Structure-Activity Correlations of Substituted 3(2 *H*)Furanones Chemically Related to the Bleaching Herbicide Flurtamone

Gerhard Sandmann and Peter Böger

Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, P.O. Box 5560, D-78434 Konstanz, Bundesrepublik Deutschland

Z. Naturforsch. **48c**, 312–316 (1993); received December 9, 1992

Bleaching Herbicide, Flurtamone

The bleaching herbicide flurtamone shows a concentration-dependent inhibition of phytoene desaturase activity in a cell-free assay. Structure-activity studies were carried out with 26 derivatives. For this purpose, I_{50} values for inhibition of formation of colored carotenoids in cultures of *Synechococcus* and with some selected compounds *in vitro* inhibition data were measured. For *meta*-substituted 4-phenyl derivatives a quantitative structure-activity correlation was calculated. Regression analysis showed a significant contribution of lipophilicity π and steric parameter B_2 of the substituents to inhibitory activity of these derivatives. In addition, the steric requirement of 4-phenyl substituents was further revealed by the substitution pattern at different positions. In general, mono substituents at the meta position were most favorable and only the very small fluoro group was allowed as a second substituent. When the ligands at position 2 of the furanone heterocycle were modified, phenyl or *para*-fluorophenyl groups were most advantageous. Among the available compounds with different alkyl substitutions of the 5-amino group, the ethyl derivative showed the highest inhibitory activity whereas the one with the branched isopropyl group was quite inactive.

Several bleaching herbicides which interfere with phytoene desaturation have been developed [1]. Based on their mode of action we can distinguish between different groups. The majority of compounds belongs to a type which directly inhibits phytoene desaturase *in vitro* [2]. For the study of their properties a cyanobacterial model system has been developed and extensively used [1]. Benzoylcyclohexanediones resemble another type which only shows bleaching effects including phytoene accumulations when applied to higher plants [3].

Among the compounds of the first group there are the 3(2 *H*)furanones with flurtamone as the representative herbicide. The mode of action of flurtamone was established with cress seedlings and a cyanobacterial model system all showing decrease of colored carotenoids and accumulation of phytoene [4]. Furthermore, direct interaction of flurtamone with phytoene desaturase was demonstrated by inhibition of this purified enzyme from *Synechococcus* which was overproduced in *E. coli* [5].

Reprint requests to Prof. G. Sandmann.

Verlag der Zeitschrift für Naturforschung, D-W-7400 Tübingen
0939–5075/93/0300–0312 $ 01.30/0

Qualitative structure-activity investigations have been carried out with bleaching herbicides of chemically different structures (see ref. [2] for review). They include flurtamone derivatives substituted at position 2 of the furanone ring, at the 4-phenyl ring and at the 5-amino group [6]. These studies were performed with intact plants after pre-emergence application. However, the screening data obtained as overall effects on intact plants were not suitable for quantitative structure-activity calculations and more compounds especially with other substitutions at the 4-phenyl ring are now available.

All quantitative structure-activity correlations obtained so far for bleaching herbicides were carried out by determination of carotenoid formation of unicellular cyanobacteria and with enzyme preparations from these species [2]. In the present publication we used the cyanobacterium *Synechococcus* to determine data on the interaction of substituted flurtamone derivatives with phytoene desaturase and to correlate some of these values with physicochemical properties of the modified ligands.

Materials and Methods

Cultures of *Synechococcus* PCC 7942 were grown as described [7]. Cells were harvested after

2 days and carotenoids were extracted with methanol/6% KOH (60 °C, 15 min) and partitioned into 10% diethylether in petrol. Details on determination of total carotenoids, determination of phytoene content by HPLC [8] and *in vitro* carotenogenesis were also given previously [7]. The radioactive substrate geranylgeranyl pyrophosphate used for the cell-free assay was generated by protein extracts from the *Fusarium* mutant SG4 from [2-^{14}C]mevalonic acid. Inhibition ratios (IR) for enzymatic phytoene desaturation in the presence of an inhibitor concentration of 50 nM were calculated as the ratio of radioactivity accumulated in phytoene *versus* radioactivity in β-carotene, related to the corresponding ratio of an untreated control [9].

