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Artigo

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Atividade Inibitória e Estudos de Docking da Catepsina V para Isoflavanoides de *Dalbergia Miscolobium* Benth

Resumo: Os extratos de plantas do gênero *Dalbergia* demonstram uma ampla gama de atividades biológicas, incluindo analgésica, antidiabética, anti-inflamatória e antimicrobiana. Neste trabalho, o estudo químico dos extratos das folhas e galhos de *Dalbergia miscolobium* levou ao isolamento e identificação de cinco isoflavonoides: prunetina, di-*O*-metildaidzeína, 8-*O*-metilretusina, duartina e sativan por meio de dados de ressonância magnética nuclear. A atividade de inibição enzimática desses Isoflavonoides foi avaliada na catepsina V na concentração de 100 µM. Duartin e sativan mostraram uma atividade notável contra a catepsina V, exibindo valores de inibição de 89% e 88%, respectivamente. Além disso, foram realizadas simulações de docking molecular para predizer o modo de ligação dos isoflavonoides a essa proteína e os resultados mostraram que a duartina está muito bem ligada à catepsina V e estabilizada por duas ligações de hidrogênio. Os isoflavanonoides duartina e sativan mostraram uma importante porcentagem de inibição da catepsina V, que pode ser considerada como alvo na investigação dos inibidores da catepsina V e estudos químicos adicionais de espécies de *Dalbergia* podem proporcionar novos isoflavonoides inibidores da catepsina V.

Palavras-chave: Produtos naturais; prunetina; di-*O*-metildaidzeína; 8-O-metilretusina; Duartina; sativan; atividade biológica.

Abstract

Plant extracts from *Dalbergia* genus have demonstrated a wide range of biological activities including, analgesic, antidiabetic, anti-inflammatory, and antimicrobial. In this work, the chemical study of the extracts from the leaves and branches of *Dalbergia miscolobium* led to the isolation and identification of five isoflavonoids: prunetin, di-O-methyldaidzein, 8-O-methylretusin, duartin and sativan employing nuclear magnetic resonance data. The inhibition activity of these isoflavonoids was screened against cathepsin V at a concentration of 100 µM. Duartin and sativan showed remarkable activity against cathepsin V displaying 89% and 88% inhibition values, respectively. Also, docking simulations to predict the binding mode of isoflavonoids into this protein were performed and results showed that the duartin is nicely bound to the cathepsin V and stabilized by two hydrogen bonds. The isoflavans duartin and sativan showed a significant inhibition percentage of cathepsin V, which can be considered as targets into cathepsin V inhibitors investigation and further chemical study of *Dalbergia* species may afford novels isoflavonoids cathepsin inhibitors.

Keywords: Natural products; prunetin; di-*O*-methyldaidzein; 8-O-methylretusin; duartin; sativan; biological activity.

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Inhibitory Activity and Docking Studies of Cathepsin V for Isoflavanoids from *Dalbergia miscolobium* Benth

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1. Introduction

The Fabaceae family includes approximately 20.000 species distributed in 630 genera.[1,2] The species of Fabaceae are characterized by trees, shrubs or herbs with large variability and a widespread occurrence around the world, from areas with a great deal of moisture to cold deserts.[2,3] It is the second most important family in nutritional and economic aspects, behind only the family of cereals.[4] The genus *Dalbergia* is distributed mainly in tropical and subtropical regions of Africa, America, and Asia. In those

continents, approximately 250 *Dalbergia* species have been cataloged.[5] In Brazil, the genus is found throughout different ecosystems showing 21 endemisms.[6]

Various plant extracts from *Dalbergia* have demonstrated a wide range of biological activities such as analgesic, antidiabetic, anti-inflammatory,[7] anti-anxiety, antidepressant,[8] antileprotic,[9] antimicrobial,[10,11] antioxidant,[11] antifungal,[12] among others.[7] Isoflavones are typical in Fabaceae and were evaluated by molecular docking as cathepsin-L like inhibitors.[13] Cathepsins are lysosomal cysteine peptidases implicated in many pathological conditions. These enzymes have the primary function of degrading proteins, non-selectively, inside of the lysosome and are found in various organs and tissues. Specifically, cathepsin-V (cat-V) is expressed in thymus, testis and corneal epithelium and its biological role has been associated with cancer progression, atherosclerosis, type 1 diabetes, auto-immune and neurological diseases.[14–17]

In this report, for the first time, *Dalbergia miscolobium* Benth. was explored to obtain isoflavones with inhibitory activity against cat-V. We present the isolation and characterization of five isoflavones. Cat-V inhibition tests were performed, and to explain a remarkable inhibitory activity, docking studies were performed to predict the binding mode in this protein.

