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# Synthesis and Anticancer Activity of 13-Membered Cyclic Enediynes

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We herein describe the synthesis of 15 novel 13-membered cyclic enediyne derivatives using simple and straightforward approach. Representative examples were screened for their anticancer activities on 60 different human tumor cell lines representing various histologies viz. leukemia, melanoma, and cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. The enediyne derivatives with halogen substitutions, especially fluorides were found to be active against most of the cell lines. The initial results indicates marginal to good inhibition for the growth of tumor cells for several cell lines, which shows the potential of these class of compound towards anticancer application.

**Keywords:** Anticancer / Cell lines / Enediyne

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## Introduction

Enediyne class of compounds is considered to be one of the most powerful anti-tumor and antibiotic agents [1–5]. First discovered in late 1980's, these compounds captured immediate attention of the chemists and biologists throughout the world due their complex molecular framework and novel biological activity profile [6]. All the natural enediynes such as calicheamicin [7], esperamicin [8], dynamicin [9, 10] maduroptin [1], and more recently uncialamycin [11] possess highly strained enediyne unit, a triggering device and delivery agent and under biological conditions these compounds forms diradical which is responsible for their anticancer activity [12, 13]. Some of the enediynes such as neocarzinostatin, and mylotary (a bioconjugate of monoclonal antibody and calcheamicin γ1) [14] have also been approved by FDA for the treatment of acute myeloid leukemia [15, 16]. Apart from anticancer activity, synthetic enediynes are known to exhibit cytotoxicity against various cell lines [1, 17], protein degradation activity [18], antibacterial activity [19], and topoisomerase inhibitory activity [20]. Although these compounds exhibit excellent anticancer activity but due to their modest selectivity for cancer cells, clinical use of this class of compounds has been limited. In order to improve the selectivity of the enediynes, efforts are being made to synthesize analogous compounds with better efficacy [21–26]. Very recently we have reported the synthesis and antibacterial activity of thermally stable 13-membered cyclic enediynes [19]. To this end, we report herein synthesis and anticancer activity of various 13-membered cyclic enediynes.

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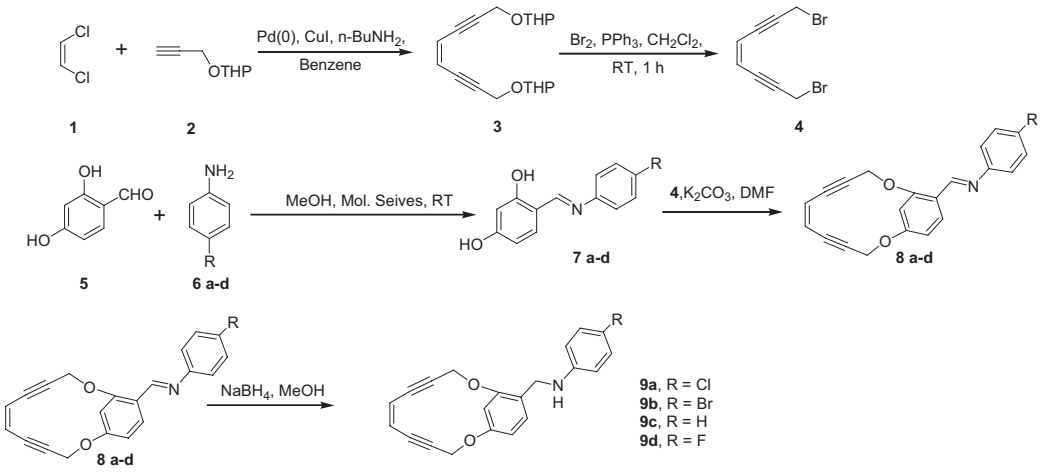
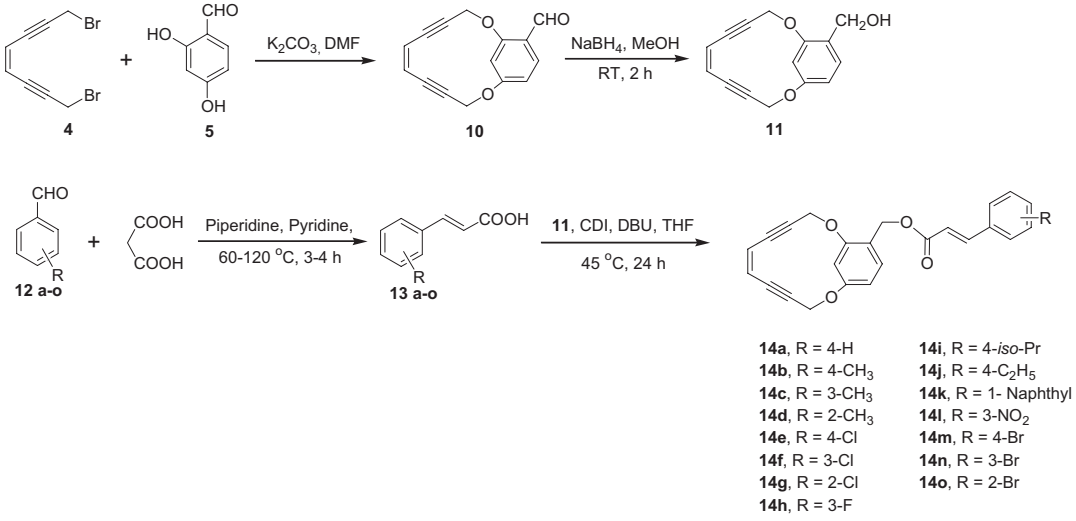
## Results and discussion

### Chemistry

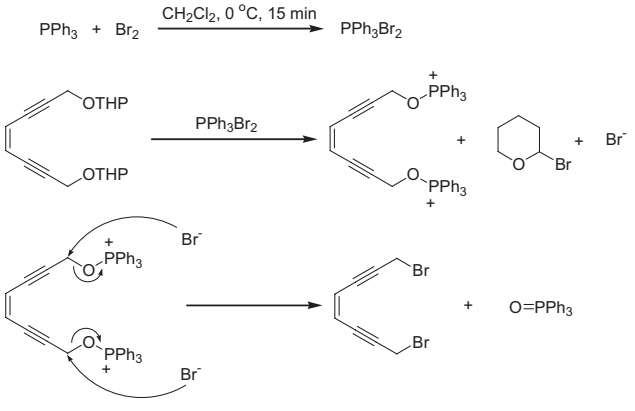
The synthesis of 13-membered cyclic substituted enediyne amines is outlined in Scheme 1 and Scheme 2 illustrates the synthesis of ester analogues of these cyclic enediynes. The multi step procedure was accomplished by using *cis*-dichloroethene **1** as starting material. The two arms of *cis*-dichloroethene were extended to form compound **3** [27] using protected propargyl alcohol via Sonagashira coupling. Compound **3** was converted to 1,8-dibromo-oct-4-ene-2,6-diyne **4** [27] using $PPh_3$ and $Br_2$. The mechanism of this reaction is depicted in Scheme 3. In the first step, triphenyl phosphine and bromine forms triphenylphosphinedibromide, which is very reactive and it replaces OTHP group




**Scheme 1.** Synthesis of 13-membered cyclic substituted enediyne amines.




**Scheme 2.** Synthesis of ester analogues of 13-membered cyclic substituted enediyne amines.




**Scheme 3.** Mechanism of the conversion of compound **3** to 1,8-dibromo-oct-4-ene-2,6-diyne **4** using $PPh_3$ and $Br_2$.

via $S_N2$ mechanism. Compound **3** is added to the mixture of $PPh_3$ and $Br_2$ only after the formation of triphenylphosphine-dibromide, so double or triple bonds remains unaffected. The cyclic enediyne imines (**8a–d**) were prepared by the coupling of compound **4** with imines **7a–d** in dry DMF in the presence of $K_2CO_3$ as a base [19]. Reduction of compounds **8a–d** by sodium borohydride in dry methanol affords compounds **9a–d** in good to moderate yield [19]. The key alcohol **11** for the synthesis of enediyne ester was synthesized by literature method as shown in Scheme 2 [19]. 3-Phenyl-acrylic acids **13a–o** were prepared by Knoevenagel condensation, followed by coupling with **11** to afford esters **14a–o** using CDI and DBU as coupling reagents in THF. All the synthesized compounds were characterized by IR, $^1$H-NMR, $^{13}$C-NMR, HRMS, and elemental analyses.

