Monatshefte für Chemie - Chemical Monthly (2020) 151:1841–1849
https://doi.org/10.1007/s00706-020-02708-0

ORIGINAL PAPER

Synthesis and cytotoxic evaluation of halogenated furanones

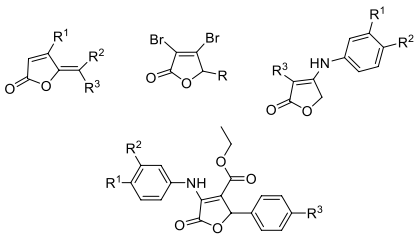
Víctor A. Castro-Torres[1] · Nadia J. Jacobo-Herrera[2] · Lidia Díaz-Sánchez[1] · Leticia Rocha-Zavaleta[3] · Patricia García-López[4] · Mariano Martínez-Vázquez[1]

Received: 10 August 2020 / Accepted: 22 October 2020 / Published online: 5 November 2020

Abstract

The objective of the current study is to evaluate the potency of halogen-furan-2(5*H*)-one-type derivatives against human cancer cell lines. Four known bromofuran-2(5*H*)-one-type derivatives, as well as five new and two known bromo-4-(phenylamino)-furan-2(5*H*)-one-type compounds and six novel and two known halogen-4-alkyl-5-phenyl-3-(phenylamino)furan-2(5*H*)-one-type derivatives, were synthesized and evaluated for their anticancer activity against prostate (PC-3) and colon (HCT-116) human cancer cell lines. The results showed that only the bromofuran-2(5*H*)-ones were cytotoxic in both cell lines. Three of these displayed particularly useful antiproliferative activities, in both cancer cells evaluated. (*E*)-5-(Bromomethylene)-furan-2-(5*H*)-one was the most active against PC-3 (IC_{50} 0.93 ± 0.02 μM) while 3,4-dibromofuran-2(5*H*)-one was the most active against HCT-116 (IC_{50} 0.4 ± 0.04 μM). Furthermore, flow cytometry studies revealed that the bromofuran-2(5*H*)-ones induced cell death by apoptosis. Also, it was found that the cytotoxic furanones induced lipid peroxidation, determined by TBARS assay. Thus, cytotoxicity of the active compounds could be associated with ROS production. Additionally, it must be taken into account that all cytotoxic compounds contain an electrophilic carbon atom in position 4, which can explain, through a non-specific reactivity with nucleophiles, the cytotoxic activity of these compounds.

Graphic abstract



Keywords Halofuranones · Antitumor agents · Cytotoxic compounds · Drug research

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00706-020-02708-0) contains supplementary material, which is available to authorized users.

✉ Nadia J. Jacobo-Herrera
nadia.jacobo@gmail.com

✉ Mariano Martínez-Vázquez
marvaz@unam.mx

[1] Instituto de Química, Universidad Nacional Autónoma de México (UNAM), Circuito Exterior S/N Circuito Exterior, C.U., Coyoacán, 04510 Mexico City, Mexico

[2] Unidad de Bioquímica, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Belisario Domínguez Secc 16, Tlalpan, 14080 Mexico City, Mexico

[3] Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), Circuito, Mario de La Cueva s/n, C.U., Coyoacán, 04510 Mexico City, Mexico

[4] Instituto Nacional de Cancerología, Av. San Fernando 22, Belisario Domínguez Secc 16, Tlalpan, 14080 Mexico City, Mexico

Introduction

With more than 18 million new cases in 2018, cancer continues to be a disease of global concern. Colon and prostate cancers have been reported to be among the top five most frequent cancers, excluding skin cancer that is not caused by melanomas [1]. Although new therapies and novel drugs have been used against cancer, medications with potent antitumor activity without aggressive side effects have yet to be discovered. There are a large number of biologically active natural and synthetic compounds that incorporate a furanone skeleton into their structures. Biological activities include antitumor, antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant. For instance, it has been reported that natural cardiac glycosides containing a furanone ring, that are in clinical use to treat heart failure and some types of cardiac arrhythmias, induce selective anticancer effects [2–4]. On the other hand, it is known that synthetic 4-(4-substituted aminobenzyl)furan-2(5*H*)-one derivatives, with an exocyclic double bond, exhibited cytotoxicity and topoisomerase I inhibitory activity against three human cancer cell lines [5]. Recently, it was reported that the inclusion of halogens in the structure of 2(5*H*)-furanones increased their cytotoxicity activity against human cancer cell lines, thus, highlighting the importance of the presence of halogen atoms in the structure of furanones [6]. Therefore, it was decided to synthesize and evaluate the cytotoxicity against PC-3 (prostate) and HCT-116 (colon) human cancer cell lines of three series of halogenated furanones. The first consisted of four known bromofuran-2-(5*H*)-one-type derivatives (**1**–**4**). The second consisted of five original (**7**–**11**) and two known (**5**, **6**) bromo-4-(phenylamino)-furan-2-(5*H*)-one-type compounds and the third one of six novel (**15**–**19**) and two known (**12**, **13**) halogen-4-alkyl-5-phenyl-3-(phenylamino)furan-2-(5*H*)-one-type derivatives.

