Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 1073–1083

BIOORGANIC & MEDICINAL CHEMISTRY

Structure–Activity Relationship of Aza-Steroids as PI-PLC Inhibitors

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Received 16 August 2000; accepted 7 November 2000

Abstract—A number of aza-steroids were synthesized as potent phosphatidylinositol phospholipase C (PI-PLC) inhibitors. The epimeric mixtures 22,25-diazacholesterol (**8a**) and 3β-hydroxy-22,25-diazacholestane (**8b**) were among the most active of these inhibitors, with IC_{50} values of 7.4 and 7.5 μM, respectively. The 20α epimer, **8a2** ($IC_{50}=0.64$ μM), whose stereochemistry at C-20 coincides with that of cholesterol, was found 50 times more potent than the 20β epimer, **8a1** ($IC_{50}=32.2$ μM). In diaza-estrone derivatives, the 3-methoxy group on the aromatic A-ring of **23** exhibited moderate PI-PLC inhibitory activity ($IC_{50}=19.7$ μM), while compound with a free hydroxyl group (**21**) was inactive. However, in diaza-pregnane derivatives, epimers with a 3-hydroxyl group (**8a**, $IC_{50}=7.4$ μM) exhibited more potent PI-PLC inhibitory activity than their counterparts with 3-methoxyl group on the non-aromatic A-ring (**26**, $IC_{50}=17.4$ μM). We have illustrated in our previous publication that 3-hydroxyl-6-aza steroids are potent PI-PLC inhibitors.[3] However, simultaneous presence of the 6-aza and 22,25-diaza moieties in one molecule as in **13**, led to loss of activity. Epimeric mixture **8a** showed selective growth inhibition effects in the NCI in vitro tumor cell screen with a mean GI_{50} value (MG-MID) of 5.75 μM for 54 tumors. © 2001 Published by Elsevier Science Ltd.

Introduction

Phosphatidylinositol and its phosphorylated derivatives represent less than 6–8% of the components of the membrane of eukaryotic cells.[1a] Until recently, they had been considered as relatively inert substances. However, it has been recognized that inositol phospholipids are crucially involved in the receptor-mediated activation of signal transduction pathways initiated by action of hormones and growth factors.[1] Phosphatidylinositol phospholipase C (PI-PLC) is a key enzyme located at the inner side of cell membrane and catalyzes hydrolysis of a minor membrane phospholipid, phosphatidylinositol (4,5)-biphosphate ($PI(4,5)P_2$), as a result of activation of receptors at the cell surface. The resulting products are the second messengers, inositol (1,4,5)-triphosphate ($I(1,4,5)P_3$), which releases Ca^{2+} from intracellular stores to increase intracellular free Ca^{2+} concentration; and diacylglycerol, which activates the Ca^{2+} and phospholipid-dependent protein serine/threonine kinase, protein kinase C. Together, the increase in intracellular free Ca^{2+} and the activation of protein kinase C result in a series of profound cellular changes, such as DNA synthesis, cell proliferation, and neuronal activity.

Abnormal functions of PIPLC have been linked[2] to cancer and Alzheimer's disease. Increased PI-PLC activity has been reported in a number of human tumors, especially in the more aggressive malignant tumors.[3–6] The growth inhibitory effect of tamoxifen on $GH_4\ C_1$ cancer cells has been linked to inhibition of PI-PLC.[7] Therefore, selective small molecule inhibitiors of over activated PI-PLC signaling pathways may provide potential therapies for cancer.

We have recently described 6-aza-steroids as potent PI-PLC inhibitors.[3] The most active of these compounds, 3β-hydroxy-6-aza-cholestane (**1**, Fig. 1) showed an IC_{50} of less than 1.8 μM against PI-PLC; it also showed significant growth inhibition effects on HT-29 colon cancer cells ($IC_{50}=1.3$ μM) and MCF-7 breast cancer cells ($IC_{50}=1.3$ μM). Structure–activity relationships revealed that the free amino group at the 6-position played a crucial role in PIPLC inhibition, and the presence of the

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0968-0896/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0968-0896(00)00302-3

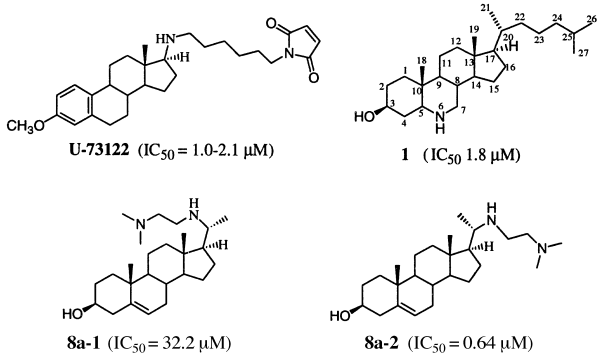
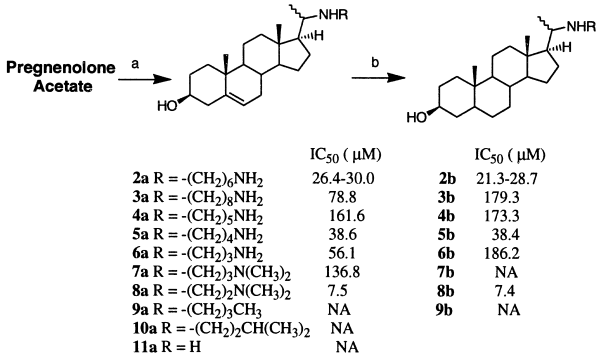


Figure 1.



	IC_{50} (μM)		IC_{50} (μM)
2a R = -$(CH_2)_6NH_2$	26.4-30.0	**2b**	21.3-28.7
3a R = -$(CH_2)_8NH_2$	78.8	**3b**	179.3
4a R = -$(CH_2)_5NH_2$	161.6	**4b**	173.3
5a R = -$(CH_2)_4NH_2$	38.6	**5b**	38.4
6a R = -$(CH_2)_3NH_2$	56.1	**6b**	186.2
7a R = -$(CH_2)_3N(CH_3)_2$	136.8	**7b**	NA
8a R = -$(CH_2)_2N(CH_3)_2$	7.5	**8b**	7.4
9a R = -$(CH_2)_3CH_3$	NA	**9b**	NA
10a R = -$(CH_2)_2CH(CH_3)_2$	NA		
11a R = H	NA		

Scheme 1. Reagents and conditions: (a) (i) RNH_2, *p*-TsOH, toluene, reflux; (ii) 1.2 equiv $NaBH_4$, CH_3OH/THF, pH 6; K_2CO_3 in CH_3OH/H_2O; or NH_4OAc, $NaB(CN)H_3$, CH_3OH/THF; (b) H_2, 5% Pd/C, AcOH.

hydrophobic cholesteryl side chain and the 3β-hydroxy group are also critical for inhibitory activity.

Comparing the structure of compound **1** with that of the well studied diaza steroidal PI-PLCγ inhibitor, U73122 (Fig. 1, $IC_{50} = 1.0–2.1$ μM),[8] lead us to reason that other steroidal derivatives with amino groups on the side chain and/or in the ring system might have improved inhibitory potencies. Herein, we report a series of aza-steroid derivatives of different ring features and amino group arrangements synthesized from readily available starting materials, as PI-PLC inhibitors and potential antitumor and anticancer agents.

Chemistry

The syntheses[9] of 3β-hydroxy-20-aza-pregnene derivatives **2a**–**10a** and 3β-hydroxy-20-aza-pregnane derivatives **2b**–**10b** are illustrated in Scheme 1. Pregnenolone acetate was first treated with primary amines and a catalytic amount of *p*-TsOH in toluene under reflux overnight to form the intermediate imines. Treatment of the resulting imines with 1.2 equiv $NaBH_4$ in methanol and THF afforded the corresponding 3β-hydroxy-20-aza-pregnene derivatives **2a**–**10a** in 80–85% yield. The ratio of 20β/20α isomers was about 1.5–2.5:1, as determined by ^1H NMR spectroscopy. Pregnenolone acetate was treated with NH_4OAc in methanol/THF, and $NaB(CN)H_3$ at pH = 6 to give compound **11a** in 85% yield (20β : 20α ≈ 3:1). Hydrogenation of aza-steroids **2a**–**9a** in glacial acetic acid with 10% Pd/C at room temperature gave the saturated 20-aza steroids **2b**–**9b** in 80–90% yield.

