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Structure–activity relationships of some 3-substituted-4-hydroxycoumarins as HIV-1 protease inhibitors

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Abstract

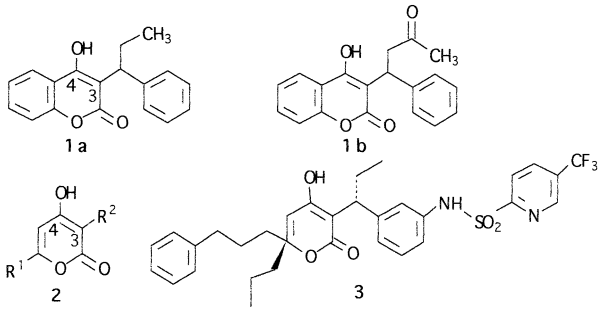
The screening of the HIV-1 protease (PR) inhibitory activity (IC–50) of various substituted 3-phenyl-4-hydroxycoumarins, 3-benzyl-4-hydroxycoumarins, 3-phenoxy-4-hydroxy-coumarins, 3-benzenesulfonyl-4-hydroxycoumarins and 3-(7-coumarinyloxy)-4-hydroxycoumarins was performed. The data indicate the importance of substituents at positions 5 and 7 of the coumarin ring on the inhibitory potency of the HIV-1-PR.

Keywords: 4-Hydroxycoumarins; Structure-HIV-1; Protease inhibition

1. Introduction

The availability of new chemotherapeutic agents for the treatment of the human acquired immune deficiency syndrome (AIDS) constitutes an important target due to the worldwide spreading of this infection. Since the isolation of the causative human immunodeficiency virus (HIV), favourable results were obtained with the use of inhibitors of the virally encoded enzymes reverse transcriptase (RT) and protease (PR), which are indispensable, respectively for the viral replication and maturation. Indeed, the clinical use of associations of HIV–RT or HIV–PR inhibitors (combination therapy) showed to present a good efficiency leading to the decrease of the viral load and to the increase of the number of CD4 lymphocytes. However, these drugs became less efficient and led to therapeutic failures [1] due particularly to the ability of the virus to generate resistant mutants [2–4].

The available peptidomimetic protease inhibitors [5] present low oral bioavailibility and their synthesis involves expensive multiple steps [6]. Therefore, the need for new nonpeptidomimetic drugs devoid from economic and pharmacokinetic drawbacks led to the identification of the 3-substituted-4-hydroxycoumarins Phenprocoumon **1a** [7], Warfarin **1b**, substituted 4-hydroxy-2-pyrone derivatives of type **2** as possible first generation HIV-PR inhibitors [8–14] and more recently, the sulfamide containing derivative **3** as a second generation inhibitor [15].



R^1, R^2 : aryl, arylthio, benzylthio, aralkyl, alkyl

Since the first crystallographic determination of the 3D structure of HIV-1 protease [16,17] showing that the active HIV-1-PR is formed by two assembled monomers in a C_2-symmetric axis, a lot of works concerning the structure of HIV-1 protease-inhibitor complexes were published. More particularly, the work of Thaisrivongs

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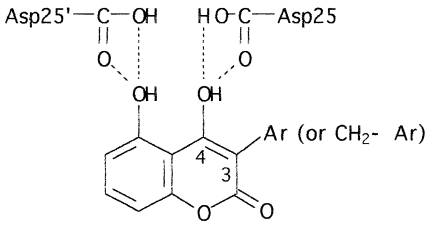
et al. [18], describing the structure of protease-hydroxycoumarin complex by X-ray diffraction method (2UPJ, Protein Data Bank), showed that the 3-substituted 4-hydroxycoumarins interact with various sites of the enzyme: the two key catalytic aspartic acid residues Asp25 and Asp25′ through hydrogen bonds with the 4-hydroxy group, the NH group of the two isoleucine Ile50 and Ile50′ with the lactone oxygens through additional hydrogen bonds and the hydrophobic S1, S1′, S2 pockets with the side chains [12,19,20].

