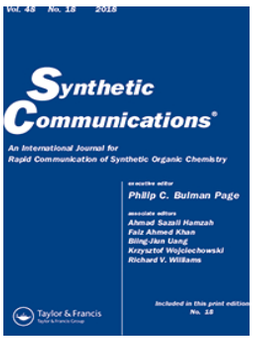

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New potential antitumor quinazolinones derived from dynamic 2-undecyl benzoxazinone: Synthesis and cytotoxic evaluation

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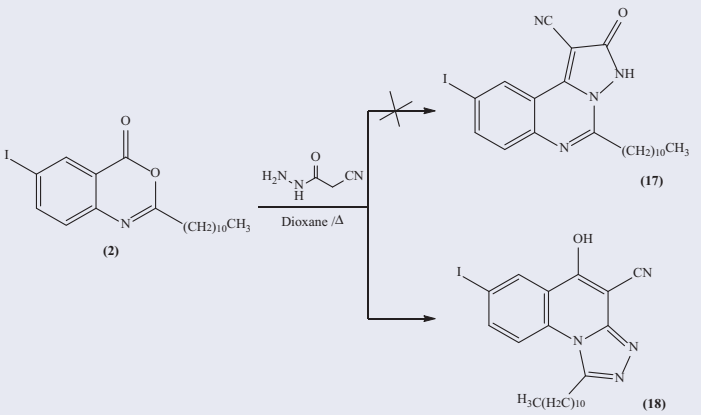
ABSTRACT

Since the quinazoline and its derivatives have been considered as a novel class of cancer chemotherapeutic agents that show promising activity against different tumors, a new series of 6-iodo-2-undecylquinazolin-4(3*H*)-ones were prepared via reaction of 6-iodo-2-undecyl-4*H*-benzoxazin-4-one with nitrogen nucleophiles, namely, primary amines, 4-amino antipyrine, hydrazine hydrate, diamines, ethanol amine, and/or hydrazide derivatives and screened for their antitumor activity *in vitro* against a panel of three human tumor cell lines namely; hepatocellular carcinoma (liver) HepG2, colon cancer HCT-116, and mammary gland breast MCF-7. Compounds **14**, **16,** and **18** showed remarkable broad spectrum antitumor activity. All compounds were fully characterized by means of IR, MS, and ^{1}H-NMR spectra.

GRAPHICAL ABSTRACT



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KEYWORDS
Cytotoxic evaluation; lauroyl chloride; quinazolinone; triazolo[4,3-a]quinoline-4-carbonitrile

Introduction

Cancer has become a major cause of human mortality and is considered a major worldwide health problem. Cancer consists of a group of cells that originated from a single

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cell with uncontrolled growth and rapid proliferation properties.[1,2] Presently, a wide range of cytotoxic drugs, either alone or in combination, are used to treat cancer, and several of these drugs are in different phases of clinical trials. Heterocyclic compounds are widely investigated as bioactive molecules and are considered important synthetic targets for the development of novel therapeutic agents.[3–5] Quinazoline is one of these heterocycles for which considerable research has been done in order to examine its biomedical applications.[6] In a number of biologically active compounds and drug molecules, the quinazoline nucleus is used as a basic framework. Due to their broad range of pharmacological activities, which include antimicrobial,[7,8] antimalarial,[9] anti-inflammatory,[10–12] anticonvulsant,[13,14] antihypertensive,[15] antioxidant,[16] anti-viral,[17] anti-HIV,[18] and anticancer,[19–22] quinazoline and its derivatives have attracted the attention of biologists and medicinal chemists. Quinazoline and its derivatives have been identified as a new class of cancer chemotherapeutic agents with significant therapeutic efficacy against solid tumors.[23–26] The Food and Drug Administration (FDA) has approved several quinazoline derivatives for clinical use as anticancer drugs. These include gefitinib, erlotinib, lapatinib, afatinib, and vandetanib (Fig. 1).[27]

