ORIGINAL RESEARCH

# Synthesis and biological evaluation of some stilbene derivatives

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**Abstract** Several *trans* and *cis* stilbenes with substitution on the olefinic bridge were synthesized and characterized by IR, NMR and mass spectroscopy in an effort to obtain substances that could be more readily formulated. All the synthesized compounds were screened against Molt4/C8, CEM and L1210 cell lines. None of these compounds were endowed with pronounced cytostatic activity. However, Schiff derivatives emerged as cytostatic agents (IC<sub>50</sub>: 0.77–10 µg/ml) that deserve further investigation.

Keywords Stilbenes · Cytostatic · Schiff · Olefine

# Introduction

Cancer is one of the main reasons of death in both men and women. One promising natural product is resveratrol (3,4',5-trihydroxy-trans-stilbene) (RSV), a phytoalexin found in grapes and in red wine. The interest in this molecule has considerably increased in the last 10–12 years. The anticancer activity of resveratrol was first revealed by its ability to reduce the incidence of carcinogen-induced development of cancers in experimental animals (Jang *et al.*, 1997; Dong, 2003). Since then, it has been demonstrated that it possesses chemopreventive and cytostatic

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J. Balzarini · E. De Clercq Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium properties via the inhibition of tumor initiation, promotion and progression (Jang et al., 1997). It has also been demonstrated that RSV inhibits ribonucleotide reductase catalyzing the rate limiting step of de novo DNA synthesis (Fontecave, 1998). It exerts a nonselective cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibition (Jang and Pezzuto, 1999). The antioxidant properties of resveratrol have been related to its polyphenolic nature, especially to the presence of hydroxyl groups (Wright et al., 2001; Stivala et al., 2001). Phenolic groups are also known as an important structural determinant for estrogen receptor (ER) binding (Sadler et al., 1998; Ekena et al., 1998; Gao et al., 1999). Various trans stilbene compounds were reported to be inhibitors of Cytochrome P450 (CYP). Resveratrol showed an inhibitory effect on human CYP1A1 and CYP1B1 (Chun et al., 1999; Chang et al., 2000). Recently, rhapontigenin, a natural hydroxystilbene, showed a strong selectivity of CYP1A1 inhibition (Chun et al., 2001), and it was found that the selectivities and inhibitory potency of stilbene compounds tested against CYP1s were sensitive to the substitution patterns on the trans stilbene template. Cushman et al. (1992) have synthesized and evaluated analogues of (Z)-1-(4-methoxyphenyl)-2-(3,4,5trimethoxyphenyl)ethene as potential cytotoxic and antimitotic agents. Lion et al. (2005) synthesized a novel family of monohydroxylated (E)-stilbenes and studied their ability to inhibit the growth and induce apoptosis in human tumor cell lines. Heynekamp et al. (2006) synthesized various substituted trans stilbene including analogue of resveratrol and proved that they inhibit the human tumor necrosis factor  $\alpha$ -induced activation of transcription factor nuclear factor kappaB. Sangjun et al. (2009) synthesized cis stilbene derivative related to VIOXX and studied for their inhibitory effects on cell cycle progression and anti-estrogenicity in human adenoma breast cancer MCF-7 cells. Moran *et al.* (2009) synthesized fluorinated analogues of resveratrol and assayed on a variety of cell lines, primarily the non-small lung carcinoma cell line DLKP-A.

In this article we performed isosteric modifications of resveratrol by keeping the stilbene backbone of RSV to design substances that could be more readily formulated by (i) replacing the hydroxyl by various functional groups and (ii) introducing substituents on olefinic carbons and (iii) Schiff bases derivatives.

## **Results and discussion**

# Chemistry

Wittig reaction was carried out by using phosphonium chloride (2) with aryl aldehyde in benzene in the presence of sodium hydride. Excess of sodium hydride was quenched by addition of methanol and then chloroform and water were added. After evaporating, organic layer

and residue were purified by preparative TLC using 5% ethanol in hexane as the eluent (**3a–f**) (Scheme 1, Table 1).

