Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2017, **15**, 9108

A diversity oriented synthesis of natural product inspired molecular libraries†

Jyoti Chauhan,[a] Tania Luthra,[a] Rambabu Gundla,[b] Antonio Ferraro,[c] Ulrike Holzgrabe [c] and Subhabrata Sen *[a]

Received 5th September 2017,
Accepted 10th October 2017

DOI: 10.1039/c7ob02230a

rsc.li/obc

Natural products are the source of innumerable pharmaceutical drug candidates and also form an important aspect of herbal remedies. They are also a source of various bioactive compounds. Herein we have leveraged the structural attributes of several natural products in building a library of architecturally diverse chiral molecules by harnessing *R*-tryptophan as the chiral auxiliary. It is converted to its corresponding methyl ester **1** which in turn provided a bevy of 1-aryl-tetrahydro-β-carbolines **2a–d**, which were then converted to chiral compounds *via* a diversity oriented synthetic strategy (DOS). In general, intermolecular and intramolecular ring rearrangements facilitated the formation of the final compounds. Four different classes of molecules with distinct architectures were generated, adding up to nearly twenty-two individual molecules. Phenotypic screening of a representative section of the library revealed two molecules that selectively inhibit MCF7 breast cancer cells with IC_{50} of ~5 μg mL^{-1} potency.

Introduction

To investigate complex biological systems, small molecules have been applied to modulate proteins by directly interacting with them.[1] In conventional genetic approaches, biological systems were assessed by developing random mutations which were then screened in search of a precise cellular phenotype.[2] Analogous to the genetic approach, large random collections of small molecules can be used to elucidate the roles of specific proteins in many biological pathways.[3] The essence of this chemical genetic approach is the design and synthesis of a library of compounds which cover large areas of biologically relevant chemical space.[4]

By virtue of binding both their biosynthetic enzymes and their target molecules, natural products necessarily reside in biologically relevant chemical space.[5] Natural product families are libraries of prevalidated, functionally diverse structures.[6] Therefore developing a library of molecules with scaffolds inspired from natural products could provide biologically active novel molecules.[7] There are quite a few reports of molecular libraries inspired from natural products.[8a–d] For example, Khan *et al.* reported the synthesis of a molecular library inspired from the marine natural product ianthelliformisamines and its biological evaluation; in another report, Singh and co-workers generated a natural product inspired β-carboline and γ-lactone based molecular hybrids and Waldmann reported the stereoselective synthesis of a natural product inspired tetrahydroindolo[2,3-*a*]-quinolizine compound library.[8c,d]

Additionally, to counter the challenge of gaining rapid access to structurally diverse natural product inspired libraries, diversity oriented synthesis (DOS) is an ideal tool.[9] This concept, discovered by Schreiber at the beginning of the last decade, is a forward directional strategy that involves the facile preparation of libraries of architecturally complex and diverse compounds from simple starting materials. DOS can be achieved through various approaches.[10] One such strategy, the reagent based approach, involves the generation of a densely functionalized central building block which in turn can be transformed into various scaffolds by subjecting them to varied tranformations.[11] Oxidative rearrangement chemistry extends interesting possibilities for DOS application, especially in reagent based approaches. In this direction, a large group of diverse reactions can be performed to generate scaffolds with diverse architectures.[12]

Herein we have reported the design and synthesis of a library of molecules based on the following natural products: perophoramidine, spirotryprostatin, harmicin and tryprostatin A and B (Fig. 1a).[13] The resulting compounds were generated

[a]*Department of Chemistry, School of Natural Sciences, Shiv Nadar University, Dadri, Chithera, GautamBudh Nagar, UP 201314, India.*
E-mail: subhabrata.sen@snu.edu.in
[b]*Department of Chemistry, Gitam Institute of Technology, GITAM University, Hyderabad, Telengana, India*
[c]*Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany*

†Electronic supplementary information (ESI) available. CCDC 1572633 and 1572513. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7ob02230a

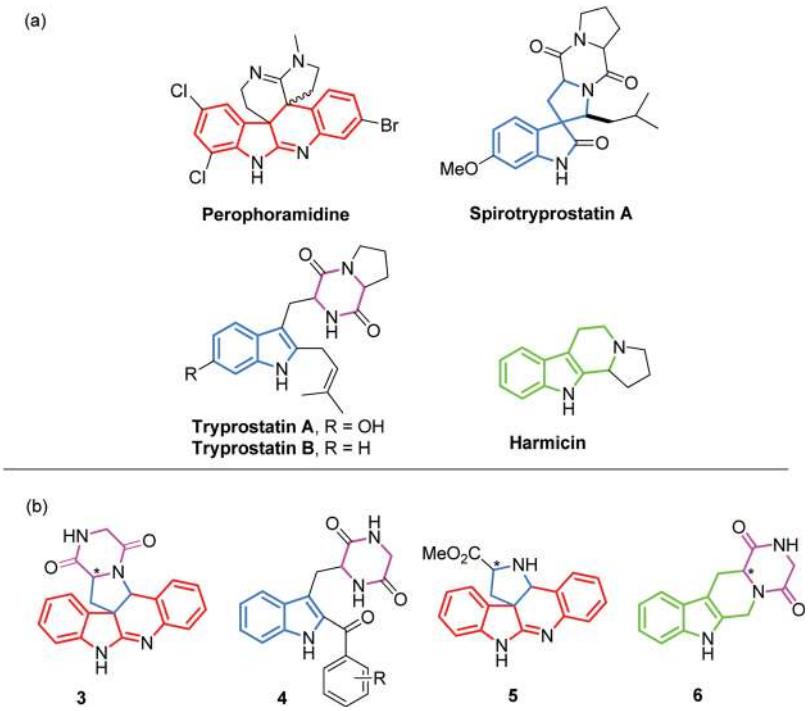


Fig. 1 Natural products and the library inspired from them.

by modifying a central tetrahydro-β-carboline moiety under various reaction conditions to generate compounds **3**, **4**, **5** and **6** (Fig. 1b). Such an effort is purely intuitive with the expectation that the process will provide novel biologically active molecules. In general, this strategy involved a DOS, *via* a novel (oxidative) inter/intramolecular ring rearrangement. A representative section of the library was screened against MCF7 cell lines to assess their ability to modulate a particular cancer phenotype.

Results and discussion

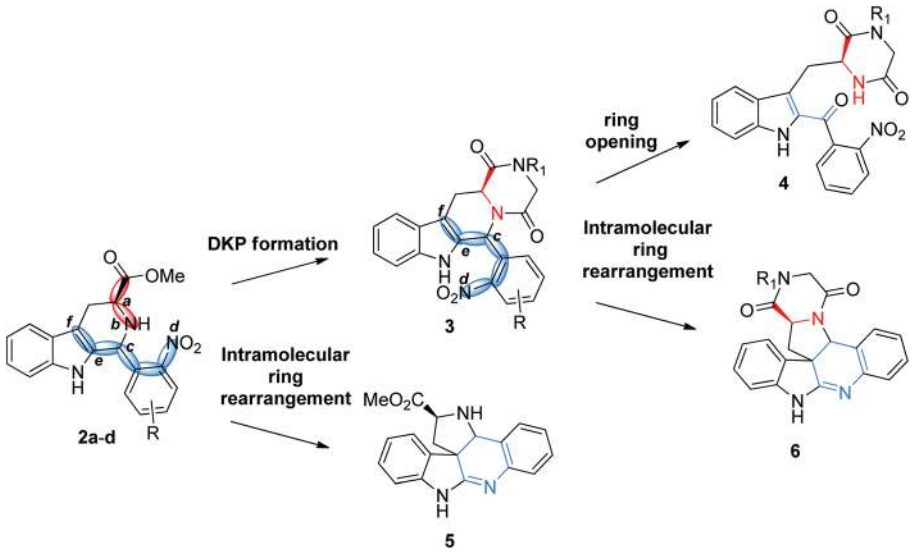
Design

The design of this library is based on tetrahydro-β-carbolines **2a–d**. They were conceived as central scaffolds because they contained several pluripotent reaction centers such as a, b, c, d and e (Scheme 1). A careful choice of the reaction conditions will enable us to harness these centers selectively to provide us with the desired scaffolds **3–6**. The design enabled the library to proliferate from one scaffold to another. For example, compound **2**, could provide **3** and **5**. Compound **3** in turn could furnish **4** and **6** (Scheme 1). Accordingly, we envisioned that compound **3** could be accessed by exploiting the reactivity of centers a and b of **2**, by sequential treatment of chloroacetyl chloride and appropriate amines (Scheme 1). Next, compound **4** could be obtained from **3** by an oxidative ring opening reaction involving centers c, e and f (Scheme 1). We further envisioned that **6** could also be accessed from **3** *via* an intramolecular ring rearrangement involving centers c, d, e and f (Scheme 1). A similar oxidative ring rearrangement of **2** again involving centers c–f could also provide compound **5** (Scheme 1).