In continuation of our program exploring the chemical reactivity of the oxazinone moiety present in 4*H*-3,1-benzoxazin-4-one derivatives with saturated aliphatic substituents at position 2 (the so-called dynamic benzoxazinones),[28–30] and derivatives with bulky substituents involving strong conjugation power (which are so-called static benzoxazinones),[31,32] undecyl group has been chosen to be the alkyl group at position-2 in the synthesized benzoxazinone and the corresponding quinazolinones which are expected to have interesting biological and medicinal activity toward different diseases. The iodo derivatives were selected for study because iodine atom has received considerable attention in organic synthesis due to its high tolerance to air and moisture, low cost, non-toxic nature and ready availability. The presence of iodine in heterocyclic compounds increases the lipophilicity of the molecules more than fluorine, chlorine or bromine (iodine atom is bigger and possesses high polarizability). In previous studies,[33,34] we have synthesized several quinazolinones derivatives. Based on our

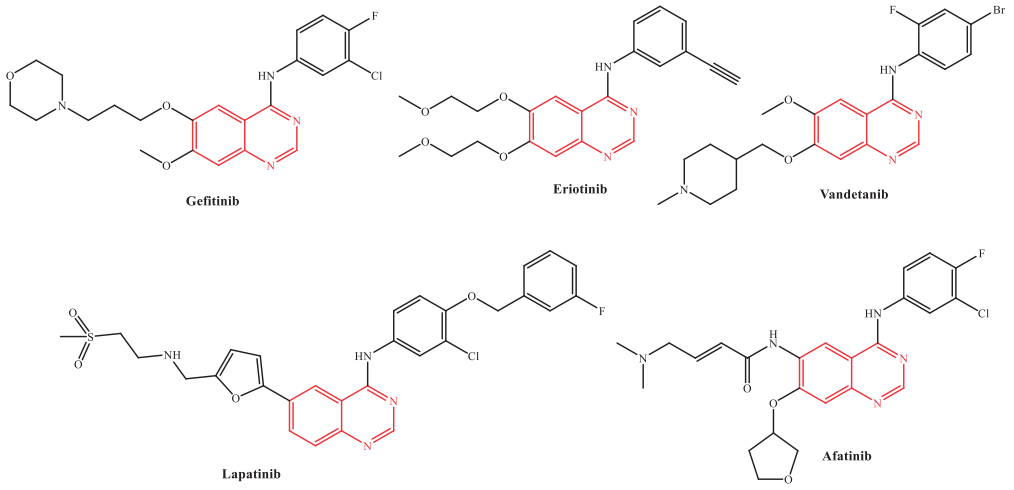


Figure 1. FDA approved quinazoline derivatives as anticancer drugs.

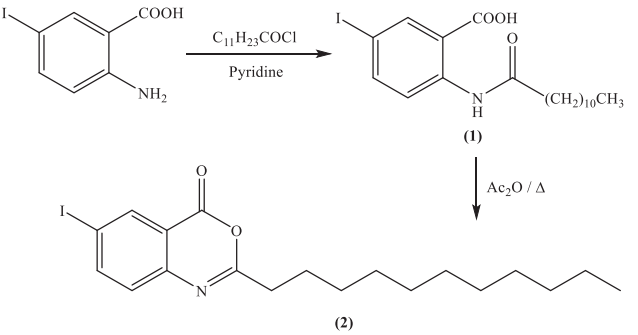
results and on literature data, the present study aimed to synthesize and evaluate the antitumor activity of some new quinazolinone derivatives.

