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Development of Dual-Acting Agents for Thromboxane Receptor Antagonism and Thromboxane Synthase Inhibition. 2. Design, Synthesis, and Evaluation of A Novel Series of Phenyl Oxazole Derivatives.

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Abstract: Synthesis and initial in vitro evaluation of a novel series of phenyl oxazole derivatives are described. An SAR study of the novel dual-acting TRA/TSI agent has revealed that the lipophilicity of the oxazole amide substituents greatly influences the TRA activity but not the TSI. The chain length of the alkenoic acid side chain affects both TRA and TSI. The optimal chain length for the combined activities was found to be n = 4 (heptenoic acid).

Thromboxane A_2 (TXA_2)[2] is an unstable endogenous arachidonic acid metabolite which plays a pivotal role in platelet aggregation and vasoconstriction and has been implicated as a contributor in cardiovascular, renal, and pulmonary disease.[3] Thromboxane receptor antagonists (TRA) and thromboxane synthase inhibitors (TSI) have been developed to treat such disorders.[4] Because of the unpredictable efficacy of these agents, theoretical arguments and preclinical observations have been made which support the concept that superior antithrombotic efficacy might be observed by using a combined TRA/TSI over either class of agent alone or aspirin.[5] Recently there has been an interest in developing agents which contain both TRA and TSI activity within a single molecule to realize this goal.[6] Previously we reported our effort to develop substituted ω-phenyl-ω-(3-pyridyl)alkenoic acids as such dual-acting agents.[7] We report here in a preliminary form the synthesis and initial biological evaluation of a novel series of phenyl oxazole derivatives which possess potent combined TRA/TSI activity in a single chemical entity.

A series of compounds **7a-f** was synthesized in 11 steps from commercially available 1,2- 1,3- or 1,4-benzenedimethanol as shown in Scheme I. Benzenedimethanol was mono-protected with *t*-butyldimethylsilyl chloride by a standard method (27-37%). The mono-protected diol was oxidized with MnO_2 in THF at reflux to afford the corresponding aldehyde (66-84%). This was then treated with 3-lithiopyridine which was generated by the reaction of 3-bromopyridine with *n*-BuLi at -78 °C in Et_2O. The carbinol thus obtained (70-89%) was oxidized to the corresponding ketone **1** by MnO_2 (95-97%). This ketone was then subjected to a Wittig reaction with 5-carboxypentylphosphonium bromide[8] and *t*-BuOK in THF at -15 to 0 °C. The *E*- and *Z*-isomers obtained could be separated by chromatography at this stage or in any of the later steps. In all cases the *Z*-isomer (less polar material) was the predominant Wittig reaction product (for the *o*- and *p*-substituted phenyl derivatives, *E/Z* = ~1:4; and for the *m*-substituted *E/Z* = ~ 1:3). Stereochemical assignment of products rested on their ^1H NMR spectra and their mobility on silica gel.[9] The heptenoic acid **2a** was esterified with CH_2N_2 (yield for two steps: *o*- 69%; *m*- 75%, *p*- 72%). Typically *E*- and *Z*- isomers were separated at this stage. Each TBS ether **2b**

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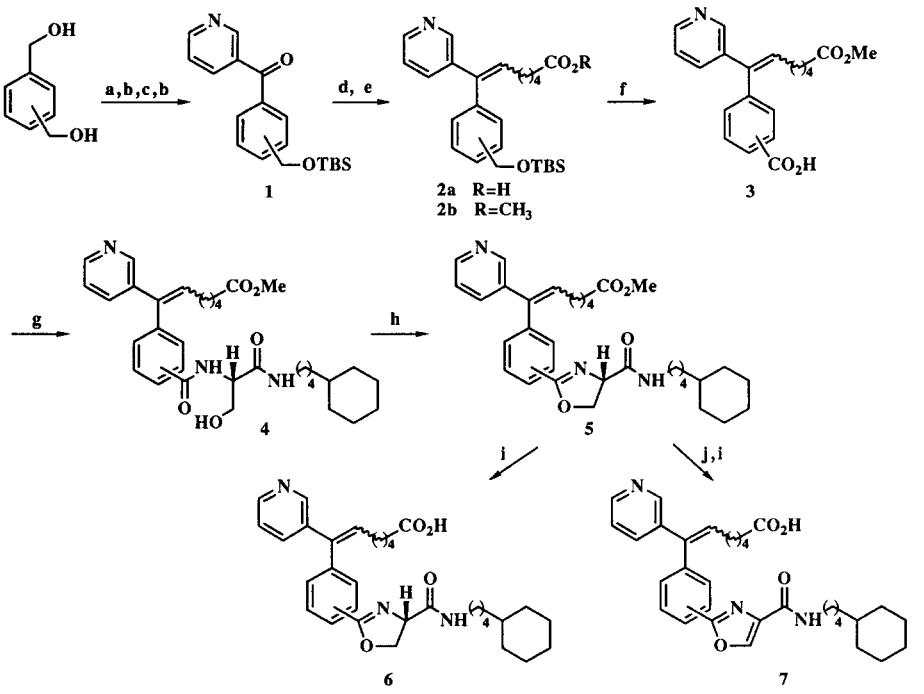
Scheme I



