7-IMIDAZOLYLALKANAMIDO-1-CARBOXYLALKYLBENZODIAZEPINE, A NOVEL SERIES OF FARNESYLTRANSFERASE INHIBITORS

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ABSTRACT: **AIM** Design, synthesis and evaluation of a series of 7-imidazolylalkanamido-1-carboxylalkylbenzodiazepine farnesyltransferase (FTase) inhibitors. **METHODS and RESULTS** Coupling of imidazolylalkylcarboxylic acids and 1-substituted 7-aminobenzodiazepines (**5a** ~ **5c**) yielded 10 new compounds (**6** ~ **12**, **16** ~ **18**) which were biologically tested against FTase using scintillation proximity assay method. **CONCLUSION** Five target compounds were found to be potential farnesyltransferase inhibitors.

KEY WORDS: benzodiazepine; farnesyltransferase; conformation analysis

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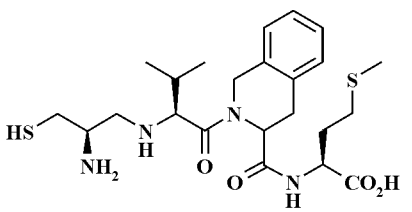
Mutation in the ras oncogene takes place in many human cancers[1]. Ras proteins function as central switches for signals given by growth factors that direct cell growth and cell differentiation[2]. To perform this function the Ras proteins have to be associated with plasma membrane, for which the proteins require post-translational modification to increase the molecular lipophilicity. One of the key events is to farnesylate a cysteine residue located in the carboxyl terminal tetrapeptide CAAX, where C stands for cysteine, A aliphatic amino acids, and X serine or methionine. The farnesylation is catalyzed by farnesyltransferase (FTase), a zinc metalloenzyme[3]. The dependence of the transforming activity of Ras on the farnesylation has led to an intensive search for inhibitors of FTase that may be therapeutic potential as anticancer agents[4].

Numerous structurally diverse classes of FTase inhibitors have been reported in literature[5]. Analyzing the reported FTase inhibitors based upon CAAX motif, we gained a knowledge of the structural feature consisting of a carboxyl group and a moiety able to coordinate zinc ion, such as mercapto group **1** or imidazole residue **2**[6~8] (Figure 1). These two pharmacophores are connected in separate distance to various scaffolds, which possess appropriate hydrophobicity and polar anchors.



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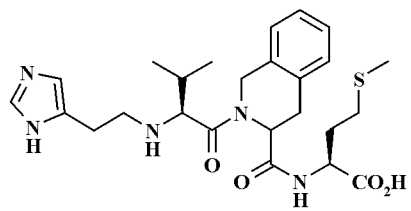

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Figure 1 Structure of compounds **1** and **2**

In this paper benzodiazepine (BZD) was selected as a scaffold structure to design novel FTase inhibitors. It is well known that BZD, as a privileged structure[9] in drug design, contains a diphenylmethyl moiety and sufficient number of anchors for linking the pharmacophores. BZD is also featured by resistance to hydrophobic collapse in water. Therefore, their potential extensive hydrophobic binding energies are available to FTase and not significantly lost in intramolecular stacking interactions[9].

To investigate the optimal disposition of carboxyl and imidazole moieties, 7-imidazolylalkan-amido-1-carboxyalkyl-