As shown in Scheme 2, treatment of **8a** with excess $NaBH_4$ (6.0 equiv) in methanol/THF, also afforded 20% of the unnatural 17α epimers. The ratio of 17α, 20α-isomer/17α, 20β-isomer was about 1:1 as determined by ^1H NMR spectroscopy. Separation of the

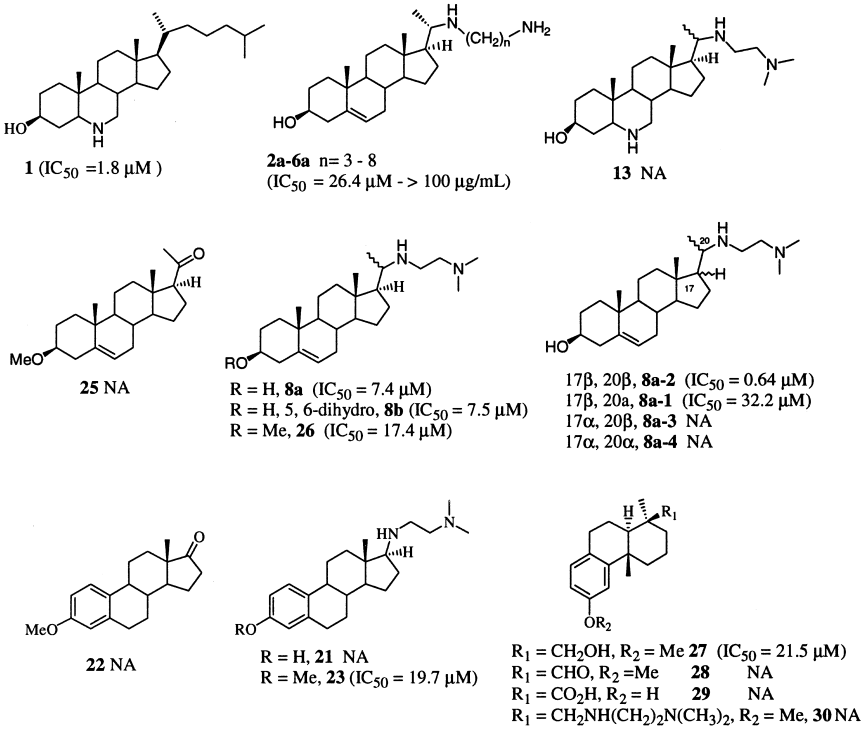
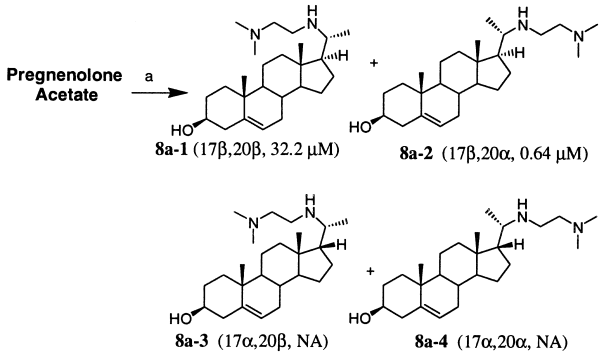


Chart 1.



Scheme 2. Reagents and conditions: (a) (i) *N,N*-dimethylethylenediamine, *p*-TsOH, toluene, reflux; (ii) 6.0 equiv $NaBH_4$, CH_3OH/THF, pH 6; K_2CO_3 in CH_3OH/H_2O.

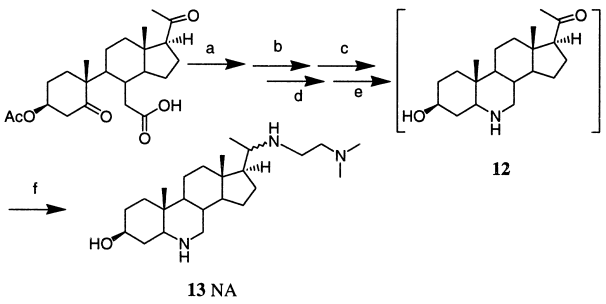
20,25-diaza-cholesterol diastereomers in mixture **8a** was performed by flash column chromatography on silica gel with ethyl acetate, petroleum ether and triethyl amine as eluent to afford the four pure diastereomers. The sequence by which these compounds being eluted were **8a1**, **8a2**, and then the two 17α epimers, **8a3** and **8a4**.

To establish the stereochemistry of the two epimers, **8a1** and **8a2**, literature precedures[10] were followed for comparison purpose. Pregnenolone acetate was treated with *N,N*-dimethylethylenediamine and $NaB(CN)H_3$ in methanol/THF at pH 6, followed by treatment with K_2CO_3 in methanol and H_2O to afford 20,25-diaza-cholesterols **8a1** and **8a2**. It has been well documented[10] that the 20β-epimer was the predominant product when 20-imines were reduced with $NaB(CN)H_3$. The ^1H NMR spectrum showed a 4:1 ratio for the two isomers, indicating that 80% of the mixture should be the 20β-amine (**8a1**) and 20% of the product should be the 20α-amine (**8a2**). On TLC, the major product, 20β-amine, was more mobile than the minor product, 20α-amine.

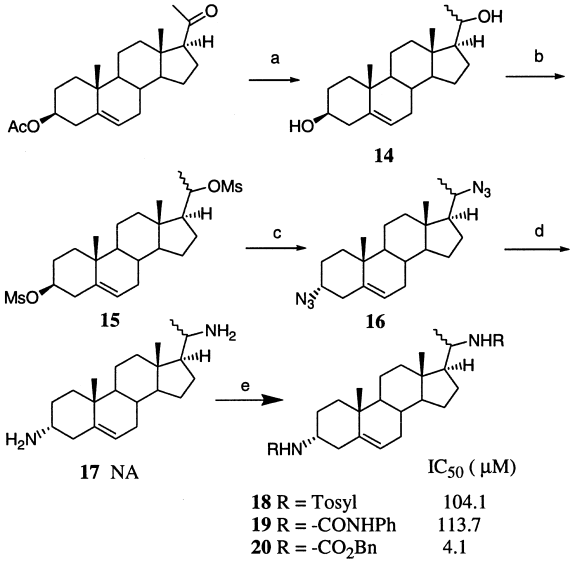
The assignment of the structures to the 17α, 20β (**8a3**) and 17α, 20α (**8a4**) isomers was not unequivocal. It was well established that the side chain of cholesteryl derivatives with 20β conformation oriented to the 'left-handed' conformation.[11] The less extended structure of 20β-amines **8a1** might account for the observed higher mobility of **8a1** compared to **8a2**. Therefore, the more mobile isomer **8a3** in the two 17α epimers was assigned the 17α, 20β stereochemistry.

The synthesis of 3β-hydroxy-6,20,25-triaza-cholestane **13** was carried out as shown in Scheme 3. Pregnenolone acetate was converted to the intermediate 3β-hydroxy-6-aza-pregnan-20-one (**12**) in six steps.[12] Reaction of ketone **12** with *N,N*-dimethylethylenediamine and $NaB(CN)H_3$ in CH_3OH/THF at pH 6 yielded 3β-hydroxy-6, 20,25-triaza-cholestane **13** in 5% yield from pregnenolone acetate. The ratio of the two isomers was 20β:20α = 3:1.

The syntheses of 3,20-diaza-pregnene derivatives **17**–**20** are illustrated in Scheme 4. Reduction of pregnenolone acetate in methanol/THF with $NaBH_4$ yielded the corresponding diol **14**[13] in 90% yield. Treatment of diol **14** with MsCl in pyridine and methylene chloride with cat. DMAP afforded the dimesylate **15**, which was dissolved in



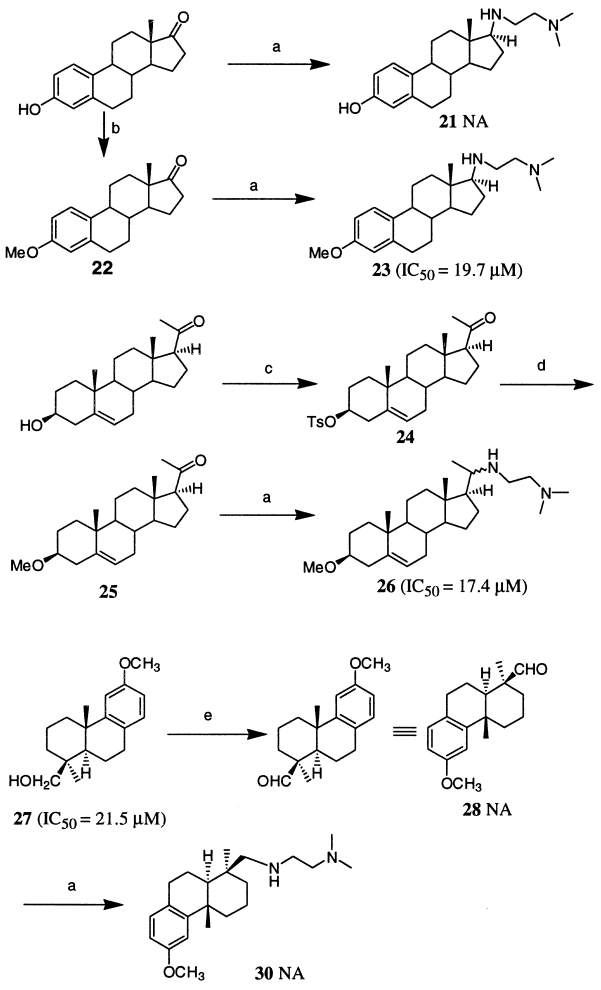
Scheme 3. Reagents and conditions: (a) $(COCl)_2/CH_2Cl_2$, pyridine; (b) NaN_3/acetone and H_2O; (c) benzene, reflux; (d) 12 N HCl/acetone; (e) 10% Pd/C/H_2, AcOH, then K_2CO_3 in methanol and H_2O; (f) *N,N*-dimethylethylenediamine, CH_3OH/THF, $NaB(CN)H_3$, pH 6.