Several derivatives containing acidic and basic functional groups were prepared in an attempt to make compounds that were more soluble in water and therefore be formulated more easily (Cushman *et al.*, 1992). Base-catalyzed condition of phenyl acetic acid (4) with aryl aldehyde (5) in the presence of triethylamine gave the carboxylic acid (**6a–c**) (Scheme 2, Table 2). Reaction of SOCl<sub>2</sub> with the carboxylic acid (6) in refluxing benzene gave the corresponding acid chlorides (7), which, upon subsequent reaction with appropriate amines, gave compounds (**8a–g**) (Scheme 2, Table 2). **9a** and **b** derivatives were prepared by compound **6** with methanol using catalytic H<sub>2</sub>SO<sub>4</sub>.

Several Schiff derivatives (12a–d) were prepared as bioisosteric compounds of stilbene by refluxing aldehyde (10) and aromatic amines (11) in toluene in a Dean stark apparatus (Scheme 3, Table 3).



3. 110	K	ĸ	MF (C)	$1C_{50}$ (µg/mi)		
				L1210	Molt4/C8	CEM
<b>3</b> a	4-F	4-OCH <sub>3</sub>	136–138	$26 \pm 10$	>200	$156\pm75$
3b	4-CH <sub>3</sub>	4-N(CH <sub>3</sub> ) <sub>2</sub>	139–140	$143 \pm 4.0$	>200	>200
3c	4-CH <sub>3</sub>	4-OCH <sub>3</sub>	143–145	>200	>200	>200
3d	4-F	2-F	115-121	$62 \pm 15$	$61 \pm 9.0$	$83 \pm 11$
3e	4-CH <sub>3</sub>	2-F	135–138	$72 \pm 1.0$	$30 \pm 7.0$	$78 \pm 18$
3f	4-F	Н	121-123	>200	>200	>200
Melphalan	-	-	-	$2.1\pm0.02$	$3.2 \pm 0.6$	$2.5\pm0.2$

IC<sub>50</sub>: 50% inhibitory concentration

Scheme 2 General procedure for the preparation of **6a–c**, **8a–g**, **9a** and **b** reagents and conditions: *a* triethyl amine, acetic anhydride, dil HCl *b* H<sub>2</sub>SO<sub>4</sub>, MeOH *c* SOCl<sub>2</sub>, benzene, *d* Amines, 2 h, rt



Biological evaluation

The effects of 18 stilbene analogues and 4 analogues of Schiff derivatives as bioisosteres of stilbene on cell growth are summarized in Tables 1, 2 and 3. This group of compounds include 6 *trans* stilbenes (**3a–f**) (Table 1), 12 *cis* stilbene derivatives with substitution on the bridge connecting two phenyl rings (**6a–c**, **8a–g**, **9a** and **b**) (Table 2), and bioisosteres of stilbenes as Schiff derivatives (**12a–f**) (Table 3).

All the *trans* stilbenes (**3a–f**) were poorly cytostatic (Table 1). The  $IC_{50}$  values ranged between 26 and >200  $\mu$ M.

Regarding the *cis* stilbenes with substitutions on the olefinic bridge (Table 2), there was not much improvement in cytostatic activity. In separate experiments, a COOH group was introduced on the olefinic carbon linkage, and this resulted in the formation of compounds **6a**, **b** and **c** (IC<sub>50</sub> 37 to >200  $\mu$ g/ml). However, when the –COOH group of the compound was converted to a methyl ester (compound **9a** and **b**) or the *N*-diethylamide

(8c), the cytostatic activity somewhat increased, as particularly noted for 8c and 9a. However, the *N*-methylamide derivatives (8a, b, f and g) were clearly less cytostatic.

In the third set of modifications, we designed Schiff derivatives as a bioisosteric replacement of *trans* stilbenes. All these compounds (**12a–d**) turned out to be much more potent than *trans* stilbenes and *cis* restricted stilbenes in inhibiting tumor cell proliferation (IC<sub>50</sub>: 0.77–10 µg/ml). Several cytostatic activities were as potent as those found for melphalan. These data justify more in-depth studies on this type of compounds by introducing a variety of modifications on the aryl moieties of these molecules.

