BIOORGANIC CHEMISTRY **26,** 269–282 (1998)
ARTICLE NO. BH981101

Inhibitors of Protein:Farnesyl Transferase and Protein:Geranylgeranyl Transferase I: Synthesis of Homologous Diphosphonate Analogs of Isoprenylated Pyrophosphate

Mark Overhand,* Hanneke R. Stuivenberg,* Elsbet Pieterman,†
Louis H. Cohen,† Rick E. W. van Leeuwen,† A. Rob P. M. Valentijn,*
Herman S. Overkleeft,* Gijs A. van der Marel,* and Jacques H. van Boom*,[1]

**Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands; and †TNO Prevention and Health, Gaubius Laboratory, P.O. Box 2215, 2301 CE Leiden, The Netherlands*

Received May 5, 1998

Novel diphosphonate homologs **7a–7c,** and their cyclic counterparts **8a–8c,** of the previously synthesized farnesyl pyrophosphate analogs **1** and **2** were prepared and tested for their inhibition potency and specificity of the enzymes PFT and PGGT-I. Compound **2** was shown to be the most potent inhibitor of PFT (IC_{50} = 0.58 ± 0.45 μM) in this series. The novel compound **7a,** the one carbon homolog of **2,** proved to be the most potent inhibitor of PGGT-I (IC_{50} = 0.98 ± 0.01 μM). The cyclic analogs **8a–8c** are generally less biologically active. The compounds **2** and **7a** are nonspecific toward inhibition of PFT and PGGT-I and may inhibit both farnesylation and geranylgeranylation processing of oncogenic proteins.

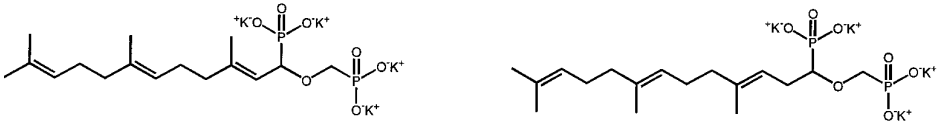
INTRODUCTION

The small GTP-binding proteins of the Ras-family play a crucial role in the transduction of growth and differentiation signals from protein–tyrosine kinase receptors to the cell nucleus (*1*). Mutated forms of Ras proteins are found in 30% of all human cancers with particularly high prevalence in colon and pancreatic carcinomas (*2*). It has become evident that oncogenic Ras proteins are locked in the active form, as result of a decreased hydrolysis rate of GTP in the GTPase cycle, leading to continuous triggering of the signaling pathways.

An essential prerequisite for the functioning of Ras proteins is its association with the plasma membrane. The latter is achieved by posttranslational modification of the Ras precursor at the C-terminus, the first step of which involves the covalent attachment of a farnesyl chain to the cysteine residue of the so-called CaaX box (C, cysteine; a, aliphatic amino acid; X, serine or methionine). The *S*-farnesylation is catalyzed by the enzyme protein:farnesyl transferase (PFT) using farnesyl pyrophosphate (C-15 terpene) as the isoprenoid donor (*3*). Inhibition of PFT prevents

[1] To whom correspondence should be addressed. Fax: 0031715274307. E-mail: boom@chem.leidenuniv.nl.