a) TBSCl, Imidazole, CH_2Cl_2. b) MnO_2, THF, reflux. c) 3-Lithiopyridine, Et_2O, -78 °C.
d) $Br^-Ph_3P^+(CH_2)_5CO_2H$, *t*-BuOK, THF, -15 - 0 °C. e) CH_2N_2, Et_2O-THF; chromatographic separation.
f) Jones reagent, acetone, 0 °C. g) N-(4-cyclohexylbutyl)-L-serinamide, WSC, HOBT, DMF, 4-methylmorpholine.
h) Ph_3P, *i*-Pr_2NEt, CCl_4,CH_3CN, r.t. i) 1N NaOH, THF-MeOH; H^+. j) NiO_2, 4A MS, PhH-dioxane, reflux.

was oxidized by Jones oxidation to the acid **3** (*E*- 47-85%, *Z*- 32-67%). The acid **3** was then coupled with either N-(4-cyclohexylbutyl)-L-serinamide or its O-TBS protected analog via activation of the carboxyl group with any of the commercially available coupling agents such as carbonyl diimidazole (CDI), dicyclohexylcarbodiimide (DCC) or water soluble 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSC) hydrochloride or methiodide. The preferred activation was best accomplished with WSC (*E*- 16-67%, *Z*- 29-89%). The oxazoline formation was effectively carried out by a modification of Miller's method[10] after TBS deprotection with *n*-$Bu_4N^+F^-$ when necessary (*E*- 67%-quantitative, *Z*- 77-95%). The final oxazole substituted phenyl derivatives **7a-f** were obtained in two steps from the oxazoline esters **5**: (1) Nickel peroxide oxidation of the oxazoline to oxazole[11] (*E*- 55-65%, *Z*- 26-57%); and (2) ester hydrolysis with aqueous NaOH in THF-MeOH (1:1) (*E*- 80%-quantitative, *Z*- 27-83%). The oxazoline substituted phenyl derivatives **6** were obtained by hydrolysis of the esters **5** with aqueous NaOH in THF-MeOH (1:1) (*E*- 71-80%, *Z*- 29-55%).

Table I lists the results of in vitro evaluation of the compounds **6a-f** and **7a-f** for their ability to antagonize the TXA_2 receptor and inhibit the thromboxane synthase. While compounds **7a-f** were our target molecules, we also tested the intermediate oxazoline acids **6a-f** because of their structural similarity to **7a-f**. We

Table I. *In Vitro* Activities of the Phenyl Oxazole Derivatives **6** and **7**.[13]

Compounds	Substitution	E/Z	TRA K_d (nM)[a]	TSI IC_{50} (nM)[b]
6a	*ortho*	*Z*	1100`	n.d.[c]
6b		*E*	1850	n.d.
6c	*meta*	*Z*	830 ± 20	452 ± 16
6d		*E*	n.d.	n.d.
6e	*para*	*Z*	51.6 ± 9.7	>10,000
6f		*E*	15.5 ± 4.0	82.1 ± 62.6
7a	*ortho*	*Z*	1500	n.d.
7b		*E*	540	n.d.
7c	*meta*	*Z*	630 ± 40	23 ± 8
7d		*E*	12.3 ± 1.5	19.0 ± 5.1
7e	*para*	*Z*	146.6 ± 26	>10,000
7f		*E*	9.9 ± 0.4	55.0 ± 17.9
CV4151[8]			8,200±400	48.7±16.0

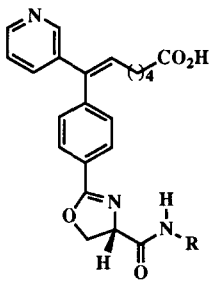
[a] Affinity receptor binding assay, see ref. 7. [b] The concentration required to inhibit by 50% the serum TXA_2 synthesis, measured in the form of TXB_2 (a stable TXA_2 metabolite) by specific radioimmunoassay. See ref. 7. [c] n.d.: not determined.

found both oxazoline and oxazole derivatives to be dual-acting TRA/TSIs. The potency of the compounds was related to the unsaturation of the oxazole ring (oxazole>oxazoline), substitution pattern of the oxazoline/oxazole on the phenyl ring (*para>meta>ortho*), and the olefin geometry of the heptenoic acid side chain (*E>Z*)[12]. In general these trends in potency were consistent in both TRA and TSI.