### Conclusion

Several *trans* and *cis* stilbenes with substitution on the olefinic bridge were synthesized in an effort to obtain substances that could be more readily formulated. None of these compounds emerged as cytostatic compounds. But

# Table 2 Compounds 6a-c, 8a-g and 9a and b



S. no	Y	R′	MP (°C)	IC <sub>50</sub> (µg/ml)		
				L1210	Molt4/C8	CEM
6a	СООН	2-Cl	105-108	$76 \pm 12$	$37 \pm 24$	93 ± 10
6b	СООН	2-F	170-172	>200	$89 \pm 2.0$	>200
6c	СООН	2-OH	139–140	>200	>200	>200
8a	CONHCH <sub>3</sub>	2-Cl	100-103	$65 \pm 25$	$77\pm0.0$	$60 \pm 1$
8b	CONHCH <sub>3</sub>	4-OCH <sub>3</sub>	182–185	$68 \pm 47$	$166 \pm 16$	$128\pm58$
8c	$CON(C_2H_5)_2$	2-Cl	60–63	$24 \pm 11$	$14 \pm 2$	$18 \pm 12$
8d	CONHC <sub>2</sub> H <sub>5</sub>	Н	99-102	$132 \pm 40$	$112 \pm 44$	$26\pm8$
8e	CONHC <sub>2</sub> H <sub>5</sub>	4-OCH <sub>3</sub>	135–138	$148 \pm 45$	$150 \pm 71$	$116 \pm 87$
8f	CONHCH <sub>3</sub>	2-F	77-80	$85 \pm 9$	$72 \pm 3$	$89 \pm 15$
8g	CONHCH <sub>3</sub>	2-OH	69–70	>200	>200	>200
9a	COOCH <sub>3</sub>	2-Cl	113–115	$25 \pm 10$	$16 \pm 1$	$19 \pm 7$
9b	COOCH <sub>3</sub>	4-OCH <sub>3</sub>	127-130	$66 \pm 46$	$71 \pm 9$	$71 \pm 46$

IC<sub>50</sub>: 50% inhibitory concentration



35-37

38-40

40-42

 $6.0\,\pm\,0.7$ 

 $2.1\,\pm\,1.4$ 

 $3.2\,\pm\,1.8$ 

 $0.77\,\pm\,0.22$ 

 $1.5\,\pm\,0.9$ 

 $16\pm8$ 

 $2.1 \pm 1.7$ 

 $4.5\pm2.6$ 

 $25\,\pm\,18$ 

IC<sub>50</sub>: 50% inhibitory concentration

2-Cl-phenyl

2-Cl-phenyl

2-thiophene

4-F-phenyl

4-Br-phenyl

3-Cl-4-F-phenyl

12b

12c

12d

Schiff derivatives emerged as interesting antiproliferative compounds against the three tumor cell lines evaluated.

### Experimental

# Chemistry

The solvents (AR grades) were obtained from Sd Fine Chem., Mumbai, and E. Merck, Mumbai. The reagents (puriss grade) were obtained from Fluka and E. Merck. FT-IR spectra were recorded in KBr powder on a Jasco V410 FT-IR spectrometer by diffuse reflectance technique. <sup>1</sup>H/<sup>13</sup>C NMR spectra were measured in CDCl<sub>3</sub> and DMSOd6 on a Bruker Ultraspec AMX 400 MHz spectrometer. The reported chemical shifts were against that of TMS. Elemental Analysis was carried out at CDRI, Lucknow on Elementar Vario EL III, Carlo Erba 1108.

### General procedure for the preparation of stilbenes 3a-f

A stirred solution of benzyl chloride 1 (2.88 g, 31.7 mmol) in acetonitrile (20 ml) was treated with triphenylphosphine (8.57 g, 32.7 mmol), and the mixture was refluxed with stirring for 12 h and then evaporated. The crude product was purified by crystallization from chloroform and ether, affording 95% yield as a white solid 2. Sodium hydride (72 mg, 3 mmol) was added in portions to a well-stirred suspension of phosphonium chloride 2 (2 mmol) and aryl aldehyde (2 mmol) in benzene (20 ml) at 0-5°C, and the mixture was allowed to warm up to room temperature. After an additional stirring for 16 h, excess sodium hydride was quenched by the addition of methanol (1 ml), after which 30 ml of chloroform and water was added. The organic and aqueous layers were then separated. Distilled of the organic layer, the residue was purified by preparative TLC using 5% ethanol in hexane as the eluent.

