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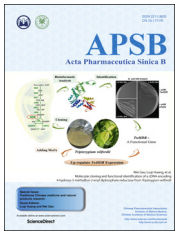

ORIGINAL ARTICLE

A novel class of apical sodium–dependent bile salt transporter inhibitors: 1-(2,4-bifluorophenyl)-7-dialkylamino-1,8-naphthyridine-3-carboxamides

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KEY WORDS

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1-(2,4-Bifluorophenyl)-7-dialkylamino-1,8-naphthyridine-3-carboxamides;
Cholesterol-lowering drug;
NC-1

Abstract The apical sodium–dependent bile acid transporter (ASBT) is the main transporter to promote re-absorption of bile acids from the intestinal tract into the enterohepatic circulation. Inhibition of ASBT could increase the excretion of bile acids, thus increasing bile acid synthesis and consequently cholesterol consumption. Therefore, ASBT is an attractive target for developing new cholesterol-lowering drugs. In this report, a series of 1-(2,4-bifluorophenyl)-7-dialkylamino-1,8-naphthyridine-3-carboxamides were designed as inhibitors of ASBT. Most of them demonstrated potency against ASBT transport of bile acids. In particular, compound **4a$_1$** was found to have the best activity, resulting in 80.1% inhibition of ASBT at 10 μmol/L.

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1. Introduction

High serum cholesterol is a main cause of coronary artery disease (CAD), which greatly increases the risk of atherosclerosis[1]. About 50% of cholesterol is eliminated from the body by conversion to bile acids. Bile acids are synthesized from cholesterol in hepatocytes and secreted through the biliary tract into the intestine[2,3]. More than 90% of the secreted bile acid is re-absorbed from the intestine and transported back to the liver *via* the enterohepatic circulation system, and re-secreted into the bile. The major mechanism for absorption of intestinal bile acids is active uptake by the apical sodium–dependent bile acid transporter (ASBT) located in the ileal brush-border membrane[3–5]. Inhibition of ASBT reduces re-absorption of bile acids, thus increasing bile acid synthesis and consequently cholesterol consumption[6,7]. Because ASBT is localized on the apical membrane of the lumen in the ileum, inhibitors can block ASBT activity without entering the circulation system. Therefore, ASBT is an attractive target for developing new cholesterol-lowering drugs[8–11]. At present, several ASBT inhibitors have been developed that are effective in animal models, which include S-1647, R-146224, 264W94 and SC-435 (Fig. 1)[12–15].

Our laboratory has been dedicated to the investigation and development of ASBT inhibitors and has obtained a series of compounds with good activity[16]. NC-1, a compound obtained by extensive screening in our lab, was found to have potent ASBT inhibitory activity. It showed 30.5% inhibition of ASBT at 10 μmol/L in an *in vitro* assay. NC-1 has a scaffold of 1-aryl-1,8-naphthyridine which is very similar to R-146224. To develop more potent ASBT inhibitors, we optimized the structure of NC-1 in this study. Using combinatorial principles, a 3-carboxamide was introduced to NC-1. At the same time, we observed that the 7 position of R-146224 was a quaternary ammonium salt with a long linker, while NC-1 held a chlorine in the corresponding position. It has been reported that a tertiary amine or a quaternary ammonium salt substituted in this position could exhibit the same potency as seen in the most commonly reported ASBT inhibitors[17–20]. Thus, in order to simplify the structure, we replaced the chlorine atom with dimethylamine or diethylamine with reference to SC-435. Finally, twenty-three 1-(2,4-bifluorophenyl)-7-dialkylamino-1,8-naphthyridine-3-carboxamides were designed, synthesized through a three-step process and evaluated for their ASBT inhibitory activity by a radioactive binding assay (Fig. 2).

2. Results and discussion

2.1. Chemistry

The synthetic pathways to this series of target compounds are shown in Scheme 1. Nucleophilic substitution of 1-(2,4-bifluorophenyl)-6-fluoro-7-chloro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-ethyl formate **1** with dimethylamine or diethylamine in the presence of triethylamine in tetrahydrofuran (THF) gave 7-aminonaphthyridine-3-ethyl formate **2a** and **2b**. Hydrolysis of the esters **2a** and **2b** in a NaOH-H_2O-EtOH system yielded the corresponding naphthyridine-3-carboxylic acid **3a** and **3b**. Coupling of the naphthyridine-3-carboxylic acid **3a** and **3b** with commercially available substituted anilines in the presence of isobutyl chloroformate and triethylamine in dry dichloromethane afforded the target compounds $\mathbf{4a_1}$–$\mathbf{4a_{13}}$ and $\mathbf{4b_1}$–$\mathbf{4b_{10}}$. All the target structures were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectrometry (MS).

2.2. ASBT inhibition assay

The inhibitory activity of the compounds was tested *in vitro* against ASBT by a radioisotope-based assay[21]. A human ASBT expression construct was prepared as previously described[22]. The inhibitory activity was expressed as inhibition (%) in 10 μmol/L (Table 1). The values are the average of three independent experiments with S-1647 as a positive control in each experiment.

