Electronic Supplementary Material (ESI) for RSC Advances. This journal is © The Royal Society of Chemistry 2016

Supporting Information

Strained Alkyne Substituted Near Infrared BF₂ Azadipyrromethene Fluorochrome

Dan Wu,^[a] Shane Cheung,^[a] Corry James O'Sullivan,^[a] Yinghua Gao,^[b] Zhi-long Chen,^[b] Donal F. O'Shea^{[a]*}

[a] Department of Pharmaceutical and Medicinal Chemistry Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland

E-mail: donalfoshea@rcsi.ie

[b] Department of Pharmaceutical Science & Technology, College of Chemistry and Biology, Donghua University, Shanghai 201620, China

Table of contents

General Information and Materials	S2
Fig. S1: Photophysical property of 3a	S3
Fig. S2: Normalized absorption and emission spectra of 5, 6, 8, 9, 10	S4
Fig. S3: MALDI-QTOF mass spectra	S 5
Fig. S4: Imaging of 9 and 10 with living HeLa cells	S7
Fig. S5: In vivo imaging of 10	S10
Fig. S6: HPLC trace of compound 9 and compound 10	S11
¹ H and ¹³ C NMR spectra	S12

General Information and Materials

Synthetic reaction conditions and yields are not optimized. All reactions involving air-sensitive reagents were performed under nitrogen in oven-dried glassware using syringe-septum cap technique. All solvents were purified and degassed before use. Chromatographic separation was carried out under pressure on Apollo 60/40 silica gel and Merck alumina 90 using flash-column techniques. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel coated aluminum plates (60 Merck F_{254}) using UV light (254 nm) as visualizing agent. Unless it is specified, all reagents were used as received without further purifications. ¹H NMR and ¹³C NMR spectra were recorded at room temperature at 400 MHz and 100 MHz respectively, and calibrated using residual non-deuterated solvent as an internal reference. Chemical shifts are reported in parts-per-million (ppm). On the basis of NMR and reverse phase HPLC all final compounds are >95% pure. MALDI-TOF MS analysis used a 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene] malononitrile (DCTB) matrix. The peptide *cyclo*[Arg-Gly-Asp-D-Phe-Lys (azide)] was purchased from Peptides International.



Fig. S1. Abs and emission properties of 3a in CrEL/water solution.

^a0.1% CrEL used.

Fig. S2. Normalized absorption and emission spectrum of 5, 6, 8, 9 and 10.^a



^a Spectra for **5**, **6** and **8** were recorded in CHCl₃, **9** and **10** were recorded in water/1% SDS.



^aSpectra for **5**, **6** and **8** were recorded in CHCl₃, **9** and **10** were recorded in water/1%SDS.

Fig. S3.

HRMS MALDI-QTOF of compound 5.

Q-TOF20160825MF006 78 (2.148) AM (Cen,4, 80.00, Ar,10000.0,1568.68,0.70); Sm (SG, 1x5.00); Sb (15,10.00); Cm (40:83-(65:68+77:81))											
100 %= 652.02: 0=	23 657.9888 661. 355.0 660.0	1539 	60 670.272	672.2747 20	673.2784 675.0	79.9834 685.119 680.0 6	96 687.977 	.3 689.9731 	693.2036 m/z 695.0		
Minimum: Maximum:		5.0	50.0	-1.5 500.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm) Formula				
672.2747	672.2719	2.8	4.2	24.0	150.7	0.0	C39 H35	5 N4 O4	F2 B		

HRMS MALDI-QTOF of compound 6.

Q-TOF20160825MF007 86 (1.811) AM (Cen,4, 80.00, Ar,10000.0,1568.68,0.70); Sm (SG, 1x5.00); Sb (15,10.00); Cm (3:93-(49:54+84:92))												
100 558.067 0 556.0 5	560.0781 561 3 559.0789 58.0 560.0 5	.0798 564.076 	1 566.0949 ⁵ 	57 67.0984 57 568.0 57	1.2145 572.2181 0.2129 573. 70.0 572.0	2244 	576.1523 576.0	577.16 	45 579	9.1798 80.0	582.06 582.0	37 583.0641
Minimum: Maximum:		5.0	50.0	-1.5 500.0								
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT	(Norm)	Formu	ıla			
572.2181	572.2195	-1.4	-2.4	23.0	171.8	0.0		C34	H27	В	N4 O	2 F2

HRMS MALDI-QTOF of compound 8.

Q-TOF20160825MF008 14 (0.258) AM (Cen,4, 80.00, Ar,10000.0,1568.68,0.70); Sm (SG, 1x5.00); Sb (15,10.00); Cm (3:56-(13:16+29:47)) TOF MS LD-6.09e+003

100 748 % 746.289	3.3022 9 749.3074	332.0638	1020,1045	1058.07151	157.3628		1425,3372	2 1568	3.6774	173	3.5570) 1881	.2175	195 ⁻	1.4762 m/ 7
700	800	900	1000	1100	1200	1300	1400	1500	1600	1700)	1800		1900	2000
Minimum: Maximum:			5.0	50.0	-1.5 500.0										
Mass	Calc. Ma	ass	mDa	PPM	DBE		i-FIT	i-FIT	(Norm)	Form	ıla				
748.3022	748.3032	2	-1.0	-1.3	28.0		123.4	0.0		C45	H39	В	N4	04	F2

HRMS MALDI-QTOF of compound 4.

