

The examination is being carried out on the **following application documents**

Description, Pages

1-67 as published

Claims, Numbers

1-15 filed in electronic form on 05-04-2016

Drawings, Sheets

1/12-12/12 as published

The following documents have been cited in the international search report; the numbering will be adhered to in the rest of the procedure.

D1 WO 2012/061409 A1 (MARSHALL EDWARDS, INC) 10 May 2012
(2012-05-10)

D2 US 2012/251630 A1 (ALVERO ET AL.) 4 October 2012 (2012-10-04)

D3 WO 2005/049008 A1 (NOVOGEN RESEARCH PTY LTD) 2 June 2005
(2005-06-02)

D4 WO 2006/032086 A1 (NOVOGEN RESEARCH PTY LTD) 30 March 2006
(2006-03-30)

1 Novelty (Art. 54 EPC)

The presently claimed compounds of formula (I) are novel over the art because of the presence of the three substituents R¹⁰, R¹¹ and R¹² on the phenyl ring attached in position 3 of the benzopyranyl ring (none of these substituents can be hydrogen).

Document D1 discloses a family of compounds (those of formula (II)) overlapping with the presently claimed compounds. The mandatory presence of the three substituents R¹⁰, R¹¹ and R¹² can be considered as a novel selection thereof. The specific compounds of Document D1 do not possess the corresponding three substituents mentioned above.

Documents D2, D3 and D4 disclose compounds which do not have three substituents on the phenyl ring attached in position 3 of the benzopyranyl ring.

2 Inventive Step (Art. 56 EPC)

Document D1 is considered as the closest prior art since it discloses a family of compounds which are useful for the treatment of cancer and whose chemical structure overlap with that presently claimed.

As indicated above, the present family of compounds is considered as a selection of Document D1. This document indeed teaches that any compound of formula (II), i.e. also those having three substituents R_2 , R_5 and R_6) are useful in the treatment of cancer. Although the specific compounds cited in D1 have only one substituent on said phenyl ring, the skilled person would expect from compounds having two or three substituents the same effect. Document D2 which discloses structurally close compounds useful for the same therapeutic provide specific compounds with two substituents.

In the light of this prior art, the presence of an inventive step can only be acknowledged if it can be shown that the distinguishing feature, i.e. the presence of **three** substituents on the phenyl ring attached in position 3 of the benzopyran, leads to an unexpected effect.

The specification contains comparative data on page 63 but with a compound having only one substituent on the phenyl ring attached in position 3 of the benzopyranyl ring. It is considered that for instance compounds 3 or 7 of Document Document D2, which only differ from those presently claimed in the absence of a third substituent, are structurally closer to those presently claimed. Consequently comparative data with this type of compounds should be provided to establish the presence of an inventive step.

In the absence of these data, the requirements of Art. 56 EPC are not fulfilled.

3 Miscellaneous (e.g. Art. 83, 84 EPC)

Claim 1 encompasses an undetermined number of compounds which are defined only by reference to a desired functional activity, namely the compounds being "prodrugs". This functional term does not give a specific technical guidance for the ascertainment of the scope of the claim as to which derivatives are suitable "prodrugs" having the desired function without imposing an unreasonable burden, and thus can be seen as a mere invitation to the skilled person to perform a research program in order to find the

suitable variants in contravention of Article 84 EPC (cf. T 279/07, point 2.1 of the decision). Further, there is no general common knowledge of this kind available to the skilled person, and the application does not provide sufficient disclosure (Article 83 EPC).

Claim 9 and 10 include all the features of claim 1. Hence, these claims should be reformulated as a claim dependent on claim 1 (see Rule 43(4) EPC and Guidelines F-IV, 3.4).

European Patent Office
D-80298 München
GERMANY

5 February 2017

By online filing

Dear Sirs

European Patent Application No. 15746211.0-1452
Novogen Ltd.
Our ref: YCJ79790P.EPP

Reference is made to the communication pursuant to Rules 70(2) and 70a(2) EPC dated 28 July 2016, deemed received 7 August 2016, which set a deadline of six months from notification of the communication for confirming that the applicant wishes to proceed with the application and for responding to the issues raised in the European search opinion.

In response, the applicant confirms that it does wish to proceed further with this application, and looks forward to receiving the results of substantive examination in due course.

In addition, we provide the following comments and amendments in reply to the issues raised in the European search opinion.

Amendments

New claims 1-15 are enclosed to replace the claims at present on file. A tracked changes version of the claims is also attached for ease of reference, to show the changes as made to the claims at present on file.

Claim 1 has been limited to cover certain specific options for the R¹⁰-R¹² groups, based on PCT claims 10 and 11. The remaining claims have been revised for consistency.

The new claims are fairly based on the PCT application as filed, as follows:

Claim	Basis
1	PCT claims 1, 10, 11
2	PCT claim 2
3	PCT claim 4
4	PCT claim 5

5	PCT claim 12
6	PCT claim 14
7	PCT claim 15
8	PCT claim 17
9	PCT claim 19
10	PCT claim 30
11	PCT claim 31
12	PCT claim 32
13	PCT claim 35
14	PCT claim 46
15	PCT claim 51

We also enclose new description pages 1, 3-6, 8, 11-15, 19-21, 28-33, 37, 40 and 67. The remaining description pages are unchanged.