### (E)-1-(4-Fluorophenyl)-2-(4-methoxyphenyl)-ethene (3a)

Yield 25%; FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3042–3017, 2924–2844, 1604, 1573, 1511 and 1463. Anal. Calcd for C<sub>15</sub>H<sub>13</sub>OF: C, 78.93; H, 5.74. Found: C, 78.25; H, 5.55.

# (*E*)-1-(4-Methylphenyl)-2-(4-dimethylaminophenyl)ethene (**3b**)

Yield 23%; Anal. Calcd for  $C_{17}H_{19}N$ : C, 86.03; H, 8.07; N, 5.90 Found: C, 86.25; H, 7.89; N, 6.05. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3197–3021, 2916–2808, 1600, 1528, 1437 and 1363. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ ; 7.42 (4H, m), 7.13 (2H, d,

J = 8 Hz), 6.99 (1H, d), 6.92 (1H, d), 6.71 (2H, d, J = 8.8 Hz), 2.98 (6H, s,  $-N(CH_3)_2$ ), 2.35 (3H, s, CH<sub>3</sub>).

### (E)-1-(4-Methylphenyl)-2-(4-methoxyphenyl)-ethene (3c)

Yield 25%; Anal. Calcd for C<sub>16</sub>H<sub>16</sub>O: C, 85.68; H, 7.19. Found: C, 85.34; H, 6.99. FT-IR  $v_{\text{max}}$  cm<sup>-1</sup> (KBr): 3049–3021, 2954–2916, 1603, 1515, 1439 and 1377. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ ; 7.44 (2H, d, J = 8.8 Hz), 7.38 (2H, d, J = 8 Hz), 7.15 (2H, d, J = 8 Hz), 7.01 (1H, d, J = 16.4 Hz), 6.93 (1H, d, J = 16.4 Hz), 6.89 (2H, d, J = 8.8 Hz), 3.83 (3H, s, OCH<sub>3</sub>), 2.36 (3H, s, -CH<sub>3</sub>).

### (E)-1-(4-Fluorophenyl)-2-(2-fluorophenyl)-ethene (3d)

Yield 26%; Anal. Calcd for  $C_{14}H_{10}F_2$ : C, 77.77; H, 4.66. Found: C, 77.34; H, 4.29. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3077–3055, 2918, 1590, 1484 and 1438.

# (E)-1-(4-Methylphenyl)-2-(2-fluorophenyl)-ethene (3e)

Yield 24%; Anal. Calcd for  $C_{15}H_{13}F$ : C, 84.88; H, 6.17. Found: C, 84.64; H, 5.99. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3081–3056, 2953–2919, 1590, 1483 and 1437.

### (E)-1-(4-Fluorophenyl)-2-phenyl-ethene (3f)

Yield 22%; Anal. Calcd for  $C_{14}H_{14}F$ : C, 84.82; H, 5.59. Found: C, 84.67; H, 5.25. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3080–3023, 2953–2849, 1593, 1508 and 1448.

# *General procedure for the preparation of compounds* (*6a–c*)

A mixture of phenyl acetic acid 4 (2 mmol) substituted benzaldehyde 5 (2 mmol) and triethylamine (0.5 ml) in acetic anhydride (5 ml) was heated under reflux for 12 h and poured into hot saturated sodium carbonate solution (50 ml) and left overnight. The mixture was extracted with ether (2  $\times$  50 ml), the ether extracts were discarded, and the aqueous solution was acidified with dilute HCl, after which the precipitated product was filtered and dried. Recrystallization from ethyl acetate–hexane gave the pure products.

# (E)-3-(2-Chlorophenyl)-2-phenyl-prop-2-enoic acid (6a)

Yield 33%; Anal. Calcd for C<sub>15</sub>H<sub>11</sub>O<sub>2</sub>Cl: C, 69.64; H, 4.29. Found: C, 69.25; H, 4.15. FT-IR  $v_{\text{max}}$  cm<sup>-1</sup> (KBr): 3250–2500, 1685, 1425 and 1266. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ ; 8.20 (1H, s), 7.37 (1H, d, J = 8 Hz), 7.31–7.26 (3H, m), 7.21–7.19 (2H, m), 7.15 (t, J = 15.2 Hz), 6.90 (1H, t, J = 15.2 Hz), 6.76 (1H, d, J = 8 Hz). MS: m/z (%) 259 (M + 1).

