Biocatalytic N-Halogenation Enabled by Vanadium-Dependent Haloperoxidases

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Abstract:

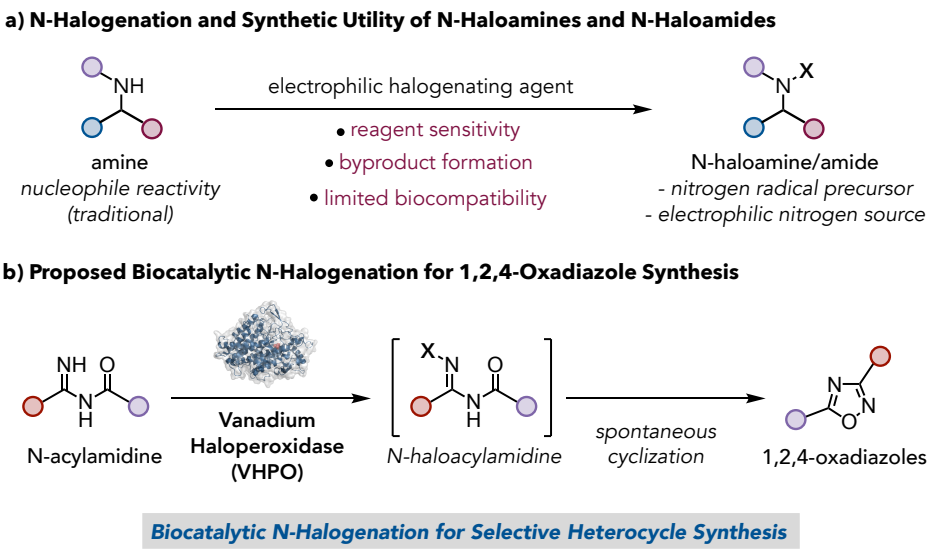
Nitrogen-containing compounds are valuable synthetic intermediates and targets in nearly every chemical industry. While methods for nitrogen-carbon and nitrogen-heteroatom bond formation have primarily relied on nucleophilic nitrogen atom reactivity, molecules containing nitrogen-halogen bonds allow for reactivity through electrophilic or radical mechanisms at the nitrogen center. Despite the growing synthetic utility of nitrogen-halogen-containing compounds, selective catalytic strategies for their synthesis are largely underexplored. We recently discovered that the vanadium-dependent haloperoxidase (VHPO) class of enzymes are a suitable biocatalyst platform for nitrogen-halogen bond formation. Herein, we show that VHPOs perform selective halogenation of a range of substituted benzamidine hydrochlorides to produce the corresponding N'-halobenzimidamides. This biocatalytic platform is applied to the synthesis of 1,2,4-oxadiazoles from the corresponding N-acylbenzamidines in high yield and with excellent chemoselectivity. Finally, the synthetic applicability of this biotechnology is demonstrated in an extension to nitrogen-nitrogen bond formation and the chemoenzymatic synthesis of the Duchenne muscular dystrophy treatment, ataluren.

Introduction:

Nitrogen-containing compounds are ubiquitous synthetic intermediates and targets in the pharmaceutical, agricultural, and materials industries.[1-3] Their synthesis is traditionally reliant on synthetic sequences that capitalize on a nucleophilic nitrogen atom in direct alkylation,[4] reductive amination,[5] or cross-coupling reactions.[6] In recent years, methods for changing the reactivity profile of the nitrogen atom have relied upon the synthesis of nitrogen-centered radical or electrophilic nitrogen precursors. One strategy for accessing these reactivities is through the

generation of a nitrogen-halogen (N-X) bond.[7] Despite the reported synthetic utility of N-X bond-containing intermediates in organic chemistry, the synthesis of these compounds relies upon the treatment of a nitrogen-containing precursor with electrophilic halogenating agents that are often air- and moisture-sensitive. Moreover, these reagents are bioincompatible, limiting the synthetic potential of using N-X bond-containing intermediates in chemoenzymatic synthesis (Scheme 1a).

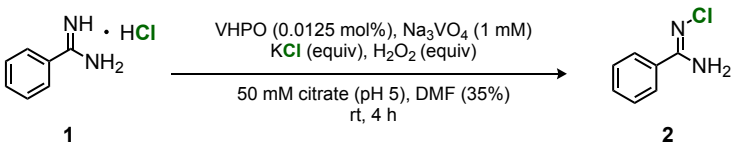
Enzymes are an attractive option for N-X bond formation because of their efficiency, selectivity, and sustainability parameters.[8] Despite these attractive features, nature is void of enzymes that have conclusively demonstrated N-X bond formation capabilities.[9,10] The discovery of enzymes that perform this type of reaction would not only provide a catalyst platform for the synthesis of N-halogenated compounds, but enable their use in unnatural biosynthesis. Among the host of halogenase enzymes found in nature,[11] the vanadium haloperoxidase (VHPO) class of enzymes have seen increased attention because of their robust operational parameters including their tolerance to high temperatures and a range of organic solvents.[12] We postulated that VHPOs could be used for N-halogenation-induced cyclization of N-acylamidines to generate 1,2,4-oxadiazoles – an important structural entity in a broad range of biologically active molecules (Scheme 1b).[13,14]



Scheme 1. (a) N-halogenation and synthetic utility of N-haloamines and N-haloamides. (b) Proposed biocatalytic N-halogenation for 1,2,4-oxadiazole synthesis.

