Scaffold Diversity Synthesis Delivers Complex, Structurally, and Functionally Distinct Tetracyclic Benzopyrones

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Complexity-generating chemical transformations that afford novel molecular scaffolds enriched in sp^3 character are highly desired. Here, we present a highly stereoselective scaffold diversity synthesis approach that utilizes cascade double-annulation reactions of diverse pairs of zwitterionic and non-zwitterionic partners with 3-formylchromones to generate highly complex tetracyclic benzopyrones. Each pair of annulation partners adds to the common chroman-4-one scaffold to build two new rings, supporting up to four contiguous chiral centers that include an all-carbon quaternary center. Differently ring-fused benzopyrones display different biological activities, thus demonstrating their immense potential in medicinal chemistry and chemical biology research.

1. Introduction

The dynamic control of biological functions at various levels through genetic, enzymatic, as well as chemical means has years of evolutionary support to make life happen and perpetuate. Many enzymes orchestrate complex and sophisticated biosynthetic designs to create natural products, which bring the life-supporting biological functions into reality. Understandably, the conserved protein binding sites are more familiar to the secondary metabolites than pure synthetic molecules and, thereby, natural products remain a major source of drug candidates.[1] Likewise, molecules embodying core scaffolds of natural products are also expected to deliver biologically intriguing small molecules.[2] In fact, structural diversity remains the most decisive factor to influence the performance of a compound collection to yield hit and lead molecules.[3] The emerging role of unbiased cell-based and phenotypic screenings to identify cell-permeable and bioactive small molecules[4] further demands a greater structural diversity in a screening collection.[5] Chemists, therefore, seek to develop concise syntheses approaches that could effectively create small molecules representing a number of molecular frameworks based on natural product scaffolds and/or privileged ring systems.[6]

Natural products embodying diverse carbo- and heterocyclic rings around a common scaffold often exhibit various and distinct biological activities, as depicted for polycyclic benzopyrones in Figure 1.[7] Chemical transformations that build up mo-
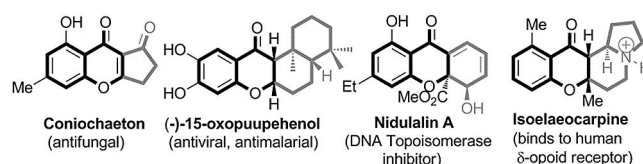

Figure 1. Natural products embodying diversely ring-fused benzopyrones display diverse biological activities.

lecular complexity on privileged scaffolds could bequeath intriguing biological activities to the resulting molecules and are, therefore, highly desired.[4b, 8] However, often structural complexity is compromised for long multistep synthesis sequences that drastically reduce the overall efficiency of the process. In particular, generation of natural-product-based molecular architectures that are rich in sp^3 character and decorated with a number of stereogenic centers[9] remain a daunting synthetic challenge. Recently, we reported a stereoselective cascade double-annulation reaction sequence that utilized easily accessible substrates to build tetracyclic benzopyrones.[10] In this novel and concise synthesis strategy, in situ generated diverse pairs of zwitterions could add in tandem to 3-formyl-chromones and generated the highly complex tetracyclic benzopyr-

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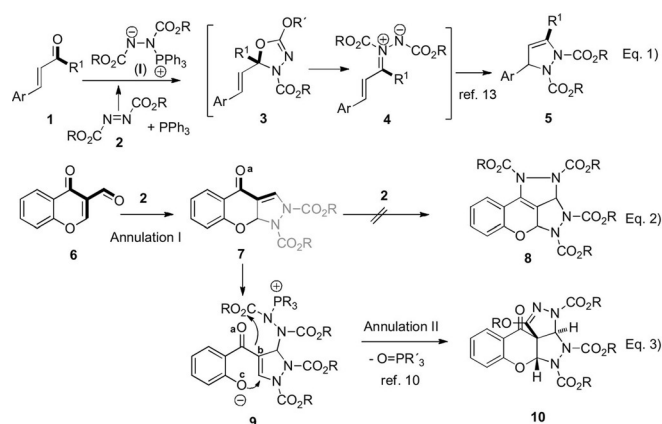
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ones. In the reactions, two new rings that emanated from each zwitterionic dipole employed, were decorated with three contiguous chiral centers including an all-carbon-quaternary center. Here, we provide our further endeavors in utilizing the double-annulation reactions to form diverse and complex benzopyrones. We also unravel how the structural diversity on chromone scaffold brings intriguing and distinct biological activities to the resulting small molecules.[11]
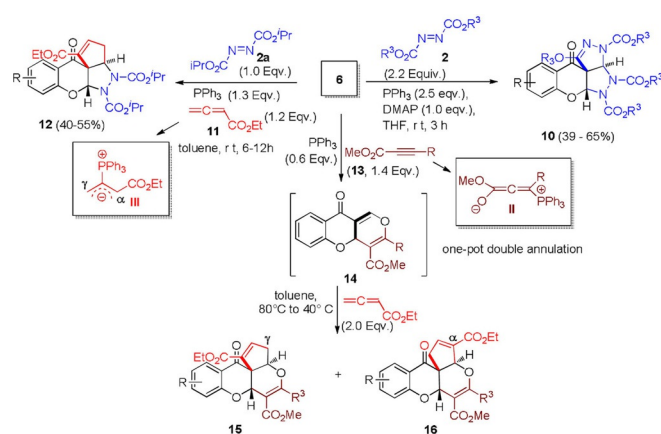
2. Results and Discussion