0045-2068/98 $25.00



1 **2**

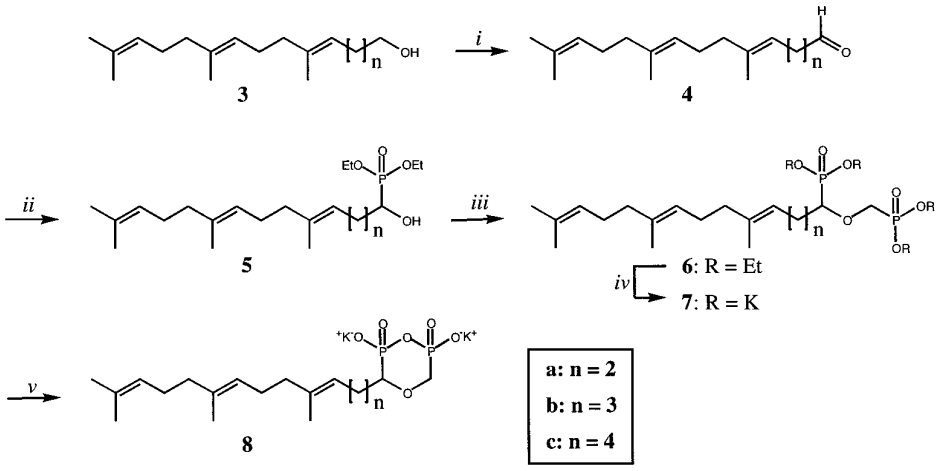
FIG. 1. Structure of the PFT specific inhibitor **1** and its one-carbon homolog compound **2,** a nonspecific inhibitor of PFT and PGGT-I.

the association of oncogenic Ras with the plasma membrane and consequently disrupts its signaling function (*4*). For this reason PFT is considered an important target for the development of anticancer therapeutics. Numerous enzyme inhibitors based on the CaaX box, farnesyl pyrophosphate as well as combinations thereof have been reported (*5*). Furthermore, several natural products have shown potent inhibitory activity against PFT (*5*).

Inhibitors of a similar isoprenylating enzyme, i.e., protein:geranylgeranyl transferase I (PGGT-I), can also function as an antiproliferative agent in cancer chemotherapy (*6*). PGGT-I covalently links a geranylgeranyl (C-20 terpene) moiety to the cysteine residue of CaaL (L, leucine) terminating proteins (e.g., brain heterotrimeric G proteins, Cdc42, and Ras related small GTP-binding proteins) (*7*). Several geranylgeranylated proteins are involved in cell cycle progression and also in this case these proteins become biologically active upon membrane association.

It has been demonstrated that under certain conditions PFT and PGGT-I show mixed specificities (*8*). Thus, PFT can transfer geranylgeranyl as well as farnesyl moieties, using the corresponding isoprenoid pyrophosphate as donors, to certain acceptors having a C-terminal methionine (*9*). Moreover, purified PGGT-I can transfer both geranylgeranyl and farnesyl groups to protein or peptide substrates ending with leucine (*10*). For instance, the oncogenic Ras related GTPase TC21 protein functions as a substrate for both PGGT-I and PFT *in vitro* (*11*). It was demonstrated that transformation of cells by oncogenic TC21 was not inhibited by a specific PFT inhibitor and it has been suggested that inhibition of PFT may cause isoprenylation by PGGT-I with restoration of signaling function (*8*, *11a*). Furthermore, Ki-, K-, and N-Ras proteins are geranylgeranylated when the farnesylation process is inhibited by specific PFT inhibitors (*11b*, *c*). These findings indicate that the functioning of certain oncogenic proteins can be more effectively blocked by an inhibitor for both farnesylation and geranylgeranylation events.

As part of an ongoing program directed toward the development of inhibitors of isoprenyl pyrophosphate consuming enzymes, we previously described the synthesis of compound **1,** as well as its homolog **2** (Fig. 1) (*12f*). Compounds **1** and **2** are inhibitors of PFT with IC_{50} values of 1.08 ± 0.23 and 0.58 ± 0.45 μM, respectively. Interestingly, compound **1** does not inhibit PGGT-I ($IC_{50} > 100$ μM) while compound **2** is also an inhibitor of PGGT-I (IC_{50} 1.15 ± 0.13 μM) (*12h*). Thus, compound **2,** obtained by elongation of PFT-specific inhibitor **1** with one methylene group, not only inhibits PFT but also PGGT-I, indicating that longer carbon analogs of compound **2** may show a higher inhibitory potential toward PGGT-I.



