Supporting Information

Synthesis and biological evaluation of nitric oxide-releasing hybrids from gemcitabine and phenylsulfonyl furoxans as anti-tumor agents

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Supporting Information

1. The IC_{50} values of 6a-m against five human cancer cell lines

Table S1. The IC_{50} values of **6a-m** against five human cancer cell lines.

Compound	*In vitro* inhibition of human cancer cells proliferation (IC_{50}[a], μM)				
	HepG2	HCT-116	SW-620	A549	SGC7901
Gemcitabine	2.97 ± 0.32	7.33 ± 0.63	5.62 ± 0.47	2.69 ±0.28	3.58 ± 0.33
JS-K	7.42 ± 0.59	3.75 ± 0.44	5.16 ± 0.63	ND[b]	ND
6a	>12.5	>12.5	>12.5	>12.5	ND
6b	11.6 ± 0.32	>12.5	9.62 ± 0.44	>12.5	ND
6c	>12.5	12.3 ± 1.03	11.8 ± 0.67	11.5 ± 0.81	ND
6d	10.8 ± 0.66	>12.5	10.5 ± 0.96	>12.5	ND
6e	8.73 ± 0.71	9.86 ± 0.92	9.09 ± 0.75	>12.5	11.5 ± 1.02
6f	>12.5	>12.5	>12.5	>12.5	ND
6g	11.2 ± 0.73	>12.5	>12.5	>12.5	ND
6h	>12.5	>12.5	>12.5	>12.5	ND
6i	>12.5	>12.5	>12.5	>12.5	ND
6j	10.8 ± 0.97	12.1 ± 0.92	>12.5	11.7 ± 0.84	ND
6k	11.6 ± 0.89	10.8 ± 1.11	>12.5	>12.5	ND
6l	>12.5	>12.5	12.4 ± 0.85	>12.5	ND
6m	>12.5	>12.5	>12.5	>12.5	ND

[a] The inhibitory effects of individual compounds on the proliferation of cancer cell lines were determined by the MTT assay. The data are the mean values of IC_{50} from at least three independent experiments. [b] Not detected.

2. Synthesis

Chemical synthesis materials and instruments

Melting points of individual compounds were determined on a Mel-TEMP II melting point apparatus and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance spectrometer at 300 K, using TMS as an internal standard. MS spectra were recorded on a Mariner Mass Spectrum (ESI). Element analysis was performed on an Eager 300 instrument. Analytical and preparative thin-layer chromatography (TLC) were performed on silica gel GF/UV 254, and the chromatograms were conducted on silica gel (200–300 mesh, Merck) and visualized under UV light at 254 and 365 nm. All analytical grade chemicals and solvents were purchased from commercial sources and used without further purification in our laboratory. Solutions after reactions and extractions were concentrated using a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Compounds with a purity of >95% were determined by high-performance liquid chromatography, and could be used for subsequent experiments. Gemcitabine **7**, dipyridamole, and compound **1** were commercially available, and **5a-m** were synthesized, as previously described.[18, 21]

Synthesis of hybrids from gemcitabine and phenylsulfonyl furoxans

4-(2-((3-Carboxypropanoyl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(6a).

To a solution of compound **5a** (0.29 g, 1.00 mmol) in CH_2Cl_2 (5 mL), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) were added. The mixture was stirred at 40 °C for 5 h and TLC (PE/EtOAc = 1:4, v/v) indicated that the starting material was totally consumed. The reaction mixture to pour into 50 mL water,

extracted with CH_2Cl_2 (3 × 30 mL) , and the extract was dried over Na_2SO_4, and concentrated under reduced pressure to give the crude product and was purified by silica gel column chromatography (PE/EtOAc = 4:1, v/v, as the eluant) to afford light yellow solid, 0.35 g, yield: 90.0 %. MS (ESI) m/z = 387 $[M + H]^+$.

4-(3-((3-Carboxypropanoyl)oxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-Oxide (6b).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5b** (0.30 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g , 1.20 mmol) in 92.0 % yield as a light yellow solid 0.37 g. MS (ESI) m/z = 401 $[M + H]^+$.

4-(4-((3-Carboxypropanoyl)oxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(6c).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5c** (0.31 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 88.0 % yield as a light yellow solid 0.36 g. MS (ESI) m/z = 415 $[M + H]^+$.

4-(3-((3-Carboxypropanoyl)oxy)-2-methylpropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (6d).
The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5d** (0.31 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 85.0 % yield as a light yellow solid 0.35 g. MS (ESI) m/z = 415 $[M + H]^+$.

4-(4-(((3-Carboxypropanoyl)oxy)methyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (6e).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5e** (0.31 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 89.0 % yield as a light yellow solid 0.36 g. MS (ESI) m/z = 411 $[M + H]^+$.

4-((5-((3-Carboxypropanoyl)oxy)pentyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (6f).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5f** (0.31 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 85.0 % yield as a light yellow solid 0.36 g. MS (ESI) m/z = 429 $[M + H]^+$.

