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Synthesis of fluorescent derivatives of wortmannin and demethoxyviridin as probes for phosphatidylinositol 3-kinase

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Abstract—Fluorescent analogs were synthesized of the potent PI 3-kinase inhibitors, wortmannin and demethoxyviridin. The esterification of 11-deacetylwortmannin, 17-hydroxywortmannin, and demethoxyviridin with the fluorescent carboxylic acids NBD-sarcosine and 7-dimethylaminocoumarin-4-acetic acid generated six novel fluorescent esters. Potent inhibition of PI 3-kinase-α was observed for the derivatives of 11-desacetylwortmannin and demethoxyviridin.

The steroidal fungal metabolites wortmannin (**1**)[1,2] and demethoxyviridin (**2**)[3] are potent inhibitors of phosphatidylinositol 3-kinase,[4–7] a protein involved in cellular signal transduction pathways,[8] to which they bind covalently through the reaction of their electrophilic furan moieties.[9] Fluorescent derivatives of these substances would be useful in detecting this enzyme by sensitive methods such as fluorescence microscopy or fluorescence activated cytometry and would enable experiments with fluorescent energy transfer.

A number of considerations went into the design of these fluorescent probes. Covalent attachment of the fluorophores was most conveniently accomplished by esterification of the kinase inhibitors with fluorescent carboxylic acids. However, because it has no free hydroxyl groups, wortmannin (**1**) itself could not be used directly as a substrate for esterification, and two of its chemical derivatives, 11-deacetylwortmannin (**3**) and 17-hydroxywortmannin (**4**),[10] were used instead (Fig. 1). Because the appended functionality might interfere with enzyme binding, fluorophores were selected that were small in size, but still possessed favorable fluorescence properties.[11] Two suitable fluorescent carboxylic acids were chosen, the green fluorescent NBD-sarcosinate (**5**, Abs 465 nm, Em 535 nm)[12] and the blue fluorescent 7-dimethylaminocoumarin-4-acetic acid (DMACA, **6**, Abs 370 nm, Em 460 nm).[13]

The esterifications were carried out on a 12 μmol scale in 1.5 ml dichloromethane, using 3 molar equivalents of the fluorescent acid, 3–4 equiv 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride as the coupling reagent, and 0.3 equiv *p*-dimethylaminopyridine. After 24 h. at room temperature, the reaction mixtures were applied directly to preparative silica gel TLC plates and developed with ethyl acetate. The isolated yields for the NBD-sarcosinates were good (**7** = 62%; **8** = 60%; **9** = 67%). However, the reactions for the DMACA derivatives gave significantly lower yields, (**10** = 24%; **11** = 3%; **12** = 30%). The reaction products were thoroughly characterized by HRMS[14] and NMR (Table 1). Evaluation by the oxidative burst bioassay[15] using guinea pig neutrophils[16,17] demonstrated wortmannin-like bioactivity for the new compounds (data

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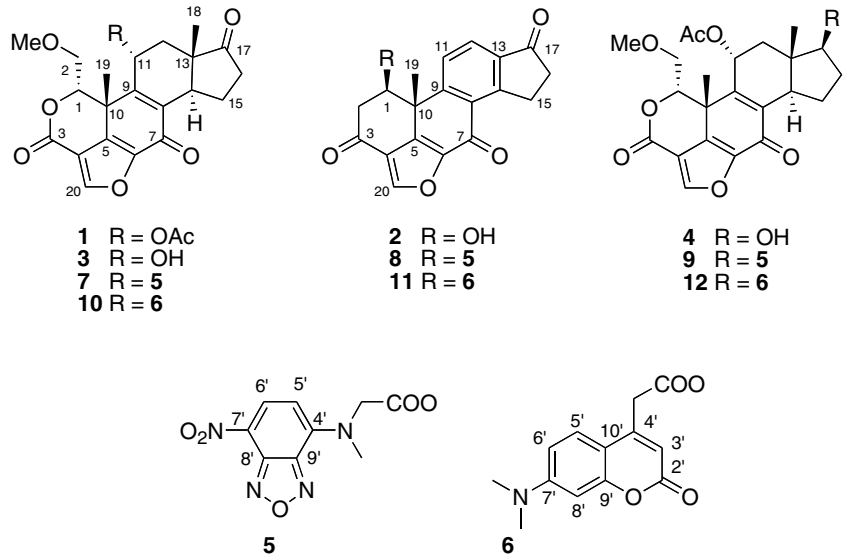

