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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and anti-breast cancer properties of butyric ester tethered dihydroartemisinin-isatin hybrids

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Abstract

Fifteen novel butyric ester tethered dihydroartemisinin-isatin hybrids **4a-d** and **5a-k** were designed, synthesized, and evaluated for cytotoxicity against four human breast cancer cell lines, including MCF-7, MDA-MB-231, MCF-7/ADR and MDA-MB-231/ADR using the MTT method. A significant part of them were active against the four tested cancer cell lines, and the representative hybrid **5b** (IC_{50}: 1.27 μM) was 14.88 -> 78.74 times more active than adriamycin (IC_{50}: 18.90 μM), DHA (IC_{50}: 28.28 μM) and ART (IC_{50}: > 100 μM) against MCF-7 breast cancer cells, whereas hybrid **5c** (IC_{50}: 2.39 and 3.95 μM) was superior to adriamycin (IC_{50}: 3.38 and >100 μM), DHA (IC_{50}: 48.80 and 82.78 μM) and ART (IC_{50}: >100 and >100 μM) against MDA-MB-231 and MDA-MB-231/ADR breast cancer cell lines. Moreover, the selected hybrids (IC_{50}: >100 μM) displayed non-cytotoxicity towards normal MCF-10A breast cells, and the SI values of hybrids **5b,c** were >78.74 and >41.84 respectively, demonstrating their excellent selectivity and safety profiles. Accordingly, hybrids **5b,c** could serve as promising anti-breast cancer candidates and deserved further preclinical evaluations.

Keywords dihydroartemisinin · isatin · hybrid molecules · breast cancer · drug resistance

Introduction

Breast cancer, originates as a result of aberrant cell division in the breast, remains the most prevalent malignancy and the leading cause of cancer deaths [1, 2]. Currently, standard treatment approaches for breast cancers are surgery accompanied by chemotherapy or radiotherapy, and around 70% of patients with early-stage are curable [3, 4]. Thus, chemotherapeutics occupies an important position for the treatment of breast cancer. However, advanced breast cancer is still an incurable disease with available therapeutics mainly due to its heterogeneity and limited immunogenicity [5, 6]. Additionally, drug-resistant especially multidrug-resistant cancer cells are responsible for most breast cancer fatalities [7, 8]. Therefore, innovation of more effective anti-breast cancer therapeutics constitutes a crucial need.

Dihydroartemisinin (DHA, Fig. 1), an active metabolite of artemisinin (ART), owns a sesquiterpene lactone and possesses remarkable and selective anticancer properties against diverse cancers including breast cancer [9, 10]. Mechanistically, DHA derivatives could exert anticancer effects through various molecular mechanisms, inclusive of

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promoting ferroptosis and autophagy, inducing apoptosis, stimulating cell cycle inhibition, inhibiting angiogenesis, mediating the tumor-associated signaling pathways, destroying cancer metastasis and invasion, and regulating tumour microenvironment [11, 12]. Notably, DHA hybrids not only demonstrated profound anticancer activity in vitro and in vivo with the potential to overcome drug resistance, but also showed excellent safety and tolerability profiles [13–16]. Hence, DHA hybrids are useful scaffolds for the discovery of novel anticancer candidates.

Isatin, widely distributed in natural kingdom, is a well-known pharmacologically active scaffold [17, 18]. Isatin hybrids possessed significant therapeutic effect on breast cancers including drug-resistant forms, good selectivity and low toxic side effects [19, 20]. Recent studies demonstrated that DHA-isatin hybrids exhibited promising antiproliferative activity against both drug-sensitive and drug-resistant cancer cell lines [21–24]. The structure-activity relationships (SARs) illustrated that the linker between DHA and isatin moieties had great impact on the antiproliferative activity [21–24]. However, only alkyl and 1,2,3-triazole were investigated as linkers in previous studies. As a continuous program to investigate the influence of the nature of the linkers, a series of butyric ester tethered DHA-isatin hybrids were designed, synthesized and evaluated for their antiproliferative activity against both drug-sensitive (MCF-7 and MDA-MB-231) and multidrug-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines in this paper.