As shown in Table 1, most of the newly synthesized derivatives exhibited ASBT inhibitory activity and most of them showed better activity than the lead compound NC-1. Particularly, $\mathbf{4a_1}$ exhibited an inhibition of 80.1% towards ASBT which was more potent than S-1647. Substitution at 7 positions of the 1,8-naphthyridine scaffold was considered an important factor in the activity. Indeed, compounds $\mathbf{4a_1}$, $\mathbf{4a_2}$ and $\mathbf{4a_3}$ with dimethylamino groups (R^1) showed better activity than the corresponding substituted diethylamino analogues $\mathbf{4b_1}$, $\mathbf{4b_2}$ and $\mathbf{4b_3}$, respectively. In addition, compounds $\mathbf{4a_{12}}$, $\mathbf{4a_{13}}$ and $\mathbf{4b_{10}}$ with an electron-donating group (R^2) substituted on the 3-carboxamide phenyl ring showed observably lower potency than other corresponding electron-withdrawing group analogues. So R^2 with an electron-withdrawing group was of greatest benefit for ASBT inhibitory activity.

3. Conclusions

In conclusion, a series of novel 1-(2,4-bifluorophenyl)-7-dialkylamino-1,8-naphthyridine-3-carboxamides was designed, synthesized and the inhibitory activities for ASBT were assessed. Although the ASBT inhibitory activity for this group of compounds was not as high as expected, it is certain that 1-(2,4-bifluorophenyl)-7-dialkylamino-1,8-naphthyridine-3-carboxamides indeed possess ASBT inhibitory activity. Further structural modifications are necessary and in progress in our laboratory.

4. Experimental

4.1. General experimental procedures

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were

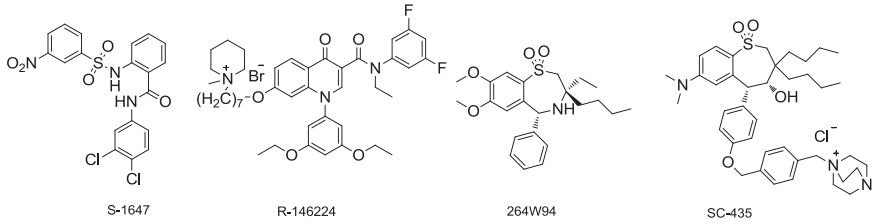


Figure 1 Structures of apical sodium–dependent bile acid transporter (ASBT) inhibitors.

uncorrected. MS were taken in ESI mode or APCI mode on an Agilent 1100 LC–MS system (Agilent, Palo Alto, CA, USA). Nuclear magnetic resonance spectroscopy (^{1}H NMR and ^{13}C NMR) were performed using a 400 MHz Bruker ARX-400 spectrometers (Bruker Bioscience, Billerica, MA, USA) with $CDCl_3$ as solvent and TMS as an internal standard. (*J* values are in Hz. Chemical shifts are expressed in ppm downfield from internal standard TMS.) All the starting materials were obtained from commercially available sources and used without further purification, unless otherwise specified. Yields were not optimized.

4.2. *The synthesis of details of target compounds*

4.2.1. *1-(2,4-Bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-ethyl formate (**2a**)*

To a solution of 1-(2,4-bifluorophenyl)-6-fluoro-7-chloro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-ethyl formate (**1**, 10.0 g, 26.13 mmol) in THF (50 mL) was added dimethylamine hydrochloride (3.2 g, 39.20 mmol) and then triethylamine (7.92 g, 78.39 mmol). The reaction mixture was stirred for 10 h at room temperature and then filtered. The filtrate was concentrated and diethyl ether (20 mL) was added to the mixture. After stirred for 0.5 h, the resulting solid was filtered. The filter cake was dried to give **2a** (81.6% yield) as a white solid; mp: 190.0–191.3 °C. ^{1}H NMR ($CDCl_3$) δ: 1.39–1.42 (3 H, m), 3.03 (6 H, s), 3.04 (3 H, s), 4.36–4.41 (2 H, m), 7.02–7.07 (2 H, m), 7.37–7.43 (1 H, m), 8.08 (1 H, d, J = 13.48 Hz), 8.38 (1 H, s). HR-MS *m/z* Calcd. $C_{19}H_{16}O_3N_3F_3$ $[M+H]^+$ 392.1216, Found 392.1216.

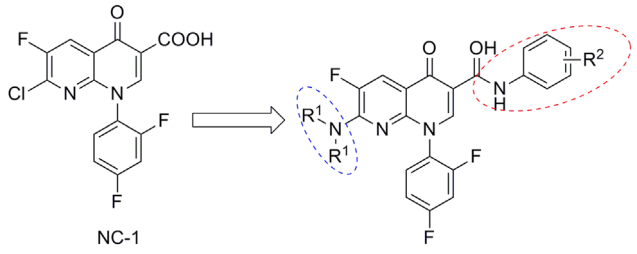
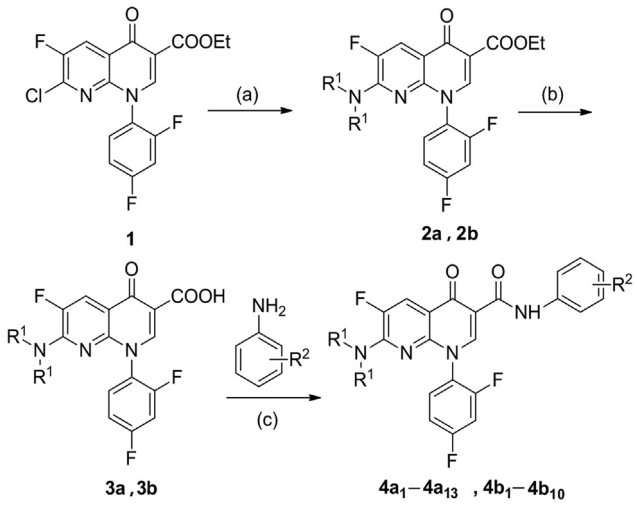


Figure 2 Design of 1-(2,4-bifluorophenyl)-7-dialkylamino-1,8-naphthyridine-3-carboxamides.



