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ORIGINAL ARTICLE

# Synthesis and antitumor evaluation of (aryl)methyl-amine derivatives of dehydroabietic acid-based B ring-fused-thiazole as potential PI3K/AKT/mTOR signaling pathway inhibitors

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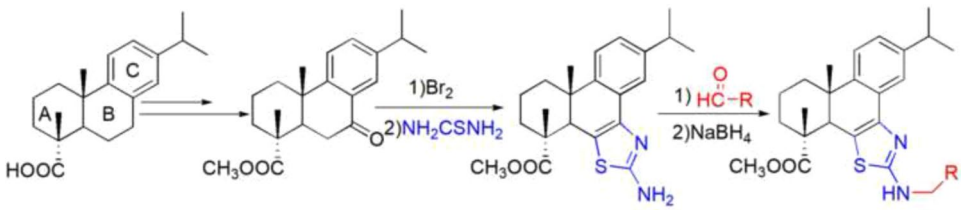
## Abstract

In an attempt to search for new natural product-based antitumor agents, a series of novel (aryl)methyl-amine derivatives of dehydroabietic acid-based B ring-fused-thiazole were designed and synthesized. The primary bioassay showed that compounds **5r** and **5s** presented certain inhibitory activity against cancer cells, weak cytotoxic activity against normal cells, and inhibitory activity against PI3K/AKT/mTOR signaling pathway. The binding modes and the binding site interactions between the active compounds and the target proteins were predicted preliminarily by the molecular docking method.

## Graphic abstract




Synthetic route of (aryl)methyl-amide derivatives of dehydroabietic acid-based B ring-fused-thiazole

**Keywords** Dehydroabietic acid · Thiazole · Antitumor · Activity · Synthesis

## Introduction

Rosin, the distillation residue of pine resins, is an abundantly available natural product and a traditional medicine. However, Rosin is used mainly as an agent in paper sizing, printing inks, soup, cosmetics, adhesives, and emulsifiers.

Nai-Yuan Chen and Yu-Lan Xie have contributed equally to this work.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s11030-020-10081-7) contains supplementary material, which is available to authorized users.

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Its essentially bioactive property as secondary metabolites against potential herbivores and pathogens has not still been sufficiently developed for pharmaceutical use [1, 2]. Thus, in order to enhance the added value of rosin, the development of rosin components for medical utilization has been studied continually in recent decades [3].

Dehydroabietic acid (DHA) is one of the components of rosin in small content, but can be readily obtained from disproportionated rosin which is one of the modified products of rosin. Recent reports indicated that DHA and its derivatives exhibited a broad spectrum of biological activities, such as antimicrobial [4], antitumor [5], antifungal [6], antiviral [7], inflammatory [8], antiprotozoal [9], anti-aging [10] and BK channel opening activities [11]. Therefore, DHA has been proved to be a promising starting material in the synthesis of physiological or pharmacological compounds.

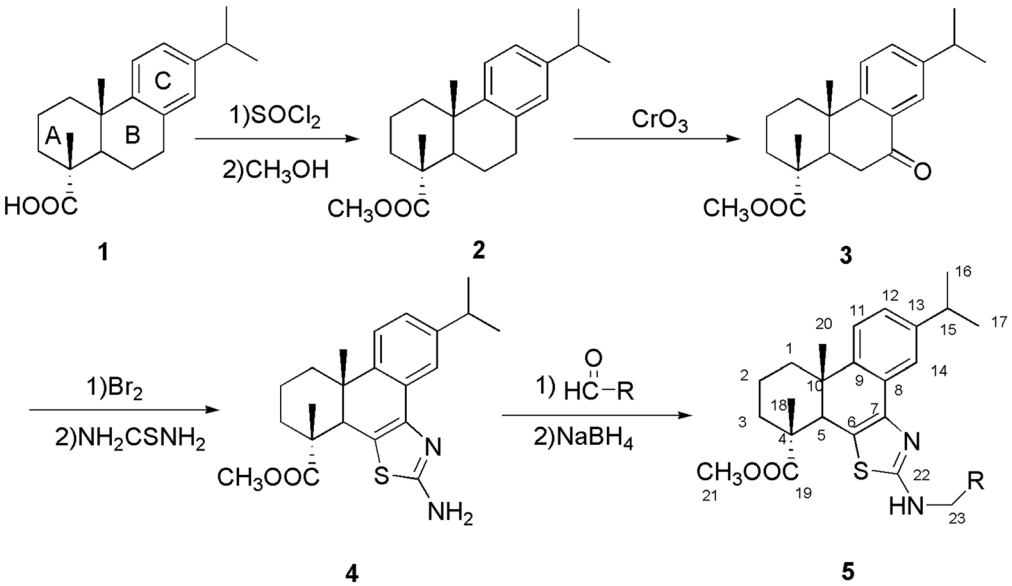
In our previous studies, a series of dehydroabietic acid-based B ring-fused-thiazole amides were synthesized, some of which showed notable antifungal activities [12]. In addition, thiazole moiety is an important scaffold in many biologically active compounds and has been found in many anticancer drugs such as epothilones, ixabepilone, bleomycin, and tiazofurin [13]. Some compounds with thiazole moiety were reported as kinase inhibitors of PI3K signaling pathway which provides strong growth and survival signals to tumor cells [14, 15].

Inspired by the above facts and in continuation of our interest in designing and developing new B ring-fused-thiazole derivatives of dehydroabietic acid, a series of (aryl)methyl-amine derivatives of dehydroabietic acid-based B ring-fused-thiazole were designed and synthesized in this study. The cytotoxic activities of target compounds against human hepatoma cells HepG2, human oral squamous cell SCC9, and human embryonic kidney cell 293T were detected by CCK8 method. The inhibitory activity against PI3K/AKT/mTOR signaling pathway was studied by western blotting analysis. The possible interaction modes between the target compounds and the signaling pathway proteins were evaluated by molecular docking study.

## Results and discussion

### Chemistry

The synthetic approaches adopted to afford the target compounds are outlined in Scheme 1. Compounds **1**, **2,** and **3** were prepared according to the studies [4, 16]. Subsequently, compound **3** was treated with bromine and then with thiourea in one pot to give compound **4**. The yield of this step is better than that of our previous work in which the bromide formed in the reaction was purified [12]. Then, the target compounds **5a**–**5t** were synthesized in good yields by condensation reaction of compound **4** with corresponding aldehydes followed by reduction reaction with sodium borohydride in one pot.




**5a**: R=phenyl; **5b**: R=2-$CH_3$-phenyl; **5c**: R=3-$CH_3$-phenyl; **5d**: R=4-$CH_3$-phenyl; **5e**: R=2-$CH_3O$-phenyl; **5f**: R=3-$CH_3O$-phenyl; **5g**: R=4-$CH_3O$-phenyl;**5h**: R=2-F-phenyl; **5i**: R=3-F-phenyl; **5j**: R=4-F-phenyl; **5k**: R=2-Cl-phenyl; **5l**: R=3-Cl-phenyl; **5m**: R=4-Cl-phenyl; **5n**: R=2-Br-phenyl;**5o**: R=3-Br-phenyl; **5p**: R=4-Br-phenyl; **5q**: R=furan-2-yl; **5r**: R=1H-pyrrol-2-yl; **5s**: R=pyridin-3-yl; **5t**: R=6-Cl-pyridin-3-yl

**Scheme 1** Synthetic route of (aryl)methyl-amide derivatives of dehydroabietic acid-based B ring-fused-thiazole **5a**–**5t**

The identities of the target compounds were confirmed using physicochemical analytical methods. $^{1}$H NMR spectra revealed that the amino-group proton exhibited a characteristic broad single signal in the 5.90–5.32 ppm range. The aromatic protons of the dehydroabietic acid scaffold showed signals at 7.73–7.63, 7.21–7.17, and 7.16–7.12 ppm. The methyl ester group protons showed a single signal in the 3.66–3.62 ppm range. The protons of the methyl bound with amino-group exhibited quartet of doublets or quartet signals in the 4.66–4.48 ppm range. The $^{13}$C NMR spectra signals of all the target compounds were matched with the number of the carbon types. The high-resolution mass spectra confirmed the expected molecular weights of the final products.

## Antitumor activity

The synthesized target compounds **5a**–**5t** were evaluated for their in vitro cytotoxic activities against the two human cancer cell lines (HepG2 and SCC9) and the human normal cell (293T) by CCK-8 assay [17]. The results are summarized in Table 1.