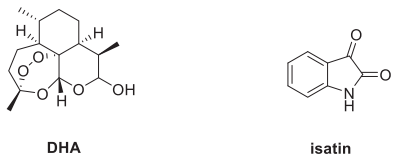


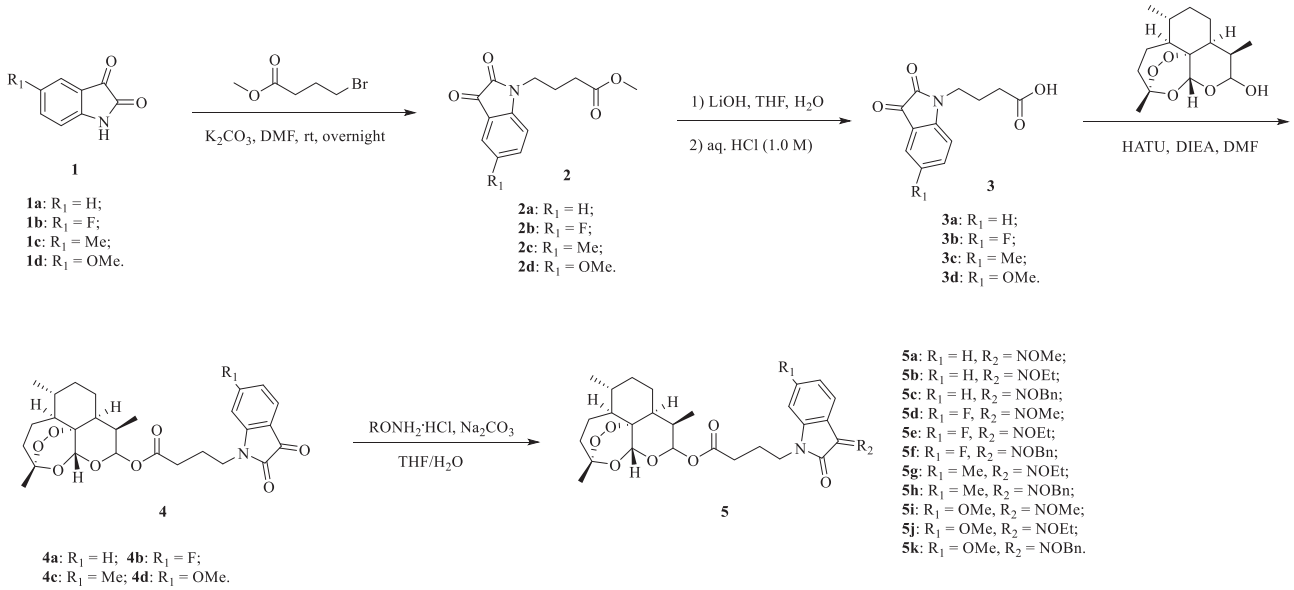
Fig. 1 Chemical structures of and DHA and isatin

Results and discussion

The detailed synthetic route of butyric ester tethered DHA-isatin hybrids was depicted in Scheme 1. Alkylation of isatins **1a-d** with methyl 4-bromobutanoate in presence of potassium carbonate (K_2CO_3) provided methyl 4-(*N*-1-isatin)butanoates **2a-d**, which were then hydrolysized by lithium hydroxide (LiOH) generated 4-(*N*-1-isatin)butanoic acids **3a-d**. Esterfication of acids **3a-d** with DHA in presence of 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) yielded desired DHA-isatin hybrids **4a-d**. Finally, imidization of DHA-isatin hybrids **4a-d** with methoxyamine/ethoxyamine/benzyloxyamine hydrochlorides generated C-3 modified DHA-isatin hybrids **5a-k**. The structures and yields were listed in Table 1.

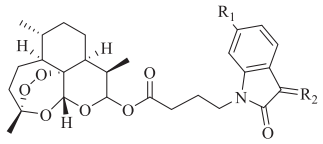
The desired butyric ester tethered DHA-isatin hybrids **4a-d** and **5a-k** were characterized by high resolution mass spectrometry (HRMS), proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR), and the corresponding analytical spectra were included in the Supplementary Information section.

Take the hybrid **5e** for an example, the typical signals in ^{1}H NMR were as follows: 0.78 (d, 3H), 0.90 (d, 3H) and 1.46 (s, 3H) belong to -CH_3 at C-3, C-6 and C-9 position of DHA moiety, whereas 5.38 (s, 1H) and 5.74 (d, 1H) belong to C-10 and C-12 positions, respectively; 6.80–6.91 (m,



Scheme 1 Synthetic route of butyric ester tethered DHA-isatin hybrids **4a-d** and **5a-k**

Table 1 The structures and yields of butyric ester tethered DHA-isatin hybrids **4a-d** and **5a-k**



Hybrids	R_1	R_2	Yield
4a	H	O	61%
4b	F	O	58%
4c	Me	O	73%
4d	OMe	O	47%
5a	H	NOMe	79%
5b	H	NOEt	66%
5c	H	NOBn	68%
5d	F	NOMe	81%
5e	F	NOEt	78%
5f	F	NOBn	89%
5g	Me	NOEt	67%
5h	Me	NOBn	93%
5i	OMe	NOMe	88%
5j	OMe	NOEt	79%
5k	OMe	NOBn	87%

1H), 7.03-7.09 (m, 1H), 7.62-7.66 (m, 1H) belong to C-4, C-6 and C-7 position of isatin moiety. In ^{13}C NMR spectrum, 91.82 (C12), 80.10 (C12a), 51.86 (C5a), 45.22 (C8a), 37.49 (C6), 36.35(C4), 34.21(C7), 30.92(C9), 25.93(C15), 24.89(C5), 24.89(C8), 20.22 (C13), 12.14 (C14). For the HRMS, m/z Calcd for $C_{29}H_{37}FN_2O_8Na$ $[M + Na]^+$: 583.2426; Found: 583.2405. Based on the above analysis, the structure of hybrid **5e** was correct.