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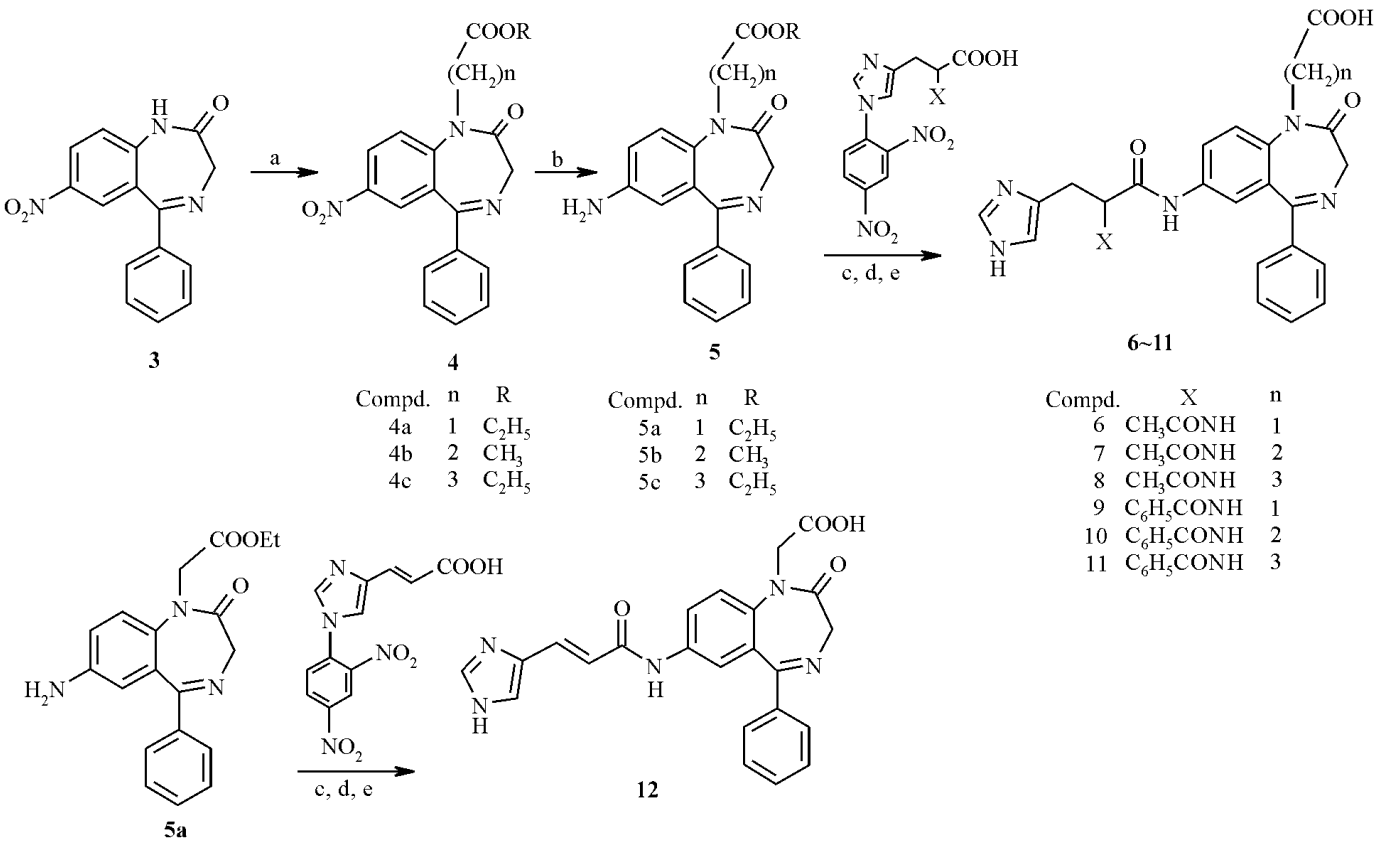
1 ,3- dihydro- 5- phenyl- 2H- 1 ,4- benzodiazepin- 2- ones with varied alkylene groups were synthesized. The route of synthesis is shown in Figure 2. 7- Nitro- 1 ,3- dihydro- 5- phenyl- 2H- 1 ,4- benzodiazepin- 2- one **3** was alkylated by ethyl α- bromoacetate (or methyl β- bromopropionate , or ethyl γ- bromobutyrate) to give the corresponding 1- alkoxycarbonyl- alkyl- 7- nitro- BZD **4a , 4b** or **4c** , which were reduced to 7- amino derivatives **5a , 5b** or **5c** . The amines were condensed with protected acylated histidines[10] by DCC to afford the protected amides , which were then deprotected and hydrolyzed to yield the target compounds **6** ~ **12** .