### (E)-3-(2-Fluorophenyl)-2-phenyl-prop-2-enoic acid (6b)

Yield 44%; Anal. Calcd for  $C_{15}H_{11}O_2F$ : C, 74.37; H, 4.58. Found: C, 74.09; H, 4.41. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3200–2600, 1682 and 1620. <sup>1</sup>H-NMR (CDCl3)  $\delta$ ; 8.15 (1H, s), 7.38–7.30 (3H, m), 7.25–7.21 (3H, m), 7.06–7.01 (1H, m), 6.81–6.74 (2H, m). MS: *m/z* (%) 243 (M + 2).

## (E)-3-(2-Hydroxyphenyl)-2-phenyl-prop-2-enoic acid (6c)

Yield 46%; Anal. Calcd for  $C_{15}H_{12}O_3$ : C, 74.99; H, 5.03. Found: C, 75.12; H, 4.79. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3405, 3109–3051, 2970, 1713, 1613, 1454 and 1262.

# General procedure for the preparation of compounds (8*a*–*g*)

A mixture of carboxylic acid **6** (0.5 mmol) and thionyl chloride (1 ml) and benzene (10 ml) was refluxed for 6 h. The excess thionyl chloride and benzene were removed at reduced pressure, and the residue **7** was subsequently mixed with amines (5 ml) and kept at room temperature for 2 h. The precipitated product was filtered, washed subsequently with 2% NaOH solution and water and dried. The product was purified with suitable solvent.

# (E)-N-Methyl-3-(2-chlorophenyl)-2-(phenyl)prop-2-enamide (8a)

Yield 46%; Anal. Calcd for  $C_{16}H_{14}ONCl$ : C, 70.72; H, 5.19; N, 5.15. Found: C, 70.15; H, 5.07; N, 5.21. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3320, 3078–3023, 2940–2931, 1654, 1617, 1403 and 1269. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ ; 8.01 (1H, s), 7.35–7.33 (3H, m), 7.26 (2H, s), 7.20–7.18 (2H, m), 7.10–7.06 (1H, m), 6.85 (1H, t, J = 18.8 Hz), 6.65 (1H, d, J = 7.6 Hz), 5.55 (1H, s, br, NH, D<sub>2</sub>O exchangeable), 2.88 (3H, s, –CH<sub>3</sub>). MS: m/z (%) 272 (M + 1).

# (E)-N-Methyl-3-(4-methoxyphenyl)-2-(phenyl)prop-2-enamide (**8b**)

Yield 48%; Anal. Calcd for C<sub>17</sub>H<sub>17</sub>NO<sub>2</sub>: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.19; H, 6.25; N, 5.29. FT-IR  $v_{\text{max}}$  cm<sup>-1</sup> (KBr): 3442, 3099–3006, 2958–2836, 1661, 1599, 1514, 1460 and 1405. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ ; 7.82 (1H, s), 7.48–7.42 (3H, m), 7.26–7.24 (2H, m), 6.90 (2H, d, J = 8.8 Hz), 6.64 (2H, d, J = 8.8 Hz), 5.44 (1H, s, br, NH, D<sub>2</sub>O exchangeable), 3.74 (3H, s, OCH<sub>3</sub>), 2.84 (3H, s, CH<sub>3</sub>).

(E)-N-Diethyl-3-(2-chlorophenyl)-2-(phenyl)prop-2-enamide (8c)

Yield 23%; Anal. Calcd for C<sub>19</sub>H<sub>20</sub>NOCl: C, 72.72; H, 6.42; N, 4.46. Found: C, 72.02; H, 6.99; N, 4.51. FT-IR  $v_{\text{max}}$  cm<sup>-1</sup> (KBr): 3442, 3077–3020, 2986–2873, 1621, 1573, 1470 and 1433. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ ; 7.39 (1H, d, J = 8 Hz), 7.25–7.21 (5H, m), 7.16–7.11 (1H, m), 6.98–6.95 (1H, m), 6.79 (1H, s), 3.48 (2H, d, J = 8 Hz), 3.36 (2H, d, J = 8 Hz), 1.18 (3H, t, J = 12 Hz), 1.02 (3H, t, J = 12 Hz).