Addition of a tertiary phosphine to diazadicarboxylate (**2**) generates a zwitterion, known as Huisgen's zwitterion (**I**, Scheme 1), that has been used as a dipole in various cycloaddi-

Scheme 2. Cascade double annulation of 3-formylchromones with two different zwitterions to form complex benzopyrones (previous work).[10]

Scheme 1. Design of cascade annulations providing diversely ring-fused and complex benzopyrones (previous work).[10]

tion/annulation reactions.[12] A reaction of chalcones (**1**) with Huisgen's zwitterion (**I**) was reported to form pyrazolines **5** [Eq. (1), Scheme 1].[13] In the reaction, the zwitterionic dipole (**I**) reacted with a ketone functional group to form an intermediary iminium dipole (**4**), which cyclizes to form a pyrazoline ring. We have successfully used commercially available 3-formyl chromones in the synthesis of natural-product-inspired small molecules.[14] We wondered how Huisgen's zwitterion would react with 3-formyl chromones (**6**), which bear two carbonyl functionalities. Unlike the reaction of **2** with chalcones **1** [Eq. (1)], which consumes the ketone moiety in forming pyrazoline **5**, the plausible adduct **7** formed by addition of **I** to 3-formylchromone (**6**) still keeps an α,β-unsaturated ketone that may entail another annulation reaction. Although zwitterion **I** generally does not undergo conjugated additions,[12, 15] another deoxygenative cyclization of **I** to **7** might yield adduct **8** [Eq. (2), Scheme 1]. Also, a plausible SN2' addition of **I** to **7** might lead to an intermediary phenoxide **9** that holds different nucleophilic sites (a to c) for diverse possible cyclization pathways, leading to either **8** or another class of tetracyclic benzopyrone **10** [Eq. (3), Scheme 1].[14a, 16] We observed that the reaction of two equivalents of Huisgen's zwitterions with 3-formylchrmone led to an exclusive and completely stereoselective

formation of adduct **10**, and no trace of plausible adduct **8** was observed (Scheme 1).

Reaction conditions were optimized to provide highly complex tetracyclic benzopyrones **10**, which were obtained as single diastereoisomers (>99% de). A compound collection was, thus, generated by using differently substituted 3-formylchromones and diazadicarboxylates. The cascade double-annulation reaction sequence generated tetracyclic benzopyrones containing three contiguous chiral centers, with one of them an *all-carbon quaternary* center and in a completely stereoselective fashion (Scheme 2). Stereoselectivity in the cascade double annulations is plausibly dictated by the steric factors along with the preferred *cis*-ring fusions in the ensuing adducts to avoid a ring strain. Thus, SN2'addition of the second zwitterion (**I**) to tricyclic intermediate **7** forms the phenoxide **9**, which undergoes a conjugated addition that happens from the least hindered face (*anti*- to addition of zwitterion **I** to **7**), providing another anion that attacks the *N*-ester and eliminates phosphine oxide to form another five-membered ring. The latter prefers a *cis*-ring fusion over a strained *trans*-ring fusion and, therefore, the double annulations works with excellent diastereoselectivity (Scheme 1).

A one-pot procedure was also developed to incorporate two different rings fused to chromone scaffold by using two different zwitterionic dipoles generated in situ from DIAD and allene ester (**11**), respectively. Thus, benzopyrones **12** obtained through this procedure support four different fused rings and a highly three-dimensionally complex scaffold (Scheme 2).

We further explored a different combination of annulation reactions to build complex tetracyclic benzopyrones. To this end, we used the [4+2] annulation reaction of zwitterion **II** (generated by addition of phosphine to acetylene carboxylates **13**) with chromones **6** as the first catalytic annulation,[16] leading to adduct **14**, followed by another [3+2] annulation with the allene ester (**11**)-derived zwitterion (**III**) through the catalytic addition of phosphine. α- and γ-addition of the allene derived zwitterion (**III**) to the first annulation adduct **14** results in the formation of regioisomeric adducts **15** and **16**. Thus, a one-pot procedure was developed with consecutive phos-
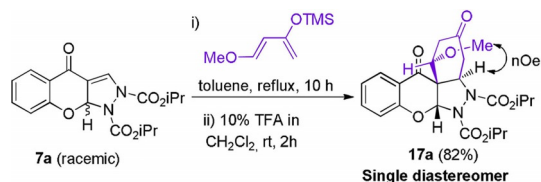
Scheme 3. A stereoselective Diels–Alder annulation reaction of Danishefsky's diene with adduct **7a**.

Scheme 4. A stereoselective one-pot double-annulation reaction of 3-formyl-chromones (**6**) with DIAD and Danishefsky's diene to from benzopyrones **17**.

phine-catalyzed annulations that engaged chromone substrates to form tetracyclic benzopyrones **15**–**16** (Scheme 3).

In all of the tetracyclic benzopyrones, a five-membered ring was generated in the second annulation reaction; for instance, formation of **10** from **9** in Scheme 1. We were curious to realize different modes of annulation or cycloaddition reactions on the first annulation adduct, so that more complex and structurally diverse benzopyrones could be generated. However, not all possible cycloaddition reaction conditions or substrates are compatible with cascade sequential annulation reaction conditions. Therefore, we planned to make use of the annulation adduct of 3-formylchromone and Huisgen's zwitterion (**I**) that is, **7**, as the substrate for accessing further scaffold diversity.

