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Graphical Abstract

A concise diastereoselective approach to enantioenriched substituted piperidines and their in vitro cytotoxicity evaluation

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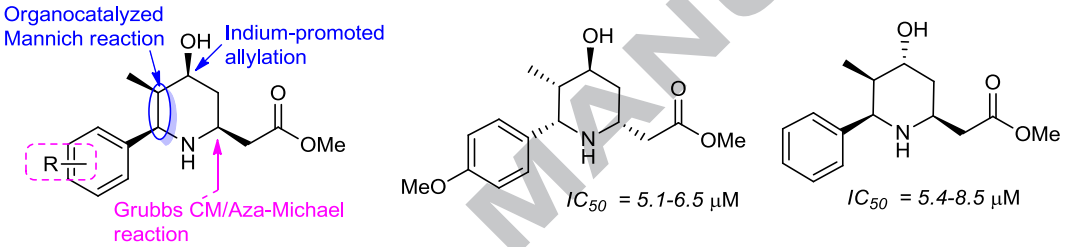

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ABSTRACT

A library of diversely stereo-oriented, highly substituted 2,6-*cis* piperidine derivatives were synthesized, and evaluated for their anticancer activity in cancer cells that included A549 (lung cancer, CCL-185), MCF7 (breast cancer (HTB-22), DU145 (prostate cancer (HTB-81), and HeLa (cervical cancer, CCL-2). One stereo-variant emerged as a promising candidate for further design based structure-activity studies.

The non-racemic functionalized piperidine scaffolds have received renewed attention owing to their broad spectrum of biological and pharmaceutical properties.[1] Functionalized piperidine sub-units are found to be part of naturally occurring alkaloids and anti-tumor antibiotic molecules.[2] Simple derivatives of the substituted piperidine molecule *L*-pipecolic acid, are a constituents of plants, fungi and human physiological fluids.[3] In particular, C-2 and C-6 substituted piperidine molecules originated from extracts of *Dendrobatesspeciosus* (Panamanian poison frogs) have showed a range of activities such as cytotoxic, antifungal, hemolytic and *anti*-HIV properties[4] (Figure 1).

The configuration of stereogenic centers in the piperdine ring has considerable influence on its biological and pharmaceutical activity. Stereogenic functional group variations in 6-membered azacycles are expected to accrue good activity; as a consequence, they are exhibiting noteworthy differences in their biological profiles.[5] The development of simple and efficient methods for the synthesis of enantiomerically pure substituted piperidine derivatives from readily available starting materials is always a demanding task. Consistent with our continued interest in developing catalytic enantioselective routes to enantioenriched small molecules,[6] we describe the preparation of new variants of highly substituted non-racemic functionalized homopipecolic derivatives and chemotherapeutical activity.

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RESULTS AND DISCUSSIONS

A number of impressive methods have been reported for accessing enantioenriched substituted piperidine molecules. Included among them are i) the use of readily available resident chiral amine as a nitrogen source[7] ii) chiral pool derived routes[8] and iii) various catalytic routes.[9] While exploring numerous methods, we have focused on the organocatalyzed asymmetric Mannich reaction that constitutes one of the atom-economic efficient strategies for providing the chiral β-amino carbonyl motif in either *syn*- or *anti*-selective fashion.[10] Organocatalyzed asymmetric reaction products having formyl functionality that could be used for tandem reactions with appropriate pro-nucleophiles are especially interesting.[11] In principle, three stereogenic centers could be realized using a tandem Mannich / indium promoted allylation sequence. Further, a Grubbs cross-metathesis with methyl acrylate and *aza*-Michael reaction is expected to provide highly substituted optically active piperidine derivatives.

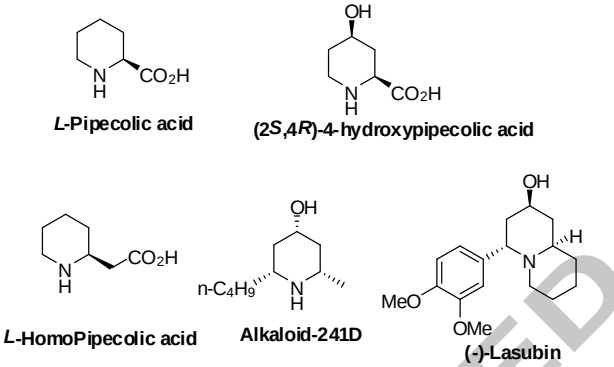
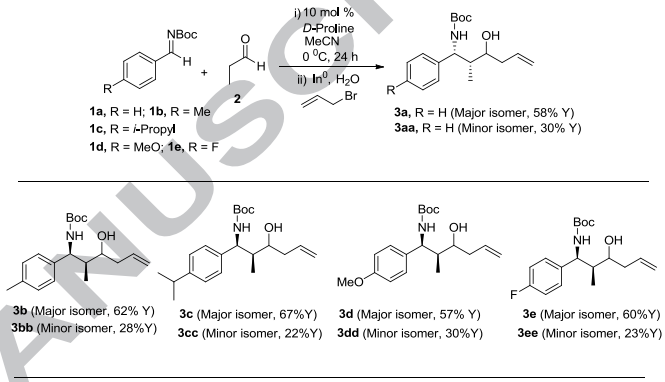