2. Experimental

2.1. Apparatus, materials, and chemicals

NMR spectra were recorded on a Bruker DRX-400 or a Varian 400 MHz (^{1}H: 400 MHz; ^{13}C: 100 MHz) instrument using $CDCl_3$ (TMS as an internal reference) and CD_3OD as solvents (Sigma Aldrich). The column chromatography was performed using silica gel 60 (70-230 mesh), flash silica gel (230-400 mesh), and Sephadex LH-20. The high-performance liquid chromatography (HPLC) analysis were performed in an Agilent G1311C-1260 with a quaternary pump coupled to a UV-Vis detector with a diode array (DAD, model G1315D-1260) using a C-18 Phenomenex column (250 mm × 4.5 mm, 5 µm) on analytical mode and (250 mm × 9.4 mm, 5 µm) on semi-preparative mode. The GC-MS analyses were performed in a Shimadzu, model GCMS QP5000, equipped with a DB-5 capillary column (30 m × 0.25 mm). The high-resolution mass spectra were obtained in an FT-ICR-MS device from MS Bruker Daltonics, model solariX.

The deionized water was purified using a Millipore Milli-Q Plus system (Bedford, MA, USA). Hydrochloric acid (HCl) from Vetec® (Rio de Janeiro, RJ, Brazil), sodium hydroxide (NaOH) from Synth® (Diadema, SP, Brazil), acetonitrile, *n*-hexane, ethanol, ethyl acetate and methanol from JT Baker® (Mexico City, MX, Mexico). All commercially available chemicals and reagents were purchased from Aldrich Chemical Co. and Sigma and used without further purification.

2.2. Plant Material

Leaves and branches from *Dalbergia miscolobium* were collected in São Carlos city (*campus* of the Federal University of São Carlos - UFSCar, São Paulo, Brazil) in May 2011. The plant was identified by a botanist, Maria Inês Salgueiro Lima. A voucher specimen (8371) has been deposited in the herbarium of the Botanical Department – UFSCar.

2.3. Extraction and isolation

Dried leaves (192 g) and branches (684 g) of *D. miscolobium* were crushed and extracted with ethanol in an Ika Ultra Turrax for 5 min at 20000 rpm at room temperature (thrice with 200 g dried weight each). The liquid ethanol extracts were combined, filtered, concentrated under reduced pressure and resuspended in a mixture of ethanol:ultra-pure water (1:3, v/v) followed by fractionation through a liquid-liquid partition with *n*-hexane for leaves (HL) and branches (HB) followed by ethyl acetate only for branches (EB).

HL (5.0 g) was subjected to column chromatography (2.5 cm × 16 cm) with silica gel 60 in an elution gradient starting with *n*-hexane:ethyl acetate (4:1, v/v), followed by *n*-hexane:ethyl acetate (1:1, v/v), ethyl acetate (100%), and then methanol (100%), which allowed the collection of seven fractions after TLC analysis (named sequentially from HL1 to HL7). The HL2 fraction (227.5 mg) was subjected into a column using flash silica gel (2.0 cm × 18.0 cm) starting with *n*-hexane:ethyl acetate (9:1, v/v), followed

by ethyl acetate (100%) and methanol (100%), yielding nine subfractions after TLC analysis.

