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**Dossier "AIDS"**

# Anti-HIV activity of *N*-1-adamantyl-4-aminophthalimide

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**Summary** – The discovery of new leads acting via novel modes of action in the treatment of the human immunodeficiency virus (HIV), the causative agent of AIDS, remains a challenge. Along this line we synthesized and evaluated a series of *N*-substituted 4-aminophthalimides which were designed according to the models of thalidomide, phenytoin (PHT) and ameltolide. From a series of 24 compounds only *N*-1-adamantyl-4-aminophthalimide was endowed with anti-HIV-1 and –HIV-2 activity in CEM cell cultures.

**phthalimide \ phenytoin \ thalidomide \ anti-HIV activity \ AIDS**

## INTRODUCTION

The replicative cycle of the human immunodeficiency virus (HIV), the causative agent of AIDS, offers many potential therapeutic targets. According to the different key steps of this cycle, many strategies were used in the design of new chemotherapeutic agents of AIDS [1, 3]. So far, only reverse transcriptase and aspartylprotease inhibitors have been approved for clinical use. Another strategy aimed at interfering with the interaction of the virally encoded glycoprotein gp120 with the cellular CD4 receptor of the host, has generated promising compounds which disrupt this interaction. They include polyanionic compounds, such as suramin, aurintricarboxylic acid and various sulfated polysaccharides [2]. However, these compounds are confronted with bioavailability problems.

Phenytoin (PHT) (fig 1), also a membrane-reactive drug that has been used in antiepileptic therapy for more than 50 years, has been reported by Lehr and Zimmer [4] to inhibit HIV binding to CD4 positive lymphocytes. These authors proposed that PHT induces the host-cell membrane fluidification that would decrease CD4 receptor availability for ligand interaction [5]. Complementarily, it was proposed that PHT suppresses the influx of $Ca^{2+}$ ions that occurs shortly after HIV infection [6]. More recently, based on this consideration, Comber et al [7] have synthetized PHT derivatives. One of them shows a moderate anti-HIV-1 activity in CEM cells ($EC_{50}$ = 53 µM).

We decided first to take ameltolide (fig 1) as a lead, since this membrane-acting compound has the same antiepileptic profile as PHT. The anticonvulsivant activity of both compounds is mediated by interaction with sodium and calcium voltage dependent channels [8, 9, 10, 11] However, in vivo studies have shown that ameltolide was rapidly inactivated by metabolic activation of

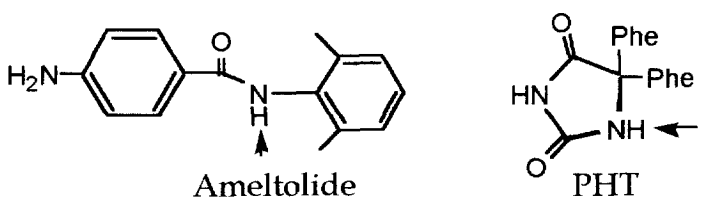


**Fig 1.** Ameltolide and phenytoin (PHT).

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the N-H bond of the amide moiety (fig 1, pointer on ameltolide and PHT) to CO-N-Cl [12]. To circumvent this problem, we *rigidified* the amide bond into an imide bond. Phenylphthalimide derivatives were in this way obtained. The phthaloyl analog of ameltolide, the 4-amino-*N*-(2,6-dimethylphenyl)-phthalimide retains the antiepileptic activity of ameltolide and PHT, and possesses a better bioavailability than ameltolide [13].

The phthaloyl analogs contain a phthalimide moiety which is also present in thalidomide, a sedative. Recently, the anti-HIV-1 activity of this molecule has been reported [14]. This anti-HIV activity was only detected in a stimulated monocytoid cell-line. We synthetized *N*-1-phenylphthalimide, *N*-adamantylbenzamide and *N*-1-adamantyl phthalimide derivatives and evaluated these compounds against HIV-1 and HIV-2 in CEM cells.

In this article, we report their anti-HIV activity and the corresponding structure-activity relationships of an original series of phthaloyl-containing compounds.

## MATERIALS AND METHODS

### Chemistry

The target compounds were synthesized by reacting a phthalic anhydride derivative with the appropriate aniline for the *N*-1-phenylphtha-limide derivatives, and with 1-adamantanamine for *N*-1-adamantylphthalimide, in refluxing acetic acid; compound 2 and the *N*-1-phenyl-4-aminophthalimide derivatives were obtained by reducing the nitro group by hydrogenation using palladium on charcoal as catalyst. The 4-chlorophthaloyl and 4-hydroxyphthaloyl derivatives were prepared by standard methods via the diazonium salt generated in situ from the 4-amino derivative. All compounds were pure in thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analyses and gave spectroscopic analysis ($^{1}H$ and $^{13}C$ nuclear magnetic resonance [NMR] and infrared [IR] analyses) and microanalyses consistent with their structure.

