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Chromopynones are "Pseudo Natural Product" Glucose Uptake Inhibitors Targeting Glucose Transporters GLUT-1 and -3

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Abstract:

The principles guiding design and synthesis of bioactive compounds based on natural product (NP) structure, such as Biology Oriented Synthesis (BIOS), are limited by their partial coverage of NP-like chemical space of existing NPs and retainment of bioactivity in the corresponding compound collections. Here we propose and validate a concept to overcome these limitations by de novo combination of NP-derived fragments to structurally unprecedented "pseudo natural products". Pseudo NPs inherit characteristic elements of NP structure yet enable the efficient exploration of areas of chemical space not covered by NP-derived chemotypes, and may possess novel bioactivities. We provide proof-of-principle by design, synthesising and investigating the biological properties of chromopynone pseudo NPs that combine biosynthetically unrelated chromane- and tetrahydropyrimidinone NP fragments. We show that chromopynones define a truly novel glucose uptake inhibitor chemotype that selectively targets glucose transporters

GLUT-1 and -3, inhibits cancer cell growth and promises to inspire new drug discovery programs aimed at tumor metabolism.

Main Text

The discovery of novel small molecule chemotypes with unprecedented or unexpected bioactivity may require the design and synthesis of novel chemical matter, *a priori* endowed with biological relevance. Corresponding design strategies will be particularly efficient if they draw from previous insight gained with established biologically relevant compound classes, such as natural products (NPs).

Biology Oriented Synthesis (BIOS) of natural product-inspired compound classes[1-2] and related approaches[3] actively embrace this principle. In BIOS natural product scaffolds are structurally simplified to less complex, synthetically accessible scaffolds which retain characteristic properties, kind of bioactivity and, therefore, biological relevance of the guiding NPs. However, the BIOS approach faces general chemical and biological limitations.

NPs occupy only a relatively small fraction of total natural product-like chemical space.[4] Yet transcriptionally silent and, therefore, unexploited biosynthesis pathways exist,[5-7] and currently operating pathways can be reassembled into new artificial biosynthesis pathways.[8] Thus, inspiration by NP structure in principle can go far beyond the scaffolds of currently known NP classes that define the chemical basis of BIOS. BIOS arrives at compound classes that often retain the kind of bioactivity and, therefore, most likely the target classes of the guiding NPs thereby restricting exploration of biological space.[9]

These limitations could possibly be overcome if BIOS was united with a principle that enables efficient synthetic coverage of larger chemical space such as fragment-based compound design.[10-11] We have identified NP fragments which represent NP structure and properties,[12] and NPs themselves may have fragment size,[13] or can be degraded to fragment-type compounds.[14] *De novo* combination of such NP fragments may yield unprecedented compound classes that

inherit characteristic elements of NP structure and properties, and, thereby, biological relevance, yet go beyond the areas of chemical space explored by nature. Corresponding compound collections will differ fundamentally from BIOS collections and define new classes of natural product inspired compounds. Such conceptually novel "*pseudo natural products*" will not be accessible by existing biosynthesis pathways and promise to have unexpected bioactivity and targets.

Here we provide proof-of-principle for the "pseudo natural product" concept by means of design, synthesis and biological investigation of chromopynones, a pseudo NP class that unites the biosynthetically unrelated chromane-[15] and tetrahydropyrimidinone NP fragments[16-18] (Figure 1a and 1b). Biological investigation of a synthesized compound collection revealed that the chromopynones define a truly novel, structurally unprecedented glucose uptake inhibitor chemotype that selectively targets glucose transporters GLUT-1 and GLUT-3 and promises to inspire new drug discovery programs aimed at modulation of tumor metabolism.

Results

Design and Synthesis of a Chromopynone "Pseudo Natural Product" Collection

To design a representative pseudo NP class we considered basic guidelines which we expect to be applicable in a general sense. First, since chirality is a defining property of many NPs and stereogenic content correlates with bioactivity[19-20] it was planned to combine NP fragments in a novel three-dimensional scaffold amenable to efficient synthetic modification and decoration for example through a bridge-forming, bipodal connection. In addition, we sought to combine NP fragments with complementary heteroatoms, in particular nitrogen and oxygen to create structural diversity and difference from the guiding NPs. As a third design criterion and in order to maximize the biological relevance of pseudo NPs we considered combining NP fragments which are embedded in NPs with diverse bioactivities. Finally, we reasoned that combination of biosynthetically unrelated fragments should be beneficial for novel bioactivity, since

biosynthetically different NPs will have emerged from biosynthesis cascades composed of different enzymes, such that the corresponding NPs will encode different structural parameters for binding to proteins.

Natural products embodying the chromane or chromene fragments occur widely in nature and are endowed with a multitude of bioactivities (for representative examples see Figure 1).[15] It was planned to combine this oxygen-rich fragment with a biosynthetically unrelated, nitrogen-containing fragment, such as a tetrahydropyrimidinone (THPM) which defines the structural core of an antibiotic class.[18] The novel scaffold should contain stereogenic centres and be readily accessible via an efficient synthesis route.