The complete phytochemical study also allowed the identification of the steroids, such as triterpenes α-amyrin, (**9**), β-amyrin (**10**), lupeol (**11**) and the diterpene phytol (**12**) were provided in a mixture (37.5 mg) from the subfraction HL2.3. From subfraction HL2.5, sitosterol (**6**), campesterol (**7**), and stigmasterol (**8**) were also obtained in a mixture (5.7 mg). The structures of these substances are presented in **Figure 1**. The HL4 fraction (43.1 mg) was subjected to a column chromatography using flash silica gel (1.5 cm × 16.0 cm), starting with *n*-hexane:ethyl acetate (3:1, v/v) followed by ethyl acetate (100%) and then methanol (100%). Eight subfractions were obtained after analysis by TLC. The subfraction HL4.4 (6.7 mg) was subjected to chromatographic separation in semi-preparative HPLC, leading to the isolation of isoflavonoid prunetin (**1**, 2.4 mg).

HB (0.4 g) was fractionated in a silica gel 60 column chromatography (2.5 cm × 18 cm) starting with *n*-hexane:ethyl acetate (4:1, v/v), followed by *n*-hexane:ethyl acetate (7:3, v/v), ethyl acetate (100%) and then methanol (100%). After analysis by TLC, eight fractions were combined and named sequentially from HB1 to HB8. The HB3 fraction (57.3 mg) was subjected to column chromatography using flash silica gel (2.0 cm × 20.0 cm), initially using *n*-hexane:ethyl acetate (9:1, v/v), followed by *n*-hexane:ethyl acetate (1:1, v/v), ethyl acetate (100%) and finally methanol (100%). Sitosterol (**6**), campesterol (**7**), and stigmasterol (**8**) were again obtained in a mixture (2.7 mg) from subfraction HB3.1. The fraction HB4 (75.1 mg) was subjected to column chromatography using flash silica gel (2.0 cm × 20.0 cm) starting with *n*-hexane:ethyl acetate (8.5:1.5, v/v), followed by *n*-hexane:ethyl acetate (1:1, v/v), ethyl acetate (100%) and finally methanol (100%). Eight subfractions were obtained after analysis by TLC. Subfraction HB4.4 (17.9) was purified by exclusion chromatography with a bed column (1.5 cm × 50.0 cm) using Sephadex LH-20 as the stationary phase and isocratic elution with methanol. After analysis by TLC, seven subfractions were combined. Subfraction HB4.4.4 (5.7 mg) was purified using semi-preparative HPLC affording di-*O*-methyldaidzein (**2**, 1.4 mg).

EB fraction (3.5 g) was subjected to column chromatography with Sephadex LH-20 as the stationary phase (3.5 cm × 48.0 cm) and isocratic elution with methanol (100%) was used. Eight fractions were obtained after TLC analysis named sequentially from EB1 to EB8. EB3 fraction (167.2 mg) was fractionated in a column with Sephadex LH-20 (2.0 cm × 125.0 cm) with an isocratic elution using methanol (100%). Ten subfractions were obtained after analysis by TLC (EB3.1 to EB3.10). Subfraction EB3.9 (4.2 mg) was purified through semi-preparative HPLC, leading to the isolation of 8-*O*-methylretusin (**3**, 1.4 mg). Fraction EB5 (619.0 mg) was thrice purified through exclusion chromatography with Sephadex LH-20 (2.0 cm × 125.0 cm) as the stationary phase and isocratic elution with methanol (100%), which yielded 6.2 mg of duartin (**4**) and 1.1 mg of sativan (**5**). The latter was obtained after purification by semi-preparative HPLC.

For all HPLC analysis, the elution system started with water:methanol (9:1, v/v) to 100% methanol at 40 min, returning to the initial proportion at 46 min, and maintaining it for 50 min. A flow rate of 4 mL min^{-1} was used.

2.4. NMR data

The structures of all isolated isoflavonoids were established using NMR data, which are presented below:

2.4.1. *Prunetin (5,4'-dihydroxy-7-methoxyisoflavone)* (1): ^{1}H NMR (400 MHz, CD_3OD): δ_H 8.12 (*s*, H-2), 7.38 (*d*, 8.8, H-6' and H-2'), 6.84 (*d*, *J* = 8.8, H-5' and H-3'), 6.55 (*d*, *J* = 2.4, H-8), 6.37 (*d*, *J* = 2.4, H-6), 3.89 (*s*, 7-OCH_3).

