Synthesis of thiazole-fused diosgenin derivatives as potential narrow-spectrum antibiotics

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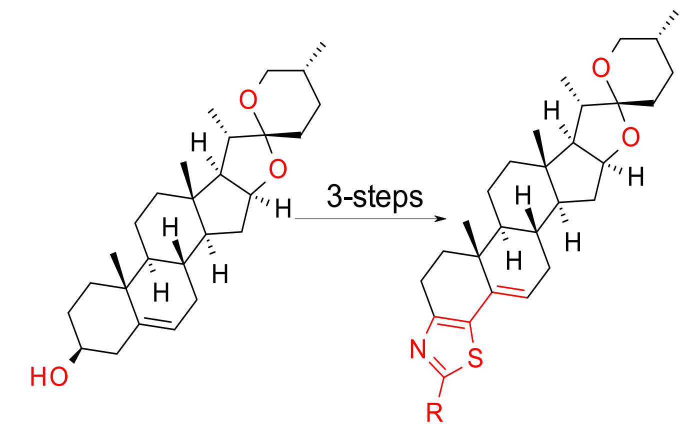
Abstract: Diosgenin, a hydrolyzed product of phytosteroid saponin, is widely researched for its medicinal properties. In an effort to find bioactive molecules, 25 novel thiazole-fused diosgenin molecules have been synthesized by an efficient reaction protocol. These compounds were tested for their anticancer and antimicrobial properties. Some of the molecules were found to be potent antimicrobial agents against *Bacillus subtilis*. Furthermore, the hit compounds are non-toxic to human cell lines.



> Novel natural product derivatives
> 25 thiazole-fused disogenin compounds
> Hit anti-*Bacillus subtilis* agent
> Non-toxic to human cell lines

Introduction

Steroidal derivatives are well known for their broad spectrum therapeutic use and have been used for cancer treatment for a long time.[1] Potent pharmacological properties of steroidal molecules made the medicinal chemists modify them with different heterocycles to discover novel drugs.[2] Diosgenin is a C_{27} spiro-acetal containing natural steroid sapogenin found in *Dioscorea*, *Trigonella*, and *Smilax* plants and it is used to synthesize different steroidal drugs.[3] Diosgenin itself possesses interesting therapeutic potency including antimicrobial,[4] and antiproliferative activities.[5–9] Due to its valuable therapeutic properties, the chemical derivatization of diosgenin has attracted the interest of many researchers. Most of the structural modifications of diosgenin were performed at C-3 or at C-26. Yin et al. esterified the hydroxy group at C-3 of the diosgenin reacting with dicarboxylic acids followed by the synthesis of amide derivatives resulting the formation of potent cytotoxic agents.[3] Hamid et al. have opened the spiro-acetal ring of diosgenin by reductive cleavage using sodium cyanoborohydride in acetic acid to get a primary alcohol at C-26.[10] Further derivatization formed potent compounds against human tumor cell lines with IC_{50} ranging from 10.9-20.0 μM. Deng. et al. have generated imidazolium salt at C-6 and C-3 of the diosgenin (**Figure 1**). They found several potent cytotoxic agents, and the most potent compounds were found with IC_{50} ranging from 0.49 to 0.79 μM against several tumor cell lines such as HL-60, A549, SMMC-7721, MCF-7, and SW480.[11] Rahman et al. have generated triazole attached derivatives at C-3 of the diosgenin.[12] All the derivatives showed good cytotoxicity values with IC_{50} ranging from 5 to17 μM against breast, lung, and colon cancer cell lines. Triazole-attached diosgenin derivatives were also developed at C-6.[13] The derivatives showed promising cytotoxic potential against T-cellular leucosis, human monocytes, breast cancer, prostate cancer, and glioblastoma. 1,3,4-Oxadiazole and 1,3,4-thiadiazole moieties bearing diosgenin were developed and the thiadiazole derivatives showed stronger cytotoxicity than oxadiazole-containing derivatives.[14] 3-Pyridyl substituted thiadiazole diosgenin derivative was found as the most potent against HepG2 and A549 with 11.7 and 3.9 μM IC_{50} values. There are several heterocycle-containing steroids approved as drugs are available such as Zytiga (abiraterone acetate): a pyridine-containing for prostate cancer,[15] Emflaza: an oxazole-containing for Duchenne muscular dystrophy,[16] galeterone: a benzimidazole-containing for prostate cancer,[17]and stanozolol: a pyrazole-containing synthetic anabolic steroid for treating hereditary angioedema[18]. Our lab has developed several thiazole-fused steroid derivatives such as thiazolo-androstenone,[19,20] thiazolo-ethisterone, cholestenone, and progestenone[21] as antineoplastic agents and thiazole-fused nootkatone as antibacterial agents[22] (**Figure 2**). The thiazole ring is very important in drug discovery and many thiazole-containing approved drugs are available in the market such as sulfathiazole: an antimicrobial drug, nizatidine: an anti-ulcer drug, talipexole: a potent dopamine receptor ligand, pramipexole: Perkinson's disease drug and so on.[23,24] 2-Aminothiazole is one of the most substituted thiazoles found in natural products and approved drugs.[25,26] The presence of

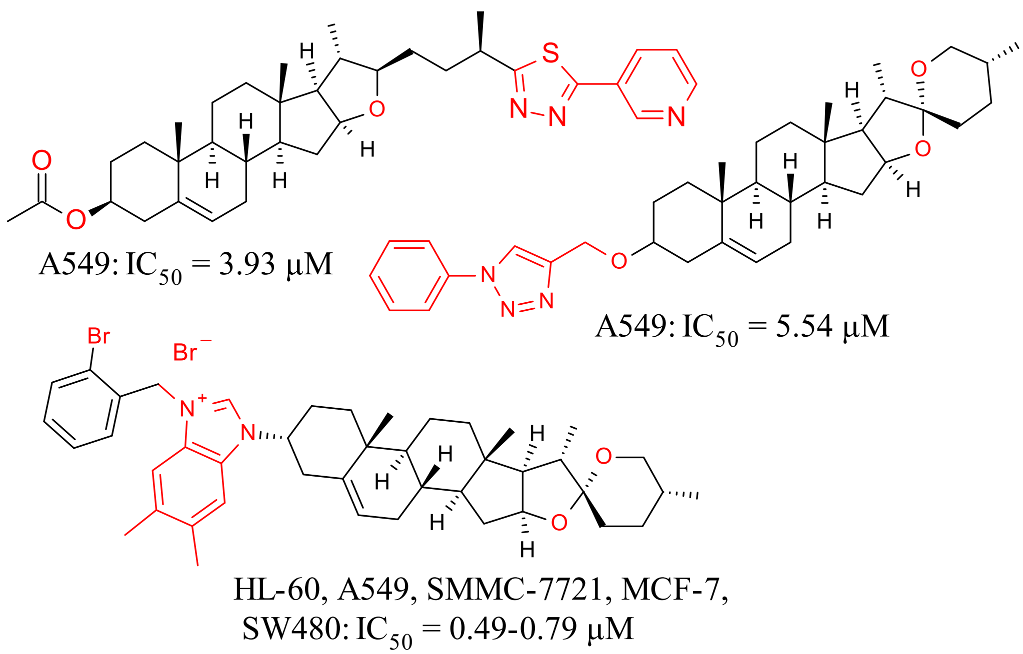


Figure 1. Representative example of heterocycle containing diosgenin as potent anticancer agents

amino groups with thiazole accelerates the metabolic process of the thiazole containing drugs.[27] Realizing the importance of thiazole nucleus in drug discovery, we envisioned to synthesize thiazole-fused diosgenin derivatives and investigating their anticancer and antimicrobial properties.