In the first case, adduct **7** was exposed to a Diels–Alder reaction with Danishefsky's diene. Besides targeting a six-membered ring fused to chromone, this reaction was of further interest to prove the proposed cascade reaction sequence of S_N2'addition of nucleophiles to first annulation adduct (**7**), followed by chromone ring opening and a stereoselective cyclization to afford tetracyclic benzopyrone. Had this not been the case, even a completely stereoselective Diels–Alder reaction of Danishefsky's diene with racemic α-β-unsaturated ketone-adduct (**7a**) would afford a mixture of two diastereomers. To our delight, the reaction afforded cycloadduct (**17a**) in very high yield and as a single diastereomer, thereby supporting the proposed cascade reaction sequence in double-annulation reactions to form tetracyclic benzopyrones. The relative stereochemistry was supported by nOe NMR experiment (Scheme 3) and further corroborated by X-ray crystal structure analysis of **17e** (see the Supporting Information).[17]

We further optimized the reaction conditions for a one-pot sequential double-annulation reaction, wherein all commercially available reagents, that is, 3-formylchromones (**6**), DIAD, and Danishefsky's diene, were employed to generate differently substituted tetracyclic benzopyrones **17a**–**e** supporting two six-membered rings, a carbacycle, and an oxa-cycle fused to a pyrazolidine heterocycle and bearing four consecutive stereogenic centers. However, the yields in a one-pot operation were only moderate, despite the fact that excellent stereoselectivity was maintained (Scheme 4).

Azides are another class of dipoles that potentially can be used in cascade sequential double-annulation reactions.[18] The first annulation adduct (**7**) of 3-formylchromone with a Huisgen's zwitterion does not bear basic nitrogen atoms, and the plausible adducts emanating from double annulation with azides would have only one possibly basic nitrogen that can also be decorated with various substitutions to modulate its
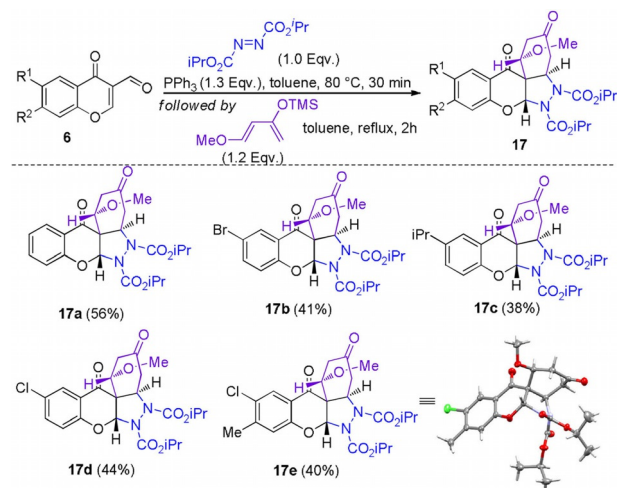
basicity. Intrigued by the complex structure of this possible adduct supporting two five-membered rings with five nitrogen atoms and fused to a privileged chromone scaffold, we attempted a one-pot double-annulation reaction. Thus, addition of DIAD and PPh₃ to 3-formylchromone in toluene at 80° C formed the desired intermediate **7a**, to which 2-azido benzyl acetate (**18a**) was added and the mixture was heated at 110° C for another 3 h. To our pleasure, the desired double-annulation adduct **19a** was isolated in moderate yield. Interestingly, although the expected diastereoselectivity in this double annulation was achieved, the reaction also followed a completely regioselective second annulation with azide and no trace of other regioisomer was detected. By using different 2-azido derivatives, tetracyclic benzopyrones **19b**–**d** were obtained in moderate yields (Scheme 5 a).

The yield of this one-pot procedure, however, could not be improved. On the other hand, employing the intermediate **7a** in the annulation reaction with 2-azido benzyl acetate afforded a better yield, and changing the solvent to DMF also afforded a cleaner reaction. Therefore, different alkyl azides supporting different functional groups were used in the annulation reaction with intermediate **7** to generate a collection of tertracyclic benzopyrones **19a**–**l** (Scheme 5 b).

