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New 5-(2-ethenylsubstituted)-3(2*H*)-furanones with in vitro antiproliferative activity

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Abstract—A convenient route to new 3(2*H*)-furanones is described through hydrogenolysis and subsequent acidic hydrolysis of isoxazoles. The antiproliferative activity of title compounds were evaluated against leukemia-, carcinoma-, neuroblastoma-, and sarcoma-derived human cell lines in comparison to the natural compound geiparvarin. The structure activity relationship indicated that the maximum in vitro antiproliferative activity correlates with the presence of a heterocyclic ring on the ethenyl moiety.

1. Introduction

In the last years, increasing attention has been devoted to the study of 3(2*H*)-furanones that were found to be present in a number of natural antitumoral agents such as geiparvarin, jatrophone, eremantholides and chinolone.[1] The ability of these compounds to act as alkylating agents by means of conjugate addition (Michael acceptors) seems to be related to their biological activity.[1] The same literature revealed that model synthetic derivatives such as 2,2-dimethyl-5-isopropenyl-3(2*H*)-furanone have been shown to exhibit significant in vitro activity. Our initial investigations in this field were directed towards the synthesis of geiparvarin analogues containing the pharmacophore 3(2*H*)-furanone ring system;[2,3] strong antiproliferative activity compounds and selective MAO-B inhibitors have been obtained by substitution of the methyl group on the allyloxy bridge of geiparvarin with an hydrogen atom (Fig. 1A). With the aim to verify the relevance of the coumarinyloxy moiety, we wish to report here the synthesis and the antiproliferative activity of new 5-(2-ethenylsubstituted)-3(2*H*)-furanones (Fig. 1B) via a convenient, general approach to the key intermediates α′-hydroxy-1,3-diketones.

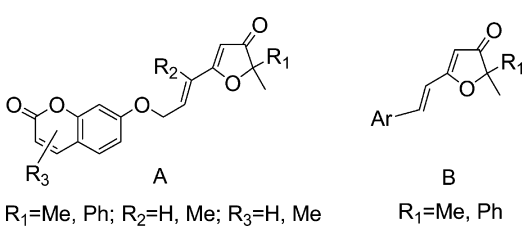
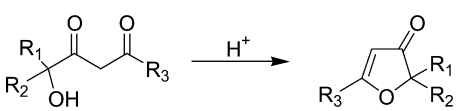


Figure 1.

2. Results and discussion

2.1. Chemistry

As reported in the literature,[4] the most efficient route to 3(2*H*)-furanone ring system involves the acid-catalyzed cyclization–dehydration process of an appropriately substituted α′-hydroxy-1,3-diketone as depicted in Scheme 1. A serious problem is constituted by the scarce availability of α′-hydroxy-1,3-diketones for which there are no satisfactory general synthetic procedures. Our recent studies on the synthesis and biological properties of geiparvarin analogues led us to devise a convenient alternative pathway to substituted 3(2*H*)-furanones through the isoxazole chemistry.[2] In fact, catalytic hydrogenation of isoxazoles under mild conditions gives rise to the breaking of the N–O linkage, thus providing β-aminoenones which can be considered as synthetic equivalents of α′-hydroxy-1,3-diketones (Scheme 2).



Scheme 1.

Keywords: 3(2*H*)-furanones; ethenyl-3(2*H*)-furanones; antitumoral compounds.

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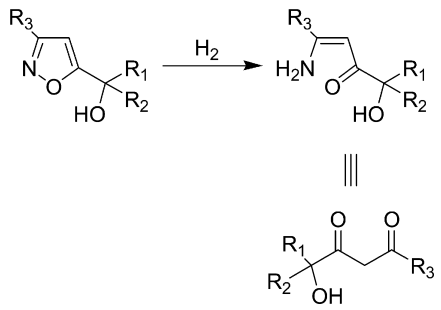
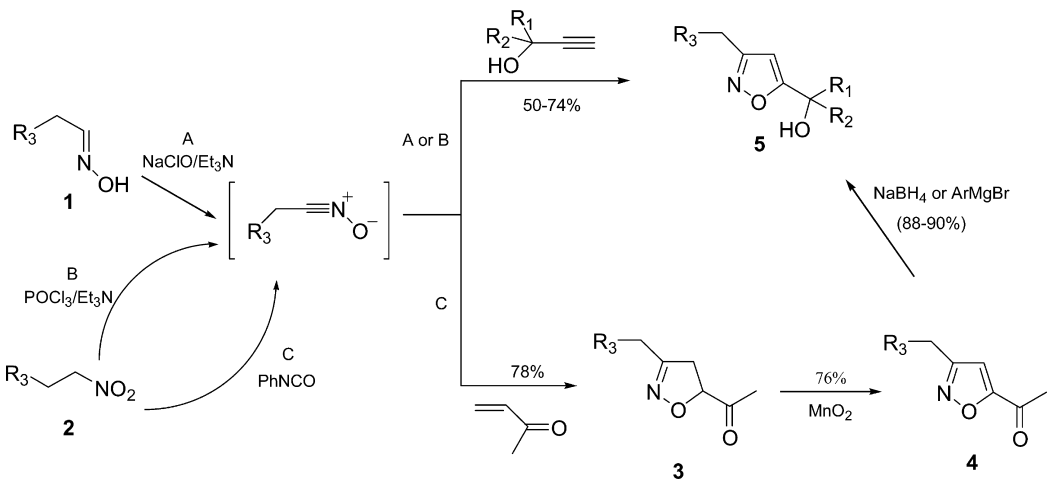


Scheme 2.

the presence of 10% palladium on charcoal and subsequent acidic hydrolysis with hydrochloric acid (1 M, pH<1) gave the desired 3(2*H*)-furanones through a cyclisation reaction with ammonia expulsion (Scheme 4).[7]

It is interesting to note that for compound **6b**, differently from **6a** (R_1=H, R_2=Me), an equilibrium was observed between the keto and enol form (80:20, respectively, via ^{1}H NMR) in $CDCl_3$ as depicted in Scheme 5.

As expected, in DMSO-d_6 solution only the keto form was observed.



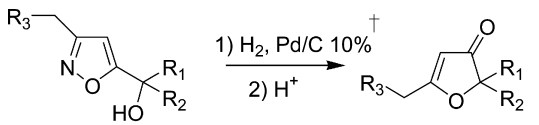
Scheme 3.

Thus, after the in situ preparation of the nitrile oxide from suitable nitro derivatives (method B) or from oxime derivatives (method A), the subsequent [3+2] cycloaddition reaction with commercially available alkynols directly provides the required isoxazolylalcohols. Alternatively, the same compounds **5** can be obtained by cycloaddition with alkyl vinyl ketones under Mukaiyama conditions[5] (method C), oxidation of the intermediate isoxazolines **3** and reduction of the resulting 3-alkyl-5-acyl-isoxazoles **4** as reported in the literature[6] (Scheme 3). The choice between the proposed routes (Table 1) to the isoxazolylalcohols **5** depends on the availability of the starting materials. In particular, the synthesis of compounds with R_2=Ar is easily achieved by reaction of acylisoxazoles **4** with Grignard reagents.

Submission of compounds **5** to catalytic hydrogenation in

Table 1. Routes to compounds **5** and relative yields

Compounds	Route	Yield (%)
5a R_1=R_3=H, R_2=Me	A or B	42–49
5a R_1=R_3=H, R_2=Me	C	52
5b R_1=R_3=H, R_2=Ph	A or B	58–62
5c R_1=R_2=Me, R_3=H	A or B	40–50
5d R_1=Me, R_2=Ph, R_3=H	A or B	56–74
5e R_1=Me, R_2=*p*-F-Ph, R_3=H	C	53
5f R_1=H, R_2=R_3=Me	A or B	50–52
5f R_1=H, R_2=R_3=Me	C	51
5g R_1=R_2=R_3=Me	A or B	55
5h R_1=R_3=Me, R_2=Ph	A or B	52–55