Taking into consideration these structural features of the active HIV-1-PR and in order to contribute to a better understanding of the structure–activity relationships of the inhibitory activity of 4-hydroxycoumarinic derivatives, we decided the preparation of some sets of 3-substituted 4-hydroxycoumarins with the objective to investigate the influence of some modifications in the way of simplification:

- influence of a phenyl ring directly attached to the 3-position of 4-hydroxycoumarin or through a methylene group or an oxygen or a sulfonyl group, in order to examine if flexibility is necessary for this region;
- presence of various substituents on the aromatic ring of the 3-substituted 4-hydroxycoumarins (hydroxy, methoxy, benzyloxy);
- the influence of a sterically hindered (7-coumarinyloxy) substituent at 3-position of 4-hydroxycoumarin;
- finally, as the 4-hydroxy group of the coumarin forms a hydrogen bond with the key catalytic aspartic acid residues (Asp 25 and Asp 25′) of the HIV-PR, we studied the 4,5-dihydroxylated coumarins **4b** and **5b** considering that the presence of a second hydroxy group could lead to increase the strength of hydrogen bond and hence increase the inhibitory activity.

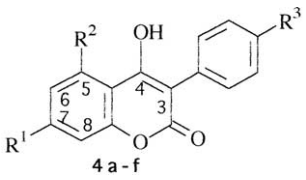


4b, 5b

Therefore, the preparation of substituted 3-phenyl-4-hydroxycoumarins **4a**–**f**, 3-benzyl-4-hydroxycoumarins **5a**–**d**, 3-phenoxy-4-hydroxycoumarins **5e**–**f**, 3-arylsulfonyl-4-hydroxycoumarins **5g**–**h**, and 3-(7-coumarinyloxy)-4-hydroxycoumarins **6a**–**c** was performed and the determination of their IC50 HIV-1 antiprotease activity determined.

The structures of the studied derivatives **4a**–**f**, **5a**–**f** and **6a**–**c** and the data related to their inhibitory activities (IC50) are indicated in Tables 1–3.

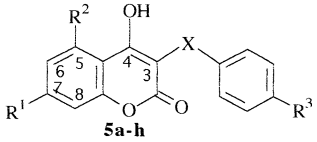
Table 1
IC50 OF 3-phenyl-4-hydroxycoumarins



4 a-f

	R^1	R^2	R^3	IC_{50} (μM)
4a	OH	H	H	5.0
4b	H	OH	H	31
4c	OCH_3	H	H	11
4d	OCH_3	H	OCH_3	3.5
4e	OH	H	OH	2.0
4f	$O–CH_2–C_6H_5$	H	H	7.0

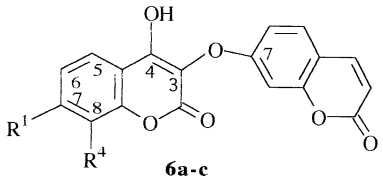
Table 2
IC50 of 3-benzyl-, 3-phenoxy- and 3-arylsulfonyl-4-hydroxycoumarins



5a-h

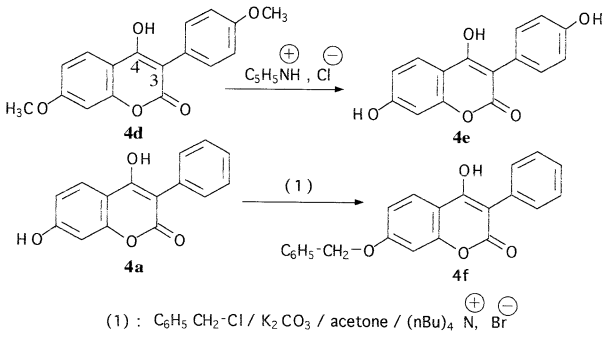
	R^1	R^2	R^3	X	IC_{50} (μM)
5a	OH	H	H	CH_2	9.5
5b	H	OH	H	CH_2	NI
5c	OCH_3	H	H	CH_2	2.4
5d	OCH_3	H	OCH_3	CH_2	3.0
5e	OCH_3	H	H	O	61
5f	OCH_3	H	Cl	O	8.0
5g	OCH_3	H	CH_3	SO_2	38% at 50 μM
5h	OCH_3	H	Cl	SO_2	28

Table 3
IC50 of 3-(7-coumarinyloxy)-4-hydroxycoumarins



6a-c

	R^1	R^4	IC_{50} (μM)
6a	OCH_3	H	4.0
6b	OH	CH_3	9.0
6c	$O–CH_2–C_6H_5$	CH_3	7.5



Scheme 1.

2. Synthesis

Most of the 3-substituted-4-hydroxycoumarins **4a**–**f**, **5a**–**f** and **6a**–**c** were prepared by described procedures: 4,7-dihydroxy-3-phenylcoumarin (**4a**), 4-hydroxy-7-methoxy-3-phenylcoumarin (**4c**) [21,22], 4,5-dihydroxy-3-phenylcoumarin (**4b**) [23], 4-hydroxy-7-methoxy-3(4-methoxyphenyl)coumarin (**4d**) [24].