Incorporating structural diversity,[19] including ring diversity to any compound class is aimed to enhance its potential for functional diversity, that is, to provide diversely bioactive molecules that can be used as tools to pursue chemical biology or drug-discovery goals.[11] To determine the potential of these significantly complex and diverse tetracyclic benzopyrones, the collection was evaluated in two different cell-based screenings (Figure 2).[20] In the first case, their neuritogenic/neurotrophic potential was assessed by treating primary neuronal cultures derived from hippocampi of E18/E19 Sprague Dowley rats with 10 μM solutions of the molecules for 2 days (see details in the Supporting Information).[21] After staining with a membrane dye, the overall membrane was quantified spectrophotometrically as an indirect measure of neurite outgrowth (Figures S1 and S2).
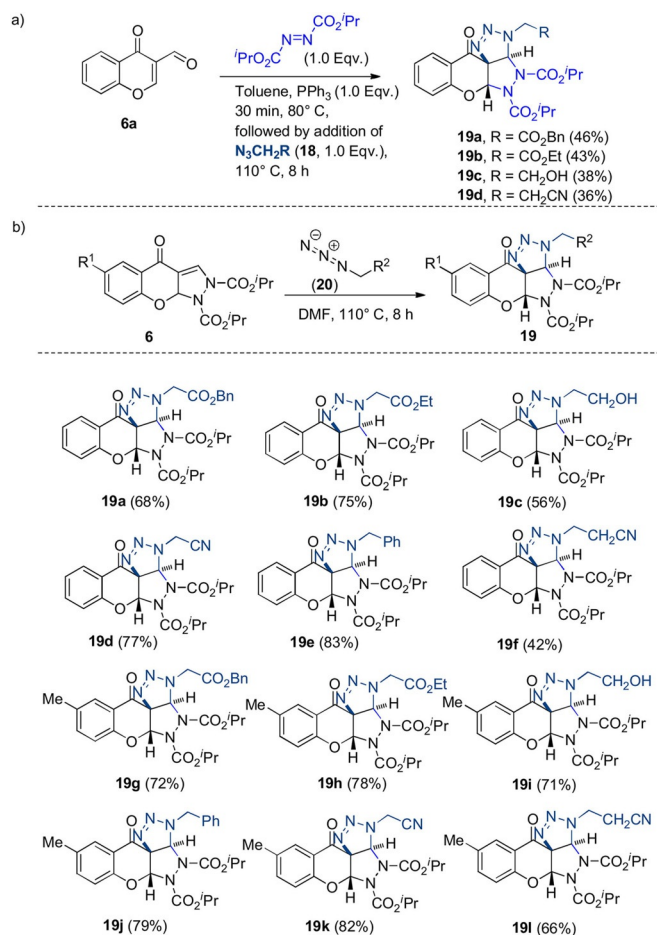
Scheme 5. a) A one-pot double-annulation reaction of 3-formylchromone with DIAD and 2-azido derivatives; b) Direct annulation of azides with adduct **7** to yield tetracyclic benzopyrones **19**.
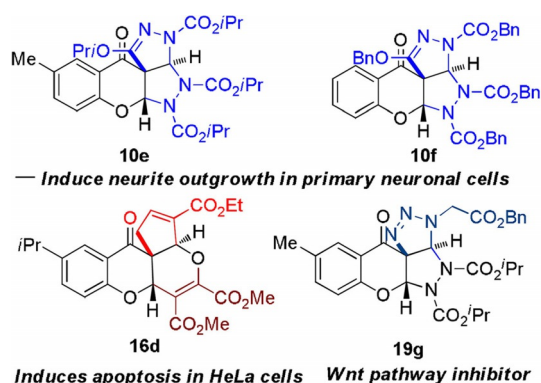

Figure 2. Structurally and functionally diverse tetracyclic benzopyrones.

Several of the diversely ring-fused benzopyrones increased the neuronal membrane amount, in particular, benzopyrones **10** embodying the pyrazolo[3,4-*c*]pyrazole ring-system (see Figure S2). Benzopyrones **10 e** and **10 f** were further analyzed, regarding neuronal differentiation and maturation (Figure 3 a). For this, cells were treated with the compounds for 2 or 7 div (where div = days in vitro). After fixation and staining for βIII
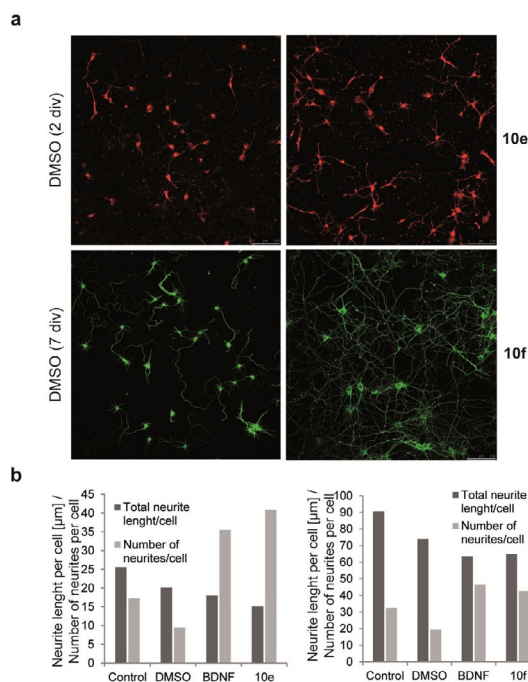

Figure 3. Enhanced neurite outgrowth induced by benzopyrones. a) Primary rat hippocampal neurons were treated with DMSO as a control or benzopyrone **10 e** (2 div) or **10 f** (7 div). Cells were fixed and stained with a membrane dye (red; for 2 div) or for βIII tubulin (green, for 7 div) and images were acquired using a 10× objective. Scale bar: 100 μm. b) The shape of the cytoskeleton was traced using βIII tubulin and was converted in a length unit. Mean values from five images per condition are shown.

tubulin, the number of neurites and the neurites' length was determined (see the Supporting Information), as compared to DMSO (negative control) and brain-derived neurotrophic factor (BDNF, positive control, Figure 3 b). Interestingly, while **10 e** increased the number of neurites after 2 div, which reflects a neurotrophic activity similar to BDNF, **10 f** led to an increase in neurite length.[22] Tubulin and actin dynamics are known to be involved in neurite outgrowth.[23] To detect any influence of the **10 e** and **10 f** on actin and tubulin, we examined their activity on the cellular cytoskeleton as well as on tubulin and actin in vitro polymerization and in tubulin regrowth assay in cells (Figures S3–S5 and details in the Supporting Information). However, neither of them showed any influence on the actin and tubulin cytoskeleton. The molecular events and mechanisms underlying a neuron's capability to form neurites, the number of neurites per cell, and the sites of neurite initiation are largely unknown. Therefore, benzopyrones **10 e** and **10 f** represent interesting probes for further exploration in neurobiology research.[24]