2.4.2. *Di-O-methyldaidzein (7,4'-dimethoxyisoflavone)* (2): ^{1}H NMR (400 MHz, CD_3OD): *d* 8.22 (*s*, H-2), 8.11 (*d*, *J* = 9.6, H-5), 7.48 (*d*, *J* = 8.8, H-6' and H-2'), 7.08 (*m*, H-6 and H-8 *overlapped*), 6.98 (*d*, *J* = 9.6, H-5' and H-3'), 3.95 (*s*, 7-OCH_3), 3.83 (*s*, 4'-OCH_3).

2.4.3. *8-O-Methylretusin (8,4'-dimethoxy-7-hydroxyisoflavone)* (3): ^{1}H NMR (400 MHz, CD_3OD): *d* 8.21 (*s*, H-2), 7.78 (*d*, *J* = 8.8, H-5), 7.48 (*d*, *J* = 8.8, H-6' and H-2'), 6.99 (*d*, *J* = 8.8, H-5' and H-3'), 6.94 (*d*, *J* = 8.8, H-6), 3.94 (*s*, 8-OCH_3), 3.83 (*s*, 4'-OCH_3).

2.4.4. *Duartin (7,5'-dihydroxy-8,4',6'-trimethoxyisoflavan)* (4): ^{1}H NMR (400 MHz, CD_3OD): *d* 6.70 (*d*, *J* = 8.6, H-3'), 6.65 (*d*, *J* = 8.6, H-5), 6.60 (*d*, *J* = 8.6, H-2'), 6.37 (*d*, *J* = 8.6, H-6), 4.29 (*ddd*, *J* = 10.2, 3.2 and 2.0, H-2 eq), 3.97 (*td*, *J* = 10.4 and 0.8, H-2 ax), 3.40-3.47 (*m*, H-3), 2.91 (*dd*, *J* = 15.6 and 11.0, H-4 ax), 2.80 (*dd*, *J* = 15.6 and 4.4, H-4 eq), 3.84 (*s*, 6'-OCH_3), 3.83 (*s*, 4'-OCH_3), 3.78 (*s*, 8-OCH_3).

2.4.5.*Sativan(7-hydroxy-2',4'-dimethoxyisoflavan)* (5): ^{1}H NMR (400 MHz, CD_3OD): *d* 7.04 (*d*, *J* = 8.4, H-6'), 6.87 (*d*, *J* = 8.4, H-5), 6.54 (*d*, *J* = 2.4, H-3'), 6.48 (*dd*, *J* = 8.6 and 2.4, H-5'), 6.31 (*dd*, *J* = 8.2, and 2.4, H-6), 6.22 (*d*, *J* = 2.4, H-8), 4.19 (*ddd*, *J* = 10.4, 3.2 and 2.0, H-2ax), 3.64 (*m*, H-2eq), 3.49-3.41 (*m*, H-3), 2.92 (*ddd*, *J* = 15.6, 10.8 and 0.8, H-4ax), 2.77 (*ddd*, *J* = 15.6, 5.2 and 1.2, H-4eq), 3.83 (*s*, 2'-OCH_3), 3.77 (*s*, 4'-OCH_3).

2.5. Inhibitory activity studies

Recombinant human cat-V was produced using the *Pichia pastoris* expression system as previously described.[18] The molar concentration of the enzyme was determined by active site titration with E-64 following the conditions previously described.[19] Stock solutions of the flavonoids were prepared at a concentration of 1 mM in dimethyl sulfoxide and the inhibitors were screened against the cat-V at the concentration of 100 μM. The *in vitro* enzyme inhibition experiments were carried out in triplicate (in 96-well black plates) as previously described.[20] The final volume of the reaction mixture was 200 mL, kept under stirring. Each well contained 191 mL of a 100 mM sodium acetate buffer pH 5.5 containing 5 mM EDTA and 5 mM dithioerythritol (DTE), 2 mL of 1 mM Z-Phe-Arg-MCA dissolved in dimethyl sulfoxide, 5 mL of sample, and 32 nM of cat-V. The enzyme was activated for 5 min with DTE at 27 °C, and then the reaction mixture was incubated for 5 min with the sample. The reactions were started by the addition of the fluorogenic substrate 4-methylcoumaryl-7-amide (MCA) and measurements were conduct using the Molecular Devices Spectra MAX GEMINI XS (excitation 355 nm, and emission 460 nm). Control assays were performed without inhibitor (negative control) and in the presence of the irreversible inhibitor for cysteine peptidase, E-64 (positive control). The percentage of inhibition was calculated according to the equation:% inhibition = $100 \times (1 - V_i / V_o)$, where V_i and V_o are initial velocities (enzyme activities) determinate in presence and in the absence of inhibitor, respectively.