### Anticancer activity

The anticancer activities of selective compounds were evaluated using 60 different human tumor cell lines representing various histologies viz. leukemia, melanoma, and cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. The cyclic enediyne **9d** and its ester analogues **14f**, **14h**, **14k**, and **14l** were screened at the concentration of 10 μM on all the cell lines. A brief summary of the results on selective cell lines is described in Table 1. Compound **9d**, having fluoro substitution on aromatic ring, showed marginal activity against most of the cell lines. It showed activity in all leukemia cell lines where significant inhibition was observed for cell line MOLT-4 (40%). This compound showed maximum activity for leukemia cell lines HL-60 (43%). The ester analogous **14f** and **14h**, both having halogen substitution on aromatic rings showed inhibition for most of the cell lines compared to naphthyl and nitro substituted **14k** and **14l**, respectively. Compounds **14k** and **14l** were totally inactive in all of the melanoma, ovarian and renal cancer cell lines, however marginal activity was observed in few cell lines in other panels. Compound **14h** with fluoro substitution on aromatic ring was marginally active in most of the cell lines including all the leukemia, ovarian and renal cell lines showed significant inhibition for leukemia cell lines K-562 (52%), SR (71%), colon cell line HT29 (37%), melanoma cell line LOX IMVI (37%), and breast cancer cell line MDA-MB-468 (48%). The compound **14f** was also active in all the leukemia cell lines with highest activity for cell line SR with 32% inhibition in tumor cell growth. Interestingly, all the five cyclic enediynes showed inhibition for MDS-MB-468 breast cancer cell line (Table 1). The One-dose data of the compounds **9d**, **14f**, **14h**, **14k**, **14l** is reported as a mean graph of the percent growth of treated cells. The number reported for the One-dose assay is growth relative to the no-drug control, and relative to the time zero number of cells. This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0). For example, a value of 100 means no growth inhibition. A value of 40 would mean 60% growth inhibition. A value of 0 means no net growth over the course of the experiment. A value of −40 would mean 40% lethality. A value of −100 means all cells are dead. The one-dose data of all the screened compounds is given in Figs. S1–S5 (see Supporting Information).

**Table 1.** Single dose screening of selected compounds at 10 μM on various tumor cell lines.

| Panel/Cell lines | Compounds (% Inhibition) | | | | |
|---|---|---|---|---|---|
| | **9d** | **14f** | **14h** | **14k** | **14l** |
| *Leukemia* | | | | | |
| CCRF-CEM | 19 | 16 | 29 | | |
| HL-60(TB) | 43 | 18 | 10 | | |
| K-562 | | 18 | 52 | | |
| MOLT-4 | 40 | 27 | 27 | 17 | 16 |
| RPMI-8226 | 32 | 16 | 20 | | |
| SR | | 37 | 71 | | 11 |
| *Non-Small Cell Lung Cancer* | | | | | |
| A549/ATCC | | 10 | 10 | 12 | |
| HOP-62 | | | 12 | | |
| HOP-92 | | | 20 | | |
| NCI-H226 | 13 | | | | |
| NCI-H23 | 12 | | 10 | | |
| NCI-H522 | 34 | 17 | 11 | 15 | 14 |
| *Colon Cancer* | | | | | |
| HCT-116 | 11 | 11 | 29 | | |
| HT29 | 10 | 11 | 37 | | 10 |
| *CNS Cancer* | | | | | |
| SF-268 | 18 | | | | |
| SF-539 | 17 | | | | |
| SNB-19 | | | 13 | | |
| SNB-75 | 12 | 19 | | 29 | 18 |
| U251 | | | 12 | | |
| *Melanoma* | | | | | |
| LOX IMVI | | 12 | 37 | | |
| MALME-3M | 15 | | | | |
| SK-MEL-2 | 20 | | | | |
| SK-MEL-5 | 31 | | | | |
| UACC-257 | 12 | 11 | | | |
| UACC-62 | 22 | 11 | 12 | | |
| *Ovarian Cancer* | | | | | |
| IGROV1 | 24 | 18 | 10 | | |
| OVCAR-3 | | | 15 | | |
| OVCAR-4 | | | 15 | | |
| OVCAR-8 | | | 25 | | |
| SK-OV-3 | | | 10 | | |
| *Renal Cancer* | | | | | |
| A498 | | 23 | 17 | | |
| UO-31 | 29 | 20 | 12 | | |
| *Breast Cancer* | | | | | |
| MCF7 | 29 | | | | |
| T-47D | 18 | 17 | | | |
| MDA-MB-468 | 22 | 21 | 48 | 14 | 25 |

## Conclusion

We have successfully synthesized 15 novel cyclic enediyne derivatives using straightforward and well established synthetic protocols. The selective compounds were screened for their anticancer activity which showed marginal to good inhibition for the growth of tumor cells for several cell lines. To the best of our knowledge, this the first report where such an extensive screening for this class of compounds has been done on diversified human tumor cell lines. These initial results show the potential of this class of compound towards anticancer application. However, extensive screening and SAR needs to be done in this direction which is underway in our group.

## Experimental

### Chemistry

All of the chemicals used in the synthesis were purchased from Sigma-Aldrich and were used as such. Thin layer chromatography was used to monitor the progress of the reactions. All the compounds were purified over silica gel column. Solvents were distilled before using for purification purposes. IR spectra were recorded using Perkin-Elmer FT-IR spectrophotometer and the values are expressed as $\lambda_{max}$ $cm^{-1}$. Mass spectral data were recorded on a Jeol (Japan) JMS-DX303 and micromass LCT, Mass Spectrometer/Data system. The $^1$H-NMR was recorded on Bruker Spectrospin spectrometer at 300 MHz, while $^{13}$C-NMR was recorded on Bruker Spectrospin spectrometer at 75.5 MHz and Jeol ECX at 100 MHz using TMS as an internal standard. The chemical shift values are recorded on δ scale and the coupling constants (*J*) are in Hz. Elemental analysis were performed on a Carlo Erba Model EA-1108 elemental analyzer and data of C, H and N is within ±0.4% of calculated values.

Compounds **10** and **11** were prepared by previously reported method [24]. 3-Phenyl-acrylic acid derivatives **13a**, **13b**, **13d**, **13e**, **13i**, **13j**, **13k** [28] **13m** [29], and **13o** [30] were synthesized by literature method. Rests of acids were prepared by condensation of malonic acid with corresponding benzaldehydes following the literature procedure.