SCHEME 1. (i) DMSO, oxalyl chloride, NEt_3. (ii) $NaP(O)(OEt)_2$, ether. (iii) BuLi, $TfOCH_2P(O)(OEt)_2$. (iv) 1, TMSBr, *sym*-collidine; 2, KOH. (v) 1, TMSBr, *sym*-collidine; 2, DCC, tributylamine/pyridine; 3, Dowex-K^+.

Here we report on the synthesis and biological evaluation of the homologous diphosphonates **7a–7c** and the corresponding cyclic compounds **8a–8c** (Scheme 1).

RESULTS AND DISCUSSION

The synthetic route toward the diphosphonates **7a–7c** and the cyclic counterparts **8a–8c** started (*13*) with the conversion of commercially available (*E*, *E*)-farnesol into the homologated alcohols **3a–3c** (see Scheme **1**). Swern oxidation of alcohols **3a–3c,** followed by reaction of the crude aldehydes **4a–4c** with $NaP(O)(OEt)_2$ in ether gave racemic α-hydroxy farnesylphosphonates **5a–5c** in 65–70% yield, based on **3a–3c**. Reaction of the alcoholate of compounds **5a–5c,** generated *in situ* with *n*-butyllithium in tetrahydrofuran at −78°C, with diethyl phosphonomethyltriflate (*14*) yielded the fully protected target molecules **6a–6c** in 65–70%. Removal of the phosphonate ethyl ester groups of **6a–6c** proceeded smoothly with trimethylsilyl bromide (TMSBr) in the presence of *sym*-collidine and subsequent *in situ* basic hydrolysis of the intermediate trimethylsilyl esters. The crude products were purified by CHP20P-gel chromatography (*15*) to afford the diphosphonates **7a–7c** in 55–60% yield. Novel farnesyl pyrophosphate analogs **7a–7c** were fully characterized (see Experimental). The ^{31}P NMR spectrum of compound **7a,** for instance, showed two singulets at 16.6 and 19.7 ppm.

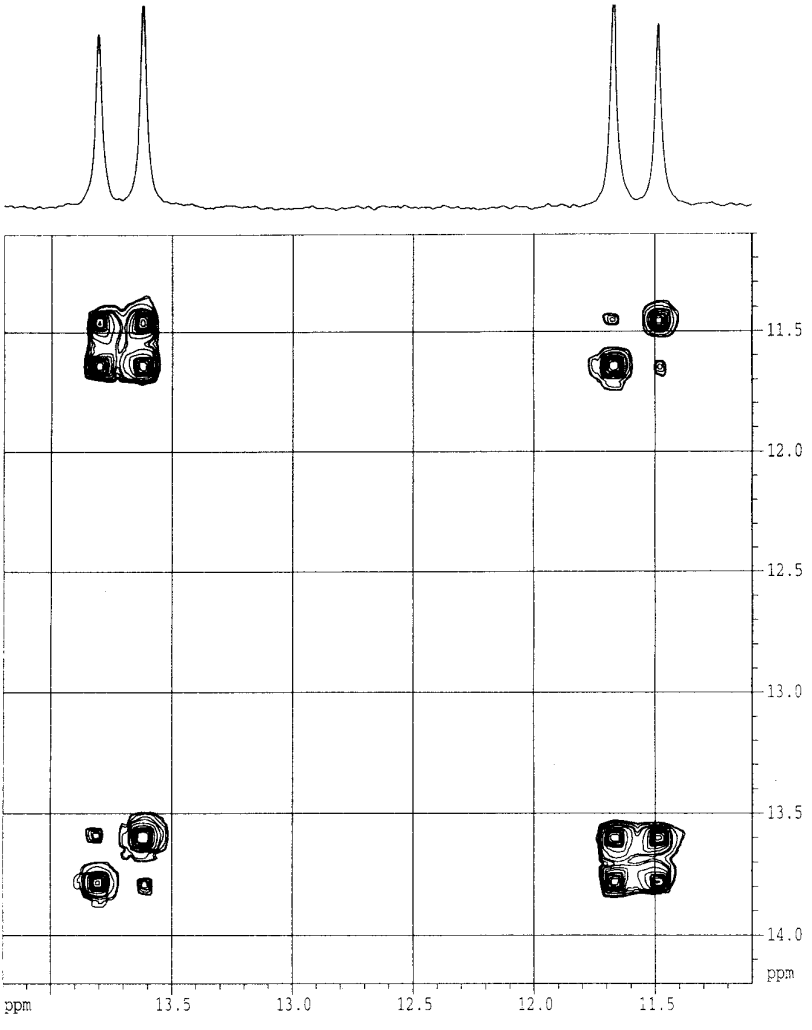
Transformation of **6a–6c** into the cyclic compounds **8a–8c** could be effected by the following (*12g*) three-step one-pot procedure: transesterfication with TMSBr/*sym*-collidine, hydrolysis with aqueous methanol, and treatment with dicyclohexyl carbodiimide in tributylamine/pyridine. Cyclic diphosphonate analogs **8a–8c** were

