Synthesis and Hypoglycaemic Activity of Some New Flavone Derivatives Part 1: Flavonylsulphonylurea Derivatives

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Summary

A new series of flavonyl-3′-sulphonylurea derivatives (**1–7**) was prepared by reaction of several isocyanates with flavone-3′-sulphonamide (**III**). The prepared compounds were tested for their insulinotropic activities in INS-1 cells. Compounds **1** and **4** were able to increase insulin release in the presence of 8.3 mM glucose at least at the higher concentrations used.

Introduction

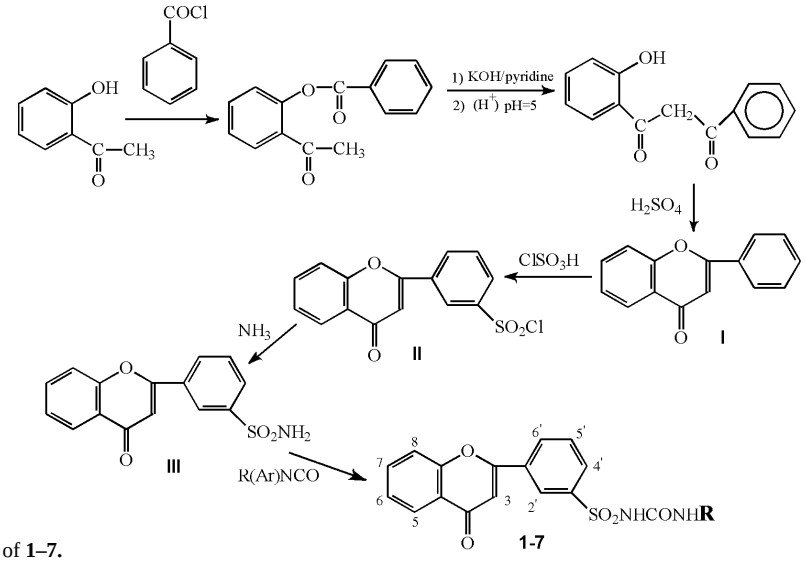
Type 2 diabetes is characterised by abnormalities of insulin secretion and by insulin resistance of major target tissues [1,2]. The sulphonylurea class of oral antidiabetic agents that are largely used in the treatment of type 2 diabetes act via a specific receptor as secretagogues and increase the availability of insulin [3, 4]. In this study, we report the synthesis and insulin releasing activities of some sulphonylurea derivatives containing flavone ring system that are already known to have antidiabetic [5–7] and aldose reductase inhibitor activity [8–11].

Results and Discussion

Non-substituted flavone (**I**) was prepared by the Baker-Venkataraman method. Flavone-3′-sulphonyl chloride (**II**) was obtained by **I** in chlorosulphonic acid. The sulphonyl chloride group of flavone was converted into sulphonamide in NH_3 [12]. Flavone-3′-sulphonylurea derivatives (**1–7**) were synthesised by substituted isocyanates and flavone-3′-sulphonamide (**III**) (Scheme 1).

The structure of flavone-3′-sulphonamide (**III**) was elucidated by ^{1}H NMR, Mass spectral data and IR findings. In addition the X-ray analysis data of **III** also prove these findings [12].

All spectral data were in accordance with assumed structures of **1–7**. IR spectra of the compounds showed γ-pyron C=O stretching bonds at 1622–1664 cm^{-1}. In ^{1}H NMR spectra, it has seen the characteristic protons belonging the flavone ring at the various δ ppm values. Flavone 5-H proton of all the compounds except **6** was observed at lower field between 7.97–8.39 ppm than other flavone ring protons due to the effect of the CO group of γ-pyron. Synthesised compounds **1**, **2**, **4**, and **6** have molecular ion (M^+) peak, the other not. The characteristic fragmentation ions of flavone and sulphonylurea moiety of all the compounds are shown (**a**, **b**,