A tracked changes copy of the full description is attached, to assist the examiner with seeing the changes made. The revisions made are to ensure the description is consistent with the claims and to remove the term "incorporated by reference", as well as acknowledging the cited art D1-D4.

It is submitted that no subject matter has been added that extends beyond the content of the application as filed and that the amendments therefore comply with Article 123(2) EPC.

In addition, no subject matter is abandoned by means of amendments made to the original claim set. The applicant reserves the right to reintroduce deleted subject matter into the present application and/or to pursue protection for this subject matter by the filing of divisional applications.

Comments

Inventive Step

In the European search opinion it was suggested that the claims were new over D1-D4 but lacked an inventive step over D1 in light of D2.

The search opinion noted that D1 discloses a broad family of compounds indicated as useful for the treatment of cancer. However, as noted by the examiner, the specific

compounds disclosed in D1 all have only one substituent on the aryl ring at position 3. Meanwhile, D2 discloses compounds that contain a mono- or di-substituted aryl ring at position 3.

The present claimed invention is directed to compounds which have a very specific tri-substituted aryl ring appended to position 3 of a benzopyran scaffold and a further aryl ring at position 4.

Specifically, the present claimed invention requires a tri-substituted aryl ring at position 3 and further requires that on this tri-substituted ring the R¹⁰ (para) substituent is OH, and that the R¹¹ and R¹² (ortho/meta) substituents are independently selected from the group consisting of: OH, OMe, C1-C4 alkyl and F.

This specific substitution pattern is not taught or suggested in the cited art.

There is no reason why, without hindsight, the skilled person would have modified the teachings of D1 or D2 to reach the claimed invention.

D1 leads the skilled person towards contemplating mono-substituted products, as these are what are exemplified. D1 certainly does not provide any teaching regarding the relevance of the substitution pattern for the aryl ring at position 3. There is nothing in D1 that even indicates that there might be any technical significance to the number of substituent groups.

In general, the skilled person is conservative and has no inventive activity. Why would he make a product more complex, with a more complicated substitution pattern, unless he was confident there would be an advantage associated with this? In fact, he would see that the specifically exemplified compounds of D1 all have only a single substituent group on the aryl ring at position 3 and would be led towards this arrangement.

Therefore the skilled person reading D1 has no prompting to genuinely contemplate using a tri-substituted ring, let alone the specific substitution pattern of the claimed invention.

Meanwhile, D2 leads the skilled person towards contemplating mono- or di-substituted products. D2 defines the R₂, R₅ and R₆ groups on the aryl ring at position 3 such that tri-substitution is not permitted; R₆ is hydrogen.

Thus to provide a tri-substituted product goes directly against the teachings of D2. D2 only teaches mono- and di-substituted products for use as being effective against cancer recurrence.

Therefore there is nothing in D2 that in any way indicates that there might be any reason to further increase the number of substituent groups. There is no hint that a tri-substitution pattern might be worth trying. Quite the opposite. The skilled person is told that he must not have tri-substitution. He is told that the R₆ group must be hydrogen.

Surely if a tri-substituted ring was obvious, the inventors of D2 would have thought of this? The fact that the authors of D2 explicitly teach only mono- and di-substituted

products gives the skilled person a very clear and direct teaching that these are what work.

Thus, without hindsight, the skilled person has no prompting at all to think about using a tri-substituted ring. That would be contrary to the teachings of D2.

Therefore when assessing D2 based on what it actually discloses and without impermissible use of hindsight, it cannot be seen as obvious to arrive at any product where there are three substituent groups on the aryl ring at position 3. Certainly it would not be obvious for the skilled person to reach a tri-substituted product with the specific substitution pattern of the claimed invention.

When considering whether or not claimed subject-matter constitutes an obvious solution to an objective technical problem, the question to be answered is whether or not the skilled person, in the expectation of solving the problem, would have modified the teaching in the closest prior art document in the light of other teachings in the prior art so as to arrive at the claimed invention (T 1014/07). So the point is not whether the skilled person **could have** arrived at the invention by modifying the prior art, or even whether the changes required are minor or major, but rather whether, in expectation of the advantages actually achieved (i.e. in the light of the technical problem addressed), he would have done so **because of promptings in the prior art** (T 219/87, T 455/94, T 414/98).

These promptings in the art are missing. In fact, D1 leads the skilled person towards mono-substitution and D2 leads the skilled person towards mono- or di-substitution and explicitly away from tri-substitution. Neither D1 nor D2 in any way prompts the skilled person to think about the use of a tri-substituted aryl ring at position 3 wherein on this tri-substituted ring the R¹⁰ (para) substituent is OH, and that the R¹¹ and R¹² (ortho/meta) substituents are independently selected from the group consisting of: OH, OMe, C1-C4 alkyl and F. That is not obvious from the cited art teachings.

Furthermore, and significantly, the specific substitution pattern as claimed is associated with an unexpected technical benefit.

As the examiner noted in the search opinion, the patent application as filed includes data which shows the benefit of the tri-substituted products as compared to mono-substituted products.

However, the examiner noted that there was no data regarding the benefit of the tri-substituted products as compared to a di-substituted product.