The synthesis of some other target compounds **16** ~ **18** is shown in Figure 3 . Coupling of amine **5** with imidazolylpropionic chloride[11] and hydrolyzing the esters afforded the target compounds **16** ~ **18** . The total yields and melting points of all the target compounds are listed in Table 1 .

All the target compounds were identified by NMR and HRMS , the spectral data of which are shown in Table 1 .



a. K_2CO_3/ethyl bromoacetate(or ethyl 3- bromo propinate or ethyl 4-bromobutanate);
b. $SnCl_2H_2O$; c. DCC/HOBt ; d. $HSCH_2CH_2OH$; e.$LiOH/CH_3COOH$

Figure 2 Route of synthesis of compounds **4a ~ c , 5a ~ c** and **6 ~ 12**

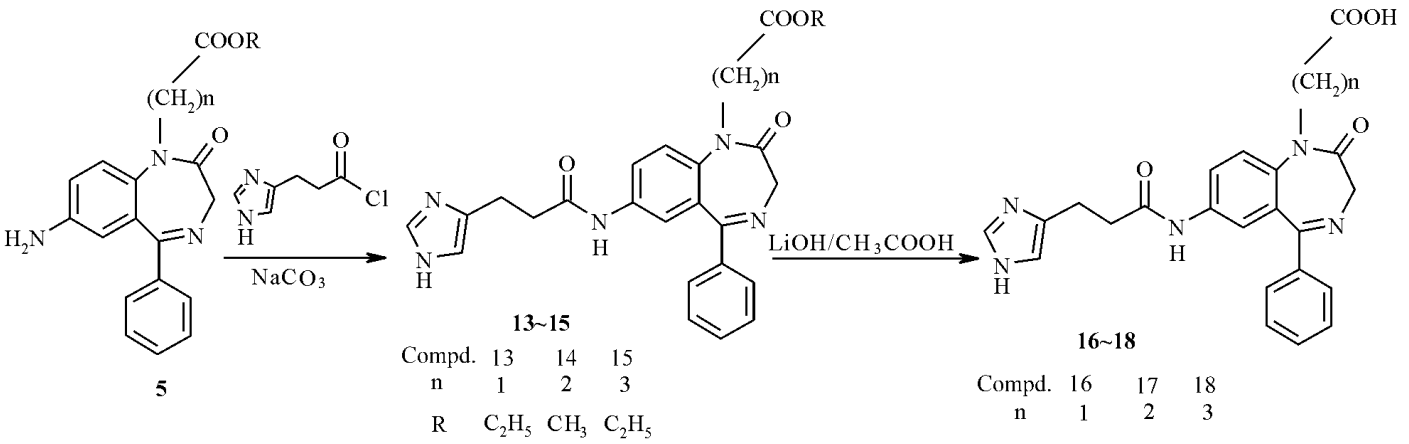


Figure 3 Route of synthesis of compounds **13 ~ 18**

Table 1 Spectral data , yields and melting points of the synthetic compounds 6 ~ 12 and 16 ~ 18