2.6.Theoretical section

The binding mode of duartin (**4**) was predicted via molecular docking technique using software package Gold Suite 5.1 (Genetic Optimization for Ligand Docking 5.1) (CCDC Software Limited), which revealed that this isoflavan **4** showed similar complementarity as well as APC-3316 in the active site of cat- V (PDB code 1FH0).

GOLD was used by a GoldScore fitness function, which is a molecular mechanism like for the calculation of binding positions of the ligand. Docking simulations were performed inside a sphere of 7 Å radius centered at the ligand in chain A, and using the pattern parameters available, a population of 100 conformers, 100.000 operations, 95 mutations and 95 crossovers. The docked lowest-energy structure has a root mean square deviation (RMSD) of 1.462Å with respect to the corresponding crystal structure of the complex.

3. Results and Discussion

3.1. Compound identification

As a part of ongoing search for bioactive compounds from Brazilian flora, *Dalbergia miscolobium* Benth. was submitted to chromatographic processes, which led to the isolation of five isoflavonoids prunetin (**1**), di-*O*-methyldaidzein (**2**), 8-*O*-methylretusin (**3**), duartin (**4**) and sativan (**5**) (**Figure 1**).

The typical olefinic oxymethine hydrogens at C-2 of the isoflavones derivatives were observed at δ_H 8.13, 8.22 and 8.21 in the ^{1}H-NMR spectrum of compounds **1**, **2** and **3**, respectively (Figures S2). Compounds **4** and **5** showed typical resonances for oxymethylene and benzylic protons at C-2 and C-4, respectively, which confirmed **4** and **5** as isoflavans derivatives. After 1D and 2D NMR experiment, and comparison with those of reported in the literature,[21–25] these isoflavonoids were identified as prunetin (**1**), di-*O*-methyldaidzein (**2**), 8-*O*-methylretusin (**3**), duartin (**4**) and sativan (**5**). The stereochemistry of **4** and **5** was defined by comparison of their specific rotation with those of reported in the literature. Their ^{1}H-NMR data recovered in deuterated methanol are also reported herein.

Prunetin (**1**) is an important anti-inflammatory agent and is commonly found in species from the genus *Prunus*.[24,26,27] Di-*O*-methyldaidzein (**2**) is usually isolated from *Maackia* species.[22] The compound 8-*O*-methylretusin (**3**) has already been