The 4,7-dihydroxy-3-(4-hydroxyphenyl)coumarin **4c** was obtained from the corresponding dimethoxy derivative **4d** by demethylation using pyridine hydrochloride. The 4-hydroxy-3-phenyl-7-benzyloxycoumarin (**4f**) was prepared by benzylation of the corresponding hydroxylated compound **4a** using benzyl chloride as reagent in acetone as solvent, potassium carbonate as base and tetrabutylammonium bromide as phase transfer catalyst (Scheme 1).

The 3-benzyl-4,7-dihydroxycoumarin (**5a**), 3-benzyl-4-hydroxy-7-methoxycoumarin (**5c**) [25], 3-benzyl-4,5-dihydroxycoumarin (**5b**) [26], 3-(4-methoxybenzyl)-4-hydroxy-7-methoxycoumarin (**5d**) [27], 3-phenoxy-4-hydroxy-7-methoxycoumarin (**5e**) [28,29] are known and their synthesis achieved by the described procedures. The synthesis of 3-(4-chlorophenoxy-4-hydroxy-7-methoxycoumarin (**5f**) is obtained by thermal condensation of diethyl-(4-chlorophenoxy-malonate with *O*-methylresorcine [29] (Scheme 2).

The synthesis of 3-arylsulfonyl-4-hydroxycoumarins **5g**–**h**, will be reported in a coming publication [30].

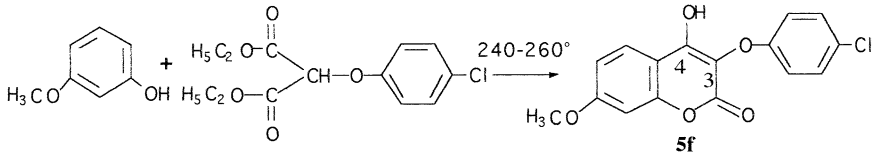
Compound **6a** is known and its preparation performed by a described procedure [31]. The derivative **6b** is obtained by the same route than **6a** by thermal condensation of diethyl 7-coumarinyloxy malonate with 2-methylresorcine. The synthesis of **6c** was achieved by benzylation of the corresponding hydroxy-derivative **6b** using the previous alkylation route (Scheme 3).

The chemical shifts of the ^{13}C NMR spectra of these derivatives are in agreement with those already reported for 3-substituted-4-hydroxycoumarins [32,33].

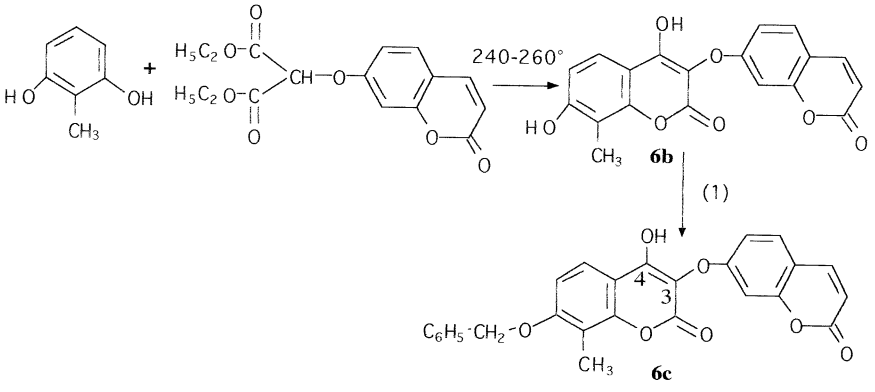
3. Experimental

3.1. Chemistry

The purity of all the compounds was routinely checked on the 'Riedel-de Haën 60 F_{254} special' silica gel plates (0.2 mm) and spots were located by UV lamp and or by iodine vapors. M.p.s were taken on a Kofler bench and are uncorrected. Analyses (C,H) are within ± 0.5% of the theoretical values.



Scheme 2.



Scheme 3.

The infrared spectra (ν in cm^{-1}) of the 3-substituted 4-hydroxycoumarins are recorded on a Bruker 'Vector22' spectrophotometer. They present characteristic bands of the hydroxyl and the conjugated carbonyl group of the lactone.

The ^1H NMR spectra were recorded on a Bruker AC300 in $CDCl_3$ or DMSO-d_6 using tetramethylsilane (TMS) as internal reference. Chemical shifts δ are in ppm. Splitting patterns are described as follows: (s) singlet; (d) doublet; (t) triplet; (q) quadruplet; (m) multiplet.

