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Coumarins as Potential Inhibitors of DNA Polymerases and Reverse Transcriptases. Searching New Antiretroviral and Antitumoral Drugs

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Abstract: Human Immunodeficiency Virus (HIV) is the viral agent of Acquired Immunodeficiency Syndrome (AIDS), and at present, there is no effective vaccine against HIV. Reverse Transcriptase (RT) is an essential enzyme for retroviral replication, such as HIV as well as for other RNA infectious viruses like Human T lymphocyte virus. Polymerases act in DNA metabolism, modulating different processes like mitosis, damage repair, transcription and replication. It has been widely documented that DNA Polymerases and Reverse Transcriptases serve as molecular targets for antiviral and antitumoral chemotherapy. Coumarins are oxygen heterocycles that are widely distributed throughout the plant kingdom. Natural coumarins have attraction due to their bioactive properties such as tumor promotion inhibitory effects, and anti-HIV activity. Coumarins and derivates exhibit potent inhibitory effects on HIV-1 replication in lymphocytes and compounds isolated from *Calophyllum inophyllum* or DCK derivates showed inhibitory activity against human RT. Furthermore, natural isocoumarins isolated from cultures of fungi or hydroxycoumarins were able to inhibit human DNA polymerase. In view of their importance as drugs and biologically active natural products, and their medicinally useful properties, extensive studies have been carried out on the synthesis of coumarin compounds in recent years. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), a class of antiretroviral chemotherapeutic agents, act by binding to an allosteric pocket showing, generally, low toxicity. This work tries to summarize the investigation about natural and synthetic coumarins with the ability to inhibit key enzymes that play a crucial role in DNA metabolism and their possible application as antiretroviral and antitumoral agents.

Keywords: Cancer, coumarins, DNA polymerase, inhibitors, *in silico*, HIV, reverse transcriptase.

INTRODUCTION

Natural products and derivatives are promising candidates for drug discovery and they still continue playing a crucial role in future drug development programs [1, 2].

Around the world, tremendous resources are being invested in prevention, diagnosis, and treatment of cancer and AIDS (acquired immunodeficiency syndrome). Cancer is the second leading cause of death in Europe and North America, accounting for 8.2 million deaths in 2012 around the world. Globally, an estimated 35.0 million people were living with HIV (Human Immunodeficiency Virus) in 2013 and 3.2 million of these were children [3]. Discovery and development of anticancer and antiviral agents are the key focus of several pharmaceutical companies as well as nonprofit government and non-government organizations.

At the end of the 1990s, a novel onco-pharmacological universe was discovered, with the use of targeted therapeutics made of receptor-specific small or large molecules. Immediately, the

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antitumor pharmacology community embraced the new concept and this vision of molecular target-based drug discovery (or reverse pharmacology) became a standard [4].

Today, the main objective of secondary metabolites research is aimed at finding bioactive molecules and interpretation of the mechanisms that are responsible for a given biological activity. This quest requires multidisciplinary teams that include pharmacologists, botanists, theoretical chemists, physicians, experimental chemists, biologists and molecular biologists. This work tries to summarize some of the recent achievements regarding coumarins as inhibitors against DNA Polymerases (DNA Pol) and Reverse Transcriptases (RT) and their possible application as antiretroviral and antitumoral agents.

NATURAL PRODUCTS AND DRUG DEVELOPMENT

The science of pharmacognosy grew in order to provide a scientific description of natural materials used in medicine. Herbs formed the bulk of these remedies. As chemical techniques improved, the active constituents were isolated from plants and were structurally characterized. In due course, many of these products were synthesized in the laboratory. Sometimes, more active, better tolerated drugs were produced by chemical modifications (semi-synthesis), or by total synthesis of analogues of the active principles [5].

At least 80% of the worlds' population in developing countries use plant materials as their source of primary health care [6]. A significant portion of the currently available pharmaceuticals in clinical use is comprised of drugs derived from higher plants [7].

The most interesting aspect in the chemistry of natural products is that it allows scientists to use the skeletons provided by nature, with its constant chirality and well-known structures as basic molds for the production of compounds with new functionalizations or hybrid systems. The concept of molecular simplification is directed towards the preparation of bioactive analogues. This is based on known structural features that are responsible for a specific bioactivity or the pharmacophore involved [8], but the generation of new defined stereogenic centers is an arduous task [9].

Drug resistance, both naturally acquired and intrinsic, has been shown to be a common cause of treatment failure. Resistance has often been demonstrated to occur by mutations or altered expression of the protein target. The understanding of the molecular basis of malignancy can provide us the success course for rational drug design. We now understand that in the case of cancer, the pathology is caused and driven by mutations in DNA or altered signal traduction pathways that normally operate to regulate life and death in healthy cells. Also several mutations in the genome of HIV virus and others retroviruses develop new kinds of resistant mutants [10, 11].

Identification of active cytotoxic compounds led to the development of anticancer therapeutics during several decades. The recent growth in molecular sciences and the advances in genomics, metabolomics and proteomics have generated several potential new drug targets, leading to changes in the paradigms of anticancer drug discovery toward molecularly targeted therapeutics. These shifting paradigms have not only resulted in the greater involvement of biological scientists in the drug discovery process but also have required changes in the screening and clinical evaluation of drug candidates [12, 13].

