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Discovery of small molecule inhibitors of xyloglucan endotransglucosylase (XET) activity by high-throughput screening

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ABSTRACT

Small molecules (xenobiotics) that inhibit cell-wall-localised enzymes are valuable for elucidating the enzymes' biological roles. We applied a high-throughput fluorescent dot-blot screen to search for inhibitors of *Petroselinum* xyloglucan endotransglucosylase (XET) activity *in vitro*. Of 4216 xenobiotics tested, with cellulose-bound xyloglucan as donor-substrate, 18 inhibited XET activity and 18 promoted it (especially anthraquinones and flavonoids). No compounds promoted XET in quantitative assays with (cellulose-free) soluble xyloglucan as substrate, suggesting that promotion was dependent on enzyme–cellulose interactions. With cellulose-free xyloglucan as substrate, we found 22 XET-inhibitors – especially compounds that generate singlet oxygen ($^1O_2$) e.g., riboflavin (IC_{50} 29 μM), retinoic acid, eosin (IC_{50} 27 μM) and erythrosin (IC_{50} 36 μM). The riboflavin effect was light-dependent, supporting $^1O_2$ involvement. Other inhibitors included tannins, sulphydryl reagents and triphenylmethanes. Some inhibitors (vulpinic acid and brilliant blue G) were relatively specific to XET, affecting only two or three, respectively, of nine other wall-enzyme activities tested; others [e.g. (−)-epigallocatechin gallate and riboflavin] were non-specific. *In vivo*, out of eight XET-inhibitors bioassayed, erythrosin (1 μM) inhibited cell expansion in *Rosa* and *Zea* cell-suspension cultures, and 40 μM mycophenolic acid and (−)-epigallocatechin gallate inhibited *Zea* culture growth. Our work showcases a general high-throughput strategy for discovering wall-enzyme inhibitors, some being plant growth inhibitors potentially valuable as physiological tools or herbicide leads.

1. Introduction

Xyloglucan endotransglucosylase (XET), a homo-transglycanase enzyme activity found in all land plants and in some charophytic algae (Eklöf and Brumer, 2010; Fry et al., 1992; Nishitani and Tominaga, 1992; Rose et al., 2002; Vissenberg et al., 2000; Franková and Fry, 2013), acts *in vivo* during the initial assembly (Thompson et al., 1997) and subsequent re-structuring (Thompson and Fry, 2001) of the xyloglucan–cellulose network in primary cell walls. XET is one of two activities exhibited by a class of proteins known as XTHs (xyloglucan endotransglucosylase/hydrolases), 33 of which are encoded in the *Arabidopsis thaliana* genome (Nishitani, 2005; Eklöf and Brumer, 2010); the second activity is xyloglucan endohydrolase (XEH), which is the predominant activity of a minority of XTHs (Shi et al., 2015). All known plant XTHs belong to CAZy class GH16 (Nishitani, 2005; Strohmeier et al., 2004). A related hetero-transglycanase activity, MXE (mixed-linkage-glucan:xyloglucan endotransglucosylase), has been detected in *Equisetum* and certain charophytes (Fry et al., 2008). Other homo-transglycanase activities potentially acting on plant cell walls include trans-β-mannanase (Schröder et al., 2009) and trans-β-xylanase (Franková and Fry, 2011; Derba-Maceluch et al., 2014). It is likely that the various transglycanases play biologically important roles in plants (Franková and Fry, 2013). Methods for assaying diverse transglycanase activities have been reviewed and extended (Franková and Fry, 2015).

Abbreviations: IC_{50}, concentration required for 50% inhibition (e.g. of XET); SR, sulphorhodamine; XET, xyloglucan endotransglucosylase (activity); XGO, xyloglucan oligosaccharide; XTH, xyloglucan endotransglucosylase/hydrolase (protein); XLLG, non-fucosylated xyloglucan nonasaccharide ($Gal_2.Xyl_3.Glc_4$); XXFG, fucosylated xyloglucan nonasaccharide ($Fuc.Gal.Xyl_3.Glc_4$); XXXGol, reduced heptasaccharide of xyloglucan ($Xyl_3.Glc_3.glucitol$).

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Table 1
Summary of all putative 'hits' for effects of xenobiotics on parsley XET activity.

Plate	Well	Dot-blot result	^{3}H XET assay results					Xenobiotic compound	Fig.	Class	β–Gal result	β–Xyl result
			#1	#2	#3	#4	IC_{50} (μM)					
Coagulant effect in dot-blots												
P2, P3	A5, D10	c, p	ii	ii			380	(–)-Epigallocatechin gallate †	6	Tan	ii i	i i
P2	B6	c, p	–	–				Apigenin		F		
P2	B7	c, p	i	i			520	Baicalein	6	F		
P2	B9	c, p						Brassinin		U		
P2	C1	c, p	i	i			610	Hesperetin	6	F		
P3	A2	c, 0	–					(±)-Taxifolin		F		
P3	A12	c, p	i	i			>700	Luteolin	6	F		
P3	B5	c, 0	ii	ii			580	Phloretin	6	F		
P4	B10	c, p	–	–				(–)-Quinic acid	9	U		
P4	F11	c, 0						Hippuric acid		U		
P4	H10	c, 0	–	–				Nigerose		U		
P4	H11	c, 0	–	–				Nigerotetraose		U		
P6	F7	c, i	ii	ii			520	Phenolphthalein	6	TM		
Inhibitory effect in dot-blots												
P2, L3	A12, C8	i	i	i	i		310	4-Chloromercuribenzoic acid	6	SH		
P2	H7	i	i	i				Mycophenolic acid †	6	U	–	i
P4	E9	i	–	–				Caffeic acid		U		
P4	G7	i						Sodium tetraborate decahydrate		U		
P5	C9	i	iii	iii				Brilliant blue G †		TM	i	i
P5	C10	i			iii			Brilliant blue R	6	TM		
P5	C11	i			ii			Bromocresol purple	6	TM		
P5	C12	i	i	i			270	Bromocresol	6	TM		
P5	D1	i	ii	ii			27	Eosin Y	6	X		
P5	D2	i	ii	ii			36	Erythrosin B †	6	X	iii	ii
L3	C3	i			ii			Ebselen †	6	SH	iii	i
L3	D2	i			i			Phenylmercuric acetate	6	SH		
L6	D6	i			i			Silver nitrate		SH		
L7	C10	i				i		*N'*-(1*E*)-(5-Bromofuran-2-yl)methylidene-2-(3-methylphenoxy)acetohydrazide	6	U		
L7	G9	i				i		Epibrassinolide	6	U		
L8	D8	i			0			4-Bromo-*N*-[(2,3,4-trimethoxyphenyl)methyl]aniline		U		
L15	E8	i				i		3-{[3-(4-Hydroxyphenyl)-3-oxopropanoyl]amino}-4-[methyl(octadecyl)amino]benzoic acid	6	U		
L27	H4	i				0		2-(4-nitro-2-thienyl)-2,3-dihydro-1*H*-benzo[*d*]imidazole		U		

(*continued on next page*)