido)-2-(chloroacetamido)-anthraquinone (**8**)*

The pure compound was obtained as a yellow powder (yield 67%). Mp: 203–204 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1689 (CO), 3318 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.42 (s, 2H), 7.58–7.67 (m, 3H), 7.88–7.92 (m, 2H), 8.09–8.18 (m, 4H), 8.24 (d, J = 8.7 Hz, 1H), 8.40 (d, J = 8.7 Hz, 1H), 9.86 (s, 1H), 10.58 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.19, 125.76, 126.28, 126.95, 127.79, 127.95, 128.40, 128.51, 129.56, 130.43, 132.00, 132.22, 133.97, 134.23, 134.34, 134.50, 139.44, 165.42, 166.35, 181.55, 183.77. HRMS (EI) m/z: calcd $[M]^+$, 418.0720 ($C_{23}H_{15}ClN_2O_4^+$); found, 418.0716.

*6.2.15. 1-(2-Methylbenzamido)-2-(chloroacetamido)-anthraquinone (**9**)*

The pure compound was obtained as a yellow powder (yield 47%). Mp: 179–180 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1664 (CO), 3277 (NH). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.52 (s, 3H), 4.20 (s, 2H), 7.48–7.50 (m, 2H), 7.79–7.83 (m, 2H), 7.99–8.01 (m, 2H), 8.26–8.33 (m, 4H), 10.08 (s, 1H), 12.35 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 21.39, 43.12, 124.08, 125.16, 125.60, 127.24, 127.63, 128.91, 129.06, 131.71, 132.07, 132.91, 132.22, 133.32, 133.81, 134.29, 134.35, 134.83, 136.53, 139.15 165.44, 168.03, 181.68, 187.58. HRMS (EI) m/z: calcd $[M]^+$, 432.0877 ($C_{24}H_{17}ClN_2O_4^+$); found, 432.0877.

*6.2.16. 1-(3-Methylbenzamido)-2-(chloroacetamido)-anthraquinone (**10**)*

The pure compound was obtained as a brown powder (yield 56%). Mp: 202–203 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1671 (CO), 3233 (NH). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.52 (s, 3H), 4.20 (s, 2H), 7.48–7.50 (m, 2H), 7.80–7.83 (m, 2H), 7.99–8.01 (m, 2H), 8.26–8.32 (m, 4H), 10.08 (s, 1H), 12.36 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 21.39, 43.12, 124.07, 125.15, 125.60, 126.30, 127.24, 127.63, 128.91, 129.06, 131.70, 132.08, 132.91, 133.30, 133.81, 134.29, 134.36, 136.84, 136.51, 139.15, 165.45, 168.03, 181.69, 187.58. HRMS (EI) m/z calcd $[M]^+$, 432.0877 ($C_{24}H_{17}ClN_2O_4^+$); found, 432.0873.

*6.2.17. 1-(4-Methylbenzamido)-2-(chloroacetamido)-anthraquinone (**11**)*

The pure compound obtained as a brown powder (yield 66%). Mp: 198–199 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1666 (CO), 3230 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.43 (s, 3H), 4.43 (s, 2H), 7.41 (d, J = 7.8 Hz, 2H), 7.88–7.91 (m, 2H), 8.01 (d, J = 8. 1 Hz, 2H), 8.10–8.13 (m, 1H), 8.16–8.19 (m, 1H), 8.24 (d, J = 8.7 Hz, 1H), 8.39 (d, J = 9.0 Hz, 1H), 9.84 (s, 1H), 10.58 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 20.90, 43.53, 123.39, 124.92, 126.62, 127.00, 127.64, 129.26, 129.91, 131.06, 131.42, 132.33, 132.41, 133.01, 133.73, 134.20, 135.90, 143.22, 164.83, 167.15, 181.07, 187.00. HRMS (EI) m/z: calcd $[M]^+$, 432.0877 ($C_{24}H_{17}ClN_2O_4^+$); found, 432.0881.

*6.2.18. 1-(2-Fluorobenzamido)-2-(chloroacetamido)-anthraquinone (**12**)*

The pure compound was obtained as a yellow brown powder (yield 31%). Mp: 245–246 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1666 (CO), 3261 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.41 (s, 2H), 7.40–7.47 (m, 2H), 7.65–7.72 (m, 1H), 7.86–7.93 (m, 2H), 8.03–8.19 (m, 3H), 8.24 (d, J = 8.4 Hz, 1H), 8.37 (d, J = 8.7 Hz, 1H), 9.83 (s, 1H), 10.48 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.10, 116.36, 116.65, 124.71, 125.86, 126.29, 126.94, 127.83, 128.50, 128.96, 130.43, 130.98, 132.23, 133.80, 133.92, 134.24, 134.35, 134.51, 139.27, 162.97, 165.41, 181.55, 183.82. HRMS (EI) m/z: calcd $[M]^+$, 436.0626 ($C_{23}H_{14}ClFN_2O_4^+$); found, 436.0630.