4-((4-((3-Carboxypropanoyl)oxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(6g).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5g** (0.33 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 91.2 % yield as a light yellow solid 0.39 g. MS (ESI) m/z = 431 $[M + H]^+$.

4-(2-(2-((3-Carboxypropanoyl)oxy)ethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (6h).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5h** (0.35 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 90.8 % yield as a light yellow solid 0.41 g. MS (ESI) m/z = 449 $[M + H]^+$.

4-(2-(3-Carboxypropanamido)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(6i).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5i** (0.26 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 88.0 % yield as a light yellow solid 0.34 g. MS (ESI) m/z = 386 $[M + H]^+$.

4-(3-(3-Carboxypropanamido)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (6j).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5j** (0.30 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 89.5 % yield as a light yellow solid 0.36 g. MS (ESI) m/z = 400 $[M + H]^+$.

4-(4-(3-Carboxypropanamido)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(6k).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5k** (0.31g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 91.5 % yield as a light yellow solid 0.38 g. MS (ESI) m/z = 400 $[M + H]^+$.

4-(2-(3-Carboxy-*N*-methylpropanamido)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (6l).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5l** (0.30 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 92.7 % yield as a light yellow solid 0.37 g. MS (ESI) m/z = 400 $[M + H]^+$.

4-((5-(3-Carboxypropanamido)pentyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxid(6m).

The target product was synthesized, using a method similar to that used for the preparation of **6a**, compound **5m** (0.31 g, 1.00 mmol), DMAP (0.06 g, 0.50 mmol) and succinic anhydride (0.12 g, 1.20 mmol) in 91.5 % yield as a light yellow solid 0.39 g. MS (ESI) m/z = 428 $[M + H]^+$.

4-Amino-1-((2*R*,4*R*,5*R*)-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)pyrimidin-2(1H)-one(8).

To a solution of compound **7** (2.63 g, 10.00 mmol) in DMF (200 mL), TBDMS-Cl (6.00 g, 40.00 mmol) and imidazole (2.72 g, 40.00 mmol) were added. The mixture was stirred at room temperature for 5 h and TLC (PE/EtOAc = 4:1, v/v) indicated that the starting material was totally consumed. The reaction mixture to pour into 500 mL water, extracted with CH_2Cl_2 (3 × 50 mL), and the extract was dried over Na_2SO_4, and concentrated under reduced pressure to give the crude product and was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 1:20, v/v, as the eluant) to afford white solid 4.28 g, yield: 87.3%. ESI-MS (m/z): 491 $[M+H]^+$.

4-(2-((4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (9a).

To a solution of compound **6a** (0.19 g, 0.50 mmol) in CH_2Cl_2 (10 mL), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) were added. The mixture was stirred at room temperature under N_2 for 2 h and TLC (PE/EtOAc = 1:4, v/v) indicated that the starting material was totally consumed. Compound **8** (0.25 g, 0.50 mmol) was added to the reaction mixture at room temperature under N_2 for 18 h until the TLC indicated that the starting material was totally consumed. The reaction mixture to pour into 50 mL water, extracted with CH_2Cl_2 (3 × 50 mL), and the extract was dried over Na_2SO_4, and concentrated under reduced pressure to give the crude product and was purified by silica gel column chromatography (PE/EtOAc = 1:4, v/v, as the eluant) to afford light yellow solid 0.22 g, yield: 52.0%. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.18 (s, 1H, NH), 8.02 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.00 (d, *J* = 7.5 Hz, 1H, CH=C<u>H</u>N), 7.73 (t, 1H, Ar-H), 7.21 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.22 (d, 1H, C<u>H</u>=CHN), 5.76 (m, 1H, NCH), 5.31 (m, 1H, HOC<u>H</u>), 4.59 (m, 3H, SiOCH, OCH_2), 4.40 (m, 1H, SiOCH), 3.98 (m, 1H, OCH), 3.80 (m, 1H, CH_2OSi), 3.65 (m, 1H, CH_2OSi), 2.74 (t, 2H, $OCCH_2$), 2.63 (t, 2H, $OCCH_2$), 0.89 (m, 18H, 6 × CH_3), 0.11 (s, 12H, 4 × CH_3); ESI-MS (m/z): 860 $[M+H]^+$.

4-(3-((4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(9b).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6b** (0.20 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 57.0 % yield as a light yellow solid 0.25 g. ESI-MS (m/z): 874 $[M+H]^+$.

4-(4-((4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(9c).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6c** (0.21 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 51.0 % yield as a light yellow solid 0.23 g. ESI-MS (m/z): 888 $[M+H]^+$.

4-((4-((4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)butan-2-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (9d).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6d** (0.21 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 53.0 % yield as a light yellow solid 0.24 g. ESI-MS (m/z): 888 $[M+H]^+$.

4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-4-((4-((4-((1-((2*R*,4*R*,5*R*)-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(9e).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6e** (0.21 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 49.3 % yield as a light yellow solid 0.22 g. ESI-MS (m/z): 884 $[M+H]^+$.

