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Synthesis and cancer cell cytotoxicity of substituted xanthenes

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ABSTRACT

A series of substituted xanthenes was synthesized and screened for activity using DU-145, MCF-7, and HeLa cancer cell growth inhibition assays. The most potent compound, **9g** ([*N*,*N*-diethyl]-9-hydroxy-9-(3-methoxyphenyl)-9*H*-xanthene-3-carboxamide), was found to inhibit cancer cell growth with IC_{50} values ranging from 36 to 50 μM across all three cancer cell lines. Structure–activity relationship (SAR) data is presented that indicates additional gains in potency may be realized through further derivatization of the compounds (e.g., the incorporation of a 7-fluoro substituent to **9g**). Results are also presented that suggest the compounds function through a unique mechanism of action as compared to that of related acridine and xanthone anticancer agents (which have been shown to intercalate into DNA and inhibit topoisomerase II activity). A structural comparison of these compounds suggests the differences in function may be due to the structure of the xanthene heterocycle which adopts a nonplanar conformation about the pyran ring.

1. Introduction

Xanthones and xanthenes are tricyclic dibenzopyrans with diverse physicochemical and pharmacological properties. Although a large number of xanthone-based natural products have been isolated and characterized,[1–3] the development of therapeutic agents that take advantage of this unique heterocyclic structure has been limited. Xanthones are active against a variety of pathogens and show promise for use as antioxidants, antineoplastics, vasodilators, and anti-inflammatories.[4–15] In most cases, the mechanism of action is not known, greatly complicating the drug discovery process. Recently, Na and co-workers reported the synthesis of several xanthone-based analogs that were cytotoxic to cancer cells in vitro.[4] These compounds contained reactive epoxides which were shown to block topoisomerase II mediated relaxation of DNA. This mechanism of action suggests the planar xanthone structure interacts with DNA, perhaps through intercalation, directing the reactive epoxides to the site of alkylation. While a number of other studies have appeared that show substituted xanthones are cytotoxic to cancer cells,[5–9] a common mechanism of action has not yet emerged.

Xanthones are structurally related to the xanthenes and xanthen-9-ols as shown in Figure 1. Xanthenes are commonly used in the production of fluorescent dyes, including fluorescein, rhodamine, and the eosins. Some evidence has surfaced that suggests the xanthene core structure also may be useful in the design and development of pharmacological agents.[16–18] Starting from 9-carboxy xanthene, Naya et al. synthesized and evaluated the structure–activity relationships of a series of 2,9-disubstituted xanthenes with potent activity as chemokine receptor (CCR1) antagonists.[16] In an earlier study, Ornstein et al. reported a 9-xanthylmethyl amino acid conjugate that was shown to be a selective and potent antagonist of the metabotropic glutamate receptor.[17] Most recently, our lab described the synthesis of a 3-piperazinyl substituted xanthen-9-ol with low micromolar activity against West Nile Virus.[18] Although only a small selection of

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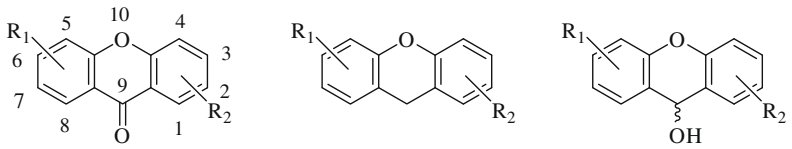


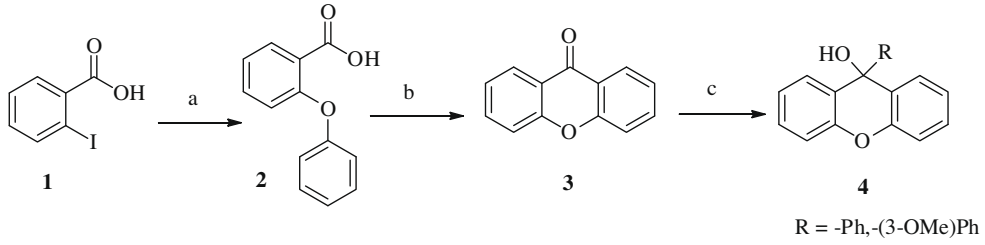
Figure 1. Xanthone, xanthene, and xanthenol structures.

representative structures were evaluated, the synthetic scheme described used a xanthone intermediate, providing an efficient route to obtaining substituted xanthenes. Compounds of this type have also been synthesized using aryne insertion reactions of benzaldehydes and benzoates.[19,20]

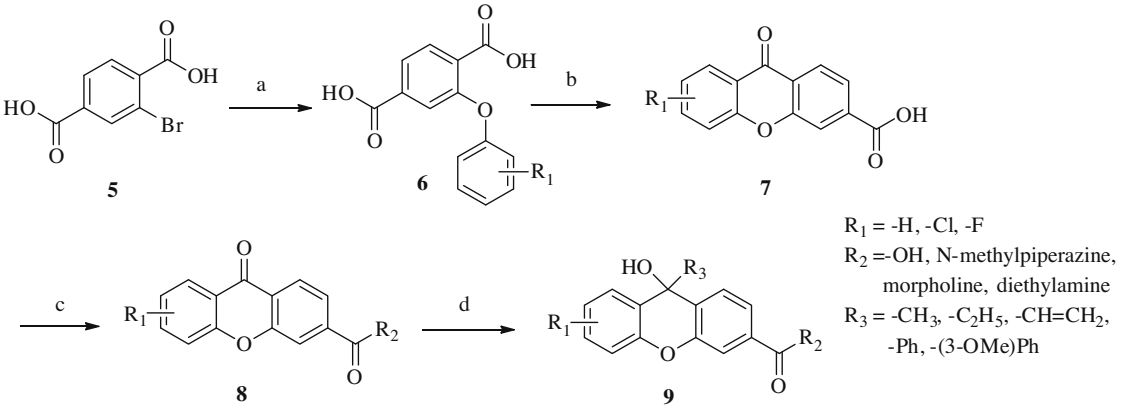
Here, we describe the synthesis of a series of substituted xanthenes using a modified Ullmann–Goldberg reaction commonly used in the synthesis of acridones and acridines.[21,22] The approach couples phenols to aryl halides in the presence of copper to generate aryl ethers. Cyclization to the xanthone is then accomplished via Friedel–Crafts-like acylation. One advantage to this approach is that the intermediate xanthone can be both evaluated for biological activity and further converted to the xanthene by any number of routes. In this report, we use a Grignard reaction to obtain a library of di- and tri-substituted 9-alkyl xanthenols for evaluation as potential anticancer agents. The cytotoxicity of the compounds against three well known cancer cell lines (DU-145, MCF-7, and HeLa) is reported and compared with previous work on a related series of substituted acridines. Some insights to the potential mechanism of action of the compounds are given using molecular modeling techniques and screening data derived from topoisomerase II activity.

