Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 601–605

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of 5,6-Dihydro-11*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-ylidene)-1-piperidine-*N*-cyanoguanidine Derivatives as Inhibitors of Ras Farnesyl Protein Transferase

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Received 24 October 2001; accepted 26 November 2001

Abstract—A series of novel *N*-cyanoguanidine tricyclic farnesyl protein transferase (FPT) inhibitors was prepared. Replacement of a piperidine amide-group with a *N*-cyanoguanidine functionality increased FPT activity. X-ray crystal structure determination of **42** complexed with FPT revealed differences in the interactions of the amide and *N*-cyanoguanidine groups with the protein.

Due to recent advances in the understanding of signal transduction pathways leading to oncogenesis, new targets for drug discovery are being identified.[1] In particular, oncogenic ras proteins are important targets for anticancer drug discovery. A ras associated protein for which there has been considerable medicinal chemistry effort recently, resulting in several compounds presently in clincial trials, is farnesyl protein transferase (FPTase).[2] Ras proteins require covalent modification by an isoprenoid lipid, farnesyl pyrophosphate (FPP) in the case of FPT, for proper membrane attachment and biological activity.[3] FPTase catalyzes the post-translational farnesylation of a cysteine residue located in the CAAX sequence present at the C-terminus of the ras protein.[4] Inhibition of this post-translational process is therefore an attractive target for antitumor drug development that could yield novel, noncytotoxic antitumor agents.[5]

As part of a program aimed at improving the potency of 5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine-11-ylidene)-1-piperidine based farnesyl transferase inhibitors (FTI),[6] we focused our efforts on modifying the functionality on the piperidine nitrogen. The importance of groups at the piperidine nitrogen for FPT inhibitory activity in the tricyclic series of compounds was previously demonstrated with the amide,[7] urea,[8] and sulfonamide[9] functionalities. Each of these groups showed good FPT activity with the amide group being the most potent (Fig. 1). However, removal of these functionalities to obtain the NH piperidine **1** resulted in substantial loss of FPT potency. Transposition of the carbonyl group of the amide series of compounds to give ketone **3** also resulted in substantial loss in FPT activity, showing the importance of carbonyl functionality position for improved FPT inhibition.[10]

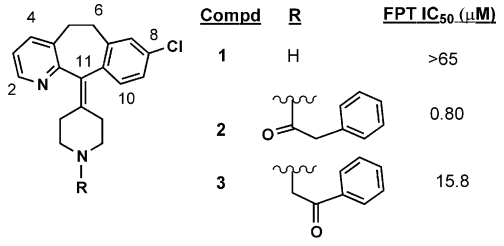

Figure 1. Comparison of various piperidine substitutions.

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0960-894X/02/$ - see front matter
PII: S0960-894X(01)00826-5

The *N*-cyanoguanidine functionality has been previously utilized as an amide replacement in a number of therapeutic areas.[11] We now report the synthesis of a number of *N*-cyanoguanidine tricyclic derivatives with improved FPT activity over that of the corresponding amide derivatives. We utilized the 3-bromo-substituted tricyclic ring system which previously resulted in enhanced FPT activity.[12] Two general methods, A and B (Fig. 2) were explored to prepare the *N*-cyanoguanidine analogues depending upon the R group. Method A started with formation of the thiourea by reacting the piperidine nitrogen with an alkyl isothiocyanate. The requisite thiourea **6** was then treated with Pb(II) cyanamide to give the corresponding *N*-cyanoguanidine **7**.[13] This method proved useful for the preparation of several aniline type derivatives but did not provide a useful intermediate whereby a number of different *N*-cyanoguanidine derivatives could be prepared. In addition there are a limited number of alkyl isothiocyanates available commercially. We therefore utilized Method B which started with the formation of a more generally useful thiomethyl-*N*-cyanoamidine intermediate **5**.[14] Reaction of the thiomethyl-*N*-cyanoamidine intermediate **5** with various primary and secondary amines gave the desired *N*-cyanoguanidine compounds in good yield. The reaction conditions were modified depending upon the amine used for displacement of the thiomethyl group. Very nucleophilic amines provided the *N*-cyanoguanidine in good yields by reaction of **5** with the amine in DMF. Less reactive amines, such as anilines, required the formation of the sodium salt of the amine with sodium hydride in DMF to obtain good yields.

The *N*-cyanoguanidine compounds prepared were divided into aliphatic, aryl, and pyridyl series as shown in Tables 1–3. The FPT inhibitory activity was determined by the ability of the compounds to inhibit the transfer of [^{3}H]-farnesyl from farnesyl pyrophosphate to H-ras-CLVS and expressed as IC_{50}s.[15] As shown in Table 1, the unsubstituted *N*-cyanoguanidine **17** was the least active in the series. Addition of alkyl groups to the nitrogen increased inhibitor potency with increasing chain length of the alkyl group in compounds **13**–**16**. Incorporation of heteroatoms or a heterocycle ring on the alkyl chain led to even more potent FPT inhibitors with double-digit nanomolar IC_{50}s as shown by **18**–**21**. The piperidine series **8**–**10** was the most active as exemplified by **8** (IC_{50} = 6 nM). Elimination of the piperidine nitrogen to give a cyclohexyl ring resulted in retained activity for **11**. A 10-fold loss in activity was observed when a methylene was introduced between the cyclohexyl ring and nitrogen to give **12**.