*6.2.19. 1-(3-Fluorobenzamido)-2-(chloroacetamido)-anthraquinone (**13**)*

The pure compound was obtained as a yellow powder (yield 47%). Mp: 197–198 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1671 (CO), 3211 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.43 (s, 2H), 7.48–7.55 (m, 1H), 7.63–7.70 (m, 1H), 7.86–7.96 (m, 4H), 8.10–8.13 (m, 1H), 8.15–8.19 (m, 1H), 8.26 (d, J = 8.7 Hz, 1H), 8.44 (d, J = 8.7 Hz, 1H), 9.85 (s, 1H), 10.49 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.21, 114.61, 114.91, 118.63, 118.90, 124.18, 126.12, 126.30, 126.96, 128.14, 128.24, 128.84, 130.37, 130.64, 130.74, 132.23, 134.35, 136.55, 139.96, 162.43, 165.54, 181.55, 183.41. HRMS (EI) m/z: calcd $[M]^+$, 436.0626 ($C_{23}H_{14}ClFN_2O_4^+$); found, 436.0623.

*6.2.20. 1-(4-Fluorobenzamido)-2-(chloroacetamido)-anthraquinone (**14**)*

The pure compound was obtained as a greenish brown powder (yield 62%). Mp: 184–185 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1672 (CO), 3213 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.42 (s, 2H), 7.44 (t, J = 9.0 Hz, 2H), 7.88–7.91 (m, 2H), 8.09–8.18 (m, 4H), 8.25 (d, J = 8.7 Hz, 1H), 8.42 (d, J = 8.7 Hz, 1H), 9.84 (s, 1H), 10.51 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.23, 115.33, 115.62, 125.97, 126.33, 126.98, 128.07, 128.25, 129.24, 130.40, 130.64, 130.82, 132.25, 134.25, 134.38, 134.56, 139.79, 165.44, 165.52, 181.58, 183.58. HRMS (EI) m/z: calcd $[M]^+$, 436.0626 ($C_{23}H_{14}ClFN_2O_4^+$); found, 436.0625.

*6.2.21. 1-(2-Chlorobenzamido)-2-(chloroacetamido)-anthraquinone (**15**)*

The pure compound was obtained as a yellowish green powder (yield 46%). Mp: 193–194 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1671 (CO), 3227 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.43 (s, 2H), 7.57–7.60 (m, 3H), 7.89–7.94 (m, 2H), 8.08–8.19 (m, 3H), 8.26 (d, J = 8.7 Hz, 1H), 8.37 (d, J = 8.7 Hz, 1H), 9.76 (s, 1H), 10.44 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.16, 126.16, 126.32, 126.93, 127.19, 127.87, 128.07, 128.37, 128.53, 129.49, 130.14, 130.63, 130.76, 131.73, 134.24, 134.34, 134.56, 135.29, 139.44, 165.36, 165.54, 181.55, 183.44. HRMS (EI) m/z: calcd $[M]^+$, 452.0331 ($C_{23}H_{14}Cl_2N_2O_4^+$); found, 436.0325.

*6.2.22. 1-(3-chlorobenzamido)-2-(chloroacetamido)-anthraquinone (**16**)*

The pure compound was obtained as a yellow powder (yield 51%). Mp: 188–189 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1678 (CO), 3235 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.43 (s, 2H), 7.72–7.64 (m, 2H), 7.87–7.91 (m, 2H), 8.02–8.19 (m, 4H), 8.26 (d, J = 8.4 Hz, 1H), 8.45 (d, J = 8.4 Hz, 1H), 9.79 (s, 1H), 10.49 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.28, 126.11, 126.24, 126.36, 126.79, 127.01, 127.88, 128.08, 128.37, 128.74, 130.36, 130.53, 131.73, 132.27, 133.33, 134.26, 134.60, 136.28, 140.17, 165.23, 165.64, 181.61, 183.36. HRMS (EI) m/z: calcd $[M]^+$, 452.0331 ($C_{23}H_{14}Cl_2N_2O_4^+$); found, 436.0330.

*6.2.23. 1-(4-chlorobenzamido)-2-(chloroacetamido)-anthraquinone (**17**)*

The pure compound was obtained as a yellow powder (yield 67%). Mp: 224–225 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1678 (CO), 3235 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.42 (s, 2H),

7.68–7.71 (m, 2H), 7.88–7.91 (m, 2H), 8.08–8.18 (m, 4H), 8.25 (d, $J = 8.4$ Hz, 1H), 8.43 (d, $J = 8.7$ Hz, 1H), 9.86 (s, 1H), 10.52 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.23, 126.06, 126.33, 126.96, 128.21, 128.61, 129.06, 129.93, 130.40, 131.71, 132.25, 132.92, 134.25, 134.38, 134.56, 136.91, 139.87, 165.50, 165.53, 181.58, 183.51. HRMS (EI) m/z: calcd $[M]^+$, 452.0331 ($C_{23}H_{14}Cl_2N_2O_4^+$); found, 436.0333.

