Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 1109–1116 **1109**
DOI: 10.1021/jm901384s

Structure−Activity Studies of Urea, Carbamate, and Sulfonamide Derivatives of Acylfulvene

Trevor C. McMorris,[†] Ramesh Chimmani,[†] Kashinatham Alisala,[†] Michael D. Staake,[†] Gangadasu Banda,[†] and Michael J. Kelner*,[‡]

[†]*Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093-0358 and*
[‡]*Department of Pathology, UCSD Medical Center, 200 West Arbor Drive, Mail Code 8320, San Diego, California 92103-8320*

Received September 17, 2009

Illudin S and M (**1**, **2**) are highly toxic sesquiterpenes found in the basidiomycete *Omphalotus illudens*. Illudins have a low therapeutic index, but acylfulvene derivatives display potent in vivo antitumor activity against a variety of multidrug resistant tumors. The lead acylfulvene (**4**), irofulven (**5**), in a randomized phase IIB clinical trial significantly increased overall survival in patients with metastatic hormone-refractory prostate cancer who failed prior treatment with two different standard chemotherapeutic regimens. Irofulven is unique, as the primary allylic hydroxyl group can undergo displacement with a variety of nucleophiles to produce analogues that retain key functional groups required for biological activity including the reactive cyclopropylmethyl carbinol and α,β-unsaturated ketone. As described here, we synthesized a variety of urea, carbamate, and sulfonamide derivatives that retain key functional groups and display potent biological activity toward target solid tumor cells in vitro but are relatively nontoxic toward a nontarget B-cell derived cell line.

Introduction

Illudins S and M (**1**, **2**)[1,2] are highly toxic sesquiterpenes found in the basidiomycete *Omphalotus illudens*. Illudins and their derivatives were evaluated for antitumor activity in the National Cancer Institute Developmental Therapeutics Program. Illudin M significantly increased the life span of rats with Dunning leukemia but had a low therapeutic index in solid tumor systems.[3,4] The acylfulvene derivatives of illudins, however, display potent in vivo antitumor activity against a variety of multidrug resistant solid tumors. The lead acylfulvene, clinically known as irofulven, in a randomized phase IIB clinical trial significantly increased overall survival in patients with metastatic hormone-refractory prostate cancer who previously failed salvage treatment with docetaxotere.[5]

The compounds behave as alkylating agents and at room temperature react spontaneously with thiol nucleophiles, such as cysteine or glutathione (GSH[a]).[6] The optimum pH for this reaction is about 6. Toxicity to myeloid leukemia cells (HL 60) can be modulated by altering glutathione levels in the cells. The reaction of illudin S with GSH is illustrated in (Figure 1).[7] Michael type addition to the α,β-unsaturated ketone gives a cyclohexadiene intermediate that is rapidly converted to a more stable aromatic product. This necessitates opening of the cyclopropane which results in reaction with nucleophiles such as water, DNA, and protein.[7] Enzymatic reduction of illudin S leads to a similar reactive intermediate and to a stable aromatic product. Nicotinamide adenosine dinucleotide phosphate (NADPH) is the coenzyme involved in this bioreductive alkylation (Figure 1).[8]

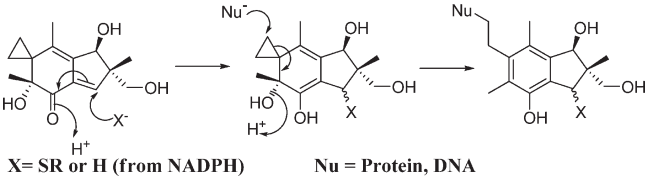


Figure 1

The ability of acylfulvenes to undergo bioreductive activation to a reactive intermediate that is susceptible to nucleophilic attack by DNA appears to be clinically relevant. Recent studies indicate that acylfulvenes selectively alkylate DNA to produce N^3-deoxyadenosine and N^7-deoxyguanosine adducts, and there is direct correlation between the production of these adducts and drug cytotoxicity.[9] Recently it was demonstrated that acylfulvene derivatives can function as both reversible and irreversible inhibitors of cellular glutathione.[10]

Three derivatives of illudins have been studied extensively. Dehydroilludin M (**3**, Chart 1) is obtained readily by oxidation of illudin M (**2**),[11] whereas acylfulvene (**4**) is obtained simply by adding dilute sulfuric acid to an aqueous solution of illudin S.[12] On further treatment of illudin S with formaldehyde solution, a hydroxymethyl acylfulvene (**5**), now renamed irofulven, is obtained.[13] The latter, irofulven, has demonstrated significant antitumor activity in several phase II clinical trials.

Dehydroilludin M reacts more slowly with GSH than illudin M and is 100 times less toxic to HL 60 cells. Likewise, acylfulvene reacted more slowly than illudin S and was much less toxic to HL 60 cells. As anticipated, enzymatic reduction of acylfulvene (NADPH, rat liver cytosol) occurred much

*To whom correspondence should be addressed. Phone: (619) 543-5976. Fax: (619) 543-3730. E-mail: mkelner@ucsd.edu.

[a]Abbreviations: Ac_2O, acetic anhydride; DABCO, 1,4-diazabicyclo-(2,2,2,)octane; DMSO, dimethyl sulfoxide; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; EtOAc, ethyl acetate; GSH, reduced glutathione; MeOH, methanol; NADPH, nicotinamide adenosine dinucleotide phosphate; PPh_3, triphenylphosphine; *t*-BuOH, tertiary butanol; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

Published on Web 01/12/2010

Chart 1

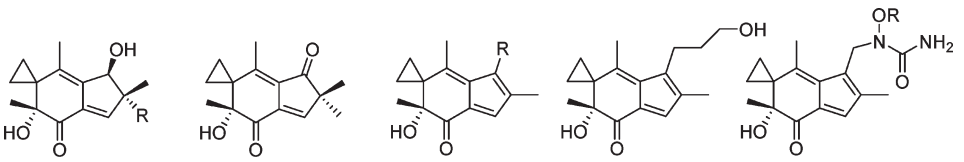
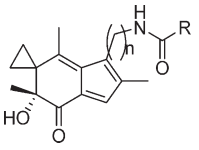


Table 1. Cytotoxicity IC_{50} Values (nM) for Urea Derivatives ($X \pm SD$, $N = 3$)



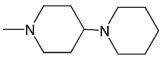
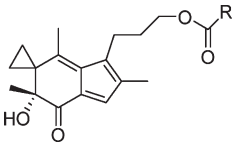
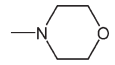
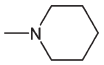
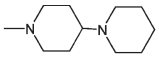
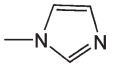
Compound	R	n	MV522 (48h) target	8392 (48h) nontarget	Nontarget to Target cell ratio
13a	-NH–CH_2-CH_2-Cl	1	110±10	>8,500	> 77
13b	-NH–CH_2-CH_2-OH	1	190±20	>9,000	> 47
13c	-NH–OH	1	190±20	9,800±1000	> 51
13d	-NH–OMe	1	270±10	>9,400	> 34
14a	-NH –CH_2-CH_2-Cl	3	170±10	5,000±700	> 29
14b	-NH–CH_2-CH_2-OH	3	<80	>2,800	> 35
14c	-NH–OH	3	150±20	>9,000	> 60
14d		3	150±20	9,000±4,500	60
Irofulven (5)	Not applicable		170±20	76±4	2.2

Table 2. Cytotoxicity IC_{50} Values (nM) for Carbamate Derivatives ($X \pm SD$, $N = 3$)



Compound	R	MV522 (48h) target	8392 (48h) nontarget	Nontarget to Target cell ratio
16a	-NH–OH	460±60	3,300±600	7.2
16b	-NH–CH_2-CH_2-F	670±240	26,000±6,000	38
16c	-NH–CH_2-CH_2-Cl	57±8	4,700±1,2000	82
16d	-NH–CH_2-CH_2-Br	730±140	6,600±900	9
16e	-NH–CH_2-CH_2-OH	70±20	28,000±4,000	400
16f		1,900±450	62,200±1,300	33
16g		1,200±250	7,100±1,700	6
16h		200±30	610+120	3
16i		1,000±150	38,000±2,100	38
Irofulven (5)		170±20	76±4	2.2

more slowly than with illudin S, yielding the corresponding aromatic product.[8]

Irofulven, while similar in properties to acylfulvene, is unique because of the reactivity of the primary allylic hydroxyl group. Displacement of the hydroxyl takes place with a variety of nucleophiles giving products retaining the reactive cyclopropylmethyl carbinol and α,β-unsaturated ketone. Well over 100 such derivatives have been prepared and tested for biological activity.[14,15] Thus, we have been able to make structure−activity correlations of these compounds. We have also included in our study compounds derived from the reaction of acylfulvene and acrolein which afforded a three carbon functionalized side chain as in **6**. The primary hydroxyl in **6**, while not as reactive as the corresponding group in irofulven, nevertheless leads to compounds with potent antitumor activity, both in vitro in xenograft models and in phase II clinical trials.