The examiner noted that compounds 3 and 7 of D2 could be seen as structurally closer to the claimed invention.

In response, in support of the technical effect for the tri-substituted materials, we therefore present **Annex A**, which sets out the results when four di-substituted compounds were tested and compared with a tri-substituted compound according to the invention. The first two of the di-substituted compounds that were tested correspond with compounds 3 and 7 of D2.

Annex A shows that the benzopyran derivatives with the specific claimed tri-substitution pattern are more effective in terms of their activity against a range of cancer cell types than products with a di-substitution pattern. Specifically, low IC50 values are seen in tests for activity across a range of cancer cell types, indicating that the compounds are strong candidates for use in treatment, because they are effective at low concentrations.

In particular, from Annex A it can be seen that whilst all of the tested compounds had some effect against cancer cells, for the tri-substituted product according to the invention (which is compound 9 in the present application) the IC50 value was 0.5 µM or less for every cancer cell type. Seven different cancer cell lines were tested. In contrast, for the di-substituted materials, the IC50 values ranged from 0.8 to greater than 30 µM. Thus none of the di-substituted products was as effective against any individual cell line, nor was any of the substituted products as consistently effective across the range of cell lines.

In fact, for every cancer cell type the IC50 value for the most effective of the tested di-substituted materials was at least twice as high as the IC50 value for for the tri-substituted product according to the invention. Therefore for all of the cancer cell types tested, the tri-substituted product was always significantly more effective than the di-substituted products.

When comparing the compounds SBP-391 and SBP-392 that are equivalent to compounds 3 and 7 of D2 with the product of the invention, it can be seen that for every cancer cell type the IC50 value for the di-substituted materials according to D2 was at least 2.4 times as high as the IC50 value for for the tri-substituted product according to the invention.

Thus the benzopyran derivatives with the specific claimed tri-substitution pattern are technically beneficial as compared to both mono- substituted materials (as shown in the application as filed) and di-substituted materials (as shown in Annex A). This therefore supports the fact that the claimed materials are non-obvious from D1 and D2, which do not disclose, teach or suggest the claimed benzopyran derivatives with their specific claimed tri-substitution pattern.

As the examiner will appreciate, the case law of the Boards of Appeal have established that to determine whether the claimed invention, starting from the closest prior art and the objective technical problem, would have been obvious to the skilled person, it is necessary to apply the "could-would approach". This means asking not whether the skilled person **could** have carried out the invention, but whether he **would** have done so in the hope of solving the underlying technical problem or in the expectation of some improvement or advantage (T 2/83, OJ 1984, 265; T 90/84, T 7/86, OJ 1988, 381; T 200/94, T 885/97).

There is absolutely no teaching in D1 or D2 which is such that the skilled person would have modified the prior art benzopyran derivatives such that they instead had a tri-substitution on the aryl ring at position 3. There certainly is no reason why the skilled person would have been motivated to use tri-substitution at this ring with the substitution pattern as claimed, wherein the R¹⁰ (para) substituent is OH, and the R¹¹ and R¹² (ortho/meta) substituents are independently selected from the group consisting of: OH, OMe, C1-C4 alkyl and F.

There is nothing in the cited art that would give the skilled person an expectation of some improvement or advantage associated with this specific claimed tri-substitution pattern. To the contrary, reading D1 and D2 leads the skilled person towards mono-and di-substitution on the aryl ring at position 3.

There is no reason from D1 or D2, when read alone or in combination, to believe that the particular claimed benzopyran derivatives would be notably effective in terms of their activity against a range of cancer cell types.

It is of course well established by the case law of the Boards of Appeal that an *ex post facto* approach must be avoided when assessing inventive step. This is re-affirmed in the Guidelines for Examination, G-VII, 8. This applies especially to inventions which at first sight seem obvious, to combination inventions and where the proposed solution is supposedly "simple". Correct application of the problem and solution approach avoids this inadmissible *ex post facto* analysis which draws on knowledge of the invention (T 24/81, OJ 1983, 133; T 564/89, T 645/92, T 795/93).

When assessed without hindsight, it can be appreciated that the claimed invention is non-obvious. Neither D1 nor D2 points the skilled person towards the specific benzopyran derivatives that are claimed. There is no motivation provided to change the substitution pattern on the aryl ring at position 3, and certainly not to be the specific combination of groups now required.

Accordingly, there is an inventive step over D1 and D2.

Formal Matters - Clarity

It was suggested that claim 1 was unclear due to the term "prodrugs", which was stated as providing a definition by reference to functional activity without there being a definition as to which derivatives are "prodrugs".

In claim 1 the term "prodrugs" has been deleted and therefore this objection is addressed.

It was suggested that claims 9 and 10 should depend from claim 1, because they include all the features of claim 1.

In the new claims, claim 9 and claim 10 are both presented as dependent from claim 1. Therefore this objection is addressed

Formal Matters - Description

The description has been revised to be consistent with the claims, to remove the term "incorporated by reference", as well as to acknowledge the cited art D1-D4.

Conclusion

It is submitted that the present response addresses all of the issues raised to date and that the application is in allowable form. We therefore request issuance of the Rule 71(3) EPC Communication.

However, in the event that the examiner has any outstanding concerns we look forward to receipt of a first Communication under Article 94 (3) EPC.