2. Chemistry

The synthesis of xanthenes proceeds through the generation of the xanthone intermediate as shown in Schemes 1 and 2. The first step of the pathway utilizes a modified Ullmann condensation reaction of 2-iodobenzoic acid (**1**) or bromoterephthalic acid (**5**) with various phenols in the presence of catalytic Cu(0) to form 2-phenoxybenzoic acid (**2**) or *O*-phenylsalicylic acid derivatives **6**. The use of a non-nucleophilic base, such as 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU), was found to be essential for the coupling reaction with phenol to form the diarylethers. Cyclization of 2-phenoxybenzoic acid (**2**) and *O*-phenylsalicylic acid derivatives **6** with concd H_2SO_4 at 100 °C afforded xanthone (**3**) and 3-carboxyxanthone derivatives **7**, respectively. Refluxing **7** with $SOCl_2$ produced the acid chloride intermediate. Subsequent reaction of the acid chloride with an amine afforded the 3-carboxamidexanthone derivatives **8**. Xanthone (**3**) and 3-carboxamidex-



Scheme 1. Synthesis of the 9-alkylxanthen-9-ols. Reagents and conditions: (a) phenol, Cu, CuI, pyridine, DBU, DMF, reflux; (b) H_2SO_4, 100 °C; (c) MgBrR, THF, −78 °C 1 h to rt 5 h.



Scheme 2. Synthesis of the di- and tri-substituted xanthenes. Reagents and conditions: (a) phenol, Cu, CuI, pyridine, DBU, DMF, reflux; (b) H_2SO_4, 100 °C; (c) $SOCl_2$, reflux, secondary amine, CH_2Cl_2, 0 °C; (d) $MgBrR_3$, THF, −78 °C 1 h to rt 5 h.

anthone derivatives **8** were reacted with different Grignard reagents at −75 °C to yield xanthene derivatives **4** and **9**, respectively.

3. Results and discussion

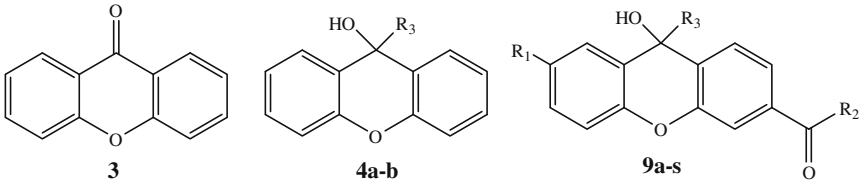
The substituted xanthenes and corresponding activity against prostate (DU-145), breast (MCF-7), and cervical (HeLa) cancer cell proliferation are reported in Table 1. While limited, the library was designed to provide some structure–activity relationship data to help guide the design of additional xanthene derivatives. The unsubstituted xanthone (**3**) and 9-phenyl-xanthenol (**4a**) were synthesized for comparison and notably showed no activity. The simple addition of a 3-methoxy group to the 9-phenyl ring of **4a**, however, was found to have a dramatic impact on activity suggesting this group may contribute to activity. Further analysis of the activity with respect to the R_3 substitution shows that the 3-methoxyphenyl group is generally favored. One notable exception is with compound **9b** which contains a vinyl group in the R_3 position. In regards to the R_2 substitution, it was found that replacement of the *N*-methylpiperazine with the diethylamine (**9f** and **9g**, respectively) improved activity against all three cancer cell lines by several folds. Comparing **4a**, **9d**, **9f**, and **9h** (which all contain the 9-phenyl substituent) reveals a clear trend indicating that the diethylamino group contributes the most favorable to activity. Additionally, it was found that activity increases in both the chloro- and fluoro-series when R_3 contains a bulky phenyl or 3-methoxyphenyl substitution. At the R_1 position the fluoro substitutions show the best activity, suggesting that additional gains in potency may be achieved by adding a fluoro group to the diethylamine **9g**.

The effect of the compounds on the catalytic activity of human topoisomerase II has been examined using a DNA relaxation assay. As shown in Figure 2, no activity in the relaxation assays was detected. These results are somewhat surprising given the results reported by Na and co-workers that indicated reactive xanthone derivatives, which include reactive epoxides, inhibit topoisomerase II activity.[4] The differences in their effects on the topoisomerase II activity might be due to the presence of reactive epoxides. Additionally, the compounds in the current study were designed, in part, to possess some of the same structural features found in our recently published work highlighting acridine-based anticancer agents.[23] While both the acridines and xanthones have been shown to intercalate into DNA,[4,24] the xanthenes did not intercalate into DNA (Fig. 3). This observation suggests that the xanthenes studied here function through an alternative mechanism. A structural comparison of xanthenol **9e**, xanthone **3**, and a representative acridine-based structure is given in Figure 4. The structures were model built interactively and subsequently energy minimized using quantum mechanics calculations.[25] While the acridine and xanthone structures adopt a planar conformation that is well suited to DNA intercalation, the xanthen-9-ol buckles about the pyran ring and adopts a boat-like conformation thus orienting the phenyl ring perpendicular to the plane of the heterocycle. This deviation from planarity may, in part, explain the lack of DNA binding noted for the xanthenes when compared to related acridines and the parent xanthones.

4. Conclusion

This study has reported the synthesis and cytotoxicity of a small library of substituted xanthenes. Although the activity of the compounds is modest, it is important to point out that the xanthenes described here are novel anticancer agents. Given our previous work on substituted acridines and the data reported by Woo et al. for xanthones,[4,23,24] the failure of these compounds to inter-

Table 1
Cancer cell cytotoxicities for the substituted xanthenes and related compounds[a]



3 **4a-b** **9a-s**

Compd	R_1	R_2	R_3	DU-145	MCF-7	HeLa
3	—	—	—	>500	>500	>500
4a	—	—	Ph	>500	>500	>500
4b	—	—	–Ph(3-OMe)	59 ± 4	82 ± 6	85 ± 15
9a	–H	*N*-Methylpiperazine	Methyl	119 ± 4	115 ± 13	121 ± 12
9b	–H	*N*-Methylpiperazine	$-CH=CH_2$	63 ± 3	74 ± 2	74 ± 0.3
9c	–H	*N*-Methylpiperazine	$-C_2H_5$	195 ± 5	218 ± 6	213 ± 5
9d	–H	*N*-Methylpiperazine	–Ph	>300	169 ± 2	170 ± 4
9e	–H	*N*-Methylpiperazine	–Ph(3-OMe)	139 ± 9	125 ± 7	111 ± 2
9f	–H	Diethylamine	–Ph	59 ± 3	62 ± 4	67 ± 2
9g	–H	Diethylamine	–Ph(3-OMe)	36 ± 3	50 ± 4	44 ± 1
9h	–H	Morpholine	–Ph	125 ± 4	134 ± 9	120 ± 6
9i	–H	Morpholine	–Ph(3-OMe)	163 ± 7	151 ± 30	153 ± 38
9j	–Cl	*N*-Methylpiperazine	Methyl	241 ± 0.5	160 ± 9	264 ± 14
9k	–Cl	*N*-Methylpiperazine	$-CH=CH_2$	208 ± 12	>500	285 ± 25
9l	–Cl	*N*-Methylpiperazine	$-C_2H_5$	145 ± 15	180 ± 3	153 ± 3
9m	–Cl	*N*-Methylpiperazine	–Ph	117 ± 6	95 ± 9	158 ± 2
9n	–Cl	*N*-Methylpiperazine	–Ph(3-OMe)	92 ± 5	72 ± 2	94 ± 6
9o	–F	*N*-Methylpiperazine	Methyl	>300	208 ± 4	>300
9p	–F	*N*-Methylpiperazine	$-CH=CH_2$	>300	254 ± 8	230 ± 16
9q	–F	*N*-Methylpiperazine	$-C_2H_5$	139 ± 2	138 ± 3	127 ± 5
9r	–F	*N*-Methylpiperazine	–Ph	84 ± 4	62 ± 9	109 ± 6
9s	–F	*N*-Methylpiperazine	–Ph(3-OMe)	55 ± 0.4	46 ± 2	85 ± 4

[a] IC_{50} values (μM).

