Journal of Molecular Graphics and Modelling 44 (2013) 91–103

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Is the conformational flexibility of piperazine derivatives important to inhibit HIV-1 replication?

Cátia Teixeira[a,*], Nawal Serradji[a], Souad Amroune[a], Karen Storck[b], Christine Rogez-Kreuz[b], Pascal Clayette[b], Florent Barbault[a], François Maurel[a]

[a] Univ Paris Diderot, Sorbonne Paris Cité, ITODYS, UMR 7086, CNRS, 15 rue Jean Antoine de Baïf, F-75205 Paris, France
[b] Laboratoire de Neurovirologie, Bertin Pharma, CEA, F-92265 Fontenay aux Roses, France

ARTICLE INFO

Article history:
Accepted 5 May 2013
Available online 13 May 2013

Keywords:
HIV-1 gp120
BMS-378806
Piperazine derivatives
Conformational flexibility
Docking and molecular dynamics

ABSTRACT

The conserved binding site of HIV-1 gp120 envelope protein, an essential component in the viral entry process, provides an attractive antiviral target. The structural similarities between two piperazine derivatives: PMS-601, showing a dual activity for anti-PAF and anti-HIV activity, and BMS-378806, known to inhibit HIV-1 gp120, motivated us to merge important structural features of the two compounds. Novel piperazine derivatives were synthesized and evaluated *in vitro* concerning their ability to inhibit HIV-1 replication in *in vitro* infected lymphocytes. We described an approach that combines molecular docking, molecular dynamics, MM-PBSA calculations and conformational analysis to rationally predict piperazine derivatives binding mode with HIV-1 gp120. We also inquired about the conformational adaptability of the molecules, upon complex formation, and its importance to their respective inhibitory activity. The analysis suggested that the impact of the flexibility of these molecules revealed to be more important, in the context of drug design, than it has generally been assumed. These new insights at the atomic level might be useful to design inhibitors with improved antiviral activity.

1. Introduction

Attachment of the human immunodeficiency virus (HIV-1) to the cell surface is the first step of the virus cycle and it is mediated through the binding of the glycoprotein gp120 on the virion surface to a CD4 receptor on the host cell. Thus, the HIV-1 gp120 envelope protein is an essential component in the multi-tiered viral entry process and, despite the diversity in other gp120 domains, the conserved binding site that interacts with CD4 receptor provides an attractive antiviral target [1].

BMS-378806 (**1**, Fig. 1), a substituted piperazine compound bearing a 4-methoxy-7-azaindole group, protects target cells from HIV-1 infection at nanomolar levels [2]. Despite the controversy regarding the binding site on HIV-1 gp120 where this compound acts [3,4], more and more studies arisen to be consistent with the premise that BMS-378806 interacts with the CD4-binding pocket (called Phe43 cavity) of gp120 [5–7]. Thereby, by binding to gp120, BMS-378806 blocks the attachment of the virus to CD4 receptor. Given its good bioavailability, low protein binding and the promising potent antiviral activity, further development of other members of this class of compounds, piperazine derivatives, is certainly warranted and remains the subject of active research.

A decade ago, some of us found that PMS-601 (**2**, Fig. 1), a piperazine derivative substituted on nitrogen atoms with trimethoxyphenyl groups leading to "cache-oreilles" (ear-muff) electronic distribution, presents a dual activity with IC_{50} values of 8 and 11 μM for anti-platelet activation factor (anti-PAF) and anti-HIV activity, respectively. Additionally, the compound did not show cytotoxic events at 1000 μM in monocyte-derived macrophages [8]. We also established that: (i) the presence of a carbamate function was favourable to the antiviral activity of PMS-601 and (ii) the lipophilicity of the substituent on the piperazine cycle seemed to be less important for the anti-PAF activity than for the antiviral one. Although the mode of action responsible for the anti-HIV activity of PMS-601 is not clearly defined, it is a promising lead compound for the treatment of HIV infection and neuroAIDS, particularly, by combining its anti-PAF and anti-HIV-1 effects [9].

The structural similarities between PMS-601 and BMS-378806 (shortly named from now on BMS), motivated us to merge important structural features of the two compounds and engage in the development of novel piperazine derivatives (**8a**,**b**, Fig. 1) as potential antiretroviral agents. Hypothetically, molecules bearing key structural motif belonging to both PMS-601 and BMS would present an antiviral activity, more interesting than PMS-601, and using an

* Corresponding author. Current address: CICECO, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, P-3810-193 Aveiro, Portugal. Tel.: +351 234372571.
E-mail address: ca.teixeira@ua.pt (C. Teixeira).

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http://dx.doi.org/10.1016/j.jmgm.2013.05.003