No.	^{1}HNMR (DMSO-d_6)	MS	Yield/ %	MP/ ℃
6	1 .888 s ,2 .727 ~ 2 .935 m ,3 .709 ~ 3 .736 d ,3 .977 ~ 4 .031 d ,4 .208 ~ 4 .262 d J = 16 .2 Hz , 4 .471 ~ 4 .541 m ,6 .736 s ,7 .388 ~ 7 .786 m ,8 .7 ~ 8 .8 b ,10 .950 b	EI-HRMS :(M^+) 488 .1829	23 .8	2463 ~ 248
7	1 .844 s ,2 .133 ~ 2 .260 m ,2 .726 ~ 2 .937 octet ,3 .676 ~ 3 .712 d J = 10 .8 Hz ,3 .749 ~ 3 .845 d , 4 .249 ~ 4 .345 d ,4 .464 ~ 4 .553 m ,6 .772 s ,7 .385 ~ 8 .283 m ,10 .950 b	FAB-HRMS :(M + H) 503 .2023	27 .5	195 ~ 197
8	1 .768 s ,1 .501 ~ 1 .702 m ,2 .750 ~ 2 .905 m ,3 .358 ~ 3 .64 m ,3 .660 ~ 3 .690 m ,4 .102 ~ 4 .147 d , 4 .477 ~ 4 .512 m ,6 .695 s ,7 .382 ~ 7 .902 m	FAB-HRMS :(M + 2Li) 528 .2453	27 .0	200 ~ 202
9	2 .949 ~ 3 .027 m ,3 .775 ~ 3 .810 d J = 10 .5 Hz ,4 .404 ~ 4 .703 m ,6 .841 s ,7 .422 ~ 8 .650 m , 8 .683 b ,10 .307 b	EI-HRMS :(M^+) 550 .2009	26 .5	226 ~ 228
10	2 .281 ~ 2 .293 m ,3 .011 ~ 3 .039 octet ,3 .696 ~ 3 .771 d J = 10 .5 Hz ,3 .779 ~ 3 .873 m ,4 .297 ~ 4 .393 m ,4 .481 ~ 4 .516 d J = 10 .5 Hz ,4 .683 ~ 4 .727 m ,6 .827 s ,7 .385 ~ 8 .690 m ,8 .713 s ,10 .334 b	FAB-HRMS :(M + H) 565 .2193	13 .6	189 ~ 191
11	1 .469 ~ 1 .538 m ,1 .603 ~ 1 .690 m ,1 .903 ~ 1 .969 m ,3 .018 ~ 3 .076 octet ,3 .717 ~ 3 .751 d J = 10 .2 Hz ,3 .599 ~ 3 .687 m ,4 .169 ~ 4 .263 m ,4 .497 ~ 4 .531 d J = 10 .2 Hz ,4 .470 ~ 4 .731 m , 6 .829 s ,7 .415 ~ 7 .974 m ,8 .687 b ,10 .356 b	FAB-HRMS :(M + H) 579 .2352	17 .8	194 ~ 196
12	3 .710 ~ 3 .744d J = 10 .2 Hz ,3 .858 ~ 3 .913 d J = 16 .5 Hz ,4 .210 ~ 4 .270 d J = 16 .5 Hz , 4 .494 ~ 4 .528 d J = 10 .2 Hz ,6 .693 ~ 6 .744 d J = 15 .3 Hz ,7 .338 ~ 7 .856 m ,10 .416 b	FAB-HRMS :(M + 2Li) 441 .1686	21 .4	202 ~ 204
16	2 .483 ~ 2 .546 m ,2 .712 ~ 2 .761 m ,3 .683 ~ 3 .717 dJ = 10 .2 Hz ,3 .927 ~ 3 .982 d J = 16 .5 Hz ,4 .176 ~ 4 .231 d J = 16 .5 Hz ,4 .475 ~ 4 .509 d J = 10 .2 Hz ,6 .679 s ,7 .292 ~ 7 .720 m ,10 .268 b ,12 .139 b	FAB-HRMS :(M + H) 432 .1652	34 .8	218 ~ 220
17	2 .242 ~ 2 .361 m ,2 .493 ~ 2 .566 m ,2 .718 ~ 2 .768 m ,3 .678 ~ 3 .712 d J = 10 .2 Hz ,4 .464 ~ 4 .498 d J = 10 .2 Hz ,3 .770 ~ 3 .863 m ,4 .283 ~ 4 .377 m ,6 .690 s ,7 .394 ~ 7 .886 m ,10 .079 s	FAB-HRMS :(M + H) 446 .1810	38 .0	160 ~ 162
18	1 .434 ~ 1 .528 quintet ,1 .591 ~ 1 .679 quintet ,1 .901 ~ 2 .000 m ,2 .530 ~ 2 .579 m ,2 .731 ~ 2 .781 m , 3 .586 ~ 3 .674 m ,4 .148 ~ 4 .244 m ,3 .704 ~ 3 .738 d J = 10 .2 Hz ,4 .485 ~ 4 .519 dJ = 10 .2 Hz , 6 .701 s ,7 .394 ~ 7 .902 m ,10 .148 b	EI-HRMS :(M^+) 459 .1851	33 .7	128 ~ 130

