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Synthesis and Evaluation of Substituted 4-(*N*-Benzylamino)cinnamate Esters as Potential Anti-Cancer Agents and HIV-1 Integrase Inhibitors

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Abstract. Encouraging selectivity and low micromolar activity against HeLa cervical carcinoma ($IC_{50} \geq 3.0\ \mu M$) and the aggressive MDA-MB-231 triple negative breast carcinoma ($IC_{50} \geq 9.6\ \mu M$) cell lines has been exhibited by a number of readily accessible 4-(*N*-benzylamino)cinnamate esters. The potential of the ligands as HIV-1 integrase inhibitors has also been examined.

Keywords: 4-(*N*-benzylamino)cinnamate esters, anti-cancer agents, HIV-1 integrase inhibitors

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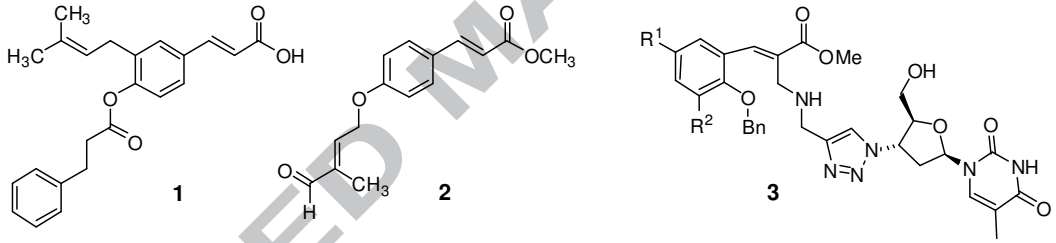
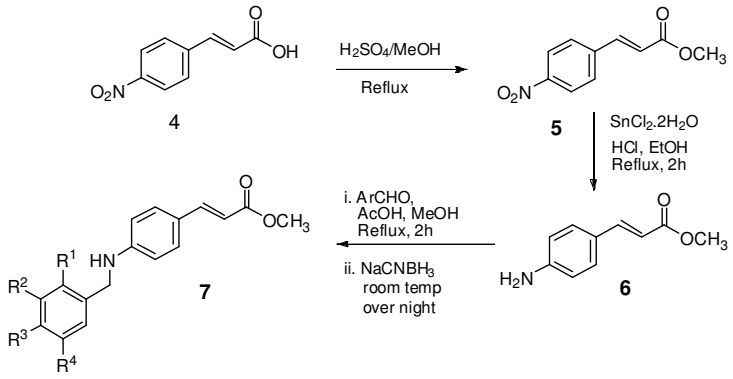
Biologically active cinnamic acid derivatives are widely distributed in nature, and cinnamic acid itself plays important roles in phenylpropanoid and shikimate biosynthetic pathways.[1] Baccharin **1**, a cinnamic acid derivative isolated from Brazilian green propolis, has been shown to selectively inhibit Human Type-5 17β-Hydroxysteroid Dehydrogenase (AKR1C3), a proposed target for breast and prostate cancer therapy, at nanomolar levels.[2] *O*-Prenylated cinnamate esters (*e.g.*, compound **2**) have shown modest activity against K562 cancer cells,[3] while cinnamyl hydroxyamides and 2-aminoanilides have been reported to exhibit potent apoptotic and cytodifferentiation activity.[4] The potential of cinnamic acid derivatives has been recognised not only as therapeutics for the treatment of tuberculosis, malaria, cardiovascular diseases and cancer,[5,6] but also as HIV-1 integrase (IN) inhibitors.[7] In our research on novel HIV-1 enzyme inhibitors, we have reported the preparation and bioassay of cinnamate ester–AZT conjugates **3** as potential, dual-action HIV-1 integrase/reverse transcriptase (IN/RT) inhibitors.[8,9] We now report the HIV-1 IN inhibition- and anti-cancer activities of a series of novel 4-(*N*-benzylamino)cinnamate esters.



The 4-(*N*-benzylamino)cinnamate esters **7a-i** were accessed from 4-nitrocinnamic acid **4** *via* a four-step pathway, involving the methyl ester **5**[10] and its 4-amino analogue **6**[11] (Scheme 1). Esterification of 4-nitrocinnamic acid **4** afforded the methyl ester **5** in 84% yield and subsequent reduction of the nitro group using stannous chloride dihydrate gave methyl 4-aminocinnamate **6** in 77% yield. Condensation of the amino derivative **6** with a series of benzaldehyde and salicylaldehyde derivatives gave the corresponding imines, which were not isolated but reduced *in situ* using sodium cyanoborohydride to afford, following column chromatography, the targeted 4-(*N*-benzylamino)cinnamate esters **7a-i** in yields ranging from 60% to 84%. The 4-(*N*-benzylamino)cinnamate esters **7a-i** are all new compounds and were fully characterised using NMR and HRMS techniques. Assignment of an (*E*)-configuration to

the cinnamate double bond is supported by the large coupling constant (J = *ca.* 15.8 Hz) between the vinylic protons which typically resonate at *ca.* 6.2 and 7.5 ppm.