4-((5-((4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)pentyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(9f).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6f** (0.21 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 50.8 % yield as a light yellow solid 0.22 g. ESI-MS (m/z): 902 $[M+H]^+$.

4-(2-(2-((4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)ethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (9g).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6g** (0.22 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 55.0 % yield as a light yellow solid 0.25 g. ESI-MS (m/z): 904 $[M+H]^+$.

4-(4-(2-((4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)ethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (9h).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6h** (0.22 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 48.7 % yield as a light yellow solid 0.23 g. ESI-MS (m/z): 936 $[M+H]^+$.

4-(2-(4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanamido)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(9i).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6i** (0.18 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 51.5 % yield as a light yellow solid 0.22 g. ESI-MS (m/z): 859 $[M+H]^+$.

4-(3-(4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanamido)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(9j).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6j** (0.20 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 47.8 % yield as a light yellow solid 0.21 g. ESI-MS (m/z): 873 $[M+H]^+$.

4-(4-(4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanamido)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(9k).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6k** (0.21 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 55.0 % yield as a light yellow solid 0.24 g. ESI-MS (m/z): 887 $[M+H]^+$.

4-(2-(4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-N-methyl-4-oxobutanamido)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(9l).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6l** (0.20 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 48.0 % yield as a light yellow solid 0.21 g. ESI-MS (m/z): 873 $[M+H]^+$.

4-((5-(4-((1-((2*R*,4*R*,5*R*)-4-((Tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3,3-difluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanamido)pentyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(9m).

The target product was synthesized, using a method similar to that used for the preparation of **9a**, compound **6m** (0.21 g, 0.50 mmol) and **8** (0.25 g, 0.50 mmol), NHS (0.07 g, 0.60 mmol), EDCI (0.12 g, 0.60 mmol), DMAP (0.01 g, 0.10 mmol) in 59.0 % yield as a light yellow solid 0.26 g. ESI-MS (m/z): 901$[M+H]^+$.

4-(2-((4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)ethoxy)-3(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10a).

Compound **9a** (0.20 g, 0.23 mmol) was treated with TBAF (1M solution in THF, 5 mL). The reaction mixture was stirred for 0.5 h at 0 °C under nitrogen atmosphere. After completion of the reaction, THF was removed under reduced pressure and the liquid crude product was purified by column chromatography over silica gel (CH_2Cl_2/MeOH = 20:1, v/v, as the eluant) to afford light yellow solid product 0.13 g, yield: 86.7%. Analytical data for **10a**: mp: 172-175 °C; ESI-MS (m/z): 632 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.10 (s, 1H, NH), 8.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.02 (d, *J* = 7.5 Hz, 1H, CH=C<u>H</u>N), 7.90 (t, 1H, Ar-H), 7.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.34 (d, 1H, C<u>H</u>=CHN), 6.13 (m, 1H, NCH), 5.33 (m, 1H, HOC<u>H</u>), 4.48 (t, 2H, OCH_2), 4.42 (t, 2H, OCH_2), 3.89 (m, 1H, OCH), 3.80 (m, 1H, C<u>H</u>$_2$OH), 3.65 (m, 1H, C<u>H</u>$_2$OH), 2.78 (t, 2H, $OCCH_2$), 2.58 (t, 2H, $OCCH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.27 (NHCO), 172.63 (COO), 163.27 (OC=N), 159.35 (NC=N), 154.66 (NCO), 145.20 (N<u>C</u>H=CH), 137.74 (Ar–C), 136.51 (Ar–C), 130.46 (Ar–C), 128.76 (Ar–C), 123.17 (CF), 110.99 (SC=N), 96.38 (NCH=<u>C</u>H), 84.38 (NCHO), 81.51 (OCH), 71.46 (CHOH), 67.94 (OCH_2), 62.38 (OCH_2), 59.24 (CH_2OH), 31.82 (O C<u>C</u>H$_2$) , 28.59 (OC<u>C</u>H$_2$); Anal. Calcd for $C_{23}H_{23}F_2N_5O_{12}S$: C, 43.74; H, 3.67; N, 11.09; Found: C, 43.53; H, 3.77; N, 11.21.

4-(3-((4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofura-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10b).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9b** (0.20 g, 0.23 mmol) in 82.8 % yield as a light yellow solid 0.12 g. Analytical data for **10b**: mp: 182-185 °C; ESI-

MS (m/z): 646 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.18 (s, 1H, NH), 8.26 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.07 (d, *J* = 7.5 Hz, 1H, CH=CHN), 7.88 (t, 1H, Ar-H), 7.29 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.32 (d, 1H, CH=CHN), 6.16 (m, 1H, NCH), 5.30 (m, 1H, HOCH), 4.43(t, 2H, OCH_2), 4.06 (t, 2H, OCH_2), 3.88 (m, 1H, OCH), 3.82 (m, 1H, CH_2OH), 3.61 (m, 1H, CH_2OH), 2.68 (t, 2H, $OCCH_2$), 2.56 (t, 2H, $OCCH_2$), 2.11 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.39 (NHCO), 172.54 (COO), 163.28 (OC=N), 159.24 (NC=N), 154.61 (NCO), 145.23 (NCH=CH), 137.78 (Ar–C), 136.52 (Ar–C), 130.41 (Ar–C) , 128.88 (Ar–C), 123.17 (CF), 110.91 (SC=N), 96.35 (NCH=CH), 84.56 (NCHO) , 81.53 (OCH), 71.52 (CHOH), 63.97 (OCH_2), 61.53 (CH_2OH), 31.80 ($OCCH_2$), 28.59 ($OCCH_2$), 28.32 (CH_2); Anal. Calcd for $C_{24}H_{25}F_2N_5O_{12}S$: C, 44.65; H, 3.90; N, 10.85; Found: C, 44.38; H, 4.08; N, 11.00.