It was found that the newly synthesized compounds showed weak to moderate cytotoxic activities against the two human cancer cell lines and displayed weak cytotoxic activity against the normal cell 293T. Among the target compounds, **5r** and **5s** showed the best cytotoxic activity against HepG2 with half-inhibitory concentration ($IC_{50}$) values of 24.41 ± 0.26 and 22.92 ± 0.24 μM, respectively, better than those of DHA and positive control 5-fluorouracil. Moreover, compound **5s** exhibited the best cytotoxic activity against SCC9, better than that of DHA and positive control 5-fluorouracil. This result of $IC_{50}$ indicated that DHA could be modified according to the strategy in this study to form new derivatives with better antitumor activity. In addition, the compounds with pyrrole moiety (**5r**) and unsubstituted pyridine moiety (**5s**) displayed better cytotoxic activities than other compounds. The compound with ortho-chloro-substituted pyridine moiety (**5t**) showed much weaker cytotoxic activity than compound **5s**. This result suggested that the cytotoxic activities of compounds may be affected by the electron donating ability of R group.

**Table 1** In vitro cytotoxic activities of compounds **5a**–**5t**

| Compound | $IC_{50}$ (μmol/L) | | |
|---|---|---|---|
| | HepG2 | SCC9 | 293T |
| **5a**(R = Ph) | > 100 | > 100 | > 100 |
| **5b**(R = 2$CH_3$-Ph) | > 100 | > 100 | > 100 |
| **5c**(R = 3$CH_3$-Ph) | > 100 | > 100 | > 100 |
| **5d**(R = 4$CH_3$-Ph) | > 100 | > 100 | > 100 |
| **5e**(R = 2$CH_3O$-Ph) | > 100 | > 100 | > 100 |
| **5f**(R = 3$CH_3O$-Ph) | > 100 | 51.13 ± 0.40 | > 100 |
| **5g**(R = 4$CH_3O$-Ph) | > 100 | 60.13 ± 0.69 | > 100 |
| **5h**(R = 2F-Ph) | > 100 | > 100 | > 100 |
| **5i**(R = 3F-Ph) | > 100 | 56.28 ± 0.28 | > 100 |
| **5j**(R = 4F-Ph) | > 100 | 96.08 ± 0.38 | > 100 |
| **5k**(R = 2Cl-Ph) | > 100 | > 100 | > 100 |
| **5l**(R = 3Cl-Ph) | > 100 | 82.62 ± 0.99 | > 100 |
| **5m**(R = 4Cl-Ph) | > 100 | 51.93 ± 0.61 | > 100 |
| **5n**(R = 2Br-Ph) | > 100 | > 100 | > 100 |
| **5o**(R = 3Br-Ph) | > 100 | > 100 | > 100 |
| **5p**(R = 4Br-Ph) | > 100 | 56.68 ± 0.28 | > 100 |
| **5q**(R = furan-2-) | > 100 | 82.72 ± 0.24 | > 100 |
| **5r**(R = 1*H*-pyrrol-2-) | 24.41 ± 0.26 | 44.15 ± 0.49 | 76.25 ± 0.50 |
| **5s**(R = pyridin-3-) | 22.92 ± 0.24 | 21.79 ± 0.34 | 68.64 ± 0.24 |
| **5t**(R = 6-Cl-pyridin-3-) | 91.72 ± 0.27 | 95.04 ± 0.23 | > 100 |
| **1**(DHA) | > 100 | 76.66 ± 0.30 | > 100 |
| 5-Fluorouracil | 32.74 ± 0.17 | > 100 | > 100 |

$IC_{50}$ values are taken as means standard deviation from three experiments. HepG2 is human hepatoma cells; SCC9 is human oral squamous cell; 293T is human embryonic kidney cell

## PI3K/AKT/mTOR signaling pathway inhibitory activity of 5r and 5s

To investigate the PI3K/AKT/mTOR signaling pathway inhibitory activity of the two effective compounds, in the treatment group, HepG2 cells were treated with **5r** or **5s** in concentrations of 20 μM and 40 μM. The HepG2 cells untreated were served as blank control. The protein expressions of p-PI3K, p-AKT, p-mTOR, mTOR, p-S6K, p-S6, p-4EBP1, S6, 4EBP1, and α-tubulin were detected by the western blot method. The result is shown in Fig. 1.

In the treatment group, the expression levels of the phosphorylated proteins, p-PI3K, p-AKT, p-mTOR, p-S6K, p-S6, and p-4EBP1 suffered a significant decrease, while the total mTOR and S6 expressions were decreased greatly and slightly, respectively. The expression levels of total 4EBP1 and α-tubulin were almost not affected by the treatments with compounds, compared with the untreated control. Generally, this result showed that compounds **5r** and **5s** could inhibit the expressions of the phosphorylated proteins of PI3K/AKT/mTOR signaling pathway and the phosphorylation of the downstream effectors S6 and 4EBP1.

## Molecular docking studies

In an attempt to study the possible interaction mode between compounds (**5r** or **5s**) and the active sites of PI3K, AKT, and mTOR, molecular docking was conducted using AutoDock Tools. Each docking result contained 50 conformations predicted, and the conformation with the lowest binding energy was chosen as representative to analysis.

The representative docking results of compounds within PI3K are shown in Fig. 2. The compound **5r** binds to PI3K

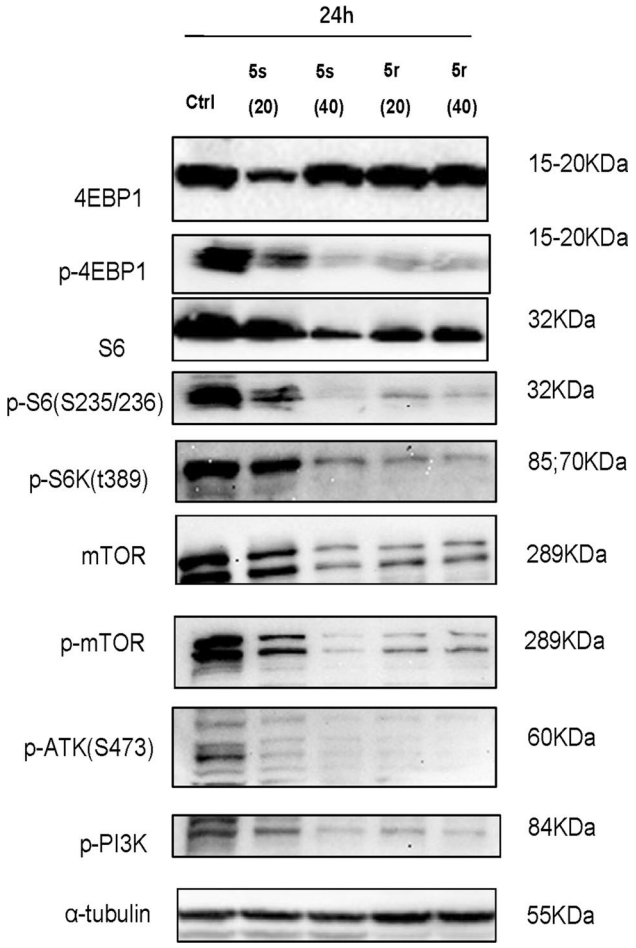



**Fig. 1** The protein expressions affected by the treatment with **5r** and **5s**

possibly by interacting with 15 residues through 13 non-bonding interactions and 2 hydrogen bonds (Fig. 2a, b). Some adenosine triphosphate (ATP)-binding sites reported were found in the interacting residues [18], such as Ala805, Ser806, Asp964, Tyr867, Glu880, and Lys890. The S atom of thiazole moiety and the N atom of pyrrole ring form hydrogen bonds with Lys890 and Ala885, respectively. The binding energy of this conformation is − 7.04 kcal/mol. The compound **5s** binds to PI3K possibly by interacting with 14 residues through 13 non-bonding interactions and 1 hydrogen bond (Fig. 2c, d). Ser806, Asp950, Ala805, Asp964, Glu880, Val882, Trp812, and Lys890 were found in the interacting residues reported as (ATP)-binding site. The S atom of thiazole moiety forms a hydrogen bond with Lys890. The binding energy of this conformation is − 6.81 kcal/mol.

The representative docking results of compounds within AKT are shown in Fig. 3. The compound **5r** binds to AKT possibly by interacting with 16 residues through 15 non-bonding interactions and 1 hydrogen bond (Fig. 3a, b). Compared with other reported ATP competitive inhibitors [18], the interacting residues Ala177, Val164, Met281, Phe438, and Glu234 were found as the common interacting sites. The O atom of carbonyl group forms a hydrogen bond with Lys185. The binding energy of this conformation is − 7.88 kcal/mol. The compound **5s** binds to AKT possibly by interacting with 15 residues through 14 non-bonding interactions and 1 hydrogen bond (Fig. 3c, d). Compared with other reported ATP competitive inhibitors, the interacting residues Glu234, Phe438 and Val164 were found as the common sites. The N atom of thiazole moiety forms a hydrogen bond with Arg4 (B). The binding energy of this conformation is − 9.15 kcal/mol.

