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Combinatorial Synthesis and Biological Evaluation of Isoxazole-Based Libraries as Antithrombotic Agents[†]

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Abstract—The 3-substituted phenyl-5-isoxazolecarboxaldehydes have been identified as activated aldehydes for the generation of isoxazole-based combinatorial libraries on solid phase through automation. Three highly functionalized isoxazole-based libraries comprising of 32, 96 and 45 compounds each have been synthesized in parallel format using Baylis Hillman reaction, Michael addition, reductive amination and alkylation reactions. With an objective of lead generation all the three libraries were evaluated for their antithrombin activity in vivo. © 2002 Elsevier Science Ltd. All rights reserved.

The synthesis of highly functionalized libraries of organic molecules through solid-phase organic synthesis has been recognized as a valuable tool for lead discovery in the search of novel chemotherapeutic agents. The isoxazole ring system, which can be easily obtained by [3 + 2] cycloadditions of nitrile oxides with alkynes, is of particular interest since it forms part of various biodynamic agents. The biological activities associated with this ring system could be attributed to facile cleavage of the N–O bond, which could lead into more reactive species such as imines, amino alcohols or α,β-unsaturated ketones. Some of the biological activities ascribed to isoxazole derivatives includes antithrombotic, PAF antagonist, hypolipidemic, nootropic, immunomodulator, antiviral, antiobesity and CNS modulation.[1] The substituted isoxazoles, are also considered to be important synthons due to their versatility towards chemical transformations to useful synthetic intermediates such as 1,3-dicarbonyl, 1,3-iminocarbonyl and γ-amino alcohols. The significance of this class of molecules gets further impetus due to their involvement as intermediates in the synthesis of various natural products.[2] Though there are several reports pertaining to the synthesis functionalized isoxazole heterocycle on the resin,[3,4] the use of substituted 5-isoxazolecarboxaldehydes for the generation of isoxazole-based libraries is hitherto unreported. In our endeavor to explore the synthetic utility of substituted 5-isoxazolecarboxaldehydes on solid phase we have carried out automated solid-phase parallel synthesis of three isoxazole-based libraries utilizing this molecule. All the libraries were subjected to in vivo evaluation for antithrombotic activity. The details of our studies are reported here.

All reactions reported herein were first optimized in the PP syringes followed by the synthesis of library through automation on Advanced Chemtech MOS 496 Ω. Cleavage of various 5–7 mg samples of resin bound derivatives with subsequent HPLC, FAB MS and PMR of the resultant products accompanied each step during optimization. All compounds synthesized through automation were subjected to FAB MS to confirm the presence of the desired product.

The synthesis of library as shown in Scheme 1 involved Baylis Hillman (BH) reaction of substituted 5-isoxazolecarboxaldehyde followed by Michael addition. As other reactive aldehydes,[5] in our earlier report[6,7] utilizing 5-isoxazolecarboxaldehyde as scaffold we have observed that this aldehyde also undergoes fast BH reaction. However, as the aldehyde was being loaded on the solid support utilizing the hydroxyl group on the phenyl residue at position 3 of the isoxazole moiety, the

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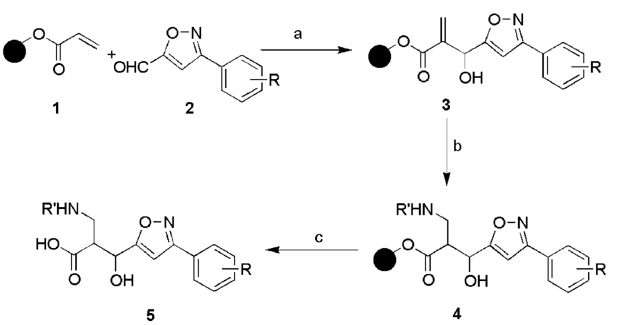


Scheme 1. (a) DABCO, DMSO, 3 h; (b) R′NH$_2$, DMSO, 5 h; (c) 5% TFA in DCM, 20 min. R = H, 4-CH$_3$, 2-Cl or 4-OCH$_2$Ph; R′ = CH(CH$_3$)CH$_2$CH$_3$, (CH$_2$)$_3$N(C$_2$H$_5$)$_2$, cyclohexyl, (CH$_2$)$_2$(morpholin-4-yl), (CH$_2$)$_8$CH$_3$, (CH$_2$)$_2$(4-chlorophenyl), CH$_2$(4-chlorophenyl), CH$_2$(4-fluorophenyl).