4-(4-((4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(10c).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9c** (0.20 g, 0.23 mmol) in 81.6 % yield as a the light yellow solid 0.12 g. Analytical data for **10c**: mp: 205-208 °C; ESI-MS (m/z): 660 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.12 (s, 1H, NH), 8.23 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.02 (d, *J* = 7.5 Hz, 1H, CH=CHN), 7.89 (t, 1H, Ar-H), 7.23 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.33 (d, 1H, CH=CHN), 6.17 (m, 1H, NCH), 5.31 (m, 1H, HOCH), 4.41 (t, 2H, OCH_2), 4.08 (t, 2H, OCH_2), 3.89 (m, 1H, OCH), 3.80 (m, 1H, CH_2OH), 3.65 (m, 1H, CH_2OH), 2.72 (t, 2H, $OCCH_2$), 2.60 (t, 2H, $OCCH_2$), 1.80 (m, 2H, CH_2), 1.68 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.34 (NHCO), 172.58 (COO), 163.22 (OC=N), 159.29 (NC=N), 154.63 (NCO), 145.29 (NCH=CH), 137.70 (Ar–C), 136.58 (Ar–C), 130.49 (Ar–C) , 128.77 (Ar–C), 123.16 (CF), 110.90 (SC=N), 96.33 (NCH=CH), 84.54 (NCHO), 81.51 (OCH), 71.50 (CHOH), 63.94 (OCH_2), 59.28 (CH_2OH), 31.79 ($OCCH_2$) , 28.54 ($OCCH_2$), 25.05 (CH_2), 24.93 (CH_2); Anal. Calcd for $C_{25}H_{27}F_2N_5O_{12}S$: C, 45.52; H, 4.13; N, 10.62; Found: C, 45.29; H, 4.28; N, 10.80.

4-((5-((4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)pentyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10d).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9d** (0.20 g, 0.23 mmol) in 86.3 % yield as a light yellow solid 0.14 g. Analytical data for **10d**: mp: 195-197 °C; ESI-MS (m/z): 660 $[M+H]^+$; ^{1}H NMR (d^6-DMSO, 300 MHz, δ ppm): 11.16 (s, 1H, NH), 8.27 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.04 (d, *J* = 7.5 Hz, 1H, CH=CHN), 7.86 (t, 1H, Ar-H), 7.28 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.39 (d, 1H, CH=CHN), 6.12 (m, 1H, NCH), 5.36 (m, 1H, HOCH), 4.38 (t, 2H, OCH_2), 4.00 (t, 1H, OCH), 3.83 (m, 1H, OCH), 3.80 (m, 1H, CH_2OH), 3.67 (m, 1H, CH_2OH), 2.77 (t, 2H, $OCCH_2$), 2.57 (t, 2H, $OCCH_2$), 1.89 (m, 2H, CH_2), 1.21 (m, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.39 (NHCO), 172.52 (COO), 163.29 (OC=N), 159.26 (NC=N), 154.61 (NCO), 145.26 (NCH=CH), 137.71 (Ar–C), 136.52 (Ar–C), 130.44 (Ar–C), 128.71 (Ar–C), 123.12 (CF), 110.93 (SC=N), 96.37 (NCH=CH), 84.51 (NCHO), 81.50 (OCH), 71.52 (CHOH), 69.97 (OCH), 60.97 (OCH_2), 59.29 (CH_2OH), 31.79 ($OCCH_2$), 28.51 ($OCCH_2$), 29.08 (CH_2), 20.05 (CH_2); Anal. Calcd for $C_{25}H_{27}F_2N_5O_{12}S$: C, 45.52; H, 4.13; N, 10.62; Found: C, 45.79; H, 4.27; N, 10.82.