### Anti-HIV evaluation

CEM (human T-lymphocyte) cell cultures were suspended at 400,000 cells/mL of culture medium and infected with HIV-1($III_B$) or HIV-2(ROD) strains at 100 $CCID_{50}$/mL. Then, 100 µL of the infected cell suspension was transferred to 200 µL plate wells containing 100 µL of serially diluted test compound solutions. After 4 days of incubation at 37 °C, cell cultures were assessed for syncytium formation as previously described [15]. Cytostatic assays were based on counting of proliferating CEM cells. The inhibitory effects of the test compounds on cell viability were determined as follows : 100 µL of a CEM cell culture containing 40,000 cells was added to 200 µL plate wells containing 100 µL of a serially diluted test compound solutions. After 4 days of incubation at 37 °C, the number of viable cells was counted with an automated Coulter counter (Coulter Electronics, Harspenden Hertz, UK). To this end, the 200 µL cell suspensions were further suspended in 20 mL of Diluid (JT Baker, Deventer, The Netherlands) before being counted.

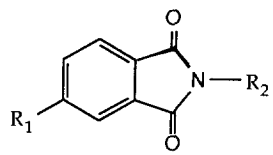
## RESULTS AND DISCUSSION

Table I summarizes the anti-HIV-1 and anti-HIV-2 activity and cytotoxicity of the compounds synthesized in this study.

The *N*-1-adamantyl-4-aminophthalimide (2) exhibited antiviral activity (50 % effective concentration ($EC_{50}$) = 4.78 µg/mL). Cytotoxicity was observed at the compound concentration that was very close to the $EC_{50}$. The presence of the amino group and the adamantyl moiety appears critical for the observed anti-HIV activity of compound 2, since the replacement of the amino group on position 4 of the phthalimide moiety with a hydrogen (1) or the substitution of the adamantyl group with a hydrogen (24) results in a complete loss of its anti-HIV activity. Among *N*-1-adamantylphthalimide derivatives, the amino group could not be replaced by another group without a complete loss of anti-HIV activity (3, 4, 5, 6); this was accompanied for compound 3 by an three-fold increase of toxicity. The place of the amino group on the phthaloyl moiety appears also important since the 3-aminophthalyl derivatives show an $EC_{50}$ (> 4 µg/mL) corresponding to the $CC_{50}$ (50 % cytotoxic concentration, 6.85 µg/mL).

In exploring the influence of the large adamantyl moiety on the anti-HIV activity, we first decided to test the anti-HIV activity of *N*-1-phenyl-4-aminophthalimide derivatives. For this purpose, various substituents were placed on the phenyl moiety (more than 80 were evaluated). Although all of them were found inactive, a relationship could be drawn between the size of the substituents at position 2 and 6 of the phenyl moiety (10,

**Table I.** Anti-HIV-1 and HIV-2 activity in CEM Cells of some *N*-1-Phenylphthalimide and *N*-1-Adamantylphthalimide derivatives.




| | *substituents* | | *$EC_{50}$[a]* | *CEM (μg/mL)* | *$CC_{50}$[b] (μg/mL)* |
|---|---|---|---|---|---|
| *Compd* | *$R_1$* | *$R_2$* | *HIV-1* | *HIV-2* | |
| 1 | H | Adamantyl | > 200 | > 200 | > 200 |
| 2 | $NH_2$ | Adamantyl | 4.7 ± 0.1 | 8 ± 0.0 | 10.3 ± 1.8* |
| 3 | OH | Adamantyl | > 4 | > 4 | 3.71 ± 1.03 |
| 4 | $CH_3$ | Adamantyl | > 100 | > 100 | > 100 |
| 5 | Cl | Adamantyl | > 8 | 35 | 35 ± 7.8 |
| 6 | $NO_2$ | Adamantyl | > 100 | > 100 | > 100 |
| 7 | $NH_2$ | 2-MeCyclohexyl | > 8 | > 8 | 14 ± 4.9 |
| 8 | $NH_2$ | 5,6,7,8 Tetrahydronaphtyl | > 8 | > 8 | 26 ± 3.9 |
| 9 | H | 2,6-Me2Phe | > 200 | > 200 | 56 ± 1.4 |
| 10 | $NH_2$ | 2,6-Me2Phe | > 8 | > 8 | 16.6 ± 1.4 |
| 11 | OH | 2,6-Me2Phe | > 8 | > 8 | 20 ± 14 |
| 12 | $OCH_3$ | 2,6-Me2Phe | > 40 | > 40 | > 200 |
| 13 | Cl | 2,6-Me2Phe | > 40 | > 40 | 32 ± 23 |
| 14 | $NO_2$ | 2,6-Me2Phe | > 200 | > 200 | 155 |
| 15 | COOH | 2,6-Me2Phe | > 8 | > 40 | 6.4 ± 4.2 |
| 16 | $NH_2$ | 2,6-Me2Phe | >1.6 | > 1.6 | 3.9 ± 0.1 |
| 17 | $NH_2$ | 2,6-Isopropyl2Phe | > 0.32 | > 0.32 | 0.75 ± 0.1 |
| 18 | $NH_2$ | 2,6-Cl2Phe | > 1.6 | >1.6 | 5.65 ± 1.24 |
| 19 | $NH_2$ | 2-Cl-6-MePhe | > 8 | > 8 | 14.3 ± 5.0 |
| 20 | $NH_2$ | 2-$NH_2$-6-MePhe | > 40 | > 40 | 72 ± 25 |
| 21 | $NH_2$ | 2-ClPhe | > 40 | > 40 | 90 ± 7.1 |
| 22 | $NH_2$ | 2-MePhe | > 40 | > 40 | 103 ± 41 |
| 23 | $NH_2$ | 2-$NH_2$Phe | > 40 | > 40 | 125 |
| 24 | $NH_2$ | H | > 40 | > 40 | 80 ± 1.4 |

