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Short communication

Bifunctional derivative of *p*,*p*′-dichlorochalcone Part III. Synthesis and study for cytotoxic activity of a new compound, 2-[2,2-bis(4-chlorophenyl)ethyl]-2-(4-chlorophenyl)-thiazolidin-4-one from *p*,*p*′-dichlorochalcone

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Abstract

The synthesis of 2-(2,2-bis(4-chlorophenyl)ethyl]-2-(4-chlorophenyl)-thiazolidin-4-one (**3**) from *p*,*p*′-dichlorochalcone (**1**) via 1,3,3-tris(4-chlorophenyl)-propan-1-one (**2**) using thioglycollic acid in the presence of ammonium carbonate is described. Structural assignment, stereochemistry and biological assay are discussed.

Keywords: organic synthesis; chalcone; 1,3,3-tris(4-chlorophenyl)propan-1-one; thiazolidin-4-one; ^{1}H-NMR; ^{13}C-NMR; cytotoxic activity

1. Introduction

A survey of the literature reveals that in recent years the interest in thiazolidin-4-ones for medical applications is increasing strongly. Substituted thiazolidin-4-ones show anti-platelet activating factor [1], anticonvulsant [2], antidiarrhoeal [3], antihistaminic [4], antimicrobial [5], oxygenase inhibitory [6] and calcium antagonist activity [7]. As part of our continuing programme in the search of biologically active compounds with nitrogen and sulphur containing heterocycles, we have synthesised 2-[2-carboxymethylthio-2-(4-chlorophenyl)ethyl]-2-(4-chlorophenyl)thiazolodin-4-one as a novel compound [8].

In continuation of the above mentioned work, we report here the synthesis of a new compound 2-[2,2-bis(4-chlorophenyl)ethyl]-2-(4-chlorophenyl)thiazolidin-4-one (**3**) from *p*,*p*′-dichlorochalcone (**1**) via 1,3,3-tris(4-chlorophenyl)propan-1-one (**2**) using thioglycollic acid in the presence of ammonium carbonate. Screening results are also summarised for cytotoxic activity against 60 cell lines of nine types of human cancers: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast.

2. Results and discussion

The synthesis of compound **3** was performed in two steps (Fig. 1): compound **2** was first prepared following a published procedure [9] by adding an excess of chlorobenzene to a suspension of chalcone **1** and

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anhydrous aluminium chloride (molar ratio, 1:3) as colourless crystalline needles in 66% yield. The adduct **2** was then condensed with thioglycollic acid and ammonium carbonate (molar ratio, 1:1.30:5.25). The cyclocondensation was carried out by refluxing the reaction mixture in dry benzene for 40 h with azeotropic removal of water. Purification of the products by column chromatography over silica gel followed by crystallisation afforded compound **3** as colourless globules in 58.6% yield.

The structures of **2** and **3** have been confirmed by IR, DCIMS, ^{1}H- and ^{13}C-NMR spectra and by a comparison with the NMR spectra of an analogous previously described compound [8]. From additional HETCOR, long-range HETCOR and 2D-NOE spectra (Tables 1 and 2) for compound **3**, a most preferred conformation as presented in Fig. 2 could be deduced. Especially, according to Karplus [10], the coupling constants $J_{1'up,2'}$ and $J_{1'dn,2'}$ indicate a synclinal position of H-2′ relative to the diastereotopic hydrogen atoms H-1′$_{up}$ and H-1′$_{dn}$. This is further confirmed by a NOE-correlation of H-2′ with both H-1′$_{up}$ and H-1′$_{dn}$.

3. Cytotoxic activity against malignant human tumour cells

Compound **3** was screened for cytotoxic activity [11] at five different concentrations against 60 cell lines of nine types of human cancers: leukaemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. A 48-h continuous drug exposure protocol was used and a sulphorhodamine B protein assay was used to estimate cell viability or growth. Results (Table 3) are expressed as $\log_{10}$ GI50 which is the drug concentration (M) causing a 50% reduction in the net protein increase in control cells during the drug incubation. The new compound shows activity only at higher concentration. Noteworthy results are obtained in the case of melanoma, colon and renal cancers where the reduction in growth is 52, 80 and 91%, respectively.