Efficacy of various compounds was assessed from the differential of toxicity in lung carcinoma MV522 cells and in non target B 8392 human cells. The compounds are listed in Tables 1−3.

In earlier papers we had explored the high susceptibility of the allylic hydroxyl to S_N1 displacement by a variety of nucleophiles under acidic conditions. There were indications that biological activity of derivatives could be modified by altering the polarity of the substituent group; more polar groups appeared to enhance activity.

It occurred to us that hydroxyurea would be a good candidate nucleophile. In the event, the hydroxyl group of irofulven was rapidly replaced by hydroxyurea when the compounds were dissolved in a 1:1 mixture of 2 M H_2SO_4 and acetone at room temperature in 1 h. The yield of alkylated product **7** was 83%.

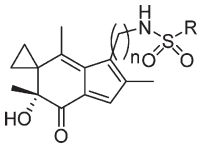
The efficacy of **7** was similar to that of irofulven, and it was somewhat less toxic. The acetate **8**, prepared from **7** with acetic anhydride (Ac_2O), 4-(dimethylamino)pyridine (DMAP), was more toxic than that of irofulven. These results were recently reported.[16]

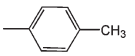
We were thus prompted to examine further urea derivatives listed in Table 1. The study has been extended to carbamate derivatives (Table 2) and also sulfonamide derivatives (Table 3).

Chemistry

The syntheses of urea, carbamate, and sulfonamide derivatives of acylfulvene were readily accomplished using known procedures. Treatment of the amines **9**, **10**[17] with 4-nitrophenylchloroformate in CH_2Cl_2 in the presence of 1,4-diazabicyclo(2,2,2,)octane (DABCO) gave the activated

Table 3. Cytotoxicity IC_{50} Values (nM) for Sulfonamide Derivatives ($X \pm SD$, $N = 3$)



Compound	R	n	MV522 (48h) target	8392 (48h) nontarget	Nontarget to Target cell ratio
17a	$-CH_3$	1	200±90	>9,300	> 46
17b	$-NH_2$	1	300±20	>9,300	> 31
18a	$-CH_3$	3	280±50	42,000±1,3000	> 150
18b		3	580±150	2,900±600	5
18c	$-NH_2$	3	76±17	54,000±20,000	710
18d	-NH–OH	3	8±1	250±80	31
Irofulven (5)	**Not applicable**		170±20	76±4	2.2

carbamates **11**, **12** which on subsequent treatment with appropriate amine yielded the urea derivatives **13a**−**d**, **14a**−**d** (Scheme 1).[18]

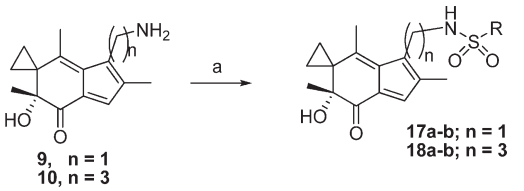
The preparation of carbonate derivatives was achieved in two steps. Acylfulvene (**6**) was reacted with 4-nitrophenylchloroformate in the presence of DABCO in CH_2Cl_2 at 0 °C, giving the activated carbonate **15** which on treatment with an appropriate suitable amine afforded the corresponding carbamate derivatives **16a**−**h** (Scheme 2).[19]

Sulfonamide derivatives **17a**,**b** and **18a**,**b** were obtained by treating amines **9**, **10** with the appropriate sulfonyl chloride in the presence of DABCO in CH_2Cl_2 at 0 °C (Scheme 3).[20]

Sulfamide derivatives were prepared by reacting chlorosulfonyl isocyanate with *tert*-butanol (*t*-BuOH) in presence of diisopropyl ethylamine in CH_2Cl_2 at 0 °C (Scheme 4).[21]

A hydroxysulfamide derivative was prepared by reacting the key intermediate **21** with **6** under Mitsunobu conditions to afford compound **22** which was then fully deprotected using trifluoroacetic acid (TFA) with 5% of water to yield *N*-hydroxysulfamide derivative **18d**.[21] The key intermediate was prepared in single steps outlined in Scheme 5.

Scheme 3[a]



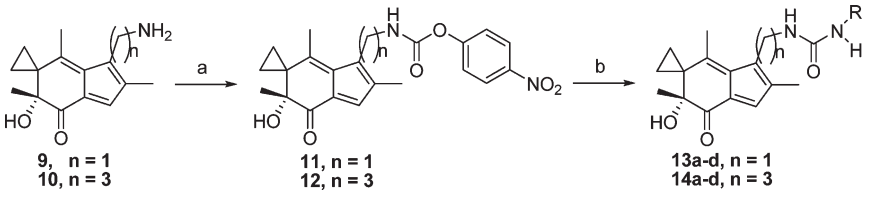
[a] Reagents and conditions: (a) R-SO_2Cl, DABCO, CH_2Cl_2, 0 °C.

Antitumor Activity

For cytotoxicity studies, the compounds were dissolved in cell culture grade dimethyl sulfoxide (DMSO) (1 mg/mL stock solution), and the solutions were diluted in sterile 10% DMSO/phosphate-buffered saline just prior to addition to cultures of the MV522 lung adenocarcinoma or 8392 B-cells. Control cells received equal amounts of the DMSO/phosphate-buffered saline. Analogues of irofulven selectively target and kill tumor cells by apoptosis at 24−48 h after drug exposure.[22] Therefore, cell survival was determined at 48 h by use of trypan blue exclusion studies as previously described in detail.[3,23]

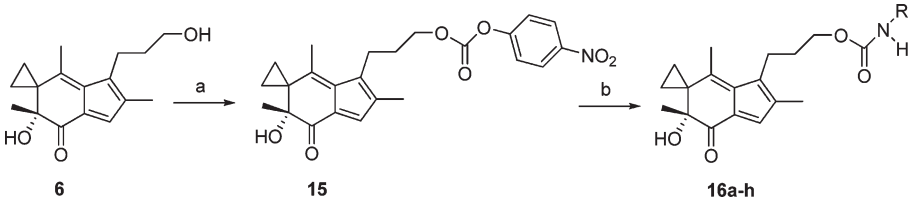
A total of 23 novel compounds were assayed for overall toxicity and selective antitumor activity using conventional cell culture techniques. Overall cytotoxicity was studied by continuous 48 h exposure to the target human MV522 lung adenocarcinoma and the nontarget 8392 B-cell lymphoma/leukemia lines. Cell survival was assessed by trypan blue exclusion technique. For comparison, the cytotoxicity data for irofulven are provided from previous studies.[14] All compounds showed considerable greater toxicity in MV522 cells compared to B 8392 cells in the 48 h cytotoxicity assay. We had previously noted that one of the parameters predicting in vivo activity in xenograft models was the ratio of nontarget B 8392 cytotoxicity (IC_{50}) to the target MV522 cytotoxicity (IC_{50}). All of the analogues displayed favorable cytotoxicity ratios compared to the parent irofulven (**5**) compound

Scheme 1[a]



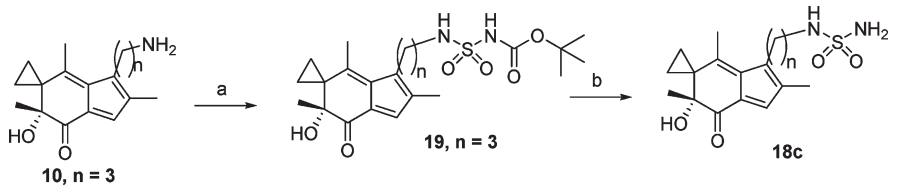
[a] Reagents and conditions: (a) 4-nitrophenyl chloroformate, DABCO, CH_2Cl_2, 0 °C; (b) R-NH_2, DIPEA, CH_2Cl_2, 0 °C.