The representative docking results of compounds within mTOR are shown in Fig. 4. The compound **5r** binds to mTOR possibly by interacting with 17 residues through 15 non-bonding interactions and 2 hydrogen bonds (Fig. 4a, b). Compared with other reported ATP competitive inhibitors [18], the interacting residues Gly2238, Val2240, Met2345, Asp2357, Ile2237, Ile2356, Leu2185, Trp2239, Asp2195, and Glu2190 were found as the common sites. The N atom of amino moiety and pyrrole ring form hydrogen bonds with Glu2190 and Asp2195, respectively. The binding energy of this conformation is − 8.39 kcal/mol. The compound **5s** binds to mTOR possibly by non-bonding interaction with 14 residues (Fig. 4c, d). Compared with other reported ATP competitive inhibitors, the interacting residues Leu2185, Ile2237, Tyr2225, Asp2357, Ile2356, Gly2238, Val2240, Trp2239, and Met2345 were found as the common sites. The binding energy of this conformation is − 8.99 kcal/mol.

These results indicated that compounds **5r** and **5s** may inhibit the PI3K/AKT/mTOR signaling pathway by acting as ATP competitive inhibitor.

## Conclusions

In summary, 20 new (aryl)methyl-amine derivatives of dehydroabietic acid-based B ring-fused-thiazole were synthesized and their in vitro cytotoxic activities were investigated by CCK-8 assay against HepG2, SCC9, and 293T. Compounds **5r** and **5s** presented certain inhibitory activity against cancer cells and weak cytotoxic activity against normal cells. The western blot assay showed that these two compounds can inhibit the PI3K/AKT/mTOR signaling pathway. According to the result of molecular docking study, the compounds tested may inhibit the pathway through ATP competition. The moieties integrated into the skeleton of DHA, such as amino thiazole moiety and nitrogen heterocycles, may play as an important role in the inhibitory activity of the compounds. Compounds **4**, **5r** and **5s** could be chosen as the leading compounds for further modification to search new antitumor agents.

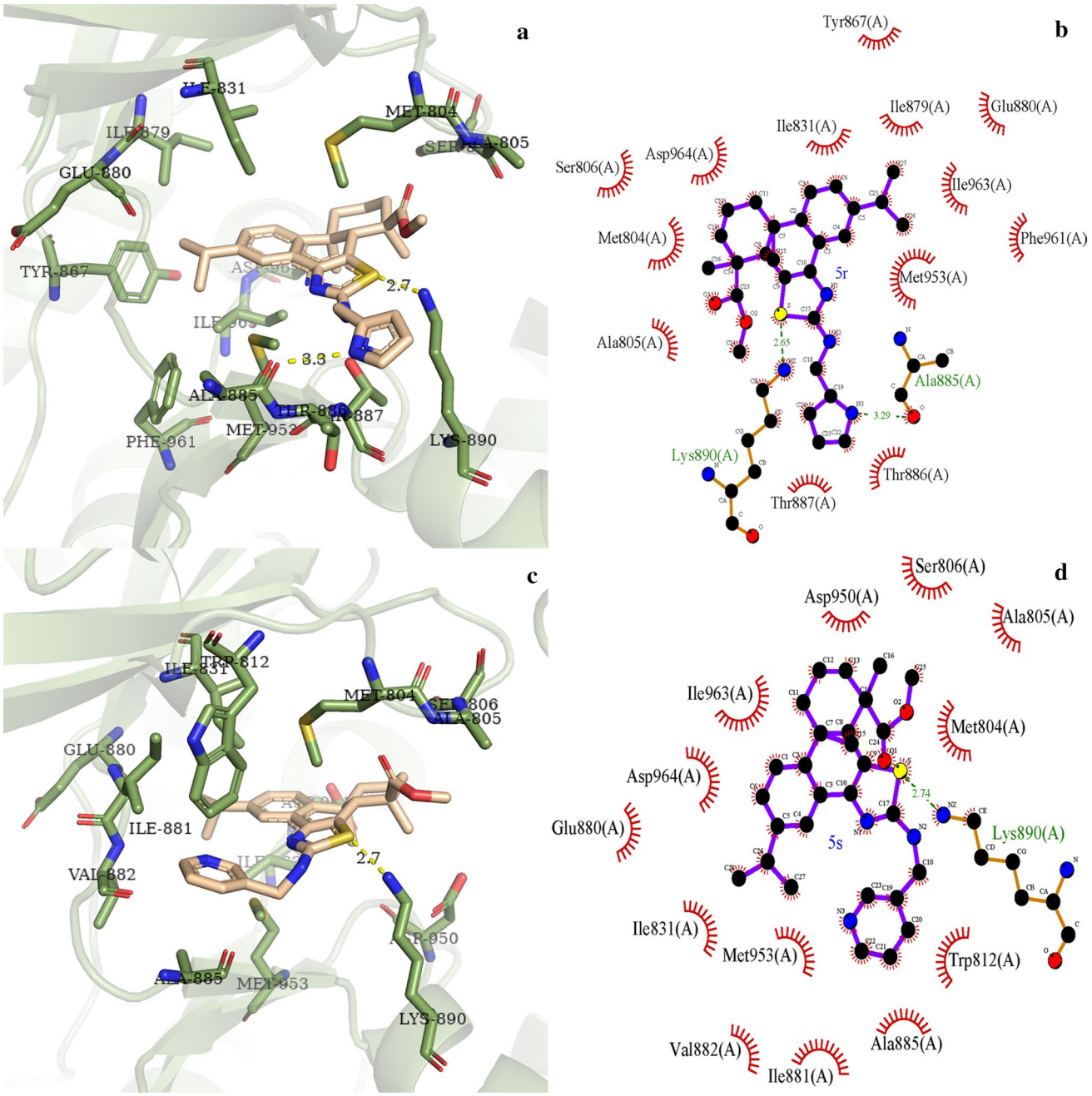



**Fig. 2** **a** 3D conformation of **5r** docked in PI3K. **b** Predicted interactions between **5r** and the amino acids of PI3K. **c** 3D conformation of **5s** docked in PI3K. **d** Predicted interactions between **5s** and the amino acids of PI3K

## Materials and methods

### Chemistry

The melting points (m.p.) were determined on an X-5A digital melting point apparatus (Gongyi Kerui Instruments, Gongyi, China). NMR spectra were recorded in $CDCl_3$ on a Bruker AVANCE III HD500 spectrometer (Bruker, Switzerland), and chemical shifts were expressed in ppm ($\delta$) downfield relative to TMS as an internal standard; NMR abbreviations: $s$ = singlet, $d$ = doublet, $t$ = triplet, $q$ = quartet, $m$ = multiplet. MS spectra were obtained by means of the electrospray ionization (ESI) on a high-resolution mass spectrometer UPLC I-CLASS-XEVOG2-XSQTOF (Waters, Milford, USA). Reaction products were isolated by using column chromatography on silica gel column (200–300 mesh). All chemicals and reagents were purchased from commercial suppliers and used as received.

