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New 2-bromomethyl-8-substituted-benzo[*c*]chromen-6-ones. Synthesis and biological properties

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Abstract—New 2-bromomethyl-8-substituted-benzo[*c*]chromen-6-ones have been synthesized and their bioactive properties have been evaluated on different enzymatic models: serine proteases (trypsin and α-chymotrypsin), HIV aspartyl protease, nitric oxide synthase and a panel of protein kinases. These new derivatives can provide upon chemical or enzymatic attack, very reactive quinonimine methide intermediates, which could be utilized for the design of enzyme inhibitors. We found that some of these new derivatives exhibit modest inhibitory activities on the studied enzyme models, but it could be improved after structure optimization.

1. Introduction

Among the 877 new small-molecule entities worldwide introduced as drugs during the 1981–2002 period, 61% can be traced to or were inspired by natural products.[1] These include natural products, natural products derivatives, synthetic compounds with natural-product-derived pharmacophores and synthetic compounds designed on the basis of knowledge gained from a natural product (natural product mimic). Coumarins occur naturally in many food sources including citrus fruits, herbs and vegetables.[2] Isocoumarins are secondary metabolites derived from acetate pathway and are structurally related to coumarins but with an inverted lactone ring.[3] Both scaffold has demonstrated interesting biological activities on different biological targets involved in a wide range of pathologies (neurodegenerative disorders, cancer and infectious diseases).

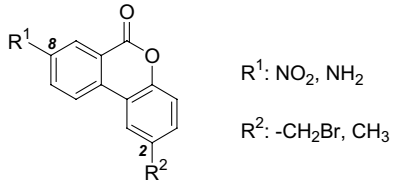

Figure 1. 2,8-Disubstituted-benzo[*c*]chromen-6-one scaffold general structure.

In this paper, we report the design, synthesis and bioactive properties of a novel scaffold: **benzo[*c*]chromen-6-one** (Fig. 1) which can be seen as a *coumarin* as well as an *isocoumarin* nucleus. When such scaffold is substituted at the 2- and 8-positions by various specific groups, its resulting chemical reactivity could confer to these new entities interesting inhibitory enzymatic properties. The first challenge to be faced was to synthesize such reactive analogues. Indeed, 2-bromomethyl-8-substituted-benzo[*c*]chromen-6-one analogues **6b** and **7b** are very reactive compounds, which could react chemically or enzymatically with any kind of nucleophilic species through the possible quinonimine methide intermediate formation during the course of the chemical synthesis

Keywords: Substituted-benzo[*c*]chromen-6-one; Coumarin–isocoumarin; Privileged structure.
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or enzymatic assay. It has been reported that bioactive compounds based on the quinonimine methide mechanism were potent protease inhibitors of biological interest.[4]

2. Chemistry

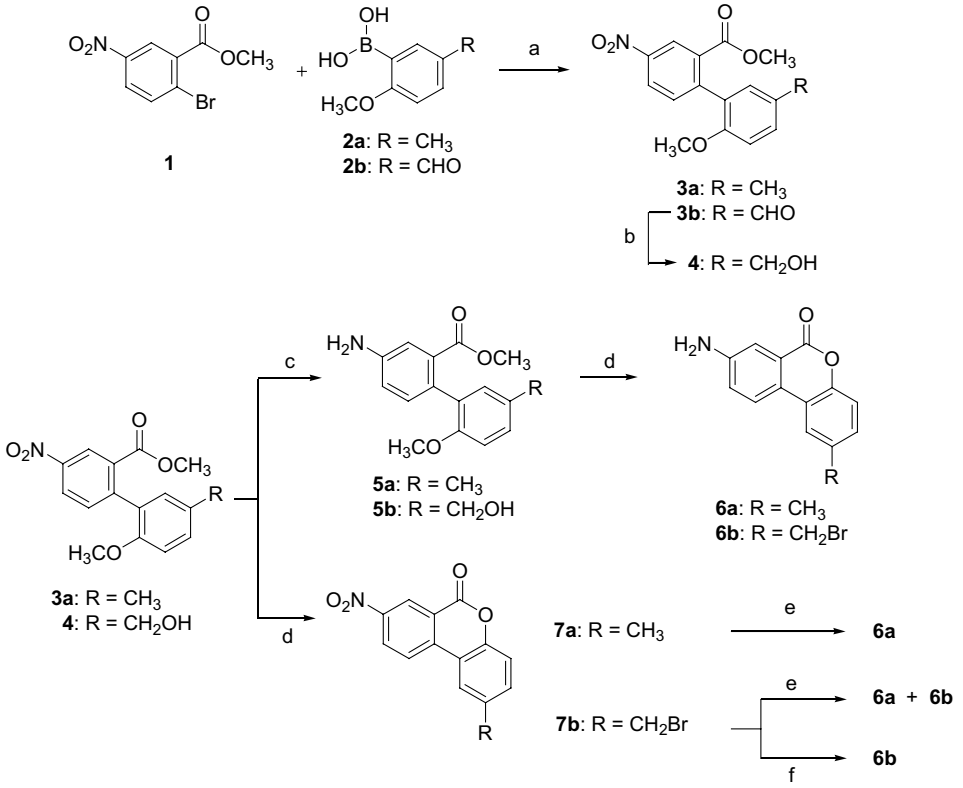
Scheme 1 summarizes the whole synthesis used for the described compounds. The first step involved a Suzuki-type coupling reaction between a common bromoaryl substrate **1** and various aryl boronic acids **2a** and **2b**, which were commercially available. 2-Methoxyphenyl boronic acid derivatives reacted with methyl 2-bromo-5-nitrobenzoate in the presence of the catalytic system 1,1′-bis(diphenylphosphino)-ferrocene, [1,1′-bis(diphenylphosphino)-ferrocene]-dichloropalladium II complex and potassium acetate in refluxing dioxane to yield the biphenyl esters **3a** and **3b**, respectively. Aromatic aldehyde **3b** was then reduced into the corresponding primary alcohol **4** by using borane–methyl sulfide complex (BMS). Nitro compounds **3a** and **4** were catalytically hydrogenated into the corresponding aniline derivatives **5a** and **5b**, in good yields. Treatment of each ester derivatives **3a**, **4**, **5a** and **5b** with boron tribromide resulted in an efficient cleavage of the methoxy-ether group, followed by a spontaneous lactonization reaction, leading to the corresponding benzo[*c*]chromen-6-ones **6a**, **6b**, **7a** and **7b**. It should be underlined that during the process of this reaction, the adventitious bromination of benzyl alcohol **4** occurred simultaneously.