Likewise, the current collection of highly-effective drugs for fighting HIV infection is one of the major successes of modern drug design. Two types of drugs are used for blocking the action of RT and stopping HIV infection. One type is a modified nucleotide with a missing connector, such as the drug AZT (azidothymidine). These are used by the enzyme like normal nucleotides and added to the growing chain. But, since they are missing a site for connecting the next nucleotide, the synthesis of the DNA chain is stopped. The other type of drug binds on the back side of the enzyme and changes the shape of the active site, blocking its action [14, 15].

COUMARINS

Coumarin (1,2-benzopyrone; 2-*H*-1-benzopyran-2-one; *cis*-*o*-coumarinic acid lactone; coumarinic anhydride; *tonka* bean camphor) is a white crystalline solid (mol. wt 146.15, mp 68 °C; bp 297 °C).

The compound consists of an aromatic ring fused to a condensed lactone ring (for structure see Fig. **1**) [16].

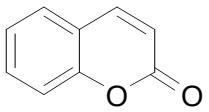

Fig. (1). Chemical structure of coumarin.

Coumarins have been found to be distributed extensively in various types of flora and in all parts of the plants. They have also been reported in microorganisms and animals. The simple hydroxylated and methoxylated coumarins in the free state or as glycosides occur widely in different plant families, but as the structural complexity of the compound increases, they seem to be restricted more and more to familial occurrence. The coumarin content of the plants may vary in different stages of their growth [17].

Coumarin derivatives have long been identified as powerful anticoagulant drugs [18], and many biological activities have been described for coumarin derivatives, including their ability to inhibit a variety of enzymes, e.g. monoamine oxidases [19], cytochrome P450 [20, 21], Human Immunodeficiency Virus type 1 (HIV-1) Reverse Transcriptase [22-25], and the ability of coumarins to inhibit the mutagenicity of cellular xenobiotics [26].

Furthermore, these moieties can also be considered as versatile building blocks and excellent intermediates for the synthesis of various transcendental compounds (e.g., benzo- and dibenzofurans, fluorescent triazole-coumarins, polycyclic coumarins, etc.) [27], and they can even be included inside materials, like polymers [28, 29]. Moreover, these widely distributed derivatives are reported to have various biological activities, such as: insecticidal; anthelminthinc; hypnotic; antifungal [30]; and antimicrobian [31], and are well-known to be HIV Protease (PR) and Reverse Transcriptase inhibitors [32].

DNA METABOLISM RELATED ENZYMES

Reverse Transcriptases

RT is a key enzyme, which plays an essential and multifunctional role in the replication of HIV-1 and is thus considered to be an attractive target for inhibition of HIV replication (Fig. **2**) [33]. This protein executes several different functions. As indicated by its name, it can build different DNA strands based on an RNA template. This reaction is carried out in the polymerase active site, which is formed by two sets of arms that surround the RNA and DNA together. This structure shares a right hand shaped domain with all known polymerases. RT performs a remarkable feat, reversing the normal flow of genetic information. The polymerases used to make DNA and RNA into cells, are very accurate and make very few mistakes [34]. This is essential because they are the wardens of our genetic information, and mistakes may be passed on to our offspring. On the other hand, RT is a promiscuous enzyme and makes a lot of mistakes, up to about one in every 2,000 bases. We might think that this would cause severe problems. But, in fact, this high error rate turns out to be an advantage for the virus under drug treatment [35]. The errors allow HIV to mutate rapidly; finding drug resistant strains in a matter of a few weeks after the treatment begins. Fortunately, the recent developments of treatments that combine several drugs (HAART) are often effective in combating this problem. Since the virus is simultaneously attacked by several different drugs, it cannot mutate to evade all of them at the same time [36-38].

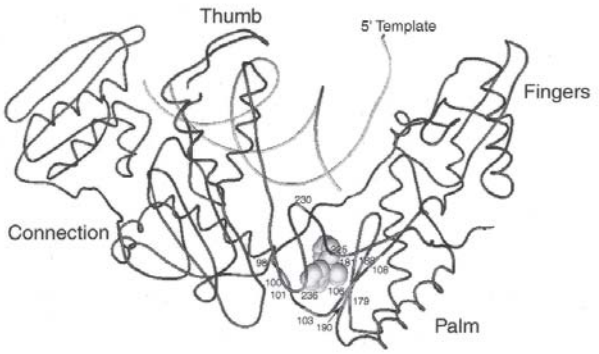

Fig. (2). Structural model of HIV-1 reverse transcriptase labeled with non-nucleoside RT inhibitor and principal residues in resistance mutations, taken with permission from "*The Genetic Basis of HIV-1 Resistance to Reverse Transcriptase and Protease Inhibitors. Shafer RW, Kantor R, Gonzales MJ. AIDS Rev 2000; 2: 211-28"*.

DNA Polymerases

DNA replication is the process of duplicating DNA to generate two copies of an organism's genetic information. This complex biological process is catalyzed by DNA Pol that add mononucleotides into a growing primer using

nucleic acid templates as a guide for directing each incorporation event. These proteins have an essential role in genome duplication, but they are also crucial for protecting the cell against the effects of DNA damage. DNA replication is absolutely essential for the proliferation and survival of all forms of life, ranging from simple viruses and bacteria to more complex organisms including humans [35].

Hyperproliferative diseases such as cancer, autoimmune conditions, and viral/bacterial infections are associated with uncontrollable DNA replication. Inhibiting this essential biological process provides an obvious therapeutic target against these diseases [39-41].