resulting product was invariably 3-(4-hydroxyphenyl)-isoxazole derivative. It was therefore envisaged that if the same synthetic strategy is employed using activated alkene on the solid support followed by incorporation of various substituted 5-isoxazolecarboxaldehydes, then it would be possible to generate diversity at the phenyl residue as well.

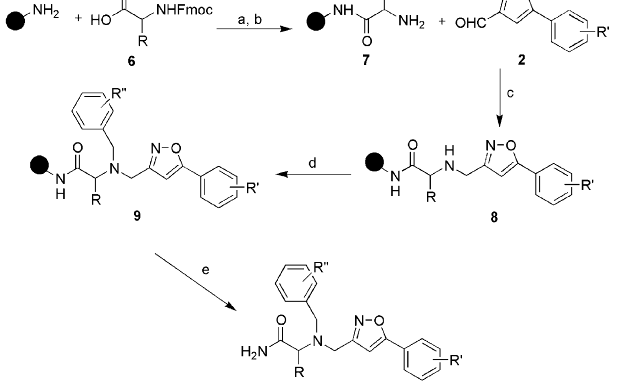
Therefore in the first instance acrylic acid was loaded on 2-chlorotrityl resin using standard procedure to obtain the acrylate **1**. Treatment of resin 1 with DABCO and 3-substituted-phenyl-5-isoxazolecarboxaldehydes (**2**) in DMSO led to the formation of BH adducts (**3**) within 3 h in quantitative yields and high purity. The Michael addition of primary amines to these BH adducts followed by cleavage from the solid support furnished various 3-substituted amino-2[1-hydroxy-1-(3-substituted-phenyl-isoxazol-5yl) methyl] propionic acid derivatives (**5**) (Table 1) as diastereomeric mixtures in excellent yields and purity (**7**). The scope and limitation of the method has been investigated by synthesizing a library of 32 compounds in parallel format using four aldehydes and eight amines. The purity of these compounds ranged from 76–91% based on analytical HPLC. Structure, yields and purity of some of the representative compounds have been summarized in Table 1 and the PMR data of a representative compound after purification by RP HPLC (t_R = 17.2 min; C-18 column: 4.6×250 mm; using a linear gradient of A: 0.1% A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile, from 70 to 40% A over 45 min) has also been described.[8]

Table 1. Structure, yields and purities of representative compounds from library based on structure **5**

Entry	R	R′	Yield[a]	Purity[b]	FABMS[c]
1	H	(CH$_2$)$_3$NEt$_2$	72	83	375 (M$^+$)
2	H	(CH$_2$)$_8$CH$_3$	81	79	388 (M$^+$)
3	4-CH$_3$	(CH$_2$)$_2$(morpholin-4-yl)	69	74	389 (M$^+$)
4	4-CH$_3$	(CH$_2$)$_2$(4-Cl-C$_6$H$_4$)	91	88	414 (M$^+$)
5	2-Cl	CH(CH$_3$)CH$_2$CH$_3$	66	77	353 (M$^+$)
6	2-Cl	Cyclohexyl	96	85	378 (M$^+$)
7	4-OCH$_2$Ph	CH$_2$(4-Cl-C$_6$H$_4$)	69	84	492 (M$^+$)
8	4-OCH$_2$Ph	CH$_2$(4-F-C$_6$H$_4$)	71	87	476 (M$^+$)

[a] Crude yields.
[b] As determined by analytical HPLC.
[c] All compounds in the library gave the corresponding M$^+$ + 1 in the FABMS(+).

