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Discovery of nonbenzamidine factor VIIa inhibitors using a biaryl acid scaffold

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Dedicated to the memory of Steven M. Seiler, Ph.D. Steve's untimely passing will not diminish the impact of his drug discovery research or the admiration of his fellow scientists

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ABSTRACT

In this Letter, we describe the synthesis of several nonamidine analogs of biaryl acid factor VIIa inhibitor **1** containing weakly basic or nonbasic P1 groups. 2-Aminoisoquinoline was found to be an excellent surrogate for the benzamidine group (compound **2**) wherein potent inhibition of factor VIIa is maintained relative to most other related serine proteases. In an unanticipated result, the *m*-benzamide P1 (compounds **21a** and **21b**) proved to be a viable benzamidine replacement, albeit with a 20–40 fold loss in potency against factor VIIa.

Thromboembolic cardiovascular disease remains a leading cause of hospitalization and mortality in developed countries.[1] Existing anticoagulant and antiplatelet agents are effective in treating thromboembolic disorders; however, associated bleeding side effects, narrow therapeutic indices, and limitations in route of administration continue to highlight the need for agents with an improved balance in efficacy, safety and convenience.[2] A current area of inquiry is the development of selective inhibitors of the tissue factor/factor VIIa complex (TF/FVIIa), a key initiator of coagulation, which may offer antithrombotic efficacy with preserved hemostasis.[3]

Under normal physiological conditions, membrane associated tissue factor (TF) is not exposed to blood flow, but becomes exposed appropriately upon vascular injury. However, exposure of TF can be significantly increased under pathological conditions, such as through the rupture of atherosclerotic plaque or in post-operative settings. Exposure of TF to blood leads to the formation of the catalytically active TF/FVIIa complex. TF/FVIIa then initiates coagulation by activation of factor X–Xa, both directly by proteolytic action on factor X and indirectly by converting factor IX–IXa which binds to surface bound VIIIa to form the tenase complex, which in turn activates factor X.[4]

Factor VIIa alone is a weakly active trypsin-like serine protease, and becomes fully competent in the TF/FVIIa complex. Like other trypsin-like serine proteases, factor VIIa has a conserved aspartic acid in the bottom of the S1 binding pocket.[5] At the time of our entry into the field, the bulk of reported small-molecule factor VIIa inhibitors were peptidic in origin and obtained potency through the use of strongly basic guanidine or amidine P1 groups, features that hamper oral bioavailability. A notable nonpeptidic exception is compound **1**, reported by Ono Pharmaceuticals.[6] As part of our interest in the development of selective factor VIIa inhibitors, we have focused extensively on identifying weakly basic or nonbasic P1 groups applicableacross multiple chemotypes. In this Letter, we report the synthesis of nonamidine analogs of **1**, leading to

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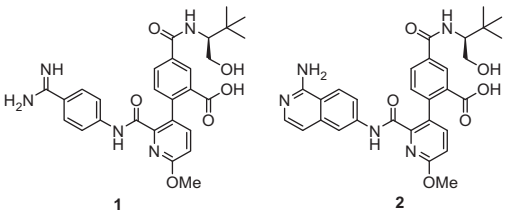
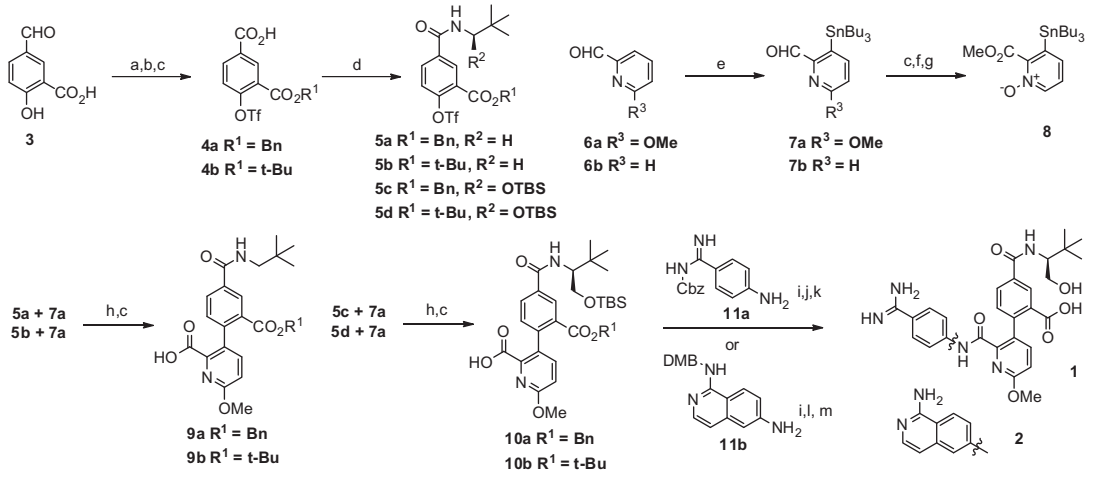