Another attempt to reach final compound **6b** was performed starting from 2-bromomethyl-8-nitro-benzo-[*c*]chromen-6-one intermediate **7b**. Reduction of the nitro intermediate **7b** appears to be a straightforward synthesis of the desired amino analogue **6b**. We found that this reduction led to the corresponding methyl analogue **6a** by loss of the bromine atom possibly according to a similar mechanism suggested in Scheme 2. Surprisingly, the reduction of the lactone was not observed during the elimination of bromine atom.

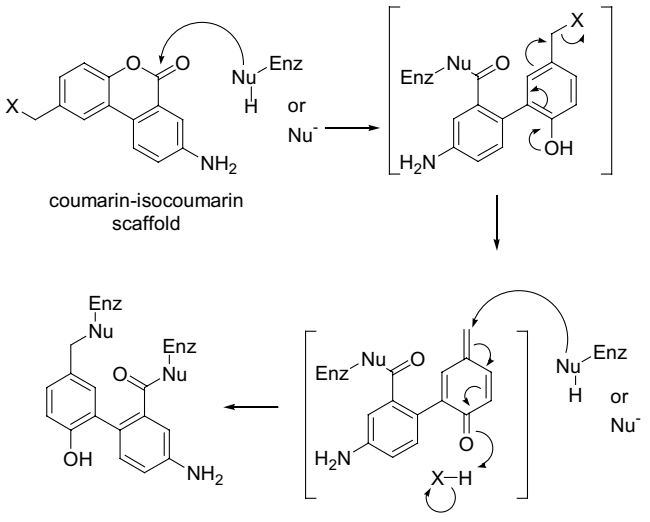
Intermediates and final compounds of both series were fully characterized by ^{1}H NMR, ^{13}C NMR and MS.[5]

3. Enzymatic properties and discussion

The enzymatic properties of the novel analogues **6a**, **6b**, **7a** and **7b** were investigated on representative examples of enzymatic models: serine proteases (α-chymotrypsin and trypsin), HIV aspartyl protease,[6] nitric oxide synthase (NOS)[7] and a panel of protein kinases.[8] Among this panel of protein kinases, glycogene synthase kinase (GSK-3) is of particular interest since it represents an attractive therapeutic target for the treatment of



Scheme 1. Reagents and conditions: (a) $PdCl_2$ dppf, dppf, KOAc, dioxane, reflux, 15 h; 73% (**3a**), 76% (**3b**); (b) BH_3/DMS, THF, rt, 1 h; quantitative; (c) H_2/Pd/C, THF, rt, 1 h; 89% (**5a**), quantitative (**5b**); (d) BBr_3, CH_2Cl_2, −78 °C, 2 h then CH_3OH; 80% (**6a**), 60% (**6b**), 77% (**7a**), 87% (**7b**); (e) H_2/Pd/C, CH_3OH, rt, 1 h; 80% (**6a**) from **7a**, mixture of **6a** (53%) and **6b** (31%) from **7b**; (f) Fe, NH_4Cl, EtOH/H_2O (5/3, v/v), reflux, 2 h, 83% (**6b**).



Scheme 2.

numerous serious pathologies, including Alzheimer's disease, stroke, bipolar disorders, chronic inflammatory processes, cancer and type II diabetes.[9]

The enzymatic inhibitory properties of the new 2-bromomethyl-8-substituted-benzo[*c*]chromen-6-ones are summarized in Table 1.

