EFFECT OF 2-[3-(TRIFLUOROMETHYL)PHENYL]-4*H*-FURO[3,2-*b*]PYRROLE-5-CARBOXHYDRAZIDES ON PHOTOSYNTHETIC PROCESSES

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Abstract: A new series of carboxhydrazides **6-8** was synthesized under microwave irradiation by reaction of carboxhydrazide **1** with heterocyclic aldehydes **2-4** in the presence of *p*-toluenesulfonic acid in ethanol. *N*-Benzoylcarboxhydrazide **9** was prepared by reaction of **1** with benzoylchlorid **5** in THF at room temperature. The effects of **6-9** on inhibition of photosynthetic electron transport in spinach chloroplasts and chlorophyll content in the antialgal suspensions of *Chlorella vulgaris* were investigated.

Keywords: 2-[3-(trifluoromethyl)phenyl]furo[3,2-b]pyrrole-5-carboxhydrazide, Chlorella vulgaris, spinach chloroplasts, photosynthetic electron transport

1. Introduction

Carboxhydrazides and their derivatives represent an interesting class of compounds which exhibits antimicrobial (EL-SHAAER *et al.*, 1998), antifungal (DUTTA *et al*., 1986), analgesic and anti-inflammatory (SANTOS *et al.*, 1997) activities. The presence of trifluoromethyl group in the molecule leads often to the biologically active compounds. Many heterocycles bearing the trifluoromethyl group possess antiprotozoal (NAVARRETE-VAZQUEZ *et al.*, 2006), antimalarial (MADRID *et al*., 2005) or antibacterial (WOLFART *et al*., 2004) activities.

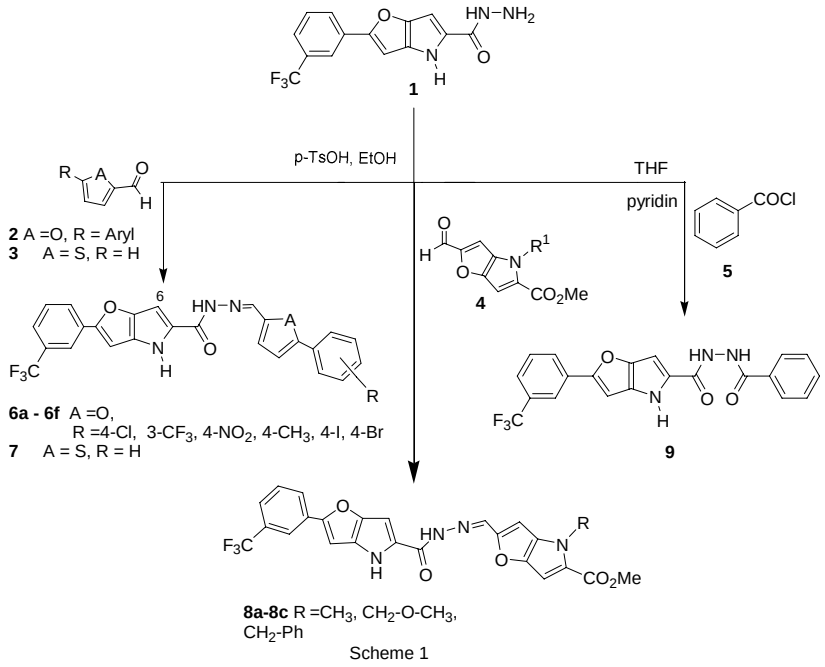
Substituted furans are structural units in natural products and pharmaceuticals (KRUTOŠÍKOVÁ, 1996) and have been widely used as synthetic intermediates (LIPSHUTZ, 1986). 5-Substituted furan-2-carboxaldehydes and some of their derivatives show antibacterial (SHINDHAR *et al*., 1980) or antiviral (SHIGETAKA *et al.*, 1970) activities

The present study is a follow-up paper to our previous research dealing with the synthesis and reactions of furo[3,2-*b*]pyrrole system and the study of its biological activity (GAŠPAROVÁ *et al*., 2005; MONCMAN, 2006).

2. Materials and methods

2.1 Chemistry

Scheme 1 shows the synthetic pathway to hydrazides **6-9.**



Scheme 1

2.1.1 General procedure for synthesis of 6-8

The mixture carboxhydrazide **1** (0.3 g, 1 mmol), 5-arylfuran-2-carboxaldehyde **2** (or thiophen-2-carboxaldehyde **3**, methyl 2-formylfuro[3,2-*b*]pyrrole-5-carboxylates **4**) (0.21 g, 1 mmol) and catalytic amount of *p*-toluenesulfonic acid in ethanol (5 cm^3) was irradiated in microwave oven at 90W for 0.5-2 min. After cooling, the solid product was filtered off, dried and crystallized from ethanol to give 51-87 % yields of products.

