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In a search for selective inhibitors of carbonic anhydrases: coumarin and its bioisosteres – synthesis and derivatization

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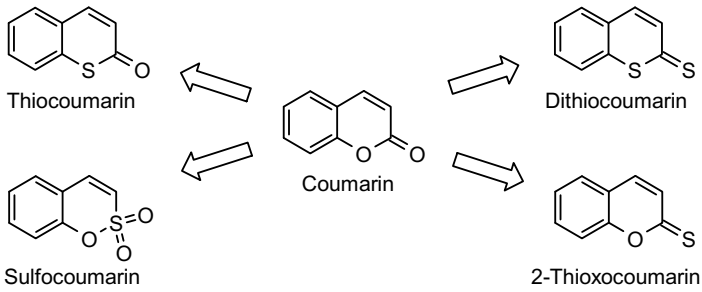


This review describes the preparation and derivatization of coumarin and its bioisosteres – thioxocoumarin, thiocoumarin, dithiocoumarin, and sulfocoumarin derivatives triggered by natural product and utilized in the investigation of inhibitors of carbonic anhydrases with a focus on cancer-associated isoforms of carbonic anhydrases.

Keywords: carbonic anhydrases, coumarin, dithiocoumarin, sulfocoumarin, thiocoumarin, thioxocoumarin.

Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous metalloenzymes which are present in most of the living organisms and catalyze simple reaction – reversible hydration of carbon dioxide to hydrogen carbonate and a proton.[1]

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

CAs are involved in many physiological and pathological processes, like respiration and CO_2 transport, pH regulation, electrolyte secretion, bone resorption, calcification, tumorigenicity, and other processes.[2] Among 15 human α-CAs isoforms, CA IX and CA XII are overexpressed in a variety of tumors and, therefore, are established as anticancer drug targets.[3] Although many CA inhibitors containing mostly sulfonamide or sulfamate groups as the zinc-binding moiety, like clinically used drugs acetazolamide or topiramate, respectively (Fig. 1), are known, there is a serious lack of isoform-selective CA inhibitors.[2]

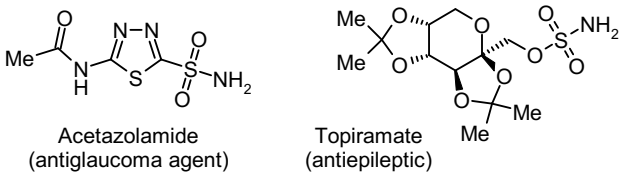


Figure 1. Clinically used non-selective CA inhibitors.

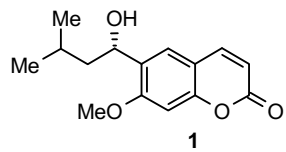
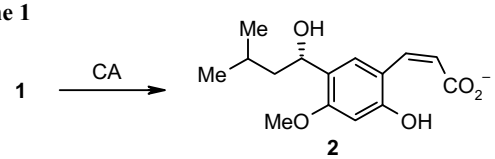


Figure 2. Coumarin isolated from plant *Leionema ellipticum*.

A discovery of the ability of coumarin derivative **1** (Fig. 2), isolated from the Australian plant *Leionema ellipticum*, to inhibit CA triggered the investigation of coumarin derivatives as CA inhibitors.[4,5]

Even though natural product **1** did not show the desired selectivity towards CA IX and CA XII, where the off-targets are the ubiquitous CA I and CA II, the synthetic derivatives of coumarin exhibited very promising to excellent selectivity towards CA IX and CA XII in nanomolar range.[5] It is important to notice that coumarin do not have a distinct zinc-binding group. However it was demonstrated by protein-ligand X-ray crystallography that the coumarin lactone ring undergoes ring opening in the presence of CA (Scheme 1)

Scheme 1



and binds in a form of hydroxycinnamic acid **2** near the active center without directly binding to zinc atom.

In this way coumarin and its bioisosteres are considered as prodrugs.