Scheme 2[a]



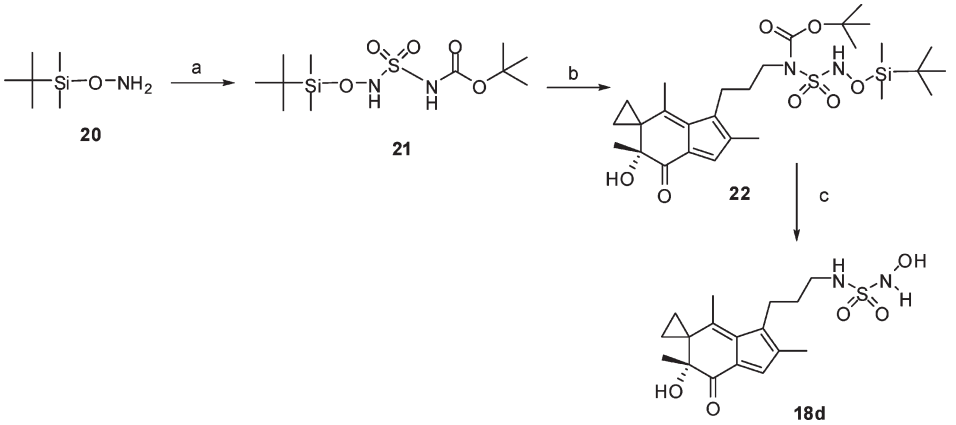
[a] Reagents and conditions: (a) 4-nitrophenyl chloroformate, Py, DMAP, CH_2Cl_2, 0 °C; (b) R-NH_2, DIPEA, CH_2Cl_2, 0 °C.

Scheme 4[a]



[a] Reagents and conditions: (a) chlorosulfonyl isocyanate, *t*-BuOH, CH_2Cl_2, 0 °C; (b) TFA, anisole, CH_2Cl_2, room temp.

Scheme 5[a]



[a] Reagents and conditions: (a) chlorosulfonyl isocyanate, *t*-BuOH, CH_2Cl_2, 0 °C; (b) **6**, PPh_3, DIAD, THF, 0 °C; (c) TFA−H_2O, 5%.

There does not appear to be a correlation between cytotoxicity toward the target MV522 cells and chain length, as evidenced by comparing **13a** and **13c** versus **14a** and **14c** (Table 1) or by comparing **17a** and **17b** versus **18a** and **18c** (Table 3). Presumably a longer side chain would be required to reveal the effect of a nonpolar chain. In regard to the type of heteroatom in the chain, there appears to be little difference between substituting nitrogen (urea derivatives) for oxygen (carbamate derivatives), as evidenced by comparing **14a**, **14b**, and **14d** (Table 1) versus **16c**, **16e**, and **16h** (Table 2). The substitution of a sulfur for carbon to produce sulfonamide derivatives, however, results in a marked increase in cytotoxicity as evidenced by comparing the urea derivative **14c** and carbamate derivative **16a** versus the sulfonamide derivative **18d** (Table 3). Indeed, the sulfonamide derivative **18d** is one of the most cytotoxic analogues we have synthesized to date. It is nearly equivalent in cytotoxicity toward MV522 cells compared to the parent compound illudin S.[23] Because selective toxicity against target MV522 cells to nontarget 8392 cells is a reliable indication of in vivo efficacy, all the urea derivatives and the majority of both the carbamate and sulfonamide derivatives are clearly worthy of further development. Indeed, two compounds (**16e** and **18c**) displayed ratios nearly two logs higher than irofulven (**5**) and a log higher than ratios for other analogues. Preliminary MV522 xenograft studies indicate that several of the described analogues (**16e**, **18a**, **18c**) are active and induce tumor shrinkage at doses that are nontoxic to the animals (unpublished observations). Given their stability, ease of synthesis, and potent activity toward the MV522 target cells, both of these compounds are viable candidates for clinical trials.

Experimental Section

General. Melting points are uncorrected. ^1H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively. All chromatography was carried out with silica gel (Davisil 230−425 mesh, Fisher Scientific). Analytical TLC was carried out on Whatman 4420 222 silica gel plates. Reactions were routinely monitored by TLC. Purity of the compounds was determined by ThermoFinnigan LCQDECA and NMR spectroscopy. All the compounds were ≥95% pure based on NMR and analytical HPLC data using chromatography conditions as previously described.[24] Yields were calculated by taking into account recovered starting materials.

(*R*)-3′-(Aminomethyl)-6′-hydroxy-2′,4′,6′-trimethylspiro[cyclopropane-1,5′-inden]-7′(6′*H*)-one (9). A synthesis and yield of this key intermediate were previously published.[17] ^1H NMR ($CDCl_3$ + CD_3OD) δ 0.72 (m, 1H), 1.08 (m, 1H), 1.32 (s, 3H), 1.34 (m, 1H), 1.48 (m, 1H), 2.03 (s, 3H), 2.12 (s, 3H), 4.07, 4.13 (each d, J = 12 Hz, 2H), 7.05 (s, 1H); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 9.52, 12.62, 14.43, 16.0, 26.84, 36.2, 38.04, 76.55, 94.40, 126.9, 134.21, 137.28, 145.77, 161.18, 198.68; MS ESI m/z 368 $(M + Na)^+$.

(*R*)-3′-(3-Aminopropyl)-6′-hydroxy-2′,4′,6′-trimethylspiro[cyclopropane-1,5′-inden]-7′(6′*H*)-one (10). A synthesis and yield of this key intermediate were previously published.[17] ^1H NMR (CD_3OD) δ 0.77 (m, 1H), 1.06 (m, 1H), 1.33 (s, 3H), 1.42 (m, 2H), 1.84 (m, 2H), 2.07 (s, 3H), 2.11 (s, 3H), 2.79 (m, 2H), 2.97 (t, J = 7.8 Hz, 2H), 7.07 (s, 1H); ^{13}C NMR (CD_3OD) δ 9.5, 13.1, 14.5, 16.7, 25.4, 27.5, 29.4, 38.6, 40.4, 77.4, 127.8, 135.5, 136.7, 139.7, 141.1, 159.7, 198.6.

(*R*)-4-Nitrophenyl (6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)methylcarbamate (11). To a solution of compound **9** (244 mg, 0.68 mmol) in 5.0 mL of CH_2Cl_2 was added DABCO (152.7 mg, 1.36 mmol). The resulting solution was cooled to 0 °C. Then *p*-nitrophenylchloroformate (161.6 mg, 0.8 mmol) was added. The reaction mixture was stirred at 0 °C for 3 h. Then the reaction mixture was partially concentrated and then subjected to column chromatography (50% ethyl acetate (EtOAc)−hexanes) to give 200.7 mg of **11** (72%) as a yellow liquid. ^1H NMR ($CDCl_3$) δ 0.74 (m, 1H), 1.10 (m, 1H), 1.27 (m, 1H), 1.37 (s, 3H), 1.54 (m, 1H), 2.04, (s, 3H), 2.17 (s, 3H), 3.89 (s, 1H), 4.47 (m, 2H), 5.11 (brs, 1H), 7.11 (s, 1H), 7.31 (d, J = 9.2 Hz, 2H), 8.24 (d, J = 9.2 Hz, 2H).