Scheme 4. Reagents and conditions: (a) $NaBH_4$, CH_3OH, THF, 96%; (b) MsCl, pyridine, CH_2Cl_2, 90%; (c) NaN_3, acetone, H_2O, 85%; (d) $LiAlH_4$, THF 63%; (e) (i) TsCl, pyridine, CH_2Cl_2, **18**, 78%; (ii) phenyl isocyanate, THF, **19**, 95%; (3) benzyl chloroformate, THF, CH_2Cl_2, Et_3N, **20**, 81%.

acetone and treated with NaN_3/water to give diazide **16** in quantitative yield. Reduction of **16** with $LiAlH_4$ in THF afforded 3α,20-diamine **17** (20β:20α ≈ 3:1). Reaction of diamine **17** with *p*-toluenesulfonyl chloride, phenyl isocyanate, and benzyl chlorformate gave tosylate **18**, urea **19**, and carbamate **20**, respectively.

The syntheses of diaza-estrone derivatives **21** and **23** followed the same procedure, and are illustrated in Scheme 5. Estrone was allowed to react with $(CH_3O)_2SO_2$ in ethanol and NaOH in water to give 3-methoxy-estra-1,3,5(10)-triene-20-one (**22**)[15] in 57% yield. Treatment of estrone or compound **22** with *N,N*-dimethylethylenediamine and $NaB(CN)H_3$ in CH_3OH/THF at pH 6 gave diaza-estrone derivatives **21** or **23** in 85 and 80% yield, respectively. In these reactions, only 17β-epimers were found. Reaction of pregnenolone with TsCl in pyridine gave a quantitative amount of ester **24**. Reflux of compound **24** in methanol afforded pregnenolone methyl ether **25**[16] in 80% yield. Reductive amination of **25**, using the same procedure described previously, gave 22,25-diaza-cholesterol methyl ether **26** in 86% yield (Scheme 5).



Scheme 5. Reagents and conditions: (a) *N*,*N*-dimethylethylenediamine, CH_3OH/THF, $NaB(CN)H_3$, pH 6, 80–85%; (b) $(CH_3O)_2SO_2$, ethanol, NaOH in water, 57%; (c) TsCl, pyridine, CH_2Cl_2, 90%; (d) methanol, reflux, 95%; (e) $(COCl)_2$, DMSO, Et_3N, 85%.

The synthesis of diaza-podocarpatriene derivative **29** is illustrated in Scheme 5). Swern oxidation of 12-methoxy-podocarpatrien-16-ol (**27**) gave the corresponding aldehyde **28** in 85% yield. Reductive amination of **27** with *N*,*N*-dimethylethylenediamine using $NaB(CN)H_3$ gave diaza-podocarpatriene derivative **29** in 80% yield.

Results and Discussion

Structure–activity relationships

In the series of diaza-steroids with two free amino groups **2a**–**6a** and **2b**–**6b** (Scheme 1), 3β-hydroxy-20-aza-(*N*-hexylamine)-pregnene (**2a**, IC_{50} = 26.0–30.0 μM) and 3β-hydroxy-20-aza-(*N*-hexylamine)-pregnane (**2b**, IC_{50} = 21.3–28.7 μM) with six carbons between the two amino groups gave the best PLC inhibition. Compounds with longer side chains, as in 3β-hydroxy-20-aza-(*N*-octylamine)-pregnene (**3a**, IC_{50} = 78.8 μM) and 3β-hydroxy-20-aza-(*N*-octylamine)-pregnane (**3b**, IC_{50} = 179.3 μM) with eight carbon atoms between the two amino groups exhibited much less PI-PLC inhibition. With five, four and three carbons between the two amino groups in the side chains, compounds **4a**/**4b**, **5a**/**5b**, **6a**/**6b** also showed reduced inhibitory activities (IC_{50} = 38.6–186.2 μM, Scheme 1).

It is interesting that when the terminal amino group was masked by methyl groups, with two carbon atoms between the two amino group, **8a** and **8b**, the most potent PIPLC inhibition (IC_{50} = 7.5, 7.4 μM, respectively) was observed. However, when the number of carbon atoms was increased to three as in **7a** and **7b**, the activity dropped significantly (Scheme 1). Without the terminal amino group, (**9a**), 3β-hydroxy-20-aza-(*N*-butane)-pregnane (**9b**), and 20-aza-cholesterol **10a** were inactive. 3β-Hydroxy-20-aza-pregn-5-ene (**11a**), also showed no activity. It was observed that compounds (**2a**/**2b**, **5a**/**5b** and **8a**/**8b**), with even numbers of carbon atoms between the two amino groups in the side chain, gave significantly better inhibitory activities than those compounds (**4a**/**4b**, **6a**/**6b**, and **7a**/**7b**) with odd numbers of carbon atoms (Scheme 1). Better complementary hydrogen bonding arrangements of compounds **2a**/**2b**, **5a**/**5b** and **8a**/**8b** and their PIPLC bind site might account for these differences in activities. Saturation of the double bond at C-5 did not affect the inhibition potencies of compounds with good PIPLC inhibitory activity, such as **2a**, **5a** and **8a**. However, removal of the C-5 double bond greatly reduced inhibition activity of those compounds (**3a** and **6a**) with moderate PIPLC inhibitory activity (Scheme 1). This may indicate amplification of unfavorable orientation by saturation of the double bond.

It has been documented previously that the side-chain stereochemistry at C-20 had dramatic effects on biological activity, with the natural 20α isomers exhibiting more potent activities.[17,18] To differentiate PI-PLC inhibition activities, the two epimers, 20β,25-diaza-cholesterol (**8a1**) and 20α,25-diaza-cholesterol (**8a2**) were separated by flash column chromatography on silica gel, with ethyl acetate, petroleum ether and triethyl amine as eluent; amine **8a1** was the more mobile component. As shown in Scheme 2, the PIPLC inhibitory activities of these two epimers were significantly different, with compound **8a2** (IC_{50} = 0.64 μM) being 50 times more active than **8a1** (IC_{50} = 32.2 μM). This result is consistent with the previous observation on the hypocholesterolemic activity of this series that with a natural 20α stereocenter as that of cholesterol, epimer **8a2** has better biological activity.[17] Furthermore, when the stereochemistry of C-17 was changed from the natural β as in cholesterol to α as in 17α-20β,25-diaza-cholesterol (**8a3**) and 17α-20α,25-diaza-cholesterol (**8a4**), the PIPLC inhibitory activities were dramatically reduced. It seems that departure from the natural stereochemistry of cholesterol leads to marked reduction in biological activity.

We previously reported that 3β-hydroxy-6-aza-cholestane (**1**) was a potent PI-PLC inhibitor (IC_{50} = 1.8 μM) primarily due to the presence of the hydroxyl and the amino groups.[3] 3β-Hydroxy-6,20,25-triaza-cholestane (**13**) was synthesized in an attempt to combine the 6-aza feature of compound **1** and 20,25-diaza feature of compound **8a** into one molecule. Unfortunately, the resulting combination lead to acute loss of activity (Scheme 3).

This observation suggests that the hydrophobic side chain in compound **1** contributes significantly to its binding affinity to PIPLC. Compounds **1** and **8a2** might not bind to the same site of the enzyme or they might orient completely differently to find the most favorable hydrophobic and hydrophilic complements if they do bind to the same site in the enzyme. Similarly, compound **17**, with simultaneous presence of amino groups at C-3 and C-21, showed no PIPLC inhibitory activity. Among the derivatives of diamine **17**, including *p*-tosylate **18**, urea **19**, and carbamate **20**, only carbamate **20** exhibited significant PIPLC inhibition with an IC_{50} value of 4.1 μM (Scheme 4). Again, this compound might bind to a completely different site from those of compounds **1** and **8a2**.

It was previously observed that removal of 3-methoxy group in U73122 resulted in a pronounced loss of inhibitory activity.[9] To test the effects of a 3-methoxy group in an ring A aromatic aromatic in the diaza series, diaza-estrane derivative **21**, diaza-3-methoxy-estrane derivative **23** and diaza-3-methoxy-aza-pregnane derivative **26** were synthesized. As shown in Scheme 5, with the presence of aromatic A-ring, compound **23** containing the 3-methoxy group and the diaza-side chain showed an IC_{50} value of 19.7 μM, while compound **21**, with a 3-hydroxyl group, was not active. However, in the aza-pregnane PIPLC inhibitor series, with the 3-methoxy group, 20, 25-diaza-cholesterol-methyl ester (**26**) was less potent ($IC_{50}=17.9$ μM) than its 3-hydroxy counterpart, **8a** ($IC_{50}=7.9$ μM).

(+)-Podocarpic acid (**29**) is a representative member of the podocarpene diterpenoids isolated from *Podocarpus capressina* var. *imbricata*.[19] Random screening of our in house chemical library in the PIPLC in vitro assay revealed 2-methoxy-podocarpatriene-16-ol (**27**) as a moderate PIPLC inhibitor ($IC_{50}=21.5$ μM), whereas (+)-podocarpic acid (**29**, Chart 1) and 12-methoxy-podocarpatrien-16-al (**28**) were not active. In hope of discovery of potent non-steroidal PI-PLC inhibitors, we synthesized diaza-podocarpatriene derivative **30**. Unfortunately, addition of diaza side chain as in **30** led to loss of inhibition. Thus, the methylene hydroxyl group in **27** must contribute greatly to its observed activity.

