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Original article

Antiproliferative effect of Baylis–Hillman adducts and a new phthalide derivative on human tumor cell lines

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Abstract

In this work we report our results concerning the study on the in vitro antiproliferative activity of 18 Baylis–Hillman adducts and some derivatives against a panel of humor tumor cell lines. A brief qualitative structure–activity relationship study indicated that carbon-carbon double bond and the presence of an electron-withdrawing substituent at the aromatic ring are essential for the activity. A quinoline–phthalide derivative has exhibited a potent effect on the proliferation of all cell lines. It is interesting to note their special cytotoxic activity against NCIADR cell line.

Keywords: Baylis–Hillman adducts; Antiproliferative activity; Cancer; Phthalide

1. Introduction

Cancer is a serious pathology and a substantial number of new antineoplastic agents have been discovered. Considerable insight has been gained into the mechanisms by which many of these compounds affect cellular growth and this knowledge have been used to the design of new chemotherapic drugs [1].

Interaction of antineoplastic drugs with cellular targets involves various types of chemical binding. Cellular glutathione (GSH) levels were found to be higher in human cancer cell lines than in a normal cell [2,3] The ability to modulate cellular GSH levels to probe the importance of this tripeptide in various drugs or radiation interactions has come from the elegant work of Griffith and Meister [4] and Williamson et al. [5] who has introduced compounds that either inhibit or stimulate GSH synthesis. More specifically, McCarthy et al. [6] have investigated the reactivity of selected acrylate esters toward biological nucleophiles and they found a significant interaction between these compounds and glutathione rather than deoxyribonucleosides. Since we have involved with the synthesis, structural modifications and biological evaluation of β-hydroxyacrylates, known as Baylis–Hillman adducts, we decided to investigate the effect of a set of acrylates (**1**–**18**) and some derivatives **19**–**27** on proliferation of eight cancer cell lines (Fig. 1).

2. Chemistry

The broad application of Baylis–Hillman reaction allowed us to prepare a variety of derivatives with different substituents at the aromatic ring as well heteroaromatic adducts (Scheme 1). Compounds **1**–**18** were prepared by the condensation between the appropriated aldehydes (**28**) and methyl acrylate (**29**) under sonication, according to published procedures [7]. Adducts were obtained in 40–90% yields and 1,4-diazabicycled[2.2.2] octane (DABCO) was used as catalyst. All spectra data were in according to the precedent literature [8]. Hydrogenated derivatives **19**–**22** were prepared by heterogeneous catalytic hydrogenation (Scheme 1) of the correspondent adduct **8**, **10**–**12** [9]. The resulting diastereomeric mixture (*anti*/*syn*) was purified by

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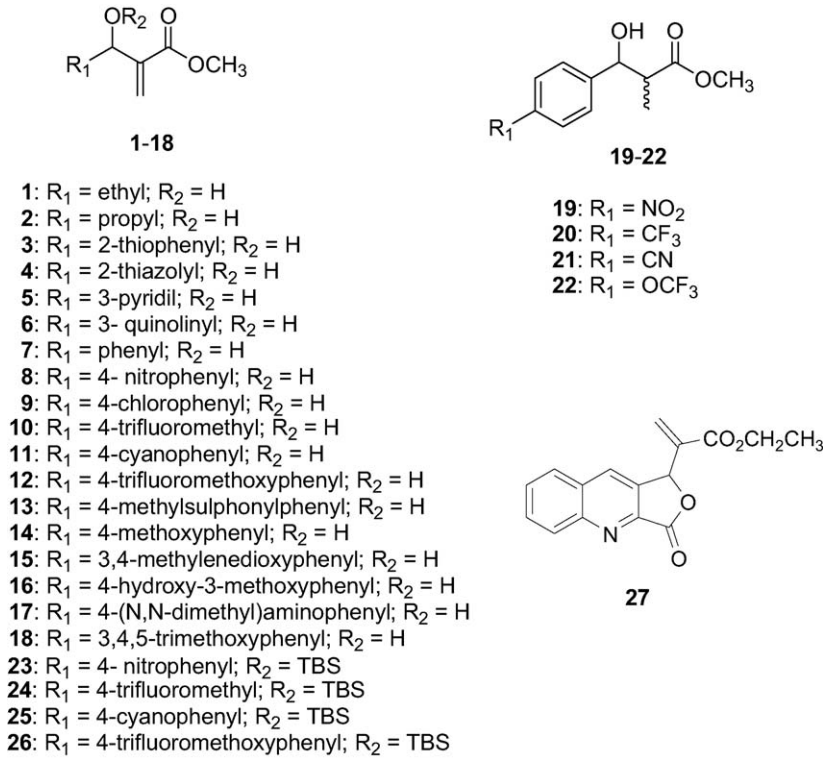
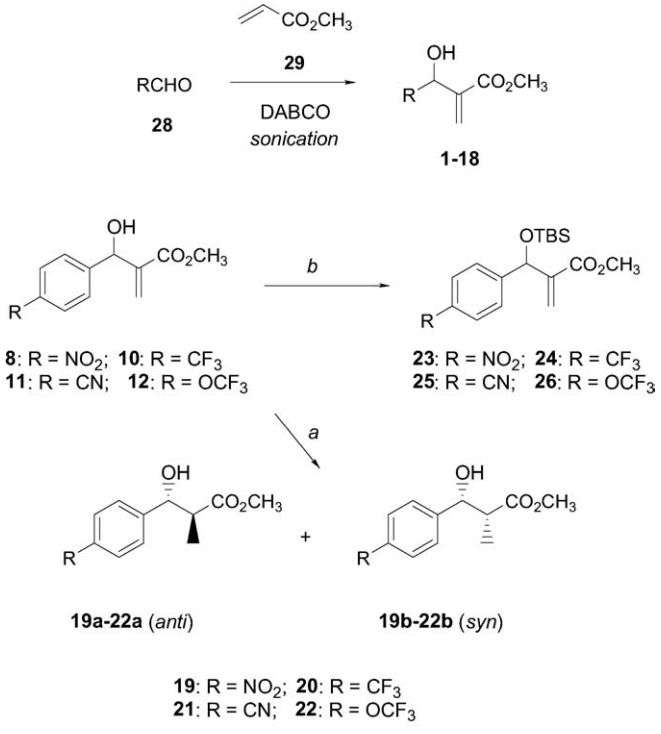