### (E)-N-Ethyl-3-(phenyl)-2-(phenyl)-prop-2-enamide (8d)

Yield 44%; Anal. Calcd for  $C_{17}H_{16}NOCl$ : C, 71.45; H, 5.64; N, 4.90. Found: C, 69.98; H, 5.25; N, 5.01. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3329, 3077–3018, 2974–2871, 1654, 1528, 1493 and 1261.

# (E)-N-Ethyl-3-(4-methoxyphenyl)-2-(phenyl)prop-2-enamide (8e)

Yield 44%; Anal. Calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>2</sub>: C, 76.84; H, 6.81; N, 4.98. Found: C, 76.02; H, 6.53; N, 5.01. FT-IR  $v_{\text{max}}$  cm<sup>-1</sup> (KBr): 3303, 3053–3004, 2966–2842, 1650, 1606, 1525, 1459 and 1349. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ ; 7.81 (1H, s), 7.49–7.43 (3H, m), 7.27–7.25 (2H, m), 6.90 (2H, d, J = 8.8 Hz), 6.65 (2H, d, J = 8.8 Hz), 5.41 (1H, s, br, NH, D<sub>2</sub>O exchangeable), 3.74 (3H, s, OCH<sub>3</sub>), 3.37–3.30 (2H, q, CH<sub>2</sub>), 1.07 (3H, t, J = 14.5 Hz, CH<sub>3</sub>).

# (E)-N-Methyl-3-(2-fluorophenyl)-2-(phenyl)prop-2-enamide (8f)

Yield 45%; Anal. Calcd for C<sub>16</sub>H<sub>14</sub>NOF: C, 75.28; H, 5.53; N, 5.49. Found: C, 69.89; H, 5.36; N, 5.35. FT-IR  $v_{\text{max}}$  cm<sup>-1</sup> (KBr): 3316, 3079–3022, 2933, 1656, 1619, 1454 and 1271. <sup>1</sup>H-NMR (CDCl<sub>3</sub>):  $\delta$ ; 8.01 (1H, s), 7.43–7.39 (3H, m), 7.24–7.22 (2H, m), 7.17–7.15 (1H, m), 7.01–6.97 (1H, m), 6.74 (1H, t, J = 15.2 Hz), 6.61 (1H, t, J = 15.2 Hz), 5.53 (1H, s, br, NH, D<sub>2</sub>O exchangeable), 2.86 (3H, s, –CH<sub>3</sub>).

(E)-N-Methyl-3-(2-hydroxyphenyl)-2-(phenyl)prop-2-enamide (**8g**)

Yield 38%; Anal. Calcd for C<sub>16</sub>H<sub>15</sub>NO<sub>2</sub>: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.19; H, 5.55; N, 5.61. FT-IR  $v_{\text{max}}$  cm<sup>-1</sup> (KBr): 3406, 3106–3057, 2966–2929, 1714, 1667, 1612, 1488 and 1454. <sup>1</sup>H-NMR (CDCl3)  $\delta$ ; 8.01 (1H, s), 7.35–7.33 (3H, m), 7.26 (2H, s), 7.20–7.18 (2H, m), 7.10–7.06 (1H, m), 6.85 (1H, t, *J* = 18.8 Hz), 6.65 (1H, d,

J = 7.6 Hz), 5.55 (1H, s, br, NH, D<sub>2</sub>O exchangeable), 2.88 (3H, s, -CH<sub>3</sub>).

# General procedure for the preparation of compounds 9a and b

Concentrated H<sub>2</sub>SO<sub>4</sub> (0.5 ml) was added to a stirred solution of carboxylic acid (0.5 mmol) in absolute methanol (20 ml) and the mixture was heated under reflux for 6 h. Excess of methanol was removed by evaporation, and the residue was poured into ice water. The product was extracted with ether (2 × 20 ml), and the combined extracts were washed with 2% aqueous NaOH solution (2 × 50 ml) followed by water (200 ml). Evaporation of ether layer gave the desired product.

# (*E*)-*Methyl*-3-(2-*chlorophenyl*)-2-(*phenyl*)-*prop*-2-*enoate* (**9***a*)

Yield 48%; Anal. Calcd for  $C_{16}H_{13}O_2Cl$ : C, 70.46; H, 4.80. Found: C, 69.99; H, 4.98. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3057, 2991–2848, 1704, 1570, 1496 and 1387.