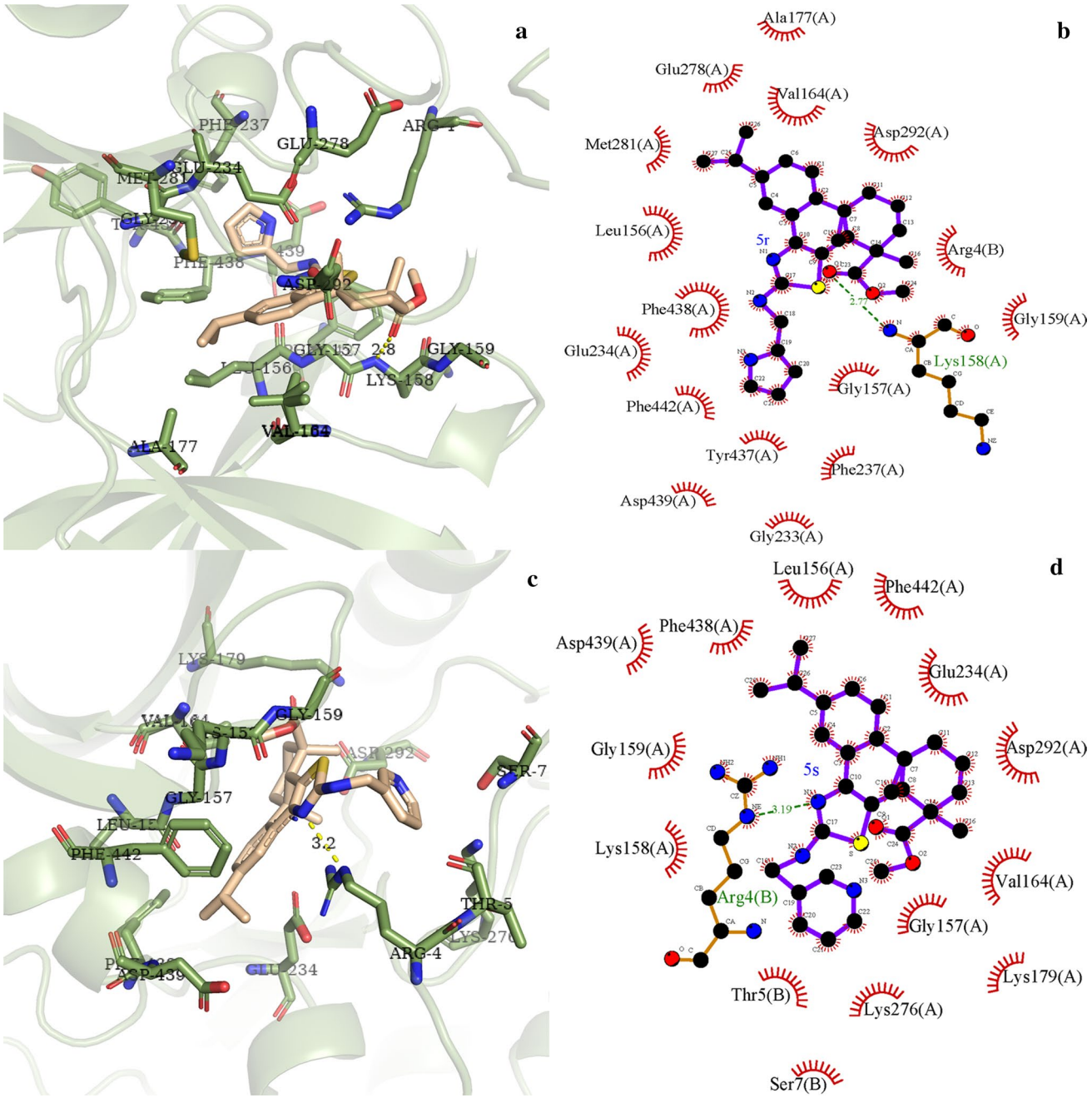



**Fig. 3** **a** 3D conformation of **5r** docked in AKT. **b** Predicted interactions between **5r** and the amino acids of AKT. **c** 3D conformation of **5s** docked in AKT. **d** Predicted interactions between **5s** and the amino acids of AKT

Compounds **1**, **2,** and **3** were prepared according to the studies [4, 16].

### Preparation of (7bS,11R)-methyl 2-amino-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate(4)

A mixture of glacial acetic acid (1.4 mL) and $Br_2$ (1.1 mL, 21.4 mmol) was gradually added to the solution of compound **3** (5.0 g, 15 mmol) in glacial acetic acid (5.0 mL) at 60 °C. Then, the mixture was continuously stirred for 10 min followed by adding thiourea (2.25 g, 30 mmol). Later, the reacting mixture was heated to 105 °C for 12 h. After that, the mixture was evaporated in vacuo to remove acetic acid and then dissolved in $CH_2Cl_2$ followed by washing with excess aqueous $NaHCO_3$ solution three times. After removing $CH_2Cl_2$, the residue was purified by column chromatography on silica gel

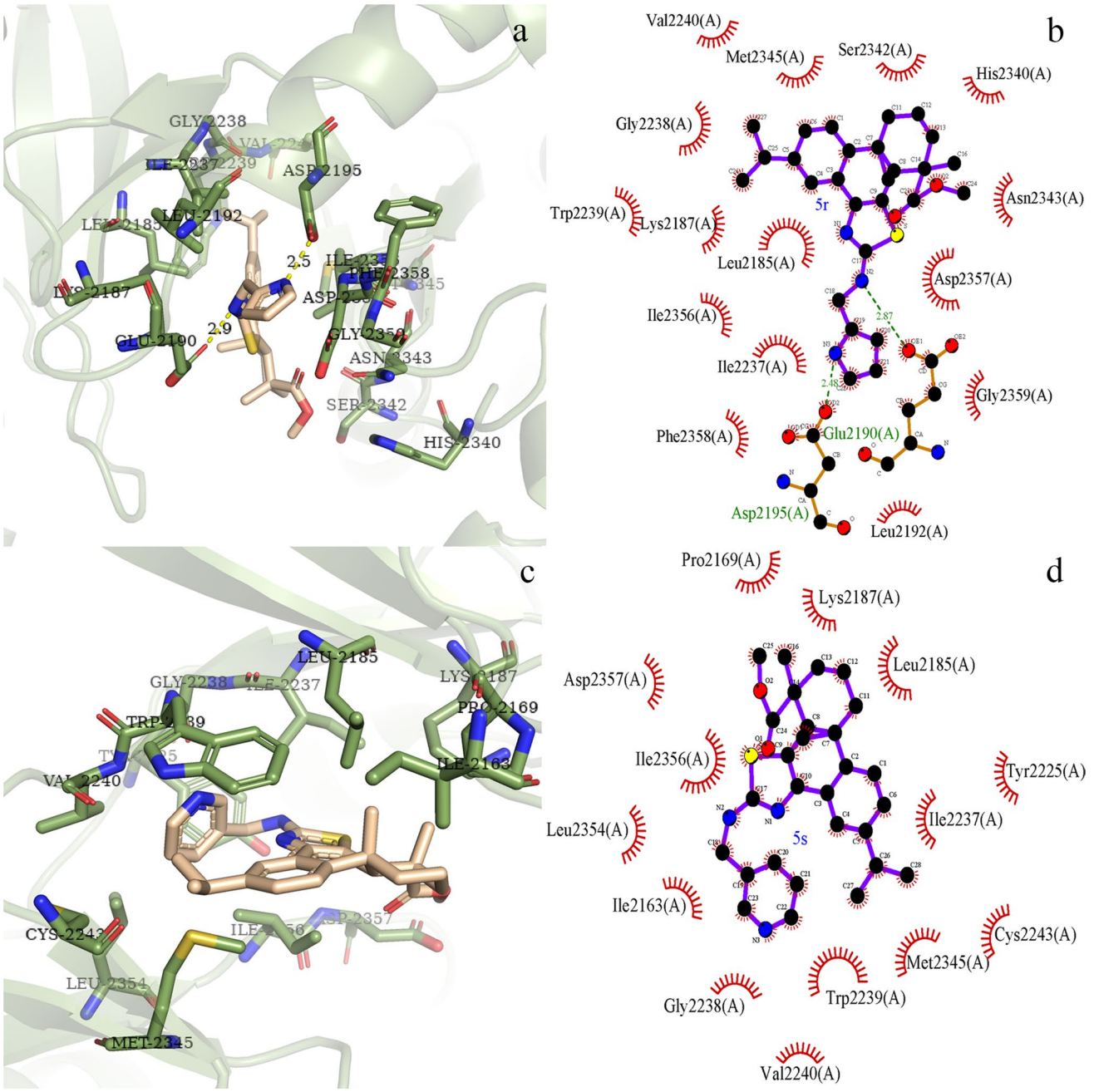



**Fig. 4** **a** 3D conformation of **5r** docked in mTOR. **b** Predicted interactions between **5r** and the amino acids of mTOR. **c** 3D conformation of **5s** docked in mTOR. **d** Predicted interactions between **5s** and the amino acids of mTOR

(petroleum ether–ethyl acetate, 5:1) to afford compound **4** as white powder, Yield 65%.

### Synthesis of (aryl)methyl-amine derivatives of dehydroabietic acid-based B ring-fused-thiazole 5a–5t

Compound **4** (0.1 g, 0.26 mmol) and corresponding aldehydes (0.3 mmol) were dissolved in anhydrous methanol (1.5 mL) and heated to 60 °C for 4 h. Then, sodium borohydride was gradually added to the reaction mixture till the color of solution became no longer lighter, and the solution was evaporated in vacuo to remove solvent followed by dissolving in $CH_2Cl_2$. After washing with deionized water and drying with anhydrous sodium sulfate, the resulting solution was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 10:1) to afford target compounds **5a**–**5t**.