1: R_1 = ethyl; R_2 = H
2: R_1 = propyl; R_2 = H
3: R_1 = 2-thiophenyl; R_2 = H
4: R_1 = 2-thiazolyl; R_2 = H
5: R_1 = 3-pyridil; R_2 = H
6: R_1 = 3- quinolinyl; R_2 = H
7: R_1 = phenyl; R_2 = H
8: R_1 = 4- nitrophenyl; R_2 = H
9: R_1 = 4-chlorophenyl; R_2 = H
10: R_1 = 4-trifluoromethyl; R_2 = H
11: R_1 = 4-cyanophenyl; R_2 = H
12: R_1 = 4-trifluoromethoxyphenyl; R_2 = H
13: R_1 = 4-methylsulphonylphenyl; R_2 = H
14: R_1 = 4-methoxyphenyl; R_2 = H
15: R_1 = 3,4-methylenedioxyphenyl; R_2 = H
16: R_1 = 4-hydroxy-3-methoxyphenyl; R_2 = H
17: R_1 = 4-(N,N-dimethyl)aminophenyl; R_2 = H
18: R_1 = 3,4,5-trimethoxyphenyl; R_2 = H
23: R_1 = 4- nitrophenyl; R_2 = TBS
24: R_1 = 4-trifluoromethyl; R_2 = TBS
25: R_1 = 4-cyanophenyl; R_2 = TBS
26: R_1 = 4-trifluoromethoxyphenyl; R_2 = TBS

Fig. 1. Baylis–Hillman adducts and derivatives synthesized and biologically evaluated.



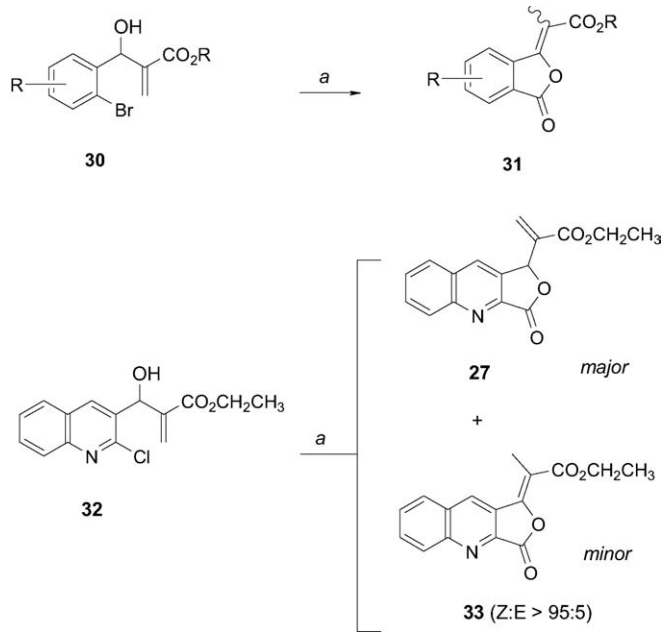
Scheme 1. (a) H_2, 5% Pd/C, EtOAC, 1 atm. Yields and diastereomeric ratio: **19** (83%, 7:1 *syn*/*anti*); **20** (72%, 6:1 *syn*/*anti*); **21** (68%, 4:1 *syn*/*anti*); **22** (78%, 6:1 *syn*/*anti*). (b) TBSCl, DMF, imidazole, room temperature. Yields: **23** (95%); **24** (86%); **25** (88%); **26** (90%).

column chromatography and both diastereoisomers were tested. *O*-silyl-derivatives **23**–**26** were obtained by treatment of the corresponding adduct with *tert*-butyldimethylsilylchloride in the presence of imidazol.