In parallel, the compound collection was screened in human cervical cancer HeLa cells at a concentration of 30 μm to determine their cytotoxicity, influence on cytoskeleton, and mitosis.[6b] Among four benzopyrone subclasses (**10**, **12**, **15–16**, **19**), only **16 a–d** were found to be cytotoxic (Table S1 and Figure S6). Regioisomeric analogues **15** did not display any influence on HeLa cells. Compound **16 d** was the most potent compound with an IC_{50} for inhibition of cell viability of 8.6 ± 0.6 μm (Figure 4 a). In addition, the African green monkey BSC-1 cells
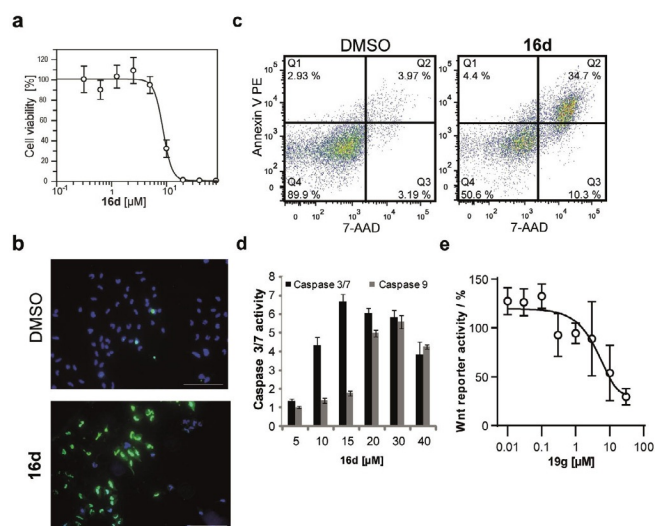
Figure 4. Benzopyrone **16 d** induces apoptosis in HeLa cells (a–d). a) Cells were treated for 48 h with different concentrations of **16 d**. Cell viability was determined by means of the WST-1 reagent. Data are shown as mean values ($n = 4$) $\pm$ SD and were normalized to DMSO. b) Cells were treated for 18 h with 20 μm **16 d**. Fragmentation of DNA (green color) was detected by means of a TUNEL assay. Nuclei were stained with DAPI (blue). Scale bar: 200 μm. c) HeLa cells were treated with 20 μm **16 d** for 18 h. Cells were analyzed for Annexin V PE and 7-AAD using FACS. d) Cells were treated for 18 or 6 h with different concentration of **16 d** prior to detection of caspase −3/7 or −9 respectively. For details, see the Supporting Information. e) **19 g** inhibits WNT-3a-responsive reporter gene expression in HEK293 cells.

responded to the compound with an IC_{50} (viability) of 18.1 ± 1.2 μm (see Figure S7).

Time-lapse experiments revealed morphological changes indicative of apoptosis-like membrane blebbing and cell shrinkage (see Movies S1 and S2). To address the form of cell death by **16 d**, we performed terminal transferase dUTP nick end labeling (TUNEL) to detect fragmented DNA and, thus, apoptotic cells. Treatment with 20 μm of **16 d** increased the number of TUNEL-positive cells (Figure 4b). Apoptotic cell death was further confirmed after Annexin V PE and 7-AAD staining as well as fluorescence-activated cell sorting (FACS). Compound **16 d** (20 μm) increased the number of late apoptotic cells (high Annexin V-PE and 7-AAD intensity: 34.7%) compared to DMSO (3.97%) and other compounds of the same scaffold class (Figures 4 c and S8). Increased activation of caspase 3/7 and caspase 9 by **16 d** in different cell lines confirmed the induction of apoptosis by **16 d** (Figures 4 d and S9).[25]

Signaling pathways govern and control a range of biological functions and are often abrogated in a disease state like cancer. Small-molecule modulators of different signaling pathways are important tool compounds for deeper biological investigations. Wnt/β-catenin is an evolutionary conserved signal transduction cascade that controls a number of biological processes, such as regulation of cell proliferation, migration and polarity, tissue regeneration, and stem-cell renewal, and is also a major pathway with relevance to the establishment of cancer.[26] Wnt signaling modulators are widely employed to dissect signal progression through the pathway.[27] In canonical Wnt signaling, the absence of a Wnt signal results in a low pro-

tein level of the central player β-catenin. In the nucleus, transcription factors of the TCF/LEF family inhibit transcription of Wnt target genes by recruiting histone deacetylases. Upon Wnt activation, β-catenin accumulates in the cytoplasm and translocates to the nucleus, where it associates with TCF/LEF and recruits transcriptional coactivators and chromatin remodeling complexes to initiate the expression of Wnt target genes. In many epithelial cancers, the Wnt pathway is constitutively active as a result of mutations in different components of the pathway. Therefore, novel small-molecule modulators of Wnt pathway are required not only to dissect the complex signal pathway and enhance the general understanding of the associated biology, but also as novel drug candidates against certain cancer types that are directly influenced by the active Wnt pathway.