Scheme 1. General synthesis of **1–7.**

	1	2	3	4	5	6	7
R	C_2H_5	$CH_2CH{=}CH_2$	$CH(CH_3)_2$	$n.C_4H_9$			

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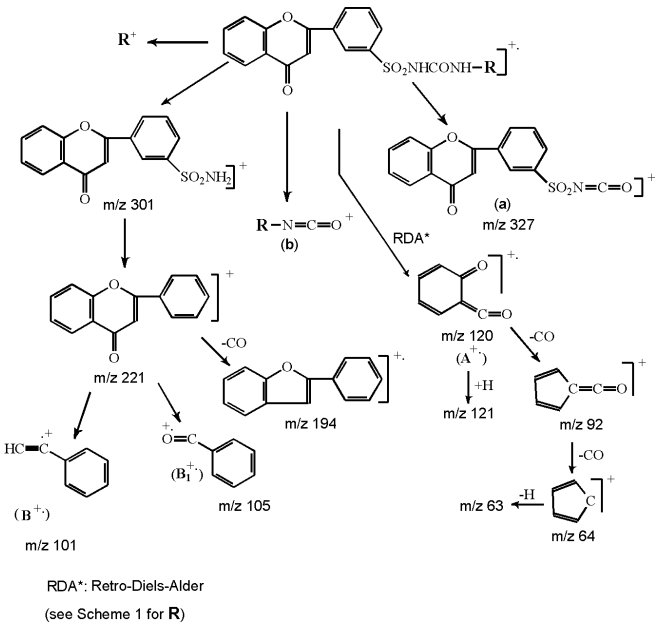
Table 1. Spectral data of **1–7**.

No	^{1}H NMR (ppm)	MS (70 eV) *m/z*	IR (cm^{-1}) (γ-pyron CO)
1	0.95 (t, 3H, CH_3), 3.00–3.05 (m, 2H, CH_2), 6.30 (broad s,1H, CONH), 6.94 (s, 1H, 3-H), 7.45 (ddd, 1H, $J_{6,5}$=$J_{6,7}$=7.34 Hz, 6-H), 7.66–7.77 (m, 3H, 5′, 7, 8-H), 8.01 (s, 1H, 2′-H), 8.08 (dd, 2H, 4′, 6′-H), 8.25 (d, 1H, $J_{5,6}$=7.42 Hz, 5-H), 8.51 (s, 1H, SO_2NH)	372(M+),327,301,221, 194,121,120,105,101, 92,71,64,63, 56 (100%)	1655
2	3.66 (t, 2H, NH*CH_2*), 4.99–5.09 (dd, 2H, CH=*CH_2*), 5.69–5.79 (m, 1H, CH_2-*CH*=CH_2), 6.55 (t, 1H, CONH), 6.99 (s, 1H, 3-H), 7.49 (ddd, 1H, $J_{6,5}$=$J_{6,7}$=7.31 Hz, 6-H), 7.70–7.83 (m, 3H, 5′, 7, 8-H), 8.05 (s, 1H, 2′-H), 8.1–8.14 (dd, 2H, 4′, 6′-H), 8.29 (d, 1H, $J_{5,6}$=7.88 Hz, 5-H), 8.56 (s, 1H, SO_2NH)	386(M+),328,327,301, 221,194,121,120,105, 101,92,84,64(100%), 63, 57	1645
3	1.02 (d, 6H, CH_3), 3.65–3.75 (m, 1H, CH), 6.00 (broad s, 1H, CONH), 6.86 (s, 1H, 3-H), 7.41 (ddd, 1H, $J_{6,5}$=$J_{6,7}$=7.55 Hz, 6-H), 7.60–7.78 (m, 3H, 5′, 7, 8-H), 7.80 (s, 1H, 2′-H), 8.08 (dd, 2H, 4′, 6′-H), 8.14 (d, 1H, $J_{5,6}$=7.73 Hz, 5-H), 8.49 (s, 1H, SO_2NH)	327,301,221,194,121,120, 105,101, 92(100%),85, 64, 63	1653
4	0.74 (t, 3H, CH_3), 1.04–1.31 (m, 4H, *$(CH_2)_2$*CH_3), 2.93 (m, 2H, NH*CH_2*), 6.69 (t, 1H, CONH), 7.11 (s, 1H, 3-H), 7.52 (ddd, 1H, $J_{6,5}$=$J_{6,7}$=6.79 Hz, 6-H), 7.76–7.85 (m, 3H, 5′, 7, 8-H), 7.90 (s, 1H, 2′-H), 8,0–8.10 (dd, 2H, 4′,6′-H) 8.39 (d, 1H, $J_{5,6}$=7.86 Hz, 5-H), 8.52 (s, 1H, SO_2NH)	400(M+),328,327,301, 221,194,121, 120 (100%), !05, 101, 99, 92, 73, 64, 63, 57	1664
5	1.06–1.71 (m, 11H, cyclohexyl protons), 6.20 (d, 1H, CONH), 6.93 (s, 1H, 3-H), 7.45 (ddd, 1H, $J_{6,5}$=$J_{6,7}$=7.52 Hz, 6-H), 7.65–7.76 (m, 3H, 5′, 7, 8-H), 7.96 (s, 1H, 2′-H), 8.09 (dd, 2H, 4′, 6′-H), 8.23 (d, 1H, $J_{5,6}$=8.01 Hz, 5-H), 8.50 (s, 1H, SO_2NH)	328,327,301,285,221, 194,125,121,120,105, 101, 99, 92(100%),64, 63, 57	1648
6	6.72 (ddd, 1H, $J_{c,b}$=7.34 Hz, c), 6.86 (s, 1H, 3-H), 7.04 (ddd, 2H, $J_{b,a}$=7.54 Hz, bb′), 7.40 (ddd, 1H, $J_{6,5}$=$J_{6,7}$=7.66, 6H), 7.45 (dd, 2H, aa′), 7.53 (ddd, 1H, $J_{7,8}$=$J_{7,6}$=7.81 Hz, 7-H), 7.61 (d, 1H, $J_{8,7}$=8.38 Hz, 8-H), 7.72 (ddd, 1H, $J_{5',4'}$=$J_{5',6'}$=7.04 Hz, 5′-H), 7.91 (s, 1H, 2′-H), 7.94 (s, 1H, CONH), 7.97 (d, 1H, $J_{5,6}$=7.97 Hz, 5-H), 8.06–8.09 (m, 2H, 4′, 6′-H), 8.49 (s, 1H, SO_2NH)	420(M+),328,327,301, 285,221,194,121,120, 119,105,101,93,92,64, 63 (100%)	1622
7	2.15 (s, 3H, CH_3), 6.85 (d, 2H, J_{ba}=8.30 Hz, bb′), 6.89 (s, 1H, 3-H), 7.29 (d, 2H, J_{ab}=8.34 Hz, aa′), 7.43 (ddd, 1H, $J_{6,7}$=$J_{6,5}$=7.35 Hz, 6-H), 7.55 (ddd, 1H, $J_{7,6}$=$J_{7,8}$=7.81 Hz, 7-H), 7.65 (d, 1H, $J_{8,7}$=8.33 Hz, 8-H), 7.75 (ddd, 1H, $J_{5',4'}$=$j_{5',6'}$=7.11 Hz, 5′-H), 7.99 (s, 1H, 2′-H), 8.01 (d, 1H, 5-H), 8.08 (d, 2H, 4′, 6′-H), 8.48 (s, 1H, SO_2NH)	328, 327, 301, 285, 221, 194, 133 (100%), 121, 120, 107, 105, 101, 92, 64, 63	!624