Palladium-mediated cyclocarbonylation reaction of Baylis–Hillman adducts has been investigated in our laboratory [10]. Adducts **30** derived from *ortho*-bromo aldehydes are sequentially treated with $Pd_2(dba)_3$ and carbon monoxide to provide tetrasubstituted olefins **31** (Scheme 2). Otherwise, phthalide **27** could be isolated as major product (60% yields) when adduct **32** was submitted to these conditions. Tetrasubstituted olefin **33** (Z:E > 95:5) was obtained as minor product. The configuration of the major diastereoisomer was unambiguously determined by NOE experiments. Compounds **27** and **33** have not been described in the literature.

3. Antiproliferative assay

National Cancer Institute (NCI) established a primary screen in which compounds are tested in vitro to determine their growth inhibitory properties against 60 different human tumor cell lines organized in subpanels representing melanoma, leukemia, and cancer of breast, prostate, lung, colon, ovary, kidney and brain. The experimental procedures have been described in detail [11]. Following the NCI preclinical antitumor drug discovery screen, UACC62 (melanoma), MCF7 (breast), NCI460 (lung, non-small cells), OVCAR



Scheme 2. (a) $Pd_2(dba)_3$, CO, 2 atm, 18 h, 70–85% yields; **27** and **33** (*Z*/*E* > 95:5), 60% and 22% yields, respectively.

(ovarian), PC03 (prostate), HT29 (colon) and 786-0 (renal) cell lines were used. Since multidrug resistance phenomenon is a relevant therapeutic problem, we have also evaluated the antiproliferative activity of our synthesized compounds against NCI-ADR cell lines, a breast cells expressing a multiple drugs resistance phenotype. Stock cultures of cell lines, kindly provided by National Cancer Institute (NCI), were grown in a medium containing 5 ml of RPMI 1640 (GIBCO BRL, Life Technologies) and supplemented with 5% of fetal bovine serum (GIBCO BRL, Life Technologies). For details, see Section 6.1.

4. Results and discussion

Aromatic compounds were significantly more active than non-aromatic ones in all concentrations. Heteroaromatic substances exhibited either minimum or no cytotoxic effect. In order to quantify their effects, IC_{50} values were calculated (Graph pad Prisma). These data are summarized in Table 1.

Initial structure–activity relationship (SAR) studies were focused on the effects of the substitutions in the aromatic ring. Compounds with electron-withdrawing substituents (**8**–**12**) showed enhanced activity compared with those bearing electron-donating substituents (**14**–**18**). As we can see from Table 1, nitro (**8**), trifluoromethyl (**10**), cyano (**11**) and trifluoromethoxy (**12**) derivatives showed a pronounced effect on the all cell lines proliferation. These results suggest that the electronic features of the various substituents have an effect on how easily the drug can interact with biological molecules. Otherwise, NCIADR cell line was the most sensitive and its proliferation was affected by exposition on test-compounds, including the less active compounds **2**, **7** and **17** [12].

With the aim to proof the relevance of α,β-unsaturated carbonyl moiety, hydrogenated derivatives (**19**–**22**) of the most active compounds were tested. Neither of them inhibited cell proliferation at any concentration. This lack of activity is not

Table 1
Cytotoxic activities of Baylis–Hillman adducts and some derivatives compounds against a panel of human tumor cell lines

Compounds	IC_{50} (μM) of cell lines [a]							
	UACC62	MCF7	NCIADR	786-0	NCI460	PC-03	OVCAR	HT29
1	51	Na	Na	6.8	18.7	na	na	na
2	5.4	25	3.7	26	36	34	24	31
3	20	9.3	19.5	Na	31.6	na	53.4	64
4	42.7	35.7	20	Na	22	na	43.7	61.8
5	na	na	Na	Na	Na	na	na	na
6	11	15.5	5.7	55.9	18.9	21.3	10.9	21.6
7	9.5	3.1	3.9	61	40	41	40	27
8	3.8	14	2	7.1	4.3	4.1	3.2	21
9	11	25	24	31	26	10	16	25
10	4.3	4.2	1.7	3.9	30	39	19	9.1
11	2.3	0.4	1.2	2.5	1.5	1.4	4.2	4.6
12	31	3.7	3.6	21.5	13.8	6.4	13	0.5
13	3.9	na	na	3.1	Na	na	21.2	2.4
14	na	88	na	88	Na	86	58	na
15	3.9	3.9	32	Na	42	42	43	51
16	86	na	Na	Na	Na	na	na	47
17	na	6.7	5.5	Na	Na	12.3	na	na
18	na	na	na	26	Na	na	na	21.9
23	4.2	13.8	2.3	7.5	4.5	4.0	3.5	20.8
24	4.6	3.9	1.7	3.9	29.4	38.8	18.2	8.7
25	1.9	0.7	1.7	2.2	1.8	1.3	4.1	4.4
26	31.4	4.1	3.9	21.1	13.7	6.1	13.3	0.9
27	2.0	2.7	2.3	4.1	3.5	1.3	1.7	2.7
32	na	10.9	8.1	Na	Na	9.6	na	na
33	6.4	21	5.5	10.8	Na	na	na	18.1