Figure 1. Structures of compounds **1** and **2**.

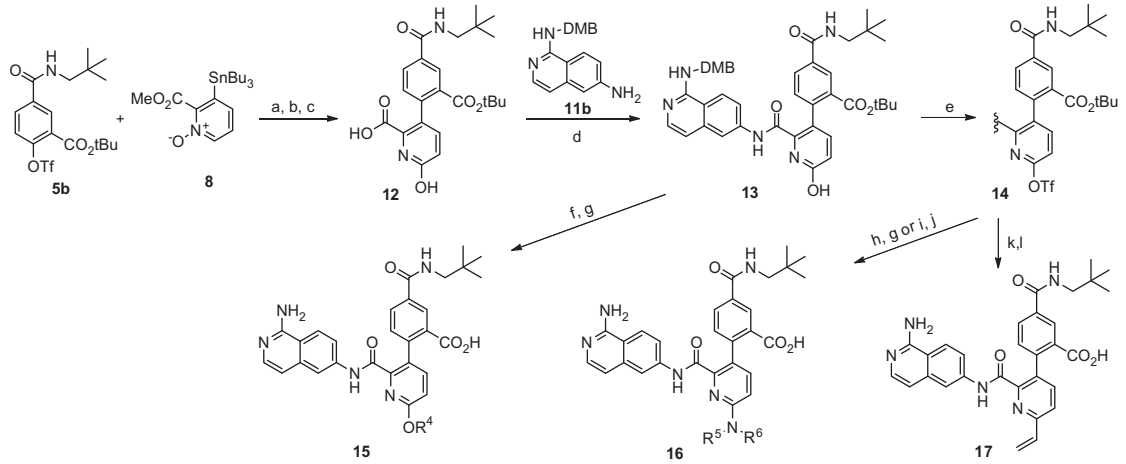
the identification of the potent factor VIIa inhibitor **2**, utilizing a novel 2-aminoisoquinoline P1 group (Fig. 1).

General methods for the preparation of 2-(pyridin-3-yl)benzoic acid analogs of compound **1**, are described in Scheme 1, following a previously described route.[7] Intermediates **4a** and **4b** were prepared in three steps starting with esterification of 5-formyl salicylic acid with benzylbromide or *t*-butanol. Treatment with Tf_2O followed by oxidation of the aldehyde with sodiumchlorite then provided intermediates **4a** and **4b**. EDC mediated coupling of the carboxylic acids to neopentylamine provided triflates **5a** and **5b**. DCC mediated coupling of the carboxylic acids to (*S*)-1-(*tert*-butyldimethylsilyloxy)-3,3-dimethylbutan-2-amine provided triflates **5c** and 5**d**. Tributyl stanannes **7a** and **7b** were prepared from picolinaldehydes **6a** and **6b** following methods described by Comins and Kelly.[8] Compound **7b** was then transformed to compound **8** in three steps beginning with conversion to the carboxylic acid by treatment with sodium chlorite. Esterification by treatment with MeI and Cs_2CO_3 followed by *m*CPBA oxidation provided the methyl *N*-oxo-3-stannyl picolinate **8**.