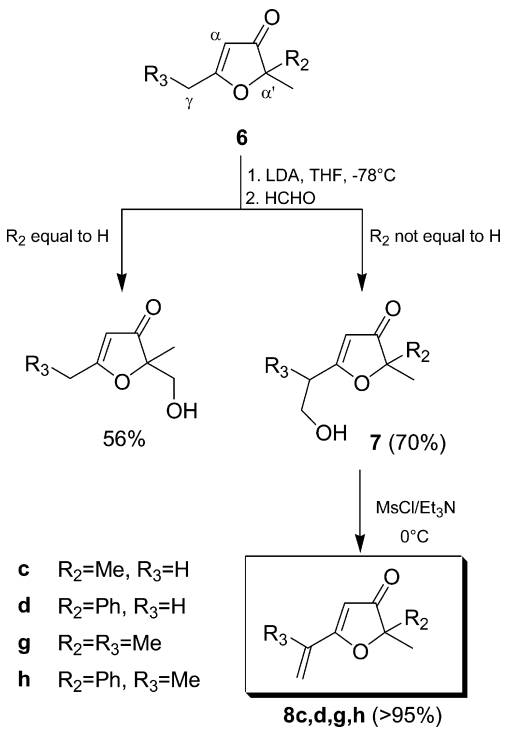


5a	R_1=R_3=H, R_2=Me	**6a** (77%)
5b	R_1=R_3=H, R_2=Ph	**6b** (70%)
5c	R_1=R_2=Me, R_3=H	**6c** (70%)
5d	R_1=Me, R_2=Ph, R_3=H	**6d** (73%)
5e	R_1=Me, R_2=*p*-F-Ph, R_3=H	**6e** (63%)
5f	R_1=H, R_2=R_3=Me	**6f** (88%)
5g	R_1=R_2=R_3=Me	**6g** (72%)
5h	R_1=R_3=Me, R_2=Ph	**6h** (80%)

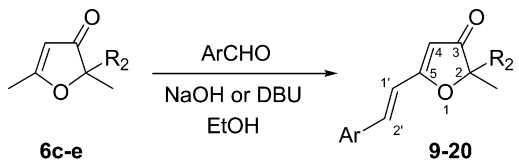
† Reaction catalysed by Rh/Al_2O_3 for compound **5b**

Scheme 4.

Scheme 5.



Scheme 6.



9 R_2=Me, Ar=1-naphtyl (70%)
10 R_2=Me, Ar=2-naphtyl (80%)
11 R_2=Me, Ar=4-quinolinyl (36%)
12 R_2=Ar=Ph (54%)
13 R_2=Ph, Ar=4-fluorophenyl (54%)
14 R_2=Ph, Ar=1-naphtyl (37%)
15 R_2=Ph, Ar=2-naphtyl (36%)
16 R_2=Ph, Ar=4-quinolinyl (40%)
17 R_2=4-fluorophenyl, Ar=Ph (60%)
18 R_2=Ar=4-fluorophenyl (60%)
19 R_2=4-fluorophenyl, Ar=4-pyridinyl (40%)
20 R_2=4-fluorophenyl, Ar=4-quinolinyl (44%)

Scheme 7.

Recently, we investigated the reaction between 3(2*H*)-furanones and cumarinyloxyaldehydes that provided a new entry to geiparvarin analogues.[3] We wish now to point out that this procedure can be applied only to furanones having R_2 not equal to H (Scheme 6). Thus, for compounds **6c**,**d**,**g**,**h** the procedure can be conveniently extended to the synthesis of 2,2-disubstituted-5-alkenyl-3(2*H*)-furanones **8** by reaction with formaldehyde and subsequent dehydration of the intermediate alcohols **7** in nearly quantitative yields (≥95%). This procedure can be considered to be a more convenient and general alternative to that one reported by Smith[1] for **8c** and **8g**.

After proving the formation of the carbanion on the γ-carbon of furanones in absence of an hydrogen atom at position 2, we turned our attention to the extension of such a reaction to the synthesis of new ethenyl substituted furanones, employing aromatic and heteroaromatic aldehydes. Thus, following the procedure reported by Felman *et al.*,[8] the new derivatives **9**–**20** have been synthesized (Scheme 7).

We wish to point out here that the applied procedure is remarkably efficient from the stereoselectivity point of view: the *E*-diastereoisomer being the unique product for all the obtained compounds **9**–**20**. Stereochemical assignment was achieved on the basis of H-1′/H-2′ coupling constant values (~16 Hz) from the ^{1}H NMR spectra. To this end, unambiguous assignment of H-1′ and H-2′ resonances is however compulsory. This attribution was reached by ^{13}C NMR experiments as follows. Apart from chemical shifts consideration on C-2′ and C-1′ (e.g. signal at 139.0 and 116.1 ppm in compound **12**), C-2′ resonances are easily assigned on the basis of fully coupled C–H spectra. The high-frequency signal always appears as a doublet of triplets of doublets due to long-range couplings to *ortho*-protons and H-1′, whereas the low-frequency one is a doublet of doublets of doublets (appearing as a doublet of pseudo-triplets) owing to couplings to H-2′ and H-4. Now, 2D NMR experiments (HSQC, HMBC) allowed us not only to unambiguously identify H-2′ and H-1′ (7.68 and 6.95 ppm, respectively, in compound **12**), but also to establish the conformational situation of the dienic system. In fact, NOEDIF experiments by irradiation of H-4 always gave positive nOes only on H-1′ (as confirmed by NOESY experiments, τ_{mix}=400 ms), thus proving the *s-trans* dienic conformation. Finally, for the fluorinated compounds **13** and **18**, the value of an uncommon long-range coupling constant ($^6J_{C,F}$=2.24 Hz, as in 4-fluorostirene)[9] led us to establish the coplanarity between the aromatic and vinylic system.

2.2. Biological results

The antiproliferative activity of analogues **9**–**20** was evaluated against leukemia-, carcinoma-, neuroblastoma- and sarcoma-derived human cell lines in comparison to the natural compound geiparvarin. The most potent derivatives are compounds **11**, **16**, **19** and **20** (Table 2), that show in all cell lines a comparable activity with respect to that one of the lead compound. The structure activity relationships indicate that the maximum antiproliferative activity in vitro correlates with the presence of an heterocyclic ring (pyridine or quinoline). Derivatives bearing a naphthalene ring present a very low activity or are devoid of significant cytotoxic activity. Introduction of a phenyl ring on the furanone moiety does not influence the activity in a significant way.

Drug resistance is a relevant therapeutic problem caused by the emergence of tumor cells possessing different mechanisms which confer resistance to a variety of anticancer drugs. Among the more common mechanisms are those related to the over expression of glycoproteins capable to mediate the efflux of different drugs such as doxorubicin and vinblastine or to alter contents of target enzymes (topoisomerase I and II). Therefore, it was interesting to

Table 2. Cytotoxicity activity of derivatives **9**–**20** against a panel of human tumor cell lines

Compounds	IC_{50} (μM)[a] of cell lines[b]				
	K562	HL-60	HT-1080	A549	SHSY5Y
9	17.4±1.4	>40	34.0±2.3	n.d.	33.4±1.3
10	n.d	>40	>40	>40	36.8±1.4
11	7.7±0.3	10.9±0.6	17.0±1.8	>40	16.1±3.9
12	>40	20.1±1.8	>40	>40	>40
13	24.0±1.6	15.7±1.4	>40	>40	40.4±0.7
14	21.5±3.1	26.3±4.4	25.5±3.0	n.d.	>40
15	n.d.	>40	23.0±2.5	n.d.	30.4±3.8
16	5.2±0.2	8.6±0.4	8.6±0.3	n.d.	7.3±0.6
17	17.5±1.2	21.7±1.6	>40	>40	>40
18	25.1±2.4	24.5±1.3	>40	>40	>40
19	5.1±0.25	6.3±0.7	12.8±2.4	15.4±1.2	9.5±0.9
20	2.9±0.1	7.2±1.3	10.7±2.4	12.6±1.1	7.8±0.5
Geiparvarin	9.2±0.5	6.3±0.6	9.8±0.9	11.5±2.8	5.7±1.6

[a] IC_{50} concentration of compound required to inhibit the cellular growth by 50% after 72 h of drug exposure, as determined by MTT assay.
[b] Human cell lines: K562 human erythroblastic leukemia; HL-60 human promyelocityc leukemia; HT-1080 fibrosarcoma; A549 small cell lung carcinoma; SHSY5Y neuroblastoma.