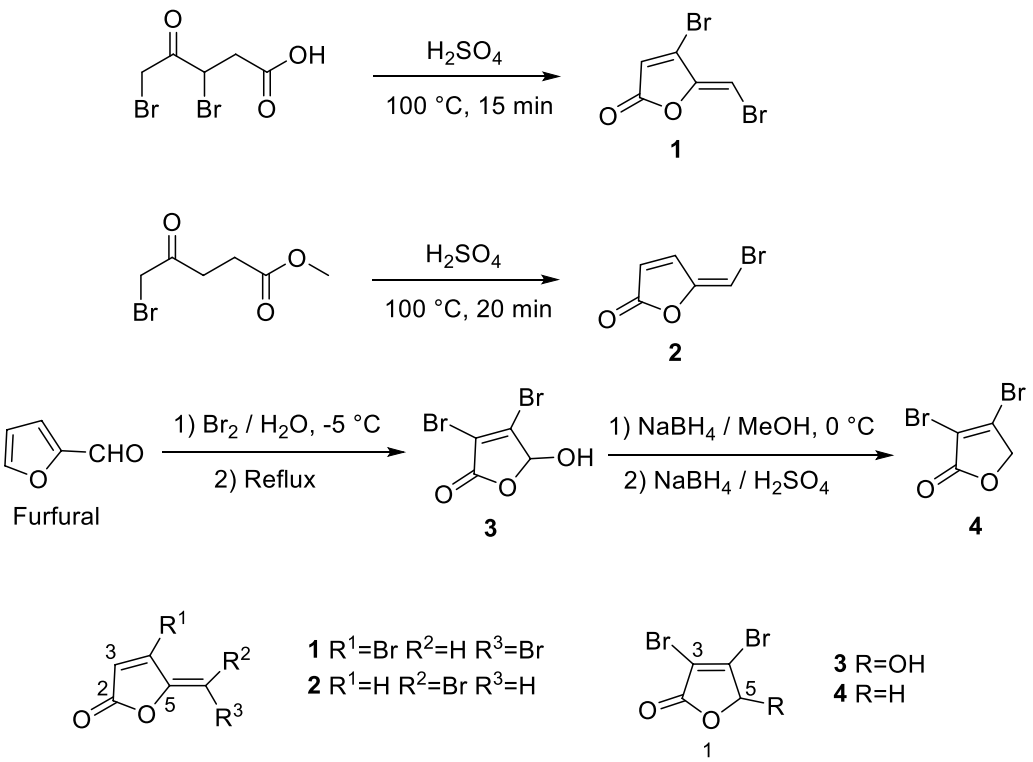
Results and discussion

Chemistry

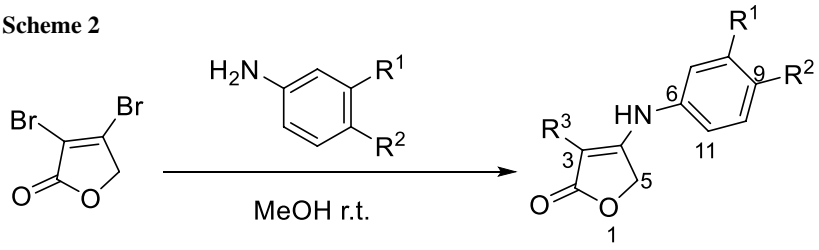
The synthesis of derivative **1** was done by reacting 3,5-dibromo-4-oxopentanoic acid with sulfuric acid [7]. While the compound **2** was obtained from the 5-bromo-4-oxopentanoic acid methyl ester [8, 9]. The mucobromic acid (**3**) and the 3,4-dibromofuran-2(5*H*)-one (**4**) were obtained from furfural [10, 11] (Scheme 1) (see supplemental files).

The compounds **5**–**11** were obtained by reaction of **4** with the respective mono- or di-halogenated aniline in methanol (Scheme 2). The compounds **5** and **6** had been previously synthesized [12].

Scheme 1



Scheme 2



5 R^1=Cl R^2=H R^3=Br
6 R^1=Br R^2=H R^3=Br
7 R^1=F R^2=H R^3=Br
8 R^1=F R^2=F R^3=Br
9 R^1=Cl R^2=Cl R^3=Br
10 R^1=I R^2=H R^3=H
11 R^1=*p*-phenylazoaniline R^2=H R^3=Br

As an unexpected result, compound **10** does not have a bromine atom in position C3, like furanones **5**–**9** and **11**. In the **10** ^{1}H NMR spectrum, an AA'BB' system for the aromatic protons was observed. In addition, a singlet at 5.33 ppm assigned to proton H3 which correlate with a signal at 85.05 ppm in the ^{13}C NMR spectrum was observed. Additionally, an interaction between H3 and C2 and C5 was observed in the **10** HMBC spectrum (see supplemental files). It appears that in 4-(phenylamino) furan-2-(5*H*)-one-type compounds the bromine atom at C3 is required for cytotoxic activity since **10**, in this series, was the least active. The lack of the presence of an bromine atom at C3 alter the electrophilic nature of the furanone ring, which is reflected not just in the lower activity of **10** but also in the null cytotoxic activity of compounds **12**–**19**.

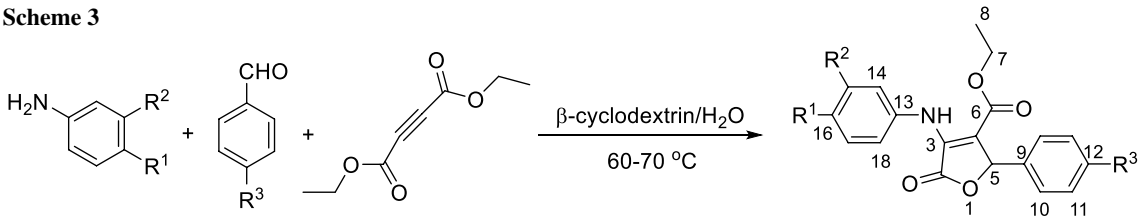
The synthesis of furanones **12**–**19** was conducted using a one-pot reaction between diethyl acetylene dicarboxylate, an aromatic amine which can have different substituents and an aromatic aldehyde that can also be substituted. Green chemistry conditions were managed in this reaction since an oligosaccharide (β-cyclodextrin) was used as catalyst, and water was the solvent used (Scheme 3) (see supplemental files).

The furanones **12** and **13** were previously synthetized [13]. The ^{1}H and ^{13}C 2D spectra of each synthesized compounds are in the supplementary files. The structures of known furanones **1**–**6**, **12**, **13** were confirmed by comparison of their ^{1}H and ^{13}C NMR data with those previously published [7–11]. The structures of the new compounds **7**–**11** and **14**–**19** were deduced by 2D NMR experiments data (see supplementary material).

