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NOVEL TRICYCLIC AMINOACETYL AND SULFONAMIDE INHIBITORS OF RAS FARNESYL PROTEIN TRANSFERASE

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Abstract: Novel tricyclic FPT inhibitors with submicromolar FPT activity are described. Greatly enhanced FPT activity is realized with phthaloyl derivatized amino compound **2k**, which showed FPT inhibitory activity of IC_{50} = 0.66 μM. Sulfonamides **5g** and **50** were also found to be potent FPT inhibitor. SAR resulting from a variety of tricyclic amino acids and sulfonamide derivatives is discussed.

Introduction

In recent years, the understanding of biochemical events involved in tumorigenesis has greatly increased. One particular area that has received considerable attention is that of Ras p21 mediated signal transduction.[1a-c]

p21 Ras proteins (H, K, and N) are small guanine nucleotide binding proteins that transduce information from the inner surface of the cell membrane to the nucleus, thereby, playing a major role in controlling cell growth and differentiation. It does this through a 'biochemical regulatory switch' that works in the following way: when ras p21 is GTP-bound, signal transduction occurs; however, when it is GDP-bound the signal is terminated. In normal cell signaling, inactivation of the ras p21 occurs through hydrolysis of the bound GTP to GDP by its intrinsic GTPase activity. However, in mutated forms of Ras the GTPase activity is impaired. This results in mutant ras remaining complexed to GTP leading to unregulated cell proliferation and malignant transformation.[2a-d] The mutated ras gene is found in 30% of all human carcinomas including 90% of pancreatic adenocarcinomas and 50% of human colon tumors.[3a-b]

In order for Ras p21 proteins to exert their normal as well as oncogenic effects, they must be bound to the inner surface of the plasma membrane. This membrane binding occurs by the following defined sequence of post-translational modifications: (a) farnesylation of the cysteine residue at the carboxyl terminal tetrapeptide CaaX motif (where C = cysteine, a = aliphatic amino acid, and X = Ser or Met); (b) proteolytic removal of the three amino acids distal to cysteine; and (c) carboxy methylation of the new cysteine carboxyl terminus. Farnesyl protein transferase (FPT) catalyzes the transfer of the farnesyl group from farnesyl diphosphate (an intermediate of cholesterol biosynthesis) to p21 ras proteins.[2b,4a-c] Inhibition of FPT represents a possible method for preventing association of Ras p21 to the cell membrane, thereby blocking its cell-transforming capabilities. Such inhibitors may have therapeutic potential as anticancer agents.[5]

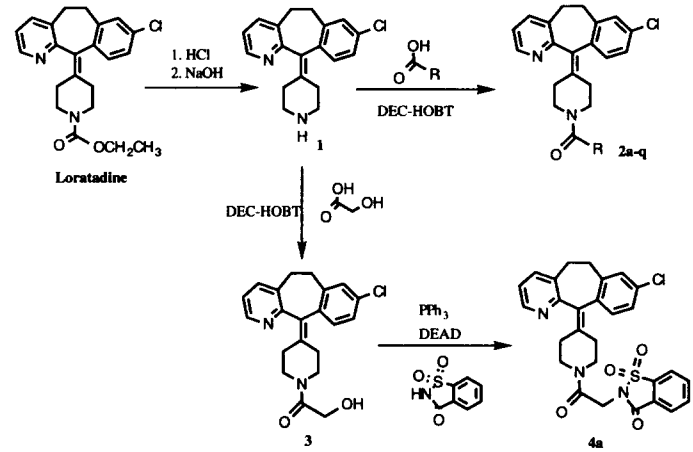
Inhibition of FPT by CaaX peptides,[6] peptidomimetics,[7] and farnesyl diphosphate mimics[8] has been extensively studied. Although a number of these compounds have shown significant FPT inhibitory activity,

most of them exhibited poor activity in cell based assays mainly because they had proteolytically cleavable peptide bonds or possessed easily oxidizable sulfhydryl groups.[9]

Recently, we reported the discovery of SCH 44342, a tricyclic amide as a novel nonsulfhydryl, nonpeptidic FPT inhibitor with an FPT IC_{50} of 0.25 μM.[10] We now report the discovery of a new class of tricyclic FPT inhibitors that are derived from amino acids and sulfonamides. These FPT inhibitors are also nonpeptidic and do not have any free sulfhydryl groups, possibly making them more stable in vivo.

