Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 255–259

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Cyclic Acid Anhydrides as a New Class of Potent, Selective and Non-Peptidic Inhibitors of Geranylgeranyl Transferase

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Received 20 August 2001; accepted 30 October 2001

**Abstract**—Cyclic acid anhydrides possessing a lipid chain have been shown to be a new class of non-peptidic inhibitors of geranylgeranyl protein-transferase type I (GGPTase-I). 

Mutations of the *ras* oncogene are found in 50% of colon and 90% of pancreatic carcinomas, as well as in lung cancers, and in 25% of all human tumors.[1] Inhibition of the prenylation of Ras protein offers a new approach to cancer therapy.[1,2] Protein prenylation (C-terminal addition of farnesyl or geranylgeranyl isoprenoid units), is effected by one of three cellular enzymes: farnesyl-protein transferase (FPTase), geranylgeranyl-protein transferase type I (GGPTase-I) or GGPTase-II. The farnesylation of Ras (p21) protein, catalyzed by farnesyl-protein transferase (FPTase), occurs at the C-terminal cysteine residue and is the crucial modification for oncogenicity.[3] Conversely, inhibition of farnesylation prevents localization of oncogenic Ras at cell membrane and blocks Ras-induced cell transformation.[4] Thus, Ras-dependent tumor growth can be blocked by suitable inhibitors of FPTase. However, the Ras status of human tumor cell lines does not correlate with growth inhibition by FPTase inhibitors, and many additional observations indicate that prenylation of proteins other than Ras, notably those of the Rho family,[5] is central to the anti-transforming mechanism of FPTase inhibitors.[6]

While many FPTase inhibitors have been reported,[7] and some are being evaluated as antitumor agents, only a few GGPTase ones are known, and most are peptidic.[8] Rarely is their selectivity over FPTase high.[8,9] The importance of potential anticancer drugs that inhibit both geranylgeranylation and farnesylation has recently been emphasized.[10–12] We disclose here a new class of potent and highly selective inhibitors of geranylgeranyl transferase, namely certain cyclic and lipidic anhydrides **5** fused to a second (cyclohexene) ring (Fig. 1). Some are highly selective for the inhibition of GGPTase-I (over FPTase), again a rare feature for peptidomimetics and to our knowledge not previously reported for non-peptidomimetic inhibitors.

Inhibitors of GGPTase are currently receiving increasing attention for several reasons. First, cell cycle progression from $G_1$ to S phase has been shown to be controlled by geranylgeranylated proteins, and not farnesylated protein, in mouse fibroblasts.[8a] This also applies to cells transformed with H-Ras, $K_B$-Ras and Raf oncogenes. A specific peptidomimetic GGPTase-I inhibitor arrested human tumor cells in $G_0$–$G_1$, and showed the potential of GGPTase-I inhibitors for restoring growth arrest in cells that lack the functional tumor suppressor gene p53.[8b] Secondly, K-Ras4B, the protein encoded by the most frequently mutated *ras* gene in human cancers, is not very sensitive to FPTase inhibitors.[11] In contrast, a GGPTase-I inhibitor blocked K-Ras4B processing and oncogenic signaling.[11] This suggests that K-Ras4B may be prenylated by both GGPTase-I and FPTase, and therefore that a combination of GGPTase-I and FPTase inhibitors may be required for the inhibition of tumor growth dependent on K-Ras4B.[12] Thirdly, a combination antitumor therapy of GGPTase-I inhibitors with taxol or cisplatin can be effective.[9] Fourthly, K-*ras* is the Ras gene which is often mutated in human tumors,[13] and there is now

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0960-894X/02/$ - see front matter 
PII: S0960-894X(01)00718-1

strong evidence that inhibition of K-Ras prenylation requires both an FPTase and a GGPTase-I inhibitor.[12b,c] Both or either type of inhibitor could be relevant to a given therapy, and the new compounds described below have been assessed for inhibition of GGPTase-I. Structures were designed on the basis that anhydride or succinate might mimic the phosphate groups of FPP or GPP, and that respective FPTase versus GGPTase activity could be favored by adjustment of the lipid chain length (and degree of unsaturation), as is shown to be the case.