*6.2.24. 1-[2-(Trifluoromethyl)benzamido]-2-(chloroacetamido)-anthraquinone (**18**)*

The pure compound was obtained as a yellowlish green powder (yield 32%). Mp: 248–250 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1671 (CO), 3282 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.38 (s, 2H), 7.80 (t, $J = 7.4$ Hz, 1H), 7.90–7.97 (m, 4H), 8.13–8.29 (m, 4H), 8.37 (d, $J = 8.4$ Hz, 1H), 9.72 (s, 1H), 10.49 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.32, 126.27, 126.33, 126.61, 126.63, 126.87, 128.07, 128.13, 128.53, 128.64, 128.71, 129.01, 130.66, 130.76, 132.18, 132.59, 134.23, 134.35, 134.58, 139.39, 165.31, 166.00, 181.45, 183.24. HRMS (EI) m/z: calcd $[M]^+$, 486.0594 ($C_{24}H_{14}ClF_3N_2O_4^+$); found, 486.0587.

*6.2.25. 1-[3-(Trifluoromethyl)benzamido]-2-(chloroacetamido)-anthraquinone (**19**)*

The pure compound was obtained as a yellow powder (yield 48%). Mp: 190–191 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1670 (CO), 3288 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.38 (s, 2H), 7.84–7.93 (m, 3H), 8.04 (d, $J = 7.5$ Hz, 1H), 8.08–8.11 (m, 1H), 8.16–8.19 (m, 1H), 8.27 (d, $J = 9.0$ Hz, 1H), 8.38–8.49 (m, 3H), 9.90 (s, 1H), 10.58 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.25, 124.55, 124.66, 126.16, 126.32, 126.63, 126.96, 127.98, 128.39, 128.48, 128.55, 129.82, 130.34, 132.07, 132.25, 134.22, 134.37, 134.55, 135.27, 140.30, 165.24, 165.63, 181.56, 183.25. HRMS (EI) m/z: calcd $[M]^+$, 486.0594 ($C_{24}H_{14}ClF_3N_2O_4^+$); found, 486.0596.

*6.2.26. 1-[4-(Trifluoromethyl)benzamido]-2-(chloroacetamido)-anthraquinone (**20**)*

The pure compound was obtained as a brown powder (yield 57%). Mp: 203–204 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1666 (CO), 3285 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.43 (s, 2H), 7.88–7.91 (m, 2H), 8.01 (d, $J = 8.4$ Hz, 2H), 8.08–8.11 (m, 1H), 8.16–8.19 (m, 1H), 8.25–8.32 (m, 3H), 8.45 (d, $J = 8.3$ Hz, 1H), 9.90 (s, 1H), 10.60 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.25, 125.53, 125.77, 126.27, 126.95, 128.11, 128.39, 128.66, 128.93, 130.37, 131.56, 131.99, 132.26, 134.24, 134.39, 134.57, 138.02, 140.12, 165.42, 165.61, 181.57, 183.35. HRMS (EI) m/z: calcd $[M]^+$, 486.0594 ($C_{24}H_{14}ClF_3N_2O_4^+$); found, 486.0597.

*6.2.27. 1-[2-(Phenyl)acetylamido]-2-(chloroacetamido)-anthraquinone (**21**)*

The pure compound was obtained as a brown powder (yield 34%). Mp: 184–185 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1667 (CO), 3282 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.86 (s, 2H), 4.36 (s, 2H), 7.23–7.93 (m, 5H), 7.89–7.93 (m, 2H), 8.11–8.20 (m, 3H), 8.31 (d, $J = 8.7$ Hz, 1H), 9.66 (s, 1H), 10.25 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 42.51, 43.34, 125.59, 126.26, 126.53, 126.83, 128.15, 128.86, 129.30, 129.66, 130.30, 132.16, 134.22, 134.29, 134.52, 135.35, 142.78, 145.07, 165.25, 170.57, 181.51, 183.25. HRMS (EI) m/z: calcd $[M]^+$, 432.0877 ($C_{24}H_{17}ClN_2O_4^+$); found, 432.0885.

*6.2.28. 1-[2-(4-Fluorophenyl)acetylamido]-2-(chloroacetamido)-anthraquinone (**22**)*

The pure compound was obtained as a yellowish brown powder (yield 37%). Mp: 213–214 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1667 (CO), 3279 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.86 (s, 2H), 4.37 (s, 2H), 7.17 (t, $J = 9.0$ Hz, 2H), 7.42–7.47 (m, 2H), 7.90–7.93 (m, 2H), 8.11–8.23 (m, 3H), 8.32 (d, $J = 8.7$ Hz, 1H), 9.64 (s, 1H), 10.20 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 41.48, 43.03, 114.69, 114.97, 125.67, 126.26, 126.79, 127.79, 128.15, 128.28, 129.14, 130.45, 131.42, 131.53, 132.23, 134.27, 134.50, 139.18, 165.23, 170.70, 181.54, 183.46. HRMS (EI) m/z: calcd $[M]^+$, 450.0783 ($C_{24}H_{16}ClFN_2O_4^+$); found, 450.0782.