Scheme 1 The synthesis of 1-(2,4-bifluorophenyl)-7-dialkylamino-1,8-naphthyridine-3-carboxamides **4a$_1$**–**4a$_{13}$**, **4b$_1$**–**4b$_{10}$**. Reagents and conditions: (a) $HN(R^1)_2$, Et_3N, THF, r.t.; (b) NaOH, EtOH, reflux; (c) Et_3N, isobutylchloroformate, CH_2Cl_2, r.t.

4.2.2. *1-(2,4-Bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-ethyl formate (**2b**)*

Compound **2b** was obtained as a white solid (83.2% yield) from compound **1** as described for **2a**; mp: 146.8–147.9 °C. ^{1}H NMR ($CDCl_3$) δ: 1.05 (6 H, t, J=6.8 Hz), 1.40 (3 H, t, J=7.2 Hz), 3.35–3.41 (4 H, m), 4.40 (2 H, dd, J_1=14.4 Hz, J_2=4.8 Hz), 7.01–7.08 (2 H, m), 7.45 (1 H, dd, J_1=13.6 Hz, J_2=8 Hz), 8.08 (1 H, d, J=13.6 Hz), 8.42 (1 H, s). HR-MS *m/z* Calcd. $C_{21}H_{20}O_3N_3F_3$ $[M+H]^+$ 420.1529, Found 420.1530.

4.2.3. *1-(2,4-Bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**3a**)*

To a solution of **2a** (5.0 g, 12.78 mmol) in alcohol (30 mL) was added 10% NaOH (10 mL). The mixture was heated to 80 °C for 10 h and concentrated. The reaction mixture was diluted with water (12 mL), adjusted to pH 6 with 6 mol/L hydrochloric acid. After stirred for 0.5 h the precipitate was filtered and washed with water to pH 7. The filter cake was dried to give **3a** (89.3% yield) as a greyish white solid; mp: 225.4–226.6 °C. ^{1}H NMR ($CDCl_3$) δ: 3.10 (6 H, s), 7.04–7.10 (2 H, m), 7.35–7.39 (1 H, m), 8.06 (1 H, d, J=12.8 Hz), 8.65 (1 H, s), 14.90 (1 H, s). HR-MS *m/z* Calcd. $C_{17}H_{12}O_3N_3F_3$ $[M+H]^+$ 364.0903, Found 364.0901.

4.2.4. *1-(2,4-Bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**3b**)*

Compound **3b** was obtained as a greyish white solid (87.6% yield) from compound **2b** as described for **3a**; mp: 171.3–172.5 °C. ^{1}H NMR ($CDCl_3$) δ: 1.07 (6 H, t, J=6.8 Hz), 3.42 (4 H, t, J=6.8 Hz), 7.04–7.10 (2 H, m), 7.35–7.39 (1 H, m), 8.05 (1 H, d, J=13.6 Hz), 8.64 (1 H, s). HR-MS *m/z* Calcd. $C_{19}H_{16}O_3N_3F_3$ $[M+H]^+$ 392.1216, Found 392.1218.

4.2.5. *N-(3,5-bifluorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_1$**)*

To a solution of **3a** (0.5 g, 1.38 mmol) in dry dichloromethane (5 mL) was added triethylamine (0.42 g, 4.14 mmol) and then stirred for 0.5 h at room temperature. Isobutyl chloroformate (0.38 g, 2.76 mmol) was added at 0 °C and then stirred for 1 h. The mixture was upswing to room temperature and 3,5-difluorophenylamine (0.21 g, 1.66 mmol) was added. After stirred for 10 h the precipitate was filtered and washed with little diethyl ether. The filter cake was dried to give **4a$_1$** (71.5% yield) as a white solid; mp: 278.2–279.5 °C. ^{1}H NMR ($CDCl_3$) δ: 3.07 (6 H, s), 6.51–6.57 (1 H, m), 7.03–7.11 (2 H, m), 7.34–7.39 (2 H, m), 7.41–7.45 (1 H, m), 8.06 (1 H, d, J=13.2 Hz), 8.71 (1 H, s), 12.43 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.10, 164.44, 162.78, 162.01, 161.85, 159.19, 156.65, 150.63, 148.27, 146.39, 145.69, 145.53, 140.77, 129.99, 119.68, 113.01, 112.62, 112.22, 112.00, 105.04, 103.45, 103.16, 98.95, 39.72, 39.64. HR-MS *m/z* Calcd. $C_{23}H_{15}O_2N_4F_5$ $[M+H]^+$ 475.1188, Found 475.1188.

4.2.6. *N-(3,5-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_2$**)*

Obtained as a white solid (68.7% yield) from compound **3a** as described for **4a$_1$**; mp: 293.3–294.2 °C. ^{1}H NMR ($CDCl_3$) δ: 3.08

Table 1 The structures and ASBT inhibition of compounds **4a$_1$**–**4a$_{13}$** and **4b$_1$**–**4b$_{10}$**.