Biaryl intermediates **9a** and **9b** and **10a** and **10b** were prepared following general Stille coupling conditions where appropriately substituted triflates **5a–d** and stannane **7a** were treated with $PdCl_2(PPh_3)_2$ in the presence of CuO in DMF. Oxidation with sodium chlorite then provided the carboxylic acids **9a** and **9b** and **10a** and **10b**. Coupling of **10a** with the mono-Cbz protected 4-amidinoaniline **11a** using DCC and catalytic DMAP in DCM followed by removal of the TBS group with 3:1 HOAc/water and catalytic hydrogenation afforded compound**1**. In similar fashion,



Scheme 1. Preparation of derivatives **1** and **2**. Reagents and conditions: (a) BnBr, $NaHCO_3$, DMF, 78% or *t*-BuOH, DCC, DMAP, THF, 93%; (b) Tf_2O, pyridine, DCM, −10 °C, 83%; (c) $NaClO_2$, $NaH_2PO_4{\cdot}H_2O$, 2-methyl-2-butene, *t*BuOH, CH_3CN, H_2O, 87%; (d) neopentylamine, EDAC, HOAt, NMM, DCM, 92% or ((*S*)-1-(*tert*-butyldimethylsilyloxy)-3,3-dimethylbutan-2-amine), DCC, HOBt, DMAP, Et_2O, 92%; (e) *N*,*N*,*N*′-trimethylethylenediamine, *n*BuLi (2.5 M in hexanes), tributyltin chloride, THF, 53%; (f) MeI, Cs_2CO_3, DMF, 72%; (g) mCPBA, DCM, 95%; (h) $PdCl_2(PPh_3)_2$, CuO, DMF, 110 °C, 68–74%; (i) DCC, HOBt, DMAP, DMF, 53–85%; (j) AcOH–H_2O (3:1), 70%; (k) H_2, Pd/C, 1 N HCl, MeOH, 90%; (l) TFA, anisole; (m) 1 N NaOH, 1,4-dioxane, 50% for two steps.



Scheme 2. Preparation of generic analogs **15** and **16** and compound **17**. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuO, DMF, 110 °C, 74–82%; (b) $(CF_3CO)_2O$, DMF, 95%; (c) 1 N NaOH, THF, H_2O, 86%; (d) EDAC, HOAt, DMAP, DMF, 69%; (e) *N*-phenyltrifluoromethanesulfonimide, TEA, DCM, 81%; (f) R^4-OH, DIAD, PPh_3, THF; (g) TFA, DCM, 59–88% for two steps; (h) $R^5(R^6)NH$, DMSO, 100 °C; (i) $Bz(R^6)NH$, DMSO, 100 °C; (j) conc H_2SO_4, 28–34% for two steps; (k) tributyl(vinyl)tin, $PdCl_2(PPh_3)_2$, CuO, DMF, 110 °C, 77%; (l) TFA, anisole, 78%.

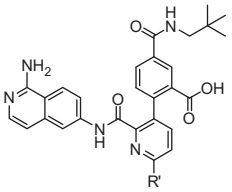
compound **2** was prepared from **10a** by coupling with the 2,4-dimethoxybenzyl protected aminoisoquinoline **11b**[9] and subsequent deprotection with TFA/anisole followed by ester hydrolysis using 1 N NaOH. Using similar chemistry as described above, intermediates **9a** and 9**b** were used to prepare compounds **18** and **19**. Compounds **20a** and **20b** and **21a** and **21b** were prepared by coupling of Boc protected 2-aminobenzimidazole or 3-aminobenzamide to the appropriate biaryl acid followed by deprotection.

Intermediate **12**, Scheme 2, was preparedby coupling triflate **5b** and stannane **8** by treatment with $PdCl_2(PPh_3)_2$ in the presence of CuO in DMF. Treatment with trifluoroacetic anhydride to rearrange the *N*-oxide to the pyridone, followed by hydrolysis with sodium hydroxide provided the pyridone carboxylic acid **12**. Coupling to the protected aminoisoquinoline **11b** provided compound **13**. Ether formation following standard Mitsunobu protocols and deprotection provided compounds **22**–**29** (Table 2). Conversion of **13** to triflate **14** provided access to compounds **30**–**32** (Table 2) through displacement with primary or secondaryamines followed by global deprotection with TFA or H_2SO_4. Compound **17** was obtained from triflate **14** by palladium coupling with tributyl(vinyl)stannane and subsequent deprotection.

Discussion: Following the general methods of Scheme 1, a number of compounds having various weakly basic and neutral P1 groups, such as benzamides, phenyl sulfones, sulfonamides, anilines, phenols, and heterocycles were synthesized and screened for activity against human factor VIIa and a panel of related serine proteases: factor IXa, factor Xa, factor XIa, thrombin, trypsin, urokinase (uPA) and tissue plasminogen activator (tPA).