# (E)-Methyl-3-(4-methoxyphenyl)-2-(phenyl)-prop-2-enoate (**9b**)

Yield 45%; Anal. Calcd for  $C_{17}H_{16}O_3$ : C, 76.10; H, 6.10. Found: C, 75.98; H, 5.79. FT-IR  $v_{max}$  cm<sup>-1</sup> (KBr): 3055, 2954–2837, 1704, 1597, 1513 and 1385.

# *General procedure for the preparation of Schiff derivatives* (12*a*–*d*)

A solution of aldehyde **10** (1 mmol) and aniline **11** (1 mmol) in toluene (5 ml) were heated to reflux in a Dean Stark apparatus for 16 h. After the solvent was removed in vacuo, the crude product was recrystallised from ethanol to give 45–50% yield.

# (E)-3-Chloro-N-(2-chlorobenzyledene)-4-fluoro benzenamine (**12a**)

Yield 45%; Anal. Calcd for C<sub>13</sub>H<sub>8</sub>Cl<sub>2</sub>N: C, 58.24; H, 3.01; N, 5.22. Found: C, 58.01; H, 2.99; N, 5.31. IR (KBr) cm<sup>-1</sup>: 3089–2996, 2922, 1620 and 1494. <sup>1</sup>H-NMR (CDCl<sub>3</sub>):  $\delta = 8.89$  (1H, s), 8.20 (1H, d, J = 8 Hz), 7.46–7.43 (2H, m), 7.41–7.36 (1H, m), 7.33–7.31 (1H, m), 7.21–7.12 (2H, m). MS: m/z (%): 268 (M).

# (E)-N-(2-Chlorobenzyledene)-4-fluorobenzenamine (12b)

Yield 51%; Anal. Calcd for  $C_{13}H_9CIN$ : C, 66.82; H, 3.88; N, 5.99. Found: C, 66.79; H, 3.58; N, 6.31. FT-IR  $v_{max}$ 

cm<sup>-1</sup> (KBr): 3075–2997, 2918–2848, 1617 and 1498. <sup>1</sup>H-NMR (CDCl<sub>3</sub>):  $\delta = 8.9$  (1H, s), 8.22 (1H, d, J = 7.6 Hz), 7.47–7.35 (3H, m), 7.27–7.23 (2H, m), 7.13–7.08 (2H, m), 6.85 (1H, t, J = 18.8 Hz), 6.65 (1H, d, J = 7.6 Hz), 5.55 (1H, s, br, NH), 2.88 (3H, s, –CH<sub>3</sub>).

# (E)-N-(2-Chlorobenzyledene)-4-bromobenzenamine (12c)

Yield 52%; Anal. Calcd for C<sub>13</sub>H<sub>9</sub>ClNBr: C, 53.01; H, 3.08; N, 4.75. Found: C, 52.75; H, 2.94; N, 4.94. FT-IR  $v_{\text{max}}$  cm<sup>-1</sup> (KBr): 3061–3018, 2918–2848, 1618 and 1484. <sup>1</sup>H-NMR (CDCl3):  $\delta = 8.92-8.90$  (1H, m), 7.26–7.22 (1H, m), 7.56–7, 52 (2H, m), 7.47–7.38 (2H, m), 7.26 (1H, d, J = 9.6 Hz), 7.16–7.12 (2H, m).

# (E)-3-Chloro-4-fluoro-N-(thiophen-2-ylmethylene)benzenamine (12d)

Yield 56%; Anal. Calcd for C<sub>11</sub>H<sub>7</sub>ClFNS: C, 55.12; H, 2.94; N, 5.84. Found: C, 55.01; H, 3.01; N, 5.55. FT-IR  $v_{\text{max}}$  cm<sup>-1</sup> (KBr): 3060, 2954, 2886, 1615 and 1492. <sup>1</sup>H-NMR (CDCl3):  $\delta = 8.53$  (1H, s), 7.54 (1H, d, J = 5.2 Hz), 7.50 (1H, d, J = 3.6 Hz), 7.29–7.26 (1H, m), 7.17–7.08 (3H, m).

