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Syntheses and antibacterial activity of phendioxy substituted cyclic enediynes

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Abstract—Syntheses and antibacterial activity of 13-membered 1,3-phendioxy substituted cyclic enediynes are reported. The compounds were screened against gram-positive and gram-negative strains and some of the compounds exhibit potent antibacterial activity.

Enediyne chemistry has captured the imagination of the chemist and biologist throughout the world since the discovery of natural product enediynes such as calicheamicin, esperamicin, maduroptin, dynamicin, and more recently uncialamycin.[1–4] Some of the natural product enediynes are three order of magnitude more potent than other anticancer drugs. The anticancer activity of these compounds is due to the presence of highly unsaturated 1,5-diyne-3-ene unit that undergoes cycloaromatization and generates benzene-1,4-diradical, which cleaves the DNA.[5,6] Unfortunately, due to modest selectivity for cancer cells clinical use of this class of compounds has been limited. In order to improve the biological activity of the enediynes, efforts are being made to synthesize analogous compounds with better efficacy.[7–12] Apart from anticancer activity, synthetic enediynes are known to exhibit cytotoxicity against various cell lines,[13,14] protein degradation activity[15] and topoisomerase inhibitory activity.[16] Natural product enediynes are known to exhibit potent antibacterial activity against gram-positive and gram-negative strains.[17] To the best of our knowledge, none of the synthetic enediynes were evaluated for antibacterial activity. To this end, we report syntheses and antibacterial activity of substituted 13-membered phendioxy cyclic enediynes.

Scheme 1 illustrates two different strategies for the preparation of the 13-membered cyclic enediynes.

Reaction of 2,4-dihydroxybenzaldehyde (**2**) with 1,8-dibromo-oct-4-ene-1,6-diyne (**5**)[18] in presence of K_2CO_3 afforded desired 13-membered cyclic enediyne (**7**) in 62% yield, which on reduction with $NaBH_4$ in MeOH affords corresponding alcohol (**8**). However, under identical reaction conditions, reaction of resorcinol (**1**) with 1,8-dibromo-oct-4-ene-1,6-diyne (**5**)[18] results in the decomposition of the dibromoenediyne precursor. Alternatively, the desired 13-membered cyclic enediyne (**6**) was synthesized by different approach, using propargyl bromide and resorcinol as starting material (Scheme 1).

Reaction of resorcinol (**1**) with propargyl bromide in dry DMF in the presence of K_2CO_3 leads to the formation of **3** in 92% yield. Sonogashira coupling of 1.0 equivalents of **3** with *cis*-1,2-dichloroethylene over a $Pd(PPh_3)_4$ catalyst in the presence of CuI and $BuNH_2$ generates **6** in 66% yield. The identity of these compounds was confirmed spectroscopically. Compounds **6**, **7**, and **8** were tested against gram-positive and gram-negative strains and these compounds exhibit potent antibacterial activity (Table 1). In order to study the effect of different substituents in the aromatic ring on antibacterial activity, we planned to derivatise compound **7**. Surprisingly, reaction of cyclic enediyne **7** with primary amines did not work even at elevated temperatures. Then desired enediynes (**13**–**20**) were prepared by different approach as shown in Scheme 3. Imines (**9**–**12**) used for the synthesis of cyclic enediynes were prepared as shown in Scheme 2. Reaction of equimolar amount of aldehyde (**2**) with different amines, under anhydrous conditions, yielded substituted imines in good yields. All imine

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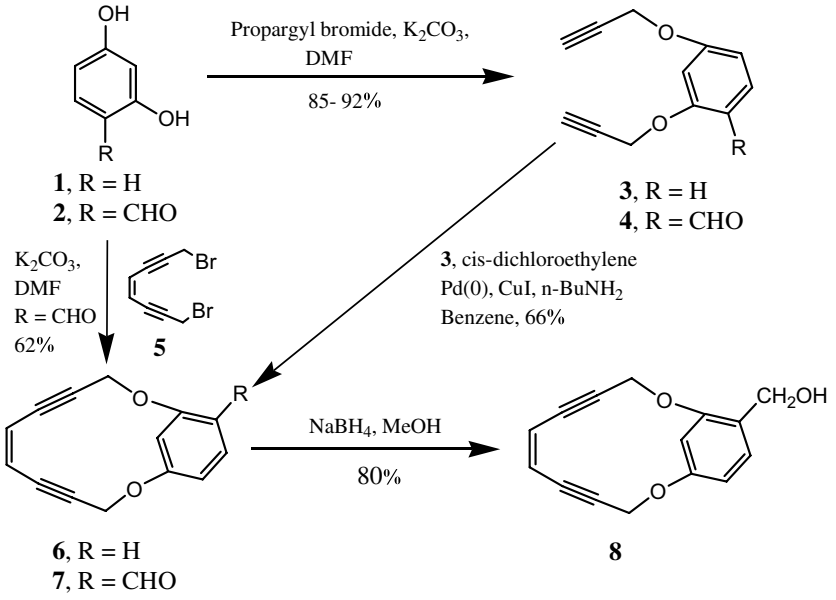