NCI 60-human tumor cell lines screen

The in vitro cytotoxicity of epimeric mixture **8a** (20α:20β = 1:1) was evaluated at the National Cancer Institute against a panel of 60 human tumor cell lines representing nine different cancer types. The GI_{50}, TGI and GI_{50} values for some sensitive subpanels are shown in Table 1. Mixture **8a** showed a mean GI_{50} value (MG-MID) of 5.75 μM for 54 tumors. It was more selective against SR leukemia cancer cell line, HT-29 and SW-620 colon cancer cell lines, and LOX IMVI melanoma cancer cell lines. It also showed some selective inhibition on MDA-MB-435 breast cancer cell, DU-145 and PC-3 prostate cancer. This is consistent with the observation that PI-PLC activity is found to be increased in a number of human tumors, like human breast cancers, human non-small cell lung cancer and colon cancer.[3,4] However, we cannot rule out cytotoxicity contributions by inhibiting other crucial enzymes, such as 24,25-reductase operating in the terminal stage of cholesterol synthesis.[20]

Table 1. NCI 60-human cell line screen of compound **8a**

Cell lines	8a		
	GI_{50}[a]	TGI[b]	LC_{50}[c]
Leukemia			
K-562	4.17	11.5	>25.0
SR	0.003	7.94	>25.0
Colon cancer			
HT-29	4.90	>25.0	>25.0
SW-620	4.47	12.5	>25.0
Melanoma			
LOX IMVI	1.51	5.01	12.9
MALME-3M	4.68	8.30	15.1
Ovarian cancer			
OVCAR-8	4.26	7.94	14.8
Prostate cancer			
PC-3	4.68	8.51	15.1
DU-145	3.09	6.92	15.8
Breast cancer			
HS 578T	5.12	11.7	>25.0
MDA-MB-435	4.27	9.12	19.4
MDA-N	4.47	9.33	>25.0
MG-MID[d]	5.75	15.1	22.4

[a]GI_{50} represents the compound concentration (μM) required to achieve 50% inhibition of tumor cell growth.
[b]TGI represents the compound concentration (μM) required to achieve total growth inhibition of tumor cell.
[c]LC_{50} represents the compound concentration (μM) that is lethal to the survival of 50% tumor cell.
[d]MG-MID represents the calculated mean GI_{50}, TGI and LC_{50} for all panels.

Conclusion

A series of aza-steroids have been synthesized as potent PI-PLC inhibitors. The most active compound 20α,25-diaza cholesterol **8a2** ($IC_{50}=0.64$ μM), whose stereochemistry at C-20 coincides with that of cholesterol, showed 50 times more potent PI-PLC inhibition activity than that of the 20β epimer, **8a1** ($IC_{50}=32.2$ μM). The unnatural 17α-diaza isomers **8a3** and **8a4** were essentially inactive. These observations suggest that retention of the natural stereochemistry of cholesterol is important for the PIPLC inhibitory activity in this series of aza-steroids. Simultaneous presence of the active 6-aza and 22, 25-diaza characteristics of two lead compounds (**1** and **8a**) in one molecule, **13**, led to loss of activity.

It was also observed that the 3-methoxy group played a key role in PI-PLC inhibition by diaza-estrone derivatives. Replacing the 3-methoxy group on the aromatic A-ring of compound **23** ($IC_{50}=19.7$ μM, Chart 1) with a hydroxyl group, as in compound **21**, led to loss of activity. This is analogues to the previously reported results for U73122 and its inactive 3-hydroxyl counterpart.[21] However, in diaza-pregnane derivatives,

compounds with a 3-hydroxyl group (**8a**, $IC_{50} = 7.4$ μM, Chart 1) exhibited more potent PI-PLC inhibitory activity than those with a 3-methoxy group in their non-aromatic A-ring (**26**, $IC_{50} = 17.4$ μM, Chart 1).

The side-chain-diaza inhibitors represented by **8a2** might bind to PIPLC in a different orientation or even to a different site from 6-aza cholesterol (**1**). As shown in Chart 1, Pregn-5-en-3, 20 diamino-bis(benzyloxy-carbamate) **20** and 12-methoxy-podocarpatriene-16-ol (**27**) represent two more unique leads for PIPLC inhibition. Different from the phosphatidylinositol ether lipid analogues[22] which likely inhibited PIPLC by mimicing its natural substrate (PIP2), the mechanism by which this series of aza-steroids inactive PIPLC is currently unknown. Epimeric mixture **8a** (20α:20β = 1:1) showed potent growth inhibition effects in the NCI in vitro tumor cell screen with a mean GI_{50} value (MG-MID) of 5.75 μM for 54 tumors, indicating the potential of using PIPLC as a molecular target for cancer drug discovery.

Experimental

Starting materials were purchased from Aldrich unless otherwise indicated. Thin layer chromatography analysis (TLC) was performed on aluminum sheets precoated with 0.2 mm of silica gel containing 60F254 indicator. Flash chromatography was run using 230–400 mesh silica gel. Reverse phase high performance liquid chromatography (HPLC) was run on a Phenomenex® LUNA 5μ C18 semi-preparative column. TLC routinely checked the homogeneity of all compounds on silica gel plates, and by HPLC. Fourier transformed infrared spectra were obtained on a Nicolet 520 FTIR spectrometer. ^1H (300 or 400 MHz), ^{13}C (75 or 100 MHz) NMR and DEPT spectra were recorded on either a Varian Gemini-300 or on a Varian XL-400 spectrometer. High-resolution mass spectrum (EI or FAB) were recorded on a VG Analytical 70-SE mass spectrometer equipped with a 11-250J data system. Melting points are uncorrected. Elemental analyses were performed by Atlantic Microlab, Norcross, GA.

Syntheses of 3β-hydroxy-20-aza-pregnene analogues (2a–8a) and 3β-hydroxy-20-aza-pregnane analogues (2b–8b). General procedure: To a solution of 1 g of pregnenolone acetate in 150 mL toluene was added 4 equiv amine and cat. *p*-TsOH. The reaction mixture was refluxed overnight with continuous extraction using a Dean-Stack water separation trap. When the reaction is finished, the solvent was removed under reduced pressure and the resulted residue was dissolved in THF, then 1.2 equiv $NaBH_4$ in 5 mL methanol was added dropwise. The mixture was stirred at rt for 3 h. The solvent was then removed completely in vacuo, and the residue obtained was extracted with 10% HCl and ethyl acetate. The solution was made alkaline with 10% NaOH solution, resulting in precipitates which was filtered, washed well with water to afford the corresponding 20-aza-pregnene analogues **2a**–**8a** as white gums. Hydrogenation of compounds **2a**–**8a** (200 mg) was carried out in 30 mL glacial acetic acid with 10% Pd on carbon as catalyst at atmospheric pressure for 4 h. The solvent was then removed under reduced pressure, and the residue obtained was resuspended in water and the pH was adjusted to 8. The solution was then extracted with chloroform and the organic layer was washed with water. Removal of solvent in vacuo afforded the saturated 3β-hydroxy-20-aza-pregnane analogues **2b**–**8b** as white gums.

3β-Hydroxy-20-aza-(*N*-hexylamine)-pregn-5-ene (2a). ^1H NMR ($CDCl_3$, 300 MHz) δ 0.66, 0.69 (1.2:1 (2s, $2CH_3$, 18-H)), 0.95, 1.07 (1:1.2 (2d, $J = 6.6$ Hz, $2CH_3$, 21-H)), 0.98 (s, CH_3, 19-H), 2.43 (m, 2H), 2.40, 2.51 (2m, each 1H), 2.66 (t, $J = 7.2$ Hz, 2H), 3.49 (m, 1H), 5.32 (m, 1H); CIMS *m*/*z* (relative intensity) 417 (50, $M^+ + 1$), 399 (25, $M^+ + 1\text{-}H_2O$); HRMS *m*/*z* calcd for $C_{27}H_{49}N_2O$ 417.3845 ($M^+ + 1$), found 417.3877.

3β-Hydroxy-20-aza-(*N*-hexylamine)-pregnane 2b. ^1H NMR ($CDCl_3$, 300 MHz) δ 0.81, 0.84 (1:1.2 (2s, $2CH_3$, 18-H)), 0.89 (s, CH_3, 19-H), 0.96, 1.17 (1.2:1 (2d, $J = 6.6$ Hz, $2CH_3$, 21-H)), 2.38 (m, 1H), 2.51 (m, 1H), 2.62 (t, $J = 7.2$ Hz, 2H), 3.58 (m, 1H); CIMS *m*/*z* (relative intensity) 419 (50, $M^+ + 1$), 401 (25, $M^+ + 1\text{-}H_2O$); HRMS *m*/*z* calcd for $C_{27}H_{51}N_2O$ 419.4003 ($M^+ + 1$), found 419.3973.