[a] Values are means of three experiments; na = not active.

dependent of the relative configuration of diastereoisomers, which were separately tested (**19a**–**22a**,**b**).

Recently, Donadel et al. [13] reported the *tert*-butyl dimethylsilyl group as an enhancer of drug cytotoxicity against human tumor cells. So, we planned to test some *O*-silylated derivatives (**23**–**26**) of the most active adducts **8, 10**–**12**. We found that *O*-silyl protection of hydroxyl group (derivatives **23**–**26**) did not affect the antiproliferative effect on the most cell lines in a significant way (IC_{50} values ranging ± 0.4 μM compared to the unprotected compounds).

Moreover, we have been involved with the synthesis of phthalides, a large number of natural and unnatural enol-lactones are known and many of them show important biological activity [14]. In order to gain more knowledge about the structural requirements for the antiproliferative effect of this set of acrylates we envisaged cyclic derivatives **31** as potential candidates and we tested compounds **27** and **33**. In this kind of substrate the α,β-unsaturated moiety related to the biological effect was maintained and the hydroxyl group blocked as a lactone. As we can see from Table 1, the new phthalide **27** proved to be very active against all cell lines proliferation, with IC_{50} values ranging 1.7–4.1 μM. On the other hand, the minor product **33** did not show significant activity. This result confirms the importance of the substitution pattern of the carbon-carbon double bond for the activity. It is worth to note that adduct **32** has not effect on the cell proliferation.

5. Conclusions

In this study we have demonstrated the in vitro antiproliferative activity of some Baylis–Hillman adducts against eight human tumor cell lines. The structure–activity relationships indicated that the maximum antiproliferative activity correlates with the presence of electron-withdrawing groups in the aromatic ring. Derivatives bearing electron-donating groups present a very low activity or are devoid of significant cytotoxic activity. Protection of hydroxyl group as *tert*-butyl dimethylsilyl ether does not influence the activity in a significant way. The activity of these compounds can be due to the ability of the double carbon-carbon bond interacts with biological nucleophiles, since the corresponding hydrogenated derivatives are devoid of activity. Preliminary studies on toxicity in mice have demonstrated that compounds were well tolerated by the animals (LD_{50} ~1.4 g kg^{-1}), except cyano derivative (**11**), and there were no signs of weight loss or other adverse effects [15,16]. Phthalide derivative **27** exhibited a potent antiproliferative effect on all cell lines. The low activity of the corresponding tetrasubstituted derivative **33** can be due to steric and electronic effects blocking the biological nucleophiles access.

Our results are very promising and it is also particularly interesting to note that the majority of test-compounds, mainly with an electron-withdrawing substituted compounds proved to be fully inhibitory to NCIADR cell line proliferation. The following points will be studied in our laboratory: the mechanism by which tested compounds achieve their inhibitory effects on cell proliferation and experiments in vivo (hollow fiber) employing homochiral compounds.

6. Experimental protocols

6.1. Biological assay

6.1.1. Antiproliferative assay

The cells were maintained in RPMI-1640 medium, supplemented with 10% fetal calf serum (FCS) at 37 C with 5% CO_2. The medium was changed every 2 days until the cells reached confluence, at which point they were subcultured. The adherent cell lines were detached from the culture flasks by adding 0.5 ml trypsin solution 0.025%. The trypsin was inactivated by adding 5 ml of 5% FCS in RPMI-1640 medium. After counting, the cells were diluted to appropriate seeding densities and transferred to 96-well microtiter plates in a fixed volume of 100 μM per well. The seeding densities were as follows: 6.5×10^4 (MCF-7), 5×10^4 (NCIADR, 786-0, HT-29), 4×10^4 (NCI.460, PC03), 3×10^4 (UACC62), and 7×10^4 (OVCAR03) cells ml^{-1}. This initial cell concentration was determined from individual growth curves and ensured that the cells would be in the logarithmic growth throughout the experiment. The microtiter plates containing cells were pre-incubated for 24 h at 37 °C to allow stabilizations prior to addition (100 μl) of samples as well as doxorubicin (DOX). The plates were incubated with the samples for 48 h at 37 C and 5% CO_2. Adducts **1**–**18** and derivatives were tested at four concentrations (0.25, 2.5, 25, and 250 μg ml^{-1}), each in triplicate wells. The stock solution was diluted with complete medium containing 50 μg of gentamicin ml^{-1}.