Table 3. Effect of new 5-(2-ethenylsubstituted)-3(2*H*)-furanones **11**, **16**, **19** and **20** on wild type and drug-resistant cells

Compounds	IC_{50} (μM)[a] of cell lines[b]			
	CEM	CEM/Vbl_{100}	LoVo	LoVo/Doxo
11	3.2±0.3	1.5±0.2	18.9±1.6	4.7±0.4
16	2.1±0.1	0.9±0.1	14.0±1.3	2.4±0.4
19	1.8±0.6	0.5±0.2	6.0±1.0	3.4±0.2
20	2.4±0.4	0.2±0.07	6.6±2.7	3.7±0.5
Geiparvarin	1.8±0.1	4.8±0.7	9.2±1.3	5.6±0.6

[a] IC_{50} concentration of compound required to reduce cell growth by 50% after 72 h of incubation. Values are expressed as mean±SEM of least three independent experiments.
[b] LoVo, human intestinal adenocarcinoma; CEM human lymphoblastic leukemia.

investigate whether the test compounds were inhibitory to drug resistant cell lines. Thus, we evaluated the activity of the most potent compounds against the human colon adenocarcinoma cell line LoVo and its subline which is resistant to doxorubicin (LoVo/Doxo); this cell line is resistant to a number of intercalating agents such as various anthracyclines, mitoxantrone and ametantrone.[10] CEM, a lymphoblastoid cell line and its subline CEM/Vbl_{100}, are selected under continuous treatment with vinblastine and showed a classical multidrug-resistance phenotype and overexpressed the *mdr1* gene. Interestingly, all the compounds proved to be fully inhibitory to these resistance cell lines, as shown in Table 3, thus suggesting that they are not subject to the pump mediating efflux of antitumor drugs.

3. Conclusions

In summary, a convenient route to 3(2*H*)-furanones has been realized through hydrogenolysis and subsequent acidic hydrolysis of isoxazole derivatives. This process provides a convenient general method for the synthesis of compounds **6a**–**h**, which are often difficult to obtain by the methods already reported. Condensation of these compounds with aromatic or heteroaromatic aldehydes allowed us to obtain compounds **9**–**20**. The substitution of the coumarinyloxy moiety with phenyl or naphtyl ring results in an almost complete loss of activity when compared to geiparvarin. Instead, substitution with a pyridyl or a quinolinyl ring provides better results: the cytotoxic activity is now absolutely comparable to that of the lead compound. The most active compounds in these series are demonstrated not to be cross resistant against multidrug-resistant cell lines.

4. Experimental

4.1. General

Melting points were measured using a Büchi apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 (operating at 50.29 MHz for ^{13}C) and on Varian Gemini 300 (operating at 75.43 MHz for ^{13}C) instruments in the Fourier transform mode at 21±0.5°C in $CDCl_3$. Chemical shifts (δ) are in ppm relative to TMS as secondary internal reference standard; coupling constants are in Hz. Attributions of ^{13}C NMR resonances were performed on the basis of 'gated decoupled' or DEPT experiments. Mass spectra were registered with a Carlo Erba QMD 1000 instrument at 70 eV. IR spectra were recorded with a Perkin–Elmer 881 spectrophotometer. Elemental analyses were obtained by Elemental Analyzer Perkin–Elmer 240C apparatus. Silica gel plates (Merck F_{254}) and silica gel 60 (Merck 230–400 mesh) were used for analytical TLC and for flash chromatography, respectively. Solvents were removed under reduced pressure. γ-Manganese dioxide and lithium diisopropylamide, 2.0 M solution in heptane/tetrahydrofuran/ethylbenzene, were purchased from Sigma-Aldrich.

4.1.1. 1-(3-Ethyl-5-isoxazolyl)-1-ethanone 4 (R_3=Me). *Method C*. A solution of nitropropane **2** (8.6 g, 95 mmol) and triethylamine (20 drops) in dry benzene (20 mL) was added dropwise to a solution of phenyl isocyanate (20.7 g, 174 mmol) and 3-buten-2-one (6.7 g, 95 mmol) in dry benzene (35 mL). After stirring for 1 h, the reaction mixture was refluxed for an additional hour, cooled and checked. The solid was filtered off, and the filtrate was concentrated to give a yellow oil which was distilled to afford pure 1-(3-ethyl-4,5-dihydro-5-isoxazolyl)-1-ethanone (**3** R=Me) (78%); 55–56°C/0.078 mm Hg [lit.[11] 104–106°C/10 Torr].

Compound **3** (2.2 g, 15.7 mmol) in dry benzene (50 mL) and active γ-manganese dioxide (5.0 g) were refluxed for 3 h, while the water formed was removed by means of a Dean–Stark trap. The end of the reaction was monitored by ^1H NMR. The solid was filtered through Celite and washed with the same solvent. Evaporation of the filtrate left a compound which was purified by flash-chromatography (ethyl acetate/petroleum ether=1:3, v/v as eluant, yield 76%) and identified as the corresponding 1-(3-ethyl-5-isoxazolyl)-1-ethanone (**4**) identical to the product obtained by Tamariz *et al.*[12]

4.2. General procedure for the preparation of isoxazolylalcohols 5a–d,f–h

Method A. A solution of $POCl_3$ (3.93 mL, 42.2 mmol) in

$CHCl_3$ (12 mL) was added dropwise under stirring to a cooled (10°C) solution of alkynol (60 mmol), nitroethane or nitropropane (42.2 mmol) and triethylamine (12.90 mL, 93 mmol) in $CHCl_3$ (35 mL). The resulting solution was allowed to reach room temperature, stirred for 15 h and then washed with water (2×20 mL) and saturated aqueous $NaHCO_3$ (2×15 mL). After drying over sodium sulfate and filtering, the solvent was removed under reduced pressure and the dark residual oil distilled.

Method B. A 5% aqueous solution of sodium hypochlorite (54.4 mL) was added, over 2 h, to a solution of oxime (23.0 mmol), alkynol (10.0 mmol) and triethylamine (0.320 mL, 2.3 mmol) in chloroform (25 mL) at 0°C. The mixture was allowed to warm to 4°C and stirred overnight. The layers were separated, and the aqueous layer was extracted with chloroform (4×15 mL). The combined chloroform extracts were washed with saturated aqueous sodium chloride (60 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure to give an oil that was purified by flash-chromatography.

4.2.1. (3-Methyl-5-isoxazolyl)-1-ethanol 5a. Using the general procedure A or B 3-butyn-2-ol gave **5a** (42–49%) as colourless oil;[8] bp 62–63°C/0.04 mm Hg; EI-MS *m/z* (%) 127 (M^+, 10), 112 (42), 96 (43), 84 (98), 71 (100), 56 (38), 43 (80).

4.2.2. (3-Methyl-5-isoxazolyl)(phenyl)-methanol 5b. Using the general procedure A or B 2-phenyl-3-butyn-2-ol gave **5b** (58–62%). Ethyl acetate/diethyl ether=1:2, v/v as eluant for flash-chromatography. IR (neat) 3600–3100 (OH), 1601, 1416 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.43–7.35 (m, 5H, Ar), 5.96 (dq, 1H, ^4J=0.8, 0.4 Hz, H-4), 5.88 (m, 1H, CH), 2.25 (d, 3H, ^4J=0.4 Hz, 3-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 173.2 (s, C-5), 159.7 (s, C-3), 139.6 (C-1′), 128.6 (C-4′), 128.7 (C-3′, C-5′), 126.5 (C-2′, C-6′), 102.4 (d, C-4), 69.2 (d, C–OH), 11.4 (q, 3-CH_3); EI-MS *m/z* (%) 189 (M^+, 38), 147 (55), 105 (100), 77 (79), 84 (41). Anal. calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40. Found: C, 70.13; H, 5.76; N, 7.52.

4.2.3. 2-(3-Methyl-5-isoxazolyl)-2-propanol 5c. Using the general procedure A or B 2-methyl-3-butyn-2-ol gave **5c** (40–50%) as colourless oil; bp 60–62°C/0.015 mm Hg [lit.[13] 115–116°C/22 Torr]; IR (neat) 3700–3000 (OH), 1595, 1420 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.97 (s, 1H, H-4), 2.26 (s, 3H, 3-CH_3), 1.59 (s, 6H, 2×2-CH_3); EI-MS *m/z* (%) 141 (M^+, 5), 126 (50), 56 (50), 43 (100).

4.2.4. 1-(3-Methyl-5-isoxazolyl)-1-phenyl-1-ethanol 5d. Using the general procedure A or B 2-phenyl-3-butyn-2-ol gave **5d** (56–74%) as colourless oil;[8] bp 132–134°C/0.07 mm Hg; IR (neat) 3700–3100 (OH), 1596, 1445, 1415, 764, 711, 699 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.48–7.26 (m, 5H, Ar), 6.00 (s, 1H, H-4), 2.67 (br s exch, 1H, OH), 2.28 (s, 3H, 3-CH_3), 1.94 (s, 3H, CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 176.4 (s, C-5), 159.6 (s, C-3), 144.3 (s, C-1′), 128.3 (d, C-2′, C-6′), 127.7 (d, C-4′), 125.0 (d, C-3′, C-5′), 101.9 (d, C-4), 72.6 (s, C–OH), 29.1 (q, CH_3), 11.3 (q, 3-CH_3); EI-MS *m/z* (%) 203 (M^+, 2), 188 (98), 161 (40), 110 (47), 105 (100), 77 (57), 43 (77).

