RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Journal Name

RSCPublishing

ARTICLE

Phosphate tricyclic coumarin analogs as steroid sulfatase inhibitors: synthesis and biological activity

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Witold Kozak,[a] Mateusz Daśko,[a] Maciej Masłyk,[b] Jerzy S. Pieczykolan,[c] Bartłomiej Gielniewski,[b] Janusz Rachon,[a] and Sebastian Demkowicz*[a]

In the present work, we report convenient methods for the synthesis and biological evaluation of phosphate tricyclic coumarin derivatives as potential steroid sulfatase inhibitors. The described synthesis includes the straightforward preparation of 7-hydroxy-2,3-dihydro-1*H*-cyclopenta[c]chromen-4-one, 3-hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[c]chromen-6-one and 3-hydroxy-8,9,10,11-tetrahydro-7*H*-cyclohepta[c]chromen-6-one modified with various phosphate moieties. The inhibitory effects of the synthesized compounds were tested on STS isolated from human placenta as well as the MCF-7, MDA-MB-231 and MDA-MB-435S cancer cell lines. Most of the new STS inhibitors possessed IC_{50} values between 21 to 159 μM. In the course of our investigation, the largest inhibitory effects in the STS enzyme assays were observed for the three compounds **9p**, **9r** and **9s**, with IC_{50} values of 36.4, 37.8 and 21.5 μM, respectively (IC_{50} value of 1.0 μM for the 665-COUMATE used as a reference). The compound **9r**, exhibited the highest potency against MCF-7, an estrogen receptor positive (ER+) cell line, with a GI_{50} value of 24.7 μM. The structure-activity relationships of the synthesized coumarin derivatives with the STS enzyme are discussed.

Introduction

Breast cancer is a major cause of mortality, and there is an urgent need for novel treatment strategies. The World Health Organization (WHO) lists estrogens as one of the most important factors inducing the development of breast cancer. In females in industrialized countries, breast cancer is the most frequently diagnosed cancer. Estimates show that in the United States, more than 190,000 new cases of breast cancer were diagnosed and more than 40,000 deaths occurred from this disease in 2009.[1] One strategy for the treatment of hormone-dependent breast cancer (HDBC) involves inhibitors to prevent the synthesis of estrogens in peripheral tissues.[2] In recent years, there has been intensive research toward finding novel effective inhibitors of STS, an enzyme involved in the biosynthesis of estrogen in the mammary gland. Approaches to design effective STS inhibitors include three different categories of compounds: alternative substrates (including competitive reversible inhibitors), reversible inhibitors, and irreversible inhibitors.[3] Initial reports on the synthesis and biological evaluation of STS inhibitors appeared in the 1970s and were focused on natural and synthetic unconjugated steroids.[4] In the 1980s, a series of 2-(hydroxyphenyl)indole sulfates were reported as the first class of STS inhibitors. Among them, compound **1**, with an IC_{50} value of 80 μM, exhibits the highest activity. In the 1990s, intensive research on natural and synthetic steroid derivatives with potent activity against STS has continued. During this time, Evan's research group reported 5-androstene-3β,17β-diol-3-sulfate **2**, which showed potent activity toward STS with an IC_{50}= 2 μM.[5] Because of numerous side effects from the production of estrogenic metabolites that bind to the ER, these compounds have not been utilized in hormone therapy for breast cancer.

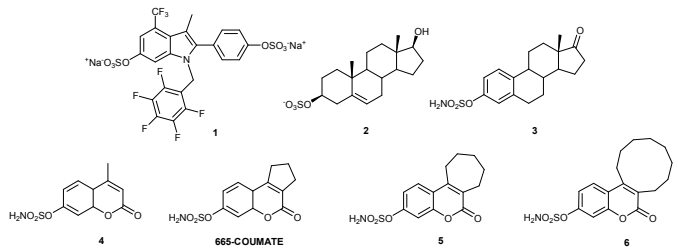


Fig. 1 Chemical structures of STS inhibitors.

There has been a concerted effort by various research groups to find an effective, reversible STS inhibitor that does not show

estrogenic action. A series of estrone sulfate (E1S) analogues with different functional groups has been synthesized, and these functional groups include: sulfonates, sodium methylenesulfonate, sulfonamide, sulfonyl halides, methylenesulfonyl groups, phosphates and phosphonates.[6] The most promising compound was estrone-3-O-sulfamate **3** (EMATE), which exhibited very high activity in MCF-7 cells, with an IC_{50} value of 65 pM.[7] Unfortunately, due to its estrogenic properties, clinical trials for EMATE have been discontinued. An important class of compounds that exhibits high activity against STS are the coumarin derivatives. In contrast to EMATE, they exhibit fewer adverse effects and much weaker estrogenic properties. Coumarin analogs are classified as irreversible inhibitors whose effects are time and concentration dependent. The first potent inhibitor based on the coumarin scaffold was 4-methylcoumarin-7-O-sulfamate **4** (COUMATE), which exhibited good activity with an IC_{50} value of 380 nM when evaluated against placental microsomes.[8] Further modification of its structure led to a wide range of tricyclic coumarin derivatives that mimic the ABC rings of the natural substrate. These compounds demonstrated inhibitory activity 100 times more potent than COUMATE. For example, 667-COUMATE **5** (currently in clinical trials) and 6610-COUMATE **6** have demonstrated potent activity toward STS with IC_{50} values of 8 nM and 1 nM, respectively.[9]

Results and discussion

Molecular modeling

Prior to docking calculations, the ligands were constructed using Portable HyperChem 8.0.7 Release (Hypercube Incorporation), and each was optimized using a MM+ force field and the Polak – Ribiere conjugate gradient algorithm terminating at a gradient of 0.05 kcal/mol/Å. The X-ray structure of human STS (Protein Data Bank accession code 1P49) was prepared for docking using the following procedure. First, the water molecules from crystallization were removed from the structure. The catalytic amino acid FGly75 (formyloglycine) was then converted to the gem-diol form using the Protein Preparation Wizard module, delivered with Maestro (Schrödinger, LLC, New York, NY). Hydrogen atoms were built onto the structure and optimized using the OPLS-AA force field. Docking of the optimized ligands to the prepared structure of human STS was carried out with Autodock Vina 1.1.2 software (The Molecular Graphics Laboratory, Scripps Research Institute). For all of the docking studies, a grid box size of 30 Å x 30 Å x 30 Å, centered on the Cβ atom of amino acid 75, was used. The best poses for a particular ligand were visually inspected. Illustrations of the 3D model were generated using VMD 1.9 (University of Illinois at Urbana - Champaign). Preliminary docking studies were carried out in order to assess the capability of the designed inhibitors to interact with the ligand binding domain (LBD) of STS. Figure 2 shows a putative enzyme-ligand complex prior to the presumed inactivation of STS elicited by selected phosphate tricyclic coumarin derivatives and reference compound. As predicted, designed new STS inhibitors dock in a similar manner to the mode of reported sulfamate-based STS inhibitor (665-COUMATE) (see Fig. 1) with the phosphate groups directed toward the catalytic cavity of STS where the coordinating Ca^{2+} ion and formylglycine in its gem-diol form are located.

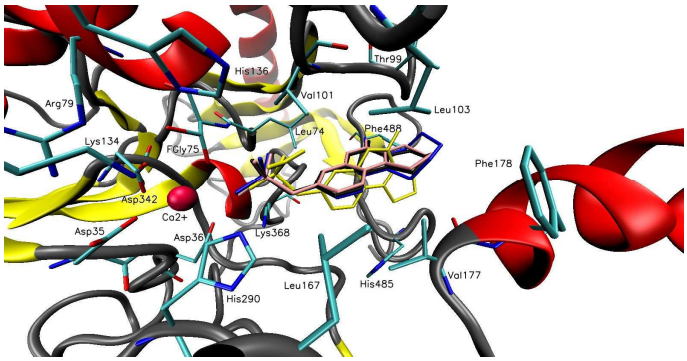


Fig. 2 Docked binding modes for compounds **9p** (brown), **9r** (black), **9s** (blue) and **665 COUMATE** (yellow).