The antiproliferative activity of butyric ester tethered DHA-isatin hybrids **4a-d** and **5a-k** against MCF-7, MDA-MB-231, MCF-7/ADR and MDA-MB-231/ADR (Purchased from Procell) breast cancer cell lines were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The half maximal inhibitory concentration (IC_{50}) values, selectivity index (SI: $IC_{50(MCF\text{-}10A)}$/$IC_{50(MCF\text{-}7)}$) and resistance index (RI: $IC_{50(MDA\text{-}MB\text{-}231/ADR)}$/$IC_{50(MDA\text{-}MB\text{-}231)}$) were presented in Table 2.

From Table 2, it can be seen that some of the synthesized hybrids (IC_{50}: 1.27–52.16 μM) showed considerable activity against MCF-7, MDA-MB-231, MCF-7/ADR and MDA-MB-231/ADR breast cancer cell lines, and the activity was superior to these of ART (IC_{50}: >100 μM) and DHA (IC_{50}: 14.85–82.78 μM). The SAR illustrated that introduction of electron-withdrawing (fluoro) or electron-donating (methyl and methoxy) substituents into C-5 position of isatin moiety decreased the activity; for hybrids without substituent at C-5

Table 2 The antiproliferative activity of butyric ester tethered DHA-isatin hybrids **4a-d** and **5a-k**

Hybrid	Antiproliferative activity (IC_{50}: μM)			
	MCF-7	MDA-MB-231	MCF-7/ADR	MDA-MB-231/ADR
4a	13.63	14.87	12.38	49.56
4b	13.11	50.01	>100	>100
4c	>100	32.91	>100	>100
4d	18.09	15.13	25.88	20.07
5a	19.10	>100	>100	52.16
5b	1.27	15.06	>100	26.78
5c	>100	2.39	>100	3.95
5d	6.07	>100	>100	>100
5e	15.89	8.12	>100	>100
5f	18.29	>100	>100	>100
5g	>100	>100	>100	>100
5h	>100	>100	>100	>100
5i	>100	>100	15.13	>100
5j	>100	>100	>100	>100
5k	>100	>100	>100	>100
ART[a]	>100	>100	>100	>100
DHA[b]	28.28	48.80	14.85	82.78
ADR[c]	18.90	3.38	>100	>100

[a]ART: Artemisinin

[b]DHA: Dihydroartemisinin

[c]ADR: Adriamycin

position of isatin fragment, benzoxime at C-3 position could enhance the antiproliferative activity against MDA-MB-231 and MDA-MB-231/ADR breast cancer cell lines, whereas ethoxime was favorable to the activity against MCF-7 cells; for 5-methoxyisatin derivatives, incorporation of methoxime, ethoxime and benzoxime led to significant loss of activity against all tested breast cancer cell lines.

The hybrids which were active against MCF-7 or MDA-MB-231 cancer cells were selected for evaluation of their cytotoxicity towards normal MCF-10A breast cells *via* MTT assay, and the results were presented in Table 3. All of the evaluated nine hybrids (IC_{50}: >100 μM) displayed lower cytotoxicity than adriamycin (IC_{50}: 68.8 μM) against normal MCF-10A breast cells, demonstrating their excellent safety profile. In addition, the selectivity index (SI: $IC_{50(MCF\text{-}10A)}$/$IC_{50(MCF\text{-}7)}$ or $IC_{50(MCF\text{-}10A)}$/$IC_{50(MDA\text{-}MB\text{-}231)}$) values of the selected hybrids were >5.23, revealing their good selectivity.

Amongst them, hybrid **5b** (IC_{50}: 1.27 μM) was 14.88 - > 78.74 times superior to adriamycin (IC_{50}: 18.90 μM), DHA (IC_{50}: 28.28 μM) and ART (IC_{50}: >100 μM) against MCF-7 breast cacner cells; hybrid **4a** (IC_{50}: 12.38 μM) was more potent than adriamycin (IC_{50}: > 100 μM), DHA (IC_{50}: 14.85 μM) and ART (IC_{50}: >100 μM) against MCF-7/ADR cells; hybrid **5c** (IC_{50}: 2.39 and 3.95 μM) demonstrated

Table 3 The cytotoxicity and selectivity index values of butyric ester tethered DHA-isatin hybrids

Hybrids	Cytotoxicity (IC_{50}: μM)	Selectivity index (SI)
4a	>100	>7.33
4b	>100	>7.62
4d	>100	>5.52
5a	>100	>5.23
5b	>100	>78.74
5c	>100	>41.84
5d	>100	>14.47
5e	>100	>6.29
5f	>100	>5.46
ART	>100	--
DHA	>100	>3.53
ADR	68.80	3.64