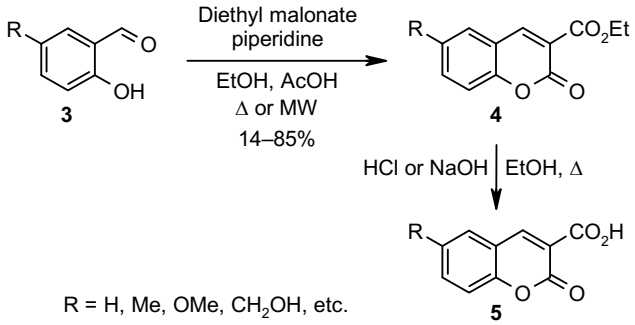
It was also demonstrated that other natural products containing coumarin motive exhibit good to excellent CA IX and CA XII inhibition selectivity and activity.[6]

Taking into account discovery of coumarin series as CA IX and CA XII selective inhibitors and their significance in anticancer drug discovery, this minireview highlights synthesis and derivatization of coumarin and its bioisosteres used in the investigation of CA inhibition in recent years.

Synthesis of coumarin derivatives

After the first promising CA inhibition results with naturally occurring and commercially available coumarin derivatives, the preparation of synthetic analogs was performed. A well-established Knoevenagel-type reaction between diethyl malonate and salicylaldehyde **3** was used for coumarin lactone ring formation (Scheme 2). The desired derivatives **4** were isolated in broadly varying yields, most probably due to different isolation and purification techniques used, including crystallization and recrystallizaton.[7–9]

Scheme 2

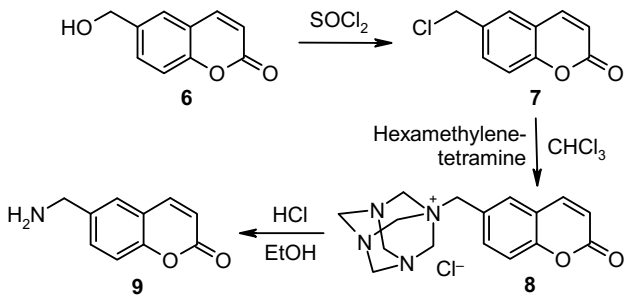


In order to improve the water solubility of coumarin derivatives, the hydrolysis of ester group was reported under acidic or basic conditions (Scheme 2). Even though the authors do not provide details on the yields of obtained compounds **5**, there is no report of lactone ring opening under basic saponification or decarboxylation under acidic hydrolysis of ester.[7–9]

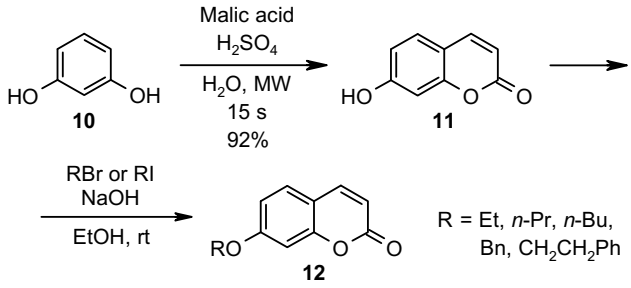
Since the first results on CA inhibition were obtained with 3-unsubstituted coumarin derivatives and there were indications that bulky substituents at position 6 are responsible for better selectivity towards CA IX and CA XII the synthesis of compound **8** containing an urotropine moiety as a somewhat extreme case was reported (Scheme 3). First, 6-hydroxymethylcoumarin (**6**) was treated with $SOCl_2$, thus obtaining corresponding chloride **7** which in turn was treated with urotropine (hexamethylenetetramine). The obtained salt **8** was further used for the synthesis of amino derivative **9** by treatment with HCl.[7,10]

Umbelliferone or 7-hydroxycoumarin (**11**) is a natural product which can be easily synthesized from resorcinol (**10**) and malic acid under acidic conditions using MW in excellent yield (Scheme 4).[11]

Scheme 3



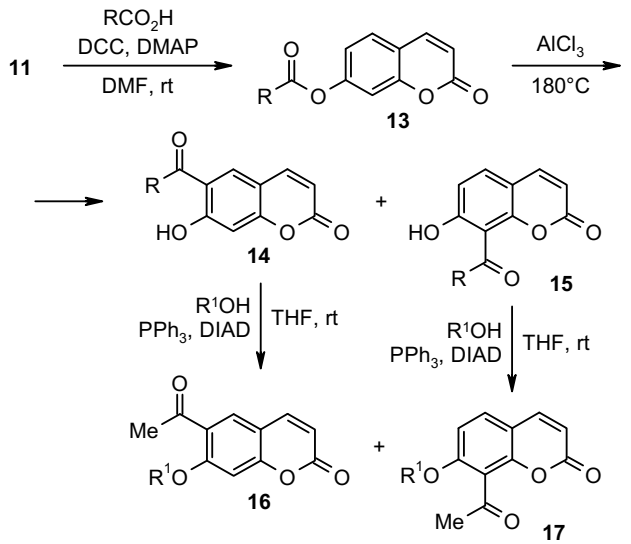
Scheme 4



To explore the influence of substitution at position 7, simple alkylation of umbelliferone (**11**) with alkyl halides was performed in the presence of NaOH (Scheme 4). In this way Maresca and coauthors easily obtained a series of compounds **12** for the CA screening purposes.[7]