4-((4-((4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10e).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9e** (0.20 g, 0.23 mmol) in 79.6 % yield as a light yellow solid 0.12 g. Analytical data for **10e**: mp: 209-211 °C; ESI-MS (m/z): 656 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.11 (s, 1H, NH), 10.53 (s, 1H, OH), 8.23 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.02 (d, *J* = 7.5 Hz, 1H, CH=CHN), 7.75 (t, 1H, Ar-H), 7.23 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.31 (d, 1H, CH=CHN), 6.17 (m, 1H, NCH), 5.26 (m, 3H, HOCH, OCH_2), 4.84 (t, 2H, OCH_2), 3.89 (m, 1H, OCH), 3.80 (m, 1H, CH_2OH), 3.63 (m, 1H, CH_2OH), 2.73 (t, 2H, $OCCH_2$), 2.63 (t, 2H, $OCCH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.23 (NHCO), 171.99 (COO), 163.20 (OC=N), 158.47 (NC=N), 154.65 (NCO), 145.26 (NCH=CH), 137.44 (Ar–C), 136.69 (Ar–C), 130.52 (Ar–C), 128.82 (Ar–C), 123.16 (CF), 111.14 (SC=N), 96.37 (NCH=CH), 84.46 (NCHO), 81.46 (OCH), 79.81 (C≡C), 68.83 (CHOH), 59.52 (OCH_2), 59.29 (OCH_2), 59.26 (CH_2OH), 31.65 ($OCCH_2$) , 28.26 ($OCCH_2$); Anal. Calcd for $C_{25}H_{23}F_2N_5O_{12}S$: C, 45.80; H, 3.54; N, 10.68; Found: C, 45.59; H, 3.76; N, 10.87.

4-((5-((4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)pentyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10f).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9f** (0.21 g, 0.23 mmol) in 75.8 % yield as a light yellow solid 0.12 g. Analytical data for **10f**: mp: 218-220 °C; ESI-MS (m/z): 674 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.11 (s, 1H, NH), 8.25 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.03 (d, *J* = 7.5 Hz, 1H, CH=CHN), 7.87 (t, 1H, Ar-H), 7.26 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.34 (d, 1H, CH=CHN), 6.19 (m, 1H, NCH), 5.32 (m, 1H, HOCH), 4.47 (t, 2H, OCH_2), 4.11 (t, 2H, OCH_2), 3.89 (m, 1H, OCH), 3.82 (m, 1H, CH_2OH), 3.66 (m, 1H, CH_2OH), 2.78 (t, 2H, $OCCH_2$), 2.55 (t, 2H, $OCCH_2$), 1.83 (m, 2H, CH_2), 1.62 (m, 2H, CH_2), 1.52 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.33 (NHCO), 172.56 (COO), 163.21 (OC=N), 159.24 (NC=N), 154.61 (NCO), 145.26 (NCH=CH), 137.73 (Ar–C), 136.56 (Ar–C), 130.41 (Ar–C), 128.75 (Ar–C), 123.11 (CF), 110.93 (SC=N), 96.31 (NCH=CH), 84.51 (NCHO), 81.52 (OCH), 71.53 (CHOH), 65.92 (OCH_2), 65.22 (OCH_2), 59.21 (CH_2OH), 31.78 ($OCCH_2$), 28.57 ($OCCH_2$), 27.05 (CH_2), 24.93 (CH_2), 20.19 (CH_2); Anal. Calcd for $C_{26}H_{29}F_2N_5O_{12}S$: C, 46.36; H, 4.34; N, 10.40; Found: C, 46.11; H, 4.42; N, 10.58.

4-(2-(2-((4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)ethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10g).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9g** (0.20 g, 0.22 mmol) in 87.2 % yield as a light yellow solid 0.13 g. Analytical data for **10g**: mp: 200-203 °C; ESI-MS (m/z): 676 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.11 (s, 1H, NH), 8.23 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.02 (d, *J* = 7.5 Hz, 1H, CH=CHN), 7.74 (t, 1H, Ar-H), 7.23 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.33 (d, 1H, CH=CHN), 6.17 (m, 1H, NCH), 5.31 (m, 1H, HOCH), 4.51 (m, 2H, OCH_2), 4.16 (m, 2H, OCH_2,), 3.89 (m, 1H, OCH), 3.81 (m, 3H, OCH_2, CH_2OH), 3.69 (m, 3H, OCH_2, CH_2OH), 2.71 (t, 2H, $OCCH_2$), 2.59 (t, 2H, $OCCH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.25 (NHCO), 172.62 (C, COO), 163.21 (OC=N), 159.34 (NC=N), 154.64 (NCO), 145.22 (NCH=CH), 137.73 (Ar–C), 136.58 (Ar–C), 130.46 (Ar–C) , 128.76 (Ar–C), 123.16 (CF), 110.96 (SC=N), 96.35 (NCH=CH), 84.31 (NCHO) , 81.52 (OCH), 71.30 (CHOH), 68.87 (OCH_2), 68.25 (OCH_2), 63.88 (OCH_2), 59.27 (CH_2OH), 31.73 ($OCCH_2$) , 28.43 ($OCCH_2$); Anal. Calcd for $C_{25}H_{27}F_2N_5O_{13}S$: C, 44.45; H, 4.03; N, 10.37; Found: C, 44.23; H, 4.30; N, 10.51.