A network of interactions is evident between the phosphate groups and neighboring amino acid residues (Asp35, Asp36, FGly75, Arg79, Lys134, His136, His290, Asp342 and Lys368). The hydrophobic five, six and seven-membered ring of coumarin scaffolds are in close proximity to several lipophilic amino acids (Leu74, Thr99, Val101, Leu103, Leu167, Val177, Phe178, His485 and Phe488), which are implicated in substrate recognition. All the candidates to be synthesized were able to bind STS, at least theoretically. Regarding the predicted binding energies, these were the most favourable in the case of phosphorus acid esters of tricyclic coumarin **9p**, **9r**, **9s** (predicted binding energies of -4.3, -4.7 and -4.9 kcal/mol, respectively). The docked binding energy of these compounds were compared to 665-COUMATE used as reference (-5.1 kcal/mol). These results suggest that increasing the size of the hydrophobic ring in the coumarin scaffold could favour the binding by the establishment of stronger hydrophobic interactions with the cavity delimited by lipophilic amino acids in the enzyme pocket. Furthermore, the arrange of phosphate groups and their distance to the active site of STS suggest that a phosphate group transfer to FGly75 may be crucial during the inactivation mechanism.

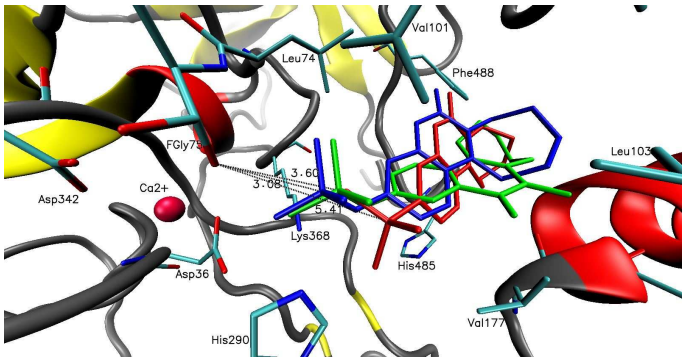


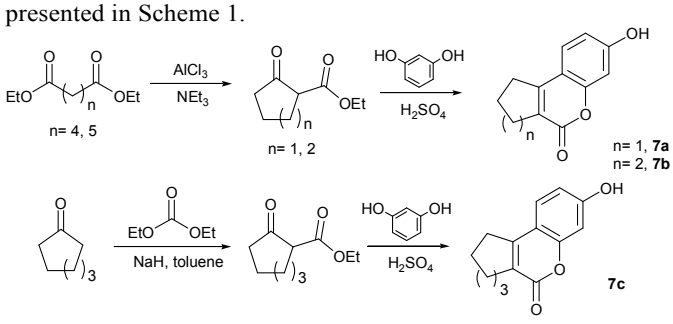
Fig. 3 Docked binding modes and distance to FGly75 for compounds **9s** (blue), **11** (green) and **16** (red).

The presence of a phosphonic acid group in 3-position (compound **16**) and lactam moiety (compound **11**) led to a decrease of the docked binding energy (predicted binding energies of -4.4 and -3.8 kcal/mol, respectively). In addition, the distance of functional groups in 3-position of compound **16** (5.41 Å) and **11** (3.60 Å) to catalytic amino acid FGly75 is longer (compared to **9s** = 3.08 Å) which may indicate a decreased ability of these analogs to interact with the active site of STS.

Chemistry

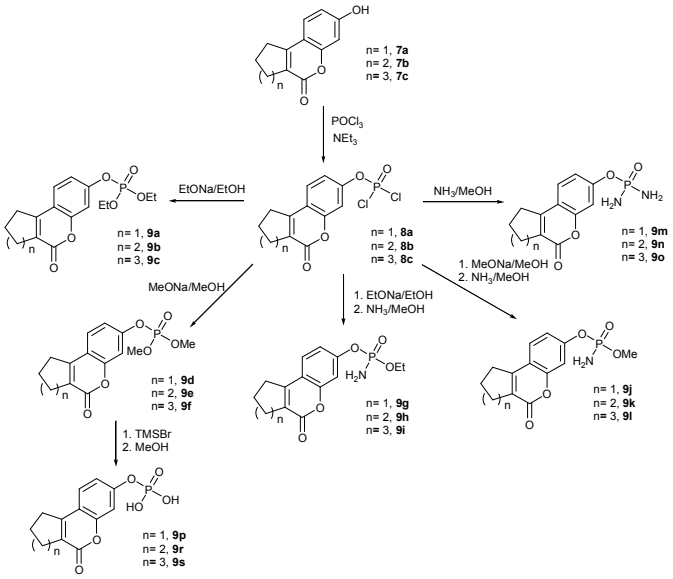
The synthesis of coumarin and their derivatives has been the subject of extensive research over many decades. Many convenient synthetic methods have been described including Pechmann,[10] Perkin,[11] Knoevenagel condensation,[12] Reformatsky[13] and Wittig reactions.[14] The Pechmann condensation is one of the most common procedures for the preparation of coumarin and its derivatives. This method involves the reaction between a β-ketoester and phenol in the presence of an acidic catalyst. With substituted phenols, electron-donating groups are required for the straightforward preparation of the coumarin ring. Sulfuric acid and other Brønsted and Lewis acids, including $ZrCl_4$,[15] $InCl_3$,[16] PPA,[17] $BiCl_3$,[18] $Yb(OTf)_3$,[19] p-TsOH[20] and AgOTf,[21] are widely used as catalysts.

As a result of the previous molecular modeling studies, we decided to synthesize 7-hydroxy-2,3-dihydro-1*H*-cyclopenta[c]chromen-4-one, 3-hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[c]chromen-6-one and 3-hydroxy-8,9,10,11-tetrahydro-7*H*-cyclohepta[c]chromen-6-one modified with different phosphate groups. First, we prepared the corresponding β-ketoesters as the starting materials for the synthesis of tricyclic coumarin scaffolds. Ethyl 2-oxocyclopentanecarboxylate and ethyl 2-oxocyclohexanecarboxylate were obtained *via* Dieckmann cyclization from the corresponding adipic or pimelic acid diethyl esters by treatment with triethylamine and aluminium trichloride. Ethyl 2-oxocycloheptacarboxylate was prepared via the reaction of commercially available cycloheptanone with diethyl carbonate in the presence of sodium hydride. Treatment of ethyl 2-oxocyclopentanecarboxylate, ethyl 2-oxocyclohexanecarboxylate or ethyl 2-oxocycloheptacarboxylate with resorcin in concentrated sulfuric acid afforded the Pechmann condensation products (**7a-c**) in good yields. The detailed synthesis of the scaffolds is presented in Scheme 1.



Scheme 1 Synthesis of coumarin scaffolds.

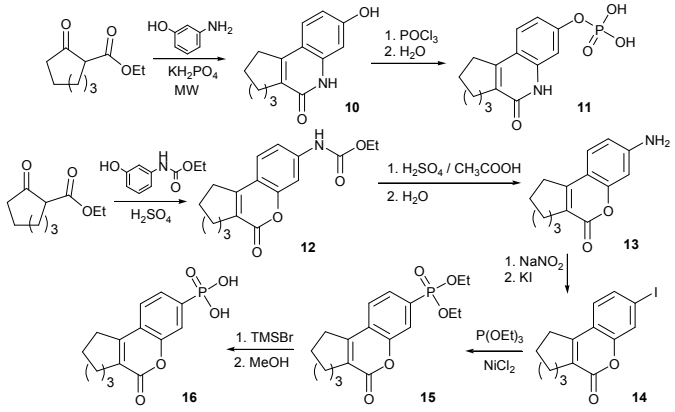
Next, functionalization of the hydroxyl group of the synthesized scaffolds was performed. The stable and readily available 7-hydroxy-2,3-dihydro-1*H*-cyclopenta[c]chromen-4-one, 3-hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[c]chromen-6-one and 3-hydroxy-8,9,10,11-tetrahydro-7*H*-cyclohepta[c]chromen-6-one **7a-c** were treated with $POCl_3$ in the presence of triethylamine at 0°C to yield phosphoryl dichloride derivatives **8a-c**. Phosphoryl dichlorides **8a-c** were treated *in situ* at 0°C with nucleophilic agents including MeONa, EtONa or NH_3/MeOH in different stoichiometric ratios to obtain the corresponding phosphate coumarin derivatives **9a-o** in good yield (Scheme 2). Finally, compounds **9d**, **9e** and **9f** were transformed into the corresponding phosphoric acid derivatives **9p**, **9r** and **9s** by reaction with bromotrimethylsilane (TMSBr).