(*R*)-4-Nitrophenyl 3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)propylcarbamate (12). To a solution of compound **10** (81.4 mg, 0.21 mmol) in 5.0 mL of CH_2Cl_2 was added DABCO (42.2 mg, 0.42 mmol). The resulting solution was cooled to 0 °C. Then *p*-nitrophenylchloroformate (50.7 mg, 0.25 mmol) was added. The resulting reaction mixture was stirred at 0 °C for 5 h. Then the reaction mixture was partially concentrated and then subjected to column chromatography (50% EtOAc−hexanes) to give 62.5 mg of **12** (68%) as a yellow liquid. ^{1}H NMR ($CDCl_3$) δ 0.67 (m, 1H), 1.06 (m, 1H), 1.32 (m, 1H), 1.35 (s, 3H), 1.46 (m, 1H), 1.93 (m, 2H), 2.05 (s, 6H), 2.75−2.82 (m, 2H), 4.23, (m, 2H), 7.12 (s, 1H), 7.33 (d, J = 9.2 Hz, 2H), 8.22 (d, J = 9.2 Hz, 2H).

(*R*)-1-(2-Chloroethyl)-3-((6′-hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)methyl)urea (13a). To a solution of compound **11** (21.4 mg, 0.052 mmol) and 2-chloroethylamine (24.4 mg, 0.210 mmol) in CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (14.4 mg. 0.112 mmol) at room temperature. The solution was stirred for about 2 h and then partially concentrated in high vacuum and subjected to column chromatography (2% methanol (MeOH) in EtOAc) to give 11.7 mg of **13a** (78%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 1H), 1.05 (m, 1H), 1.32 (m, 1H), 1.35 (s, 3H), 1.45 (m, 1H), 2.01 (s, 3H), 2.09 (s, 3H), 3.52 (m, 2H), 3.60 (t, J = 4.8 Hz, 2H), 3.86 (brs, 1H), 4.32 (each dd, J = 10.8 Hz, 2H), 4.58 (brs, 1H), 5.09 (brs, 1H), 7.04 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.6, 13.0, 14.4, 16.0, 27.5, 36.9, 37.7, 42.1, 45.1, 76.2, 129.8, 134.9, 138.4, 142.7, 157.4, 160.0, 197.9; MS (ESI) m/z 373 $(M + Na)^+$.

(*R*)-1-((6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)methyl)-3-(2-hydroxyethyl)urea (13b). To a solution of compound **11** (20.0 mg, 0.048 mmol) and ethanolamine (14.6 mg, 0.24 mmol) in CH_2Cl_2 (2.5 mL) was added *N*,*N*-diisopropylethylamine (12.4 mg, 0.096 mmol) at 0 °C. The solution temperature was raised to room temperature. After 15 min the solution was partially concentrated in high vacuum and then subjected to column chromatography (5% MeOH−EtOAc) to give 11.3 mg of **13b** (83%) as an orange-red gum. ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 1H), 1.05 (m, 1H), 1.31 (m, 1H), 1.32 (s, 3H), 1.45 (m, 1H), 2.01 (s, 3H), 2.08 (s, 3H), 3.32 (q, J = 4.8 Hz, 2H), 3.67 (t, J = 5.20 Hz, 2H), 3.96 (brs, 1H), 4.30 (m, 2H), 4.84 (brs, 1H), 5.22 (m, 1H), 7.02 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.5, 13.0, 14.4, 15.9, 27.5, 29.6, 36.8, 37.7, 43.1, 63.1, 76.2, 129.8, 134.9, 138.3, 142.6, 158.9, 160.0, 197.9; MS (ESI) m/z 333 $(M + H)^+$.

(*R*)-1-Hydroxy-3-((6′-hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)methyl)urea (13c). To a solution of compound **11** (35.5 mg, 0.086 mmol) and hydroxylamine hydrochloride (17.9 mg, 0.258 mmol) in CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (22.2 mg, 0.172 mmol) at 0 °C. After 1 h the solution was partially concentrated in high vacuum and then subjected to column chromatography (1% MeOH−EtOAc) to give 19.6 mg of **13c** (73%) as an orange-yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.71 (m, 1H), 1.08 (m, 1H), 1.30 (m, 1H), 1.35 (s, 3H), 1.48 (m, 1H), 2.00 (s, 3H), 2.11 (s, 3H), 4.39 (m, 2H), 5.86 (brs, 1H), 7.09 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.5, 13.1, 14.4, 16.0, 27.5, 36.1, 37.8, 76.2, 126.9, 128.7, 134.9, 138.4, 143.2, 159.8, 161.4, 197.9; MS (ESI) m/z 327 $(M + Na)^+$.

(*R*)-1-((6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)methyl)-3-methoxyurea (13d). To a solution of compound **11** (15.0 mg, 0.036 mmol) and methoxylamine hydrochloride (12 mg, 0.144 mmol) in CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (9.3 mg, 0.072 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for about 2 h. Then the solution was partially concentrated in high vacuum and then subjected to column chromatography (1% MeOH−EtOAc) to give 11.39 mg of **13d** (72%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.69 (m, 1H), 1.09 (m, 1H), 1.33 (m, 1H), 1.37 (s, 3H), 1.51 (m, 1H), 2.02 (s, 3H), 2.14 (s, 3H), 3.64 (s, 3H), 3.92 (brs, 1H), 4.26 (m, 2H), 5.51 (brs, 1H), 7.11 (s, 1H), 7.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.5, 13.1, 14.4, 16.0, 27.5, 36.1, 37.8, 64.3, 76.2, 126.9, 128.9, 134.8, 138.5, 143.2, 159.4, 159.8, 197.8; MS (ESI) m/z 341 $(M + Na)^+$.

(*R*)-1-(2-Chloroethyl)-3-(3-(6′-hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)propyl)urea (14a). To a solution of compound **12** (17.4 mg, 0.039 mmol) and 2-chloroethylamine (10.6 mg, 0.156 mmol) in CH_2Cl_2 (2.5 mL) was added *N*,*N*-diisopropylethylamine (20.16 mg, 0.156 mmol) at room temperature. The solution was stirred for about 2 h and then partially concentrated in high vacuum and subjected to column chromatography (2% MeOH−EtOAc) to give 11.7 mg of **14a** (78%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 1H), 1.03 (m, 1H), 1.31 (m, 1H), 1.34 (s, 3H), 1.45 (m, 1H), 1.67 (m, 2H), 2.02 (s, 6H), 2.66 (m, 2H), 3.21 (m, 2H), 3.53 (q, J = 4.4 Hz, 2H), 3.61 (t, J = 5.8 Hz, 2H), 3.9 (brs, 1H), 4.58 (brs, 1H), 4.84 (brs, 1H), 7.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.1, 13.0, 13.9, 16.0, 25.1, 27.1, 30.9, 37.4, 40.4, 42.1, 45.1, 75.9, 94.4, 135.9, 136.2, 139.1, 139.6, 157.6, 157.7, 197.1; MS (ESI) m/z 401 $(M + Na)^+$.

(*R*)-1-(3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)propyl)-3-(2-hydroxyethyl)urea (14b). To a solution of compound **12** (24.6 mg, 0.056 mmol) and ethanolamine (13.68 mg, 0.224 mmol) in CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (14.47 mg, 0.112 mmol) at 0 °C. The solution was stirred for about 40 min at 0 °C and then partially concentrated in high vacuum and subjected to column chromatography (5% MeOH−EtOAc) to give 14.9 mg of **14b** (73%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 1H), 1.03 (m, 1H), 1.29 (m, 1H), 1.34 (s, 3H), 1.45 (m, 1H), 1.67 (m, 2H), 2.02 (s, 6H), 2.67 (m, 2H), 3.21 (m, 2H), 3.31 (t, J = 5.2 Hz, 2H), 3.68 (t, J = 4.8 Hz, 2H), 7.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.1, 13.0, 14.0, 16.0, 25.2, 27.5, 30.8, 37.5, 40.5, 43.3, 63.3, 76.0, 94.4, 136.0, 136.3, 139.6, 157.9, 159.3, 170.4, 197.2; MS (ESI) m/z 361 $(M + H)^+$.