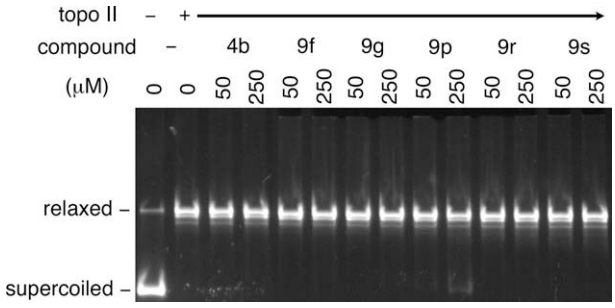


Figure 2. Representative results of topoisomerase II relaxation assay for the substituted xanthenes.

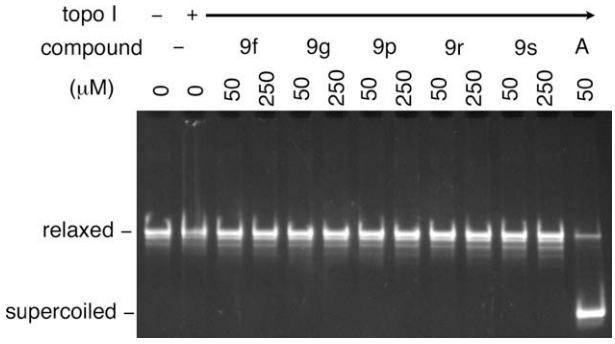


Figure 3. Representative results of DNA intercalation assay for the substituted xanthenes. **A**, amsacrine.

calate into DNA or block topoisomerase II activity is somewhat surprising. A comparison of the acridine, xanthone, and xanthene structures, however, indicates this failure is directly related to differences in the structures of these compounds. Whereas the acridine and xanthone structures adopt flat, highly conjugated ring systems, the xanthenes buckle about the pyran ring, placing the 9-phenyl substituent in a perpendicular orientation relative to the tricyclic ring system. The position of this phenyl ring would certainly disrupt intercalation and may explain the lack of DNA binding noted for the xanthenes. This hypothesis could be evaluated by preparing a 9-benzylidene derivative of **4b** to further determine the effect of planarity on function and activity.

An analysis of the IC_{50} values also has shown a correlation exists between xanthene substitution and the inhibition of cancer cell proliferation in vitro. In addition, no significant preference for cancer cell type was noted. These findings support the general hypothesis that these compounds function through specific as opposed to non-specific binding in mediating cytotoxicity. While the chemistry presented here is limited, the SAR suggests that some gains in activity should be obtainable through further derivatization of the xanthene scaffold. In particular, fluoro derivatives coupled with 9-(3-methoxyphenyl) substitutions may prove promising in reducing the IC_{50} values to the sub-micromolar range. One issue not addressed here, but central to exploring both the potential mechanism of action of the compounds and future SAR studies is chirality. The xanthenes tested in this study are all racemates, suggesting that further gains in potency may be realized by resolving the enantiomers. Enantioselectivity is a key measure of specificity in evaluating mechanisms of action and would provide much needed insight to understanding the function of these compounds in inhibiting cancer cell growth.

5. Experimentals

5.1. General

Unless mentioned otherwise all the starting materials and solvents were purchased from commercially available sources. Water was purified via a Millipore filtration system. Column chromatography was conducted using Silica Gel 60 (40–63 μm). ^{1}H nuclear magnetic resonance spectra were collected on Varian Mercury 600 MHz instrument using $CDCl_3$ and DMSO-d_6 and acetone-d_6 as solvents. High-resolution mass spectra (HRMS) were collected from a TOF-ESI Agilant LC–MS and analyzed using the Analyst QS software. All the reactions were monitored using thin layer chromatography (TLC) using EMD Chemical Silica Gel 60 F254 on aluminum sheets.

5.1.1. 2-Phenoxybenzoic acid (2)

2-Iodo benzoic acid (**1**) (3.10 g, 12.4 mmol) was added to 100 mL of dimethylformamide (DMF), followed by phenol (2.37 g, 25.2 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (5.2 mL, 36.9 mmol), pyridine (0.2 mL), copper(0) (0.1 g), and copper(I) iodide (0.1 g). The reaction was heated to reflux and monitored via TLC. After 2 h, TLC showed that all of the 2-iodo benzoic acid was consumed. The reaction was cooled and diluted with 1 M HCl (500 mL) until no more precipitate had formed. The resulting precipitates were filtered and washed with water (100 mL) and dried under vacuum to give desired product (2.41 g, 93.0%) as brown so-

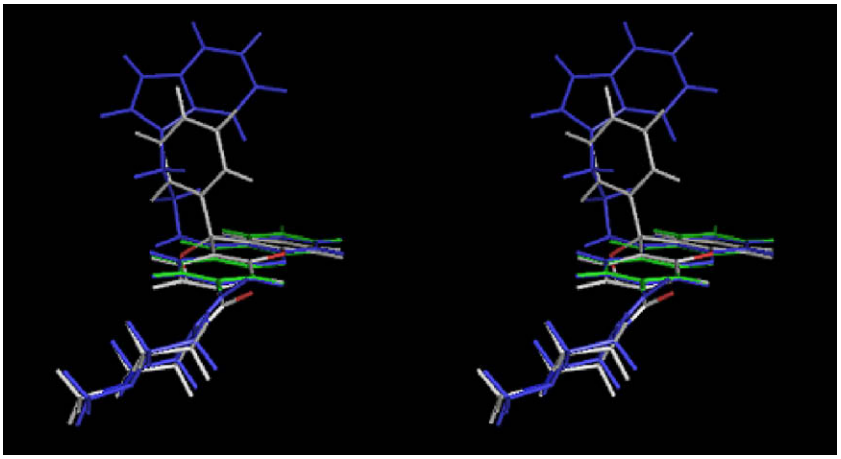


Figure 4. Stereoview of xanthenol **9e**, xanthone (**3**) (green), and a representative acridine structure (blue) taken from Refs. 23.[26] The structures are overlaid based on an RMS fit of the tricyclic ring system.

lid: R_f = 0.5 (90:10 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.87–7.81 (m, 9H), 12.1 (s (br s, 1H); APCI-MS: m/z 213.16 $[M-H]^-$. $C_{13}H_9O_3$ (214.06).