Scheme 2 Synthesis of phosphate coumarin derivatives **9a**-**9s**.

To further study the structure-activity relationships, we synthesized two additional compounds, **11** and **16**. The detailed synthesis is shown in Scheme 3. Preparation of 6-oxo-5,7,8,9,10,11-hexahydro-6*H*-cyclohepta[c]quinolin-3-yl dihydrogen phosphate **11** involved two steps: reaction of ethyl 2-oxocycloheptacarboxylate with 3-aminophenol in the presence of KH_2PO_4, as an acidic catalyst, to yield 3-hydroxy-5,7,8,9,10,11-hexahydro-cyclohepta[c]quinolin-6-one **10** and subsequent phosphorylation of its 3-hydroxyl group. 6-Oxo-6,7,8,9,10,11-hexahydro-cyclohepta[c]chromen-3-yl phosphonic acid **16** was obtained using reported methods to transform the functional groups. First, the synthesis of 3-amino-8,9,10,11-tetrahydro-7*H*-cyclohepta[c]chromen-6-one **13** was performed by following the procedure published by Atkins and Bliss.[22] Treatment of urethane-protected 3-aminophenol with ethyl 2-oxocycloheptacarboxylate in the presence of sulfuric acid afforded coumarin derivative **12** in good yield. Deprotection of 3-carbethoxy-8,9,10,11-tetrahydro-7*H*-

cyclohepta[c]chromen-6-one **12** was accomplished with a 1:1 mixture of concentrated sulfuric acid and acetic acid to yield compound **13**. Preparation of 3-iodo-8,9,10,11-tetrahydro-7*H*-cyclohepta[c]chromen-6-one **14** was achieved *via* the Sandmeyer reaction of compound **13** with sodium nitrite and potassium iodide.[23] Treatment of 3-iodo-8,9,10,11-tetrahydro-7*H*-cyclohepta[c]chromen-6-one **14** with triethyl phosphite in the presence of anhydrous nickel(II) chloride quantitatively afforded derivative **15**,[24] which was then hydrolyzed to 6-oxo-6,7,8,9,10,11-hexahydro-cyclohepta[c]chromen-3-yl phosphonic acid **16** using TMSBr.



Scheme 3 Synthesis of lactam **11** and phosphonic acid derivatives **16**.

For all studied compounds, the effects of scaffold modification through introduction of the lactam moiety and the phosphonic acid group in the 3-position of the coumarin are discussed.

STS enzyme assays

The aim of our work was to evaluate the structure-activity relationship of the synthesized coumarin derivatives with the STS enzyme. The effects of the ring size of the coumarin scaffolds, the presence of a lactone or lactam moiety and the type of phosphate group were studied. The affinity of all synthesized compounds for STS was determined using an *in vitro* STS assay, following reported methods.[25,26] For the *in vitro* assays, STS enzyme was extracted from human placenta and purified using 3-step chromatography. After purification, the fractions were used directly as an enzyme source in the *in vitro* activity assays. Table 1 shows a summary of the results.

No.	IC_{50}[μM]	No.	IC_{50}[μM]	No.	IC_{50}[μM]
9a	73.0±5.2	**9h**	111.0±7.6	**9o**	52.5±4.5
9b	92.5±6.4	**9i**	108.0±10.1	**9p**	36.4±3.2
9c	63.9±5.8	**9j**	87.3±7.1	**9r**	37.8±2.8
9d	74.7±6.2	**9k**	73.3±6.0	**9s**	21.5±3.2
9e	98.4±7.0	**9l**	159.2±19.4	**11**	79.2±8.2
9f	47.5±4.1	**9m**	87.8±7.7	**16**	76.5±7.9
9g	81.1±7.2	**9n**	110.5±8.4	**665-COUMATE**	1.0±0.3

Table 1 Activities of the synthesized compounds and a reference inhibitor (665-COUMATE) in STS enzyme assays

In the course of the investigation, we found that the coumarin derivatives with the highest activity contained a hydrophobic seven-membered ring in their structures. This correlation was observed for all synthesized compounds. Compounds **9p**, **9r** and **9s** exhibited the highest inhibitory properties toward STS with IC_{50} values of 36.4, 37.8 and 21.5 μM, respectively (IC_{50} value of 1.0 μM for 665-COUMATE as the reference compound).

Compounds **16** contained a phosphonic acid group in the 3-position and **11** with lactam moiety were much less effective compared to compound **9s**. It suggests that predicted a longer distance (confirmed by docking studies) of their functional groups to catalytic amino acid FGly75 may be responsible for a decreased ability of these analogs to interact with the active site of STS.

Cancer cell viability assay

We examined the effects of the synthesized compounds on the viability of MCF-7, MDA-MB-231 and MDA-MB-435S cancer cell lines. Compounds **9p**, **9r**, **9s** and **11** were assayed for their ability to inhibit the proliferation of three breast cancer cell lines *in vitro*. The results obtained from this screening are presented in Table 2. Three compounds, **9r**, **9s** and **11**, showed the highest potency against MCF-7, an estrogen receptor positive (ER+) cell line, with GI_{50} values of 24.7, 63.8 and 57.3 μM, respectively.

No.	GI_{50} [μM]		
	MCF-7	**MDA-MB-231**	**MDA-MB-435S**
9p	320.1±10.3	524.1±43.8	115.8±6.7
9r	24.7±1.4	138.7±5.5	114.8±8.2
9s	63.8±2.2	87.1±4.8	127.2±7.1
11	57.3±1.5	63.3±3.6	103.0±9.2

Table 2 Antiproliferative activities of selected compounds

Only compound **9p** did not show activity against this cell line. Its weaker influence on MCF-7 viability can be attributed to the much lower activity of compounds with 5-membered rings in the coumarin scaffolds, which was observed in the enzyme assays. When estrogen receptor negative (ER-) cell lines were used (MDA-MB-231 and MDA-MB-435S), almost all compounds demonstrated poor antiproliferative activity, with GI_{50} values of < 80 μM. Compound **11**, which contains a lactam moiety in its scaffold structure, did not show significantly higher activity. This compound exhibited moderate activity, with a GI_{50} of 63.3 μM.

Experimental

Materials and methods

Pimelic acid, adipic acid, cycloheptanone, resorcin, and phosphorus oxychloride, are commercially available from Aldrich. Ethyl 2-oxocyclopentanecarboxylate, ethyl 2-oxocyclohexanecarboxylate, and ethyl 2-

oxocycloheptanecarboxylate were synthesized using modified versions of the described procedures. Tetrahydrofuran was dried and distilled using standard procedures. Melting points (uncorrected) were determined with a Stuart Scientific SMP30 apparatus. NMR spectra were recorded on a Varian Gemini 500 MHz spectrometer. Chemical shifts are reported in ppm relative to the residual solvent peak ($CDCl_3$ = 7.26 ppm for ^1H, 77.0 ppm for ^{13}C, DMSO-d_6 2.49 ppm for ^1H, 39.5 ppm for ^{13}C) or to an external standard (85% H_3PO_4 = 0 for ^{31}P). Coupling constants are given in Hertz. IR spectra were measured on a Nicolet 8700. Elemental analysis was performed using CHNS-Carlo Erba EA-1108. Column chromatography was performed using silica gel 60 (230-400 mesh, Merck). Preparative thin-layer chromatography was performed with Polygram SIL G/UV_{254} silica gel (Macherey-Nagel).

General method for the synthesis of tricyclic coumarin modified by phosphoric acid diethyl or dimethyl ester groups

To an ice-cooled solution of phosphorus oxychloride (0.354 g, 2.3 mmol) in dry THF (7 mL) was added a solution of the corresponding tricyclic coumarin (2.3 mmol) in THF dropwise, followed by triethylamine (0.233 g, 2.3 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 1 h. The triethylamine hydrochloride precipitate was removed by filtration, and sodium alkoxide (4.6 mmol) (freshly prepared by the addition of methanol or ethanol to 60% NaH dispersed in mineral oil) was added. The reaction mixture was stirred for 30 min and a precipitate of NaCl formed. The solution was filtered, and the solvent was evaporated. The resulting residue was crystallized from 2-propanol or purified by column chromatography using CH_2Cl_2 as eluent to give the desired product.