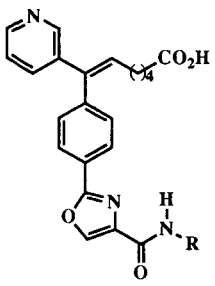
Next, the effect of amide substituents was investigated (Table II). N-alkylated phenyl oxazole amides **8-11** were prepared according to the synthetic route described above.[13] The calculated logP[14] of these amides suggests that increased TRA activity of both oxazoline intermediates and oxazoles is associated with increased lipophilicity. Lipophilicity of the amide substituents, however, has little effect on the TSI activity of the oxazoles.

We have also investigated whether the length of alkenoic acid side chain influences the TRA/TSI activity. During the course of our synthetic effort to prepare this series of compounds, we found that a Wittig reaction of an oxazole amide substituted aromatic ketone such as **16** preferentially produced an (*E*)-alkenoic acid.[15] We therefore modified the above synthetic route and synthesized compounds **17** as shown in Scheme II. The keto ester **13** was prepared in two steps from commercially available methyl 4-formylbenzoate which was treated with 3-lithiopyridine in Et_2O at -78 °C to room temperature, followed by MnO_2 oxidation in THF at reflux in 60% yield. Hydrolysis of the ester and subsequent amide coupling with DL-serine methyl ester which was activated with WSC in the presence of 1-hydroxybenzotriazole hydrate (HOBT) and N-methylmorpholine (NMM) in DMF at room temperature provided the amide **14** in 81% yield. Oxazoline formation via a

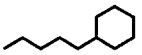
Table II. N-Substituent Effect of Phenyl Oxazole Amides on TRA/TSI Activities[a]



Oxazoline



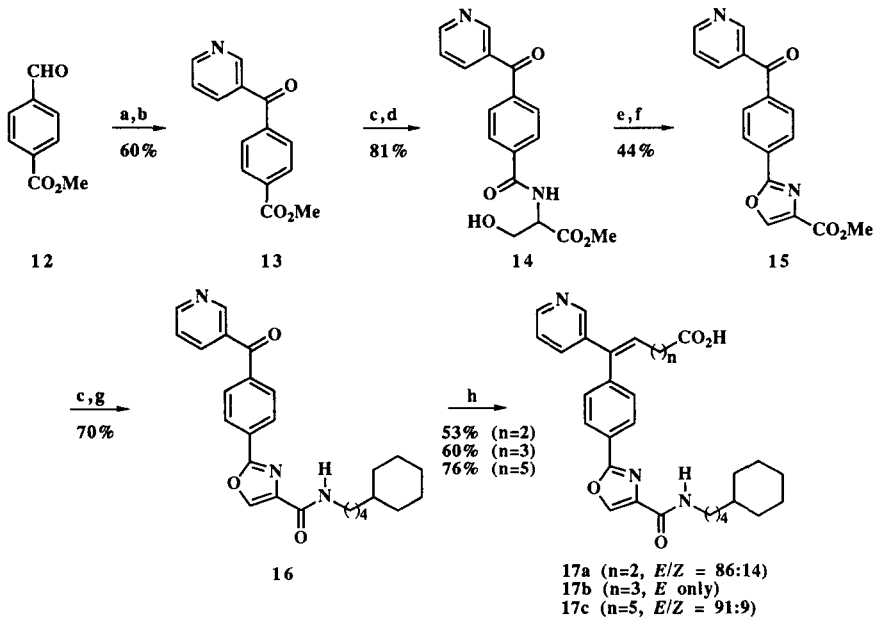
Oxazole

		Oxazoline		Oxazole		
		TRA	TSI	TRA	TSI	
entry	R	Kd (nM)	IC_{50}(nM)	Kd (nM)	IC_{50}(nM)	clogP[b]
8		15.5 ± 4.0	82.1 ± 62.6	9.9 ± 0.4	55.0 ± 17.9	7.35
9		224.5 ± 54.9	---	153.1 ± 19.6	31.2 ± 14.7	5.23
10		1122.6 ± 176.1	---	791.2 ± 194.6	25.0 ± 6.47	4.09
11		378.6 ± 5.7	---	135.2 ± 6.8	24.7 ± 9.6	4.88