The synthesis of dihydropyrimidinones (DHPM) by a variant of the Biginelli multicomponent reaction can be combined with intramolecular attack by an appended nucleophile (Figure 1c and Figure 2a), ester hydrolysis and decarboxylation to a bicyclic scaffold which contains both the chromane, and the tetrahydropyrimidinone (THPM) fragments.[21] We expanded the scope of this stepwise transformation to substituted ureas, and developed a robust, modular and telescoped process requiring only one final chromatographic purification. Acetylated 2-hydroxybenzaldehydes, substituted ureas and methyl acetoacetate in the presence of TMSCl[22] yielded DHPM intermediates **4** in good yields (76-85%, see Supplementary Information, Experimental for Compounds). Removal of the acetate under mild conditions to induce the cyclisation, methyl ester hydrolysis with lithium hydroxide and thermal decarboxylation in the presence of lithium salts could readily be combined, resulting in a highly efficient, modular and telescoped one-pot synthesis. The synthesis combines four linear steps, requires minimal handling and only one late stage purification step. The intramolecular cyclisation is stereospecific, as it can only occur from the same face of the approaching nucleophilic phenol, resulting in the ultimate isolation of products **5**-**48** as single diastereomers (see Supplementary Information, Experimental for Compounds for further details). A range of substituents on the benzaldehydes, including halogens and unsaturated alkyl groups (Supplementary Table 1, entries

9, 12, and 15) were tolerated. Nucleophilic hydroxyl groups and sulfur nucleophiles did not interfere with the cyclisation step, affording the corresponding intermediates in similar yield (Supplementary Table 1, entries 16, and 48). Compound **5** (Figure 2c; Supplementary Table 1, entry 1) was converted to various amides (Figure 2c and Supplementary Table 1, entries 17-39). Alkyl- or phenyl- substituted ureas and thioureas, but not disubstituted ureas (Figure 2a and Supplementary Table 1, entries 2-7 and 45) were well tolerated. *O*-alkylation (Figure 2b and Supplementary Table 1, entry 40) of the hydroxyl group of **20** yielded further derivatives. Overall, 44 compounds were synthesized and termed chromopynones (Figure 2 and Supplementary Table 1).

Cheminformatic Analyses of the Chromopynones

In order to compare the chemical space occupied by the chromopynones, natural product-likeness scores as introduced by P. Ertl *et al.*[23] (Figure 3a and Supplementary Figure 12) were calculated for the chromopynones, NPs listed in ChEMBL,[24] compound collections synthesized according to the BIOS logic (see the Supplementary Figure 3 for structures) and compounds in DrugBank, representing marketed and experimental drugs.[25] The chromopynones display a narrow score distribution in an area which is sparsely covered by NPs and BIOS collections (Figure 3a). Therefore, they structurally differ from both groups. This finding seems counterintuitive since the chromopynones structurally combine NP-fragments. However, the novel combination realized in the chromopynones does not exist in NP scaffolds, which implies that the NP score should be different from the majority of NPs. Furthermore, introduction of nitrogen atoms (by analogy to the higher proportion of N in drugs[26] as compared to NPs) may yield a lower score. In fact, *in silico* replacement of nitrogen atoms by oxygen or carbon atoms in the scaffold of chromopynone **6,** thereby generating close analogues with different heteroatom composition, results in increasing NP scores (see Figure S2d). Inclusion of the NP scores calculated for compounds in DrugBank revealed that despite their difference, chromopynone pseudo-NPs and BIOS compounds display NP-scores in the range of many approved and experimental drugs. In

addition, 84% of the chromopynones fall into Lipinksi "Rule-of-5" space (Figure 3b, and Supplementary Table 4). The design principle to combine an oxygen-rich scaffold with a nitrogen-rich fragment may eventually render these pseudo-NPs structurally closer to drugs and less related to NP structures. Notably, this drug-likeness may also induce advantageous physical properties for drug discovery programs. This comparison suggests that the properties of the chromopynones are more than merely the sum of the individual NP-fragment components. An analysis of principles moments of inertia (PMI)[27] of the chromopynones to analyse their molecular shape and size revealed that these compounds display a wide distribution of PMI values similar to chromane- and THPM-containing NPs and thus a higher three-dimensional character compared to commercially available compound collections.[28] This observation suggests that molecular diversity of NPs is conserved through the process of deconstruction into the NP-derived fragments and recombination into the pseudo-NPs.

Collectively, these data suggest that chromopynones have a novel scaffold which lies outside the chemical space covered by NPs. This observation reflects the fact that chromopynones are not accessible *via* biosynthesis. Chromopynones occupy a distinct portion of chemical space accessible by synthesis guided by the principle of fusing biosynthetically unrelated NP-fragments in novel arrangements. These pseudo-natural products are endowed with advantageous properties, as they display NP-scores similar to those of approved drugs and since the majority of the collection falls into Lipinski space.

Biological Investigation of the Chromopynones - Glucose Uptake Inhibition

Pseudo-NPs may display very different bioactivities than the individual guiding NPs from which the newly combined fragments were derived. Therefore, this chemocentric approach requires a wider biological activity investigation to reveal potential biological effects, similar to the biological evaluation of newly discovered NPs, which in general are investigated in multiple bioassays to uncover their potential bioactivity. Beyond this experimental approach, in principle

the bioactivity of pseudo NPs could be hypothesized by means of current computational target prediction. Such methods could for instance suggest known biological targets by comparing NP-fragments to synthetic drugs.[29] The necessity to evaluate pseudo NPs in multiple assays to identify novel bioactivity in the future may be overcome by phenotyping based on high-content technologies.[30] Such techniques may efficiently cover larger areas of biological space in one experimental approach, and lead to *e.g.* quantifiable bioactivity fingerprints for compounds which may indicate particular targets.

Chromopynones were investigated in several cell-based assays monitoring cell signaling, *e.g.,* the Wnt and Hedgehog pathway, or metabolic processes like autophagy and glucose uptake. These assays monitor macroscopic changes and effects such as, reporter gene expression or osteoblast differentiation for the Wnt and Hedgehog pathway respectively, or LC3 puncta formation following induction of autophagy. This investigation revealed that chromopynones selectively inhibited glucose uptake in HCT116 cells (no activity was observed up to 10 μM in the other assays). Elevated glucose uptake and aerobic glucose metabolism (glycolytic phenotype) are characteristics of many cancers and contribute to their increased demand of energy and nutrients.[31] Among the Class 1 glucose transporters (GLUT-1-4),[32-33] in particular GLUT-1 and -3 are strongly upregulated in the majority of cancers (*e.g.* lung, brain, breast, bladder, cervical, colorectal, esophageal, hepatocellular, ovarian, renal cell, pancreatic and prostate cancer),[34] and have been linked to poor survival and tumor aggressiveness.[34-35] Therefore, potent and selective small molecule inhibitors of glucose uptake are in high demand but have only rarely been reported.[36-37]

Eighteen of the 44 chromopynones inhibited uptake of the non-metabolisable glucose analogue 2-deoxy-glucose (2-DG)[38] with IC_{50} values in the range of 0.9 to 23 μM (Table 1 and Supplementary Table 1) with (±)-Chromopynone-1 (compound **11**, Table 1, entry 2) being most active with an IC_{50} of 0.92 ± 0.25 μM. The compound did not inhibit the downstream hexokinase demonstrating direct interference with glucose uptake (Supplementary Figure 9).