Scheme 1.

Table 1. Antibacterial activity of cyclic enediynes

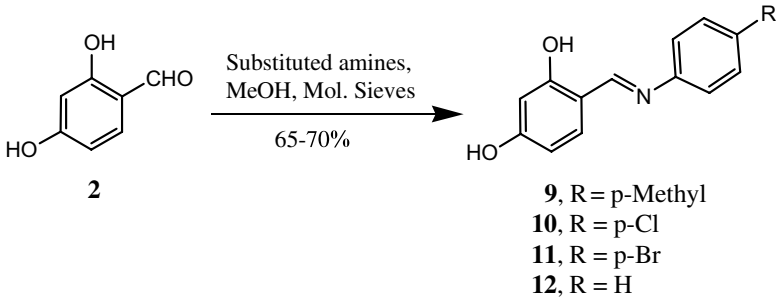
S. No.	MIC (μg/mL)			
	P. aeruginosa (ATCC, 11775)	*E. coli* (ATCC, 25668)	*S. aureus* (clinical isolate)	*S. typhimurium* (clinical isolate)
3	>1000	>1000	>1000	>1000
4	>1000	>1000	>1000	>1000
6	na	4	na	8
7	na	256	8	128
8	na	8	4	128
13	na	256	na	128
14	nd[a]	nd[a]	nd[a]	nd[a]
15	na	na	na	128
16	128	128	128	64
17	32	4	16	128
18	na	64	na	512
19	na	128	na	na
20	16	4	8	16
21[b]	1	2	2	0.5

na, MIC no activity up to 512 μg/mL.
[a] MIC not determined.
[b] Tetracycline was used as a reference compound.

derivatives were purified over SiO_2 column and characterized spectroscopically.

Reaction of substituted imines (**9**–**12**) with 1,8-dibromo-oct-4-ene-1,6-diyne (**5**)[18] in presence of K_2CO_3 in dry DMF leads to the formation of desired products in moderate yields as shown in Scheme 3. Reaction completes in 8–10 h and excess of DMF from the reaction mixture was removed at reduced pressure. The residue thus obtained was washed with water and crude product was purified over SiO_2 column. The cyclic imine enediynes (**13**–**16**) were reduced to amine derivatives (**17**–**20**) using $NaBH_4$ in methanol. After usual work-up, these compounds were purified by SiO_2 column and characterized spectroscopically.[19]