3β-Hydroxy-20-aza-(*N*-octylamine)-pregn-5-ene (3a). ^1H NMR ($CDCl_3$, 300 MHz) δ 0.66, 0.69 (1:1 (2s, $2CH_3$, 18-H)), 0.94, 1.05 (1:1. (2d, $J = 6.0$ Hz, $2CH_3$, 21-H)), 0.98 (s, CH_3, 19-H), 2.64, 2.78 (2m, each 1H), 3.49 (m, 1H), 5.32 (m, 1H); CIMS *m*/*z* (relative intensity) 445 (50, $M^+ + 1$), 427 (25, $M^+ + 1\text{-}H_2O$); HRMS *m*/*z* calcd for $C_{29}H_{53}N_2O$ 445.4158 ($M^+ + 1$), found 445.4175.

3β-Hydroxy-20-aza-(N-octylamine)-pregnane (3b). ^1H NMR ($CDCl_3$, 300 MHz) δ 0.81, 0.84 (1:1.2 (2s, $2CH_3$, 18-H)), 0.89 (s, CH_3, 19-H), 0.96, 1.17 (1.2:1 (2d, $J = 6.6$ Hz, $2CH_3$, 21-H)), 2.38 (m, 1H), 2.51 (m, 1H), 2.62 (t, $J = 7.2$ Hz, 2H), 3.58 (m, 1H); CIMS *m*/*z* (relative intensity) 447 (50, $M^+ + 1$), 429 (25, $M^+ + 1\text{-}H_2O$); HRMS *m*/*z* calcd for $C_{29}H_{55}N_2O$ 447.4316 ($M^+ + 1$), found 447.4318.

3β-Hydroxy-20-aza-(*N*-pentylamine)-pregn-5-ene (4a). ^1H NMR ($CDCl_3$, 300 MHz) δ 0.66, 0.69 (1:1.3 (2s, $2CH_3$, 18-H)), 0.92, 1.03 (1.2:1 (2d, $J = 6.0$ Hz, $2CH_3$, 21-H)), 0.98 (s, CH_3, 19-H), 2.24 (m, 2H), 2.40, 2.51 (2m, each 1H), 2.62 (t, $J = 7.2$ Hz, 2H), 3.51 (m, 1H), 5.31 (m, 1H); CIMS *m*/*z* (relative intensity) 403 (50, $M^+ + 1$), 385 (25, $M^+ + 1\text{-}H_2O$); HRMS *m*/*z* calcd for $C_{26}H_{47}N_2O$ 403.3690 ($M^+ + 1$), found 403.3694.

3β-Hydroxy-20-aza-(*N*-pentylamine)-pregnane (4b). ^1H NMR ($CDCl_3$, 300 MHz) δ 0.81, 0.84 (1:1.2 (2s, $2CH_3$, 18-H)), 0.89 (s, CH_3, 19-H), 0.96, 1.17 (1.2:1 (2d, $J = 6.6$ Hz, $2CH_3$, 21-H)), 2.38 (m, 1H), 2.51 (m, 1H), 2.62 (t, $J = 7.2$ Hz, 2H), 3.58 (m, 1H); CIMS *m*/*z* (relative intensity) 405 (50, $M^+ + 1$), 387 (25, $M^+ + 1\text{-}H_2O$); HRMS *m*/*z* calcd for $C_{26}H_{49}N_2O$ 405.3846 ($M^+ + 1$), found 405.3817.

3β-Hydroxy-20-aza-(*N*-butylamine)-pregn-5-ene (5a). ^1H NMR ($CDCl_3$, 300 MHz) δ 0.65, 0.68 (1:1.2 (2s, $2CH_3$,

18-H)), 0.94, 1.05 (1.2:1 (2d, $J=5.4$ Hz, $2CH_3$, 21-H)), 0.98 (s, CH_3, 19-H), 2.20 (m, 2H), 2.40, 2.53 (2m, each 1H), 2.66 (m, 2H), 3.49 (m, 1H), 5.31 (m, 1H); CIMS m/z (relative intensity) 389 (50, $M^+ + 1$), 371 (35, $M^+ + 1\text{-}H_2O$); HRMS m/z calcd for $C_{25}H_{45}N_2O$ 389.3533 ($M^+ + 1$), found 389.3490.

3β-Hydroxy-20-aza-(*N*-butylamine)-pregnane (5b). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81, 0.84 (1:1.2 (2s, $2CH_3$, 18-H)), 0.89 (s, CH_3, 19-H), 0.96, 1.17 (1.2:1 (2d, $J=6.6$ Hz, $2CH_3$, 21-H)), 2.38 (m, 1H), 2.51 (m, 1H), 2.62 (t, $J=7.2$ Hz, 2H), 3.58 (m, 1H); CIMS m/z (relative intensity) 391 (50, $M^+ + 1$), 373 (25, $M^+ + 1\text{-}H_2O$); HRMS m/z calcd for $C_{25}H_{47}N_2O$ 391.3688 ($M^+ + 1$), found 391.3658.

3β-Hydroxy-20-aza-(*N*-propylamine)-pregn-5-ene (6a). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.66, 0.68 (1:1.5 (2s, $2CH_3$, 18-H)), 0.94 (d, $J=6$ Hz, CH_3, 21-H), 0.98 (s, CH_3, 19-H), 2.23 (m, 2H), 2.50, 2.65, 2.81 (3m, each 1H), 3.49 (m, 1H), 5.31 (m, 1H); CIMS m/z (relative intensity) 375 (50, $M^+ + 1$), 357 (25, $M^+ + 1\text{-}H_2O$); HRMS m/z calcd for $C_{24}H_{43}N_2O$ 375.3377 ($M^+ + 1$), found 375.3364.

3β-Hydroxy-20-aza-(*N*-propylamine)-pregnane (6b). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81, 0.84 (1:1.2 (2s, $2CH_3$, 18-H)), 0.89 (s, CH_3, 19-H), 0.96, 1.17 (1.2:1 (2d, $J=6.6$ Hz, $2CH_3$, 21-H)), 2.38 (m, 1H), 2.51 (m, 1H), 2.62 (t, $J=7.2$ Hz, 2H), 3.58 (m, 1H); CIMS m/z (relative intensity) 377 (50, $M^+ + 1$), 359 (25, $M^+ + 1\text{-}H_2O$); HRMS m/z calcd for $C_{24}H_{45}N_2O$ 377.3533 ($M^+ + 1$), found 377.3490.

3β-Hydroxy-20-aza-(*N*-propanyl-*N*′-dimethylamine)-pregn-5-ene (7a). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.66, 0.69 (1:1.5 (2s, $2CH_3$, 18-H)), 0.85, 1.06 (1.5:1 (2d, $J=6.6$ Hz, $2CH_3$, 21-H)), 0.98 (s, CH_3, 19-H), 2.23 (m, 2H), 2.55, 2.59 (1.5:1 (2s, each $2CH_3$)), 2.74 (m, 2H), 3.49 (m, 1H), 5.32 (m, 1H); CIMS m/z (relative intensity) 403 (50, $M^+ + 1$), 385 (25, $M^+ + 1\text{-}H_2O$); HRMS m/z calcd for $C_{26}H_{47}N_2O$ 403.3690 ($M^+ + 1$), found 403.3658.

3β-Hydroxy-20-aza-(*N*-propanyl-*N*′-dimethylamine)-pregnane (7b). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81, 0.84 (1:1.2 (2s, $2CH_3$, 18-H)), 0.89 (s, CH_3, 19-H), 0.96, 1.17 (1.2:1 (2d, $J=6.6$ Hz, $2CH_3$, 21-H)), 2.38 (m, 1H), 2.51 (m, 1H), 2.62 (t, $J=7.2$ Hz, 2H), 3.58 (m, 1H); CIMS m/z (relative intensity) 405 (50, $M^+ + 1$), 387 (25, $M^+ + 1\text{-}H_2O$); HRMS m/z calcd for $C_{26}H_{49}N_2O$ 405.3847 ($M^+ + 1$), found 405.3804.

20,25-Diaza-cholesterol (8a). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.63, 0.68 (1:1 (2s, $2CH_3$, 18-H)), 0.96, 1.10 (1:1 (2d $J=6.6$ Hz, $2CH_3$, 21-H)), 0.98 (s, CH_3, 19-H), 2.22, 2.28 (1:1 (2s, $2CH_3$)). 2.60 (2m, 2H), 2.84 (m, 1H), 3.48 (m, 1H), 5.32 (m, 1H); CIMS m/z (relative intensity) 389 (80, $M^+ + 1$), 371 (80, $M^+ + 1\text{-}H_2O$), 283(100); HRMS m/z calcd for $C_{25}H_{44}N_2O$ 388.3454, found 388.3480.

3β-Hydroxy-20,25-diaza-cholestane (8b). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81, 0.84 (1:1.2 (2s, $2CH_3$, 18-H)), 0.89 (s, CH_3, 19-H), 0.96, 1.17 (1.2:1 (2d, $J=6.6$ Hz, $2CH_3$, 21-H)), 2.38 (m, 1H), 2.51 (m, 1H), 2.62 (t, $J=7.2$ Hz, 2H), 3.58 (m, 1H); CIMS m/z (relative intensity) 391 (50, $M^+ + 1$), 373 (25, $M^+ + 1\text{-}H_2O$); HRMS m/z calcd for $C_{25}H_{47}N_2O$ 391.3690 ($M^+ + 1$), found 391.3677.