The glucose uptake inhibiting activity was not shared by 2801 compounds containing the chromane fragment or substructure and by 1270 compounds containing the THPM fragment or substructure contained in our in-house library (Supplementary Figure 1). Thus, the chromopynones constitute independent bioactive entities with bioactivities not shared by the individual NP-fragments.

Structure-activity Relationship for Glucose Uptake Inhibition

For inhibition of glucose uptake by the chromopynones a clear structure-activity relationship emerged. Thus, the substitution pattern around the fused phenyl ring was important for activity and potency (Table 1, entries 13-18) with the 10-ethoxy-substituted compound (Chromopynone-1) being the most potent (Table 1, entry 2). Minimal modifications of the central scaffold core like methylation at the 5-position (Table 1, entry 20), exchange of oxygen to sulfur at the 1- or 4- positions (Supplementary Table 1, entries 41 and 42), or introduction of an ethyl substituent at the 3-position (Table 1, entry 19) led to a decrease in activity. The position of the amide substituent on the *N*-phenyl group (compare **8**, Table 1, entry 1 to Chromopynone-1, Table 1, entry 2) and the structure of the phenethyl amides (compare **21-25,** Table 1, entries 3-7 to other arylethyl derivatives, Supplementary Table 1, entries 23-28) was important. Extension of the amide substituent is possible (see **26,** Table 1, entry 8 and **36,** Table 1, entry 11) but introduction of an ester (see **37,** Table 1, entry 12) or a tertiary amide (see **40** and **43**) is disadvantageous. Based on the structure-activity correlation analysis compound **11** (Chromopynone-1) was chosen for further in-depth analysis. Enantiomer separation and independent synthesis utilising an enantioselective variant of the Biginelli reaction[39] (see Supplementary Information, Enantioselective Biginelli Reaction for further details) revealed that the (-)-(*R*,*R*)-enantiomer (see Figure 4 for the structure) is more active with an IC_{50} of 412 nM (± 120 nM) for inhibition of 2-DG uptake in HCT116 cells.

GLUT isoform selectivity

Selectivity for inhibition of the two most cancer relevant glucose transporters, GLUT-1 and -3,[40-41] was assessed employing the human colorectal adenocarcinoma DLD-1 cells which express mainly GLUT-1 and GLUT-3 (as reported by the CCLE database)[42] and an isogenic cell line DLD-1-*GLUT1*$^{(-/-)}$ with homozygous deletion of *GLUT1*. This cell line expresses GLUT-3 at *ca* 3-fold higher level than the parent DLD-1 cells (see Figures S6 and S7). (-)-(*R*,*R*)-Chromopynone-1 inhibited 2-DG uptake in DLD-1-*GLUT1*$^{(-/-)}$ cells with IC_{50} = 322 nM and with IC_{50} = 162 nM for DLD-1 cells (Figure 4 and Supplementary Table 2), *i.e.* the compound targets both GLUT-1 and -3.

Selective transient overexpression of GLUT-1 to GLUT-4 in CHO cells should lead to selective increase of IC_{50} if the respective overexpressed isoforms were targeted by (-)-(*R*,*R*)-Chromopynone-1.[43] Overexpression of GLUT-1 or GLUT-3 respectively (see Supplementary Figures 4 and 5) indeed led to an increased IC_{50} for (-)-(*R*,*R*)-Chromopynone-1 as compared to mock-transfected cells (Figure 4 and Supplementary Table 2, entries 3, 4 and 6). A similar shift was not observed after overexpression of GLUT-2 and GLUT-4 (see Supplementary Figure 8 and Supplementary Table 2, entries 5 and 7). These findings suggest that (-)-(*R*,*R*)-Chromopynone-1 selectively interacts with GLUT-1 and GLUT-3 but not, or only much weaker with GLUT-2 and GLUT-4.

Inhibition of cell growth

The growth of the cancer cell lines MIA PaCa2 (pancreatic cancers) and HCT116 (colon cancer) was inhibited by (-)-(*R*,*R*)-Chromopynone-1 in the presence of high (25 mM, standard cell culture conditions) or physiological (5 mM) glucose concentration. Growth of MIA PaCa2 cells was inhibited with half-maximal growth inhibition (GI_{50}) values of 2.8 μM (25 mM glucose) and 0.6 μM (5 mM glucose) demonstrating the increased potency when glucose is not available in excess (Figure 5a). (-)-(*R*,*R*)-Chromopynone-1 suppressed as well the growth of HCT116 cells with GI_{50}

values of >25 μM (25 mM glucose) or 3.8 μM (5 mM glucose) (Figure 5b). The difference in GI_{50} values for both cell lines is in agreement with their known different dependency on glucose.[44-45] These findings indicate that inhibition of glucose uptake by (-)-(*R*,*R*)-Chromopynone-1 translates into reduced cell growth.

The inhibition of glucose uptake *via* the glucose transporters GLUT-1 and -3 and the fact that chromopynones define an unprecedented glucose uptake inhibitor chemotype provide proof-of-principle for the concept that combination of NP fragments in novel arrangements may enable exploration of chemical space not covered by currently existing NPs and identification of novel bioactivities and targets for NP-inspired compounds.

