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Phenol based Tripeptide Inhibitors of Ras Farnesyl Protein Transferase

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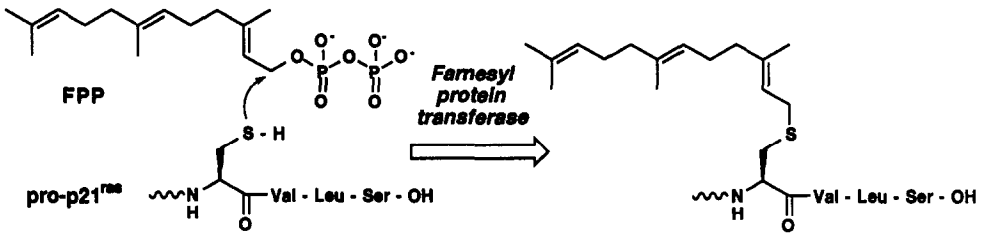
Abstract: *Preparation and activity of phenol tripeptides, a new class of inhibitors of ras farnesyl protein transferase (FPT), is described. Based on the inhibitory potency of meta-phenol 11 (I_{50} = 29 uM) and the ortho-benzyl ether analog 7 (I_{50} = 63 uM), regioisomeric hybrid analogs 18 and 25 were designed and evaluated as partial bisubstrate analog inhibitors of FPT (18, I_{50} = 157 uM; 25, I_{50} = 137 uM).*

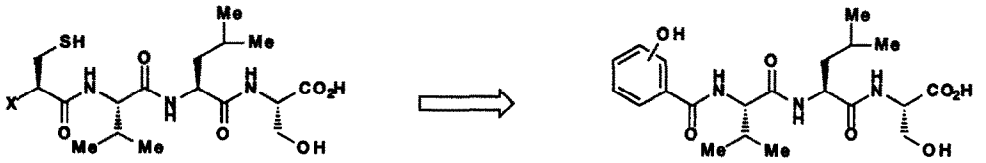
An agent that would interfere with ras function could lead to the discovery of rationally designed anti-cancer agents.[1] Several points of intervention of ras function are feasible. One approach would be to block the membrane localization process of ras protein, an event which appears to be critically required for efficient cell transformation activity.[2] Farnesylation of C-terminal cysteine side chain of $p21^{ras}$ is the first and essential step in a series of post-translational modifications involved in the overall migration of protein from the cytoplasm to the inner surface of the plasma membrane, after which it participates in mitogenic signaling.[3]



Farnesyl-protein transferase (FPT) is the enzyme that catalyzes this S-prenylation process. The reaction in question involves participation of two substrates, namely, the ras protein and the prenyl group donor farnesyl pyrophosphate (FPP). Inhibition of FPT has recently attracted attention as a potential approach toward discovery of novel anti-tumor agents.[4] Tetrapeptide CAAX based sequences have proved to be the minimal requirement for efficient binding.[5] A structural feature critical for activity for all peptide based FPT inhibitors has been the presence of a cysteine or a cysteine like thiol group in these molecules.[6]

Our efforts in this area have centered on discovering non-sulfhydryl, and non-peptidic or less peptidic inhibitors of FPT. The removal of an amino group from the tetrapeptide CVLS **1a** (I_{50} = 1 uM) is well tolerated, whereas substitution of its thiol group by a hydroxyl has deleterious effect on activity (SVLS, I_{50} >360 uM).[6,7] This equipotency of the desamino analog **1b** to its parent compound alongwith the mandatory requirement of a mercaptan group led us to propose tripeptidic phenols **2** as novel inhibitors of farnesyl transferase (Scheme 1). The acidity of a phenolic hydroxyl group is similar to that of a free sulfhydryl group (pKa = 10), and may serve as a suitable replacement for the -SH group in CVLS. In addition, the aromatic ring of a phenol moiety conformationally restricts the movement of the hydroxyl group, and each regioisomer (*ortho*, *meta* and *para*) enables positioning the hydroxyl group in a different region of well defined three dimensional space.