[a]The thromboxane receptor antagonism (TRA) and thromboxane synthase inhibition (TSI) were determined in triplicate using a human platelet binding assay and human serum levels of TXB_2, see ref 7.
[b]The clogP program provided values for the octanol/water partition coefficient, see ref 14.

triflate[16] was carried out with triflic anhydride in the presence of phenyl sulfoxide and K_3PO_4 in CH_2Cl_2 at -78 to 0 °C in 75% yield. Oxidation of the oxazoline with MnO_2[17] in benzene at reflux yielded the oxazole ester **15** in 58% yield. Hydrolysis of the ester and amide coupling with 4-cyclohexylbutylamine furnished the ketone **16** in 70% yield. A Wittig reaction of the ketone with (ω-carboxypropyl or pentyl)triphenylphosphonium bromide which was treated with 1.0 M *t*-BuOK in THF at 0 °C provided the final compounds **17a-c** (53~76%).[13] In vitro activities of the N-cyclohexylbutyl oxazole amide derivatives thus prepared are listed in Table III. The data show that the alkenoic acid side chain length has affected both TRA and TSI activities. The heptenoic acid derivative (**7f**, n = 4) appears to maintain the optimal chain length for both TRA and TSI potency. The existence of an optimal distance between the pyridyl nitrogen and carboxylic acid terminus for TSI potency has been well documented.[8,18] Main and coworkers[19] have optimized the distance between the pharmacophores of TRA as well as of TSI in their development of TRA/TSI agents. Our present and previous data[7] as well as these literature precedents confirm that the optimal alkenoic acid side chain for TRA/TSI activities of our series also falls within 8.5-10 Å.

Scheme II



(a) 3-Lithiopyridine, Et_2O, -78 °C to r.t., 4 hr. (b) MnO_2, THF, reflux, overnight. (c) 1N NaOH, MeOH/THF (1:1); H^+. (d) DL-Serine Methyl Ester, WSC, HOBT, NMM, DMF, 0 °C to r.t. (e) $(TfO)_2O$, Ph_2SO, K_3PO_4, CH_2Cl_2, -78 to 0 °C, 2 hr. (f) MnO_2, PhH, reflux. (g) 4-Cyclohexylbutylamine, WSC, HOBT, NMM, DMF, 0 °C to r.t. (h) $Br^-Ph_3P^+(CH_2)_{n+1}CO_2H$, 1.0 M *t*-BuOK, THF, 0 °C, 2 hr.

Table III. Effects of Carboxylic Acid Side Chain Length on TRA/TSI Activities[a]

entry	n	TRA Kd (nM)	TSI IC_{50} (nM)
17a	2	93.3 ± 6.7	3167 ± 745
17b	3	33.3 ± 1.7	230 ± 170
7f	4	9.9 ± 0.4	55.0 ± 17.9
17c	5	110 ± 20	38.0 ± 11.1

[a]The thromboxane receptor antagonism (TRA) and thromboxane synthase inhibition (TSI) were determined in triplicate using a human platelet binding assay and human serum levels of TXB_2. See ref. 7.

Further SAR study and the fuller extent of the effect of lipophilicity on the biological activity of these phenyl oxazole amide derivatives will be reported in due course.

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13. Satisfactory spectral and analytical data were obtained. For example, **7f**: mp 52-56 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (br s, 1H), 8.47 (d, J = 2.7 Hz, 1H), 8.27 (s, 1H), 8.04 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.2 Hz, 2H), 7.23 (m, 1H), 7.12 (t, J = 5.9 Hz, 1H), 6.18 (t, J = 7.5 Hz, 1H), 3.43 (ddd, J = 6.9, 6.8, 6.6 Hz, 2H), 2.31 (dd J = 7.2, 6.9 Hz, 2H), 2.19 (ddd, J = 7.3, 7.2, 7.1 Hz, 2H), 1.68-0.82 (m, 21H); FDMS 530 (M+1). Anal. ($C_{32}H_{39}N_3O_4 \cdot 0.2C_2H_4O_2$) C, H, N.
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