6.1.2. Sulforhodamine B (SRB) assay

This non-clonogenic methodology permits a highly sensitive protein with a straight relationship to cell culture. The SRB assay was done as described by Skehan et al. Briefly, the cells were fixed with 50% TCA (sigma) at 4 C (50 μl per well, final concentration 10%) for 1 h. The supernatant was then discarded and the plates were washed five times with tap water. The cells were stained for 30 min with 0.4% SRB in 1% acetic acid (50 μl per well) and subsequently washed 4 × with 1% acetic acid to remove unbound dye. The plates were air-dried and protein-bound dye was solubilized with 150 μl (100 mM) of Trizma buffer. The resulting optical density was read in a multiwell plate reader at 540 nm.

6.1.3. Data calculations

Absorbances were calculated using the Excell® program (Microsoft Office package) and the mean ± S.E. were calculated for four wells in triplicate samplings. The background absorbances were subtracted from the appropriate control and drug–blank measurements. To assess the effect of Baylis–Hillman adducts **1**–**18** and their derivatives on cell growth, three measurements were obtained at time zero (T_0) values for all cells at the beginning of incubation, and control (C) and test

(T) values at the end of incubation without and with the test substance, respectively. For T value T_0 (cytostatic effect), the calculation was $100 \times [(T - T_0)/C - T_0]$. While for $T < T_0$ (cytocidal effect), the calculation was $100 \times [(T - T_0)/T_0]$. The IC_{50} values (test substance concentration eliciting 50% inhibition) were determined by non-linear regression analysis. These results presented here refer to a representative experiment since all assays were run in triplicate and the average standard error was always < 5%.

6.2. Chemistry

General Procedures. The ^1H and ^{13}C spectra were recorded on a Varian GEMINI BB-300 at 300 and 75 MHz, respectively. IR spectra were recorded using an equipment Nicolet model impact 410. Yields were determined from GC analyses on HP6890 equipment with a flame ionization detector, using a HP-5 capillary (cross linked 5% phenyl methyl siloxanes, 28 m) column.

All Baylis–Hillman reactions were sonicated in an ultrasonic cleaner UNIQUE model GA 1000 (1000 W, 25 kHz). Spectral data of adducts **1**–**18** are in accord to the literature [8,9]. Manipulations and reactions were not performed under dry atmospheres or employing dry solvents, unless otherwise specified.

6.2.1. 2-[(2-Chloro-quinolin-3-yl)-hydroxy-methyl]-acrylic acid ethyl ester (**32**)

A mixture of (2-chloro-quinolin-3-yl)-carboxaldehyde (2 mmol), ethyl acrylate (8 mmol, 0.7 ml) and DABCO (1.3 mmol, 145.8 mg) and 1 ml of dichloromethane was sonicated for 15 h. After chromatographic column (ethyl acetate/hexane, 30:70), adduct **32** was obtained as a tinged yellow solid (94% yield). m.p. 75–77 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 1.24 (t, $J = 7.3$ Hz, 3H, C*H*$_3$CH$_2$), 4.24 (q, $J = 7.3$ Hz , 2H, C*H*$_2$ CH$_3$), 5.63 (s, 1H, C*H*OH), 6.05 (s, 1H, C*H* double bond), 6.40 (s, 1H, C*H* double bond), 7.57 (m, 1H, aromatic), 7.74 (m, 1H, aromatic), 7.85 (d, $J = 8.6$ Hz, 1H, aromatic), 8.02 (d, $J = 8.6$ Hz, 1H, aromatic), 8.38 (s, 1H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.4, 149.3, 147.0, 140.3, 137.0, 132.6, 130.5, 128.2, 127.8, 127.4, 127.2, 127.1, 69.3, 61.3, 14.0. Anal. Calcd for $C_{15}H_{14}ClNO_3$: C 61.76%, H 4.84%. Found C 61.81%, H 4.87%.

6.2.2. General procedure for the hydrogenation reaction

To a suspension of 5% Pd-C (10 mol %) in ethyl acetate (5 ml) was added, under nitrogen, a solution of adduct (1 mmol) in ethyl acetate (5 ml). Then, the reaction atmosphere was changed by hydrogen and the reaction mixture stirred at room temperature. After a period for reaction completion (20 min to 2 h), the suspension was filtered over a pad of celite and the solvent removed under reduced pressure. Analysis by GC did not indicate the presence of the starting adduct.