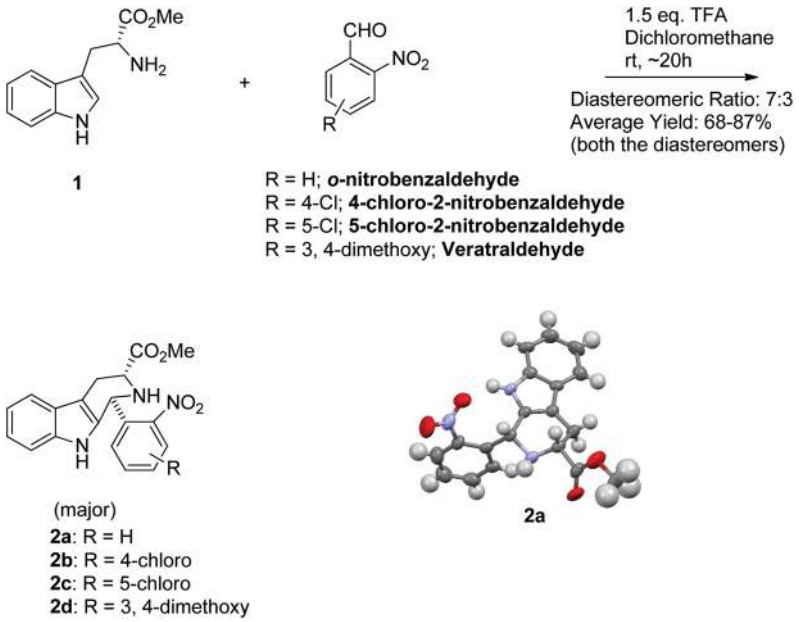
Chemistry

With the design in place, we embarked on the synthesis of the molecular library. To begin with, the central tetrahydro-β-carboline building blocks **2a–d** were synthesized *via* a Pictet Spengler[13f] reaction between **1** (obtained by methylation of *R*-tryptophan) and the appropriately substituted *o*-nitrobenzaldehydes (*i.e.* *o*-nitrobenzaldehyde, 4-chlorobenzaldehyde, 5-chlorobenzaldehyde and veratraldehyde) (Scheme 2). A typical reaction involved refluxing **1**, 1.5 equiv. of trifluoroacetic acid and 1.2 equiv. of *o*-nitrobenzaldehyde in acetonitrile. The desired products were obtained as 1 : 1 diastereomeric mixtures. From the desired products that were generated, we opted to proceed with compound **2a** (in a bid to prove this modular concept this was a random choice) which was isolated *via* column chromatography. Accordingly, single crystal X-ray of **2a** (refer to the ESI†) indicated that the methyl ester and the aryl moiety were in *syn* conformation. Compounds **2b–d** were generated in a similar fashion and were taken forward to proliferate the library (Scheme 2).

The first set of molecules, **3a–g**, that we envisioned possessed tetrahydro-β-carboline diketopiperazine (DKP) scaffolds. Accordingly, **2a** was treated with chloroacetyl chloride to provide amide **9a** which was further treated with methyl amine to facilitate an *in situ* nucleophilic substitution followed by lactamization (with the ester) to generate the desired hybrid **3a** in 58% yield. In a similar fashion, **2a**, after reaction with chloroacetyl chloride ($ClCH_2COCl$), was treated with various



Scheme 1 Design of the hybrid library.

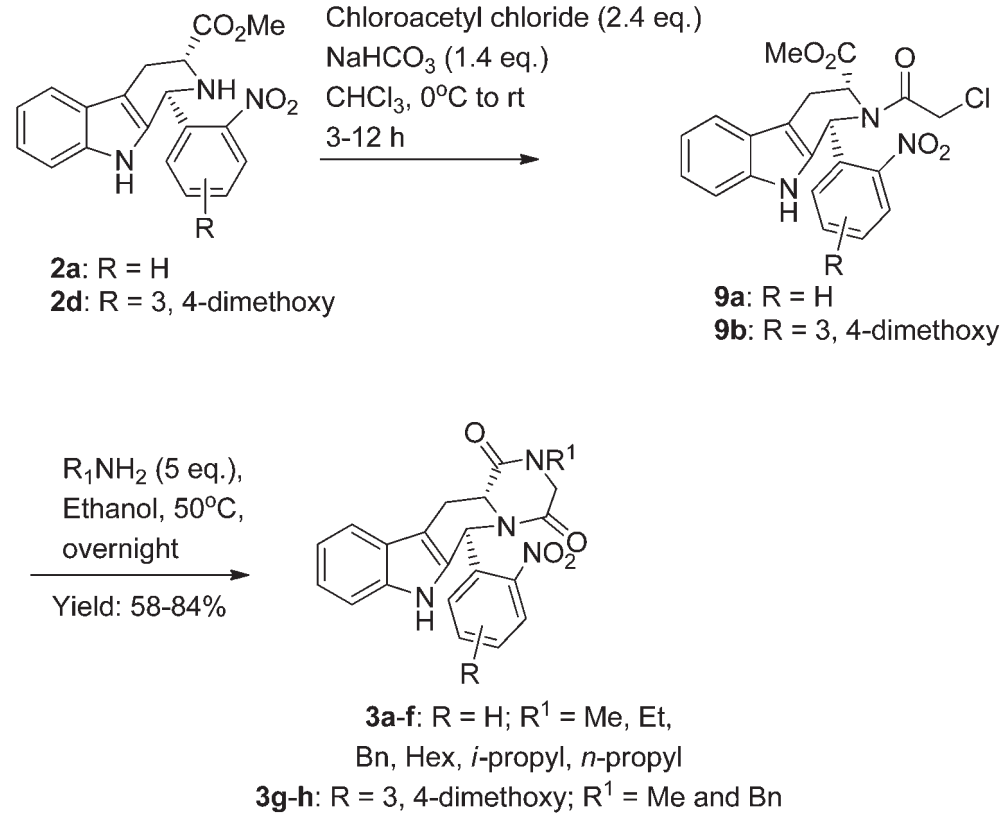


Scheme 2 Synthesis of the central scaffolds **2a–d**.

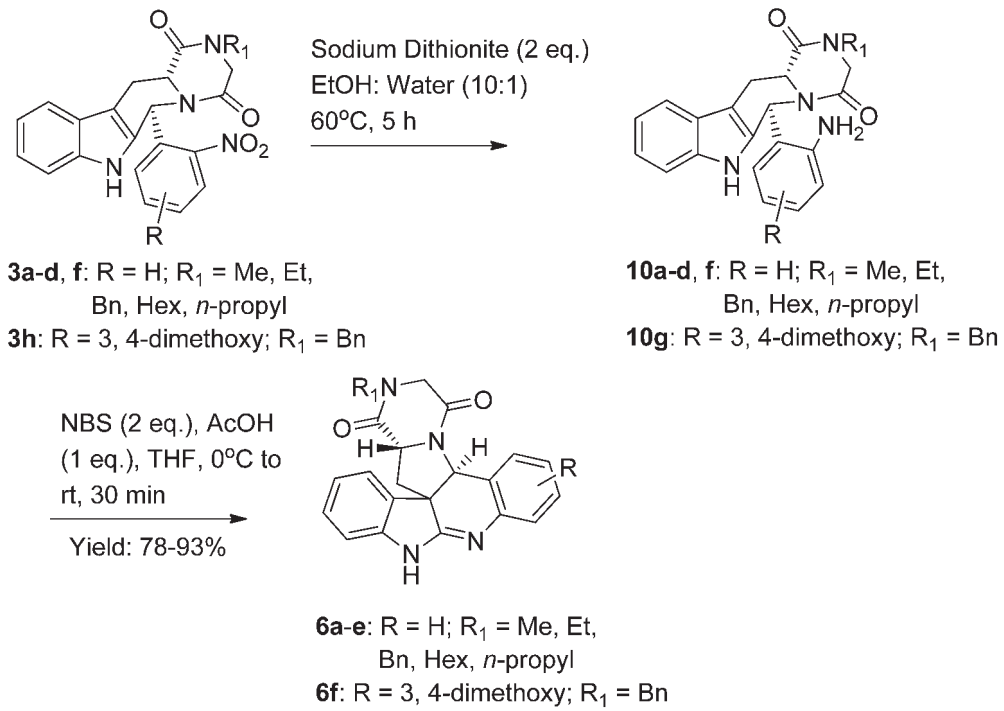
other amines, *i.e.* ethyl, benzyl, hexyl, propyl and isopropyl, to afford **3b–f**. Accordingly **2d** provided **3h** (Scheme 3).

Next, as envisioned during the design of the molecules, sodium dithionate mediated nitro reduction of **3a–d**, **f** and **g** facilitated the amine intermediates **10a–d**, **f** and **g** (Scheme 4). The reduction was extremely facile with no formation of any by-products. Consequently, amines **10a–d**, **f** and **g** were used in the next step without further purification. To promote the intramolecular oxidative ring rearrangement of these amines, a variety of oxidants such as *t*-butoxychloride (*t*-BuOCl), *N*-bromosuccinimide (NBS), sodium tungstate (dehydrate) ($Na_2WO_4{\cdot}2H_2O$), lead tetraacetate ($Pb(OAc)_4$) and osmium tetroxide (OsO_4) were explored with **10a** as the model substrate. To our extreme gratification, NBS furnished the best yield (~91%) of the desired hybrid **6a** which is the major diastereomer as a 70 : 30 mixture of the products. It was separated from the mixture by column chromatography. With the optimized conditions in hand, the other hybrids, **6b–f**, were also synthesized in moderate to excellent yields (Scheme 4).