**(7bS,11R)-methyl 2-(benzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d] thiazole-11-carboxylate (5a)**

Yield 74%, white solid, m.p.79.5–80.6 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (d, $J$ = 1.6 Hz, 1H, H-14), 7.44–7.28 (m, 5H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 5.45 (s, 1H, NH), 4.52 (ddd, $J$ = 33.9, 14.2, 5.4 Hz, 2H, H-23), 3.71 (d, $J$ = 6.8 Hz, 1H, H-5), 3.63 (s, 3H, $COOCH_3$), 2.94 (dq, $J$ = 13.8, 6.9 Hz, 1H, H-15), 2.29 (dd, $J$ = 8.5, 5.9 Hz, 1H, $H_{1\text{-e}}$), 1.91–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.58 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 2.2 Hz, 6H, H-16, H-17), 1.15 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.51, 167.79, 146.99, 146.35, 144.23, 137.80, 130.05, 128.68, 127.82, 127.69, 125.41, 122.13, 121.82, 120.04, 52.42, 49.98, 46.66, 46.57, 39.20, 37.38, 35.35, 33.83, 24.14, 23.89, 21.75, 18.28, 17.60. Mass spectrum (ESI), $m/z$: 475.2240 $[M+H]^+$ Caled $C_{29}H_{34}N_2O_2S$ 474.2341.

**(7bS,11R)-methyl 2-(2-tolylmethanamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d] thiazole-11-carboxylate (5b)**

Yield 67%, white solid, m.p. 178.6–179.1 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (d, $J$ = 1.4 Hz, 1H, H-14), 7.39 (d, $J$ = 7.2 Hz, 1H, H-Ph), 7.27–7.20 (m, 3H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 5.22 (s, 1H, NH), 4.49 (qd, $J$ = 13.7, 5.2 Hz, 2H, H-23), 3.73 (s, 1H, H-5), 3.65 (s, 3H, $COOCH_3$), 2.95 (hept, $J$ = 6.9 Hz, 1H, H-15), 2.43 (s, 3H, $CH_3$-Ph), 2.33–2.27 (m, 1H, $H_{1\text{-e}}$), 1.92–1.76 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.59 (s, 3H, H-19), 1.30 (dd, $J$ = 6.9, 1.6 Hz, 6H, H-16, H-17), 1.16 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.54, 167.63, 146.98, 146.42, 144.25, 136.59, 135.55, 130.55, 130.09, 128.65, 127.93, 126.18, 125.41, 122.12, 121.84, 119.97, 52.44, 48.03, 46.67, 46.57, 39.21, 37.41, 35.36, 33.84, 24.14, 23.90, 21.76, 19.10, 18.29, 17.62. Mass spectrum (ESI), $m/z$: 489.2557 $[M+H]^+$ Caled $C_{30}H_{36}N_2O_2S$ 488.2497.

**(7bS,11R)-methyl 2-(3-tolylmethanamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d] thiazole-11-carboxylate (5c)**

Yield 82%, white solid, m.p. 153.5–154.8 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (d, $J$ = 1.4 Hz, 1H, H-14), 7.24 (dt, $J$ = 17.8, 7.6 Hz, 3H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.15–7.11 (m, 2H, H-12, H-Ph), 5.43 (s, 1H, NH), 4.48 (ddd, $J$ = 35.6, 14.1, 5.5 Hz, 2H, H-23), 3.73 (s, 1H, H-5), 3.63 (s, 3H, $COOCH_3$), 2.95 (hept, $J$ = 6.9 Hz, 1H, H-15), 2.38 (s, 3H, $CH_3$-Ph), 2.30 (dd, $J$ = 8.6, 5.8 Hz, 1H, $H_{1\text{-e}}$), 1.91–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.59 (s, 3H, H-19), 1.30 (dd, $J$ = 6.9, 2.1 Hz, 6H, H-16, H-17), 1.15 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.52, 167.82, 146.98, 146.36, 144.24, 138.37, 137.71, 130.08, 128.57, 128.56, 128.42, 125.39, 124.87, 122.13, 121.83, 119.99, 52.41, 49.98, 46.67, 46.57, 39.20, 37.39, 35.36, 33.84, 24.15, 23.90, 21.75, 21.40, 18.29, 17.60. Mass spectrum (ESI), $m/z$: 489.2466 $[M+H]^+$ Caled $C_{30}H_{36}N_2O_2S$ 488.2497.

**(7bS,11R)-methyl 2-(4-tolylmethanamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d] thiazole-11-carboxylate (5d)**

Yield 75%, white solid, m.p. 81.0–82.7 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (d, $J$ = 1.8 Hz, 1H, H-14), 7.31 (d, $J$ = 8.0 Hz, 2H, H-Ph), 7.18 (d, $J$ = 8.3 Hz, 3H, H-11, H-Ph), 7.12 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 5.37 (d, $J$ = 5.1 Hz, 1H, NH), 4.47 (ddd, $J$ = 31.8, 13.9, 5.5 Hz, 2H, H-23), 3.72 (s, 1H, H-5), 3.64 (s, 3H, $COOCH_3$), 2.94 (dq, $J$ = 13.8, 6.9 Hz, 1H, H-15), 2.37 (s, 3H, $CH_3$-Ph), 2.29 (dd, $J$ = 8.5, 5.8 Hz, 1H, $H_{1\text{-e}}$), 1.91–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.58 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 2.0 Hz, 6H, H-16, H-17), 1.15 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.53, 167.79, 149.14, 146.98, 146.35, 144.23, 137.39, 134.73, 130.09, 129.34, 127.83, 125.38, 122.12, 121.83, 119.97, 52.41, 49.78, 46.67, 46.57, 39.20, 37.39, 35.36, 33.83, 24.14, 23.89, 21.75, 21.13, 18.29, 17.60. Mass spectrum (ESI), $m/z$: 489.2734 $[M+H]^+$ Caled $C_{30}H_{36}N_2O_2S$ 488.2497.

**(7bS,11R)-methyl 2-(2-methoxybenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5e)**

Yield 72%, white solid, m.p. 235.8–236.4 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.67 (d, $J$ = 1.7 Hz, 1H, H-14), 7.39 (dd, $J$ = 7.4, 1.5 Hz, 1H, H-Ph), 7.30 (dt, $J$ = 8.0, 1.8 Hz, 1H, H-Ph), 7.17 (d, $J$ = 8.0 Hz, 1H, H-11), 7.11 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 6.94 (ddd, $J$ = 12.3, 9.4, 4.5 Hz, 2H, H-Ph), 5.60 (s, 1H, NH), 4.49 (qd, $J$ = 14.4, 6.1 Hz, 2H, H-23), 3.89 (s, 3H, $CH_3O$-Ph), 3.71 (s, 1H, H-5), 3.65 (s, 3H, $COOCH_3$), 2.95 (hept, $J$ = 6.9 Hz, 1H, H-15), 2.34–2.24 (m, 1H, $H_{1\text{-e}}$), 1.90–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.59 (s, 3H, H-19), 1.30 (dd, $J$ = 6.9, 1.6 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.58, 168.15, 157.58, 146.93, 146.33, 144.22, 130.14, 129.71, 129.00, 125.94, 125.27, 122.09, 121.82, 120.41, 119.81, 110.30, 55.30, 52.41, 46.67, 46.54, 46.02, 39.18, 37.38, 35.37, 33.83, 24.16, 23.88, 21.73, 18.30, 17.61. Mass spectrum (ESI), $m/z$: 505.2514 $[M+H]^+$ Caled $C_{30}H_{36}N_2O_3S$ 504.2247.

**(7bS,11R)-methyl 2-(3-methoxybenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5f)**

Yield 86%, white solid, m.p. 116.7–117.6 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (d, $J$ = 1.8 Hz, 1H, H-14), 7.30–7.27 (m, 1H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 6.99 (dd, $J$ = 9.4, 4.9 Hz, 2H, H-Ph), 6.86 (dd, $J$ = 8.2, 2.4 Hz, 1H, H-Ph), 5.45 (s, 1H, NH), 4.50 (ddd, $J$ = 33.2, 14.2, 5.4 Hz, 2H, H-23), 3.82 (s, 3H, $CH_3O$-Ph), 3.72 (s, 1H, H-5), 3.63 (s, 3H, $COOCH_3$), 2.94 (dq, $J$ = 13.8, 6.9 Hz, 1H, H-15), 2.29 (dd, $J$ = 8.6, 5.9 Hz, 1H, $H_{1\text{-e}}$), 1.92–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.58 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 2.0 Hz, 6H, H-16, H-17), 1.15 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.51, 167.76, 159.90, 146.99, 146.36, 144.23, 139.41, 130.06, 129.71, 125.41, 122.14, 121.83, 120.07, 120.05, 113.36, 113.18, 55.25, 52.42, 49.96, 46.66, 46.57, 39.20, 37.39, 35.35, 33.84, 24.14, 23.89, 21.75, 18.28, 17.60. Mass spectrum (ESI), $m/z$: 505.2535 $[M+H]^+$ Caled $C_{30}H_{36}N_2O_3S$ 504.2247.

