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Novel β-(imidazol-4-yl)-β-amino acids: solid-phase synthesis and study of their inhibitory activity against geranylgeranyl protein transferase type I

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Abstract—Solid-phase synthesis of imidazolyl-β-amino acid derivatives is described. Several analogs demonstrated moderate inhibition of geranylgeranyl protein transferase type I (GGPT I).

The prenyl transferases are a class of enzymes that is involved in post-translational modification of the membrane associated proteins.[1] These enzymes catalyze the transfer of a farnesyl or geranylgeranyl group to a C-terminal cysteine residue contained in a C-terminal tetra peptide signal sequence, frequently referred to as a CAAX motif.[2] Many of these proteins such as the Ras and Rho family of proteins regulate cell growth by signal transduction. Mutated *ras* genes encoding activated Ras occur in 20–30% of all human cancers. Such mutated Ras proteins lose their intrinsic GTPase activity and remain constitutively activated with bound GTP, resulting in uncontrolled cell growth. Mutations in the K-*ras* isoform are most relevant to human cancers in particular pancreatic, colon, and lung cancers, which exhibit approximately 90%, 40%, and 25% incidence of K-*ras* mutations, respectively. The most important step for the functioning of Ras is the membrane attachment, which is aided by farnesylation via the enzyme farnesyl transferase.[3] At least two inhibitors of farnesyl protein transferases (FTIs) Zarnestra™ (Johnson & Johnson) and Sarasar™ (Schering-Plough) remain in advanced stages of clinical development despite early setbacks.[4] Alternative prenylation of mutated K-Ras by geranylgeranyl protein transferase type I (GGPT I) is however considered a potential mechanism for resistance to FTIs in an important population of tumors harboring K-*ras* mutations.[5] Therefore, the combination of a GGPT I inhibitor and a an FTI was considered a therapeutic strategy for human tumors harboring K-*ras* gene mutations.[6] Unfortunately, conflicting data surround the combined use of FTIs and inhibitors of GGPT I with at least one group reporting severe toxicity with combined use of FTIs and GGPT I inhibitors.[7] Also, there have been several reports that GGPT I inhibitors alone can produce significant antitumor effects in animal tumor models.[8] As the number of published GGPT I inhibitors is limited, it would be useful to develop additional chemical series of GGPT I inhibitors to further explore the role of this enzyme in tumor growth and resolve the conflicts that currently exist in the published data.

We had undertaken synthesis of diverse libraries of 4-methane amino imidazole derivatives as part of an effort toward discovery of new antifungal agents.[9] A majority of initial library of compounds belonged to the sulfonamide type analogs (**1**, Fig. 1), due to their favorable activity in in vitro screens for antifungal activity. During

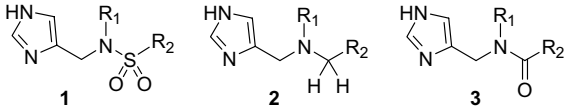

Figure 1.

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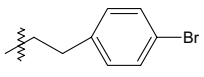
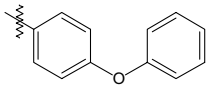
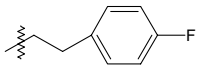
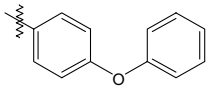
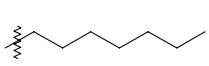
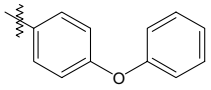
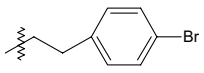
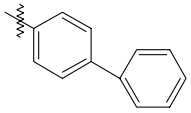
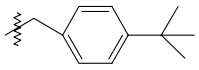
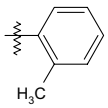
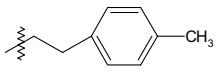
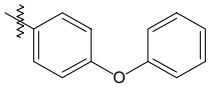
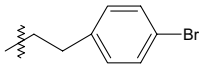
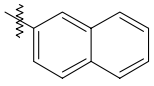
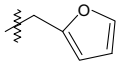
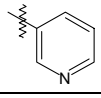
Table 1. Initial screening data for compounds **2** against GGPT I