In another synthetic strategy, it was envisaged that if the free amino group of the amino acid is made to react with 3-substituted-phenyl-5-isoxazolecarboxaldehyde through reductive amination followed by alkylation of the NH group, then an isoxazole-based library with three-point diversity could be generated. Thus initially an amino acid was loaded on the Rink Amide AM resin using TBTU/DIPEA method to yield **7**. After the deprotection of the Fmoc group, reductive alkylation of the resin bound amino acid with 3-substituted-phenyl-5-isoxazolecarboxaldehydes in the presence of NaCNBH$_3$ resulted in the formation of secondary amine derivative (**8**). Finally, it was reacted with substituted benzyl bromides and cleaved to obtain highly functionalized isoxazole derivatives (**10**) (Scheme 2).[9] The general applicability of this method has been exemplified by synthesizing two libraries through automaton. In the first library four amino acids, three aldehydes and eight halides were used as diversity elements to generate 96 compounds (4×3×8) while in the second five amino acids, three aldehydes and three halides were utilized to furnish 45 compounds (5×3×3) in parallel format. Structure, yields and purity of some of the representative compounds have been summarized in Table 2 and the PMR data of one of the compound after purification by RP HPLC (t_R = 28 min; C-18 column: 4.6×250 mm; using a linear gradient of A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile, from 75 to 35% A over 45 min) has also been described.[9]



10 (96 and 45 examples)

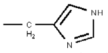
Scheme 2. (a) TBTU, DIPEA, DMF, 5 h; (b) 20% piperidine: DMF mix, 25 min; (c) NaCNBH$_3$, TMOF, AcOH, 2 h; (d) (R″)C$_6$H$_4$CH$_2$Br, DBU, DMSO, 5 h; (e) 95% TFA/DCM mix. 96 membered: R = CH$_2$Ph (**A1**),  (**A2**), (CH$_2$)$_4$NH$_2$ (**A3**), CH$_2$CH(CH$_3$)$_2$ (**A4**); R′ = H (**B1**), 4-CH$_3$ (**B2**), 2-Cl (**B3**); R′ = H (**C1**), 4-CN (**C2**), 4-NO$_2$ (**C3**), 4-Br (**C4**), 3-Br (**C5**), 4-Me (**C6**), 3-Me (**C7**), 3-NO$_2$ (**C8**). 45-membered: R = H (**A5**), (CH$_2$)$_3$NHC(=NH)NH$_2$ (**A6**), CH$_2$OH (**A7**), CH$_2$C$_6$H$_4$(4-OH) (**A8**), (CH$_2$)$_2$CONH$_2$ (**A9**); R′ = H (**B1**), 4-CH$_3$ (**B2**), 2-Cl (**B3**); R′ = H (**C1**), 4-CN (**C2**), 4-Br (**C4**).

Table 2. Structure, yields and purities of representative compounds from libraries based on structure **10**

Entry	R	R′	R″	Yield[a]	Purity[b]	FAB MS[c]
1	CH_2Ph	2-Cl	4-NO_2	62	92	492 (M^+)
2	CH_2-imidazol-4-yl	4-CH_3	4-CH_3	67	74	430 (M^+)
3	$(CH_2)_4NH_2$	H	3-Br	70	86	472 (M^+)
4	$CH_2CH(CH_3)_2$	2-Cl	3-NO_2	61	82	458 (M^+)
5	$(CH_2)_3NHC(=NH)NH_2$	4-CH_3	4-Br	61	77	513 (M^+)
6	CH_2OH	2-Cl	4-CN	72	87	410 (M^+)
7	$CH_2C_6H_4$(4-OH)	H	H	69	91	427 (M^+)
8	$(CH_2)_2CONH_2$	4-CH_3	H	54	75	407 (M^+)
9	H	4-CH_3	H	86	93	335 (M^+)

[a] Crude yields.

[b] As determined by analytical HPLC.

[c] All compounds in two libraries gave the corresponding $M^+ + 1$ in the FABMS(+).

Biological activity

All the compounds obtained were evaluated for their antithrombotic activity in vivo. Swiss mice (20–25 g, from CDRI animal colony) were used in a group of at least 10 animals each. Thrombosis was induced by infusion of a mixture of 15 μg collagen and 5 μg adrenaline in a volume of 100 μL into the tail vein of each mouse. This resulted either death or hind limb paralysis of 100% animals. The compounds were administered at 30 μmol/kg by oral route 1 h prior to the thrombotic challenge. The antithrombotic effects of these compounds were assessed by the percentage protection offered by these agents to mice from death or paralysis following thrombotic challenge using aspirin as a standard.

