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SYNTHESIS OF NOVEL SUBSTITUTED PYRIDINES AS INHIBITORS OF ENDOTHELIN CONVERTING ENZYME-1 (ECE-1)

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Abstract: A series of bi-aryl pyridine carboxylic acids has been prepared and evaluated as inhibitors of ECE-1. The analogs were prepared by Pd catalyzed cross couplings of halogenated pyridines with heteroaryl organo -boranes, -tinate or -zincate derivatives.

Introduction: The endothelins (ET's) are a family of potent vasoconstrictive bicyclic peptides that are produced by cleavage of their precursor, big-ET's, between Trp^{21} and Val^{22}/Ile^{22} by endothelin converting enzymes (ECE's).[1] ET exerts its effects by acting upon specific ET receptors and has been implicated in several human disease states including hypertension, renal failure, cerebral vasospasm, and ischemia.[2] Recently, many small molecule ET antagonists have been disclosed and reviewed.[3] The development of ECE inhibitors offers an alternative approach to ET antagonists in the treatment of many human diseases.[4] Early ECE-1 inhibitors were peptide like compounds designed around the weak ECE-1 inhibitor phosphoramidon.[5] More recently less peptide like inhibitors have been reported with nanomolar inhibitory activity for ECE-1.[6]

The compound WS75624 B, **3a** (Table 1), is one of two structural isomers which were isolated from the fermentation broth of *Saccharothrix* sp. No. 75624 and reported as nonpeptide inhibitors of ECE.[7] The structure was determined by 2-D NMR spectral analysis and chemical evidence.[8] We recently published a synthetic route to ± **3a** which exhibits the same ECE-1 inhibitory activity as the natural product.[9] In order to study the SAR of similar molecules, our original synthesis was modified to allow the preparation of a variety of related analogs. Utilizing Pd catalyzed couplings afforded an improved route to ± **3a**, as well as a number of novel biaryl compounds.

Chemistry: Previously ± **3a** was synthesized in 14 steps from kojic acid through a bromoacetyl intermediate, **1** (Scheme 1).[9] The final steps involved coupling **1** with an alkylhydroxy thioamide to form the

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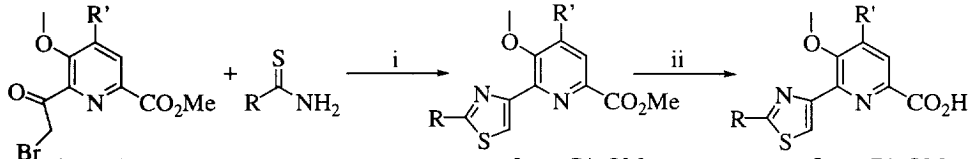
thiazole ring followed by hydrolysis to the acid, ± **3a**. Compounds **1** and **13** also underwent coupling with various thioamides and thioureas to give analogs **3b–e** and **22a–e,** respectively.

Scheme 1.



1, R'=OMe
13, R'=H

2a–e, R'=OMe
21a–e, R'=H

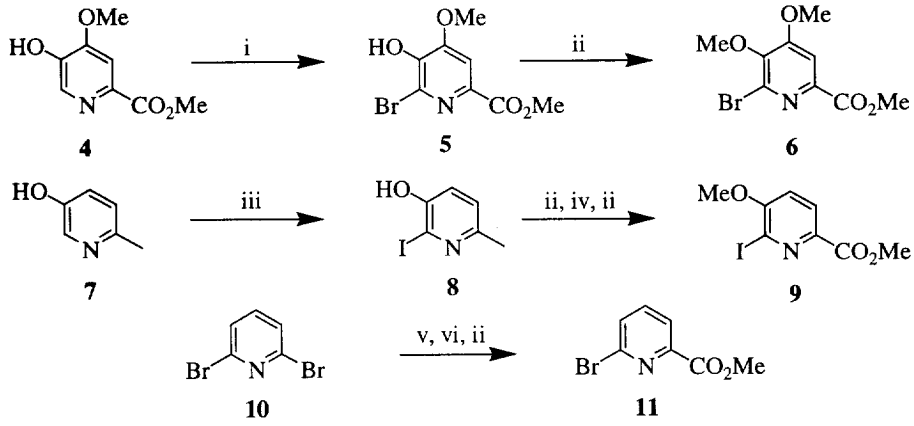
3a–e, R'=OMe
22a–e, R'=H

Reaction Conditions: (i) methanol reflux, overnight; (ii) 1.0 M NaOH/dioxane (1:1), 2 h, HCl.