2.1.2 Synthesis of 9

The solution of carboxhydrazide **1** (0.5 g, 1.6 mmol) in THF (5 cm^3) and catalytic amount of pyridin was cooled in the ice bath to 0 °C. Then benzoylchlorid **5** (0.18g, 1.3 mmol) was added and the reaction mixture was stirred at room temperature for 5 h. The solid product was filtered off, dried and crystallized from ethanol to give 60 % of product.

2.2 Study of inhibition of photosynthetic electron transport in spinach chloroplasts

Spinach chloroplasts were prepared according to WALKER (1980). The effect of the compounds **5**-**7** on the inhibition of photosynthetic electron transport (PET) in

spinach chloroplasts was investigated spectrophotometrically in the presence of electron acceptor 2,6-dichlorophenol indophenol (DCPIP) (30 μmol.dm^{-3}). Before measurements, the chloroplasts were resuspended in phosphate buffer (20 mmol.dm^{-3}; pH = 7.2) containing 5 mmol.dm^{-3} $MgCl_2$ and 15 mmol.dm^{-3} NaCl. The chlorophyll content in the suspension was adjusted to 30 mg.dm^{-3}. Samples were irradiated at 25 °C with a halogen lamp (250 W) at a distance of 1 dm. A 4 cm water filter was used to prevent overheating of the samples. The PET-inhibitory activity of the compounds studied was expressed in term of IC_{50} values as their negative logarithms thus, corresponding to molar concentrations of inhibitors causing a 50% decrease of oxygen evolution rate (OER) with respect to the untreated control sample. Due to lower aqueous solubility of the compounds studied, these were dissolved in dimethyl sulfoxide. The effect of DMSO on OER in the suspensions of spinach chloroplasts was in the range of experimental error and could be neglected.

2.3 Study of chlorophyll content in Chlorella vulgaris

The algae *Chlorella vulgaris* were statically cultivated (photoperiod: 16h light / 8h dark; illumination: 5 000 lx; temperature: 23 ±1 °C) in liquid cultivation medium (pH = 7.2) (KRÁĽOVÁ *et al*., 1998). The effect of the compounds applied at three stepwise increasing concentrations (10, 50 and 100 μmol.dm^{-3}) on the total chlorophyll content of algal suspension was determined after 7 days of cultivation spectrophotometrically (Kontron Uvikon 800) and after extraction into methanol according to WELLBURN, 1994. The chlorophyll content in the suspensions at the beginning of cultivation was 0.1 mg.dm^{-3}. The effect of the compounds studied and applied in the concentration range 1–100 μmol.dm^{-3} on the content of chlorophyll in the suspensions was expressed as the percentage from the corresponding value obtained for the control (Table 2).

3. Results and discussion

3.1 Study of inhibition of photosynthetic electron transport in spinach chloroplasts

HILL and SCARISBRICK (1940) showed that isolated chloroplasts and chloroplast fragments could release O_2 in the light if they were given a suitable acceptor for the electrons being removed from H_2O. DCPIP (2,6-dichlorophenol indophenol) is often used as an synthetic electron acceptor for this reaction for measuring oxygen evolution rate (OER) in isolated plant chloroplasts (e.g. ŠERŠEŇ *et al*., 1990).

PET-inhibitory activity is exhibited by many compounds possessing X = C-NH group with a sp^2 hybridized carbon atom i.e. ureas, triazines or anilides (KRÁĽOVÁ *et al*., 1999; MILETIN *et al*., 2001). Due to formation of hydrogen bonds between this group and the target proteins in photosynthetic centers of thylakoid membranes, changes in protein conformation may occur resulting in inhibition of photosynthetic electron transport (DRABER *et al*., 1991).