4-(4-(2-((4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanoyl)oxy)ethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide
(10h).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9h** (0.20 g, 0.21 mmol) in 81.0 % yield as a light yellow solid 0.12 g. Analytical data for **10f**: mp: 199-201 °C; ESI-MS (m/z): 708 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.12 (s, 1H, NH), 9.22 (s, 1H, OH), 8.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.05 (d, *J* = 7.5 Hz, 1H, CH=CHN), 7.77 (t, 1H, Ar-H), 7.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.03 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.67 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.33 (d, 1H, CH=CHN), 6.18 (m, 1H, NCH), 5.31 (m, 1H, HOCH), 4.24 (t, 2H, OCH_2), 4.13 (t, 2H, CH_2Ar), 3.89 (m, 1H, OCH), 3.81 (m, 1H, CH_2OH), 3.65 (m, 1H, CH_2OH), 2.73 (t, 2H, $OCCH_2$), 2.56 (t, 2H, $OCCH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.27 (NHCO), 172.48 (COO), 163.22 (OC=N), 158.98 (NC=N), 156.32 (Ar–C), 154.65 (NCO), 145.24 (NCH=CH), 137.47 (Ar–C), 136.69 (Ar–C), 130.93 (Ar–C), 130.50 (Ar–C), 130.19 (Ar–C), 128.98 (Ar–C), 120.07 (CF), 116.27 (Ar–C), 111.66 (SC=N), 96.36 (NCH=CH), 84.54 (NCHO), 81.53 (OCH), 68.89 (CHOH), 65.44 (OCH_2), 59.27 (CH_2OH), 34.08 (CH_2), 31.76 ($OCCH_2$), 28.54 ($OCCH_2$); Anal. Calcd for $C_{29}H_{27}F_2N_5O_{12}S$: C, 49.22; H, 3.85; N, 9.90; Found: C, 49.01; H, 4.03; N, 10.09.

4-(2-(4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanamido)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(10i).
The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9i** (0.20 g, 0.23 mmol) in 76.5% yield as a light yellow solid 0.11g. Analytical data for **10f**: mp: 152-155 °C; ESI-MS (m/z): 631 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.12 (s, 2H, NH), 8.23 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.18 (d, *J* = 7.5 Hz, 1H, CH=CHN), 7.75 (t, 1H, Ar-H), 7.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.34 (d, 1H, CH=CHN), 6.19 (m, 1H, NCH), 5.30 (m, 1H, HOCH), 4.27 (t, 2H, OCH_2), 3.91 (m, 1H, OCH), 3.80 (m, 1H, CH_2OH), 3.65 (m, 1H, CH_2OH), 3.61 (t, 2H, $NHCH_2$), 2.71 (t, 2H, $OCCH_2$), 2.52 (t, 2H, $OCCH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.22 (NHCO), 173.09 (NHCO), 163.24 (OC=N), 158.42 (NC=N), 154.66 (NCO), 145.24 (NCH=CH), 137.49 (Ar–C), 136.62 (Ar–C), 130.51 (Ar–C), 128.43 (Ar–C), 124.39 (CF), 121.19 (SC=N), 96.33 (NCH=CH), 84.11 (NCHO), 81.56 (OCH), 68.89 (CHOH), 65.71 (CH_2O), 59.27 (CH_2OH), 51.99 ($HNCH_2$), 31.72 ($OCCH_2$), 29.27 ($OCCH_2$); Anal. Calcd for $C_{23}H_{24}F_2N_6O_{11}S$: C, 43.81; H, 3.84; N, 13.33; Found: C, 43.59; H, 3.91; N, 13.44.

4-(3-(4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanamido)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(10j).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9j** (0.20 g, 0.23 mmol) in 77.0 % yield as a light yellow solid 0.11 g. Analytical data for **10j**: mp: 181-183 °C; ESI-MS (m/z): 645 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 11.11 (s, 2H, NH), 8.25 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.24 (d, *J* = 7.5 Hz, 1H, CH=CHN), 7.25 (t, 1H, Ar-H), 7.23 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.33 (d, 1H, CH=CHN), 6.18 (m, 1H, NCH), 5.31 (m, 1H, HOCH), 4.19 (t, 2H, OCH_2), 3.90 (m, 1H, OCH), 3.81 (m, 1H, CH_2OH), 3.67 (m, 1H, CH_2OH), 3.63 (t, 2H, $NHCH_2$), 2.70 (t, 2H, $OCCH_2$), 2.58 (t, 2H, $OCCH_2$), 1.24 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.27 (NHCO), 173.04 (NHCO), 163.22 (OC=N), 158.47 (NC=N), 154.65 (NCO), 145.26 (NCH=CH), 137.44 (Ar–C), 136.69 (Ar–C), 130.52 (Ar–C), 128.42 (Ar–C), 124.35 (CF), 121.14 (SC=N), 96.35 (NCH=CH), 84.05 (NCHO), 81.51 (OCH), 68.87 (CHOH), 65.74 (CH_2O), 59.27 (CH_2OH), 51.91 ($HNCH_2$),

31.77 (OC$\underline{C}$H$_2$), 29.28 (OC$\underline{C}$H$_2$), 28.32 (CH$_2$); Anal. Calcd for $C_{24}H_{26}F_2N_6O_{11}S$: C, 44.72; H, 4.07; N, 13.04; Found: C, 44.42; H, 4.28; N, 13.24.

