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Organocatalytic Multicomponent Reaction for the Acquisition of a Selective Inhibitor of mPTPB, a Virulence Factor of Tuberculosis

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Abstract

Mycobacterium protein tyrosine phosphatase B (mPTPB) is essential for the survival and persistence of *Mycobacterium* in the host. Thus small molecule inhibitors of mPTPB are potential anti-TB agents. We developed an efficient organocatalytic multicomponent reaction (MCR) between pyrrole, formaldehyde and aniline, affording a potent and selective mPTPB inhibitor with IC_{50} at 1.5 μM and >50-fold specificity. Our studies provide a successful example of using organocatalysis as a discovery tool for the acquisition of PTP inhibitors.

Organocatalysis is a very useful tool for the preparation of various chiral and non chiral molecules, owing to the mild reaction conditions, low cost, and environmental consciousness.[1,2,3] A recent trend in organocatalysis is organocatalyzed multicomponent reaction (MCRs)[4] affording novel and complex molecules with multiple stereocenter controls, which is highly desirable in modern organic and medicinal chemistry.[5] Examples in this subject include three-component domino condensations,[6] Biginelli reactions,[7] and Mannich reactions[8] catalyzed by various organocatalysts to yield important novel amine building blocks and heterocycles. We are interested in applying these advanced synthetic strategies to the discovery of protein tyrosine phosphatase (PTP) inhibitors, which possess enormous potential therapeutic values for many human diseases.

Tuberculosis (TB) is a major worldwide threat to public health, with approximately 9 million new cases and 1.8 million deaths each year in the world.[9] No new anti-TB drugs have been developed in close to 40 years.[10] The inadequate efficacy, lengthy treatment, and multi-drug resistant TB underscore the urgency of developing new and more effective therapies.[11] mPTPB has emerged as a novel anti-TB target. It is secreted by *Mtb* into the cytoplasm of macrophages, where it mediates mycobacterial survival in the host and serves as a virulence factor.[12,13] Small molecules that inhibit mPTPB hence possess great potentials as novel anti-TB agents. Unfortunately, only a handful of mPTPB inhibitors have been reported,[14] and many of them lack the required potency and selectivity, due to the challenge in acquiring selective PTP inhibitory agents targeting the conserved active site.[15] Moreover, these molecules were acquired through multiple fragments appending procedures, which unavoidably introduce high molecular weight and lipophilicity, and thus are not appropriate as lead compounds.

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Pyrroles are favourable substrates in organic chemistry due to their high reactivity towards electrophilic aromatic substitutions and Diels-Alder reactions.[16] Pyrrole is also a privileged structure motif that exists in various biologically active molecules such as drugs and natural products. Compound **1** and several analogues have been reported to inhibit PTP1B at micromolar range (Figure 1).[17] Unfortunately, this class of compounds exhibited no selectivity against other PTPs, which is a common issue in the field due to the highly conserved active sites in over 100 PTP family members. In addition, compound **1** also exhibits poor stability. We envisaged that the poor stability is probably due to the high reactivity of the pyrrole ring, and that substitutions at the pyrrole reactive sites may mask its reactivity and hence increase its stability. More importantly, fragments added through the substitution reactions may not only enhance its binding affinity to PTPs, but also improve its specificity, as targeting both PTP active site and nearby peripheral site by two or more fragments is a proven strategy in acquiring potent and selective PTP inhibitors.[15,18] To these ends, we sought to develop a pyrrole Mannich type reaction that couples the pyrrole, an amine and an aldehyde or ketone, which should be very useful for preparing pyrrole-based libraries that are potential PTP inhibitors with improved potency and specificity.

To begin our study, we designed **2** (Table 1) as the parent pyrrole compound, which, after hydrolysis, afforded compound **3** with a salicylic acid group serving as a nonhydrolazble p-Tyr mimetic.[19] **3** is a moderately selective inhibitor against mPTPB with an IC_{50} at 2.9 μM.[20] Subsequently, MCR Mannich reaction between **2**, formaldehyde and aniline was studied as the model reaction to probe the optimal conditions prior to the library generation. The reaction was first carried out in a range of solvents using HOAc as a catalyst. CH_2Cl_2 stands out as the most optimal solvent in affording both mono- and di-alkylated products in a combined 74% conversion (entry 1, Table 1). In exploring for alternative acids as catalysts, we found that this reaction was very sensitive to the acidity of catalysts. For example, TFA catalyzed reaction provided a complex mixture with the complete consumption of pyrrole (entry 2, Table 1), weaker acids such as proline, PTSA and benzoic acid, and inorganic acid HCl afforded products in zero to low conversions (entry 3–6, Table 1). In contrast, methoxyacetic acid catalyzed reaction slightly more efficiently than acetic acid, but with low selectivity for **4a** (entry 7, Table 1). We also evaluated N,N-di[3,5-di(trifluoromethyl)phenyl]thiourea, a frequently used organocatalyst,[21] and it showed no capability to catalyze this reaction (entry 8, Table 1). Increasing acetic acid from 20 mol% to 100 mol% did not show much improvement in total conversion, however, the selectivity for product **4a** was increased by 1.7-fold (entry 1 vs entry 9, Table 1), and further increase of acetic acid in large excess resulted in a complex mixture with trace product. Finally, using 2 equiv. of pyrrole, 2 equiv. of HCHO, and 1 equiv. of aniline greatly improved the selectivity for **4a** with 85% isolated yield (entry 10, Table 1). And reaction using 1 equiv. of pyrrole, 3 equiv. of HCHO, and 3 equiv. of aniline in the presence of methoxyacetic acid after extended time afford **5** as the sole product in 75% isolated yield. Thus we were able to obtain mono and di-alkylated products by fine-tuning the catalysts and the relative ratios of reactants.