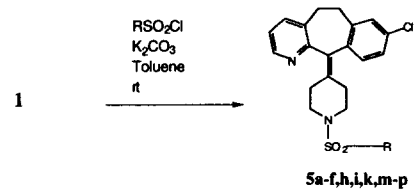
Hydrolysis of Loratadine[11] in boiling hydrochloric acid gave amine **1** that was then coupled to a variety of protected amino acids to afford compounds of type **2a-q** (Scheme 1). Compound **2a** was obtained from BOC- hydrolysis of compound **2b** using trifluoroacetic acid or HCl in dioxane. In a similar manner compound **2h** was obtained from compound **2i**. Saccharin derivative, compound **4a**, was obtained first by coupling tricyclic amine **1** with glycolic acid to obtain compound **3**, which was subsequently reacted with saccharin using Mitsunobu protocol to give the desired derivative **4a** (Scheme 1).[12]

Scheme 1



Synthesis of the sulfonamides was carried out as outlined in Scheme 2; thus, amine **1** was reacted with appropriate sulfonyl chloride in the presence of K_2CO_3 in dry toluene to give target sulfonamides **5a-p**.

Scheme 2

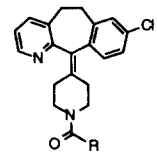


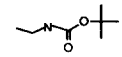
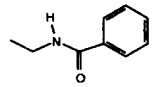
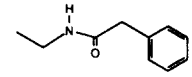
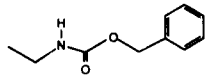
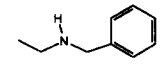
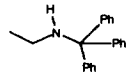
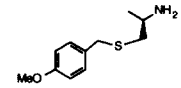
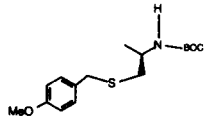
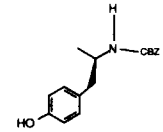
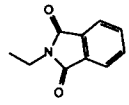
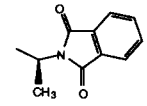
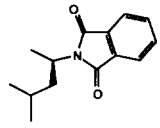
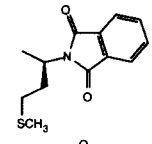
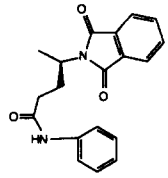
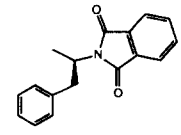
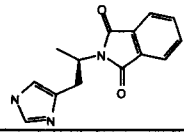
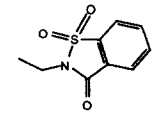
Sulfonamide **5g** was obtained from the reaction of **1** with 2-chloroethylsulfonyl chloride with subsequent elimination of hydrogen chloride in situ. Compound **5j** was obtained from $NaBH_4$ reduction of tricyclic ketone **5i** as a mixture of endo and exo alcohols. Carboxylic acid **5l** was obtained by sodium hydroxide hydrolysis of sulfonamide **5k**.

Results and Discussion

Compounds thus prepared were tested for their ability to inhibit the transfer of tritiated farnesyl from farnesyl diphosphate to Ras-CVLS a process that is mediated by farnesyl protein transferase (FPT) using conditions previously described.[10,13] Biological data for amino acetyl derived compounds and the sulfonamides are reported in Tables 1 and 2, respectively.

Table 1. Tricyclic Amino Acetyl Based - FPT Inhibitors



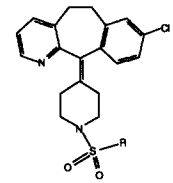
Entry	R	FPT(IC_{50}) μM	Entry	R	FPT(IC_{50}) μM
2a		22.3	2b		6.8
2c		8.8	2d		8.4
2e		8.5	2f		9.6
2g		>10	2h		6.29
2i		3.05	2j		1.2
2k		0.66	2l		4.6
2m		>10	2n		4.7
2o		4.8	2p		>10
2q		3.6	4a		2.0

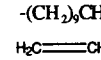
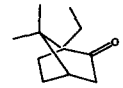
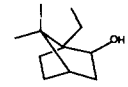
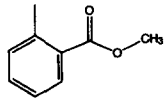
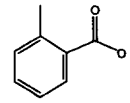
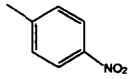
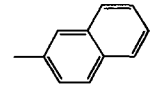
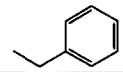
The Amino Acetyl Based FPT Inhibitors