4.2.5. 1-(3-Ethyl-5-isoxazolyl)-1-ethanol 5f. Using the general procedure A or B 3-butyn-2-ol gave **5f** (50–52%) as colourless oil; bp 71–72°C/0.04 mm Hg; IR (neat) 3700–3000 (OH), 1602, 1424 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.01 (s, 1H, H-4), 4.90 (q, 1H, ^3J=6.6 Hz, CH), 3.51 (br s exch, 1H, OH), 2.60 (q, 2H, ^3J=7.8 Hz, $CH_{2a,b}CH_3$), 1.50 (d, 3H, ^3J=6.6 Hz, 2-CH_3), 1.18 (t, 3H, ^3J=7.8 Hz, CH_2CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 175.1 (s, C-5), 165.1 (s, C-3), 99.8 (d, C-4), 62.9 (d, C–OH), 21.8 (q, $CHCH_3$), 19.5 (t, CH_2), 12.6 (q, CH_2CH_3); EI-MS *m/z* (%) 141 (M^+, 4), 126 (8), 98 (46), 71 (37), 66 (100). Anal. calcd for $C_7H_{11}NO_2$: C, 59.56; H, 7.85; N, 9.92. Found: C, 59.53; H, 7.86; N, 9.93.

4.2.6. 2-(3-Ethyl-5-isoxazolyl)-2-propanol 5g. Using the general procedure A or B 2-methyl-3-butyn-2-ol gave **5g** (55%) as colourless oil;[2] bp 65–68°C/0.05 mm Hg; IR (neat) 3700–3000 (OH), 1598, 1416 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.99 (s, 1H, H-4), 2.65 (q, 2H, ^3J=7.6 Hz, CH_2CH_3), 1.59 (s, 6H, 2×2-CH_3), 1.24 (t, 3H, ^3J=7.6 Hz, CH_2CH_3); EI-MS *m/z* (%) 155 (4, M^+), 140 (79), 138 (6), 112 (39), 85 (42), 68 (70), 59 (64), 43 (100).

4.2.7. 1-(3-Ethyl-5-isoxazolyl)-1-phenyl-1-ethanol 5h. Using the general procedure A or B 2-phenyl-3-butyn-2-ol gave **5h** (52–55%) as light yellow oil; 120–121°C/0.04 mm Hg; IR (neat) 3600–3100 (OH), 1591, 1420 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.45–7.28 (m, 5H, Ar), 6.00 (s, 1H, H-4), 2.64 (q, 2H, ^3J=7.6 Hz, CH_2CH_3), 1.98 (s, 3H, 2-CH_3), 1.24 (t, 3H, ^3J=7.6 Hz, CH_2CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 176.2 (s, C-5), 165.0 (s, C-3), 144.3 (s, C-1′), 128.4 (d, C-2′, C-6′), 127.8 (d, C-4′), 125.0 (d, C-3′, C-5′), 100.5 (d, C-4), 72.8 (s, C–OH), 29.2 (q, CH_3), 19.5 (t, CH_2CH_3), 12.5 (q, CH_2CH_3); EI-MS *m/z* (%) 217 (M^+, 1), 202 (38), 105 (100), 77 (27), 68 (19). Anal. calcd for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.45. Found: C, 71.79; H, 6.97; N, 6.42.

4.2.8. 1-(4-Fluorophenyl)-1-(3-methyl-5-isoxazolyl)-1-ethanol 5e. To a solution of isoxazole **4** (R_3=H) (2.50 g, 20 mmol) in diethyl ether (130 mL) at 0°C was added a solution of (*p*-fluorophenyl)magnesium bromide (37 mmol) in diethyl ether (15 mL) dropwise. After addition, the reaction mixture was stirred 30 min and then neutralized to pH 7 with 1 M hydrochloric acid. The separated aqueous layer was extracted with diethyl ether (3×25 mL). The combined ethereal extracts were washed with saturated aqueous sodium chloride (10 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give a solid (3.99 g, 18 mmol, 90%). An analytical sample (yellowish solid), identical (^1H NMR) to the product described by Felman (amber oil),[8] was obtained by flash chromatography (ethyl acetate/petroleum ether=1:2, v/v as eluant); mp 76–77°C; IR (KBr) 3600–3100 (OH), 2995, 1597, 1507, 1224, 1160, 848, 788 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.46–7.39 (m, 2H, Ar), 7.07–6.98 (m, 2H, Ar), 5.99 (s, 1H, H-4), 3.23 (br s, exch, 1H, OH), 2.28 (s, 3H, 3-CH_3), 1.92 (s, 3H, CH_3); ^{13}C NMR (50.29 MHz, $CDCl_3$): 176.1 (s, C-5), 162.2 (d, C-4′), 159.7 (s, C-3), 140.1 (d, C-1′), 126.9 (dd, C-2′, C-6′), 115.1 (dd, C-3′, C-5′), 101.9 (d, C-4), 72.3 (s, C-6) 29.3 (q, CH_3), 11.3 (q, 3-CH_3); EI-MS *m/z* (%) 221 (M^+,3), 206 (98), 179 (13), 123 (100), 95 (21), 43 (87). Anal. calcd for

$C_{12}H_{12}FNO_2$: C, 65.15; H, 5.47; N, 6.33. Found: C, 64.93; H, 5.76; N, 6.09.

4.2.9. (3-Methyl-5-isoxazolyl)-1-ethanol 5a. Starting from compound **4** (R_3=H). $NaBH_4$ (0.5 g, 13.2 mmol) was added, in small portions and under stirring, to a cooled (−10°C) solution of 5-acetyl-3-methylisoxazole (**4**) (0.4 g, 3.2 mmol) in ethanol (16 mL) and the mixture was warmed to room temperature. After 1 h the solvent was removed under vacuum and the residue was treated with water and acidified with HCl 5 M. The solution was extracted with methylene chloride (3×15 mL) and the organic layer was dried over sodium sulfate. Removal of the solvent left compound **5a** as a pale yellow oil (88%) that was purified by distillation.

4.2.10. 1-(3-Ethyl-5-isoxazolyl)-1-ethanol 5f. Starting from compound **4** (R_3=Me) and operating as above for **5a**. Yield 86%.

4.3. General procedure for the synthesis of 3(2*H*)-furanones 6

A solution of compound **5** (13.8 mmol) in methanol (5 mL) was added under nitrogen to a suspension of 10% palladium on charcoal (0.7 g) in methanol (25 mL). The reaction mixture was then hydrogenated for 10 h. The catalyst was filtered off and washed with methanol (2×5 mL). Removal of the solvent under vacuum left a white solid that was treated under stirring with water (10 mL) containing 1 M aqueous hydrochloric acid (20 mL) for 3 h. The mixture was then neutralized with solid $NaHCO_3$ and saturated with NaCl. Extraction with diethyl ether (5×10 mL), removal of the solvent and distillation furnished pure **6**.

4.3.1. 2,5-Dimethyl-3(2*H*)-furanone 6a. Colourless oil (77%); bp 74–75°C/25 mm Hg [lit.[14] 88°C/15 Torr]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.39 (br s, 1H, H-4), 4.47 (q, 2H, ^3J=7.0 Hz, H-2), 2.23 (br s, 3H, 5-CH_3), 1.42 (d, ^3J=7.0 Hz, 2-Me).

4.3.2. 5-Methyl-2-phenyl-3(2*H*)-furanone 6b. Yellowish oil (70%); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.15–7.20 (m, 10 H, Ar), 6.12 (q, 1H, ^4J=0.5 Hz, H-4, enol form), 5.49 (q, 1H, ^4J=0.7 Hz, H-4, keto form), 5.36 (q, 1H, ^4J=0.7 Hz, H-2, keto form), 5.27 (s, 1H, OH, enol form), 2.33 (dd, 3H, ^4J=^5J=0.7 Hz, 5-Me, keto form), 2.20 (d, 3H, ^4J=0.5 Hz, 5-CH_3, enol form); EI-MS *m*/*z* (%) 174 (M^+, 100), 145 (20), 131 (27), 105 (55), 77 (53). Anal. calcd for $C_{11}H_{10}O_2$: C, 75.84; H, 5.79. Found: C, 75.93; H, 5.61.