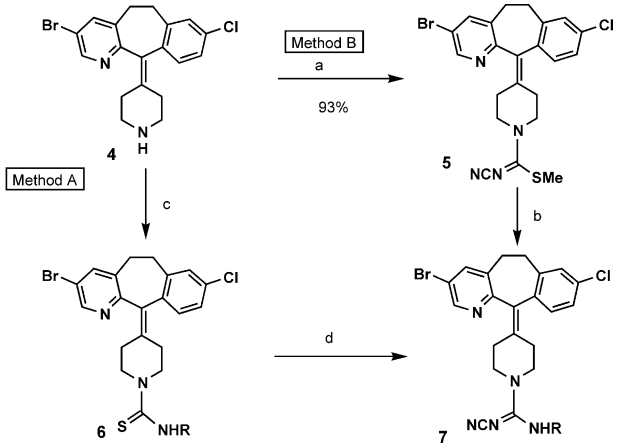
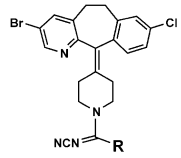


Figure 2. Conditions and reagents: (a) NCN=$(SMe)_2$, EtOH, reflux; (b) 1 equiv of amine Na salt in DMSO, or amine in DMF @ 85 °C; (c) RNCS; (d) PbNCN.

Table 1. Aliphatic series of *N*-cyanoguanidine derivatives[23]



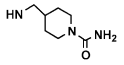
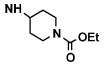
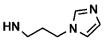
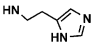
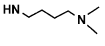
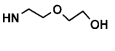
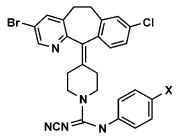
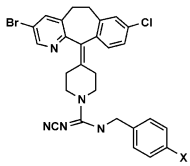
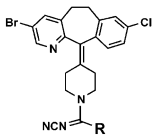
Compd	R	FPT IC_{50} (nm)
8		6
9		18
10		42
11		20
12		199
13		105
14		124
15		155
16		260
17	NH_2	2600
18		35
19		58
20		74
21		84
22		170

Table 2. Phenyl series of *N*-cyanoguanidine derivatives[23]







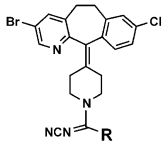
Compd	X	FPT IC_{50} (nm)
23	CN	32
24	F	38
25	OCH_3	43
26	NO^2	62
27	SO_2Me	82
28	H	116
29	IPr	150
30	OCF_3	320

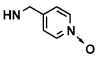
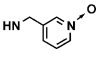
Compd	X	FPT IC_{50} (nm)
31	NMe_2	100
32	H	230
33	OCH_3	310

Compd	R	FPT IC_{50} (nm)
34		38
35		15
36		240

The phenyl series (Table 2) showed no improvement in potency when compared to the aliphatic series. Introduction of a methylene between the phenyl ring and nitrogen, as with the cyclohexyl compounds **11** and **12**, also resulted in reduced FPT activity. This is apparent when comparing the phenyl series compounds **34** with **36**, **28** with **32**, and **25** with **33**. With the exception of **25**, the most active compounds in this series incorporated an electron withdrawing group in the phenyl ring (**23**–**27**). The *p*-trifluoromethoxyphenyl **30** was the least effective FPT inhibitor in this series.

Table 3. Pyridine series of *N*-cyanoguanidine derivatives[23]



Compd	R	FPT IC_{50} (nm)
37		13
38		15
39		29
40		< 110
41		43
42		6
43		12
44		30

Since an increase in activity with incorporation of a heteroatom in the ring (Table 1) was observed, a number of pyridine compounds (Table 3) were explored. In contrast to the observations with the phenyl and aliphatic series of compounds, the opposite pattern of activity is observed when comparing the pyridine series **37**–**44**. Introduction of a methylene between the pyridine ring and the nitrogen increases the FPT activity of this series of compounds. For example, the 4-aminomethyl substituted pyridine **37** is substantially more active than its desmethylene counterpart **40**. The same is true when comparing the 3-substituted aminomethyl pyridine **38** with its desmethylene counterpart **41**, although the difference in activity is not as pronounced. Oxidation of the pyridine ring to give **42** and **43** resulted in slightly increased FPT activity, giving the first single digit nanomolar inhibitor in this series. The addition of two carbons between the pyridine ring and the *N*-cyanoguanidine functionality to give **44** resulted in roughly a 2-fold loss in FPT activity as compared to **37**. This further suggests that a single methylene spacer leads to optimal FPT inhibition in the pyridine series.