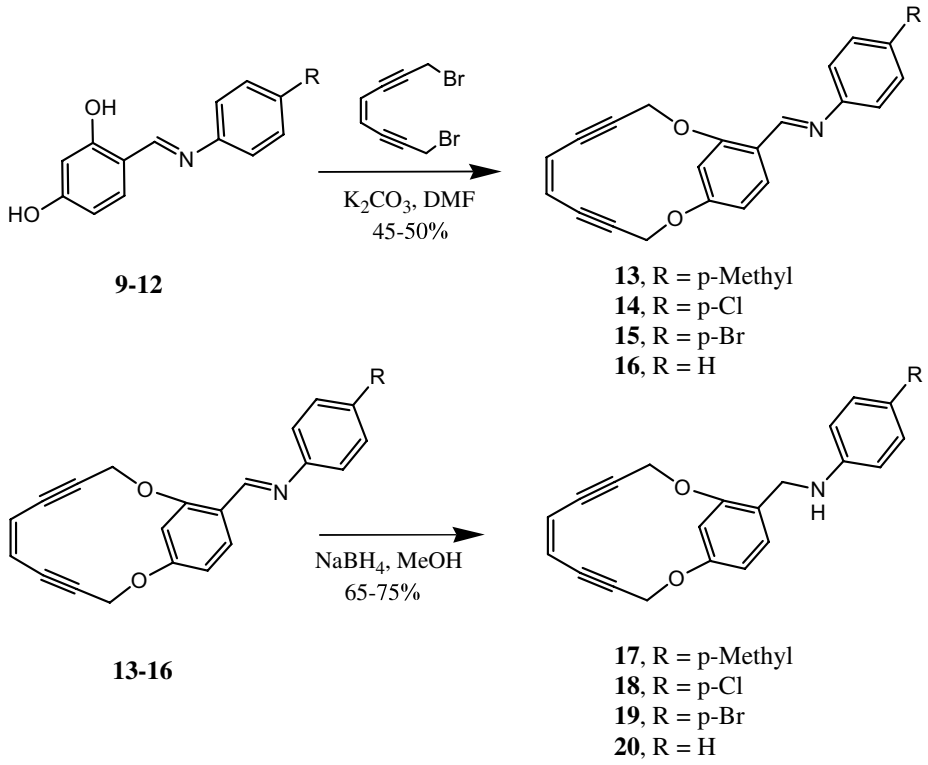
In vitro antibacterial activity: Antimicrobial susceptibility testing was carried out using National Committee for Clinical Laboratory Standards (NCCLS) microdilution broth assay. Briefly, the bacterial strains were grown in Mueller Hinton Broth (HIMEDIA) until exponential growth was achieved. Test compounds were dissolved in DMSO/Water to make a series of two fold dilution. 90 μl of $2–7 \times 10^5$ CFUs/mL of bacterial sample per mL of Mueller–Hinton broth (HIMEDIA) was dispensed into 96-well polypropylene microtitre plate (SIGMA). Then 10 μl of serially diluted compounds was added. The microtitre plates were incubated overnight at 37 °C and the absorbance was read at 630 nm. Uninoculated Mueller–Hinton broth was used as negative control. The tests were carried out in triplicate. The minimum inhibitory concentration (MIC) is taken



2

9, R = p-Methyl
10, R = p-Cl
11, R = p-Br
12, R = H

Scheme 2.



9-12

13, R = p-Methyl
14, R = p-Cl
15, R = p-Br
16, R = H

13-16

17, R = p-Methyl
18, R = p-Cl
19, R = p-Br
20, R = H

Scheme 3.

as the lowest concentration of compound that inhibits 50% growth of microorganism. The compounds were evaluated against gram-positive and gram-negative bacterial strains. Minimum inhibitory concentration (MIC) of each compound is shown in Table 1.

Compounds **6**, **7** and **8** displayed varied activity against *Escherichia coli* and *Salmonella typhimurium* but only compounds **7** and **8** (in which R group is –CHO and $–CH_2OH$, respectively) exhibit potent activity against *Salmonella aureus*. Higher activity of **8** as compared to **7** confers that terminal hydroxyl group is somehow playing a role in killing mechanism. In the series of cyclic enediyne compounds (**13**–**16**) compounds **13** and **16** displayed weak activity against *E. coli* and *S. typhimurium* but activity in case of compound **16** (where R = H) increases twofold higher than compound **13**. All of the cyclic amine endiynes displayed higher MIC values as compared to their cyclic imine counterparts. For example, compound **17** possess much lower MIC value than compound **13**. In similar manner compound **20** has much higher antimicrobial activity than compound **16**. Moreover only compounds **17** and **20** have potent activity against *Pseudomonas aeruginosa*. This suggests unsaturation around nitrogen is not required for antimicrobial activity of these synthetic compounds. Tetracycline is used as standard drug for antimicrobial activity. All tested compound showed varied activity against different bacterial strains. The antimicrobial activity data indicate that compounds **6**, **8**, **17**, and **20** were most effective and others displayed moderate activity while **15**, **18**, and **19** exhibited scarce activity. Compound **20** was found to be the most active among this series. In order to understand the origin of antibacterial activity of these cyclic enediynes, antibacterial activity of control compounds **3**, and **4** were determined. Compound **3** has been used as an intermediate in the synthesis of cyclic enediyne (**6**), and simple

resorcinol ethers (**3**, **4**)[20,21] were found inactive upto 1000 μg/mL. So, we believe that the antibacterial activity of these cyclic enediynes is due to the presence of enediyne moiety, not due to the resorcinol ether. Moreover studies are currently underway to further optimize these compounds against bacterial infection.