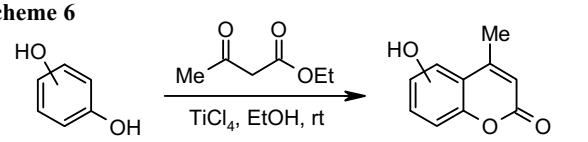
Supuran and coauthors also took advantage of acetylation reaction followed by the Fries rearrangement. Umbelliferone (**11**) first was reacted with acetic or propionic acid in the presence of a coupling agent. Two esters **13** obtained in this way were further submitted to the Fries rearrangement. In both cases two regioisomers **14** and **15** were obtained in a ratio 1:9, which were separable by flash chromatography. From the separated regioisomers in reaction with corresponding alcohols under Mitsunobu conditions two series of coumarin derivatives **16** and **17** were obtained (Scheme 5).[12]

Scheme 5



To obtain potentially valuable building blocks for further exploration of CA inhibition, 4-methyl-substituted hydroxycoumarins **19**–**21** were synthesized under the Pechmann condensation conditions (Scheme 6).[13]

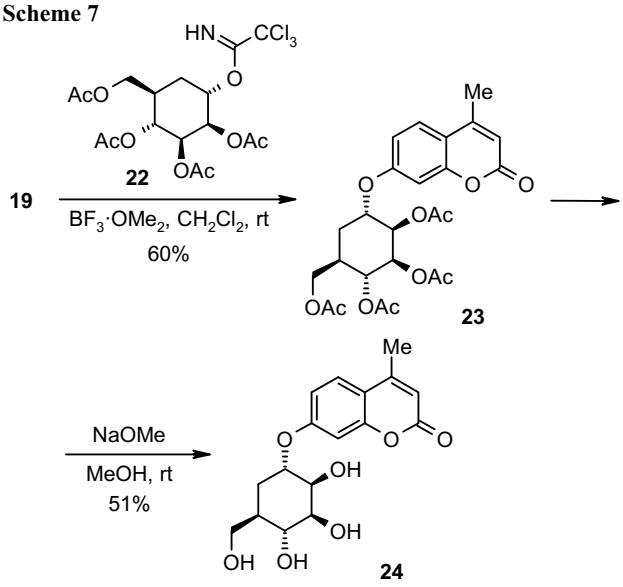
Scheme 6



Since CA inhibition screening revealed that 4-methyl-substituted hydroxycoumarins **19**–**21** are CA IX selective inhibitors with activities in nanomolar range, a further exploitation of these building blocks followed.

Glycosyl moieties incorporated in sulfonamide-based CA inhibitors previously showed very good results in CA inhibition tests,[14–17] therefore, glycosyl fragments were chosen as promising moieties to be incorporated in one molecule with 4-methylcoumarin. To obtain the desired compound **23**, a pentaacetylated trichloroacetimidate derivative of mannose **22** was coupled with 7-hydroxycoumarin **19** followed by deprotection of the mannose moiety in intermediate **23** (Scheme 7).[18] Introducing by this approach various sugar motives, the authors obtained a series of glycosyl derivatives of coumarin.