With reaction conditions towards either **4a** or **5** at hand, we proceeded to expand the substrate scope by treating pyrrole **2** with paraformaldehyde and various amines. Generally, reactions went well with aromatic amines, such as aniline derivatives and naphthyl amines, and products can be received in moderate to good yields. For aniline derivatives, substituents at *o*, *m*, and *p* positions and with either electron donating or withdrawing properties are tolerated (Figure 2). Unlike many similar reactions where substituents at 2 position was not feasible,[8] our studies indicate broader opportunity in choosing anilines for this reaction, and we attribute it to the small size of formaldehyde. Indeed, when we used larger aldehydes, i.e. acetaldehyde and benzaldehyde, the reaction did not proceed, even under elevated temperature, extended time, and with stronger acid catalysts. Aliphatic amines are usually not applicable in Mannich reaction,[8] due to the sluggish imine formation. Thus it is not surprising that propyl amine, benzyl amine, diethyl amine and piperidine did not afford any product under our reaction conditions. Nevertheless, our goals are not limited to develop a Mannich reaction, but more importantly, to use it as a tool to develop more potent, selective, and stable PTP inhibitors. We anticipated that, after hydrolysis, the N-salicylic acid moiety would occupy the active site, while the added aniline moiety would target an adjacent secondary site, with the methylene from formaldehyde serving as a linker.[18]

4a was converted to **6a** under hydrolysis conditions (Scheme S1, Supplementary Information), which was observed in good yield by LC-MS analysis. However, once the reaction mixture was acidified by 2M HCl or NH_4Cl prior to extraction, we were not able to observe the product, indicating that it decomposed rapidly even at weakly acidic conditions. The reason could be that the basic aniline nitrogen is easily protonated, and this internal proton donor at the right orientation could promote the degradation of pyrrole. To reduce the basicity of this nitrogen, we decided to protect it with a small group with electron withdrawing ability. Hence compound **4a** was reacted with acetic anhydride to give compound **7a**, and hydrolyzed to afford product **8a** in excellent yield (Scheme S1, supplementary information). As expected, compound **8a** indeed showed much improved stability.

Encouraged by the results, all of the Mannich adducts obtained in Figure 2 were protected by acetyl group, hydrolyzed and purified by reverse phase HPLC, to afford products in good yields and high purities. All hydrolyzed products display much improved stability compare to **3**, and most of them exhibit good inhibitory activity under the same assay conditions (Figure 3).[20] Although no profound influence on activity, substituents at the *meta* position are generally more beneficial than at *ortho* and *para* positions. Among them, **8f** is the most potent mPTPB inhibitor, with an IC_{50} at 1.5 μM. Meanwhile bis Mannich adduct **9** exhibited no activity at 100 μM (page 19, Supplementary Information), suggesting that the second aniline fragment may disrupt the binding to mPTPB. Additionally, we reasoned that the introduction of one or two halogen atoms into the pyrrole ring may be helpful in improving potency, specificity and stability. Though brominations using either Br_2 or NBS gave complex mixture, iodinations successfully afforded mono-iodinated **10a** and di-iodinated **10b**, by using I_2 and ICl, respectively. The hydrolyzed compounds **11a** and **11b** (Scheme S2, Supplementary Information) indeed exhibited improved stability over parent compound **3**, unfortunately they are several fold less potent than **8f** from our MCR Mannich reactions as mPTPB inhibitors.