	R^1	R^2	R^3	R^4
7a	OH	H	H	H
7b	OH	OH	H	H
7c	H	OH	OH	H
7d	OH	H	H	Cl
7e	OH	H	H	Br
7f	OH	H	H	NO_2
7g	OH	OMe	H	H
7h	OH	Cl	H	Cl
7i	OH	Br	H	Br

Scheme 1

The 4-(*N*-benzylamino)cinnamate esters **7a-i** were each tested against both the HeLa cervical carcinoma and MDA-MB-231 triple negative breast carcinoma cell lines, using paclitaxel as a positive control. The results are summarised as IC_{50} values in Table 1, and it is apparent that all of the ligands exhibit a measure of activity against the HeLa cells. Five of them (**7a**, **7d**, **7e**, **7f** and **7g**), however, have IC_{50} values well below 20 μM. Each of these five compounds contain an *ortho*-hydroxy group (R^1 = OH); three of them contain an electron-withdrawing substituent (**7d**: R^4 = Cl; **7e**: R^4 = Br; **7f**: R^4 = NO_2), while the most active ligand **7g** (IC_{50} = 3.04 μM) contains a *meta*-methoxy substituent (R^2 = OMe). The *meta*-hydroxy analogue **7b** (R^2 = OH), however, exhibits a much higher IC_{50} value (68.2 μM).

Perhaps, even more significant is the observed activity of most of the ligands against the aggressive MDA-MB-231 triple negative breast carcinoma cells. All three of the compounds (**7f, 7h** and **7i**) which exhibit IC_{50} values below 20 μM contain an *ortho*-hydroxy group (R^1 = OH) and an electron-withdrawing substituent on the aromatic carbon C-5 (**7f**: R^4 = NO_2; **7h**: R^4 = Cl; **7i**: R^4 = Br); the slightly more active ligands **7h** (IC_{50} = 9.61 μM) and **7i** (IC_{50} = 11.5 μM), however, contain an additional, matching *para*-halogen substituent (**7h**: R^3, R^4 = Cl; **7i**: R^3, R^4 = Br). Of particular interest are the ligands which exhibit:

i) promising activity against *both* cell lines, *viz.*, **7a**, **7f**, **7h** and **7i**; and

ii) a measure of selectivity against the HeLa cells relative to the MDA-MB-231 cells, *viz.*, **7d**, **7e** and **7g**.

The structural features which typically characterise the most active ligands thus include the presence of an *ortho*-hydroxy group (R^1 = OH) and appropriately located, electronegative aromatic substituents (R^2 = Br, Cl and/or R^4 = Br, Cl, NO_2).

Table 1. Anti-cancer activity (IC_{50} at μM) of compounds **7a-i** against HeLa cervical carcinoma and MDA-MB-231 triple negative breast carcinoma cell lines.

	HeLa cervical carcinoma cell activity		**MDA-MB-231 triple negative breast carcinoma cell activity**	
Compound	IC_{50} (μM)	R^2	IC_{50} (μM)	R^2
7a	7.168	0.9302	31.69	0.8918
7b	68.19	0.9677	32.6	0.8839
7c	78.12	0.9248	36.63	0.9754
7d	8.558	0.7284	86.41	0.7402
7e	7.821	0.8617	109.4	0.9095
7f	15.97	0.8976	17.4	0.9104
7g	3.042	0.86	73.68	0.9665
7h	25.88	0.917	9.611	0.9713
7i	35.4	0.09815	11.5	0.8533
Paclitaxel [a]	19.87 nM	0.9409	3.388 nM	0.9141

[a] Positive control.

The 4-(*N*-benzylamino)cinnamate esters **7a-i**, all of which showed no significant toxicity against HEK293 human embryonic kidney cells (Table 2), were also examined for their capacity to inhibit the HIV-1 integrase (IN) enzyme. Disappointingly, only one of the ligands (**7c**) showed statistically significant activity against HIV-1 IN (an average of 43.2% residual enzyme activity at a concentration 20 μM), relative to the established inhibitor, raltegravir, which was used as a positive control (0.67% residual activity).[12]

Access to a series of nine, novel 4-(*N*-benzylamino)cinnamate esters has been achieved in good yield in four steps. A number of the compounds show encouraging activity, at low micromolar concentrations, against HeLa cervical carcinoma and the aggressive MDA-MB-231 triple negative breast carcinoma cell lines – in some cases with interesting selectivity against the HeLa cells relative to the MDA-MB-231 cells. The compounds, generally, exhibit minimal HEK293 toxicity at 100 μM, but only one shows > 50% inhibition of HIV-1 integrase at a concentration of 20 μM.

Table 2. HIV-1 IN enzyme activity at inhibitor concentrations of 20 µM and HEK 293 cell viability data (at 100 µM) for compounds **7a-i** .

	HIV-1 IN activity at 20 µM[a]		**HEK293 viability at 100 µM**	
Compound	% Activity	SD	% Cell viability	SD
Negative control[b]	100	0.07		
Positive control[c]	0.67	0.01		
7a	105.9	0.06	94.4	0.17
7b	73.6	0.19	82.35	1.32
7c	43.2	0.09	113.6	0.44
7d	78.6	0.08	92.36	0.04
7e	73.8	0.16	98.11	0.94
7f	87.3	0.001	108.01	3.14
7g	90	0.02	114.12	0.9
7h	102.5	0.08	106.67	11.69
7i	96.7	0.08	114.33	10.09

[a] Mean of duplicate results. [b] No inhibitor present. [c] Raltegravir for HIV-1 IN; emetine for HEK 293 cells.

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13. Experimental (synthetic and bioassay) details and NMR spectroscopic data are provided in Supplementary Data files I and II, respectively.