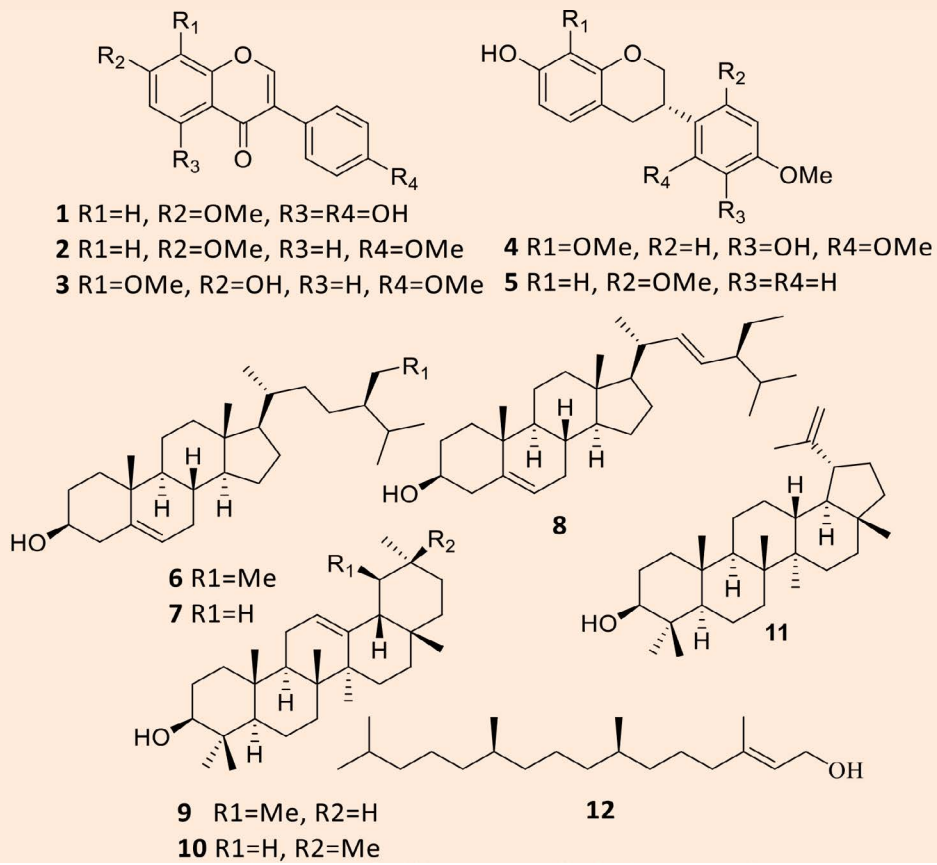

Figure 1. Compounds identified from *D. miscolobium*

described in other *Dalbergia* species and showed antibacterial activity.[28] The moderate antitumoral agent duartin (**4**)[29] has been described in *Machaerium* species[25] and sativan (**5**) is reported as an active compound against tuberculosis.[30,31] Furthermore, in the course of the phytochemical procedure, the steroids sitosterol (**6**), campesterol (**7**) and stigmasterol (**8**) along with the terpenes derivatives *α*-amyrin (**9**), *β*-amyrin (**10**), lupeol (**11**) and phytol (**12**) were also isolated and identified according to spectroscopic data.[32–34]

3.2. Inhibitory activity

Tests of the inhibitory activity against cat-V were performed for the isolated isoflavonoids. The compounds duartin (**4**) and sativan (**5**) showed remarkable results with inhibitions percentage of 89 and 88%, respectively at a concentration of 100 mM (**Table 1**). 8-*O*-methylretusin (**3**) also showed a critical inhibition ability (57%).

These results express the percentage of inhibition in only one concentration tested and not determining the real potency of inhibition of the compounds. To verify the existence of interactions between the compounds and the tested enzyme, molecular docking studies were carried out to suggest the interactions that justify the inhibition percentages.

3.3. Molecular docking studies of isoflavonoids

Two domains are presented in cat-V structure, with similar size, in which one is structurally related to the R-domain of papain consisting primarily of a twisted β-sheet and the second one is related to the papain L-domain and comprises three R-helices. The catalytic site of cat-V is in a wedge-shaped cleft at the interface between the R- and L-domains and the crucial amino acids in the catalytic machinery are Cys25 and His159. The His159 has a crucial role supporting the cysteine to keep its deprotonated state and protonating the leaving amine. Finally, it is also worth noting that the indole ring of Trp177 lies above His159 and Asn175 and seems to shield these residues from the solvent.[35,36]

As described by Somoza *et al.*,[35] by analyzing the crystallographic structure of complex between cat-V and the irreversible inhibitor APC-3316

Table 1. Percentage of cathepsin V inhibition for the isolated isoflavonoids from *D. miscolobus*

Compound	Cathepsin V inhibition (%)
prunetin (**1**)	38
di-*O*-methyldaidzein (**2**)	26
8-*O*-methylretusin (**3**)	57
duartin (**4**)	89
sativan (**5**)	88

bound in the active site, it was possible to identify the important molecular interactions, which must be considered in the investigation of duartin (**4**) binding mode prediction. Concerning the critical interactions observed in APC-3316/cat-V complex, three hydrogen bonds keep the APC-3316 structure tightly fitting. These interactions occur with the oxygen atom of the sulphonyl group in this inhibitor and Trp189, NH amide and Asp162, and carbonyl in amide moiety and Gly68, as illustrated in **Figure 2**.