5.1.2. Xanthen-9-one (3)

2-Phenoxy-terephthalic acid (**2**) (3.0 g, 14.0 mmol) was added to 100 mL of H_2SO_4 and heated to 100 °C while being stirred for 3 h. The starting material dissolved upon heating. The reaction was cooled and poured over ice (300 mL), producing a gray solid that was then filtered and washed with water and dried. The crude was purified by column chromatography (SiO_2, 95:5 CH_2Cl_2/MeOH) to give desired product (1.80 g, 67.0%) as white solid: mp 179–180 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.46 (t, 2H, J = 7.2 Hz), 7.64 (d, 2H, J = 9.0 Hz), 7.85 (t, 2H, J = 7.2 Hz), 8.17 (d, 2H, J = 7.8 Hz); APCI-MS: m/z 197.06 $[M+H]^+$. $C_{13}H_8O_2$ (196.05).

5.1.3. 9-Phenyl-xanthen-9-ol (4a)

Xanthen-9-one (**3**) (0.52 g, 2.70 mmol) was taken into dry THF and cooled to −78 °C. Phenyl magnesium bromide (1.10 g, 6.0 mmol) was added into the reaction with continued stirring while maintaining the temperature of reaction at −78 °C in 30 min. The reaction was warmed to rt with continued stirring overnight. The reaction was quenched with the addition of satd NH_4Cl followed by extraction with EtOAc (2 × 50 mL). The organic layers were combined and washed with water (20 mL). The organic layer was then dried over $MgSO_4$ evaporated in vacuo. Crude product was purified by column chromatography (Sio_2, 95:5 CH_2Cl_2/MeOH) to afford the desired product (0.40 g, 56.0%) as white solid: mp 143–145 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.73 (br s, 1H), 7.07 (t, 2H, J = 15 Hz), 7.11 (t, 1H, J = 13.2 Hz), 7.19–7.30 (m, 8H), 7.35 (d, 2H, J = 7.8 Hz); APCI-MS: m/z 275.10 $[M+H]^+$. $C_{19}H_{14}O_2$ (274.09).

5.1.4. 9-(3-Methoxyphenyl)-xanthen-9-ol (4b)

Xanthen-9-one (**3**) (0.52 g, 2.7 mmol) was reacted with 3-methoxyphenyl magnesium bromide (1.10 g, 6.0 mmol) using the same procedure described for **4a** to afford title compound (0.73 g, 89.0%) as white solid: mp 127–128 °C; R_f = 0.64 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.23 (s, 3H), 6.19 (d, 1H, J = 8.4 Hz), 6.25 (dd, 1H, J = 10.2, 2.4 Hz), 6.28 (s, 1H), 6.49 (br s, 1H), 6.62–6.68 (m, 3H), 6.75 (d, 2H, J = 8.4 Hz), 6.85 (dd, 2H, J = 15.6, 7.2 Hz), 6.91 (d, 2H, J = 7.8 Hz); APCI-MS: m/z 303.10 $[M-H]^-$. $C_{20}H_{16}O_3$ (304.11).

5.1.5. 2-Phenoxyterephthalic acid (6a)

Bromotherethphalic acid (**5**) (10.0 g, 41.0 mmol) was reacted with phenol (7.7 g, 82.0 mmol) using the same procedure described for **2** to afford the title compound (9.70 g, 93.0%) as greenish solid: R_f = 0.5 (80:20 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.91 (d, 2H, J = 7.8 Hz), 7.15 (t, 1H, J = 7.2 Hz), 7.42 (dd, 2H, J = 12, 7.3 Hz), 7.71 (d, 1H, J = 7.8 Hz), 7.87 (d, 1H, J = 9.6 Hz), 8.12 (s, 1H), 13.28 (br s, 2H); ESI-MS: m/z 257.06 $[M-H]^-$. $C_{14}H_{10}O_5$ (258.05).

5.1.6. 2-(4-Chlorophenoxy) terephthalic acid (6b)

Bromotherethphalic acid (**5**) (10.0 g, 40.8 mmol) was reacted with 4-chloro phenol (10.5 g, 81.6 mmol) using the same procedure described for **2** to afford the title compound (11.0 g, 91.0%) as greenish solid; R_f = 0.4 (80:20 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.98 (dd, 2H, J = 9.0, 2.4 Hz), 7.40 -7.41 (m, 2H), 7.77–7.78 (m, 1H), 7.89 (d, 1H, J = 7.8 Hz), 8.01 (s, 1H), 13.2 (br s, 2H); APCI-MS: m/z 293.02 $[M+H]^+$. $C_{14}H_9O_5Cl$ (292.04).

5.1.7. 2-(4-Fluorophenoxy) terephthalic acid (6c)

Bromotherethphalic acid (**5**) (10.0 g, 40.8 mmol) was reacted with 4-fluoro phenol (9.20 g, 81.6 mmol) using the same procedure described for **2** to afford the title compound (9.10 g, 81.0%) as greenish solid: R_f = 0.36 (80:20 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.01 (dd, 2H, J = 8.8, 2.6 Hz), 7.35–7.39 (m, 2H), 7.79 (d, 1H, J = 8.2 Hz), 7.92 (d, 1H, J = 7.8 Hz), 8.14 (br s, 1H), 12.8 (br s, 2H); APCI-MS: m/z 275.05 $[M-H]^-$. $C_{14}H_9O_5Cl$ (276.04).

5.1.8. Xanthen-9-one-3-carboxylic acid (7a)

2-Phenoxy-terephthalic acid (**6a**) (2.70 g, 10.6 mmol) was added to H_2SO_4 (100 mL) and heated to 100 °C while being stirred for 3 h on a water bath. After the completion of reaction, the reaction was cooled and poured over ice (300 mL) producing a gray solid that was then filtered, washed with water and dried to give desired product (1.98 g, 68.0%) as black solid: R_f = 0.48 (90:10 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.47 (t, 1H, J = 7.2 Hz), 7.66 (d, 1H, J = 8.4 Hz), 7.86–7.91 (m, 2H), 8.04 (s, 1H), 8.16 (d, 1H, J = 7.8 Hz), 8.23 (d, 1H, J = 13.8 Hz); APCI-MS: m/z 239.04 $[M-H]^-$. $C_{14}H_8O_4$ (240.04).

5.1.9. 2-Chloro-xanthen-9-one-6-carboxylic acid (7b)

2-(4-Chlorophenoxy) terephthalic acid (**6b**) (5.0 g, 17.1 mmol) was treated with H_2SO_4 using the same procedure described for **7a** to afford the title compound (3.67 g, 78.0%) as dark green solid: R_f = 0.43 (90:10 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.73 (d, 1H, J = 9.0 Hz), 7.89–7.93 (m, 2H), 8.01 (s, 1H), 8.05 (s, 1H), 8.07 (d, 1H, J = 2.4 Hz), 8.23 (d, 1H, J = 3.6 Hz); APCI-MS: m/z 273.01 $[M-H]^-$. $C_{14}H_7O_4Cl$ (274.00).