These protein assemblies contain highly coordinated moving parts, whose functions are in general temporally and spatially regulated by a series of ordered conformational changes that are powered by chemical energy derived from hydrolysis of nucleoside triphosphates. They fold into a conformation resembling a human right hand composed of three distinct domains designated as *palm*, *thumb*, and *fingers;* this structure is shared by all kinds of polymerases known, including reverse transcriptases [35]. The high degree of structural and sequence conservation of these domains between eukaryotic, prokaryotic, and viral polymerases suggests that these proteins derive from a common ancestor gene [42].

PCR (polymerase chain reaction) inhibitors generally exert their effects through direct interaction with DNA or interference with different DNA Pol. Direct binding of agents to single stranded or double-stranded DNA can prevent amplification and facilitate the isolation of inhibitor and DNA. Usually, inhibitors can interact directly with a DNA Pol to block enzyme activity. Moreover, but not commonly, DNA Pol have cofactor requirements that can be the target of inhibition [43].

These enzymes are being regarded as attractive targets for the development of specific inhibitors of DNA repair and DNA replication in cancer cells, because its degree of proliferation is usually faster than normal and healthy cells [44].

Within the cancer drug development arena, coumarin-type compounds have attracted an increasing amount of interest recently. Several studies have reported a number of coumarins, natural or synthetic, with marked cytotoxic activities [45], and DNA gyrase was mainly inhibited by quinolines and coumarins [46].

REVERSE TRANSCRIPTASE INHIBITORS

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), a group of structurally diverse compounds, have been reported to directly inhibit the enzyme in an allosteric fashion by binding to a pocket near the polymerase active site, causing distortion of the three-dimensional structure of the protein and inhibiting its catalytic function [47]. Until now, many classes of NNRTIs have been identified, and the most well-known inhibitors are: nevirapine, delavirdine, and efavirenz, (Fig. **3**) approved for the treatment of HIV-1 infection. However, NNRTI-containing regimens are compromised by rapid emergence of drug-resistant strains carrying the amino acid mutations surrounding the NNRTI binding pocket, making the continuous search for new drugs indispensable [48].

Different coumarins have been described as NNRTIs principally, and in this review they can be divided into three structural groups.

a) Tetracyclic Dipyranocoumarins

The naturally derived dipyranocoumarins, calanolides and inophyllums have been established as non-nucleoside-specific inhibitors of HIV Reverse Transcriptase. These are isolated from various species of *Callophyllum* belonging to *Clusiaceae* family, a genus primarily found in Malaysia [49].

They have the common tetracyclic dipyranocoumarin arrangement, displaying different groups, which are mainly substituted at the C-4 position. Additionally, these compounds can be named as inophyllums when a phenyl group is present at C-4 position [50].

The first natural product with potent activity against HIV-1 RT was dipyranocoumarin (+)-calanolide A (Fig. **4**). This compound was isolated from the tropical rainforest tree *Calophyllum lanigerum* var. *austrocoriaceum* (Clusiaceae) in 1992 by Kasmann *et al.* [51].

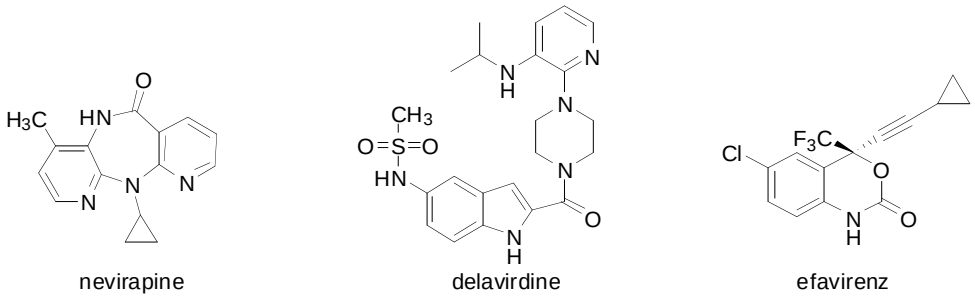

Fig. (3). The most well-known NNRTIs.

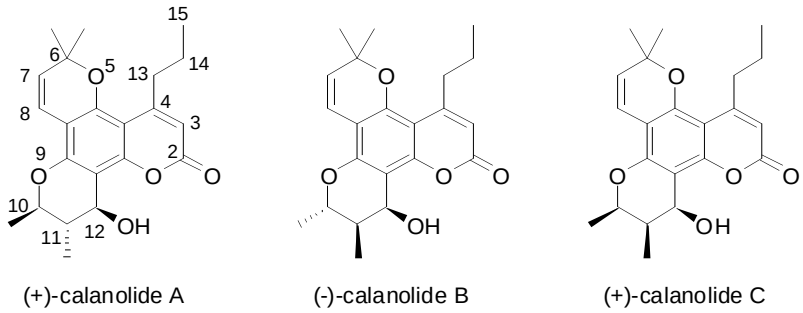

Fig. (4). Different kinds of calanolides showing its particular stereochemistry.

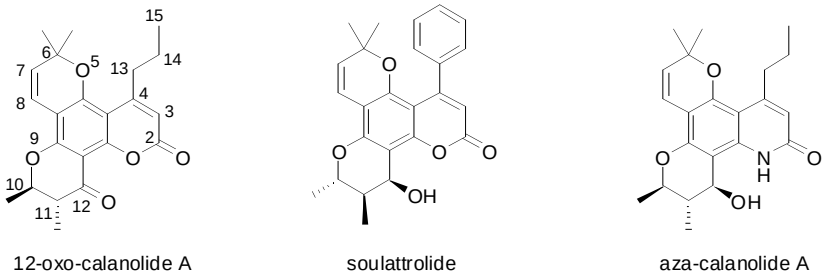

Fig. (5). Calanolide derivatives.

