Cosalane and its Analogues: A Unique Class of Anti-HIV Agents

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Abstract: Cosalane and related compounds are a peculiar group of anti-HIV agents with activities against a broad range of viral targets, such as viral entry and reverse transcriptase (RT). Cosalane and its analogues having anionic pharmacophore inhibit the binding of gp120 to CD4 as well as the fusion of the viral envelope with the cell membrane. The alkenyldiarylmethanes (ADAMs), characterized by the lack of the steroidal moiety of cosalane, are a unique class of non-nucleoside reverse transcriptase inhibitors (NNRTIs) that have potential value in the treatment of HIV infection. In this article, the structural modifications, structure-activity relationship (SAR) studies and/or crystallographic studies of cosalane related derivatives as potent antiviral agents were reviewed, which will be beneficial to the discovery of next generation cosalane derivatives with improved antiviral potency, metabolic stability and bioavailability.

Keywords: cosalane, AIDS, HIV, inhibitors, gp120, CD4, reverse transcriptase (RT), non-nucleoside RT inhibitors (NNRTIs), ADAMs.

1. INTRODUCTION

Today, the acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) remains a worldwide deadly disease [1]. Though the highly active antiretroviral therapy (HAART) currently used to treat HIV infection slow or halt disease progression, the long-term sustainable effects are unpredictable, increased toxicities can occur due to drug-drug interactions in the multiple drug therapies. In addition, the drug resistance is likely to become an escalating problem due to the propensity of HIV to rapidly mutate and misuse of drug therapy, making the identification and development of alternative drugs with novel action mechanism a high research priority [2-5].

One way of overcoming or diminishing drug resistance issues is the development of a multifunctional molecule that can interact with several viral targets, and which may be an effective inhibitor of different stages of viral life cycle, since resistance to multitarget inhibitors would necessitate multiple mutations [6]. Cosalane **(1),** the subject of the present review, is such a multifunctional molecule which prevents the cytopathic effect of HIV by inhibiting HIV-1 reverse transcriptase (RT), protease, gp120-CD4 interaction as well as inhibition of a post attachment event prior to reverse transcription [7]. Also, cosalane proved to be a potent HIV inhibitor with a broad range of activity against a variety of laboratory, clinical, and drug-resistant HIV-1 isolates, HIV-2, and Rauscher murine leukemia virus [7].

Cosalane **(1)** was obtained from the structurally modification of the aurintricarboxylic acid (ATA), a multifunctional antiviral molecule with potency against HIV (as a nonspecific attachment inhibitor), vesicular stomatitis virus, vaccinia virus [8], the severe acute respiratory syndrome coronavirus [9-11], and avian influenza virus neuraminidase [12]. ATA also shows antithrombotic effect [13], inhibiting NF-κB-DNA binding [14,15], inhibiting DNase(s) involved in apoptosis [16], inhibiting cytokine-induced JAK-STAT signal pathways [17,18] and inhibiting apoptosis [19]. Conceptually, replacing the quinine methide moiety of the ATA with an alkene chain linked to cholestane and attaching two chlorine atoms *ortho* to the phenolic hydroxyl groups of the two salicylic acid units yielded cosalane (Fig. **1**).

However, the poor oral bioavailability (<1%), resistance to hepatic metabolism (intravenous administration) [20] and limited diffusion across Caco-2 cell monolayers [21] of this highly lipophilic compound prompted researchers to undertake extensive chemical modifications effect aimed at overcoming these shortcomings.

Initial structural modifications indicated that the cholestane portion of cosalane functioned as a lipophilic accessory appendage to escort the dichlorodisalicylmethane pharmacophore to a lipid environment (Fig. **2**) [22-24], which may be useful in the design of additional cosalane analogues of potential usage (as HIV entry inhibitors) in the treatment of AIDS.

To date, cosalane derivatives have been gradually developed into two types of potent anti-HIV compounds by Cushman M and his coworkers: multifunctional viral entry inhibitors and alkenyldiarylmethanes (ADAMs) based non-nucleoside reverse transcriptase inhibitors (NNRTIs) with a unique binding mode. In principle, mechanistically novel inhibitors should not be cross-resistant to the existing anti-HIV drugs and would find great utility in drug combinations aimed at salvage or, eventually, first-line treatment. Consequently, the brief stories of ATA and cosalane derivative as bioactive molecules have been described in an excellent review [25]. Though they have been exploited over a period

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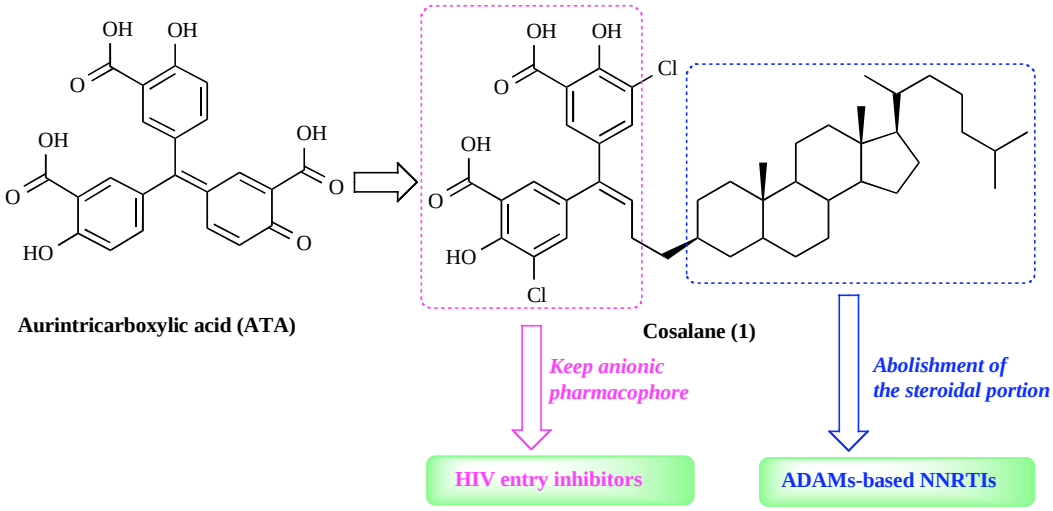


Fig. (1). The discovery and development of cosalane (**1**), which should conceptually be considered as an ATA derivative (missing one salicylic acid part) conjugated to cholestane.

spanning one or two decades, they are likely to continue to find new applications for a long time.