(*R*)-1-Hydroxy-3-(3-(6′-hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)propyl)urea (14c). To a solution of compound **12** (36.1 mg, 0.082 mmol) and hydroxylamine hydrochloride (22.8 mg, 0.328 mmol) in CH_2Cl_2 (3.0 mL) was added *N*,*N*-diisopropylethylamine (21.2 mg, 0.164 mmol) at 0 °C. The solution was stirred for about 3 h and then partially concentrated in high vacuum and subjected to column chromatography (1% MeOH−EtOAc) to give 20.9 mg of **14c** (76%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.67 (m, 1H), 1.04 (m, 1H), 1.30 (m, 1H), 1.34 (s, 3H), 1.44 (m, 1H), 1.73 (m, 2H), 2.03 (s, 6H), 2.69 (m, 2H), 3.22 (m, 2H), 7.12 (s, 1H); MS (ESI) m/z 401 $(M + Na)^+$.

(*R*)-*N*-(3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)propyl)-1,4′-bipiperidine-1′-carboxamide (14d). To a solution of compound **12** (20.5 mg, 0.046 mmol) in DMF (0.5 mL) was added 4-piperidinopiperidine (15.4 mg, 0.092 mmol) at 0 °C. After 30 min, the solution was concentrated in high vacuum. The residue was purified by column chromatography (10% methanol−EtOAc) to yield 14.8 mg of **14d** (67%) as an orange-red gum. ^{1}H NMR ($CDCl_3 + CD_3OD$) δ 0.65 (m, 1H), 1.04 (m, 1H), 1.28 (m, 1H), 1.33 (s, 3H), 1.45 (m, 5H), 1.60 (m, 4H), 1.68 (m, 2H), 1.83 (m, 2H), 2.02, (s, 6H), 2.45 (m, 1H), 2.64 (m, 2H), 2.71 (m, 4H), 3.27 (m, 2H), 3.92 (brs, 2H), 4.50 (t, J = 4.5 Hz, 1H), 7.10 (s, 1H); MS (ESI) m/z 468 $(M + H)^+$.

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)propyl 4-Nitrophenylcarbonate (15). To a solution of compound **6** (453 mg, 1.65 mmol) in 5.0 mL of CH_2Cl_2 was added DABCO (370.3 mg, 3.3 mmol). The resulting solution was cooled to 0 °C. Then *p*-nitrophenylchloroformate (370 mg, 1.65 mmol) was added, and the mixture was stirred at the same temperature for about 5 h. The reaction mixture was concentrated partially and then subjected to column chromatography (50% EtOAc−hexanes) to give 478 mg of **15** (66%) as a yellow liquid. ^{1}H NMR ($CDCl_3$) δ 0.68 (m, 1H), 1.07 (m, 1H), 1.25 (m, 1H), 1.36 (s, 3H), 1.46 (m, 1H), 1.93 (m, 2H), 2.04 (s, 3H), 2.06 (s, 3H), 2.8

(m, 2H), 3.92 (s, 1H), 4.32, (m, 2H), 7.13 (s, 1H), 7.36 (d, $J = 9.2$ Hz, 2H), 8.22 (d, $J = 9.2$ Hz, 2H).

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propylhydroxycarbamate (16a). To a solution of compound **15** (53.4 mg, 0.121 mmol) and hydroxylamine hydrochloride (12.6 mg, 0.181 mmol) in CH_2Cl_2 (3.0 mL) was added *N*,*N*-diisopropylethylamine (62.5 mg, 0.484 mmol) at 0 °C. The solution temperature was raised to room temperature. After 4.0 h the solution was partially concentrated and then subjected to column chromatography (40% EtOAc−hexanes) to give 34.0 mg of **16a** (81%) as an orange gum. ^{1}H NMR ($CDCl_3$) δ 0.7 (m, 1H), 1.07 (m, 1H), 1.34 (m, 1H), 1.37 (s, 3H), 1.46 (m, 1H), 1.93 (m, 2H), 2.05 (s, 6H), 2.75−2.80 (m, 2H), 3.96 (brs, 1H), 4.23 (m, 2H), 6.92 (brs, 1H), 7.15 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.3, 13.1, 14.1, 16.1, 24.0, 27.6, 29.5, 37.5, 65.5, 76.6, 125.4, 135.0, 136.2, 138.8, 139.8, 157.5, 158.9, 196.9; MS (ESI) m/z 356 $(M + Na)^+$.

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl 2-Fluoroethylcarbamate (16b). To a solution of compound **15** (50.1 mg, 0.114 mmol) and 2-fluoroethylamine hydrochloride (22.6 mg, 0.228 mmol) in DMF (1.0 mL) was added triethylamine (28.78 mg, 0.285 mmol) at 0 °C. After 30 min the solution was concentrated in high vacuum. The residue was purified by column chromatography (40% EtOAc−hexanes) to yield 39.8 mg of **16b** (96%) as a yellow syrup. ^{1}H NMR ($CDCl_3$) δ 0.65 (m, 1H), 1.04 (m, 1H), 1.31 (m, 1H), 1.34 (s, 3H), 1.44 (m, 1H), 1.84−1.77 (m, 2H), 2.03 (s, 6H), 2.73−2.67 (m, 2H), 3.44, 3.51 (m, 2H), 3.96 (brs, 1H), 4.09 (m, 2H), 4.42, 4.53 (m, 2H), 5.05 (brs, 1H), 7.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.0, 12.9, 13.9, 15.9, 24.0, 27.5, 29.6, 37.4, 41.3, 64.4, 75.9, 82.0, 82.3, 125.8, 136.0, 136.3, 139.0, 139.8, 157.5, 197.3; MS (ESI) m/z 364 $(M + H)^+$.

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl 2-Chloroethylcarbamate (16c). To a solution of compound **15** (95.6 mg, 0.217 mmol) and 2-chloroethylamine (37.5 mg, 0.325 mmol) in DMF (0.5 mL) was added *N*,*N*-diisopropylethylamine (33.6 mg, 0.260 mmol) at 0 °C. The solution temperature was raised to room temperature. After 30 min the solution was partially concentrated in high vacuum and then subjected to column chromatography (40% EtOAc−hexanes) to give 86.5 mg of **16c** (71%) as an orange-red gum. ^{1}H NMR ($CDCl_3$) δ 0.67 (m, 1H), 1.05 (m, 1H), 1.30 (m, 1H), 1.36 (s, 3H), 1.47 (m, 1H), 1.82 (m, 2H), 2.04 (s, 6H), 2.72 (m, 2H), 3.53 (t, $J = 5.2$ Hz, 2H) 3.62 (d, $J = 5.2$ Hz, 2H), 4.11 (m, 2H), 5.09 (brs, 1H), 7.12 (s, 1H); MS (ESI) m/z 402 $(M + Na)^+$.

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl 2-Bromoethylcarbamate (16d). To a solution of compound **15** (100.3 mg, 0.228 mmol) and 2-bromoethylamine (70.5 mg, 0.344 mmol) in DMF (1.0 mL) was added *N*,*N*-diisopropylethylamine (36.97 mg, 0.286 mmol) at 0 °C. The solution temperature was raised to room temperature. After 30 min the solution was partially concentrated in high vacuum and then subjected to column chromatography (40% EtOAc−hexanes) to give 84.6 mg of **16d** (87%) as an orange-red gum. ^{1}H NMR ($CDCl_3$) δ 0.67 (m, 1H), 1.06 (m, 1H), 1.31 (m, 1H), 1.36 (s, 3H), 1.45 (m, 1H), 1.84 (m, 2H), 2.04 (s, 6H), 2.73 (m, 2H), 3.48 (t, $J = 5.20$ Hz, 2H) 3.59 (d, $J = 5.2$ Hz, 2H), 4.11 (m, 2H), 5.29 (brs, 1H), 7.13 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.3, 13.1, 14.1, 16.1, 24.3, 27.7, 29.8, 32.8, 37.7, 42.9, 64.7, 76.2, 125.8, 136.5, 140.1, 157.7, 197.3; MS (ESI) m/z 447 $(M + Na)^+$.