The *in vitro* FTase inhibitory activities were primarily determined against FTase using the SPA assay[3]. The substrates used were ^{3}H-farnesylpyrophosphate and a biotin-linked K-Ras (B) decapeptide (Lys-Lys-Ser-Lys-Thr-Lys-Cys-Val-Ile-Met) . The results are listed in Table 2 .

Table 2 Inhibitory activities of compounds (6 ~ 12 and 16 ~ 18) on FTase *in vitro* (c : 1×10^{-6} mol·L^{-1})

Compd .	Percent inhibition/ %	Compd .	Percent inhibition/ %	Compd .	Percent inhibition/ %
6	18 .60	**10**	41 .50	**16**	61 .00
7	30 .10	**11**	8 .80	**17**	37 .50
8	31 .50	**12**	34 .30	**18**	56 .66
9	50 .90				

In Table 2 , compounds **9** , **16** and **18** displayed rather high activities compared with the others . There are no distinct correlation between the activity with changes both in the carbon number at the 1-position and the substituents variation at the 7-position . The reported inhibitory percentage of only one concentration for each compound can not convey the whole picture of the activity , which is to be investigated further .

The X-ray crystallography of compound **15** was elucidated in Figure 4 , the coordinates of which served as starting conformation in structure-activity relationship analysis . The conformation of CAAX stripped off from CAAX and FTase complex is also shown in Figure 4 . The alignment of the structure of **15** with that of CAAX indicated that the pharmacophore dispositions between them are comparable as shown in Figure 4 . The length of the molecule of **15** is 1 .40 nm from N^{π} of imidazol to carboxylate , CAAX is 1 .20 nm from thiol to carboxylate . It was found that when the thiol was overlapped with the imidazole ring while the two carboxyl groups were superimposed each other , the two phenyl groups can be overlaped with the residues of Val and Ile which showed hydrophobic features . Compound **18** , the acid of **15** , structurally close to CAAX , showed potent activity against FTase among the whole series , suggesting that it can presumably bind to FTase well through induced-fit action .