In this review, we will focus our interest and attention on the structural modifications and structure-activity relationship (SAR) studies of cosalane related derivatives as potent antiviral agents, which would provide valuable insight for further optimization.

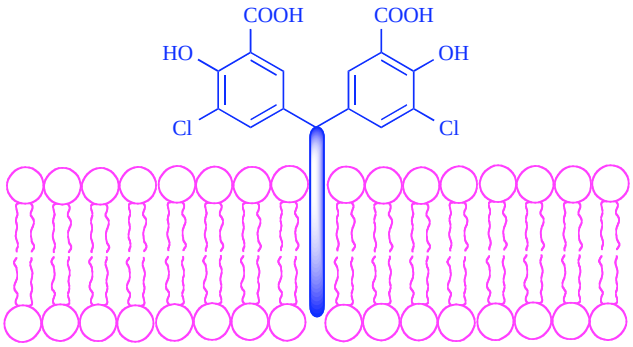


Fig. (2). Schematic diagram of cosalane cholestane moiety as a lipophilic accessory appendage.

2. COSALANE ANALOGUES AS INHIBITORS OF VIRAL ENTRY

One stage of the HIV life cycle that presents targets for promising therapeutic intervention is the entry of virus into host cells, initiated by the interaction of viral envelope glycoprotein gp120 with the CD4 molecule on the cell surface [26,27].

Cosalane was reported as a inhibitor targeted HIV entry process. Shortly thereafter, its new analogues **2** and **3**, having an extended polyanionic pharmacophore, resemble cosalane in their ability to inhibit attachment and fusion [28,29]. The compound (**2**) displayed an EC_{50} of 0.55 μM against the replication of HIV-1_{RF} in CEM-SS cells, which represents a significant increase in antiviral efficacy over cosalane itself (EC_{50} = 5.1 μM) [28]. In contrast to cosalane itself, both compounds are either very weak or inactive as inhibitors of RT, protease, and integrase in enzymic assay [28].

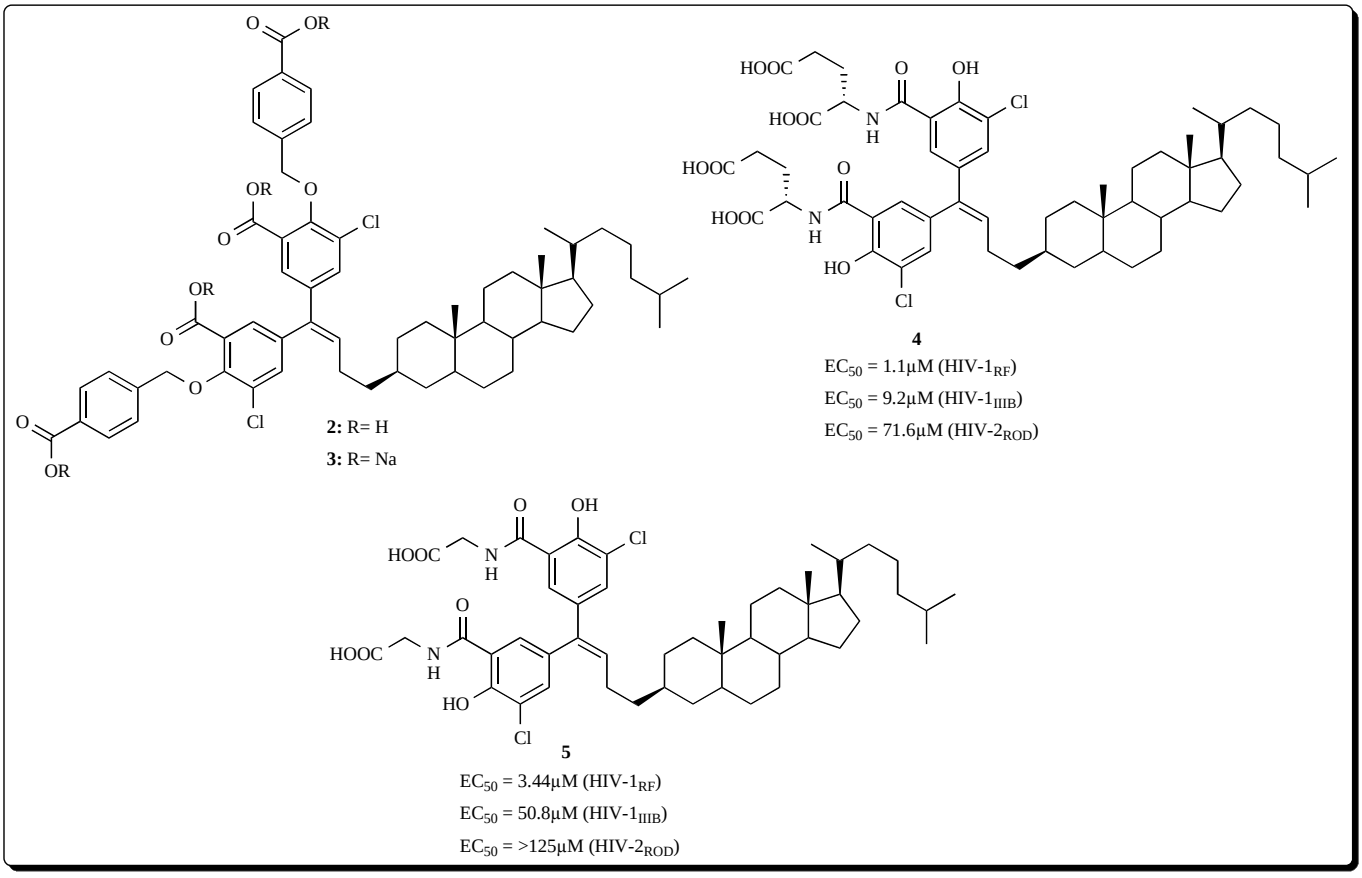
The interactions of cosalane and its analogues with CD4 is considered to involve ionic binding of negatively charged carboxylates of these inhibitors with positively charged residues on the CD4 surface. In cosalane tetracarboxylate analogues, the optimal potency results when the two distal and the two proximal carboxylates are separated by eight atoms [30].