Compd.	R^1	R^2	Inhibition (%)[a]
4a$_1$	Methyl	3,5-Difluoro	80.1±1.5
4a$_2$	Methyl	3,5-Dichloro	62.5±2.1
4a$_3$	Methyl	2,4-Dichloro	51.7±2.6
4a$_4$	Methyl	2,3-Dichloro	49.8±3.3
4a$_5$	Methyl	2,5-Dichloro	47.6±1.9
4a$_6$	Methyl	3-Chloro	45.4±4.1
4a$_7$	Methyl	3-Chloro-4-fluoro	53.2±2.8
4a$_8$	Methyl	3-Trifluoromethyl-4-methyl	52.6±1.2
4a$_9$	Methyl	3-Difluoromethoxy	50.5±3.4
4a$_{10}$	Methyl	2-Methyl-3-trifluoromethyl	48.3±4.2
4a$_{11}$	Methyl	3,4-Dichloro	50.2±1.1
4a$_{12}$	Methyl	3,5-Dimethoxy	19.6±3.1
4a$_{13}$	Methyl	4-Methoxy	18.5±3.7
4b$_1$	Ethyl	3,5-Difluoro	58.1±4.6
4b$_2$	Ethyl	3,5-Dichloro	44.3±2.9
4b$_3$	Ethyl	2,4-Dichloro	46.5±5.1
4b$_4$	Ethyl	2,3-Dichloro	47.2±2.5
4b$_5$	Ethyl	2,5-Dichloro	45.7±3.9
4b$_6$	Ethyl	3-Chloro	42.9±2.8
4b$_7$	Ethyl	3-Chloro-4-fluoro	44.3±4.1
4b$_8$	Ethyl	2-Methyl-3-trifluoromethyl	27.1±2.3
4b$_9$	Ethyl	3,4-Dichloro	35.6±3.4
4b$_{10}$	Ethyl	4-Methoxy	13.7±2.6
NC-1			30.5±2.8
S-1647			77.9±3.3

[a]Values represent the percent inhibition of ASBT at 10 μmol/L of the test compounds and are the average of three independent experiments. Each value represents the mean±SD.

(6 H, s), 7.03–7.10 (3 H, m), 7.40–7.46 (1 H, m), 7.73 (2 H, s), 8.06 (1 H, d, J=13.6 Hz), 8.72 (1 H, s), 12.43 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.04, 162.78, 150.63, 150.53, 148.27, 146.38, 145.70, 145.52, 140.46, 135.03, 129.98, 129.88, 123.64, 119.66, 119.44, 118.56, 112.98, 112.57, 112.21, 112.02, 105.28, 105.02, 104.79, 39.74, 39.66. HR-MS *m/z* Calcd. $C_{23}H_{15}O_2N_4F_3Cl_2$ $[M+H]^+$ 507.0597, Found 507.0596.

*4.2.7. N-(2,4-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_3$**)*

Obtained as a white solid (65.3% yield) from compound **3a** as described for **4a$_1$**; mp: 281.3–282.6 °C. ^{1}H NMR ($CDCl_3$) δ: 3.08 (6 H, s), 7.03–7.10 (2 H, m), 7.23–7.24 (1 H, m), 7.39–7.44 (2 H, m), 8.16 (1 H, d, J=13.6 Hz), 8.58 (1 H, d, J=8.8 Hz), 8.74 (1 H, s), 12.61 (1 H, s). ^{13}C NMR ($CDCl_3$) δ:176.13, 162.88, 150.62, 148.24, 146.48, 145.67, 145.53, 134.89, 130.00, 128.97, 128.58, 127.47, 124.40, 122.99, 119.99, 119.77, 113.11, 113.02, 112.19, 111.96, 105.28, 105.05, 104.78, 39.73, 39.65. HR-MS *m/z* Calcd. $C_{23}H_{15}O_2N_4F_3Cl_2$ $[M+H]^+$ 507.0597, Found 507.0597.

*4.2.8. N-(2,3-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_4$**)*

Obtained as a white solid (67.2% yield) from compound **3a** as described for **4a$_1$**; mp: 283.6–284 °C. ^{1}H NMR ($CDCl_3$) δ: 3.08 (6 H, s), 7.03–7.10 (2 H, m), 7.21 (2 H, d, J=4.8 Hz), 7.39–7.45 (1 H, m), 8.18 (1 H, d, J=13.6 Hz), 8.53–8.56 (1 H,m), 8.74 (1 H, s), 12.67 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.08, 163.00, 150.59, 148.23, 146.56, 145.66, 145.52, 137.80, 132.89, 129.95, 124.90, 122.50, 120.39, 120.00, 119.78, 113.11, 113.04, 112.14, 111.95, 105.26, 105.02, 104.76, 39.71, 39.64. HR-MS *m/z* Calcd. $C_{23}H_{15}O_2N_4F_3Cl_2$ $[M+H]^+$ 507.0597, Found 507.0597.

*4.2.9. N-(2,5-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_5$**)*

Obtained as a white solid (72.6% yield) from compound **3a** as described for **4a$_1$**; mp: 260.0–261.3 °C. ^{1}H NMR ($CDCl_3$) δ: 3.08 (6 H, s), 7.01–7.10 (3 H, m), 7.34 (1 H, d, J=8.4 Hz), 7.39–7.45 (1 H, m), 8.16 (1 H, d, J=13.2 Hz), 8.71 (1 H, d, J=2.4 Hz), 8.74 (1 H, s), 12.65 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.08, 162, 96, 150.61, 148.24, 146.59, 145.67, 145.52, 136.95, 132.99, 130.01, 129.88, 124.08, 122.11, 121.88, 119.98, 119.76, 113.11, 112.93, 112.21, 119.98, 105.28, 105.02, 104.79, 39.73, 39.65. HR-MS *m/z* Calcd. $C_{23}H_{15}O_2N_4F_3Cl_2$ $[M+H]^+$ 507.0597, Found 507.0598.

