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Anti-HIV activity of some synthetic lignanolides and intermediates

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Abstract—The evaluation of the anti-HIV-1 activity of synthetic lignanolides and their intermediates is reported. The antiviral activity was studied through luciferase-based assays targeting the HIV-1 promoter activation induced by either, the HIV-1 Tat protein or the cellular transcription factor NF-κB, both known as crucial factors in HIV-1 replication. Among the compounds tested, three of them **2**, **4** and **13** were further analysed for their anti-HIV-1 activity by recombinant virus assays, showing a suitable profile for development of novel anti-HIV-1 drugs.

The detailed characterisation of the HIV-1 genome and precise knowledge about the role of viral proteins and cellular factors involved in viral replication[1] have opened new avenues to design and develop new antiviral drugs for the treatment of AIDS.[2] To date, commercially available drugs against HIV are directed against viral proteins (i.e., retrotranscriptase, protease and transmembrane gp41 fusion protein) but their efficiency is actually limited by the toxicity associated to such therapies and the appearance of HIV-1 resistance.[2] Thus, new biomolecules impairing the function of other viral or cellular proteins required for efficient HIV-1 replication should be considered in the search of new anti-HIV-1 agents. Among those proteins, the cellular transcription factor NF-κB and the HIV-1 Tat regulatory protein represent attractive and potential targets.[3–5]

The Rel/NF-κB family of transcription factors is involved in different processes such as embrionic development, apoptosis control, regulation of inflammation and activation of the immune system.[6] In addition NF-κB is the major inducible regulatory element involved in LTR transactivation and HIV-1 replication in CD4 lymphocytes.[7–9] The HIV-1 Tat protein is a strong *trans*-activator of the viral LTR promoter by at least two mechanisms. The first one is mediated by Tat interaction with TAR,[10] resulting in an overall 100-fold increase of the transcription rate, thus promoting RNA synthesis, protein expression and subsequent virion spread. The second mechanism is based on the interaction of Tat with cellular transcription factors bound to the enhancer region of HIV-1 LTR.[11] Accordingly, a functional interaction between Tat and NF-κB has been described.[12]

Inhibition of HIV-1-gene regulation by targeting both Tat and NF-κB signalling pathways would result in crippling crucial stages in the cycle of viral replication. In addition, simultaneous targeting of both Tat and NF-κB could provide better expectancy against drug resistance than that attained in current AIDS therapy.[3–5]

In an attempt to discover novel anti-HIV drug leads, we have tested several families of natural and synthetic compounds by means of a multi-target screening system, based on the capacity to inhibit NF-κB and Tat-dependent activation of the HIV-1 LTR promoter. To perform the screening of anti-Tat and anti-NF-κB activities, we have used two stably transfected cell lines,

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namely 5.1, a lymphoid T cell line, in which the HIV-1 LTR is activated by TNFα-induced NF-κB activation, and HeLa-Tat-Luc cells, in which the HIV-1 LTR is directly activated by the HIV-1 Tat protein.[13–16] Those compounds considered active in both assays, inhibiting >50% (NF-κB assay) and >30% (Tat assay), were subsequently submitted to evaluation through a HeLa-Tet-ON assay to discard nonspecific luciferase inhibitory activity.[17,18] The impact of these compounds was also evaluated in an original viral replication assay developed in our laboratory using HIV-1 clones, in which luciferase reporter genes have been cloned. In this model, the inhibition of viral replication is directly evaluated through the measurement of luciferase activity in cell lysates infected with single-cycle viral clones, in the presence or absence of the different compounds.[19]

In this paper, we communicate the results of assaying a number of lignan analogues and synthetic intermediates, taking into consideration that lignans have been reported to display antiviral properties,[20] as well as several other pharmacological activities.[21] We selected for these evaluations only a few representative substances, displaying low cytotoxicity[22] and containing in their structures naphthalene and carbazole moieties,[23] not present in natural lignans.

According to their main structural fragments, the compounds evaluated (Fig. 1) can be grouped into the following classes: (Het)aryldithiane-butanolides (**Type I**: compounds **1**, **2**), arylmethanol-(het)aryldithiane-butanolides (**Type II**: **3**, **4**), dithiane-cyclolignanolides (**Type III**: **5**, **6**, **7**), arylmethanol-(het)arylcarbonyl-butanolides (**Type IV**: **8**, **9**) and cyclolignan related compounds (**Type V**: **10**, **11**, **12**, **13**). All of them were assayed as racemic or diastereomeric mixtures.