If at any stage during the examination of this application the examiner contemplates refusal of the application Oral Proceedings are hereby requested as a precaution. However, we would prefer that the application progresses via the usual channels of Communications under Article 94(3) EPC and/or informal telephone conversations.

We look forward to the results of substantive examination in due course.

Yours faithfully

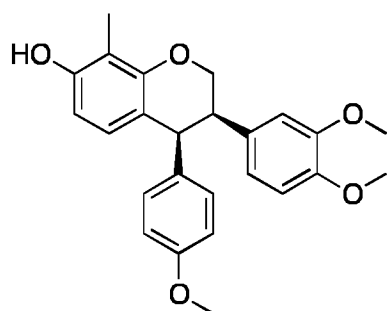
(signed electronically)

Yvonne C Johnson – Professional Representative
Barker Brettell LLP

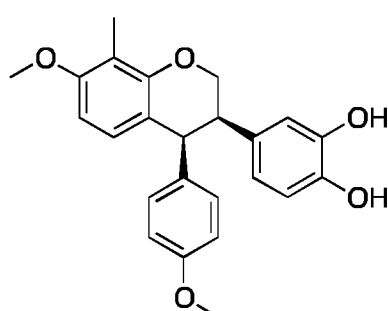
ANNEX A

A number of compounds, as set out below, were synthesised and tested for anti-proliferative activity. These compounds were chosen to compare the efficacy of benzopyrans that contain a di-substituted aryl ring at position 3 with benzopyrans that contain a tri-substituted aryl ring at position 3.

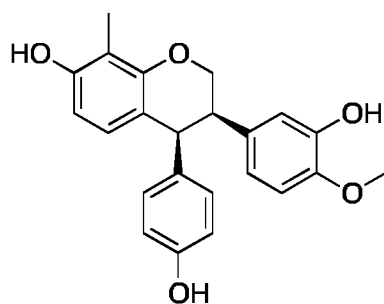
GAD-305 is compound 9 disclosed in the patent application PCT AU2015/050040 as filed.