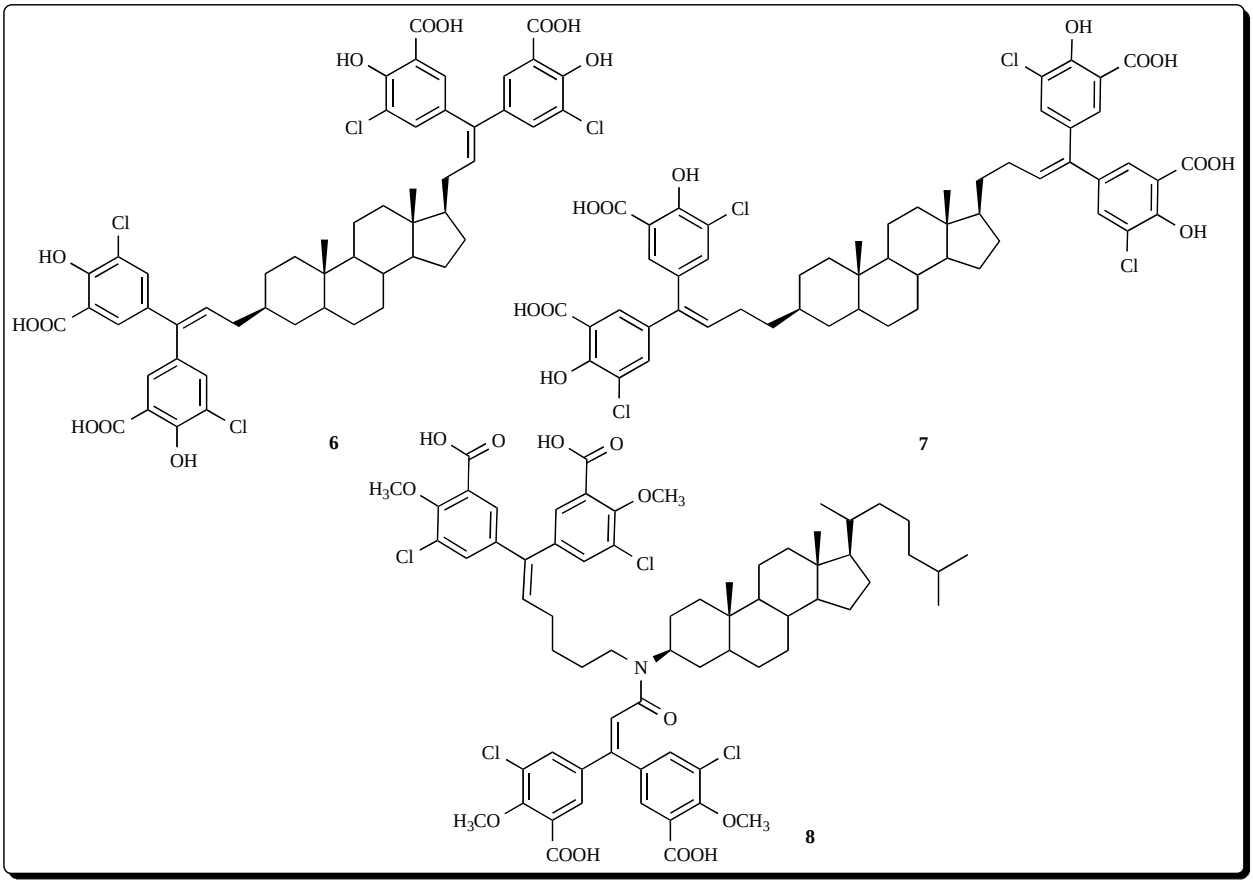
In a series of cosalane-amino acid conjugates containing glycine, glutamic acid, aspartic acid, leucine, β-alanine, and phenylalanine residues, the glutamic acid congener **4** proved to be the most active one [31,32]. It was more active than the glycine derivative **5** in all three systems tested (EC_{50}: 9.2 versus 50.8 μM against HIV-1_{IIIB}, 1.1 μM versus 3.4 μM against HIV-1_{RF}, and 71.6 versus >125 μM against HIV-2_{ROD}) [32], which indicates that the addition of two carboxyl groups to those already present in the glycine derivative **5** results in a general improvement in anti-HIV potency [32].

In general, these amino acid derivatives were more potent against HIV-1_{RF} in CEM-SS cells than they are vs HIV-1_{IIIB} in MT-4 cells, and they also showed anti-HIV-2_{ROD} activity in MT-4 cells [32]. Moreover, compound **4** had an absolute oral bioavailability of 5.10 ± 1.51% in rats [33].

Cosalane analogues **6-8**, incorporating two fragments of the dichlorodisalicylmethane pharmacophore were synthesized and evaluated against HIV-1_{RF}, HIV-1_{IIIB}, and HIV-2_{ROD} in cell culture. As illustrated in Table **1**, the attachment of the second pharmacophore did not affect the anti-HIV activity significantly, suggesting that the two pharmacophores acted independently, and one at a time, with positively charged amino acid side chains present on the surface of gp120 and CD4 [34].