Figure 1. Structures of PI 3-kinase inhibitors.

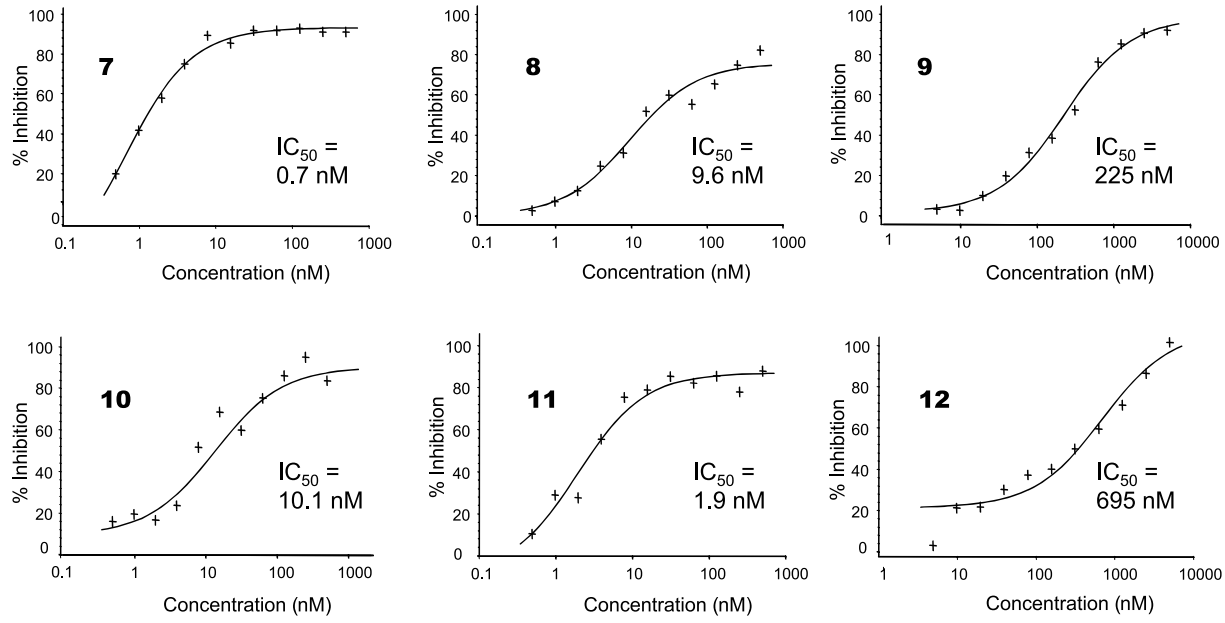

Figure 2. Inhibition of PI 3-kinase a by fluorescent derivatives **7**–**12**.

not shown). Enzymatic assay of the fluorescent inhibitors using recombinant PI 3-kinase p110α/p85α complex[18] indicated lower activity for the derivatives of 17-hydroxywortmannin (Fig. 2, **9** and **12**), while the remaining compounds (**7**, **8**, **10**, and **11**) were found to have potencies rivaling that of wortmannin (**1**), which has an IC_{50} of 1.2 nM in this assay.[19] These results are consistent with experiments in which extension at position-11 and -17 of wortmannin led to the retention and loss of bioactivity, respectively.[20,21] The bioactivity trends are also consistent with crystallographic data that show substituents at position-1 and -11 point out into the solvent, while position-17 is deep in the binding pocket.[8] Of the fluorescent derivatives prepared here, wortmannin 11-NBD-sarcosinate (**7**) possesses a number of favorable properties: it can be prepared in good yield, it fluoresces at a higher wavelength and is excited in the visible range, and it is a very potent binder to PI 3-kinase (IC_{50} = 0.7 nM). After this synthesis was completed, fluorescent analogs of wortmannin bearing BODIPY and rhodamine fluorophores at position-11 were reported.[22–25] The potency of these other derivatives is significantly lower than wortmannin or not reported.