17β-20β,25-Diaza-cholesterol (8a1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.69 (s, CH_3, 18-H), 0.98 (s, CH_3, 19-H), 1.09 (d, $J=6.6$ Hz, CH_3, 21-H), 2.25 (s, $2CH_3$), 2.60 (m, 1H), 2.84 (m, 1H), 3.48 (m, 1H), 5.36 (m, 1H). CIMS m/z (relative intensity) 389 (40, $M^+ + 1$), 371 (60, $M^+ + 1\text{-}H_2O$), 283 (100). HRMS m/z calcd for $C_{25}H_{45}N_2O$ 389.3533 ($M^+ + 1$), found 389.3538. Anal. ($C_{25}H_{44}N_2O + 1.5H_2O$) C, H, N.

17β-20α,25-Diaza-cholesterol (8a2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.65 (s, CH_3, 18-H), 0.98 (s, CH_3, 19-H), 1.21 (d, $J=6.6$ Hz, CH_3, 21-H), 2.24 (s, $2CH_3$), 2.60 (2m, 2H), 2.84 (m, 1H), 3.48 (m, 1H), 5.33 (m, 1H). CIMS m/z (relative intensity) 389 (60, $M^+ + 1$), 371 (100, $M^+ + 1\text{-}H_2O$). HRMS m/z calcd for $C_{25}H_{45}N_2O$ 389.3533 ($M^+ + 1$), found 389.3534. Anal. ($C_{25}H_{44}N_2O + 1.5H_2O$) C, H, N.

17α-20β,25-Diaza-cholesterol (8a3). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.72 (s, CH_3, 18-H), 0.97(s, CH_3, 19-H), 0.99 (d, $J=6.6$ Hz, CH_3, 21-H), 2.60 (s, $2CH_3$), 2.79 (m, 2H), 3.00 (m, 1H), 3.46 (m, 1H), 5.36 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): 52.35, 52.18, 21.71, 20.17, 19.36 (5 CH_3); 64.82, 42.25, 42.16, 37.30, 33.74, 32.41, 31.63, 26.13, 22.58, 21.08 (10 CH_2); 121.68, 71.72, 54.22, 53.70, 52.93, 50.16, 32.21 (7 CH); 140.72, 43.22, 36.59 (3 C). CIMS m/z (relative intensity 389 (70, $M^+ + 1$), 371 (60, $M^+ + 1\text{-}H_2O$), 330 (100); HRMS m/z calcd for $C_{25}H_{44}N_2O$ 388.3454 ($M^+ + 1$), found 388.3480. Anal. ($C_{25}H_{44}N_2O + 1.5H_2O$) C, H, N.

17α-20α,25-Diaza-cholesterol (8a4). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.75 (s, CH_3, 18-H), 0.98 (s, CH_3, 19-H), 1.06 (d, $J=6.6$ Hz, CH_3, 21-H), 2.65 (s, $2CH_3$), 2.80 (m, 2H), 3.06 (2m, 2H), 3.46 (m, 1H), 5.33 (m, 1H). CIMS m/z (relative intensity) 389 (40, $M^+ + 1$), 371 (65, $M^+ + 1\text{-}H_2O$). HRMS m/z calcd for $C_{25}H_{45}N_2O$ 389.3533 ($M^+ + 1$), found 389.3557. Anal. ($C_{25}H_{44}N_2O + 1.5H_2O$) C, H, N.

3β-Hydroxy-20-aza-(*N*-butane)-pregn-5-ene (9a). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.67, 0.70 (1.2:1 (2s, $2CH_3$, 18-H)), 0.91 (t, $J=7.2$ Hz, CH_3), 0.96, 1.07 (1:1.2 (2d, $J=6.6$ Hz, $2CH_3$, 21-H)), 0.98 (s, CH_3, 19-H), 2.59 (m, 1H), 2.86 (m, 1H), 3.51 (m, 1H), 5.32 (m, 1H). CIMS m/z (relative intensity) 374(85, $M^+ + 1$), 356 (100, $M^+ + 1\text{-}H_2O$). HRMS m/z calcd for $C_{25}H_{44}NO$ 374.3423 ($M^+ + 1$), found 374.3425.

3β-Hydroxy-20-aza-(*N*-butane)-pregnane (9b). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.64, 0.66 (1:1 (2s, $2CH_3$, 18-H)), 0.73, 1.09 (1:1 (2d, $J=6.6$ Hz, $2CH_3$, 21-H)), 0.77 (s, CH_3, 19-H), 0.89 (t, $J=8.0$ Hz, CH_3), 2.41 (m, 3H), 2.65 (m, 2H), 3.58 (m, 1H). CIMS m/z (relative intensity) 376 (100, $M^+ + 1$), 358 (40, $M^+ + 1\text{-}H_2O$). HRMS m/z calcd for $C_{25}H_{45}NO$ 376.3581 ($M^+ + 1$), found 376.3550.

3β-Hydroxy-20-aza-cholestan-5-ene (10a). ^{1}H NMR ($CDCl_3$ 300 MHz) δ 0.67, 0.69 (1:1.2 (2s, $2CH_3$, 18-H)), 0.87, 0.88 (1.2:1 (2d, $J=6.6$ Hz, 26, 27-CH_3)), 0.97, 1.08 (1.2:1 (2d, $J=6.3$ Hz, CH_3, 21-H)), 1.02 (s, CH_3, 19-H), 3.49 (m, 1H), 5.32 (m, 1H). CIMS *m/z* (relative intensity) 388 (85, $M^+ + 1$), 370 (100, $M^+ + 1$-H_2O). HRMS *m/z* calcd for $C_{26}H_{46}NO$ 388.3580 ($M^+ + 1$), found 388.3574.

3β-Hydroxy-20-aza-pregn-5-ene (11a). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.70, 0.72 (2.5:1 (2s, CH_3, 18-H)), 0.98 (s, CH_3, 19-H), 1.00 (d, $J=6.6$ Hz, CH_3, 21-H), 2.81 (m, 1H), 3.50 (m, 1H), 5.32 (m, 1H). CIMS *m/z* (relative intensity) 318 ($M^+ + 1$, 40) for $C_{21}H_{36}NO$, 310 (100, $M^+ + 1$-H_2O).

General procedures for reductive amination using NaB(CN)H_3

Ketone precursor (200 mg) was dissolved in 10 mL methanol, and 6 equiv *N*,*N*-dimethylethylene diamine was added. pH of the solution was adjusted to ~6 by adding glacial acetic acid. Then 10 mL THF and 1.1 equiv $NaBH_3CN$ in methanol was added, and the reaction mixture was stirred under reflux overnight. After evaporation of the solvent in vacuo, the resulted residue was resuspended in 10 mL water, and the pH was adjusted to ~8. The solution was extracted with chloroform, and the combined organic layer was washed with water and dried over anhydrous Na_2SO_4. After evaporation, the residue was washed with petroleum ether to give the corresponding amine.

3β-Hydroxy-6,20,25-triaza-cholestane (13). 3β-Acetoxy-5-oxo-5,6-*seco*-pregnan-6-oic acid 1 g (2.5 mmol) was allowed to react with oxalyl chloride in dichloromethane and cat. pyridine to give its acid chloride. The solvent was evaporated and the residue was dissolved in acetone. Then 10% NaN_3/water was added dropwise to afford acyl azide. Formation of the intermediate acid chloride and acyl azide was confirmed by their characteristic IR absorptions at 1801 cm^{-1} and 2134 cm^{-1}, respectively. Curtis rearrangement of the acyl azide was carried out in benzene at reflux to yield the isocyanate (ν 2275 cm^{-1}), which, without further purification, was treated with 12 N HCl in acetone at reflux and after hydrogenation in acetic acid on 5% Pd/C gave the corresponding aza-ketone **12**. After being washed with petroleum ether, crude ketone **12** was allowed to undergo reductive amination using NaB(CN)H_3. Following the procedures described above, the crude amine product **13** in chloroform was further purified by precipitation and filtration. Amine **13** was precipitated from its chloroform solution by adding 2 M HCl. The solid obtained from filtration was resuspended into water, and pH of the solution was adjusted to ~8. The solution was extracted again with chloroform, and the combined organic layer was washed with water, dried over anhydrous Na_2SO_4. Removal of the solvent gave the corresponding 3β-hydroxy-6,20,25-triaza-chholestane **13** (60 mg, 5% from the acid) as a white gum: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.51, 0.53 (1:1.2, (2s, $2CH_3$, 18-H)), 0.70 (s, CH_3, 19-H), 0.70, 0.80 (1.2:1, (2d, $J=6.6$ Hz, CH_3, 21-H)), 2.03 (s, $2CH_3$), 2.23 (m, 2H), 2.50, 2.80 (2m, 2H), 3.40 (m, 1H). CIMS *m/z* (relative intensity) 392 (100, $M^+ + 1$), 374 (60, $M^+ + 1$-H_2O), 333 (40). HRMS *m/z* calcd for $C_{24}H_{46}N_3O$ 392.3641 ($M^+ + 1$), found 392.3634. Anal. ($C_{24}H_{45}N_3O + 1.5H_2O$) C, H. N.