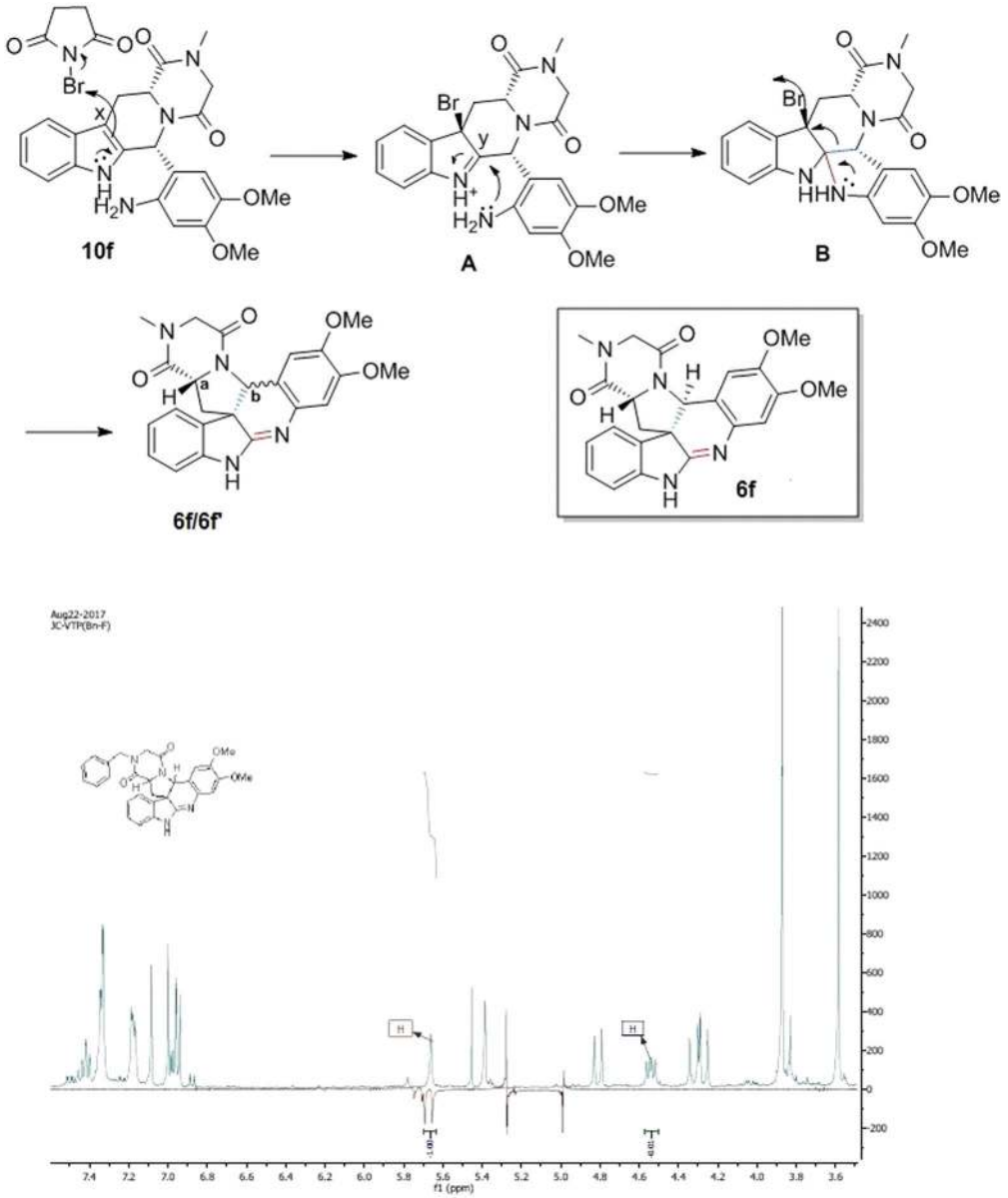
The rationale behind the transformation of the resulting hybrids **6a–f** from the corresponding amine intermediate could be explained by the putative mechanism of the intramolecular ring rearrangement of **6f**, as depicted in Scheme 5 below. The reaction involved the treatment of **10f** with NBS as



Scheme 3 Synthesis of the tetrahydro-β-carboline-DKP hybrids **3a–g**.



Scheme 4 Synthesis of hybrids **6a–f**.

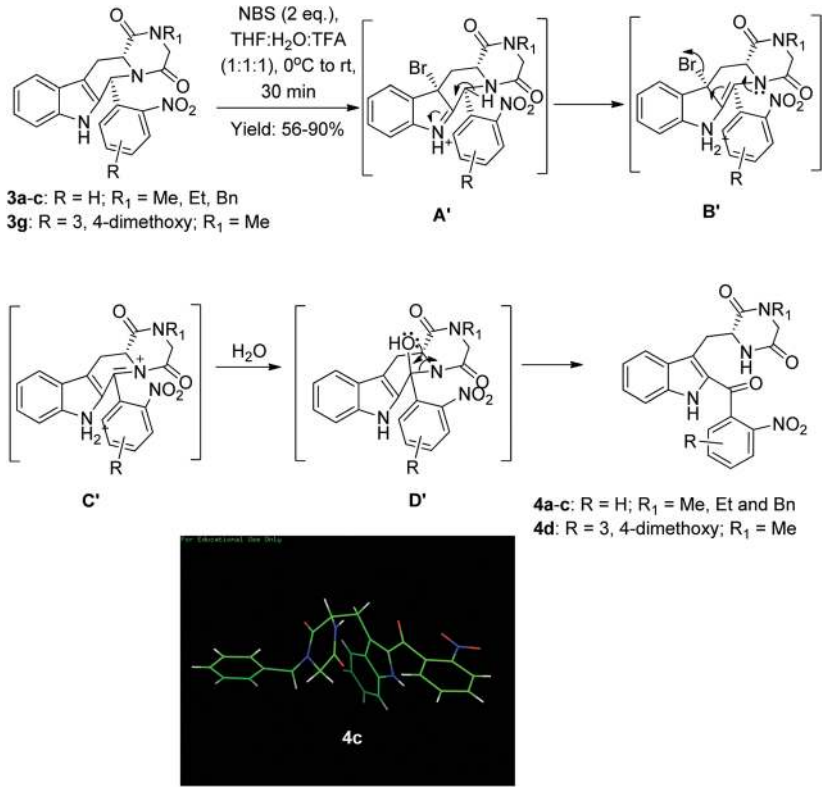


Scheme 5 Putative mechanism of the intramolecular ring rearrangement to generate **6**.

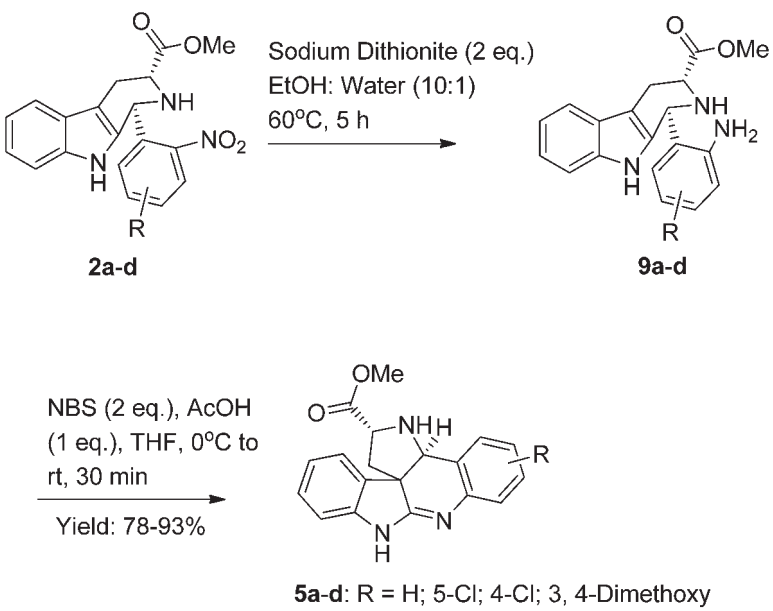
an oxidant, which brominates at C_X of **10f** to generate **A**. The bromination is expected to occur from the β face to avoid the steric interaction from the DKP and 2-nitroaryl moiety. A nucleophilic attack by the aromatic amine at C_Y generated **B** followed by a final bond rearrangement prompted by the imine formation, where the bond shift happened from the opposite face of the bromide functionality (in a fashion similar to SN_2 reactions) to afford the desired products **6f/f'** as a 70 : 30 mixture of diastereomers (Scheme 5) from which the major diastereomer **6f** was isolated by column chromatography. 1D-NOESY experiment of **6f** provided the relative configuration of the stereocenters **a** and **b** as *S* and *R*, respectively (Scheme 5).

In a bid to further diversify our scaffold library, **3a–g** were treated with NBS in the presence of tetrahydrofuran : water : trifluoroacetic acid (1 : 1 : 1). Initially the bromination occurred at C_3 of the indole skeleton to afford the bromo iminium intermediate **A′**, which subsequently led to the formation of enamine **B′**. Elimination of the bromide on **B′** generated **C′** followed by the attack of the water molecule to afford hemiaminal **D′**. **D′** then underwent ring opening to furnish the desired hybrids **4** (Scheme 6).

Finally compounds **5a–d** were synthesized, *via* a similar ring rearrangement as in compounds **6a–g**, from the starting substrates **2a–d** by their subsequent reduction with sodium dithionite (to generate **9a–d**) followed by treatment with NBS and acetic acid. Here too we obtained the major diastereomers **5a–d** from a 80 : 20 mixture, by column chromatography in 60–72% yield (Scheme 7).



Scheme 6 Synthesis of hybrids **4a–g**.



Scheme 7 Synthesis of hybrid scaffolds **5**.