The original synthesis was limited in that the bromoacetyl intermediate, **1**, originated from a low yield radical acylation and relatively few heteroaryl rings can be formed from the bromoacetyl moiety. An improved route to 4-thiazole pyridine compounds, and related heteroaryls, involved Pd catalyzed couplings of halogenated pyridine compounds.

Several halogenated pyridines were prepared to study the importance of the methoxy groups in the 4 and 5 positions (Scheme 2). Direct halogenation of the pyridine ring ortho to the nitrogen was aided by the presence of a hydroxy group in the meta position. Compound **4** was cleanly brominated by pyridinium tribromide to give **5** in 94% yield. The di-methoxy intermediate, **6**, was then prepared by methylation with MeI and Cs_2CO_3 in 79% yield. Iodination of 2-methyl-5-pyridinol, **7**, with elemental iodine and sodium

Scheme 2.

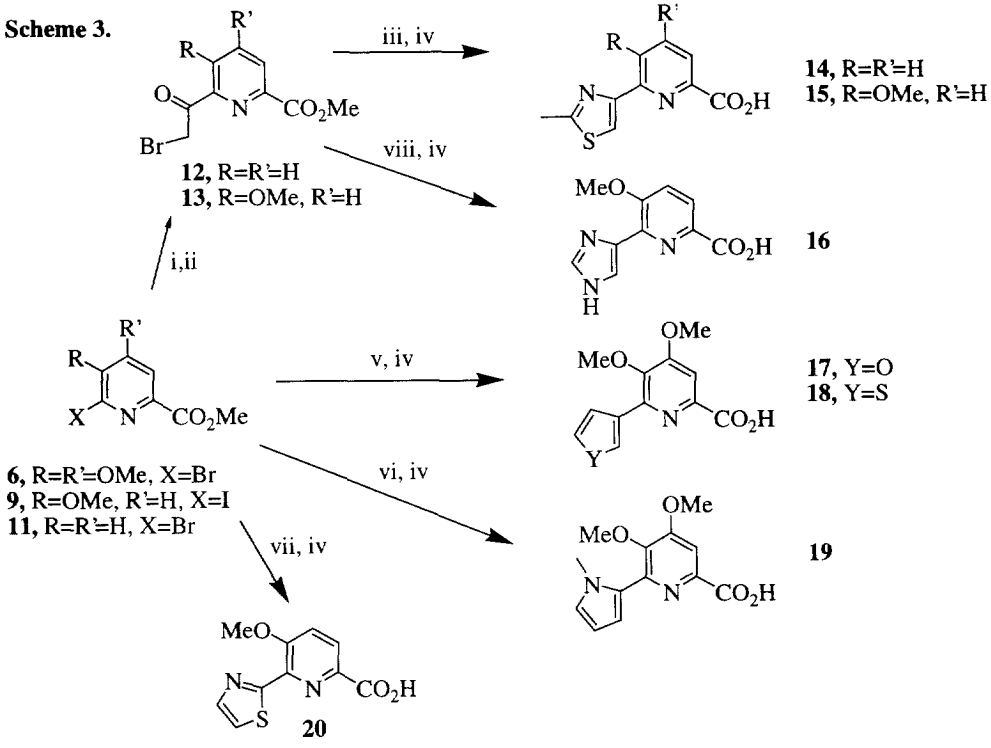


Reaction Conditions: (i) pyridine tribromide, pyridine, rt, 1 h; (ii) MeI, Cs_2CO_3, DMF, 55 °C, overnight; (iii) I_2, $NaHCO_3$, THF/H_2O, 24 h; (iv) $KMnO_4$, H_2O, 90 °C, 3 h; (v) n-BuLi, THF, -78 °C, 15 min; (vi) CO_2, HCl.

bicarbonate gave **8** in 81% yield.[10] The pyridinol was then methylated as above followed by oxidation of the methyl group to the acid with $KMnO_4$.[11] The desired intermediate, **9**, was then obtained in 41% overall yield from **8** by esterification of the acid with MeI/Cs_2CO_3. The 4,5-dihydro compound, **11**, was prepared from 2,6-dibromopyridine by mono-lithiation and quenching with CO_2 followed by esterification of the resulting acid to give ester, **11**, in 31% yield.