6.2.2.1. Methyl-3-(4-nitrophenyl)-3-hydroxy-2-methylpropanoate (**19**).

(Hexane/acetate 90:10, 83% yield, 7:1 *syn*/*anti*). ^1H NMR (300 MHz, $CDCl_3$): diastereoisomer syn δ 1.15 (d, $J = 6.6$ Hz, 3H, C*H*$_3$–CH), 2.53–2.72 (m, 1H, C*H*C=O), 3.55 (s, 3H, OC*H*$_3$ ester), 4.72 (d, $J = 6.6$ Hz, 1H, C*H*OH); 6.62 (d, $J = 8.0$ Hz, 2H aromatics), 7.07 (d, $J = 8.4$ Hz, 2H aromatics); diastereoisomer *anti*: δ 0.86 (d, $J = 6.98$ Hz, 3H, C*H*$_3$–CH), 2.53–2.72 (m, 1H, C*H*C=O), 3.55 (s, 3H, OC*H*$_3$ ester), 4.58 (d, $J = 9.1$ Hz, 1H, C*H*OH), 6.62 (d, $J = 8.0$ Hz, 2H aromatics), 7.07 (d, $J = 8.4$ Hz, 2H aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): diastereoisomer *syn* δ 173.6, 149.5, 145.7, 130.1, 121.8, 73.4; 51, 38.5, 15; diastereoisomer *anti* δ 176, 149.5, 145.7, 130.2, 121.8, 73; 49.9, 44.3, 16.4. Anal. Calcd for $C_{11}H_{13}NO_5$: C 55.23%, H 5.48%. Found C 55.20%, H 5.47%.

6.2.2.2. Methyl-3-(4-trifluoromethylphenyl)-3-hydroxy-2-methylpropanoate (**20**).

(Hexane/acetate 95:5, 72% yield, 6:1 *syn*/*anti*). ^1H NMR (300 MHz, $CDCl_3$): diastereoisomer *syn* δ 1.13 (d, $J = 6.0$ Hz, 3H, C*H*$_3$-CH), 2.55–2.72 (m, 1H, C*H*C=O), 3.62 (s, 3H, OC*H*$_3$ ester), 5.23 (d, $J = 6.5$ Hz, 1H, C*H*OH), 7.15–7.31 (m, 4H aromatics); diastereoisomer *anti*: δ 0.91 (d, $J = 6.7$ Hz, 3H, C*H*$_3$-CH), 2.53–2.71 (m, 1H, C*H*C=O), 3.6 (s, 3H, OC*H*$_3$ ester), 4.76 (d, $J = 8.9$ Hz, 1H, C*H*OH); 7.18–7.31 (m, 4H aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): diastereoisomer *syn* δ 173.1, 139.1, 133, 128.4, 128.1; 127.7, 73.2; 51, 39.5; 15; diastereoisomer *anti* δ 174.6, 139.2, 133.1, 128.6, 128.2; 127.5, 73.4; 51.3, 44.3; 16.1. Anal. calcd for $C_{12}H_{13}FO_3$: C 54.96%, H: 5.00%. Found C 54.94%, H 5.04%.

6.2.2.3. Methyl-3-(4-cyanophenyl)-3-hydroxy-2-methylpropanoate (**21**).

(Hexane/acetate 90:10, 68% yield, 4:1 *syn*/*anti*). ^1H NMR (300 MHz, $CDCl_3$): diastereoisomer *syn* δ 1.14 (d, $J = 6.9$ Hz, 3H, C*H*$_3$-CH), 2.59–2.75 (m, 1H, C*H*C=O), 3.62 (s, 3H, OCH$_3$ ester), 4.69 (d, $J = 6.5$ Hz, 1H, C*H*OH), 7.37 (d, $J = 8$ Hz, 2H aromatics), 7.5 (d, $J = 8$ Hz, 2H aromatics); diastereoisomer *anti*: δ 0.91 (d, $J = 7$ Hz, 3H, C*H*$_3$-CH), 2.58–2.73 (m, 1H, C*H*C=O), 3.68 (s, 3H, OCH$_3$ ester), 4.64 (d, $J = 8.8$ Hz, 1H, C*H*OH), 7.38 (d, $J = 8$ Hz, 2H aromatics), 7.48 (d, $J = 8$ Hz, 2H aromatics); ^{13}C NMR (75.4 MHz, $CDCl_3$): diastereoisomer *syn* δ 171.2, 143, 130.1, 129, 117.8, 112.6, 72.9, 51.2, 39.1, 15.1. diastereoisomer *anti* δ 171.5, 143.1, 131, 129.2, 117.6, 112.7, 72.5, 51, 40.1, 16. Anal. Calcd for $C_{12}H_{13}NO_3$: C 65.74%; H 5.98%. Found C 65.75%, H 5.94%.

