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Pyrrolidinyl pyridone and pyrazinone analogues as potent inhibitors of prolyl oligopeptidase (POP)

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ABSTRACT

We report the synthesis and in vitro activity of a series of novel pyrrolidinyl pyridones and pyrazinones as potent inhibitors of prolyl oligopeptidase (POP). Within this series, compound **39** was co-crystallized within the catalytic site of a human chimeric POP protein which provided a more detailed understanding of how these inhibitors interacted with the key residues within the catalytic pocket.

Prolyl oligopeptidase (POP: E.C. 3.4.21.26) is a serine protease first identified in 1971 in human uterus as an oxytocin-cleaving enzyme.[1] It has since been found to be widely expressed in various tissues with the highest concentrations found in the brain.[2] Three different types of POP have been described in mammals: cytoplasmic,[2c,e,3] serum,[4] and membrane.[5] Although POP has been thoroughly characterized enzymatically and structurally its exact physiological role still remains obscure. However, due to its structure, which includes a β-propeller domain, POP catalysis is controlled by a gating filter mechanism which only allows smaller proteins to gain access to the catalytic site (30 amino acids or less).[6] POP has been shown to cleave internal proline containing peptides at the C-terminus.[7] A number of bioactive peptides which contain an internal proline have been identified as potential substrates for POP, although many of these have not been validated in vivo. Interestingly, many of these are neuropeptides, such as substance P, thyrotropin releasing hormone, arginine vasopressin and oxytocin.[8] It is not surprising then that studies have linked POP to various neurodegenerative disorders like bipolar disorder, amnesia, and Alzheimer's and Parkinson's disease.[9] It has also been shown to be involved in inositol-1,4,5-triphosphate signaling.[8] Not surprisingly this has led, since the late 1970s, to the search for specific potent inhibitors of POP. Indeed, many small molecule POP inhibitors have now been reported,[10] for example, *Z*-pro-prolinal,[11] S-17092[12] and JTP-4819[13] (Fig. 1).

We report herein our efforts to identify novel POP inhibitors. The goal was to not only identify potent novel inhibitors, but attempt to remove as much of the peptidic nature as possible from these inhibitors. Many of the previously reported POP inhibitors

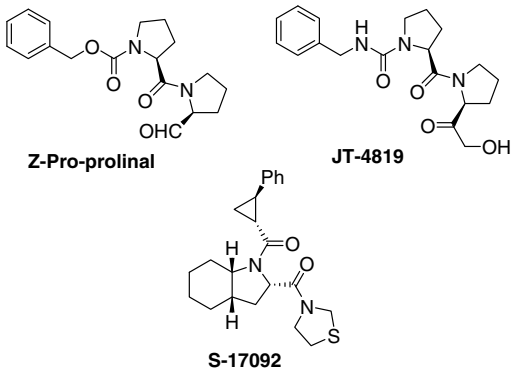

Figure 1. Examples of known POP inhibitors.

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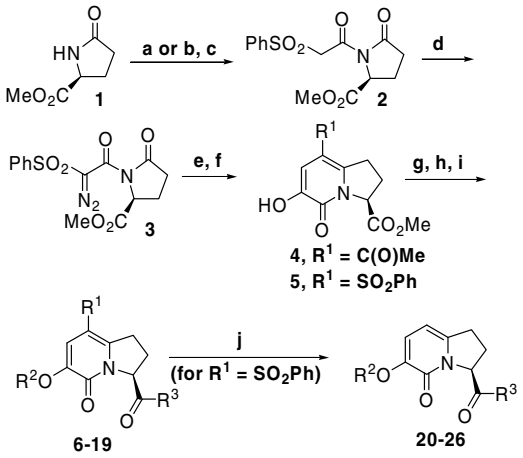
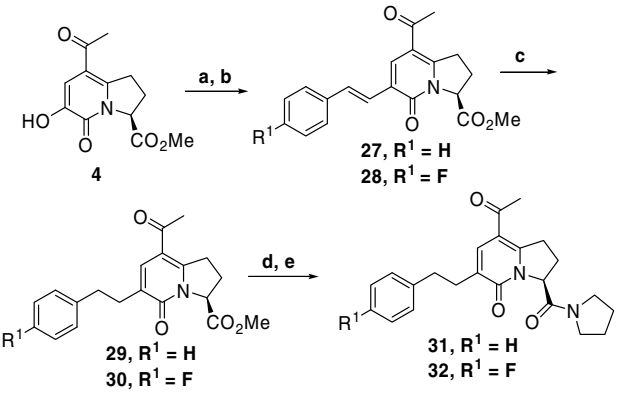