#	R1	R2	% Inh. at 10 μM
2a			88
2b			78
2c			40
2d			77
2e			24
2f			72
2g			69
2h			23

the course of this work, we also synthesized smaller libraries of analogs where the $-SO_2$-group in **1** was replaced by a $-CH_2$-group (**2**) as well as carboxamides **3**. Such compounds were readily prepared on solid support via reductive alkylation of resin bound 4-methaneamine imidazole.[9] We initiated a screening campaign on all available library compounds of types **1**, **2**, and **3** against GGPT I in an assay using a K-Ras peptide substrate (biotin-KKKKKKSKCVIM). While none of the library members of structure type **3** and only a few of structure type **1** showed activity several members of library type **2** demonstrated inhibitory activity at the screening dose of 10 μM (Table 1). Among the later only compounds containing hydrophobic biphenylether, biphenyl, or naphthyl moieties were associated with better activity than other R_2 groups.

The biphenylether analog **2a** had an IC_{50} of 256 nM, while closely related **2b** had an IC_{50} of 460 nM. They were essentially inactive against farnesyl transferase (IC_{50}: >10 μM). Considering that the biphenylether may constitute part of a potential pharmacophore, we first attempted to modify or replace the 4-imidazolyl group in **2a**. Replacement of this group in **2a** with other heterocycles such as the thiazole or pyridines (**7**, **8**, **9**, or **10**; Fig. 2) resulted in complete loss of activity, indicating the hypothesized role of the imidazole in binding to catalytic zinc atom in the active site of the enzyme. Substituting the imidazole ring with a methyl group either at the 1-position or the 2-position also caused loss of all activity (**4**, **5**; Fig. 2). A similar result was obtained when the substitution pattern was changed to the 2-imidazolyl (**6**; Fig. 2).

During these synthetic efforts, we learned that libraries of type **1** and **2**, resembled nonthiol peptidomimetic inhibitors of FPT disclosed in patents by Merck in 1997 and published later.[10] Significant diversity existed in the R_1 and R_2 groups in our initial screening library for HTS. We therefore looked for an alternative way to insert a novel feature on the lead structures **2** to improve potency.

We hypothesized that juxtaposition of a carboxyl functionality close to zinc coordinating imidazolyl moiety would lead to novel and potentially very selective inhibitors of prenyl transferases. Focus was on the novel β-amino acid derivatives **11** and **12** with an eventual goal to develop bi-substrate type inhibitors via the carboxyl handle. Developing a parallel synthesis method for such interesting structures that allow generation of numerous single pure compounds was also of interest for broad screening purposes (Fig. 3).

β-Amino acids have indeed found utility as bioactive compounds and in research on oligomeric moieties such as β-peptides.[11,12] While several methods exist for synthesis of β-amino acid structures, particularly relating to the antitumor agent taxol, antifungal cyclodepsipep-

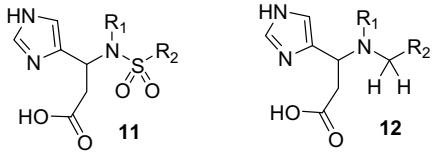


Figure 3.