We then compared the effect of direct replacement of the amide functionality with the *N*-cyanoguanidine

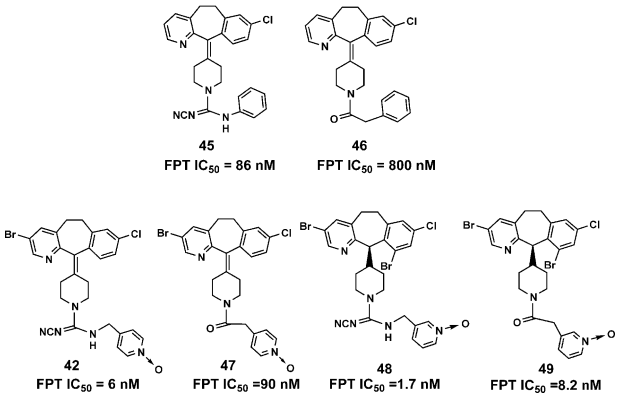


Figure 3. Comparison of amide and *N*-cyanoguanidine functionalities.[23]

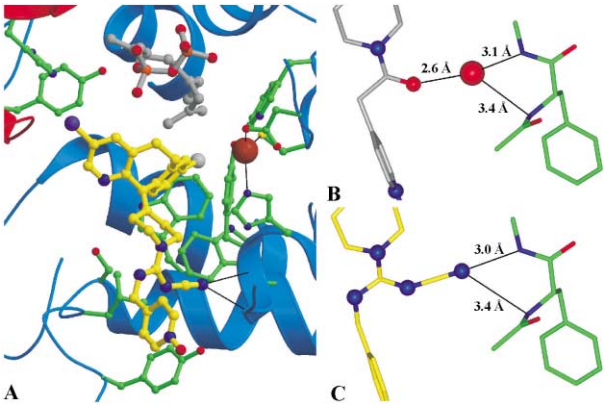


Figure 4. (A) The crystal structure of **42** (yellow) and FPP (gray) bound to the α-subunit (red), β-subunit (blue) of FPT. (B) A close-up view of the amide **47** (gray) bound to FPT (green).[22] The ordered solvent molecule is shown as the red sphere. (C) A close-up view of the *N*-cyanoguanidine **42** (yellow) bound to FPT (green).

functionality using three different halogenated tricyclic ring systems. As shown in Figure 3, the phenyl-*N*-cyanoguanidine **45** shows a 10-fold improvement in FPT activity over that of the corresponding amide **46**.[16] A similar comparison in the 3-bromo-8-chloro series shows the 4-aminomethylpyridine-*N*-oxide **42** as 15 times more active than the amide **47**.[17] Finally, comparing the incorporation of the *N*-cyanoguanidine functionality into one of the most active series of compounds, the 3,10-dibromotricyclic ring series of compounds[18] with a single bond at C-11 to give **48**, resulted in a compound with 5-fold enhanced FPT activity over that of **49**.[19]

In an attempt to explain the increased affinity of the *N*-cyanoguanidine containing compounds, the 2.1 Å X-ray crystal structure of **42** bound to FPT was determined. The compound spans the active site making interactions with residues from the α-subunit, β-subunit, and FPP (Fig. 4a). The overall observed binding mode is similar to compounds previously described containing the amide functionality.[20] There are however, differences in the interactions with FPT between the amide and the *N*-cyanoguanidine groups. In the compounds containing an amide functionality, an ordered water molecule bridges the compound and FPT (Fig. 4b). This water donates a hydrogen bond to the amide carbonyl and accepts two hydrogen bonds from the backbone amides of residues Phe 360β and Tyr 361β. In contrast, the *N*-cyanoguanidine directly accepts two hydrogen bonds from the backbone amide of Phe 360β and Tyr 361β (Fig. 4c). Thus, the bridging water seen in the amide compounds is displaced in the *N*-cyanoguanidine compounds. In a different protein, the displacement or release of an ordered water molecule has resulted in increases in affinity by 5- to 20-fold through entropic effects.[21] Thus, in the tricyclic FPT inhibitors it appears that the 5- to 20-fold increase in affinity of the *N*-cyanoguanidine containing compounds compared to the amide containing compounds is a result of the displacement of an ordered water molecule.

References and Notes

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23. IC_{50} values were determined from single dose–response curves. Data points were run in duplicate. A standard FPT inhibitor (related to the compounds described) was run with each set of compounds. The range of IC_{50} values for the standard was 0.8–2.2 nM over 33 separate determinations on different days. The mean IC_{50} for this compound was 1.5 ± 0.4 nM (mean + standard deviation). The coefficient of variation for the assay (comparing no inhibitor control values) was typically on the order of 6.8%.