4.3.3. 2,2,5-Trimethyl-3(2*H*)-furanone 6c. Colourless oil (70%); bp 57–58°C/0.05 mm Hg [lit.[8] 100–120°C/1 mm Hg]; IR (neat) 1696 (C=O), 1599 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.33 (q, 1H, ^4J=0.7 Hz, H-4), 2.19 (d, 3H, ^4J=0.7 Hz, 5-CH_3), 1.35 (s, 6H, 2×2-CH_3); ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 206.7 (s, C-3), 187.6 (s, C-5), 101.2 (d, C-4), 88.2 (s, C-2), 22.2 (q, 2-CH_3), 16.4 (q, 5-CH_3); EI-MS *m*/*z* (%) 126 (M^+, 81), 68 (100).

4.3.4. 2,5-Dimethyl-2-phenyl-3(2*H*)-furanone 6d. Colourless oil (73%);[8] bp 100–102°C/0.08 mm Hg; IR (neat) 2982, 1704 (C=O), 1599, 1385, 1342, 961, 697 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.47–7.33 (m, 5H, Ar), 5.41 (d, 1H, ^4J=0.7 Hz, H-4), 2.35 (d, 3H, ^4J=0.7 Hz, 5-CH_3), 1.74 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$): 204.8 (s, C-3), 188.5 (s, C-5), 138.1 (s, C-1′), 128.5 (d, C-2′, C-6′), 128.0 (d, C-4′), 124.5 (d, C-3′, C-5′), 102.2 (d, C-4), 90.4 (s, C-2), 24.3 (q, 2-CH_3), 17.0 (q, 5-CH_3); EI-MS *m*/*z* (%) 188 (M^+, 67), 105 (49), 104 (38), 77 (68), 68 (46), 51 (38), 43 (85), 40 (100).

4.3.5. 2,5-Dimethyl-2-(4-fluoro)phenyl-3(2*H*)-furanone 6e. Colourless oil (63%);[8] bp 78–80°C/0.07 mm Hg; IR (neat) 2982, 2933, 1702 (C=O), 1604, 1228, 834 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.51–7.43 (m, 2H, Ar), 7.08–6.99 (m, 2H, Ar), 5.41 (d, 1H, ^4J=0.7 Hz, H-4), 2.34 (d, 3H, ^4J=0.7 Hz, 5-CH_3), 1.72 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 204.5 (s, C-3), 188.6 (s, C-5), 162.3 (d, C-4′), 133.8 (d, C-1′), 126.3 (dd, C-2′, C-6′), 115.2 (dd, C-3′, C-5′), 102.0 (d, C-4), 89.7 (s, C-2), 24.3 (q, 2-CH_3), 16.7 (q, 5-CH_3); EI-MS *m*/*z* (%) 206 (M^+, 70), 123 (54), 122 (62), 95 (67), 68 (100), 43 (63), 39 (66).

4.3.6. 5-Ethyl-2-methyl-3(2*H*)-furanone 6f. Yield: 88%; bp 64–66°C/15 mm Hg [lit.[14] 66–68°C/15 Torr]; IR (neat) 1697 (C=O), 1591 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.41 (br s, H-4), 4.47 (qdd, 1H, ^3J=7.0 Hz, ^5J=^4J=1.0 Hz, H-2), 2.49 (m, 2H, ^3J=7.5 Hz, ^4J=0.7 Hz, ^5J=1.0 Hz, H-1′$_a$ and H-1′$_b$), 1.42 (dd, 3H, ^3J=7.0 Hz, 2-CH_3), 1.21 (dd, 3H, ^3J=7.5 Hz, CH_2CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 205.7 (s, C-3), 195.0 (s, C-5), 101.8 (d, C-4), 82.5 (d, C-2), 24.2 (t, CH_2), 16.3 (q, 2-CH_3), 10.2 (q, CH_3); EI-MS *m*/*z* (%) 126 (M^+, 45), 97 (2), 82 (23), 81 (20), 54 (100).

4.3.7. 2,2-Dimethyl-5-ethyl-3(2*H*)-furanone 6g. Yield: 72%;[2] bp 48–50°C/0.07 mm Hg; IR (neat) 1700 (C=O), 1591 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.33 (t, 1H, ^4J=0.8 Hz, H-4), 2.48 (qd, 2H, ^3J=7.6 Hz, ^4J=0.8 Hz, CH_2CH_3), 1.35 (s, 6H, 2×2-CH_3), 1.20 (t, 3H, ^3J=7.6 Hz, CH_2CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 207.5 (s, C-3), 193.0 (s, C-5), 100.1 (d, C-4), 88.5 (s, C-2), 24.2 (t, CH_2CH_3), 22.8 (q, 2×2-CH_3), 10.2 (q, CH_2CH_3); EI-MS *m*/*z* (%) 140 (M^+, 58), 82 (38), 81 (43), 54 (100).

4.3.8. 5-Ethyl-2-methyl-2-phenyl-3(2*H*)-furanone 6h. Yield: 80%; bp 87–88°C/0.02 mm Hg; IR (neat) 3063, 2979, 1705 (CO), 1600 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.49–7.39 (m, 5H, Ar), 5.39 (dd, 1H, ^4J=0.8 Hz, H-4), 2.61 (qd, 2H, ^3J=8.0 Hz, ^4J=0.8 Hz, H-1′a and H-1′b), 1.17 (s, 3H, 2-CH_3), 1.29 (dd, 3H, ^3J=8.0 Hz, CH_2CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 204.6 (s, C-3), 193.6 (s, C-5), 138.3 (s, C-1′), 128.4 (d, C-2′, C-6′), 128.2 (d, C-4′), 124.5 (d, C-3′, C-5′), 100.3 (d, C-4), 89.9 (s, C-2), 24.3 (q, 2-CH_3), 24.1 (t, CH_2CH_3), 10.2 (q, CH_2CH_3); EI-MS *m*/*z* (%) 202 (28, M^+), 187 (3), 173 (7), 105 (60), 77 (83), 54 (100). Anal. calcd for $C_{13}H_{14}O_2$: C, 72.20; H, 6.98. Found: C, 77.21; H, 6.99.

4.4. General procedure for the synthesis of 3(2*H*)-furanones 8c,d,g,h

A 2.0 M lithium diisopropylamide (LDA) solution in heptane/tetrahydrofuran/ethylbenzene (0.75 mL) was added under nitrogen to a solution of the furanone **6** (1.5 mmol) in dry tetrahydrofuran (10 mL) at −78°C. The

solution was stirred for 30 min, and a slow stream vapour of formaldehyde (0.100 g, 3 mmol) was passed through the stirred mixture at −78°C. After 3 h the reaction was quenched at −78°C by being poured into ether (10 mL) containing ammonium chloride (0.11 g) and filtered. The organic layer was separated and dried over Na_2SO_4. Removal of the solvent under vacuum left an yellow oil that was purified by flash-chromatography (ethyl acetate/diethyl ether=1.5:1, v/v as eluant, yield 70%) and identified as the corresponding alcohol **7**. To a solution of the alcohol **7** (0.6 mmol) in dry tetrahydrofuran (8 mL) was added triethylamine (0.5 mL, 3.6 mmol); the mixture was cooled to 0°C and a solution of methanesulfonyl chloride (0.25 mL, 3.2 mmol) in dry tetrahydrofuran (6 mL) was added. The resulting suspension was stirred at 0°C for 90 min and then filtered through a pad of Celite with diethyl ether. Removal of the solvent under vacuum gave pure **8**.

4.4.1. 2,2-Dimethyl-5-vinyl-3(2*H*)-furanone 8c. Yellowish oil (95%); IR (neat) 1692, 1638, 1175, 962, 933 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.50 (dd, 1H, ^3J=10.9, 17.4 Hz, H-1′), 6.18 (ddd, 1H, ^3J=17.4 Hz, ^2J=1.2 Hz, ^5J=0.4 Hz, H-$2'_{trans}$), 5.67 (ddd, 1H, ^3J=10.9 Hz, ^2J=1.2 Hz, ^5J=0.4 Hz, H-$2'_{cis}$), 5.48 (br s, 1H, H-4), 1.38 (s, 6H, 2×2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 217.2 (s, C-3), 181.0 (s, C-5), 126.3 (d, C-1′), 125.0 (t, C-2′), 101.8 (d, C-4), 88.4 (s, C-2), 22.9 (q, 2-CH_3); EI-MS *m/z* (%) 138 (M^+, 44), 123 (1), 110 (3), 95 (3), 80 (23), 52 (100). Anal. calcd for $C_8H_{10}O_2$: C, 69.54; H, 7.30. Found: C, 69.33; H, 7.54.