Moreover, the fragments hPhe, Phe, and *N*-methylpiperazine in the inhibitor structure are in the S1, S2 and S3 pockets of the protease, respectively, while the sulfone phenyl group is in the S1' region. The amino acids residues Asn64, Gly23, Gly65 and Cys63 are present in the S1 region. The specificity of the papain family members of cysteine proteases is intrinsically related to S2 region and, this is a deep slot located in the R domain. This portion is occupied with Ala133, Gly160 and Met68, while the sides with Lys155, Asn156, Leu157, Phe67, Asp158 and Gly66 amino acids. Considering the sides and bottom of the S3 portion, these regions present the residues Phe67, Arg70, Gln61, Asn64 and Gly59, and Asn60, Gly65 and Gly66, respectively. Furthermore, *N*-methylpiperazine fragment in the inhibitor structure is in this region. Finally, the S1' pocket of cathepsin V, which is involved in the potency improvement but not in the selectivity of novel inhibitors, also comprehend amino acids Trp177, His159, Asp158 and Ala136 and receives the sulfone phenyl moiety of APC-3316 (**Figure 2**).

To better understand the potency of duartin (**4**), we proceeded to analyse their interactions with cat-V. The molecular docking was carried out by simulation of duartin (**4**) into the cat-V catalytic

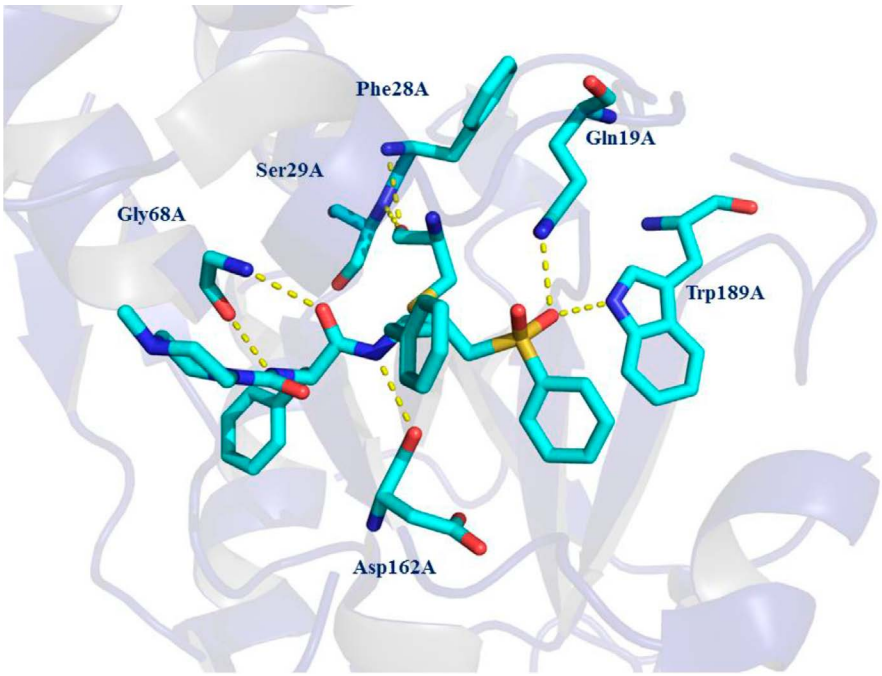

Figure 2. APC-3316/cathepsin V complex and key amino acids residues in stick. Light blue, carbon atoms; blue, nitrogen; red, oxygen; sulfur, yellow