#### *1,8-Bis-(tetrahydropyran-2-yloxy)-oct-4-ene-2,6-diyne (**3**) [27]*

The title compound was prepared by literature method [27]. A mixture of $Pd(PPh_3)_4$ (0.95 g, 0.82 mmol), CuI (0.78 g, 4.12 mmol), *n*-butylamine (7.5 mL, 100 mmol), anhydrous benzene (5 mL), and 2-prop-2-ynyloxy-tetrahydro-pyran (**2**) (6.06 g, 43.2 mmol) was stirred at room temperature for 15 min under inert atmosphere. A solution of *cis*-dichloroethene (2.0 g, 20.8 mmol) (**1**) in anhydrous benzene (2 mL) was added to this reaction mixture drop wise (Scheme 1). The resulting reaction mixture was stirred for 15 h at 45°C. After the completion of reaction excess of solvent removed under reduced pressure, and the crude product thus obtained was purified by $SiO_2$ column using ethyl acetate/hexane as an eluent. Yield: 91%; Yellow viscous liquid ($R_f = 0.5$, 20% ethyl acetate/hexane); DSC: 151.57°C; IR (KBr, $cm^{-1}$): 2942, 2870, 2210, 1440, 1389, 1344, 1201, 1119, 1078, 1023; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 1.42–1.85 (m, 12H, 6 C*H*$_2$), 3.54 (m, 2H), 3.85 (m, 2H), 4.45 (s, 4H), 4.78 (s, 2H), 5.85 (s, 2H); $^{13}$C-NMR (75.5 MHz, $CDCl_3$): 18.60, 25.10, 30.00, 54.30, 61.60, 82.60, 92.60, 96.30, 119.00; ESI-HRMS calculated for $C_{18}H_{24}O_4$: 304.1675. Found: 305.1682 ($M^+ + H$).

#### *1,8-Dibromo-oct-4-ene-2,6-diyne (**4**) [27]*

The title compound was prepared after [27]. $PPh_3$ (5.176 g, 19.7 mmol) was dissolved in dichloromethane (20 mL) and to this solution of $Br_2$ (3.153 g, 19.7 mmol) in dichloromethane (5 mL) was added drop wise under inert atmosphere at 0°C. After 15 min solution of compound **3** (2.0 g, 6.57 mmol) was added to this reaction mixture at the same temperature (Scheme 1). After the complete addition reaction mixture was allowed to stir for 1 h at room temperature. The crude product was purified over silica gel column using 2% ethyl acetate/hexanes as an eluent. Yield: 88%; dark brown liquid ($R_f = 0.6$, 10% ethyl acetate/hexane); DSC: 120°C; IR (KBr, $cm^{-1}$): 3052, 2924, 2856, 2106, 1586, 1440, 1336, 1247, 1161, 1099; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.12 (s, 4H, 2 C*H*$_2$Br), 5.96 (s, 2H, C*H*=C*H*); ESI-HRMS calculated for $C_8H_6Br_2$: 259.8836. Found: 260.8883($M^+ + H$), 262.2472 ($M^+ + 2$), 264.7164 ($M^+ + 4$).

#### *4-[(4-Chloro-phenylimino)-methyl]-benzene-1,3-diol (**7a**)*

To a stirred solution of 2,4-dihydroxybezaldehyde (1.0 g, 7.24 mmol) in (MeOH or EtOH, 20 mL), 4-chloroaniline (0.92 g, 7.24 mmol) was added and reaction mixture was stirred at 65–75°C. Progress of reaction was monitored by TLC. After completion of reaction, excess of solvent was removed from the reaction mixture and product thus obtained was purified by crystallization or over $SiO_2$ column. All dihydroxy imines (**7b–d**) were synthesized similarly. Yield: 75%; mp 129–130°C; IR (KBr, $cm^{-1}$): 3421, 2924, 1624, 1495; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 6.31 (s, 1H) 6.60 (d, 2H, $J = 8.3$ Hz), 7.04 (d, 2H, $J = 8.2$ Hz), 7.43–7.48 (m, 2H), 8.79 (s, 1H), 13.20 (s, 2H); ESI-HRMS calculated for $C_{13}H_{10}ClNO_2$: 247.0400. Found: 248.0475 ($M^+ + H$), 250.0361 ($M^+ + 2$).

#### *4-[(4-Bromo-phenylimino)-methyl]-benzene-1,3-diol (**7b**)*

Yield: 73%; mp 135°C; IR (KBr, $cm^{-1}$): 3436, 2950, 1624, 1491; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 6.28 (s, 1H, Ar-*H*) 6.50 (d, 2H, $J = 8.3$ Hz, Ar-*H*), 7.11 (d, 2H, $J = 8.3$ Hz, Ar-*H*), 7.50–7.59 (m, 2H, Ar-*H*), 8.78 (s, 1H, C*H*=N), 13.68 (s, 2H, 2 O*H*); ESI-HRMS calculated for $C_{13}H_{10}BrNO_2$: 290.9895. Found: 291.9865 ($M^+ + H$), 293.9860 ($M^+ + 2$).

#### *4-Phenyliminomethyl-benzene-1,3-diol (**7c**)*

Yield: 60%; mp 106–108°C; IR (KBr, $cm^{-1}$): 3374, 2872, 1627, 1498; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 6.30 (s, 1H, Ar-*H*) 7.25–7.43 (m, 5H, Ar-*H*), 7.45–7.54 (m, 2H, Ar-*H*), 8.79 (s, 1H, C*H*=N), 13.54 (s, 2H, 2 O*H*); ESI-HRMS calculated for $C_{13}H_{11}NO_2$: 213.0790. Found: 214.0778 ($M^+ + H$).

#### *4-[(4-Fluoro-phenylimino)-methyl]-benzene-1,3-diol (**7d**)*

Yield: (79.09%), mp 145°C (decomposed); IR (KBr, $cm^{-1}$): 3425, 2923, 2854, 1629, 1464; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 6.31 (s, 1H, Ar-*H*), 6.42 (d, 2H, $J = 7.9$ Hz, Ar-*H*), 7.24 (d, 2H, $J = 7.7$ Hz, Ar-*H*), 7.40–7.43 (m, 2H, Ar-*H*), 8.76 (s, 1H, C*H*=N), 13.36 (s, 2H, 2 O*H*); ESI-HRMS calculated for $C_{13}H_{10}FNO_2$: 231.0696. Found: 232.0687 ($M^+ + H$).

#### *4-Chloro-N-[(E)-(6Z)-2,11-dioxabicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8 diyn-13-ylmethyl idene]aniline (**8a**) [19]*

A mixture of **7a** (0.5 g, 2.02 mmol) and $K_2CO_3$ (2.79 g, 20.2 mmol) in 15 mL dry DMF was stirred at room temperature for 25 min under nitrogen atmosphere. To this suspension 1,8-dibromooct-4-ene-2,6-diyne (**4**) (0.53 g, 2.02 mmol) in 10 mL dry DMF was added drop wise and reaction mixture was stirred at room temperature under nitrogen atmosphere for 13 h. After completion of the reaction the reaction mixture was poured in 50 mL water and product was extracted with $CHCl_3$ (6 × 20 mL). Combined organic layer was washed with distilled water (8 × 75 mL) and finally dried over anhydrous $Na_2SO_4$. After filtration excess of solvent was removed under reduced pressure. The crude product was purified over $SiO_2$ column using ethyl acetate in hexanes as an eluent. Yield: 45%; mp 198–200°C (dec.);

DSC: 182.70°C; IR (KBr, $cm^{-1}$): 2924, 2854, 2202, 1606, 1457, 1376, 1266, 1100; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.97 (s, 2H, C$H_2$OAr), 5.01 (s, 2H, C$H_2$OAr), 5.92 (s, 2H), 6.68 (m, 1H, Ar-*H*), 7.13 (m, 1H, Ar-*H*), 7.33 (d, 2H, *J* = 8.5 Hz, Ar-*H*), 7.37 (m, 1H, Ar-*H*), 8.11 (d, 2H, *J* = 8.6 Hz, Ar-*H*), 8.75 (s, 1H, C*H*=N); ESI-HRMS calculated for $C_{21}H_{14}ClNO_2$: 347.0713. Found: 348.0724 ($M^+$ + H), 350.0676 ($M^+$ + 2).