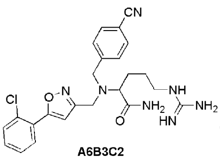
Table 3. In vivo antithrombotic activity and increase in the bleeding time[a] of compounds belonging to libraries represented by structure **10**

Compd	% Protection	Compd	% Protection
A1B1C1	30	**A6B2C1**	10
A1B3C1	30	**A6B3C1**	40
A1B3C2	50 (0)	**A6B1C2**	50 (31)
A1B3C3	20	**A6B2C2**	40
A1B1C4	20	**A6B3C2**	90 (87.5)
A1B2C6	50 (0)	**A6B1C4**	80 (25)
A2B1C1	50 (81)	**A6B2C4**	40
A2B1C2	50 (80)	**A7B2C1**	60 (79)
A2B1C3	60 (30)	**A7B1C2**	30
A2B1C5	40	**A7B2C2**	60 (41)
A2B2C5	10	**A7B3C2**	10
A2B1C6	40	**A7B1C4**	30
A2B2C8	60 (40)	**A7B1C1**	60 (58)
A3B2C1	40	**A7B3C4**	70 (50)
A3B2C2	40	**A8B2C1**	60 (20)
A3B1C3	30	**A8B2C2**	20
A3B2C5	40	**A8B3C2**	40
A3B1C6	20	**A8B1C4**	20
A3B2C8	20	**A8B3C4**	60
A4B1C1	30	**A9B1C2**	70 (62)
A4B1C2	20	**A9B3C4**	20
A4B1C6	20	**Aspirin**	40±1 (110±7)
A4B2C7	60 (NT)		
A5B2C1	20		

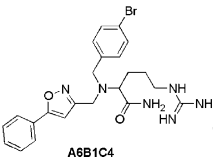
[a] Values appearing in brackets represent the percentage increase in the bleeding time.

In the first library represented by structure **5**, all compounds were found to be inactive. In the second 96-membered library represented by structure **10**, only mild activity was observed in the derivatives with leucine residue as one of the diversity element. But in the third 45 membered library also represented by structure **10** various hits were observed. The results of the in vivo antithrombotic activity of active compounds only from these two libraries are summarized in Table 3. The structure–activity relationship indicates that nearly all the derivatives incorporating arginine as the diversity element in the amino acid position elicit good biological response. The compounds having tyrosine, serine and histidine residues followed them in the activity profile. However compounds having glycine, glutamic acid, phenylalanine and leucine did not show any significant activity. The substitution on the phenyl group at the 3-position of isoxazole did not make any significant impact on the activity but it was observed that compounds with 2-chlorophenyl were found to show higher order of activity than simple phenyl or 4-methylphenyl. On the other hand, the benzyl group did not follow any definite pattern though in the third library compounds with 4-cyanophenyl-methyl show better activity.

All the compounds exhibiting more than 50% protection were also tested[10] for increase in the bleeding time at the same dosage of 30 μM. The result of percentage protection against the bleeding time is summarized in Table 3. Thus the most active compounds after combining both results are **A6B3C2**, **A6B1C4** and **A9B1C2**.



A6B3C2



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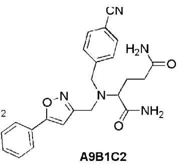

A9B1C2

In conclusion, we have successfully utilized 3-substituted-phenyl-5-isoxazolecarboxaldehydes for the generation of isoxazole-based libraries using robust reactions, which could be easily performed on an automated synthesizer. The hits for the antithrombotic activity identified in these libraries are being optimized further.