To assess the potential of tetracyclic benzopyrones modulation of Wnt pathway, the compounds were exposed to HEK293 cells that were stably transfected with the human Frizzled-1 receptor and a TOPFLASH-driven luciferase reporter gene.[28] Dose-response analyses were carried out for hit compounds, for which cell viability remained above 80% with respect to control experiments. To rule out any direct inhibition of the reporter Firefly luciferase or interference with transcription or translation, hit compounds were assayed for modulation of luciferase in HEK293 cells with constitutive luciferase expression. Pleasingly, the compound collection revealed the tetracyclic benzopyrone bearing triazole ring **19 g** as a moderate (IC_{50} of ca. 5.5 μm, Figure 4 e) yet structurally novel Wnt-pathway inhibitor.

3. Conclusions

In summary, the cascade double-annulation approach was successfully explored as a complexity-generating transformation to build novel tetracyclic benzopyrone scaffolds. Different pairs of zwitterionic and non-zwitterionic annulation partners were successfully employed to create diversely ring-fused benzopyrones. Overall, the two tandem annulations transformed the structurally flat, commercially available chromones (**6**) into highly sp^3-enriched new molecular frameworks, with high efficiency and excellent stereoselectivity. The small collection of diversely ring-fused benzopyrones displayed functional diversity in providing small molecules possessing neuro-enhancing abilities in primary neurons and apoptosis-inducing activity in different cancer cell lines, as well as delivering a structurally novel class of Wnt pathway inhibitors. We believe these results will inspire the chemists to further explore and exploit the immense potential of cascade multi-annulation strategies to achieve natural product-like structural complexity and diversity and as means of discovering interesting small molecules to investigate different biological processes.

Experimental Section

General procedure for the synthesis of tetracyclic benzopyrones **19**:
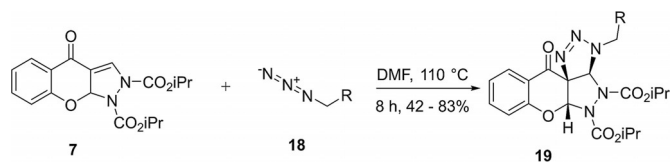
To a solution of Huisgen's adduct (1.0 equiv) in DMF (3 mL mmol^{-1}) azido-compound (1.5 equiv) was added. The reaction mixture was heated to 110 °C and stirred at the same temperature for 8 h. After completion of the reaction, as confirmed by TLC and mass analysis, it was cooled to room temperature and quenched with water (10 mL). The aqueous solution was extracted with Et$_2$O (3×20 mL) and the combined extract was washed with water, dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure to give the crude compound. The crude compound was purified by column chromatography on silica gel using EtOAc/Pet ether (10–20 %) as eluents to give the tetracyclic benzopyrone skeleton in 42–83 % yield.
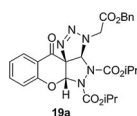
19 a: ^{1}H NMR (500 MHz, CDCl3) $\delta = 7.86$ (dd, $J = 7.9$, 1.6 Hz, 1 H), 7.53 (ddd, $J = 8.6$, 7.5, 4.3 Hz, 1 H), 7.33–7.22 (m, 5 H), 7.09 (t, $J = 7.4$ Hz, 1 H), 7.01 (d, $J = 8.3$ Hz, 1 H), 6.54 (s, 1 H), 6.01 (s, 1 H), 5.17 (q, $J = 12.2$ Hz, 2 H), 4.95–4.81 (m, 3 H), 4.33 (d, $J = 15.2$ Hz, 1 H), 1.19 (dd, $J = 6.3$, 1.8 Hz, 6 H), 1.14 (d, $J = 6.1$ Hz, 6 H); ^{13}C NMR (126 MHz, CDCl3) $\delta = 182.7$, 167.4, 158.7, 152.9, 137.8, 135.1, 128.6, 128.5, 128.3, 127.7, 123.4, 118.9, 118.7, 96.8, 90.5, 75.9, 71.9, 67.3, 47.6, 29.7, 26.9, 21.9, 21.8, 21.7, 21.6; HRMS [ESI]: calcd for C$_{27}$H$_{30}$N$_5$O$_8$ [M+H+]: 552.2089, found: 552.2088.
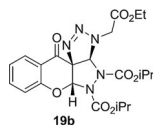
19 b: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.93 (dd, $J = 7.9$, 1.7 Hz, 1 H), 7.65–7.54 (m, 1 H), 7.19–7.12 (m, 1 H), 7.08 (d, $J = 8.3$ Hz, 1 H), 6.60 (s, 1 H), 6.07 (s, 1 H), 5.03–4.93 (m, 2 H), 4.88 (d, $J = 17.9$ Hz, 1 H), 4.34 (d, $J = 15.0$ Hz, 1 H), 4.26 (tdd, $J = 10.8$, 7.1, 3.7 Hz, 2 H), 1.33–1.22 (m, 15 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 183.1, 167.7, 158.9, 138.0, 127.9, 123.6, 119.2, 118.9, 97.0, 90.7, 76.2, 72.1, 62.0, 47.8, 22.0, 21.9, 21.9, 14.3; HRMS [ESI]: calcd for C$_{22}$H$_{28}$N$_5$O$_8$ [M+H$^+$]: 490.1932, found: 490.1932.
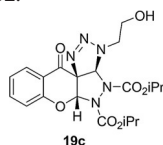
19 c: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.89 (d, $J = 7.9$ Hz, 1 H), 7.60 (t, $J = 7.8$ Hz, 1 H), 7.15 (t, $J = 7.6$ Hz, 1 H), 7.07 (d, $J = 8.4$ Hz, 1 H), 6.58 (s, 1 H), 5.92 (s, 1 H), 5.00 (dd, $J = 13.2$, 7.0 Hz, 2 H), 4.11 (s, 1 H), 3.92–3.87 (m, 3 H), 1.38–1.19 (m, 12 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 183.9, 158.9, 138.2, 127.9, 123.7, 119.2, 118.9, 96.1, 90.6, 77.4, 72.4, 60.6, 22.0, 21.9, 21.9, 21.8; HRMS [ESI]: calcd for C$_{20}$H$_{26}$N$_5$O$_7$ [M+H$^+$]: 448.1827, found: 448.1822.
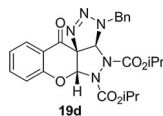
19 d: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.81 (d, $J = 7.9$ Hz, 1 H), 7.51 (t, $J = 8.0$ Hz, 1 H), 7.30 (d, $J = 4.3$ Hz, 4 H), 7.26–7.22 (m, 1 H), 7.06 (t, $J =$