### *4-Bromo-N-[(E)-(6Z)-2,11-dioxabicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8-diyn-13-ylmethyl idene]aniline (**8b**) [19]*

Yield: 34%; mp 197–200°C (dec.); DSC: 188.92°C; IR (KBr, $cm^{-1}$): 2924, 2861, 2202, 1606, 1579, 1457, 1363, 1265, 1100, 1002; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.97 (s, 2H, C$H_2$OAr), 5.07 (s, 2H, C$H_2$OAr), 5.93 (s, 2H), 6.71 (m, 1H, Ar-*H*), 7.08 (m, 1H, Ar-*H*), 7.37 (m, 1H, Ar-*H*), 7.48 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 8.11 (d, 2H, *J* = 8.7 Hz, Ar-*H*), 8.74 (s, 1H, C*H*=N); ESI-HRMS calculated for $C_{21}H_{14}NO_2Br$: 391.0208. Found: 392.0212 ($M^+$ + H), 394.0178 ($M^+$ + 2).

### *N-[(E)-(6Z)-2,11-Dioxabicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8-diyn-13-ylmethyl idene] aniline (**8c**) [19]*

Yield: 30%; mp 159–160°C (dec.); DSC 141.86°C; IR (KBr, $cm^{-1}$): 2923, 2856, 2202, 1603, 1588, 1503, 1452, 1370, 1264, 1098, 1004; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.95 (s, 2H, *CH*$_2$OAr), 4.99 (s, 2H, C$H_2$OAr), 5.91 (s, 2H,), 6.62–6.70 (m, 2H, Ar-*H*), 7.17–7.24 (m, 5H, Ar-*H*), 7.36 (m, 1H, Ar-*H*), 8.13 (s, 1H, C*H*=N); $^{13}$C-NMR (100 MHz, $CDCl_3$): 56.85, 56.97, 86.72, 86.99, 90.62, 91.06, 102.24, 111.95, 112.29, 115.06, 121.01, 122.56, 122.96, 123.04, 129.00, 129.24, 15.65, 157.53, 162.45; ESI-HRMS calculated for $C_{21}H_{15}NO_2$: 313.1103. Found: 314.1118 ($M^+$ + H).

### *4-Fluoro-N-[(E)-(6Z)-2,11-dioxabicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8-diyn-13-ylmethyl idene]aniline (**8d**) [19]*

Yield: 32%; mp 180–182°C (dec.); DSC: 188.90°C; IR (KBr, $cm^{-1}$): 3354, 2922, 2861, 2211, 1580, 1467, 1371, 1270, 1090; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 5.00 (s, 2H, C$H_2$OAr), 5.05 (s, 2H, C$H_2$OAr), 5.96 (s, 2H), 6.71 (dd, 1H, *J* = 8 Hz, 1.5 Hz, Ar-*H*), 7.10 (d, 2H, *J* = 6 Hz, Ar-*H*), 7.18 (d, 2H, *J* = 6 Hz, Ar-*H*), 7.36 (d, 1H, *J* = 1.5 Hz, Ar-*H*), 8.12 (d, 1H, *J* = 6.6 Hz, Ar-*H*), 8.78 (s, 1H, C*H*=N); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ = 56.87, 57.13, 86.74, 87.03, 90.98, 91.07, 102.11, 112.29, 115.55, 115.78, 119.09, 122.29, 122.38, 122.57, 122.69, 129.14, 155.39, 157.51, 160.06; ESI-HRMS calculated for $C_{21}H_{14}FNO_2$: 331.1009. Found: 332.1013 ($M^+$ + H).

### *4-Chloro-N-[(6Z)-2,11-dioxabicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8-diyn-13-ylmethyl] aniline (**9a**) [19]*

To a solution of **8a** (0.20 g, 0.57 mmol) in 10 mL dry methanol or dry THF, $NaBH_4$ (0.065 g, 1.71 mmol) was added at room temperature and reaction mixture was stirred for 2 h. After completion of reaction excess of solvent was removed under reduced pressure and crude product was purified by $SiO_2$ column using ethyl acetate/hexane as an eluent. Yield: 60%; mp 185–187°C; DSC: 143.27°C; IR (KBr, $cm^{-1}$): 3383, 2920, 2864, 2211, 1593, 1502, 1460, 1270, 1098, 1006; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.01 (br, 1H, N*H*); 4.22 (s, 2H, NC$H_2$Ph), 4.89 (s, 2H, C$H_2$OAr), 4.97 (s, 2H, C$H_2$OAr), 5.90 (s, 2H), 6.54–6.61 (m, 2H, Ar-*H*), 7.11 (m, 2H, Ar-*H*), 7.22 (m, 2H, Ar-*H*), 7.38 (s, 1H, Ar-*H*); ESI-HRMS calculated for $C_{21}H_{16}ClNO_2$: 349.0870. Found: 350.0876 ($M^+$ + H), 352.0851 ($M^+$ + 2).

### *4-Bromo-N-[(6Z)-2,11-dioxabicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8-diyn-13-ylmethyl] aniline (**9b**) [19]*

Yield: 71%; mp 186–190°C (decomposed); DSC: 149.26°C; IR (KBr, $cm^{-1}$): 3401, 2921, 2854, 2202, 1593, 1501, 1450, 1268, 1097, 1006; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.12 (s, 2H, NC$H_2$Ph), 4.68 (brs, 1H, *NH*); 5.01 (s, 2H, C$H_2$OAr), 5.12 (s, 2H, C$H_2$OAr), 5.92 (s, 2H), 6.13–6.21 (m, 2H, Ar-*H*), 6.52 (m, 2H, Ar-*H*), 7.16 (s, 2H, Ar-*H*); 7.30 (m, 1H, Ar-*H*); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ = 40.59, 56.71, 57.93, 86.47, 91.27, 91.86, 103.89, 111.49, 112.28, 114.52, 120.86, 121.78, 122.54, 122.75, 130.22, 131.81, 132.01, 147.24, 154.59, 156.78; ESI-HRMS calculated for $C_{21}H_{16}BrNO_2$: 393.0364. Found: 394.0356 ($M^+$ + H), 396.0340 ($M^+$ + 2).

### *N-[(6Z)-2,11-Dioxabicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8-diyn-13-ylmethyl]aniline (**9c**) [19]*

Yield: 45%; mp 159–162°C (decomposed); DSC: 139.19°C; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.18 (s, 2H, NC$H_2$Ph); 4.53 (br, 1H, N*H*), 4.81 (s, 2H, C$H_2$OAr), 4.89 (s, 2H, C$H_2$OAr), 5.82 (s, 2H), 6.56–6.59 (m, 5H, Ar-*H*), 7.09–7.31 (m, 3H, Ar-*H*); ESI-HRMS calculated for $C_{21}H_{17}NO_2$: 315.1259. Found: 316.1270 ($M^+$ + H).