## Chemistry

At physiological pH, the biologically active form of chaetomellic acid A, a potent Ras FPTase inhibitor,[4] is thought to be the dianion **1**, rather than the corresponding anhydride **2**. In contrast, we show that the anhydrides **5** are *more* potent GGPTase inhibitors than either the corresponding acids **3** or esters **4**. Anhydrides **5b**–**5d**[14,15] were synthesized via a cycloaddition strategy (Scheme 1).[14] For **5c**, farnesol was derivatized as its tetrahydropyranyl group[16] which then underwent elimination[17] in the presence of $KOBu^t$ to give the 2-substituted

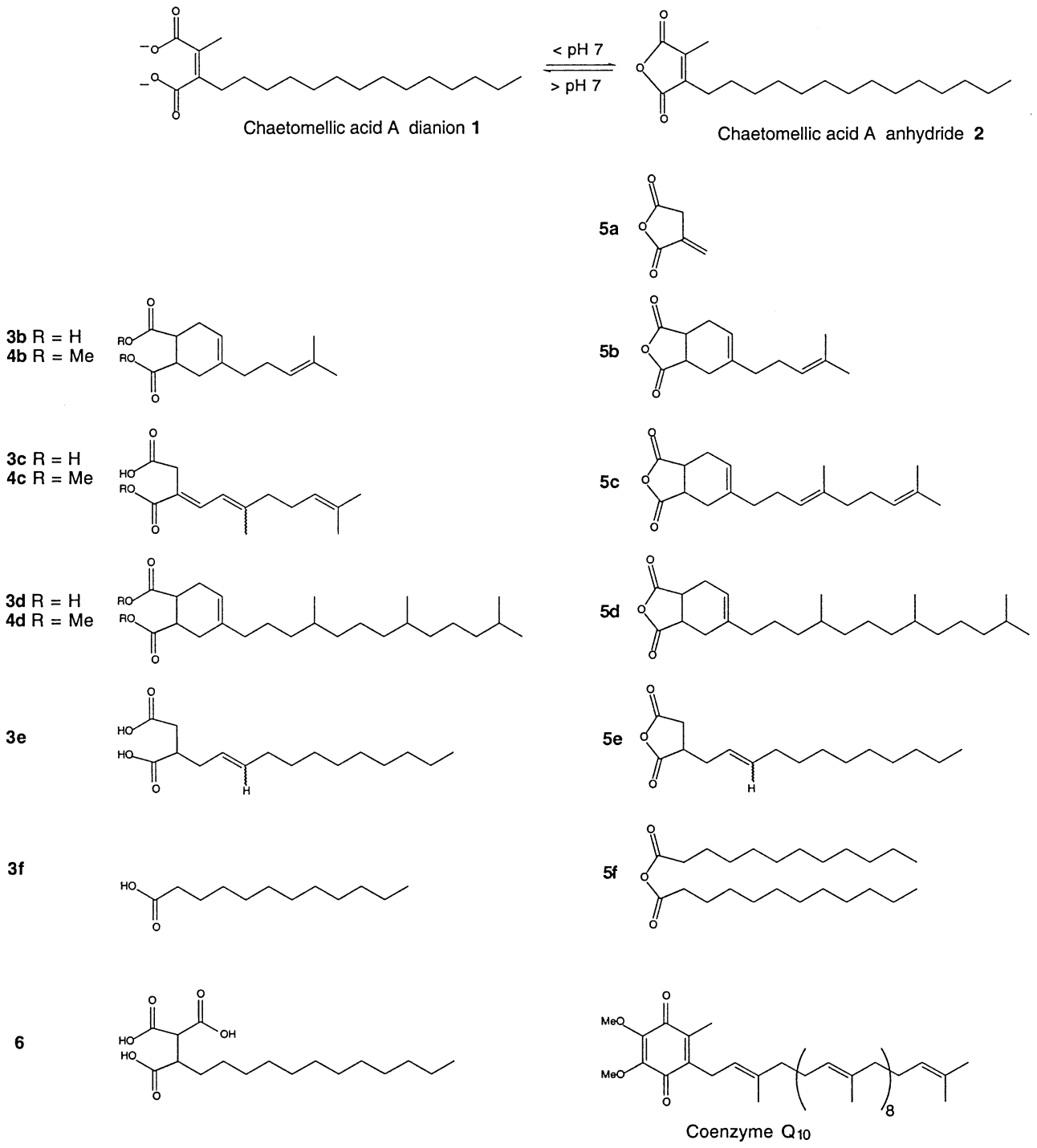


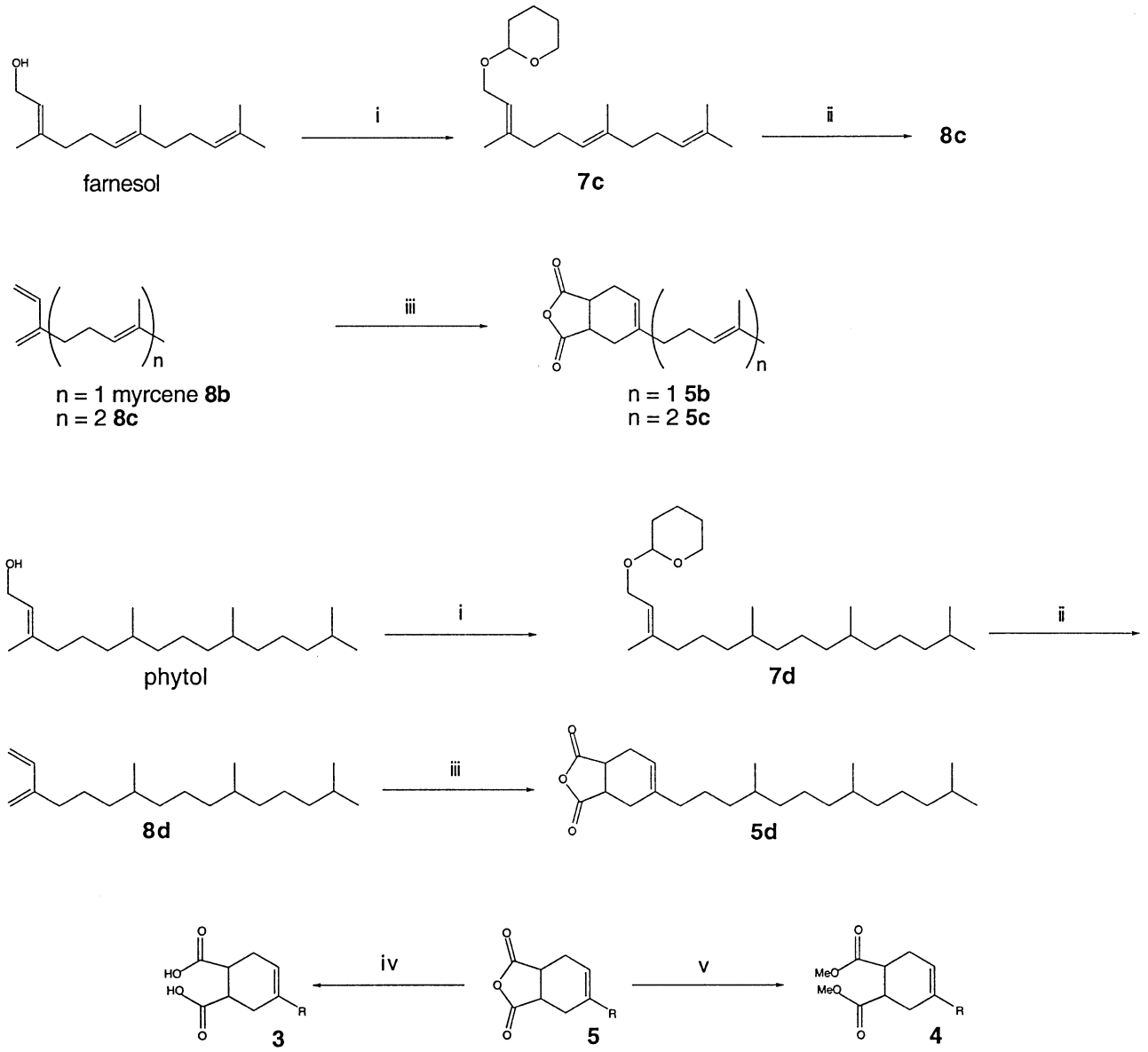

**Figure 1.** Fused anhydrides and related carbonyl compounds.

buta-1,3-dienes **8c**. Using the same sequence, phytol was converted into **5d**. Anhydride **5b** was prepared directly from γ-myrcene and maleic anhydride. Diels–Alder reactions were similarly conducted with the buta-1,3-dienes **8c** and **8d**, and maleic anhydride (2.0 equiv, 0.01 mol% methylrhenium trioxide,[18] 20 °C, 18 h) to give the adducts **5b** (52%), **5c** (54%), **5d** (71%), respectively, after purification by column chromatography. Anhydride **5c** retained the *(E)*-configuration of the initial farnesol. All three anhydrides possessed a *cis*-ring junction, following from the rules of cycloaddition. Since the phytol was a naturally occurring mixture of configurations about the two methine center on the chain, **5d** was obtained as a corresponding 2:1 mixture.

The anhydrides **5b–5d** were hydrolyzed (5% aqueous KOH, 20 °C, 18 h) to give the corresponding succinic acids **3b–3d** in respective yields of 78, 81 and 65%. Reaction of anhydrides **5b–5d** with 5% v/v sulfuric acid/methanol (18 h reflux) gave the corresponding dimethyl esters **4b–4d** in respective yields of 68, 67 and 74%. $^{13}C$ NMR and NOE experiments were consistent with *cis*-1,2-disubstitution on the cyclohexene ring.