Results and discussion

Synthesis of thiazole fused diosgenin

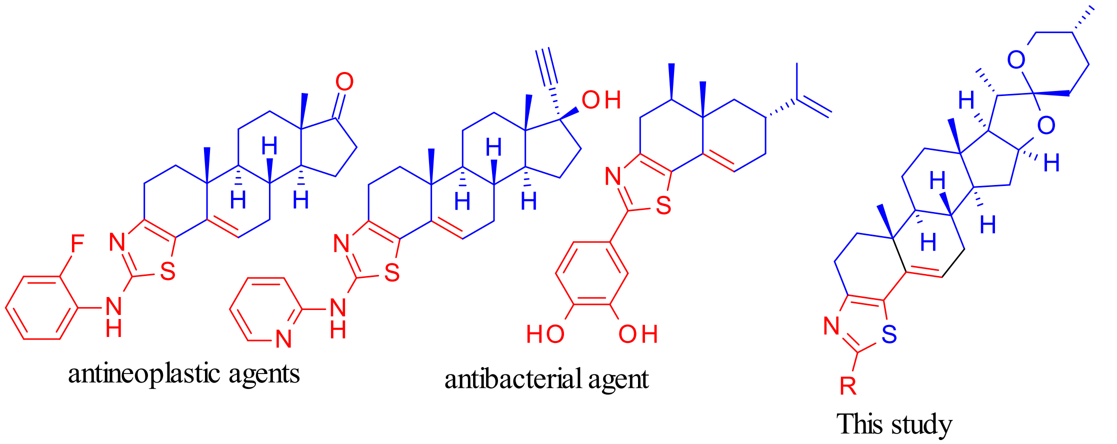
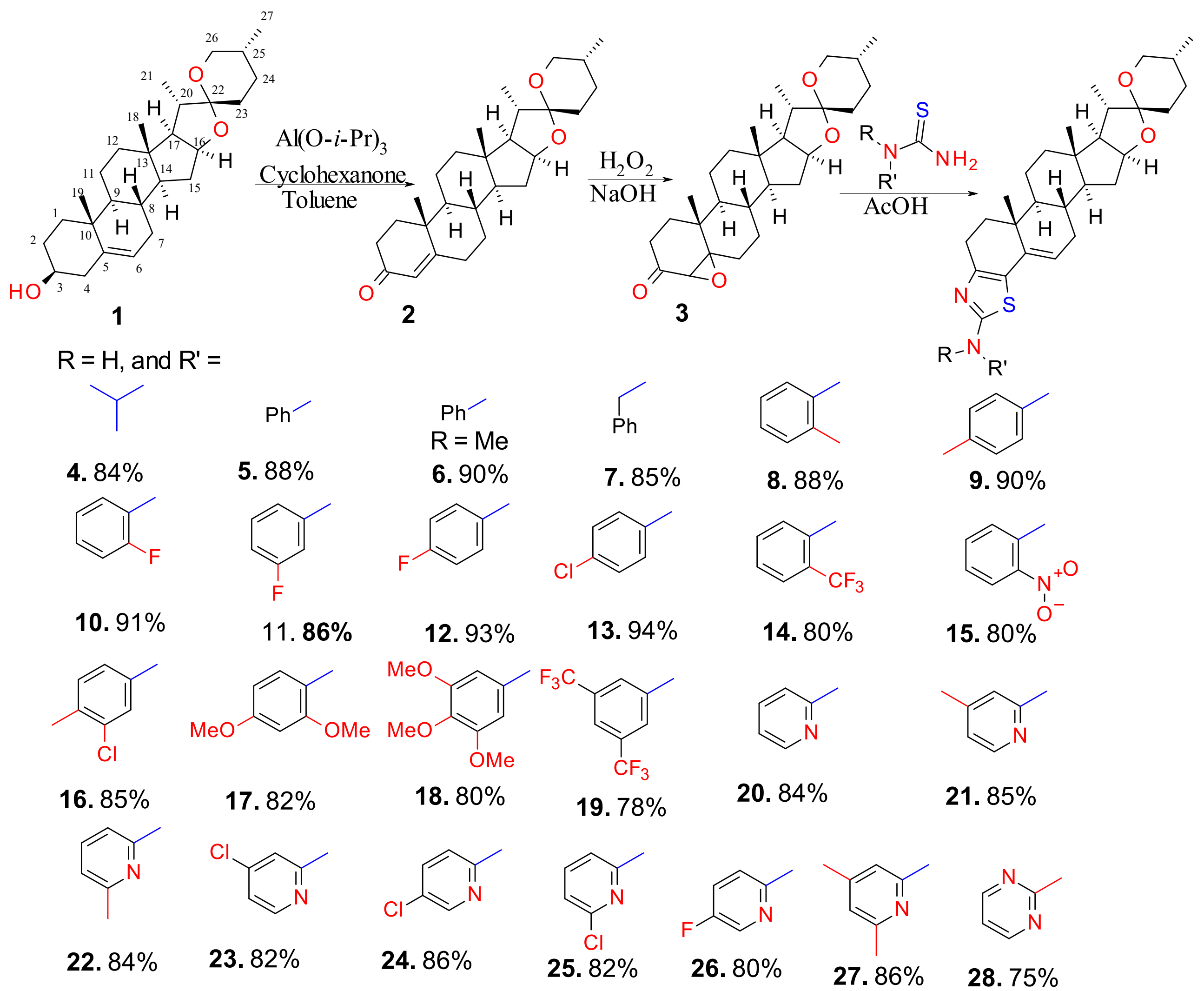


Figure 2: Design of thiazole-fused diosgenin molecules based on our previous results.

We applied the Oppenauer oxidation to the commercially available diosgenin (**1**) and the secondary alcohol at C-3 was transformed into ketone. Due to energetic stability of *α,β*-unsaturated ketone than *β,γ*-unsaturated ketone, the double bond at C-5 is shifted to C-4 providing an enone functional group containing diosginone (**2**).[28] An epoxide was generated by using NaOH/H_2O_2 in between C-4 and 5 yielding an epoxy-ketone functional group containing diosgenin (**3**).[29] The epoxy-ketone containing diosgenin (**3**) was reacted with thiourea derivatives in acetic acid solvent to make thiazole-fused diosgenin derivatives. The acetic acid generated hydrogen bonds with the oxygens of the ketone and epoxide and facilitated the nucleophilic attack by the thiourea. The reaction mechanism involved the synthesis of isothiourea via intermolecular S_N2 reaction and subsequent intramolecular nucleophilic addition formed the five-membered thiazole ring and finally elimination of water molecule provided the expected products.[30] Initially, we reacted with isopropyl thiourea with epoxy-diosgenin (**3**) and it provided the desired amino thiazole-fused diosgenin product (**4**) with 84% yield (**Scheme 1**). Encouraged by the result, we reacted with phenyl, *N*-methyl-*N*-phenyl, and *N*-benzyl thiourea and all reactions provided the desired products (**5**, **6** and **7**) with 85-90% yield. We have reacted electron donating 2-methyl and 4-methyl substituted phenyl thioureas and they provided the desired products (**8** and **9**) with 88% and 90% yield respectively. We reacted mild electron withdrawing groups such as fluorine and chlorine substituted phenyl thioureas and they also provided the expected products (**10**, **11**, **12** and **13**) with very good yields. The strong electron withdrawing groups such as trifluoromethyl and nitro-containing phenyl thioureas furnished the reactions efficiently with 80% products (**14** and **15**). 3-Chloro-4-methyl substituted phenyl thiourea also provided 85% product (**16**). The electron-donating, 2,4-dimethoxy (**17**) and 3,4,5-trimethoxy (**18**) substituted thioureas provided 82% and 80% yields respectively. The electron-withdrawing, 3,5-ditrifluoromethy phenyl substituted thiourea also furnished the product (**19**) in 78% yield. Realizing the importance of heterocycles in medicinal chemistry, we reacted pyridyl thioureas with epoxy-diosgenin (**3**). Initially, we reacted with *N*-pyridyl thiourea and it provided an 84% yield of the expected product (**20**). Encouraged by the results, we reacted mild electron donating 4-methyl (**21**) and 6-methyl (**22**) substituted pyridyl thioureas and they provided the desired products (**21** and **22**) with good yields. Then we reacted with an electron-withdrawing 4-chloro (**23**), 5-chloro (**24**), 6-chloro (**25**), and 5-fluoro (**26**) substituted pyridyl thiourea and they all provided desired (**23**, **24**, **25**, and **26**) products in 80-86% yields. 4,6-Dimethyl substituted pyridyl also reacted efficiently and 86% yield of the product (**27**) was obtained. Last but not least, pyrimidinyl thiourea also reacted well and provided a 75% yield of the expected product (**28**).



Scheme 1. Synthesis of fused-thiazolo diosgenin derivatives (**4-28**)

Cytotoxicity studies

To evaluate the cytotoxicity of the thiazolo-diosgenin molecules, we sent all thiazolo-diosgenin molecules to the Development Therapeutics Program (DTP) of the National Cancer Institute[31] to test against NCI-60 cancer cell lines. The compounds did not show any significant cytotoxicity against the cancer cell lines (**Figure 3**).