4-oxo-1,2,3,4-tetrahydro-cyclopenta[c]chromen-7-yl diethyl phosphate 9a. Yield 37 %, mp 80-81 °C; ν_{max} (KBr)/cm^{-1} 1720, 1608, 1568, 1506, 1392, 1269, 1149, 1051, 1018, 897, 751, 666; ^1H NMR δ_H (500 MHz, DMSO) 1.26 (6H, t, *J* 7.3, CH_3), 2.10 (2H, quin, *J* 7.6, CH_2), 2.74 (2H, t, *J* 7.1, CH_2), 3.06 (2H, t, *J* 7.6, CH_2), 4.18 (4H, quin, *J* 7.8, CH_2), 7.21-7.27 (2H, m, Ar-H), 7.62 (1H, d, *J* 8.8, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 159.4, 156.5, 154.9, 152.6 (d, J_{P-C} 6.6), 127.5, 126.8, 117.0 (d, J_{P-C} 4.4), 116.2, 108.5 (d, J_{P-C} 5.3), 65.4 (d, J_{P-C} 6.2), 32.4, 30.9, 22.6, 16.6 (d, J_{P-C} 6.6); ^{31}P NMR δ_P (202 MHz, DMSO) -5.69. Anal. Calcd for: $C_{16}H_{19}O_6P$: C, 56.81; H, 5.66. Found: C, 56.94; H, 5.74.

6-oxo-7,8,9,10-tetrahydro-*6H*-benzo[c]chromen-3-yl diethyl phosphate 9b. Yield 41 %, oil; ν_{max} (KBr)/cm^{-1} 1720, 1608, 1573, 1508, 1388, 1261, 1159, 1048, 1016, 902, 749, 683; ^1H NMR δ_H (500 MHz, $CDCl_3$) 1.36 (6H t, *J* 7.1, CH_3), 1.76-1.88 (4H, m, CH_2), 2.56 (2H, t, *J* 6.1, CH_2), 2.75 (2H, t, *J* 5.9, CH_2), 4.17-4.29 (4H, m, CH_2), 7.14-7.19 (2H, m, Ar-H), 7.51 (1H, d, *J* 8.3, Ar-H); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 161.7, 152.9, 152.1 (d, J_{P-C} 6.6), 146.9, 124.6, 123.2, 117.6, 116.4 (d, J_{P-C} 5.3), 108.5 (d, J_{P-C} 5.7), 65.2 (d, J_{P-C} 6.1), 25.5, 24.2, 21.7, 21.5, 16.3 (d, J_{P-C} 7.0); ^{31}P NMR δ_P (202 MHz, $CDCl_3$) -5.63. Anal. Calcd for: $C_{17}H_{21}O_6P$: C, 57.95; H, 6.01. Found: C, 58.07; H, 6.09.

6-oxo-6,7,8,9,10,11-hexahydro-cyclohepta[c]chromen-3-yl diethyl phosphate 9c. Yield 35%, mp 88-90 °C; ν_{max} (KBr)/cm^{-1} 1703, 1606, 1566, 1508, 1381, 1267, 1157, 1050, 1007, 897, 764, 683; ^1H NMR δ_H (500 MHz, $CDCl_3$) 1.27 (6H, t, *J* 7.1, CH_3), 1.46-1.52 (2H, m, CH_2), 1.54-1.62 (2H, m, CH_2), 1.78-1.86 (2H, m, CH_2), 2.74-2.80 (2H, m, CH_2), 2.92-2.98 (2H, m, CH_2), 4.18 (4H, quin, *J* 7.3, CH_2), 7.18-7.24 (2H, m, Ar-H), 7.93 (1H, d, *J* 8.8, Ar-H); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 161.4, 154.1, 153.4, 152.5 (d, J_{P-C} 6.6), 127.7, 127.1, 117.4, 116.9 (d, J_{P-C} 4.8), 108.5 (d, J_{P-C} 5.3), 65.4 (d, J_{P-C} 6.1), 32.0, 28.1, 26.8, 25.8, 25.2, 16.6 (d, J_{P-C} 6.1); ^{31}P NMR δ_P (202 MHz, $CDCl_3$) -5.69. Anal. Calcd for: $C_{18}H_{23}O_6P$: C, 59.01; H, 6.33. Found: C, 59.15; H, 6.41.

4-oxo-1,2,3,4-tetrahydro-cyclopenta[c]chromen-7-yl dimethyl phosphate 9d. Yield 43 %, mp 147-148 °C; ν_{max} (KBr)/cm^{-1} 1714, 1608, 1568, 1508, 1395, 1273, 1136, 1020, 899, 783, 667; ^1H NMR δ_H (500 MHz, DMSO) 2.10 (2H, quin, *J* 7.6, CH_2), 2.74 (2H, t, *J* 7.3, CH_2), 3.05 (2H, t, *J* 7.6, CH_2), 3.83 (6H, d, *J* 11.7, CH_3), 7.21-7.29 (2H, m, Ar-H,), 7.61 (1H, d, *J* 8.3, Ar-H,); ^{13}C NMR δ_C (125 MHz, DMSO) 159.4, 156.5, 154.9, 152.5 (d, J_{P-C} 6.4), 127.5, 126.8, 117 (d, J_{P-C} 4.8), 116.4, 108.5 (d, J_{P-C} 5.3), 55.8 (d, J_{P-C} 6.6), 32.4, 30.9, 22.6; ^{31}P NMR δ_P (202 MHz, DMSO) -3.43. Anal. Calcd for: $C_{14}H_{15}O_6P$: C, 54.20; H, 4.87. Found: C, 54.35; H, 4.96.

6-oxo-7,8,9,10-tetrahydro-*6H*-benzo[c]chromen-3-yl dimethyl phosphate 9e. Yield 48 %, mp 79-81 °C; ν_{max} (KBr)/cm^{-1} 1703, 1610, 1574, 1506, 1387, 1288, 1144, 1024, 906, 775, 683; ^1H NMR δ_H (500 MHz, $CDCl_3$) 1.77-1.88 (4H, m, CH_2), 2.53-2.59 (2H, m, CH_2), 2.72-2.78 (2H, m, CH_2), 3.88 (6H, d, *J* 11.2, CH_3), 7.12-7.20 (2H, m, Ar-H), 7.52 (1H, d, *J* 7.3, Ar-H); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 161.6, 152.9, 151.9 (d, J_{P-C} 6.6), 146.8, 124.7, 123.3, 117.8, 116.3 (d, J_{P-C} 4.8), 108.5 (d, J_{P-C} 5.3), 55.5 (d, J_{P-C} 6.1), 25.5, 24.2, 21.7, 21.5; ^{31}P NMR δ_P (202 MHz, $CDCl_3$) -3.33. Anal. Calcd for: $C_{15}H_{17}O_6P$: C, 55.56; H, 5.28. Found: C, 55.69; H, 5.36.

6-oxo-6,7,8,9,10,11-hexahydro-cyclohepta[c]chromen-3-yl dimethyl phosphate 9f. Yield 44 %, mp 114-116 °C; ν_{max} (KBr)/cm^{-1} 1705, 1608, 1566, 1500, 1379, 1268, 1140, 1035, 897, 773, 683; ^1H NMR δ_H (500 MHz, $CDCl_3$) 1.56-1.62 (2H, m, CH_2), 1.62-1.70 (2H, m, CH_2), 1.86-1.93 (2H, m, CH_2), 2.86-2.94 (4H, m, CH_2), 3.88 (6H, d, *J* 11.2, CH_3), 7.16-7.20 (2H, m, Ar-H), 7.63 (1H, d, *J* 9.8, Ar-H); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 162.0, 153.5, 153.4, 152.2 (d, J_{P-C} 6.1), 128.2, 125.6, 117.5, 116.3 (d, J_{P-C} 4.8), 108.7 (d, J_{P-C} 5.7), 55.4 (d, J_{P-C} 6.1), 32.2, 28.4, 27.0, 25.8, 25.2; ^{31}P NMR δ_P (202 MHz, $CDCl_3$) -3.38. Anal. Calcd for: $C_{16}H_{19}O_6P$: C, 56.81; H, 5.66. Found: C, 56.98; H, 5.78.