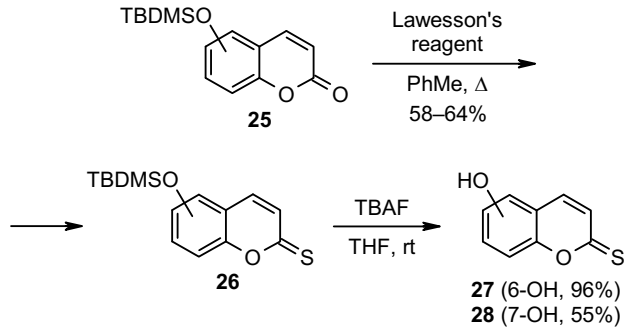
Scheme 7



Synthesis of thioxocoumarin derivatives

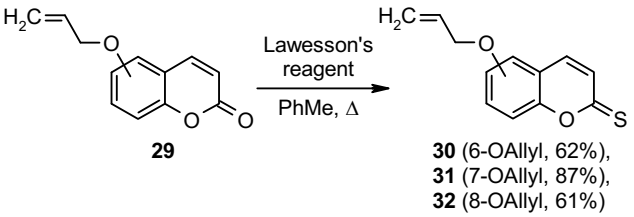
Thioxocoumarin or 2*H*-chromene-2-thione is considered one of coumarin bioisosteres in which carbonyl group in lactone ring is replaced by thiocarbonyl group. Lawesson's reagent (LR) is broadly used for the transformation of carbonyl group into thiocarbonyl one. Since reactions with LR are performed under mild conditions, LR was an obvious choice to transform coumarin into thioxocoumarin. In this way, silyl-protected hydroxycoumarins **25** were transformed into corresponding thioxocoumarins **26** in acceptable yields. Following elimination of silyl groups provided hydroxycoumarins **27** and **28** in good yields (Scheme 8) for CA screening experiments.[19]

Scheme 8



To broaden the variety of substituents in thioxocoumarin for CA screening as well as to obtain potentially valuable building blocks for further derivatization, allyl derivatives of coumarin **29** were treated with LR as described above. In this way, a small series of thioxocoumarins **30**–**32** in good yields was obtained (Scheme 9).[19]

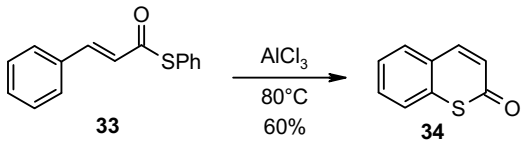
Scheme 9



Synthesis of thiocoumarin derivatives

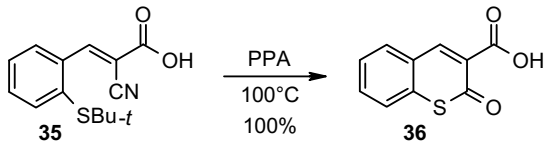
Thiocoumarin or 2*H*-thiochromen-2-one can be considered as another bioisostere of coumarin in which oxygen in lactone ring is replaced by sulfur. Taking into account its similarity to coumarin, thiocoumarins also were subject of CA inhibition studies.[7,20] To obtain desired thiocoumarin Zhong et al. utilized previously described reaction where thiocoumarin **34** was obtained in cyclization of α,β-unsaturated thioester **33** (Scheme 10).[21]

Scheme 10



In order to obtain 3-substituted thiocoumarin **36**, other previously reported method was used. Treating derivative of cinnamic acid **35** with polyphosphoric acid (PPA) provided the desired thiocoumarin **36** in quantitative yield (Scheme 11).[22]

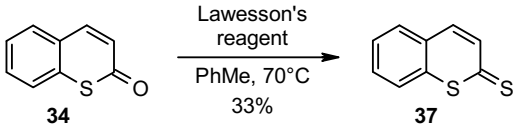
Scheme 11



Synthesis of dithiocoumarin derivatives

Dithiocoumarin (2*H*-thiochromene-2-thione) is an eventual bioisostere of coumarin where both oxygen atoms in lactone ring are replaced with sulfur. It is important to notice that dithiocoumarins in general are a little studied class of compounds with very limited number of literature references and one method of synthesis. Following the only method available for the synthesis of dithiocoumarin, thiocoumarin **34** was treated with LR, and the desired compound **37** was isolated in 33% yield (Scheme 12).[20]

Scheme 12



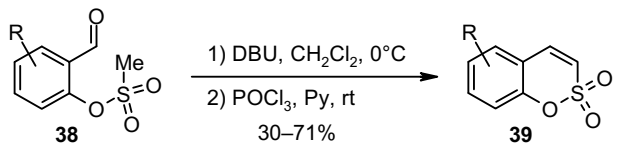
Synthesis of sulfocoumarin derivatives

Sulfocoumarins or 1,2-benzoxathiine 2,2-dioxides are other class of compounds which was considered as bioisosteres of coumarin. In the case of sulfocoumarin, carbonyl group of coumarin lactone ring is replaced with sulfonyl group.