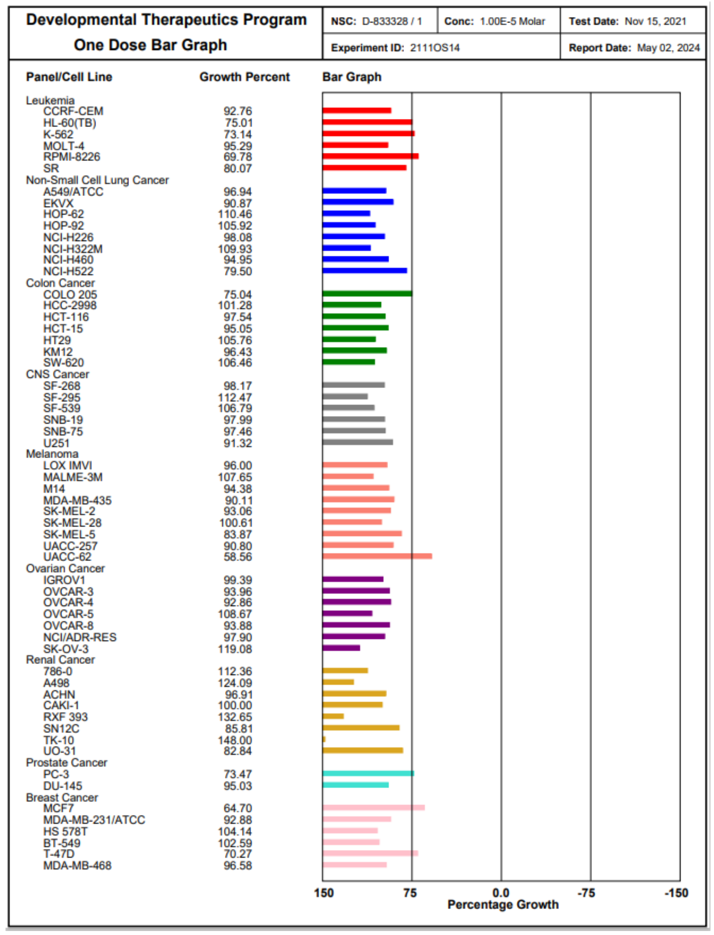


Figure 3. Cytotoxicity data for compound **26** against NCI-60 cancer cell lines

Antimicrobial Studies

In the pursuit of determining the antibacterial efficacy of diosgenin derivatives, all 25 compounds were subjected to antibacterial testing against a spectrum of Gram-positive and Gram-negative bacterial strains through minimum inhibitory concentration (MIC). Encouragingly, a subset of diosgenin derivatives exhibited notable narrow-spectrum potency against the bacterial strain *Bacillus subtilis* (**Table 1**). Of particular significance, compound (**26**) demonstrated an inhibitory activity against *B. subtilis*, with an MIC recorded at 16 µg/mL. Additionally, compounds (**4**, **7**, **8**, and **25**) exhibited the antibacterial efficacy, albeit at a slightly higher MIC value of 32 µg/mL. These findings constitute promising initial results indicative of the potential for these derivatives to serve as foundational scaffolds for the development of novel antibacterial agents. The *Bacillus* genus encompasses a diverse array of bacteria grouped into two groups , *B. subtilis* group and the *Bacillus cereus* group.[32] This bacterial genus includes aerobic spore bearers, with some species possessing pathogenic potential towards humans. Notably, *Bacillus anthracis*, causative agent of anthrax, presents a significant public health concern, primarily transmitted through infected livestock. Moreover, species such as *B. cereus* and, *B. subtilis* have been implicated in various human infections, encompassing bacteremia, septicemia, endocarditis, and meningitis, as well as infections affecting diverse anatomical sites including wounds, ears, eyes, respiratory, urinary, and gastrointestinal tracts.[33,34] Besides this, *B. cereus* is also associated with food poisoning leading to diarrheal syndrome.[32] This implies that *Bacillus* genus of bacteria are important pathogenic bacteria and needs an outlook for the development of antibacterials.

The elucidation of the antibacterial activity of these novel scaffolds against closely related *Bacillus* species holds profound implications for the screening and discovery of potent therapeutic agents targeting pathogenic bacteria.

Table 1. Antibacterial Properties (MIC, µg/mL) of synthesized compounds against Bacteria

Antimicrobial screening of the compounds Antibiotic-resistant *Staphylococcus aureus* ATCC 700699 (Sa99), antibiotic susceptible *Enterococuus faecalis* ATCC 29212 (Ef12), *Bacillus subtilis* ATCC 6623 (Bs), *Acinetobacter baumannii* ATCC 1705 (Ab05), *A. baumannii* BAA 747 (Ab747), *Pseudomonas aeruginosa* ATCC (Pa33), *Escherichia coli* ATCC 25922 (Ec22), *Klebsiella pneumioniae* ATCC (Kp03)									
Compound	Sa99	Efs12	Bs	Ab05	Ab747	Ab05	Pa33	Ec22	Kp03
4	>32	>32	**32**	>32	>32	>32	>32	>32	>32
7	>32	>32	**32**	>32	>32	>32	>32	>32	>32
8	>32	>32	**32**	>32	>32	>32	>32	>32	>32
25	>32	>32	**32**	>32	>32	>32	>32	>32	>32
26	>32	>32	**16**	>32	>32	>32	>32	>32	>32
Van	4	4	**4**	-	-	-	-	-	-
Colistin	-	-	**-**	2	2	2	1	1	1

Experimental methods

General Methods

All reactions were carried out in the standard air atmosphere in round bottom flasks. All reacting materials, reagents, and solvents were purchased from Fischer Scientific (Hanover Park, IL, USA) and Oakwood Chemical (Estill, SC, USA). No chemicals were further purified. Proton (^{1}H), and Carbon (^{13}C) spectra were recorded on JEOL with 400 MHz for ^{1}H, 101 MHz for ^{13}C in $CDCl_3$ and TFA-*d* solvents. ^{1}H NMR spectra are described in chemical shifts (δ, ppm) and multiplicity are designated as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd =doublet of doublets of doublets, td = triplet of doublets, m = multiplet. The infrared (IR) spectral data were obtained in Nicolet iS 10 FTIR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) by making KBr pellets of the compounds. The Brucker Apex II-FTMS was used to obtain High Resolution Mass Spectroscopy (HRMS) data.

Synthesis of diosginone (**2**)

A mixture of 1 mmol diosgenin (**1**), 1.5 mL cyclohexanone, and 40 mL toluene solvent was taken in a 50 mL round bottle flask. The reaction was refluxed for 30 min with a Dean-Stark to remove moisture and then 0.30 mmol Al-isopropoxide was added to the reaction mixture. Then the reaction continued with reflux for 3 h. After that, the solvent was removed through the Dean-Stark, and a solid product was collected. The solid product underwent liquid-liquid extraction using dichloromethane and water. The dichloromethane layer was collected and passed through sodium sulfite. Subsequent rotary evaporation of the dichloromethane yielded pure diosginone (**2**) in 70% yield.

Synthesis of epoxy-diosginone (**3**)

Diosginone (**2**, 1 mmol), dichloromethane (3 mL), methanol (4 mL), 10% NaOH (0.28 mL), and 30% H_2O_2 (0.56 mL) were taken in a 50 mL round bottom flask sequentially and stirred for 24 h. Then the dichloromethane and methanol were evaporated from the reaction mixture by using a rotary evaporator. Then the solid product was vacuum-dried to get epoxy-diosginone (**3**). In case of impurity, recrystallization with methanol provided pure epoxy-diosginone (**3**).

Synthesis of thiazolo-diosgenin

A mixture of epoxy-diosginone (0.5 mmol), thiourea derivative (0.55 mmol), and acetic acid (5 mL) were taken in a round bottom flask and heated the reaction at 100 °C for 12 h. Then sodium bicarbonate was added slowly to quench the reaction and water was added to make precipitate. The precipitates were washed thoroughly with distilled water and vacuum dry provided pure thiazolo-diosgenin compounds. In case of impurities, recrystallization with acetonitrile provided the pure products.

Antibacterial studies

Overnight cultures of the bacterial strains were prepared from a single isolated colony. Diosgenin compounds, synthesized chemically, along with commercially available vancomycin and colistin, were used as test compounds. These compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted in cation-adjusted Mueller Hinton Broth (Ca-MHB) media to achieve standard starting concentrations. Minimum inhibitory concentration (MIC) was determined using the standard broth microdilution method, following Clinical and Laboratory Standards Institute (CLSI) guidelines with slight modifications as reported previously.[35,36] A standard concentration of diosgenin derivatives was serially diluted 2-fold down a 96-well plate column and bacterial suspension was added to it. The plates were then incubated at 37 ºC for 20 h. MIC values, denoting the lowest compound concentration inhibiting visible bacterial growth, were determined. All the MIC assays were performed in triplicates with vancomycin and 1% DMSO as positive and negative controls, respectively.

Conclusions

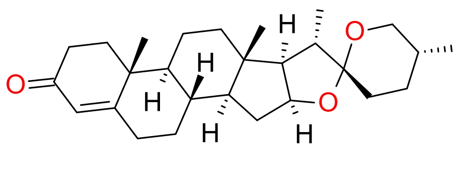
We have efficiently synthesized a series of thiazole-fused diosgenin derivatives. Although, a number of thiazole derivatives have been reported in the literature, we are reporting for the first time 4,5-carbon fused heterocycles of diosgenin. These novel compounds are non-toxic to human cell lines. Some of these compounds showed narrow-spectrum antibacterial properties with moderate activity against *Bacillus subtilis* with MIC values as low as 16 μg/mL.