With this product as template, Ze-Qi Xu *et al.* [52] prepared an oxo-derivate using a combination of aldol/Mitsunobu reactions (Fig. **5**) [53, 54]. Not only does this 12-oxo-calanolide A have one less chiral center than calanolide A, but both enantiomers, namely (+)-2 and (-)-2, have also been found to be active against HIV-1 RT and SIV-RT (Simian Immunodeficiency Virus RT), showing IC_{50} values lower than 10 μM. Furthermore, using 12-oxocalanolide A as starting material, Xue *et al.* prepared 10-chloromethyl-11-demethyl-12-oxo-calanolide A [55, 56] which has shown an excellent EC_{50} value of 7.4 *n*M against wild-type HIV-1 and EC_{50} = 0.46 *n*M using the HIV-1 Y181C mutant strain. These strains that carry the viral Reverse Transcriptase Y181C amino acid mutation (mutations at codon 181) are associated with high-level resistance to most of the NNRTIs employed. Natural mutants or modified RT by site-directed mutagenesis are excellent models for the development of efficient novel NNRTIs. Looking at these results, it would be interesting to elucidate the mechanism of action for such structures as possible NNRTIs.

The highest inhibitory activity of HIV-1 RT has been observed for calanolides A and B that possess *trans* configuration between methyl groups at C-10 and C-11, while other configurations led to a reduction in potency, like calanolide C, as proposed by Huerta-Reyes *et al.* [57]. These authors have isolated soulattrolide (Fig. **5**) from *Calophyllum brasiliense* leaves and tested its bioactivity against HIV-1 RT. Soulattrolide also exhibits the pharmacophoric ring D, without the *β-cis* orientation of methyl groups on C-10 and C-11 like calanolide C. The chemical and biological properties of plants

belonging to the genus *Calophyllum*, and specially *Calophyllum brasiliense,* highlight the importance of natural sourcing, but structural modifications to obtain more active substances are still necessary [58, 59]. Madhava Sharma *et al.* have incorporated an interesting modification, replacing oxygen in ring B with a nitrogen atom, leading to aza-calanolide (Fig. **5**). This stable quinolinone ring system might provide greater stability and bio-availability in physiological environments like cytosol, increasing the anti-HIV activity compared to the natural product calanolide A [60].

b) Pyranocoumarins

Khellactone is also a naturally derived coumarin, which shows an interesting number of biological activities like anti-tumor promoting, anti-platelet aggregation, and the most well-known anti-HIV. A lot of naturally derived khellactone coumarins have been discovered to date, but suksdorfin and derivates have shown the best therapeutic index (Fig. **6**). Chemically, it is a dihydroseselin-type angular pyranocoumarin obtained from the methanolic extract of *Lomatium suksdorfii* fruit. It suppressed viral replication in separate acute HIV- 1 infections of H_9 lymphocyte cell lines with an average EC_{50} value of 2.6 μM. This coumarin also suppressed acute HIV-1 infections in fresh peripheral blood mononuclear cells, monocyte/macrophages and U-937 cells, a pro-monocyte cell line [61, 62].

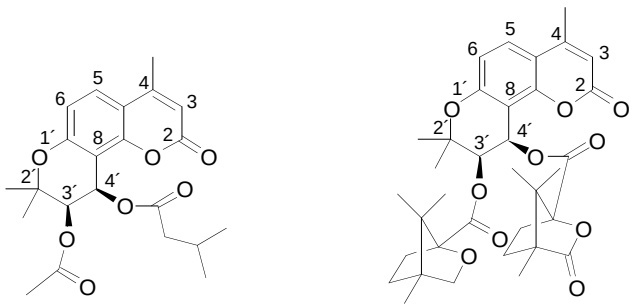

Fig. (6). Khellactone analogues.

Dicamphanoyl khellactone (DCK) is a coumarin analogue (Fig. **6**) that can accurately inhibit HIV-1 replication, but DCK does not inhibit RNA dependent DNA synthesis. However, a kind of HIV reverse transcriptase inhibitor-resistant strain, HIV-1/RTMDR1, is resistant to DCK. Thus, it is possible that HIV-1 RT may be the target of DCK. Li Huang *et al.* [63] prepared a chromone derivate called 3´R,4´R-di-*O*-(-)-camphanoyl-2-ethyl-2´,2´-dimethyldihydropyrano[2,3-f]chromone (DCP8), belonging to DCP family compounds, which was effective against HIV-1/RTMDR1, indicating that DCK can inhibit the DNA-dependent DNA polymerase activity of HIV-1 RT. More specifically, a preliminary mechanism of action-related studies indicated that a DCK analogue (4-methyl DCK,) inhibits the activity of HIV-RT through inhibition of DNA-dependent DNA polymerase activity, in contrast to currently available NNRTIs that block HIV-RT by stopping the RNA-dependent DNA polymerization [64].

