Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 4405–4408

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Stable Analogues of Geranylgeranyl Diphosphate Possessing Improved Geranylgeranyl versus Farnesyl Protein Transferase Inhibitory Selectivity

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Received 28 May 2003; revised 30 July 2003; accepted 12 September 2003

Abstract—Phosphonoacetamido(oxy) groups have proven to be good mimics of the diphosphate portion in geranylgeranyl protein transferase I (GGTase I) inhibitors. The introduction of small alkyl groups (Me, Et) into the diphosphate mimic moiety caused a further decrease in collateral farnesyl protein transferase (FTase) inhibitory activity, thereby improving GGTase I over FTase selectivity.

Introduction

Signal transduction of extracellular stimuli activating cell proliferation is brought about by small intracellular G-proteins belonging to the Ras superfamily, which become activated by anchorage to intracellular and plasma membranes via a posttranslationally added lipophilic isoprenyl group, such as farnesyl (Ras proteins) and geranylgeranyl (Rho, Rac, and Cdc42 proteins).[1] The enzymes involved in this activation step are farnesyl transferase (FTase) and geranylgeranyl transferase (GGTase), respectively. Aberrant signalling through Ras pathways occurs in several types of cancer, where mutated Ras accumulates in its GTP-bound active form and causes uncontrolled cell proliferation.[2] For these reasons, molecules able to target the Ras pathway in any of its stages are potentially useful in anti-cancer therapies.[1] Over the past few years, a great deal of attention has been devoted to FTase inhibitors, since Ras proteins involved in cell hyper-proliferation seemed to be exclusively activated by farnesylation. Several strategies have been followed: (a) designing compounds that mimic the carboxy-terminal CAAX motif of Ras proteins; (b) using molecules that compete with the farnesyl diphosphate (FPP analogues); (c) making drugs that act both as FPP and CAAX mimics.[3] Several compounds have proven to efficiently inhibit farnesylation of Ras proteins in vitro, showing some reversal of tumor mass when tested in animal models, accompanied by an impressively low toxicity. Unfortunately, the same success was not achieved in early attempts to treat human patients affected by cancer. In fact, it was later found that in malignant cells, some mutant forms of Ras (K- and N-Ras) can be geranylgeranylated by GGTase I when FTase is blocked, circumventing the antiproliferative effect of FTase inhibitors.[4] Therefore, new GGTase I inhibitors have been synthesized[5] and some of them have shown a potent anticancer activity.[6]

Recently, GGTase I inhibitors have displayed other interesting therapeutic properties.[7] They have proven to be effective in reducing geranylgeranylation of small G-proteins in osteoclast, thus reducing bone resorption

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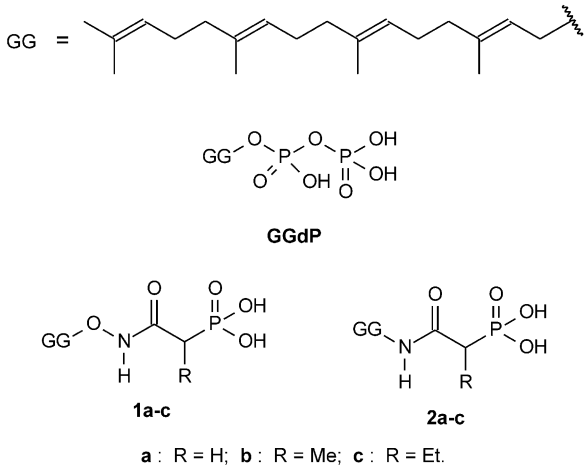
doi:10.1016/j.bmcl.2003.09.035

and showing a potential for treatment of osteoporosis.[7b,c] Besides, they have also shown an inhibitory effect on unwanted smooth muscle cell proliferation accompanying reocclusion of arteries occurring in atherosclerosis.[7d] All these aspects make the search for new and selective GGTase inhibitors an on-going challenge for medicinal chemists.

Our research has been focused on the design of stable analogues of the diphosphate moiety in GPP, attached to a geranylgeranyl isoprenoid portion. We had previously found that the (phosphonoacetamido)oxy group, as in **1a**, was one of the most effective for this purpose.[5h] We then performed a more detailed structure/activity relationship investigation within this class of molecules, and we herein report the development of new selective GGTase I inhibitors (**1b**,**c** and **2a–c**), structurally related to **1a**.



Compounds **1b** and **1c** derive from **1a** by replacement of one of the methylene hydrogen atoms of the (phosphonoacetamido)oxy group by a methyl or an ethyl group, respectively. These substitutions should make it possible to verify whether the increase in steric hindrance within this portion of the molecule might increase enzyme selectivity without affecting the inhibition potency. Moreover, the related series **2a–c**, where the (phosphonoacetamido)oxy groups of **1a–c** have been replaced by phosphonoacetamido ones, then lacking an oxygen atom in the phosphate mimic portion, should also prove to be an important means for the optimization of the polar moiety in this class of molecules.