Discussion

We introduce the concept to combine NP fragments in new arrangements to "pseudo natural products" in order to explore larger areas of NP-like, biologically relevant chemical space not covered by nature. Pseudo NPs resemble and contain different elements of NP structure but are not accessible by existing biosynthetic pathways and promise to have unexpected bioactivity and biological targets. We note that the term "pseudo natural product" has been used before by Suga *et al.* to characterize *in vitro* synthesized cyclopeptides[46-47] and by Oshima *et al.* who intercepted biosynthetic pathways to obtain hybrid alkaloid-like compounds with novel bioactivity.[48-49]

We validate the "pseudo natural product" concept by the design, synthesis and evaluation of chromopynones which unite fragments representative for biosynthetically unrelated NPs containing the chromene or chromane structural element,[15] and the tetrahydropyrimidinone fragment (Figure 1).[16, 18]

Chromopynone design emphasized stereogenic character, since bioactivity often correlates with three-dimensional structure,[50-51] and combination of fragments with complementary heteroatom content derived from biosynthetically unrelated NPs to guarantee structural novelty and difference to NPs. Such combinations not found in nature should enable modulation of biological

targets not shared by the guiding NPs and introduce favourable physicochemical properties, in particular balanced hydrophobic and polar character. Thus, the chromane fragment contains carbon and oxygen atoms and an aromatic ring, whereas the tetrahydropyrimidinone fragment is aliphatic and introduces nitrogen atoms. Fragment combination was planned to employ an efficient synthetic strategy that generates stereogenic centers in a complexity-generating manner, resembling the often complex structures of NPs. These design criteria were met by a variant of the Biginelli multicomponent and multistep reaction cascade which was combined in a one-pot process with subsequent cyclization and decarboyxlation steps to yield a collection of 44 structurally diverse chromopynones.

Comparison of NP scores for the chromopynones, BIOS compound collections, NPs and drugs and drug candidates revealed that the chemical space occupied by the chromopynones differs from the space defined by NPs and BIOS compounds. They rather resemble NP scores of drugs and comply with the rule of five. Since the combined fragments represent NP structures, this difference appears unexpected. However, it reflects that this fragment combination is novel, *i.e.* not found in nature. This analysis indicates that *de novo* combination of biosynthetically unrelated fragments in novel arrangements indeed allows to explore areas of NP-like chemical space not or only sparsely covered by NPs. Combination of NP fragments with different heteroatom content appears to be particularly important, *i.e.* oxygen-rich (as found more frequently in NPs than in drugs) with nitrogen-rich fragments (as found more frequently in drugs). Future pseudo NP design, in general, might favour such combinations, *e.g.* novel combinations of alkaloid- with terpene- or polyketide fragments.

Biological investigation of the chromopynones in different cell-based assays, monitoring biological signaling and metabolic processes, revealed that members of this pseudo NP class selectively inhibit glucose uptake in cells. Compounds with chromane- or tetrahydropyrimidinone structure only, did not inhibit glucose uptake which demonstrates that this novel bioactivity is the result of fragment combination. Subsequent target validation

experiments revealed that the most potent compound, termed (-)-(*R*,*R*)-Chromopynone-1, selectively and potently targets glucose transporters GLUT-1 and -3 and, thereby, inhibits tumor cell growth.

Many cancers have an increased demand of glucose to meet their bioenergetics and biosynthetic requirements and upregulate expression of glucose transporter expression, in particular GLUT-1 and -3.[35] Inhibition of glucose uptake has been proposed as a novel opportunity for the treatment of cancer, and novel GLUT inhibitors are in high demand. Very recently, potent small molecules targeting GLUTs, including a selective GLUT-1 inhibitor have been described.[37] However, inhibitors simultaneously and selectively targeting GLUT-1 and -3 may be most advantageous and even required for full efficacy,[52] yet dual selective GLUT-1/-3 inhibitors have not been reported to date. (-)-(*R*,*R*)-Chromopynone-1 defines a structurally novel, unprecedented GLUT-inhibitor with the desired selectivity.

Our results provide proof of principle for the concept to combine compound collection synthesis based on biological relevance, *i.e.* BIOS, with fragment-based compound discovery to inspire design and synthesis of biologically relevant "pseudo natural product" collections with both novel NP-like structure and novel bioactivity. Pseudo-NPs retain characteristic structural elements of NP scaffolds and NP properties, but they allow to explore uncharted areas of natural product inspired chemical space and to expand target space accessible to NP-inspired compounds. The chromopynone example demonstrates that pseudo-NPs may be accessible by rapid and efficient chemical synthesis, and that pseudo-NPs can yield modulators of protein function with unprecedented chemotype. The discovery that the chromopynone pseudo NPs define a novel glucose uptake inhibitor chemotype that selectively targets glucose transporters GLUT-1 and GLUT-3 and thereby inhibits tumor cell growth promises to inspire new drug discovery programs aimed at modulation of tumor metabolism.

Methods:

General method for the preparation of chromopynones: In a typical example, trimethylchlorosilane (770 μL, 6 mmol, 6 eq.) was added dropwise to a stirred solution of the urea (1 mmol, 1 eq.), aldehyde (1 mmol, 1 eq.) and methylacetoacetate (180 μL, 1.5 mmol, 1.5 eq.) in DMF (1 mL, 1 M), and the resulting mixture was stirred at r.t. After 18 h, the reaction was quenched with H_2O (2 mL) and diluted with EtOAc (40 mL). The organic layer was washed sequentially with H_2O (5×20 mL), sat. aq. LiCl solution (1×20 mL) and sat. aq. NaCl solution (1×20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a crude product. Saturated $NaHCO_3$ aq. solution (10 mL) was added to a stirred solution of the crude dihydropyrimidinone in MeOH (10 mL) and the resulting suspension was heated to 40 ˚C. After 16 h the reaction mixture was allowed to cool to r.t. and was concentrated *in vacuo*. The crude was diluted with THF-H_2O (1:1, 10 mL, 0.1 M), LiOH (15 eq.) was added and the reaction mixture was heated to 40 ˚C. After 18 h, the reaction mixture was allowed to cool to r.t. and was concentrated *in vacuo* to half volume. The reaction mixture was acidified to pH = 1-2, by slow addition of 1 M aq. HCl solution and was heated to 80 ˚C (probe temperature 82 ˚C). After 6 h, the reaction mixture was allowed to cool to r.t. and extracted with $CHCl_3$-MeOH (8:2, 5×20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a crude product. Purification by flash column chromatography eluting with 2-4% MeOH in CH_2Cl_2 or preperative HPLC eluting with 10-100% MeCN in H_2O (with or without 0.1% TFA) afforded the reported chromopynones. See Supplementary Information, General Procedure E for further details.