An E138K mutation in the NNRTIs binding pocket of HIV-1 RT offers resistance to DCK and its chromone derivative, DCP8. This E138 change in the NNRTI binding pocket is located in the p51 subunit of the p51/p66 HIV-1 RT heterodimer. Li Huang *et al.* postulate that DCK could bind to the p51 subunit and interfere with viral DNA replication. Structure-activity relationship (SAR) study and pharmacophore analysis based on DCP (Fig. **7**) suggested that the planar ring system is an important pharmacophore to maintain anti-HIV activity against both wild-type and multi-drug resistant HIV strains [65]. Moreover, Ting Zhou *et al.* synthesized 3,3-dimethyldihydropyrano-[2,3-*c*]xanthen-7(1*H*)-one (DCX) derivatives (Fig. **7**) [64]. They suggested that the extended conjugated system of the pyranoxanthone skeleton facilitates the interaction of the small DCX molecule within the retroviral binding pocket, consequently leading to enhanced anti-HIV activity and selectivity, and converting DCX analogues in a more promising new class of anti-HIV agents. A critical view of these cases clearly shows an evolutionary pathway through the synthesis of novel anti-HIV drugs; taking a simple natural coumarin framework such as khellactone as a template, DCK, DCP and DCX analogues were prepared, in that order.

The last kind of kellactone analogues could be a ring-C opened DCK analogues, called seco-DCKs (Fig. **7**). These seco-compounds were screened against HIV-1_{NL4-3} and a RTMDR, showing excellent drug-like properties. The seco-DCKs have a simplified skeleton, fewer hydrogen-bond acceptors and lower log P values, resulting in increased water solubility and better pharmacokinetic properties, compared with traditional DCKs [66].

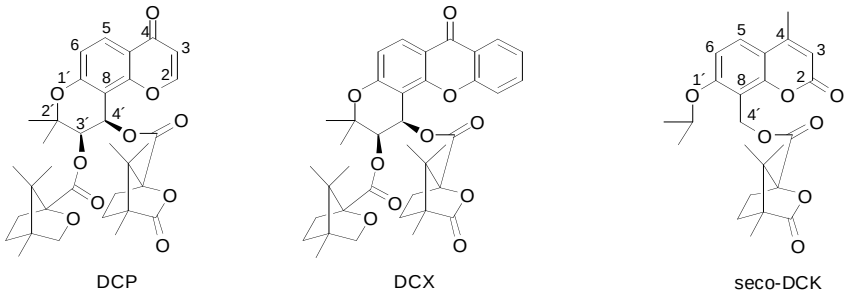

Fig. (7). DCK derivatives obtained by chemical transformations.

DCK is a unique HIV-1 RT inhibitor that inhibits the DNA-dependent DNA polymerase activity. The mechanistic and structural distinctiveness of DCK and DCP analogues opened a new way for scientists to discover more effective, more potent and novel anti-HIV drugs for AIDS therapy.

(c) Miscellaneous Coumarins

Mammea coumarins are characterized by a simple 5,7-dioxygenated coumarin skeleton bearing a phenyl, or an alkyl chain on C-4, acyl and prenyl (free or cyclized) substituents on either C-6 or C-8 positions (Fig. **8**). They are common constituents of the *Mammea* and *Mesua* species (Clusiaceae) [67]. Reyes-Chilpa *et al.* were the first authors to propose *mammea* type coumarins as possible anti-HIV agents. They used mixtures of two isomers which differ only by the kind of acyl substituent (3-methylbutyryl or 2-methylbutyryl) attached to C-8. None of the compounds tested was able to inhibit HIV-1 RT. In the case of *mammea* type coumarins, their inactivity could be explained by the lack of ring D (2,3-dimethylcroman-4-ol ring) attached to C-7 and C-8, and present in the calanolides, inophyllums, and cordatolides.

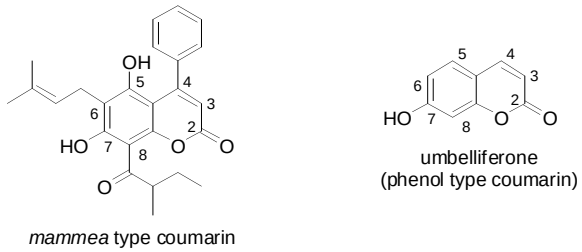

Fig. (8). *Mammea* type and phenol coumarins.

A single dose of coumarin derivatives like phenol coumarin umbelliferone and enol coumarin 4-hydroxycoumarin (Fig. **8**) or a benzyl coumarin such as warfarin over free virus exhibit a dose-dependent inhibitory effect on viral replication and reduction in RT activity. Unfortunately, no complete inhibition of viral production was observed, making these compounds possible substrates for further reactions in the search of new antiviral agents [68].

HIV-1 RT has some interaction with complementary oligodeoxynucleotide (ODN) primers at the 5´-end of the *t*RNA binding site as well as at the 3´-end of the primer. ODN derivatives could contain specific intercalating groups, such as coumarins, covalently linked to the 5´- end (Fig. **9**). The introduction of a coumarin derivative, the 1-(3-aminopropoxy)-2-ethyl-3*H*-naphto [2,1-b] pyran-3-one, and a chromone derivative, the 2-[3-(aminopropyl)amino]-8-isopropyl-5-methyl-4-oxo-4*H*-1-benzopyran-3carbaldehyde], into the 5´-end of a non-complementary ODN allowed these compounds to act as effective primers, enhancing the affinity with *Km* values that were three orders of magnitude lower [69].

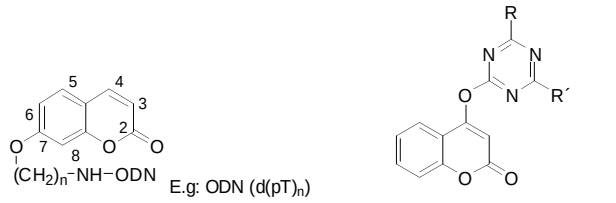

Fig. (9). ODN and triazinyl coumarin derivatives.