7.6 Hz, 1 H), 6.99 (d, $J = 8.4$ Hz, 1 H), 6.55 (s, 1 H), 5.70 (s, 1 H), 5.25 (d, $J = 15.4$ Hz, 1 H), 5.02–4.91 (m, 1 H), 4.91–4.81 (m, 1 H), 4.62 (d, $J = 16.0$ Hz, 1 H), 1.28–1.22 (m, 6 H), 1.12 (d, $J = 6.1$ Hz, 6 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 183.9, 160.8, 158.9, 156.7, 138.1, 136.6, 135.3, 129.0, 128.2, 127.8, 123.6, 119.2, 118.9, 96.3, 93.2, 90.8, 76.1, 72.0, 50.6, 22.0, 22.0, 21.9, 21.8; HRMS [ESI]: calcd for C$_{25}$H$_{28}$N$_5$O$_6$ [M+H$^+$]: 494.2034, found: 494.2028.
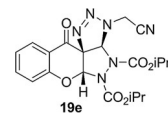
19 e: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.91 (dd, $J = 7.9$, 1.6 Hz, 1 H), 7.68–7.56 (dd, $J = 8.0$, 4.1 Hz, 1 H), 7.17 (t, $J = 8.0$ Hz, 1 H), 7.10 (d, $J = 8.3$ Hz, 1 H), 6.65 (s, 1 H), 5.89 (s, 1 H), 5.10–4.90 (m, 2 H), 4.83 (d, $J = 17.2$ Hz, 1 H), 4.60 (d, $J = 17.5$ Hz, 1 H), 1.31–1.20 (m, 12 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 182.1, 158.9, 153.1, 138.5, 127.9, 125.7, 123.9, 119.0, 113.9, 98.3, 90.4, 75.4, 73.0, 72.9, 34.9, 22.0, 21.9, 21.9, 21.8, 15.5; HRMS [ESI]: calcd for C$_{20}$H$_{23}$N$_6$O$_6$ [M+H$^+$]: 443.1674, found: 443.1673.
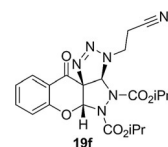
19 f: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.89 (dd, $J = 7.9$, 1.5 Hz, 1 H), 7.60 (ddd, $J = 8.4$, 7.3, 1.7 Hz, 1 H), 7.18–7.13 (m, 1 H), 7.07 (d, $J = 8.3$ Hz, 1 H), 6.61 (s, 1 H), 5.85 (s, 1 H), 4.92–5.06 (m, 2 H), 4.18–4.08 (m, 1 H), 4.06–3.91 (m, 1 H), 2.94 (t, $J = 7.3$ Hz, 2 H), 1.32–1.25 (m, 12 H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 182.9, 158.9, 152.9, 138.3, 127.9, 123.8, 119.1, 118.9, 117.2, 97.1, 90.4, 76.2, 72.6, 43.0, 29.5, 22.1, 21.99, 21.9, 21.9, 17.8. HRMS [ESI]: calcd for C$_{21}$H$_{25}$N$_6$O$_6$ [M+H$^+$]: 457.1830, found: 457.1827.
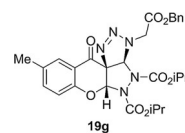
19 g: ^{1}H NMR (400 MHz, CDCl3) δ 7.71 (d, $J = 1.9$ Hz, 1 H), 7.40 (dd, $J = 8.5$, 2.3 Hz, 1 H), 7.38–7.32 (m, 5 H), 6.98 (d, $J = 8.5$ Hz, 1 H), 6.58 (s, 1 H), 6.06 (s, 1 H), 5.24 (q, $J = 12.2$ Hz, 2 H), 5.01–4.89 (m, 3 H), 4.40 (d, $J = 17.2$ Hz, 1 H), 2.35 (s, 3 H), 1.33–1.10 (m, 12 H); 13C NMR (101 MHz, CDCl3) δ 183.1, 167.6, 156.9, 139.1, 135.3, 133.4, 128.8, 128.7, 128.6, 127.4, 118.8, 118.6, 97.1, 90.7, 76.1, 72.1, 67.5, 47.8, 22.2, 22.0, 21.9, 21.9, 20.7; HRMS [ESI]: calcd for C28H32N5O8 [M+H+]: 566.2245, found: 566.2245.
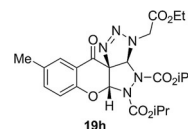
19 h: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.71 (d, $J = 1.0$ Hz, 1 H), 7.40 (dd, $J = 8.5$, 1.8 Hz, 1 H), 6.98 (d, $J = 8.2$ Hz, 1 H), 6.57 (s, 1 H), 6.05 (s, 1 H), 5.04–4.91 (m, 2 H), 4.88 (d, $J = 17.9$ Hz, 1 H), 4.34 (d, $J = 14.6$ Hz, 1 H), 4.26 (tdd, $J = 10.8$, 7.2, 3.6 Hz, 2 H), 2.34 (s, 3 H), 1.30 (t, $J = 7.1$ Hz, 3 H), 1.28–1.22 (m, 12 H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 183.2, 167.7, 156.9, 139.1, 133.4, 127.4, 125.7, 118.8, 118.6, 97.0, 90.7, 76.2, 72.0, 61.9, 47.8, 30.5, 29.9, 22.2, 22.0, 21.9, 21.9, 20.6, 14.3; HRMS [ESI]: calcd for C$_{23}$H$_{30}$N$_5$O$_8$ [M+H$^+$]: 504.2089, found: 504.2087.