Results and discussion

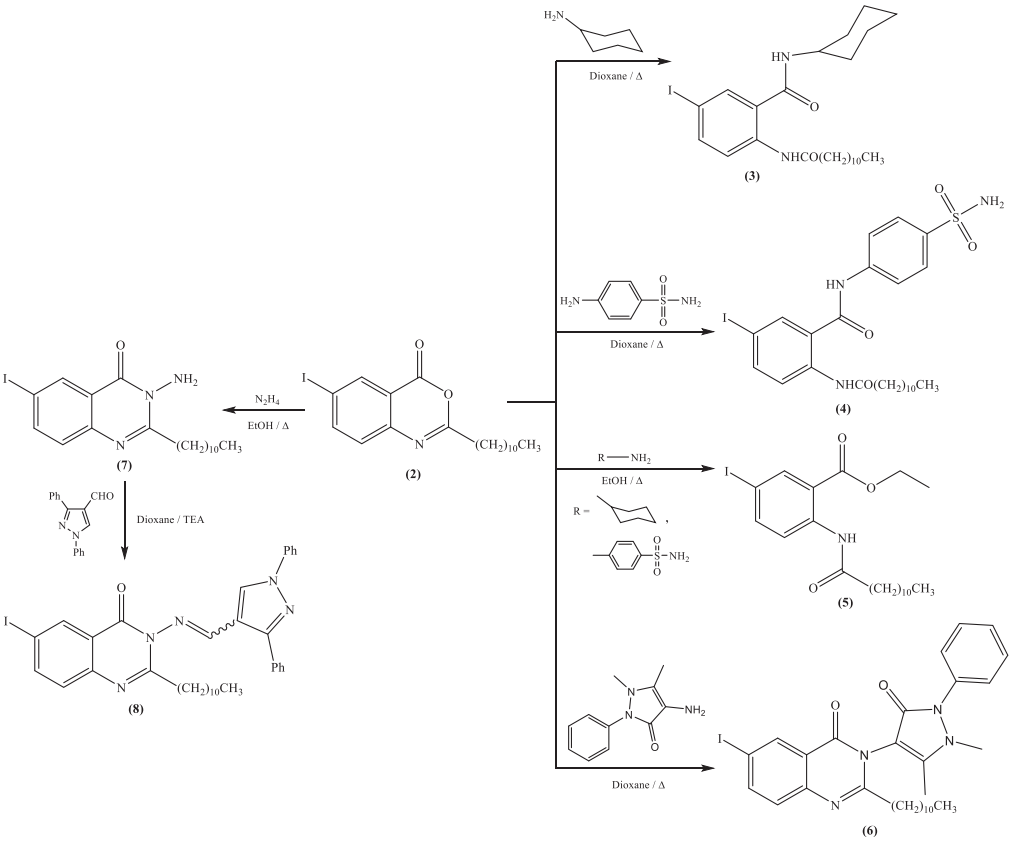
The aim of this work was to design and synthesize novel quinazolinone derivatives to evaluate their anticancer activity. Thus, the reaction of 2-amino-5-iodobenzoic acid with lauroyl chloride in the presence of pyridine at room temperature afforded the amide analog **1** followed by ring closure in freshly distilled acetic anhydride to obtain the key intermediate 6-iodo-2-undecyl-4*H*-benzo[d][1,3]oxazin-4-one **2** (Scheme 1). The structure of compound **2** was confirmed by complete analysis of IR, ^{1}H NMR, and mass spectrum besides the correct elemental analysis. Thus, the IR spectrum shows absorption bands for carbonyl group of oxazinone at 1764 cm^{-1} and C=N at 1643 cm^{-1}. ^{1}H NMR spectrum (DMSO-d_6) of compound **2** revealed the presence of signals corresponding to aromatic protons (3H) as multiplet in the region at δ 8.52–7.30 ppm beside the signals characteristic for the aliphatic chain which appeared as follows: 2.67 (t, 2H), 1.82 (m, 2H), 1.36-1.2 (m, 16H), 0.88 (t, 3H).

The starting intermediate 6-iodo-2-undecyl-4*H*-benzo[d][1,3]oxazin-4-one **2** was allowed to react with primary amines such as cyclohexyl amine and/or sulfanilamide in an attempt to obtain 3-substituted-quinazolin-4-ones in different reaction conditions, in all cases; the reaction afforded the diamides **3** and **4** instead. Attempts to cyclize the diamides **3** and **4** to the corresponding 4-(3*H*)-quinazolin-4-one using a variety of reaction conditions, including fusion, were not successful. On the other hand, the ester **5** was obtained upon reaction of compound **2** with primary amines in refluxing ethanol (Scheme 2). ^{1}H NMR spectrum of compound **5** revealed the existence of triplet signal at 1.43 ppm and quartet signal at 4.4 ppm characteristic for ethyl protons of ester group which consistent with the structure **5**. In contrast, the reaction of **2** with 4-aminoantipyrine in boiling dioxane afforded the cyclized product 3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-6-iodo-2-undecylquinazolin-4(3*H*)-one **6** (Scheme 2).