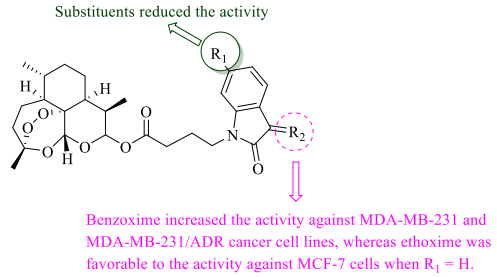


Fig. 2 The SAR of butyric ester tethered DHA-isatin hybrids

profound activity activity against MDA-MB-231 and MDA-MB-231/ADR breast cancer cell lines, and the activity was higher than these of adriamycin (IC_{50}: 3.38 and >100 μM), DHA (IC_{50}: 48.80 and 82.78 μM) and ART (IC_{50}: >100 and >100 μM). Moreover, the SI values of hybrids **5b,c** were >78.74 and >41.84 respectively, proving their excellent safety profile. Accordingly, hybrids **5b,c** could serve as promising candidates for further preclinical evaluations.

Conclusion

In conclusion, fifteen butyric ester tethered DHA-isatin hybrids were designed, synthesized and evaluated for their antiproliferative activity against MCF-7, MDA-MB-231, MCF-7/ADR, and MDA-MB-231/ADR breast cancer cell lines. The SAR (Fig. 2) revealed that (1) regardless of electron-withdrawing or electron-donating group especially methoxy group at C-5 position of isatin moiety reduced the activity; (2) for C-5 unsubstituted hybrids, introduction of benzoxime into C-3 position of isatin skeleton was beneficial for the activity against MDA-MB-231 and MDA-MB-231/ADR breast cancer cell lines, whereas ethoxime improved the activity against MCF-7 cacner cells.

In particular, the representative hybrid **5b** (IC_{50}: 1.27 μM) was 14.88->78.74 times more active than adriamycin (IC_{50}: 18.90 μM), DHA (IC_{50}: 28.28 μM) and ART (IC_{50}: >100 μM) against MCF-7 breast cancer cells, whereas hybrid **5c** (IC_{50}: 2.39 and 3.95 μM) was superior to adriamycin (IC_{50}: 3.38 and >100 μM), DHA (IC_{50}: 48.80 and 82.78 μM) and ART (IC_{50}: > 100 and >100 μM) against MDA-MB-231 and MDA-MB-231/ADR breast cancer cell lines. Additionally, both of them (IC_{50}: >100 μM) displayed non-cytotoxicity towards normal MCF-10A breast cells, and the SI values were >78.74 and >41.84 respectively, indicating their excellent selectivity and safety profiles. Thus, hybrids **5b,c** were potential anti-breast cancer candidates and deserved further preclinical evaluations.

Experimental section

Materials

^{1}H NMR and ^{13}C NMR spectra were determined on a Varian Mercury-600 spectrometer in $CDCl_3$ or acetone-d_6 using tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI) mass spectra were obtained on a MDSSCIEXQ-Tap mass spectrometer. Unless otherwise noted, the reagents were obtained from commercial supplier and were used without further purification. MCF-7, MDA-MB-231, MCF-7/ADR and MDA-MB-231/ADR breast cancer cell lines were purchased from Procell.

Synthesis

To the suspension of isatins **1a-d** (50 mmol) in DMF (50 mL), K_2CO_3 (150 mmol) was added. The mixture was stirred at room temperature for 1 h, and then methyl 4-bromobutanoate (70 mmol) was added. The mixture was stirred overnight at room temperature, and then filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel chromatography eluted with PE to PE: EA = 2: 1 to provide methyl 4-(N-1-isatin) butanoates **2a-d**. The mixture of 4-(N-1-isatin)butanoates **2a-d** (50 mmol) and LiOH (100 mmol) in a mixture of THF (100 mL) and H_2O (50 mL) was stirred at room temperature overnight, and then the pH was adjusted to 4.0 by 1 M HCl. The precipitate was collected and washed with H_2O (100 mL). The solid was dried under reduced pressure to give 4-(N-1-isatin)butanoic acids **3a-d**. The mixture of 4-(N-1-isatin)butanoic acids **3a-d** (20 mmol), HATU (30 mmol) and DIEA (10 mL) in DMF (100 mL) was stirred at room temperature overnight, and then concentrated *in vacuo*. The residue was purified by silica gel chromatography eluted with PE to PE: EA = 1: 1 to give the desired DHA-isatin hybrids **4a-d**. To a solution of DHA-isatin