Given the increased potency and stability, we proceeded to study compound **8f**'s specificity to mPTPB over selected members of the PTP super family, including mPTPA, PTP1B, SHP2, CD45, PTPα, and MKP5. As shown in Table 2, **8f** is highly selective for mPTPB, exhibiting a greater than 50-fold preference for mPTPB over all PTPs examined, marking compound **8f** as one of the most selective mPTPB inhibitors reported to date. In comparison, compound **3** has only 4 and 5-fold selectivity against mPTPA and PTP1B, respectively. These results show that PTP inhibitors generated from the MCR Mannich reaction not only exhibit increased stability, potency, but also greatly improved specificity.

In conclusion, we have successfully developed an efficient organocatalyzed MCR Mannich type reaction between pyrrole, paraformaldehyde, and anilines. By fine-tuning the catalysts and reactants ratios, we were able to obtain mono- and di-alkylated products in good yields. More importantly, these reactions enabled us to identify PTP inhibitors, which have increased stability, potency, and selectivity. In particular, compound **8f** has an IC_{50}value at 1.5 μM against mPTPB, with >50-fold selectivity over a large panel of PTPs. The low molecular weight and compact structure render **8f** a good lead molecule for anti-TB drug discovery targeting mPTPB. Our studies provide a successful example in using organocatalysis as a tool to discover enzyme inhibitors. Given that a vast array of biologically active molecules containing pyrrole, furan, and indole moieties can serve as substrates in organocatalytic reactions, this study should have a broader impact on enzyme inhibitor discovery beyond the PTP target class.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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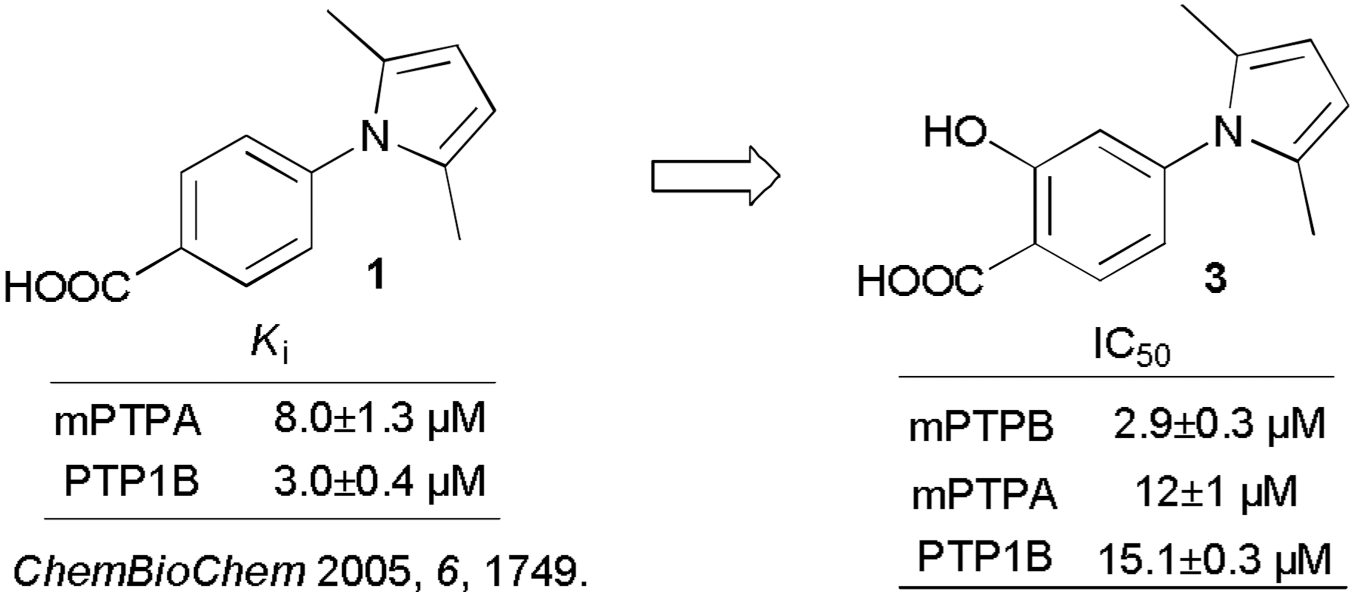
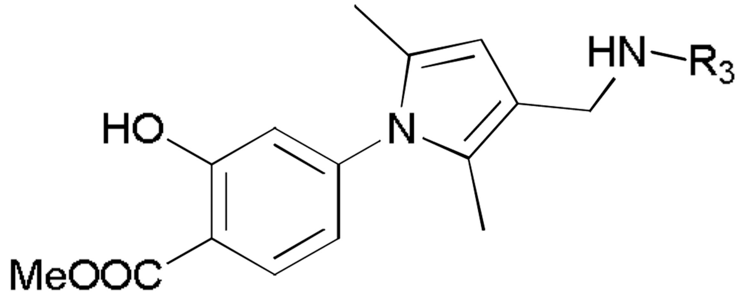
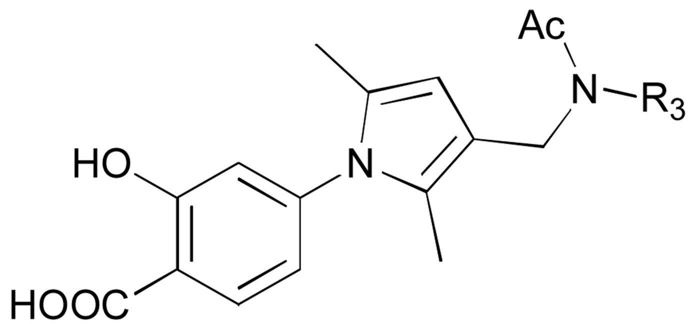