Hydrazinolysis of the benzoxazinone **2** using hydrazine hydrate in boiling ethanol yielded 3-amino quinazolinone derivative **7**, via hetero ring opening followed by cyclization. The IR spectrum of compound **7** showed bands at 3319, 3210, and 1673 cm^{-1} attributed to NH_2 and C=O groups. While the ^{1}H NMR spectrum revealed the NH_2 as a singlet at 4.84 ppm. Condensation of 3-amino quinazolinone derivative **7** with



Scheme 1. Synthesis of 6-iodo-2-undecyl-4H-benzo[d][1,3]oxazin-4-one 2.



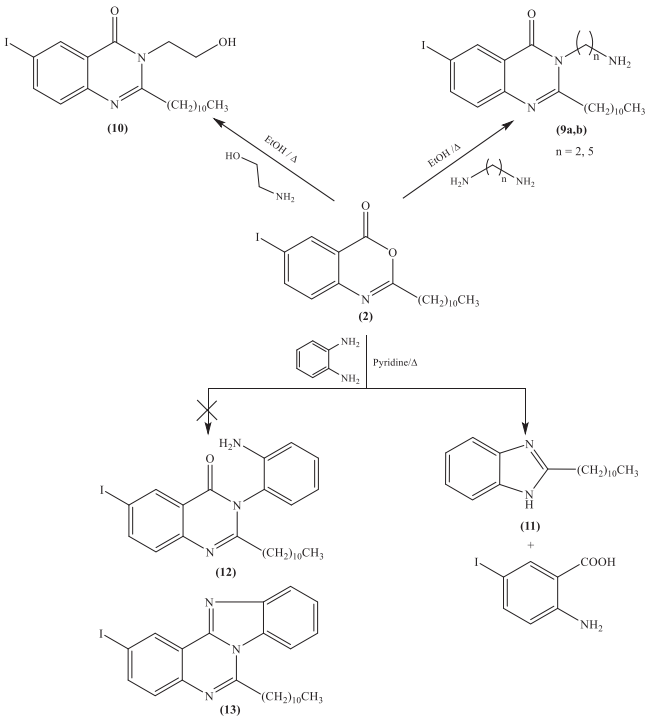
Scheme 2. Reaction of benzoxazinone 2 with primary amines and hydrazine hydrate.

1,3-diphenyl pyrazole-4-carboxaldehyde in dioxane in the presence of catalytic amount of triethylamine afforded the arylidene derivatives **8**. The IR and ^{1}H NMR spectra of compound **8** were devoid of any signals for the NH_2 group (Scheme 2).

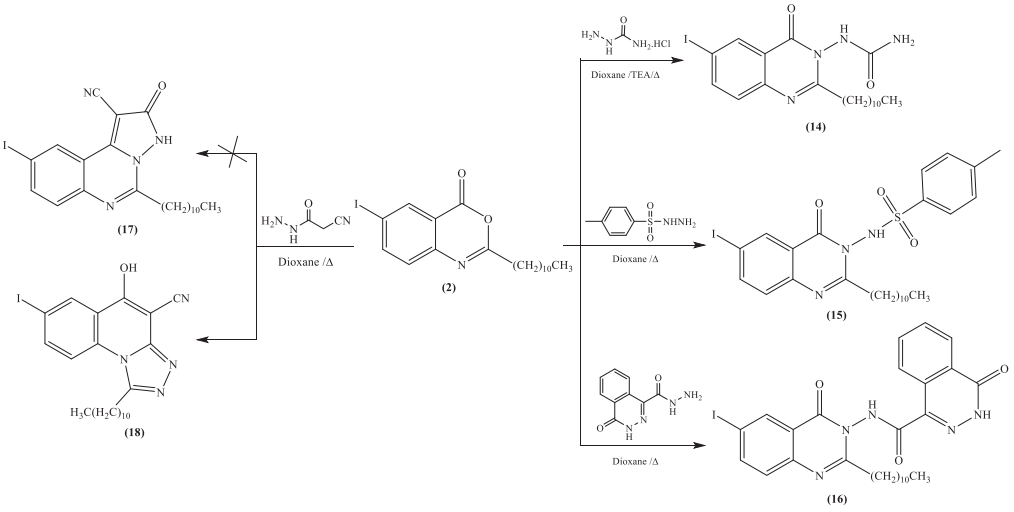
The reactivity of benzoxazinone **2** towards bident nucleophiles such as 1,2-diaminoethane, 1,5-diaminopentane, and ethanolamine was also investigated. Thus, the reaction of **2** with 1,2-diaminoethane and/or 1,5-diaminopentane in refluxing ethanol afforded the quinazolinone derivatives **9a,b** (Scheme 3). Similarly, ethanolamine reacted with compound **2** to give 3-(2-hydroxyethyl)-6-iodo-2-undecylquinazolin-4(3*H*)-one **(10)** (Scheme 3).