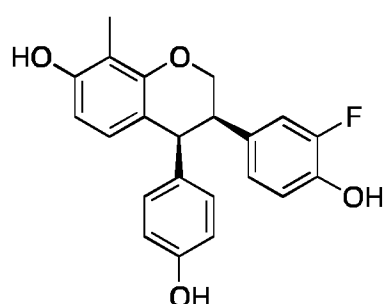
SBP-391



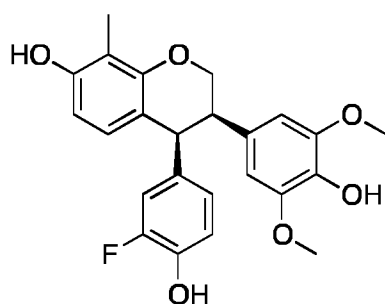
SBP-392



SBP-393



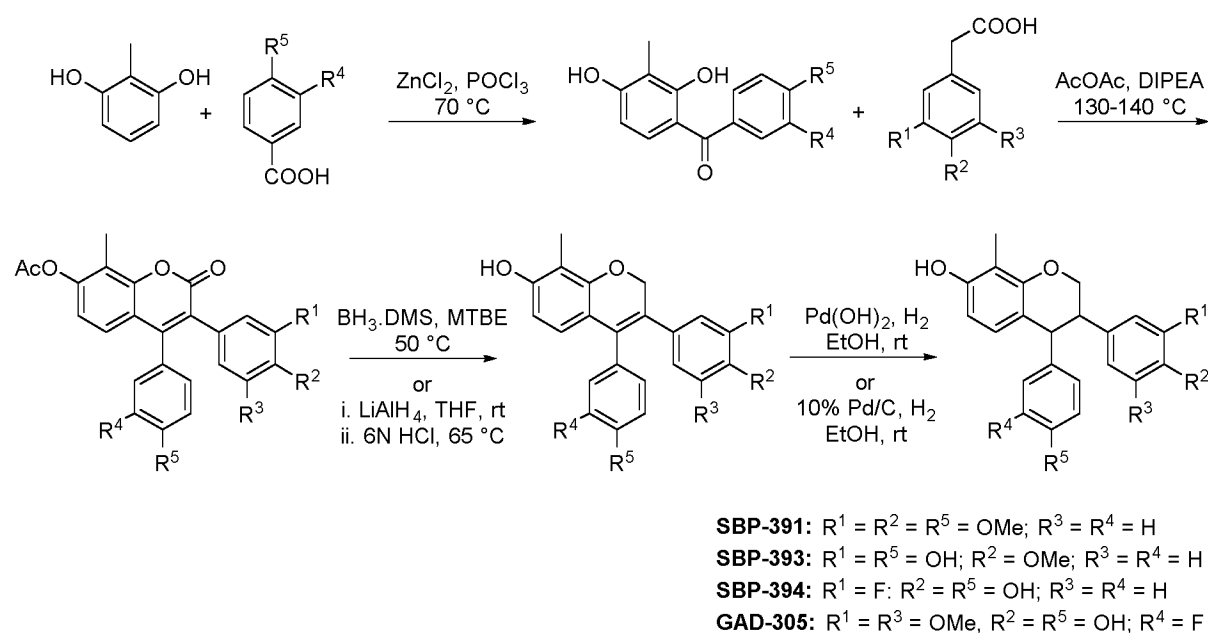
SBP-394



GAD-305

Compound Synthesis

Scheme 1. Preparation of Compounds SBP-391, SBP-393, SBP-394 and GAD-305

*Preparation of (2,4-dihydroxy-3-methylphenyl)(4-methoxyphenyl)methanone*

A solution of 4-methoxybenzoic acid (2.45 g, 16.1 mmol) in POCl_3 (10 mL) was stirred at room temperature for 10 minutes. Then 2-methylresorcinol (2.00 g, 16.1 mmol) was added, followed by ZnCl_2 (4.83 g, 35.4 mmol). The reaction mixture was heated to 70 °C for 2 h. After completion of the reaction based on TLC, the reaction mixture was cooled to room temperature, then poured into crushed ice and stirred for 10 minutes. The resulting precipitate was filtered, washed with water and petroleum ether and dried to obtain (2,4-dihydroxy-3-methylphenyl)(4-methoxyphenyl)methanone (3.50 g, 84%). LCMS: m/z 259.38 $[\text{M}+\text{H}]^+$.

Other analogues prepared by this method:

(2,4-dihydroxy-3-methylphenyl)(4-hydroxyphenyl)methanone (91%). LCMS: m/z 245.34 $[\text{M}+\text{H}]^+$.

(2,4-dihydroxy-3-methylphenyl)(3-fluoro-4-hydroxyphenyl)methanone (93%). LCMS: m/z 263.37 $[\text{M}+\text{H}]^+$.

Preparation of 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2-oxo-2H-chromen-7-yl acetate

To a stirred solution of (2,4-dihydroxy-3-methylphenyl)(4-methoxyphenyl)methanone (1.00 g, 3.87 mmol) in Ac_2O (5 mL) was added 2-(3,4-dimethoxyphenyl)acetic acid (760

mg, 3.87 mmol), followed by DIPEA (3.1 mL, 18 mmol) was added drop wise over a period of 20 minutes. The reaction mixture was heated to 140 °C and stirred for 16 h. The progress of the reaction was monitored by TLC and LCMS. After complete consumption of the starting material, the reaction mixture was poured into ice cold water and extracted with ethyl acetate. The organic layer was washed with brine solution, dried over sodium sulphate and concentrated under reduced pressure to obtain the crude product. The crude compound was purified by column chromatography on 100-200 mesh silica gel, using 20-25% ethyl acetate in petroleum ether as an eluent, to afford 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2-oxo-2H-chromen-7-yl acetate (600 mg, 34%). LCMS: m/z 461.25 $[M+H]^+$.

Other analogues prepared by this method:

4-(7-acetoxy-3-(3-acetoxy-4-methoxyphenyl)-8-methyl-2-oxo-2H-chromen-4-yl)phenyl acetate (16%). LCMS: m/z 517.29 $[M+H]^+$.

4-(7-acetoxy-3-(4-acetoxy-3-fluorophenyl)-8-methyl-2-oxo-2H-chromen-4-yl)phenyl acetate (16%). LCMS: m/z 505.30 $[M+H]^+$.

3-(4-acetoxy-3,5-dimethoxyphenyl)-4-(4-acetoxy-3-fluorophenyl)-8-methyl-2-oxo-2H-chromen-7-yl acetate (40%). LCMS: m/z 565.43 $[M+H]^+$.

Preparation of 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2H-chromen-7-ol

To a stirred solution of 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2-oxo-2H-chromen-7-yl acetate (0.60 g, 1.3 mmol) in MTBE (12 mL) was added $BH_3 \cdot DMS$ (8.4 mL, 140 mmol) drop wise at room temperature. The mixture was stirred at 50 °C for 2 h. The progress of the reaction was monitored by TLC and LCMS. After complete consumption of the starting material, the reaction mixture was cooled to room temperature and poured into ice cold 6 N HCl solution (50 mL). The reaction mixture was extracted with ethyl acetate, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2H-chromen-7-ol (0.6 g, crude) which was used without further purification. LCMS: m/z 405.44 $[M+H]^+$.

Other analogues prepared by this method:

3-(3-hydroxy-4-methoxyphenyl)-4-(4-hydroxyphenyl)-8-methyl-2H-chromen-7-ol (82%). LCMS: m/z 377.25 $[M+H]^+$.

3-(3-fluoro-4-hydroxyphenyl)-4-(4-hydroxyphenyl)-8-methyl-2H-chromen-7-ol (91%). LCMS: m/z 365.21 $[M+H]^+$.

Preparation of 4-(3-fluoro-4-hydroxyphenyl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-8-methyl-2H-chromen-7-ol

3-(4-acetoxy-3,5-dimethoxyphenyl)-4-(4-acetoxy-3-fluorophenyl)-8-methyl-2-oxo-2H-chromen-7-yl acetate (10.0 g, 17.7 mmol) in THF (50 mL) was added to a stirred solution of LAH (3.36 g, 95.6 mmol) in THF (50 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 8 h. 6 N HCl (80 mL) was added slowly, and then the reaction mixture was heated at 60 °C for 12 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature. The organic layer was separated and the aqueous layer

was extracted with ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford the crude product. The crude compound was triturated with ethanol to afford 4-(3-fluoro-4-hydroxyphenyl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-8-methyl-2H-chromen-7-ol (3.0 g, 40%). LCMS: m/z 425.42 $[M+H]^+$.