Figure 1. Substituted piperidine molecules

At the outset, we envisaged that a one-pot reaction could be used to install a hydroxy-methyl-amino stereogenic centers with pendant terminal olefin functionality. To this end, an organocatalyzed asymmetric Mannich reaction developed by List et. al.[10a] followed by Barbas[11] Mannich-allyation reaction sequence was adopted. After some experimentation (Supporting Information), the desired transformation was achieved to give *syn-anti* **(3a)** and *syn-syn* **(3aa)** products as separable diastereomers (Figure 2). The ratio of *syn-anti* to *syn-syn* product (66:34) ratio was determined from the weight of the isolated diastereomers. A stepwise reaction sequence showed that the stereochemical information introduced in the first organocatalytic step was preserved in the reaction[9d] by yielding *syn* Mannich products in 99:1 *dr* with 98% *ee*.[12] Thus, it is the indium promoted allylation that provided poor diastereoselectivity. The relative stereochemistry of the **3a** and **3aa** were not assigned at this juncture but advanced further.

With the optimized reaction conditions, the scope of the transformation was examined with various substituted aromatic aldehydes. As shown in Figure 2, substituted aromatic aldehydes with electron rich and poor functional groups were reacted smoothly, affording the desired products in fair to good yields with the same high level of diastereoselectivity. Compounds **3b-3e**, were generated using *L*-proline catalyst under otherwise identical conditions. Next, to prepare *anti-syn* and *anti-anti* variants, we employed a previously described *anti*-Mannich strategy[10b] using amido-sulfone **1f** and **2** in the presence of 20 mol% of (*S*)-α, α-bis[3,5-bis(trifluoromethyl)phenyl]-2-pyrrolidine methanol and subsequent indium promoted allylation under identical conditions as reported (vide infra). The resulting products, *anti-syn* **3f** and *anti-anti* **3ff**, showed similar diastereo selctivities (Figure 3).

Figure 2. Synthesis of β-amino allyl alcohols produced by Mannich / indium promoted allylation[a]



a) Isolated yield.

Following successful preparation of diastereo divergent hydroxyl-methyl-amino variant compounds, we have focused on one-pot Grubbs cross-metathesis and *aza*-Michael reaction.[13] The cross metathesis reaction between **3a** and methylacrylate using second-generation Grubbs catalyst proved to be ineffective in several organic solvents. However, the use of 5 mol% of second-generation Grubbs catalyst in the absence of solvent gave the α, β-unsaturated ester in 85% yield (Figure 4).

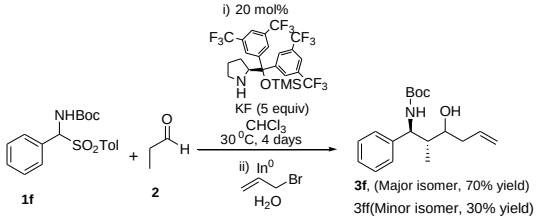
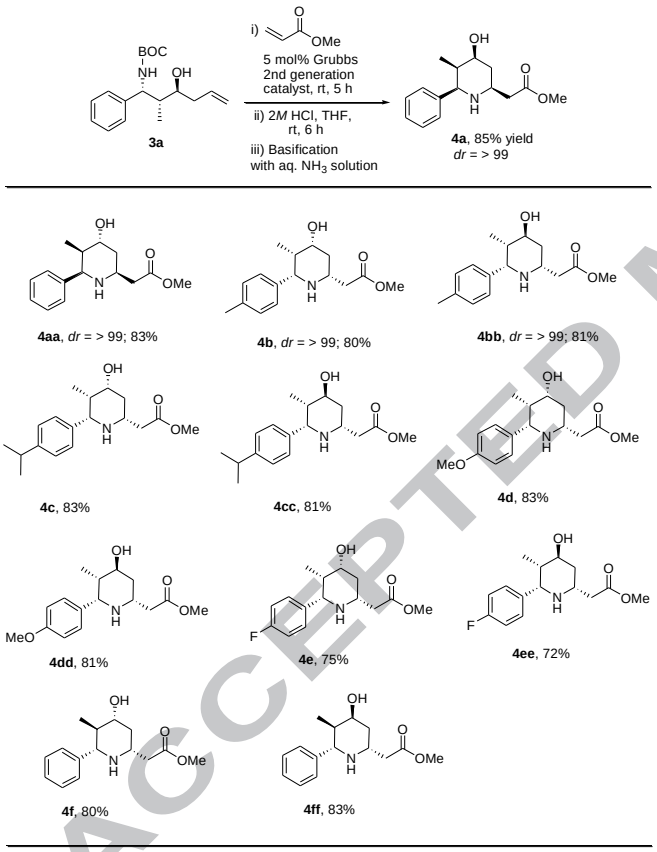