It is worth to mention that before considering of sulfocoumarin as potential CA inhibitor there was no reliable method found in the literature for the synthesis of this class of compounds.

As the first reliable and well-reproducible method of synthesis of sulfocoumarins, strong organic base-promoted intramolecular condensation of mesyl derivatives of salicylaldehyde **38** was reported. By utilizing such organic base as DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) a first series of sulfocoumarins **39** was obtained. It has to be noted that in almost all cases a mixture of sulfocoumarin **39** and 4-OH-containing product of addition was obtained. Therefore, to complete the condensation, the mixture was additionally treated by $POCl_3$ (Scheme 13).[23]

Scheme 13



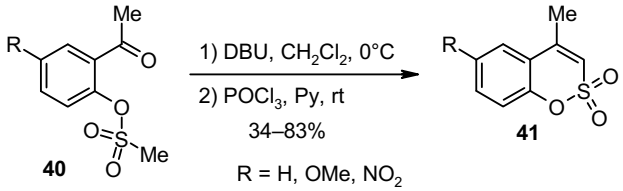
R = H, 6-NO_2, 6-OMs, 6-Br, 6-OBn, 8-OMe, etc.

The authors have obtained a monocrystal of 6-bromosulfocoumarin **39** and by single-crystal X-ray structural analysis unequivocally proved the scaffold of sulfocoumarin.[23]

It was also demonstrated that utilizing conditions described above intramolecular condensation takes place in corresponding acetophenone derivatives **40** providing 4-methyl-substituted sulfocoumarins **41** (Scheme 14).[24]

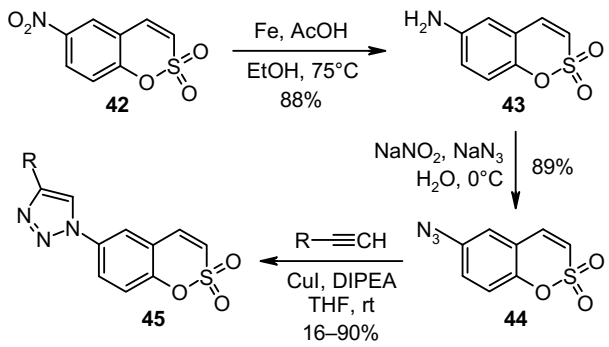
Since in the previous studies coumarin derivatives with substituents at position 6 exhibited better activities and selectivity towards cancer-associated CA IX and CA XII the authors primarily focused on the derivatization of sulfocoumarin at position 6.

Scheme 14



As a first choice, the preparation of 4-substituted 1,2,3-triazol-1-yl derivatives was considered the most suitable because of the simplicity of click chemistry. For this purpose, nitro derivative **42** was reduced to the corresponding aniline **43** (Scheme 15). Utilization of iron as reducing agent was found suitable for this substrate, because using Pd/C–H_2 along with reduction of nitro group partial reduction of double bond in oxathiine ring was observed. In the next step, diazotation followed by treatment with sodium azide was performed and azide building block **44** was isolated in very good yield. Treatment of azide with various ethyne derivatives under click chemistry conditions provided a series of triazolyl derivatives **45**.[23,25,26]

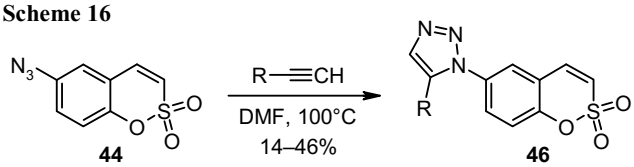
Scheme 15



R = CH_2OH, COOEt, CH_2NEt_2, Ph, 4-$MeOC_6H_4$, 3-$MeOC_6H_4$, etc.

In order to obtain 5-substituted 1,2,3-triazol-1-yl derivatives, a Ru-catalyzed ethyne cycloaddition reaction was tried. However, the authors claim that the thermal Huisgen cycloaddition reaction often provided formation of the desired triazole with a better isomer ratio than the Ru-catalyzed one (Scheme 16). In all cases, triazolyl derivatives **46** were separated by column chromatography.[26]

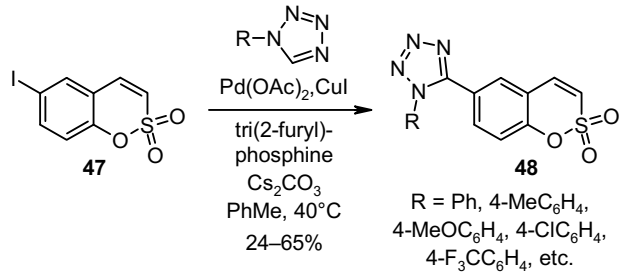
Scheme 16



R = Ph, 4-$MeOC_6H_4$, 3-$MeOC_6H_4$, 4-FC_6H_4, 4-ClC_6H_4, etc.