Introduction of an amido moiety or an amino group into the alkenyl linker chain of cosalane led to analogues **9**, **10**. The antiviral activity results showed that the replacement of the 1' and 2' carbons in the linker chain of cosalane by an


2: R= H
3: R= Na
4
EC50 = 1.1μM (HIV-1RF)
EC50 = 9.2μM (HIV-1IIIB)
EC50 = 71.6μM (HIV-2ROD)
5
EC50 = 3.44μM (HIV-1RF)
EC50 = 50.8μM (HIV-1IIIB)
EC50 = >125μM (HIV-2ROD)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7
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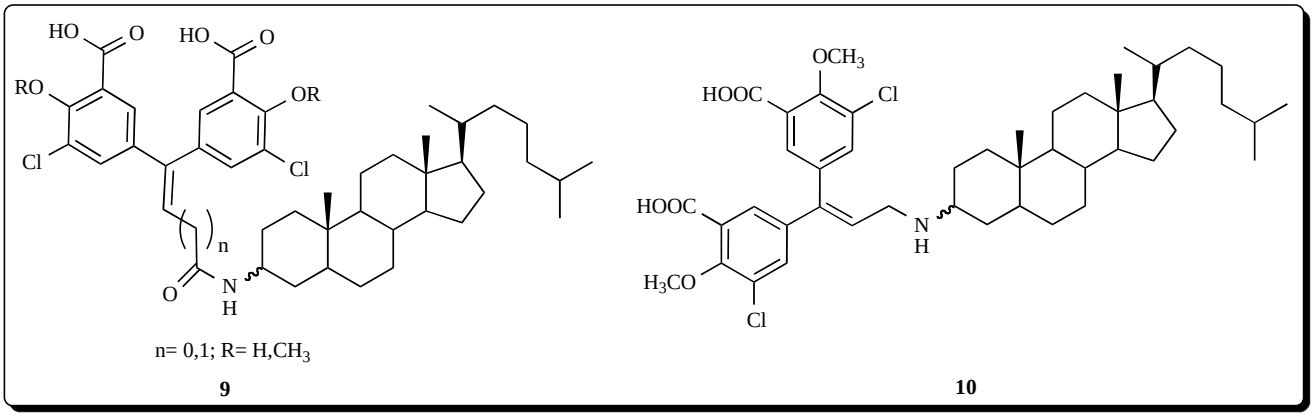
Table 1. Anti-HIV Activities of Cosalane (1) and Double Pharmacophore Analogues [34]

Compds	EC_{50} (μM)			CC_{50} (μM)	
	HIV-1_{RF} (CEM-SS cells)	HIV-1_{IIIB} (MT-4 cells)	HIV-2_{ROD} (MT-4 cells)	CEM-SS cells	MT-4 cells
1	5.1 ± 2.1	3.0 ± 0.18	4.0 ± 2.1	>200	>125
6	12 ± 2.4	14 ± 0.69	50 ± 2.7	50 ± 14	115
7	1.3 ± 0.2	20 ± 3.0	74 ± 19	130 ± 19.5	>125
8	6.9 ± 1.7	9.9 ± 0.90	8.2 ± 1.8	58 ± 12	38 ± 12



amido moiety (compound **9**) was generally tolerated. The length of the linker and the stereochemistry of the substituted groups at *C*-3 of the steroidal ring had significant influence on the anti-HIV activity. Unfortunately, incorporation of an amino group into the linker (compound **10**) completely abolished the antiviral potency [35].

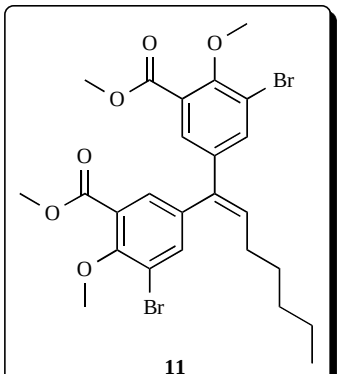
As described above, these cosalane derivatives were merely regarded as entry inhibitors which block nonspecific attachment and fusion of HIV virions with host. Undoubtedly, in the future studies, only the combination of the previous SAR conclusions of cosalane derivatives with the structural biology information of drug target involved in HIV entry process could contribute to the discovery of specific entry inhibitors *via* rational chemical modifications.

3. MODIFICATION OF COSALANE AS ADAMS-TYPED NNRTIS

HIV-1 NNRTIs are important component of the combination therapy (HAART) because of their unique antiviral potency, high specificity and low cytotoxicity [36-38]. Nevertheless, drug resistance is still a key cause of failure for their anti-HIV infection efficacy. Three earlier used NNRTIs in clinical (efavirenz, nevirapine and delavirdine) could effectively inhibit replication of the wild-type virus, but they are less effective against several key mutant strains that confer resistance to current NNRTIs, such as Y181C, K103N, Y188C, and L100A [37]. Though the newly approved etravirine shows improved potency against many commonly observed NNRTIs-resistant viruses, its pharmacokinetic profiles is not satisfactory [39, 40]. Therefore, there is an urgent need for the design and development of novel NNRTIs with improved drug resistance profiles and satisfactory pharmacokinetic properties [41].