*6.2.29. 1-[(2-Furoyl)amido]-2-(chloroacetamido)-anthraquinone (**23**)*

The pure compound was obtained as a brown powder (yield 63%). Mp: 211–212 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1667 (CO), 1710 (C=C), 3260 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.43 (s, 2H), 6.77–6.79 (m, 1H), 7.38 (d, $J = 3.0$ Hz, 1H), 7.88–7.92 (m, 2H), 8.06 (d, $J = 0.9$ Hz, 1H), 8.11–8.18 (m, 2H), 8.22 (d, $J = 8.7$ Hz, 1H), 8.39 (d, $J = 8.7$ Hz, 1H), 9.93 (s, 1H), 10.60 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.26, 112.51, 115.71, 125.74, 126.37, 127.05, 127.56, 128.59, 128.82, 130.37, 132.27, 134.26, 134.47, 134.61, 139.34, 157.15, 165.60, 181.60, 184.03. HRMS (EI) m/z: calcd $[M]^+$, 408.0513 ($C_{21}H_{13}ClN_2O_5^+$); found, 408.0518.

*6.2.30. 1-[(2-Thiophenecarbonyl)amido]-2-(chloroacetamido)-anthraquinone (**24**)*

The pure compound was obtained as a brown powder (yield 55%). Mp: 190–191 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1673 (CO), 1728 (C=C), 3288 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.45 (s, 2H), 7.30–7.33 (m, 1H), 7.88–7.94 (m, 4H), 8.10–8.20 (m, 2H), 8.24 (d, $J = 8.4$ Hz, 1H), 8.42 (d, $J = 6.9$ Hz, 1H), 9.45 (s, 1H), 10.53 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.25, 125.95, 126.36, 127.01, 128.04, 128.24, 128.37, 128.88, 130.36, 132.26, 134.26, 134.42, 134.60, 139.08, 139.79, 161.17, 165.60, 181.61, 183.59. HRMS (EI) m/z: calcd $[M]^+$, 424.0285 ($C_{21}H_{13}ClN_2O_4S^+$); found, 424.0288.

*6.2.31. 1-[(5-Isoxazolecarbonyl)amido]-2-(chloroacetamido)-anthraquinone (**25**)*

The pure compound was obtained as a yellowish brown powder (yield 52%). Mp: 212–213 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1598 (C=N), 1673 (CO), 3268 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.43 (s, 2H), 7.31 (d, $J = 1.8$ Hz, 1H), 7.88–7.92 (m, 2H), 8.08–8.18 (m, 2H), 8.27 (d, $J = 8.7$ Hz, 1H), 8.49 (d, $J = 8.7$ Hz, 1H), 8.88 (d, $J = 1.8$ Hz, 1H), 10.03 (s, 1H), 10.69 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.25, 107.12, 126.35, 126.66, 126.82, 126.98, 127.84, 128.56, 130.17, 132.24, 134.18, 134.42, 134.59, 140.46, 151.99, 155.50, 162.77, 165.79, 181.50, 183.06. HRMS (EI) m/z: calcd $[M]^+$, 409.0465 ($C_{20}H_{12}ClN_3O_5^+$); found, 409.0472.

*6.2.32. 1-[(2,5-Dimethyl-3-furoyl)amido]-2-(chloroacetamido)-anthraquinone (**26**)*

The pure compound was obtained as a yellow powder (yield 63%). Mp: 207–208 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1676 (CO), 3167 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.33 (s, 3H), 2.52 (s, 3H), 4.44 (s, 2H), 6.70 (s, 1H), 7.89–7.92 (m, 2H), 8.13–8.19 (m, 2H), 8.21 (d, $J = 8.7$ Hz, 1H), 8.34 (d, $J = 8.7$ Hz, 1H), 9.83 (s, 1H), 10.29 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 12.74, 13.04, 43.14, 105.21, 116.20, 125.29, 126.29, 126.98, 128.82, 129.93, 130.50, 132.23, 134.23, 134.39, 134.52, 138.67, 149.74, 156.05, 162.99, 165.30, 181.55, 184.22. HRMS (EI) m/z: calcd $[M]^+$, 436.0826 ($C_{23}H_{17}ClN_2O_5^+$); found, 436.0832.

*6.2.33. 1-[2-(Phenoxy)acetylamido]-2-(chloroacetamido)-anthraquinone (**27**)*

The pure compound was obtained as a yellowish green powder (yield 55%). Mp: 217–218 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1681 (CO), 3182 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.40 (s, 2H), 4.82 (s, 2H), 7.02 (t, $J = 6.0$ Hz, 1H), 7.16 (d, $J = 7.5$ Hz, 2H), 7.35–7.40 (m, 2H), 7.90–7.93 (m, 2H), 8.13–8.18 (m, 2H), 8.20 (d, $J = 8.7$ Hz, 1H), 8.33 (d, $J = 8.4$ Hz, 1H), 9.86 (s, 1H), 10.58 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.12, 67.24, 114.94, 118.49, 121.48,

125.60, 126.29, 126.89, 127.44, 128.64, 129.54, 132.22, 134.21, 134.37, 134.52, 135.11, 138.87, 157.66, 165.41, 168.03, 181.50, 183.94. HRMS (EI) *m/z*: calcd $[M]^+$, 448.0826 ($C_{24}H_{17}ClN_2O_5^+$); found, 448.0824.