#### Cytostatic activity

The methodology for measuring the cytostatic activity in Molt 4/C8, CEM and L1210 assays has been published previously (Baraldi *et al.*, 2004). In brief, varying concentrations of compounds were incubated at 37°C with the cells for 72 (human Molt 4/C8 or CEM T-lymphocytes) or 48 h (murine L1210 cells). After the incubation period, the cell number was counted by a coulter counter (Harpenden Herz, UK).

#### References

- Baraldi PG, Del Carmen Nunez M, Tabrizi MA, De Clercq E, Balzarini J, Bermejo J, Estévez F, Romagnoli R (2004) Design, synthesis, and biological evaluation of hybrid molecules containing α-methylene-γ-butyrolactones and polypyrrole minor groove binders. J Med Chem 47:2877–2886
- Chang TK, Lee WB, Ko HH (2000) *Trans*-resveratrol modulates the catalytic activity and mRNA expression of the procarcinogenactivating human cytochrome P450 1B1. Can J Physiol Pharmacol 78:874–881
- Chun YJ, Kim MY, Guengerich FP (1999) Resveratrol is a selective human cytochrome P450 1A1 inhibitor. Biochem Biophys Res Commun 262:20–24
- Chun YJ, Ryu SY, Jeong TC, Kim MY (2001) Mechanism based inhibition of human cytochrome P450 1A1 by rhapontigenin. Drug Metab Dispos 29:389–393
- Cushman M, Nagarathnam D, Gopal D, He HM, Lin CM, Hamel E (1992) Synthesis and evaluation of analogs of (Z)-1-(4-

methoxyphenyl)-2-(3, 4, 5-trimethoxyphenyl) ethene as potential cytotoxic and antimitotic agents. J Med Chem 35:2293–2306

- Dong Z (2003) Molecular mechanism of the chemopreventive effect of resveratrol. Mutation Res 523–524:145–150
- Ekena K, Katzenellenbogen JA, Katzenellenbogen BS (1998) Determinants of ligand specificity of estrogen receptor-alpha: estrogen versus androgen discrimination. J Biol Chem 273:693–699
- Fontecave M (1998) Ribonucleotide reductases and radical reactions. Cell Mol Life Sci 54:684–695
- Gao H, Katzenellenbogen J, Garg R, Hansch C (1999) Comparative QSAR analysis of estrogen receptor ligands. Chem Rev 99: 723–744
- Heynekamp JJ, Weber WM, Hunsaker LA, Gonzales AM, Orlando RA, Deck LM, Jagt DL (2006) Substituted *trans*-stilbenes, including analogues of the natural product resveratrol, inhibit the human tumor necrosis factor alpha-induced activation of transcription factor nuclear factor kappaB. J Med Chem 49(24): 7182–7189
- Jang M, Pezzuto JM (1999) Cancer chemopreventive activity of resveratrol. Drugs Exp Clin Res 25:65–77
- Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275: 218–220

- Lion CJ, Matthews CS, Stevens MF, Westwell AD (2005) Synthesis, antitumor evaluation, and apoptosis-inducing activity of hydroxylated (E)-stilbenes. J Med Chem 48(4):1292–1295
- Moran BW, Anderson FP, Devery A, Cloonan S, Butler WE, Varughese S, Draper SM, Kenny PT (2009) Synthesis, structural characterisation and biological evaluation of fluorinated analogues of resveratrol. Bioorg Med Chem 17(13):4510–4522
- Sadler BR, Cho SJ, Ishaq KS, Chae K, Korach KS (1998) Threedimensional quantitative structure-activity relationship study of nonsteroidal estrogen receptor ligands using the comparative molecular field analysis/cross-validated r<sup>2</sup>-guided region selection approach. J Med Chem 41:2261–2267
- Sangjun S, de Jong E, Nijmeijer S, Mutarapat T, Ruchirawat S, van den Berg M, van Duursen MB (2009) Induction of cell cycle arrest in human MCF-7 breast cancer cells by *cis*-stilbene derivatives related to VIOXX. Toxicol Lett 186(2):115–122
- Stivala LA, Savio M, Carafoli F, Perucca P, Bianchi L et al (2001) Specific structural determinants are responsible for the antioxidant activity and the cell cycle effects of resveratrol. J Biol Chem 276:22586–22594
- Wright JS, Johnson ER, Dilabio GA (2001) Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, application to major families of antioxidants. J Am Chem Soc 123:1173–1183