Phenotyping screening

The original natural products that inspired our molecules have a rich legacy as anticancer compounds. Subsequently we decided to screen our molecules for anticancer activity. Due to the paucity of the cell lines, a representative section of the library of molecules was screened against MCF7 (breast cancer cells; Table 1). Doxorubicin, a potent cancer drug, was used as

Table 1 Inhibition of proliferation of MCF7 cancer cells

Entry	Compound #	IC_{50} [a] (μg mL^{-1}) MCF7
1	**3a**	16.28
2	**3b**	9.21
3	**3e**	12.88
4	**3f**	9.21
5	**4g**	15.95
6	**6a**	5.01
7	**6b**	9.58
8	**6c**	7.02
9	**6d**	8.32
10	**6e**	5.33
11	**6f**	14.01
12	**5a**	28.91
13	Doxorubicin	1.81

[a] The results shown are expressed as mean ± SEM of three independent assays.

the positive control. From the screening it was evident that our library of molecules was efficacious against MCF7 cells (Table 1). Among the compounds screened, **6a** and **6e** (comprised of indoloquinolone, spirooxindole and DKP scaffolds) inhibited the proliferation of MCF7 cells with an IC_{50} of ~5 μg mL^{-1}. To investigate whether our compounds deploy their anticancer activity through cytotoxicity, we screened them against MCF10A, a normal cell line. For **6a** and **6e**, the dose required to induce cytotoxicity was ~100 fold higher than their respective IC_{50} concentration (Table 1). These anticancer hits at low μg mL^{-1} levels definitely emphasize the merits of our DOS library, since the presumptions from such unbiased, early scaffold explorations are modest (double digit μg mL^{-1} potency). This further underlines the "significance" of the hybrid aspect of our library and the power of the DOS approach to deliver hit and sometimes lead structures.

Conclusion

We have reported a DOS strategy based primarily on the oxidative ring rearrangement and ring opening reactions to synthesize a library of chiral molecules with high architectural diversity. An extremely versatile tetrahydro-β-carboline motif was harnessed as the central building block. The molecules inspired from diverse natural products are essentially comprised of four distinct architectures. This modular strategy demonstrated an efficient 2 step/scaffold ratio. Phenotypic screening against MCF7 breast cancer cells displayed selective low μmolar inhibitory activity by two compounds against MCF7 cell proliferation. This effort demonstrates the utility of an unbiased approach like diversity oriented synthesis in discovering novel bioactive scaffolds. The low μmolar hits against MCF7 obtained from this endeavor can be utilized further to develop drug candidates for breast cancer through protein deconvolution and subsequent structure–activity relationship studies.

Experimental section

General

All reactions were carried out under an N_2 or O_2 atmosphere, as specified. Column chromatography was performed on silica gel (100–200 mesh), and the reactions were monitored by thin layer chromatography (TLC, Silica gel 60 F_{254}), using UV light to visualize the course of the reaction. ^{1}H NMR and ^{13}C NMR spectra were recorded with tetramethylsilane as an internal standard at ambient temperature, unless otherwise indicated, with Bruker 400 MHz instruments at 400 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR spectroscopy. The splitting patterns are designated as singlet (s), broad singlet (br. s), doublet (d), and triplet (t). The splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). Mass spectrometry analysis was performed with a 6540 UHD Accurate-Mass QTOF LC–MS system (Agilent Technologies) equipped with an Agilent 1290 LC system procured by the Department of Chemistry, School of Natural Sciences, Shiv Nadar University, Uttar Pradesh 201314, India. HPLC experiments were carried out in an Agilent Eclipse Plus C18 column. SEM and EDX analyses were carried out using a Zeiss EVO 18 Special system. ICP-MS experiment was carried out using an Element XR system. TEM analysis was carried out using a Tecnai G2 F30 (300 kV) system.

Representative experimental procedures for Pictet Spengler reactions of *R*-tryptophan and appropriate aldehydes (2a–d)

To a solution of L-tryptophan methyl ester in dichloromethane (DCM) was added substituted 2-nitrobenzaldehydes with trifluoroacetic acid (TFA) and allowed to stir at room temperature for 24 h. After completion of the reaction, the reaction mixture was concentrated under reduced pressure and quenched with a saturated solution of sodium bicarbonate ($NaHCO_3$). It was then extracted with ethyl acetate (EtOAc) (3 × 20 mL). The organic layer was washed with brine solution and dried over sodium sulphate (Na_2SO_4) and concentrated over reduced pressure to obtain the crude product. It is purified with column chromatography.

(1*R*,3*S*)-Methyl-1-(2-nitrophenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (2a). Following the general procedure, the desired compound **2a** was generated as a yellow solid with a yield of 89% (purified *via* flash column chromatography with ethyl acetate–hexane (2 : 8) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.86 (dd, J = 1.2, 8 Hz, 1H), 7.73 (dd, J = 1.2, 8 Hz, 1H), 7.57–7.50 (m, 2H), 7.44 (m, 1H), 7.60–7.23 (m, 1H), 7.18–7.11 (m, 2H), 5.71 (s, 1H), 3.96 (dd, J = 4, 10.8 Hz, 1H), 3.83 (s, 3H), 3.27 (dd, J = 2.4, 15.2 Hz, 1H), 3.06 (m, 1H), 2.95 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.92, 150.26, 136.45, 133.61, 133.40, 131.80, 129.03, 126.88, 123.77, 122.36, 119.86, 118.45, 111.17, 109.66, 56.58, 53.18, 52.48, 25.49. HRMS (EI+) m/z calcd for $C_{19}H_{17}N_3O_4$ $[M]^+$: 352.1292, found: 352.1291.

(1*R*,3*S*)-Methyl-1-(4-chloro-2-nitrophenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (2b). Following the general procedure, the desired compound **2b** was generated as

a light yellow solid with a yield of 79% (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 9) as the eluent). ^{1}H NMR (400 MHz; $CDCl_3$): 8.21 (s, 1H); 7.79–7.78 (d, J = 4 Hz, 1H); 7.54–7.52 (d, J = 8 Hz, 1H); 7.50–7.48 (m, 2H); 7.24–7.15 (m, 3H); 5.94 (s, 1H); 4.09–4.05 (m, 1H); 3.83 (s, 3H); 3.31–3.26 (s, 1H); 3.17–3.10 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 169.38, 149.62, 137.13, 136.88, 134.09, 133.63, 128.49, 126.20, 125.73, 125.58, 123.54, 120.35, 118.56, 111.70, 108.62, 53.32, 52.80, 50.57, 22.30. $[M + H]^+$ calculated for ($C_{19}H_{16}N_3ClO_4$) 386.0902, found 386.0928.

(1*R*,3*S*)-Methyl-1-(5-chloro-2-nitrophenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (2c). Following the general procedure, the desired compound **2c** was generated as a light yellow solid with a yield of 72% (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 9) as the eluent). ^{1}H NMR (400 MHz; $CDCl_3$): 8.24 (s, 1H); 7.68–7.65 (d, J = 12 Hz, 2H); 7.54–7.52 (d, J = 8 Hz, 1H); 7.35–7.28 (m, 2H); 7.24–7.16 (m, 2H); 6.12 (s, 1H); 4.14–4.09 (m, 1H); 3.87 (s, 3H); 3.36–3.31 (s, 1H); 3.25–3.19 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 169.68, 148.03, 140.30, 136.81, 130.72, 126.22, 125.81, 123.21, 120.34, 118.44, 117.38, 111.80, 108.77, 65.88, 55.86, 53.27, 23.04. $[M + H]^+$ calculated for ($C_{19}H_{16}N_3ClO_4$) 386.0902, found 386.0929.

Methyl-1-(4,5-dimethoxy-2-nitrophenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (2d). Following the general procedure, the desired compound **2d** was generated as a yellow colored solid with a yield of 87% (purified *via* flash column chromatography with ethyl acetate–hexane (3 : 7) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.48 (s, 1H), 7.28 (s, 1H), 7.22 (s, 1H), 7.20–7.13 (m, 2H), 5.86 (s, 1H), 4.01 (d, J = 3.6 Hz, 1H), 3.99 (d, J = 4 Hz, 1H), 3.96 (s, 3H), 3.86 (s, 3H), 3.80 (s, 3H), 3.30–3.25 (m, 1H), 3.12–3.05 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.99, 153.56, 148.55, 142.52, 136.38, 133.80, 131.48, 126.97, 122.25, 119.78, 118.38, 112.10, 111.19, 109.42, 107.05, 56.68, 56.56, 56.53, 53.34, 52.47, 25.38. HRMS (EI+) m/z calcd for $C_{21}H_{21}N_3O_6$ $[M]^+$: 412.1503, found: 412.1532.

Representative experimental procedures for the synthesis of tetrahydro-β-carboline-diketopiperazine hybrids (3a–h)

To a stirred solution of compounds **2a** and **d** (1 equiv.) and sodium bicarbonate ($NaHCO_3$) (1.2 equiv.) in chloroform ($CHCl_3$), chloroacetyl chloride (2.4 equiv.) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 3 h. After completion of the reaction, which was monitored by TLC, the reaction mixture was diluted with $CHCl_3$ and washed with a saturated solution of $NaHCO_3$, brine, dried over Na_2SO_4, and evaporated under reduced pressure to obtain the crude product which was used in the next step without further purification.

The solution of the above crude compound (1 equiv.) and appropriate amine (5 equiv.) in ethanol was stirred at room temperature for 24 h. It was then concentrated under reduced pressure after the completion of the reaction. The crude product was purified with column chromatography, with ethyl acetate–hexane as the eluent.

2-Methyl-6-(2-nitrophenyl)-2,3,12,12a-tetrahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4(6*H*,7*H*)-dione (3a). Following the general procedure, the desired compound **3a** was generated as a light yellow solid with a yield of 58% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (3 : 7) as the eluent). ^{1}H NMR (400 MHz, DMSO) δ 10.53 (s, 1H), 7.96 (d, J = 7.2 Hz, 1H), 7.58 (d, J = 8 Hz, 1H), 7.50 (t, 1H), 7.43–7.39 (m, 1H), 7.33 (s, 1H), 7.31 (s, 1H), 7.06 (t, J = 6.8, 16 Hz, 1H), 7.01 (t, J = 7.2, 14 Hz, 1H), 6.71 (s, 1H), 4.50 (d, J = 7.2 Hz, 1H), 4.01 (s, 1H), 3.91 (s, 1H), 3.63–3.59 (m, 1H), 3.18 (m, 1H), 2.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 167.15, 165.70, 148.82, 138.23, 137.01, 133.81, 131.87, 128.91, 127.98, 125.70, 123.59, 121.62, 118.98, 118.19, 111.82, 106.00, 55.55, 51.52, 50.91, 32.69, 24.08, 18.55. HRMS (EI+) m/z calcd for $C_{21}H_{18}N_4O_4$ $[M]^+$: 391.1401, found: 391.1403.