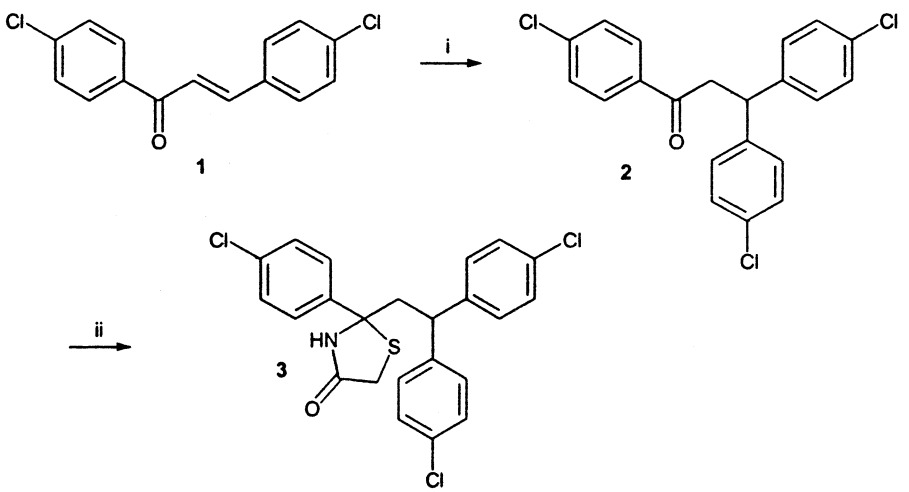

Fig. 1. Synthesis of **3**: (i) **1**/PhCl/anhyd. $AlCl_3$ (molar ratio, 1:large excess [9]:3), stirred at r.t. 2.5 h; (ii) **2**/$HSCH_2COOH$/$(NH_4)_2CO_3$ (molar ratio, 1:1.30:5.25), dry benzene, reflux, 40 h.

Table 1

H no.	δ (ppm)	Integration	Multiplicity	J (Hz)	NOE
5_{up}	3.45	1H	d	$J_{5up,5dn} = 15.42$	–
5_{dn}	3.56	1H	d	$J_{5up,5dn} = 15.42$	–
NH	8.04	1H	br s	–	–
1′$_{up}$	3.05	1H	dd	$J_{1'up,1'dn} = 14.80$; $J_{1'up,2'} = 5.03$	H-2′; H–Ar-2,6; H–Ar″-2,6
1′$_{dn}$	3.16	1H	dd	$J_{1'up,1'dn} = 14.80$; $J_{1'dn,2'} = 7.78$	H-2′; H–Ar′-2,6
2′	4.33	1H	dd	$J_{1'up,2'} = 5.03$; $J_{1'dn,2'} = 7.78$	H-1′$_{up}$; H-1′$_{dn}$; H–Ar′-2,6; H–Ar″-2,6
Ar-2,6	7.46	2H	d	$J_{Ar\text{-}2,6,Ar\text{-}3,5} = 8.86$	H-1′$_{up}$
Ar-3,5	7.31	2H	d	$J_{Ar\text{-}2,6,Ar\text{-}3,5} = 8.86$	–
Ar′-2,6	7.30	2H	d	$J_{Ar'\text{-}2,6,Ar'\text{-}3,5} = 8.40$	H-1′$_{dn}$; H-2′
Ar′-3,5	7.20	2H	d	$J_{Ar'\text{-}2,6,Ar'\text{-}3,5} = 8.40$	–
Ar″-2,6	7.36	2H	d	$J_{Ar''\text{-}2,6,Ar''\text{-}3,5} = 8.55$	H-1′$_{up}$; H-2′
Ar″-3,5	7.28	2H	d	$J_{Ar''\text{-}2,6,Ar''\text{-}3,5} = 8.55$	–

Table 2

C no.	δ (ppm)	INEPT	Long-range HETCOR correlation with
2	70.84	CH_2	H-1′$_{up}$, H-1′$_{dn}$, H–Ar-2,6
4	173.58	C	H-5$_{up}$, H-5$_{dn}$
5	33.51	CH_2	–
1′	49.91	CH_2	H-2′
2′	47.89	CH	H-1′$_{dn}$, H–Ar′-2,6, H–Ar″-2,6
Ar-1	145.21	C	H–Ar-3,5
Ar-2,6	127.91	CH	–
Ar-3,5	129.10	CH	–
Ar-4	133.69 [a]	C	–
Ar′-1	144.90 [b]	C	–
Ar′-2,6	130.52	CH	H-2′
Ar′-3,5	129.32	CH	–
Ar′-4	132.55 [a]	C	–
Ar″-1	144.25 [b]	C	–
Ar″-2,6	130.26	CH	H-2′
Ar″-3,5	129.44	CH	–
Ar″-4	132.40 [b]	C	–

[a] Assignment may be reversed.
[b] Assignment may be reversed.