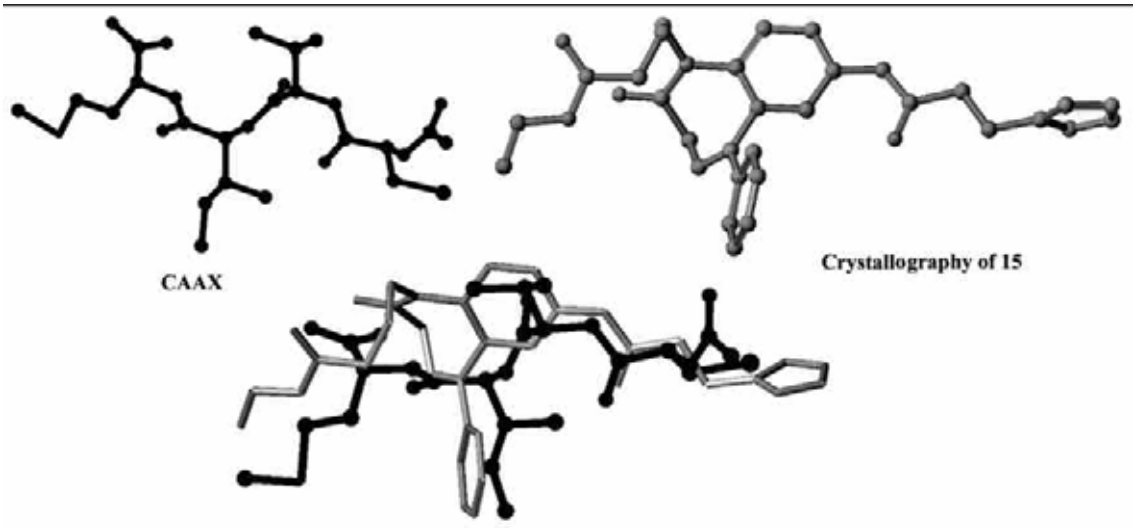


Figure 4 Alignment of **15** and CAAX

EXPERIMENTAL SECTION

Melting points were determined on the Yanaco melting point apparatus and uncorrected. ^{1}HNMR (300 MHz) spectra were recorded on a Varian Mercury 300 spectrometer using Me_4Si as internal standard. High resolution mass spectra was obtained on a ZAB-2F spectrometer. X-ray crystallography was detected on a MAC DIP 2030K spectrometer. Molecular alignment was carried out on a SGI O2 with Sybyl microsofts of Tripos Co. All flash chromatographic separations were performed by using silica gel 60 (230 ~ 400 mesh). All reagents used were of commercially analytical purity.

1 7-Nitro-1-ethoxycarbonylmethyl-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (4a)

Potassium carbonate (2.0 g, 14.4 mmol) and ethyl α-bromoacetate (1.8 g, 10.6 mmol) were added to a solution of **3** (2.0 g, 7.2 mmol) in 20 mL of acetonitrile. The mixture was stirred for 2 h at room temperature and was concentrated. Then a mixture of 50 mL of water and 100 mL of EtOAc was added, the resulting mixture was stirred for 10 min, and two layers were separated. The aqueous layer was extracted with EtOAc (100 mL × 2), and the combined organic phase was dried over Na_2SO_4. After concentration *in vacuo*, the residue was recrystallized from EtOH to give 1.8 g (82.6 %) of compound **4a**, mp 141 ~ 143 ℃.

2 7-Amino-1-ethoxycarbonylmethyl-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (5a)

A mixture of **4a** (1.8 g, 4.9 mmol) and $SnCl_2 \cdot 2H_2O$ (4.8 g, 21.6 mmol) in 30 mL of ethanol was stirred at 70 ℃ for 4 h under a nitrogen atmosphere. The reaction mixture was concentrated *in vacuo*, and then a saturated solution of Na_2CO_3 (50 mL) and CH_2Cl_2 (100 mL) was added to the residue, and stirred for 2 h. The aqueous layer was extracted with CH_2Cl_2 (100 mL × 2). The organic layer was dried over Na_2SO_4 and concentrated at reduced pressure. The crude product was recrystallized from dichloromethane and petrol ether to give 1.7 g (91.8 %) pale yellow product of **5a**, mp 164 ~ 165 ℃.

Compound **4b** or **4c** was synthesized in an analogous manner to that used for **4a**, the oilly product was not purified and reduced to **5b** or **5c**. **5b** and **5c** were prepared by the procedure described in the preparation of **5a**. The overall yield of **5b** is 53.5 %, mp 164 ~ 165 ℃, **5c** 48.4 %, mp 175 ~ 177 ℃.

3 7-(N^{α}-acetylhistidyl)imino-1-carboxymetyl-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (6)

N^{τ}-2,4-dinitrophenyl-N^{α}-acetylhistidine (1.6 g, 4.4 mmol) and HOBt (1.0 g, 7.7 mmol) were added to 20 mL of dried DMF. The solution was stirred for 10 min at 0 ~ 5 ℃, then DCC (0.72 g, 3.5 mmol) was added and stirred for 1 h at 0 ~ 5 ℃. After amino **5a** was added, the reaction mixture was stirred for 2 h at 0 ~ 5 ℃ and left overnight at room temperature. The resulting dicyclohexylurea was filtered off. A saturated solution of Na_2CO_3 (100 mL) was added to the filtrate. A pale brown solid was separated out. It was filtered and dried.