[a]50 % Effective concentration or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50 %. [b]50 % Cytostatic concentration or compound concentration required to inhibit CEM cell proliferation by 50 %; * The anti-HIV-1 and anti-HIV-2 evaluation was repeated three times independently to confirm the anti-HIV activity.

16, 17, 18 and 19) and the toxicity. The larger the size of these substituents, the more toxic was the compound. This cytotoxicity relationship emerged when the amino group at position 4 of the phthalimide moiety was present together with the substitution on position 2 and 6 of the phenyl moiety (10, 16, 17, 18 and 19). Interestingly, compound 2 shows the same cytotoxicity level as compound 10. However, in vivo studies in mice [13] have shown that compound 2 (rotorod assay, 50 % toxic dose ($TD_{50}$) => 300 mg/kg) caused significantly less neurological damage than compound 10 (rotorod assay, $TD_{50}$ =< 300 mg/kg). Moreover, the $CC_{50}$ on human embryonic cells gave values of 85 μg/mL for compound 10 and 100 μg/mL for compound 2. This means that the cytotoxicity could depend more on the model rather than the intrinsic structure of the compound.

The origin of the toxicity of compound 10 in CEM cells could be mediated by interaction of this compound with channels present in these cells (eg, $Na^+$ channels, $K^+$ channels) since it was shown by in vitro studies that compound 10 inhibited in vitro 79 % binding of 100 μM $^3$H-batrachotoxin, a well-known $Na^+$ channel blocker, to $Na^+$ channel (GB Brown, personal communication). The spacial relationship between the 4-

aminophthaloyl moiety and the adamantyl system is important for the anti-HIV activity. Indeed, the non-rigidified form of 2, the 4 amino-*N*-(adamantyl)benzamide is totally inactive ($EC_{50}$ => 20 μg/mL; $CC_{50}$ = 19.8 μg/mL). Thus, the anti-HIV activity of compound 2 was unexpected. This compound was also devoid of any antiepileptic activity in mice.

The antiviral target of compound 2 is so far unknown. Kaplan et al have reported that thalidomide elicited its anti-HIV activity by accelerating the decay of the tumor necrosis factor (TNF)-α mRNA [16] in the monocytoid cell-line U937. However, other groups [17, 18, 19, 20] have found that phenylphthalimide derivatives and thalidomide itself have a potentiating effect on the TNF-α production in the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) stimulated human leukemia cell-line HL-60. Recently, this discrepancy was partially explained by different investigators. Some authors [20, 23] have found that the effect of *N*-phenyltetrafluorophthalimide analogs depended on the cell-lines used, and others that it also depended on stimulating agents [24]. TNF-α production was increased in HL-60 cells but decreased in THP-1 cells. No explanation could be hitherto found to explain the cell-line-dependent action of thalidomide and its analogs on the TNF-α production. Moreover, these compounds are ineffective on the TNF-α production in phorbol 12-myristate 13-acetate (PMA) stimulated-and chronically HIV-infected lymphoid T-cell lines (ACH-2) [16]. Hence it is noteworthy that compound 2, a thalidomide analog possesses an anti-HIV activity towards a non-stimulated de novo infected lymphoid T-cell line (CEM).

Considering the structure of compound 2, the target should be a non-classical one. It could be used as a tool to identify a new target in the HIV life cycle. Other phthalyl analogs are currently synthetized in order to increase the antiviral potency of the compound.

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