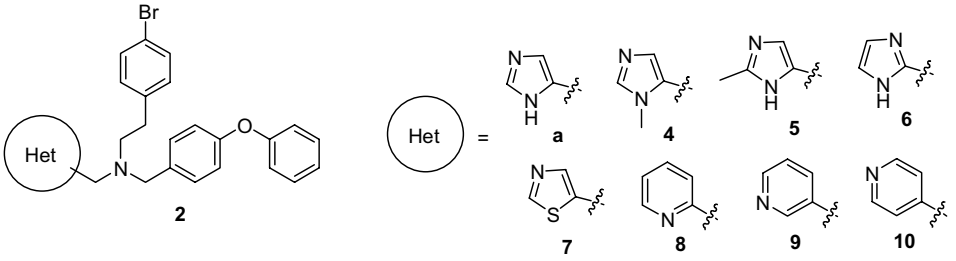
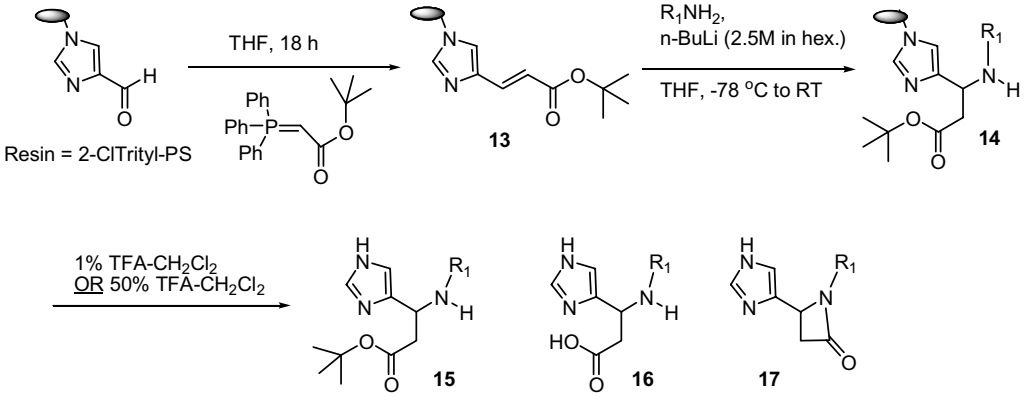


Figure 2.

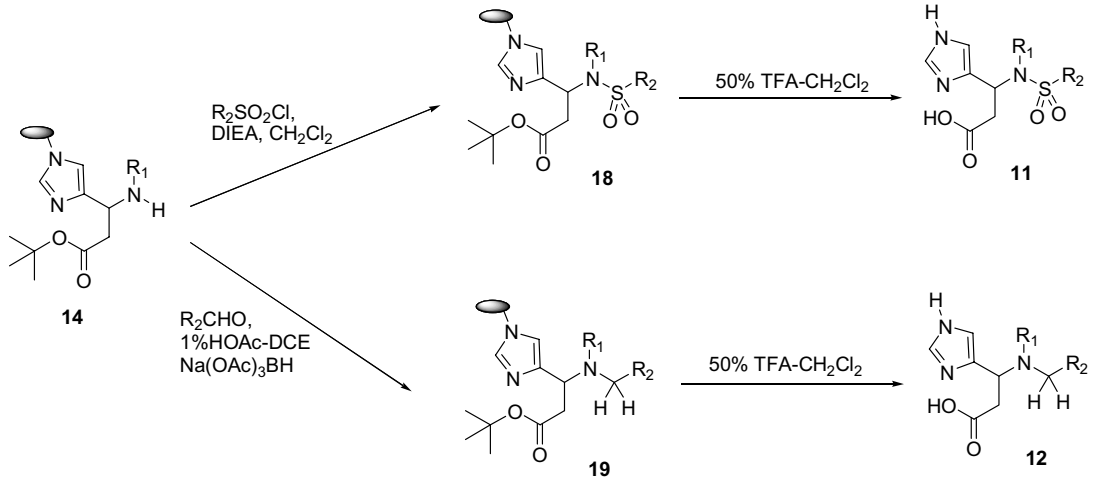


Scheme 1. Aza-Michael reaction toward synthesis of β-amino acids on solid support.

tide (±)-jasplakinolide, etc., new synthetic methods are of interest.