4. Experimental

Reagents and solvents were of commercial grade and were used without further purification. Column chromatography was performed on silica gel 60–120 mesh LR (25049). Melting points were determined in a Koffler hot-plate apparatus and are uncorrected. IR spectra were recorded in a Perkin–Elmer 621 spectrophotometer in KBr pellets. ^{1}H- and ^{13}C-NMR spectra were recorded in a Varian unity 400 spectrometer in acetone-d_6, with TMS as the internal standard. DCI-mass spectra were recorded in a Ribermag R10-10B quadrupole mass spectrometer, using ammonia as reagent gas. HRMS values were recorded in a quadrupole-time of flight mass spectrometer (Qtof2, Micromass, Manchester, UK) equipped with a standard electrospray ionisation (ESI) interface. Samples were dissolved in acetone (p.a.) (**2** 1.2 mg mL^{-1}; **3** 1.0 mg mL^{-1}) and diluted 1/10 in methanol (HPLC grade) containing 0.1% of acetic acid. Using a Harvard syringe pump (model 22) these solutions were infused at a flow rate of 1 μL min^{-1}. To enhance the $[M + Na]^+$ signal of **2** 10 μL of a NaI solution (2 μg/μl in 50:50 isopropyl alcohol/H_2O) was added.

4.1. p,p′-Dichlorochalcone (1)

Compound **1** was prepared exactly as reported in our previous paper [8].

4.2. 1,3,3-Tris(4-chlorophenyl)propan-1-one (2)

To a suspension of *p,p′*-dichlorochalcone (**1**) (990 mg, 3.57 mmol) and anhydrous aluminium chloride (1450 mg, 10.9 mmol) in chlorobenzene (8 mL), chlorobenzene (25 mL) was added slowly with stirring at room temperature. After complete addition, the reaction mixture was stirred for 2 h and worked up as usual. The products on crystallisation from benzene yielded **2** as white crystalline needles, 920 mg (66%), m.p. 155 °C, R_f 0.58 (pet. ether (40–60°)–benzene, 9:1 v/v).

ν_{max} 1677 (C=O), 1590 (phenyl), 1485, 1400, 1264, 1209, 1090, 1013, 838, 774 cm^{-1}; δ_H 8.04 (2H, d, $J = 8.69$ Hz, Ar–H-2,6) [12], 7.51 (2H, d, $J = 8.69$ Hz, Ar–H-3,5), 7.39 (4H, d, $J = 8.69$ Hz, Ar′/Ar′–H-3,5), 7.28 (4H, d, $J = 8.69$ Hz, Ar′/Ar′–H-2,6), 4.78 (1H, t, $J = 7.32$ Hz, H-3), 3.92 (2H, d, $J = 7.32$ Hz, H-2); δ_C 196.95 (C-1), 144.09 (Ar′/Ar′–C-1) [12], 139.68 (Ar–C-4), 136.66 (Ar–C-1), 132.56 (Ar′/Ar′–C-4), 130.68 (Ar–C-2,6), 130.47 (Ar′/Ar′–C-2,6), 129.64 (Ar–C-3,5), 129.32 (Ar′/Ar′–C-3,5), 45.77 (C-3), 44.58 (C-2); DCIMS (NH_3); *m/z*: 401/403/405/407 $[M + NH_4]^+$, 389/391/393/395 $[M + H]^+$, 235/237/239 [(*p*-ClC$_6$-

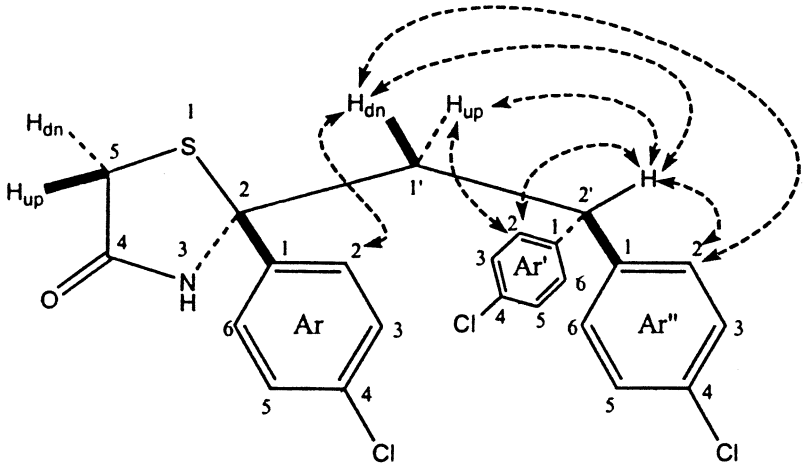

Fig. 2.