4.4.2. 2-Methyl-2-phenyl-5-vinyl-3(2*H*)-furanone 8d. Yellowish oil (95%);[1] IR (neat) 3064, 2932, 1702, 1591 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.49–7.28 (m, 5H, Ar), 6.59 (dd, 1H, ^3J=17.4 Hz, ^4J=10.8 Hz, H-1′), 6.35 (dd, 1H, ^3J=17.4 Hz, ^2J=1.2 Hz, H-$2'_a$), 5.77 (dd, 1H, ^3J=10.8 Hz, ^4J=1.2 Hz, H-$2'_b$), 5.52 (br s, 1H, H-4), 1.76 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 204.6 (s, C-3), 181.4 (s, C-5), 138.4 (s, C-1″), 128.5 (d, C-2″, C-6″), 128.1 (d, C-4″), 126.1 (d, C-1′), 125.3 (t, C-2′), 124.5 (d, C-3″, C-5″), 102.1 (d, C-4), 89.9 (s, C-2), 24.3 (q, 2-CH_3); EI-MS *m/z* (%) 200 (M^+, 54), 185 (5), 172 (5), 157 (4), 105 (31), 77 (35), 52 (100). Anal. calcd for $C_{13}H_{12}O_2$: C, 77.98; H, 6.04. Found: C, 77.87; H, 6.12.

4.4.3. 2,2-Dimethyl-5-isopropenyl-3(2*H*)-furanone 8g. Yellowish solid (94%); mp 62–63°C [lit.[1] 28°C/0.05 Torr]; IR (KBr) 1689, 1627, 1550, 1178, 853 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.94 (m, 1H, H-$2'_a$), 5.52 (br s, 1H, H-4), 5.40 (dq, 1H, ^2J=1.5 Hz, ^4J=1.5 Hz, H-$2'_b$), 2.00 (dd, 3H, ^4J=1.5 Hz, ^4J=0.9 Hz, 1′-CH_3), 1.38 (s, 6H, 2×2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 207.6 (s, C-3), 183.4 (s, C-5), 133.8 (s, C-1′), 121.3 (t, C-2′), 100.0 (d, C-4), 88.4 (s, C-2), 23.0 (q, 2-CH_3), 18.8 (q, 1′-CH_3); EI-MS *m/z* (%) 152 (M^+, 21), 124 (3), 109 (3), 94 (7), 66 (100).

4.4.4. 5-Isopropenyl-2-methyl-2-phenyl-3(2*H*)-furanone 8h. Yellowish solid (93%): mp 52–53°C; IR (KBr) 3030, 2991, 1695 (C=O), 1631 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.87–7.30 (m, 5H, Ar), 6.13 (dq, 1H, ^2J=1.9 Hz, ^4J=0.8 Hz, H-$2'_a$), 5.56 (br s, 1H, H-4), 5.50 (dq, 1H, ^2J=1.9 Hz, ^4J=1.5 Hz, H-$2'_b$), 2.06 (dd, 3H, ^4J=1.5, 0.8 Hz, 1′-CH_3), 1.76 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 204.9 (s, C-3), 183.7 (s, C-5), 138.5 (s, C-1″), 133.6 (s, C-1′), 128.5 (d, C-2″, C-6″), 128.0 (d, C-4″), 124.5 (d, C-3″, C-5″), 121.6 (t, C-2′), 100.2 (d, C-4), 90.0 (s, C-2), 24.4 (q, 2-CH_3), 18.9 (q, 1′-CH_3); EI-MS *m/z* (%) 214 (M^+, 16), 199 (2), 186 (4), 171 (4), 105 (20), 77 (28), 66 (100). Anal. calcd for $C_{14}H_{14}O_2$: C, 78.48; H, 6.59. Found: C, 78,51; H, 7.00.

4.4.5. 2,2-Dimethyl-5-[(*E*)-2-(1-naphtyl)ethenyl]-3(2*H*)-furanone 9. Sodium hydroxide (0.028 g, 0.7 mmol) was added to a solution of 1-naphthaldehyde (1.4 mmol) and **6c** (1.3 mmol) in ethanol (10 mL). The resulting solution was stirred 5 h. The solution was concentrated under reduced pressure to dryness. The resulting oil was partitioned between diethyl ether (10 mL) and saturated aqueous sodium chloride (5 mL). After the layers were separated, the ethereal layer was washed with saturated aqueous sodium chloride (5 mL), dried over magnesium sulphate, filtered and concentrated to a dark solid which was purified by flash-chromatography (ethyl acetate/petroleum ether=1:5 v/v as eluant). Yellowish crystals; yield 70%; mp 118–119°C (petroleum ether); IR (KBr) 3054, 1690 (C=O), 1617, 1555, 1149, 978 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.34 (d, 1H, ^3J=15.8 Hz, H-2′), 8.22–7.48 (m, 7H, Ar), 6.97 (d, 1H, ^3J=15.8 Hz, H-1′), 5.65 (s, 1H, H-4), 1.51 (s, 6H, 2×2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 206.9, 181.2, 135.4, 133.6, 131.9, 131.3, 130.5, 128.8, 126.8, 126.2, 125.4, 124.8, 123.1, 118.8, 102.1, 88.6, 23.1; EI-MS *m/z* (%) 264 (M^+, 26), 236 (14), 205 (16), 178 (100), 165 (22), 150 (47). Anal. calcd for $C_{18}H_{16}O_2$: C, 81.79; H, 6.10. Found: C, 81.55; H, 6.37.

4.4.6. 2,2-Dimethyl-5-[(*E*)-2-(2-naphtyl)ethenyl]-3(2*H*)-furanone 10. Operating as for **9**, starting from 2-naphthaldehyde the 3(2*H*)-furanone **10** was obtained as a yellowish crystals after purification by flash-chromatography (ethyl acetate/petroleum ether=1:5 v/v as eluant); yield 80%; mp 147.5–148.5°C (petroleum ether); IR (KBr) 3052, 1693 (C=O), 1631, 1555, 1382, 1176 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.92 (d, 1H, ^3J=15.8 Hz, H-2′), 7.97–7.40 (m, 7H, Ar), 6.97 (d, 1H, ^3J=15.8 Hz, H-1′), 5.64 (s, 1H, H-4), 1.48 (s, 6H, 2×2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 206.7, 181.3, 138.8, 134.0, 133.2, 132.1, 129.8, 128.7, 128.4, 127.7, 127.2, 126.7, 123.2, 116.5, 101.8, 88.4, 23.1; EI-MS *m/z* (%) 264 (M^+, 35), 235 (10), 205 (15), 177 (100), 151 (94). Anal. calcd for $C_{18}H_{16}O_2$: C, 81.79; H, 6.10. Found: C, 81.93; H, 6.24.

4.4.7. 2,2-Dimethyl-5-[(*E*)-2-(4-quinolinyl)ethenyl]-3(2*H*)-furanone 11. 1,8-Diazabicyclo[5.4.0]undecen-7-ene (DBU, 0.02 mL, 0.13 mmol) was added to a solution of 4-quinolinecarboxyaldehyde (1.0 mmol) and **6c** (1.0 mmol) in ethanol (10 mL). The reaction mixture was heated to 80°C for 3 h. After the reaction solution was allowed to cool at room temperature, saturated aqueous sodium chloride (40 mL) was added. The aqueous layer was extracted with diethyl ether (3×10 mL). The organic layer was washed with saturated aqueous sodium chloride (5 mL), dried over magnesium sulphate, filtered and concentrated to a dark solid which was purified by flash-chromatography (ethyl acetate/petroleum ether=5:1 v/v as eluant). Yellowish crystals; yield 36%; mp 116–117°C (petroleum

ether); IR (KBr) 3042, 1687 (C=O), 1634, 1562, 1234, 976 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.95 (m, 1H, H-3″), 8.22 (d, 1H, ^3J=16.0 Hz, H-2′), 8.22–8.18 (m, 2H, H-5″, H-8″), 7.82–7.75 (m, 1H, H-6″/H-7″), 7.69–7.60 (m, 2H, H-7″/H-6″, H-2″), 7.11 (d, 1H, ^3J=16.0 Hz, H-1′), 5.72 (s, 1H, H-4), 1.50 (s, 6H, 2×2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 206.8, 179.7, 150.0, 148.7, 140.0, 132.3, 130.2, 129.7, 127.2, 125.9, 123.1, 122.4, 117.8, 103.8, 88.8, 23.1, 10.4; EI-MS *m/z* (%) 265 (M^+, 9), 206 (14), 179 (100), 151 (27). Anal. calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 77.17; H, 5.56; N, 5.09.