*4.2.10. N-(3-chlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_6$**)*

Obtained as a white solid (69.3% yield) from compound **3a** as described for **4a$_1$**; mp: 265.1–266.5 °C. ^{1}H NMR ($CDCl_3$) δ: 3.09 (6 H, s), 7.05–7.12 (2 H, m), 7.27–7.31 (2 H, m), 7.45 (1 H, d, J=6.4 Hz), 7.60 (1 H, d, J=8.4 Hz), 7.95 (1 H, s), 8.10 (1 H, d, J=13.6 Hz), 8.78 (1 H, s), 12.34 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.16, 162.59, 148.26, 147.10, 146.29, 145.69, 145.52, 139.82, 134.52, 129.98, 129.86, 123.84, 120.49, 119.73, 119.51, 118.38, 113.03, 112.96, 112.19, 111.97, 105.26, 105.00, 104.76, 39.73, 39.65. HR-MS *m/z* Calcd. $C_{23}H_{16}O_2N_4F_3Cl$ $[M+H]^+$ 473.0986, Found 473.0988.

*4.2.11. N-(3-chloro-4-fluorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_7$**)*

Obtained as a white solid (62.1% yield) from compound **3a** as described for **4a$_1$**; mp: 270.0–271.2 °C. ^{1}H NMR (CDCl3) δ: 3.08 (6 H, s), 7.04–7.13 (3 H, m), 7.41 (1 H, s), 7.53 (1 H, d, J=7.2 Hz), 7.98 (1 H, d, J=6 Hz), 8.08 (1 H, d, J=13.2 Hz), 8.74 (1 H, s), 12.30 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.14, 162.54, 155.69, 153.25, 150.62, 148.26, 146.24, 145.54, 135.37, 129.87, 122.37, 120.98, 119.90, 119.69, 119.47, 116.58, 116.36, 112.99, 112.80, 112.21, 111.95, 105.27, 105.04, 104.77, 39.73, 39.66. HR-MS *m/z* Calcd. $C_{23}H_{15}O_2N_4F_4Cl$ $[M+H]^+$ 491.0892, Found 491.0892.

*4.2.12. N-(3-trifluoro-4-methylphenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_8$**)*

Obtained as a white solid (68.7% yield) from compound **3a** as described for **4a$_1$**; mp: 227.4–228.9 °C. ^{1}H NMR ($CDCl_3$) δ: 2.45 (3 H, s), 3.08 (6 H, s), 7.04–7.09 (2 H, m), 7.24–7.26 (1 H, m), 7.39–7.44 (1 H, m), 7.85 (1 H, d, J=8.4 Hz), 8.02 (1 H, s), 8.08 (1 H, d, J=13.6 Hz), 8.76 (1 H, s), 12.32 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.18, 162.62, 150.53, 148.27, 146.26, 145.70, 145.53, 136.56, 132.41, 131.65, 129.96, 129.35, 129.05, 125.74, 123.23, 119.72, 119.50, 117.98, 113.04, 112.98, 112.18, 111.96, 105.26, 105.03, 104.77, 39.73, 39.65. HR-MS *m/z* Calcd. $C_{25}H_{18}O_2N_4F_6$ $[M+H]^+$ 521.1406, Found 521.1407.

*4.2.13. N-(3-difluoromethoxyphenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_9$**)*

Obtained as a white solid (73.6% yield) from compound **3a** as described for **4a$_1$**; mp: 204.7–205.5 °C. ^{1}H NMR ($CDCl_3$) δ: 3.08 (6 H, s), 6.55 (1 H, t, J=7 4 Hz), 6.87 (1 H, d, J=8.0 Hz), 7.04–7.10 (2 H, m), 7.32 (1 H, t, J=8.0 Hz), 7.42 (1 H, d, J=5.2 Hz), 7.49–7.52 (1 H, m), 7.77 (1 H, s), 8.08 (1 H, d, J=13.6 Hz), 8.77 (1 H, s), 12.35 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.17, 162.67, 151.80, 146.32, 145.53, 140.11, 129.97, 129.88, 119.73, 119.50, 118.67, 117.11, 116.10, 114.52, 113.53, 113.07, 112.95, 112.15, 111.93, 111.36, 105.26, 105.03, 104.77, 39.73, 39.65. HR-MS *m/z* Calcd. $C_{24}H_{17}O_3N_4F_5$ $[M+H]^+$ 505.1293, Found 505.1290.

*4.2.14. N-(3-trifluoromethyl-2-methylphenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_{10}$**)*

Obtained as a white solid (52.5% yield) from compound **3a** as described for **4a$_1$**; mp: 208.7–209.5 °C. ^{1}H NMR ($CDCl_3$) δ: 2.57 (3 H, s), 3.08 (6 H, s), 7.03–7.10 (2 H, m), 7.31 (1 H, t, J=8.0 Hz), 7.43(2 H, d, J=8.0 Hz), 8.13 (1 H, d, J=13.6 Hz), 8.42 (1 H, d, J=6.8 Hz), 8.77 (1 H, s), 12.22 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.34, 164.40, 162.81, 161.77, 159.10, 156.69, 150.62, 148.25, 146.40, 145.54, 138.49, 130.00, 127.57, 126.03, 125.92, 121.50, 119.85, 119.63, 113.20, 113.03, 112.19, 111.96, 105.26, 105.03, 104.77, 39.73, 39.65. HR-MS *m/z* Calcd. $C_{25}H_{18}O_2N_4F_6$ $[M+H]^+$ 521.1407, Found 521.1406.