Optimization and Results:

Our investigation began by screening a structurally diverse collection of VHPOs for the conversion of benzamidine hydrochloride (**1**) to N'-chlorobenzimidamide (**2**).[15] The biocatalysts investigated included the chloroperoxidase from *Curvularia inaequalis* (*Ci*VCPO)[16] and vanadium bromoperoxidases from *Corallina officinalis* (*Co*VBPO),[17] *Corallina pilulifera* (*Cp*VBPO),[18] and *Acaryochloris marina* (*Am*VBPO).[12b] Using a 0.0125 mol% enzyme loading, sodium orthovanadate (Na_3VO_4, 1 mM), and 1.0 equivalent each of potassium chloride (KCl) and hydrogen peroxide (H_2O_2) in citrate buffer (50 mM, pH = 5) and *N*,*N*-dimethylformamide (DMF, 35% v/v) as co-solvent, *Ci*VCPO performed the desired reaction in 93% yield in 4 hours (Figure 1, Entry 1). There was no detectable product formation for all bromoperoxidases interrogated (Figure 1, Entries 2-4). Control experiments were performed in sequence to confirm the necessity of each of the reaction components. These experiments confirmed the requirement of *Ci*VCPO, Na_3VO_4, and H_2O_2 in the reaction. Notably, when KCl was excluded, an 80% yield of **2** was produced (Figure 1, Entry 8). It was concluded that an equivalent of halide salt was being generated through neutralization of the hydrochloride salt of **1** in solution. Noting the lack of requirement for exogenous chloride ion in the reaction, it was removed from our standard reaction conditions for the remainder of the study. Upon simply increasing the H_2O_2 loading to 3.0 equivalents, the reaction performed in 93% yield without the addition of KCl (Figure 1, Entry 9). The yield was not affected with increased H_2O_2 loadings up to 8.0 equivalents (SI Figure S1).

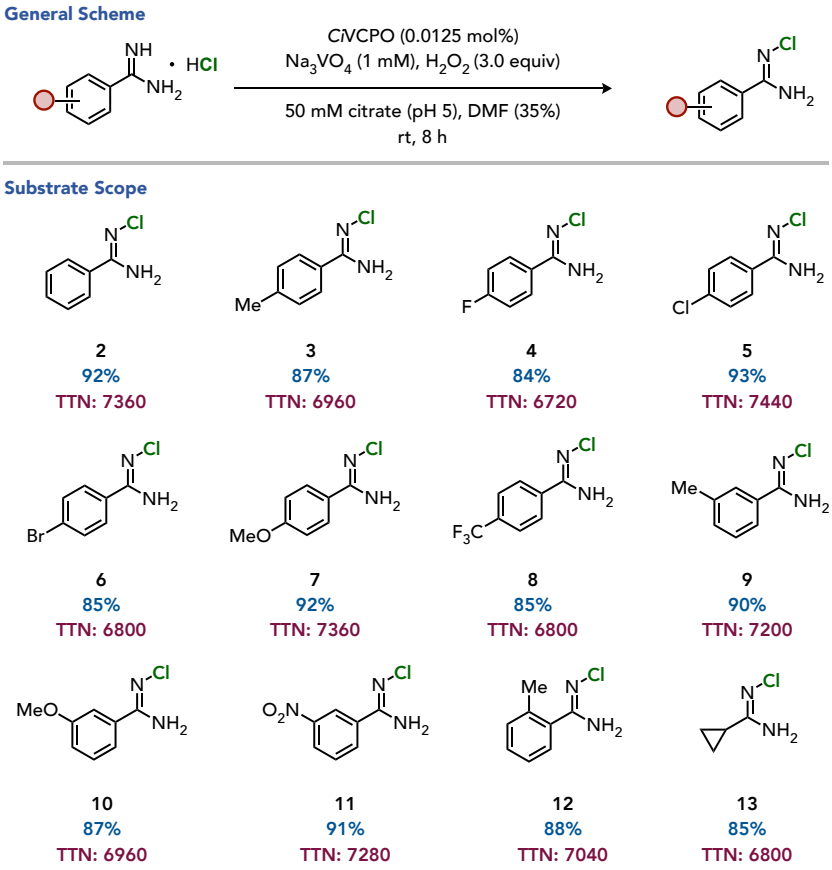


1 **2**

entry	enzyme and loading	KCl	H_2O_2	yield (%)
1	0.0125 mol% *Ci*VCPO	1.0 equiv	1.0 equiv	93
2	0.0125 mol% *Co*VBPO	1.0 equiv	1.0 equiv	0
3	0.0125 mol% *Cp*VBPO	1.0 equiv	1.0 equiv	0
4	0.0125 mol% *Am*VBPO	1.0 equiv	1.0 equiv	0
5	no enzyme (w/ Na_3VO_4)	1.0 equiv	1.0 equiv	0
6	0.0125 mol% *Ci*VCPO (no Na_3VO_4)	1.0 equiv	1.0 equiv	0
7	0.0125 mol% *Ci*VCPO	1.0 equiv	0.0 equiv	0
8	0.0125 mol% *Ci*VCPO	0.0 equiv	1.0 equiv	80
9	0.0125 mol% *Ci*VCPO	0.0 equiv	3.0 equiv	93