The reaction of benzoxazinones with anilines containing a reactive functional group at the *ortho* position adds another dimension to the basic transformation, that further cyclization to another heterocyclic system is possible. Benzoxazinones are able to transfer its carbon-2 and its attached substituents to another molecule.[35] An interesting example of this is the reaction of the benzoxazinone **2** with *o*-phenylenediamine in refluxing pyridine in which 2-undecyl-1*H*-benzo[*d*]imidazole **11** was formed in high yield along with a small amount of 5-iodoanthranilic acid rather than compound **12** or **13**. The ^{1}H NMR ($CDCl_3$) spectrum of compound **11** revealed the presence of signals down field for (NH) proton as singlet at 12.1 ppm, aromatic protons (4H) as multiplet in the region of δ 7.56–7.20 ppm, and signals characteristic for the aliphatic chain appeared as follows: 2.93 (t, 2H), 1.86 (m, 2H), 1.38-1.23 (m, 16H), 0.88 (t, 3H) (Scheme 3).

The proclivity of the benzoxazinone **2** for undergoing nucleophilic addition with semicarbazide hydrochloride, 4-methylbenzenesulfonohydrazide, 4-oxo-3,4-dihydrophthalazine-1-carbohydrazide, and/or 2-cyanoacetohydrazide has been investigated. Thus, the reaction of compound **2** with semicarbazide hydrochloride in refluxing dioxane in the presence of TEA afforded 1-(6-iodo-4-oxo-2-undecylquinazolin-3(4*H*)-yl)urea **14** (Scheme 4). Similarly,



Scheme 3. Reaction of benzoxazinone 2 with bident nucleophiles.



Scheme 4. Reaction of benzoxazinone 2 with some hydrazides.

when compound **2** reacted with 4-methylbenzenesulfonohydrazide in refluxing dioxane afforded *N*-(6-iodo-4-oxo-2-undecylquinazolin-3(4*H*)-yl)-4-methylbenzenesulfonamide **15**. Also, the reaction of compound **2** with 4-oxo-3,4-dihydrophthalazine-1-carbohydrazide in dioxane under reflux gave *N*-(6-iodo-4-oxo-2-undecylquinazolin-3(4*H*)-yl)-4-oxo-3,4-dihydrophthalazine-1-carboxamide **16** (Scheme 4). In contrast, the reaction of **2** with 2-cyanoacetohydrazide in boiling dioxane afforded the unexpected product 5-hydroxy-7-iodo-1-undecyl-[1,2,4]triazolo[4,3-a]quinoline-4-carbonitrile **18** rather than the pyrazolo[1,5-c]quinazoline derivative **17**.[36] The appearances of a broad band at 3437 cm^{-1} for OH group besides the absence of the frequency of the C=O group in the IR spectrum confirms the unexpected structure **18** (Scheme 4).