4-(3-(4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanamido)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10k).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9k** (0.20 g, 0.23 mmol) in 76.8 % yield as a light yellow solid 0.12 g. Analytical data for **10k**: mp: 198-201°C; ESI-MS (m/z): 659 $[M+H]^+$; ^{1}H NMR (DMSO-d$_6$, 300 MHz, δ ppm): 11.16 (s, 2H, NH), 8.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.09 (d, *J* = 7.5 Hz, 1H, CH=C$\underline{H}$N), 7.75 (t, 1H, Ar-H), 7.22 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.38 (d, 1H, C$\underline{H}$=CHN), 6.19 (m, 1H, NCH), 5.32 (m, 1H, HOC$\underline{H}$), 4.14 (t, 2H, OCH$_2$), 3.90 (m, 1H, OCH), 3.82 (m, 1H, C$\underline{H}_2$OH), 3.65 (m, 1H, C$\underline{H}_2$OH), 3.59 (t, 2H, NHC$\underline{H}_2$), 2.72 (t, 2H, OCCH$_2$), 2.59 (t, 2H, OCCH$_2$), 1.66 (m, 2H, CH$_2$), 1.11 (m, 2H, CH$_2$); ^{13}C NMR (DMSO-d$_6$, 75 MHz, δ ppm): 173.25 (NHCO), 173.08 (NHCO), 163.21 (OC=N), 158.43 (NC=N), 154.69 (NCO), 145.25 (N$\underline{C}$H=CH), 137.43 (Ar–C), 136.67 (Ar–C), 130.58 (Ar–C), 128.49 (Ar–C), 124.38 (CF), 121.18 (SC=N), 96.36 (NCH=$\underline{C}$H), 84.07 (NCHO), 81.50 (OCH), 68.83 (CHOH), 65.71 (CH$_2$O), 59.25 (CH$_2$OH), 51.90 (HNCH$_2$), 31.89 (OC$\underline{C}$H$_2$) , 29.48 (OC$\underline{C}$H$_2$), 28.39 (CH$_2$), 28.30 (CH$_2$); Anal. Calcd for $C_{25}H_{28}F_2N_6O_{11}S$: C, 45.59; H, 4.29; N, 12.76; Found: C, 45.28; H, 4.48; N, 12.88.

4-(2-(4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-N-methyl-4-oxobutanamido)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(10l).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9l** (0.20 g, 0.23 mmol) in 66.9 % yield as a the light yellow solid 0.10 g. Analytical data for **10l**: mp: 174-176 °C; ESI-MS (m/z): 645 $[M+H]^+$; ^{1}H NMR (DMSO-d$_6$, 300 MHz, δ ppm): 11.11 (s, 1H, NH), 8.25 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.24 (d, *J* = 7.5 Hz, 1H, CH=C$\underline{H}$N), 7.25 (t, 1H, Ar-H), 7.23 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.33 (d, 1H, C$\underline{H}$=CHN), 6.18 (m, 1H, NCH), 5.32 (m, 1H, HOC$\underline{H}$,), 4.19 (t, 2H, OCH$_2$), 3.90 (m, 1H, OCH), 3.81 (m, 1H, C$\underline{H}_2$OH), 3.67 (m, 1H, C$\underline{H}_2$OH), 3.63 (t, 2H, NC$\underline{H}_2$), 2.72 (t, 2H, OCCH$_2$), 2.58 (t, 2H, OCCH$_2$), 1.24 (m, 3H, CH$_3$); ^{13}C NMR (DMSO-d$_6$, 75 MHz, δ ppm): 173.28 (NHCO), 173.04 (NHCO), 163.14 (OC=N), 158.47 (NC=N), 154.58 (NCO), 145.13 (N$\underline{C}$H=CH), 145.07 (Ar–C), 136.69 (Ar–C), 130.52 (Ar–C), 125.40 (Ar–C), 123.38 (CF), 119.95 (SC=N), 96.36 (NCH=$\underline{C}$H), 84.54 (NCHO), 81.30 (OCH), 68.80 (CHOH), 64.38 (CH$_2$O), 59.16 (CH$_2$OH), 51.86 (HNCH$_2$), 31.61 (OC$\underline{C}$H$_2$), 29.50 (NCH$_3$), 28.37 (OC$\underline{C}$H$_2$); Anal. Calcd for $C_{24}H_{26}F_2N_6O_{11}S$: C, 44.72; H, 4.07; N, 13.04; Found: C, 44.46; H, 4.19; N, 13.25.

4-((5-(4-((1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-oxobutanamido)pentyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide(10m).