Chemistry

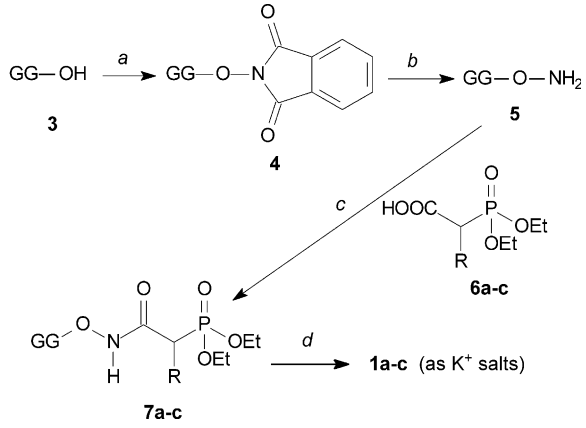
The synthetic route for the preparation of compounds **1a–c** (Scheme 1) started from geranylgeraniol **3**, which was subjected to a Mitsunobu reaction with *N*-hydroxyphthalimide to give *N*-(geranylgeranyloxy)-phthalimide **4**. Hydrazinolysis of the phthalimido portion afforded free oxyamine **5**, which was then subjected to a condensation reaction, promoted by 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole, with the appropriate phosphonoacetic acid derivative (**6a–c**). The diethyl phosphonates thus obtained (**7a–c**)[8] were submitted to hydrolysis: treatment with bromotrimethylsilane and 2,4,6-collidine, and then aqueous potassium hydroxide, gave compounds **1a–c** as the dipotassium salts.[9]

Compounds **2a–c** were synthesized as shown in Scheme 2. Geranylgeranyl bromide **8** was used to alkylate potassium phthalimide, to give *N*-(geranylgeranyl)phthalimide **9**. Removal of the phthalimido group by hydrazinolysis yielded geranylgeranylamine **10**, which was then condensed with the appropriate phosphonoacetic acid derivative (**6a–c**) under the same conditions described above. Final hydrolysis of the diethyl phosphonate derivatives **11a–c**[10] afforded the target compounds **2a–c** as the dipotassium salts.[11]

Biological Results and Discussion

The in vitro inhibition assays of GGTase I and FTase were performed as previously described[12] with some modifications.[13] The inhibitory activity of compounds **1a–c** and **2a–c** is reported in Table 1 as their IC_{50}, the concentration at which GGTase and FTase activity was inhibited by 50%.

An analysis of the results shows that, in the '(phosphonoacetamido)oxy' series (**1a–c**), the introduction of small

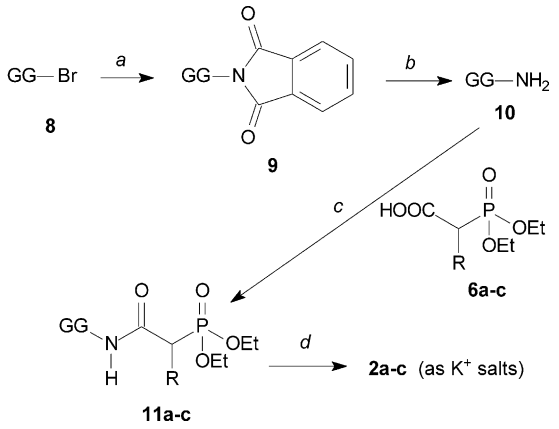


Scheme 1. Reagents and conditions: (a) *N*-hydroxyphthalimide, Ph_3P, DEAD, THF, rt; (b) $NH_2NH_2{\cdot}H_2O$, EtOH, rt; (c) 1-Hydroxybenzotriazole, EDC, THF, rt; (d) (1) TMS-Br, 2,4,6-collidine, CH_2Cl_2, rt; (2) 1 N aqueous KOH.

Table 1. Inhibitory activity of compounds **1a–c** and **2a–c** on geranylgeranyl protein transferase I (GGTase I) and farnesyl protein transferase (FTase)

Compd	IC_{50} (nM)[a]		Selectivity[b]
	GGTase I	FTase	
1a	66±8	3500±700	53
1b	151±30	>10,000	>66
1c	146±20	>10,000	>68
2a	275±40	5000±1000	18
2b	111±15	>10,000	>90
2c	566±75	>10,000	>18

[a]Values are reported as the mean±range or SD of 2–3 independent experiments.
[b]Fold (GGTase over FTase).