Acknowledgments

This work was supported by the Arkansas INBRE program, supported by a grant from the National Institute of General Medical Sciences, (NIGMS), P20 GM103429 (MAA) from the National Institutes of Health, ABI mini-200028 grant resources, NSF MRI grant (Award Number: MRI #2117138) (MAA) for 400 MHz NMR spectrometer.

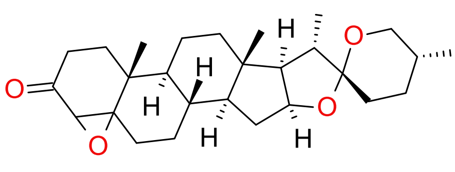
Experimental data

(1*S*,2*S*,4*S*,5'*R*,6*R*,7*S*,8*R*,9*S*,12*S*,13*R*)-5',7,9,13-tetramethylspiro[5-oxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icos-17-ene-6,2'-tetrahydropyran]-16-one (2). White powder (329mg, 70%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.70 (s, 1H), 4.38 (q, J = 7.5 Hz, 1H), 3.46-3.43 (m, 1H), 3.34 (t, J = 10.9 Hz, 1H), 2.44-2.23 (m, 4H), 2.02-1.96 (m, 2H), 1.88-1.35 (m, 14H), 1.32-1.24 (m, 1H), 1.17 (s, 3H), 1.15-0.99 (m, 3H), 0.95-0.91 (m, 3H), 0.79-0.76 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 199.6, 171.3, 124.0, 109.4, 80.7, 66.9, 62.1, 55.7, 53.8, 41.7, 40.4, 39.7, 38.7, 35.7, 35.3, 34.0, 32.9, 32.2, 31.8, 31.4, 30.3, 28.9, 20.9, 17.5, 17.2, 16.4, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{27}H_{40}O_3$ $[M+H]^+$ = 413.305 found 429.2995



2

(1*S*,2*S*,4*S*,5'*R*,6*R*,7*S*,8*R*,9*S*,12*S*,13*R*)-5',7,9,13-tetramethylspiro[5,18 dioxahexacyclo[10.9.0.0^{2,9}.0^{4,8}.0^{13,19}.0^{17,19}]henicosane-6,2'-tetrahydropyran]-16-one (3). White powder (300 mg, 85%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.41-4.35 (m, 1H), 3.47-3.31 (m, 2H), 2.96 (s, 1H), 2.31-1.95 (m, 4H), 1.86-1.52 (m, 11H), 1.47-0.99 (m, 11H), 0.95-0.94 (m, 3H), 0.77 (d, J = 6.5 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 206.9, 109.4, 80.6, 70.3, 67.0, 62.7, 62.1, 55.7, 46.6, 41.7, 40.7, 39.5, 37.3, 34.7, 32.6, 31.8, 31.4, 30.6, 30.3, 29.8, 28.9, 26.1, 21.4, 19.1, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{27}H_{40}O_4$ $[M+H]^+$ = 429.2999 found 429.2995



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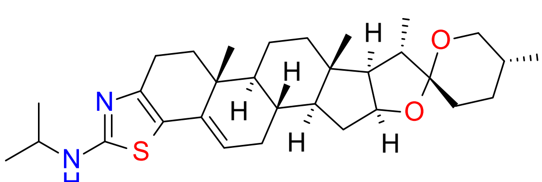
(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-isopropyl-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (4). Pink solid powder (428 mg, 84%); IR (KBr pellet, cm^{-1}) 3436, 2950, 1120, 1155; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.34 (s, 1H), 4.43-4.38 (m, 1H), 3.47-3.33 (m, 2H), 2.56 (d, J = 6.2 Hz, 2H), 2.20 (dt, J = 18.2, 4.8 Hz, 1H), 2.02-1.59 (m, 14H), 1.50-1.04 (m, 12H), 1.00-0.95 (m, 6H), 0.81-0.76 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7,



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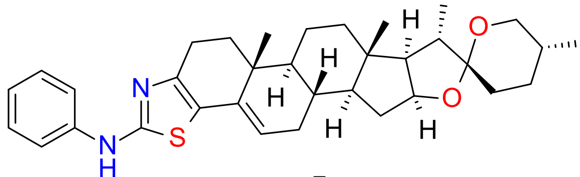
141.8, 136.2, 117.7, 117.5, 109.4, 80.9, 66.9, 62.1, 56.6, 49.0, 47.9, 41.7, 40.4, 39.8, 36.8, 34.0, 31.9, 31.4, 31.2, 30.4, 28.9, 22.7, 22.5, 22.4, 22.2, 18.8, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{31}H_{46}N_2O_2S$ $[M+H]^+$ = 511.3353, found 511.3350.

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-phenyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (5). Light yellow solid (478 mg, 88%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.47-7.31 (m, 4H), 7.04 (s, 1H), 5.42 (s, 1H), 4.41 (s, 1H), 3.46-3.37 (m, 2H), 2.67 (s, 2H), 2.29-1.60 (m, 14H), 1.45-1.17 (m, 7H), 1.03-0.96 (m, 6H), 0.80-0.78 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 162.3, 145.2, 140.4, 136.6, 129.5, 123.1, 120.6, 118.7, 118.3, 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.4, 32.0, 31.9, 31.4, 31.2, 30.4, 28.9, 24.2, 21.3, 18.8, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{34}H_{44}N_2O_2S$ $[M+H]^+$ = 545.3196, found 545.3202.



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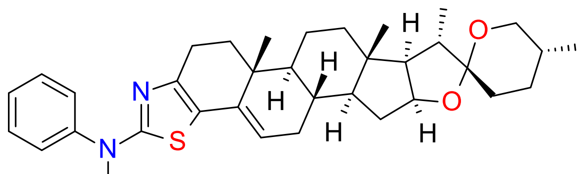
(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*,2,5',19,21-pentamethyl-*N*-phenyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (6). Pink solid powder (502 mg, 90%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41-7.34 (m, 4H), 7.26-7.24 (m, 1H), 5.26 (s, 1H), 4.39-4.38 (m, 1H), 3.48 (s, 3H), 3.44-3.32 (m, 2H), 2.67 (s, 2H), 1.96-1.59 (m, 11H), 1.46-1.05 (m, 7H), 0.96-0.95 (m, 5H), 0.78-0.76 (m, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.3, 146.4, 145.9, 136.8, 129.8, 126.7, 125.5, 120.4, 117.6, 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 40.3, 39.8, 36.6, 34.5, 31.9, 31.8, 31.4, 31.2, 30.4, 28.9, 24.2, 21.2, 18.8, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{35}H_{46}N_2O_2S$ $[M+H]^+$ = 559.3353, found 559.3355.



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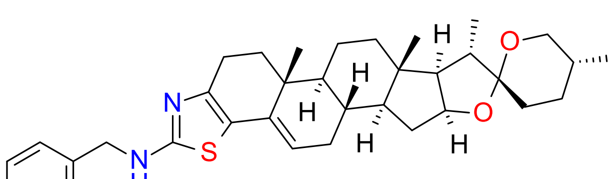
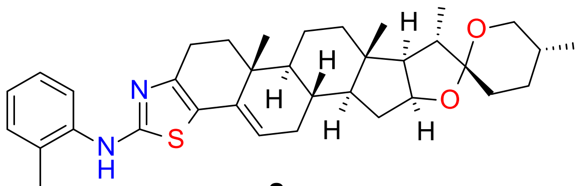
(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-benzyl-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (7). Light yellow powder (474 mg, 85%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.27 (m, 5H), 5.32 (s, 1H), 4.39 (s, 3H), 3.44-3.33 (m, 2H), 2.57 (s, 2H), 2.31-1.59 (m, 15H), 1.43-1.09 (m, 6H), 0.99-0.95 (m, 6H), 0.79-0.77 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 144.1, 137.3, 136.5, 128.8, 127.8, 127.6, 119.1, 117.6, 109.4, 80.9, 66.9, 62.2, 56.6, 50.0, 48.0, 41.7, 40.4, 39.8, 36.8, 34.2, 31.9,



7

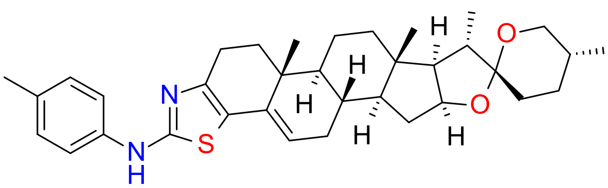
31.5, 31.2, 30.4, 28.9, 23.5, 21.9, 21.2, 18.8, 17.2, 16.4, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{35}H_{46}N_2O_2S$ $[M+H]^+$ = 559.3353, found 559.3361.