The pale brown product was dissolved in 10 mL of 2-mercaptoethanol and stirred for 2 h at room temperature, to which was added a saturated solution of Na_2CO_3 (50 mL). A pale yellow solid was obtained by filtration and dried. The crude product was purified by flash chromatography (10 % CH_3OH and 2 % AcOH in CH_2Cl_2

as eluant) to give 1.0 g pale yellow solid.

The product was dissolved in a mixture of 8 mL of methanol and 2 mL of water. Lithium hydroxide (0.3 g, 7.2 mmol) was added to the solution and stirred for 2 h at room temperature. Then the reaction mixture was acidified with AcOH and concentrated *in vacuo*. The residue was purified by flash chromatography (30 % CH_3OH and 2 % AcOH in CH_2Cl_2 as eluant), decolorized with charcoal and cryodesiccated to give 0.35 g (23.8 %, overall yield from **5a**) of white solid **6**, mp 246 ~ 248 ℃.

The method of preparing compounds **7** ~ **12** is similar to that of compound **6**.

4 7-(3-imidazol-4-ylpropionamido)-1-carboxymetyl-1,3-dihydro-5-phenyl-2H-1,4-benzodia-zepin-2-one (16)

3-Imidazol-4-ylpropionic acid (1 g, 7.1 mmol) was dissolved in 15 mL thionyl chloride and allowed to stand overnight at room temperature. Excess thionyl chloride was removed under reduced pressure.

The prepared 3-imidazol-4-ylpropionic chloride was dissolved in a mixture of 30 mL of dichloromethane and 10 mL of DMF, and stirred at 0 ℃, to which was slowly added at 0 ℃ a solution of NEt_3 (1 mL, 7.3 mmol) and the amine **5a** (2.4 g, 7.1 mmol) in 20 mL of dichloromethane. The reaction mixture was stirred for 12 h at room temperature. Removal of the solvents was followed by the addition of 50 mL of saturated solution of Na_2CO_3. The solid was collected by filtration dried, and purified by flash chromatography (30 % CH_3OH and 2 % AcOH in CH_2Cl_2 as eluant) to give 2.0 g gray solid.

The solid was dissolved in a mixture of 30 mL of methanol and 10 mL of water, to which was added lithium hydroxide (0.9 g, 20 mmol) and stirred for 2 h at room temperature. Then the reaction mixture was acidified with AcOH and concentrated *in vacuo*. The resulting solid was purified by flash chromatography (30 % CH_3OH and 2 % AcOH in CH_2Cl_2 as eluant), decolorized with charcoal and cryodesiccated to give 0.35 g (34.8 %, overall yield from **5a**) white solid of **16**, mp 218 ~ 220 ℃.

The method of preparing compounds **17** and **18** is similar to that of compound **16**.

5 Biological Testing

The *in vitro* FTase inhibitory activities were primarily determined against FTase using the scintillation proximity assay method according to the procedure described by Yuval Reiss, *et al*[4].

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7- (咪唑-4-烷基酰胺基)-1 ,3-二氢-1-羧基烷基-5-苯基-2 H-1 ,4-苯并二氮杂䓬-2-酮——一类新的法呢基蛋白转移酶抑制剂

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摘要 : 目的　设计并合成新结构类型的法呢基蛋白转移酶抑制剂 。方法　本文结合法呢基蛋白转移酶(FTase) 的作用机理和已有 FTase 抑制剂结构特征 ,设计了一类以苯并二氮杂䓬为分子骨架 ,一端连接有可与锌离子配位结合的咪唑基 ,另一端连接不同长度的末端含羧基的侧链的化合物 。此类化合物模拟了 FTase 配体之一 CAAX 四肽片段 ,共合成 10 个此类新化合物(**6 ~ 12 ,16 ~ 18**) ,并对其进行体外生物活性测定 。结果　所有新目的化合物均经^1 HNMR 和 HRMS 方法确证结构 。结论　对 FTase 抑制活性测定结果表明其中 5 个化合物(**9 ,10 ,16 ~ 18**) 有较强的抑制活性 。

关键词 : 法尼基蛋白转移酶(FTase) ; 苯并二氮杂䓬 ; 构象分析