Scheme 1. Reagents and conditions: (a) (phenylsulfonyl)acetyl chloride, benzene, reflux; (b) (phenylthio)acetyl chloride, benzene, reflux; (c) oxone, MeOH/H_2O; (d) 4-acetamidobenzenesulfonyl azide, NEt_3, CH_3CN, 0 °C to rt; (e) $Rh_2(OAc)_4$, methyl vinyl ketone or phenyl vinyl sulfone, benzene, reflux; (f) *p*-TsOH·H_2O, benzene, reflux; (g) R_2Br, K_2CO_3, DMF, 105 °C; (h) LiOH·H_2O, dioxane/water; (i) HATU, DIEA, DMF, pyrrolidine or pyrazole; (j) Raney Ni 2800, ethanol, reflux.

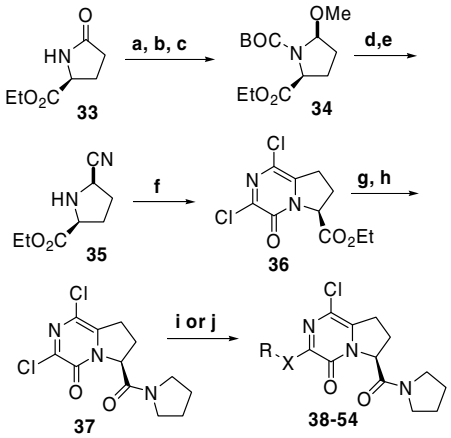
utilize an acyl-prolyl-pyrrolidine structure, although there are reports where P2 prolyl mimetics have been used successfully to identify potent inhibitors.[10e,g] Our initial synthetic efforts utilized a structure based design (SBD) approach using the published liganded porcine structure (1QFS) reported by Polgar et al.[6a] This entailed inspection of this X-ray crystal structure, utilization of reported SAR, and new chemotype generation followed by a docking calculation using the program MVP.[14] Results of the docking calculation were visually inspected, and ideas were docked iteratively until a satisfactory solution was generated. One of the chemotypes that was designed via this approach was the pyridone template shown in Scheme 1. It was felt that this chemotype would accomplish two things: (1) remove some of the peptidic nature of the molecule and (2) lock the P2-P3 portion of the molecule thereby rigidifying it and making it more drug-like.

The synthesis into these compounds utilized the prior work reported by Padwa et al.[15] Starting with the methyl ester of L-pyroglutamic acid **1**, acylation with (phenylthio)acetyl chloride followed by sulfide oxidation to the sulfone via oxone provided compound **2**. It was later found that the commercially available (phenylsulfonyl)acetic acid when converted to the acid chloride (benzene, oxalyl chloride) followed by acylation as before provided **2** in very good yield (85% vs 45%), thus eliminating the oxone oxidation step which proved to be somewhat capricious in our hands. The diazoimide was then synthesized via a diazo transfer reaction with 4-acetamidobenzenesulfonyl azide under basic conditions yielding diazosulfone **3**. The dipolar cycloaddition was carried out as previously described[15] using $Rh_2(OAc)_4$ in refluxing benzene whereby the dipolarophile chosen was either methyl vinyl ketone or phenyl vinyl sulfone. Interestingly, it was found that after several hours of reflux, removal of the catalyst followed by the addition of a catalytic amount of pTsOH·H_2O and additional reflux provided more consistent yields of the desired pyridones **4** and **5**. The 3-hydroxypyridones were then alkylated (K_2CO_3, DMF, 105 °C) followed by ester hydrolysis and amide formation (HATU, DMF, iPr_2NEt) providing carboxamides **6–19**. The phenylsulfone moiety could then be removed under Raney nickel conditions generating the C-5 hydridopyridones **20**–**26**. In order to gain access to the C-3 carbon linked analogues, the 3-hydroxypyridone was triflated and then reacted under Suzuki conditions to provide the styrenyl products **27** and **28**. The alkene was then hydrogenated under an atmosphere of hydrogen yielding the phenethyl derivatives **29** and **30**. These were then converted to the pyrrolidinyl carboxamides **31** and **32**, employing the same conditions as described previously (Scheme 2).