The following derivatives 3-(4-methoxyphenyl)-4-hydroxy-7-methoxycoumarin (**4d**) [24], 3-(phenoxy)-4-hydroxy-7-methoxycountarin (**5f**) [29], 3-(7-coumarinyloxy)-4-hydroxy-7-methoxycoumarin (**6a**) [31], diethyl *p*-chlorophenoxymalonate [34] and diethyl (7-coumarinyloxy)malonate [35] are prepared according to the described procedures.

3.1.1. Synthesis of the new 3-substituted 4-hydroxycoumarins 3-(4-hydroxyphenyl)-4,7-dihydroxycoumarin (**4e**) ($C_{15}H_{10}O_5$)

This compound was obtained from 3-(4-methoxyphenyl)-4-hydroxy-7-methoxycoumarin (**4d**) by demethylation.

In a round bottom flask, fitted with a stirring magnetic bar, a mixture of 2 g of 3-(4-methoxyphenyl)-7-methoxy-4-hydroxycoumarin (**4d**) and l6 g pyridine hydrochloride were heated at 180 °C in an oil bath during 6 h. After cooling, water (50 ml) was added and the reaction mixture extracted with EtOAc (3 × 50 ml). The resulting organic solution was washed with water (2 × 50 ml), dried over sodium sulfate and evaporated. The remaining solid was recrystallized.

Yield: 92%; m.p.: 327 °C in EtOH–water (9/1). IR: 3311, 3069, 1670. ^1H NMR ($CDCl_3$): 6.6–7.6 (m, 6H, arom) 7.9 (d, 1H, H-5); 9.4 (s, 1H, OH), 10.4 (s, 1H, OH).

3.1.2. 3-phenyl-4-hydroxy-7-benzyloxycoumarin (**4f**) ($C_{22}H_{16}O_4$)

The preparation of this compound was achieved by refluxing under anhydrous conditions 4,7-dihydroxy-3-phenylycoumarin (**4a**) (254 mg, 1.0 mmol), benzylchloride (1.52 g, 1.2 mmol) in dry C_3H_6O as solvent (100 ml), K_2CO_3 as base (1.4 g, 10.0 mmol) and a pinch of tetrabutylammonium bromide as phase transfer catalyst, during 4 h (end of the reaction monitored by TLC.

Yield: 95%, m.p: 227 °C in EtOH–water (9/1). IR: 3063, 1671, 1610. ^1H NMR ($CDCl_3$): 5.2 (s, 2H, CH_2), 7.1 (d, 2H, H-6, H-8), 7.3–7.5 (m, 10H, arom.) 7.9 (d, 1H, H-5).

3.1.3. 3-(4-chlorophenoxy)-4-hydroxy-7-methoxycoumarin (**5g**) ($C_{16}H_{11}O_5Cl$)

Prepared by thermal condensation of diethyl (4-chlorophenoxy)malonate [34] with *O*-methylresorcine according to a reported procedure [29]. M.p.: 257 °C (EtOH). IR: 3025, 1680, 1605, 1486 (C=C aromatic). ^1H NMR ($CDCl_3$): 3.9 (s, 3H, OCH_3), 6.5–7.5 (m, 6H, arom.), 7.8 (d, 1H, H-5).

3.1.4. 3-(7-coumarinyloxy)-4,7-dihydroxy-8-methylcoumarin (**6b**) ($C_{19}H_{12}O_7$)

This compound is prepared by thermal condensation of diethyl (7-coumarinyloxy)malonate (3.04 g, 10 mmol) and 2-methylresorcine (1.24 g, 10 mmol) at 250 °C, during 4 h. After cooling, the product was washed twice with a small portion (5 ml) of Et_2O to remove impurities and the derivative **6a** recrystallized in an EtOH–THF water mixture (8/1/1). Yield: 80%; m.p.: 231 °C; IR: 3256, 1726, 1672, 1593. ^1H NMR (DMSO-d_6): 2.15 (s, 3H, CH_3), 6.3–8.1 (m, 7H, arom.), 10.4 (s, 1H, OH, exchangeable with D_2O; the other OH group is mixed in the aromatic protons and is also exchangeable with D_2O).

3.1.5. 3-(7-coumarinyloxy)-4-hydroxy-7-benzyloxy-8-methyl-coumarin (**6c**) ($C_{26}H_{18}O_7$)

The preparation of compound **6c** was performed with the same procedure than compound **4f**, by refluxing under anhydrous conditions compound **6b** (1.0 mmol), benzylchloride (2.2 mmol), K_2CO_3 as base (10 mmol) and a pinch of tetrabutylammonium bromide as phase transfer catalyst in dry C_3H_6O as solvent (100 ml) during 4 h (end of the reaction monitored by TLC). Yield: 95%; m.p.: 228–229 °C in EtOH–THF–water mixture (8/1/1). IR: 3297, 3089, 1712, 1607. ^1H NMR (DMSO-d_6): 2.15 (s, 3H, CH_3), 5.2 (s, 2H CH_2). 6.3–8.1 (m, 7H, arom.).