hybrids **4a-d** (1.0 mmol) and methoxyamine/ethoxyamine/benzyloxyamine hydrochlorides (2.0 mmol) in a mixture of THF (20 mL) and H_2O (10 mL), Na_2CO_3 (5.0 mmol) was added. The mixture was stirred at 40 °C for 12 h, and then cooled to room temperature. The mixture was extracted with DCM (30 mL × 3). The combined organic layers were washed with H_2O (40 mL) and brine (40 mL) in sequence, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluted with PE to PE: EA = 1: 1 to give the desired DHA-isatin hybrids **5a-k**.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(2,3-dioxoindolin-1-yl)butanoate (4a)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.78–0.97 (m, 7H), 1.20–1.43 (m, 7H), 1.56–1.58 (m, 1H), 1.65–1.78 (m, 2H), 1.82–1.84 (m, 2H), 1.94–2.03 (m, 2H), 2.28–2.33 (m, 1H), 2.42–2.58 (m, 3H), 3.68–3.77 (m, 2H), 5.39 (s, 1H), 5.74 (d, J = 8.0 Hz, 1H), 6.94–7.07 (m, 2H), 7.53–7.58 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) 183.44, 171.88, 158.25, 150.78, 138.78, 138.55, 126.64, 126.45, 123.85, 123.77, 117.66, 110.67, 110.29, 104.52, 92.25, 91.83, 80.11, 51.86, 45.21, 39.29, 39.24, 37.48, 37.40, 36.20, 34.07, 31.70, 30.94, 30.56, 26.94, 22.14, 22.10, 22.00, 20.22, 12.16. HRMS-ESI: m/z Calcd for $C_{27}H_{33}NO_8Na$ $[M + Na]^+$: 522.2098; Found: 522.2065.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(5-fluoro-2,3-dioxoindolin-1-yl)butanoate (4b)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.87–1.06 (m, 7H), 1.25–1.52 (m, 7H), 1.64–1.68 (m, 1H), 1.74–1.83 (m, 2H), 1.90–1.94 (m, 1H), 2.00–2.09 (m, 3H), 2.37–2.43 (m, 1H), 2.50–2.62 (m, 3H), 3.74–3.88 (m, 2H), 5.48 (s, 1H), 5.84 (d, J = 8.0 Hz, 1H), 7.14 (dd, J = 8.0, 4.0 Hz, 1H), 7.32 (dd, J = 4.0, 2.0 Hz, 1H), 7.40 (td, J = 8.0, 2.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 182.87, 171.66, 160.17, 158.64, 158.01, 146.86, 125.22, 125.06, 118.16, 118.10, 112.61, 112.36, 111.96, 111.91, 104.66, 92.31, 91.66, 80.12, 51.86, 46.20, 39.33, 37.31, 36.19, 34.06, 31.70, 30.82, 25.99, 24.68, 22.01, 21.99, 20.21, 12.14. HRMS-ESI: m/z Calcd for $C_{27}H_{32}FNO_8Na$ $[M + Na]^+$: 540.2004; Found: 540.1961.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(5-methyl-2,3-dioxoindolin-1-yl)butanoate (4c)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.78–0.97 (m, 7H), 1.21–1.43 (m, 7H), 1.55–1.58 (m, 1H), 1.66–1.74 (m, 2H), 1.81–1.85 (m, 1H), 1.91–2.00 (m, 3H), 2.21–2.54 (m, 7H), 3.63–3.77 (m, 2H), 5.39 (s, 1H), 5.74 (d, J = 4.0 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 7.34–7.36 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) 183.70, 171.86, 168.33, 148.61, 139.07, 133.56, 126.77, 117.68, 110.34, 104.80, 92.22, 91.62, 80.10, 51.86, 45.21, 39.23, 37.30, 36.21, 34.08, 31.71, 30.98, 26.96, 24.89, 22.14, 22.01, 20.67, 20.22, 12.14. HRMS-ESI: m/z Calcd for $C_{28}H_{35}NO_8Na$ $[M + Na]^+$: 536.2255; Found: 536.2248.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(5-methoxy-2,3-dioxoindolin-1-yl)butanoate (4d)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.78–0.97 (m, 7H), 1.20–1.43 (m, 7H), 1.54–1.58 (m, 1H), 1.66–1.74 (m, 2H), 1.81–1.85 (m, 1H), 1.94–2.00 (m, 3H), 2.28–2.33 (m, 1H), 2.40–2.52 (m, 3H), 3.62–3.82 (m, 5H), 5.38 (s, 1H), 5.74 (d, J = 4.0 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 7.07 (d, J = 2.0 Hz, 1H), 7.12 (dd, J = 8.0, 2.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 183.81, 171.68, 158.30, 156.61, 144.69, 126.08, 117.95, 111.61, 109.63, 104.62, 92.25, 91.82, 80.10, 55.99, 51.66, 45.20, 39.22, 37.30, 36.20, 34.07, 31.70, 30.93, 25.95, 24.31, 22.11, 22.00, 20.21, 12.14. HRMS-ESI: m/z Calcd for $C_{28}H_{35}NO_9Na$ $[M + Na]^+$: 552.2204; Found: 552.2182.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-(methoxyimino)-2-oxoindolin-1-yl)butanoate (5a)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.19–1.43 (m, 7H), 1.54–1.58 (m, 1H), 1.64–1.73 (m, 2H), 1.81–1.84 (m, 1H), 1.91–1.99 (m, 3H), 2.28–2.33 (m, 1H), 2.49–2.61 (m, 3H), 3.67–3.79 (m, 2H), 4.22 (s, 3H, NOMe), 5.39 (s, 1H), 5.73 (d, J = 4.0 Hz, 1H), 6.92 (d, J = 4.0 Hz, 1H), 6.98 (t, J = 4.0 Hz, 1H), 7.34 (t, J = 4.0 Hz, 1H), 7.88 (d, J = 4.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.71, 163.80, 143.68, 143.61, 132.77, 127.95, 122.93, 116.76, 108.97, 104.49, 92.13, 91.60, 80.10, 64.75, 61.86, 45.23, 38.94, 37.29, 36.22, 34.09, 31.71, 31.03, 26.96, 24.89, 22.36, 22.00, 20.22, 12.16. HRMS-ESI: m/z Calcd for $C_{28}H_{36}N_2O_8Na$ $[M + Na]^+$: 551.2364; Found: 551.2318.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-(ethoxyimino)-2-oxoindolin-1-yl)butanoate (5b)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.19–1.27 (m, 2H), 1.31–1.42 (m, 8H), 1.52–1.57 (m, 1H), 1.64–1.72 (m, 2H), 1.81–1.83 (m, 1H), 1.91–1.99 (m, 3H), 2.28–2.33 (m, 1H), 2.40–2.52 (m, 3H), 3.70–3.80 (m, 2H),