An improved route (Scheme 3) to the bromoacetyl derivative, **13**, involved Pd catalyzed coupling of the halogenated pyridine, **6**, with ethoxyvinyl tin in dioxane at 100 °C, followed by reaction of the ethoxyvinyl compound with NBS to give **13** in 53% overall yield.[11] Compound **12** was synthesized in an analogous fashion from the bromo pyridine, **11**. The reaction of the bromoacetyl compounds **12** and **13** with thioacetamide followed by hydrolysis gave **14** and **15**, respectively. The imidazole analog, **16**, was prepared in 26% yield from **13** by reaction with formamide[12] followed by hydrolysis. Replacement of the 4-thiazole with 3-furan or 3-thiophene was accomplished with Suzuki coupling of **6** with the corresponding boronic acids in the presence of $Pd(PPh_3)_4$. Hydrolysis of the esters gave compounds **17** and **18** in 33% and 37% yield,

Scheme 3.



Reaction Conditions: (i) tributylethoxyvinyltin, $Pd(PPh_3)_2Cl_2$, dioxane, 100 °C, overnight; (ii) NBS, THF, H_2O, rt, 10 min; (iii) thioacetamide, MeOH, reflux, overnight; (iv) 1.0 M NaOH/ dioxane, 1:1, 2 h; (v) heteroarylboronic acid, toluene/methanol (1:1), $Pd(PPh_3)_4$, 2 M Na_2CO_3, reflux 45 min; (vi) methyl-2-trimethylstannyl pyrrole, dioxane, $Pd(PPh_3)_2Cl_2$, 100 °C, overnight; (vii) thiazole, n-BuLi, THF -78 °C, 45 min, $ZnBr_2$, **9**, reflux 1h; (viii) formamide, heat.

respectively. The bromide, **6**, underwent coupling with 1-methyl-2-trimethylstannyl pyrrole[13] in the presence of $Pd(PPh_3)_2Cl_2$ to give the 2-methylpyrrole analog. After hydrolysis, the acid, **19**, was obtained in 38% overall yield from **6**. The thiazol-2-yl compound was prepared from the iodopyridine, **9**, by coupling of the 2-thiazolylzinc bromide formed in situ by quenching a THF solution of 2-lithiothiazole[14] with $ZnBr_2$. Using $Pd(PPh_3)_4$ as the catalyst, the coupling was carried out in refluxing THF to give the ester which was then hydrolyzed to the acid, **20**, in 23% overall yield.

A series of 2-substituted thiazo-4-yl compounds (Table 1)[15] with aromatic containing side chains were prepared from the bromoacetyl, **13** (Scheme 1). The esters, **21a–e**, resulted from the coupling of **13** with the corresponding thioamide in refluxing methanol. A convenient source of the various thioamides were commercially available amides, which reacted with Lawesson's reagent in THF at room temperature[9] to give the desired thioamides in 60-80% yields. The desired acids, **22a–e**, resulted from hydrolysis of the esters in 50-80% yield from **13**.

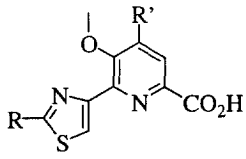
Biochemistry: The membrane fraction of CHO cells stably expressing human recombinant ECE-1 was prepared as earlier described[16] and used as the source of ECE-1. The ECE-1 assay and IC_{50} determinations have been previously described.[17]

Results: Initial modifications were performed on the 2-position of the thiazole ring. The long chain hydroxy-alkyl substituent in the natural product was shown to be unnecessary for ECE-1 inhibitory activity[16,17] as both the des-hydroxy, **3b**, and 2-methyl, **3c**, compounds retained the same activity. The 2-position unsubstituted compound, **3d**, and 2-N-methyl, **3e**, were slightly less potent. Similarly, simplification of the pyridine substitution pattern found that removal of the 4-methoxy, **15**, did not affect activity. However, removal of both methoxy groups, **14**, decreased potency approximately tenfold.