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-(*o*-tolyl)spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (8). Grey solid powder (491 mg, 88%); IR (KBr pellet, cm^{-1}) 3423, 2949, 1220, 1154; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.47 (m, 1H), 7.25-7.18 (m, 2H), 7.09-7.06 (m, 1H), 5.35 (s, 1H), 4.41-4.38 (m, 1H), 3.47-3.33 (m, 2H), 2.64-2.63 (m, 1H), 2.29 (s, 3H), 2.21-1.59 (m, 14H), 1.50-1.05 (m, 7H), 1.02-0.95 (m, 6H), 0.79-0.76 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 143.1, 138.6, 136.2, 131.3, 131.0, 127.1, 125.4, 121.6, 119.2, 118.4, 109.4, 80.9, 66.9, 62.1, 56.6, 47.9, 41.7, 40.4, 39.8, 36.8, 34.1, 31.9, 31.4, 31.2, 30.4, 28.9, 23.2, 21.8, 21.3, 18.8, 18.0, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{35}H_{46}N_2O_2S$ $[M+H]^+$ = 559.3353, found 559.3358.



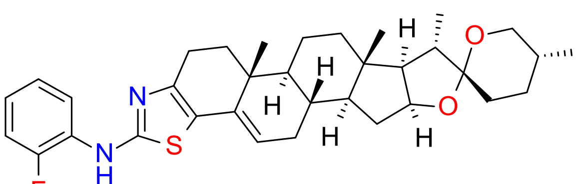
8

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-(*p*-tolyl)spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (9). Brick red powder (502 mg, 90%); IR (KBr pellet, cm^{-1}) 3439, 2948, 1219, 1154; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20-7.12 (m, 4H), 5.40 (s, 1H), 4.42-4.38 (m, 1H), 3.45-3.34 (m, 2H), 2.65 (s, 2H), 2.31 (s, 3H), 2.22-2.16 (m, 1H), 1.98-1.60 (m, 13H), 1.48-1.08 (m, 7H), 1.03-0.95 (m, 6H), 0.80-0.77 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.5, 142.0, 137.4, 136.0, 133.6, 130.1, 119.1, 118.6, 118.5, 109.4, 80.9, 67.0, 62.1, 56.6, 47.9, 41.7, 40.4, 39.8, 36.8, 34.6, 34.0, 31.9, 31.4, 31.2, 30.4, 28.9, 22.8, 21.7, 21.0, 18.8, 17.3, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{35}H_{46}N_2O_2S$ $[M+H]^+$ = 559.3353, found 559.3356.



9

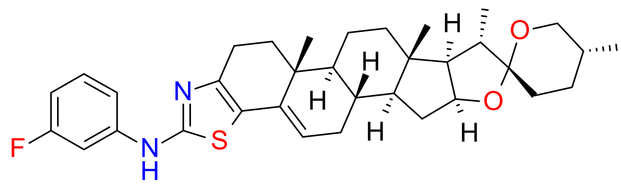
(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(2-fluorophenyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (10). Grey powder (512 mg, 91%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29-7.23 (m, 1H), 7.07-6.99 (m, 2H), 6.72 (td, J = 8.3, 1.9 Hz, 1H), 5.47 (dd, J = 4.5, 2.7 Hz, 1H), 4.41 (dd, J = 15.0, 7.3 Hz, 1H), 3.47-3.34 (m, 2H), 2.65-2.61 (m, 2H), 2.24-2.17 (m, 1H), 2.04-1.60 (m, 13H), 1.52-1.08 (m, 7H), 1.02 (s, 3H), 0.96 (d, J = 6.9 Hz, 3H), 0.80-0.77 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5 ($^1J_{C\text{-}F}$ = 245.2 Hz),



10

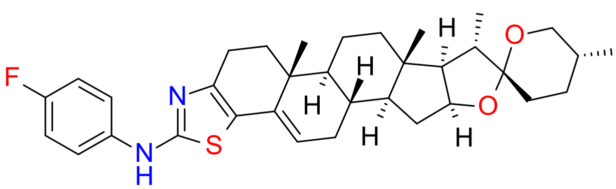
162.5, 143.0, 141.6 ($^3J_{C\text{-}F}$ = 10.6 Hz), 136.0, 130.7 ($^3J_{C\text{-}F}$ = 9.6 Hz), 119.9, 119.0, 113.5 ($^4J_{C\text{-}F}$ = 2.9 Hz), 109.6 ($^2J_{C\text{-}F}$ = 21.2 Hz), 109.4, 104.9 ($^2J_{C\text{-}F}$ = 25.5 Hz), 80.9, 66.9, 62.1, 56.6, 47.9, 41.7, 40.4, 39.8, 36.8, 34.0, 31.9, 31.8, 31.4, 31.2, 30.4, 28.9, 23.1, 21.7, 18.8, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{34}H_{43}FN_2O_2S$ $[M+H]^+$ = 563.3102, found 563.3095.

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(3-fluorophenyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (11). Light yellow powder (483 mg, 86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01-7.98 (m, 1H), 7.14-7.05 (m, 2H), 6.94 (s, 1H), 5.47 (s, 1H), 4.43-4.40 (m, 1H), 3.45-3.36 (m, 2H), 2.71 (s, 2H), 2.24-2.19 (m, 1H), 2.00-1.61 (m, 13H), 1.52-1.13 (m, 7H), 1.05-0.97 (m, 6H), 0.82-0.78 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 160.2, 152.0 ($^1J_{C\text{-}F}$ = 243.2 Hz), 145.7, 136.5, 128.8 ($^3J_{C\text{-}F}$ = 10.6 Hz), 124.8 ($^4J_{C\text{-}F}$ = 3.4 Hz), 122.5 ($^3J_{C\text{-}F}$ = 7.2 Hz), 121.9, 118.9, 118.5, 115.1 ($^2J_{C\text{-}F}$ = 19.2 Hz), 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.4, 32.0, 31.9, 31.4, 31.2, 30.4, 28.9, 24.2, 21.3, 18.8, 17.2, 16.5, 14.6; ^{19}F decoupled ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.2, 152.0, 145.7, 136.5, 128.8, 124.8, 122.5, 121.9, 118.9, 118.5, 115.2, 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.4, 32.0, 31.9, 31.5, 31.2, 30.4, 28.9, 24.2, 21.3, 18.8, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{34}H_{43}FN_2O_2S$ $[M+H]^+$ = 563.3102, found 563.3100.



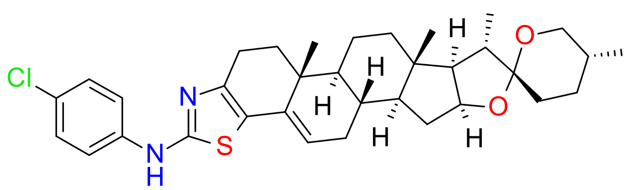
11

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(4-fluorophenyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (12). Light yellow solid (522 mg, 93%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.24 (s, 2H), 7.03-7.00 (m, 2H), 5.39 (s, 1H), 4.40-4.39 (m, 1H), 3.44-3.33 (m, 2H), 2.62-2.61 (m, 2H), 2.21-2.15 (m, 1H), 2.07-1.59 (m, 14H), 1.47-1.07 (m, 6H), 1.01-0.95 (m, 6H), 0.80-0.76 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 163.3, 159.1 ($^1J_{C\text{-}F}$ = 243.7 Hz), 144.9, 136.6, 136.4, 121.3 ($^3J_{C\text{-}F}$ = 7.7 Hz), 120.2, 118.4, 116.2 ($^2J_{C\text{-}F}$ = 22.6 Hz), 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.3, 31.9, 31.8, 31.4, 31.2, 30.4, 28.9, 24.0, 21.3, 18.8, 17.2, 16.5, 14.6. ^{19}F-decoupled ^{13}C-NMR (101 MHz, $CDCl_3$) δ 163.3, 159.1, 144.9, 136.6, 136.4, 121.3, 120.3, 118.5, 116.2, 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.3, 31.9, 31.8, 31.5, 31.2, 30.4, 28.9, 24.0, 21.3, 18.8, 17.2, 16.5, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{34}H_{43}FN_2O_2S$ $[M+H]^+$ = 563.3102, found 563.3105.