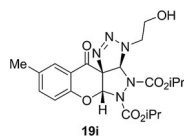
19i: ^{1}H ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.59 (s, 1H), 7.33 (dd, $J=8.5$, 2.1 Hz, 1H), 6.91 (d, $J=8.5$ Hz, 1H), 6.48 (s, 1H), 5.85 (s, 1H), 4.98–4.85 (m, 2H), 4.05 (s, 1H), 3.90 (dd, $J=10.6$, 7.8 Hz, 1H), 3.83 (d, $J=13.8$ Hz, 2H), 2.26 (s, 3H), 1.30–1.14 (m, 12H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 183.8, 156.7, 139.0, 133.2, 127.1, 118.6, 118.4, 95.90, 90.4, 77.2, 72.1, 60.4, 29.7, 21.8, 21.7, 21.7, 21.6, 20.4; HRMS [ESI]: calcd for C$_{21}$H$_{28}$N$_5$O$_7$ [M+H$^+$]: 462.1983, found: 462.1980.
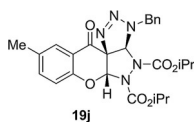
19j: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.65 (d, $J=1.8$ Hz, 1H), 7.40–7.27 (m, 6H), 6.95 (d, $J=8.4$ Hz, 1H), 6.58 (s, 1H), 5.75 (s, 1H), 5.32 (d, $J=15.3$ Hz, 1H), 5.09–4.97 (m, 1H), 4.92 (dt, $J=12.5$, 6.3 Hz, 1H), 4.68 (d, $J=15.0$ Hz, 1H), 2.31 (s, 3H), 1.34–1.28 (m, 6H), 1.18 (d, $J=6.1$ Hz, 6H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 184.0, 157.0, 153.2, 139.1, 135.3, 133.3, 131.5, 129.0, 128.2, 127.2, 118.9, 118.6, 96.3, 90.7, 76.1, 71.9, 50.6, 22.0, 22.0, 21.9, 21.8, 20.6; HRMS [ESI]: calcd for C$_{26}$H$_{30}$N$_5$O$_6$ [M+H$^+$]: 508.2191, found: 508.2186.
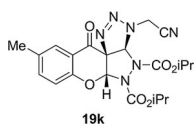
19k: ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.71 (s, 1H), 7.45 (dd, $J=8.5$, 2.1 Hz, 1H), 7.02 (d, $J=8.4$ Hz, 1H), 6.63 (s, 1H), 5.89 (s, 1H), 5.02 (ddd, $J=23.9$, 12.2, 6.0 Hz, 2H), 4.85 (d, $J=15.9$ Hz, 1H), 4.61 (d, $J=17.5$ Hz, 1H), 2.36 (s, 3H), 1.38–1.20 (m, 12H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 182.02, 156.7, 152.9, 139.4, 133.5, 127.2, 118.6, 118.5, 113.7, 98.2, 90.2, 75.2, 72.6, 34.7, 21.9, 21.8, 21.7, 21.7, 20.4; HRMS [ESI]: calcd for C$_{21}$H$_{25}$N$_6$O$_6$ [M+H$^+$]: 457.1830, found: 457.1828.
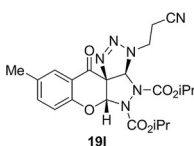
19l: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.61–7.58 (m, 1H), 7.34 (dd, $J=8.5$, 2.3 Hz, 1H), 6.91 (d, $J=8.3$ Hz, 1H), 6.51 (s, 1H), 5.78 (s, 1H), 4.99–4.86 (m, 2H), 4.12–4.02 (m, 1H), 3.94 (dt, $J=14.2$, 7.2 Hz, 1H), 2.88 (t, $J=7.3$ Hz, 2H), 2.26 (s, 3H), 1.27–1.18 (m, 12H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 183.0, 156.9, 153.0, 139.4, 133.6, 127.3, 118.8, 118.7, 117.4, 97.1, 90.4, 76.2, 72.5, 43.0, 22.0, 22.0, 21.9, 21.9, 20.6, 17.8; HRMS [ESI]: calcd for C$_{22}$H$_{27}$N$_6$O$_6$ [M+H$^+$]: 471.1887, found: 471.1984.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

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[17] CCDC1824805 (**17 e**) contains the supplementary crystallographic data for this paper. This data can be obtained free of charge from The Cam-

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