Scheme 1



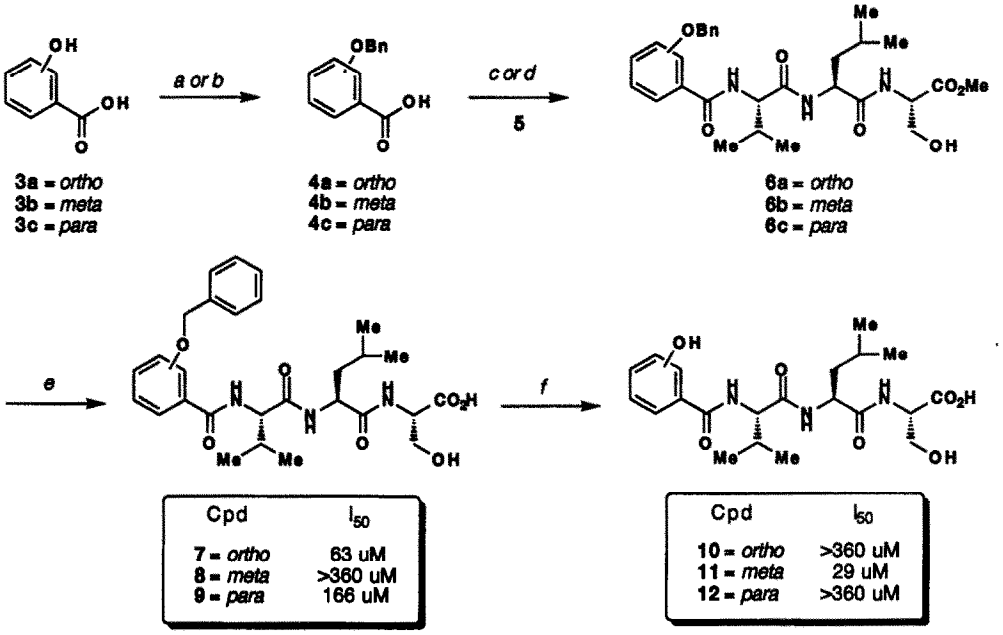
1a R = NH_2
1b R = H

2

Preparation of all three regioisomeric phenols **2** was undertaken in our study. Hydroxy benzoic acids **3a-c** were converted to their benzyl ethers **4a-c** either by direct kinetic alkylation (**4b**, **4c**) of the dianion with 1.1 equivalents of benzyl bromide or by dibenzylation of **3a** followed by saponification of the resulting benzyl ester to **4a**. Coupling of acids **4a-c** with the tripeptide H-VLS-OMe **5** proved to be slightly problematic. For example, with the *para* isomer **4c**, attempts with ethyl-3-(dimethylaminopropyl)-carbodiimide (EDC) were unrewarding, and the rearranged N-acylurea was the only major product (81%) isolated when dicyclohexyl carbodiimide (DCC) was used as an activating reagent. Alternatively, carbonyl diimidazole (CDI) provided **6c** in moderate yields (42%) alongwith minor amounts of the diacylated side product (8%).[8] Under similar conditions, the *meta* isomer **4b** provided the desired product **6b** in modest

yields (29%), and was accompanied by formation of substantial amounts of the dicoupled product (17%). Not surprisingly, very little product was obtained with the sterically demanding *ortho* acid **4a**. These results suggested that CDI was not a very suitable reagent for activation and coupling of benzoic acids. Amongst several alternate coupling procedures that were investigated, best yields of the desired monoproduct **6a** (75%) were obtained when **4a** was treated with $(COCl)_2$/DMF, and the crude chloride was reacted directly with the tripeptide **5**. Hydrolysis of **6a-c** yielded the benzyl ethers **7-9**, which upon hydrogenolysis afforded the final products **10-12** respectively in an uneventful fashion.

Scheme 2



Reagents: a) NaH (2.2), BnBr (1.1) b) i) K_2CO_3, BnBr, reflux ; ii) NaOH, reflux c) **5** HCl.H-VLS-OCH_3, CDI, DIPEA d) $(COCl)_2$, DMF, HCl.H-VLS-OCH_3, DIPEA e)1N NaOH, (HP-20) f) H_2, 10 % Pd/C