4.48 (q, $J = 4.0$ Hz, 1H), 5.38 (s, 1H), 5.74 (d, $J = 4.0$ Hz, 1H), 6.92 (d, $J = 4.0$ Hz, 1H), 6.98 (t, $J = 4.0$ Hz, 1H), 7.34 (t, $J = 8.0$ Hz, 1H), 7.90 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.73, 163.64, 143.86, 143.64, 132.68, 127.85, 122.89, 116.69, 108.90, 104.49, 92.13, 91.60, 80.10, 72.99, 61.60, 45.23, 38.91, 37.29, 36.22, 34.09, 31.71, 31.06, 26.94, 24.89, 22.38, 22.00, 20.22, 14.73, 12.15. HRMS-ESI: m/z Calcd for $C_{29}H_{38}N_2O_8Na$ $[M + Na]^+$: 565.2520; Found: 565.2484.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-((benzyloxy)imino)-2-oxoindolin-1-yl)butanoate (5c)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.18–1.42 (m, 7H), 1.53–1.57 (m, 1H), 1.64–1.72 (m, 2H), 1.80–1.83 (m, 1H), 1.90–1.98 (m, 3H), 2.27–2.33 (m, 1H), 2.39–2.52 (m, 3H), 3.65–3.78 (m, 2H), 5.37 (s, 1H), 5.46 (s, 2H), 5.72 (d, $J = 8.0$ Hz, 1H), 6.91–6.95 (m, 2H), 7.28–7.33 (m, 4H), 7.37–7.39 (m, 2H), 7.86 (d, $J = 4.0$ Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.72, 163.63, 143.98, 143.69, 136.27, 132.82, 128.52, 128.46, 128.44, 128.35, 128.19, 128.12, 122.99, 116.81, 108.96, 104.50, 92.16, 91.61, 80.11, 79.36, 61.67, 45.23, 38.96, 37.29, 36.22, 34.09, 31.71, 31.04, 25.95, 24.89, 22.36, 22.01, 20.22, 12.16. HRMS-ESI: m/z Calcd for $C_{34}H_{40}N_2O_8Na$ $[M + Na]^+$: 627.2647; Found: 627.2617.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-(methoxyimino)-5-fluoro-2-oxoindolin-1-yl)butanoate (5d)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.20–1.42 (m, 7H), 1.53–1.57 (m, 1H), 1.64–1.72 (m, 2H), 1.80–1.84 (m, 1H), 1.91–1.98 (m, 3H), 2.26–2.33 (m, 1H), 2.37–2.50 (m, 3H), 3.66–3.77 (m, 2H), 4.22 (s, 3H, NOMe), 5.37 (s, 1H), 5.74 (d, $J = 8.0$ Hz, 1H), 6.80 (d, $J = 4.0$ Hz, 1H), 7.14 (d, $J = 4.0$ Hz, 1H), 7.71 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.73, 163.66, 143.80, 141.43, 133.03, 132.49, 128.09, 116.78, 108.71, 104.49, 92.11, 91.80, 80.10, 64.89, 51.57, 46.23, 38.97, 37.29, 36.22, 34.09, 31.71, 31.05, 25.94, 24.68, 22.38, 22.00, 20.96, 20.22, 12.14. HRMS-ESI: m/z Calcd for $C_{28}H_{38}FN_2O_9$ $[M + H_3O]^+$: 565.2455; Found: 565.2496.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-(ethoxyimino)-5-fluoro-2-oxoindolin-1-yl)butanoate (5e)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77-0.96 (m, 7H), 1.19–1.44 (m, 10H), 1.56–1.58 (m, 1H), 1.64–1.73 (m, 2H), 1.81–1.84 (m, 1H), 1.90–1.97 (m, 3H), 2.28–2.33 (m, 1H), 2.39–2.51 (m, 3H), 3.65–3.79 (m, 2H), 4.60 (q, $J = 4.0$ Hz, 2H), 5.38 (s, 1H), 5.74 (d, $J = 4.0$ Hz, 1H), 6.80–6.91 (m, 1H), 7.03–7.09 (m, 1H), 7.62–7.66 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 176.29, 171.72, 163.42, 159.83, 158.04, 143.09, 139.60, 118.91, 118.76, 118.69, 118.53, 116.36, 116.24, 116.03, 115.42, 115.25, 109.64, 109.50, 109.30, 104.32, 92.18, 91.82, 80.10, 73.44, 73.36, 51.86, 45.22, 39.07, 39.04, 37.49, 37.30, 36.35, 36.21, 34.21, 34.08, 31.71, 30.92, 30.62, 25.93, 24.89, 22.83, 22.26, 22.00, 20.22, 14.71, 13.18, 12.14. HRMS-ESI: m/z Calcd for $C_{29}H_{37}FN_2O_8Na$ $[M + Na]^+$: 583.2426; Found: 583.2405.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-((benzyloxy)imino)-5-fluoro-2-oxoindolin-1-yl)butanoate (5f)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.21–1.42 (m, 7H), 1.54–1.58 (m, 1H), 1.64–1.72 (m, 2H), 1.81–1.85 (m, 1H), 1.90–1.98 (m, 3H), 2.28–2.33 (m, 1H), 2.39–2.52 (m, 3H), 3.64–3.80 (m, 2H), 5.38 (s, 1H), 5.46 (s, 2H), 5.74 (d, $J = 4.0$ Hz, 1H), 6.88 (dd, $J = 8.0$, 4.0 Hz, 1H), 7.06 (td, $J = 8.0$, 2.0 Hz, 1H), 7.58 (dd, $J = 8.0$, 2.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.84, 163.27, 159.61, 158.02, 143.68, 139.73, 136.95, 128.69, 128.62, 128.52, 119.18, 119.02, 116.27, 116.21, 115.62, 115.45, 109.71, 109.66, 104.62, 92.18, 91.82, 80.10, 79.89, 51.66, 45.22, 39.07, 37.30, 36.21, 34.09, 31.71, 30.92, 26.94, 24.69, 22.25, 22.01, 20.22, 12.16. HRMS-ESI: m/z Calcd for $C_{34}H_{39}FN_2O_8Na$ $[M + Na]^+$: 645.2583; Found: 645.2561.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-(ethoxyimino)-5-methyl-2-oxoindolin-1-yl)butanoate (5g)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.20–1.43 (m, 10H), 1.53–1.57 (m, 1H), 1.64–1.73 (m, 2H), 1.81–1.84 (m, 1H), 1.91–1.98 (m, 3H), 2.26–2.33 (m, 4H), 2.37–2.61 (m, 3H), 3.66–3.78 (m, 2H), 4.58 (q, $J = 4.0$ Hz, 2H), 5.37 (s, 1H), 5.74 (d, $J = 8.0$ Hz, 1H), 6.80 (d, $J = 8.0$ Hz, 1H), 7.14 (d, $J = 8.0$ Hz, 1H), 7.72 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.73, 163.88, 143.82, 141.33, 132.83, 132.43, 128.43, 115.89, 108.63, 104.49, 92.10, 91.80, 80.10, 72.93, 51.87, 45.23, 38.94, 37.29, 36.22, 34.10, 31.71, 31.09, 25.95, 24.89, 22.40, 22.01, 21.02, 20.22, 14.81, 12.15. HRMS-ESI: m/z Calcd for $C_{30}H_{40}N_2O_8Na$ $[M + Na]^+$: 579.2677; Found: 579.2654.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-((benzyloxy)imino)-5-methyl-2-oxoindolin-1-yl)butanoate (5h)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.18–1.42 (m, 7H), 1.53–1.57 (m, 1H), 1.64–1.72 (m, 2H),