The results of the evaluation are shown in Table 1. Related to the NF-κB activity, it can be seen that compounds **1**, **3**, and **8**–**13** showed potent inhibitory activity (>60%) in this assay, whereas compounds **2**, **4**, **6** and **7** demonstrated lower activity (20–60% inhibition). As most of the compounds tested showed high inhibition in the NF-κB assay, we analysed their anti-Tat activity in the HeLa-TAT-Luc cell assay.[16]

Compounds **1**–**4**, **7**, **8**, **10** and **12** showed Tat inhibitory activity in a dose dependent manner and compounds **9** and **11** were toxic. However, within this group, compounds **1**, **3**, **8**, **10** and **12** also showed nonspecific inhibitory activity in the luciferase assay on HeLa-Tet-On-Luc cells, in which the luciferase gene is under the control of an artificial promoter regulated by doxycycline.

In the structural sense, the most potent but unspecific and more toxic derivatives **8**–**12** and also those closer to natural cytotoxic lignans, were those carrying a ketone group on the skeleton. Most of the remaining compounds contain the 1,3-dithiane and the γ-lactone moieties as common structural features. Among them, the naphthalene derivatives **1** and **3** were highly active but unspecific in the HeLa-Tet-ON assay and compounds **5**, **6** were almost inactive. On the other hand, the carbazole derivatives **2** and **4**, respectively, lacking or carrying the bulky hydrophobic trimethoxyphenyl residue, were the most interesting compounds. These compounds as well as the lactone **13**, resulting specific in the HeLa-Tet-ON assay and being less toxic in the necrosis MT2 assay,[24] were further submitted for anti-HIV evaluation through a recombinant virus assay (RVA. Table 2).[25] It is interesting to note that within the short family of assayed compounds, **2**, **4** and **13** are those most dissimilar to natural lignans.

As it can be seen, a fair antiviral effect was observed in RVA experiments. Interestingly, compounds **2** and **13** showed a marked effect, a result that correlates with their respective and selective strong anti-Tat (**2**) or NF-κB inhibitory (**13**) activities. This fact also agrees well

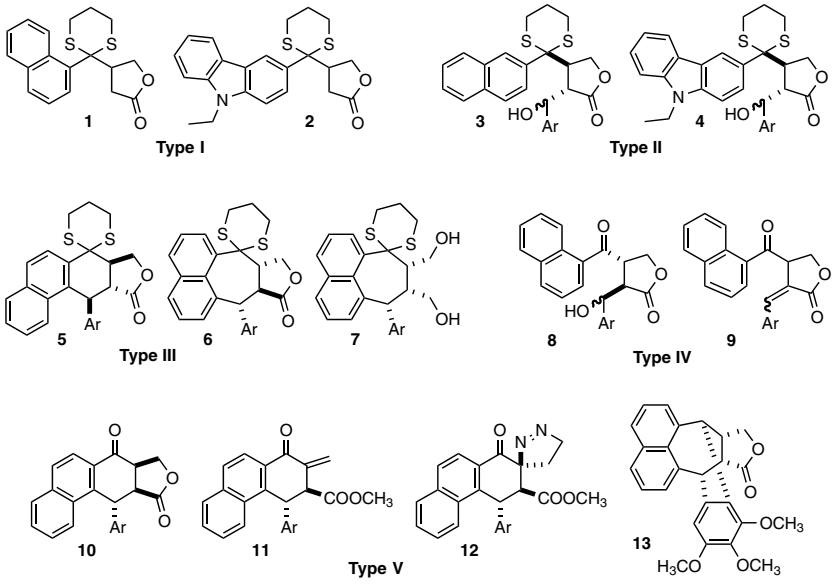

Figure 1. Lignan analogues and intermediates evaluated as anti-HIV agents (Ar = 3,4,5-trimethoxyphenyl).

Table 1. Results of anti-HIV evaluations for compounds **1**–**13**

Compound		**5.1/TNF** (Inhib. %)[a] μM			HeLa-Tat-Luc (Inhib. %)[a] μM			HeLa-Tet-ON (Inhib. %)[b] μM		Necrosis MT2 (24 h)[c] μM	
		25	50	100	25	50	100	25	50	25	50
I	**1**	3.0	24.2	**81.0**	6.0	9.7	25.6	**S**	**u**	2.9	3.5
	2	−7.1	30.0	17.6	**43.4**	**41.5**	**40.1**	**S**	**S**	4.9	4.2
II	**3**	**77.0**	**57.2**	**65.9**	**36.3**	**38.8**	**30.9**	**u**	**u**	11.4	14.6
	4	38.5	39.7	43.7	11.9	20.3	**30.7**	**S**	**S**	4.6	4.0
III	**5**	17.6	10.8	−11.7	−9.1	−9.4	−19.0	***nt***	***nt***	6.9	5.9
	6	21.8	38.0	23.6	−35.9	−39.7	−40.9	***nt***	***nt***	10.3	10.5
	7	43.5	35.2	25.2	11.7	17.6	16.4	***nt***	***nt***	7.9	6.7
IV	**8**	37.5	**64.2**	**t**	1.6	18.0	**65.0**	**u**	**u**	**32.1**	**45.7**
	9	1.5	24.9	**t**	11.6	**t**	**t**	**u**	**u**	6.2	7.6
V	**10**	32.9	25.8	**95.5**	−0.6	**60.0**	**43.0**	**S**	**u**	5.5	4.5
	11	**96.7**	**98.9**	**t**	**56.2**	**t**	**t**	**S**	**u**	**95**	**98**
	12	32.9	**87.1**	**92.0**	**38.8**	**42.2**	**46.0**	**u**	**u**	**30.0**	**43.1**
	13	**73.6**	**56.1**	***nt***	−53.1	−52.9	−53.1	**S**	**S**	7.8	11.3