5.1.10. 2-Fluoro-xanthen-9-one-6-carboxylic acid (7c)

2-(4-Fluorophenoxy) terephthalic acid (**6c**) (5.0 g, 18.1 mmol)-was treated with H_2SO_4 using the same procedure described for **7a** to afford the title compound (2.90 g, 62.0%) as dark green solid: R_f = 0.42 (90:10 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.71–7.80 (m, 2H), 7.83 (d, 1H, J = 7.2 Hz), 7.93 (d, 1H, J = 7.8 Hz), 8.01 (s, 1H), 8.05 (s, 1H), 8.23 (d, 1H, J = 8.4 Hz); APCI-MS: m/z 257.01 $[M-H]^-$. $C_{14}H_7O_4F$ (258.03).

5.1.11. 3-(4-Methylpiperazine-1-carbonyl)-xanthen-9-one (8a)

Thionyl chloride (25 mL) was added to a flask containing xanthen-9-one-3-carboxylic acid (**7a**) (0.26 g, 1.10 mmol) and was set to reflux for 1 h. Excess thionyl chloride was removed under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C on an ice bath. Next, *N*-methylpiperazine (0.22 mL, 1.60 mmol) was added dropwise while stirring. The reaction was allowed to reach rt with continued stirring for 1 h. An additional 30.0 mL of CH_2Cl_2 was added, and the reaction was extracted with 1 M HCl. The aqueous layer was made basic with 5% NaOH and extracted into EtOAc, dried with $MgSO_4$, and concentrated in vacuo to afford the desired product (0.25 g, 71.0%) as slightly yellow solid: R_f = 0.7 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.34 (s, 3H), 2.38 (br s, 2H), 2.53 (br s, 2H), 3.45 (br s, 2H), 3.85 (br s, 2H), 7.38–7.43 (m, 2H), 7.51–7.55 (m, 2H), 7.75 (m, 1H), 8.35 (d, 1H, J = 7.2 Hz), 8.37 (d, 1H, J = 8.4 Hz); APCI-MS: m/z 323.21 $[M+H]^+$. $C_{19}H_{18}O_2$ (322.13).

5.1.12. [N,N-Diethyl]-xanthen-9-one-3-carboxamide (8b)

Thionyl chloride (25 mL) was added to a flask containing xanthen-9-one-3-carboxylic acid (**7a**) (0.26 g, 1.10 mmol) and was set to reflux for 1 h. Excess thionyl chloride was removed under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C on an ice bath. Next, Diethylamine (1.7 mL, 1.60 mmol) was added dropwise while stirring. The reaction was allowed to reach rt under continued stirring for about 1 h. An additional 30 mL of CH_2Cl_2 was added, and the reaction was extracted with 1 M HCl. The organic layer was dried with $MgSO_4$, and concentrated in vacuo to afford desired product (0.22 g, 68.0%) as

slightly yellow solid: R_f = 0.64 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.12 (br s, 3H), 1.24 (br s, 3H), 3.17 (br s, 2H), 3.43 (br s, 2H), 7.22–7.45 (m, 6H), 7.71 (d, 1H, J = 7.4 Hz); APCI-MS: m/z 294.12 $[M-H]^-$. $C_{18}H_{17}O_3$ (295.12).

5.1.13. 3-(Morpholine-4-carbonyl)xanthen-9-one (8c)

Xanthen-9-one-3-carboxylic acid (**7a**) (0.26 g, 1.08 mmol) was reacted with $SOCl_2$ (25 mL) followed by the addition of morpholine (1.3 mL, 1.6 mmol) using the same procedure described for **8a** to afford the title compound (0.78 g, 62.0%) as slightly yellow solid: R_f = 0.72 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.47 (br s, 4H), 3.48 (br s, 4H), 3.42 (br s, 2H), 7.18–7.23 (m, 3H), 7.36 (dd, 1H, J = 12.6, 3.0 Hz), 7.68 (d, 1H, J = 7.8 Hz); APCI-MS: m/z 310.08 $[M+H]^+$. $C_{18}H_{17}O_3$ (309.10).

5.1.14. 2-Chloro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (8d)

2-Chloro-xanthen-9-one-6-carboxylic acid (**7b**) (2.0 g, 7.3 mmol) was reacted with $SOCl_2$ (80 mL) followed by *N*-methyl piperazine (1.22 mL, 11.0 mmol) using the same procedure described for **8a** to afford the title compound (1.56 g, 64.0%) as slightly yellow solid: R_f = 0.6 (95:5 (95:5 CH_2Cl_2/MeOH)); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.34 (s, 3H), 2.37 (br s, 2H), 2.51 (br s, 2H), 3.42 (br s, 2H), 3.73 (br s, 2H), 7.38–7.43 (m, 4H), 7.65 (d, 1H, J = 7.6 Hz), 7.81 (d, 1H, J = 7.8 Hz); APCI-MS: m/z 357.19 $[M + H]^+$. $C_{19}H_{17}ClN_2O_3$ (356.09).

5.1.15. 2-Fluoro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (8e)

2-Fluoro-xanthen-9-one-6-carboxylic acid (**7c**) (0.32 g, 1.20 mmol) was reacted with $SOCl_2$ (10 mL) followed by *N*-methyl piperazine (0.18 mL, 1.60 mmol) using the same procedure described for **8a** to afford the title compound (0.28 g, 66.0%) as a slightly yellow solid: R_f = 0.65 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.34 (s, 3H), 2.37 (br s, 2H), 2.51 (br s, 2H), 3.42 (br s, 2H), 3.73 (br s, 2H), 7.18–7.23 (m, 4H), 7.36 (dd, 1H, J = 12.6, 3.0 Hz), 7.68 (d, 1H, J = 7.8 Hz); APCI-MS: m/z 341.17 $[M + H]^+$. $C_{19}H_{17}FN_2O_3$ (340.12).

5.1.16. 3-(4-Methylpiperazine-1-carbonyl)-9hydroxy-9-methyl-9H-xanthene (9a)

3-(4-Methylpiperazine-1-carbonyl)-xanthen-9-one (**8a**) (0.50 g, 1.50 mmol) was reacted with methyl magnesium bromide (0.55 g, 3.30 mmol) using the same procedure described for **4a** to afford title compound (0.35 g, 57.0%) as an off white solid: mp 94–98 °C; R_f = 0.6 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.48 (s, 3H), 2.02 (s, 3H), 2.17 (br s, 4H), 3.24 (br s, 2H), 3.45 (br s, 2H), 5.48 (s, 1H), 6.89 (s, 1H), 6.98 (d, 1H, J = 8.4 Hz), 7.02–7.07 (m, 2H), 7.17–7.18 (m, 1H), 7.64–7.68 (m, 2H); ^{13}C NMR (acetone-d_6, δ ppm): 168.589, 149.601, 149.490, 137.079, 130.848, 129.482, 128.679, 127.221, 126.986, 123.791, 122.142, 115.989, 114.762, 65.547, 56.518, 56.507, 54.997, 45.540, 34.919, 34.420; APCI-MS: m/z 339.17 $[M+H]^+$. $C_{20}H_{22}N_2O_3$ (338.16).