### *4-Fluoro-N-[(6Z)-2,11-dioxabicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8-diyn-13-ylmethyl] aniline (**9d**) [19]*

Yield: 65%; mp 157–160°C (decomposed); DSC: 124.31°C; IR (KBr, $cm^{-1}$): 3400, 2922, 2871, 2202, 1605, 1507, 1454, 1266, 1216, 1099, 1003; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.21 (s, 2H, NC$H_2$Ph), 4.89 (s, 2H, C$H_2$OAr), 4.97 (s, 2H, C$H_2$OAr), 5.02 (brs, 1H, N*H*); 5.92 (s, 2H), 6.55–6.62 (m, 2H, Ar-*H*), 7.89 (m, 2H, Ar-*H*), 7.24 (m, 2H, Ar-*H*), 7.38 (s, 1H, Ar-*H*); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ = 43.59, 56.83, 57.89, 86.45, 86.67, 91.34, 91.87, 103.99, 111.50, 113.84, 115.40, 115.62, 122.45, 129.12, 130.30, 130.60, 148.82, 154.61, 155.38; ESI-HRMS calculated for $C_{21}H_{16}FNO_2$: 333.1165. Found: 334.1156 ($M^+$ + H).

### *3-m-Tolyl-acrylic acid (**13c**)*

To a stirred solution of malonic acid (1.29 g, 12.5 mmol) in pyridine (7 mL) at room temperature was added *m*-tolualdehyde (1.0 g, 8.32 mmol) in pyridine (7 mL). Piperidine (2 mL) was added to the solution. The reaction mixture was heated at 118–120°C for 4 h and was cooled to 0°C. Then 3 N HCl was added to adjust the pH value to 3, and the mixture was stirred at 0°C for another 1 h. After suction filtration, the filtrate was crystallized in acetone to yield the title compound. Yield: 88%; mp: 115–118°C; IR (KBr, $cm^{-1}$): 2923, 2832, 2545, 1683, 1624, 1423, 1312, 987, 812, 495; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 2.3 (s, 3H, PhC$H_3$), 6.38 (d, 1H, *J* = 15 Hz), 7.22 (m, 3H, Ar-*H*), 7.67 (d, 1H, *J* = 8 Hz), 7.78 (d, 1H, *J* = 15 Hz), 12.43 (brs, 1H, COO*H*) ESI-HRMS calculated for $C_{10}H_{10}O_2$: 162.0681. Found: 163.0651 ($M^+$ + H).

### *3-(3-Chloro-phenyl)-acrylic acid (**13f**)*

Yield: 83%; mp: 176–177°C; IR (KBr, $cm^{-1}$): 2969, 2826, 2715, 2607, 1686, 1633, 1586, 1489, 1419, 1251; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 6.57 (d, 1H, *J* = 15 Hz), 7.38–7.45 (m, 2H, Ar-*H*), 7.53 (d, 1H, *J* = 15 Hz), 7.63–7.65 (m, 1H, Ar-*H*), 7.78 (s, 1H, Ar-*H*), 12.50 (brs, 1H, COO*H*); ESI-HRMS calculated for $C_{10}H_7ClO_2$: 182.0135. Found: 183.0181 ($M^+$ + H), 185.4398 ($M^+$ + 2).

### *3-(2-Chloro-phenyl)-acrylic acid (**13g**)*

Yield: 80%; mp: 209–210°C; IR (KBr, $cm^{-1}$): 2924, 2835, 2525, 1686, 1621, 1590, 1470, 1440, 1278; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 6.57 (d, 1H, *J* = 15 Hz), 7.35–7.45 (m, 2H, Ar-*H*), 7.51 (d, 1H, *J* = 15 Hz), 7.84–7.92 (m, 2H, Ar-*H*), 12.65 (brs, 1H, COO*H*); ESI-HRMS calculated for $C_{10}H_7ClO_2$: 182.0135. Found: 183.0132 ($M^+$ + H), 185.6241 ($M^+$ + 2).

### *3-(3-Fluoro-phenyl)-acrylic acid (**13h**)*

Yield: 85%; mp: 167°C; IR (KBr, $cm^{-1}$): 2969, 2826, 2715, 2607, 1686, 1633, 1586, 1489, 1419, 1251; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 6.57 (d, 1H, *J* = 15 Hz), 7.20–7.26 (m, 1H), 7.43–7.60 (m, 4H), 12.65 (brs, 1H, COO*H*); ESI-HRMS calculated for $C_{10}H_7FO_2$: 166.0433. Found: 167.0642 ($M^+$ + H).

### *3-(3-Nitro-phenyl)-acrylic acid (**13l**)*

Yield: 75%; mp: 203°C; IR (KBr, $cm^{-1}$): 3082–2539, 1689, 1631, 1523, 1418, 1361, 1306, 1224; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 6.52 (d, 1H, *J* = 15 Hz), 7.45 (d, 2H, *J* = 9 Hz), 7.54 (d, 1H, *J* = 15 Hz), 7.70 (d, 2H, *J* = 9 Hz), 12.46 (s, 1H, COO*H*); ESI-HRMS calculated for $C_9H_7NO_4$: 193.0374. Found: 194.0358 ($M^+$ + H).

### *3-(3-Bromo-phenyl)-acrylic acid (**13n**)*

Yield: 83%; mp: 174°C; (KBr, $cm^{-1}$): 2921–2604, 1686, 1630, 1420, 1318, 1221, 978, 783; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 6.67 (d, 1H, *J* = 15 Hz), 7.38–7.42 (m, 2H, Ar-*H*), 7.67 (d, 1H, *J* = 15 Hz), 7.70–7.72 (m, 1H, Ar-*H*), 7.78–7.90 (s, 1H, Ar-*H*), 12.50 (brs, 1H, COO*H*); ESI-HRMS calculated for $C_{10}H_7BrO_2$: 225.9629. Found: 226.9631 ($M^+$ + H), 228.6247 ($M^+$ + 2).

### *3-(3-Fluoro-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14h**)*

To a stirred solution of acid **13h** (100 mg, 0.56 mmol) in dry THF (10 mL), CDI (130 mg, 8.42 mmol) was added in dry THF under inert atmosphere. The mixture was stirred at 45°C for 15 min. The solution of **11** (90 mg, 0.56 mmol) and DBU (90 mg, 0.61 mmol) in dry THF (10 mL) was then added quickly. The resulting mixture was stirred at 45°C for 6 h. Excess of solvent was evaporated to afford a residue, which was subjected to column chromatography to provide compound **14h**. Yield: 55%; DSC: 141.35°C; IR (film, $cm^{-1}$): 3062, 2923, 2853, 2208, 1705, 1639, 1612, 1586, 1510, 1458, 1323; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.92 (s, 2H, C$H_2$OAr), 4.99 (s, 2H, C$H_2$OAr), 5.24 (s, 2H, ArC$H_2$O), 5.91 (s, 2H, C*H*=C*H*), 6.43 (d, 1H, *J* = 15 Hz, C*H*COO), 6.64 (m, 1H, Ar-*H*), 7.04–7.10 (m, 1H, Ar-*H*), 7.18–7.41 (m, 5H, Ar-*H*), 7.62 (d, 1H, *J* = 15 Hz, C*H*-Ar); $^{13}$C-NMR (75.5 MHz, $CDCl_3$) δ = 57.18, 57.56, 61.67, 86.60, 86.74, 91.43, 91.58, 103.81, 111.58, 114.44, 116.92, 117.20, 118.29, 119.57, 122.10, 122.28, 124.00, 130.44, 131.84, 143.42, 155.42, 157.76, 166.57; ESI-HRMS calculated for $C_{29}H_{17}FO_4$: 388.1111. Found: 389.1642 ($M^+$ + H); Anal. Calcd for $C_{29}H_{17}FO_4$: C, 74.22; H, 4.41. Found: C, 74.33; H, 4.57.