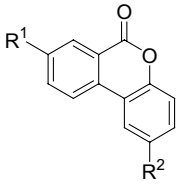
Chemically, we observed that compound **7b** is highly sensitive to nucleophiles (hydride ion) leading to the loss of the bromine atom. In contrast, when derivatives **6b** and **7b** are submitted to nucleophilic enzymatic attack by serine or aspartyl proteases, only weak inhibitory effects are observed. Nevertheless, some specific inhibitory effects could be of interest since 2-bromomethyl-8-substituted-benzo[*c*]chromen-6-ones can be the starting point for the design of more active compounds. For example, compound **7b** is inactive on α-chymotrypsin as well as trypsin but it is slightly active on HIV aspartyl protease (IC_{50} = 10 to 50 μM). With this compound as starting point, it could be possible through a series of iterative cycles of structure-based design, to enhance the HIV inhibitory activity. Such observations have been reported in the case of the 5,6-dihydro-4-hydroxy-2-pyrone sulphonamide class.[10] Indeed, the very potent HIV protease inhibitor Tipranavir (ED_{90} = 0.1 μM) was identified from a compound, which initially displays weak antiviral activity (ED_{50} = 300 μM).

The synthesized compounds were also tested as potential NOS (I, II, III) inhibitors according to a standard procedure. The rate of NO formation is measured by standard method using N-ω-hydroxy-L-arginine (NOHA) as endogenous substrate and classical NOS inhibitor 7-nitro-imidazole (7-NI) as reference.[11] Only compounds **6a**, **6b** and **7b** displayed some NOS inhibitory activities (Table 2), which are low compared to that of the reference compound 7-NI. Nevertheless, it can be observed that compound **7b**, which displayed an IC_{50} value of 60 μM on iNOS, appears to be more selective for iNOS than for the other two models nNOS and eNOS. Replacement of the nitro group in position 8 of compound **7b** by an amino group **6a** led to a drop-off in the observed inhibitory activity, indicating that the 8-nitro substituent on the 2-bromomethyl-8-substituted-benzo[*c*]chromen-6-one scaffold seems to be required for NOS inactivation. Moreover, the presence of the bromomethyl group at the 2-position (**6b**, **7b**) favoured inhibitory activity since its replacement by a methyl group (**6a**, **7a**) completely abolished the inhibitory activity. This result confirms that 2-bromomethyl-8-substituted-benzo[*c*]chromen-6-one scaffold can be seen as a potential building block, which could lead after optimization to potent and selective NOS inhibitors depending on the position and the structure of the 'dressing substituents'.

The inhibitory activity of this scaffold was also examined on a panel of 29 protein kinases, and in particular on serine/threonine kinases involved in the control of several regulatory protein.[13] Assays were performed according to previously described procedures.[14,15] At 10 μM, only compound **6b** notably reduced the activity of the following kinases: MAPKAP-K1a, PKBΔ ph, SGK, p70 S6K and DYRK 1A.

In conclusion, compound **6b** bearing an amino group at the 8-position represents an interesting scaffold for the

Table 1. Serine and HIV-1 aspartyl proteases inhibitory activities



Compound	R^1	R^2	Serine proteases		Aspartyl protease
			α-Chymotrypsin	Trypsin	HIV-1 protease
			IC_{50} (μM)		
6a	$-NH_2$	$-CH_3$	Inactive (100 μM)	>100	~100
6b	$-NH_2$	$-CH_2Br$	>100	~100	10–50
7a	$-NO_2$	$-CH_3$	>100	>100	10–50
7b	$-NO_2$	$-CH_2Br$	>100	>100	10 ± 5

Table 2. Nitric oxidase synthase inhibitory activities

Compound	R^1	R^2	nNOS[a] (μM)	iNOS[a] (μM)	eNOS[a] (μM)
6a	$-NH_2$	$-CH_3$	900 ± 200	150 ± 30	600 ± 10
6b	$-NH_2$	$-CH_2Br$	200 ± 60	110 ± 30	120 ± 40
7a	$-NO_2$	$-CH_3$	n.d.	n.d.	n.d.
7b	$-NO_2$	$-CH_2Br$	250 ± 50	60 ± 10	120 ± 20
7NI[b]	—	—	15 ± 3	15 ± 3	8 ± 2

n.d.: not determinated.

[a] nNOS: neuronal expressed NOS; iNOS: inductible NOS; eNOS: endothelial NOS.[12]

[b] 7-Nitroimidazole, as reference inhibitor.

design of bioactive drugs directed to quite a wide number of biological targets. Indeed, substitution of the bromine atom at the 2-position by an hydrogen abolished any of the observed biological activities on the different models. 2-Bromomethyl-8-substituted-benzo[*c*]chromen-6-one can be considered as a privileged structure and could be a good starting framework for a drug discovery program.

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