Our synthetic effort began with anchoring of 4-formyl imidazole to 2% cross-linked PS-resin via the convenient 2-Cl trityl linker (Nova BioChem). Conditions were optimized for quantitative loading. Horner–Emmons condensation was conducted on resin bound aldehyde with an excess of *tert*-butylcarboxy triethyl phosphonoacetate in THF (Scheme 1). This reaction went cleanly to provide the (*E*)-α,β-unsaturated ester **13** as evidenced by HPLC–MS and NMR study of cleaved resin samples. Transformation was deemed quantitative. Aza-Michael reaction on this material was then attempted with various amines. Simple incubation of the ester with a variety of amines in DMF, DMSO, or other polar solvents in temperatures of up to 100 degrees did not show any addition at all. Lithium amides have been utilized in conjugate additions to α,β-unsaturated systems for generation of chiral β-amino acids.[13] Employing this literature protocol, we generated lithium amides by pre-treating several amines with *n*-BuLi in THF at −78 °C. The resulting lithium amides were then added via cannula under N_2 to a pre-swelled suspension of resin **13** in THF maintained at −78 °C. After a brief period, the mixture was warmed to room temperature followed by a careful quench and wash procedure. Cleavage of a sample with 5% TFA–CH_2Cl_2 revealed that desired conjugate addition have indeed taken place. The addition reaction was however incomplete for most amines utilized in initial pilot runs as revealed by HPLC. In order to circumvent pitfalls associated with syringe/cannula transfer of air-sensitive lithium amides, particularly in library synthesis mode, we tried the reverse addition next. Thus, resin **13** was pre-swelled and agitated under intermittent purges of Argon and then added via pipettes to the lithium amides generated and maintained under nitrogen atmosphere at −78 °C. After a brief period of incubation at −78 °C, the reaction mixtures were warmed up gradually to room temperature. This change actually led to a much higher degree of transformation to the desired β-amino acids.

A total of 20 diverse amines participated well in the aza-Michael addition. Cleavage after this addition under mild-TFA conditions produced the *tert*-butyl ester products **15**. Cleavage with 25–50% TFA–CH_2Cl_2 produced the deprotected β-amino acid products **16**. In general aliphatic primary amines gave the best yields. Anilines did participate in the Michael addition, but were suscep-



Scheme 2. Synthesis of β-amino acids based libraries on solid support.