Cytotoxicity and antitumor evaluation

Most of the synthesized compounds were screened for their anticancer activity *in vitro* against three representative cell lines, hepatocellular carcinoma (HePG-2), colon cancer HCT-116, and mammary gland breast cancer (MCF-7). The results were expressed as growth inhibitory concentration (IC_{50}) values, which represent the compounds concentrations required to produce a 50% inhibition of cell growth after 72 h of incubation, compared with untreated controls (Table 1, Fig. 2). In general, the cytotoxic activity of the tested compounds ranged from very strong to weak activity. Compound **16** showed approximately equal activity to the DOX as a standard for the three representative cell lines with an IC_{50} value of 4.34 ± 1.2, 5.16 ± 1.4, and 7.9 ± 0.5 µg/mL. The optimal results were observed for compounds **14** and **16** (very strong activity) with an IC_{50} value of 9.62 ± 0.9 and 4.34 ± 1.2 µg/mL for HePG-2 cell line, respectively. Compounds **16** and **18** exhibited very strong activity with an IC_{50} value of 5.16 ± 1.4 and 7.49 ± 0.6 µg/mL for HCT-116 cell line, respectively. Compounds **14, 16,** and **18** showed very strong activity with an IC_{50} value of 7.89 ± 0.8, 7.9 ± 0.5**, and** 9.91 ± 0.9 µg/mL for MCF-7 cell line. Compounds **15** and **18** showed strong activity towards HePG-2 cell with an IC_{50}

Table 1. Cytotoxicity (IC_{50}) of the tested compounds on different cell lines.

	IC_{50} (µg/ml)[a]		
Compound no.	HePG2	HCT-116	MCF-7
1	>100	>100	94.02 ± 5.1
2	25.94 ± 2.2	33.42 ± 2.3	27.34 ± 2.0
3	44.89 ± 3.1	50.92 ± 2.9	40.53 ± 2.8
4	64.13 ± 3.6	70.46 ± 3.8	51.32 ± 3.5
5	74.11 ± 3.9	86.60 ± 4.4	67.16 ± 3.9
6	55.63 ± 3.4	68.49 ± 3.8	47.56 ± 3.3
7	47.34 ± 3.3	54.95 ± 3.2	43.88 ± 3.1
8	>100	>100	88.41 ± 4.5
9a	31.12 ± 2.3	35.19 ± 2.4	28.13 ± 2.2
9b	79.64 ± 4.0	75.31 ± 3.9	63.20 ± 3.6
10	17.08 ± 1.6	15.68 ± 1.3	12.98 ± 1.2
11	23.85 ± 1.9	28.46 ± 1.8	18.07 ± 1.5
14	9.62 ± 0.9	11.24 ± 1.1	7.89 ± 0.8
15	16.73 ± 1.4	21.87 ± 1.7	39.50 ± 2.7
16	4.34 ± 1.2	5.16 ± 1.4	4.05 ± 1.6
18	13.78 ± 1.1	7.49 ± 0.6	9.91 ± 0.9
DOX	4.50 ± 0.2	5.23 ± 0.3	4.17 ± 0.2

DOX: doxorubicin.
[a]IC_{50} (µg/mL): 1–10 (very strong); 11–20 (strong); 21–50 (moderate); 51–100 (weak); above 100 (non-cytotoxic).

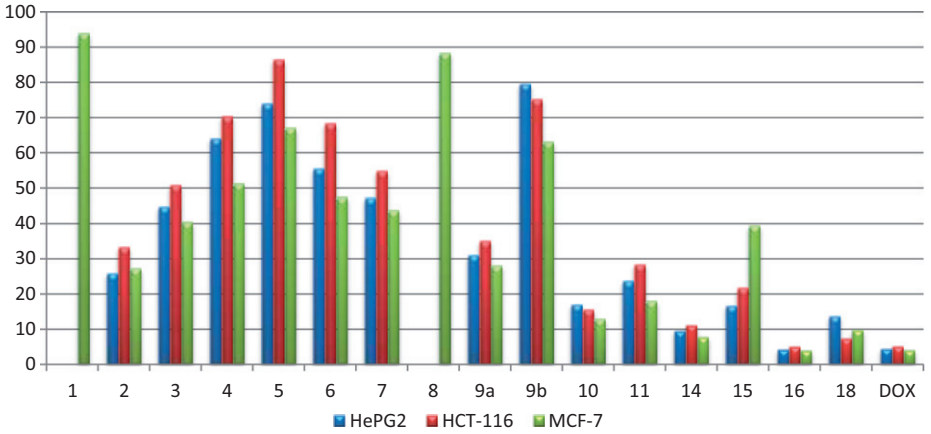


Figure 2. Cytotoxic activity of the tested compounds on different cell lines.