**(7bS,11R)-methyl 2-(4-methoxybenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5g)**

Yield 77%, white solid, m.p. 72.3–72.8 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (s, 1H, H-14), 7.33 (d, $J$ = 8.4 Hz, 2H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 1.8 Hz, 1H, H-12), 6.90 (d, $J$ = 8.4 Hz, 2H, H-Ph), 5.46 (d, $J$ = 28.9 Hz, 1H, NH), 4.43 (ddd, $J$ = 31.1, 13.8, 5.2 Hz, 2H, H-23), 3.82 (s, 3H, $CH_3O$-Ph), 3.72 (s, 1H, H-5), 3.64 (s, 3H, $COOCH_3$), 2.99–2.90 (m, 1H, H-15), 2.35–2.24 (m, 1H, $H_{1\text{-e}}$), 1.91–1.76 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.58 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 2.1 Hz, 6H, H-16, H-17), 1.15 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.52, 167.76, 159.16, 146.97, 146.32, 144.24, 130.08, 129.84, 129.20, 125.38, 122.12, 121.82, 119.95, 114.04, 55.30, 52.42, 49.47, 46.67, 46.57, 39.20, 37.40, 35.36, 33.83, 24.15, 23.90, 21.75, 18.29, 17.61. Mass spectrum (ESI), $m/z$: 505.2535 $[M+H]^+$ Caled $C_{30}H_{36}N_2O_3S$ 504.2247.

**(7bS,11R)-methyl 2-(2-fluorobenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5h)**

Yield 89%, white solid, m.p. 127.6–128.1 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (d, $J$ = 1.9 Hz, 1H, H-14), 7.50 (td, $J$ = 7.6, 1.6 Hz, 1H, H-Ph), 7.33–7.27 (m, 1H, H-Ph), 7.17 (d, $J$ = 8.0 Hz, 1H, H-11), 7.16–7.07 (m, 3H, H-12, H-Ph), 5.45 (s, 1H, NH), 4.59 (qd, $J$ = 14.8, 5.7 Hz, 2H, H-23), 3.71 (s, 1H, H-5), 3.64 (s, 3H, $COOCH_3$), 2.99–2.91 (m, 1H, H-15), 2.29 (dd, $J$ = 8.6, 5.9 Hz, 1H, $H_{1\text{-e}}$), 1.91–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.58 (s, 3H, H-19), 1.30 (dd, $J$ = 6.9, 2.6 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.50, 167.45, 162.04, 160.08, 146.96, 146.40, 144.20, 130.15, 129.44, 125.44, 125.08, 124.17, 122.11, 121.81, 120.21, 115.54, 52.42, 46.65, 46.58, 43.66, 39.19, 37.37, 35.34, 33.82, 24.14, 23.89, 21.75, 18.27, 17.59. Mass spectrum (ESI), $m/z$: 493.2198 $[M+H]^+$ Caled $C_{29}H_{33}ClN_2O_2S$ 492.2247.

**(7bS,11R)-methyl 2-(3-fluorobenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5i)**

Yield 93%, white solid, m.p. 132.8–133.6 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.67 (d, $J$ = 1.8 Hz, 1H, H-14), 7.33 (td, $J$ = 7.9, 5.9 Hz, 1H, H-Ph), 7.23–7.09 (m, 4H, H-Ph, H-12, H-11), 7.00 (td, $J$ = 8.3, 2.1 Hz, 1H, H-Ph), 5.56 (d, $J$ = 5.1 Hz, 1H, NH), 4.53 (ddd, $J$ = 34.3, 14.8, 5.5 Hz, 2H, H-23), 3.71 (s, 1H, H-5), 3.62 (s, 3H, $COOCH_3$), 2.94 (hept, $J$ = 6.9 Hz, 1H, H-15), 2.29 (dd, $J$ = 8.6, 5.9 Hz, 1H, $H_{1\text{-e}}$), 1.91–1.73 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.57 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 2.9 Hz, 6H, H-16, H-17), 1.15 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.49, 167.56, 163.98, 162.02, 147.00, 146.39, 144.22, 140.61, 130.20, 125.48, 123.27, 122.16, 121.81, 120.24, 114.73, 114.44, 52.41, 49.32, 46.65, 46.58, 39.19, 37.36, 35.34, 33.82, 24.13, 23.88, 21.75, 18.27, 17.59. Mass spectrum (ESI), $m/z$: 493.2198 $[M+H]^+$ Caled $C_{29}H_{33}ClN_2O_2S$ 492.2247.

**(7bS,11R)-methyl 2-(4-fluorobenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5j)**

Yield 90%, white solid, m.p. 75.1–76.3 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.67 (d, $J$ = 1.8 Hz, 1H, H-14), 7.41–7.35 (m, 2H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 7.08–7.01 (m, 2H, H-Ph), 5.57 (s, 1H, NH), 4.48 (qd, $J$ = 14.3, 5.2 Hz, 2H, H-23), 3.71 (s, 1H, H-5), 3.62 (s, 3H, $COOCH_3$), 2.94 (dq, $J$ = 13.8, 6.9 Hz, 1H, H-15), 2.34–2.25 (m, 1H, $H_{1\text{-e}}$), 1.90–1.76 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.57 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 2.7 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.49, 167.62, 163.26, 161.31, 146.99, 146.35, 144.23, 133.63, 130.00, 129.52, 125.47, 121.79, 120.12, 115.57, 52.41, 49.18, 46.65, 46.56, 39.19, 37.37, 35.34, 33.83, 24.14, 23.88, 21.75, 18.27, 17.59. Mass spectrum (ESI), $m/z$: 493.2303 $[M+H]^+$ Caled $C_{29}H_{33}ClN_2O_2S$ 492.2247.

**(7bS,11R)-methyl 2-(2-chlorobenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5k)**

Yield 87%, white solid, m.p. 166.8–167.4 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (d, $J$ = 1.8 Hz, 1H, H-14), 7.58–7.53 (m, 1H, H-Ph), 7.43–7.40 (m, 1H, H-Ph), 7.28–7.25 (m, 2H, H-Ph), 7.17 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 5.57 (d, $J$ = 5.7 Hz, 1H, NH), 4.63 (qd, $J$ = 15.1, 5.9 Hz, 2H, H-23), 3.70 (s, 1H, H-5), 3.63 (s, 3H, $COOCH_3$), 2.94 (hept, $J$ = 6.9 Hz, 1H, H-15), 2.29 (dd, $J$ = 8.6, 6.1 Hz, 1H, $H_{1\text{-e}}$), 1.90–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.57 (s, 3H, H-19), 1.30 (dd, $J$ = 6.9, 2.5 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.50, 167.45, 146.96, 146.41, 144.20, 135.42, 133.70, 130.05, 130.03, 129.63, 129.00, 126.90, 125.45, 122.12, 121.81, 120.16, 52.43, 47.58, 46.65, 46.59, 39.19, 37.37, 35.34, 33.82, 24.15, 23.89, 21.75, 18.27, 17.60. Mass spectrum (ESI), $m/z$: 509.2016 $[M+H]^+$ Caled $C_{29}H_{33}ClN_2O_2S$ 508.1951.

**(7bS,11R)-methyl 2-(3-chlorobenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5l)**

Yield 89%, white solid, m.p. 166.5–167.1 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.67 (d, $J$ = 1.9 Hz, 1H, H-14), 7.42 (s, 1H, H-Ph), 7.29 (d, $J$ = 6.2 Hz, 3H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 5.90–5.65 (m, 1H, NH), 4.50 (ddd, $J$ = 39.6, 14.9, 5.0 Hz, 2H, H-23), 3.72 (d, $J$ = 6.1 Hz, 1H, H-5), 3.62 (s, 3H, $COOCH_3$), 3.00–2.86 (m, 1H, H-15), 2.35–2.25 (m, 1H, $H_{1\text{-e}}$), 1.89–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.57 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 3.5 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.48, 167.56, 147.01, 146.36, 144.22, 140.08, 134.50, 129.97, 129.90, 127.87, 127.77, 125.85, 125.48, 122.17, 121.81, 120.22, 52.42, 49.23, 46.65, 46.58, 39.19, 37.36, 35.34, 33.83, 24.15, 23.88, 21.76, 18.27, 17.59. Mass spectrum (ESI), $m/z$: 509.2231 $[M+H]^+$ Caled $C_{29}H_{33}ClN_2O_2S$ 508.1951.