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl 2-Hydroxyethylcarbamate (16e). To a solution of compound **15** (139.6 mg, 0.317 mmol) in DMF (1.0 mL) was added ethanolamine (23 mg, 0.380 mmol) at 0 °C. The solution temperature was raised to room temperature. After 15 min the solution was partially concentrated in high vacuum and then subjected to column chromatography (40% EtOAc−hexanes) to give 109.5 mg of **16e** (95%) as an orange-red gum. ^{1}H NMR ($CDCl_3$) δ 0.68 (m, 1H), 1.06 (m, 1H), 1.30 (m, 1H), 1.35 (s, 3H), 1.45 (m, 1H), 1.80 (m, 2H), 2.04 (s, 6H), 2.73 (m, 2H), 3.36 (d, $J = 4.8$ Hz, 2H), 3.73 (t, $J = 5.2$ Hz, 2H), 4.11 (t, $J = 6.0$ Hz, 2H), 5.05 (brs, 1H), 7.12 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 8.9, 12.7, 13.8, 15.8, 24.0, 27.4, 29.5, 37.3, 43.2, 61.9, 64.2, 75.9, 125.4, 135.7, 136.3, 138.9, 139. 8, 157.1, 157. 6, 197.1; MS (ESI) m/z 360 $(M - H)^+$.

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl Morpholine-4-carboxylate (16f). To a solution of compound **15** (46.4 mg, 0.105 mmol) in DMF (1.0 mL) was added morpholine (22.8 mg, 0.262 mmol) at 0 °C. Then the mixture was warmed to room temperature. After 1 h the solution was concentrated in high vacuum. The residue was purified by column chromatography (50% EtOAc−hexanes) to yield 36.4 mg of **16f** (84%) as a yellow syrup. ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 1H), 1.05 (m, 1H), 1.30 (m, 1H), 1.34 (s, 3H), 1.44 (m, 1H), 1.83 (m, 2H), 2.03 (s, 6H), 2.73−2.67 (m, 2H), 3.45 (s, 4H), 3.64 (s, 4H), 4.13 (m, 2H), 7.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.0, 12.9, 13.8, 15.8, 24.1, 27.4, 29.6, 37.4, 43.8, 64.9, 66.5, 75.9, 125.5, 135.4, 136.2, 139.0, 139.7, 155.2, 157.3, 197.0; MS (ESI) m/z 410 $(M + Na)^+$.

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl Piperidine-1-carboxylate (16g). To a solution of compound **15** (34.8 mg, 0.079 mmol) in DMF (1.0 mL) was added piperidine (13.9 mg, 0.164 mmol) at 0 °C. After 30 min the solution was concentrated in high vacuum. The residue was purified by column chromatography (20% acetone−hexanes) to yield 28.9 mg of **16g** (94%) as an orange-red gum. ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 1H), 1.04 (m, 1H), 1.28 (m, 1H), 1.34 (s, 3H), 1.45 (m, 1H), 1.51 (m, 4H), 1.58 (m, 4H), 1.81 (m, 2H), 2.03 (s, 6H), 2.70 (m, 2H), 3.40 (m, 4H), 4.09−4.13 (m, 2H), 7.12 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.0, 12.8, 13.8, 15.8, 24.3, 25.6, 27.5, 29.7, 37.4, 44.7, 64.5, 75.9, 125.4, 135.7, 136.3, 139.1, 139.7, 155.5, 157.4, 197.0; MS (ESI) m/z 386 $(M + H)^+$.

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl 1,4′-Bipiperidine-1′-carboxylate (16h). To a solution of compound **15** (48.6 mg, 0.11 mmol) in DMF (1.0 mL) was added 4-piperidinopiperidine (37 mg, 0.22 mmol) at 0 °C. After 30 min the solution was concentrated in high vacuum. The residue was purified by column chromatography (10% methanol−EtOAc) to yield 45.8 mg of **16h** (88%) as an orange-red gum. ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 1H), 1.03 (m, 1H), 1.29 (m, 1H), 1.31 (s, 3H), 1.45 (m, 4H), 1.58 (m, 4H), 1.81 (m, 2H), 2.02 (s, 6H), 2.41 (m, 1H), 2.48 (m, 4 H), 2.66−2.76 (m, 6H), 4.10 (m, 2H), 7.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.0, 12.8, 13.8, 15.8, 24.2, 24.5, 26.1, 27.5, 29.7, 31.5, 37.4, 43.5, 50.1, 62.4, 64.6, 75.9, 125.4, 135.6, 136.2, 139.1, 139.7, 155.1, 157.4, 197.0. MS (ESI) m/z 386 $(M + H)^+$.

(*R*)-3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl 1*H*-Imidazole-1-carboxylate (16i). To a solution of compound **6** (70.0 mg, 0.255 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added carbodiimidazole (49.4 mg, 0.304 mmol). The reaction mixture was stirred at room temperature for 3 h and then purified by column chromatography (50% EtOAc−hexanes) to yield 57.8 mg of **16i** (60%) as an orange-yellow syrup. ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 1H), 1.26 (m, 1H), 1.29 (m, 1H), 1.34 (s, 3H), 1.97 (m, 2H), 2.04 (s, 6H), 2.79 (m, 2H), 4.44 (t, $J = 6.0$ Hz, 2H), 7.07 (s, 1H), 7.10 (s, 1H), 7.39 (s, 1H), 8.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.1, 12.9, 13.9, 15.9, 23.9, 27.4, 29.1, 37.4, 67.6, 75.9, 116.9, 125.8, 130.7, 134.1, 136.0, 136.9, 138.9, 140.1, 157.5, 197.0; MS (ESI) m/z 369 $(M + H)^+$.

(*R*)-*N*-((6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)methyl)methanesulfonamide (17a). To a solution of compound **9** (145.6 mg, 0.405 mmol) in 2.0 mL of DMF/CH_2Cl_2 (1:3) was added DABCO (91 mg, 0.811 mmol). The resulting solution was cooled to 0 °C. Then methanesulfonyl chloride (69.5 mg, 0.607 mmol) was added. The reaction mixture was stirred for 2 h at 0 °C, then partially concentrated and purified by silica gel column chromatography to afford compound **17a** as a deep-red gum in 48% yield (62.8 mg).

^{1}H NMR ($CDCl_3$) δ 0.71 (m, 1H), 1.09 (m, 1H), 1.34 (s, 3H), 1.37 (m, 1H), 1.49 (m, 1H), 2.12 (s, 3H), 2.14 (s, 3H), 2.92 (s, 3H), 3.85 (brs, 1H), 4.27 (m, 2H), 4.34 (m, 1H), 7.01 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.6, 13.1, 14.5, 16.4, 27.4, 34.5, 37.9, 39.3, 40.1, 76.25, 127.2, 134.3, 138.0, 143.3, 160.5, 197.9; MS (ESI) m/z 350 $(M + Na)^+$.

(*R*)-*N*-((6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)methyl)sulfamide (17b). To a solution of compound **9** (135.4 mg, 0.377 mmol) and sulfamyl chloride (87 mg, 0.754 mmol) in anhydrous CH_2Cl_2/DMF (3.0/0.5 mL) was added *N*,*N*-diisopropylethylamine (146.2 mg, 1.13 mmol) at −20 °C. The reaction mixture was warmed to room temperature, stirred for 6 h, concentrated in high vacuum, and then subjected to column chromatography to give 29.8 mg of compound **17b** (24%) as a yellow solid. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.69 (m, 1H), 1.06 (m, 1H), 1.31 (s, 3H), 1.33 (m, 1H), 1.45 (m, 1H), 2.09 (s, 3H), 2.10 (s, 3H), 4.21 (m, 2H), 4.58 (br, 1H), 7.04 (s, 1H); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 9.5, 12.8, 14.4, 16.1, 27.3, 37.7, 39.4, 76.2, 127.6, 134.5, 138.1, 143.2, 160.7, 198.1; MS (ESI) m/z 325 $(M + H)^+$.