### *3-Phenyl-acrylic acid 2,11-dioxa-bicyclo[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14a**)*

Yield: 50%; IR (KBr, $cm^{-1}$): 2926, 1708, 1638, 1612, 1591, 1510, 1270, 1168; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.84 (s, 2H, C$H_2$OAr), 4.91 (s, 2H, C$H_2$OAr), 5.17 (s, 2H, ArC$H_2$O), 5.83 (s, 2H, C*H*=C*H*), 6.63 (d, 1H, *J* = 15 Hz, C*H*COO), 6.56–6.60 (m, 1H, Ar-*H*), 7.18 (s, 1H, Ar-*H*), 7.29–7.31 (m, 3H, Ar-*H*), 7.34 (d, 1H, *J* = 3 Hz), 7.42–7.45 (m, 2H, Ar-*H*), 7.60 (d, 1H, *J* = 15 Hz, C*H*-Ar); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ = 57.21, 57.54, 61.50, 86.58, 86.70, 91.46, 91.56, 103.84, 111.58, 118.58, 118.44, 122.12, 122.23, 128.05, 128.83, 130.23, 131.80, 134.40, 144.86, 155.38, 157.68, 166.98; ESI-HRMS calculated for $C_{24}H_{18}O_4$: 370.1205. Found: 371.1219 ($M^+$ + H); Anal. cacld for $C_{24}H_{18}O_4$: C, 77.82; H, 4.90. Found: C, 77.99; H, 4.70.

### *3-p-Tolyl-acrylic acid 2,11-dioxa-bicyclo[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14b**)*

Yield 40%; DSC: 167.18°C; IR (KBr, $cm^{-1}$): 2924, 1709, 1609, 1509, 1271, 1165, 1003, 821, 752; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 2.29 (s, 3H, PhC$H_3$), 4.83 (s, 2H, C$H_2$OAr), 4.91 (s, 2H, C$H_2$OAr), 5.16 (s, 2H, ArC$H_2$O), 5.83 (s, 2H, C*H*=C*H*), 6.32 (d, 1H, *J* = 15 Hz, C*H*COO), 6.56–6.59 (m, 1H, Ar-*H*), 7.09–7.11 (m, 2H, Ar-*H*), 7.18 (s, 1H, Ar-*H*), 7.29–7.34 (m, 3H, Ar-*H*), 7.58 (d, 1H, *J* = 15 Hz, C*H*-Ar); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ = 21.44, 57.24, 57.55, 61.40, 86.57, 86.69, 91.49, 91.56, 103.90, 111.59, 116.98, 118.56, 122.11, 122.19, 128.05, 129.56, 131.67, 131.76, 140.64, 144.87, 155.36, 157.64, 167.17; ESI-HRMS calculated for $C_{25}H_{20}O_4$: 384.1362. Found: 385.1592 ($M^+$ + H); Anal. cacld for $C_{25}H_{20}O_4$: C, 78.11; H, 5.24. Found: C, 78.29; H, 5.44.

### *3-m-Tolyl-acrylic acid 2,11-dioxa-bicyclo[10.3.1]-hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14c**)*

Yield: 43%; IR (KBr, $cm^{-1}$): 3054, 2924, 2854, 1707, 1636, 1610, 1275, 1235, 1158, 1008; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 2.35 (s, 3H, PhC$H_3$), 4.91 (s, 2H, C$H_2$OAr), 4.98 (s, 2H, C$H_2$OAr), 5.23 (s, 2H, ArC$H_2$O), 5.90 (s, 2H, C*H*=C*H*), 6.43 (d, 1H, *J* = 15 Hz, C*H*COO), 6.64 (m, 1H, Ar-*H*), 7.17–7.41 (m, 5H, Ar-*H*), 7.65 (d, 1H, *J* = 15 Hz, C*H*-Ar), 7.76 (d, 1H, *J* = 9 Hz, Ar-*H*); ESI-HRMS calculated for $C_{25}H_{20}O_4$: 384.1362. Found: 385.1314 ($M^+$ + H); Anal. cacld. for $C_{25}H_{20}O_4$: C, 78.11; H, 5.24. Found: C, 78.01; H, 5.04.

### *3-o-Tolyl-acrylic acid 2,11-dioxa-bicyclo[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14d**)*

Yield: 39%; IR (film, $cm^{-1}$): 2922, 2847, 1709, 1601, 1449, 1261, 1164, 1013, 802; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 2.36 (s, 3H, PhC$H_3$), 4.84 (s, 2H, C$H_2$OAr), 4.92 (s, 2H, C$H_2$OAr), 5.17 (s, 2H, ArC$H_2$O), 5.83 (s, 2H, C*H*=C*H*), 6.29 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.57 (m, 1H, Ar-*H*), 7.13–7.35 (m, 5H, Ar-*H*), 7.45 (d, 1H, *J* = 6 Hz, Ar-*H*), 7.90 (d, 1H, *J* = 15 Hz, C*H*COO); ESI-HRMS calculated for $C_{25}H_{20}O_4$: 384.1362. Found: 385.1612 ($M^+$ + H); Anal. cacld. for $C_{25}H_{20}O_4$: C, 78.11; H, 5.24. Found: C, 78.50; H, 5.11.

### *3-(4-Chloro-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14e**)*

Yield: 41%; IR (KBr, $cm^{-1}$): 2958, 2925, 1708, 1269, 1166, 1000, 822, 750; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.91 (s, 2H, C$H_2$OAr), 4.95 (s, 2H, C$H_2$OAr), 5.23 (s, 2H, ArC$H_2$O), 5.90 (s, 2H, C*H*=C*H*), 6.41 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.63–6.67 (m, 5H, Ar-*H*), 7.32 (d, 1H, *J* = 9 Hz, Ar-*H*), 7.42 (d, 1H, *J* = 9 Hz, Ar-*H*), 7.61 (d, 1H, *J* = 15 Hz, C*H*COO); ESI-HRMS calculated for $C_{24}H_{17}ClO_4$: 404.0815. Found: 405.0842 ($M^+$ + H), 407.9164 ($M^+$ + 2); Anal. cacld. for $C_{24}H_{17}ClO_4$: C, 71.20; H, 4.23. Found: C, 71.05; H, 4.37.

### *3-(3-Chloro-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14f**)*

Yield: 54%; DSC: 142.22°C; IR (film, $cm^{-1}$): 2924, 2853, 1708, 1609, 1508, 1314, 1267, 1168, 1007, 759; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.91 (s, 2H, C$H_2$OAr), 4.99 (s, 2H, C$H_2$OAr), 5.25 (s, 2H, ArC$H_2$O), 5.90 (s, 2H, C*H*=C*H*), 6.43 (d, 1H, *J* = 18 Hz, C*H*-Ar), 6.64 (m, 1H, Ar-*H*), 7.23–7.41 (m, 5H, Ar-*H*), 7.58–7.61 (m, 1H, Ar-*H*), 8.09 (d, 1H, *J* = 15 Hz, C*H*COO); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ = 57.12, 57.52, 61.68, 86.58, 86.72, 91.40, 91.55, 103.71, 111.52, 118.20, 119.06, 122.11, 122.29, 126.16, 127.80, 130.06, 131.84, 134.82, 136.21, 143.18, 155.39, 157.72, 166.52; ESI-HRMS calculated for $C_{24}H_{17}ClO_4$: 404.0815. Found: 405.0871 ($M^+$ + H), 407.8521 ($M^+$ + 2); Anal. cacld. for $C_{24}H_{17}ClO_4$: C, 71.20; H, 4.23. Found: C, 71.45; H, 4.40.