Biological assay

The cytotoxicity of furanones **1**–**19** was evaluated against HCT-116 (human colon carcinoma) and PC-3 (human

Scheme 3



12 R^1=H R^2=H R^3=H
13 R^1=H R^2=H R^3=Cl
14 R^1=Br R^2=H R^3=H
15 R^1=F R^2=H R^3=Cl
16 R^1=Br R^2=H R^3=Cl
17 R^1=I R^2=H R^3=Cl
18 R^1=I R^2=H R^3=H
19 R^1=F R^2=F R^3=Cl

prostate cancer) cell lines by sulforhodamine assay. Compounds **1**–**4** were cytotoxic in both lines, especially furanones **1**, **2**, and **4** were more active than cisplatin. The structures of the **1**, **2** suggest that their cytotoxicity could be by the typical 1,4 attack of a nucleophilic compound such as amine or alcohol of an enzyme that could induce cell death [14]. This proposal is supported by the fact that all cytotoxic compounds contain an electrophilic carbon atom in position 4. On the other hand, it has been published that some furan-2(5*H*)-one-type compounds induce cell death by production of ROS [15]. In our case, there is a positive correlation between the cytotoxic activity of furanones **1**–**4** and their ROS production indicated by lipoperoxidation activity, measured by TBARS assay (Fig. 1). In contrast, furanones **7** and **15** did not show intense lipoperoxidation activity, and their cytotoxic activity was low. Then compounds **1**–**4** may induce cell death by production of ROS as well as its electrophilic characteristics. Separate mention deserves the compounds **14** and **15** that showed moderate cytotoxicity against HCT-116. However, the addition of a chlorine or fluorine atom eliminates the activity as in furanones **16** or **19** (Table 1).

On the other hand, to evaluate the safety of **1**–**4** compounds, the cytotoxic effect on non-cancerous primary fibroblast cells (HGF-1) was assessed. **1** and **3** showed a moderated cytotoxicity while **2** and **4** had a minimum impact on the growth of this primary cell culture at 50 µM dose (Table 2).

The low cytotoxicity of **3** (mucobromic acid) in non-cancer cells had previously been determined in a human lymphoblastoid TK6 cell line where at the 100 µM dose it showed marginal activity. However, **3** at a dose of 4000 µM induced 80% of inhibition of TK6 cells growth [17].

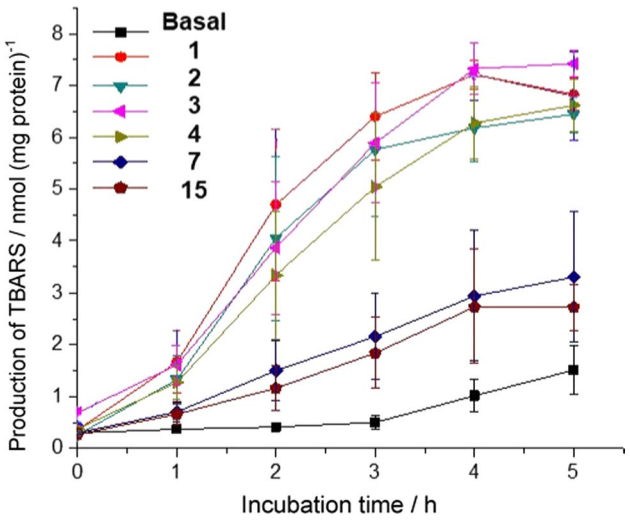


Fig. 1 Lipoperoxidation induced by furanones **1**–**4**, **7**, and **15**

Table 1 Cytotoxic activity of furanones **1**–**19** against PC3 and HCT-116 expressed as the IC_{50}

Entry	IC_{50}/µM	
	PC-3	HCT-116
Cisplatin	15.85 ± 0.05	5.56 ± 0.06
1	1.13 ± 0.02	1.12 ± 0.1
2	0.93 ± 0.02	1.5 ± 0.09
3	26.25 ± 0.02	14.06 ± 0.08
4	1.31 ± 0.03	0.4 ± 0.04
5	56.25 ± 0.01	75.0 ± 0.05
6	65.1 ± 0.03	74.3 ± 0.09
7	60.0 ± 0.05	56.25 ± 0.01
8	45.2 ± 0.02	33.2 ± 0.05
9	66.7 ± 0.03	43.3 ± 0.07
10	> 100 ± 0.01	78.5 ± 0.09
11	43.2 ± 0.03	65.3 ± 0.01
12	85.3 ± 0.01	> 100
13	62.3 ± 0.03	55.43 ± 0.03
14	56.25 ± 0.08	20.0 ± 0.04
15	78.75 ± 0.05	30.0 ± 0.01
16	> 100	> 100
17	> 100	> 100
18	> 100	> 100
19	> 100	> 100

Experiments were repeated at least in three occasions and results are expressed in ± SEM

Although furanones **5**–**11** were less cytotoxic than compounds **1**–**4**, there is some data on the structure–activity relationship that is worth highlighting. The first thing that stands out is that the activity of compounds **5**–**11** is different in each cancer line used. Taking advantage that **5**–**9** and **11** derivatives, only differ in C4 substitution so the variation of the activity of the compounds will depend on the chemical nature of this residue. In the PC-3 line (prostate) the most active compound in this series was the *p*-phenylazoaniline derivative **11** (IC_{50} 43.2 ± 0.03 µM), followed by the difluoro derivative **8** (IC_{50} 45.2 ± 0.02 µM), a result that contrasts with that

Table 2 Cytotoxic activities of compounds **1**–**4** against HGF-1 cells, after 48 h of treatment [16]

Entry	Inhibition/% in the growth of HGF-1 cells
	50 µM
1	68.95
2	NC
3	16.75
4	NC

NC non-cytotoxic

obtained by the dichloro derivative **9** (IC_{50} 66.7 ± 0.03 μM) which was the least active. Mono-halogenated compounds **6** (IC_{50} 65.1 ± 0.03 μM) and **7** (IC_{50} 60.0 ± 0.05 μM) were not more active than **11** despite **7** containing a fluorine atom. In the HCT-116 (colon) lines, the most active compound was **8** (IC_{50} 33.2 ± 0.05 μM), a result that indicates the importance of two fluorine atoms for cytotoxicity in both cell lines. Dichloro derivative **9** (IC_{50} 43.3 ± 0.07 μM) turned out to be more active in the HCT-116 cell line than in the prostate line. The other compounds showed similar activities which indicates that the chemical variation of the substituent in C4 was not crucial for the cytotoxic activity.