The target product was synthesized, using a method similar to that used for the preparation of **10a**, compound **9m** (0.21 g, 0.23 mmol) in 77.8 % yield as a the light yellow solid 0.12 g. Analytical data for **10m**: mp: 210-213 °C; ESI-MS (m/z): 673 $[M+H]^+$; ^{1}H NMR (DMSO-d$_6$, 300 MHz, δ ppm): 11.11 (s, 2H, NH), 8.26 (d, 2H, *J* = 7.5 Hz, Ar-H), 8.05 (d, *J* = 7.5 Hz, 1H, CH=C$\underline{H}$N), 7.79 (t, 1H, Ar-H), 7.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.37 (d, 1H, C$\underline{H}$=CHN), 6.18 (m, 1H, NCH), 5.30 (m, 1H, HOC$\underline{H}$), 4.18 (t, 2H, OCH$_2$), 3.91 (m, 1H, OCH), 3.80 (m, 1H,

CH_2OH), 3.66 (m, 1H, CH_2OH), 3.52 (t, 2H, NHCH_2), 2.70 (t, 2H, OCCH_2), 2.53 (t, 2H, OCCH_2), 1.69 (m, 2H, CH_2), 1.41 (m, 2H, CH_2), 1.19 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 173.23 (NHCO), 173.09 (NHCO), 163.23 (OC=N), 158.45 (NC=N), 154.68 (NCO), 145.20 (NCH=CH), 137.41 (Ar–C), 136.67 (Ar–C), 130.56 (Ar–C), 128.42 (Ar–C), 124.33 (CF), 121.14 (SC=N), 96.37 (NCH=CH), 84.09 (NCHO), 81.52 (OCH), 68.81 (CHOH), 65.79 (CH_2O), 59.24 (CH_2OH), 51.97 (HNCH_2), 31.99 (OCCH_2), 29.68 (OCCH_2), 28.66 (CH_2), 28.38 (CH_2), 24.37 (CH_2); Anal. Calcd for $C_{26}H_{30}F_2N_6O_{11}S$: C, 46.43; H, 4.50; N, 12.49; Found: C, 46.21; H, 4.68; N, 4.61.

3. Cytotoxicity Assay

Human lung cancer cell (A549), hepatocellular carcinoma cells (HepG2), human colon cancer cell (HCT-116, SW-620), and human gastric carcinoma cells (SGC7901) at 10^4 cells per well were cultured in 4% FBS DMEM in 96-well flat-bottom microplates overnight. The cells were incubated in triplicate with, or without, different concentrations of each test compound for 48h. During the last 4 h incubation, 30 μL of tetrazolium dye (MTT) solution (5 mg/mL^{-1}) was added to each well. The resulting MTT-formazan crystals were dissolved in 150 μL DMSO, and absorbance was measured spectrophotometrically at 570 nm using an ELISA plate reader. The inhibition induced by each test compound at the indicated concentrations was expressed as a percentage. The concentration required for 50% inhibition (IC_{50}) was calculated usingthe software (GraphPadPrism Version 4.03).

4. Nitrate/nitrite measurement *in vitro* Assay

The levels of nitrate/nitrite formed from individual compounds in the cells were determined by the colorimetric assay using the ni-trate/nitrite colorimetric assay kit (Beyotime, China), according to the manufacturer's instructions. Briefly, HepG2 cells, HCT-116 and SW-620 cells (5×10^6/ well) were treated in triplicate with 100 μM of one of the compounds (**10b-e**, **10j**, **10k**, and gemcitabine) for 24 h. The cells were harvested lyzed. The cell lysates were mixed with Griess for 30–300 min, followed by measuring at 540 nm. The cells treated with diluent were used as negative controls for the background levels of nitrate/nitrite production, while with sodium nitrate at different concentrations was used as positive controls for the standard curve.

5. Intracellular NO Release Measurement Using DAF-FM DA

DAF-FM DA (Beyotime) was used as a fluorescent indicator of intracellular NO. When cells grown in a 96-well plate reached 80% confluence, they were washed with PBS. After being loaded with 5 μM DAF-FM DA at 37 °C for 20 min, the cells were rinsed three times with PBS and incubated with test compounds for 24 h. NO production was measured with the flow cytometer with excitation and emission wavelengths of 495 and 515 nm, respectively.

6. Flow cytometry assay of cell apoptosis Assay

HepG2 cells were cultured overnight and incubated in triplicate with the test compound or vehicle for 48 h. The cells were harvested, and stained with FITC-Annexin V/PI (BioVision) at room temperature for 15 min. The percentage of apoptotic cells was determined by flow cytometry (Beckman Coulter) analysis.

7. Western blot Assay

The mechanisms of the cell apoptosis and the inhibitory activity of Ras-related signaling were determined by western blot assay. HepG2 cells at 1.5×10^5/mL were treated with indicated concentration of **10e**, gemcitabine, or vehicle control for 8 h. After harvested and lyzed, the cell lysates (50 μg/lane) were separated by SDS-PAGE (12% gel) and transferred onto nitrocellulose membranes. After blocked with 5% fat-free milk, the target proteins were probed with anti-Bax, anti-Bcl-2, anti-Caspase3, anti-Parp-1 and anti-β-actin antibodies (Cell Signaling, Boston), respectively. The bound antibodies were detected by HRP-conjugated second antibodies and visualized using the enhanced chemiluminescent reagent. The relative levels of each signaling event to control β-actin were determined by densimetric scanning.