Table 2. Representative examples, yield and initial screening data

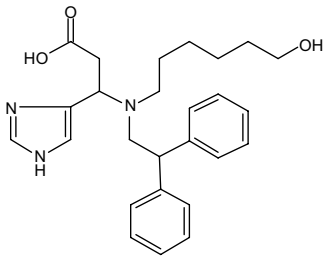
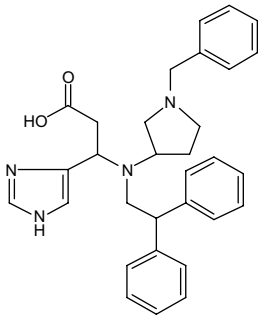
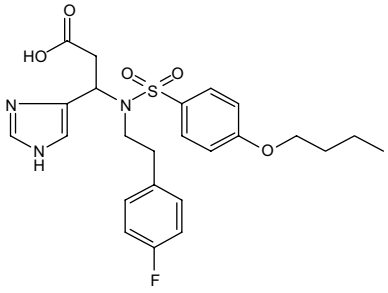
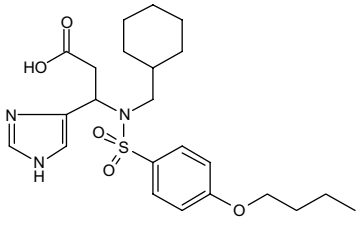
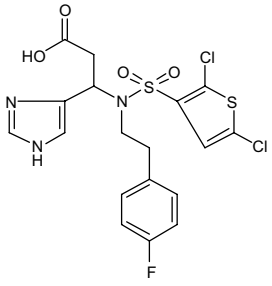
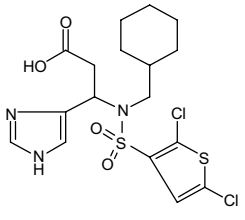
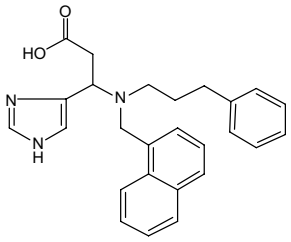
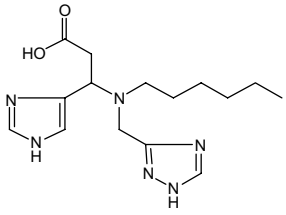
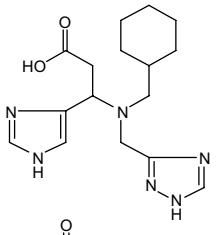
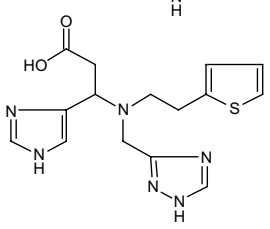
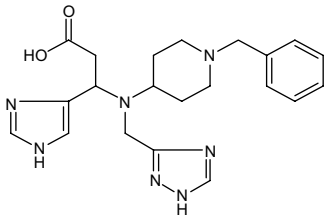
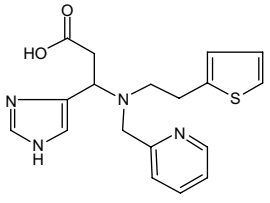
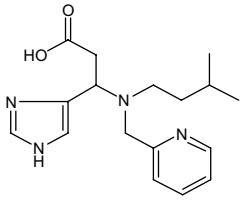
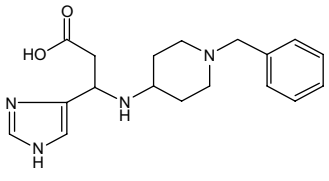
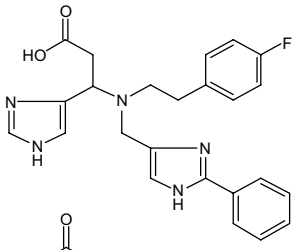
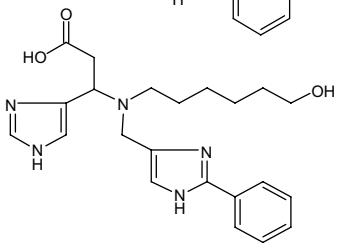
#	Structure	Purity (%) RP-HPLC (214 nM)	Yield (%)[a]	% Inh. at 10 μM
12a		100	5.9	0
12b		100	5.6	18
11a		96.5	22.1	78
11b		100	26.7	30
11c		100	11.5	53
11d		100	19.7	21

Table 2 (*continued*)

#	Structure	Purity (%) RP-HPLC (214 nM)	Yield (%)[a]	% Inh. at 10 μM
12c		95.8	7.7	74
12d		91.1	8.7	3
12e		100	26.4	8
12f		93.9	16.7	16
12g		100	21.4	11
12h		96.9	24.9	11
12i		84.9	19.3	10
16a		100	39.9	21

(*continued on next page*)

Table 2 (*continued*)

#	Structure	Purity (%) RP-HPLC (214 nM)	Yield (%)[a]	% Inh. at 10 μM
12j		85.5	17.9	35
12k		100	7.0	0

[a] Isolated yield of purified products from the entire 4-step synthetic sequence.

tible toward β-lactam (**17**) formation giving β-amino acid products in only very modest yields after purification. Very hindered amines including branched alkyl amines led to β-lactam products almost exclusively. Piperazine like secondary amines on the other hand gave good yields of β-amino acid products.