Apoptosis was assessed for compounds **1**–**4**, due to their cytotoxicity in both cell lines. The apoptosis induction by **1**–**4** furanones in PC-3 (prostate) and HCT-116 (colon) cells was determined by double staining with annexin V/PI. The results showed that in HCT cells, all the compounds evaluated induced principally early apoptosis. On the other hand, in the PC-3 cells, furanones **1** and **2** induced early apoptosis, and **3** and **4** increased the later apoptosis (Fig. 2, Table 3).

Table 3 Percentages of apoptosis induced by IC_{50} of each compound

Apoptotic cells/% 48 h treatment	HCT-116	PC-3
Vehicle	7.12 ± 0.1	0.18 ± 0.02
Cisplatin	60.84 ± 0.4	56.09 ± 0.1
1	48.40 ± 0.2	35.30 ± 0.4
2	60.21 ± 0.1	34.19 ± 0.2
3	68.53 ± 0.2	74.9 ± 0.2
4	57.41 ± 0.08	65.12 ± 0.2

The numbers represent the average of three independent experiments ± SD

Conclusion

In this work, the synthesis and cytotoxic evaluation of 19 halogenated furanones are reported. Five (**7**–**11**) that belong to 4-(phenylamino)-furan-2-(5*H*)-one-type compounds and six (**14**–**19**) that belong to 4-alkyl-5-phenyl-3-(phenylamino)-furan-2-(5*H*)-one-type derivatives are new

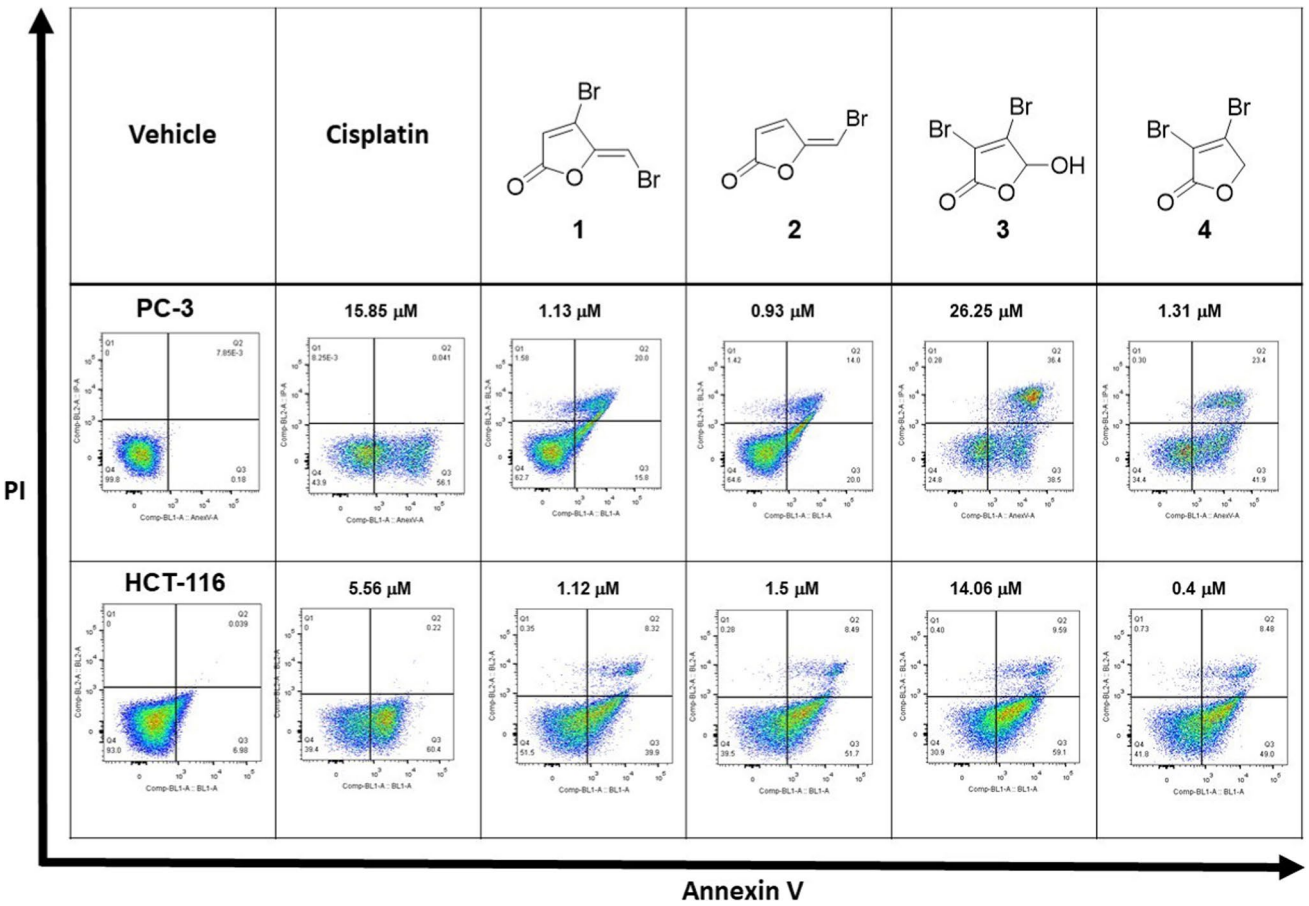


Fig. 2 PC-3 and HCT-116 apoptosis induction by furanones **1**–**4** IC_{50} (μM), determined by flow cytometric assay (annexin V/PI)

structures. The furanones **1**–**6**, **12**, **13** have already been synthesized [7–11].

The results showed that only furanones **1**–**4** were active against the two human cancer cell lines evaluated. These findings indicate that a furanone structure with lipoperoxidation activities and electrophilic properties is more critical to the cytotoxic effect than the presence of halogen atoms in larger structures. The selectivity of **1**–**4** against cancer cells was demonstrated by the low or null cytotoxic activity of these furanones against non-cancerous primary fibroblast cells (HGF-1) [16]. The relative ease in the synthesis of the cytotoxic furanones **1**–**4** makes these compounds suitable candidates for further study. Although **1**, known as C30, has been extensively used in quorum sensing (QS) studies, to our knowledge, this is the first time that **1** is reported as a cytotoxic agent [18].