5.1.17. 3-(4-Methylpiperazine-1-carbonyl)-9-hydroxy-9-vinyl-9H-xanthene (9b)

3-(4-Methylpiperazine-1-carbonyl)-xanthen-9-one (**8a**) (0.50 g, 1.5 mmol) was reacted with vinyl magnesium bromide (0.44 g, 3.3 mmol) using the same procedure described for **4a** to afford title compound (0.20 g, 37.0%) as an off white solid: mp 145–147 °C; R_f = 0.52 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.11 (s, 3H), 2.27 (br s, 2H), 2.37 (d, 2H, J = 16.8 Hz), 2.48 (br s, 4H), 4.01 (br s, 1H), 4.90 (d, 1H, J = 16.8 Hz), 5.1 (d, 1H, J = 10.4 Hz), 5.99–6.05 (m, 1H), 7.19 (q, 2H, J = 8.0 Hz), 7.35 (t, 1H, J = 7.2 Hz), 7.56 (d, 1H, J = 7.2 Hz), 7.67 (d, 1H, J = 7.8 Hz), 7.71 (s, 1H), 7.75 (d, 1H, J = 7.8 Hz); APCI-MS: m/z 351.17 $[M + H]^+$. $C_{21}H_{22}N_2O_3$ (350.16).

5.1.18. 3-(4-Methylpiperazine-1-carbonyl)-9-ethyl-9-hydroxy-9H-xanthene (9c)

3-(4-Methylpiperazine-1-carbonyl)-xanthen-9-one (**8a)** (0.30 g, 0.90 mmol) was reacted with ethyl magnesium bromide (0.27 g, 2.0 mmol) using the same procedure described for **4a** to afford title compound (0.28 g, 46.0%) as an off white solid: mp 148–152 °C; R_f = 0.52 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.36 (t, 3H, J = 7.2 Hz), 1.83 (q, 2H, J = 7.6 Hz), 2.03 (s, 3H), 2.13 (br s, 4H), 2.24 (br s, 4H), 4.89 (s, 1H), 6.98–7.21 (m, 5H), 7.60 (dd, 1H, J = 9.0, 1.8 Hz), 7.64 (d, 1H, 7.8 Hz); ESI-MS: m/z 353.20 $[M+H]^+$. $C_{21}H_{24}N_2O_3$ (352.18).

5.1.19. 3-(4-Methylpiperazine-1-carbonyl)-9-hydroxy-9-phenyl-9H-xanthene (9d)

3-(4-Methylpiperazine-1-carbonyl)-xanthen-9-one (**8a**) (0.50 g, 1.50 mmol) was reacted with phenyl magnesium bromide (0.60 g, 3.3 mmol) using the same procedure described for **4a** to afford title compound (0.30 g, 57.0%) as an off white solid: mp 187–189 °C; R_f = 0.71 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.17 (s, 3H), 2.25 (br s, 2H), 2.32 (br s, 2H), 3.30 (br s, 2H), 3.59 (br s, 2H), 6.81 (s, 1H), 7.07–7.14 (m, 3H), 7.15–7.26 (m, 5H), 7.30–7.34 (m, 2H), 7.39 (d, 1H, 8.4 Hz); ^{13}C NMR (DMSO-d_6, δ ppm): 168.581, 149.995, 149.713, 149.504, 137.019, 130.275, 129.847, 129.557, 129.423, 128.946, 128.690, 128.578, 127.205, 127.150, 126.316, 124.326, 122.525, 116.569, 115.107, 69.308, 55.290, 54.888, 47.719, 46.272, 42.147; APCI-MS: m/z 401.20 $[M+H]^+$. $C_{25}H_{24}N_2O_3$ (400.17).

5.1.20. 3-(4-Methylpiperazine-1-carbonyl)-9-hydroxy-9-(3-methoxyphenyl)-9H-xanthene (9e)

3-(4-Methylpiperazine-1-carbonyl)-xanthen-9-one (**8a**) (0.50 g, 1.5 mmol) was reacted with 3-methoxy phenyl magnesium bromide (0.52 g, 2.5 mmol) using the same procedure described for **4a** to afford title compound (0.54 g, 81.0%) as off an white solid: mp 165–167 °C; R_f = 0.56 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.05 (s, 3H), 2.21 (s, 3H), 2.34 (br s, 4H), 2.85 (br s, 1H), 3.41 (br s, 2H), 3.63 (br s, 2H), 7.11–7.15 (m, 3H), 7.19–7.21 (m, 2H), 7.26–7.32 (m, 3H), 7.38 (d, 2H, J = 7.8 Hz), 7.46 (d, 1H, J = 8.4 Hz); APCI-MS: m/z 431.18 $[M + H]^+$. $C_{26}H_{26}N_2O_4$ (430.18).

5.1.21. [N,N-Diethyl]-9-hydroxy-phenyl-9H-xanthene-carboxamide (9f)

[*N*,*N*-Diethyl]-xanthen-9-one-3-carboxamide (**8b**) (0.41 g, 1.4 mmol) was reacted with phenyl magnesium bromide (0.50 g, 2.80 mmol) using the same procedure described for **4a** to afford title compound (0.30 g, 58.0%) as an off white solid: mp 139–142 °C; R_f = 0.76 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.12 (br s, 3H), 1.24 (br s, 3H), 2.91 (s, 1H), 3.27 (br s, 2H), 3.53 (br s, 2H), 7.01 (d, 1H, J = 7.8 Hz), 7.08 (t, 1H, J = 7.2 Hz), 7.16 (s, 1H), 7.18–7.20 (m, 2H), 7.26–7.32 (m, 4H), 7.36–7.39 (m, 3H); APCI-MS: m/z 374.19 $[M+H]^+$. $C_{24}H_{24}NO_3$ (373.44).

5.1.22. [N,N-Diethyl]-9-hydroxy-(3-methoxyphenyl)-9H-xanthene-carboxamide (9g)

[*N*,*N*-Diethyl]-xanthen-9-one-3-carboxamide (**8b**) (0.41 g, 1.4 mmol) was reacted with 3-methoxy phenyl magnesium bromide (0.59 g, 2.8 mmol) using the same procedure described for **4a** to afford title compound (0.36 g, 65.0%) as an off white solid: mp >250 °C; R_f = 0.64 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.12 (br s, 3H), 1.23 (br s, 3H), 2.96 (s, 1H), 3.26 (br s, 2H), 3.53 (br s, 2H), 3.76 (s, 3H), 6.73 (dd, 1H, J = 9.6, 1.8 Hz), 6.86 (d, 1H, J = 7.8 Hz), 7.01 (d, 1H, J = 7.8 Hz), 7.06–7.09 (m, 1H),

7.14–7.20 (m, 2H), 7.26 (s, 1H), 7.29–7.40 (m, 4H); APCI-MS: *m*/*z* 404.19 $[M+H]^+$. $C_{25}H_{25}NO_4$ (403.18).

5.1.23. 3-(Morpholino-4-carbonyl)-9-hydroxy-9-phenyl-9H-xanthene (9h)

3-(Morpholine-4-carbonyl)xanthen-9-one (**8c**) (0.44 g, 1.40 mmol) was reacted with phenyl magnesium bromide (0.57 g, 3.12 mmol) using the same procedure described for **4a** to afford title compound (0.38 g, 68.0%) as a white solid: mp >250 °C; R_f = 0.56 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 3.65 (br s, 8H), 7.10–7.23 (m, 6H), 7.26–7.33 (m, 4H), 7.39–7.41 (m, 3H), 7.45–7.48 (m, 1H); APCI-MS: *m*/*z* 388.15 $[M+H]^+$. $C_{24}H_{21}NO_4$ (387.15).