Table 1. 600 MHz ^{1}H and 151 MHz ^{13}C NMR assignments ($CDCl_3$)

	7	**8**	**9**	**10**	**11**	**12**
	5.07 d 7.1	5.54 dd 5.4,10.5	4.76 dd 1.7, 7.0	4.73 dd 1.4,6.8	5.46 dd 5.4,10.7	4.75 dd 1.6,7.0
1	**88.8**	**74.6**	**88.8**	**88.6**	**73.9**	**88.9**
	3.53 dd 1.1,11.5	3.20 dd 5.4,18.3	3.44 dd 1.6,11.2	3.43 dd 1.4,11.5	3.17 dd 5.4,18.3,	3.45 dd 1.6,11.0
	3.02 dd 7.1,11.6	3.04 dd 10.7,18.3	2.98 dd 7.1,11.2	2.92 dd 7.0,11.3	2.91 dd 10.7,18.1	3.01–2.95 m
2	**73.5**	**42.6**	**72.9**	**73.1**	**42.5**	**72.9**
3	**157.4**	**186.7**	**157.6**	**157.4**	**187.0**	**157.6**
4	**114.2**	**122.8**	**114.3**	**114.2**	**122.8**	**114.3**
5	**142.6**	**141.5**	**142.8**	**142.6**	**141.6**	**142.7**
6	**144.8***	**146.5**	**144.9**	**144.8**	**146.5**	**144.9**
7	**172.4**	**172.7**	**172.7**	**172.3**	**172.7**	**172.7**
8	**141.2**	**130.2**	**140.9**	**141.1**	**130.0**	**141.1**
9	**148.5**	**152.9**	**148.7**	**148.7**	**152.8**	**148.6**
10	**40.9**	**40.6**	**40.7**	**40.7**	**40.5**	**40.7**
			6.05 ddd			
	6.18 dt 2.5,8.4	8.08 d 8.2	3.1,7.1,9.6	6.19 dt 2.3,7.8	7.46 d 8.1	6.03 dt 3.1,7.4
11	**72.3**	**126.9**	**70.2**	**71.1**	**126.7**	**70.2**
	2.66 dd 7.6,12.9		2.37 dd 7.1,11.7	2.46 dd 7.4,13.2		2.41 dd 7.3,12.0
	1.69 dd 8.8,12.7	8.14 d 8.2	1.44 dd 9.6,11.7	1.52 dd 8.2,13.2	7.38 d 8.1	1.48 dd 10.1,11.8
12	**36.2**	**127.8**	**39.9**	**36.0**	**127.3**	**40.1**
13	**49.1**	**138.0**	**44.7**	**49.2**	**137.7**	**44.8**
	2.92 ddd		2.60 ddd	2.80 ddd		2.60 ddd
	2.5,5.9,12.7		3.0.7.1,12.3	2.2,5.9,12.6		2.8,6.8,12.4
14	**44.2**	**158.4**	**43.9**	**43.9**	**158.2**	**44.0**
	3.23–3.16 m	2.83–2.73	2.88–2.80 m	3.13–3.05 m		2.79–2.86 m
	2.06 tt 9.2,12.9	m (2H)	1.81–1.71 m	2.10–2.01 m	2.77–2.67 m (2H)	1.77–1.68 m
15	**22.9**	**36.3**	**24.7**	**22.9**	**36.2**	**24.7**
		3.85 ddd			3.79 ddd	
		5.1,6.4,19.7			4.3,7.4,19.6	
	2.60 dd 8.5,19.4	3.73 ddd	2.26–2.36 m	2.55 dd 8.7,19.5	3.67 ddd	2.33–2.25 m
	2.28 dt 19.5,9.2	4.8,7.0,19.7	1.69–1.61 m	2.18 dt 19.5,9.2	4.0,7.6,19.6	1.64–1.54 m
16	**35.6**	**28.6**	**27.5**	**35.7**	**28.5**	**27.4**