In conclusion, we have shown for the first time that synthetic enediynes can have potential in the treatment of microbial infections apart from their use in the anticancer drug discovery programme.

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19. *Synthesis of 1,3-bis-prop-2-ynyloxy-benzene* (**3**). Mixture of resorcinol (1.0 g, 9.1 mmol) and K_2CO_3 (12.5 g, 90.5 mmol) was stirred in 40 mL dry DMF at room temperature for 30 min. Then propargyl bromide (2.32 g, 24.4 mmol) was added dropwise and reaction mixture was stirred at room temperature for 7 h. The progress of reaction was monitored by TLC, and reaction mixture was extracted with $CHCl_3$ (8 × 30 mL) and H_2O (8 × 300 mL). Combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The excess of solvent was evaporated under vacuo. Residue was purified by column chromatography (15% ethyl acetate in hexane). Yield 1.55 g (92%); IR (KBr, cm^{-1}): 2923, 2122, 1148; ^1H NMR (300 MHz, $CDCl_3$): 2.62 (s, 2H), 4.75 (s, 4H), 6.62–6.78 (m, 3H), 7.19–7.23 (m, 1H); MS (*m*/*z*): 186 (M^+), 161, 147.
Synthesis of 2,11-dioxa-bicyclo [10.3.1] hexadeca-1(15),6,12(16),13-tetra-ene-4,8-diyne (**6**). To a suspension of $Pd(PPh_3)_4$ (224 mg, 0.19 mmol), CuI (164 mg, 0.86 mmol) and *n*-butylamine (1.58 g, 21.5 mmol) in anhydrous benzene (30 mL), solution of **3** (800 mg, 4.3 mmol) in 5 mL benzene was added dropwise and reaction mixture was stirred for 20 min. Then *cis*-dichloroethylene (420 mg, 4.3 mmol) was added by syringe under nitrogen atmosphere at 40 °C and reaction mixture was stirred for 13 h at same temperature. Reaction mixture was washed with cold hexanes and solvent was removed under reduced pressure. The crude product thus obtained was purified by SiO_2 column (10% ethyl acetate in hexane). Yield 600 mg (66%); IR (KBr, cm^{-1}): 2922, 2211, 1148; ^1H NMR (300 MHz, $CDCl_3$): 4.86 (s, 4H), 5.90 (s, 2H), 6.43 (d, J = 7.4 Hz 1H), 6.63–6.66 (m, 2H), 7.18 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): 57.2 (OCH_2), 81.49 (Cquart), 92.49 (Cquart), 103.06 (CH), 108.48 (CH), 111.80 (CH), 130.06 (CH), 130.34 (CH), 159.25 (Cquart); MS (*m*/*z*): 210 (M^+); Anal. calcd for $C_{14}H_{10}O_2$: C,79.98; H, 4.79. Found: C, 80.09; H, 4.99.
Synthesis of 2,11-dioxabicyclo [10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8-di-yne-13 carboxaldehyde (**7**). To a solution of 2,4-dihydroxybenzaldehyde (2.0 g, 14.5 mmol), K_2CO_3 (10 g, 72.5 mmol) in 25 mL dry DMF, solution of 1,8-dibromo-oct-4-ene-1,6-diyne[18] (3.98 g, 15.2 mmol) in 5 mL DMF was added dropwise. The reaction mixture was stirred at ambient temperature for 8 h. The reaction mixture was extracted with $CHCl_3$ (6 × 25 mL), and the combined organic layer was washed with water (6 × 250 mL). The organic layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The crude product was purified over silica gel column using 10% ethyl acetate in hexanes as an eluent. Yield 2.14 g (62%); mp. 138 °C; IR (KBr, cm^{-1}): 2932, 2225, 1690; ^1H NMR (300 MHz, $CDCl_3$): 5.00 (s, 2H), 5.07 (s, 2H), 5.95 (s, 2H), 6.64 (m, 1H), 7.36 (m, 1H), 7.83 (d, J = 6 Hz, 1H), 10.29 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): 57.03 (OCH_2), 57.45 (OCH_2), 87.45 (Cquart), 87.72 (Cquart), 90.98 (Cquart), 91.11 (Cquart), 101.58 (CH), 112.41 (CH), 120.11 (Cquart), 123.41 (CH), 131.05 (CH), 160.89 (Cquart), 162.92 (Cquart), 188.48 (Cquart); MS (*m*/*z*): 238 (M^+); Anal. calcd for $C_{15}H_{10}O_3$: C, 75.62; H, 4.23. Found: C, 75.91; H, 4.42.
Synthesis of (2,11-dioxabi-cyclo[10.3.1] hexadeca1(15),6,12(16),13-tetra-ene-4,8-diyn-13 yl)methanol (**8**). To a solution of **7** (0.4 g, 1.6 mmol) in 20 mL dry methanol, sodium borohydride (0.2 g, 7.1 mmol) was added at 20 °C and reaction mixture was stirred for 1 h. Solvent from the reaction mixture was removed under reduced pressure and crude product was purified by column chromatography (30% ethyl acetate in hexanes). Yield: 322 mg (80%); mp. 135 °C; IR (KBr, cm^{-1}): 3433, 2208, 1590, 1447, 1268, 1099; ^1H NMR (300 MHz, $CDCl_3$): 1.98 (br s, 1H), 4.64 (s, 2H, CH_2OH), 4.90 (s, 2H, OCH_2), 4.99 (s, 2H, OCH_2), 5.90 (s, 2H), 6.62 (d, J = 6Hz, 1H), 7.22 (m, 1H), 7.38 (s, 1H); MS (*m*/*z*): 240 (M^+), 223; Anal. calcd for $C_{15}H_{12}O_3$: C,74.99; H, 5.03. Found: C, 74.81; H, 5.22.
Synthesis of (2,11-Dioxa-bicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4,8-diyn-13- ylmethylene)-p-tolyl-amine (**13**). Mixture of *p*-tolylimino-methyl-benzene-1,3-diol (**9**) (430 mg, 1.9 mmol) and K_2CO_3 (2.63 g, 19.1 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 (**5**)[18] (500 mg, 1.9 mmol) in 10 mL dry DMF was added dropwise 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 8% ethyl acetate in hexanes as an eluent. Yield: 297 mg(48%). mp 123–125 °C. IR (KBr, cm^{-1}): 2923, 2854, 2202, 1592, 1460, 1377, 1264, 1095; ^1H NMR (300 MHz, $CDCl_3$): 2.36 (s, 3H), 4.97 (s, 2H), 5.01 (s, 2H), 5.93 (s, 2H), 6.71 (dd, $J = 8$ Hz, 1.5 Hz, 1H), 7.10 (d, $J = 6$ Hz, 2H), 7.18 (d, $J = 6$ Hz, 2H), 7.36 (d, $J = 1.5$ Hz, 1H), 8.12 (d, $J = 6.6$ Hz, 1H), 8.78 (s, 1H); MS (*m*/*z*): 240 (M_+); Anal. calcd for $C_{22}H_{17}NO_2$: C, 80.71; H, 5.23; N, 4.28; Found: C, 81.02; H, 5.38; N, 4.49.