Table 1. Inhibition of photosynthetic electron transport in spinach chloroplasts.

compound	R	log(1 / IC_{50}) [mol.dm^{-3}]	IC_{50} [mmol.dm^{-3}]
6a	4-Cl	**3.8975**	**0.127**
6b	3-CF_3	**4.1513**	**0.071**
6c	4-NO_2	**4.0244**	**0.095**
6d	4-CH_3	2.8936	1.278
6e	4-I	**3.7615**	**0. 173**
6f	4-Br	3.0702	0.851
7	-	3.2194	0.604
8a	CH_3	2.2948	5.180
8b	CH_2OCH_3	2.2857	5.178
8c	CH_2Ph	3.2857	0.518
9	-	3.6583	2.220

IC_{50} values of the standards, used for testing of herbicidal as well as antialgal activity (KUBICOVÁ *et al.*, 2003; CONRAD *et al.*, 1993), related to inhibition of photosynthetic electron transport in plant chloroplasts determined for these herbicides that act in the photosystem 2, varied in the range 0.25 – 0.79 μmol.dm^{-3} for atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), 0.40 – 3.98 μmol.dm^{-3} for simazine (2-chloro-4,6-bis(ethylamino)-1,3,5-triazine) and 0.032-0.200 μmol.dm^{-3} for diurone (1-(3,4-dichlorophenyl)-3,3-dimethylurea), as described by FEDTKE (1982).

In comparison with these values, carboxhydrazides **6 – 9** showed relatively low inhibitory effect on photosynthetic electron transport (PET) in spinach chloroplasts (Table 1). The most effective inhibitors were compounds **6a** (R = 4-Cl), **6b** (R = CF_3), **6c** (R = 4-NO_2) and **6e** (R = 4-I). The influence of the electron acceptor properties of R substituent on PET-inhibitory activity was not significant.

3.2 Study of chlorophyll content in Chlorella vulgaris

In the concentration range (1 – 100 μmol.dm^{-3}) the majority of carboxhydrazides **6 – 9** only slightly reduced chlorophyll content in statically cultivated algal suspensions of *Chlorella vulgaris* (Table 2). The most effective inhibitors were compounds **6c** (R = 4-NO_2) a **6e** (R = 4-I).

Table 2. Effect of compounds **6-8** on chlorophyll content in algal suspensions of *Chlorella vulgaris*

Compound	R	Concentration (μmol.dm^{-3})	Concentration of chlorophyll (mg .dm^{-3})	Average in 10 – 100 μM % of control
control		0	7.333	
6a	4-Cl	100	5.561	84.3±4.8
		50	6.424	
		10	6.342	

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Compound	R	Concentration ($\mu mol.dm^{-3}$)	Concentration of chlorophyll ($mg.dm^{-3}$)	Average in 10 – 100 μM % of control
6b	3-CF_3	100 50 10	6.610 6.629 6.836	91.3±1.7
6c	4-NO_2	100 50 10	1.877 2.609 5.539	**30.9***
6d	4-CH_3	100 50 10	6.516 6.691 6.964	91.7±3.1
6e	4-I	100 50 10	3.552 4.979 5.741	**100***
6f	4-Br	100 50 10	5.917 5.909 6.379	82.8±3.7
7	-	100 50 10	7.217 6.682 5.917	90.1±8.9
8a	CH_3	100 50 10	5.714 6.570 6.946	87.4±7.6
8b	CH_2OCH_3	100 50 10	4.655 6.317 6.222	78.2±4.3
8c	CH_2Ph	100 50 10	6.958 6.400 6.409	93.2±5.1
9	-	100 50 10	5.833 8.564 6.267	81.7±3.3

* $IC_{50\ in}$ μM

4. Conclusions

N′-{[5-(R-Phenyl)furan-2-yl]methylene}-2-[3-(trifluoromethyl)phenyl]-4*H*-furo [3,2-*b*] pyrrole-5-carboxhydrazides **6a – 6f**, *N*′-[(thiophen-2-yl)methylene]-2-[3-(trifluoromethyl) phenyl] - 4*H* – furo [3,2-*b*] pyrrole – 5 - carboxhydrazide **7**, N′ - {[5 - (methoxycarbonyl) – 4 - R^1 – furo [3,2-*b*] pyrrol – 2 - yl] methylene} – 2 - [3 - (trifluoromethyl) phenyl]-4*H*-furo[3,2-*b*]pyrrole-5-carboxhydrazides **8a – 8c** and *N*′-benzoyl-2-[3-(trifluoromethyl)phenyl]-4*H*-furo[3,2-*b*]pyrrole-5-carboxhydrazide **9** showed relative-ly low inhibitory effect on the photosynthetic electron transport of spinach chloroplasts and only slightly reduced chlorophyll content in statically cultivated algal suspensions of *Chlorella vulgaris*. The most effective inhibitors of the PET were compounds **6a** (R = 4-Cl), **6b** (R = CF_3), **6c** (R = 4-NO_2) and **6e** (R = 4-I). Carboxhydrazides **6c** (R = 4-NO_2) a **6e** (R = 4-I) were proved as the best inhibitors of the chlorophyll content in suspensions of *Chlorella vulgaris*.

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