Pregn-5-en-3,20-diol (14). 3-Acetoxy-pregn-5-en-20-one (500 mg, 1.39 mmol) was treated with $LiAlH_4$ (106 mg, 2.79 mmol) in THF at 0 °C and stirred for 1 h. The excess amount of $LiAlH_4$ was quenched with ethyl acetate and the mixture was washed with 10% HCl. The organic layer was dried over $MgSO_4$ and concentrated to afford crude pregn-5-en-3,20-diol **14** as colorless solid (425 mg, 96%): mp 234–237 °C (lit.[14] 236-238 °C) which was used without further purification: FTIR (KBr) 3600–3200, 3000–2800, 1670, 1452, 1375, 1057 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz) δ 5.30 (d, $J=3.9$ Hz, 1H), 4.65 (d, $J=4.2$ Hz, 1H), 4.15 (d, $J=8.4$ Hz, 1H), 3.50 (m, 1H), 3.27 (m, 1H), 2.55.92 (m, 20H), 1.03 (d, $J=6.3$ Hz, 3H), 0.98 (s, 3H), 0.72 (s, 3H); CIMS *m/z* (relative intensity) 374(85, $M^+ + 1$), 356 (100, $M^+ + 1$-H_2O); HRMS *m/z* calcd for $C_{25}H_{44}NO$ 374.3423 ($M^+ + 1$), found 374.3425.

Pregn-5-en-3,20-diamine (17). Crude **14** (400 mg, 1.26 mmol) was dissolved in CH_2Cl_2 (3 mL) and pyridine (1 mL, 12.6 mmol), and mesyl chloride (490 μL, 6.33 mmol) was added to the solution at 0 °C. After stirring for 2 h, the mixture was diluted with water and extracted with methylene chloride. The combined organic layer was dried over $MgSO_4$ and concentrated to afford crude pregn-5-en-3,20-diol dimesylate **15** (568 mg): FTIR (neat film) 3068, 2944, 2898, 1608, 1536, 1487, 1353, 1173, 938, 903, 758, 689 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.42 (m, 1H, =CH), 4.82 (m, 1H, CH-OMs), 4.52 (m, 1H, CH-OMs); FABMS *m/z* (relative intensity) 475 ($M^+ + 1$, 25) for $C_{23}H_{38}O_6S_2$, 379 (100).

Crude **15** (550 mg, 1.16 mmol) was dissolved in DMF (5 mL) with excess amount of sodium azide (760 mg, 11.7 mmol) and heated to 80 °C overnight. The mixture was diluted with water after cooling and extracted with ethyl ether. The combined organic layer was dried over $MgSO_4$ and concentrated to afford crude pregn-5-en-3,20-diazide **16** (321 mg): FTIR (neat film) 2940, 2098 (strong), 1453, 1270, 1228 cm^{-1}. Crude **16** (230 mg, 0.62 mmol) was reduced with $LiAlH_4$ in THF to afford **17** (98 mg, 50%) or by hydrogenation with Pd/C in a 10:5:2 mixture of methanol/ethyl ether/acetic acid to afford **17** (123 mg, 63%) as yellowish solid: mp 147–149 °C (lit.[13c] 148 °C); FTIR (neat film) 3375 (strong), 2934, 1585, 1460, 1387 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz,) δ 5.33 (m, 1H), 3.12 (t, $J=3$ Hz, 1H, $CHNH_2$-3), 2.76 (m, 1H, $CHNH_2$-20), 2.54 (d, $J=15$ Hz, 1H), 1.90.96 (m, 23H), 1.08 (d, $J=6.6$ Hz, 3H), 0.97 (s, 3H) 0.65 (s, 3H); EIMS *m/z* (relative intensity) 316 (M^+, 3), 301 (4), 273 (95), 44.0 (100); FABMS *m/z* (relative intensity) 317 ($M^+ + 1$, 100).

Bis(*N'*-*p*-toluenesulfonyl)-pregn-5-en-3,20-diamine (18). To a solution of **17** (50 mg, 0.158 mmol) was added *p*-toluenesulfonyl chloride (151 mg, 0.79 mmol) and pyridine (128 μL, 1.58 mmol) at rt. After stirring for 2 days, the reaction mixture was treated with brine and extracted with ethyl acetate three times. The combined

organic layer was dried over $MgSO_4$, concentrated and purified by column chromatography (ethyl acetate/hexane, 1/2) to afford **18** (77 mg, 78%) as colorless solid: mp > 300 °C (decompose); FTIR (neat film) 3283, 2938, 2877, 1435, 1334, 1165, 1097 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz,) δ 7.74 (dd, $J = 3.3$ Hz, 8.1 Hz, 4H), 7.28 (d, $J = 8.1$ Hz, 4H), 5.17 (d, $J = 5.1$ Hz, 1H), 4.74 (d, $J = 9.0$ Hz, 1H), 4.46 (d, $J = 8.7$ Hz, 1H), 3.52 (m, 1H), 3.23 (m, 1H), 2.41 (s, 6H), 2.48.33 (m, 1H), 1.91.79 (m, 19H), 1.01 (d, $J = 6.6$ Hz, 3H), 0.89 (s, 3H), 0.59 (s, 3H); FABMS *m/z* (relative intensity) 625 ($M^+ + 1$, 45), 471 (19), 454 (76), 429 (25), 323 (53), 283 (100), 257 (24).

Bis(*N'*-phenylaminocarbonyl)-pregn-5-en-3,20-diamine (19). A solution of **17** (52 mg, 0.164 mmol) and phenylisocyanate (38 μL, 0.345 mmol) in THF (3 mL) was stirred for 3 days under nitrogen at rt. The reaction mixture was concentrated and washed with ethyl ether to afford a white solid **19** (86 mg, 95%), mp 195–197 °C; FTIR (KBr) 3352 (strong) 3062, 2935, 1939, 1852, 1788, 1654, 1601, 1542, 1444, 1313, 1232, 894, 754, 693 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz,) δ 7.25 (2s, 4H), 7.18 (t, $J = 7.2$ Hz, 4H), 6.87 (t, $J = 6.6$ Hz, 2H), 5.34 (m, 1H), 3.91 (m, 1H), 3.74 (m, 1H), 2.57 (m, 1H), 2.00–0.95 (m, 23H), 1.12 (d, $J = 6.6$ Hz, 3H), 1.00 (s, 3H), 0.71 (s, 3H); FABMS *m/z* (relative intensity) 555 ($M^+ + 1$, 100), 462 (11), 419 (27), 323 (9), 283 (7); HRMS *m/z* calcd for $C_{35}H_{47}N_4O_2$ 555.3699 ($M^+ + 1$), found 555.3669.

Pregn-5-en-3,20-diamino-bis(benzyloxycarbamate) (20). To a solution of **17** (52 mg, 0.164 mmol) in CH_2Cl_2 (3 mL) were added benzyl chloroformate (70 μL, 0.493 mmol) and triethylamine (120 μL, 0.86 mmol) under nitrogen at rt. After stirring for 3 days, the mixture was treated with brine and extracted with ethyl acetate. The combined organic layer was dried over $MgSO_4$, concentrated and purified by column chromatography (THF/methylene chloride/hexane, 1/3/11) to afford **20** as colorless solid (78 mg, 81%): mp 64–67 °C; FTIR (neat film) 3431, 3332, 3067, 3032, 2941, 1699 (strong), 1530, 1504, 1456, 1335, 1264, 1241, 1221, 1072, 1031, 739, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.33 (m, 10H), 5.33 (m, 1H), 5.06 (m, 4H), 4.88 (d, $J =$ Hz, 1H), 4.58 (d, $J = 8.7$ Hz, 1H), 3.92 (brs, 1H), 3.70 (m, 1H), 2.56 (d, $J = 14.7$ Hz, 1H), 1.94.07 (m, 19H), 1.16 (d, $J = 6.6$ Hz, 3H), 0.98 (s, 3H), 0.70 (s, 3H); FABMS *m/z* (relative intensity) 585 ($M^+ + 1$, 100), 541 (12), 495 (11), 449 (17), 432 (11), 342 (13), 283 (18), 255 (8); HRMS *m/z* calcd for $C_{37}H_{49}N_2O_4$ 585.3692 ($M^+ + 1$), found 585.3724.

Diaza derivative of estrone (21). Following the general procedures described above for reductive amination using $NaB(CN)H_3$, estrone (500 mg, 1.85 mmol) gave the corresponding diaza derivative as amorphous solid (540 mg, 85%): ^1H NMR ($CDCl_3$, 300 MHz) δ 7.11 (d, $J = 5.1$ Hz, 1H), 6.60 (dd, $J = 5.1$, 3.0 Hz, 1H), 6.69 (d, $J = 3.0$ Hz, 1H), 2.80 (m, 2H), 2.79 (m, 1H), 2.40 (m, 1H), 2.21 (s, 6H), 0.70 (s, 3H); EIMS *m/z* (relative intensity) 342 (M^+, 20), 327 (M^+- CH_3, 5), 284 (95), 58 (100); HRMS (EI) *m/z* calcd for $C_{22}H_{34}N_2O$ 342.2671, found 344.2668.