Figure 3. Synthesis of β-amino allyl alcohols from *anti*-Mannich reaction.

Finally, the one-pot Grubbs cross metathesis / *aza*-Michael reaction sequence between terminal olefin **3a** and methylacrylate was performed by the exposer of the metathesis product to acidic and then basic conditions. The initial product **4a** was obtained in an encouraging 40% yield. In a survey of bases, aqueous ammonia was found to increase the yield of **4a** to 85% as a single diastereomer. Various other substrates were submitted to the successful conditions and the results are shown in **Figure 4**. In all cases, exclusive *cis* diastereomer was obtained.

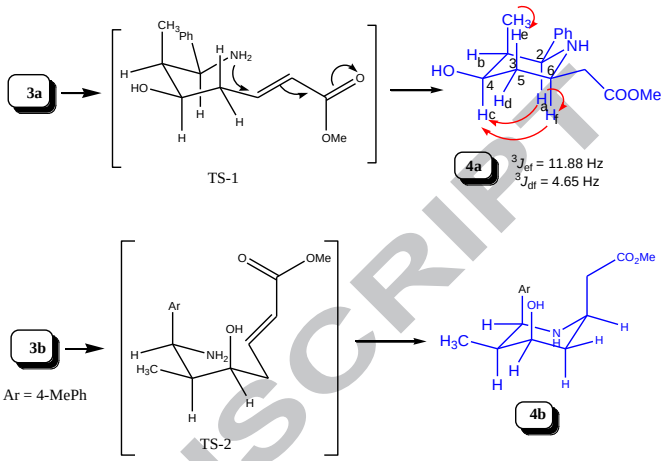
The relative stereochemistry of C_2, C_3, C_4 and C_6 centers of **4a** were assigned by NMR studies such as 1D including selective ^{1}H-^{1}H homo-nuclear decoupling NMR, and 2D-NOESY and HSQC analysis. In compound **4a**, the observed strong *nOe* cross-peaks of H_a-H_c, H_c-H_f, and H_f-H_a protons and the scalar coupling constants ($^3J_{ab}$ = 2.87 Hz, $^3J_{ef}$ = 11.88 Hz, and $^3J_{df}$ = 4.65 Hz) unequivocally showed that H_a and H_f protons are in a *syn* orientation (**Figure 5**). The stereochemical outcome of **3a** can be explained on the basis of 6-*exo-trig* cyclization wherein, substituents at positions 2 and 5 are likely to adopt the equatorial orientations **TS-1**, leading to the *cis*-diastereomer **4a (Figure 5)**.[7c] In case of **3b**, the substituents at positions 2 and 5 are likely to adopt the axial orientations **TS-2**, leading to the *cis*-diastereomer **4b**.

Figure 4. Synthesis of enantioenriched piperedine derivatives[a,b,c,d]



a) All reactions were carried out using 2 mmol scales. b) Isolated yield. c) The relative stereochemistry of C_2, C_3, C_4 and C_6 centers of **3a** was unambiguously assigned by NMR studies and all other by analogy. d) In all cases, exclusive one diastereomer was observed.

Figure 5. Nuclear Overhauser effect (*nOe*) analysis of **4a** and *Aza*-Michael reaction by 6-*exo-trig* cyclization of **4a** and **4b**.