Experimental

All reagents were acquired from Sigma-Aldrich, except tetronic acid which was purchased from Alfa Aesar Chemicals. All solvents (acetone, ethyl acetate, dichloromethane, hexane, ethanol, methanol) used were previously distilled. Diisopropyl ether was acquired from Sigma-Aldrich. Deuterated chloroform ($CDCl_3$) and dimethylsulfoxide-d_6 were purchased from Cambridge Isotope Laboratories, Inc.

TLC was performed using DC-Fertigfolien ALUGRAMR Xtra SIL G/UV254 20 × 20 cm. Normal phase flash column chromatography was performed using Silica gel 60 Kieselgel 60 0.063–0.2 mm.

^{1}H, ^{13}C, DEPT-135, HSQC, and HMBC NMR were recorded on a Bruker Fourier Spectrometer 300 MHz. Chemical shift values are expressed in δ (ppm) relative to tetramethyl silane (TMS, $\delta = 0.00$ ppm for ^{1}H NMR and $\delta = 77.0$ ppm for ^{13}C NMR). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (J; Hz), and integration. The exact mass was calculated by a Jeol AccuTOF JMST100LC-DART apparatus. Infrared spectra were acquired in FT-IR Bruker spectrometer (KBr pellet).

HPLC was performed in Agilent Liquid Chromatograph equipped with a Waters 2996 diode array. A column Synergi Polar-RP 80A 150 × 2.0 mm 4 um was used. Methanol was the initial eluent and water after 30 min. Flow = 0.2 cm^3/min, wavelength 254 or 240 nm, sample solvent: acetonitrile or methanol.

(*Z*)-4-Bromo-5-(bromomethylene)furan-2-(5*H*)-one (**1**) [7], (*E*)-5-(bromomethylene)furan-2-(5*H*)-one (**2**) [8, 9], as well as 3,4-dibromo-5-hydroxyfuran-2(5*H*)-one (**3**) and 3,4-dibromofuran-2(5*H*)-one (**4**) [10, 11] were synthesized according to literature.

General procedure for the synthesis of furanones 5–11

General procedure for the synthesis of furanones **5**–**11** was conducted as previously reported with slight modifications [12] (Scheme 2). To a solution of 1.0 mmol of **4** in 10 cm^3 of dry methanol was added 2.0 mmol of an aromatic amine. The solution was left 24 h at room temperature. Afterward, the solvent was evaporated under reduced pressure, then 20 cm^3 of CH_2Cl_2 was added and washed with water (3 × 5 cm^3). The organic layer was dried over anhydrous Na_2SO_4, filtrated, and the solvent evaporated under reduced pressure. The crude residue was recrystallized from $CHCl_3$/hexane, affording the respective solid furanones. Compounds **7**, **8**, and **10** were purified by open column chromatography packed with silica and eluted with hexane/EtOAc 7:3 mixture.

3-Bromo-4-[(4-chlorophenyl)amino]furan-2(5*H*)-one (5) $R_f = 0.55$ (hex/EtOAc 7:3, v/v); yield: 72%; m.p.: 125–126 °C; HPLC purity: 95%. The NMR analysis was in accordance with previously published data [12] (for 2D NMR experiments see supplementary material).

3-Bromo-4-[(4-bromophenyl)amino]furan-2(5*H*)-one (6) $R_f = 0.55$ (hex/EtOAc 7:3, v/v); yield: 76%; m.p.: 145–147 °C; HPLC purity: 99%. The NMR analysis was in accordance with previously published data [12] (for 2D NMR experiments see supplementary material).

3-Bromo-4-[(4-fluorophenyl)amino]furan-2(5*H*)-one (7, $C_{10}H_8BrFNO_2$) $R_f = 0.6$ (hex/EtOAc 7:3, v/v); yield: 81%; m.p.: 128–130 °C; HPLC purity: 99%; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.13$ (s, 2H), 7.12 (s, 2H), 6.77 (s, 1H), 4.81 (s, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 169.46$, 161.16 (d, $J = 124$ Hz), 160.65, 133.09, 125.06 (d, $J = 8.6$ Hz), 117.11 (d, $J = 23$ Hz), 77.83, 67.05 ppm; IR (KBr): $\bar{\nu} = 3285$, 1723, 1626, 1593, 1189, 506 cm^{-1}; MS (DART): *m/z* calcd for $^{12}C_{10}{}^{1}H_{8}{}^{81}Br_{1}{}^{19}F_{1}{}^{14}N_{1}{}^{16}O_{2}$ ([M + H]$^+$]): 273.97020, found 273.96988 (for 2D NMR experiment see supplementary material).

3-Bromo-4-[(3,4-difluorophenyl)amino]furan-2(5*H*)-one (8, $C_{10}H_7BrF_2NO_2$) $R_f = 0.45$ (hex/EtOAc 7:3, v/v); yield: 70%; m.p.: 140–142 °C; HPLC purity: 91%; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 9.62$ (s, 1H), 7.5–7.34 (m, 2H), 7.12 (dt, $J = 8.4$, 4.0 Hz, 1H), 5.08 (s, 2H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 169.96$, 161.77, 150.11 (dd, $J = 174.1$, 13 Hz), 146.87 (dd, $J = 171.8$, 13 Hz), 135.59 (d, $J = 5.5$ Hz), 119.92 (dd, $J = 6.3$, 3.3 Hz), 118.26 (d, $J = 18$ Hz), 112.64 (d, $J = 19.8$ Hz), 75.44, 67.80 ppm; IR (KBr): $\bar{\nu} = 3239$, 1727, 1632, 1602, 1524 cm^{-1}; MS (DART): *m/z* calcd for $^{12}C_{10}{}^{1}H_{7}{}^{79}Br_{1}{}^{19}F_{2}{}^{14}N_{1}{}^{16}O_{2}$ ([M + H]$^+$]): 289.96282, found

289.96302 (for 2D NMR experiment see supplementary material).