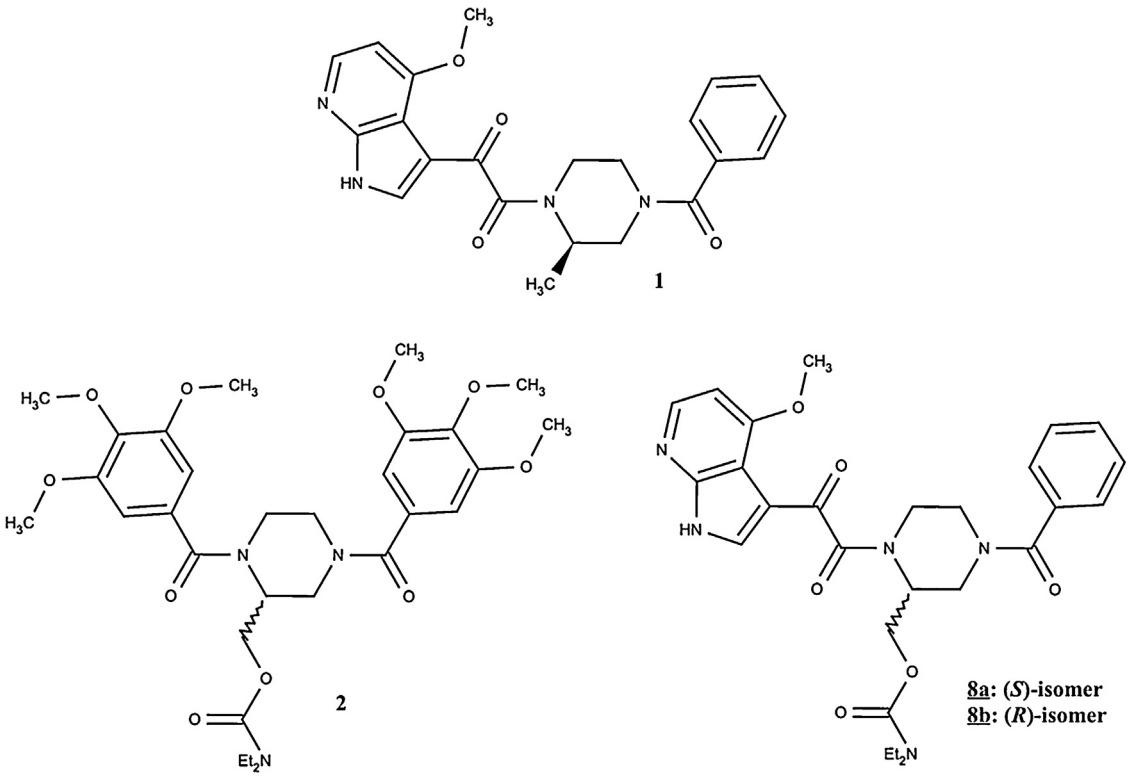

Fig. 1. Structures of BMS-378806 (**1**), PMS 601 (**2**) and synthesized compounds **8a** and **8b**.

identified mode of action, *i.e.*, the inhibition HIV-1 entry into cells by targeting the viral protein gp120. In this regard, novel piperazine derivatives were synthesized (Supp. Information, Scheme S1) and evaluated *in vitro* concerning their ability to inhibit HIV-1 replication in *in vitro* infected lymphocytes (Table 1).

None of the compounds showed significant anti-HIV-1 replication (Table 1). In order to understand the possible causes for this lack of activity and, consequently, design a second generation of compounds with an improved profile, molecular modelling studies were conducted. Specifically, we attempted to address the following questions: do the piperazine derivatives, **8a** and **8b**, bind to HIV-1 gp120 in a similar way that does BMS? How tightly the ligands bind to HIV-1 gp120? Which are the residues that establish stronger interactions with the ligands? How does the inhibitor conformational flexibility affect its affinity towards the target?

To answer to these questions, we used an approach combining molecular docking, molecular dynamics, MM-PBSA calculations and conformational analysis to rationally predict the binding mode of piperazine derivatives with HIV-1 gp120, inquire about the conformational adaptability of the molecules, upon complex formation, and its impact on their respective inhibitory activity. Since there is no crystal structure of a complex between gp120 and BMS, we first had to predict its binding mode by using molecular docking combined with molecular dynamics. It is important to refer that Kong and co-workers already carried out a previous study of rational prediction of the binding mode of BMS with HIV-1 gp120 wild-type (wt) [10]. In this study, they found that BMS inserts the azaindole ring deeply into the protein active site. Kong and co-workers put a lot of efforts into modelling protein's flexibility but they neglected the one of the ligand as their primary objective was to determine the precise gp120 binding site of BMS-378806. In fact, they assumed that BMS is rather rigid and the methoxy group on the azaindole was the only bond set as rotatable. However, it is well known that flexible molecules are deformed when binding to proteins, and thus, may bind as different conformers to them. The degree of deformation appears to depend somewhat upon the number of rotors in the ligand. Since BMS presents a high degree of flexibility it should be take into account during the molecular modelling studies. So, in order to validate our results for the predicted binding mode of BMS, we also performed the same molecular modelling procedure using the gp120 S375W as this mutation completely abates the inhibitory activity of BMS [7]. By running the same procedure with the mutant, we expected to observe the loss of the interactions between the residues of the binding site and BMS when compared to the results with the wild-type. Once we validated our procedure, we applied the same protocol for the novel piperazine derivatives, compounds **8a** and **8b**, which were also synthesized and their inhibition of HIV-1 binding determined.

To our knowledge this is the first study concerning the impact of the conformational adaptability of piperazine derivatives on their inhibition of HIV-1 gp120. The results obtained suggested that the impact of the flexibility of these molecules revealed to be more important, in the context of drug design, than it has generally been assumed. These new insights, at atomic level into the binding mode of piperazine derivatives and the impact of the flexibility of such molecules, might be useful to design improved inhibitors and make molecular modelling stand out as an important research tool for understanding experimental results.

Table 1
Anti-HIV-1-LAI effects and cytotoxicity of the tested compounds on activated PBMC *in vitro* infected by HIV-1-LAI.

Compound	ED_{50} (μM)	ED_{90} (μM)	CC_{50} (μM)	CC_{90} (μM)
AZT	0.03	0.12	–	–
BMS	<0.04	0.04	>125	>125
8a	>125	>125	>125	>125
8b	>125	>125	>125	>125

Results are expressed as ED_{50} and ED_{90}, concentration of drugs that decreases the HIV replication of 50% and 90%, respectively. CC_{50} and CC_{90}, concentration of drugs to reduce the viable cell number by 50% and 90%, respectively.