The BOC-protected glycine tricyclic derivative, compound **2b**, had an FPT activity of 6.8 μM. On removal of the Boc group, the resulting amino compound **2a** lost a substantial amount of its FPT activity (IC_{50} = 22.3 μM). Derivatization of the amino group with groups such as benzoyl (entry **2c**), benzyl carbonyl (entry **2d**), CBZ (entry **2e**), or benzyl (entry **2f**) did not have significant effect on FPT activity (6.8-9.0 μM). FPT activity was lost on introduction of the bulky trityl group as shown by compound **2g**. 4-Methoxylbenzyl-*S*-cysteine with the amino group protected as BOC gave compound **2i** that had an FPT activity of 3.05 μM. As in the case of glycine derivatives, removal of the BOC group gave the free amino compound **2h** that was less active than compound **2i.** Coupling amine **1** with CBZ-tyrosine afforded compound **2j**, which had an FPT activity of 1.2 μM; more potent than the CBZ-protected glycine derivative, **2e**, which had an FPT activity of 8.5 μM.

In a different series, we looked at a number of phthaloyl protected amino acids. Coupling tricyclic amine **1** to phthaloyl glycine provided compound **2k**, the most potent compound in this series with a FPT activity of 0.66 μM. The alanine derivative, compound **2l** was less active (4.6 μ) while the leucine and

Table 2. Tricyclic Sulfonamide FPT Inhibitors



Entry	R	FPT(IC_{50} μM)	Entry	R	FPT(IC_{50} μM)
5a	$-CH_3$	1.9	**5b**	$-CH_2CH_3$	5.2
5c	$-(CH_2)_3CH_3$	4.8	**5d**	$-(CH_2)_7CH_3$	>12
5e	$-(CH_2)_9CH_3$	Inactive	**5f**	$-(CH_2)_{10}CH_3$	Inactive
5g		1.0	**5h**		1.9
5i		11.4	**5j**		8.2
5k		10.6	**5l**		>11
5m		>12	**5n**		>12
5o		1.0	**5p**		13.0

phenyl alanine derivatives, compounds **2m** and **2p**, respectively, were not active at 10 μM. Histidine derived phthaloyl analogue, compound **2q**, had activity of 3.6 μM; this was an interesting result since the corresponding phenyl alanine derivative, compound **2p** had an IC_{50} greater than 10 μM.

The Sulfonamides Inhibitors

In the case of alkyl sulfonamides, it was clear that compounds with carbon chain length of 1-4 were active within 2-5 μM range (entries **5a-5c**); however, when the C-chain length was larger than 7 the resulting sulfonamides were inactive (compounds **5d-5f**). The vinyl sulfonamide derivative, compound **5g**, was one of the best inhibitors in this series; it is possible that in addition to the vinyl group being a small alkyl substituent, the presence of an electron rich system gave it an added advantage for inhibition of FPT. This was further supported by the activity exhibited by the dimethyl amino compound **5h** with FPT activity of 1.9 μM.

Introduction of carbocyclic groups, such as norbornyl systems, entries **5i** and **5j**, resulted in active compounds (i.e., 11.4 and 8.2 μM, respectively), this was rather surprising since in the case of the bulky C-alkyl chains (compounds **5d-5f**) the resulting sulfonamides were inactive. We would have expected these bulky carbocylic sulfonamides to have as low activity as the long chain aliphatics, however this was not the case.

In the case of aryl sulfonamides, mixed activity was observed; for example, in the case where the substitution was methylbenzoate the resulting sulfonamide **5k** had an activity of 10.6 μM, however the hydrolyzed form of **5k**, compound **5l**, was dramatically less active (IC_{50} > 11 μM). 4-Nitrobenzene sulfonamide **5m** and the 2-naphthalene sulfonyl derivative **5n** were also found not to be active at 12 μM; it is conceivable that the naphthaloyl group could have been too bulky a group to fit in the enzyme pocket. Introducing a smaller aryl group such as thiophene gave sulfonamide **5p**, which had an activity of 13.0 μM, whereas, the benzyl sulfonamide **5o** was a very potent FPT inhibitor with FPT activity of 1.0 μM.

In summary, we have developed novel amino acetyl and sulfonamide FPT inhibitors that are active in the low micromolar range. Derivatization of the amino group with a phthaloyl moiety resulted in enhanced potency. Having a free amino group resulted in decreased activity. The vinyl and the benzyl sulfonamides were also potent FPT inhibitors. Compounds discussed in this paper are nonpeptidic and do not contain sulfhydryl groups; properties that might make them more pharmacokinetically stable. These compounds have also been found to be poor inhibitors of geranygeranyl protein transferase (GGPT), a closely related enzyme to FPT. For example, compounds **2a**, **2j**, **4a**, and **50** did not inhibit GGPT at 40 μM.

Acknowledgments

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