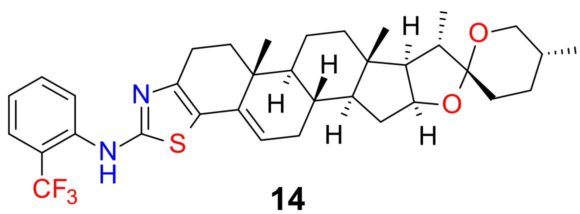
12

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(4-chlorophenyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (13). Red powder (544 mg, 94%); IR (KBr pellet, cm^{-1}) 3446, 2950, 1230, 1154; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.26 (m, 4H), 5.42 (s, 1H), 4.41 (t, J = 6.8 Hz, 1H), 3.46-3.35 (m, 2H), 2.66 (s, 2H) , 2.22-2.16 (m, 1H), 1.98-1.60 (m, 13H), 1.45-1.13 (m, 7H), 1.03 (s, 3H), 0.97 (d, J = 6.6 Hz, 3H), 0.79 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.6, 145.3, 139.0, 136.4, 129.5, 127.9, 121.1, 119.8, 118.7, 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.3, 31.9, 31.8, 31.4, 31.2, 30.4, 28.9, 24.2, 21.3, 18.8, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{34}H_{43}ClN_2O_2S$ $[M+H]^+$ = 579.2806, found 579.2814.



13

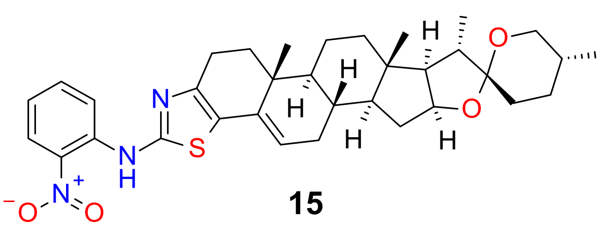
(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-[2-(trifluoromethyl)phenyl]spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (14). Light yellow solid (489 mg, 80%); IR (KBr pellet, cm^{-1}) 3453, 2951, 1598, 1220, 1156; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.5 Hz, 1H), 7.60-7.50 (m, 2H), 7.12 (t, J = 7.6 Hz, 1H), 5.44 (s, 1H), 4.41 (q, J = 7.4 Hz, 1H), 3.47-3.33 (m, 2H), 2.70-2.66 (m, 2H), 2.20 (dt, J = 18.0, 4.8 Hz, 1H), 2.02-1.60 (m, 13H), 1.48-1.07 (m, 7H), 1.04-0.95 (m, 6H), 0.81-0.76 (m, 6H); ^{13}C-NMR (101 MHz,$CDCl_3$) δ 161.0, 145.4, 138.6, 136.4, 133.3, 126.8 ($^3J_{C\text{-}CF3}$ = 5.3 Hz), 124.1 ($^3J_{C\text{-}CF3}$ = 273.1 Hz), 123.0, 122.2, 120.9, 119.2, 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.3, 32.0, 31.9, 31.4, 31.2, 30.4, 28.9, 24.1, 21.2, 18.8, 17.2, 16.5, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{35}H_{43}N_2O_2S$ $[M+H]^+$ = 613.3070, found 613.3079.



14

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-(2-nitrophenyl)spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (15).

Red powder (471 mg, 80%); IR (KBr pellet, cm^{-1}) 3480, 2945, 2871, 1613, 1588, 1240, 1146; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.58 (s, 1H), 8.67 (d, J = 8.6 Hz, 1H), 8.23 (d, J = 8.5 Hz, 1H), 7.62-7.59 (m, 1H), 6.99 (dd, J = 8.3, 7.3 Hz, 1H), 5.56-5.55 (m, 1H), 4.42 (dd, J = 15.0, 7.2 Hz, 1H), 3.49-3.45 (m, 1H), 3.37 (t, J = 10.8 Hz, 1H), 2.83-2.68 (m, 2H), 2.27-2.20 (m, 1H), 2.04-1.98 (m, 2H), 1.90-1.57 (m, 11H), 1.51-1.12 (m, 7H), 1.05 (s, 3H), 0.97 (d, J = 6.9 Hz, 3H), 0.82-0.78 (m, 6H); ^{13}C NMR (101 MHz,



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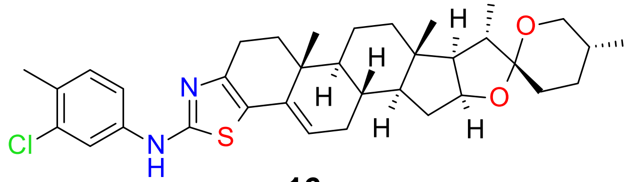
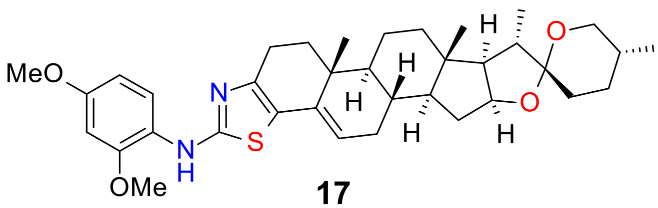
$CDCl_3$) δ 157.8, 146.4, 138.1, 136.5, 136.2, 134.0, 126.3, 124.8, 120.6, 120.5, 119.2, 109.4, 80.9, 67.0, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.3, 32.0, 31.9, 31.5, 31.2, 30.4, 28.9, 24.4, 21.3, 18.8, 17.2, 16.5, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{34}H_{43}N_3O_4S$ $[M+H]^+$ = 590.3047, found 590.3039.

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(3-chloro-4-methyl-phenyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (16). Grey solid (504 mg, 85%); IR (KBr pellet, cm^{-1}) 3431, 2949, 1220, 1154; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 1H), 7.16-7.09 (m, 2H), 5.43 (s, 1H), 4.41 (q, J = 7.3 Hz, 1H), 3.47-3.34 (m, 2H), 2.67-2.66 (m, 2H), 2.31 (s, 3H), 2.23-2.17 (m, 1H), 2.02-1.60 (m, 14H), 1.48-1.08 (m, 6H), 1.03-0.96 (m, 6H), 0.81-0.77 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 161.9, 145.3, 139.2, 136.5, 134.9, 131.5, 130.6, 120.9, 119.2, 118.6, 117.0, 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.3, 32.0, 31.9, 31.5, 31.2, 30.4, 28.9, 24.1, 21.3, 19.5, 18.8, 17.2, 16.5, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{35}H_{45}ClN_2O_2S$ $[M+H]^+$ = 593.2963, found 593.2958.



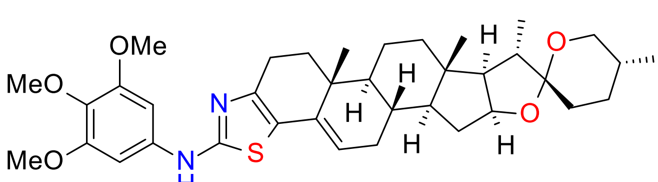
16

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(2,4-dimethoxyphenyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (17). Red powder (495 mg, 82%); IR (KBr pellet, cm^{-1}) 3424, 2949, 1539, 1209, 1157; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 9.4 Hz, 1H), 6.48-6.46 (m, 2H), 5.39 (s, 1H), 4.40 (q, J = 7.4 Hz, 1H), 3.82-3.78 (m, 6H), 3.47-3.33 (m, 2H), 2.68-2.67 (m, 2H), 2.19 (dt, J = 17.8, 4.8 Hz, 1H), 2.02-1.59 (m, 14H), 1.51-1.08 (m, 6H), 1.03-0.95 (m, 6H), 0.80-0.77 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.8, 157.0, 151.3, 143.8, 136.4, 123.1, 120.8, 119.3, 118.1, 109.4, 103.8, 99.4, 80.9, 66.9, 62.1, 56.6, 55.9, 55.7, 48.0, 41.7, 40.4, 39.8, 36.8, 34.2, 31.9, 31.8, 31.4, 31.2, 30.4, 28.9, 23.5, 21.2, 18.8, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{36}H_{48}N_2O_4S$ $[M+H]^+$ = 605.3407, found 605.3410.