Planar structures of coumarins could increase π-stacking interactions between nucleotides and easily convert these complexes into better and strong inhibitors. Taking into account the idea of a more specific recognition, Mahajan *et al.* synthetized new 2-(coumarin-4-yloxy)-4,6-(substituted)-*s*-triazine products (Fig. **9**). This is because X-ray studies of complex NNRTIs/RT have shown that these

compounds maintain a similar conformational butterfly-like' shape and appear to function as π-electron donors to aromatic side-chain residues surrounding the binding pocket. They proposed a Het–NH–Ph–U motif as fundamental structure for binding, where Het is an aromatic heterocycle and U is an unsaturated, hydrophobic group, such as triazinyl derivatives, obtaining moderate results against HIV-RT mutant strains RES056: (K103N and Y181C) [47].

Fig. **10** shows a Docking study using an alkenylcoumarin against polymerase active site that we carried out. Looking for a dual recognition, Olomola *et al.* have obtained a series of 3-alkynylmethylcoumarins coupled with AZT (Fig. **11**). These hybrid products act as dual-action HIV-1 protease and non-nucleoside reverse transcriptase inhibitors. Docking different ligands into the non-nucleoside binding pocket of HIV-1 RT and HIV-1 PR, they found potential hydrogen-bonding interactions with amino acid residues and the place that coumarin moiety occupies. Surprisingly, it is the same cavity as the approved drug efavirenz [70, 71].

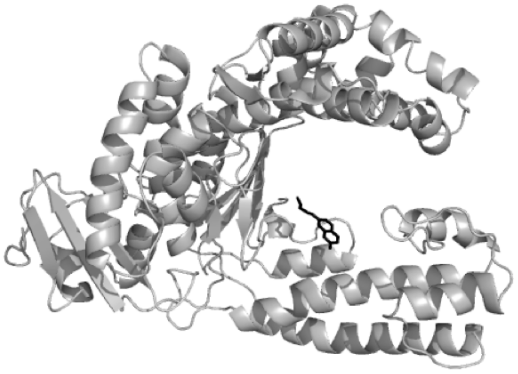

Fig. (10). Docked conformations of an alkenylcoumarin against polymerase active site.

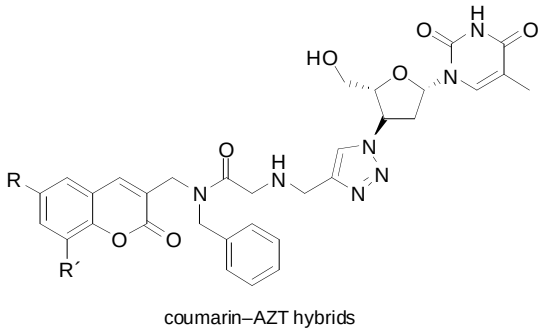

Fig. (11). Hybrid coumarins with dual-action inhibition.

The dihydroxycoumarin scaffold shows attracting properties as a possible pharmacophore in the recognition of RNase H domain, blocking the ribonuclease H function. In HIV RT the active sites of DNA polymerase and RNase H activities are located in distinct protein domains separated by over 50 Å [72].

Himmel *et al.* analyzed a group of potent dihydroxycoumarins able to inhibit RNase H activity from HIV-1 RT (Fig. **12**) [73]. Using Docking analyses, they found that the deprotonation of the coumarin ring *ortho* hydroxyl (8 position) was necessary to provide an optimal binding mode.

Taking this into account, we synthesized five related dihydroxycoumarins using ionic liquids (Fig. **12**). Their inhibitory activity against Myeloid Murine Leukemia Virus (MMLV) RT and *Taq* DNA polymerase was evaluated, showing that 4-methyl-7-hydroxycoumarin and 4-methyl-5,7-dihydroxycoumarin inhibited the retro-transcription event. Besides, two of them inhibited the replication process. These results clearly show the role of free hydroxycoumarins as a vital moiety in MMLV-RT recognition [74].

Finally we could envisage the coumarin bioactivity against MMLV-RT. Searching for a possible pharmacophore, we have assumed that the coumarin nucleus can be responsible for this inhibition (Fig. **12**) with a IC_{50} value of 38.62 μM. The presence of a Michael acceptor might be a potential target for nucleophiles present in Lys or Cys residues, like the amino or thionyl groups. Moreover, the hydrophobic and planar structure of coumarin could place it allosterically, for example at some hydrophobic pocket, changing the protein three-dimensional conformation. Likewise, a simple 3-allyl derivate was also active, indicating that in reverse transcription simple molecules like coumarin are able to inhibit this process [75].

DNA POLYMERASE INHIBITORS

Since DNA Pol has been identified as a significant target enzyme in antitumoral research, several compounds have shown activity against replication events. These include natural, synthetic and modified natural products. However, few coumarins have been reported as non-nucleosides polymerases inhibitors. Only in recent years have researches paid a little attention to these compounds

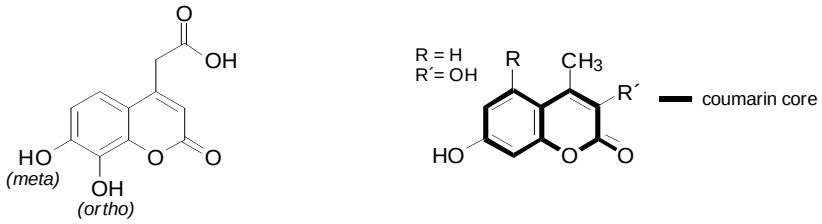

Fig. (12). Dihydroxycoumarins and coumarin core.

as active, and their usefulness for clarifying the biological mechanism and functions of DNA polymerases.