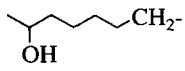
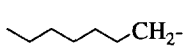
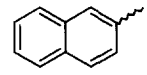
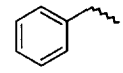
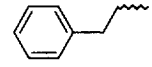
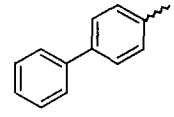
The importance of the thiazol-4-yl substitution was demonstrated by its replacement with various heteroaryl groups (Scheme 2). Whereas the unsubstituted thiazole-4-yl compound **3d** showed ECE-1 inhibition of 4.3 μM, the corresponding thiophene-3-yl, **17**, furan-3-yl, **18**, and 1-methylpyrrol-2-yl, **19**, all showed IC_{50}'s greater than 50 μM. The unsubstituted thiazol-2-yl, **20**, did show activity (24 μM) but significantly less than the original thiazol-4-yl substitution. The only other heteroaryl, besides the thiazoles, to show activity was the imidazole analog, **16**, with ECE-1 IC_{50} = 2.4 μM.

Aryl substitutions on the 2-position of the thiazole gave analogues of similar potency. The phenyl, **22a**, and 2-napthyl, **22b**, derivatives resulted in no significant change from the methyl compound, **15**. Addition of an alkyl spacer between the phenyl and the 2-position of the thiazole modestly increased potency. The benzyl and phenylethyl derivatives, **22c** and **22d**, gave analogues with slightly submicromolar potency. The biphenyl derivative, **22e**, was approximately ten fold less potent than the other analogues. Although the

Table 1[15]. ECE-1 Inhibitory Data



3a–e, R'= OMe **22a–e**, R'= H

Compound	R	$IC_{50}(\mu M)$	Compound	R	$IC_{50}(\mu M)$
3a		1.3	**22a**		1.3
3b		1.3	**22b**		1.5
3c	CH_3-	1.4	**22c**		0.86
3d	H-	4.3	**22d**		0.98
3e	MeHN-	2.6	**22e**		10

Variation of heteroaryl (Scheme 3)

Compound	$IC_{50}(\mu M)$	Compound	$IC_{50}(\mu M)$
14	10	**18**	>50
15	1.4	**19**	>50
16	2.4	**20**	24
17	>50		

results are not shown, all of the corresponding esters of these pyridine carboxylic acids were devoid of any ECE-1 inhibitory activity.

The relatively flat SAR in this series caused us to examine these compounds more intensely. When compound **15** was incubated with an aqueous solution of zinc chloride a stable tridentate crystal resulted with the zinc molecule equally spaced between the pyridine nitrogen, carboxylate oxygen and the thiazole nitrogen[18], suggesting these analogues bind quite efficiently to zinc. Further work will be required to determine whether these compounds actually bind to the active site of ECE-1 or elsewhere on the enzyme.

Conclusions: A convenient route to novel pyridine bi-aryl compounds was developed from halogenated pyridines and various organometallic heteroaryls. Either pyridyl bromides or iodides served as organic electrophiles in the Pd-catalyzed coupling with heteroaryl organo-boranes, -tinate, or -zincate derivatives to

give the desired bi-aryl compounds. This methodology also led to an improvement in the synthesis of WS75624 B, ± **3a**.

Several heteroaryls were coupled to the pyridine, however the thiazole-4-yl moiety was found to be most active. Only modest improvement in activity was realized by changing the 2-substitution pattern from that found in the natural product. The relatively flat SAR and the compounds propensity for binding to zinc has caused us to abandon further synthetic work on these analogues. The exact mode of binding for these analogues is still being examined.

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17. X-ray structure determined in house by Dr. Ron Rubin.
18. All final compounds had satisfactory ^{1}H NMR, MS, and microanalysis (C, H, N to ± 0.4 %). For example: **22d**: 33% yield from **13**. ^{1}H NMR (DMSO-d_6): δ 3.06 (t, J = 7.5 Hz, 2H), 3.36 (t, J = 7.5 Hz, 2H), 4.02 (s, 3H), 7.10-7.22 (m, 5H), 7.39 (d, J = 8.5 Hz, 1H), 8.01 (s,1H), 8.14 (d, J = 8.5 Hz, 1H), acid proton missing. Microanalysis ($C_{18}H_{16}N_2O_3S$): calc'd: C= 63.51, H= 4.74, N= 8.23; found: C= 63.14, H= 4.56, N= 8.05. MS (APCI), $[M+1]^+$= 341.1. mp= 104-106 °C.