			4.84 dd 7.8,8.8			4.87 dd 7.8,9.2
17	**215.6**	**205.7**	**81.9**	**215.9**	**205.6**	**81.0**
	0.94 (3H)		0.87 s (3H)	0.92 (3H)		0.77 s (3H)
18	**14.5**		**12.9**	**14.9**		**12.7**
	1.79 s (3H)	1.81 s (3H)	1.72 s (3H)	1.73 s (3H)	1.65 s (3H)	1.71 s (3H)
19	**26.4**	**26.5**	**26.6**	**26.4**	**26.2**	**26.6**
	8.29 s	8.27 s	8.22 s	8.25 s	8.25 s	8.22 s
20	**150.2**	**148.3**	**149.8**	**150.1**	**148.2**	**149.8**
2′				**161.4**	**161.3**	**161.6**
3′				6.02 s **110.9**	6.19 s **111.1**	6.06 s **110.8**
4′	**145.0***	**144.8***	**145.2***	**147.1**	**107.9**	**148.2**
	6.31 d 8.8	6.32 d 8.8	6.25 d 8.8	7.34 d 9.0	7.41 d 8.8	7.39 d 8.8
5′	**102.7**	**102.9**	**102.4**	**124.9**	**125.1**	**125.1**
	8.51 d 8.8	8.50 d 8.8	8.51 d 8.8	6.64 dd 2.5,9.0	6.63 dd 2.5,8.8	6.67 dd 2.6,9.0
6′	**135.0**	**134.9**	**135.0**	**109.3**	**109.4**	**109.1**
7′	**124.8**	**124.9**	**124.5**	**153.2**	**153.5**	**153.1**
				6.53 s	6.65 d 2.5	6.55 d 2.5
8′	**144.8***	**144.7***	**144.9***	**98.5**	**98.6**	**98.5**
9′	**144.5***	**144.5***	**144.5***	**156.0**	**156.3**	**156.0**
10′				**108.0**	**147.0**	**108.3**
	5.05 d 18.8	5.38 d 18.6		3.83 d 15.8	3.92 d 15.2	
	4.89 d 18.8	5.05 d 18.6	4.92 br s (2H)	3.72 d 15.8	3.86 d 15.2	3.71 s (2H)
CH_2CO_2	**57.2**	**57.5**	**57.3**	**38.2**	**39.2**	**38.4**
CH_2CO_2	**167.8**	**167.3**	**168.3**	**168.2**	**167.2**	**169.1**
			2.13 s (3H)			2.13 s (3H)
$MeCO_2$			**21.0**			**21.1**
$MeCO_2$			**169.5**			**169.4**
	3.45 s (3H)	3.47 s (3H)	3.46 br s (3H)	3.08 s (6H)	3.12 s (6H)	3.09 s (6H)
NMe	**42.1**	**42.1**	**42.2**	**40.1**	**40.2**	**40.1**
	3.27 s (3H)		3.17 s (3H)	3.22 s (3H)		3.19 s (3H)
OMe	**59.6**		**59.4**	**59.7**		**59.4**

All ^{1}H signals are 1 H unless specified otherwise, the coupling constants are given in Hz, and all ^{13}C data are in boldface. The assignments were determined by HSQC and HMBC 2-D NMR experiments. Signals that could not be securely assigned are marked with an asterisk.

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