17

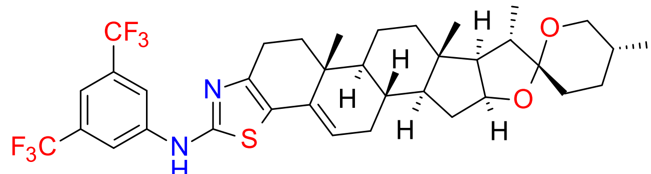
(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-(3,4,5-trimethoxyphenyl)spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (18). Grey powder (507 mg, 80%); IR (KBr pellet, cm^{-1}) 3407, 2952, 1610, 1234, 1155; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.56 (s, 2H), 5.41 (s,



18

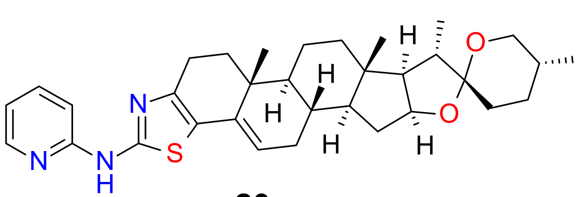
1H), 4.41 (m, 1H), 3.85-3.80 (m, 9H), 3.46-3.33 (m, 2H), 2.66-2.62 (m, 2H), 2.23-2.15 (m, 1H), 2.02-1.59 (m, 11H), 1.51-1.08 (m, 7H), 1.03 (s, 3H), 0.96 (d, J = 6.9 Hz, 3H), 0.80-0.76 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.2, 153.8, 145.1, 136.8, 136.5, 134.1, 120.2, 118.3, 109.4, 97.2, 80.9, 66.9, 62.1, 61.1, 56.6, 56.2, 48.0, 41.7, 40.4, 39.8, 36.8, 34.3, 31.9, 31.8, 31.4, 31.2, 30.4, 28.9, 24.2, 21.3, 18.8, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{37}H_{50}N_2O_5S$ $[M+H]^+$ = 635.3513, found 635.3519.

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-[3,5-bis(trifluoromethyl)phenyl]-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (19). Light yellow solid (530 mg, 78%); IR (KBr pellet, cm^{-1}) 3445, 2954, 1622, 1210, 1149; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 2H), 7.45 (s, 1H), 5.51 (s, 1H), 4.43 (q, J = 7.4 Hz, 1H), 3.48-3.34 (m, 2H), 2.74-2.70 (m, 2H), 2.26-2.19 (m, 1H), 2.03-1.99 (m, 2H), 1.90-1.61 (m, 10H), 1.53-1.10 (m, 7H), 1.05 (s, 3H), 0.97 (d, J = 6.9 Hz, 3H), 0.82-0.77 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 159.7, 145.4, 141.9, 136.2, 132.7 ($^2J_{\text{C-CF3}}$ = 33.2 Hz), 123.3 ($^1J_{\text{C-CF3}}$ = 272.6 Hz), 122.5, 119.9, 117.1, 115.3, 109.5, 80.9, 67.0, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.2, 32.0, 31.9, 31.4, 31.2, 30.4, 28.9, 24.2, 21.3, 18.8, 17.2, 16.5, 14.6; ^{19}F decoupled ^{13}C-NMR (101 MHz, $CDCl_3$) δ 159.7, 145.4, 141.9, 136.2, 132.7, 123.3, 122.5, 119.9, 117.1, 115.3, 109.5, 80.9, 67.0, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.8, 34.2, 32.0, 31.9, 31.4, 31.2, 30.4, 28.9, 24.2, 21.3, 18.8, 17.2, 16.5, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{36}H_{42}F_6N_2O_2S$ $[M+H]^+$ = 681.2943, found 593.2950.



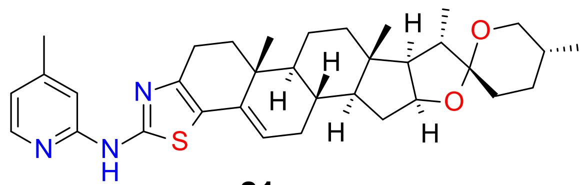
19

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-(2-pyridyl)spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (20). Grey powder (457 mg, 84%); IR (KBr pellet, cm^{-1}) 3458, 2950, 1604, 1220, 1152; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.54 (s, 1H), 6.84 (d, J = 4.1 Hz, 2H), 5.65 (s, 1H), 4.42 (s, 1H), 3.45-3.35 (m, 2H), 2.70 (s, 2H), 2.26-2.20 (m, 1H), 2.00-1.61 (m, 13H), 1.49-1.14 (m, 7H), 1.03-0.95 (m, 6H), 0.81-78 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 158.3, 151.6, 147.1, 143.1, 137.8, 136.7, 123.1, 118.3, 116.4, 110.7, 109.4, 80.9, 66.9, 62.1, 56.7, 48.1, 41.7, 40.4, 39.9, 36.7, 34.5, 32.0, 31.9, 31.5, 31.2, 30.4, 28.9, 23.9, 21.3, 18.8, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{33}H_{43}N_3O_2S$ $[M+H]^+$ = 546.3149, found 546.3152.



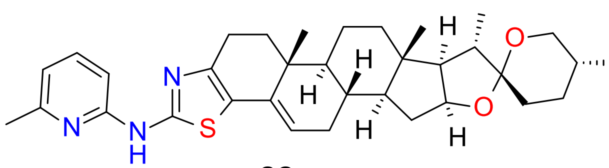
20

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-(4-methyl-2-pyridyl)spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.02,10.05,9.014,21.016,20]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (21). White powder (475 mg, 85%); IR (KBr pellet, cm^{-1}) 3470, 2948, 1620, 1220, 1154; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 5.6 Hz, 1H), 6.70 (s, 2H), 5.65 (s, 1H), 4.41 (s, 1H), 3.46-3.37 (m, 2H), 2.69 (s, 2H), 2.51 (s, 3H), 2.27-2,22 (m, 1H), 1.99-1.60 (m, 13H), 1.48-1.14 (m, 7H), 1.02-0.97 (m, 6H), 0.81-0.78 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.7, 156.3, 150.3, 140.9, 138.1, 136.5, 122.5, 118.5, 116.0, 109.4, 107.9, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.7, 34.3, 32.0, 31.9, 31.5, 31.2, 30.4, 28.9, 23.8, 23.2, 21.3, 18.7, 17.3, 16.5, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{34}H_{45}N_3O_2S$ $[M+H]^+$ = 560.3305, found 560.3307.



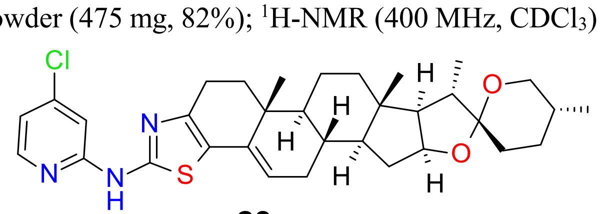
21

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-(6-methyl-2-pyridyl)spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (22). Grey solid powder (469 mg, 84%); ^{1}H-NMR (400 MHz, CDCl3) δ 8.17 (d, J = 5.3 Hz, 1H), 6.76-6.70 (m, 2H), 5.66-5.64 (m, 1H), 4.40 (q, J = 7.4 Hz, 1H), 3.47-3.34 (m, 2H), 2.66-2.62 (m, 2H), 2.30 (s, 3H), 2.26-2.18 (m, 1H), 2.03-1.59 (m, 13H), 1.52-1.06 (m, 7H), 1.00-0.95 (m, 6H), 0.80-77 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 159.7, 151.0, 149.4, 146.3, 139.6, 136.0, 122.0, 118.9, 118.6, 111.7, 109.4, 80.9, 66.9, 62.1, 56.6, 47.9, 41.7, 40.4, 39.8, 36.6, 34.0, 32.0, 31.9, 31.4, 31.2, 30.4, 28.9, 22.4, 22.1, 21.3, 18.7, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{34}H_{45}N_3O_2S$ $[M+H]^+$ = 560.3305, found 560.3303.



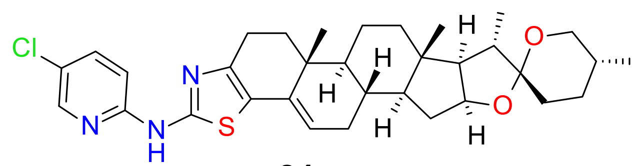
22

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(4-chloro-2-pyridyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (23). Grey solid powder (475 mg, 82%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 5.5 Hz, 1H), 6.94 (s, 1H), 6.85 (d, J = 5.5 Hz, 1H), 5.64 (s, 1H), 4.42-4.37 (m, 1H), 3.47-3.33 (m, 2H), 2.64 (d, J = 6.4 Hz, 2H), 2.21 (dt, J = 17.6, 4.7 Hz, 1H), 2.02-1.59 (m, 12H), 1.50-1.05 (m, 7H), 1.00-0.93 (m, 6H), 0.80-0.77 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 159.1, 152.1, 147.7, 145.1, 140.5, 136.1, 122.8, 119.1, 117.2, 111.0, 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.6, 34.1, 31.9, 31.8, 31.4, 31.2, 30.4, 28.9, 22.6, 21.9, 18.7, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{33}H_{42}ClN_3O_2S$ $[M+H]^+$ = 580.2759, found 580.2760.