1.80–1.84 (m, 1H), 1.90–1.97 (m, 3H), 2.22 (s, 3H, -Me), 2.28–2.33 (m, 1H), 2.38–2.52 (m, 3H), 3.64–3.77 (m, 2H), 5.37 (s, 1H), 5.46 (s, 2H), 5.74 (d, $J = 8.0$ Hz, 1H), 6.80 (d, $J = 4.0$ Hz, 1H), 7.12 (d, $J = 4.0$ Hz, 1H), 7.27–7.33 (m, 3H), 7.38–7.39 (m, 2H), 7.69 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.72, 163.86, 144.16, 141.48, 136.82, 133.09, 132.43, 128.72, 128.59, 128.35, 128.30, 115.84, 108.69, 104.49, 92.11, 91.60, 80.10, 79.23, 51.57, 45.24, 38.98, 37.30, 36.23, 34.10, 31.72, 31.08, 25.93, 24.80, 22.39, 22.01, 21.01, 20.22, 12.15. HRMS-ESI: m/z Calcd for $C_{35}H_{42}N_2O_8Na$ $[M + Na]^+$: 641.2833; Found: 641.2795.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(5-methoxy-3-(methoxyimino)-2-oxoindolin-1-yl)butanoate (5i)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.19–1.42 (m, 7H), 1.54–1.57 (m, 1H), 1.64–1.73 (m, 2H), 1.81–1.85 (m, 1H), 1.90–1.99 (m, 3H), 2.28–2.33 (m, 1H), 2.37–2.52 (m, 3H), 3.63–3.77 (m, 5H), 4.42 (s, 3H, NOMe), 5.38 (s, 1H), 5.74 (d, $J = 8.0$ Hz, 1H), 6.79–6.91 (m, 2H), 7.50 (d, $J = 2.0$ Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.72, 163.36, 156.82, 143.89, 137.41, 117.77, 116.31, 114.45, 109.47, 104.49, 92.13, 91.80, 80.09, 64.80, 56.00, 51.57, 45.23, 38.99, 37.29, 36.22, 34.09, 31.71, 31.03, 25.95, 24.69, 22.35, 22.00, 20.22, 12.15. HRMS-ESI: m/z Calcd for $C_{29}H_{38}N_2O_9Na$ $[M + Na]^+$: 581.2470; Found: 581.2440.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-(ethoxyimino)-5-methoxy-2-oxoindolin-1-yl)butanoate (5j)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.21–1.43 (m, 10H), 1.52–1.57 (m, 1H), 1.64–1.73 (m, 2H), 1.81–1.84 (m, 1H), 1.91–1.98 (m, 3H), 2.28–2.33 (m, 1H), 2.38–2.52 (m, 3H), 3.64–3.78 (m, 5H), 4.48 (q, $J = 8.0$ Hz, 2H), 5.38 (s, 1H), 5.74 (d, $J = 8.0$ Hz, 1H), 6.76–6.90 (m, 2H), 7.53 (d, $J = 2.0$ Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.75, 163.30, 156.78, 143.72, 137.29, 117.29 116.47, 114.65, 109.38, 104.30, 92.12, 91.61, 80.10, 73.04, 55.95, 51.57, 45.23, 38.97, 37.29, 36.22, 34.09, 31.72, 31.05, 25.95, 22.35, 22.01, 20.22, 14.74, 12.15. HRMS-ESI: m/z Calcd for $C_{30}H_{40}N_2O_9Na$ $[M + Na]^+$: 595.2626; Found: 595.2605.