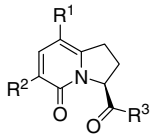
Scheme 2. Reagents and conditions: (a) Tf_2NPh, NEt_3, CH_2Cl_2, 0 °C to rt; (b) 2-arylvinylboronic acid, dppf·$PdCl_2$·CH_2Cl_2, 2 M aq Na_2CO_3, toluene, EtOH, 80 °C; (c) H_2 (1 atm), dioxane/EtOH; (d) LiOH·H_2O, dioxane/water; (e) pyrrolidine, HATU, DIEA, DMF.

Based on the modeling of the pyridone in the catalytic site, it appeared that insertion of nitrogen at the 4 position of the pyridone ring would not be deleterious in regard to POP inhibitory activity. In order to test this hypothesis the pyrazinone core was synthesized utilizing the known procedure.[16] The synthesis started with the ethyl ester of L-pyrroglutamic acid which was protected with di-*tert*-butyldicarbonate under basic conditions followed by reduction with $LiBEt_3H$ in THF and methanolysis in the presence of a catalytic amount of sulfuric acid to give BOC protected pyrrolidine **34**. Pyrrolidine **34** was then treated with trimethylsilyl cyanide and BF_3·Et_2O followed by exposure to TFA removing the BOC protecting group yielding cyanopyrrolidine **35**. The crude material was then condensed with oxalyl chloride in toluene at 85 °C providing 3,5-dichloropyrazinone **36**. The resulting pyrazinone was then hydrolyzed (LiOH·H_2O, dioxane, H_2O) followed by amide formation as described previously to generate pyrrolidinyl carboxamide **37**. Nucleophilic addition via alcohols or amines occurred exclusively at the C-3 position of the pyrazinone ring producing the desired 5-chloropyrazinones **38**–**54** (Scheme 3).



Scheme 3. Reagents and conditions: (a) Boc_2O, DMAP, CH_3CN, 0 °C to rt; (b) $LiEt_3BH$, THF, −78 °C; (c) H_2SO_4, MeOH; (d) Me_3SiCN, BF_3·Et_2O, CH_2Cl_2, −78 °C; (e) TFA, CH_2Cl_2; (f) $(COCl)_2$, toluene, 85 °C; (g) LiOH·H_2O, dioxane/water; (h) pyrrolidine, HATU, DIEA, DMF; (i) ROH, K_2CO_3, DMF (100 °C) or CH_3CN (reflux); (j) RNH_2, EtOAc, reflux.

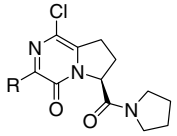
Table 1
Prolyl oligopeptidase inhibiton data for pyridones **6**–**26**, **31** and **32**