Table 1 shows data for compounds containing P1 groups with factor VIIaIC_{50}'s below 0.5 μM. The results demonstrate that, in the context of the 2-(pyridin-3-yl) benzoic acid scaffold, 2-aminoisoquinoline, 2-aminobenzimidazole, and *m*-benzamide can replace benzamidine while retaining significant inhibition of factor VIIa. The 2-aminoisoquinoline compounds **2** and **19** are equipotent to **1** and **18**, and likewise show good selectivity against related serine proteases, with the exceptions of factor IXa and factor Xa where selectivity is only modest. By comparison, the 2-aminobenzimidazole compounds **20a** and **20b** are 12- and 15-fold less potent against factor VIIa than **1** and **18**, respectively. Selectivity is generally observed with the notable exception of factor IXa, where compounds **20a** and **20b** were found to be moderately morepotent than factor VIIa. Interestingly, the neutral *m*-benzamide P1 (compounds **21a** and **21b**) proved to be a viable benzamidine replacement, albeit

Table 2
Factor VIIa inhibition of compounds **17** and **22**–**32**[10]



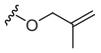
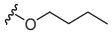
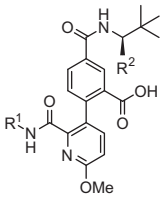
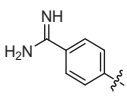
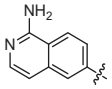
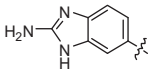
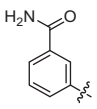
Compound	R^1	FVIIa IC_{50} (μM)
17		0.023
22		0.011
23		0.0125
24		0.111
25		0.034
26		0.113
27		0.437
28		0.357
29		0.499
30		0.101
31		0.051
32		0.009

Table 1
Factor VIIa inhibition for compounds with varied P1 groups[10]



Compd	R^1	R^2	FVIIa	IC_{50} (μM)						
				FIXa	FXa	FXIa	Thrombin	Trypsin	μPA	tPA
1		CH_2OH	0.005	0.044	0.035	3.9	24.0	1.6	5.9	>33
18		H	0.011	0.051	0.10	4.6	21.7	0.64	15.5	>33
2		CH_2OH	0.007	0.066	0.27	10.9	>33	7.9	>33	>33
19		H	0.013	0.180	1.7	7.2	>33	10.8	31.5	>33
20a		CH_2OH	0.060	0.013	>33	>33	>33	>33	>33	>33
20b		H	0.164	0.067	>33	20.8	>33	>33	>33	>33
21a		CH_2OH	0.175	20	>33	>33	>33	>33	>33	>33
21b		H	0.265	—	—	—	—	—	—	—

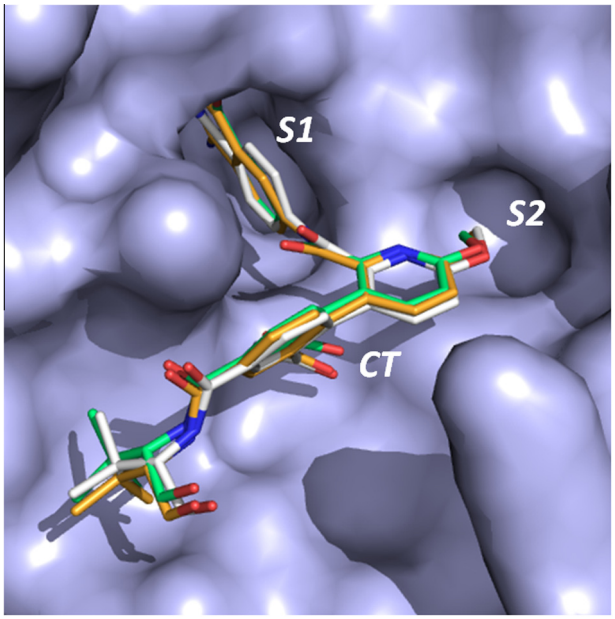


Figure 2. Overlay of **1** (white), **2** (green), and **21a** (orange) extracted from their crystallographic complexes with factor VIIa.[11] The surface was generated using the cognate protein structure bound to **1**. Graphics in Figures 2–4 were generated using the program PyMol.[15]