General method for the synthesis of tricyclic coumarin modified by ethyl or methyl phosphoroamidate groups

To an ice-cooled solution of phosphorus oxychloride (0.354 g, 2.3 mmol) in dry THF (7 mL) was added a solution of the tricyclic coumarin (2.3 mmol) in THF dropwise, followed by triethylamine (0.233 g, 2.3 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 1 h. The triethylamine hydrochloride precipitate was removed by filtration, and

sodium alkoxide (2.3 mmol) (freshy prepared by addition of methanol or ethanol to 60% NaH dispersed in mineral oil) was added. After 30 min, a solution of NH_3 in methanol (5 mL) was added. Then, the reaction mixture was concentrated under vacuum. The resulting residue was dispersed in cold methanol and filtered, and solvent was evaporated under vacuum. The crude product was crystallized from ethyl acetate.

4-oxo-1,2,3,4-tetrahydro-cyclopenta[c]chromen-7-yl ethyl phosphoroamidate 9g. Yield 34 %, mp 187-189 °C; ν_{max} (KBr)/cm^{-1} 3340, 1710, 1610, 1566, 1508, 1392, 1261, 1151, 1030, 940, 883, 714, 667; ^{1}H NMR δ_H (500 MHz, DMSO) 1.25 (3H, t, *J* 7.1, CH_3), 2.10 (2H, quin, *J* 7.6, CH_2), 2.74 (2H, t, *J* 7.3, CH_2), 3.06 (2H, t, *J* 7.8, CH_2), 4.06 (2H, m, CH_2), 5.19 (2H, d, $J_{P\text{-}N}$ 7.3, NH_2), 7.17-7.25 (2H, m, Ar-H), 7.59 (1H, d, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 158.9, 156.1, 154.2, 153.2 (d, $J_{P\text{-}C}$ 6.2), 126.4, 125.4, 116.8 (d, $J_{P\text{-}C}$ 4.8), 114.7, 107.8 (d, $J_{P\text{-}C}$ 5.3), 62.2 (d, $J_{P\text{-}C}$ 5.7), 31.7, 21.9, 16.0 (d, $J_{P\text{-}C}$ 6.6); ^{31}P NMR δ_P (202 MHz, DMSO) 8.02. Anal. Calcd for: $C_{14}H_{16}NO_5P$: C, 54.37; H, 5.21; N, 4.53. Found: C, 54.22; H, 5.09; N, 4.69.

6-oxo-7,8,9,10-tetrahydro-*6H*-benzo[c]chromen-3-yl ethyl phosphoroamidate 9h. Yield 36 %, mp 167-170 °C; ν_{max} (KBr)/cm^{-1} 3354, 1695, 1609, 1558, 1506, 1387, 1257, 1161, 1034, 930, 891, 725, 683; ^{1}H NMR δ_H (500 MHz, DMSO) 1.24 (3H, t, *J* 7.1, CH_3), 1.70-1.77 (4H, m, CH_2), 2.40-2.49 (2H, m, CH_2), 2.76-2.78 (2H, m, CH_2), 4.02-4.07 (2H, m, CH_2) 5.18 (2H, d, $J_{P\text{-}N}$ 7.3, NH_2), 7.16-7.18 (2H, m, Ar-H), 7.70 (1H, d, *J* 8.0, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 160.5, 152.7 (d, $J_{P\text{-}C}$ 6.1), 152.0, 147.2, 125.1, 121.3, 116.6 (d, $J_{P\text{-}C}$ 4.8), 116.1, 107.6 (d, $J_{P\text{-}C}$ 4.8), 62.1 (d, $J_{P\text{-}C}$ 5.3), 24.7, 23.6, 21.1, 20.7, 16.0 (d, $J_{P\text{-}C}$ 7.0); ^{31}P NMR δ_P (202 MHz, DMSO) 8.03. Anal. Calcd for: $C_{15}H_{18}NO_5P$: C, 55.73; H, 5.61; N, 4.33. Found: C, 55.86; H, 5.69; N, 4.18.

6-oxo-6,7,8,9,10,11-hexahydro-cyclohepta[c]chromen-3-yl ethyl phosphoroamidate 9i. Yield 32 %, mp 163-165 °C; ν_{max} (KBr)/cm^{-1} 3316, 1687, 1608, 1566, 1508, 1382, 1246, 1169, 1039, 951, 889, 733, 681; ^{1}H NMR δ_H (500 MHz, DMSO) 1.25 (3H, t, *J* 7.1, CH_3), 1.40-1.60 (4H, m, CH_2), 1.75-1.90 (2H, m, CH_2), 2.70-2.80 (2H, m, CH_2), 2.85-3.00 (2H, m, CH_2), 4.00-4.10 (2H, m, CH_2), 5.19 (2H, d, $J_{P\text{-}N}$ 6.8, NH_2), 7.10-7.20 (2H, m, Ar-H), 7.88 (1H, d, *J* 8.8, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 161.6, 154.3, 153.8 (d, $J_{P\text{-}C}$ 6.2), 153.4, 127.0, 126.6, 117.4 (d, $J_{P\text{-}C}$ 5.3), 116.5, 108.5 (d, $J_{P\text{-}C}$ 5.3), 62.9 (d, $J_{P\text{-}C}$ 5.7), 32.0, 28.1, 26.7, 25.9, 25.3, 16.7 (d, $J_{P\text{-}C}$ 7.0); ^{31}P NMR δ_P (202 MHz, DMSO) 8.03. Anal. Calcd for: $C_{16}H_{20}NO_5P$: C, 56.97; H, 5.98; N, 4.15. Found: C, 57.13; H, 6.07; N, 4.32.

4-oxo-1,2,3,4-tetrahydro-cyclopenta[c]chromen-7-yl methyl phosphoroamidate 9j. Yield 36 %, mp 164-166 °C; ν_{max} (KBr)/cm^{-1} 3323, 1707, 1610, 1510, 1394, 1263, 1155, 1049, 964, 887, 793, 664; ^{1}H NMR δ_H (500 MHz, DMSO) 2.1 (2H, quin, *J* 7.6, CH_2), 2.73 (2H, t, *J* 7.3, CH_2), 3.04 (2H, t, *J* 7.6, CH_2), 3.68 (3H, d, *J* 11.2, CH_3), 5.24 (2H, d, $J_{P\text{-}N}$ 7.3, NH_2), 7.17-7.24 (2H, m, Ar-H,), 7.58 (1H, d, *J* 8.3, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 159.6, 156.7, 154.9, 153.8 (d, $J_{P\text{-}C}$ 5.7), 127.1, 126.2, 117.5 (d, $J_{P\text{-}C}$ 5.3), 115.5, 108.5 (d, $J_{P\text{-}C}$ 5.3), 53.7 (d, $J_{P\text{-}C}$ 5.7), 32.4, 30.9, 22.6; ^{31}P NMR δ_P (202 MHz, DMSO) 14.09. Anal. Calcd for: $C_{13}H_{14}NO_5P$: C, 52.89; H, 4.78; N, 4.74. Found: C, 52.75; H, 4.69; N, 4.55.

6-oxo-7,8,9,10-tetrahydro-*6H*-benzo[c]chromen-3-yl methyl phosphoroamidate 9k. Yield 28 %, mp 183-187 °C; ν_{max} (KBr)/cm^{-1} 3311, 1699, 1608, 1508, 1386, 1258, 1159, 1055, 960, 885, 789, 680; ^{1}H NMR δ_H (500 MHz, DMSO) 1.65-1.80 (4H, m, CH_2), 2.37-2.43 (2H, m, CH_2), 2.70-2.80 (2H, m, CH_2), 3.68 (3H, d, *J* 11.2, CH_3), 5.22 (2H, d, $J_{P\text{-}N}$ 7.3, NH_2), 7.14-7.20 (2H, m, Ar-H), 7.69 (1H, d, *J* 8.3, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 160.5, 152.6 (d, $J_{P\text{-}C}$ 6.1), 152.0, 147.1, 125.1, 121.4, 116.6 (d, $J_{P\text{-}C}$ 4.8), 116.2, 107.6 (d, $J_{P\text{-}C}$ 5.3), 53.0 (d, $J_{P\text{-}C}$ 5.7), 24.7, 23.6, 21.1, 20.7; ^{31}P NMR δ_P (202 MHz, DMSO) 9.35. Anal. Calcd for: $C_{14}H_{16}NO_5P$: C, 54.37; H, 5.21; N, 4.53. Found: C, 54.22; H, 5.10; N, 4.31.