(*R*)-*N*-(3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl)methanesulfonamide (18a). To a solution of compound **10** (19.4 mg, 0.05 mmol) in 2.0 mL of CH_2Cl_2 was added DABCO (8.4 mg, 0.075 mmol). The resulting solution was cooled to 0 °C. Then methanesulfonyl chloride (8.6 mg, 0.075 mmol) was added. The reaction mixture was stirred for 15 min at 0 °C, partially concentrated, and purified by silica gel column chromatography to afford the expected compound **18a** as an orange-yellow liquid in 69% yield (12.2 mg). ^{1}H NMR ($CDCl_3$) δ 0.68 (m, 1H), 1.06 (m, 1H), 1.31 (m, 1H), 1.35 (s, 3H), 1.46 (m, 1H), 1.77 (m, 2H), 2.04 (s, 6H), 2.71 (m, 2H), 2.95 (s, 3H), 3.17 (q, J = 6.8 Hz, 2H), 4.38 (brs, 1H), 7.12 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.3, 13.2, 14.1, 16.3, 24.9, 27.6, 31.1, 37.6, 40.4, 43.1, 75.9, 134.7, 135.9, 138.8, 139.7, 139.7, 157.7; MS (ESI) m/z 350 $(M - H)^+$.

(*R*)-*N*-(3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl)-4-methylbenzenesulfonamide (18b). To a solution of compound **10** (45.3 mg, 0.117 mmol) and DABCO (19.6 mg, 0.175 mmol) in CH_2Cl_2 (2.5 mL) was added *p*-toluenesulfonyl chloride (33.4 mg, 0.175 mmol) at 0 °C. After 1.0 h the reaction mixture was partially concentrated and then subjected to column chromatography (50% EtOAc−hexanes) to give 15.4 mg of **18b** (90%) as an orange-red liquid. ^{1}H NMR ($CDCl_3$) δ 0.64 (m, 1H), 1.05 (m, 1H), 1.27 (m, 1H), 1.33 (s, 3H), 1.35 (s, 3H), 1.44 (m, 1H), 1.66 (m, 2H), 1.95 (s, 3H), 1.97 (s, 3H), 2.42 (s, 3H), 2.64 (m, 2H), 2.96 (m, 2H), 4.59 (brs, 1H), 7.08 (s, 1H), 7.31 (d, J = 8 Hz, 2H), 7.73 (d, J = 8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 9.1, 12.9, 13.9, 16.0, 21.5, 24.9, 27.5, 30.3, 37.4, 43.0, 75.9, 125.5, 127.0, 129.7, 135.1, 136.1, 136.7, 138.9, 139.8, 143.5, 157.8, 197.1.

(*R*)-*N*-(3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro-[cyclopropane-1,5′-indene]-3′-yl)propyl)sulfamide (18c). To a solution of *tert*-butanol (30 mg, 0.41 mmol) in anhydrous CH_2Cl_2 (4.0 mL) was added chlorosulfonyl isocyanate (58 mg, 0.41 mmol) at 0 °C. After 10 min the resulting solution was then added to a solution of **10** (123.1 mg, 0.31) in *N*,*N*-diisopropylethylamine (DIPEA) (120 mg, 0.93 mmol) in anhydrous methylene chloride. The temperature was raised to room temperature, and the mixture was stirred for 3 h and then purified by column chromatography (50% EtOAc−hexanes) to yield 104.4 mg of **19** (72%) as a yellow solid. To the solution of **19** (42.4 mg, 93.8 μmol) in anhydrous CH_2Cl_2 (2.0 mL) was added a few drops of anisole followed by 1.0 mL of TFA. The reaction mixture was stirred for 45 min, then concentrated in high vacuum, then washed several times with hexanes and finally with 5% EtOAc/hexanes, and then dried under high vacuum to give 26.1 mg of compound **18c** (79%) as a yellow gum. ^{1}H NMR ($CDCl_3$) δ 0.66 (m, 1H), 1.02 (m, 1H), 1.28 (m, 1H), 1.32 (s, 3H), 1.41 (m, 1H), 1.72 (m, 2H), 2.04, (s, 6H), 2.69 (m, 2H), 3.10 (t, J = 7.2 Hz, 2H), 5.09 (brs, 1H), 7.09 (s, 1H); ^{13}C NMR ($CDCl_3$+ CD_3OD) δ 9.0, 12.9, 13.9, 15.4, 16.0, 24.9, 27.4, 30.1, 37.4, 43.1, 75.9, 135.6, 136.4, 138.9, 139.9, 157.9, 197.2; MS (ESI) m/z 353 $(M + H)^+$.

(*R*)-*N*-(3-(6′-Hydroxy-2′,4′,6′-trimethyl-7′-oxo-6′,7′-dihydrospiro[cyclopropane-1,5′-indene]-3′-yl)propyl)hydroxysulfamide (18d). To a solution of *tert*-butanol (225 mg, 3.0 mmol) in anhydrous CH_2Cl_2 (5.0 mL) was added chlorosulfonyl isocyanate (430 mg, 3.0 mmol) at 0 °C. After 20 min the resulting solution was then added to a solution of *O*-(*tert*-butyldimethylsilyl)hydroxylamine **20** (345.4 mg, 2.34 mmol) in DIPEA (910 mg, 7.035 mmol) in anhydrous CH_2Cl_2 (8.0 mL). The temperature was raised to room temperature, and the mixture was stirred for 3 h and then purified by column chromatography (50% EtOAc−hexanes) to yield 104.4 mg of **21** (47%) as a white solid. To the solution of **21** (47.2 mg, 0.184 μmol) and **6** (50.4 mg, 0.184 mmol) in anhydrous tetrahydrofuran (THF) (3.0 mL) was added triphenylphosphine (48.6 mg, 0.184 mmol) and diisopropyl azodicarboxylate (DIAD) (37.2 mg, 0.184 mmol). The reaction mixture was stirred for 30 min at 0 °C and then concentrated in high vacuum and purified by column chromatography to give 37.71 mg of compound **22** (78%) as a yellow solid. To the above compound **22** (43 mg, 0.074 mmol) was added a few drops of anisole followed by 2.0 mL of TFA−H_20, 5%. Reaction mixture was stirred for 2 h at 0 °C and then concentrated in high vacuum. The residue was purified by silica gel column chromatography to afford 18.4 mg of compound **18d** (68%) as a yellow gum. ^{1}H NMR ($CDCl_3$) δ 0.65 (m, 1H), 0.99 (m, 1H), 1.29 (m, 1H), 1.31 (s, 3H), 1.42 (m, 1H), 1.70 (m, 2H), 2.06 (s, 6H), 2.68 (m, 2H), 3.01 (t, J = 6.4 Hz, 2H), 7.09 (s, 1H); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 9.1, 12.8, 13.8, 13.9, 16.0, 24.8, 27.4, 30.3, 37.4, 43.2, 76.2, 136.0, 136.4, 140.1, 140.2, 158.4, 197.4; MS (ESI) m/z 391 $(M + Na)^+$.

Supporting Information Available: NMR spectral data of analogues **9**−**18**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Anchel, M.; Hervey, A.; Robbins, W. Antibiotic Substances from Basidiomycetes. VII. *Clitocybe illudens*. *Proc. Natl. Acad. Sci. U.S.A.* **1950**, *36*, 300–305.

(2) McMorris, T. C.; Anchel, M. The Structures of the Basidiomycete Metabolites Illudin S and Illudin M. *J. Am. Chem. Soc.* **1963**, *85*, 831–832.

(3) Kelner, M. J.; McMorris, T. C.; Beck, W. T.; Zamora, J. M.; Taetle, R. Preclinical Evaluation of Illudins as Anticancer Agents. *Cancer Res.* **1987**, *47*, 3186–3189.

(4) Kelner, M. J.; McMorris, T. C.; Taetle, R. Preclinical Evaluation of Illudins as Anticancer Agents: Basis for Selective Cytotoxicity. *J .Natl. Cancer Inst.* **1990**, *82*, 1562–1565.

(5) Berger, E. R.; Ciuleanu, T.; Hart, L.; Chi, K. N.; Alexandre, J.; Oudard, S.; Kahatt, C.; Weems, G.; Cvitkovic, E.; MacDonald, J. R. Results of a Randomized Phase II Study of Irofulven in Hormone-Refractory Prostate Cancer Patients That Have Failed First-Line Docetaxel Treatment. *J. Clin. Oncol.* **2007**, *25* (18S), 5068.

(6) McMorris, T. C.; Kelner, M. J.; Want, W.; Moon, S.; Taetle, R. On the Mechanism of Toxicity of Illudins: The Role of Glutathione. *Chem. Res. Toxicol.* **1990**, *3*, 574–579.