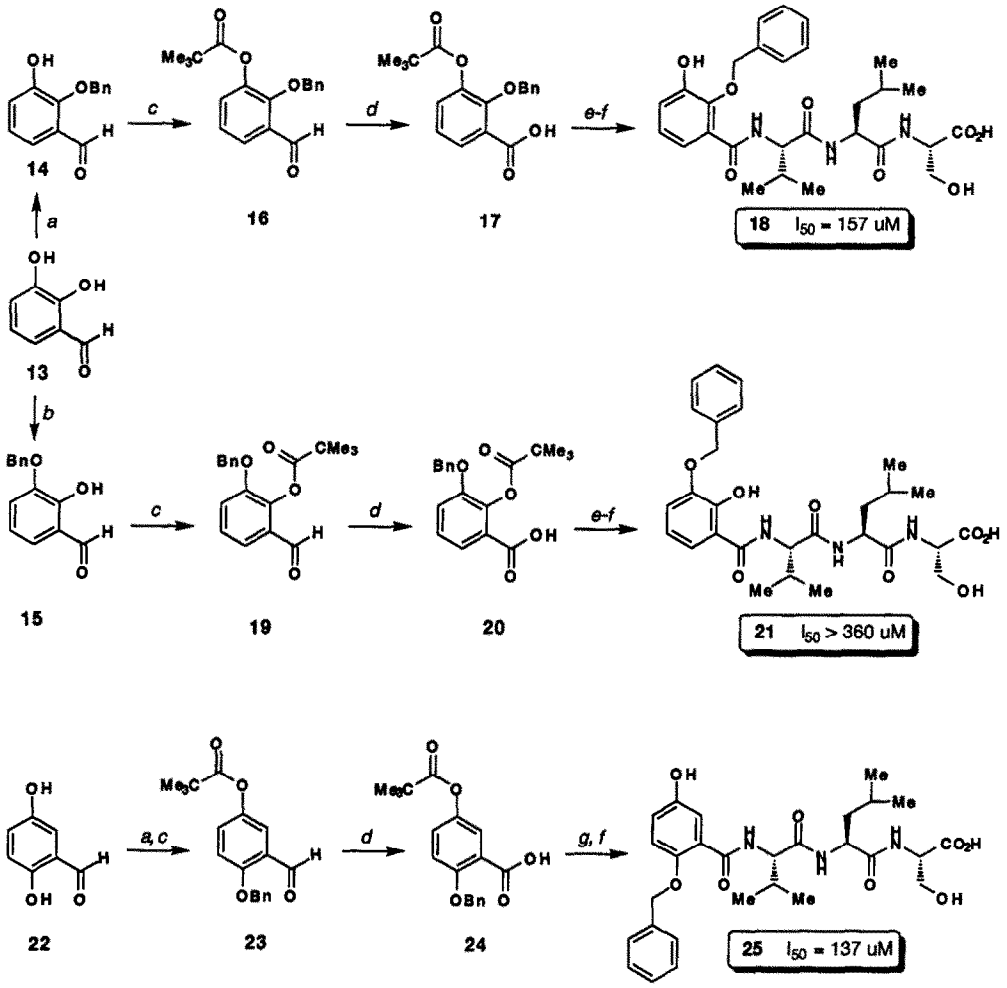
Biological data on the three regioisomeric tripeptide phenols **10-12** and their corresponding benzyl ethers **7-9** is summarized in the above Scheme. While the *ortho* and *para* tripeptide phenols **10** and **12** were found to be inactive, the *meta* isomer **11** had an I_{50} of 29 uM. Although 29 fold less active than the parent analogs **1a** and **1b** (I_{50} = 1 uM), *these are one of the first examples of peptide based non-sulfhydryl inhibitors of FPT.* Moderate activity of the *ortho* isomer **11** versus total inactivity of the other two isomers seems to indicate that the -OH group in **11** is a significant contributor to the overall observed

activity of this compound.[9] The penultimate benzyl ether precursors **7-9** were also submitted for testing. Although a benzyl group is probably a poor mimic of the farnesyl moiety, it might still be able to benefit from some degree of hydrophobic interactions prevailing in the lipid binding domain of the enzyme. In contrast to free phenols in which the *meta* isomer **11** was active, it was the *ortho* isomer **7** in benzyl ether series that showed best activity (I_{50} = 63 uM). It is possible that the benzyl group might be accomodated by the hydrophobic pocket of the enzyme in which the farnesyl group of FPP would normally reside.