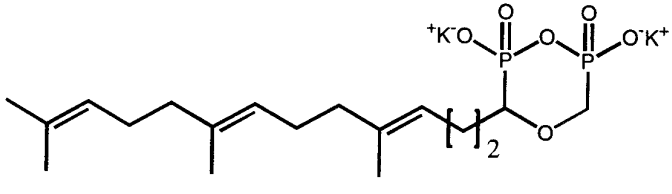
obtained in 50–55% yield after ion-exchange (Dowex-K^+) and purification by preparative HPLC. The cyclic structure of the compounds **8a–8c** was established with ^{31}P-^{31}P COSY NMR. The 242 MHz ^{31}P-^{31}P COSY NMR spectrum of cyclic phosphonate **8a** (Fig. 2), for instance, showed a cross-peak between the doublets at 11.6 and 13.7 ppm (J = 44.8 Hz) corresponding to the two phosphonate functionalities. It is of importance to note that the ^{31}P NMR spectra of the cyclic compound **8a** did not change after standing in D_2O at room temperature for several weeks, indicating that the P–O–P linkage does not readily hydrolyze under these conditions. The novel isoprenylated pyrophosphate derivatives **7a–7c** and the previously synthesized inhibitors **1** and **2** were tested on their inhibitory activity in a PFT and PGGT-I enzyme assay (*12f*, *h*). The outcome of this study is summarized in Table 1 and Fig. 3, showing the IC_{50} values as a function of chain length. Diphosphonate **2** (n = 1) is the most potent inhibitor of PFT (IC_{50} = 0.58 ± 0.45 μM) in this series. On the other hand, compound **7a** (n = 2) is the most potent inhibitor of PGGT-I, having a IC_{50} value of 0.98 ± 0.01 μM. The longer chain derivatives **7b** and **7c** have similar activity, but are less potent inhibitors of both enzymes than the compounds **2** and **7a.** The compounds **2** and **7a–7c** are nonspecific inhibitors of PFT and PGGT-I as compared with the large difference in inhibitory potential as observed with compound **1** (IC_{50} = 1.08 ± 0.23 μM in PFT and $IC_{50} > 100$ μM in PGGT-I). The IC_{50} values of the novel cyclic diphosphonates **8a–8c** in the PFT and PGGT-I are also listed in Table 1. The $IC_{50rel} = IC_{50cyclic}/IC_{50noncyclic}$ values have been calculated to compare the differences in inhibitory activity of the cyclic versus the noncyclic analogs. The cyclic diphosphonates are less potent inhibitors than their noncyclic counterparts in both enzyme assays. Chain elongation of the isoprenoid unit has a negative effect on the inhibitory activity toward PFT. In contrast, the cyclic compounds **8a–8c** all have similar inhibitory activities in the PGGT-I assay.

CONCLUSION

The earlier reported farnesyl pyrophosphate analog **1** (n = 0 in Fig. 3) showed specific inhibition of the enzyme PFT. Elongation of the isoprenyl moiety with one methylene group led to compound **2** (n = 1 in Fig. 3) being a potent inhibitor of both PFT and PGGT-I (*12h*). The novel diphosphonate analogs **7a–7c** were prepared in order to study the effect of additional isoprenoid elongation on the inhibitory potential and specificity of the enzymes PFT and PGGT-I.