4.4.8. 2-Methyl-2-phenyl-5-[(*E*)-2-phenylethenyl]-3(2*H*)-furanone 12. Operating as for **9**, starting from benzaldehyde and **6d** the 3(2*H*)-furanone **12** was obtained as a yellowish crystals after purification by flash-chromatography (ethyl acetate/petroleum ether=1:7, v/v as eluant); yield 54%; mp 85.0–85.5°C (hexane); IR (KBr) 3088, 1686 (C=O), 1629, 964, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, 1H, ^3J=16.1 Hz, H-2′), 7.62–7.32 (m, 10H, Ar), 6.95 (d, 1H, ^3J=16.1 Hz, H-1′), 5.64 (s, 1H, H-4), 1.85 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 204.0 (s, C-3), 181.6 (s, C-5), 139.0 (d, C-2′), 138.5 (s, C-1‴), 134.6 (s, C-1″), 130.3 (d, C-4″), 128.9 (d, C-3″, C-5″), 128.4 (d, C-3‴, C-5‴), 128.0 (d, C-2″, C-6″), 127.9 (d, C-4‴), 124.5 (d, C-2‴, C-6‴), 116.1 (d, C-1′), 102.0 (d, C-4), 89.9 (s, C-2), 24.2 (q, 2-CH_3); EI-MS *m/z* (%) 276 (M^+, 67), 207 (61), 128 (100), 102 (49), 77 (71). Anal. calcd for $C_{19}H_{16}O_2$: C, 82.58; H, 5.84. Found: C, 82.29; H, 5.82.

4.4.9. 5-[(*E*)-2-(4-fluorophenyl)ethenyl]-2-methyl-2-phenyl-3(2*H*)-furanone 13. Operating as for **9**, starting from 4-fluorobenzaldehyde and **6d** the 3(2*H*)-furanone **13** was obtained as a yellowish crystals after purification by flash-chromatography (ethyl acetate/petroleum ether=2:7 v/v as eluant); yield 54%; mp 118.5–119.0°C (hexane); IR (KBr) 3089, 1689 (C=O), 1629, 1592, 1230, 968 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, 1H, ^3J=16.1 Hz, H-2′), 7.58–7.31 (m, 7H, Ar), 7.14–7.09 (m, 2H, Ar), 6.86 (d, 1H, ^3J=16.1 Hz, H-1′), 5.62 (s, 1H, H-4), 1.84 (s, 3H, 2-CH_3): ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 203.9 (s, C-3), 181.4 (s, C-5), 163.8 (d, $^1J_{C,F}$=252.0 Hz, C-4″), 138.5 (s, C-1‴), 137.6 (d, C-2′), 130.9 (d, $^4J_{C,F}$=3.5 Hz, C-1″), 129.8 (dd, $^3J_{C,F}$=8.5 Hz, C-2″, C-6″), 128.4 (d, C-3‴, C-5‴), 128.0 (d, C-4‴), 124.5 (d, C-2‴, C-6‴), 116.1 (dd, $^2J_{C,F}$=22.0 Hz, C-3″, C-5″), 115.8 (dd, $^6J_{C,F}$=2.4 Hz, C-1′), 102.0 (d, C-4), 89.9 (s, C-2), 24.2 (q, 2-CH_3); EI-MS *m/z* (%) 294 (M^+,10), 225 (10), 147 (12), 146 (100), 77 (5). Anal. calcd for $C_{19}H_{15}FO_2$: C, 77.54; H, 5.14. Found: C, 77.65; H, 5.19.

4.4.10. 2-Methyl-5-[(*E*)-2-(1-naphtyl)ethenyl]-2-phenyl-3(2*H*)-furanone 14. Operating as for **9**, starting from **6d** the 3(2*H*)-furanone **14** was obtained as a yellowish crystals after purification by flash-chromatography (ethyl acetate/petroleum ether=1:5 v/v as eluant); yield 37%; mp 136–137°C (petroleum ether); IR (KBr), 3058, 1687 (C=O), 1626, 1552, 1126 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.51 (d, 1H, ^3J=15.8 Hz, H-2′), 8.28–7.24 (m, 12H, Ar), 7.06 (d, 1H, ^3J=15.8 Hz, H-1′), 5.68 (s, 1H, H-4), 1.89 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 204.2, 181.7, 138.7, 135.7, 133.8, 131.9, 131.5, 130.8, 128.9, 128.7, 128.2, 127.1, 126.4, 125.6, 125.1, 124.8, 123.2, 118.6, 102.4, 90.2, 24.4; EI-MS *m/z* (%) 326 (M^+, 2), 205 (5), 195 (18), 178 (100), 153 (24). Anal. calcd for $C_{23}H_{18}O_2$: C, 84.64; H, 5.56. Found: C, 84.86; H, 5.38.

4.4.11. 2-Methyl-5-[(*E*)-2-(2-naphtyl)ethenyl]-2-phenyl-3(2*H*)-furanone 15. Operating as for **9**, starting from 2-naphthaldehyde and **6d** the 3(2*H*)-furanone **15** was obtained as a yellowish solid after purification by flash-chromatography (ethyl acetate/petroleum ether=1:5 v/v as eluant); yield 36%; mp 105–106°C (petroleum ether); IR (KBr) 1689 (C=O), 1622, 1552, 1369 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.00–7.28 (m, 12H, Ar), 7.84 (d, 1H, ^3J=15.8 Hz, H-2′), 7.06 (d, 1H, ^3J=15.8 Hz, H-1′), 5.66 (s, 1H, H-4), 1.86 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 204.0, 181.7, 139.1, 138.6, 134.1, 133.3, 132.1, 130.1, 128.8, 128.5, 128.0, 127.8, 127.3, 126.8, 124.5, 123.2, 116.2, 102.0, 89.9, 24.3; EI-MS *m/z* (%) 326 (M^+, 10), 205 (6), 194 (72), 178 (100), 151 (67). Anal. calcd for $C_{23}H_{18}O_2$: C, 84.64; H, 5.56. Found: C, 84.43; H, 5.67.

4.4.12. 2-Methyl-2-phenyl-5-[(*E*)-2-(4-quinolinyl)-ethenyl]-3(2*H*)-furanone 16. Operating as for **11**, starting from **6d** the 3(2*H*)-furanone **16** was obtained as a yellowish crystals after purification by flash-chromatography (ethyl acetate/petroleum ether=5:1 v/v as eluant); yield 40%; mp 168–169°C (hexane); IR (KBr) 3064, 1693 (C=O), 1629, 1562, 1237, 961 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.98 (d, 1H, ^3J=4.8 Hz, H-3″), 8.39 (d, 1H, ^3J=16.0 Hz, H-2′), 8.25–8.18 (m, 2H, H-5″, H-8″), 7.84–7.76 (m, 1H, H-6″/H-7″), 7.74–7.62 (m, 1H, H-7″/H-6″), 7.65 (d, ^3J=4.8 Hz, 1H, H-2″), 7.60–7.30 (m, 5H, Ar), 7.19 (d, ^3J=16.0 Hz, 1H, H-1′), 5.77 (s, 1H, H-4), 1.88 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 203.8, 179.9, 149.6, 148.3, 139.7, 137.9, 132.4, 129.9, 129.6, 128.3, 127.9, 127.1, 125.7, 124.3, 122.8, 121.9, 117.6, 103.7, 90.0, 24.1; EI-MS *m/z* (%) 327 (M^+, 3), 206 (15), 179 (100), 151 (25). Anal. calcd for $C_{22}H_{17}NO_2$: C, 80.71; H, 5.23; N, 4.28. Found: C, 80.87; H, 5.12; N, 4.47.

4.4.13. 2-(4-Fluorophenyl)-2-methyl-5-[(*E*)-2-phenyl-ethenyl]-3(2*H*)-furanone 17. Operating as for **9**, starting from benzaldehyde and **6d** the 3(2*H*)-furanone **12** was obtained as a yellowish crystals after purification by flash-chromatography (ethyl acetate/petroleum ether=1:4 v/v as eluant); yield 60%; mp 70–71°C (petroleum ether); IR (KBr) 3082, 1695 (C=O), 1631, 1218, 985, 756, 690 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.66 (d, 1H, H-2′), 7.63–7.42 (m, 7H, Ar), 7.10–7.01 (m, 2H, Ar), 6.94 (d, ^3J=16.1 Hz, 1H, H-1′), 5.63 (s, 1H, H-4), 1.81 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 203.9 (s, C-3), 181.7 (s, C-5), 162.5 (d, $^1J_{C,F}$=246.8 Hz, C-4‴), 139.2 (d, C-2′), 134.6 (s, C-1″), 134.4 (d, $^4J_{C,F}$=3.0 Hz, C-1‴), 130.4 (d, C-4″), 129.0 (d, C-3″, C5″), 128.0 (d, C-2″, C-6″), 126.4 (dd, $^3J_{C,F}$=8.2 Hz, C-2‴, C-6‴), 116.0 (d, C-1′), 115.4 (dd, $^2J_{C,F}$=21.6 Hz, C-3‴, C-5‴), 102.0 (d, C-4), 89.5 (s, C-2), 24.5 (q, 2-CH_3); EI-MS *m/z* (%) 294 (M^+, 5), 225 (11), 129 (11), 128 (100), 127 (10), 102 (9), 77 (7). Anal. calcd for $C_{19}H_{15}FO_2$: C, 77.54; H, 5.14. Found: C, 77.36; H, 5.30.