with a 20–40 fold loss in potency against factor VIIa, which is presumably due to loss of the salt bridge interaction of the basic P1 group with ASP189 (see the Modeling and structure below). Overall selectivity was improved in that all other serine proteases including factor IXa showed IC_{50} values greater than 33 μM. It should be noted that as part of the SAR development for this series, variation of the (*S*)-*tert*-leucinol amide group was evaluated, and several examples showed comparable potency for both the (*S*)-*tert*-leucinol and related neopentyl amides (data not shown).

We then decided to explore the S2 pocket of factor VIIausing the potent and selective compound **19**, which contains the 2-amino-

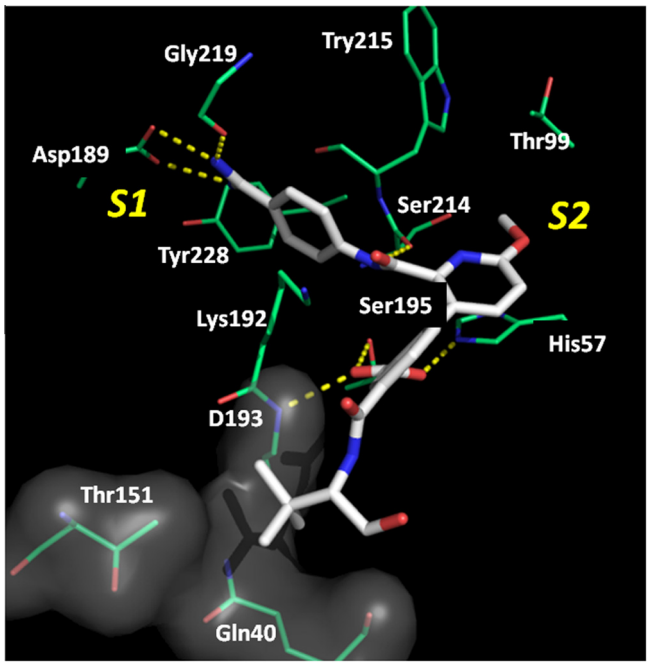


Figure 3. Crystallographic structure of **1** bound to factor VIIa with key interactions noted.

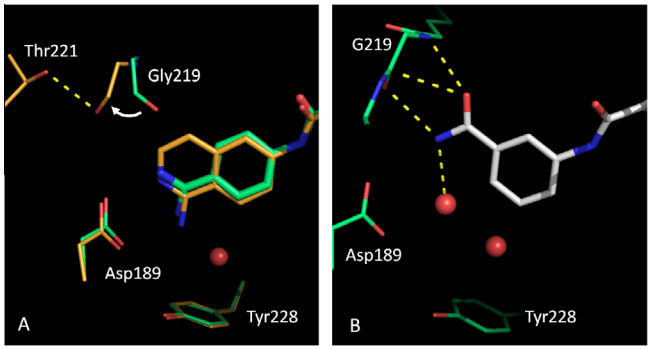


Figure 4. (A) Overlay of compounds **1** and **2** depicting key interactions in the S1 pocket. (B) Key interactions of the benzamide of **21a** in the S1 pocket of factor VIIa.

isoquinoline P1. A number of analogues were prepared as outlined in Scheme 2 in which the methoxy group in **19** was replaced by small ether groups, alkyl amine groups or vinyl (Table 2). The body of data from Table 2 shows that small groups are well tolerated, but as group size increases, factor VIIa inhibition decreases. This suggests the region of the factor VIIa binding pocket occupied by the methoxy group of **19** is sterically limited, which is consistent with the crystallographic structures described below. Similarly, it was also found that increasing the size of groups in the S2 pocket leads to improved selectivity relative to factor IXa. For example, compounds **19**,**22** and **24** containing methoxy, ethoxy and isopropoxy R2 groups have factor IXa IC_{50} values of 0.18, 1.80 and 6.70 μM, respectively (data not shown) compared to 0.051 μM for compound **18**. No significant change in inhibition was seen versus other related serine proteases as group size increased.