3-Bromo-4-[(3,4-dichlorophenyl)amino]furan-2(5*H*)-one (9, $C_{10}H_7BrCl_2NO_2$) $R_f = 0.55$ (hex/EtOAc 7:3, v/v); yield: 86%; m.p.: 163–165 °C; HPLC purity: 99%; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 9.68$ (s, 1H), 7.60 (d, $J = 8.7$ Hz, 1H), 7.55 (d, $J = 2$ Hz, 1H), 7.25 (dd, $J = 8.7$, 2.1 Hz, 1H), 5.14 (s, 2H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 169.40, 160.77, 138.42, 131.40, 130.91, 126.73, 123.74, 122.14, 76.08, 67.52 ppm; IR (KBr): $\overline{\nu} = 3232$, 1727, 1646, 1618, 1477 cm^{-1}; MS (DART): *m/z* calcd for $^{12}C_{10}{}^{1}H_{7}{}^{81}Br_{1}{}^{35}Cl_{1}{}^{37}Cl_{1}{}^{14}N_{1}{}^{16}O_{2}$ ([M + H]$^+$]): 325.89872, found 325.89867 (for 2D NMR experiment see supplementary material).

4-[(4-Iodophenyl)amino]furan-2(5*H*)-one (10, $C_{10}H_9INO_2$) $R_f = 0.50$ (hex/EtOAc 1:1, v/v); yield: 83%; m.p.: 249–251 °C; HPLC purity: 98%; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 9.79$ (s, 1H), 7.66 (d, $J = 8.2$ Hz, 2H), 7.01 (d, $J = 8.3$ Hz, 2H), 5.33 (s, 1H), 4.85 (s, 2H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 175.30, 162.70, 140.47, 138.47, 121.18, 86.82, 85.06, 68.56 ppm; IR (KBr): $\overline{\nu} = 3435$, 1698, 1612, 1552 cm^{-1}; MS (DART +): *m/z* calcd for $^{12}C_{10}{}^{1}H_{9}{}^{127}I_{1}{}^{14}N_{1}{}^{16}O_{2}$ ([M + H]$^+$]): 301.96780, found 301.96761 (for 2D NMR experiment see supplementary material).

(*E*)-3-Bromo-4-[(4-(phenyldiazenyl)phenyl)amino]-furan-2(5*H*)-one (11, $C_{16}H_{13}BrN_3O_2$) $R_f = 0.35$ (hex/EtOAc 1:1, v/v); yield: 85%; m.p.: 142–143 °C; HPLC purity: 99%; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 9.85$ (s, 1H), 7.90 (t, $J = 8.2$ Hz, 4H), 7.59 (q, $J = 6.5$ Hz, 3H), 7.57–7.42 (m, 2H), 5.27 (s, 2H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 169.86, 160.98, 152.46, 148.75, 141.83, 131.76, 129.92, 124.24, 122.91, 122.21, 77.21, 68.35 ppm; IR (KBr): $\overline{\nu} = 3434$, 1731, 1638, 1583 cm^{-1}; MS (DART +): *m/z* calcd for $^{12}C_{16}{}^{1}H_{13}{}^{81}Br_{1}{}^{14}N_{3}{}^{16}O_{2}$ ([M + H]$^+$]): 360.01707, found 360.01622 (for 2D NMR experiment see supplementary material).

General procedure for the synthesis of furanones 12–19

β-Cyclodextrin (0.226 g, 0.2 mmol) was dissolved in 30 cm^3 distilled water and stirred for 10 min. After aniline (2.0 mmol) was added and stirred for another five min more and then, 0.170 g diethyl acetylene dicarboxylate (2.0 mmol) was slowly added through an addition funnel and finally the respective aromatic aldehyde was also added (2.0 mmol). The reaction mixture was heated at 60–70 °C until completion of the reaction as indicated by TLC. The reaction mixture was cooled at room temperature and β-cyclodextrin was filtered; the aqueous phase was extracted with ethyl acetate (4×10 cm^3). The organic layers were washed with water, saturated brine solution (2×10 cm^3) and dried over anhydrous Na_2SO_4. The combined organic layers were evaporated under reduced pressure and the resulting crude product was purified by silica open column chromatography by using ethyl acetate and hexane (7:3) as eluent to give the respective furanone derivative.

Ethyl 5-oxo-2-phenyl-4-(phenylamino)-2,5-dihydrofuran-3-carboxylate (12) $R_f = 0.45$ (EtOAc); yield: 80%; m.p.: 152–153 °C; HPLC purity: 99%. NMR analysis was in accordance with previously published data [13] (for 2D NMR experiments see supplementary material).

Ethyl 2-(4-chlorophenyl)-5-oxo-4-(phenylamino)-2,5-dihydrofuran-3-carboxylate (13) $R_f = 0.40$ (EtOAc); yield: 90%; m.p.: 165–167 °C; HPLC purity: 99%. NMR analysis was in accordance with previously published data [13] (for 2D NMR experiments see supplementary material).

Ethyl 4-[(4-bromophenyl)amino]-5-oxo-2-phenyl-2,5-dihydrofuran-3-carboxylate (14, $C_{19}H_{17}BrNO_4$) $R_f = 0.35$ (EtOAc); yield 85%; m.p.: 144–147 °C; HPLC purity: 99%; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.39$ (d, $J = 8.7$ Hz, 2H), 7.36 (d, $J = 8.5$ Hz, 2H), 7.25 (dd, $J = 12.7$, 7.6 Hz, 3H), 7.20 (d, $J = 7.1$ Hz, 2H), 5.68 (s, 1H), 4.18 (q, 7.1 Hz, 2H), 1.17 (t, $J = 6.9$ Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.06, 162.87, 156.37, 135.46, 134,78, 132.03, 128,74, 127.46, 123.43, 118.94, 113.28, 61.40, 61.35, 13.91 ppm; IR (KBr): $\overline{\nu} = 1676$, 1227, 826 cm^{-1}; MS (DART +): *m/z* calcd for $^{12}C_{19}{}^{1}H_{17}{}^{81}Br_{1}{}^{14}N_{1}{}^{16}O_{4}$ ([M + H]$^+$]): 404.03205, found 404.03405 (for 2D NMR experiment see supplementary material).