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Data Availability Statement:

The materials and data reported in this study are available upon request which should be addressed to H. W.

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Author contributions

Author contributions: H. W., S. Z., and G. K., conceived and design the project. G. K. and J. C. performed the chemical synthesis. G. K., E. S. R., M. S., and S. S. performed the biological experiments. G. K., A. P., performed the chemoinformatic analyses. H. W., S. Z., G. K., E. S. R., and A. P. analysed the results. All authors discussed the results and commented on the manuscript. H. W., S. Z., A. P., and G. K., prepared the manuscript.

Competing Financial Interests

H. W. is an academic sponsor of a drug discovery project at Lead Discovery GmbH, aimed at the development of GLUT inhibitors. G. K., E. S. R., J. C., M. S., S. S., A. P., C. O., and S. Z., declare no competing financial interests.

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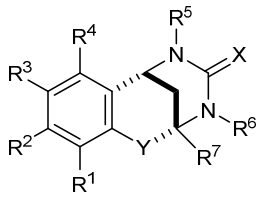
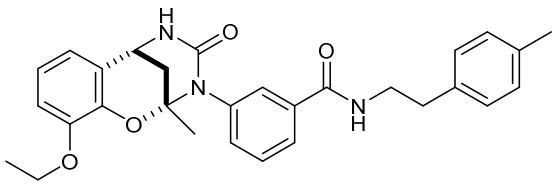
Table 1: Structure-activity relationship analysis of chromopynones.



R^2, R^3, R^4 = H



11, (±)-Chromopynone-1

Entry	Compound	R^1	R^5	R^6	R^7	X	Y	IC_{50} (μM)[a]
1	**8**	OEt	H	4-(CONH$(CH_2)_2$-4-Me-C_6H_4)-C_6H_4	Me	O	O	4.8 (±0.7)
2	**11**[b]	OEt	H	3-(CONH$(CH_2)_2$-4-Me-C_6H_4)-C_6H_4	Me	O	O	**0.9 (±0.4)**
3	**21**	OEt	H	3-(CONH$(CH_2)_2$-3,4-di-Me-C_6H_3)-C_6H_4	Me	O	O	1.8 (±0.1)
4	**22**	OEt	H	3-(CONH$(CH_2)_2$-4-Et-C_6H_4)-C_6H_4	Me	O	O	1.7 (±0.3)
5	**23**	OEt	H	3-(CONH$(CH_2)_2$-4-Br-C_6H_4)-C_6H_4	Me	O	O	2.3 (±0.5)
6	**24**	OEt	H	3-(CONH$(CH_2)_2$-3-OMe-C_6H_4)-C_6H_4	Me	O	O	6.1 (±1.4)
7	**25**	OEt	H	3-(CONH$(CH_2)_2$-4-Ph-C_6H_4)-C_6H_4	Me	O	O	5.7 (±1.6)
8	**26**	OEt	H	$(CH_2)_6CH_3$	Me	O	O	9.9 (±1.3)
9	**29**	OEt	H	3-(CONH$(CH_2)_2$-2-thiophyl)-C_6H_4	Me	O	O	15.6 (±4.1)
10	**30**	OEt	H	3-(CONH$(CH_2)_2$-3,4-di-OMe-C_6H_3)-C_6H_4	Me	O	O	11.7 (±1.2)
11	**35**	OEt	H	3-(CONHCH_2-2-furanyl)-C_6H_4	Me	O	O	> 30
12	**36**	OEt	H	3-(CONH$(CH_2)_2$O-4-Me-C_6H_4)-C_6H_4	Me	O	O	5.0 (±1.4)
13	**37**	OEt	H	3-($CO_2(CH_2)_2$-4-Me-C_6H_4)-C_6H_4	Me	O	O	3.7 (±1.2)
14	**40**	OEt	H	3-(CONH(tetrahydroisoquinolyl))-C_6H_4	Me	O	O	> 30
15	**43**	OEt	H	3-(CO-1(6-Me-indolyl))-C_6H_4	Me	O	O	> 30
16	**44**	O*i*Bu	H	3-(CONH$(CH_2)_2$-4-Me-C_6H_4)-C_6H_4	Me	O	O	2.3 (±0.5)
17	**45**	OEt	H	3-(CONH$(CH_2)_2$-4-Me-C_6H_4)- C_6H_4	Me	S	O	> 30
18	**46**	H	H	3-(CONH$(CH_2)_2$-4-Me-C_6H_4)- C_6H_4	Me	O	S	> 30
19	**48**	OEt	H	3-(CONH$(CH_2)_2$-4-Me-C_6H_4)-C_6H_4	Et	O	O	7.3 (±2.4)
20	**49**	OEt	Me	3-(CONH$(CH_2)_2$-4-Me-C_6H_4)- C_6H_4	Me	O	O	8.4 (±1.3)

a: IC_{50} values determined for the inhibition of 2-DG uptake in HCT116 cells. Data are mean values (N=3 independent experiments, n=3 independent replicates). Error bars represent mean ± SD. b: Compound **11** is (±)-Chromopynone-1. See Supplementary Table 1 for further details.