Modeling and structure: An overlay of crystallographic structures of compounds **1**, **2** and **21a** (Fig. 2) indicate that all three analogues are oriented similarly in the factor VIIa binding site.[11,12] Furthermore, the binding mode of **1** in factor VIIa is indistinguishable from that reported earlier fortrypsin.[13] The methoxy pyridine moiety occupies the S2 pocket defined by His57, Thr99 and Try215 sidechains (Fig. 3). Hydrogen bonds are observed between the carboxylic acid and His57 and Ser195 side-chains, and Gly193 oxygen. The *tert*-butyl group is bound to a small hydrophobic recess defined by amino acids Thr151, Gln40 and Asp193 and located on the prime side of the catalytic triad. This pocket, rarely discussed in the literature, is also involved in binding Leu17 sidechain of BPTI.[14]

Although the phenyl rings of the benzamidine, 2-aminoisoquinoline and benzamide groups of **1**, **2**, and **21a**, respectively, are oriented similarly in the S1 pocket, the polarinteractions vary in interesting ways (Fig. 4). The amidine moiety of **1** forms a bidentate salt bridge with Asp189 sidechain and also hydrogen bonds with Gly219 oxygen and a water located above Tyr228. The 2-aminoisoquinoline analogue, deduced from crystal contacts to be protonated, engages in similar interactions except that chemical replacement of the polar NH with an aromatic CH results in a significant rotation of the Gly219 carbonyl such that it engages in a hydrogen bond with the side chain oxygen of Thr221 (Fig. 4A). Binding of the benzamide moiety to S1 differs significantly, in that a pair of hydrogen bonds are established with Gly219 (Fig. 4B). An additional hydrogen bond is formed with a second water molecule that is otherwise displaced by compounds**1** and **2**. Interestingly, the amide carbonyl forms a close contact (3.2 Å) with Gly219 carbonyl carbon at an angle of 110°, which is in range for the geometry of the carbonyl–carbonyl polar interaction previously described by Paulini et al.[16] Taken together, these observations suggest that efficient binding in the factor VIIa S1 pocket does not require a salt

bridge with Asp189, and that the development of high affinity neutral factor VIIa inhibitors are possible, such as have been discovered for other trypsin-like serine proteases.[17]

In summary, we have identified several weakly basic or nonbasic P1 groups that can replace the highly basic benzamidine moiety contained in **1**, while maintaining factor VIIa potency. Of particular interest was the discovery of the 2-aminoisoquinoline group as an equipotent surrogate for benzamidine, but like compound **1**, these compounds displayed substantial factor IXa and Xa inhibition. Substituting aminobenzimidazole for benzamidine led to compounds with less factor Xa activity but even more potent factor IXa activity. Use of a *m*-benzamide P1 group, although less potent, indicates that a salt bridge interaction with Asp189 is not required for activity and that together with further exploration of the S2 pocket of factor VIIa may enable the discovery of new analogs devoid of factor IXa activity with improved permeability and oral bioavailability.

Acknowledgment

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10. Factor VIIa determinations were made in 0.005 M calcium chloride, 0.15 M sodium chloride, 0.001 M CHAPS, and 0.02 M HEPES buffer containing 0.1% PEG 6000 at a pH of 7.4. Determinations were made using purified human factor VIIa, $n = 2$ separate experiments (Enzyme Research Labs) at a final assay concentration of 1 nM, innovin human tissue factor (Dade Behring), and D-Ile-Pro-Arg-AFC (Enzyme Systems Products) as substrate. Inhibitor concentrations of required for half maximal effect (IC_{50}) were determined by a four-parameter logistic fit. Activity against other serine proteases was determined as describedin: Schumacher, W.; Seiler, S.; Steinbacher, T.; Stewart, A.; Bostwick, J.; Hartl, K.; Liu, E.; Oletree, M. *Eur. J.Pharmacol.* **2007**, *570*, 167–174.
11. Structures were determined by soaking crystals of des-Gla factor VIIa in complex with benzamidine with each of the inhibitors **1**, **2**, and **21a**. Resolutions of the complexes were: 2.2 Å (**1**), 1.7 Å (**2**) and 2.0 Å (**21a**).
12. Co-ordinates of **1**, **2** and **21a** have been deposited in the Protein Data Bank under ID codes 4JZD, 4JZE, and 4JZF, respectively.
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