The best inhibitor of PFT was shown to be the previously obtained compound **2** (IC_{50} = 0.58 ± 0.45 μM). The novel compound **7a,** the one-carbon homolog of compound **2,** proved to be the most potent inhibitor of PGGT-I. The higher chain elongated derivatives **7b** and **7c** are poorer inhibitors of both enzymes. In sharp contrast with the enzyme specificity for inhibition of PFT, as observed with compound **1** (n = 0 in Fig. 3), compounds **2** and **7a–7c** (n = 1–4 in Fig. 3) are nonspecific inhibitors of PFT and PGGT-I. The latter may be explained by the fact that the additional methylene units between the negatively charged phosphonate groups and





7a

FIG 2. The 243 MHz ^{31}P-^{31}P COSY NMR spectrum of compound **7a.** The observed cross-peak provides evidence for the cyclic structure.

TABLE 1
IC_{50} Values of Compounds **1,2, 7a–7c** and **8a–8c** in PFT and PGGT-I Assays

Compound	Protein:Farnesyl transferase IC_{50} (μM)	PFT IC_{50rel} = $IC_{50cyclic}/IC_{50noncyclic}$	Protein:Geranylgeranyl transferase I IC_{50} (μM)	PGGT-I IC_{50rel} = $IC_{50cyclic}/IC_{50noncyclic}$
1	1.08 ± 0.23	—	>100	—
2	0.58 ± 0.45	—	1.15 ± 0.13	—
7a	5.27 ± 1.28	—	0.98 ± 0.01	—
7b	14.7 ± 3.0	—	20.1 ± 5.7	—
7c	8.19 ± 1.55	—	18.2 ± 5.9	—
8a	18.6 ± 2.7	3.5	15.9 ± 2.7	16
8b	38.5 ± 8.0	2.6	45.8 ± 8.2	2
8c	82.9 ± 7.9	10	22.8 ± 4.8	1.25

Note. $IC_{50rel} = IC_{50cyclic}/IC_{50noncyclic}$.

the hydrophobic terpene chain as in compounds **2** and **7a–7c** induce conformational flexibility, allowing for nonspecific interaction with PFT and PGGT-I.

Preliminary experiments on the inhibition of the farnesyl pyrophosphate consuming enzyme squalene synthase (SS), involved in the cholesterol biosynthesis (*16*), showed that compounds **1, 2,** and **7a–7c** are weaker inhibitors of SS than previously synthesized compounds (*12*). Thus, both compounds **2** and **7a** are more selective toward inhibition of protein isoprenylation compared to the inhibition of cholesterol biosynthesis. Compounds **2** and **7a** have four negative charges which may hamper their cellular uptake. In order to increase the cellular permeability we prepared cyclic phosphono derivatives **8a–8c,** having only two negative charges. The cyclic compounds **8a–8c** proved to be less potent inhibitors than their noncyclic counterparts in the enzyme assays; however, it is not unlikely that this lesser activity is compensated for by a better cellular uptake.

Currently we are studying the inhibitory action of the here reported molecules and several pivaloyloxymethyl protected prodrug (*17*) derivatives on the proliferation of cultured cells. Further research will determine if nonspecific inhibitors of isoprenylating enzymes have better antitumor properties than specific inhibitors in cells where the unregulated proliferation is caused by oncogenic proteins that can either be farnesylated, geranylgeranylated, or both.

EXPERIMENTAL

1. Abbreviations

DCC, dicyclohexylcarbodiimide; EI, electron impact; FPP, farnesyl pyrophosphate; GTP, guanosine triphosphate; GGPP, geranylgeranyl pyrophosphate; IC_{50}, inhibition concentration to inhibit 50% of enzyme activity; MS, mass spectrometry; PFT, protein:farnesyl transferase; PGGT-I, protein:geranylgeranyl transferase I; Ras, rat sarcoma; TMSBr, trimethylsilyl bromide.