Fig. 1.
Structures and activities of N-Phenyl, 2,5-dimethyl pyrroles.



compound	R_3	yield	compound	R_3	yield
4a	Ph	85%	**4g**	3-OCF_3-Ph	78%
4b	4-Ph-Ph	70%	**4h**	4-*i*Pr-Ph	64%
4c	2-naphthyl	72%	**4i**	4-Cl-Ph	80%
4d	2-OMe-Ph	79%	**4j**	4-Br-Ph	83%
4e	3-F-Ph	65%	**4k**	3-Cl-4-Cl-Ph	81%
4f	3-CF_3-Ph	67%	**4l**	2-F-4-F-Ph	67%

Fig. 2.
Representative Mannich reaction products from **2**, formaldehyde and aromatic amines.



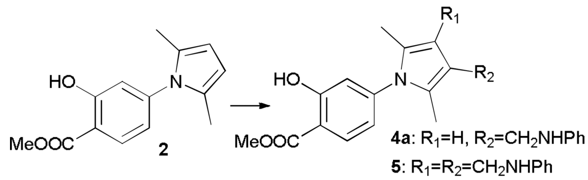
compound	R_3	IC_{50} (μM)	compound	R_3	IC_{50} (μM)
8a	Ph	10.8±0.7	**8h**	4-*i*Pr-Ph	8.5±0.3
8b	4-Ph-Ph	10.5±0.8	**8i**	4-Cl-Ph	2.6±0.3
8c	2-naphthyl	5.8±0.4	**8j**	4-Br-Ph	3.4±0.5
8d	2-OMe-Ph	14.2±0.2	**8k**	3-Cl-4-Cl-Ph	2.1±0.1
8e	3-F-Ph	3.3±0.4	**8l**	2-F-4-F-Ph	8.3±0.6
8f	$3\text{-}CF_3$-Ph	1.5±0.2	**11a**		4.4±0.1
8g	$3\text{-}OCF_3$-Ph	7.0±0.5	**11b**		6.6±0.4

Fig. 3.

Structure of hydrolyzed products from Mannich reactions and their inhibition against mPTPB.[20]

Table 1

MannichMCR between pyrrole, paraformaldehyde and aniline under various conditions[a]



Entry	Catalyst	Ratio of 4a/5/2 %[b]	Isolated yield %
1	HOAc (20 mol%)	52//22/26	
2	TFA (20 mol%)	NA	
3	Proline (20 mol%)	7/0/93	
4	PTSA (20 mol%)	21/5/74	
5	$PhCO_2H$(20 mol%)	28/13/59	
6	2 M HCl (20 mol%)	26/4/70	
7	$MeOCH_2CO_2H$ (20 mol%)	45/36/19	
8	(3,5-$(CF_3)_2$-PhNH$)_2$CS (20 mol%)	no reaction	
9	HOAc (100 mol%)	59//17/24	
10[c]	HOAc (100 mol%)	45/9/46	85 (**4a**)[e]
11[d]	$MeOCH_2CO_2H$ (100 mol%)	0/100/0	75 (**5**)

[a]the reaction was carried out at rt for 24 h in 1 mL CH_2Cl_2 with **2** (0.1mmol), paraformaldehyde (0.12mmol), aniline (0.12mmol);

[b]the ratio is based on UV absorption in LC-MS studies of crude reaction mixture.

[c]2 equiv. of pyrrole, 2 equiv. of paraformaldehyde, and 1 equiv. of aniline, were used.

[d]1 equiv. of pyrrole, 3 equiv. of formaldehyde, and 3 equiv. of aniline were used, reaction time was 48 h.

[e]based on 1 equiv. aniline

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Table 2

Specificity studies of compound **8f** against a panel of PTPs.[20]

Enzyme	IC_{50} (μM)
mPTPB	1.5±0.2
mPTPA	180±30
PTP1B	200±30
SHP2	86±7
CD45	78±7
PTPα	>> 100
MKP5	>> 100