2-Ethyl-6-(2-nitrophenyl)-2,3,12,12a-tetrahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4(6*H*,7*H*)-dione (3b). Following the general procedure, the desired compound **3b** was generated as a light brown solid with a yield of 62% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (3 : 7) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.44 (t, J = 7.2, 14.8 Hz, 1H), 7.33 (s, 1H), 7.32 (s, 1H), 7.30 (s, 1H), 7.21–7.13 (m, 2H), 6.715 (s, 1H), 4.37 (dd, J = 3.6, 11.3 Hz, 1H), 4.12 (d, J = 7.2 Hz, 1H), 4.07 (d, J = 17.2 Hz, 1H), 3.86 (s, 1H), 3.83 (t, J = 4.4, 8 Hz, 1H), 3.70 (q, J = 6.8, 13.6 Hz, 1H), 3.39–3.32 (m, 2H), 1.19 (t, J = 7.2, 4.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.78, 165.58, 149.27, 138.33, 136.54, 133.88, 131.45, 128.22, 127.98, 126.15, 124.07, 123.00, 120.23, 118.77, 111.50, 106.72, 60.54, 56.36, 52.36, 52.48, 49.37, 41.44, 24.48, 14.34, 12.09. HRMS (EI+) m/z calcd for $C_{22}H_{20}N_4O_4$ $[M]^+$: 381.1557, found: 381.1570. M. P. 98 °C.

2-Benzyl-6-(2-nitrophenyl)-2,3,12,12a-tetrahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4(6*H*,7*H*)-dione (3c). Following the general procedure, the desired compound **3c** was generated as a grey solid with a yield of 56% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (2 : 8) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.84 (d, J = 8 Hz, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.44 (t, J = 7.6, 14.8 Hz, 1H), 7.34–7.27 (m, 8H), 7.19 (t, J = 6.8, 14.4 Hz, 1H), 7.16 (t, J = 5.2, 12.8 Hz, 1H), 4.89 (d, J = 14.4 Hz, 1H), 4.89 (d, J = 14.4 Hz, 1H), 4.41 (d, J = 4 Hz, 1H), 4.12 (q, J = 7.2 Hz, 1H), 3.93 (d, J = 5.2 Hz, 1H), 3.89 (d, J = 4.4 Hz, 1H), 3.79 (d, J = 18 Hz, 1H), 3.38 (dd, J = 11.6, 16 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.54, 166.07, 149.19, 138.34, 136.51, 135.11, 133.87, 131.43, 129.16, 128.40, 128.23, 127.89, 126.12, 124.12, 123.01, 120.24, 118.74, 111.51, 106.63, 60.54, 56.34, 52.45, 49.96, 49.41, 24.59, 21.20, 14.34. HRMS (EI+) m/z calcd for $C_{27}H_{22}N_4O_4$ $[M]^+$: 467.1714, found: 467.1830.

2-Hexyl-6-(2-nitrophenyl)-2,3,12,12a-tetrahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4(6*H*,7*H*)-dione (3d). Following the general procedure, the desired compound **3c** was generated as a grey solid with a yield of 77% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (3 : 7) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.85 (d, J = 8 Hz, 1H), 7.61 (d, J = 8 Hz, 1H), 7.42 (d, J = 8 Hz, 1H),

7.33–7.30 (m, 3H), 7.21–7.130 (m, 2H), 6.72 (s, 1H), 4.38 (dd, J = 4.4, 11.2 Hz, 1H), 4.12 (d, J = 8 Hz, 1H), 4.06 (d, J = 8 Hz, 1H), 3.86 (s, 1H), 3.81 (s, 1H), 3.61–3.54 (m, 1H), 3.38–3.29 (m, 1H), 2.04 (s, 1H), 1.59 (s, 3H), 1.30 (s, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.84, 165.85, 149.25, 138.40, 136.54, 133.87, 131.44, 128.22, 127.95, 126.16, 124.10, 123.00, 120.23, 118.76, 111.50, 106.69, 56.26, 52.45, 49.91, 46.57, 31.53, 26.82, 26.48, 24.43, 22.64, 14.11. HRMS (EI+) m/z calcd for $C_{26}H_{28}N_4O_4$ $[M]^+$: 461.2183, found: 461.2209.

2-Isopropyl-6-(2-nitrophenyl)-2,3,12,12a-tetrahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4(6*H*,7*H*)-dione (3e). Following the general procedure, the desired compound **3e** was generated as a light yellow solid with a yield of 81% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (3 : 7) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.83–7.81 (m, 1H), 7.56 (d, J = 6.8 Hz, 1H), 7.48–7.46 (m, 2H), 7.34 (s, 1H), 7.32 (s, 1H), 7.23–7.15 (m, 2H), 4.83 (t, J = 6.8, 13.6 Hz, 1H), 4.47 (dd, J = 4, 11.6 Hz, 1H), 3.91 (s, 2H), 3.78 (s, 1H), 3.66 (dd, J = 4, 15.6 Hz, 1H), 2.99–2.92 (m, 1H), 1.25 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.52, 163.42, 150.57, 136.67, 132.94, 132.61, 130.07, 129.75, 126.26, 124.54, 123.18, 120.38, 118.69, 111.46, 109.23, 54.15, 53.90, 48.12, 44.58, 43.24, 29.40, 27.19, 19.04. HRMS (EI+) m/z calcd for $C_{23}H_{22}N_4O_4$ $[M]^+$: 419.1714, found: 419.1736. M. P. 86 °C.

6-(2-Nitrophenyl)-2-propyl-2,3,12,12a-tetrahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4(6*H*,7*H*)-dione (3f). Following the general procedure, the desired compound **3f** was generated as a yellow solid with a yield of 85% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (3 : 7) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.82–7.79 (m, 1H), 7.56 (d, J = 8 Hz, 1H), 7.47–7.45 (m, 2H), 7.33 (d, J = 5.2 Hz, 2H), 7.23–7.17 (m, 2H), 5.29 (s, 1H), 4.48 (dd, J = 3.6, 11.6 Hz, 1H), 4.06 (d, J = 16 Hz, 1H), 3.94 (d, J = 18 Hz, 2H), 3.66 (dd, J = 4, 15.6 Hz, 1H), 3.50–3.44 (m, 2H), 2.99–2.92 (m, 1H), 1.25 (t, J = 7.2, 14 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.97, 163.11, 150.53, 136.68, 132.94, 132.65, 130.03, 129.74, 128.91, 126.25, 124.52, 123.18, 120.39, 118.69, 111.48, 109.18, 54.05, 49.31, 48.20, 47.79, 27.33, 19.81, 11.24. HRMS (EI+) m/z calcd for $C_{23}H_{22}N_4O_4$ $[M]^+$: 419.1714, found: 419.1732. M. P. 82 °C.

6-(4,5-Dimethoxy-2-nitrophenyl)-2-methyl-2,3,12,12a-tetrahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-(6*H*,7*H*)-dione (3g). Following the general procedure, the desired compound **3g** was generated as a yellow solid with a yield of 79% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (4 : 6) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.41 (s, 1H), 7.30 (d, J = 8 Hz, 1H), 7.21–7.12 (m, 2H), 6.91 (s, 1H), 6.68 (s, 1H), 4.38 (dd, J = 4, 11.2 Hz, 1H), 4.13 (d, J = 4 Hz, 1H), 4.10 (d, J = 7.2 Hz, 1H), 3.92 (d, J = 3.2 Hz, 1H), 3.88 (d, J = 8 Hz, 1H), 3.85 (s, 3H), 3.75 (s, 3H), 3.27 (dd, J = 16, 12.4 Hz, 1H), 3.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.52, 166.32, 153.81, 148.01, 141.37, 136.42, 133.27, 131.72, 126.01, 122.94, 120.15, 118.61, 111.57, 108.47, 107.29, 106.35, 56.46, 56.37, 56.28, 33.92, 24.48. HRMS (EI+) m/z calcd for $C_{23}H_{22}N_4O_6$ $[M]^+$: 451.1612, found: 451.1635. M. P. 123 °C.

6-(4,5-Dimethoxy-2-nitrophenyl)-2-benzyl-2,3,12,12a tetrahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-(6*H*,7*H*)-dione (3h). Following the general procedure, the desired compound **3h** was generated as a light yellow solid with a yield of 88% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (4 : 6) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.43 (s, 1H), 7.33–7.27 (m, 6H), 7.25–7.14 (m, 2H), 6.95 (s, 1H), 6.70 (s, 1H), 4.74–4.63 (dd, J = 4, 11.2 Hz, 1H), 4.47–4.43 (m, 1H), 4.03–3.98 (m, 1H), 3.93–3.92 (m, 1H), 3.89–3.85 (d, J = 8 Hz, 1H), 3.87 (s, 3H), 3.72 (s, 3H), 3.41–3.33 (dd, J = 16, 12.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.68, 166.47, 153.91, 148.02, 141.09, 136.34, 135.33, 133.59, 131.68, 129.17, 128.46, 128.28, 125.99, 122.97, 120.19, 118.59, 111.58, 108.21, 107.40, 106.17, 56.47, 56.24, 56.20, 52.42, 49.85, 49.60, 29.82, 24.36. HRMS (EI+) m/z calcd for $C_{29}H_{26}N_4O_6$ $[M]^+$: 527.1925, found: 527.1948.