4.4.14. 2-(4-Fluorophenyl)-5-[(*E*)-2-(4-fluorophenyl)-ethenyl]-2-methyl-3(2*H*)-furanone 18. Operating as for **9**, starting from 4-fluorobenzaldehyde and **6e** the 3(2*H*)-furanone **18** was obtained as a yellowish crystals after purification by flash-chromatography (ethyl acetate/

petroleum ether=1:3 v/v as eluant); yield 60%; mp 108–109°C (petroleum ether); IR (KBr) 3083, 1697 (C=O), 1632, 1592, 1233, 1157, 967, 834 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.61 (d, 1H, ^3J=16.1 Hz, H-2′), 7.58–7.48 (m, 4H, Ar), 7.13–7.00 (m, 4H, Ar), 6.85 (d, 1H, ^3J=16.1 Hz, H-1′), 5.61 (s, 1H, H-4), 1.79 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 203.8 (s, C-3), 181.4 (s, C-5), 163.8 (d, $^1J_{C,F}$=252.0 Hz, C-4″), 162.4 (d, $^1J_{C,F}$=246.8 Hz, C-4‴), 137.8 (d, C-2′), 134.4 (d, $^4J_{C,F}$=3.0 Hz, C-1‴), 130.8 (d, $^4J_{C,F}$=3.5 Hz, C-1″), 129.9 (dd, $^3J_{C,F}$=8.5 Hz, C-2″, C-6″), 126.4 (dd, $^3J_{C,F}$=8.2 Hz, C-2‴, C-6‴), 116.2 (dd, $^2J_{C,F}$=22.0 Hz, C-3″, C-5″), 115.8 (dd, $^6J_{C,F}$=2.4 Hz, C-1′), 115.3 (dd, $^2J_{C,F}$=21.6 Hz, C-3‴, C-5‴), 102.0 (d, C-4), 89.4 (s, C-2), 24.4 (q, 2-CH_3); EI-MS *m/z* (%) 312 (M^+, 4), 243 (8), 147 (11), 146 (100), 120 (9), 32 (26). Anal. calcd for $C_{19}H_{14}F_2O_2$: C, 73.07; H, 4.52. Found: C, 73.11; H, 4.33.

4.4.15. 2-(4-Fluorophenyl)-2-methyl-5-[(*E*)-2-(4-pyridinyl)ethenyl]-3(2*H*)-furanone 19. Operating as for **11**, starting from 4-pyridinecarboxyaldehyde and **6e** the 3(2*H*)-furanone **19** was obtained as a yellowish crystals after purification by flash-chromatography (diethyl ether as eluant); yield 40%; mp 134–135°C (diethyl ether); IR (KBr) 3096, 1693 (C=O), 1636, 1594, 1236, 965, 843 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.69–8.67 (m, 2H, H-3″, H-5″), 7.55 (d, 1H, ^3J=16.2 Hz, H-2′), 7.52–7.46 (m, 2H, H-2‴, H-6‴), 7.43–7.41 (m, 2H, H-2″, H-6″), 7.10 (d, 1H, ^3J=16.2 Hz, H-1′), 7.07–7.01 (m, 2H, H-3‴, H-5‴), 5.71 (s, 1H, H-4), 1.79 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 203.9 (s, C-3), 180.4 (s, C-5), 162.6 (d, $^1J_{C,F}$=247.3 Hz, C-4‴), 150.6 (d, C-3″, C-5″), 141.7 (s, C-1″), 135.9 (d, C-2′), 134.0 (d, $^4J_{C,F}$=3.1 Hz, C-1‴), 126.4 (dd, $^3J_{C,F}$=8.2 Hz, C-2‴, C-6‴), 121.6 (d, C-2″, C-6″), 120.4 (d, C-1′), 115.4 (dd, $^2J_{C,F}$=21.6 Hz, C-3‴, C-5‴), 103.8 (d, C-4), 89.7 (s, C-2), 24.5 (q, 2-CH_3); EI-MS *m/z* (%) 295 (M^+, 10), 226 (21), 129 (100), 128 (23), 102 (32). Anal. calcd for $C_{18}H_{14}FNO_2$: C, 73.21; H, 4.78; N, 4.74. Found: C, 73.38; H, 5.03; N, 4.49.

4.4.16. 2-(4-Fluorophenyl)-2-methyl-5-[(*E*)-2-(4-quinolinyl)ethenyl]-3(2*H*)-furanone 20. Operating as for **11**, starting from **6e** the 3(2*H*)-furanone **20** was obtained as a yellowish crystals after purification by flash-chromatography (diethyl ether as eluant); yield 44%; mp 140.5–141.5°C (hexane); IR (KBr) 3059, 1695 (C=O), 1631, 1564, 1233, 960, 759 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.97 (d, 1H, ^3J=4.6 Hz, H-3″), 8.36 (d, 1H, ^3J=16.0 Hz, H-2′), 8.22–8.16 (m, 2H, H-5″, H-8″), 7.81–7.76 (m, 1H, H-6″/H-7″), 7.69–7.62 (m, 1H, H-7″/H-6″), 7.62 (d, ^3J=4.6 Hz, 1H, H-2″), 7.56–7.51 (m, 2H, H-2‴, H-6‴), 7.17 (d, ^3J=16.0 Hz, 1H, H-1′), 7.10–7.04 (m, 2H, H-3‴, H-5‴), 5.77 (s, 1H, H-4), 1.86 (s, 3H, 2-CH_3); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 203.9 (s, C-3), 180.2 (s, C-5), 162.6 (d, $^1J_{C,F}$=247.3 Hz, C-4‴), 150.0 (d, C-3″), 148.8 (s, C-4″a), 139.8 (s, C-1″), 134.0 (d, $^4J_{C,F}$=3.1 Hz, C-1‴), 132.9 (d, C-2′), 130.4 (d, C-5″/C-8″), 129.8 (d, C-6″/C-7″), 127.4 (d, C-7″/C-6″), 126.5 (dd, $^3J_{C,F}$=8.2 Hz, C-2‴, C-6‴), 125.92 (s, C-8″a), 123.0 (d, C-8″/C-5″), 122.0 (d, C-1′), 117.9 (d, C-2″), 115.5 (dd, $^2J_{C,F}$=21.6 Hz, C-3‴, C-5‴), 103.9 (d, C-4), 89.8 (s, C-2), 25.5 (q, 2-CH_3); EI-MS *m/z* (%) 345 (M^+, 7), 206 (20), 179 (100), 178 (57), 152 (19), 151 (27), 150 (44). Anal. calcd for $C_{22}H_{16}FNO_2$: C, 76.51; H, 4.67; N, 4.05. Found: C, 76.80; H, 4.39; N, 3.88.

4.5. Antitumor assay

Exponentially growing leukemia cells were resuspended at a density of 1×10^5 cells/mL in a complete medium (RPMI 1640 containing 10% fetal bovine serum, 100 UI/mL penicillin G and 100 μg/mL streptomycin). Cell viability was determined after 72 h at 37°C by the MTT method.[15] CEM/Vbl_{100} were kindly provided by Professor G. Basso (Department of Pediatric, University of Padova), vinblastine (100 ng/mL) was added at each passage. Activity against cell lines derived from solid tumors was evaluated in exponentially growing cultures seeded at 5×10^4 cells/mL which were allowed to adhere for 18 h to culture plates before addition of the drugs. Cell viability was determined after 72 h as described above. LoVo and LoVo/Doxo were cultured in Ham's F12 medium; doxorubicin (100 ng/mL) was added to the resistant subline at each passage. Cell growth at each drug concentration was expressed as percentage of untreated controls and the concentration resulting in 50% (IC_{50}) growth inhibition was determined by linear regression analysis.

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