(7) McMorris, T. C.; Elayadi, A. N.; Yu, J.; Hu, Y.; Kelner, M. J. Metabolism of Antitumor Hydroxymethylacylfulvene by Rat liver Cytosol. *Drug Metab. Dispos.* **1999**, *27*, 983–985.

(8) McMorris, T. C.; Elayadi, A. N.; Yu, J.; Hu, Y.; Kelner, M. J. Metabolism of Antitumor Acylfulvene by Rat Liver Cytosol. *Biochem. Pharmacol.* **1999**, *57*, 83–88.

(9) (a) Gong, J.; Vaidyanathan, V. G.; Yu, X.; Kensler, T. W.; Peterson, L. A.; Sturla, S. J. Depurinating Acylfulvene−DNA Adducts: Characterizing Cellular Chemical Reactions of a Selective Antitumor Agent. *J. Am. Chem. Soc.* **2007**, *129* (7), 2101–2111. (b) Neels, J. F.; Gong, J.; Yu, X.; Sturla, S. J. Quantitative Correlation of Drug Bioactivation and Deoxyadenosine Alkylation by Acylfulvene. *Chem. Res. Toxicol.* **2007**, *20*, 1513–1519.

(10) Liu, X.; Sturla, S. J. Profiling Patterns of Glutathione Reductase Inhibition by the Natural Product Illudin S and Its Acylfulvene Analogues. *Mol. BioSyst.* **2009**, *5*, 1013–1024.

(11) McMorris, T. C.; Kelner, M. J.; Wang, W.; Estes, L. A.; Montoya, M. A.; Taetle, R. J. Structure−Activity Relationships of Illudins

Analogs with Improved Therapeutic Index. *J. Org. Chem.* **1992**, *57*, 6876–6883.
(12) McMorris, T. C.; Kelner, M. J.; Wang, W.; Diaz, M. A.; Estes, L. A.; Taetle, R. Acylfulvenes, a New Class of Potent Antitumor Agents. *Experientia* **1996**, *52*, 75–80.
(13) (a) McMorris, T. C.; Kelner, M. J.; Wang, W.; Yu, J.; Estes, L. A.; Taetle, R. Hydroxymethylacylfulvene (HMAF) an Illudin Derivative with Superior Antitumor Properties. *J. Nat. Prod.* **1996**, *59*, 896–899. (b) Kelner, M. J.; McMorris, T. C.; Montoya, M. A.; Estes, L.; Uglik, S. F.; Rutherford, M.; Samson, K. M.; Bagnell, R. D.; Taetle, R. *Cancer Chemother. Pharmacol.* **1999**, *44*, 235–240.
(14) McMorris, T. C.; Yu, J.; Lira, R.; Dawe, R.; McDonald, J. R.; Waters, S. J.; Estes, L. A.; Kelner, M. J. Structure−Activity Studies of the Antitumor Agent Irofulven (Hydroxymethylacylfulvene) and Related Analogues. *J. Org. Chem.* **2001**, *66*, 6158–6163.
(15) McMorris, T. C.; Yu, J.; Ngo, H. T.; Wang, H.; Kelner, M. J. Preparation and Biological Activity of Amino Acid and Peptide Conjugates of Antitumor Hydroxymethylacylfulvene. *J. Med. Chem.* **2000**, *43*, 3577–3580.
(16) Kelner, M. J.; McMorris, T. C.; Alisala, K.; Staake, M. D.; Shah, A.; Kiorpes, A. K.; Webber, J. G.; Van Laar, E. S.; Waters, S. J. MGI 4184, a Novel Acylfulvene with Differential Pharmacokinetic Profile and Marked Activity in Vitro and in Vivo. Presented at the 97th Annual Meeting of American Association for Cancer Research, **2006**; Abstract 5675.
(17) McMorris, T. C.; Chimmani, R.; Guram, R.; Guram, M.; Staake, M. D.; Kelner, M. J. Synthesis and Antitumor Activity of Amine Analogs of Irofulven. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6770–6772.
(18) Scozzafava, A.; Supuran, C. T. Hydroxyurea Is a Carbonic Anhydrase Inhibitor. *Bioorg. Med. Chem.* **2003**, *11*, 2241–2246.
(19) (a) Samant, M. P.; Hong, D. J.; Croston, G.; Rivier, C.; Rivier, J. *J. Med. Chem.* **2006**, *49*, 3536–3543. (b) Matthews, S. E.; Pouton, C. W.; Threadgill, M. D. *J. Chem. Soc., Chem. Commun.* **1995**, 1809. (c) Nishiyama, T.; Ichikawa, Y.; Isobe, M. *Synlett* **2003**, *1*, 47–50.
(20) (a) Dow, R. L.; Ernest S. Paight, E. S.; Schneider, S. R.; Hadcock, J. R.; Hargrove, D. M.; Martin, K, A.; Maurer, T. S.; Nardone, N. A.; Tess, D. A.; Jardine, P. D. Potent and Selective, Sulfamide-Based Human b3-Adrenergic Receptor Agonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3235–3240. (b) Simone, G. D.; Fiore, A. D.; Menchise, V.; Pedone, C.; Antel, J.; Casini, A.; Scozzafava, A.; Wurl, M.; Supuran, C. T. Carbonic Anhydrase Inhibitors. Zonisamide Is an Effective Inhibitor of the Cytosolic Isozyme II and Mitochondrial Isozyme V: Solution and X-ray Crystallographic Studies. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2315–2320. (c) Supuran, C. T.; Casini, A.; Scozzafava, A. Protease Inhibitors of the SulfonamideType: Anticancer, Antiinflammatory, and Antiviral Agents. *Med. Res. Rev.* **2003**, *23* (5), 535–558. (d) Winum, J. Y.; Vullo, D.; Casini, A.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. Carbonic Anhydrase Inhibitors. Inhibition of Cytosolic Isozymes I and II and Transmembrane, Tumor-Associated Isozyme IX with Sulfamates Including EMATE Also Acting as Steroid Sulfatase Inhibitors. *J. Med. Chem.* **2003**, *46*, 2197–2204. (e) Fettes, K. J.; Howard, N.; Hickman, D. T.; Adah, S.; Player, M. R.; Torrence, P. F.; Micklefield, J. Synthesis and Nucleic-Acid-Binding Properties of Sulfamide- and 3′-N-Sulfamate-Modified DNA. *J. Chem. Soc., Perkin Trans. 1* **2002**, 485–495. (f) Guianvarc'h, D.; Duca, M.; Boukarim, C.; Berthier, L. K.; Léonce, S.; Pierré, A.; Pfeiffer, B.; Renard, P.; Arimondo, P. B.; Monneret, C.; Dauzonne, D. Synthesis and Biological Activity of Sulfonamide Derivatives of Epipodophyllotoxin. *J. Med. Chem.* **2004**, *47*, 2365–2374.
(21) Winum, J. Y.; Innocenti, A.; Nasr, J.; Montero, J. L.; Scozzafava, A.; Vullo, D; Supuran, C. T. Carbonic Anhydrase Inhibitors: Synthesis and Inhibition of Cytosolic/Tumor-Associated Carbonic Anhydrase Isozymes I, II, IX, and XII with *N*-Hydroxysulfamides—A New Zinc-Binding Function in the Design of Inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2353–2358.
(22) Woynarowska, B. A.; Woynarowski, J. M.; Herzig, M. C.; Roberts, K.; Higdon, A. L.; MacDonald, J. R. *Biochem. Pharmacol.* **2000**, *59*, 1217–1223.
(23) Kelner, M. J.; McMorris, T. C.; Estes, L.; Wang, W.; Samson, K. M.; Taetle, R. Efficacy of HMAF (MGI-114) in the MV522 Metastatic Lung Carcinoma Xenograft Model Nonresponsive to Traditional Anticancer Agents. *Invest. New Drugs* **1996**, *14*, 161.
(24) Brandsteterova, E.; Kelner, M. J.; McMorris, T. C.; Estes, L.; Bagnell, R.; Montoya, M. HPLC Determination of a New Anticancer Agent (Acylfulvene) in Serum. *Neoplasma* **1992**, *39*, 369.