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References and Notes

1. (a) Nantermet, P. G.; Barow, J. C.; Lundell, G. F.; Pellicore, J. M.; Rittle, K. E.; Young, M. B.; Friedanger, R. M.; Connoly, T. M.; Condra, C.; Karczewski, J.; Bednar, R. M.;

Gaul, S. L.; Gould, R. J.; Prendergast, K.; Selnick, H. G. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 319. (b) Pruitt, J. R.; Pinto, D. J.; Estrella, M. J.; Bostrom, L. L.; Knabb, R. M.; Wong, P. C.; Wright, M. R.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 685. (c) Xue, C. B.; Roderick, J.; Mousa, S. A.; Olson, R. E.; Degrado, W. F. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3499. (d) Witayak, J.; Xue, C. B.; Sielecki-Drudz, F. M.; Olson, R. E.; Degrado, W. F.; Cain, G. A.; Batt, D. G.; Pinto, D.; Hussain, M. A.; Mousa, S. A. US Patent US, 5,849,736, 1997 (Chem. Abstr. *130*, 66484v). (e) White, A. D.; Purchase, C. F., II; Picard, J. A.; Anderson, M. K.; Mueller, S. B.; Bocan, T. M. A.; Bousley, R. F.; Hamelehle, K. L.; Krause, B. R.; Lee, P.; Stanfield, R. L.; Reindal, J. F. *J. Med. Chem.* **1996**, *39*, 3908. (f) Riccardi, S.; Fusi, R.; Luberi, L.; Napoletano, M.; Masotto, C. *Drugs Future* **1995**, *20*, 584. (g) Garvey, D. S.; Carrera, G. M., Jr.; Arneric, S. P.; Edmund, L.; Lebold, S. A. US Patent US 5,409,946, 1995 (Chem. Abstr. *123*, 55872z). (h) Garvey, D. S.; Wasiacak, J. T.; Decker, M. W.; Brioni, J. D.; Buckley, M. J.; Sullivan, J. P.; Carrera, G. M.; Holladay, M. W.; Arneric, S. P.; Williams, M. *J. Med. Chem.* **1994**, *37*, 1055. (i) Ryng, S.; Glowiak, T. *J. Chem. Crystallogr.* **1998**, *28*, 373. (j) Chiarino, D.; Fantucci, M.; Carenzi, A.; Della, B. D.; Frigenio, V.; Sala, R. *Farmaco Ed. Science* **1986**, *41*, 440. (k) Diana, G. D.; Otto, M. J.; Freasuryawala, A. M.; McKinlay, M. A.; Oglesby, R. C.; Maliski, E. G.; Rossmann, M. G.; Smith, T. J. *J. Med. Chem.* **1988**, *31*, 540. (l) Lybrand, J. P.; McCammon, J. A. *J. Comput. Aided Mol. Des.* **1989**, *2*, 259. (m) MDDR in MDL database.

2. (a) Baraldi, P. G.; Basco, A.; Benetti, S.; Pollini, G. P.; Simoni, D. *Synthesis* **1987**, 857. (b) Kozikowski, A. P. *Acc. Chem. Res.* **1984**, *17*, 410.

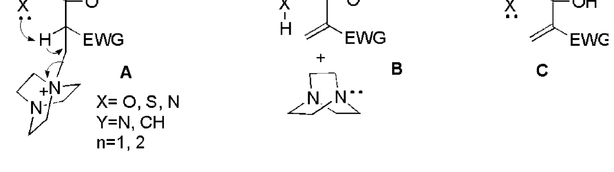
3. Shankar, B. B.; Yang, D. Y.; Girton, S.; Ganguly, A. K. *Tetrahedron Lett.* **1998**, *39*, 2447.

4. (a) Shang, Y. J.; Wang, Y. G. *Tetrahedron Lett.* **2002**, *43*, 2247. (b) Cereda, E.; Ezhaya, A.; Quai, M.; Barbaglia, W. *Tetrahedron Lett.* **2001**, *42*, 4951. (c) Park, K. H.; Kurth, M. J. *J. Org. Chem.* **1999**, *64*, 9297. (d) Park, K. H.; Kurth, M. J. *Tetrahedron Lett.* **1999**, *40*, 5841. (e) Kantorowski, E. J.; Kurth, M. J. *J. Org. Chem.* **1997**, *62*, 6797.

5. Hoffmann, H. M. R.; Rabe, J. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 795.

6. (a) Batra, S.; Rastogi, S. K.; Kundu, B.; Patra, A.; Bhaduri, A. P. *Tetrahedron Lett.* **2000**, *41*, 5971. (b) Patra, A.; Batra, S.; Kundu, B.; Joshi, B. S.; Roy, R.; Bhaduri, A. P. *Synthesis* **2001**, 276.