Figure Captions:

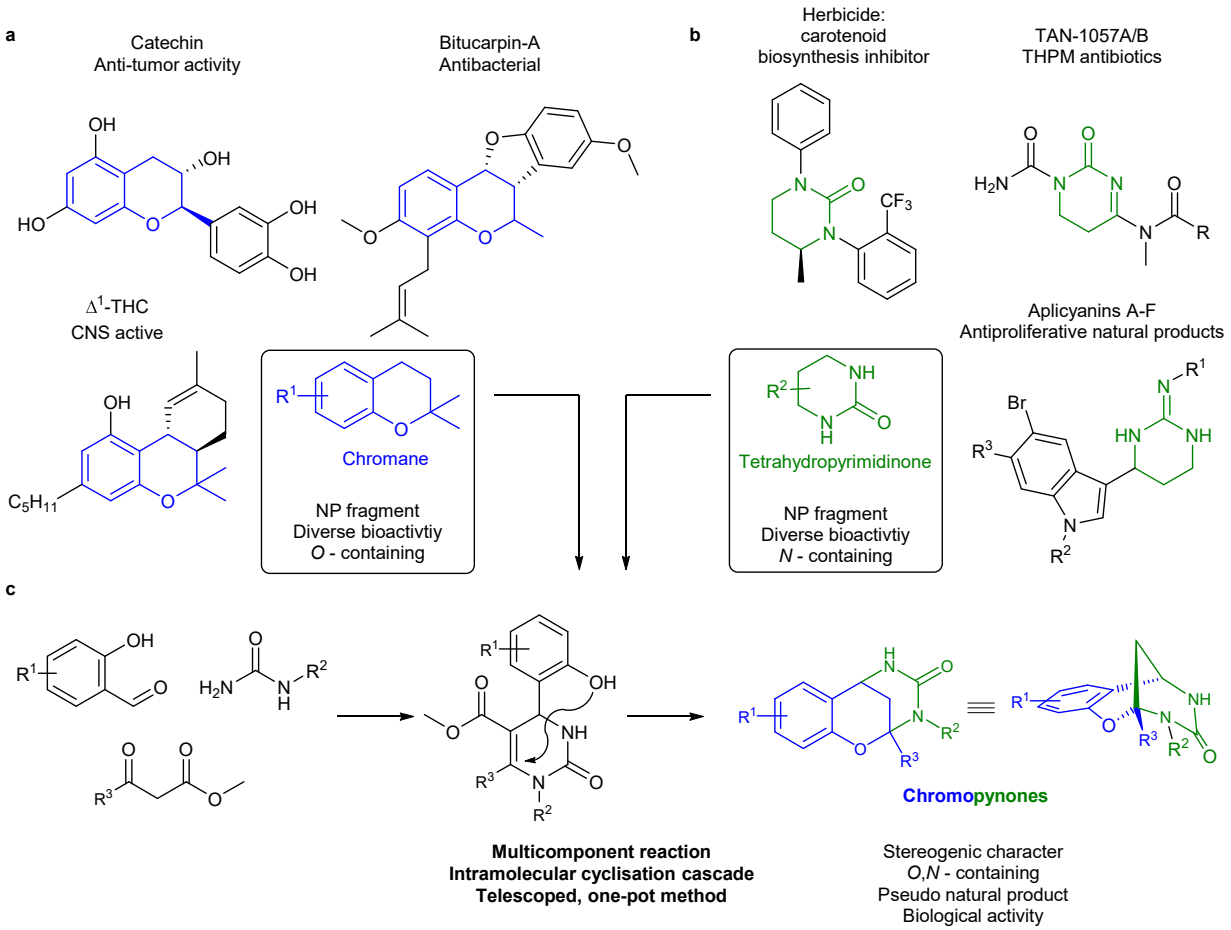


Figure 1: Design of chromopynone pseudo natural products. (a) The chromane NP-fragment and examples of diverse and bioactive chromane NPs. (b) The tetrahydropyrimidinone (THPM) NP-fragment and examples of bioactive THPM NPs and synthetic molecules. (c) The fusion of the chromane and THPM fragments using a multicomponent reaction followed by an intramolecular cyclisation.