6.2.34. 1-[2-(Phenylsulfanyl)acetylamido]-2-(chloroacetamido)-anthraquinone (**28**)

The pure compound was obtained as a brown powder (yield 59%). Mp: 161–162 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1671 (CO), 3260 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 4.10 (s, 2H), 4.43 (s, 2H), 7.18–7.22 (m, 1H),7.33–7.35 (m, 2H), 7.45 (d, $J = 7.2$ Hz, 2H), 7.86–7.93 (m, 2H), 8.08–8.16 (m, 2H), 8.21 (d, $J = 8.7$ Hz, 1H), 8.30 (d, $J = 8.7$ Hz, 1H), 9.71 (s, 1H), 10.46 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 43.82, 120.98, 126.43, 126.58, 127.00, 127.51, 128.56, 129.20, 129.52, 129.66, 131.16, 132.93, 134.81, 134.96, 135.01, 135.23, 136.69, 139.82, 166.05, 168.92, 182.23, 184.16. HRMS (EI) *m/z*: calcd $[M]^+$, 464.0598 ($C_{24}H_{17}ClN_2O_4S^+$); found, 464.0602.

6.2.35. 1-(4-Methylbenzamido)-2-[2-(dimethylamino) acetylamido]-anthraquinone (**29**)

The pure compound was obtained as a yellowish brown powder (yield 54%). Mp: 190–191 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1685 (CO), 3299 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 2.11 (s, 6H), 2.44 (s, 3H), 3.09 (s, 2H), 7.43 (d, $J = 8.1$ Hz, 2H), 7.88–7.91 (m, 2H), 8.00–8.04 (m, 2H), 8.11–8.19 (m, 2H), 8.26 (d, $J = 8.7$ Hz, 1H), 8.64 (d, $J = 8.4$ Hz, 1H), 10.16 (s, 1H), 10.54 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.86, 45.16, 62.82, 125.73, 126.30, 126.47, 127.79, 127.99, 128.12, 128.89, 129.09, 129.39, 130.93, 132.32, 134.13, 134.36, 134.43, 140.34, 142.23, 166.35, 169.14, 181.45, 183.63. HRMS (EI) *m/z*: calcd $[M]^+$, 441.1689 ($C_{26}H_{23}N_3O_4^+$); found, 441.1689.

6.2.36. 1-(4-Methylbenzamido)-2-{2-[(1,3-dioxolan-2ylmethyl)(mehyl)amino]acetylamido}- anthraquinone (**30**)

The pure compound was obtained as a yellowish powder (yield 43%). Mp: 156–157 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1672 (CO), 3282 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 2.18 (s, 3H), 2.44 (s, 3H), 2.54 (d, $J = 8.7$ Hz, 2H), 3.26 (s, 2H), 3.52–3.55 (m, 2H), 3.72–3.77 (m, 2H), 4.58 (t, $J = 4.5$ Hz, 1H), 7.43 (d, $J = 7.8$ Hz, 2H), 7.88–7.92 (m, 2H), 8.04 (d, $J = 8.1$ Hz, 2H), 8.10–8.19 (m, 2H), 8.27 (d, $J = 8.4$ Hz, 1H), 8.70 (d, $J = 8.7$ Hz, 1H), 10.15 (s, 1H), 10.53 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.87, 43.49, 59.43, 61.73, 63.91, 102.61, 125.50, 126.31, 126.48, 126.90, 127.89, 127.91, 128.06, 129.06, 129.31, 130.75, 132.33, 134.16, 134.38, 134.45, 140.42, 142.29, 166.25, 169.59, 181.46, 183.70. HRMS (EI) *m/z*: calcd $[M]^+$, 513.1900 ($C_{29}H_{27}N_3O_6^+$); found, 513.1902.