[a] Data are represented as % of inhibition over positive control (i.e., TNFα-treated cells in the case of 5.1; and untreated cells in the case of HeLa-Tat-Luc). **t**—toxic at this dose.
[b] **S**: inhibition<15%; **u**: inhibition>15%; ***nt***: not tested.
[c] Data represent the percentage of cell-death.

Table 2. Results of anti-HIV evaluations for compounds **2**, **4** and **13**

Compound	RVA activity (IC_{50} μM)	IP toxicity (CC_{50} μM)
2	4.52	>50
4	26.83	>50
13	2.29	>50

IC_{50}: concentration that reduces luciferase activity, thus RV replication, by 50%. CC_{50}: concentration that reduces cellular growth or viability of uninfected cells by 50%.

with the fair structural differences between both compounds and confirms that HIV-1 replication can be inhibited through any of the both selected targets. Moreover, compound **4**, structurally related to compound **2**, being a less potent inhibitor of either Tat or NF-κB (Table 1), still remained as a potent inhibitor of HIV-1 replication (Table 2). This effect seems to be due to the addition of the effects on both targets, though could also be due to the inhibition of other Tat-independent transcription factors in the lymphocytic cell.[26] Taking these observations into account, it will be of much interest for us to evaluate a possible anti-HIV-1 synergy with both compounds.

Altogether, our results have shown that the Tat/NF-κB physical or functional interactions represent an excellent multi-molecular system and the simultaneous targeting of both could serve to develop new anti-HIV-1 drugs. In this sense, it is clear that the transcriptional activity of the HIV promoter is a complex process mediated by an increasing number of proteins where both, the viral protein Tat and the transcription factors, are crucial elements. Several proteins have been described to activate NF-κB through a Tat dependent pathway. Such is the case of histone acetyl transferase, CBP/p300, that serves as Tat coactivator in NF-κB activation by acetylation and is also involved in TNFα-induced NF-κB activation.[27] However, other mechanisms could be involved in the effect of these lignanolides and along with the synergy evaluation, further studies to determine the specific molecular targets affected by these compounds are warranted.

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16. The HeLa-Tat-Luc contains the same reporter plasmid as 5.1 cells and the Tat gene regulated by the CMV promoter. Therefore the HIV-1 LTR is highly activated in this cell line as a consequence of high levels of intracellular Tat protein. Cells (10^5 cells/mL), seeded the day before the assay, were treated either with the CDK9 inhibitor DRB, as a positive control, or with three doses of the compounds tested. After 12 h, the cells were washed twice with PBS and the luciferase activity measured as indicated for 5.1 cells.
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24. MT2 cell death: 10^6 cells/mL were treated with the compounds for 24 h or 48 h at 37 °C. Then, collected, resuspended in PBS and analysed by flow cytometry after Propidium Iodide staining to determine the cell death percentage. All the results, showed as % of cell death or CC50 values, were calculated by GraphPad software.
25. Recombinant virus assay: The RVA assay is a reliable and sensitive test to detect anti-HIV activity because two main reasons, firstly it evaluates direct viral replication (not indirect protection of a cytopathic effect), and secondly the luciferase measurement provides a sensitive assay of HIV-1 replication (the tests is performed in a single-cycle of virus replication in 48 h). The latter is particularly important because if only partial inhibition of HIV-1 replication is achieved or the compound has a short half-life the viral escape could occur easily in the classical MTT test (multiple cycles of viral replication produced in 7 days of culture). The anti-HIV activity was assayed by means of recombinant viruses. These viruses were obtained by transfection of 293T with the NL 4.3 Luc plasmid by calcium phosphate method. Briefly, 1×10^5 MT-2 cells were seeded in 96 well microtiter culture plates and were infected with the recombinant virus, previously titrated (100,000 RLUs/well), in the presence or absence of the samples to be analysed at different concentrations, in a final volume of 200 μL of RPMI medium. The infections were incubated at 37 °C in 5% CO_2 atmosphere. At 48 h post-infection, the antiviral activity was assayed measuring luciferase activity of the infected cells with the Luciferase Assay System Kit with Reporter Lysis Buffer (Promega). Since the luciferase activity obtained is proportional to infection rate, fallen of this activity shows the antiviral effect of the sample. All the results are showed as IC_{50} values, calculated by GraphPad software.
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