3.1. Structurally Modifications of ADAMs-Based NNRTIs

Alkenyldiarylmethanes (ADAMs), structurally related to cosalane, represent a unique group of NNRTIs characterized by the lack of the steroidal portion of cosalane. Among the first series of ADAMs, compound **11** proved to be the most active and was chosen as the lead for further optimization. It inhibited HIV-1 replication in CEM cells with an EC_{50} of 7.1 μM and was active as an inhibitor of a broad panel of labora-

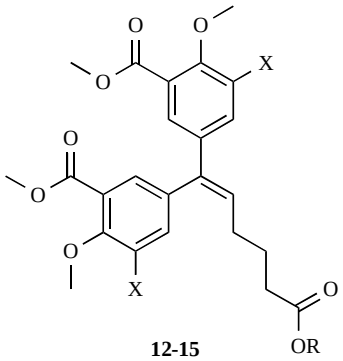


11

EC_{50} = 7.1±5.0μM

(HIV-1_{RF}/CEM-SS cells)

Table 2. Anti-HIV Activities of Cosalane Analogues (12-15)



12-15

Compds	X	R	IC_{50} (μM)	EC_{50} (μM) HIV-1_{RF} (CEM-SS cells)	SI	Refs.
12	Cl	Me	0.3	0.013	2430	[43,44]
13	Br	Me	0.3	0.0013	10000	[45]
14	Cl	Et	0.233	0.01	1600	[46]
15	Br	Et	0.359	0.007	2357	[46]

tory isolates of HIV-1 in CEM-SS and MT-4 cells, but was inactive against HIV-2. It remained active against a variety of RT mutations (at residues 103, 100, 74, 98, and at 103/181 double sites). In addition, ADAM **11** displayed synergistic action with AZT [42].

ADAMs deriatives **12-15** were also found to inhibit the cytopathic effect of several clinically relevant HIV-1 strains. They inhibited the replication of HIV-1_{RF} in CEM-SS cells with an EC_{50} value of 1.3-13 nM, while it showed lower cytotoxicity, providing a higher selective index (SI). The IC_{50} value for **12-15** against HIV-1 RT was lower than 0.4 μM (Table **2**) [43-46]. In addition, ADAM **12** could serve as an adjunctive therapy to AZT and certain NNRTIs that select for L100I resistants [43]. These pharmacology profiles spurred the persistent interest in studying the ADAMs scaffold.

However, the potential clinical utility of the ADAMs was expected to be limited by the presence of ester moieties that were rapidly metabolized by nonspecific esterases in blood plasma to the corresponding carboxylic acids. Fortunately, new synthetic methodology based on Pd-catalyzed coupling reactions, including Suzuki reaction, Sonogashira reaction, Stille reaction, *et al.*, allowed the incorporation of differently substituted aromatic rings or groups in a stereochemically defined fashion to obtain new candidates with improved metabolic stability [47,48].

Consequently, the replacement of labile esters with thioesters [49], various heterocycles [50-56], and nitriles [49,51] led to the successful development of several low micromolar and sub-micromolar ADAMs-based NNRTIs that exhibited improved metabolic stability relative to their parent compounds (Table **3**). Especially, compound **19** displayed improved metabolic stability in rat plasma ($t_{1/2}$ = 61 h) along with the potent inhibitory activity against HIV-1 RT and the reproduction of HIV-1_{RF} and HIV-1_{IIIB} in cell cultures at submicromolar concentrations [52]. Though the rat plasma half-lives of benzoxazolone derivative **24** was not improved when compared to the parent analogues, it was identified as one of the most potent derivative, which inhibited HIV-1 RT with an IC_{50} of 20 nM and inhibited the replication of both HIV-1_{RF} and HIV-1_{IIIB} strains with EC_{50} values of 30 and 90 nM, respectively [55]. Concerning inhibition of RT enzymatic activity, ADAM 25 is the most potent analogue among the investigated compounds, exhibiting an impressive IC_{50} of $\leq$ 1 nM [56].

3.2. Crystallographic Studies and Proposed Pharmacophore Model

Molecular models [55] and crystallographic structures studies (PBD code: 3IS9, 3IRX) [56] showed that ADAMs were exclusively hydrophobic in nature and adopted the typical "butterfly"-like conformation that is characteristic of many other reported NNRTIs like nevirapine, α-APA, and TIBO. Besides, ADAMs protruded from a large gap in the back side of the binding pocket, placing portions of the molecules unusually close to the polymerase active site, making the ADAMs unique among other published NNRTI-RT complexes [56].

Consequently, an pharmacophore model had been developed for the ADAM typed NNRTIs [53], which provided insight on the binding mode of ADAMs with RT. Notable features of pharmacophore model (Fig. **3**) and SAR conclusions include:

First, The aryl ring (A) situated *trans* to the side chain is buried deep in the pocket and probably has π-π stacking interaction with hydrophobic residues (F227, W229, and Y188) [56]. The aryl ring (A) also contains a fused heterocycle, at the C_3 or C_4 position, with a hydrogen bond acceptor