Q-TOF20160825MF011 28 (0.516) AM (Cen,4, 80.00, Ar,10000.0,1568.66,0.70); Sm (SG, 1x5.00); Sb (15,10.00); Cm (13:89)														TOF MS LI 8.42e+0		
100 % 831.061 0 7 7 7 7 7 7 7 7 8 30	18 + + + + + - 	848.3069 	8 866.3177 860 860	69.2975 87 14144444 870 8	77.0933 ^{892.2} 	2863 908.26 	38 917.4570 ₁₁₀ 0 920	940.202 بېرابېر 930 9	2 947 	.1732 	959.1 بېلېبې 91	1934 	바바바 970	977.19 հարկեր	925 ⊬ m/z	
Minimum: Maximum:			5.0	10.	-1. 0 500	5 .0										
Mass	Calc.	Mass	mDa	PPM	DBE	i-FIT	i-FIT	(Norm)	Form	ula						
869.2975	869.2	992	-1.7	-2.	0 28.	5 115.8	0.0		C48	H44	в	N4	07	F2	S	

HRMS MALDI-QTOF of compound 9.

Q-TOF20160825MF012 46 (1.087) AM (Cen,4, 80.00, Ar,10000.0,1568.66,0.70); Sm (SG, 1x5.00); Sb (15,10.00); Cm (7:80)													TOF MS LD-					
100 %	604.0632 605.068	85 779	9.1195	892.3071	1065.4172	1113.416	4 137.394	7			1569.659	3 1632	2.5681		187	9.5878	2.300	
500	600	700	800	900	1000	1100	1200	1300	1400	150	00 160)0	1700	180	00	1900	200	00
Minimum: Maximum:				5.0	10.0	-1. 500	5 .0											
Mass	Calc.	Mass	I	mDa	PPM	DBE		i-FIT	i	-FIT	(Norm)	Form	ula					
1113.416	4 1113.	4163		0.1	0.1	31.	5	81.9	C	0.0		C57	H60	В	N8	011	F2	S

HRMS MALDI-QTOF of compound 10.

2-TOF20150907MF012 30 (0.836) AM (Cen,4, 80.00, Ar,10000.0, 1568.66,0.70); Sm (SG, 1x5.00); Sb (15,10.00); Cm (4:35)														MS LD- 50e+004
100 % 1484 0 	.6526 	1490.7036 	1 1497.617 	498.5990 3 150 1500.0	0.6089 1503.0 	6323 1507.7114 	1515.73 ¹ 	3 ,-, ^{,,,} ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	1521. 	6571 	1523.6 	6859 15 4,-, (-,-,-) .0	26.71	50 m/z 30.0
Minimum: Maximum:			5.0	5.0	-1.5 200.0									
Mass	Calc.	Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Form	ula					
1498.5990	1498.	6026	-3.6	-2.4	41.5	61.5	0.0	C75	H83	в	N15	014	F2	S



Fig. S4. Cell imaging with compound 10

Fig. S4.1. HeLa-Kyoto cells after addition of 10 and the acquisition of a 4D video made up of z-scans acquired every sixty seconds over the course of 10 min. Increasing fluorescence on the cell surface is demonstrated in this montage summary of the video shown in movie S1. Scale bar = $10\mu m$.



Fig. S4.2. HeLa-Kyoto cells were pre-incubated with **10** for 30, 60 and 120 min and z-scans were acquired. Six slices from the bottom (A) to top (F) show the localisation of **10** after the allotted time (see movie S2-4). Scale bar = $10 \mu m$.



Fig. S4.3. After 120 min pre-incubation the fluorophore has been internalised by the cell, and the fluorescence has a more punctuated distribution. Top: Grey channel, bottom: red channel. Scale bar = $10 \ \mu$ m.



Fig. S5. In vivo imaging time course with compound 10.

Fig. S5. *In vivo* imaging of **10** using a human esophageal cancer cell line Eca-109 subcutaneous tumour model in mice. NIR fluorescence imaging at 1, 2, 4, 6, 8, 10, 12, 24 and 48 h post intravenous (i.v.) administration of **10** (excit. 640 nm, emis. 700 nm).



Fig. S6. HPLC trace of compound 9 and compound 10.

Column: reverse phase HLPC with YMC triart phenyl column and size: 150×4.6 mm I.D., particle size: S-5 µm, 12 nm hole, detection method: UV-Vis and wavelength for detection: 650 nm. Eluent CH₃CN : H₂O=70:30.

Compound 5 ¹H NMR (400 MHz, DMSO-*d*₆)









Compound 6

¹H NMR (400 MHz, DMSO- d_6)





HSQC NMR (DMSO-d₆)



Compound 8 ¹H NMR (400 MHz, CDCl₃)



¹⁹F NMR (375 MHz, CDCl₃)



¹H COSY NMR (CDCl₃)



Compound 4 ¹H NMR (400 MHz, MeOD- d_4)





¹H COSY NMR (MeOD- d_4)