Details on the phenylfuranones employed including the procedure of their synthesis are given elsewhere [6]. Quantitative structure-activity analysis was carried out with R. A. Coburn's multiple linear regression program, QSAR-PC, from Biosoft (Cambridge, U.K.). Physicochemical parameters (Hansch-Fujita hydrophobicity constants π and Verloop's steric constant (B_2) were provided by the data base of this program or taken from ref. [10]. The π value for $-OCF_2H$ was extrapolated from the values for $-OCH_3$ and $-OCF_3$ and in case of $-CO_2CH(CH_3)_2$ the π value for the *n*-propylester was used.

Results and Discussion

Flurtamone is a typical bleaching herbicide and its interaction with phytoene desaturation has already been demonstrated [4]. Fig. 1 shows the concentration-depending action of this herbicide on *in vitro* carotenoid synthesis. The assay involved the reaction sequence from geranylgeranyl pyrophosphate to β-carotene which covers the phytoene to β-carotene desaturation step catalyzed by phytoene desaturase. With increasing concentrations of flurtamone more of the substrate phytoene was accumulated and less β-carotene was formed. This *in vitro* system was used to determine the IR value [9] for a fixed concentration of herbicides.

For structure-activity investigations 26 different flurtamone analogs were provided from Chevron Chemical Company, Richmond, California. The first set of flurtamone derivatives represents 13 compounds in which the meta substituent of the 4-phenyl ring was modified (Table I). For all compounds I_{50} values for decreased formation of colored carotenoids in intact *Synechococcus* cells and for the 7 most active compounds *in vitro* IR values were determined which indicate inhibition of phytoene desaturase activity. The most inhibitory compound with lowest I_{50} values carried an $-OCF_3$ group but also the lead compound flurtamone ($-CF_3$) showed high inhibition. Replacement of this ligand by less lipophilic groups stepwise decreased herbicidal activity which was very low for the −H and −COOH derivative. Quantitative correlations of I_{50} values to physicochemical parameters were calculated. In this multiple regression analysis the only significant contributions

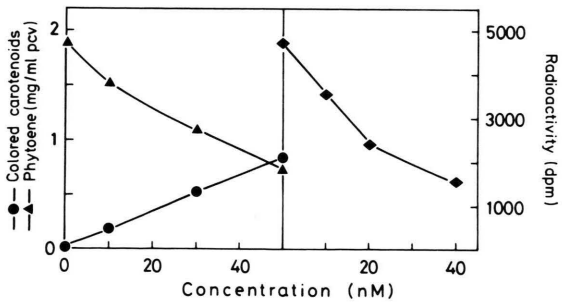
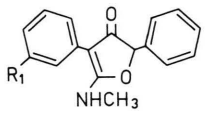


Fig. 1. *In vivo* and *in vitro* inhibition of formation of colored carotenoids by flurtamone.

Table I. Inhibition of phytoene desaturation by 4-phenyl substituted flurtamone derivatives.



	I_{50} (10^{-7} M)	IR*
R_1 = $-OCF_3$	0.11	11.35
$-CF_3$	0.32	3.02
−Br	0.72	1.87
$-OCF_2H$	1.21	1.32
$-CO_2CH_2CH_3$	1.32	1.26
$-CO_2CH(CH_3)_2$	1.43	1.35
$-OCH(CH_3)_2$	1.50	1.32
−Cl	2.31	–
$-CCl_3$	4.10	–
$-CO_2CH_3$	6.18	–
$-CH_3$	7.09	–
−H	42.8	–
−COOH	2920	–

* Determined as described in Materials and Methods for a fixed concentration of 50 nM.

to the QSAR equation was by lipophilicity π and the steric constant B_2:

$$pI_{50} = 0.515\,(\pm 0.082)\,\pi + 1.505\,(\pm 0.322)\,B_2 + 3.562\,(\pm 0.619)$$

$$n = 12;\ r = 0.96;\ s = 0.380$$

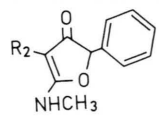
$$F = 48.34;\ t(\pi) = 6.26;\ t(B_2) = 4.68$$