Compound	R^1	R^2	R^3	IC_{50}[a] (nM)	SE[b] (nM)
S-17092	—	—	—	2	0.3
6	C(O)Me	OCH_2CF_3	Pyrrolidine	584	100
7	C(O)Me	OCH_2-4-F-Ph	Pyrrolidine	53	10
8	C(O)Me	OCH_2-4-CF_3-Ph	Pyrrolidine	28	4.1
9	C(O)Me	OCH_2-4-OMe-Ph	Pyrrolidine	140	10
10	C(O)Me	OCH_2-4-Cl-Ph	Pyrrolidine	48	50
11	C(O)Me	OCH_2-4-CN-Ph	Pyrrolidine	783	200
12	C(O)Me	OCH_2-3,5-F-Ph	Pyrrolidine	22	4
13	C(O)Me	OCH_2-3,4-F-Ph	Pyrrolidine	7	3
14	C(O)Me	OCH_2-3-Cl-4-F-Ph	Pyrrolidine	8	3
15	C(O)Me	OCH_2-3,4-Cl-Ph	pyrrolidine	28	6
16	SO_2Ph	OCH_2-3,5-F-Ph	Pyrrolidine	4	0.5
17	SO_2Ph	OCH_2-4-CF_3-Ph	Pyrrolidine	11	2
18	SO_2Ph	OCH_2-4-^{t}Bu-Ph	Pyrrolidine	270	40
19	C(O)Me	OCH_2-4-F-Ph	Pyrazole	343	60
20	H	OCH_2-4-F-Ph	Pyrrolidine	22	6
21	H	OCH_2-3,5-F-Ph	Pyrrolidine	22	5.8
22	H	OCH_2-3,4-F-Ph	Pyrrolidine	18	2
23	H	OCH_2-2,5-F-Ph	Pyrrolidine	64	7
24	H	OCH_2-4-CF_3-Ph	Pyrrolidine	34	5
25	H	OCH_2-4-^{t}Bu-Ph	Pyrrolidine	60	10
26	H	OCH_2-2,4-F-Ph	Pyrrolidine	31	6
31	C(O)Me	$(CH_2)_2$Ph	Pyrrolidine	2	0.5
32	C(O)Me	$(CH_2)_2$-4-F-Ph	Pyrrolidine	3	1

[a] A brief description of the conditions used to determine the IC50's can be found in Ref. 17.
[b] Standard error.

Table 2
Prolyl oligopeptidase inhibition data for pyrazinones **37**–**54**



Compound	R	IC_{50} (nM)	SE[a] (nM)
37	Cl	3100	600
38	OPh	1000	300
39	OCH_2-4-F-Ph	40	0.6
40	OCH_2-3,4-Cl-Ph	34	5
41	OCH_2-3,4-F-Ph	55	4
42	OCH_2-3-Cl-4-F-Ph	43	3
43	OCH_2-2-naphthalene	4000	1000
44	$O(CH_2)_2$-3,4-Cl-Ph	2200	300
45	OCH_2-4-pyridyl	800	100
46	$O(CH_2)_2$-4-F-Ph	900	200
47	OCH_2-Cyclohexyl	70	20
48	OCH_2-4-CF_3-Ph	250	10
49	OCH_2-2,3,5-F-Ph	40	10
50	OCH_2-3-Cl-Ph	30	5
51	$NH(CH_2)_2$Ph	80	10
52	$NHCH_2$Ph	12	1
53	$NHCH_2$-4-F-Ph	17	2
54	$NHCH_2$-3,4-F-Ph	15	3

[a] Standard error.

The data shown in Table 1 compare the in vitro activity of the pyridone analogues. Many of the compounds showed low nanomolar potency against POP. The substituent at R^1 tolerated bulk as can be seen by comparing compounds **12** and **16** versus **21**. In fact, compound **16** which has the greatest bulk was 5-fold more active than compound **21**. We also found that replacing C for O at R^2 increased potency by 18-fold (compound **7** vs compound **32**). Furthermore, replacing the pyrrolidine ring in compound **7** with a pyrrazole in compound **19** lowered activity by 6.5-fold. It does appear however that there are steric and electronic limitations within the aryl groups substituted off the R^2 position. Compounds **9** and **11** which contain either a 4-methoxy or 4-cyano moiety, respectively, are less active than those analogues containing less polar substituents such as the fluoro or trifluoromethyl groups. It was also noted that compound **18** was 25-fold less active than compound **17** suggesting possible steric limitations in this part of the protein.