(3*R*,5a*S*,6*R*,8a*S*,9*R*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl 4-(3-((benzyloxy)imino)-5-methoxy-2-oxoindolin-1-yl)butanoate (5k)

^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.96 (m, 7H), 1.18–1.42 (m, 7H), 1.53–1.57 (m, 1H), 1.63–1.72 (m, 2H), 1.80–1.84 (m, 1H), 1.90–1.97 (m, 3H), 2.27–2.33 (m, 1H), 2.38–2.50 (m, 3H), 3.63–3.77 (m, 5H), 5.37 (s, 1H), 5.54 (s, 2H), 5.72 (d, $J = 4.0$ Hz, 1H), 6.75–6.89 (m, 2H), 7.27–7.38 (m, 5H), 7.49 (d, $J = 2.0$ Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) 171.74, 163.38, 156.79, 144.33, 137.41, 136.29, 128.60, 128.49, 128.44, 128.35, 128.33, 117.66, 116.38, 114.71, 109.48, 104.30, 92.14, 91.61, 80.10, 79.31, 55.87, 51.57, 45.23, 39.02, 37.29, 36.22, 34.09, 31.72, 31.04, 25.95, 24.89, 22.35, 22.01, 20.22, 12.15. HRMS-ESI: m/z Calcd for $C_{35}H_{42}N_2O_9Na$ $[M + Na]^+$: 657.2783; Found: 657.2741.

In vitro antiproliferative activity evaluation

MCF-7, MDA-MB-231, MCF-7/ADR and MDA-MB-231/ADR breast cancer cells (2×10^3) were plated in each well of a 96-well plate and were allowed to adhere and spread for 24 h. The DHA-isatin hybrids **4a-d** and **5a-k** were added to a final concentration of 100 μM, and the cells were cultured for 24 h at 37 °C. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) solution (10 μL) was added to each well, and the cultures were incubated for an additional 4 h. A further 100 μL of MTT solution was added and incubation continued overnight. The absorbance at 540 nm was determined in each well with a 96-well plate reader. The growth of the treated cells was compared with that of untreated cells. Half maximum inhibitory concentration (IC50) is a measurement of the efficacy of a substance in inhibiting a specific biological or biochemical process. For a drug, IC_{50} represents the drug concentration required to inhibit the activity of tumor cells by 50% in vitro, and it is the most important parameter to compare the efficacy of a drug with that of similar drugs. In this experiment, the dose-response curve constructed by Spss22.0 software was used to calculate the inhibitory effect of the hybrid (0–100 μM) on the activity of breast cancer cells.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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