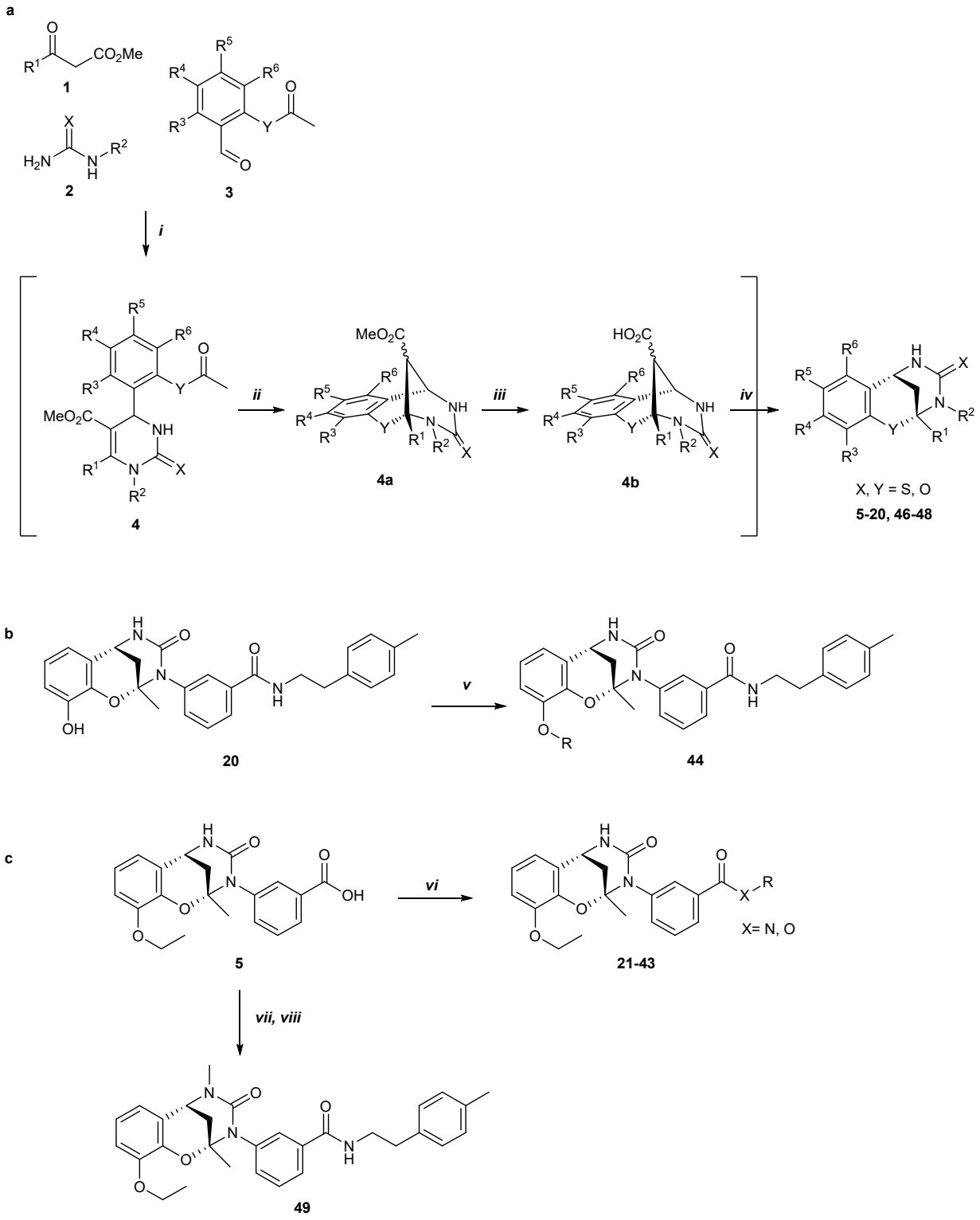


Figure 2: Synthesis of chromopynones. Conditions ***i-vi*** are also detailed in the Supplementary Information, General Experimental. (a) Telescoped process for the preparation of a chromopynone collection. Conditions: **i:** Urea (1 eq.), aldehyde (1eq.), 1,3-dicarbonyl compound (1.5 eq.), TMSCl (6 eq.), DMF, rt, 18 h; **ii:** 1) $NaHCO_3$, MeOH-H_2O, 40 ˚C, 16 h; **iii:** LiOH (15 eq.), THF-H_2O, 40 ˚C, 18 h; **iv:** 1 M HCl, pH 1-2, 80 ˚C, 6 h. (b) Preparation of chromopynone

analogue **44**. Conditions: **v:** phenol **20** (1 eq.), alkyl halide (1 eq.), K_2CO_3 (2 eq.), MeCN, reflux, 4 h. (c) Preparation of chromopynone analogues **21**-**43** and **49**. Conditions: **vi:** acid **5** (1 eq.), TBTU (1.5 eq.), DIPEA (1.5 eq.), amine (1.2 eq.), DMF, rt, 18 h; **vii:** acid **5** (1 eq.), Cs_2CO_3 (3 eq.), MeI (10 eq.), DMF, 40 ˚C, 8 h. **viii:** LiOH (15 eq.), THF-H_2O, rt, 22 h; then: TBTU (1.5 eq.), DIPEA (1.5 eq.), amine (1.2 eq.), DMF, rt, 24 h.

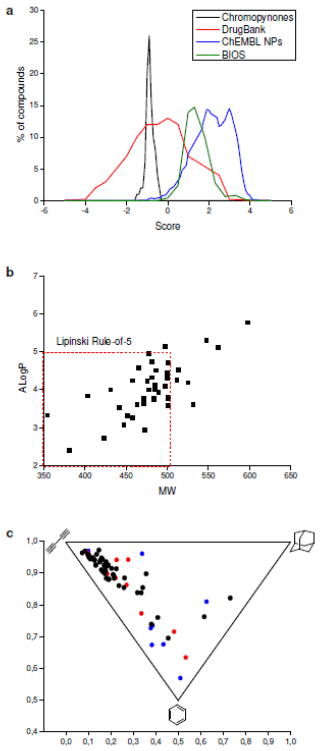


Figure 3: Chromopynones occupy a different portion of chemical space than selected BIOS collections and NPs, and may be endowed with advantageous physiochemical properties. (a) NP-likeness score comparison of NPs represented in ChEMBL (blue curve), chromopynones (black curve), DrugBank collection (red curve) and BIOS compound collections (green curve). (b) ALogP vs MW plot of chromopynones. 37 out of 44 molecules fall within Lipinski's "Rule-of-five" space. (c) PMI plot for chromopynones (black dots), chromane- (red dots) and THPM-containing (blue dots) NPs. Chromopynones display a PMI distribution similar to NPs. See Supplementary Figures 12-14 for further details.