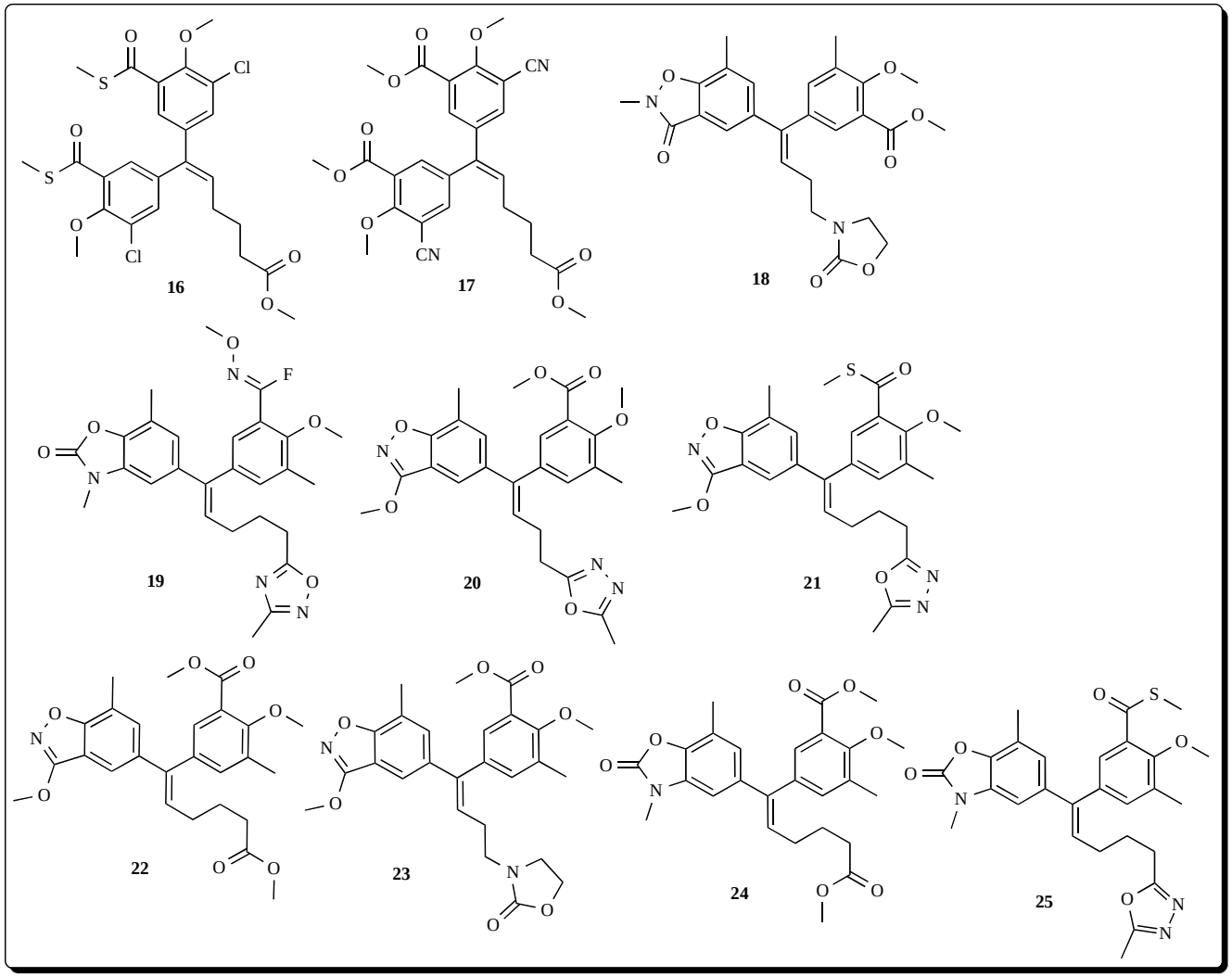


Table 3. Anti-HIV Activities and Metabolic Stability of Cosalane Analogues

Compds	EC_{50} (μM)		IC_{50} (μM)	CC_{50} (μM)		$t_{1/2}$ (min)	Refs.
	$HIV\text{-}1_{RF}$ (CEM-SS cells)	$HIV\text{-}1_{IIIB}$ (MT-4 cells)		CEM-SS cells	MT-4 cells		
12	0.013[a]	0.6[b]	0.3[a]	31.6[a]	160[b]	5.8[b]	[a][43,44] [b][49,50]
16	ND	1.8	ND	ND	>224	55.3	[49,50]
17	0.7	0.8	0.38	17	>100	12.4	[49]
18	2.7	ND	ND	16.3	ND	22.1	[51]
19	0.7	0.24	0.67	2.9	12.4	3641	[52]
20	0.36	0.42	0.39	2.8	6.4	331	[53]
21	0.05	0.14	0.47	5.7	7.0	864	[53]
22	0.04	0.02	0.91	0.5	1.09	3.46	[54]
23	0.6	>1.1	0.63	1.2	>1.1	51.4	[54]
24	0.03	0.09	0.02	5.1	16.86	1.3	[55]
25	0.07	0.18	< 0.001	3.0	3.9	238	[56]

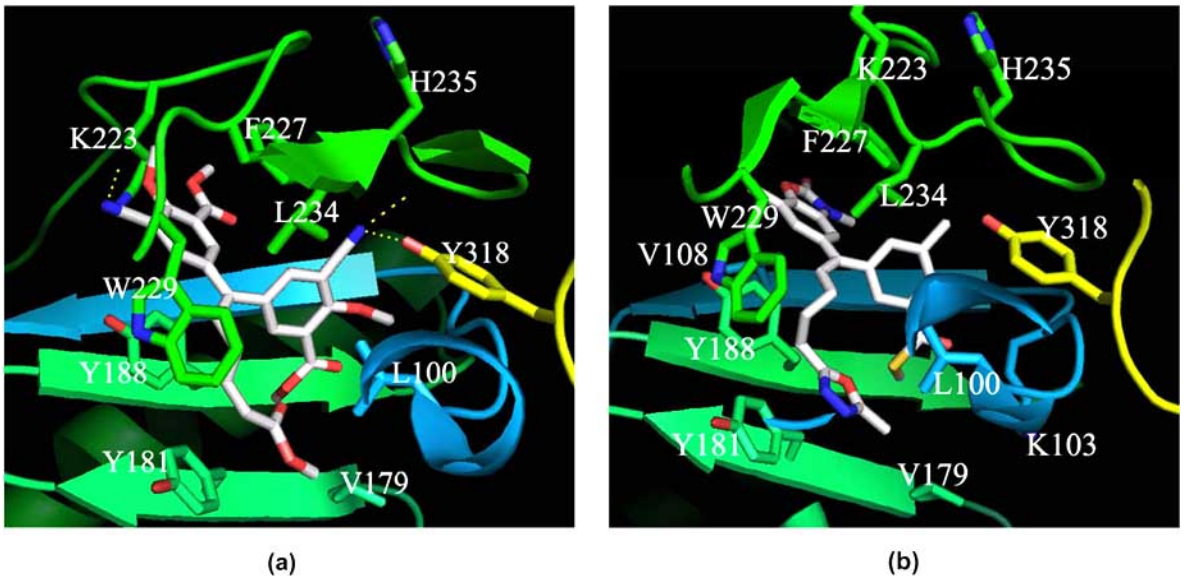


Fig. (3). Two crystal structures (3IS9, 3IRX) solved for separate complexes of ADAM NNRTIs **17** (a) and **25** (b) with HIV-1 RT [56].