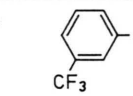
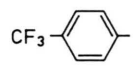
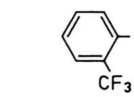
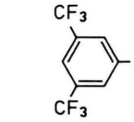
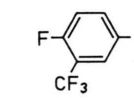
The validity of this regression was confirmed by an F test and the contributions of both independent variables by a t test which all were significant at least at the 5% level. It should be mentioned that the $-CCl_3$ derivative did not fit into this regression and was therefore omitted from the calculation. A similar correlation was calculated for log IR as dependent variable but this attempt was not successful as indicated by insignificant F and t values. This may be due to the small number of compounds which show inhibitory activity at the concentration of 50 nM used for determination of IR values. In a previous publication with substituted phenylpyridinones this *in vitro* parameter allowed for a quantitative QSAR equation which looked similar to the one obtained with I_{50} values [9].

QSAR calculations have been established already for variation of *m*-substituents in a group of bleaching herbicides, the phenylpyridazinones [11]. In this case, regression analysis showed a significant dependence between inhibition and of lipophilicity together with electronic properties of the corresponding ligands.

In Table II the effect of shifting the activating $-CF_3$ group from the meta to other positions at the 4-phenyl ring and the influence of disubstitution was investigated. CF_3 at the para position decreased herbicidal activity by a factor of about 2000. The resulting compound was even a magnitude of order less active then the corresponding unsubstituted compound (Table I). When this ligand was present at the ortho position inhibition was almost lost. Obviously, only the meta position is allowed for $-CF_3$. However, two CF_3 groups both at meta position strongly reduced herbicidal activity. Nevertheless, we found another disubstituted 4-phenyl furanone in which a second ligand exerted a positive effect. Introduction of the small and lipophilic fluorine group increased activity about 2-fold. This means that decrease of activity by disubstitution of the 4-phenyl ring [6] is not a general rule – it depends on the bulkiness of the second substituent, and suitable ligands can also have a positive effect. Alltogether, the results obtained with the derivatives of Table II are in line with the regression analysis performed with compounds of Table I. They both point to the very crucial steric requirements for ligands of the 4-phenyl ring which were introduced in order to increase herbicidal activity. Groups which are too bulky may counteract a possible positive contribution by their lipophilicity.

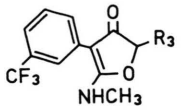
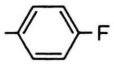
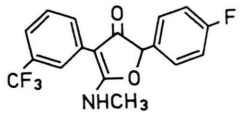
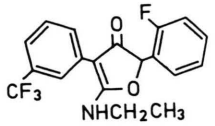
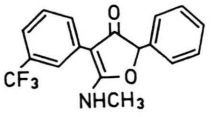
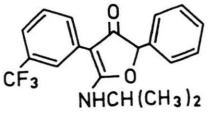
Table II. I_{50} values of trifluoromethylphenyl furanones after modifications at the 4-phenyl ring.



R_2 =	I_{50} (10^{-7} M)
	0.32
	660
	10000
	2100
	0.18

In the lead compound flurtamone position 2 of the furanone ring is substituted by phenyl. In Table III I_{50} values were compared for replacement of this ligand by heterocycles or alkyl groups. None of the monosubstitutions gave a better inhibitor. The *p*-FC_6H_4 derivative was almost as highly active as the compound with $-C_6H_5$. In the ranking of activity it was followed by a compound carrying an additional 2-furanone group which still showed reasonable activity. The compounds with a cyclohexyl or a 3-pyridyl substituent instead were even less active (about 20 times). Similar low inhibition was observed for compounds with an isopropyl group. Extremely low activity was found with a phenylfuranone carrying two methyl groups at position 2.

Table III. I_{50} values of flurtamone derivatives modified at position 2 of the furanone heterocycle (A) or substituted at the 5-amino group (B).

Structure	I_{50} (10^{-7} M)
A. 	
$R_3 = -CH(CH_3)_2$	5.04
	5.84
	1.07
	4.43
	0.44
	0.32
$-CH_3$, $-CH_3$*	1260
B. 	0.44
	0.22
	0.32
	61.7

* Disubstitution of position 2 by two methyl groups.