The fact that best activity in the phenolic and benzyl ether series was displayed by different isomers suggested some interesting possibilities for designing partial bisubstrate analogs in which a phenolic -OH group and a benzyl residue are incorporated on the same aromatic ring system. This leads to two different type of regioisomeric combinations (compounds **18** and **25**), whose syntheses are outlined in Scheme 3. The *ortho* phenol analog **21** was prepared for comparison of activity with regioisomer **18**. The key step in the preparation of such analogs would require regioselective monoalkylation of polyphenolic compounds. This should be possible for substrates in which these hydroxy groups differ considerably in their acidity, a further requirement being that the factors governing C-alkylation of mono- and di-carbanions operate in phenoxides also. We were pleased to realize that this was indeed the case.[10] With polyhydroxy benzaldehydes, *ortho* and *para* hydroxyl anions have extra stabilization through carbonyl conjugation, and thus represent thermodynamic sites, whereas *meta* hydroxyl anion is a more reactive center under kinetic conditions. Thus, treatment of 2,3-dihydroxybenzaldehyde with 1 equivalent of base and an equivalent of benzyl bromide gave the 2-benzyl ether **14**, whereas the same reaction utilizing 2.0 equivalents of NaH gave the kinetic product **15**. The position of -OH proton in the ^1H NMR spectra of the regioisomers was very typical. In **14**, it was at 6.0 ppm, whereas in **15**, it was shifted downfield at 11.0 ppm because of intramolecular H-bonding.[11] The -OH group in these regioisomers was then protected as a pivaloyl ester. The hindered pivaloyl group was chosen as a protecting group because unlike a simple acetate, it was expected to be more stable to further reaction conditions and silica gel column purifications. Aldehydes **16** and **19** were treated with Jones reagent and the resulting acids coupled with tripeptide amine H-VLS-OMe **5** via their acid chlorides. Base hydrolysis of the coupled products removed the two ester groups in one step and yielded the final products **18** and **21** respectively. Preparation of regioisomer **25** commenced with 2,5-dihydroxy benzaldehyde **22**. Treatment of **22** with one equivalent each of NaH and benzyl bromide, followed by acylation of the resulting alkylation product (42%) with pivalic anhydride yielded intermediate **23** (85%). Jones oxidation of **23** to acid **24** and its coupling with amine **5** utilizing BOP followed by basic hydrolysis gave **25**.

The two regioisomeric hybrid analogs (**18**, I_{50} = 157 uM; **25**, I_{50} = 137 uM) failed to show any improvement in activity over the parent *meta*-phenol **11** (I_{50} = 29 uM) and the *ortho*-benzyl ether **7** (I_{50} = 63 uM). Not unexpectedly, the *ortho*-phenol hybrid regioisomer **21** was inactive (I_{50} = >360 uM), reiterating the earlier observation that the hydroxyl group was strictly favored at the *meta* position of the ring (analogs **10-12**).

Scheme 3



Reagents: a) NaH (1.0), BnBr (1.0), >37 %; b) NaH (2.0), BnBr (1.0), >31% c) Me_3CCOCl,DMAP, Pyr, 40-60% d) Jones oxidation, 77-95%; e) 1) $(COCl)_2$,DMF, 2) DIPEA, HCl. H-VLS-OCH_3 **5**, 55-75% f) 1N NaOH, 60-80% g) BOP, DIPEA, HCl. H-VLS-OCH_3 **5**, 50%

In summary, the first examples of tripeptidic phenols (**10-12**), a new class of peptide based non-thiol type inhibitors of farnesyl protein transferase are reported in this communication. The activity of these compounds and their penultimate benzyl ether precursors (**6-9**) led to the design of partial bisubstrate analogs (**18, 25**), which were synthesized by selective O-alkylation of polyhydroxy benzaldehydes.

Notes and References :

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8 Diacylation refers to the product arising from simultaneous N-acylation of the amine group and O-acylation of the serine hydroxyl group of the tripeptide H-VLS-OMe **5**.

9 As pointed out by one of the referee, differences in relative acidity (pKa) of the hydroxyl groups of the *meta* isomer **11** versus the *ortho* and *para* regioisomers **10** and **12** respectively should also be taken into consideration.

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