Scheme 2. Mass fragmentations of **1–7.**

Table 2. Effects of various compounds on glucose-mediated insulin release from INS-1 cells. INS-1 cells in multi-wells were washed three times and incubated in KRBH-buffer for 90 min at 8.3 mM glucose. The results are expressed as percent insulin release at 8.3 mM glucose alone. Values obtained in the presence of 3.0 mM glucose (substimulatory concentration) and glibenclamide (1 μg/ml) in the presence of 8.3 mM glucose serve as controls. Each value represents the mean ± S.E.M., number of independent experiments in parentheses.

Compound	Insulin release (%)
Glucose [3.0 mM]	62.96 ± 5.68 (9)
Glucose [8.3 mM]	100 (9)
plus **1** [1 μg/ml]	101.6 ± 1.58 (3)
plus **1** [10 μg/ml]	160.2 ± 13.3 (3)
plus **2** [1 μg/ml]	85.63 ± 15.42 (3)
plus **2** [10 μg/ml]	118.6 ± 11.09 (3)
plus **3** [1 μg/ml]	138.7 ± 11.96 (3)
plus **3** [10 μg/ml]	85.63 ± 15.42 (3)
plus **4** [1 μg/ml]	82.84 ± 7.10 (3)
plus **4** [10 μg/ml]	152.6 ± 14.1 (3)
plus **5** [1 μg/ml]	87.26 ± 5.23 (3)
plus **5** [10 μg/ml]	92.60 ± 11.76 (3)
plus **6** [1 μg/ml]	53.35 ± 6.18 (3)
plus **6** [10 μg/ml]	107.5 ± 11.2 (3)
plus **7** [1 μg/ml]	86.29 ± 13.3 (3)
plus **7** [10 μg/ml]	124.3 ± 10.4 (3)
plus Glibenclamide [1 μg/ml]	210.4 ± 15.6 (11)

$\mathbf{A}^{+\cdot}$, $\mathbf{B}^{+\cdot}$, $\mathbf{B_1}^{+\cdot}$). Some spectral data of the compounds are summarised shown in Table 1.

Compounds **1**–**7** were evaluated for their in vitro insulin releasing activity by using INS-1 cells (Table 2). Inhibitory effects were observed using some compounds, especially using the lower concentrations of compounds **4** and **6**. There was a tendency for inhibition of insulin release with respect to compound **2** and **7**. Compound **5** exhibits no effect.

What we need are new insulin stimulatory compounds; compounds **1** and **4** were able to increase insulin release at least at the higher concentrations used.