### *3-(2-Chloro-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14g**)*

Yield: 45%; DSC: 132.44°C; IR (film, $cm^{-1}$): 2924, 2853, 1708, 1609, 1508, 1314, 1267, 1168, 1007, 759; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.92 (s, 2H, C$H_2$OAr), 4.99 (s, 2H, C$H_2$OAr), 5.24 (s, 2H, ArC$H_2$O), 5.91 (s, 2H, C*H*=C*H*), 6.44 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.64 (m, 1H, Ar-*H*), 7.30–7.41 (m, 5H, Ar-*H*), 7.49 (s, 1H, Ar-*H*), 7.60 (d, 1H, *J* = 15 Hz, C*H*COO); ESI-HRMS calculated for $C_{24}H_{17}ClO_4$: 404.0815. Found: 405.0817 ($M^+$ + H), 406.3182 ($M^+$ + 2); Anal. cacld. for $C_{24}H_{17}ClO_4$: C, 71.20; H, 4.23. Found: C, 71.31; H, 4.03.

### *3-(4-iso-Propyl-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14i**)*

Yield: 35%; IR (film, $cm^{-1}$): 2922, 2852, 1709, 1638, 1275, 1165, 1009, 819; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 1.23 (d, 6H, *J* = 6 Hz, PhCH(C$H_3$)$_2$), 2.86–2.96 (m, 1H, PhC*H*(CH$_3$)$_2$), 4.91 (s, 2H, C$H_2$OAr), 4.98 (s, 2H, C$H_2$OAr), 5.23 (s, 2H, ArC$H_2$O), 5.90 (s, 2H, C*H*=C*H*), 6.40 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.63 (m, 1H, Ar-*H*), 7.21–7.50 (m, 6H), 7.66 (d, 1H, *J* = 15 Hz, C*H*COO); ESI-HRMS calculated for $C_{27}H_{24}O_4$: 412.1675. Found: 413.1616 ($M^+$ + H); Anal. cacld. for $C_{27}H_{24}O_4$: C, 78.62; H, 5.86. Found: C, 78.80; H, 5.55.

### *3-(4-Ethyl-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14j**)*

Yield: 40%; IR (KBr, $cm^{-1}$): 2959, 1707, 1635, 1270, 1164, 1109, 1001, 821, 750; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 1.23 (t, 3H, *J* = 6 Hz, PhCH$_2$C$H_3$), 2.62 (q, 2H, *J* = 6 Hz, PhC$H_2$CH$_3$), 4.91 (s, 2H, C$H_2$OAr), 4.95 (s, 2H, C$H_2$OAr), 5.23 (s, 2H, ArC$H_2$O), 5.90 (s, 2H, C*H*=C*H*), 6.40 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.63 (m, 1H, Ar-*H*), 7.19–7.46 (m, 6H, Ar-*H*), 7.66 (d, 1H, *J* = 15 Hz, C*H*COO), ESI-HRMS calculated for $C_{26}H_{22}O_4$: 398.1518. Found: 399.1511 ($M^+$ + H); Anal. cacld. for $C_{26}H_{22}O_4$: C, 78.37; H, 5.57. Found: C, 78.52; H, 5.71.

### *3-Naphthalen-1-yl-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14k**)*

Yield: 43%; IR (film, $cm^{-1}$): 3054, 2925, 2852, 1707, 1611, 1508, 1252, 1165, 1111, 1007; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.92 (s, 2H, C$H_2$OAr), 5.01 (s, 2H, C$H_2$OAr), 5.29 (s, 2H, ArC$H_2$O), 5.90 (s, 2H, C*H*=C*H*), 6.54 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.66–6.68 (m, 1H, Ar-*H*), 7.40–7.57 (m, 5H, Ar-*H*), 7.73–7.90 (m, 3H, Ar-*H*), 8.18 (d, 1H, *J* = 6 Hz, Ar-*H*), 8.52 (d, 1H, *J* = 15 Hz, C*H*COO); ESI-HRMS calculated for $C_{28}H_{20}O_4$: 420.1362. Found: 421.1334 ($M^+$ + H).

### *3-(3-Nitro-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14l**)*

Yield: 52%; DSC: 155.38°C; IR (KBr, $cm^{-1}$): 2964, 1714, 1529, 1354, 1262, 1097, 1020, 800; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.85 (s, 2H, C$H_2$OAr), 5.93 (s, 2H, C$H_2$OAr), 5.19 (s, 2H, ArC$H_2$O), 5.84 (s, 2H, C*H*=C*H*), 6.49 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.57 (m, 1H, Ar-*H*), 7.19–7.35 (m, 4H, Ar-*H*), 7.47–7.53 (m, 1H, Ar-*H*), 7.63 (d, 1H, *J* = 15 Hz, C*H*COOH), 7.72 (d, 1H, *J* = 6 Hz, Ar-*H*); ESI-HRMS calculated for $C_{24}H_{17}NO_6$: 415.1056. Found: 416.1041 ($M^+$ + H); Anal. cacld. for $C_{24}H_{17}NO_6$: C, 69.39; H, 4.12; N, 3.37. Found: C, 69.55; H, 4.01; N, 3.49.

### *3-(4-Bromo-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14m**)*

Yield: 36%; DSC: 178.83°C; IR (KBr, $cm^{-1}$): 2926, 1715, 1638, 1509, 1226, 1163; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.91 (s, 2H, C$H_2$OAr), 4.98 (s, 2H, C$H_2$OAr), 5.23 (s, 2H, ArC$H_2$O), 5.90 (s, 2H, C*H*=C*H*), 6.42 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.63–6.66 (m, 1H, Ar-*H*), 7.32–7.41 (m, 4H, Ar-*H*), 7.49–7.52 (m, 2H, Ar-*H*), 7.60 (d, 1H, *J* = 15 Hz, C*H*COO); ESI-HRMS calculated for $C_{24}H_{17}BrO_4$: 448.0310. Found: 449.0335 ($M^+$ + H), 451.7221 ($M^+$ + 2); Anal. cacld. for $C_{24}H_{17}BrO_4$: C, 64.16; H, 3.81. Found: C, 64.02; H, 3.99.

### *3-(3-Bromo-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14n**)*

Yield: 48%; DSC: 284°C; IR (KBr, $cm^{-1}$): 2963, 2922, 2852, 1710, 1277, 1184, 1115, 1011, 820, 668; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.92 (s, 2H, C$H_2$OAr), 4.99 (s, 2H, C$H_2$OAr), 5.24 (s, 2H, ArC$H_2$O), 5.90 (s, 2H, C*H*=C*H*), 6.43 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.63 (m, 1H, Ar-*H*), 7.25–7.27 (m, 2H, Ar-*H*), 7.33 (d, 1H, *J* = 6 Hz, Ar-*H*), 7.40–7.44 (m, 2H, Ar-*H*), 7.48–7.50 (m, 1H, Ar-H), 7.58 (d, 1H, *J* = 15 Hz, C*H*COO); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ = 57.14, 57.54, 61.71, 86.59, 86.73, 91.40, 103.74, 111.54, 118.22, 119.62, 122.13, 122.96, 126.60, 130.35, 130.76, 131.86, 132.98, 136.50, 143.11, 155.41, 157.75, 166.52; ESI-HRMS calculated for $C_{24}H_{17}BrO_4$: 448.0310. Found: 449.0373 ($M^+$ + H), 451.5791 ($M^+$ + 2); Anal. cacld. for $C_{24}H_{17}BrO_4$: C, 64.16; H, 3.81. Found: C, 64.02; H, 3.99.