6-oxo-6,7,8,9,10,11-hexahydro-cyclohepta[c]chromen-3-yl methyl phosphoroamidate 9l. Yield 34 %, mp 181-184 °C; ν_{max} (KBr)/cm^{-1} 3301, 1689, 1606, 1506, 1379, 1248, 1161, 1043, 947, 891, 777, 681; ^{1}H NMR δ_H (500 MHz, DMSO) 1.40-1.65 (4H, m, CH_2), 1.75-1.90 (2H, m, CH_2), 2.70-2.80 (2H, m, CH_2), 2.85-3.00 (2H, m, CH_2), 3.68 (3H, d, *J* 11.7, CH_3), 5.22 (2H, d, $J_{P\text{-}N}$ 7.3, NH_2), 7.10-7.20 (2H, m, Ar-H), 7.89 (1H, d, *J* 8.8, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 161.6, 154.3, 153.6 (d, $J_{P\text{-}C}$ 6.1), 153.4, 127.1, 126.7, 117.4 (d, $J_{P\text{-}C}$ 5.3), 116.6, 108.5 (d, $J_{P\text{-}C}$ 5.3), 53.7 (d, $J_{P\text{-}C}$ 5.7), 32.0, 28.1, 26.7, 25.9, 25.3; ^{31}P NMR δ_P (202 MHz, DMSO) 9.35. Anal. Calcd for: $C_{15}H_{18}NO_5P$: C, 55.73; H, 5.61; N, 4.33. Found: C, 55.61; H, 5.54; N, 4.15.

General method for the synthesis of phosphorodiamidate derivatives

To an ice-cooled solution of phosphorus oxychloride (0.354 g, 2.3 mmol) in dry THF (7 mL) was added a solution of the tricyclic coumarin (2.3 mmol) in THF dropwise, followed by triethylamine (0.233 g, 2.3 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 1 h. The precipitate of triethylamine hydrochloride was removed by filtration, and a solution of NH_3 in methanol (5 mL) was added. After concentration under vacuum, the crude product was crystallized from methanol to give the desired product.

4-oxo-1,2,3,4-tetrahydro-cyclopenta[c]chromen-7-yl phosphorodiamidate 9m. Yield 33 %, mp 188-190 °C; ν_{max} (KBr)/cm^{-1} 3340, 1691, 1610, 1558, 1504, 1391, 1216, 1130, 972, 895, 729; ^{1}H NMR δ_H (500 MHz, DMSO) 2.10 (2H, quin, *J* 7.3, CH_2), 2.74 (2H, t, *J* 7.3, CH_2), 3.05 (2H, t, *J* 7.6, CH_2), 4.53 (4H, d, $J_{P\text{-}N}$ 4.4, NH_2), 7.10-7.30 (2H, m, Ar-H), 7.54 (1H, d, *J* 8.0, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 159.8, 157.0, 155.1 (d, $J_{P\text{-}C}$ 6.6), 154.9, 126.7, 125.5, 118.1 (d, $J_{P\text{-}C}$ 5.3), 114.7, 108.8 (d, $J_{P\text{-}C}$ 4.8), 32.4, 30.8, 22.7; ^{31}P NMR δ_P (202 MHz, DMSO) 16.01. Anal. Calcd for: $C_{12}H_{13}N_2O_4P$: C, 51.43; H, 4.68; N, 10.00. Found: C, 51.57; H, 4.59; N, 10.18.

6-oxo-7,8,9,10-tetrahydro-*6H*-benzo[c]chromen-3-yl phosphorodiamidate 9n. Yield 46 %, mp 188-191 °C; ν_{max} (KBr)/cm^{-1} 3364, 1683, 1610, 1562, 1504, 1386, 1217, 1140, 962, 893, 725; ^{1}H NMR δ_H (500 MHz, DMSO) 1.68-1.75 (4H, m, CH_2), 2.37-2.48 (2H, m, CH_2), 2.71-2.74 (2H, m, CH_2), 4.52 (4H, d, $J_{P\text{-}N}$ 4.4, NH_2), 7.11-7.20 (2H, m, Ar-H), 7.61 (1H, d, *J*

8.8, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 161.4, 154.6 (d, J_{P-C} 6.6), 152.7, 148.0, 125.3, 121.4, 117.9 (d, J_{P-C} 5.3), 116.0, 108.5 (d, J_{P-C} 4.8), 25.3, 24.3, 21.8, 21.5; ^{31}P NMR δ_P (202 MHz, DMSO) 15.99. Anal. Calcd for: $C_{13}H_{15}N_2O_4P$: C, 53.06; H, 5.14; N, 9.52. Found: C, 53.23; H, 5.26; N, 9.72.

6-oxo-6,7,8,9,10,11-hexahydro-cyclohepta[c]chromen-3-yl phosphorodiamidate 9o. Yield 41 %, mp 187-189 °C (with decomposition); ν_{max} (KBr)/cm^{-1} 3232, 1703, 1605, 1564, 1504, 1381, 1203, 1142, 949, 908, 729; ^{1}H NMR δ_H (500 MHz, DMSO) 1.46-1.49 (2H, m, CH_2), 1.55-1.58 (2H, m, CH_2), 1.79-1.82 (2H, m, CH_2), 2.73-2.78 (2H, m, CH_2), 2.90-2.97 (2H, m, CH_2), 4.53 (4H, d, J_{P-N} 3.9, NH_2), 7.12-7.22 (2H, m, Ar-H), 7.83 (1H, d, J 8.8, Ar-H); ^{13}C NMR δ_C (125 MHz, DMSO) 161.8, 154.9 (d, J_{P-C} 6.6), 154.6, 153.4, 126.5, 126.2, 118.0 (d, J_{P-C} 5.3), 115.8, 108.8 (d, J_{P-C} 4.8), 32.1, 28.0, 26.7, 26.0, 25.4; ^{31}P NMR δ_P (202 MHz, DMSO) 16.02. Anal. Calcd for: $C_{14}H_{17}N_2O_4P$: C, 54.55; H, 5.56; N, 9.09. Found: C, 54.68; H, 5.63; N, 9.27.

General method for the synthesis of phosphoric acid derivatives

To an ice-cooled solution of derivative **9d**, **9e** or **9f** (1 mmol) in dry DCM (7 mL) was added TMSBr (4 mmol) dropwise. The reaction mixture was stirred under a nitrogen atmosphere for 1 h. After concentration under vacuum, 5 mL of methanol was added. All solvents were then evaporated, and the crude product was crystallized from ethyl acetate to give the desired product.

4-oxo-1,2,3,4-tetrahydro-cyclopenta[c]chromen-7-yl dihydrogen phosphate 9p. Yield 75 %, mp 217-219 °C (with decomposition); ν_{max} (KBr)/cm^{-1} 2652, 1653, 1601, 1560, 1504, 1392, 1219, 1134, 1084, 1019, 964, 882; ^{1}H NMR δ_H (500 MHz, DMSO) 2.09 (2H, quin, J 7.6, CH_2), 2.73 (2H, t, J 7.3, CH_2), 3.04 (2H, t, J 7.3, CH_2), 7.14-7.18 (2H, m, Ar-H), 7.55 (1H, d, J 8.3, Ar-H), 8.80-10.90 (2H, br s, OH); ^{13}C NMR δ_C (125 MHz, DMSO) 159.6, 156.7, 154.9, 154.3, 127.1, 126.0, 117.2 (d, J_{P-C} 5.3), 115.1, 108.1 (d, J_{P-C} 5.3), 32.3, 30.8, 22.6; ^{31}P NMR δ_P (202 MHz, DMSO) -5.45. Anal. Calcd for: $C_{12}H_{11}O_6P$: C, 51.08; H, 3.93. Found: C, 51.22; H, 3.85.