value of 16.73 ± 1.4 and 13.78 ± 1.1 µg/mL, while compounds **10** and **14** showed strong activity towards HCT-116 cell with an IC_{50} value of 15.68 ± 1.3 and 11.24 ± 1.1 µg/mL, respectively. Also, strong activity towards MCF-7 cell line was observed with compounds **10** and **11** with an IC_{50} value of 12.98 ± 1.2 and 18.07 ± 1.5 µg/mL, respectively. Moderate activity towards HePG-2, HCT-116, and MCF-7 cell lines was observed with compounds **2, 3, 7, 9a, 11,** and **15**.

Structure–activity relationship (SAR)

DNA is made of chemical building blocks called nucleotides. The four types of nitrogen bases found in nucleotides are: adenine (A), thymine (T), guanine (G), and cytosine (C). The base adenine always pairs with thymine, while guanine always pairs with cytosine through a hydrogen bond. The cytotoxic activity of the tested compounds towards different cell lines depends on two factors[37,38]: (i) the formation of an intermolecular hydrogen bond with DNA bases; (ii) the positive charge on the tested compounds attracted to the negative charge on the cell wall. By comparing the experimental cytotoxicity of the compounds reported in this study to their structures, the following SAR was postulated:

Compounds **14, 16,** and **18** showed very strong activity, this is due to the presence of NH and NH_2 groups in **14**, two NH group in **16,** and one OH group in **18** which may be added to any unsaturated moiety in DNA or forming hydrogen bond with either one of the nucleobases of the DNA and causes it damage.

Compound **15** showed strong activity, this is due to the presence of NH group which can form a hydrogen bond with either one of the nucleobases of the DNA and causes it damage. Also, the presence of the SO_2Ph group as a strong electron attracting group rendered the molecule positively charged forming electrostatic attraction with the DNA nucleobases. Moreover, the SO_2 group acts on the mitotic spindle (Fig. 3).[39]

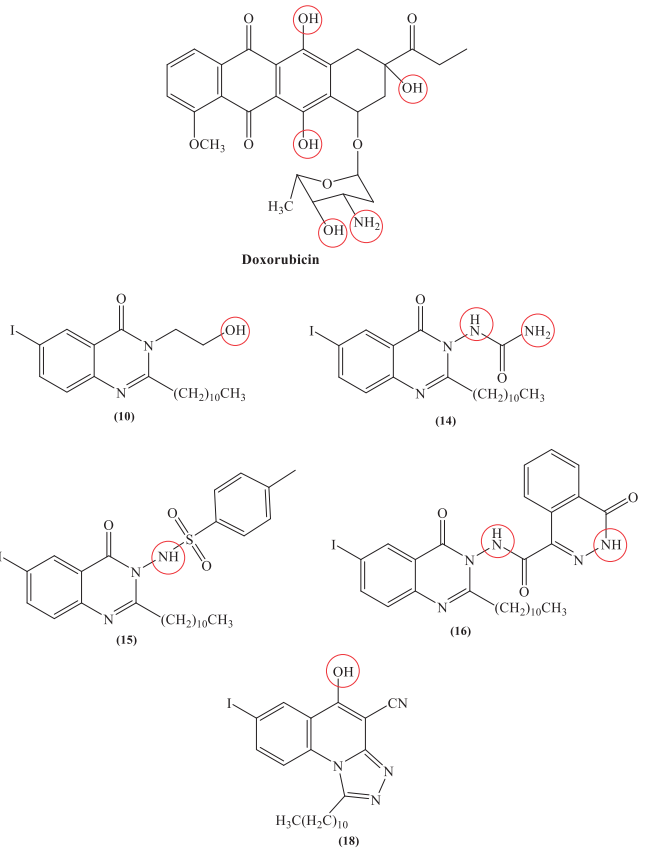


Figure 3. Structure of doxorubicin and some of the designed target compounds.