The data in Table 2 compare the in vitro activity of the pyrazinone analogues. As the R substituent increased in length the potency increased as well as can be seen by comparing compounds **37**, **38** and **39**. However, as was seen in the pyridone series, there were steric limitations as can be seen when comparing compounds **40** versus **44** and **39** versus **46** (65- and 23-fold difference, respectively, in potency). The nitrogen linked analogues were also synthesized and tested (compounds **51–54**). All were quite potent, but similar to that observed for the oxygen analogues, a drop in potency was seen in the phenethyl derivative **51**. There did appear to be a small increase in potency when going from the O-linked to N-linked compounds (**54** vs **41** and **53** vs **39**).

We were also able to solve a single X-ray crystal structure of compound **39** bound to a human chimeric protein (Fig. 2). As can be seen from the structure, the pyrrolidine ring fits into the small hydrophobic S1 pocket presumably occupying the same space as proline containing endogenous substrates. There also appear to be hydrogen bond interactions between the carboxamide carbonyl and Arg-643, and the pyrazinone carbonyl and Trp-595. The structure also shows the S3 portion of the protein to be quite hydrophobic, with the 4-fluorophenyl substituent appearing to fit nicely into this hydrophobic pocket. The C-5 chloro substituent occupies the large cavity extending away from the catalytic site. This presumably is the same region occupied by both the methyl ketone and phenyl sulfone from the pyridone series, perhaps explaining why they were equally tolerated versus their hydrogen counterparts. This also could be an area which lends itself to further modifications in the inhibitor to improve its overall drug properties as needed.

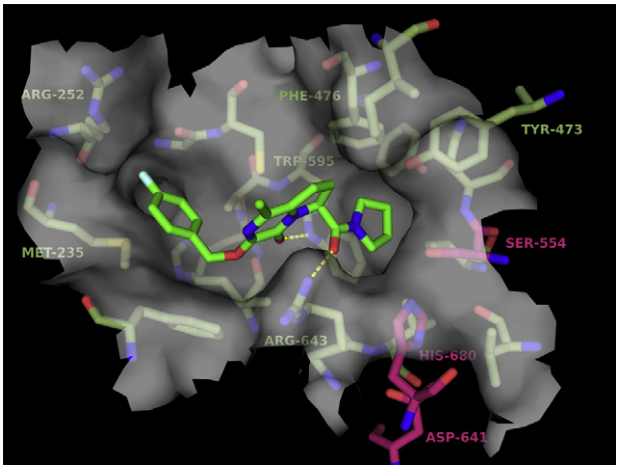


Figure 2. X-ray co-crystal structure of pyrazinone **39** (carbons highlighted in darker green) bound at the catalytic site of the human POP protein. POP carbons are colored in lighter green with the catalytic triad residues highlighted in magenta. The semi-transparent white surface represents the molecular surface while hydrogen bonds are depicted as yellow dashed lines. The coordinates have been deposited in the Brookhaven Protein Data Bank, accession number 3DDU. This figure was generated using PyMOL version 1.02 (Delano Scientific, www.pymol.org).

In conclusion, we have utilized a SBD approach to identify novel pyrrolidinyl pyridone and pyrazinone analogues, many of which were highly potent inhibitors, of POP. We were also able to solve a liganded crystal structure with compound **39** bound into the catalytic site. This structure has assisted in the understanding of how these compounds interact with the protein. Undoubtedly, this structure will provide valuable insight into further inhibitor design. We also found that the in vivo PK properties of some of these inhibitors provided higher plasma and brain levels than S-17092 (data not reported) which may provide better tool molecules for further validation work.

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17. Recombinant human POP was expressed in *E. coli.* The purified enzyme was assayed in 15 mM potassium phosphate, pH 7.1, with 25 μM *Z*-Gly-Pro-pNA (substrate). Cleavage of the peptide by POP was observed by the increase in absorbance at 385 nm. Compound (1 μL in DMSO) was incubated for 15 min in 50 μL assay buffer plus enzyme prior to initiation of the assay with 50 μL substrate in assay buffer. The equation $Y = V_{max} * (1 - (x^n/(IC_{50}^n)) + Y2$ was fit to the rate data from the concentration response curves where Y2 is the background (no enzyme) rate, V_{max} is the uninhibited rate—Y2, and IC_{50} is the concentration of compound at which the initial rate is $Y2 + (V_{max}/2)$.