6.2.37. 1-(4-Methylbenzamido)-2-{{2-{methy[2-(2-pyridyl)ethyl] amino}acetylamido}}–anthraquinone (**31**)

The pure compound was obtained as a pale yellow powder (yield 32%). Mp: 166–167 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1666 (CO), 3263 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 2.15 (s, 3H), 2.40 (s, 3H), 2.68 (d, $J = 7.2$ Hz, 4H), 3.20 (s, 2H), 7.03 (d, $J = 7.0$ Hz, 1H), 7.13–7.18 (m, 1H), 7.39 (d, $J = 8.1$ Hz, 2H), 7.60–7.65 (m, 1H), 7.89–7.93 (m, 2H), 8.03 (d, $J = 8.1$ Hz, 2H), 8.11–8.20 (m, 2H), 8.28 (d, $J = 8.7$ Hz, 1H), 8.40–8.42 (m, 1H), 8.69 (d, $J = 8.7$ Hz, 1H), 10.18 (s, 1H), 10.54 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.81, 42.45, 56.92, 61.03, 63.91, 121.62, 122.81, 125.50, 126.29, 126.44, 126.88, 127.83, 127.88, 128.07, 129.08, 129.30, 130.72, 132.33, 134.13, 134.36, 134.42, 136.24, 140.37, 142.30, 148.86, 159.40, 166.19, 169.59, 181.43, 183.74. HRMS (EI) *m/z*: calcd $[M]^+$, 532.2111 ($C_{32}H_{28}N_4O_4^+$); found, 532.2119.

6.2.38. 1-(4-Methylbenzamido)-2-[2-(tetrahydro-1H-1-pyrrolyl) acetylamido]-anthraquinone (**32**)

The pure compound was obtained as a dark brown powder (yield 39%). Mp: 193–194 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1667 (CO), 3287 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 1.32 (br, 8H), 2.43 (s, 3H), 3.27 (s, 2H), 7.42 (d, $J = 8.1$ Hz, 2H), 7.87–7.91 (m, 2H), 8.05 (d, $J = 8.1$ Hz, 2H), 8.09–8.19 (m, 2H), 8.27 (d, $J = 8.7$ Hz, 1H), 8.73 (d, $J = 8.4$ Hz, 1H), 10.12 (s, 1H), 10.54 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.86, 23.20, 53.65, 59.09, 126.30, 126.54, 126.88, 127.80, 127.88, 127.92, 129.05, 129.25, 130.59, 132.33, 134.12, 134.38, 134.42, 136.24, 140.44, 142.43, 166.26, 169.56, 181.42, 183.68. HRMS (EI) *m/z*: calcd $[M]^+$, 467.1845 ($C_{28}H_{25}N_3O_4^+$); found, 467.1840.

6.2.39. 1-(4-Methylbenzamido)-2-[(2-piperidinoacetyl)amido]-anthraquinone (**33**)

The pure compound was obtained as a yellowish green powder (yield 43%). Mp: 213–214 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1673 (CO), 3300 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 1.08 (d, $J = 3.6$ Hz, 2H), 1.19 (br, 4H), 2.33 (br, 4H), 2.43 (s, 3H), 3.07 (s, 2H), 7.43 (d, $J = 7.8$ Hz, 2H), 7.87–7.91 (m, 2H), 8.07–8.19 (m, 4H), 8.27 (d, $J = 8.7$ Hz, 1H), 8.78 (d, $J = 8.7$ Hz, 1H), 10.13 (s, 1H), 10.52 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.85, 22.80, 24.79, 54.13, 62.39, 125.19, 126.27, 126.53, 126.89, 127.84, 127.92, 128.02, 129.00, 129.22, 130.57, 132.31, 134.13, 134.36, 134.42, 140.63, 142.40, 166.25, 169.55, 181.42, 183.67. HRMS (EI) *m/z*: calcd $[M]^+$, 481.2002 ($C_{29}H_{27}N_3O_4^+$); found, 481.2000.

6.2.40. 1-(4-Methylbenzamido)-2-[(2-morpholinoacetyl)amido]-anthraquinone (**34**)

The pure compound was obtained as a yellow powder (yield 47%). Mp: 225–226 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1672 (CO), 3213 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 2.39 (br, 4H), 2.45 (s, 3H), 3.15 (s, 2H), 3.22 (br, 4H), 7.45 (d, $J = 7.8$ Hz, 2H), 7.88–7.91 (m, 2H), 8.07–8.12 (m, 4H), 8.27 (d, $J = 8.7$ Hz, 1H), 8.73 (d, $J = 8.7$ Hz, 1H), 9.99 (s, 1H), 10.61 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.95, 53.17, 61.94, 65.59, 125.73, 126.37, 126.53, 126.98, 127.82, 128.05, 128.54, 129.28, 129.46, 130.55, 132.35, 134.16, 134.42, 134.53, 140.31, 142.66, 166.32, 168.97, 181.50, 183.84. HRMS (EI) *m/z*: calcd $[M]^+$, 483.1794 ($C_{28}H_{25}N_3O_5^+$); found, 483.1793.