Ethyl 2-(4-chlorophenyl)-4-[(4-fluorophenyl)-amino]-5-oxo-2,5-dihydrofuran-3-carboxylate (15, $C_{19}H_{16}ClFNO_4$) $R_f = 0.40$ (EtOAc); yield: 70%; m.p.: 157–160 °C; HPLC purity: 96%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46–7.31 (m, 2H), 7.29–7.19 (m, 2H), 7.14 (d, $J = 8.4$ Hz, 2H), 6.97 (t, $J = 8.6$ Hz, 2H), 5.65 (s, 1H), 4.20 (q, $J = 7.1$ Hz, 2H), 1.19 (t, $J = 7.1$ Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.03, 162.8, 160.54 (d, $J = 246.9$ Hz), 156.84, 134.65, 133.69, 132.15 (d, $J = 2.9$ Hz), 129.05 (d, $J = 9.5$ Hz), 124.45 (d, $J = 8.2$ Hz), 116.15 (d, $J = 22.7$ Hz), 112.82, 61.56, 61.36, 14.12 ppm; IR (KBr): $\overline{\nu} = 3306$, 1722, 1659, 1510, 1201, 837 cm^{-1}; MS (DART +): *m/z* calcd for $^{12}C_{19}{}^{1}H_{16}{}^{35}Cl_{1}{}^{19}F_{1}{}^{14}N_{1}{}^{16}O_{4}$ ([M + H]$^+$]): 376.07519, found 376.07514 (for 2D NMR experiment see supplementary material).

Ethyl 4-[(4-bromophenyl)amino]-2-(4-chlorophenyl)-5-oxo-2,5-dihydrofuran-3-carboxylate (16, $C_{19}H_{16}BrClNO_4$) $R_f = 0.35$ (EtOAc); yield: 85%; m.p.:

170–172 °C; HPLC purity: 99%; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.57–7.53 (m, 2H), 7.47 (d, J = 8.9 Hz, 2H), 7.36 (m, 4H), 6.07 (s, 1H), 4.20–3.87 (m, 2H), 1.04 (t, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.54, 162.14, 153.89, 136.03, 135.59, 132.36, 131.57, 129.63, 128.23, 124.19, 117.64, 110.80, 59.64, 59.54, 14.01 ppm; IR (KBr): $\bar{\nu}$ = 3295, 1722, 1682, 1660, 1589, 1489, 828 cm^{-1}; MS (DART+): *m/z* calcd for $^{12}C_{19}{}^{1}H_{16}{}^{81}Br_1{}^{35}Cl_1{}^{14}N_1{}^{16}O_4$ ([M + H]$^+$]): 437.69534, found 437.69547 (for 2D NMR experiment see supplementary material).

Ethyl 2-(4-chlorophenyl)-4-[(4-iodophenyl)amino]-5-oxo-2,5-dihydrofuran-3-carboxylate (17, $C_{19}H_{17}ClINO_4$) R_f = 0.30 (EtOAc); yield: 75%; m.p.: 125–127 °C; HPLC purity: 98%; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.61 (d, J = 8.6 Hz, 2H), 7.41 (d, J = 8.6 Hz, 2H), 7.22 (s, 4H), 5.84 (s, 1H), 4.20–3.66 (m, 2H), 1.04 (t, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.98, 164.67, 151.93, 137.79, 136.85, 136.63, 132.43, 129.92, 128.52, 124.57, 114.27, 90.34, 59.80, 59.49, 14.53 ppm; MS (DART+): *m/z* calcd for $^{12}C_{19}{}^{1}H_{17}{}^{35}Cl_1{}^{127}I_1{}^{14}N_1{}^{16}O_4$ ([M + H]$^+$]): 483.97319, found 483.97370 (for 2D NMR experiment see supplementary material).

Ethyl 4-[(4-iodophenyl)amino]-5-oxo-2-phenyl-2,5-dihydrofuran-3-carboxylate (18, $C_{19}H_{16}INO_4$) R_f = 0.35 (EtOAc); yield: 75%; m.p.: 185–187 °C; HPLC purity: 99%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.71–7.47 (m, 2H), 7.42–7.04 (m, 7H), 5.69 (s, 1H), 4.18 (q, J = 7.1 Hz, 2H), 1.17 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.20, 162.93, 156.46, 138.09, 136.28, 134.86, 128.87, 127.56, 123.67, 113.44, 90.11, 61.48, 61.35, 31.71, 22.78, 14.03 ppm; IR (KBr): $\bar{\nu}$ = 1711, 1681, 1661, 1584, 1486, 1240, 823 cm^{-1}; MS (DART+): *m/z* calcd for $^{12}C_{19}{}^{1}H_{16}{}^{127}I_1{}^{14}N_1{}^{16}O_4$ ([M + H]$^+$]): 449.01240, found 449.01053 (for 2D NMR experiment see supplementary material).

Ethyl 2-(4-chlorophenyl)-4-[(3,4-difluorophenyl)amino]-5-oxo-2,5-dihydrofuran-3-carboxylate (19, $C_{19}H_{15}ClFNO_4$) R_f = 0.40 (EtOAc), yield 20%; m.p.: 145–147 °C; HPLC purity: 98%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46 (ddd, J = 9.6, 6.9, 2.2 Hz, 1H), 7.30–7.23 (m, 2H), 7.19–7.12 (m, 2H), 7.12–6.98 (m, 2H), 5.64 (s, 1H), 4.23 (q, J = 7.1 Hz, 2H), 1.21 (t, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.81, 162.66, 156.32, 151.68 (d, J = 12.6 Hz), 146.40 (d, J = 12.6 Hz), 134.75, 133.19, 132.46 (d, J = 12.7 Hz), 129.14, 128.74, 117.82, 117.60, 117.36, 113.01, 111.92, 111.77 (d, J = 21.4 Hz), 61.59, 60.94, 13.95 ppm; IR (KBr): $\bar{\nu}$ = 1711, 1681, 1661, 1584, 1486, 1240, 823 cm^{-1}; MS (DART+): *m/z* calcd for $^{12}C_{19}{}^{1}H_{15}{}^{35}Cl_1{}^{19}F_2{}^{14}N_1{}^{16}O_4$ ([M + H]$^+$]): 394.06577 found, 394.05497 (for 2D NMR experiment see supplementary material).

Cytotoxicity assay

Cell culture: Roswell Park Memorial Institute medium (RPMI-1640), FBS (fetal bovine serum, BIO-S1650-500) and Trypsin (BIO-L0931-100) were obtained from Biowest company). Dimethyl sulfoxide and Cisplatin (*cis*-diammineplatinum(II) dichloride) were obtained from Sigma-Aldrich.