5.1.24. 3-(Morpholino-4-carbonyl)-9-hydroxy-(3-methoxyphenyl)-9H-xanthene (9i)

3-(Morpholine-4-carbonyl)xanthen-9-one (**8c**) (0.25 g, 0.81 mmol) was reacted with 3-methoxy phenyl magnesium bromide (0.38 g, 1.6 mmol) using the same procedure described for **4a** to afford title compound (0.20 g, 57.0%) as a white solid: mp 111–114 °C; R_f = 0.62 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.10 (br s, 4H), 2.81 (br s, 5H), 3.75 (s, 3H), 6.06 (s, 1H), 6.80 (d, 1H, *J* = 7.8 Hz), 6.86 (d, 1H, *J* = 8.4 Hz), 7.10 (s, 1H), 7.22 (d, 2H, *J* = 7.2 Hz), 7.31 (s, 1H), 7.47 (d, 1H, *J* = 8.4 Hz), 7.54 (d, 1H, *J* = 7.2 Hz), 7.73 (d, 1H, *J* = 7.2 Hz), 7.82 (s, 1H); ESI-MS: *m*/*z* 418.13 $[M+H]^+$. $C_{25}H_{23}NO_5$ (417.16).

5.1.25. 2-Chloro-6-(4-methylpiperazine-1-carbonyl)-9-hydroxy-9-methyl-9H-xanthene (9j)

2-Chloro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (**8d**) (0.17 g, 0.5 mmol) was reacted with methyl magnesium bromide using (0.17 g, 1.10 mmol) the same procedure described for **4a** to afford title compound (0.10 g, 57.0%) as an off white solid: R_f = 0.65 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ: 1.64 (s, 3H), 2.36 (s, 3H), 2.24 (br s, 4H), 2.82 (s, 1H), 3.60 (br s, 4H), 7.10–7.22 (m, 4H), 7.48 (dd, 1H, *J* = 12.6, 4.8 Hz), 7.82 (d, 1H, *J* = 9.0 Hz); APCI-MS: *m*/*z* 373.14 $[M+H]^+$. $C_{20}H_{21}N_2O_3Cl$ (372.12).

5.1.26. 2-Chloro-6-(4-methylpiperazine-1-carbonyl)-9-hydroxy-9-vinyl-9H-xanthene (9k)

2-Chloro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (**8d**) (0.50 g, 1.40 mmol) was reacted with vinyl magnesium bromide (0.41 g, 3.30 mmol) using the same procedure described for **4a** to afford title compound (0.31 g, 57.0%) as an off white solid: mp 143–145 °C; R_f = 0.44 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 2.23 (s, 3H), 2.35 (br s, 4H), 2.82 (s, 1H), 3.43 (br s, 2H), 3.65 (br s, 2H), 5.15 (d, 2H, *J* = 16.8 Hz), 6.08–6.13 (m, 1H), 7.14–7.17 (m, 4H), 7.34 (dd, 1H, *J* = 12.6, 3.6 Hz), 7.68 (d, 1H, *J* = 8.4 Hz); APCI-MS: *m*/*z* 385.15 $[M + H]^+$. $C_{21}H_{21}N_2O_3Cl$ (384.12).

5.1.27. 2-Chloro-6-(4-methylpiperazine-1-carbonyl)-9-ethyl-9-hydroxy-xanthene (9l)

2-Chloro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (**8d**) (0.40 g, 1.1 mmol) was reacted with ethyl magnesium bromide (0.33 g, 2.5 mmol) using the same procedure described for **4a** to afford title compound (0.26 g, 61.0%) as an off white solid: mp 169–172 °C; R_f = 0.46 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 0.51 (t, 3H, *J* = 7.8 Hz), 1.96 (q, 2H, *J* = 7.5 Hz), 2.05 (br s, 1H), 2.25 (s, 3H), 2.40 (br s, 4H), 2.83 (br s, 4H), 7.13 (s, 1H), 7.17 (d, 1H, *J* = 9.0 Hz), 7.24 (d, 1H, *J* = 7.8 Hz), 7.36 (dd, 1H, *J* = 11, 2.1 Hz), 7.70 (d, 1H, *J* = 2.4 Hz), 7.77 (d, 1H, *J* = 7.8); APCI-MS: *m*/*z* 387.16 $[M+H]^+$. $C_{21}H_{23}N_2O_3Cl$ (386.14).

5.1.28. 2-Chloro-6-(4-methylpiperazine-1-carbonyl)-9-hydropxy-9-phenyl-9H-xanthene (9m)

2-Chloro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (**8d**) (0.16 g, 0.50 mmol) was reacted with phenyl magnesium bromide (0.16 g, 0.9 mmol) using the same procedure described for **4a** to afford title compound (0.15 g, 77.0%) as an off white solid: mp 124 °C; R_f = 0.52 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 2.21 (s, 3H), 2.34 (br s, 4H), 3.40 (br s, 2H), 3.63 (br s, 2H), 5.94 (s, 1H), 7.10–7.15 (m, 3H), 7.19–7.21 (m, 2H), 7.26–7.32 (m, 3H), 7.40 (d, 2H, *J* = 7.8 Hz), 7.48 (d, 1H, *J* = 7.8 Hz); APCI-MS: *m*/*z* 435.14 $[M+H]^+$. $C_{25}H_{23}N_2O_3Cl$ (434.14).

5.1.29. 2-Chloro-6-(4-methylpiperazine-1-carbonyl)-9-hydroxy-(3-methoxyphenyl)-9H-xanthene (9n)

2-Chloro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (**8d**) (0.30 g, 0.84 mmol) was reacted with 3-methoxy phenyl magnesium bromide (0.40 g, 1.85 mmol) using the same procedure described for **4a** to afford title compound (0.20 g, 67.0%) as an off white solid: mp 174–177 °C; R_f = 0.50 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 2.24 (s, 3H), 2.36 (br s, 4H), 3.44 (br s, 2H), 3.63 (br s, 2H), 3.76 (s, 3H), 5.83 (s, 1H), 6.77 (dd, 1H, *J* = 10.2, 2.4 Hz), 6.82 (d, 1H, *J* = 7.8 Hz), 7.10–7.14 (m, 4H), 7.18–7.20 (m, 2H), 7.25–7.28 (m, 1H), 7.48 (d, 1H, *J* = 7.8 Hz); APCI-MS: *m*/*z* 465.17 $[M+H]^+$. $C_{26}H_{25}N_2O_4Cl$ (464.15).

