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Select Acetophenones Modulate Flagellar Motility in *Chlamydomonas*

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Acetophenones were screened for activity against positive phototaxis of *Chlamydomonas* cells, a process that requires co-ordinated flagellar motility. The structure–activity relationships of a series of acetophenones are reported, including acetophenones that affect flagellar motility and cell viability. Notably, 4-methoxyacetophenone, 3,4-dimethoxyacetophenone, and 4-hydroxyacetophenone induced negative phototaxis in *Chlamydomonas*, suggesting interference with activity of flagellar proteins and control of flagellar dominance.

Key words: acetophenone, *Chlamydomonas*, cilia, flagella, motility, phototaxis

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Eukaryotic cilia and flagella, now collectively referred to as cilia, are specialized organelles conserved throughout all eukaryotes. Recently, the importance of cilia in the maintenance of human health has become increasingly clear. It is now thought that almost every cell in the body possesses at least one cilium during its lifetime, and it is currently recognized that many diseases and syndromes are caused by defects in ciliary assembly and/or function (1–7). Motile cilia are specialized structures that move with a whip-like or wave-like motion. These structures are capable of complex, yet carefully co-ordinated movements that, in the human body, are important for processes such as embryonic development, fertilization, clearing of respiratory airways, and circulation of fluid in the brain (1,5,8). Movement of cilia is mediated by the axoneme, a highly ordered and conserved structure found at the ciliary core that is comprised of hundreds of proteins (9,10). Overall, very little is known about the roles these proteins play in control of ciliary assembly, signaling, and motility; a better understanding of these processes is essential to diagnose, treat, and prevent disease.

In an attempt to gain insight into the workings of the cilium, we sought to identify novel chemical inhibitors of motility. Accordingly, we screened a chemical library of acetophenones (all obtained from Sigma-Aldrich) for inhibitory effects on ciliary motility.

Acetophenones were selected for their commercial availability and generally low toxicity in eukaryotic cells. In addition, several acetophenones are natural products (11) or are catabolized by microorganisms, thus they are inherently involved in biological systems (12,13). For example, in a study examining acetophenones for activity against mycobacteria, cytotoxicity was observed against three eukaryotic cell lines (14). The cytotoxicity was not correlated with the antibacterial activity, suggesting different mechanisms. One active compound, 4-nitroacetophenone, also showed antibacterial activity against several other species in a second study (15). This study suggested that external cell hydrophobicity was correlated with activity, which could explain why the compounds are inhibitory toward the very lipid-rich mycobacteria. Thus, because acetophenones have demonstrated a number of biological activities, they are excellent candidates for bioassay screens. For this study, we employed the model organism, *Chlamydomonas reinhardtii*, a unicellular green alga with two flagella used for motility. Wild-type *Chlamydomonas* cells naturally migrate toward a light source, a process termed positive phototaxis. While the phototaxis signaling pathway is not well understood, it involves detection of a photon of light by photoreceptors in the *Chlamydomonas* eyespot, followed by a transient increase in intracellular calcium, and finally, a change in flagellar waveform such that the cell moves toward the light source (16,17). More specifically, when the cell is oriented such that the eyespot is facing the light source, the *trans* flagellum (the one farthest from the eyespot) responds to the rise in intracellular calcium by increasing the amplitude of its flagellar bend, thus causing the cell to turn toward the light (Figure 1). When the eyespot is facing away from the light source, the two flagella have similar waveform, although the *cis* flagellum exhibits a slightly larger amplitude (18).

Since positive phototaxis requires flagellar motility, we performed a simple phototaxis assay to test acetophenones for inhibitory effects on flagellar motility. Wild-type *Chlamydomonas* cells (CC-125, obtained from the *Chlamydomonas* Center)[a] were grown to a density of $\sim 5 \times 10^6$ cells/mL (mid log phase) in liquid modified medium I under constant aeration and a 14-h/10-h light/dark cycle (19). Cells were then incubated with 250–500 μg/mL (1–4 mM) of acetophenone for 4–6 h under constant light and gentle shaking. Ten milliliters of cells were then poured into a 60-mm × 15-mm Petri dish, and the dish was placed into a chamber such that half of the dish was exposed to a cool, white light source and the other half