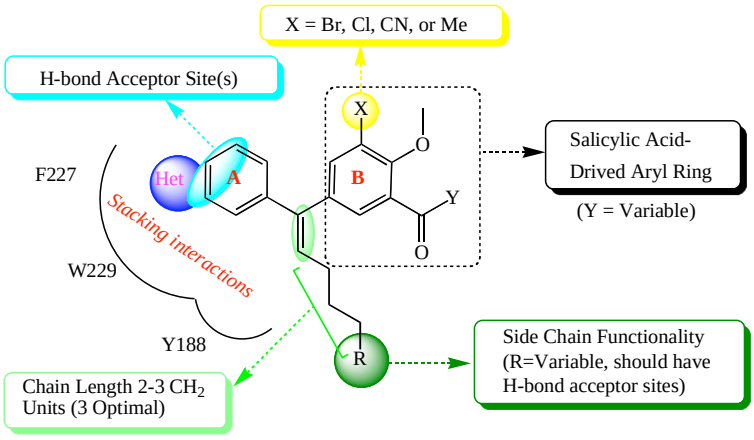


Fig. (4). Pharmacophore model for the design of potent ADAM-based NNRTIs [53].

site in the plane of the ring. Notably, a potential hydrogen bond between the nitrile of the aryl ring (A) in **17** and the side chain of K223 is observed in Fig. (**3a**). Additionally, the ester functionalities (ADAM **17**) and fused five-membered heterocycles (oxazolone group in ADAM **25**) on the A ring show similar stacking interactions with the aromatic ring of F227. In view of the observed stacking interactions between the methyl ester of **17** and F227, substitution of this functional moiety with the cyclic oxazolone in 25 may have the benefit of rigidifying the atoms in the proper planar orientation for π-π stacking with F227, which may be one reason for the improved potency of 25.

Second, the salicylic acid-derived aryl ring (B) that are oriented *cis* to the side chain are cradled by the hydrophobic residues L100, V106, V179, and L234. In additional, an hydrogen bond is formed between the nitrile of the aryl ring (B) in **17** and the side chain hydroxyl of highly conserved Y318.

Third, the alkenyl side chains of the ADAMs display significant edge-to-face interactions with the aromatic side chains of Y181, Y188, and W229, in addition to general hydrophobic interactions with the side chains of L234 and L100. Most notably, the vinyl hydrogen on 17 is nearly buried in the π cloud of W229's indole moiety, considerably increasing the interactions to this highly conserved residue (Fig. **3a**). ADAMs have chain lengths of either two or three CH_2 units, and the longer three CH_2 unit side chain is optimal. The presence of additional torsional degree of freedom in alkenyl side chains is also likely to contribute to the excellent resistance profiles of some ADAMs derivatives.

The ending of side chain must contain a small functional group (e.g. ester group or heterocycles) capable of accepting hydrogen bonds. SAR studies indicated the ester functionalities are a crucial element of the ADAM pharmacophore and probably participate in one or more hydrogen bonds with the adjacent residues.

All in all, the combination of additional hydrophobic interactions, π-π stacking, and side-chain hydrogen bonding appears to be what governs the binding of the ADAMs to RT [55,56]. In addition, the development of ADAMs-based

NNRTIs with not-conventional binding mode and novel characteristics including conformational flexibility and positional adaptability (the alkenyl side chains), forming new interactions with highly conserved residues (F318, W229), would undoubtedly be an extremely useful approach to overcome drug resisitance [41b]. Undoubtedly, the crystallographic studies and pharmacophore model which clearly defines the structural features that are required for potent RT inhibitory in the ADAMs series afford valuable clues to the future research work.

3.3. Other NNRTIs Sharing Similar Structural Similarities with ADAMs

It is well-known that, NNRTIs, although forming a wide range of chemically diverse compounds, contain many ubiquitous fragments in their structures and possess common pharmacophore model. It has been illustrated in Fig. (**5**) that ADAMs compounds shares the 'diphenyl' as common pharmacophore element with many other NNRTIs families, such as tetrahydronaphthalene lignan (**26**) [57], arylsulfonylbenzonitrile (**27**) [58], indazole (**28**) [59], benzophenone (**29**) [60], diaryl ether (**30**) [61] and nitroimidazole (**31**) [62]. Most of their derivatives possess high levels of potency against wild-type and drug-resistant HIV-1 mutations, excellent oral bioavailability and overall pharmacokinetics.

The existence of ubiquitous diphenyl motif derived from databases of known NNRTIs are very similar with the concept of 'privileged structures' [63,64] in medicinal chemistry.

Based on the fragment-based ligand discovery strategy [65], the ubiquitous motifs in different NNRTIs, can be used as templates for the generation of "privileged fragments" libraries that are able to construct high-quality NNRTIs hits. Actually, the computational tools of understanding the structural similarity of NNRTIs has been employed in the "colonization" of the existing chemical space for each molecular scaffold and in association with *de novo* drug design and classical medicinal chemistry concepts (such as molecular hybridization [66] and scaffold hopping [67]) has assisted the rational design of new NNRTIs candidates against wild-type and drug-resistant variants of HIV-1 RT. The method is believed to be effortless, and fast enough to be effectively applied to the optimization of different kinds of drug-like candidates.