Representative experimental procedures for the synthesis of hybrids (6a–g)

To a stirred solution of compounds **3a–g** (1 equiv.) in ethanol, sodium dithionate ($Na_2S_2O_4$) (10 equiv.) and potassium carbonate (K_2CO_3) (2 equiv.) were added at 50 °C under a nitrogen atmosphere. After five minutes, water was added to dissolve the solid completely and the reaction mixture was stirred for thirty minutes. The reaction progress was monitored by thin layer chromatography (TLC); after the completion of the reaction, the insoluble substance was removed by filtration and the filtrate was extracted with ethyl acetate (EtOAc) (3 × 30 mL), dried over sodium sulphate (Na_2SO_4) and evaporated under reduced pressure to obtain the reductive product, which was used in the next step without further purification.

The crude compound from the previous step was added to a mixture of tetrahydrofuran and acetic acid (1 : 1) and the resulting solution was stirred for fifteen minutes after which *N*-bromosuccinimide (NBS) (1 equiv.) was added portionwise to the stirred reaction mixture. The reaction was monitored by TLC, after completion of which, solid $NaHCO_3$ was added to quench the reaction and evaporated under reduced pressure to obtain a solid. The solid was extracted with ethyl acetate (3 × 30 mL), dried over sodium sulphate and evaporated under reduced pressure to obtain the crude compound which was further purified by column chromatography.

3-Methyl-2,3,4a,5-tetrahydroindolo[2,3-*b*]pyrazino[1′,2′:1,5]pyrrolo[3,2-*c*]quinoline-1,4(10*H*,15b*H*)-dione (6a). Following the general procedure, the desired compound **6a** was generated as a lemon yellow solid with a yield of 70% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 8 Hz, 1H), 7.72 (s, 1H), 7.45–7.41 (m, 3H), 7.36 (d, J = 8 Hz, 1H), 7.01 (s, 1H), 6.69 (d, J = 4 Hz, 1H), 5.53 (s, 1H), 4.92 (m, 1H), 4.31 (d, J = 16 Hz, 2H), 4.03 (m, 1H), 3.04 (s, 3H), 2.49 (m, 1H), 2.33 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.42, 167.13, 166.91, 154.91, 142.01, 134.88, 132.96, 130.98, 129.42, 126.86, 123.86, 122.85, 121.42, 120.90, 112.67, 65.67, 61.98, 54.74, 36.68, 33.95, 32.05. M. P. 142 °C.

HRMS (EI+) *m*/*z* calcd for $C_{21}H_{18}N_4O_2$ $[M]^+$: 359.1503, found: 359.1506.

3-Ethyl-2,3,4a,5-tetrahydroindolo[2,3-*b*]pyrazino[1′,2′:1,5]pyrrolo[3,2-*c*]quinoline-1,4(10*H*,15b*H*)-dione (6b). Following the general procedure, the desired compound **6b** was generated as a yellow solid with a yield of 72% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 7.2 Hz, 1H), 7.67 (s, 1H), 7.46–7.34 (m, 4H), 7.04–6.89 (m, 2H), 5.50 (s, 1H), 4.67 (s, 1H), 4.28 (d, J = 17.2 Hz, 2H), 4.02 (d, J = 16.8 Hz, 1H), 3.50 (d, J = 7.2 Hz, 2H), 2.7 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.69, 167.55, 167.17, 156.39, 142.34, 134.85, 132.04, 129.77, 128.92, 127.96, 123.05, 121.30, 119.23, 118.62, 112.11, 65.12, 60.47, 56.47, 51.63, 35.63, 35.29, 29.51, 24.28, 12.61. HRMS (EI+) *m*/*z* calcd for $C_{22}H_{20}N_4O_2$ $[M]^+$: 373.1659, found: 373.1684. M. P. 134 °C.

3-Benzyl-2,3,4a,5-tetrahydroindolo[2,3-*b*]pyrazino[1′,2′:1,5]pyrrolo[3,2-*c*]quinoline-1,4(10*H*,15b*H*)-dione (6c). Following the general procedure, the desired compound **6c** was generated as a yellow solid with a yield of 80% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, DMSO) δ 11.05 (s, 1H), 7.93–6.90 (m, 13H), 5.52 (d, J = 8 Hz, 1H), 4.33 (s, 2H), 3.93 (d, J = 15.6 Hz, 1H), 2.09 (d, J = 15.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.36, 167.65, 155.09, 141.94, 136.95, 136.60, 135.10, 132.26, 129.90, 128.88, 128.00, 127.44, 126.91, 125.07, 123.31, 119.32, 114.14, 110.24, 65.82, 65.63, 60.44, 56.41, 51.86, 48.02, 29.50. HRMS (EI+) *m*/*z* calcd for $C_{27}H_{22}N_4O_2$ $[M]^+$: 435.1816, found: 435.1829. M. P. 125 °C.

3-Hexyl-2,3,4a,5-tetrahydroindolo[2,3-*b*]pyrazino[1′,2′:1,5]pyrrolo[3,2-*c*]quinoline-1,4(10*H*,15b*H*)-dione (6d). Following the general procedure, the desired compound **6d** was generated as a yellow solid with a yield of 65% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.79 (d, J = 7.6 Hz, 1H), 7.56 (s, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.42 (d, J = 7.6 Hz, 1H), 6.91 (t, J = 8.4, 16.4 Hz, 2H), 5.42 (s, 1H), 4.72–4.68 (m, 2H), 4.34 (d, J = 16.8 Hz, 1H), 4.06 (d, J = 16.8 Hz, 1H), 3.54–3.47 (m, 2H), 3.15 (t, J = 6.4, 13.2 Hz, 2H), 2.69 (s, 2H), 2.56 (s, 6H), 2.2–2.09 (m, 3H). HRMS (EI+) *m*/*z* calcd for $C_{26}H_{28}N_4O_2$ $[M]^+$: 429.2285, found: 429.2311. M. P. 76 °C.

3-Propyl-2,3,4a,5-tetrahydroindolo[2,3-*b*]pyrazino[1′,2′:1,5]pyrrolo[3,2-*c*]quinoline-1,4(10*H*,15b*H*)-dione (6e). Following the general procedure, the desired compound **6e** was generated as a light solid with a yield of 56% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.78 (s, 1H), 7.69 (d, J = 6.8 Hz, 1H), 7.37 (s, 1H), 7.33 (d, J = 8.4 Hz, 1H), 6.93–6.88 (m, 2H), 5.75 (s, 1H), 5.43 (s, 1H), 4.73–4.69 (m, 2H), 4.35 (d, J = 16 Hz, 1H), 4.05 (d, J = 16.4 Hz, 1H), 3.51–3.42 (m, 2H), 1.50–1.45 (m, 2H), 0.78 (t, J = 7.2, 14.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.68, 167.68, 167.44, 156.39, 134.85, 129.84, 128.90, 127.97, 123.06, 121.31, 119.24, 118.66, 112.12, 74.58, 65.13, 60.47, 56.48, 52.00, 46.39, 35.32, 20.29, 10.63. HRMS (EI+) *m*/*z* calcd for $C_{23}H_{22}N_4O_2$ $[M]^+$: 387.1816, found: 387.1831. M. P. 95 °C.

13,14-Dimethoxy-3-benzyl-2,3,4a,5-tetrahydroindolo[2,3-*b*]pyrazino[1′,2′:1,5]pyrrolo[3,2-*c*]quinoline-1,4(10*H*,15b*H*)-dione (6f). Following the general procedure, the desired compound **6f** was generated as a white solid with a yield of 80% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.64 (d, J = 7.6 Hz, 1H), 7.39 (t, J = 7.6, 14.8 Hz, 1H), 7.08 (s, 1H), 7.01 (s, 1H), 6.91–6.86 (m, 1H), 5.75 (s, 1H), 4.70–4.65 (m, 1H), 4.05 (d, J = 17.2 Hz, 2H), 3.79 (s, 3H), 3.66 (s, 3H), 2.89 (s, 3H), 2.82 (s, 1H), 2.71 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ 168.07, 167.62, 167.62, 167.16, 155.68, 148.86, 147.42, 136.89, 134.01, 122.53, 121.73, 119.14, 117.27, 112.03, 110.61, 110.45, 65.19, 61.35, 56.20, 55.66, 55.52, 53.85, 35.74, 33.01. HRMS (EI+) *m*/*z* calcd for $C_{29}H_{26}N_4O_4$ $[M]^+$: 495.2027, found: 495.2058. M. P. 103 °C.

Representative procedure for the synthesis of hybrids 4a–d

Compounds **3a–c** and **g** (1 equiv.) in a mixture of THF : AcOH : H_2O (1 : 1 : 1) were stirred for fifteen minutes and then *N*-bromosuccinimide (1 equiv.) was added portion-wise to the stirred reaction mixture and stirring was continued for 3 h. The reaction was monitored by TLC; after the completion of the reaction, solid sodium bicarbonate ($NaHCO_3$) was added to quench the reaction and evaporated under reduced pressure to obtain a solid residue. The solid was dissolved in EtOAc, dried over sodium sulphate (Na_2SO_4) and evaporated under reduced pressure to obtain the crude compound which was further purified by column chromatography.