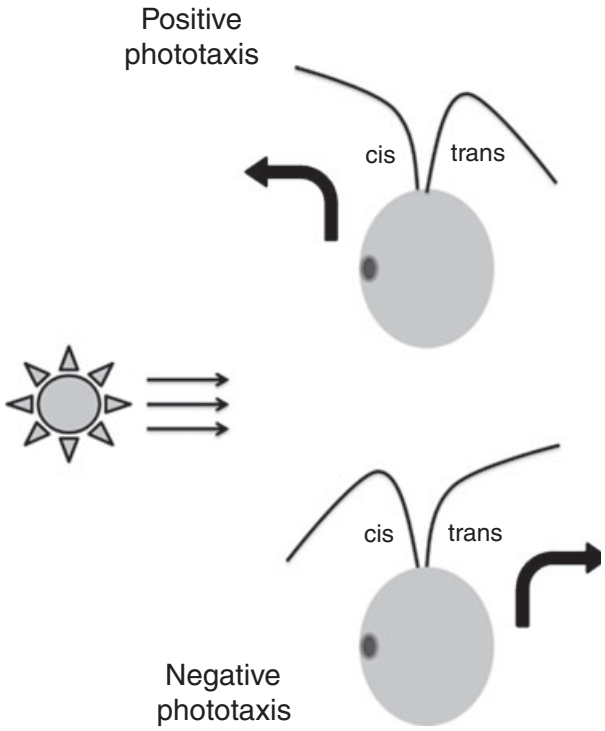


Figure 1: *Chlamydomonas* phototaxis. When the eyespot is facing the light source, the *trans* flagellum has a larger amplitude during positive phototaxis, while the *cis* flagellum has a larger amplitude during negative phototaxis (18).

was in complete darkness. After 10 min in the chamber, the dish was removed and observed for phototaxis. If the cells did not phototax, the dish of cells was returned to the phototaxis chamber for up to 30 additional minutes and observed again. Each compound was tested a minimum of three times to ensure reliability of phototaxis assay results.

Several acetophenones showed inhibitory effects on phototaxis in *Chlamydomonas*, including 3,4-dimethylacetophenone, 4-ethylacetophenone, 3-bromoacetophenone, 4-bromoacetophenone, 3,4-dichloroacetophenone, 4-piperidinoacetophenone, 4-cyclohexylacteophenone, and 4-trifluoroacetophenone (Table 1). These compounds all demonstrated a concentration-dependent effect. Since inhibition of phototaxis can be caused by a variety of disruptions within the cell, cells were plated on solid TAP (Tris–acetate–phosphate) medium following incubation with the compounds to test for cytotoxicity. Significant inhibition of cell growth, in a concentration-dependent manner, was observed for 3,4-dimethylacetophenone, 4-ethylacetophenone, 3-bromoacetophenone, 3,4-dichloroacetophenone, 4-cyclohexylacetophenone, and 4-trifluoroacetophenone, indicating that these compounds non-specifically interfere with phototaxis by disrupting overall cell viability. On the other hand, 4-bromoacetophenone and 4-piperidinoacetophenone had very little effect on cell growth, suggesting that these compounds specifically disrupt the phototaxis signaling pathway. Moreover, when these cells were examined for motility under the microscope, the cells were immotile, indicating that 4-bromoacetophenone and 4-piperidinoacetophenone interfere with flagellar function.

A number of acetophenones had no observed effect on phototaxis, including acetophenone (dihydroacetophenone), 4-methylacetophenone, 3-methoxyacetophenone, 4-fluoroacetophenone, 3-nitroacetophenone, and 4-morpholinoacetophenone (Table 1). Meanwhile, dimethyl sulfoxide (DMSO), the solvent used to prepare acetophenone stock solutions, had no effect on either phototaxis or cell viability (Table 2).

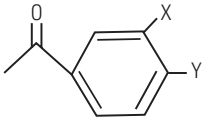
Interestingly, three compounds, 4-methoxyacetophenone, 3,4-dimethoxyacetophenone, and 4-hydroxyacetophenone, demonstrated the surprising effect of inducing negative phototaxis of *Chlamydomonas* (Table 1). Notably, 4-methoxyacetophenone and 4-hydroxyacetophenone induced almost complete negative phototaxis, while 3,4 dimethoxyacetophenone was somewhat less effective in this regard. This result suggests that the 3′ methoxy group may interfere with the reactivity of the 4′ methoxy group, and thus lessen its ability to cause negative phototaxis. The 'gain of function' result of inducing negative phototaxis indicates that these acetophenones are not grossly toxic to the cell, but rather that they specifically disrupt the phototaxis signaling pathway. Moreover, the results suggest that these compounds interfere with amplitude dominance of the *trans* flagellum, and therefore indirectly promote dominance of the *cis* flagellum (Figure 1). Three mutant strains of *Chlamydomonas* have been described that exhibit negative phototaxis because of dominance of the *cis* flagellum: *agg1*, *agg2*, and *agg3* (18,20). While the gene product of *AGG1* is unknown, *AGG2* and *AGG3* both code for flagellar proteins. Thus, defects in either Agg2p or Agg3p appear to cause reversal of flagellar dominance, resulting in negative phototaxis characteristic of the *agg2* and *agg3* mutant strains. We propose that 4-methoxyacetophenone, 3,4-dimethoxyacetophenone, and 4-hydroxyacetophenone may induce negative phototaxis of wild-type *Chlamydomonas* by interacting with Agg2p or Agg3p. Certain acetophenones have alkylating activity (21–23), thus it is possible that our active compounds are modifying Agg2p and/or Agg3p by alkylation. Additional experiments are currently underway to test this hypothesis.