4. OTHER BIOACTIVITIES OF COSALANE ANALOGUES

The 'diphenyl' template certainly deserves the title of 'privileged scaffold' in anti-HIV drug research field, respecting the fact that it is a ubiquitous fragment in various NNRTIs and the potentiality of cosalane to yield derivatives (especially, ADAMs) with multiple and peculiar antiviral activities. Besides, substituted diarylmethylenepiperidines (DAMPs, **32**) are novel conformationally restricted analogues of the ADAM series with an unidentified antiviral target [68].

Compounds **33** and **34**, are not only active as inhibitors of PDE4B2 [69], but as potent inhibitors of tubulin assembly (IC_{50} = 3.7 ± 0.3, 2.8 ± 0.2 μM, respectively), and they also inhibit the binding of colchicine to tubulin. Furthermore, they displayed submicromolar cytotoxicities against 60 human cancer cell lines [70]. They could serve as anticancer drug candidates for further optimization. It was also reported that several other cosalane analogues also inhibited RANTES (a CCR5, CCR3, and CCR1 ligand)-induced migration of human monocytes [71].

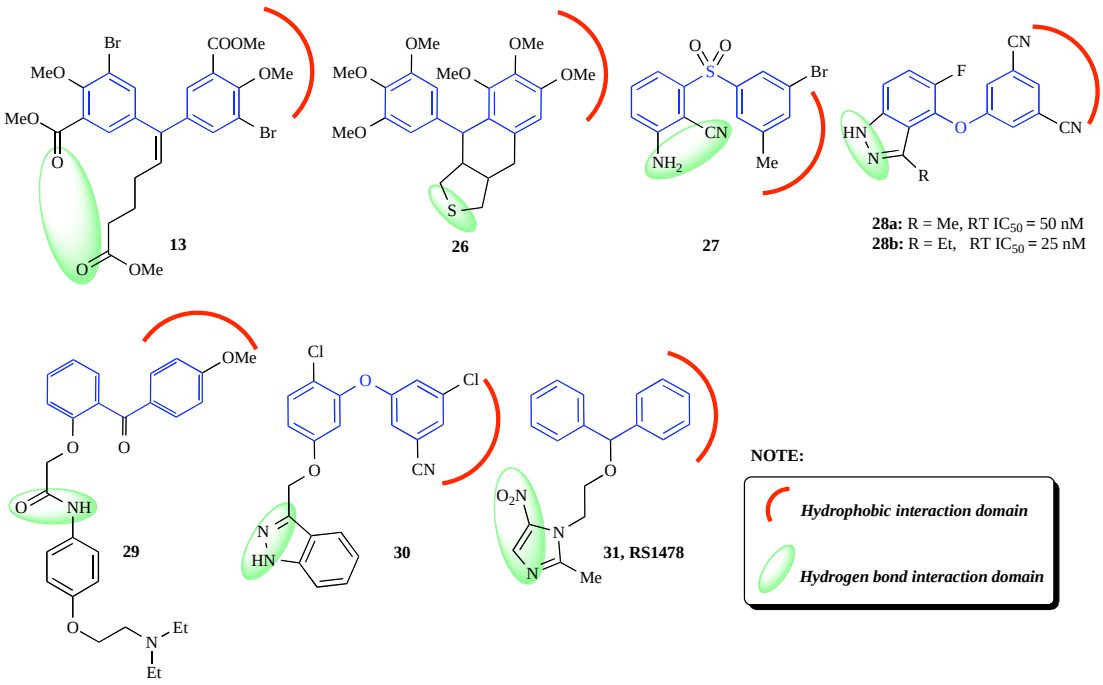
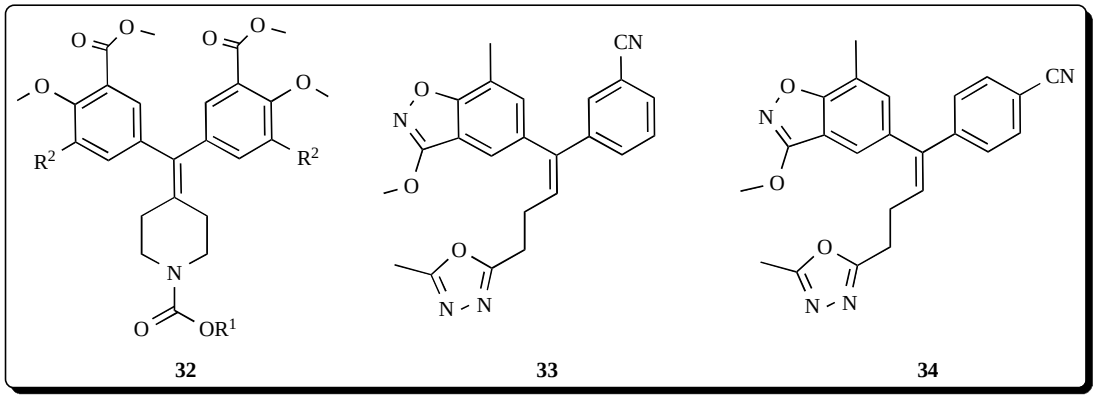


Fig. (5). Chemical structures of NNRTIs with diphenyl fragment. Structural similarities are remarkable between the ADAMs-based NNRTIs with other different chemical scaffolds.



32 **33** **34**

5. CONCLUSION

Though current available antiretroviral therapies have had a profoundly positive impact on the survival of HIV-1 infected patients, there is still an urgent need for the development of new and safer agents acting at newly emerging targets or with not-conventional mechanisms for the treatment of the patients carrying resistant viral strains. This review summarized recent progress in the discovery and development of cosalane analogues as potent viral entry inhibitors and unique NNRTIs, respectively. It was believed that current SAR conclusions and/or a number of novel characteristics of cosalane analogues, identified by the crystallographic structures studies (ADAM-based NNRTIs), will be beneficial to the discovery of next generation candidates with improved antiviral potency, metabolic stability and bioavailability.

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