7. We presume that in activated aldehydes represented by structure A, the proton abstraction in the intermediate step is aided by the heteroatom present in the molecule.



8. General procedure for **5**: To 2-chlorotrityl resin (100 mg, 1.30 mmol/g, Novabiochem) in 1 mL DMF/DCM mix (50:50 v/v) was added 6 equivalents of triethylamine followed by 4 equiv of acrylic acid. The resulting mixture was shaken at 600 rpm for 4 h. Thereafter, 1 mL of methanol was added to the reaction and it was allowed to continue for another 30 min. Subsequently, the resin was washed with DMF (4 mL×6), MeOH (4 mL×6) and DCM (4 mL×3). To this resin was added 700 μL of DMSO followed by 3 equivalents of DABCO and reaction was shaken for 30 min. To this mixture was added a solution of 4 equivalents of 3-substituted-phenyl-5-isoxazolecarbox-aldehyde in 500 μL of DMSO and the reaction was allowed to proceed for 3 h. A wash sequence as given above was again carried out to wash the resin. Further, to this resin was added a solution of 10 equiv of amine and 2 equiv of DBU simultaneously and the reaction was mixed for 5 h. Then the resin was washed and cleaved using 20% TFA in DCM for 20 min. The excess solvent was evaporated and the residue was freeze-dried using 800 μL of *tert*-butanol/water (4:1, v/v). The yields of each well ranged between 51 and 89% while the purity was between 76 and 91% as determined by analytical HPLC. [R = 4-CH$_3$, R^1 = CH$_2$C$_6$H$_4$(p)Cl; FAB MS 406 (M$^+$); ^{1}H NMR of diastereomeric mixture (300 MHz, CDCl$_3$) δ 2.35 (s, 6H, 2×CH$_3$), 3.12 (m, 4H, 2×CH$_2$), 3.65 (m, 4H, 2×CH$_2$), 3.93 (m, 2H, 2×CH), 4.18 (m, 2H, 2×CH), 6.33 (brm, 4H, 2×NH and OH), 6.51 (brs, 2H, 2×=CH), 7.18 (m, 8H, Ar–H), 7.57 (m, 8H, Ar–H).

9. General procedure for **10**: To the Rink Amide AM resin (100 mg, 0.63 mmol/g, Novabiochem) was loaded appropriate amino acids using standard TBTU/DIPEA followed by Kaiser test. After washing of the resin and deprotection of Fmoc amino acid with piperidine, TMOF (1 mL) and a solution of 4 equiv of appropriate 5-isoxazolecarboxaldehyde in 500 μL TMOF was added and the reaction was allowed to be shaken at 600 rpm for 1.5 h. To this were added 10 equiv of sodium cyano-borohydride solution in 400 μL of TMOF followed by 10 μL of acetic acid and the reaction was continued for another 30 min. The resin was thoroughly washed as above. To this resin was then added a solution of 5 equiv of appropriate benzyl bromide in 1 mL of DMF followed by 2 equiv of DBU and the reaction was worked up after 5 h. The resin was cleaved using 95% TFA in DCM (with or without scavengers as required) for 3 h. The excess TFA mix was evaporated and the residue was freeze dried as described for 5. The yields of each well ranged between 49 and 92% while the purities of the sample were found to be of the order between 79 and 95%. [R = R$''$ = H, R$'$ = 4-CH$_3$; FAB MS 335 (M$^+$); ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.44 (s, 3H, Ar–CH$_3$), 3.24 (s, 2H, CH$_2$), 3.75 (s, 2H, CH$_2$), 3.87 (s, 2H, CH$_2$), 5.44 (brs, 1H, NH$_2$), 6.46 (s, 1H, =CH), 7.08 (brs, 1H, NH$_2$), 7.33 (m, 7H, Ar–H), 7.67, 7.70 (d, 2H, J = 9 Hz, Ar–H)].

10. Dejna, E.; Callioni, A.; Quintana, A. *Thrombosis Res.* **1979**, *15*, 191.