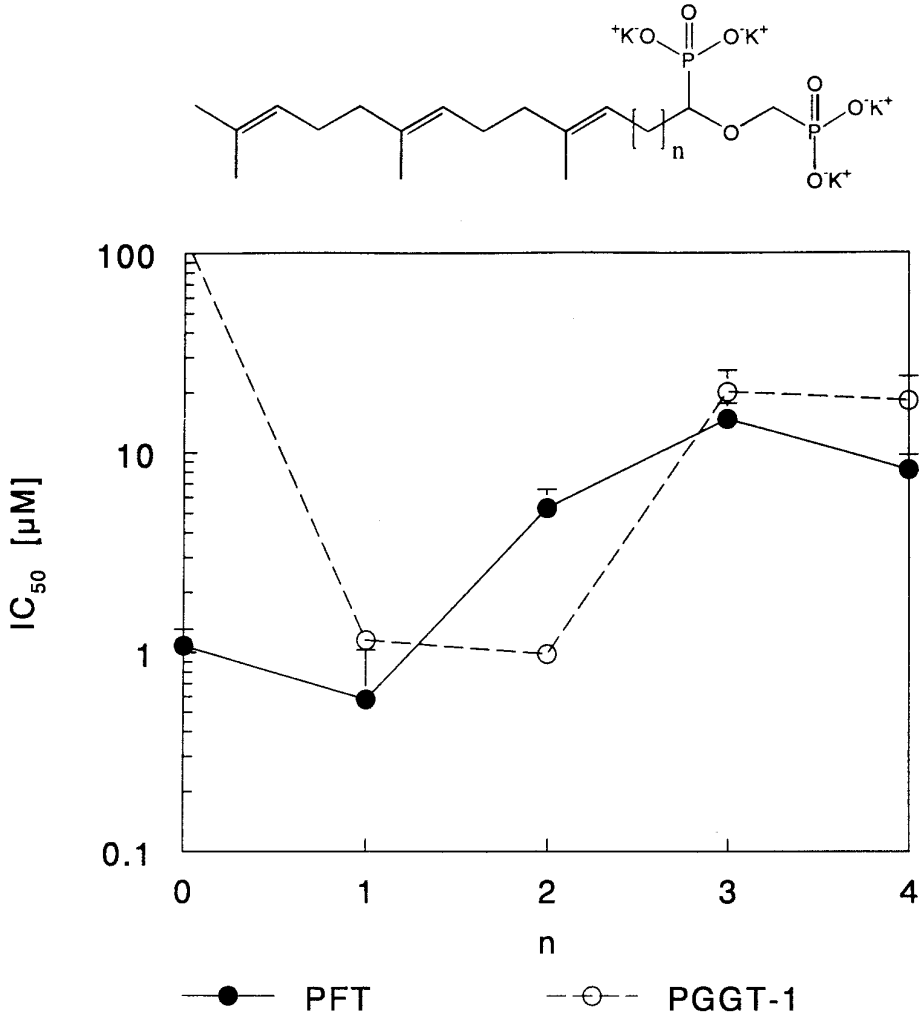


FIG. 3. *In vitro* inhibition of the enzymes PFT and PGGT-I by the synthetic analogs **1, 2,** and **7a–7c** (e.g., $n = 0$–4). The IC_{50} values in μM are plotted as a function of the chain length.

2. General

(*E*, *E*)-Farnesol was purchased from Aldrich. Toluene, dichloromethane, and diethylether were heated under reflux with P_2O_5 for 2 h prior to distillation. Toluene and ether were stored over sodium wire. Dichloromethane was stored over 4-Å molecular sieves. THF was distilled from $LiAlH_4$ directly before use. All other solvents and reagents were used as received. All reactions were carried out under a nitrogen atmosphere. TLC analysis was performed on silica gel (Schleicher & Schull, F 1500 LS 254). Visualization was performed with I_2 on silica gel. Column