Quantitative structure-activity calculations were carried out with bleaching 5-phenyl pyridinones substituted at position 3 which by arrangement in the molecule is equivalent to position 2 in our furanone derivations [9]. Based on 9 derivatives a correlation of inhibiting potential was observed with lipophilicity and electronic properties of these substituents. The number of derivatives in Table III A was too low and the π and σ_p values were not available for some of them. Therefore, a quantitative structure-activity calculation using the constants of the two parameters mentioned was not carried out.

For the determination of the best pattern of substitution of the 5-amino group only two pairs of compounds were available to assess the influence of methyl, ethyl, and isopropyl groups. The first two compounds of Table III B carried either a methyl or an ethyl group. The rest of the molecule is the same except for the position of the fluoro-substituent of the 2-phenyl group which is present in a *para* arrangement in first and in *ortho* in the second case. Disregarding this difference which should exert a negligible effect on herbicidal activity, the N-ethyl-substituted compound is about twice as active as the N-methyl derivative. Comparing the third and fourth compound of Table III B a drastic decrease of inhibition of about 200-fold was observed when the N-methyl was replaced by an isopropyl-substituted amino group. It has already been demonstrated for phenylpyrrolidones that substituents at the central ring especially at the position most distant from the keto group have a certain steric requirement [12]. Herbicidal activity was best with an ethyl group at position 4 of the pyrrol ring which resembles our results (Table III B).

Structure-activity investigations with different classes of inhibitors interacting all with the same target enzyme and binding in a common region provide pieces of data to set up a model which describes the general structural features of this type of inhibitor. For herbicides which interfere with phytoene desaturase more structure-activity results using more chemically different groups of compounds are still needed to supply the information necessary for more refined computer modelling approaches. First attempts to compare and model phytoene desaturase inhibitors have already been made [6, 13].

Acknowledgements

Due thanks are expressed to Chevron Chemical Company, especially to Dr. C. E. Ward for providing us with all the flurtamone derivatives.

[1] G. Sandmann and P. Böger, in: Target Sites of Herbicide Action (P. Böger and G. Sandmann, eds.), pp. 25–44, CRC Press, Boca Raton, FL, 1989.
[2] G. Sandmann and P. Böger, in: QSAR in the Development of Agricultural Chemicals (W. Draber and T. Fujita, eds.), pp. 357–371, CRC Press, Boca Raton, FL, 1992.
[3] G. Sandmann, I. Kumita, and P. Böger, Pestic. Sci. **30,** 353–355 (1990).
[4] G. Sandmann, C. E. Ward, W. C. Lo, J. O. Nagy, and P. Böger, Plant Physiol. **94,** 476–478 (1990).
[5] G. Sandmann and P. D. Fraser, Z. Naturforsch. **48c,** in press (1993).
[6] C. E. Ward, W. C. Lo, P. B. Pomidor, F. E. Tisdell, A. W. W. Ho, C. L. Chiu, D. M. Tuck, C. R. Bernardo, P. J. Fong, A. Omid, and K. A. Buteau, in: Synthesis and Chemistry of Agrochemicals (D. R. Baker, J. G. Fenyes, W. K. Moberg, and B. Cross, eds.), pp. 65–73, ACS Symp. Ser. 355, American Chemical Society, Washington, D.C. 1987.
[7] G. Sandmann, in: Target Assays for Modern Herbicides and Related Phytotoxic Compounds (P. Böger and G. Sandmann, eds.), pp. 15–20, Lewis Publishers, Boca Raton, FL 1993.
[8] G. Sandmann, in: Target Assays for Modern Herbicides and Related Phytotoxic Compounds (P. Böger and G. Sandmann, eds.), pp. 9–13, Lewis Publishers, Boca Raton, FL 1993.
[9] G. Sandmann, S. Kowalczyk-Schröder, H. M. Taylor, and P. Böger, Pestic. Biochem. Physiol. **42,** 1–6 (1992).
[10] C. Hansch and A. Leo, Substitution Constants for Correlation Analysis in Chemistry and Biology, Wiley-Interscience, New York 1979.
[11] G. Sandmann and P. Böger, Z. Naturforsch. **37c,** 1092–1094 (1982).
[12] P. D. Fraser, N. Misawa, H. Linden, and G. Sandmann, J. Biol. Chem. **267,** 19891–19895 (1992).
[13] P. D. Fraser, H. Linden and G. Sandmann, Biochem. J. **291,** 687–692 (1993).