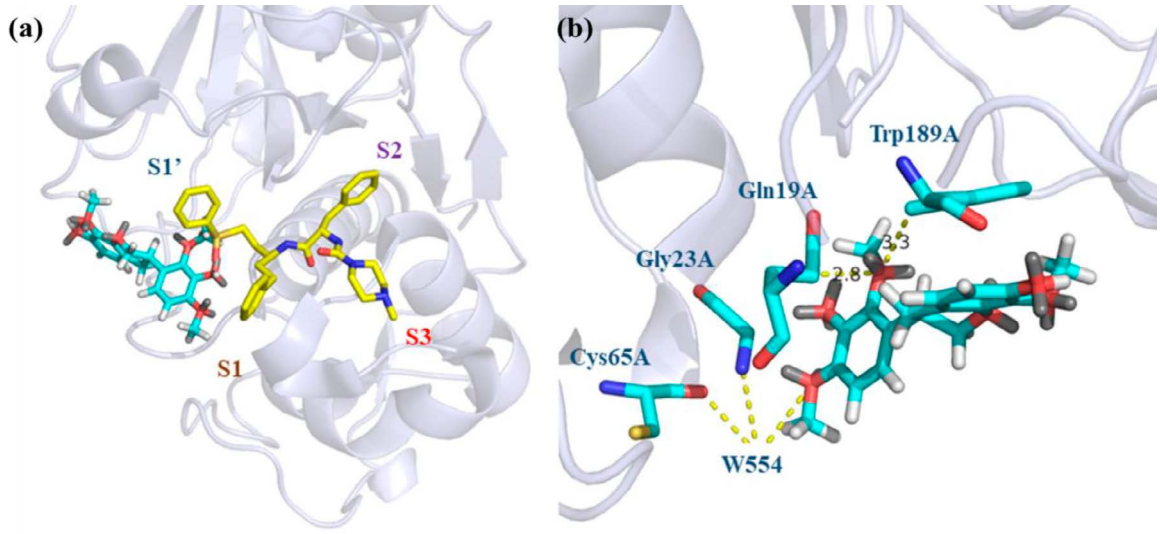

Figure 3. (a) Overview of 3D structure of cathepsin with the important regions for catalysis or inhibitor binding. The structure of duartin **(4)** (light blue, carbon) is closely on the structure of APC-3316 (yellow, carbon) showing a complementarity and a fill of S1 and S1' pocket; **(b)** Detailed view of duartin **(4)** and nearby residues in active site. Dashed lines, hydrogen bonds and distances. All interactions shown are under 4.5Å and therefore are capable of hydrogen bonding[37]

site. All docking runs were applied to the genetic algorithm of GOLD 5.1. The binding modes of duartin (**4**) in catalytic site of cat-V were depicted in **Figure 3**. All the amino acid residues which had interactions with duartin (**4**) were exhibited. In the binding mode, duartin (**4**) is nicely bound to the cat-V and stabilized by two remarkable hydrogen bonds. The oxygen atom of the methoxy group in duartin (**4**) formed a hydrogen bond with the $-NH_2$ moiety in Gln19 residue (2.8Å), as similarly observed to inhibitor APC-3316. Another notable hydrogen bond is performed by the oxygen atom of the methoxy group of duartin (**4**) and NH in indole ring present inside chain of Trp189 (3.3Å) amino acid. This interaction is equal to that carried out by APC-3316 and important for its stabilization in the cat-V active site. In this binding mode prediction, the water molecule W554 participates as a hydrogen bonding bridge between **4** and Gly23 amino acid residue which is present in the S1 region.

Furthermore, the molecular docking study results showed that the duartin (**4**) skeleton is closely on the structure of APC-3316 irreversible inhibitor showing a complementarity and a fill of S1 and S1' pocket. The docking results confirmed that the hydrogen bonding interactions may contribute to the potent biological activities. This study also contributes to understanding the interesting pharmacophoric features required for the further development of isoflavone derivatives as cat-V inhibitors.

4. Conclusions

Isoflavonoids are common in Fabaceae species. The phytochemical study of *D. miscolobus* led to the isolation of three isoflavones and two isoflavans, a part of the known terpenes and steroids derivatives. The isoflavone 8-*O*-methylretusin (**3**) showed an important inhibition percentage of cat-V (57%), even though the best results were observed for the isoflavans duartin (89%) and sativan (88%). In silico studies, the rational understanding of the potency of isoflavan duartin (**4**) allowed to identify interesting hydrogen bond interactions with amino acid residues in active site of this protein. In this attempt, 8-O-methylretusin (**3**), duartin (**4**) and sativan (**5**) can be considered as targets into cat-V inhibitors investigation and further chemical study of *Dalbergia* species may afford novels isoflavonoids cathepsin inhibitors.

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