6.2.41. 1-(4-Methylbenzamido)-2-[2-(1,4-thiazinan-4-yl) acetylamido]-anthraquinone (**35**)

The pure compound was obtained as a yellowish green powder (yield 51%). Mp: 210–211 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1677 (CO), 3234 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 2.39 (br, 4H), 2.45 (s, 3H), 3.15 (s, 2H), 3.23 (br, 4H), 7.45 (d, $J = 8.1$ Hz, 2H), 7.88–7.91 (m, 2H), 8.07–8.19 (m, 4H), 8.27 (d, $J = 8.4$ Hz, 1H), 8.73 (d, $J = 8.4$ Hz, 1H), 10.00 (s, 1H), 10.61 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.89, 53.13, 61.92, 65.54, 127.72, 126.30, 126.40, 126.92, 127.69, 127.92, 127.98, 129.22, 129.43, 130.53, 132.30, 134.12, 134.41, 134.45, 140.21, 142.61, 166.27, 168.89, 181.42, 183.84. HRMS (EI) *m/z*: calcd $[M]^+$, 499.1566 ($C_{28}H_{25}N_3O_4S^+$); found, 499.1570.

6.2.42. 1-(4-Methylbenzamido)-2-[2-(4-methylpiperazino) acetylamido]-anthraquinone (**36**)

The pure compound was obtained as a yellowish brown powder (yield 55%). Mp: 214–215 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1678 (CO), 3252 (NH). ^1H NMR (300 MHz, DMSO-d_6): δ ppm 1.76 (s, 3H), 1.96 (br, 4H), 2.38 (br, 4H), 2.43 (s, 3H), 3.13 (s, 2H), 7.45 (d, $J = 7.8$ Hz, 2H), 7.87–7.93 (m, 2H), 8.08–8.16 (m, 4H), 8.27 (d, $J = 9.0$ Hz, 1H), 8.81 (d, $J = 8.7$ Hz, 1H), 10.01 (s, 1H), 10.50 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.82, 44.73, 52.79, 53.73, 61.60, 125.02, 126.28, 126.58, 126.89, 127.77, 127.91, 128.28, 129.14, 129.21, 130.61, 132.31, 134.12, 134.38, 134.42, 140.59, 142.53, 166.29, 169.20, 181.42, 183.67. HRMS (EI) *m/z*: calcd $[M]^+$, 496.2111 ($C_{29}H_{28}N_4O_4^+$); found, 496.2115.

*6.2.43. 1-(4-Methylbenzamido)-2-{2-[4-(2-hydroxyethyl) piperazino]acetylamido}- anthraquinone (**37**)*

The pure compound was obtained as a yellowish brown powder (yield 48%). Mp: 210–211 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1677 (CO), 3264 (NH), 3314 (OH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 1.92 (t, J = 6.0 Hz, 2H), 2.03 (br, 4H), 2.39 (br, 4H), 2.43 (s, 3H), 3.01 (s, 2H), 3.26–3.30 (m, 2H), 4.29 (t, J = 5.4 Hz, 1H), 7.43 (d, J = 8.1 Hz, 2H), 7.87–7.91 (m, 2H), 8.07–8.19 (m, 4H), 8.27 (d, J = 8.7 Hz, 1H), 8.79 (d, J = 8.7 Hz, 1H), 10.00 (s, 1H), 10.50 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.93, 52.32, 52.93, 58.21, 59.10, 61.69, 125.11, 126.37, 126.72, 126.98, 127.81, 128.04, 128.33, 129.24, 129.41, 130.61, 132.36, 134.17, 134.48, 134.53, 140.68, 142.70, 166.37, 169.28, 181.50, 183.67. HRMS (EI) m/z: calcd $[M]^+$, 526.2216 ($C_{30}H_{30}N_4O_5^+$); found, 526.2219.

*6.2.44. 1-(4-Methylbenzamido)-2-[2-(4-phenylpiperazino) acetylamido]-anthraquinone (**38**)*

The pure compound was obtained as a yellowish green powder (yield 53%). Mp: 270–271 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1673 (CO), 3274 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.21 (s, 3H), 2.55 (br, 4H), 2.73 (br, 4H), 3.22 (s, 2H), 6.64 (d, J = 7.5 Hz, 2H), 6.79 (d, J = 7.2 Hz, 1H), 7.12–7.21 (m, 4H), 7.87–7.92 (m, 2H), 7.98 (d, J = 8.1 Hz, 2H), 8.08–8.19 (m, 2H), 8.29 (d, J = 8.7 Hz, 1H), 8.80 (d, J = 8.7 Hz, 1H), 10.07 (s, 1H), 10.52 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.76, 47.66, 52.81, 61.52, 115.40, 118.74, 125.16, 126.28, 126.57, 126.88, 127.84, 127.95, 128.16, 128.58, 129.05, 129.27, 130.28 132.31, 134.11, 134.42, 135.80, 140.51, 142.46, 150.77, 166.21, 169.05, 181.42, 183.65. HRMS (EI) m/z: calcd $[M]^+$, 558.2267 ($C_{34}H_{30}N_4O_4^+$); found, 558.2271.