Preparation of SBP-391, 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methylchroman-7-ol

A stirred solution of 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2H-chromen-7-ol (0.60 g, 1.5 mmol) in ethanol (10 mL) was purged with nitrogen. Then 20% Pd(OH)₂ (200 mg) was added and reaction mixture was stirred under a hydrogen balloon for 16 h. The progress of the reaction was monitored by LCMS. After complete consumption of the starting material, the reaction mixture was filtered through a bed of Celite and washed with ethanol. The resulting filtrate was concentrated under reduced pressure to give the crude product. The crude compound was purified by Prep-HPLC to afford 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methylchroman-7-ol (62 mg, 10%) as an off white solid.

¹H NMR (400 MHz, DMSO-d₆): δ 6.72 (d, J = 8.0 Hz, 1H), 6.66 (d, J = 8.4 Hz, 2H), 6.54-6.48 (m, 3H), 6.34 (d, J = 8.4 Hz, 1H), 6.30 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 6.21 (d, J = 2.0 Hz, 1H), 4.37-4.18 (m, 3H), 3.67 (s, 3H), 3.65 (s, 3H), 3.48 (s, 3H), 3.45-3.41 (m, 1H), 2.04 (s, 3H). LCMS: m/z 407.49 $[M+H]^+$.

Preparation of SBP-393, 3-(3-hydroxy-4-methoxyphenyl)-4-(4-hydroxyphenyl)-8-methylchroman-7-ol

A stirred solution of 3-(3-hydroxy-4-methoxyphenyl)-4-(4-hydroxyphenyl)-8-methyl-2H-chromen-7-ol (0.60 g, 1.6 mmol) in ethanol (10 mL) was purged with nitrogen. Then 10% Pd on carbon (300 mg) was added and the reaction mixture was stirred under a hydrogen (50 psi) for 16 h. The progress of the reaction was monitored by LCMS. After complete consumption of the starting material, the reaction mixture was filtered through a bed of Celite and washed with ethanol. The resulting filtrate was concentrated under reduced pressure to give the crude product. The crude compound was purified by Prep-HPLC to afford 3-(3-hydroxy-4-methoxyphenyl)-4-(4-hydroxyphenyl)-8-methylchroman-7-ol (42 mg, 7%) as an orange solid.

¹H NMR (400 MHz, DMSO-d₆): δ 9.04 (br s, 2H), 8.83 (br s, 1H), 6.68 (d, J = 8.0 Hz, 1H), 6.48 (d, J = 8.4 Hz, 1H), 6.45 (d, J = 8.4 Hz, 2H), 6.36 (d, J = 8.8 Hz, 2H), 6.32 (d, J = 8.0 Hz, 1H), 6.22 (d, J = 2.0 Hz, 1H), 6.11 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 4.25-4.18 (m, 2H), 4.09 (br d, J = 5.6 Hz, 1H), 3.69 (s, 3H), 3.39-3.34 (m, 1H), 2.03 (s, 3H). LCMS: m/z 379.01 $[M+H]^+$.

Other analogues prepared by this method:

SBP-394, 3-(3-fluoro-4-hydroxyphenyl)-4-(4-hydroxyphenyl)-8-methylchroman-7-ol (9%).

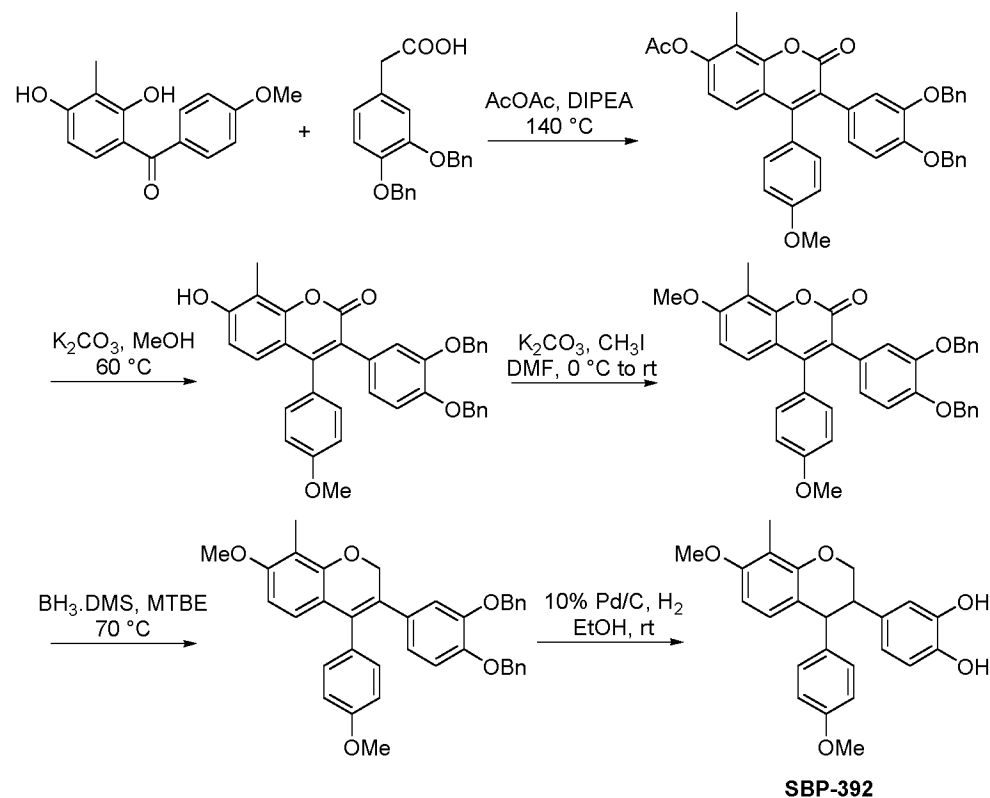
¹H NMR (400 MHz, DMSO-d₆): δ 9.56 (br s, 1H), 9.05 (br s, 2H), 6.71 (t, J = 8.8 Hz, 1H), 6.49-6.31 (m, 8H), 4.27-4.17 (m, 2H), 4.10 (d, J = 5.2 Hz, 1H), 3.42-3.36 (m, 1H), 2.03 (s, 3H). LCMS: m/z 367.45 $[M+H]^+$.