In addition to the *agg* mutants, the *mbo* class of *Chlamydomonas* mutants also exhibits negative phototaxis. However, the *mbo* mutants exhibit a symmetric, sinusoidal waveform that is characteristic of flagellar motion, whereas other strains of *Chlamydomonas* typically demonstrate a breast-stroke waveform that is typical of ciliary motion. While the sinusoidal waveform, resulting in negative phototaxis, does occur in wild-type cells under conditions of extreme light intensity, *mbo* mutants exhibit this waveform under all light conditions (24). Our observations of *Chlamydomonas* motility in the presence of 4-methoxyacetophenone, 3,4-dimethoxyacetophenone, 4-hydroxyacetophenone reveal a breast-stroke waveform and not a sinusoidal waveform; thus, negative phototaxis is most likely caused by disruption of flagellar dominance such as that observed in the *agg* mutants.

In this study, 500 μg/mL of sodium azide (NaN_3) was used to disrupt phototaxis as a positive control in the phototaxis assay (Table 2). This concentration of NaN_3 was also completely lethal

Table 1: SAR of acetophenones

SAR of acetophenones



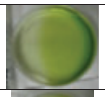
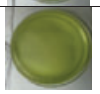
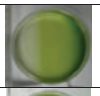
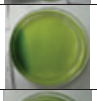
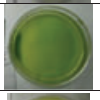
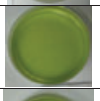
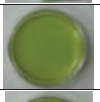
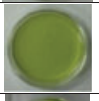
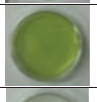
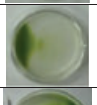
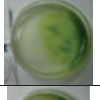
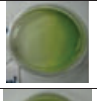
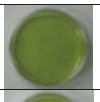
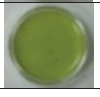
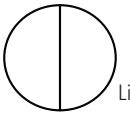
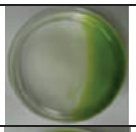
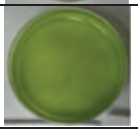
Compound	X	Y	Phototaxis	Dark / Light
Acetophenone	H	H	++	
4-Methylacetophenone	H	CH_3	++	
3,4-Dimethylacetophenone	CH_3	CH_3	None	
3-Methoxyacetophenone	OCH_3	H	++	
4-Methoxyacetophenone	H	OCH_3	−−	
3,4-Dimethoxyacetophenone	OCH_3	OCH_3	−	
4-Ethylacetophenone	H	CH_2CH_3	None	
3-Bromoacetophenone	Br	H	None	
4-Bromoacetophenone	H	Br	None	
3,4-Dichloroacetophenone	Cl	Cl	None	
4-Fluoroacetophenone	H	F	++	
4-Trifluoromethylacetophenone	H	CF_3	None	
4-Hydroxyacetophenone	H	OH	−−	
3-Nitroacetophenone	NO_2	H	++	
4-Morpholinoacetophenone	H	C_4H_8NO	++	
4-Piperidinoacetophenone	H	$C_5H_{10}N$	None	
4-Cyclohexylacetophenone	H	C_6H_{11}	None	

Table 2: Controls used in phototaxis assays

Compound	Concentration	Phototaxis	Dark  Light
Dimethylsulfoxide (C_2H_6OS)	0.5%	+ +	
Sodium azide (NaN_3)	500 μg/mL	None	

to cells, as shown by lack of cell growth on solid TAP medium. Azide is a known poison of cytochrome proteins in the electron transport chain. Moderately high concentrations of acetophenones (1–4 mM) were used in this study; however, several acetophenones still had no observed effect on either phototaxis or cell growth, demonstrating the rather low toxicity of these compounds. On the other hand, a few acetophenones were cytotoxic at this concentration, indicating that some acetophenones may have potential to be used as algicides. Further testing of biologically active acetophenones is needed to determine the minimum concentrations required for inhibition of phototaxis and cell growth.

In summary, here we present the results of a simple phototaxis assay that was used to screen acetophenones for inhibitors of flagellar motility. This novel assay has general utility to discover small molecule inhibitors of flagellar function. In a small initial screen of 17 compounds, five compounds interfered with motility, including three with the interesting ability to reverse phototaxis. Of those, the active functional groups para to the aceto group were methoxy and hydroxyl, suggesting the oxygen atom as important to the function. In addition, our data suggest that the oxygen atom must be directly bound to the phenyl ring, as 4-morpholinoacetophenone, in which the oxygen is displaced from the ring by three atoms, exhibited no effect on phototaxis. Future experiments will include screens of compounds based on the oxygen-containing pharmacophores discovered here.

Acknowledgments

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Note

[a]www.chlamy.org