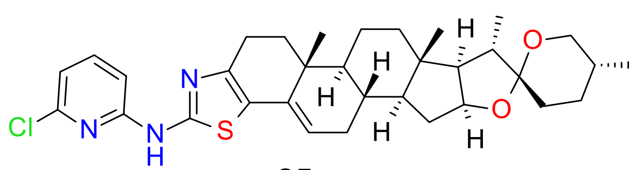
23

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(5-chloro-2-pyridyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (24). Grey solid powder (498 mg, 86%); IR (KBr pellet, cm^{-1}) 3407, 2950, 2871, 1597, 1120, 1155; ^{1}H-NMR (400 MHz, CDCl$_3$) δ 8.26 (d, *J* = 2.3 Hz, 1H), 7.51 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.86 (d, *J* = 8.7 Hz, 1H), 5.65 (d, *J* = 1.8 Hz, 1H), 4.41 (q, *J* = 7.4 Hz, 1H), 3.47-3.34 (m, 2H), 2.66 (d, *J* = 6.0 Hz, 2H), 2.23 (dt, *J* = 17.7, 4.7 Hz, 1H), 2.04-1.59 (m, 12H), 1.48-1.07 (m, 5H), 1.01-0.94 (m, 6H), 0.81-0.77 (m, 9H); ^{13}C-NMR (101 MHz, CDCl$_3$) δ 158.5, 149.7, 145.5, 137.7, 136.3, 123.8, 123.1, 119.0, 112.0, 109.4, 80.9, 66.9, 62.1, 56.6, 48.0, 41.7, 40.4, 39.8, 36.6, 34.3, 32.0, 31.9, 31.4, 31.2, 30.4, 28.9, 23.3, 22.1, 21.3, 18.8, 17.3, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{33}H_{42}ClN_3O_2S$ $[M+H]^+$ = 580.2759, found 580.2762.



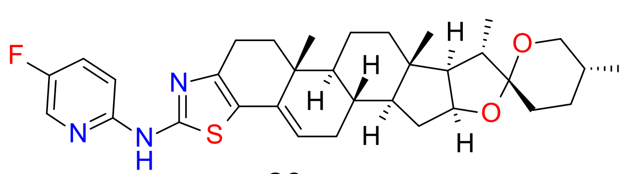
24

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(6-chloro-2-pyridyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (25). Pink solid powder (475 mg, 82%); IR (KBr pellet, cm^{-1}) 3446, 2946, 2871, 1617, 1220, 1158; ^{1}H-NMR (400 MHz, CDCl$_3$) δ 7.50-7.47 (m, 1H), 6.86-6.77 (m, 2H), 5.69 (s, 1H), 4.41 (s, 1H), 3.48-3.35 (m, 2H), 2.69 (s, 2H), 2.27-2.22 (m, 1H), 1.99-1.60 (m, 12H), 1.48-1.13 (m, 7H), 1.03-0.97 (m, 6H), 0.82-0.78 (m, 6H); ^{13}C-NMR (101 MHz, CDCl$_3$) δ 158.2, 151.6, 148.9, 142.4, 139.9, 136.3, 123.8, 119.2, 115.9, 109.4, 108.9, 80.9, 66.9, 62.2, 56.6, 48.0, 41.7, 40.4, 39.8, 36.6, 34.4, 32.0, 31.9, 31.5, 31.2, 30.4, 28.9, 23.8, 21.3, 18.7, 17.2, 16.5, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{33}H_{42}ClN_3O_2S$ $[M+H]^+$ = 580.2759, found 580.2760.



25

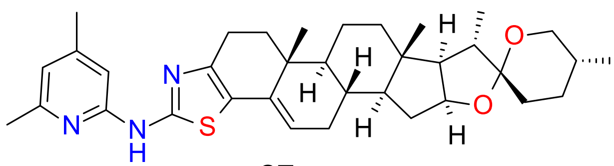
(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(5-fluoro-2-pyridyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (26). Grey solid powder (450 mg, 80%); IR (KBr pellet, cm^{-1}) 3492, 2949, 1627, 1229, 1155; ^{1}H-NMR (400 MHz, CDCl$_3$) δ 8.17 (d, *J* = 2.8 Hz, 1H), 7.35 (td, *J* = 8.4, 2.5 Hz, 1H), 6.92 (dd, *J* = 8.9, 3.4 Hz, 1H), 5.63 (d, *J* = 2.1 Hz, 1H), 4.40 (q, *J* = 7.5 Hz, 1H), 3.47-3.33 (m, 2H), 2.63 (d, *J* = 6.2 Hz, 2H), 2.22 (dt, *J* = 17.7, 4.7 Hz, 1H), 2.03-1.59 (m, 12H), 1.47-1.05 (m, 5H), 0.99-0.93 (m, 6H), 0.82-0.77 (m, 9H); ^{13}C-NMR (101 MHz, CDCl$_3$) δ 159.7, 155.2 ($^1J_{C\text{-}F}$ = 247.6 Hz), 147.5, 139.8, 136.0, 133.7 ($^2J_{C\text{-}F}$ = 26.0 Hz), 125.9 ($^2J_{C\text{-}F}$ = 20.7 Hz), 121.6, 119.0, 112.3 ($^3J_{C\text{-}F}$ = 4.33 Hz), 109.4, 80.9, 66.9, 62.1, 56.6, 47.9, 41.7, 40.4, 39.8, 36.6,



26

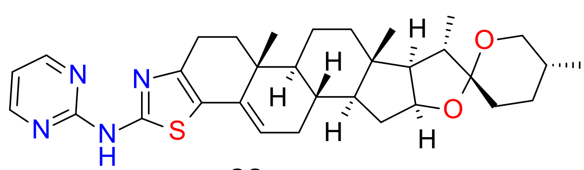
34.0, 31.9, 31.4, 31.2, 30.4, 28.9, 22.4, 22.0, 21.3, 18.7, 17.2, 16.5, 14.6. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{33}H_{42}FN_3O_2S$ $[M+H]^+$ = 564.3054, found 564.3057.

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-*N*-(4,6-dimethyl-2-pyridyl)-2,5',19,21-tetramethyl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (27). Light yellow solid (492 mg, 86%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.56 (d, J = 3.1 Hz, 2H), 5.64 (d, J = 2.2 Hz, 1H), 4.38 (dd, J = 15.0, 7.3 Hz, 1H), 3.48-3.34 (m, 2H), 2.63 (d, J = 6.1 Hz, 2H), 2.46 (s, 3H), 2.25-2.19 (m, 4H), 2.01-1.58 (m, 12H), 1.48-1.06 (m, 7H), 0.99-0.95 (m, 6H), 0.80-0.77 (m, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 159.9, 155.7, 150.3, 149.5, 139.7, 136.3, 121.9, 118.6, 117.6, 109.4, 108.4, 80.9, 66.9, 62.1, 56.5, 47.9, 41.7, 40.4, 39.8, 36.6, 34.1, 31.9, 31.5, 31.2, 30.4, 28.9, 23.6, 22.4, 22.3, 21.3, 18.7, 17.3, 16.4, 14.6; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{35}H_{47}N_3O_2S$ $[M+H]^+$ = 574.3461, found 574.3464.



27

(1*S*,2*R*,5'*R*,13*S*,14*S*,16*S*,18*R*,19*S*,20*R*,21*S*)-2,5',19,21-tetramethyl-*N*-pyrimidin-2-yl-spiro[17-oxa-8-thia-6-azahexacyclo[11.10.0.0^{2,10}.0^{5,9}.0^{14,21}.0^{16,20}]tricosa-5(9),6,10-triene-18,2'-tetrahydropyran]-7-amine (28). Grey solid (409 mg, 75%); ^{1}H-NMR (400 MHz, TFA-*d*) δ 9.09 (d, J = 3.7 Hz, 2H), 7.67-7.65 (m, 1H), 6.05 (s, 1H), 4.76 (dd, J = 14.3, 6.9 Hz, 1H), 3.79-3.77 (m, 1H), 3.53-3.48 (m, 1H), 2.94-2.83 (m, 2H), 2.37-2.17 (m, 3H), 2.03-1.50 (m, 13H), 1.39-1.22 (m, 4H), 1.08-1.06 (m, 6H), 0.91 (s, 3H), 0.82 (d, J = 5.6 Hz, 3H); ^{13}C-NMR (101 MHz, TFA-*d*) δ 157.6, 150.0, 133.6, 132.8, 127.7, 125.8, 117.0, 83.2, 67.4, 60.2, 55.9, 47.4, 41.9, 40.2, 38.8, 36.4, 32.2, 31.1, 30.5, 30.3, 29.1, 27.0, 20.5, 19.6, 16.7, 14.7, 12.0, 11.9; HRMS (ESI-FTMS Mass (m/z): calcd for $C_{32}H_{42}N_4O_2S$ $[M+H]^+$ = 547.3101, found 547.3103.



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