*4.2.15. N-(3,4-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_{11}$**)*

Obtained as a white solid (63.7% yield) from compound **3a** as described for **4a$_1$**; mp: 285.0–286.2 °C. ^{1}H NMR ($CDCl_3$) δ: 3.08 (6 H, s), 7.03–7.10 (2 H, m), 7.38 (1 H, d, J=8.8 Hz), 7.42–7.45 (1 H, m), 7.52–7.56 (1 H, m), 8.03–8.07 (2 H, m), 8.74 (1 H, s), 12.38 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.06, 162.65, 161.79, 159.06, 156.51, 150.45, 148.21, 146.30, 145.49, 138.18, 132.54, 130.32, 130.02, 126.65, 121.86, 119.51, 112.92, 112.63, 112.22, 112.00, 105.24, 105.01, 104.74, 39.73, 39.65. HR-MS *m/z* Calcd. $C_{23}H_{15}O_2N_4F_3Cl_2$ $[M+H]^+$ 507.0597, Found 507.0599.

*4.2.16. N-(3,5-bimethoxyphenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_{12}$**)*

Obtained as a white solid (62.6% yield) from compound **3a** as described for **4a$_1$**; mp: 237.2–238.2 °C. ^{1}H NMR ($CDCl_3$) δ: 3.08 (6 H, s), 3.81 (6 H, s), 6.26 (1 H, d, J=2.0 Hz), 7.03–7.09 (4 H, m), 7.41–7.44 (1 H, m), 8.08 (1 H, d, J=13.2 Hz), 8.77 (1 H, s), 12.19 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.17, 162.48, 160.39, 150.56, 150.47, 148.23, 146.22, 145.66, 145.49, 140.33, 130.01, 129.92, 119.70, 119.48, 113.17, 113.04, 112.13, 111.91, 105.20, 104.94, 104.71, 98.39, 97.13, 55.40, 39.70, 39.62. HR-MS *m/z* Calcd. $C_{25}H_{21}O_4N_4F_3$ $[M+H]^+$ 499.1587, Found 499.1588.

*4.2.17. N-(4-methoxyphenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(dimethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4a$_{13}$**)*

Obtained as a white solid (71.7% yield) from compound **3a** as described for **4a$_1$**; mp: 261.5–262.7 °C. ^{1}H NMR ($CDCl_3$) δ: 3.08 (6 H, s), 3.81 (3 H, s), 6.90 (2 H, d, J=8.8 Hz), 7.02–7.09 (2 H, m), 7.42 (1 H, s), 7.68 (2 H, d, J=8.8 Hz), 8.08 (1 H, d, J=13.6 Hz), 8.78 (1 H, s), 12.07 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.17, 162.09, 156.08, 150.57, 148.23, 146.03, 145.66, 145.49, 131.95, 130.03, 121.89, 119.72, 119.50, 114.09, 113.34, 113.03, 112.14, 111.92, 105.19, 104.93, 104.70, 55.49, 39.71, 39.63. HR-MS *m/z* Calcd. $C_{24}H_{19}O_3N_4F_3$ $[M+H]^+$ 469.1482, Found 469.1485.

*4.2.18. N-(3,5-bifluorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_1$**)*

Obtained as a white solid (62.9% yield) from compound **3b** as described for **4a$_1$**; mp: 212.0–213.4 °C. ^{1}H NMR ($CDCl_3$) δ: 1.07 (6 H, t, J=6.8 Hz), 3.37–3.44 (4 H, m), 6.53 (1 H, t, J=8.8 Hz), 7.03–7.10 (2 H, m), 7.34–7.45 (3 H, m), 8.05 (1 H, d, J=14.0 Hz), 8.70 (1 H, s), 12.47 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.11, 164.46, 164.31, 162.89, 162.01, 161.87, 149.31, 147.84, 146.24, 145.95, 145.27, 140.79, 129.98, 119.76, 119.54, 112.67, 112.58, 112.15, 111.93, 104.97, 103.52, 103.23, 99.00, 45.17, 45.10, 13.33. HR-MS *m/z* Calcd. $C_{25}H_{19}O_2N_4F_5$ $[M+H]^+$ 503.1501, Found 503.1500.

*4.2.19. N-(3,5-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_2$**)*

Obtained as a white solid (60.3% yield) from compound **3b** as described for **4a$_1$**; mp: 202.7–203.9 °C. ^{1}H NMR ($CDCl_3$) δ: 1.07 (6 H, t, J=6.8 Hz), 3.41 (4 H, t, J=6.8 Hz), 7.03–7.10 (3 H, m), 7.37–7.43 (1 H, m), 7.73 (2 H, d, J=1.2 Hz), 8.05 (1 H, d, J=14.0 Hz), 8.70 (1 H, s), 12.46 (1 H, s). ^{13}C NMR ($CDCl_3$) δ:176.08, 162.88, 149.41, 149.31, 147.84, 146.22, 145.94, 145.27, 140.48, 135.04, 130.08, 129.98, 123.67, 119.75, 119.52, 118.62, 112.66, 112.54, 112.17, 111.94, 105.21, 104.98, 104.72, 45.17, 45.10, 13.34. HR-MS *m/z* Calcd. $C_{25}H_{19}O_2N_4F_3Cl_2$ $[M+H]^+$ 535.0910, Found 535.0909.