6.2.2.4. Methyl-3-(4-trifluoromethoxyphenyl)-3-hydroxy-2-methylpropanoate (**22**).

(Hexane/acetate 85:15, 78% yield, 6:1 *syn*/*anti*). ^1H NMR (300 MHz, $CDCl_3$): diastereoisomer *syn* δ 1.15 (d, $J = 6.7$ Hz, 3H, C*H*$_3$-CH), 2.58–2.81 (m, 1H, C*H*C=O), 3.55 (s, 3H, OCH$_3$ ester), 4.78 (d, $J = 6.6$ Hz, 1H, C*H*OH), 6.72–6.78 (d, $J = 7.8$ Hz, 2H aromatics), 6.84–6.92 (d, $J = 7.9$ Hz, 2H aromatics); diastereoisomer *anti*: δ 0.89 (d, $J = 6.9$ Hz, 3H, C*H*$_3$-CH), 2.56–2.77 (m, 1H, C*H*C=O), 3.58 (s, 3H, OCH$_3$ ester), 4.64 (d, $J = 8.8$ Hz, 1H, C*H*OH), 6.74–6.77 (d, $J = 7.9$ Hz, 2H aromatics), 6.81–6.91 (d, $J = 7.9$ Hz,

2H aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): diastereoisomer *syn* δ 173.6, 146.8, 131.9, 129.3, 122.3, 119.5, 71.8, 51, 39.2, 14.3; diastereoisomer *anti* δ 174.5, 148.9, 132.9, 129.2, 122.3, 119.2, 72.1, 50.7, 38.9, 14.6. Anal. calcd for $C_{12}H_{13}F_3O_4$: C 51.8%, H: 4.71%. Found C 52.1%, H 4.69%.

6.2.3. General Procedure for the protection of Baylis–Hillman adducts as tert-butyl-dimethylsilyl ethers

A mixture of 1 mmol of the Baylis–Hillman adduct, 1.3 mmol of *tert*-butyl-dimethylsilyl chloride, 2.5 mmol of imidazole and 0.3 ml of dry *N*,*N*-dimethylformamide (DMF) was stirred at room temperature, under nitrogen for 8–18 h (monitoring by TLC). Then, the reaction medium was quenched with 10 ml of hexane. After washing and brine (3 × 5 ml, drying over $MgSO_4$ and evaporation, the residue was purified by silica gel column chromatography using hexane/acetate as eluent.

*6.2.3.1. Methyl-2-[tert-butyldimethylsilyloxy-(4-nitrophenyl)-methyl]acrylate (**23**).* (Hexane/acetate 90:10, 95% yield, 8 h). IR (ν_{max}/film) 1722 (C=O), 1630 (C=C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ –0.06 (s, 3H, OSiC*H*$_3$), 0.07 (s, 3H, OSiC*H*$_3$), 0.88 (s, 9H, OSi-t-*Bu*), 3.69 (s, 3H, OC*H*$_3$ ester), 5.68 (s large, 1H, C*H*OSi), 6.19 (s, 1H, double bond); 6.33 (s, 1H, double bond), 7.56 (d, $J = 8.8$ Hz, 2H aromatics), 8.15 (d, $J = 8.8$ Hz, 2H aromatics). Anal. Calcd for $C_{17}H_{25}NO_5Si$: C 58.09%, H: 7.17%. Found C 58.07%, H7.15%.

*6.2.3.2. Methyl-2-[tert-butyldimethylsilyloxy-(4-trifluoro-methylphenyl)-methyl]acrylate (**24**).* (Hexane/acetate 95:5, 86% yield, 16 h). IR (ν_{max}/film) 1723 (C=O), 1632 (C=C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ –0.04 (s, 3H, OSiC*H*$_3$), 0.07 (s, 3H, OSiC*H*$_3$), 0.87 (s, 9H, OSi-t-*Bu*), 3.69 (s, 3H, OC*H*$_3$ ester), 5.52 (s large, 1H, C*H*OSi), 6.15 (s large, 1H, double bond), 6.28 (s large, 1H, double bond), 7.23–7.32 (m, 4H, aromatics). Anal. Calcd for $C_{18}H_{25}F_3O_3Si$: C 57.73%, H 6.73%. Found: C 57.81%, H 6.76%.