Insulin release at 8.3 mM glucose was normalised to 100%. Control experiments were performed with either low glucose (3.0 mM) or glibenclamide (1 μg/ml) at 8.3 mM glucose, one of the leading sulphonylureas in treatment of diabetes. Substimulatory glucose (3.0 mM) has a low effect (63.0% of that at 8.3 mM glucose). 1 μg/ml glibenclamide at 8.3 mM glucose increased insulin release from 100 to 210.4%. This is to compare the effects of various compounds with that of an established control compound.

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Table 3. Reaction conditions of **1**–**7**.

No	Formula	React. time	React. temp. °C	Purification procedure	Yield %	Mp (°C)
1	$C_{18}H_{16}N_2O_5S$, $0.8H_2O$ 1-(3′-Flavone)sulphonyl-3-ethylurea	10	40	Crystallisation from ethanol	32.37	206–208
2	$C_{19}H_{16}N_2O_5S$ 1-(3′-Flavone)sulphonyl-3-allylurea	10	60	Crystallisation from ethanol	48.74	178–180
3	$C_{19}H_{18}N_2O_5S$,$1.1(CH_3)_2CHOH$ 1-(3′-Flavone)sulphonyl-3-isopropylurea	12	60	$CHCl_3$:isopropanol (1:0.1) (Flash chrom.)	62.71	173–174
4	$C_{20}H_{20}N_2O_5S$, $0.2H_2O$ 1-(3′-Flavone)sulphonyl-3-n-buthylurea	12	60	Crystallisation from ethanol	67.73	184–186
5	$C_{22}H_{22}N_2O_5S$, $0.35H_2O$ 1-(3′-Flavone)sulphonyl-3-cyclohexylurea	3	60	Crystallisation from ethanol	28.26	228–229
6	$C_{22}H_{16}N_2O_5S$,$0.3CHCl_3$,$O.1H_2O$ 1-(3′-Flavone)sulphonyl-3-phenylurea	12	60	$CHCl_3$ (Flash chrom.)	35.83	269–270
7	$C_{23}H_{18}N_2O_5S$,$0.3(CH_3)_2CHOH$,$1H_2O$ 1-(3′-Flavone)sulphonyl-3-p-tolylurea	4	60	$CHCl_3$:isopropanol (1:0.1) (Flash chrom.)	55.48	294–296

Experimental

Chemistry

Melting points were determined with a Büchi SMP-20 melting point apparatus and are uncorrected. All the instrumental analysis were performed by Instrumental Analysis Lab. of Scientific and Technical Research Council of Turkey (TUBITAK, Ankara, Turkey), with a Jasco FT/IR 420 spectrometer (IR spectra were recorded as potassium bromide discs), a Bruker AC 400 NMR spectrometer (the ^{1}H NMR spectra were measured in DMSO-d_6 and $CDCl_3$, all chemical shifts were reported as δ (ppm) values) and VG Platform II Mass spectrometer. Elementary analysis were performed on a Leco CHNS 932 analyser and satisfactory results ± 0.4% of calculated values (C, H, N, S) were obtained. For the chromatographic analysis Merck Silica Gel 60 (230–400 mesh ASTM) was used. The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, FRG) and Aldrich (Milwaukee, USA). Flavone-3′-sulphonamide (**III**) [12] was synthesised in our laboratory.

Method

A mixture of **III** [12] (0.01 mol) and NaOH (0.01 mol) in 10 ml dioxan:H_2O (1:1) was treated with appropriate isocyanates (0.02 mol) and heated. The reaction mixture was cooled and acidified with acetic acid. The precipitate was filtered. Reaction conditions are given in Table 3.

Biological Activity

Cell Culture

INS-1 cells generously provided by Dr. C. Wollheim, Geneva, Switzerland, were grown in plastic culture bottles or micro-wells for 4-6 days (half confluence:1–2x10^6 cells per ml) in RPMI medium supplemented with 10% (v/v) foetal calf serum, 100 U of penicillin per ml and 0.1 mg of streptomycin per ml. Prior to the experiment cells were washed 2 times and then incubated in Krebs-Ringer buffer containing 10 mM HEPES and 0.5% bovine serum albumin (KRBH).

Insulin Release

To measure insulin secretion, half-confluent cells in micro-wells were incubated for 90 min at 37 °C in the aforementioned KRBH buffer. Insulin released into the medium was assayed with a radioimmunoassay using rat insulin (Novo Nordisk, Bagsvaerd, Denmark) as a standard, (mono ^{125}I-Tyr A14)-porcine insulin as the labelled compound (Hoechst, Germany) and anti-insulin antibodies from Linco (St. Louis, U.S.A.). Each compound had been checked for non-interference with the insulin radioimmunoassay. The data were corrected for the effects of solubilizing compounds (ethanol, DMSO).

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