5.1.30. 2-Fluoro-6-(4-methylpiperazine-1-carbonyl)-9-hydroxy-methyl-9H-xanthene (9o)

2-Fluoro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (**8e**) (0.30 g, 0.88 mmol) was reacted with methyl magnesium bromide (0.33 g, 1.94 mmol) using the same procedure described for **4a** to afford title compound (0.20 g, 64.0%) as an off white solid: mp 143–144 °C; R_f = 0.58 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 1.51 (s, 3H), 2.11 (s, 3H), 2.23 (br s, 4H), 3.32 (br s, 2H), 3.51 (br s, 2H), 5.11 (br s, 1H), 6.96–7.10 (m, 4H), 7.35 (dd, 1H, *J* = 12.6, 3.0 Hz), 7.68 (d, 1H, *J* = 7.8 Hz); APCI-MS: *m*/*z* 357.15 $[M+H]^+$. $C_{20}H_{21}FN_2O_3$ (356.15).

5.1.31. 2-Fluoro-6-(4-methylpiperazine-1-carbonyl)-9-hydroxy-vinyl-9H-xanthene (9p)

2-Fluoro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (**8e**) (0.50 g, 1.5 mmol) was reacted with vinyl magnesium bromide (0.42 g, 3.23 mmol) using the same procedure described for **4a** to afford title compound (0.35 g, 65.0%) as an off white solid: mp 147–149 °C; R_f = 0.48 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 2.23 (s, 3H), 2.35 (br s, 4H), 3.43 (br s, 2H), 3.65 (br s, 2H), 5.13–5.16 (m, 2H), 5.51 (s, 1H), 6.11 (q, 1H, *J* = 11.0 Hz), 7.14–7.22 (m, 4H), 7.34 (dd, 1H, *J* = 12.6, 3.0 Hz), 7.67 (d, 1H, *J* = 8.4 Hz); ESI-MS: *m*/*z* 369.13 $[M+H]^+$. $C_{21}H_{21}FN_2O_3$ (358.15).

5.1.32. 2-Fluoro-6-(4-methylpiperazine-1-carbonyl)-9-ethyl-9-hydroxy-9H-xanthene (9q)

2-Fluoro-6-(4-methylpiperazine-1-carbonyl)-9*H*-xanthen-9-one (**8e**) (0.18 g, 0.53 mmol) was reacted with ethyl magnesium bromide (0.16 g, 1.2 mmol) using the same procedure described for **4a** to afford title compound (0.12 g, 63.0%) as an off white solid: mp 139–42 °C; R_f = 0.51 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 0.51 (t, 3H, *J* = 7.8 Hz), 1.96 (q, 2H, *J* = 7.4 Hz), 2.25 (s, 3H), 2.38 (br s, 4H), 2.83 (br s, 4H), 5.51 (s, 1H), 7.13 (s, 1H), 7.17 (d, 1H, *J* = 9.0 Hz), 7.24 (d, 1H, *J* = 7.8 Hz), 7.36 (dd, 1H, *J* = 11.4, 2.4 Hz), 7.70 (d, 1H, *J* = 1.8 Hz), 7.77 (d, 1H, *J* = 7.8 Hz); ^{13}C NMR ($CDCl_3$, δ ppm): 170.172, 159.324, 149.581, 149.436, 149.287, 137.922, 129.325, 129.209, 129.061, 128.897, 127.997, 126.951, 123.826, 121.393, 119.128, 116.442, 114.529, 112.149, 111.996, 70.181, 55.230, 43.281, 39.304, 14.285, 12.857; ESI-MS: *m*/*z* 371.17 $[M+H]^+$. $C_{21}H_{23}N_2O_3F$ (371.17).

5.1.33. 2-Fluoro-6-(4-methylpiperazine-1-carbonyl)-9-hydroxy-phenyl-9H-xanthene (9r)

2-Fluoro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (**8e**) (0.28 g, 0.82 mmol) was reacted with phenyl magnesium bromide (0.33 g, 1.81 mmol) using the same procedure described for **4a** to afford title compound (0.36 g, 55.0%) as an off white solid: mp 175–178 °C; R_f = 0.63 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 2.21 (s, 3H), 2.32 (br s, 4H), 3.41 (br s, 2H), 3.63 (br s, 2H), 5.94 (s, 1H), 7.10–7.15 (m, 3H), 7.19–7.21 (m, 2H), 7.26–7.32 (m, 3H), 7.40 (d, 2H, J = 7.8 Hz), 7.47(d, 1H, J = 7.8 Hz); APCI-MS: m/z 419.11 $[M+H]^+$. $C_{25}H_{23}N_2O_3F$ (418.17).

5.1.34. 2-Fluoro-6-(4-methylpiperazine-1-carbonyl)-9-hydroxy-(3-methoxyphenyl)-9H-xanthene (9s)

2-Fluoro-6-(4-methylpiperazine-1-carbonyl)xanthen-9-one (**8e**) (0.3 g, 0.88 mmol) was reacted with 3-methoxy phenyl magnesium bromide (0.41 g, 1.94 mmol) using the same procedure described for **4a** to afford title compound (0.21 g, 53.0%) as an off white solid: mp 108–111 °C; R_f = 0.52 (95:5 CH_2Cl_2/MeOH), ^{1}H NMR (600 MHz, acetone-d_6) δ 2.24 (s, 3H), 2.36 (br s, 4H), 3.44 (br s, 2H), 3.64 (br s, 2H), 3.76 (s, 3H), 5.83 (s, 1H), 6.77 (dd, 1H, J = 10.2, 2.4 Hz), 6.82 (d, 1H, J = 7.8 Hz), 7.10–7.14 (m, 4H), 7.18–7.20 (m, 2H), 7.25–7.28 (m, 1H), 7.49 (d, 1H, J = 7.8 Hz); APCI-MS: m/z 450.10 $[M+H]^+$. $C_{26}H_{25}N_2O_4F$ (448.49).

5.2. Biology

The in vitro cytotoxicity of compounds was assayed by determining their ability to inhibit the growth of the tumor cell lines. In brief, 10^4 cells/well were plated in a 96-well plate. Twenty-four hours after plating, a series of compounds in varied concentrations with 1% (v/v) DMSO in the final cell medium were used for the cell treatment (cells treated with medium containing 1% DMSO served as a control). After a 48 h treatment, the relative viable cell mass in each well was determined by using CellTiter-Blue Cell Viability Assay kit (Promega, Madison, WI), which is based on the ability of viable cells to reduce resazurin to the fluorescent end product resorufin. The IC_{50} of each candidate was determined by fitting the relative fluorescence of the cells to the drug concentration by using a dose–response model in Prism program from GraphPad Software, Inc. (San Diego, CA).

5.3. Topoisomerase activity

Standard relaxation reaction mixtures (20 μl) containing 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 200 mM potassium glutamate, 10 mM dithiothreitol, 50 μg/mL bovine serum albumin, 1 mM ATP, 0.3 μg of pBR322 plasmid DNA, two units of human topoisomerase II (Topogen), and the indicated concentrations of drug were incubated at 37 °C for 30 min. Reactions were terminated by adding EDTA to 25 mM and the DNA products were analyzed by electrophoresis through vertical 1.2% agarose gels at 2 V/cm for 15 h in TAE buffer. Gels were stained with ethidium bromide and photographed using an Eagle Eye II system (Stratagene). The DNA intercalation assay was performed as described previously.[27]

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.018.

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