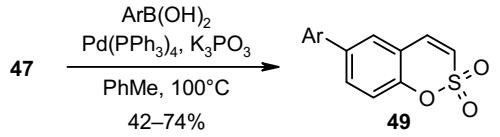
The next obvious choice for the authors was the synthesis of 6-tetrazolyl derivatives **48**. For this purpose, the C–H bond activation reaction in tetrazoles in which iodide **47** was coupled with various tetrazoles[27] was successfully adopted (Scheme 17). In this way, a series of 6-tetrazolylsulfocoumarins **48** was obtained.[28]

Scheme 17



Starting from iodide **47**, the Suzuki reaction was used to further explore the influence of substituents at position 6 of sulfocoumarin on the CA inhibition ability. Thus, a series of 6-aryl derivatives **49** was synthesized in acceptable to good yields (Scheme 18).[29]

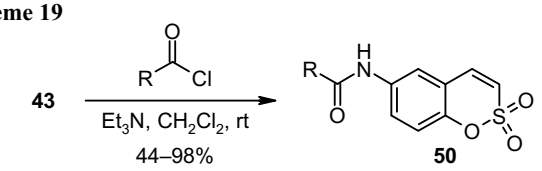
Scheme 18



Ar = Ph, 4-MeC$_6$H$_4$, 4-MeOC$_6$H$_4$, 4-FC$_6$H$_4$, 4-ClC$_6$H$_4$, etc.

Another obvious option considered was the acylation of the amino group in sulfocoumarin **43**. Performing the acylation by variety of acyl chlorides, a series of acylaminosulfocoumarins **50** was obtained (Scheme 19).[30]

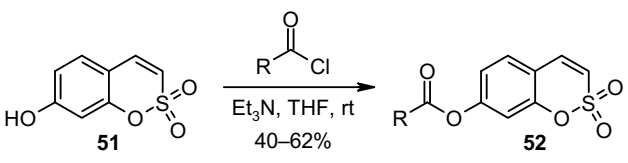
Scheme 19



R = Me, *t*-Bu, Ph, 4-MeC$_6$H$_4$, 2-thienyl, 2-furyl, 3-pyridyl, etc.

A similar approach was used in another work where simple acylation was examined with hydroxysulfocoumarin. In this case, 7-hydroxy derivative **51** was acylated by acyl chlorides, and acyloxysulfocoumarins **52** were isolated in good yields (Scheme 20).[31]

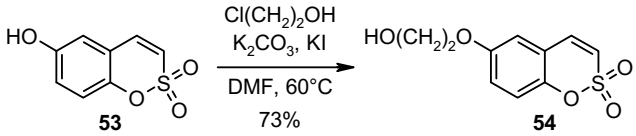
Scheme 20



R = Me, 4-ClC$_6$H$_4$, 2-BrC$_6$H$_4$

One more opportunity currently found in the literature for the derivatization of sulfocoumarins is alkylation. In this case Supuran et al. selected 6-hydroxysulfocoumarin **53** and performed alkylation, for instance, with 2-chloroethanol. In this way 6-alkyloxysulfocoumarin **54** was isolated in good yield (Scheme 21).[32]

Scheme 21



Since the discovery, more than half a decade ago, of the ability of naturally occurring coumarin to inhibit carbonic anhydrases significant efforts were dedicated to explore the synthesis of new coumarin and its bioisosteres' derivatives for the search of CA isoform-selective inhibitors. Even though for the synthesis of simple derivatives the time-proved synthetic methods were used, also new protocols have been developed, in particular, for the synthesis of coumarin bioisosteres like dithiocoumarin or sulfocoumarin. Also derivatization methods of these compounds have been explored. And finally, most of the coumarin derivatives highlighted in this review, especially with bulky substituents at position 6, have exhibited good to excellent selectivity towards cancer-associated CA IX and CA XII with activities in low nanomolar range. In this way it is demonstrated that derivatives of coumarin and its bioisosteres have great potential in future anticancer drug discovery.

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