ACTIVITY AGAINST CANCER CELLS

The stereo-divergent substituted homopipecolic ester derivatives **4a, 4aa-4f**, and **4ff** were screened for cytotoxicity activity against a panel of tumor cell lines. *In vitro* growth inhibition was assessed in 96-well plates by the standard MTT assay using doxorubicin as a standard. Four human tumor cell lines were used: A549 derived from human alveolar adenocarcinoma epithelial cells (ATCC No.CCL-185), HeLa derived from human cervical cancer cells (ATCC No. CCL-2), DU-145 derived from human prostate adenocarcinoma cells (ATCC No. HTB-81) and

Table 1. Cytotoxicity results of various enantioenriched substituted piperidine derivatives

Entry	Test Substrate	IC_{50} values[a] in (μM)			
		A549	**DU145**	**HeLa**	**MCF7**
1	**4a**	20.5	11.3	14.2	12.3
2	**4aa**	**8.5**	**6.3**	**6.5**	**5.4**
3	**4b**	29.0	49.7	13.4	12.2
4	**4bb**	97.6	330.3	NA	NA
5	**4c**	NA	110.4	NA	NA
6	**4cc**	NA	27.4	18.2	17.1
7	**4d**	23.5	12.6	11.5	10.4
8	**4dd**	**5.4**	**6.5**	**5.3**	**5.1**
9	**4e**	26.6	51.7	19.8	15.6
10	**4ee**	17.2	NA	17.4	18.2

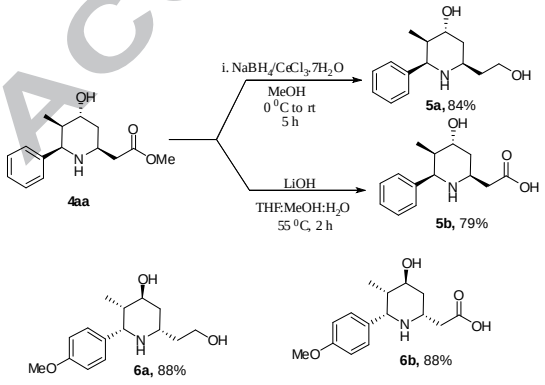
11	**4f**	34.6	64.1	35.2	35.6
12	**4ff**	69.2	NA	41.1	NA
13	**5a**	31.2	41.6	31.5	39.8
14	**5b**	28.9	46.5	33.4	23.1
15	**6a**	17.6	14.8	16.5	13.7
16	**6b**	20.1	15.7	19.9	12.5
Doxorubicin[b] (Standard control)		**0.6**	**0.6**	**0.7**	**0.6**

i. Not active; A549-Lung cancer (CCL-185); MCF7-Breast cancer (HTB-22); DU145-Prostate cancer (HTB-81); HeLa-Cervical cancer (CCL-2)

ii. (a) The IC_{50} values (50% inhibitory concentration) were calculated from the plotted absorbance data from the dose-response curves. IC_{50} values (in μM) were expressed as the average of two independent experiments. (b) Reference compound for positive control profile, MCF7 derived from human breast adenocarcinoma cells (ATCC No.HTB-22) using the MTT assays.[14]

IC_{50} values (50% inhibitory concentration) were determined as a preliminary screen, with results shown in Table 1. Compounds **4aa** and **4dd** had excellent activity against all four cell types. By comparing the 4-fluorophenyl and 4-methoxyphenyl analogues, the substitution pattern on the aromatic nucleus was shown to have an effect on the activity profile. Similarly, the configuration of piperidine stereogenic centers had an influence on the activity profile compound **4aa** and **4dd** being more cytotoxic than the corresponding diastereomers of **4a** and **4d**, respectively.

The substituted piperidine molecules have three strategic sub-structures for modifications; hence we aimed at for further improvement of activity based on the lead molecular structures of **4aa** and **4dd** that showed good activity in preliminary screening. Reduction of the ester group of **4aa** and **4dd** to the primary alcohols **5a** and **6a**, and saponification to the carboxylic acids **5b** and **6b**, was accomplished in good yields (**Scheme 1**). These more polar compounds possessing hydrogen bond donors were less active than the parent esters (Table 1).



Scheme 1. Synthesis of acid and alcohol derivatives of substituted piperidines

The activity results of **5a**, **6a**, **5b** and **6b** were shown in Table 1. The results indicate the polar functional groups shown deleterious to activity.

In conclusion, we have accomplished a concise diastereoselective approach for a library of enantioenriched substituted piperidine molecules. Strategic transformation of this two-step sequence includes a catalytic enantioselective Mannich reaction that is either *syn*- or *anti*-selective as the genesis of chirality / indium promoted allylation, and Grubbs cross-metathesis / *aza*-Michael reaction. Moreover, this strategy can be used for the synthesis of various derivatives of stereo-divergent substituted *aza*-cyclic small molecules which can be evaluated for biological activity. Interestingly, promising data presented in this study reveals that the configuration of stereogenic center plays an important role in anticancer activity. This study also provides the impetus for the next generation design and synthesis of enantioenriched substituted homopipecolic esters and amides. Further study aimed at a comprehensive understanding of stereogenicity-structure activity relationships is currently in progress.

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Supplementary data

Supplementary data associated (detailed experimental procedures for the synthesis and the *nOe* studies) with this article can be found, in the online version,

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