3.2. Biological assay

The HIV-1-protease was kindly supplied by Rhône-Poulenc Rorer. The fluorogenic substrate DABCYL-S-Q-N-Y-P-l-V-Q-EDANS was purchased from Bachem (France). The fluorescence measurements were performed using a Perkin–Elmer fluorometer. Enzymatic assays were performed in 150 mM AcONa, 1 M NaCl, pH 5.5, 3% DMSO. Inhibitors and the substrate were dissolved in DMSO before addition to the buffer.

For the determination of the IC50 values, 0.52 µl of a 3 mM solution of substrate (final concentration 5.2 µM) was added to 8.5 µl of 4–10 different concentrations of inhibitors (final volume 300 µl). The enzymatic reaction was initiated by the addition of enzyme (final concentration 7.5 nM). The increase in fluorescence at 490 nm (λ exc = 340) was monitored over a period of 8 min at 30 °C.

4. Results and discussion

The results of IC50 of protease inhibition for the studied substituted 4-hydroxycoumarins are presented in Table 1 for the 3-phenyl-4-hydroxycoumarins derivatives **4a**–**f**, Table 2 for the 3-benzyl-4-hydroxycoumarins, 3-phenoxy-4-hydroxycoumarins and 3-arylsulfonyl-4-hydroxycoumarins **5a**–**h** and Table 3 for the 3-(7-coumarinyloxy)4-hydroxycoumarins **6a**–**c**. Examination of the data obtained with the 3-phenyl-4-hydroxycoumarins **4a**–**f** (Table 1) indicates that the most active derivatives are the disubstituted compounds **4e** and **4d** (R^1, $R^3 = OH$, OCH_3).

The activities increase according to **4b** < **4c** < **4f** < **4a** < **4d** < **4e** and the least active compound **4b** presents a hydroxyl group at position 5. This result could indicate that this position is particularly sensitive and a new hydroxyl group near to the hydroxyl group in position 4 decreases the strength of the hydrogen bond between this group and Asp25/Asp25′ of HIV-1 protease. The other positions R^1 at 7 and R^3 are not very sensitive to modifications, although hydroxyl group as R^3 appeared the best substituent for activity.

The results obtained with the 3-benzyl-4-hydroxycoumarins **5a**–**d**, 3-phenoxy-4-hydroxycoumarins **5e**–**f** and 3-arylsulfonyl-4-hydroxycoumarins **5g**–**h** are indicated in Table 2.

Examination of the data shows that the most active derivatives of this group of compounds are **5e** and **5d** where R^1 at position 7 is a methoxy group and $X = CH_2$). Comparison of compounds **5c**, **5e** and **5g** shows clearly that the replacement of $X = CH_2$ by O or SO_2 is not favourable to activ-ity. However, introduction of Cl in *para* position of the 3-phenoxy group allows to restore activity at the level of **5e** where $X = CH_2$.

The comparison of compound **4a** (3-phenyl) and **5a** (3-benzyl) shows that the flexibility of this latter substituent at 3-position does not lead to a significative change in activity.

It is noteworthy that the 4,5-dihydroxy-3-benzylcoumarin **5b** has no activity, confirming the previously observed decrease in activity with its surrogate 4,5-dihydroxy-3-phenylcoumarin **4b**. These results confirm that the presence of a hydroxyl substituent at position 5 is unfavourable for the inhibition of the HIV-PR.

The results reported in Table 3 are related to the IC-50 of the 3-(7-coumarinyloxy-4-hydroxycoumarins **6a**–**c**. The data show that the presence of a second coumarin ring at position 3 allows to obtain an as good activity than that of the compound **5f**.

5. Conclusion

From this study, it could be concluded that position 5 of our molecules is a very sensitive position not allowing the introduction of an hydroxyl group. Position 3 is also a sensitive one, since direct fixation of an aromatic group or through a methylene is preferable to fixation through an oxygen or a sulfonyl group. Finally, a substitution by a Cl of this aromatic group in *para* position seems favourable to activity.

More work is now in progress in order to improve our knowledge of the structure–anti-HIV-PR activity relationships of various 3-substituted-4-hydroxycoumarins.

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