## Biological Results and Discussion

The anhydrides **5b–5f** are much more efficient inhibitors of GGPTase-I (Table 1) than the succinic acids **3b–3f**, implying a special role for the anhydride group, and indicating that rapid hydrolysis to the corresponding diacid did not occur in vitro. That the anhydride moiety is not merely acting as a delivery system is indicated by the activity of **5d** (GGPTase-I $IC_{50} = 21$ μM) versus the inactivity of the corresponding dimethyl ester **4d** (GGPTase-I $IC_{50} > 1000$ μM). This may support an




**Scheme 1.** Synthesis of fused anhydrides and their derivatives. Reagents: (i), dihydropyran, *p*-TsOH, benzene, reflux; (ii) Bu$^t$OK, 18-crown-6, THF, reflux, 2 h for **7c** and 18 h for **7d**; (iii) maleic anhydride, chloroform (for **8b**) or acetone (for **8c** and **8d**), 0.01 mol% $MeReO_3$, 20 °C, 24 h; (iv) 5% aqueous KOH, 25 °C, 18 h; (v) 5% v/v sulfuric acid/methanol (18 h reflux).

acylating role for the anhydrides. As expected, the length and nature of the lipid chain markedly influences the prenyl-protein transferase activity. The lack of an alkyl (including prenyl) chain, as in itaconic anhydride **5a**, excludes prenyl-protein transferase activity, whereas the longest chain tested (**5d**) gave excellent inhibition of GGPTase-I. Moreover, **5d** is highly selective for GGPTase-I/FPTase (50:1), and **5b** also shows good selectivity (11:1). Interestingly, a 2:1 *E*/*Z* mixture of **5c** shows excellent selectivity (1:50) for FPTase. Accordingly, the anhydrides **5b**, **5c** and **5d** could be useful as probes for the selective inhibition of either FPTase or GGPTase-I.

The uniqueness of the anhydride function is shown by comparison with a variety of related functional groups including diacids, monomethyl ester carboxylic acids,[20] the triacid **6** and $CoQ_{10}$. Thus, FPTase $IC_{50}$ values for the diacids **3b**, **3c**, and **3d** were all $>1000$ μM; that for **3e** was 402 μM. GGPTase-I $IC_{50}$ values for the monomethyl ester derived from the carboxylic acids **3c**, **3d** and **3e** were each $>1000$ μM; those for the diacids **3b** and **3c** were 900, and 420 μM, respectively. FPTase/GGPTase-I $IC_{50}$ values (ratio of μM) for various non-anhydride compounds were: **4b**, 900:500; **4c**, $>1000$:185; **4d**: $>1000$: $>1000$; CoQ, 500:1000; **6**, 166:252. Despite the lengthy prenylated chain of CoQ, and the possibility of it acting as a Michael acceptor or participating in redox processes, CoQ was not found to be a significant inhibitor of GGPTase-I.

The synthetic GGPTase-I inhibitors **5b** and **5d** are more potent than the naturally occurring chaetomellic acid A (GGPTase-I $IC_{50}=92$ μM and GGPTase-II $IC_{50}=34$ μM) and are among the most potent non-peptidic inhibitors of GGPTase-I known.[12a] They possess the advantage over peptidic enzyme inhibitors because the anhydrides **5** lack peptide bonds that could be degraded by peptidases. Thus, although a peptidic GGPTase-I inhibitor with $IC_{50}$ 50 nM has been described, effects on whole cells required micromolar concentrations.[11] One of the most potent non-peptidic GGPTases hitherto has an $IC_{50}$ of 11 μM, but with a selectivity for GGPTase-I over FPTase of only 96:11.5.[21]

In contrast to chaetomellic acid, cyclic, fused anhydrides have been shown to be much *more* active than their corresponding dicarboxylic acids or ester derivatives. Thus, anhydride **5c** is over 50 times more potent than either the diacid **3d** or dimethyl ester **4d** derivatives, as an inhibitor of GGPTase-I. Whether an anhydride can generally function as a neutral bioisostere of the charged and biologically labile diphosphate group of FPP is an important issue to be investigated. GGPTase-I inhibitors have been proposed to prevent hyperplastic remodeling and to act on vascular lesions by inducing apoptotic regression.[22] Consequently, the advent of these non-peptidic inhibitors of GGPTase-I, whose activity can be tuned by altering the lipid chain length and type, indicates potential therapeutic value and use as biochemical probes.

**Table 1.** In vitro inhibition of recombinant protein prenyltransferases[19]

| | FPTase $IC_{50}$ (μM) | GGPTase-I $IC_{50}$ (μM) |
|---|---|---|
| **5a** | $>1000$ | $>1000$ |
| **5b** | $449\pm38$ | $40.2\pm1.5$ |
| *(E)*-**5c** | $190\pm23$ | $136\pm9$ |
| 3:1*(E/Z)*-**5c** | $9\pm0.3$ | $503\pm37$ |
| **5d** | $>1000$ | $20.8\pm0.2$ |
| **5e** | $>1000$ | $401\pm8$ |
| **5f** | $854\pm80$ | $229\pm15$ |

## Acknowledgements

Financial support from the Mandeville Trust is gratefully acknowledged.

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