*6.2.45. 1-(4-Methylbenzamido)-2-{2-[4-(2-pyridyl)piperazino] acetylamido}-anthraquinone (**39**)*

The pure compound was obtained as a yellowish brown powder (yield 63%). Mp: 258–259 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1660 (CO), 3263 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.16 (s, 3H), 3.05 (br, 8H), 3.22 (s, 2H), 6.54 (d, J = 8.4 Hz, 1H), 6.66 (t, J = 6.3 Hz, 1H), 7.08 (d, J = 7.8 Hz, 2H), 7.49–7.54 (m, 1H), 7.87–7.91 (m, 2H), 7.97 (d, J = 8.1 Hz, 2H), 8.07–8.19 (m, 3H), 8.29 (d, J = 8.7 Hz, 1H), 8.82 (d, J = 9.0 Hz, 1H), 10.16 (s, 1H), 10.51 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.88, 44.21, 52.56, 61.54, 107.00, 113.11, 124.91, 126.42, 126.89, 126.99, 127.63, 128.03, 128.21, 129.01, 129.29, 132.26, 133.39, 134.19, 134.52, 134.58, 137.30, 140.73, 142.51, 147.49, 159.15, 166.26, 166.31, 181.54, 183.63. HRMS (EI) m/z: calcd $[M]^+$, 559.2220 ($C_{33}H_{29}N_5O_4^+$); found, 559.2224.

*6.2.46. 1-(4-Methylbenzamido)-2-{2-[4-(2-pyrimidinyl)piperazino] acetylamido}-anthraq uinone (**40**)*

The pure compound was obtained as a yellow powder (yield 41%). Mp: 267–268 °C (EtOH). FT-IR (KBr, $\nu_{max}cm^{-1}$): 1683 (CO), 3236 (NH). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.20 (s, 3H), 3.11 (s, 2H), 3.22 (br, 8H), 6.66 (d, J = 4.8 Hz, 1H), 7.16 (d, J = 7.8 Hz, 2H), 7.87–7.91 (m, 2H), 8.01 (d, J = 6.0 Hz, 2H), 8.08–8.19 (m, 2H), 8.28–8.34 (m, 2H), 8.32 (d, J = 7.2 Hz, 1H), 8.82 (d, J = 8.7 Hz, 1H), 10.19 (s, 1H), 10.54 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 20.98, 42.53, 43.01, 52.46, 61.44, 110.28, 111.11, 124.77, 126.32, 126.89, 127.44, 127.92, 128.14, 128.90, 129.18, 130.18, 132.26, 134.06, 134.48, 142.39, 157.65, 158.07, 161.24, 166.12, 169.08, 181.35, 183.39. HRMS (EI) m/z: calcd $[M]^+$, 560.2172 ($C_{32}H_{28}N_6O_4^+$); found, 560.2170.

6.3. Cell cultures and sulforhodamine B (SRB) assay

Human hormone-refractory prostate cancer cell lines (PC-3) were from American Type Culture Collection (Rockville, MD) [30]. 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 °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.

6.4. Cell cultures and assessment of hTERT

Non-small lung cancer cells H1299 [25] were grown in RPMI1640 media supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100 mg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. Culture media were changed every 3 days. To establish stable cell lines so that the expression of *hTERT* could be monitored by a reporter system, a ~3.3 kbp DNA fragment ranging from −3338 to +1 bp of the *hTERT* gene was subcloned upstream to a secreted alkaline phosphatase gene (SEAP) and transfectectd into H1299 by electroporation. The stable clones were selected using G418. The stable clones derived from H1299 was cultured using conditions that are similar to their parental cells.

6.5. Secreted alkaine phosphatase (SEAP) assay

Secreted alkaine phosphatase was used as the reporting system to monitor the transcriptional activity of *hTERT* [31]. Here, about 2×10^3 cells each were grown in 96-well plates and incubated at 37 °C for 24 h and changed with fresh media. Varying amounts of drugs were added and cells were incubated for another 24 h. Culture media were collected and heated at 65 °C for 10 min to inactivate heat-labile phosphatases. An equal amount of SEAP buffer (2 M diethanolamine, 1 mM $MgCl_2$, and 20 mM L-homoarginine) was added to the media and *p*-nitrophenyl phosphate was added to a final concentration of 12 mM. Absorptions at 405 nm were taken, and the rate of absorption increase was determined.

6.6. MTT assay for cell viability

The tetrazolium reagent (MTT; 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, USB) was designed to yield a colored formazan upon metabolic reduction by viable cells [32,33]. Approximately 2×10^3 cells were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C 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 were then added to the culture medium for designated various concentrations. After 48 h, an amount of 25 μL of MTT was added to each well, and the samples were incubated at 37 °C for 4 h. A 100 μL solution of lysis buffer containing 20% SDS and 50% *N,N*-dimethylformamide was added to each well and incubated at 37 °C for another 16 h. The absorbency at 550 nm was measured using an ELISA reader.

6.7. Telomere repeat amplification protocol (TRAP) assay

Telomerase activity was detected by modifying version of the TRAP protocol [2,24]. Telomerase products were resolved by 10% polyacrylamide gel electrophoresis and visualized by staining with SYBER Green. As a source of telomerase, the total cell lysates derived from lung cancer cell lines (H1299) were used. Protein concentration of the lysates was assayed using Bio-Rad protein assay kit using BSA standards.

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