**(7bS,11R)-methyl 2-(4-chlorobenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5m)**

Yield 87%, white solid, m.p. 77.3–78.8 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.66 (d, $J$ = 1.9 Hz, 1H, H-14), 7.37–7.30 (m, 4H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 2.0 Hz, 1H, H-12), 5.60 (d, $J$ = 4.7 Hz, 1H, NH), 4.49 (qd, $J$ = 14.6, 5.0 Hz, 2H, H-23), 3.71 (d, $J$ = 6.5 Hz, 1H, H-5), 3.62 (s, 3H, $COOCH_3$), 2.93 (dq, $J$ = 13.8, 6.9 Hz, 1H, H-15), 2.29 (dd, $J$ = 8.7, 5.9 Hz, 1H, $H_{1\text{-e}}$), 1.91–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.57 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 2.8 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.48, 167.60, 146.99, 146.35, 144.22, 136.42, 133.41, 129.97, 129.14, 128.77, 125.49, 122.17, 121.78, 120.18, 52.42, 49.17, 46.64, 46.56, 39.19, 37.36, 35.33, 33.82, 24.14, 23.88, 21.75, 18.26, 17.59. Mass spectrum (ESI), $m/z$: 509.2021 $[M+H]^+$ Caled $C_{29}H_{33}ClN_2O_2S$ 508.1951.

**(7bS,11R)-methyl 2-(2-bromobenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5n)**

Yield 84%, white solid, m.p. 170.9–171.4 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (d, $J$ = 1.9 Hz, 1H, H-14), 7.58 (ddd, $J$ = 16.0, 7.8, 1.3 Hz, 2H, H-Ph), 7.31 (td, $J$ = 7.5, 1.2 Hz, 1H, H-Ph), 7.21–7.16 (m, 2H, H-11, H-Ph), 7.12 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 5.60 (t, $J$ = 5.8 Hz, 1H, NH), 4.62 (qd, $J$ = 15.1, 5.9 Hz, 2H, H-23), 3.70 (s, 1H, H-5), 3.63 (s, 3H, $COOCH_3$), 2.95 (hept, $J$ = 6.9 Hz, 1H, H-15), 2.33–2.27 (m, 1H, $H_{1\text{-e}}$), 1.90–1.73 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.57 (s, 3H, H-19), 1.30 (dd, $J$ = 6.9, 2.6 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.50, 167.35, 146.96, 146.42, 144.20, 137.04, 132.91, 130.24, 130.04, 129.26, 127.52, 125.45, 123.77, 122.12, 121.81, 120.17, 52.44, 49.91, 46.65, 46.59, 39.19, 37.38, 35.34, 33.82, 24.15, 23.89, 21.76, 18.27, 17.60. Mass spectrum (ESI), $m/z$: 553.1537; 555.1518 $[M+H]^+$ Caled $C_{29}H_{33}BrN_2O_2S$ 552.1446; 554.1426.

**(7bS,11R)-methyl 2-(3-bromobenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5o)**

Yield 88%, white solid, m.p. 157.7–158.5 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.67 (d, $J$ = 1.9 Hz, 1H, H-14), 7.57 (d, $J$ = 14.6 Hz, 1H, H-Ph), 7.44 (t, $J$ = 7.2 Hz, 1H, H-Ph), 7.32 (dd, $J$ = 19.4, 7.6 Hz, 1H, H-Ph), 7.26–7.21 (m, 1H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.14–7.10 (m, 1H, H-12), 5.59 (d, $J$ = 5.7 Hz, 1H, NH), 4.50 (ddd, $J$ = 41.0, 14.8, 5.1 Hz, 2H, H-23), 3.71 (s, 1H, H-5), 3.63 (s, 3H, $COOCH_3$), 2.94 (hept, $J$ = 6.9 Hz, 1H, H-15), 2.34–2.25 (m, 1H, $H_{1\text{-e}}$), 1.91–1.74 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.57 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 3.0 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.48, 167.49, 147.01, 146.37, 144.22, 140.32, 130.79, 130.73, 130.20, 129.96, 126.34, 125.50, 122.71, 122.16, 121.81, 120.26, 52.44, 49.18, 46.65, 46.58, 39.20, 37.36, 35.34, 33.83, 24.14, 23.90, 21.76, 18.27, 17.59. Mass spectrum (ESI), $m/z$: 553.1536; 555.1519 $[M+H]^+$ Caled $C_{29}H_{33}BrN_2O_2S$ 552.1446; 554.1426.

**(7bS,11R)-methyl 2-(4-bromobenzylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5p)**

Yield 83%, white solid, m.p. 78.4–78.6 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.65 (d, $J$ = 1.9 Hz, 1H, H-14), 7.48 (d, $J$ = 8.3 Hz, 2H, H-Ph), 7.29 (t, $J$ = 4.1 Hz, 2H, H-Ph), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 5.59 (d, $J$ = 5.4 Hz, 1H, NH), 4.47 (qd, $J$ = 14.7, 5.2 Hz, 2H, H-23), 3.71 (s, 1H, H-5), 3.62 (s, 3H, $COOCH_3$), 2.93 (hept, $J$ = 6.9 Hz, 1H, H-15), 2.29 (dd, $J$ = 8.7, 5.9 Hz, 1H, $H_{1\text{-e}}$), 1.90–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.57 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 2.7 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.48, 167.57, 146.99, 146.35, 144.22, 136.95, 131.73, 129.96, 129.47, 125.50, 122.17, 121.78, 121.49, 120.20, 52.42, 49.21, 46.64, 46.57, 39.19, 37.36, 35.33, 33.82, 24.14, 23.89, 21.75, 18.26, 17.59. Mass spectrum (ESI), $m/z$: 553.1539; 555.1528 $[M+H]^+$ Caled $C_{29}H_{33}BrN_2O_2S$ 552.1446; 554.1426.

**(7bS,11R)-methyl 2-((furan-2-yl)methylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5q)**

Yield 72%, yellow solid, m.p. 77.5–78.0 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (d, $J$ = 1.9 Hz, 1H, H-14), 7.42–7.38 (m, 1H, H-furan), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dd, $J$ = 8.0, 2.0 Hz, 1H, H-12), 6.37–6.32 (m, 2H, H-furan), 5.36 (s, 1H, NH), 4.53 (qd, $J$ = 15.2, 5.0 Hz, 2H, H-23), 3.72 (s, 1H, H-5), 3.66 (s, 3H, $COOCH_3$), 2.95 (hept, $J$ = 6.9 Hz, 1H, H-15), 2.34–2.26 (m, 1H, $H_{1\text{-e}}$), 1.90–1.74 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.58 (s, 3H, H-19), 1.29 (dd, $J$ = 6.9, 1.8 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.50, 167.11, 151.11, 146.98, 146.33, 144.19, 142.31, 130.02, 125.45, 122.12, 121.83, 120.40, 110.40, 107.87, 52.44, 46.65, 46.56, 42.68, 39.19, 37.37, 35.35, 33.83, 24.13, 23.89, 21.74, 18.27, 17.59. Mass spectrum (ESI), $m/z$: 465.2224 $[M+H]^+$ Caled $C_{27}H_{32}N_2O_3S$ 464.2134.

**(7bS,11R)-methyl 2-((1H-pyrrol-2-yl)methylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5r)**

Yield 74%, yellow solid, m.p. 87.4–89.5 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.61 (d, $J$ = 59.0 Hz, 1H, NH-pyrrole), 7.73 (s, 1H, H-14), 7.21 (d, $J$ = 8.0 Hz, 1H, H-11), 7.16 (d, $J$ = 8.1 Hz, 1H, H-12), 6.76 (td, $J$ = 2.6, 1.6 Hz, 1H, H-pyrrole), 6.14 (dt, $J$ = 6.9, 2.2 Hz, 2H, H-pyrrole), 5.32 (s, 1H, NH), 4.57 (d, $J$ = 4.3 Hz, 2H, H-23), 3.73 (s, 1H, H-5), 3.66 (s, 3H, $COOCH_3$), 3.02–2.93 (m, 1H, H-15), 2.37–2.29 (m, 1H, $H_{1\text{-e}}$), 1.92–1.77 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.57 (s, 3H, H-19), 1.34–1.31 (m, 6H, H-16, H-17), 1.17 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.47, 167.89, 147.05, 145.79, 144.25, 130.09, 129.94, 125.67, 122.34, 121.52, 120.65, 118.01, 107.73, 106.80, 52.51, 46.65, 46.61, 41.77, 39.24, 37.33, 35.33, 33.78, 24.14, 23.89, 21.80, 18.26, 17.59. Mass spectrum (ESI), $m/z$: 464.2389 $[M+H]^+$ Caled $C_{27}H_{33}N_3O_2S$ 463.2293.