Synthesis of 4-chlorophenyl-2,11-dioxa-bicyclo[10.3.1] hexadeca-1(16),6,12,14-tetraene-4,8-diyn-13-yl-methyl)-amine (**18**). To a solution of 4-chlorophenyl-2,11-dioxa-bicyclo[10.3.1]hexadeca-1(16),6,12,14-tetraene-4, 8-diyn-13-yl methylene)-amine (**14**) (150 mg, 0.43 mmol) in 10 mL dry methanol, $NaBH_4$ (87 mg, 1.42 mmol) was added at room temperature and reaction mixture was stirred for 2 h. The excess of solvent was removed under reduced pressure and crude product was purified by SiO_2 (20% ethyl acetate in hexanes). Yield: 100 mg (66%); mp. 185–187 °C; ^1H NMR (300 MHz, $CDCl_3$): 4.01 (br s, 1H, NH); 4.22 (s, 2H, NCH_2Ph), 4.89 (s, 2H, OCH_2), 4.97 (s, 2H, OCH_2), 5.90 (s, 2H), 6.54–6.61 (m, 2H), 7.11 (m, 2H), 7.22 (m, 2H), 7.38 (s, 1H); MS (*m*/*z*): 349 (M^+), 224; Anal. calcd for $C_{21}H_{16}ClNO_2$: C, 72.10; H, 4.61; N, 4.00; Found: C, 72.03; H, 4.91; N, 4.25.

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