GAD-305, 4-(3-fluoro-4-hydroxyphenyl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-8-methylchroman-7-ol (60%).

¹H NMR (400 MHz, DMSO-d₆): δ 9.53 (br s, 1H), 9.12 (br s, 1H), 8.13 (br s, 1H), 6.69 (t, J = 8.8 Hz, 1H), 6.52 (d, J = 8.4 Hz, 1H), 6.35 (d, J = 8.4 Hz, 1H), 6.27-6.23 (m,

2H), 5.67 (s, 2H), 4.29-4.12 (m, 3H), 3.54 (s, 6H), 3.40-3.33 (m, 1H), 2.04 (s, 3H).
LCMS: m/z 427.00 $[M+H]^+$.

Scheme 2. Preparation of Compound SBP-392



Preparation of 3-(3,4-bis(benzyloxy)phenyl)-4-(4-methoxyphenyl)-8-methyl-2-oxo-2H-chromen-7-yl acetate

To a stirred solution of (2,4-dihydroxy-3-methylphenyl)(4-methoxyphenyl)methanone (518 mg, 2.01 mmol) in Ac_2O (7.5 mL) was added 2-(3,4-bis(benzyloxy)phenyl)acetic acid (700 mg, 2.01 mmol), followed by DIPEA (3.0 mL, 17 mmol) was added dropwise over a period of 20 minutes. The reaction mixture was heated to 140 °C and stirred for 18 h. The progress of the reaction was monitored by TLC and LCMS. After complete consumption of the starting material, the reaction mixture was poured into ice cold water and extracted with ethyl acetate. The organic layer was washed with brine solution, dried over sodium sulphate and concentrated under reduced pressure to obtain crude 3-(3,4-bis(benzyloxy)phenyl)-4-(4-methoxyphenyl)-8-methyl-2-oxo-2H-chromen-7-yl acetate (550 mg, crude) which was used without further purification. LCMS: m/z 613.03 $[M+H]^+$.

Preparation of 3-(3,4-bis(benzyloxy)phenyl)-7-hydroxy-4-(4-methoxyphenyl)-8-methyl-2H-chromen-2-one

To a stirred solution of 3-(3,4-bis(benzyloxy)phenyl)-4-(4-methoxyphenyl)-8-methyl-2-oxo-2H-chromen-7-yl acetate (500 mg, 0.816 mmol) in methanol (10 mL) was added K_2CO_3 (350 mg, 2.53 mmol) at room temperature. The reaction mixture was heated to 60 °C and stirred for 18 h. The progress of the reaction was monitored by TLC and LCMS. After complete consumption of the starting material, the reaction mixture was poured into ice cold water and extracted with ethyl acetate. The organic layer was washed with brine solution, dried over sodium sulphate and concentrated under reduced pressure to obtain the crude product. The crude compound was purified by column

chromatography on silica gel to afford 3-(3,4-bis(benzyloxy)phenyl)-7-hydroxy-4-(4-methoxyphenyl)-8-methyl-2H-chromen-2-one (100 mg, 21%). LCMS: m/z 571.47 $[M+H]^+$.

Preparation of 3-(3,4-bis(benzyloxy)phenyl)-7-methoxy-4-(4-methoxyphenyl)-8-methyl-2H-chromen-2-one

To a stirred solution of 3-(3,4-bis(benzyloxy)phenyl)-7-hydroxy-4-(4-methoxyphenyl)-8-methyl-2H-chromen-2-one (600 mg, 1.05 mmol) in DMF (5 mL) at room temperature was added K_2CO_3 (435 mg, 3.15 mmol). The mixture was stirred at room temperature for 30 min. To this mixture, methyl iodide (0.20 mL, 3.2 mmol) was added dropwise at 0 °C. The mixture was allowed to warm to room temperature and was stirred for 16 h. After complete consumption of the starting material, ice cold water was added and the reaction mixture was extracted with ethyl acetate. The organic layer was washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product. The crude product was purified by flash column chromatography on silica gel to afford 3-(3,4-bis(benzyloxy)phenyl)-7-methoxy-4-(4-methoxyphenyl)-8-methyl-2H-chromen-2-one (200 mg, 33%). LCMS: m/z 585.34 $[M+H]^+$.