(*R*)-1-Methyl-3-((2-(2-nitrobenzoyl)-1*H*-indol-3-yl)methyl)piperazine-2,5-dione (4a). Following the general procedure, the desired compound **4a** was generated as a white gummy liquid with a yield of 82% (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.97 (d, J = 3.2 Hz, 1H), 7.95 (s, 1H), 7.81 (s, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.29 (t, J = 7.2, 14.8 Hz, 1H), 7.24 (s, 1H), 7.09 (t, J = 7.2, 14.4 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 3.50 (d, J = 17.2 Hz, 1H), 3.40–3.35 (m, 2H), 2.88 (s, 3H), 2.51 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 166.00, 165.28, 162.30, 153.82, 149.26, 138.40, 136.89, 131.98, 130.36, 127.47, 125.89, 121.00, 120.09, 117.59, 112.71, 110.69, 107.31, 56.60, 56.37, 56.62, 50.51, 35.78, 32.92, 30.77. HRMS (EI+) *m*/*z* calcd for $C_{21}H_{18}N_4O_5$ $[M]^+$: 407.1350, found: 407.1372. M. P. 204 °C.

(*R*)-1-Ethyl-3-((2-(2-nitrobenzoyl)-1*H*-indol-3-yl)methyl)piperazine-2,5-dione (4b). Following the general procedure, the desired compound **4b** was generated as a colorless oil with a yield of 80% (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.23–8.19 (m, 1H), 7.81–7.75 (m, 1H), 7.72–7.67 (m, 3H), 6.32 (s, 1H), 4.35 (s, 1H), 3.86–3.81 (m, 1H), 3.45 (dd, J = 8, 16 Hz, 1H), 3.29–3.24 (m, 1H), 3.11–3.04 (m, 2H), 0.87 (t, J = 4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.66, 165.72, 165.25, 146.34, 136.91, 135.47, 134.83, 131.22, 129.30, 127.98, 127.70, 125.04, 121.86, 121.81, 119.72, 112.38, 56.84, 48.40, 41.17, 29.47, 11.46. HRMS (EI+) *m*/*z* calcd for $C_{22}H_{20}N_4O_5$ $[M]^+$: 421.1506, found: 421.1529. M. P. 175 °C.

(*R*)-1-Benzyl-3-((2-(2-nitrobenzoyl)-1*H*-indol-3-yl)methyl)piperazine-2,5-dione (4c). Following the general procedure, the desired compound **4c** was generated as a colorless oil with a yield of 80% (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.22 (d, J = 8 Hz, 1H), 7.78–7.76 (m, 2H), 7.67 (d, J = 7.6 Hz, 2H), 7.41–7.32 (m, 3H), 7.24–7.19 (m, 3H), 7.03–7.00 (m, 2H), 6.29 (s, 1H), 4.63 (d, J = 14.4 Hz, 1H), 4.46 (s, 2H), 3.99 (d, J = 14.4 Hz, 1H), 3.90 (dd, J = 4.8, 14 Hz, 2H), 2.92 (d, J = 18 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.64, 165.61, 165.39, 146.35, 134.88, 134.78, 131.22, 129.26, 128.99, 128.49, 128.18, 127.76, 125.03, 121.89, 121.84, 112.47, 56.74, 49.63, 48.33, 29.84, 29.60. HRMS (EI+) m/z calcd for $C_{27}H_{22}N_4O_5$ $[M]^+$: 483.1663, found: 483.1689. M. P. 167 °C.

(*R*)-3-((2-(4,5-Dimethoxy-2-nitrobenzoyl)-1*H*-indol-3-yl)methyl)-1-methylpiperazine-2,5-dione (4d). Following the general procedure, the desired compound **4d** was generated as a colorless oil with a yield of 71% (purified *via* flash column chromatography with ethyl acetate–hexane (1 : 1) as the eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.97 (d, J = 3.2 Hz, 1H), 7.95 (s, 1H), 7.81 (s, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.29 (t, J = 7.2, 14.8 Hz, 1H), 7.24 (s, 1H), 7.09 (t, J = 7.2, 14.4 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 3.50 (d, J = 17.2 Hz, 1H), 3.40–3.35 (m, 2H), 2.88 (s, 3H), 2.51 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 166.00, 165.28, 162.30, 153.82, 149.26, 138.40, 136.89, 131.98, 130.36, 127.47, 125.89, 121.00, 120.09, 117.59, 112.71, 110.69, 107.31, 56.60, 56.37, 56.62, 50.51, 35.78, 32.92, 30.77. HRMS (EI+) m/z calcd for $C_{23}H_{22}N_4O_7$ $[M]^+$: 467.1561, found: 467.1592. M. P. 109 °C.

Representative experimental procedures for the synthesis of hybrids (5a–c)

To a stirred solution of compounds **2a–c** (1 equiv.) in ethanol, sodium dithionate ($Na_2S_2O_4$) (10 equiv.) and potassium carbonate (K_2CO_3) (2 equiv.) were added at 50 °C under a nitrogen atmosphere. After five minutes, water was added to dissolve the solid completely and the reaction mixture was stirred for thirty minutes. The reaction progress was monitored by thin layer chromatography (TLC); after the completion of the reaction, the insoluble substance was removed by filtration and the filtrate was extracted with ethyl acetate (EtOAc) (3 × 30 mL), dried over sodium sulphate (Na_2SO_4) and evaporated under reduced pressure to obtain the reductive product, which was used in the next step without any further purification.

The crude compound from the previous step was added to a mixture of tetrahydrofuran and acetic acid (1 : 1) and the resulting solution was stirred for fifteen minutes after which *N*-bromosuccinimide (NBS) (1 equiv.) was added portion-wise to the stirred reaction mixture. The reaction was monitored by TLC after completion of which solid $NaHCO_3$ was added to quench the reaction and evaporated under reduced pressure to obtain the solid. The solid was extracted with ethyl acetate (3 × 30 mL), dried over sodium sulphate and evaporated under reduced pressure to obtain the crude compound which was further purified by column chromatography.

Methyl 2,3,8,13b-tetrahydro-1*H*-indolo[2,3-*b*]pyrrolo[3,2-*c*]quinoline-2-carboxylate (5a). Following the general procedure, the desired compound **5a** was generated as a colorless gummy solid with a yield of 64% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (6 : 4) as the eluent). ^{1}H NMR (400 MHz; $CDCl_3$): 7.81 (s, 1H); 7.43–7.41 (d, J = 8 Hz, 1H); 7.37–7.34 (dd, J = 4 Hz, 8 Hz, 1H); 7.29–7.25 (m, 2H); 7.16–7.14 (d, J = 8 Hz, 1H); 7.04–7.01 (m, 1H); 6.94–6.92 (d, J = 8 Hz, 1H); 4.67 (s, 1H); 4.53 (s, 1H); 3.91–3.87 (m, 1H); 3.75 (s, 3H); 2.70–2.63 (m, 2H); 2.12–2.07 (m, 1H). M. P. 72 °C.

Methyl-11-chloro-2,3,8,13b-tetrahydro-1*H*-indolo[2,3-*b*]pyrrolo[3,2-*c*]quinoline-2-carboxylate (5b). Following the general procedure, the desired compound **5b** was generated as a light yellow oil with a yield of 57% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (7 : 3) as the eluent). ^{1}H NMR (400 MHz; $CDCl_3$): 7.55–7.51 (t, J = 8 Hz, 2H); 7.43–7.41 (t, J = 8 Hz, 1H); 7.37–7.35 (d, J = 8 Hz, 1H); 7.29–7.23 (m, 3H); 4.75 (s, 1H); 4.35–4.30 (m, 1H); 3.61 (s, 3H); 2.67–2.62 (m, 1H); 2.27–2.22 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 173.18, 170.65, 140.49, 135.39, 133.88, 132.77, 130.05, 129.94, 126.91, 125.45, 123.01, 122.87, 119.37, 113.95, 113.95, 61.29, 57.29, 55.19, 52.72, 39.63. $[M + H]^+$ calculated for ($C_{19}H_{16}N_3ClO_2$) 354.1044, found 354.1028. M. P. 86 °C.

Methyl-12-chloro-2,3,8,13b-tetrahydro-1*H*-indolo[2,3-*b*]pyrrolo[3,2-*c*]quinoline-2-carboxylate (5c). Following the general procedure, the desired compound **5c** was generated as a light yellow oil with a yield of 75% over two steps (purified *via* flash column chromatography with ethyl acetate–hexane (7 : 3) as the eluent). ^{1}H NMR (400 MHz; $CDCl_3$): 7.49 (s, 1H); 7.43–7.41 (d, J = 8 Hz, 1H); 7.32–7.28 (t, J = 8 Hz, 1H); 7.19–7.14 (m, 2H); 7.07–7.04 (t, J = 8 Hz, 1H); 6.98–6.96 (d, J = 8 Hz, 1H); 4.58 (s, 1H); 4.29–4.24 (m, 1H); 3.62 (s, 3H); 3.50–3.45 (m, 1H); 2.55–2.50 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 173.86, 171.36, 146.79, 136.89, 135.25, 129.18, 129.02, 128.89, 128.57, 126.52, 122.81, 122.20, 121.03, 113.86, 62.04, 57.70, 54.45, 52.46, 39.27. $[M + H]^+$ calculated for ($C_{19}H_{16}N_3ClO_2$) 354.1004, found 354.1037. M. P. 84 °C.

Biological assays

Cell culture

The MCF-7 cell line derived from the pleural effusion of breast adenocarcinoma from a female patient was grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Bioservices India Pvt. Ltd) supplemented with 10% Fetal Bovine Serum (FBS, Gibco, USA) with 1% antibiotics (penicillin-10 000 units per ml, streptomycin-10 000 μg ml^{-1}, Gibco, USA). The cells were confluent at the time of experimentation (cell confluency ~80%); then the cells were dislodged from the flasks by trypsinization using 0.25% trypsin. All cell lines were purchased from NCSS (http://www.nccs.res.in).