For analog synthesis purposes, the products **14**, after adequate wash cycles were subjected to reaction with aromatic aldehydes under reductive alkylation conditions with sodium triacetoxy borohydride (Scheme 2). Reaction analysis by LC–MS revealed that desired *tert*-amines were formed as major products. Treatment with 50% trifluoroacetic acid in CH_2Cl_2 resulted in cleavage from solid support and concomitant removal of *tert*-butyl ester group. Crude products were purified by reverse phase HPLC. Proton and C-13 NMR data were obtained in addition to LC–MS and was consistent with the β-amino acid structures **12**. In a similar fashion, the resin bound β-amino acids **14** were treated with aryl sulfonyl chlorides to produce a library of sulfonamide derivatives **11** after cleavage under TFA conditions. All compounds were prepared from approximately 100 mg or ~0.1 mmol of starting resin yielding products in the range of 1 mg to up to 20 mg. Isolated percent yields from the entire sequence on support are shown in Table 2.

Screening of the β-amino acids library against GGPT I revealed moderate activity only as shown in Table 2. Library compounds containing polar groups at either R_1 or R_2 position were inactive or poorly active at the screening dose of 10 μM. Tertiary amine based analogs such as **12c** containing naphthyl or similar hydrophobic chains did show good inhibitory activity. Interestingly, several sulfonamides, which are non-zwetterionic such as **11a** were also active. Compounds with >75% inhibition were selected for a full dose response curve and IC_{50} determination. As can be seen in Table 3, the best IC_{50} value of 90 nM was associated with side chains identical to one of the original lead structures (**12l** vs

Table 3. IC_{50} values for selected compounds from the β-amino acids library

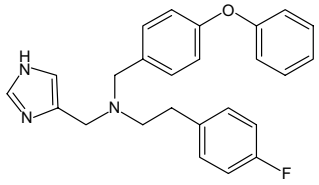
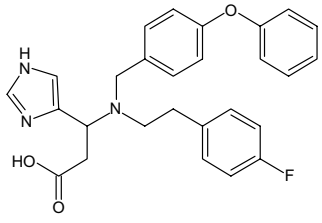
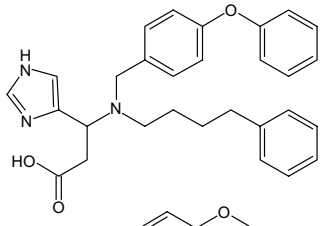
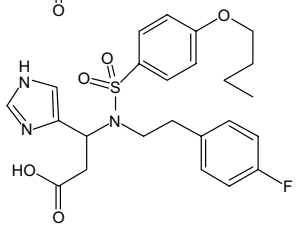
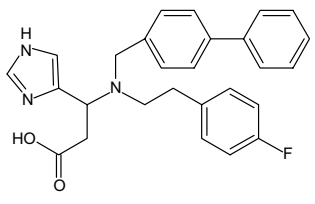
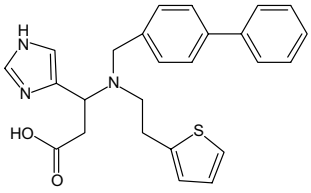
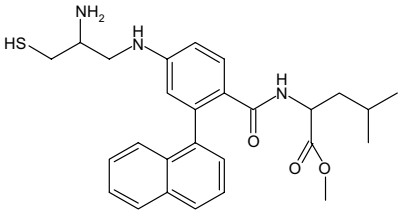
#	Structure	IC_{50} GGTase (K-Ras) μM
2b		0.46
12l		0.09
12m		0.86
11a		1.07
12n		1.1

Table 3 (*continued*)

#	Structure	IC_{50} GGTase (K-Ras) μM
12o		1.07
20		0.15

2b). The β-carboxymethylene substitution did therefore have a beneficial effect on enzyme potency in that a 5-fold improvement was achieved. This compound was as potent as reference inhibitor GGTI-298 (**20**; Table 3),[14] which we had included for comparative purposes.

In summary, we have synthesized a series of imidazol-4-yl-β-amino acids with diverse functionalities. To our knowledge, this is the first illustration of solid-phase synthesis of library molecules belonging to an important class of β-amino acids. The resulting analogs were screened against GGPT I. A moderate improvement of potency for the β-amino acids was noted.

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References and notes

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