Table 3

Type of cancer	Cell line	$\log_{10}$ GI50	Retardation of growth (%)	Concentration (M)
Leukaemia	CCRF-CEM	−5.33	–	–
	HL-60 (TB)	−5.42	31	1.0×10^{-4}
	K-562	−5.29	–	–
	MOLT-4	−5.48	5	1.0×10^{-4}
	RPMI-8226	−5.30	24	1.0×10^{-4}
	SR	−5.37	30	1.0×10^{-4}
Non-small cell	A549/ATCC	−5.22	–	–
Lung cancer	EKVX	−4.77	–	–
	HOP-62	−4.85	6	1.0×10^{-4}
	HOP-92	−4.82	9	1.0×10^{-4}
	NCI-H226	−5.01	30	1.0×10^{-4}
	NCI-H23	−5.01	–	–
	NCI-H322M	−4.35	–	–
	NCI-H460	−5.04	–	–
	NCI-H522	−5.20	–	–
Colon cancer	COLO 205	−5.32	80	1.0×10^{-4}
	HCC-2998	−4.71	–	–
	HCT-116	−5.02	–	–
	HCT-15	−4.97	–	–
	HT29	−5.02	–	–
	KM12	−4.87	–	–
	SW-620	−4.74	–	–
CNS cancer	SF-268	−4.76	–	–
	SF-295	−5.29	13	1.0×10^{-4}
	SF-539	−4.64	21	1.0×10^{-4}
	SNB-19	−4.48	–	–
	SNB-75	−4.47	–	–
	U251	−4.99	13	1.0×10^{-4}
Melanoma	LOX IMVI	−5.07	11	1.0×10^{-4}
	MALME-3M	−4.67	7	1.0×10^{-4}
	M14	−4.93	–	–
	SK-MEL-2	−4.71	4	1.0×10^{-4}
	SK-MEL-28	−4.37	–	–
	SK-MEL-5	−4.90	52	1.0×10^{-4}
	UACC-257	−4.85	–	–
	UACC-62	−4.90	14	1.0×10^{-4}
Ovarian cancer	IGROVI	−4.58	–	–
	OVCAR-3	−5.21	27	1.0×10^{-4}
	OVCAR-4	−5.17	–	–
	OVCAR-5	>-4.00	–	–
	OVCAR-8	−4.84	–	–
	SK-OV-3	−4.35	–	–
Renal cancer	786-O	−5.01	9	1.0×10^{-4}
	A498	−4.94	29	1.0×10^{-4}
	ACHN	−4.95	3	1.0×10^{-4}
	CAKI-1	−4.72	–	–
	RXF 393	−5.42	44	1.0×10^{-4}
	SN12C	−5.67	33, 91	1.0×10^{-5}, 1.0×10^{-4}
	TK-10	−4.29	–	–
	UO-31	−4.60	–	–
Prostate cancer	PC-3	−5.33	6	1.0×10^{-4}
	DU-145	−4.41	–	–
Breast cancer	MGF7	−5.38	33	1.0×10^{-4}
	NCI/ADR-RES	−5.09	–	–
	HS 578T	−4.42	–	–
	MDA-MB-435	−4.85	–	–
	MDA-N	−5.09	–	–
	BT-549	−4.30	–	–
	T-47D	−5.37	–	–
MG_MID		−4.92	–	–
Delta		0.75	–	–
Range		1.67	–	–

$H_4)_2CH]^+$, 139/141 [p-$ClC_6H_4CO]^+$; HRMS (ESI) for $C_{21}H_{25}Cl_3O$ $[M + Na]^+$: Calc. 411.0086. Found 411.0089.

4.3. 2-[2,2-Bis(4-chlorophenyl)ethyl]-2-(4-chlorophenyl)-thiazolidin-4-one (3)

To a solution of the adduct **2** (890 mg, 2.3 mmol) in dry benzene (20 mL) thioglycollic acid (280 mg, 3.0 mmol) and ammonium carbonate (1160 mg, 12.1 mmol) were added. The reaction mixture was refluxed for 40 h with stirring while collecting the generated water in an azeotropic collector, and was worked up as usual. The products on purification on a silica gel column (pet. ether (40–60°)–diethylether, 3:7 v/v) and crystallisation (benzene–acetone, 9:1 v/v) afforded white crystalline globules, 615 mg (58.6%), m.p. 170 °C, R_f 0.85 (pet. ether (40–60°)–diethylether, 1:1 v/v).

ν_{max} 3380 (NH), 1665 (–CONH), 1600, 1590 (phenyl), 1470 (S–CH_2), 1405 (C–H), 1250, 1080, 1010, 825 cm^{-1}; The δ_H and δ_C values are given in Tables 1 and 2; DCIMS (NH_3); m/z: 479/481/483/485 $[M + NH_4]^+$, 462/464/466/468 $[M + H]^+$, HRMS (ESI) for $C_{23}H_{18}Cl_3NOS$ $[M + H]^+$: Calc. 462.0253. Found 462.0264.

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[12] Ar = 1-p-Cl-phenyl, Ar′ = 3-p-Cl-phenyl.