Compound exposure for dose–response curves

15 000 cells per 200 μl of media were plated per well in 96-well plates and allowed to adhere for 18 hours. The adhered cells

were then treated with 1–100 μg ml^{-1} of compounds **3a**, **b**, **e–f**, **4g**, **6a–g**, **5a** and doxorubicin in triplicate for 24 hours. All the solutions were prepared from concentrated stock solutions (in DMSO) of the compounds.

Viability assays

After 24 hours of cell incubation in the presence or absence of each compound, cell viability was evaluated by using MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) (Sisco Research Laboratories Pvt. Ltd, India). In brief, this is a homogeneous, colorimetric method for determining the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays. MTT is bioreduced by cells into a formazan product that is soluble in the tissue culture medium. After 24 hours of compound treatment, the medium was removed and 100 μl of MTT (0.5 mg ml^{-1} in media) was added to the cells and kept in the dark for 2 hours at 37 °C and the formazan formed was dissolved in 100 μl DMSO (Dimethyl Sulfoxide, ACS).[14,15] The absorbance of the formazan product at 595 nm was measured directly from 96-well assay plates without additional processing using a multimode plate reader (Bio-Rad) (iMark, India), as absorbance is directly proportional to the number of viable cells in culture. The percentage of viable cells in each group is determined with respect to the untreated control cells.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We are grateful for the funds from Shiv Nadar University and to Dr Parthapratim Munshi for the single crystal X-ray crystallographic analyses of our samples.

References

1 (*a*) B. Schuster-Bockler and A. Bateman, *Genome Biol.*, 2008, **9**, R9; (*b*) S. Teng, T. Madej, A. Panchenko and E. Alexov, *Biophys. J.*, 2009, **96**, 2178–2188; (*c*) S. Fletcher and A. D. Hamilton, *Curr. Top. Med. Chem.*, 2007, **7**, 922–927; (*d*) L. Pagliaro, J. Felding, K. Audouze, S. J. Nielsen, R. B. Terry, C. Krog-Jensen and S. Butcher, *Curr. Opin. Chem. Biol.*, 2004, **8**, 442–449; (*e*) D. C. Fry, *Biopolymers*, 2006, **84**, 535–552; (*f*) D. C. Fry, *Curr. Protein Pept. Sci.*, 2008, **9**, 240–247.

2 E. C. Butcher, E. L. Berg and E. J. Kunkel, *Nat. Biotechnol.*, 2004, **22**, 1253–1259.

3 (*a*) R. R. Thangudu, S. H. Bryant, A. R. Panchenko and T. Madej, *J. Mol. Biol.*, 2012, **415**, 443–453; (*b*) A. Whitty and G. Kumaravel, *Nat. Chem. Biol.*, 2006, **2**, 112–118; (*c*) C. Reynes, H. Host, A. C. Camproux, G. Laconde, F. Leroux, A. Mazars, *et al.*, *PLoS Comput. Biol.*, 2010, **6**, e1000695.

4 (*a*) K. C. Nicolaou, X.-Y. Xiao, Z. Parandosh, A. Senyei and M. P. Nova, *Angew. Chem., Int. Ed. Engl.*, 1995, **36**, 2289–2291; (*b*) S. Brenner and R. A. Lerner, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 5381–5383; (*c*) J. Nielsen, S. Brenner and K. D. Janda, *J. Am. Chem. Soc.*, 1993, **115**, 9812–9813; (*d*) Q. B. Su, A. B. Beeler, E. Lobkovsky, J. A. Porco and J. S. Panek, *Org. Lett.*, 2003, **5**, 2149–2152; (*e*) P. Ertl, *J. Chem. Inf. Model.*, 2014, **54**, 1617–1622; (*f*) C. M. Garcia, S. Zimmermann, M. G. Sankar and K. Kumar, *Angew. Chem. Int. Ed.*, 2016, **55**, 7586–7605.

5 (*a*) A. L. Harvey, *Drug Discovery Today*, 2008, **13**, 894–901; (*b*) D. A. Dias, S. Urban and U. Roessner, *Metabolites*, 2012, **2**, 303–333; (*c*) D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2012, **75**, 311–335; (*d*) B. B. Mishra and V. K. Tiwari, *Eur. J. Med. Chem.*, 2011, **46**, 4769–4807; A. L. Harvey, R. Edraba-Ebel and R. J. Quinn, *Nat. Rev. Drug Discovery*, 2015, **14**, 111–129.

6 (*a*) M. Pascolutti and R. J. Quinn, *Drug Discovery Today*, 2013, **19**, 215–221; (*b*) S. J. Teague, A. M. Davis, P. D. Leeson and T. Oprea, *Angew. Chem., Int. Ed.*, 1999, **38**, 3743–3748; (*c*) G. M. Keserü and G. M. Makara, *Nat. Rev. Drug Discovery*, 2009, **8**, 203–212; (*d*) S. M. Khersonsky and Y.-T. Chang, *Comb. Chem. High Throughput Screening*, 2004, **7**, 645–652.

7 (*a*) T. S. Bugni, B. Richards, L. Bhoite, D. Cimbora, M. K. Harper and C. M. Ireland, *J. Nat. Prod.*, 2008, **71**, 1095–1098; (*b*) M. Pascolutti and R. J. Quinn, *Drug Discovery Today*, 2014, **19**, 215–221; (*c*) J. Gu, Y. Gui, G. Yuan, L. Chen, H.-Z. Lu and X. Xu, *PLoS One*, 2013, **8**, e62839; (*d*) G. L. Thomas and C. W. Johannes, *Curr. Opin. Chem. Biol.*, 2011, **15**, 516–522.

8 (*a*) T. Rodrigues, D. Reker, P. Schneider and G. Schneider, *Nat. Chem.*, 2016, **8**, 531–541; (*b*) F. A. Khan, S. Ahmad, N. Kodipelli, G. Shivange and A. Roy, *Org. Biomol. Chem.*, 2014, **12**, 3847–3865; (*c*) M. G. Sankar, L. Mantilli, J. Bull, F. Giodanetto, J. O. Bauer, C. Strohmann, H. Waldmann and K. Kumar, *Bioorg. Med. Chem.*, 2015, **23**, 2614–2620; (*d*) D. Singh, N. Devi, V. Kumar, C. C. Malakar, S. Mehra, S. Rattan, R. K. Rawal and V. Singh, *Org. Biomol. Chem.*, 2016, **14**, 8154–8166.

9 (*a*) B. M. Ibbeson, L. Laraia, E. Alza, C. J. O'Connor, Y. S. Tan, H. M. L. Davies, G. Mckenzie, A. R. Venkitaraman and D. R. Spring, *Nat. Commun.*, 2014, **5**, 3155–3162; (*b*) C. J. O'Connor, H. S. G. Beckmann and D. R. Spring, *Chem. Soc. Rev.*, 2012, **41**, 4444–4456; (*c*) D. Robbins, A. F. Newton, C. Gignoux, J.-C. Legeay, A. Sinclair, M. Rejzek, C. A. Laxon, S. K. Yalamanchili, W. Lewis, M. A. O'Connell and R. A. Stockman, *Chem. Sci.*, 2011, **2**, 2232–2235; (*d*) G. Karageorgis, S. Warriner and A. Nelson, *Nat. Chem.*, 2014, **6**, 872–876.

10 (*a*) D. S. Tan, *Nat. Chem. Biol.*, 2005, **1**, 74–84; (*b*) W. R. J. D. Galloway, A. Isidro-Llobet and D. R. Spring, *Nat. Commun.*, 2010, **1**, 80–87; (*c*) T. E. Nielsen and S. L. Schreiber, *Angew. Chem.*, 2008, **47**, 48–56.

11 E. E. Wyatt, S. Fergus, W. R. J. D. Galloway, A. Bender, D. J. Fox, A. T. Plowright, A. S. Jessiman, M. Welch and D. R. Spring, *Chem. Commun.*, 2006, **31**, 3296–3298.

12 (*a*) F. V. Singh, J. Rehbein and T. Wirth, *Chemistryopen*, 2012, **1**, 245–250; (*b*) M. Shibuya, M. Tomizawa and Y. Iwabuchi, *J. Org. Chem.*, 2008, **73**, 4750–4752; (*c*) A. Nakamura, S. Tanaka, A. Imamiya, R. Takane, C. Ohta, K. Fujimura, T. Maegawa and Y. Miki, *Org. Biomol. Chem.*, 2017, **15**, 6702–6705.

13 (*a*) J. R. Fuchs and R. L. Funk, *J. Am. Chem. Soc.*, 2004, **126**, 5068–5069; (*b*) A. D. Borthwick, *Chem. Rev.*, 2012, **112**, 3641–3716; (*c*) C.-B. Cui, H. Kayeka, G. Okada, R. Onose, G. Okada, I. Takahashi, K. Isono and H. Osada, *J. Antibiot.*, 1995, **48**, 1382; (*d*) C.-B. Cui, H. Kayeka, G. Okada, R. Onose and H. Osada, *J. Antibiot.*, 1996, **49**, 534; (*e*) T.-S. Kam and K.-M. Sim, *Phytochemistry*, 1998, **47**, 145–147; (*f*) A. Pictet and T. Spengler, *Ber. Dtsch. Chem. Ges.*, 1911, **44**, 2030–2036.

14 D. Gerlier and N. Thomasset, *J. Immunol. Methods*, 1986, **94**, 57–63.

15 F. Denizot and R. Lang, *J. lmmunol. Methods*, 1989, **89**, 271–277.