6-oxo-7,8,9,10-tetrahydro-*6H*-benzo[c]chromen-3-yl dihydrogen phosphate 9r. Yield 77 %, mp 244-248 °C (with decomposition); ν_{max} (KBr)/cm^{-1} 2655, 1651, 1604, 1564, 1504, 1388, 1223, 1138, 1105, 1038, 972, 885; ^{1}H NMR δ_H (500 MHz, DMSO) 1.69-1.78 (4H, m, CH_2), 2.39 (2H, t, J 5.9, CH_2), 2.73 (2H, t, J 5.9, CH_2), 7.13-7.15 (2H, m, Ar-H), 7.66 (1H, d, J 8.8, Ar-H), 7.80-9.80 (2H, br s, OH); ^{13}C NMR δ_C (125 MHz, DMSO) 161.2, 153.9 (d, J_{P-C} 5.7), 152.7, 147.9, 125.7, 121.9, 117.1 (d, J_{P-C} 5.3), 116.5, 107.9 (d, J_{P-C} 5.3), 25.3, 24.3, 21.8, 21.4; ^{31}P NMR δ_P (202 MHz, DMSO) -5.41. Anal. Calcd for: $C_{13}H_{13}O_6P$: C, 52.71; H, 4.42. Found: C, 52.59; H, 4.49.

6-oxo-6,7,8,9,10,11-hexahydro-cyclohepta[c]chromen-3-yl dihydrogen phosphate 9s. Yield 81 %, mp 230-233 °C (with decomposition); ν_{max} (KBr)/cm^{-1} 2656, 1645, 1599, 1556, 1504, 1381, 1236, 1140, 1109, 1029, 953, 875; ^{1}H NMR δ_H (500 MHz, DMSO) 1.47-1.56 (4H, m, CH_2), 1.75-1.85 (2H, m, CH_2), 2.76 (2H, t, J 4.9, CH_2), 2.94 (2H, t, J 4.9, CH_2), 7.13-7.15 (2H, m, Ar-H), 7.85 (1H, d, J 9.5, Ar-H), 7.95-8.90 (2H, br s, OH); ^{13}C NMR δ_C (125 MHz, DMSO) 161.6, 154.3, 153.4, 126.9, 126.6, 117.1 (d, J_{P-C} 5.3), 116.3, 108.2 (d, J_{P-C} 5.3), 32.0, 28.1, 26.7, 25.9, 25.3; ^{31}P NMR δ_P (202 MHz, DMSO) -5.43. Anal. Calcd for: $C_{14}H_{15}O_6P$: C, 54.20; H, 4.87. Found: C, 54.33; H, 4.96.

Preparation of 3-hydroxy-5,7,8,9,10,11-hexahydro-cyclohepta[c]quinolin-6-one 10

A mixture of 3-aminophenol (3 mmol), ethyl 2-oxocycloheptacarboxylate (3 mmol) and potassium dihydrogen phosphate (KH_2PO_4) (10 mol%) were placed in a teflon reactor. The mixture was irradiated for 8 min in a microwave at 400 W. After completion, the reaction mixture was poured into cold water (40 ml) and filtered. The crude product was crystallized from 2-propanol.

Yield 58%, mp 310-314 °C (with decomposition); ν_{max} (KBr)/cm^{-1} 3151, 1651, 1607, 1540, 1514, 1450, 1394, 1323, 1256, 1190, 847, 751, 692, 661; ^{1}H NMR δ_H (500 MHz, DMSO) 1.44-1.83 (6H, m, CH_2), 2.71-2.96 (4H, m, CH_2), 6.54-6.76 (2H, m, Ar-H), 7.65 (1H, d, J 8.8, Ar-H), 9.96 (1H, s, OH), 11.43 (1H, s, NH); ^{13}C NMR δ_C (125 MHz, DMSO) 162.1, 158.9, 150.0, 139.6, 129.3, 125.7, 112.5, 111.5, 100.3, 32.1, 27.8, 26.3, 25.7, 25.0.

Preparation of 6-oxo-5,7,8,9,10,11-hexahydro-6*H*-cyclohepta[c]quinolin-3-yl dihydrogen phosphate 11

To an ice-cooled solution of phosphorus oxychloride (0.354 g, 2.3 mmol) in dry THF (10 mL) was added a solution of 3-hydroxy-5,7,8,9,10,11-hexahydro-cyclohepta[c]quinolin-6-one **10** (2.3 mmol) in THF dropwise, followed by triethylamine (0.233 g, 2.3 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 1 h. The triethylamine hydrochloride precipitate was removed by filtration, and 3 mL of water was added. The reaction mixture was stirred for 2 h. The reaction mixture was then poured into 50 mL of water and extracted with hot ethyl acetate (3 x 20 mL). All organic layers were combined and dried over magnesium sulfate. The solvent was removed under vacuum, and the crude product was crystallized from 2-propanol.

Yield 20 %, mp 219-222 °C (with decomposition); ν_{max} (KBr)/cm^{-1} 2614, 1666, 1604, 1558, 1512, 1459, 1396, 1370, 1325, 1242, 1195, 944, 862, 745, 679, 624; ^{1}H NMR δ_H (500 MHz, DMSO) 1.40-1.45 (2H, m, CH_2), 1.50-1.60 (2H, m, CH_2), 1.75-1.85 (2H, m, CH_2), 2.83-2.98 (4H, m, CH_2), 3.90-6.80 (2H, br s, OH), 6.95-7.15 (2H, m, Ar-H), 7.81 (1H, d, J 9.3, Ar-H), 11.64 (1H, s, NH); ^{13}C NMR δ_C (125 MHz, DMSO) 162.3, 152.8, 150.1, 139.3, 132.6, 126.2, 116.3, 115.2, 106.7, 32.5, 28.2, 26.4, 25.9, 25.5; ^{31}P NMR δ_P (202 MHz, DMSO) -5.32. Anal. Calcd for: $C_{14}H_{16}NO_5P$: C, 54.37; H, 5.21; N, 4.53. Found: C, 54.55; H, 5.08; N, 4.27.

Preparation of 6-oxo-6,7,8,9,10,11-hexahydro-cyclohepta[c]chromen-3-yl phosphonic acid 16

To an ice-cooled solution of compound **15** (1 mmol) in dry DCM (10 mL) was added TMSBr (4 mmol) dropwise. The reaction mixture was stirred under a nitrogen atmosphere for 1

h. After concentration under vacuum, 5 mL of methanol was added. All solvents were then evaporated, and the crude product was washed with ethyl acetate to afford the desired product.

Yield 87 %, mp 229-234 °C (with decomposition); ν_{max} (KBr)/cm^{-1} 2611, 1665, 1605, 1558, 1512, 1460, 1398, 1368, 1241, 1196, 945, 863, 743, 680, 622; ^{1}H NMR δ_H (500 MHz, DMSO) 1.45-1.55 (2H, m, CH_2), 1.57-1.65 (2H, m, CH_2), 1.80-1.85 (2H, m, CH_2), 2.75-2.85 (2H, m, CH_2), 2.95-3.05 (2H, m, CH_2), 7.47-7.6 (2H, m, Ar-H), 7.95 (1H, d, *J* 3.9, Ar-H), 8.8-11.6 (2H, br s, OH); ^{13}C NMR δ_C (125 MHz, DMSO) 161.3, 153.8, 152.0 (d, J_{P-C} 19.8), 137.8 (d, J_{P-C} 180.5), 130.0, 126.6 (d, J_{P-C} 8.8), 125.5 (d, J_{P-C} 14.9), 121.8, 118.8 (d, J_{P-C} 10.5), 32.0, 27.8, 26.9, 25.7, 25.1; ^{31}P NMR δ_P (202 MHz, DMSO) 11.27. Anal. Calcd for: $C_{14}H_{15}O_5P$: C, 57.15; H, 5.14. Found: C, 57.01; H, 5.21.