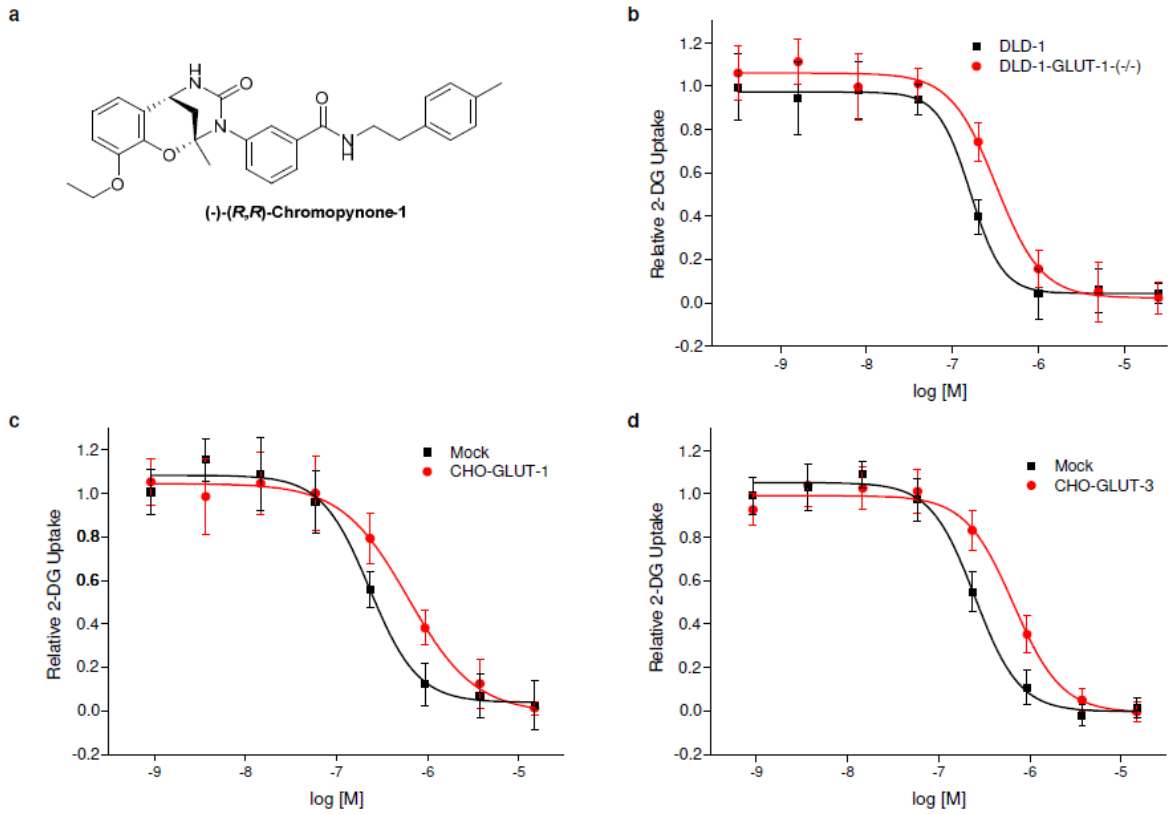


Figure 4: Modulation of 2-DG uptake of different cell lines by (-)-(*R*,*R*)-Chromopynone-1. (a) Structure of (-)-(*R*,*R*)-Chromopynone-1. (b) Dose-response curves for 2-DG uptake by DLD-1 (squares, black line, IC_{50} = 162 (±46) nM) and DLD-1-*GLUT1*$^{(-/-)}$ (circles, red line, IC_{50} = 322 (±31) nM) cells. (c) Dose-response curves for 2-DG uptake by mock CHO (squares, black line, IC_{50}=263 (±41) nM) and CHO-GLUT-1 (circles, red line, 15-fold overexpression, IC_{50} = 612 (±42) nM) cells. (d) Dose-response curves for 2-DG uptake by mock CHO (squares, black line, 9-fold overexpression, IC_{50} = 279 (±35) nM) and CHO-GLUT-3 (circles, red line, IC_{50} = 650 (±76) nM) cells. 2-DG uptake was determined by means of an enzyme-coupled assay and determination of resorufin fluorescence. Data are normalized to the DMSO control and are mean values (N=3 independent experiments, n=3 independent replicates). Error bars represent mean ± SD. See Supplementary Figure 8 for further details.

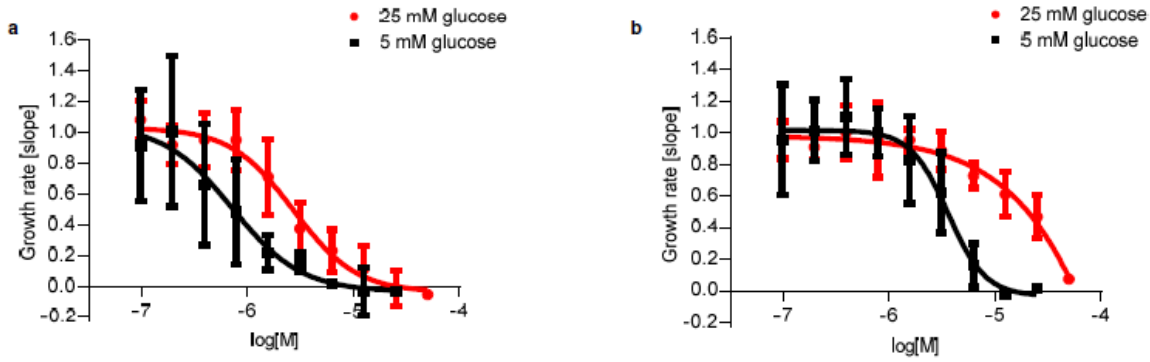


Figure 5: Modulation of proliferation of different cell lines by (-)-(*R*,*R*)-Chromopynone-1. (a) Influence of (-)-(*R*,*R*)-Chromopynone-1 on the growth of MIA PaCa2 cells at 25 (circles, red line,) or 5 (squares, black line) mM glucose (b) Influence of (-)-(*R*,*R*)-Chromopynone-1 on the growth of HCT116 cells at 25 (circles, red line,) or 5 (squares, black line) mM glucose. Growth was determined by image-based quantification of cell confluency using an IncuCyte ZOOM microscope. The concentration for 50% growth inhibition was extracted using the slope of the exponential growth phase, normalized to the growth of the DMSO control. Data are normalized to the DMSO control and are mean values (N=3 independent experiments, n=3 independent replicates). Error bars represent mean ± SD. See Supplementary Figures 10 and 11 for further details.