### *3-(2-Bromo-phenyl)-acrylic acid 2,11-dioxa-bicyclo-[10.3.1]hexadeca-1(15),6,12(16),13-tetraene-4,8-diyn-13-ylmethyl ester (**14o**)*

Yield: 39%; $^1$H-NMR (300 MHz, $CDCl_3$) δ = 4.92 (s, 2H, $CH_2$OAr), 5.00 (s, 2H, $CH_2$OAr), 5.24 (s, 2H, ArC$H_2$O), 5.91 (s, 2H, C*H*=C*H*), 6.40 (d, 1H, *J* = 15 Hz, C*H*-Ar), 6.63 (m, 1H, Ar-*H*), 7.22–7.42 (m, 5H, Ar-*H*), 7.59 (d, 1H, *J* = 6 Hz, Ar-*H*), 8.03 (d, 1H, *J* = 15 Hz, *CH*COO); ESI-HRMS calculated for $C_{24}H_{17}BrO_4$: 448.0310. Found: 449.0315 ($M^+$ + H), 451.8371 ($M^+$ + 2); Anal. cacld. for $C_{24}H_{17}BrO_4$: C, 64.16; H, 3.81. Found: C, 64.02; H, 3.99.

## Screening of NCI-60 human tumor cell lines

Details of the methodology for NCI 60 cell line screening are described at http://dtp.nci.nih.gov/branches/btb/ivclsp.html. Briefly, the panel is organized into nine sub panels representing diverse histologies: Leukemia, melanoma, and cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. The cells are grown in supplemented RPM1 1640 medium for 24 h. The test compounds were dissolved in DMSO and incubated with cells at 10 μM. The assay is terminated by addition of cold trichloroacetic acid, and the cells are fixed and stained with sulforhodamine B. Bound stain is solubilized, and the absorbance is read on an automated plate reader. The output from the single dose screen was reported as a mean graph (given in the supporting information).

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## References

[1] G. B. Jones, F. S. Fouad, *Curr. Pharm. Des.* **2002**, *8*, 2415–2440.

[2] A. L. Smith, K. C. Nicolaou, *J. Med. Chem.* **1996**, *39*, 2103–2117.

[3] K. C. Nicolaou, W.-M. Dai, *Angew. Chem. Int. Ed.* **1991**, *30*, 1387–1416.

[4] M. Kar, A. Basak, *Chem. Rev.* **2007**, *107*, 2861–2890.

[5] D. S. Rawat, J. M. Zaleski, *Synlett* **2003**, 393–421.

[6] A. Basak, S. Mandal, S. S. Bag, *Chem. Rev.* **2003**, *103*, 4077–4094.

[7] J. S. Thorson, E. L. Sievers, J. Ahlert, E. Shepard, R. E. Whitwam, K. C. Onwueme, M. Ruppen, *Curr. Pharm. Des.* **2000**, *6*, 1841–1879.

[8] G. B. Jones, G. Hynd, J. M. Wright, A. Purohit, G. W. Plourde, II, R. S. Huber, J. E. Mathews, A. Li, M. W. Kilgore, G. J. Bubley, M. Yancisin, M. A. Brown, *J. Org. Chem.* **2001**, *66*, 3688–3695.

[9] S. J. Danishefsky, M. D. J. Shair, *J. Org. Chem.* **1996**, *61*, 16–44.

[10] A. G. Myers, M. E. Fraley, N. J. Tom, S. B. Cohen, D. J. Madar, *Chem. Biol.* **1995**, *2*, 33–43.

[11] J. Davies, H. Wang, T. Taylor, K. Warabi, X.-H. Huang, R. J. Andersen, *Org. Lett.* **2005**, *7*, 5233–5236.

[12] B. Shen, W. Liu, K. Nonaka, *Curr. Med. Chem.* **2003**, *10*, 2317–2325.

[13] B. Tuesuwan, S. M. Kerwin, *Biochemistry* **2006**, *45*, 7265–7276.

[14] U. Galm, M. H. Hager, S. G. van Lanen, J. H. Ju, J. S. Thorson, B. Shen, *Chem. Rev.* **2005**, *105*, 739–758.

[15] Anonymus, Endiine, in *Römpp kompakt Basislexikon der Chemie* (Eds.: J. Falbe, M. Regitz,), 1st Edition, Vol. *11*, Georg Thieme Verlag, Stuttgart **1998**, p. 661.

[16] P. F. Bross, J. Beitz, G. Chen, X. H. Chen, E. Duffy, L. Kieffer, S. Roy, R. Sridhara, A. Rahman, G. William, R. Pazdur, *Clin. Cancer Res.* **2001**, *7*, 1490–1496.

[17] Z. Wang, Q. He, Y. Liang, D. Wang, Y.-Y. Li, D. Li, *Biochem. Pharm.* **2003**, *65*, 1767–1775.

[18] X. Liu, H. He, Y. Feng, M. Zhang, K. Ren, R. Shao, *Anti-Cancer Drugs* **2006**, *17*, 173–179.

[19] M. C. Joshi, G. S. Bisht, D. S. Rawat, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3226–3230.

[20] C.-F. Lin, P.-C. Hsieh, W.-D. Lu, H.-F. Chiub, M.-J. Wu, *Bioorg. Med. Chem.* **2001**, *9*, 1707–1711.

[21] I. Suzuki, H. Shigenaga, M. Nemoto, M. Shibuya, *Tetrahedron Lett.* **2004**, *45*, 1955–1959.

[22] W.-M. Dai, *Curr. Med. Chem.* **2003**, *10*, 2265–2293.

[23] P. J. Benites, R. C. Holmberg, D. S. Rawat, B. J. Kraft, L. J. Klein, D. G. Peters, H. H. Thorp, J. M. Zaleski, *J. Am. Chem. Soc.* **2003**, *125*, 6434–6446.

[24] A. Basak, S. S. Bag, A. Basak, *Bioorg. Med. Chem.* **2005**, *13*, 4096–4102.

[25] C.-F. Lin, Y.-H. Lo, M.-C. Hsieh, Y.-H. Chen, J.-J. Wang, M.-J. Wu, *Bioorg. Med. Chem.* **2005**, *13*, 3565–3575.

[26] D. S. Rawat, J. M. Zaleski, *J. Am. Chem. Soc.* **2001**, *123*, 9675–9676.

[27] B. Koenig, W. Pitsch, I. Dix, P. G. Jones, *Synthesis* **1996**, 446–448.

[28] D. Basavaiah, A. J. Rao, *Synth. Commun.* **2002**, *32*, 195–201.

[29] P. Ghorai, A. Kraus, M. Keller, C. Götte, P. Igel, E. Schneider, D. Schnell, G. Bernhardt, S. Dove, M. Zabel, S. Elz, R. Seifert, A. Buschauer, *J. Med. Chem.* **2008**, *51*, 7193–7204.

[30] D. R. Brittelli, *J. Org. Chem.* **1981**, *46*, 2514–2520.

 