*6.2.3.3. Methyl-2-[4-cyanophenyl-(tert-butyldimethylsilyloxy) methyl]acrylate (**25**).* (Hexane/acetate 90:10, 88% yield, 12 h). IR (ν_{max}/film) 1724 (C=O), 2240–2218 (CN), 1630 (C=C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ –0.04 (s, 3H, OSiC*H*$_3$), 0.07 (s, 3H, OSiC*H*$_3$), 0.89 (s, 9H, OSi-t-*Bu*), 3.68 (s, 3H, OC*H*$_3$ ester), 4.89 (s large, 1H, C*H*OSi), 5.66 (s large, 1H, double bond), 6.16 (s large, 1H, double bond), 7.36 (d, $J = 8$ Hz, 2H aromatics), 7.46 (d, $J = 8$ Hz, 2H aromatics). Anal. Calcd for $C_{18}H_{25}NO_3Si$: C 65.22%, H 7.60%. Found: C 65.32%, H 7.70%.

*6.2.3.4. Methyl-2-[4-trifluoromethoxyphenyl-(tert-butyldi-methylsilyloxy)methyl]acrylate (**26**).* (Hexane/acetate 90:10, 90% yield, 12 h). IR (ν_{max}/film) 1728 (C=O), 1632 (C=C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ –0.05 (s, 3H, OSiC*H*$_3$), 0.07 (s, 3H, OSiC*H*$_3$), 0.88 (s, 9H, OSi-t-*Bu*),, 3.76 (s, 3H, OC*H*$_3$ ester), 5.49 (s large, 1H, C*H*OSi), 6.11 (s large, 1H, double bond), 6.28 (s large, 1H, double bond), 6.98–6.70 (m, 4H, aromatics). Anal. Calcd for $C_{18}H_{25}F_3O_4Si$: C 55.37%, H: 6.45%. Found: C 55.41%, H 6.48%.

*6.2.4. Preparation of phthalides **27** and **33***

In a dry Fisher–Porter flask, 1.0 mol% of $Pd_2(dba)_3$ was added (Aldrich) under magnetic stirring. After closing the reactor and atmosphere exchange by argon, 1.0 ml of anhydrous and degasified 1,4-dioxane (Merck), 1.1 equivalents of dicyclohexylmethylamine (Aldrich) and 4.0 mol% of a 0.1 mol l^{-1} solution of tri-*t*-butylphosphine (Aldrich) in 1,4-dioxane were sequentially added. The resulting catalytic mixture was stirred at room temperature, until the reaction media become red-brown (~ 5 min). A solution of **32** (1.12 mmol) in anhydrous and degasified 1,4-dioxane (2.0 ml) was then injected in the Fisher–Porter reactor, and the reaction mixture stirred at room temperature, under argon by 15 min. At this point, the color was exchanged to a pale yellow. Finally, the reactor was pressurized with carbon monoxide (CO, 2 atm). The temperature was raised to 70–90 °C in the silicon bath, and the mixture stirred under these conditions. In the final hours of the reaction, a gray precipitate forms, indicating the deactivation of the catalyst. After cooling at room temperature, the reactor was carefully opened. The mixture was then filtered and the residue washed with ethyl acetate (10 ml). The combined solutions were sequentially washed with a 10% solution of HCl (10 ml), water (10 ml) and brine (10 ml). The organic phase was removed, dried over a pad of Na_2SO_4. Filtration and solvent removal furnished a mixture of phthalides **27** and **29**. Purification by silica gel column chromatography (10% ethyl acetate/hexane) provided phthalides **27** (60% yields) and **33** (22% yields). **27**: white solid, m.p. 162–164 °C; IR (neat, ν_{max}): 2982, 2921, 2847, 1772, 1711, 1629, 1376, 1335, 1286, 1123 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.34 (d, $J = 8.5$ Hz, 1H), 7.93 (d, $J = 8.0$ Hz, 1H), 7.83 (m, 1H), 7.69 (m, 1H), 6.47 (broad s, 2H), 6.08 (broad s, 1H), 4.21 (m, 2H), 2.32 (s, 3H), 1.24 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 167.4, 164.3, 149.7, 144.2, 136.7, 136.1, 136.0, 131.3, 130.9, 130.8, 129.2, 129.0, 128.1, 128.0, 76.5, 61.5, 14.0; HRMS (70 eV, *m*/*z*) Calcd for $C_{16}H_{13}NO_4$: C 67.84; H 4.63; Found C 67.79, H 4.61.

33 (*Z*-isomer): tinged yellow solid, m.p. 196–198 °C; IR (neat, ν_{max}): 2962, 2917, 1805, 1711, 1634, 1609, 1372, 1294, 1233 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.66 (s, 1H), 8.36 (d, $J = 9.0$ Hz, 1H), 8.08 (d, $J = 9.0$ Hz, 1H), 7.89 (m, 1H), 7.74 (m, 1H), 4.40 (q, $J = 7.0$ Hz, 2H), 2.32 (s, 3H), 1.43 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 166.8, 163.2, 150.6, 149.7, 144.2, 136.7, 132.1, 130.7, 129.6, 129.5, 129.4, 126.2, 114.0, 61.5, 14.4, 14.3.

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