3-Methoxy-estra-1,3,5(10)-trien-17-one (22). Estrone (1 g, 3.70 mmol) was dissolved in 20 mL ethanol by heating. To the hot solution was added a solution of 3 equiv NaOH in water and 6 equiv dimethyl sulfate. Then one more equivalent of NaOH in water was added while stirring under reflux. The reaction mixture was refluxed for 3 h, then the solvent was removed under reduced pressure. The resulted residue was resuspended in water and extracted with ethyl acetate. The combined organic layer was washed with brine and water, dried over anhydrous Na_2SO_4 and concentrated to give a crude product containing 40% starting material. Recrystallization in 10–15% methanol/acetone solution to give pure methyl ether (300 mg, 57%) as pellet: mp 160–162 °C (lit.[15] 164–165 °C): ^1H NMR ($CDCl_3$, 300 MHz) δ 7.21 (d, $J = 8.7$ Hz, 1H), 6.71 (dd, $J = 8.7$, 3.0 Hz, 1H), 6.63 (d, $J = 3.0$ Hz, 1H), 3.76 (s, 3H), 2.87 (m, 2H), 0.89 (s, 3H); EIMS *m/z* (relative intensity) 284 (M^+, 100), 269 ($M^+ - CH_3$, 5), 256 (5), 199 (50); HRMS (EI) *m/z* calcd for $C_{19}H_{24}O_2$ 284.1776, found 284.1761.

Diaza derivative of 3-methoxy-estra-1,3,5(10)-trien-17-one (23). Following the general procedures described above for reductive amination using $NaB(CN)H_3$, 3-methoxy-estra-1,3,5(10)-trien-17-one (**22**, 200 mg, 0.70 mmol) gave the corresponding diaza derivative **23** as amorphous solid (200 mg, 80%): ^1H NMR ($CDCl_3$, 300 MHz) δ 7.15 (d, $J = 9.0$ Hz, 1H), 6.64 (d, $J = 8.7$, 2.7 Hz, 1H), 6.58 (d, $J = 2.7$ Hz, 1H), 3.72 (s, 3H), 2.18 (s, 6H), 0.70 (s, 3H); EIMS *m/z* (relative intensity) 356 (M^+, 10), 341 ($M^+ - CH_3$, 5), 298 (100), 58 (90); HRMS (EI) *m/z* calcd for $C_{23}H_{36}N_2O$ 356.2828, found 356.2831.

3β-Toluene-4-sulfonyloxy-pregn-5-en-20-one (24). To a solution of pregnenolone (2 g, 6.3 mmol) in 20 mL pyridine, 5 equiv toluene sulfonyl chloride was added with cat. DMAP. After stirring at rt for 4 h, the reaction mixture was poured into ice water resulting in white precipitates. The white powder obtained from filtration was washed with water, dried in air to give quantitative amount of product **24**: ^1H NMR ($CDCl_3$ 300 MHz) δ 7.78 (d, $J = 8.7$ Hz), 7.30 (d, $J = 8.7$ Hz), 5.28 (m, 1H), 4.31 (m, 1H), 2.42 (s, 3H), 2.09 (s, 3H), 0.94 (s, 3H), 0.58 (s, 3H).

Pregnenolone methyl ether (25). 3β-Toluene-4-sulfonyloxy-pregn-5-en-20-one (**24**, 500 mg, 1.06 mmol) was dissolved in 50 mL methanol and stirred at rt for 2 h. After evaporation of the solvent, the resulted residue was resuspended in water and extracted with ethyl acetate. The combined organic layer was washed with saturated Na_2CO_3 solution, brine and water, dried over anhydrous Na_2SO_4. Removal of the solvent in vacuo gave a crude product, which was recrystalized in 10–15% water/methanol solution to give pure methyl ether (280 mg, 80%) as pellet: mp 121–123 °C (lit.[16] 123.5 °C): ^1H NMR ($CDCl_3$, 300 MHz) δ 5.33 (m, 1H), 3.33 (s, 3H), 3.02 (m, 1H), 2.51 (m, 1H), 2.34 (m, 1H), 2.10 (s, 3H), 0.97 (s, 3H), 0.60 (s, 3H); EIMS *m/z* (relative intensity) 330 (M^+, 50), 315 ($M^+ - CH_3$, 10), 298 ($M^+ - CH_3OH$), 283 (50), 43 (100); HRMS (EI) *m/z* calcd for $C_{22}H_{34}N_2O$ 330.2559, found 330.2554.

22,25-Diaza-cholesterol methyl ether (26). Following the general procedures described above for reductive amination using $NaB(CN)H_3$, pregnenolone methyl ether

25 (200 mg, 0.61 mmol) gave the corresponding diaza derivative as amorphous solid (210 mg, 86%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.26 (m, 1H), 3.26 (s, 3H), 2.96 (m, 1H), 2.13, 2.11 (2s, 1:2, 6H), 0.99, 0.89 (2d, 1:2, 3H, 21-H), 0.91 (s, 3H), 0.62, 0.60 (2s, 2:1, 3H, 18-H); EIMS *m*/*z* (relative intensity) 403 ($M^+ + 1$, 20), 402 (M^+, 10), 387 ($M^+ + 1$- CH_3, 5), 358 (10), 344 (100), 58 (90); HRMS (EI) *m*/*z* calcd for $C_{26}H_{46}N_2O$ 402.3610, found 402.3611.

12-Methoxy-podocarpatrien-16-al (28). To a solution of 12-methoxy-podocarpatrien-16-ol (1 g, 3.6 mmol) in 5 mL dichloromethane was added dropwise a cold solution of 2.2 equiv DMSO and 1.1 equiv oxalyl chloride in 5 mL dichloromethane at −60 °C. After 5 min, 5 equiv Et_3N was injected. After stirring at −60 °C for 10 min, the reaction mixture was poured into ice water and extracted with dichloromethane. The combined organic layer was washed with brine and water, dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue obtained was recrystalized in acetone/methanol to give the corresponding aldehyde as colorless needles (850 mg, 85%): mp 134–136 °C (lit.[17] 135–137 °C): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.80 (s, 1H), 6.95 (d, J = 8.7 Hz, 1H), 6.76 (d, J = 2.7 Hz, 1H), 6.70 (dd, J = 8.7, 2.7 Hz, 1H), 3.78 (s, 3H), 2.80 (m, 2H), 2.20 (m, 2H), 1.98 (m, 1H), 1.08 (s, 3H), 1.03 (s, 3H); EIMS *m*/*z* (relative intensity) 272 (M^+, 100), 229 ($M^+ - CH_3CO$, 20), 147 (65); HRMS (EI) calcd for $C_{18}H_{24}O$ 272.1776, found 272.1773.

Compound 29

Following the general procedures described above for reductive amination using $NaB(CN)H_3$, 12-methoxy-podocarpatrien-16-al (**28**, 200 mg 0.73 mmol) gave the corresponding diaza derivative **29** as amorphous solid, 200 mg, 80% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.95 (d, J = 8.7 Hz, 1H), 6.76 (d, J = 2.7 Hz, 1H), 6.69 (dd, J = 8.7, 2.7 Hz, 1H), 3.75 (s, 3H), 3.20 (m, 1H), 3.09 (m, 1H), 2.43 (s, 6H), 1.17 (s, 3H), 1.13 (s, 3H); EIMS *m*/*z* (relative intensity) 344 (M^+, 10), 328 ($M^+ - CH_4$, 5), 286 (50), 58 (100); HRMS (EI) *m*/*z* calcd for $C_{22}H_{36}N_2O$ 344.2828, found 344.2818.

PI-PLCγ in vitro inhibition assay

Inhibition of PI-PLC activity was measured as previously described[4] using bovine brain PI-PLCγ and [^{3}H]-phosphatidylinositol-(4,5)-biphosphate ([^{3}H]PIP_2) as the substrate. Unreacted [^{3}H]PtdIns(4,5) biphosphate was removed from its water soluble hydrolysis product by acid coprecipitation with bovine serum albumin, to which [^{3}H]PtdIns(4,5)biphosphate bind quantitatively. IC_{50} values were calculated from at least three independent measurements liquid scintillation counting of the supernatant. The variation from the mean value is 20% or less. NA stands for IC_{50} value larger than 100 μg/mL.

Cytotoxicity assays

The in vitro cytotoxicity assays were carried out at the National Cancer Institute (NCI). Details of the assay procedure have been reported previously.[23]

Acknowledgements

We gratefully acknowledge support of this research by the NCI/NIH (Grant 5U19 CA52995). We are grateful to Dr. Leslie T. Gelbaum for his assistance in obtaining high resolution NMR spectra, and Mr. David E. Bostwick and Ms. Sarah J. Shealy for their assistance in obtaining high resolution mass spectra.

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Elemental analysis data for compounds **1**, **8**, **9**, **13**

Compound no.		**8a1** $+1.5H_2O$	**8a2** $+1.5H_2O$	**8a3** $+1.5H_2O$	**8a4** + $1.5H_2O$	**13** $+1.5\ H_2O$
Calcd	C	72.24	72.24	72.24	72.24	68.85
	H	11.40	11.40	11.40	11.40	11.56
	N	6.74	6.74	6.74	6.74	10.04
Found	C	72.31	71.86	71.99	71.88	68.46
	H	11.25	11.40	11.24	11.24	11.32
	N	6.70	6.64	6.53	6.67	9.87

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