*4.2.20. N-(2,4-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_3$**)*

Obtained as a white solid (71.5% yield) from compound **3b** as described for **4a$_1$**; mp: 228.1–229.5 °C. ^{1}H NMR ($CDCl_3$) δ: 1.07 (6 H, t, J=6.8 Hz), 3.38–3.44 (4 H, m), 7.03–7.10 (2 H, m), 7.24 (1 H, d, J=2 Hz), 7.37–7.41 (1 H, m), 7.44 (1 H, d, J=2.4 Hz), 8.15 (1 H, d, J=13.6 Hz), 8.58 (1 H, d, J=8.8 Hz), 8.73 (1 H, s), 12.64 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.09, 162.96, 149.27, 147.78, 146.32, 145.92, 145.21, 134.92, 130.13, 128.96, 128.54, 127.46, 124.38, 122.97, 120.01, 119.78, 112.90, 112.69, 112.12, 111.86, 105.18, 104.95, 104.69, 45.12, 45.05, 13.33. HR-MS *m/z* Calcd. $C_{25}H_{19}O_2N_4F_3Cl_2$ $[M+H]^+$ 535.0910, Found 535.0912.

*4.2.21. N-(2,3-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_4$**)*

Obtained as a white solid (78.5% yield) from compound **3b** as described for **4a$_1$**; mp: 218.2–219.7 °C. ^{1}H NMR ($CDCl_3$) δ: 1.07 (6 H, t, J=6.8 Hz), 3.41 (4 H, t, J=6.8 Hz), 7.04–7.09 (2 H, m), 7.22 (2 H, d, J=5.2 Hz), 7.37–7.43 (1 H, m), 8.16 (1 H, d, J=13.6 Hz), 8.55 (1 H, t, J=4.8 Hz), 8.74 (1 H, s), 12.70 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.08, 163.10, 149.27, 147.79, 146.40, 145.92, 145.22, 137.82, 132.90, 130.03, 127.45, 124.91, 122.54, 120.41, 120.06, 119.83, 112.96, 112.11, 112.08, 111.89, 105.18,

104.95, 104.69, 45.12, 45.05, 13.34. HR-MS *m/z* Calcd. $C_{25}H_{19}O_2N_4F_3Cl_2$ $[M+H]^+$ 535.0910, Found 535.0910.

4.2.22. *N-(2,5-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_5$**)*

Obtained as a white solid (73.4% yield) from compound **3b** as described for **4a$_1$**; mp: 282.2–283.7 °C. ^{1}H NMR ($CDCl_3$) δ: 1.07 (6 H, t, J=6.8 Hz), 3.38–3.44 (4 H, m), 7.00–7.03 (1 H, m), 7.03–7.10 (2 H, m), 7.34 (1 H, d, J=8.8 Hz), 7.38–7.44 (1 H, m), 8.16 (1 H, d, J=13.6 Hz), 8.71 (1 H, t, J=2.4 Hz), 8.73 (1 H, s), 12.68 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.05, 163.04, 149.27, 147.78, 146.43, 145.92, 145.21, 136.98, 132.97, 130.13, 129.88, 124.04, 122.10, 121.87, 120.01, 119.78, 112.82, 112.69, 112.14, 111.91, 105.19, 104.96, 104.69, 45.12, 45.05, 13.33. HR-MS *m/z* Calcd. $C_{25}H_{19}O_2N_4F_3Cl_2$ $[M+H]^+$ 535.0910, Found 535.0909.

4.2.23. *N-(3-chlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_6$**)*

Obtained as a white solid (77.9% yield) from compound **3b** as described for **4a$_1$**; mp: 204.6–205.5 °C. ^{1}H NMR ($CDCl_3$) δ: 1.07 (6 H, t, J=6.8 Hz), 3.38–3.45 (4 H, m), 7.03–7.10 (3 H, m), 7.24–7.28 (1 H, m), 7.42 (1 H, d, J=5.6 Hz), 7.58 (1 H, d, J=8.0 Hz), 7.93 (1 H, s), 8.06 (1 H, d, J=13.6 Hz), 8.77 (1 H, s), 12.35 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.11, 162.67, 149.25, 147.79, 146.13, 145.91, 145.22, 139.84, 134.49, 130.14, 129.84, 123.78, 120.45, 119.74, 119.52, 118.36, 112.81, 112.68, 112.13, 111.90, 105.15, 104.92, 104.65, 45.13, 45.06, 13.34. HR-MS *m/z* Calcd. $C_{25}H_{20}O_2N_4F_3Cl$ $[M+H]^+$ 501.1299, Found 501.1296.

4.2.24. *N-(3-chloro-4-fluorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_7$**)*

Obtained as a white solid (78.1% yield) from compound **3b** as described for **4a$_1$**; mp: 183.1–184.5 °C. ^{1}H NMR ($CDCl_3$) δ: 1.08 (6 H, t, J=6.8 Hz), 3.40–3.45 (4 H, m), 7.03–7.13 (3 H, m), 7.42 (1 H, d, J=4.8 Hz), 7.56 (1 H, d, J=8.8 Hz), 7.99 (1 H, dd, J_1=6.8 Hz, J_2=2.0 Hz), 8.06 (1 H, d, J=14.0 Hz), 8.77 (1 H, s), 12.35 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.14, 162.63, 149.29, 147.83, 146.08, 145.93, 145.26, 135.36, 129.99, 122.43, 121.00, 119.94, 119.87, 119.75, 119.53, 116.60, 116.38, 112.73, 112.66, 112.14, 111.92, 105.20, 104.93, 104.70, 45.15, 45.09, 13.33. HR-MS *m/z* Calcd. $C_{25}H_{19}O_2N_4F_4Cl$ $[M+H]^+$ 519.1205, Found 519.1208.