A few years ago, Kamisuki *et al.* have shown a novel class of isocoumarins (Fig. **13**) as specific inhibitors to the X-family human polymerases such as β, λ, μ [76]. Another achievement of this work was showing that these natural products had no effect on the activities of HIV-1 RT and other related enzymes, denoting its high level of specificity.

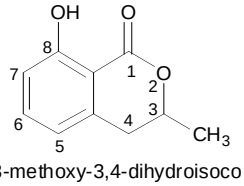

Fig. (13). Isocoumarins inhibitors against human polymerase.

Human Telomerase Reverse Transcriptase (hTERT) is a catalytic enzyme that is required for telomerase activity and cancer progression. Last year Xiao-Qin Wu *et al.* introduced the coumarin moiety in the dihydropyrazole skeleton compounds increasing the antitumor activity (Fig. **14**). They identified an unoccupied space above the plane of the coumarin ring lined with the region of high electron density in the amide backbone of the hTERT. Nonetheless, when the activity was measured against *Taq* DNA polymerase no effects were observed, determining that the principal target was telomerase [77, 78]. In view of these results, it would be interesting to assay these products against different RTs, because the recognition could be inside RT domains and not in polymerase conformation.

Some attempts in antitumoral search using hydroxycoumarins have been made. For example, Sung-Young Lee *et al.* used esculetin (6,7-dihydroxycoumarin) to suppress the proliferation of human colon cancer cells by directly targeting the transcriptional complex of β-catenin–T-cell factor; and Kaneko *et al.* have used esculetin and esculin (Fig. **15**) to prevent oxidative DNA damage in rat colon tumors [79, 80].

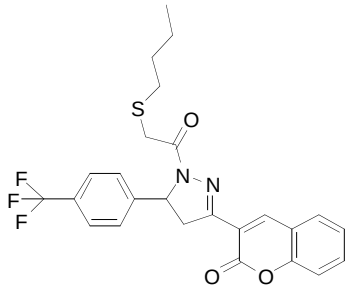

Fig. (14). Human telomerase reverse transcriptase inhibitors.

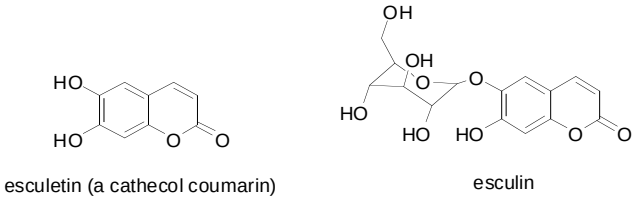

Fig. (15). Antitumoral hydroxycoumarins.

We have found two hydroxycoumarins that show DNA polymerase inhibition, called 3,4-dimethyl-7-hydroxycoumarin and 3-isopropyl-4-methyl-5,7-dihydroxycoumarin (Fig. **16**). These derivatives contain a hydrophilic part at the phenol side of the structure, but on the other face they show lipophilic moieties such as isopropyl and two methyl groups.

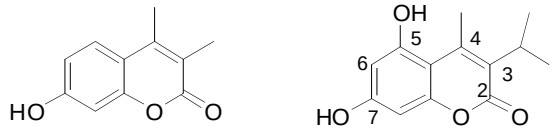

Fig. (16). Hydroxycoumarins as *Taq* DNA polymerase inhibitors.

Many kinds of compounds present allyl and isoprenyl moieties. These current natural structures act as a common pharmacophore in many types of bioactivities, and we proposed its vital importance against DNA related enzymes recognition. Likewise, Nichols *et al.* identified different coumarins and coumestans (Fig. **17**) as novel inhibitors of hepatitis C virus NS5B polymerase, and they predicted their binding in thumb pocket-1 of this protein. Furthermore, 6,8-diallyl-5,7-dihydroxycoumarin (one derivate obtained by Claisen rearrangement) was the most potent allyl-coumarin against polymerase chain reaction, indicating the importance of the allyl group (Fig. **17**) [81, 82]. We have also observed bioactivity using 3-allyl-coumarin in the inhibition of MMLV-RT [75].

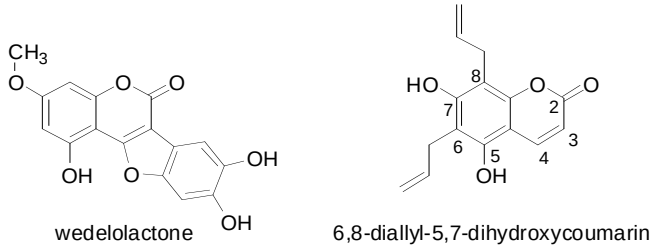

Fig. (17). Coumestans and allyl-coumarins.

Finally, we isolated 5-(3´,3´-dimethylallyloxy)-6,7-methylendioxycoumarin from *Pterocaulon* sp. and using its isoprenyl moiety as starting material we could obtain different related derivatives (Fig. **18**). Two of them were able to inhibit *Taq* DNA polymerase, sharing an hydroxyl group, denoting the importance of *γ,γ*–dimethylallyl scaffold for further modifications in the improvement of new inhibitor development [75].

IN SILICO SIMULATIONS

Molecular modeling using *in silico* simulations has become in recent decades an essential tool for the study of biomolecules like DNA, proteins or complexes enzyme/ligands.