Experimental

All melting points were taken on a Griffin and Geory melting-point apparatus and are uncorrected. IR spectra were recorded on Pye Unicam SP1200 spectrophotometer using the KBr wafer technique. ^{1}H NMR experiments were run at 300 and 400 MHz on a Varian Mercury VX-300 NMR spectrometer (Varian Inc., Palo Alto, CA) using tetramethylsilane (TMS) as an internal standard in $CDCl_3$ or dimethyl sulfoxide (DMSO-d_6). Chemical shifts are quoted as δ. EI-MS were measured on a Schimadzu-GC-MS operating at 70 eV. Elemental analyses were carried out at the Microanalytical Unit, Faculty of Science, Ain Shams University, using a Perkin-Elmer 2400 CHN elemental analyzer (PerkinElmer, Waltham, MA), and satisfactory analytical data (±0.4) were obtained for all compounds. The homogeneity of the synthesized compounds was controlled by thin layer chromatography (TLC) using aluminum sheet silica gel F_{254} (Merck, Kenilworth, NJ). The Pharmacological activity assays were carried out at Pharmacology Department, Faculty of Pharmacy, El-Mansoura University, El-Mansoura, Egypt.

2-Dodecanamido-5-iodobenzoic acid (1)

To a solution of iodoanthranilic acid (2.6 g, 10 mmole) in pyridine (50 ml), dodecanoyl chloride (2.1 ml, 10 mmole) was added dropwise with stirring at room temperature for

0.5 h. The solid product that precipitated down was filtered off, washed by cold water, dried and then recrystallized from benzene to give **1** as white crystals, mp 132–134 °C, yield 84%. Anal. calcd. for $C_{19}H_{28}INO_3$ (445.34): C, 51.24; H, 6.34; I, 28.50; N, 3.15. Found: C, 51.13; H, 6.38; I, 28.23; N, 2.99. IR (υ/cm^{-1}): br. 3339 (OH, NH), 2920, 2850 (CH aliphatic), 1674 (C = O). MS *m*/*z* (%): 445 (M, 5.4), 401 (8.9), 263 (100), 245 (58). ^{1}H NMR (DMSO-d_6) δ (ppm): 10.91 (s, 1 H, NH, exchangeable with D_2O), 8.57–7.84 (m, 3H$_{arom.}$), 2.46 (t, 2H), 1.75 (m, 2H), 1.35–1.25 (m, 16H), 0.87 (t, 3H).

Pharmacological activity

Cytotoxicity assay

The cytotoxic activity of thirteen compounds was tested against three human tumor cell lines namely hepatocellular carcinoma (liver) HePG-2, colon cancer HCT-116, and mammary gland (breast) MCF-7. The cell lines were obtained from the ATCC via the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Doxorubicin was used as a standard anticancer drug for comparison. The reagents used were RPMI-1640 medium, MTT, DMSO and Doxorubicin (Sigma Co., St. Louis, MO), and Fetal Bovine Serum (GIBCO, Paisley, UK).

The different cell lines[40,41] mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100 µg/mL streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeded[42] in a 96-well plate at a density of 1.0×10^4 cells/well at 37 °C for 48 h under 5% CO_2 incubator. After incubation, the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µL of MTT solution at 5 mg/mL was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in the volume of 100 µL was added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at an absorbance of 570 nm using a plate reader (EXL 800, BioTech, Winoosky, VT).

The relative cell viability in percentage was calculated as (A_{570} of treated samples/A_{570} of the untreated sample) × 100.

Calculation of the IC_{50} for each compound

Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer. The assay was used to examine the newly synthesized compounds. Doxorubicin was used as a standard anticancer drug for comparison. To calculate IC_{50}, you would need a series of dose–response data (e.g. drug concentrations *x*1, *x*2, *x*n and growth inhibition *y*1, *y*2, *y*n). The values of y are in the range of 0–1.

Supporting information

Full experimental details and spectroscopic data for compounds 1–18 can be accessed on the publisher's website.

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