4.2.25. *N-(3-trifluoro-2-methylphenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_8$**)*

Obtained as a white solid (55.7% yield) from compound **3b** as described for **4a$_1$**; mp: 240.8–241.9 °C. ^{1}H NMR ($CDCl_3$) δ: 1.08 (6 H, t, J=6.8 Hz), 2.57 (3 H, s), 3.40–3.44 (4 H, m), 7.04–7.10 (2 H, m), 7.31 (1 H, t, J=8.0 Hz), 7.41–7.45 (2 H, m), 8.12 (1 H, d, J=14.0 Hz), 8.43 (1 H, d, J=7.2 Hz), 8.77 (1 H, s), 12.25 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.29, 162.91, 149.38, 147.81, 146.25, 145.94, 145.24, 138.49, 130.03, 129.75, 129.46, 127.63, 126.03, 123.17, 121.51, 119.88, 119.66, 113.08, 112.68, 112.09, 111.90, 105.18, 104.91, 104.68, 45.14, 45.07, 14.08, 13.33. HR-MS *m/z* Calcd. $C_{27}H_{22}O_2N_4F_6$ $[M+H]^+$ 549.1720, Found 549.1719.

4.2.26. *N-(3,4-bichlorophenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_9$**)*

Obtained as a white solid (56.2% yield) from compound **3b** as described for **4a$_1$**; mp: 163.1–164.6 °C. ^{1}H NMR ($CDCl_3$) δ: 1.07 (6 H, t, J=6.8 Hz), 3.40–3.43 (4 H, m), 7.03–7.10 (2 H, m), 7.38–7.43 (2 H, m), 7.57 (1 H, d, J=8.0 Hz), 8.06 (2 H, d, J=13.6 Hz), 8.76 (1 H, s), 12.42 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.08, 162.74, 149.39, 147.82, 146.15, 145.92, 145.25, 138.21, 132.56, 130.37, 130.01, 126.73, 124.87, 124.70, 121.98, 119.60, 112.62, 112.12, 111.93, 105.18, 104.92, 104.69, 45.16, 45.09, 13.33. HR-MS *m/z* Calcd. $C_{25}H_{19}O_2N_4F_3Cl_2$ $[M+H]^+$ 535.0910, Found 535.0909.

4.2.27. *N-(4-methoxyphenyl)-1-(2,4-bifluorophenyl)-6-fluoro-7-(diethylamino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamide (**4b$_{10}$**)*

Obtained as a white solid (69.5% yield) from compound **3b** as described for **4a$_1$**; mp: 217.3–218.5 °C. ^{1}H NMR ($CDCl_3$) δ: 1.07 (6 H, t, J=6.8 Hz), 3.37–3.44 (4 H, m), 3.81 (3 H, s), 6.81 (2 H, d, J=8.8 Hz), 7.01–7.08 (2 H, m), 7.38–7.42 (1 H, m), 7.68 (2 H, d, J=8.8 Hz), 8.06 (1 H, d, J=13.6 Hz), 8.75 (1 H, s), 12.09 (1 H, s). ^{13}C NMR ($CDCl_3$) δ: 176.20, 162.18, 156.08, 149.20, 147.76, 145.85, 145.20, 131.98, 130.14, 121.91, 119.80, 119.58, 114.46, 114.10, 113.27, 112.67, 112.03, 111.84, 105.12, 104.88, 104.62, 55.49, 45.10, 45.03, 13.34. HR-MS *m/z* Calcd. $C_{26}H_{23}O_3N_4F_3$ $[M+H]^+$ 497.1795, Found 497.1796.

4.3. *ASBT inhibition assay*

HEK293T cells were obtained from the American Type Culture Collection (Manassas, VA, USA), and grown in MEM supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin and 10% heat-inactivated fetal bovine serum. A human ASBT expression construct was prepared as previously described[15]. HEK293T cells were seeded in 12-well plates and transiently transfected with 0.5 μg/well pcDNA3.1/ASBT or the negative control plasmid pcDNA3.1 using lipofactamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Twenty-four hours after transfection, HEK293T cells were assayed for taurocholic acid uptake as previously described with minor modifications[16]. Briefly, cells were washed twice with warm wash and uptake buffer (116 mmol/L NaCl, 5.3 mmol/L KCl, 1.1 mmol/L KH_2PO_4, 0.8 mmol/L $MgSO_4$, 1.8 mmol/L $CaCl_2$, 11 mmol/L D-dextrose/D-glucose, and 10 mmol/L HEPES, pH 7.4), and then incubated with the same buffer containing the indicated concentrations of test compounds (dissolved in dimethyl sulfoxide) and 1 μCi/mL of [^{3}H]-taurocholic acid (TCA, 0.2 μmol/L) (PerkinElmer Life Sciences) for 10 min. To terminate the transport process the plates were chilled on ice and the cells were immediately washed with ice-cold buffer three times. Cells were lysed with 0.3 mL lysis buffer (0.5% Triton X-100) and shaken vigorously for 20 min. The radioactivity of the cell lysate was counted using a MicroBeta^2 Liquid Scintillation and Luminescence Counter (PerkinElmer Life Sciences). Protein concentration of the lysate was used to normalize uptake activity.

The inhibition rate was calculated using the following formula in Eq. (1):

$$\text{Inhibition rate } (\%) = \left[\left(1-(A-C)/(B-C)\right]\right. \times 100 \quad (1)$$

where A stands for [^{3}H] uptake value of test compound in [^{3}H]-TCA buffer added to pcDNA3.1/ASBT transfected cells; B

stands for [^{3}H] uptake value of DMSO (without inhibitor) in [^{3}H]-TCA buffer added to pcDNA3.1/ASBT transfected cells; *C* stands for [^{3}H] uptake value of blank sample (DMSO) in [^{3}H]-TCA buffer added to empty vector (pcDNA3.1)-transfected cells.

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