Theoretical understanding of the interactions between proteins and their ligands is of great importance for the search of new pharmaceuticals. Generally, a structural model of the interaction site is used to place chemical moieties in favorable binding connections [83, 84].

In Docking studies, ligands are taken from a three-dimensional database, and are classified to predict their binding capacity. Here, each ligand is studied based on their complementarity with the active site, or any other potential binding site (e.g. a hydrophobic pocket near to active site) using force fields with optimal energy minimization [85, 86].

Molecular Dynamics simulations have multiple applications in important research areas. For example, it can be used to explore which conformations from a molecule or a complex are thermodynamically accessible [87]. Moreover, this technique is widely used to explore spatial conformations, e.g. in Docking/ligands studies. The free energy calculations using *in silico* simulations can be used as a powerful tool in automated processes for drugs design [88].

CONCLUSION

The search for new drugs and molecular structures of novel therapeutic products has increased considerably in recent years. The existence of diseases for which there are no effective drugs, the resistances of pathogens and the advances in molecular biology techniques have led to the identification of an increasing number of molecular targets. These factors are being attractive in search of new drugs capable of exerting more specific and potent actions. This search has been very successful, particularly in the discovery of

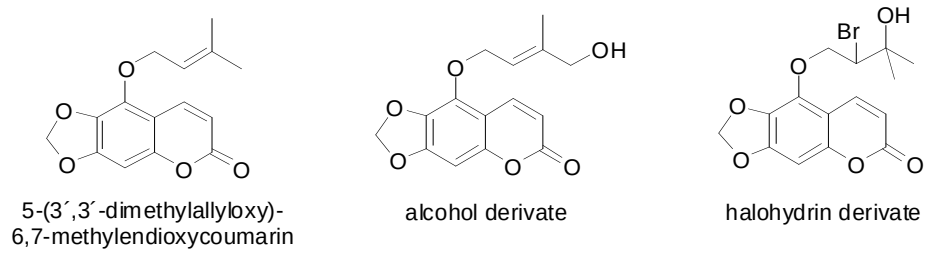

Fig. (18). Isoprenyl moiety derivatives as polymerase inhibitors.

applicable substances in modern medicine. Many drugs with major therapeutic effects come from nature or are synthesized imitating natural frameworks. Natural products have enormous structural diversity and a large number of chiral centers. Furthermore, many are relatively small and capable to cross cell membranes, and have similar properties like approved drugs. In the arena of new NNRTIs research, coumarin compounds remain an attractive option. Since the first calanolides derivatives showed activity, it has been shown that small chemical modifications in stereochemistry or the incorporation of heteroatoms become transcendental in the improvement of molecular recognition. We may even trace an imaginary time-line in the development of DCK analogues, in which regio-selective transformations and changes in the fusion of new heterocyclics seem to pave the way toward better bioactivity values. Moreover, the suksdorfin family shows a novel direction in the elucidation of enzyme mechanism, because they are capable of inhibiting the reverse transcription event but only at replicative level. Another remarkable aspect is the development of potential inhibitors that include small nucleotides sequences which could improve the molecular recognition site targeted. These inhibitors can be located towards different protein cavities where they carry out retro-transcription and replication processes. Because of this, it is necessary to gain thorough knowledge of protein three-dimensional structures, which often do not show good crystal resolution. The improvement of protein characterization technics, such as the use of solution methods like high-resolution NMR, is therefore vital.

An alternative approach also involves dual recognition by proteins, generating hybrid coumarins which include different pharmacophores. Finally, the search of new targets of action is essential; these could employ a greater number and variety of polymerases, reverse transcriptases and other enzymes with similar activities that share catalytic domains, such as the right hand shaped. In this continuous advancement in molecular recognition, carrying out *in silico* studies that allow for a more rational drug design is fundamental.

Considering that many of the currently available commercial drugs derive from natural products and that they have served as excellent "lead compounds", the search for new natural structures from medicinal plants and derivatives is still widely used as a method to find new molecules. Coumarins allow us the opportunity to evaluate completely new chemical kinds of therapeutic agents. and they also make it possible for us to find, new potentially relevant mechanisms of action. This review article sheds light on the fact that natural and synthetic coumarins play a central role in the discovery of lead compounds for the development of antitumoral and antiviral drugs.

ABBREVIATIONS

AIDS	=	Acquired immunodeficiency syndrome
AZT	=	Azidothymidine
DCK	=	Dicamphanoyl khellactone
DCP	=	Dicamphanoyl dihydropyrano chromone
DCX	=	Dicamphanoyl xanthone
DNA	=	Deoxyribonucleic acid
DNA Pol	=	DNA polymerase
HAART	=	Highly active antiretroviral therapy
HIV	=	Human immunodeficiency virus
hTERT	=	Human telomerase reverse transcriptase
MMLV	=	Myeloid murine leukemia virus
NNRTI	=	Non-nucleoside reverse transcriptase inhibitor
NMR	=	Nuclear magnetic resonance
ODN	=	Oligodeoxynucleotide
PCR	=	Polymerase chain reaction
PR	=	Protease
RNA	=	Ribonucleic acid
RT	=	Reverse transcriptase
RTMDR	=	Multiple reverse transcriptase inhibitor-resistant
SAR	=	Structure-activity relationship
SIV	=	Simian immunodeficiency virus
Taq	=	*Thermus aquaticus*
*t*RNA	=	Transcriptional RNA

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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