Preparation of 3-(3,4-bis(benzyloxy)phenyl)-7-methoxy-4-(4-methoxyphenyl)-8-methyl-2H-chromene

To a stirred solution of 3-(3,4-bis(benzyloxy)phenyl)-7-methoxy-4-(4-methoxyphenyl)-8-methyl-2H-chromen-2-one (0.20 g, 0.34 mmol) in MTBE (5 mL) was added $BH_3.DMS$ (2.8 mL, 30 mmol) drop wise at room temperature. The mixture was stirred at 50 °C for 2 h. The progress of the reaction was monitored by TLC and LCMS. After complete consumption of the starting material, the reaction mixture was cooled to room temperature and poured into ice cold 6 N HCl solution (20 mL). The reaction mixture was extracted with ethyl acetate, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford 3-(3,4-bis(benzyloxy)phenyl)-7-methoxy-4-(4-methoxyphenyl)-8-methyl-2H-chromene (0.2 g, crude) which was used without further purification. LCMS: m/z 571.47 $[M+H]^+$.

Preparation of SBP-392, 4-(7-methoxy-4-(4-methoxyphenyl)-8-methylchroman-3-yl)benzene-1,2-diol

A stirred solution of 3-(3,4-bis(benzyloxy)phenyl)-7-methoxy-4-(4-methoxyphenyl)-8-methyl-2H-chromene (40 mg, 0.071 mmol) and methane sulphonic acid (0.03 mg, cat.) in ethanol (10 mL) was purged with nitrogen. Then 10% Pd on Carbon (40 mg) was added and reaction mixture was stirred under a hydrogen (50 psi) for 24 h. The progress of the reaction was monitored by LCMS. After complete consumption of the starting material, the reaction mixture was filtered through a bed of Celite and washed with ethanol. The resulting filtrate was concentrated under reduced pressure to give the crude product. The crude compound was purified by Prep-HPLC to afford 4-(7-methoxy-4-(4-methoxyphenyl)-8-methylchroman-3-yl)benzene-1,2-diol (5 mg, 12%) as an off white solid.

^1H NMR (400 MHz, CD_3OD): δ 6.68 (d, J = 8.0 Hz, 1H), 6.61 (d, J = 8.8 Hz, 2H), 6.55 (d, J = 8.4 Hz, 1H), 6.51 (d, J = 8.8 Hz, 2H), 6.47 (d, J = 8.0 Hz, 1H), 6.18 (d, J = 2.0 Hz, 1H), 6.07 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 4.37-4.31 (m, 1H), 4.23-4.19 (m, 2H), 3.79 (s, 3H), 3.69 (s, 3H), 3.40-3.36 (m, 1H), 2.11 (s, 3H). LCMS: m/z 393.52 $[M+H]^+$.

Testing for anti-proliferative activity of the compounds

The ability of the five compounds (SBP-391 to SBP-394, and GAD-305) to inhibit the proliferation of cancer cells was tested. In this regard, testing was carried out in relation to assess the inhibition of proliferation of cancer cells representative of:

- non-small cell lung carcinoma (A549),
- ovarian cancer (A2780),
- prostate cancer (DU145 and PC3),
- triple negative breast cancer (MDA-MB-231),
- pancreatic cancer (MiaPaCa-2),
- glioblastoma multiforme (U87-MG).

A pre-determined number of cells as calculated from cell growth assays for each of the cell lines employed were seeded into their respective culture medium (using ATCC culture parameters - <http://www.atcc.org>) and cultured for 24 hours at 37 °C and 5% CO₂ in 96-well culture plates.

Once attached, each cell line was then exposed to increasing concentrations of each respective analogue (0.1, 0.3, 1, 3, 10 and 30 µM for SBP-391 – 394; 0.03, 0.1, 0.3, 1, 3, and 10 µM for GAD-305), cultured for a further 72 hours and exposed to Cell-Titre luminescent reagent (Promega, Cat# G7573, 100 µL/well) for a further 30 minutes to assess cell viability.

Luminescence was captured using an EnVision Multilabel reader and the data for each analogue concentration was normalised, as a percentage, to the no treatment control.

Dose-response curves were prepared, and half maximal effective concentration (EC₅₀) values were determined using Graph Pad Prism 6 (nonlinear regression sigmoidal dose-response variable slope).

Results

Compound ID	A549	A2780	DU145	MDA-MB-231	MIA-PACA2	PC3	U87MG
	IC50 values, µM						
SBP-391	1.6	2.3	0.9	2.1	1.2	6.1	2.7
SBP-392	17.7	22.3	>30	21.4	>30	>30	>30
SBP-393	1.2	0.8	1.0	2.8	0.9	3.0	1.9
SBP-394	1.2	0.7	0.8	2.2	1.0	1.9	1.6
GAD-305	0.5	0.2	0.3	0.3	0.5	0.5	0.4