Scheme 2. Reagents and conditions: (a) potassium phthalimide, DMF, rt; (b–d) see Scheme 1.

alkyl groups, such as a methyl (**1b**) or an ethyl (**1c**), preserves a good inhibitory potency on GGTase when compared with the reference compound **1a**, whereas the activity on FTase is completely lost, thus affording better selectivity ratios for both **1b** and **1c**, with respect to **1a**.

Removal of one oxygen atom from the polar moiety, as in the 'phosphonoacetamido' series (**2a–c**), did not cause any dramatic changes in the biological properties of these compounds. As a matter of fact, the smaller homologue **2a** shows decreased inhibitory properties on both enzymes with respect to its oxygenated counterpart. This decrease is more pronounced on GGTase, resulting in relatively low selectivity. As seen before, also in this series the introduction of a methyl group such as in **2b** causes a dramatic decline in the inhibition of FTase ($IC_{50} > 10{,}000$ nM) accompanied by a good inhibition potency on GGTase ($IC_{50} = 111$ nM), providing **2b** with a selectivity ratio higher than 90. In this series, the introduction of a more hindered ethyl group, as in **2c**, causes significant reduction in the inhibition ability on GGTase ($IC_{50} = 566$ nM), and preserves complete inactivity on FTase.

These results show that a slight increase in the steric hindrance in the diphosphate mimic portion of the reference compound **1a** improves GGTase selectivity. In fact, the introduction of a methyl (**1b**) or an ethyl (**1c**) substituent led to selectivity ratios of about 70. Among the 'phosphonoacetamido' series, the most active and selective compound proved to be the methyl-substituted **2b**, with a remarkable selectivity ratio of more than 90. To the best of our knowledge, compounds **1b**, **1c** and **2b** are among the most active and selective GGTase I inhibitors possessing a GGPP-like chemical structure, and might therefore represent a good starting-point in the design of new selective GGTase inhibitors which may potentially be developed as drugs in the therapy of cancer and of other pathologies related to uncontrolled cell proliferation.

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8. For example, compound **7b**: ^{1}H NMR ($CDCl_3$) δ 1.38 (m, 9H), 1.59 (s, 9H), 1.67 (s, 3H), 1.70 (s, 3H), 2.02 (m, 12H), 2.56 (m, 1H), 4.04 (q, 2H, $J = 7.2$ Hz), 4.06 (q, 2H, $J = 7.2$ Hz), 4.41 (d, 2H, $J = 7.2$ Hz), 5.09 (br, 3H), 5.41 (t, 1H, $J = 7.2$ Hz); MS (FAB^+) *m/e* 498 $(M+H)^+$.
9. For example, compound **1b**: ^{1}H NMR (CD_3OD) δ 1.35 (dd, 3H, $J = 15.2$, 7.2 Hz), 1.60 (s, 9H), 1.67 (s, 3H), 1.72 (s, 3H), 2.04 (m, 12H), 2.51 (dq, 1H, $J = 20.0$, 7.2 Hz), 4.40 (d, 2H, $J = 7.2$ Hz), 5.10 (br, 3H), 5.41 (t, 1H, $J = 7.2$ Hz); MS (FAB^+) *m/e* 518 $(M+H)^+$.
10. For example, compound **11b**: ^{1}H NMR ($CDCl_3$) δ 1.37 (m, 9H), 1.59 (s, 9H), 1.68 (s, 6H), 2.01 (m, 12H), 2.78 (m, 1H), 3.85 (d, 2H, $J = 7.2$ Hz), 4.03 (q, 2H, $J = 7.2$ Hz), 4.05 (q, 2H, $J = 7.2$ Hz), 5.12 (br, 3H), 5.23 (t, 1H, $J = 7.2$ Hz); MS (FAB^+) *m/e* 482 $(M+H)^+$.
11. For example, compound **2b**: ^{1}H NMR (CD_3OD) δ 1.35 (dd, 3H, $J = 15.2$, 7.2 Hz), 1.59 (s, 9H), 1.66 (s, 6H), 2.01 (m, 12H), 2.54 (dq, 1H, $J = 20.0$, 7.2 Hz), 3.79 (d, 2H, $J = 7.2$ Hz), 5.13 (br, 3H), 5.23 (t, 1H, $J = 7.2$ Hz); MS (FAB^+) *m/e* 502 $(M+H)^+$.
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13. Enzymes were incubated with [^{3}H]GGPP and recombinant H-Ras-CVLL (GGTase I), or with [^{3}H]FPP and H-Ras-CVLS (FTase), in the presence of different concentrations of inhibitors. After incubation the reaction was stopped and filtered through glass fibre filters to separate free from incorporated label.