The cytotoxicity of compounds **1**–**19** was evaluated against HCT-116 (human colon carcinoma) and PC-3 (human prostate cancer) cell lines obtained from the American Type Culture Collection (ATCC). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 IU/cm^3 G-penicilin, 100 µg/cm^3 streptomycin, and 0.25 µg/cm^3 B-amphotericin and incubated at 37 °C under a 5% CO_2 atmosphere. For experiments, cells were seeded in 96-well plates (0.7×10^5 for adherent cells in 100 mm^3 medium). After 24 h, furanones 1–20 (0.39–25 µg/cm^3) dissolved in DMSO (5%) were added to each well and incubated for 2 days (48 h) before the sulforhodamine B. Results are summarized in Table 1; cisplatin was used as a reference compound. The cytotoxicity of **1**–**4** against primary fibroblast cells (HGF-1) was evaluated according to a previous method reported [16].

TBARS assay

The experiments were performed according to a previously protocol reported [18]. Adult female rats were used under approval of NOM-062-ZOO-1999. Animals were euthanized in CO_2 chamber. The whole brain homogenized in PBS solution (0.2 g of KCl, 0.2 g of KH_2PO_4, 8 g of NaCl, and 2.16 g of $NaHPO_4 \cdot 7\ H_2O$/dm^3, pH adjusted to 7.4). It was used thiobarbituric acid (TBA) 1% in 0.05 N NaOH solution mixed with 30% trichloroacetic acid in 1:1 proportion. Protein content in brain supernatant solutions was measured using the Folin and Ciocalteu's phenol reagent. Lipid peroxidation was induced with EDTA (100 µM) according to protocol, during 5 h at one-hour intervals. 0.5 cm^3 of TBA solution were taken for each tube and cooled on ice for 10 min, centrifuged to 12 000 rpm during 5 min, and finally heated at 95 °C for 30 min. The tubes were allowed to reach room temperature and 200 mm^3 of supernatant were taken for analysis. TBARS content was analyzed using optical density at λ = 540 nm using Bio-Tek EL microplate reader. Results were analyzed using Origin 2020 software.

Annexin V and propidium iodide assay

Apoptotic cell death was determined using FITC-Annexin-V Apoptosis Detection Kit (Sigma Aldrich APOAF-50TST) in accordance with the manufacturer's protocol. HCT-116 and PC-3 cells were seeded in 6-well plates at a density

of 4.0×10^5 cells/cm^3 in 3 cm^3 of cell culture medium. Cells were treated with concentrations (1.13, 0.73, 0.93, 26.25, 1.31 μM for PC-3 and 1.12, 0.54, 1.5, 14.06, 0.4 μM for HCT-116) of each furanone (**1**–**5**) for 48 h, at 37 °C. Untreated cells were used as a control. Then, cells were washed with PBS, resuspended in the binding buffer and stained with FITC annexin-V and propidium iodide (PI), followed by incubation in the dark at 25 °C for 15 min. Finally, flow cytometry analysis of the cells was performed using Attune Acoustic Focusing Flow Cytometer (Red-Blue laser) from Thermo Scientific in National Flow Cytometry Laboratory, 20.000 cells were analyzed. Data were analyzed using FlowJo Software v10.6.2 (Fig. 1).

Statistical analysis

All experiments were assayed in triplicate ($n = 3$). Data are expressed as means ± SEM. All statistical analyses were performed using GraphPad Pro. Prism 5.0 (GraphPad, San Diego, CA). Student's t test and two-way ANOVA were employed to analyze the differences between sets of data. A p value < 0.05 was considered statistically significant.

Acknowledgements Víctor A. Castro-Torres is a doctoral student from the Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and has received CONACyT fellowship 267787. This study was supported by Grants from PAPIIT IN211819 and CONACyT 402838 and 00042. We acknowledge the technical support of MS Antonio Nieto-Camacho and MS María Teresa O. Ramírez-Apan from Instituto de Química-UNAM.

References

1. Globocan 2019. https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf. Accessed 9 May 2020
2. Lopez-Lazaro M, Pastor N, Azrak SS, Ayusco CA, Austin F, Cortes F (2005) J Nat Prod 68:1642
3. Lopez-Lazaro M (2007) Expert Opin Ther Targets 11:1043
4. Newman RA, Yang P, Pawlus AD, Keith IB (2008) Mol Interv 8:36
5. Peng CK, Zeng T, Xu XJ, Chang YQ, Hou W, Lu K, Lin H, Sun PH, Lin J, Chen WM (2017) Eur J Med Chem 127:187
6. Byczek-Wyrosteka A, Kitela R, Rumaka K, Skoniecznab M, Kasprzyckaa A, Walczaka K (2018) Eur J Med Chem 150:687
7. Manny AJ, Kjelleberg S, Kumar N, Nys R, Read RW, Steinberg P (1997) Tetrahedron 53:15813
8. MacDonald SF (1974) Can J Chem 52:3257
9. Benneche T, Lönn J, Scheie AA (2006) Synth Commun 36:1401
10. Taylor GA (1963) Org Synth Coll 4:688
11. Bellina F, Anselmi C, Viel S, Mannina L, Rossi R (2001) Tetrahedron 57:9997
12. Cunha S, Oliveira C, Sabino JR (2011) J Braz Chem Soc 22:598
13. Narayana MS, Madhav B, Vijay KA, Rama RK, Nageswar YVD (2009) Tetrahedron 65:5251
14. Hall IH, Lee KH, Mar EC, Starnes CO, Waddell TG (1997) J Med Chem 20:333
15. Zeni O, Di Pietro R, d'Ambrosio G, Massa R, Capri M, Naarale J, Juutilainen J, Scarfì MR (2007) Radiat Res 167:306
16. Hernández-Vázquez E, Miranda LD (2016) Org Biomol Chem 14:4875
17. Liviac D, Creus A, Marcos R (2009) Environ Mol Mutagen 50:413
18. Wu H, Song Z, Hentzer M, Andersen JB, Moli S, Givskov M, Høiby N (2004) J Antimicrob Chemother 53:1054
19. Kibanova A, Camacho AN, Silva JC (2009) Environ Sci Technol 43:7550