Biological assays

Enzyme purification. STS was extracted from human placenta and purified to homogeneity following a multi-step chromatography protocol as previously described.[27]

***In vitro* activity assay.** The reaction mixture, at a final volume of 100 µl, contained 20 µM Tris-HCl pH 7.4, 3 mM NPS, various concentrations of inhibitor (0.1-200 µM) and 5 U of purified enzyme (1 U is the amount of enzyme that hydrolyzes 100 µM of NPS in 1 h at 37°C). The reaction was performed at 37°C for 15 min. It was halted by the addition of 100 µl of 1 M NaOH. The absorbance of the released p-nitrophenol was measured at 405 nm using a Microplate Reader Biotek ELx800 (SERVA). IC_{50} values were calculated using GraphPad Prism software. All measurements were performed in triplicate.

Viability assay. An MTT proliferation cytotoxicity assay (Sigma-Aldrich, Munich, Germany) was used to determine the effect of the compounds on the viability of cancer cells. Cells were seeded in 96-well microtiter plates and treated with 5×10^4 - 5×10^2 µM compound or the vehicle control. Optical density was measured using a Fluorostar Optima microplate reader (BMG Labtech, Ortenberg, Germany) at 560 nm with a 650 nm reference. Viability results are expressed as a percentage of the mean control. GI_{50} values were calculated using GraphPad Prism 5 software. (GraphPad Prism Software, GraphPad Software, San Diego, California, USA, www.graphpad.com). All experiments were performed in triplicate.

Conclusions

A convenient method for the synthesis and biological evaluation of 7-hydroxy-2,3-dihydro-1*H*-cyclopenta[c]chromen-4-one, 3-hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[c]chromen-6-one and 3-hydroxy-8,9,10,11-tetrahydro-7*H*-cyclohepta[c]chromen-6-one modified by various phosphorus moieties is presented. All compounds exhibited moderate activities toward STS enzyme as well as the MCF-7, MDA-MB-231 and MDA-MB-435S cancer cell lines. In the course of the investigation, the highest inhibitory properties were observed for the phosphorus acid esters of tricyclic coumarin. Three compounds, **9p**, **9r** and **9s,** demonstrated the highest activity in the STS enzyme assays with IC_{50} values of 36.4, 37.8 and 21.5 µM, respectively (IC_{50} value of 1.0 µM for 665-COUMATE, which was used as the reference). These results clearly showed that increasing the size of the hydrophobic ring in the coumarin scaffold significantly improved the biological activity of the synthesized compounds. The introduction of a phosphonic acid moiety did not increase the inhibitory properties of compound **16**. Although the mechanism of activity is unknown, docking studies conducted to explore the potential interactions between designed compounds and the active site of STS suggest a phosphate group transfer to FGly75 (diol) during the inactivation process. Furthermore, compound **11**, which contains a lactam moiety, was a weaker inhibitor in the STS enzyme assays. It also had moderate ability to inhibit the proliferation of MCF-7 and MDA-MB-231 cancer cell lines *in vitro*. The highest potency toward the MCF-7 cell lines was demonstrated by compound **9r** with GI_{50} value of 24.7 µM.

Acknowledgements

We gratefully acknowledge the National Science Centre (Poland) for financial support (grant no. 2011/03/D/NZ7/03985).

Notes

[a] Department of Organic Chemistry, Chemical Faculty, Gdansk University of Technology, Narutowicza 11/12, 80-952 Gdansk, Poland
[b] Department of Molecular Biology, Faculty of Biotechnology and Environment Sciences, The John Paul II Catholic University of Lublin, Konstantynów 1i, 20-708 Lublin, Poland
[c] Drug Discovery Department, Adamed Group, Pieńków 149, 05-152 Czosnów, Poland

Electronic Supplementary Information (ESI) available: [NMR spectra of compounds 9a-s, 11 and 16]. See DOI: 10.1039/b000000x/

References

1 N.J. Petrelli, E.P. Winer, J. Brahmer, S. Dubey, S. Smith, C. Thomas, L.T. Vahdat, J. Obel, N. Vogelzang, M. Markman, J.W. Sweetenham, D. Pfister, M.G. Kris, L.M. Schuchter, R. Sawaya, D. Raghavan, P.A. Ganz and B. Kramer, *J. Clin. Oncol.*, 2009, **27**, 6052.
2 C.R. Ireson, S.K. Chander, A. Purohit, D.C. Parish, L.W.L. Woo, B.V.L. Potter and M.J. Reed, *British Journal of Cancer*, 2004, **91**, 1399.
3 A. Purohit and P.A. Foster, *Journal of Endocrinology*, 2012, **212**, 99.
4 J.D. Townsley, D.A. Scheel and E.J. Rubin, *Journal of Clinical Endocrinology and Metabolism*, 1970, **31**, 670.
5 T.R. Evans, M.G. Rowlands, M.C. Silva, M. Law and R.C. Coombes, *Journal of Steroid Biochemistry and Molecular Biology*, 1993, **44**, 583.
6 P.A. Foster, M.J. Reed and A. Purohit, *Anti-Cancer Agents in Medicinal Chemistry*, 2008, **8**, 732.
7 A. Purohit, M.J. Reed, N.C. Morris, G.J. Williams and B.V.L. Potter, *Ann. N. Y. Acad. Sci.*, 1996, **784**, 40.
8 L.W.L. Woo, N.M. Howarth, A. Purohit, A.M. Hatem, M.J. Reed and B.V.L. Potter, *J. Med. Chem.*, 1998, **41**, 1068.

9 B. Malini, A. Purohit, D. Ganeshapillai, L.W.L. Woo, B.V.L. Potter and M.J. Reed, *J. Steroid Biochem. Mol. Biol.*, 2000, **75**, 253.
10 M. Maheswara, V. Siddaiah, G.L.V. Damu, Y.K. Rao and C.V. Rao, *J. Mol. Cat. A: Chem.*, 2006, **255**, 49.
11 W.H. Perkin, *J. Chem. Soc*, 1968, **21**, 181.
12 H. Valizadeh and H. Gholipour, *Synth. Commun.*, 2010, **40**, 1477.
13 D.C. Dittmer, Q. Li and D.V. Avilov, *J. Org. Chem.*, 2005, **70**, 4682.
14 P.K. Upadhyay and P. Kumar, *Tetrahedron Lett.*, 2009, **50**, 236.
15 G. Smitha and C.S. Reddy, *Synth. Commun.*, 2004, **34**, 3997.
16 D.S. Bose, A.P. Rudradas and M.H. Babu, *Tetrahedron Lett.*, 2002, **43**, 9195.
17 A.J. Nadkarni and N.A. Kudav, *Ind. J. Chem.*, 1981, **20B**, 719.
18 B.P. Sachin, P.B. Ramakrishna, P.R. Vivek and D.S. Shriniwas, *Synth. Commun.*, 2006, **36**, 525.
19 L.Wang, J. Xia, H. Tian, C. Qian and Y. Ma, *Ind. J. Chem.*, 2003, **42B**, 2097.
20 T. Sugino and K. Tanaka, *Chem. Lett.*, 2001, **4**, 110.
21 W. Jie, D. Tianning, S. Wei and L. Yizhe, *Synth. Commun.*, 2006, **39**, 2949.
22 R.L. Atkins and D.E. Bliss, *J. Org. Chem.*, 1978, **43**, 1975.
23 L. Jun, Q. Liu, J. Tang, F. Perdih, K. Kranjc, *Tetrahedron Letters*, 2012, **53**, 5248.
24 L. Peyrard, S. Chierici, S. Pinet, P. Batat, G. Jonusauskas ,N. Pinaud, P. Meyrand and I. Gosse, *Org. Biomol. Chem.*, 2011, **9**, 8489.
25 A.M. Vaccaro, R. Salvioli, M. Muscillo and L. Renola, *Enzyme*, 1987, **37**, 115.
26 L.W.L. Woo, T. Jackson, A. Putey, G. Cozier, P. Leonard, K.R. Acharya, S.K. Chander, A. Purohit, M.J. Reed and B.V.L. Potter, *J. Med. Chem*., 2010, **53**, 2155.
27 F.G. Hernandez-Guzman, T. Higashiyama, Y. Osawa and D. Ghosh, *J. Steroid. Biochem. Mol. Biol.*, 2001, **78**, 441.