**(7bS,11R)-methyl 2-((pyridin-3-yl)methylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5s)**

Yield 52%, yellow solid, m.p. 83.7–84.3 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.69 (d, $J$ = 25.2 Hz, 1H, H-Py), 8.55 (dd, $J$ = 4.7, 1.4 Hz, 1H, H-Py), 7.76 (t, $J$ = 7.6 Hz, 1H, H-Py), 7.66 (d, $J$ = 1.9 Hz, 1H, H-14), 7.31–7.27 (m, 1H, H-Py), 7.17 (d, $J$ = 8.0 Hz, 1H, H-11), 7.12 (dt, $J$ = 8.0, 3.9 Hz, 1H, H-12), 5.76 (s, 1H, NH), 4.66–4.48 (m, 2H, H-23), 3.73–3.68 (m, 1H, H-5), 3.63 (s, 3H, $COOCH_3$), 2.92 (dq, $J$ = 13.7, 6.9 Hz, 1H, H-15), 2.35–2.25 (m, 1H, $H_{1\text{-e}}$), 1.92–1.74 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.56 (s, 3H, H-19), 1.28 (dd, $J$ = 6.9, 3.3 Hz, 6H, H-16, H-17), 1.14 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.47, 167.33, 149.36, 149.07, 147.00, 146.29, 144.21, 135.56, 133.64, 129.88, 125.57, 123.53, 122.18, 121.79, 120.28, 52.45, 47.17, 46.63, 46.57, 39.20, 37.35, 35.32, 33.82, 24.13, 23.89, 21.75, 18.25, 17.59. Mass spectrum (ESI), $m/z$: 476.2391 $[M+H]^+$ Caled $C_{28}H_{33}N_3O_2S$ 475.2293.

**(7bS,11R)-methyl 2-((6-chloropyridin-3-yl)methylamino)-7b,8,9,10,11,11a-hexahydro-5-isopropyl-7b,11-dimethylphenanthro[9,10-d]thiazole-11-carboxylate (5t)**

Yield 64%, yellow solid, m.p. 84.5–85.7 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.44 (s, 1H, H-Py), 7.78–7.72 (m, 1H, H-Py), 7.63 (t, $J$ = 2.7 Hz, 1H, H-14), 7.31 (d, $J$ = 8.2 Hz, 1H, H-Py), 7.18 (d, $J$ = 8.0 Hz, 1H, H-11), 7.13 (dd, $J$ = 8.0, 1.9 Hz, 1H, H-12), 5.80 (s, 1H, NH), 4.56 (q, $J$ = 15.0 Hz, 2H, H-23), 3.70 (s, 1H, H-5), 3.64 (s, 3H, $COOCH_3$), 2.99–2.88 (m, 1H, H-15), 2.29 (t, $J$ = 7.7 Hz, 1H, $H_{1\text{-e}}$), 1.89–1.75 (m, 5H, H-3, H-2, $H_{1\text{-a}}$), 1.56 (s, 3H, H-19), 1.29–1.28 (m, 6H, H-16, H-17), 1.13 (s, 3H, H-20).$^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 178.46, 167.02, 150.72, 149.14, 147.02, 146.25, 144.19, 138.47, 132.80, 129.77, 125.68, 124.22, 122.22, 121.75, 120.43, 52.48, 46.61, 46.57, 46.28, 39.20, 37.34, 35.30, 33.81, 24.12, 23.89, 21.75, 18.23, 17.59. Mass spectrum (ESI), $m/z$: 508.1620 $[M-H]^-$ Caled $C_{28}H_{32}ClN_3O_2S$ 509.1904.

## Antitumor activity

HepG2, SCC9, and 293T cells (Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China) were cultured in Dulbecco's modification Eagle's medium (Invitrogen, Carlsbad, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, USA), 100 U/mL penicillin, and 0.1 mg/mL streptomycin (Invitrogen). The cells were incubated at 37 °C in a humidified atmosphere with 5% $CO_2$.

Cell proliferation was measured with the Cell Counting Kit-8 (CCK-8) assay kit (Dojindo Corp, Kumamoto, Japan) [17]. Cells were harvested during logarithmic growth phase, seeded in 96-well plates at a density of $5 \times 10^3$ cells/well, and cultured at 37 °C in a humidified incubator (5% $CO_2$) for 24 h, followed by exposure to various concentrations of compounds for 24 h. Subsequently, 10 μL of CCK-8 (Dojindo) was added to each well; the cells were then incubated for an additional 1 h at 37 °C. Cell growth inhibition was determined by measuring the optical density value (OD) at λ = 450 nm using a microplate reader. Three independent experiments were performed. The cell growth inhibition was calculated according to the following equation:

$$\text{Growth inhibition} = \left(\frac{\text{OD of control} - \text{OD of treatment}}{\text{OD of control} - \text{OD of blank}}\right) \times 100\%$$

The $IC_{50}$ values were obtained from linear regression analysis of the concentration–response curves plotted for each tested compound.

## Western blot analysis of PI3K/AKT/mTOR signaling pathway inhibitory activity of 5r and 5s

HepG2 cells were seeded at a density of $3 \times 10^6$ cells per dish and attached for 8 h and then treated with compounds in concentrations of 0, 20, and 40 μM for 24 h. After that, cells were collected, washed with cold PBS, and lysed with lysis buffer (100 mM Tris–HCl, pH 6.8, 4% SDS, 20% glycerol) on ice for 30 min. Protein concentrations were detected using the BCA method (Beyotime Institute of Biotechnology, Haimen, China). Proteins were electrophoresed using sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE Bio-Rad, CA, USA) and transferred electrophoretically to membranes. The membranes were blocked with 5% nonfat milk at room temperature for 1 h and were incubated with primary antibodies overnight at 4 °C. The next day, membranes were washed and incubated with the appropriate fluorescence-conjugated secondary antibodies at room temperature for 1 h. Finally, membranes were washed and developed by the addition of ECL substrate (Thermo Fisher Scientific, Rockford, USA). Proteins were visualized using the intelligent gel imaging system iBright FL1000 (Thermo Fisher, Rockford, USA). Primary antibodies were as follows: anti-EBP1, anti-phospho-EBP1, anti-S6, anti-phospho-S6(S235/236), anti-phospho-S6K(t389), anti-mTOR, anti-phospho-mTOR, anti-Akt(S473), anti-phospho-PI3K, and anti-α-tubulin, which were purchased from Cell Signaling Technology, Boston, USA.

## Molecular docking studies

All docking procedures were performed using AutoDock 4.2.6 software (ADT) according to the reported paper [19]. The structures of PI3K, AKT, and mTOR were obtained from the Protein Data Bank (PDB) with PDB IDs 3L54, 3MVH, and 4JT6, respectively [18]. In the PDB file of mTOR, there are 4 chains, and two of them marked as A and B both containing an original ligand whose binding modes to those two chains show distinctive similarity. Thus, only the chain A was maintained to carry out molecular docking. All the proteins in the PDB files were cleaned by removing small molecules, ions, and original ligand in the crystal of the proteins. Then, polar hydrogen atoms were added, Gasteiger charges were computed, and the atoms were set as "Assign AD4 type" in ADT. The compounds for docking program were drawn using ChemDraw 8.0 and automatically set the torsional bonds by the AUTOTORS module in ADT. The docked sites of the protein were set in the place of the original ligand. The grid map was 40 × 40 × 40 (center of the grid box, PI3K: $x = 22.0$, $y = 15.0$, $z = 22.0$; AKT: $x = 19.0$, $y = -3.0$, $z = 28.0$; mTOR: $x = 52.0$, $y = 1.0$, $z = -47.0$). The binding energy between the docked compound and the protein was calculated using the AutoGrid program with a grid spacing of 0.375 Å by the Lamarckian genetic algorithm as a searching method. The interaction between compounds and the amino acid residues of the proteins was found by LigPlot+ 2.1 and visualized by PyMOL 2.4.0a0 software.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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