Figure 1. Optimization experiments for biocatalytic N-chlorination of benzamidine hydrochloride (**1**). Reaction conditions: **1** (4.0 µmol, 0.6 mg), VHPO (0.0125 mol%), Na_3VO_4 (1 mM), KCl (1.0 equiv), H_2O_2 (1.0–3.0 equiv), citrate buffer (50 mM, pH = 5, 100 µL), DMF (350 µL), 1 mL total reaction volume, 4 h, rt. Yields determined by HPLC based on a calibration curve. See the Supporting Information for details.

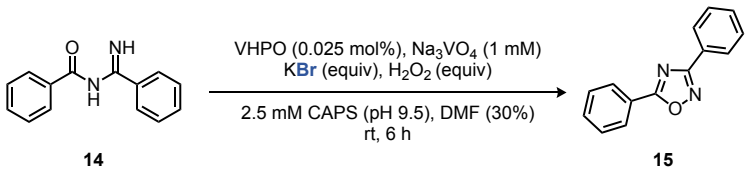
With the optimized conditions in hand, a diverse set of benzamidine hydrochlorides were investigated for their performance in the reaction. Benzamidine hydrochlorides bearing methyl-, fluoro-, chloro-, bromo-, methoxy- and trifluoromethyl substituents in the *para*-position performed in excellent yields 84-93% and with total turnover number (TTN) values of 6720-7440 (Scheme 2, **3-8**). Electron-donating methyl- and methoxy- groups and an electron-withdrawing nitro group in the *meta*-position were all tolerated in high yield from 87-91% and 6960-7280 TTN (Scheme 2, **9-11**). The reaction conditions also proved compatible with an *ortho*-methyl substituted benzamidine in 88% yield and 7040 TTN (Scheme 2, **12**). Finally, an aliphatic cyclopropyl substitution was accommodated in 85% yield and 6800 TTN (Scheme 2, **13**).



Scheme 2. Substrate scope for biocatalytic N-chlorination of benzamidine hydrochlorides. Standard reaction conditions: **substrate** (0.4 mmol), *Ci*VCPO (0.0125 mol%), Na_3VO_4 (1 mM), H_2O_2 (3.0 equiv), citrate buffer (50 mM, pH = 5), DMF (35% v/v), 8 h, rt. Yields determined by isolation. TTNs were determined by dividing the quantity of the resulting product by the concentration of the enzyme used. See the Supporting Information for a detailed procedure.

Upon completion of the substrate scope for VHPO-catalyzed N-chlorination of benzamidine hydrochlorides, we turned our attention to N-halogenation-induced cyclization of N-(imino(phenyl)methyl)benzamidine (**14**) to generate 3,5-diphenyl-1,2,4-oxadiazole (**15**). Encouraged by the effectiveness of *Ci*VCPO in the previously developed N-chlorination protocol, conversion of **14** to **15** was explored under the same conditions but resulted in no product formation. It was postulated that this class of substrates would require a higher pH medium for the desired reaction. To test this hypothesis, the same set of biocatalysts were interrogated in N-cyclohexyl-3-aminopropanesulfonic acid (CAPS) buffer (2.5 mM, pH = 9.5) and again, no activity was

observed. Screening the original set of biocatalysts under these conditions using potassium bromide (KBr) as the halide source enabled the transformation to occur across the entire collection of biocatalysts. Interestingly, *Ci*VCPO only catalyzed the desired cyclization reaction in 17% yield (Figure 2, Entry 1) and was outperformed by all bromoperoxidases used in the study (Figure 2, Entries 2-4). Among the bromoperoxidases tested, *Cp*VBPO emerged as the optimal catalyst for the transformation, providing the desired 1,2,4-oxadiazole (**15**) in 71% yield (Figure 2, Entry 3). The reaction performance was readily improved to 95% yield by doubling the KBr and H_2O_2 loading used to 2.0 and 4.0 equivalents, respectively (Figure 2, Entry 5). Control reactions were run to confirm the necessity of all the components in the reaction (Figure 2, Entries 6-9). Some other notable features of the reaction include tolerance of up to 5.0 equivalents of KBr (SI Figure S2) and successful operation in a broad range of co-solvents (SI Figure S3) with the best performance in 30% DMF (SI Figure S4). Finally, increasing the H_2O_2 loading past 4.0 equivalents lead to a notable decrease in yield (SI Figure S5).



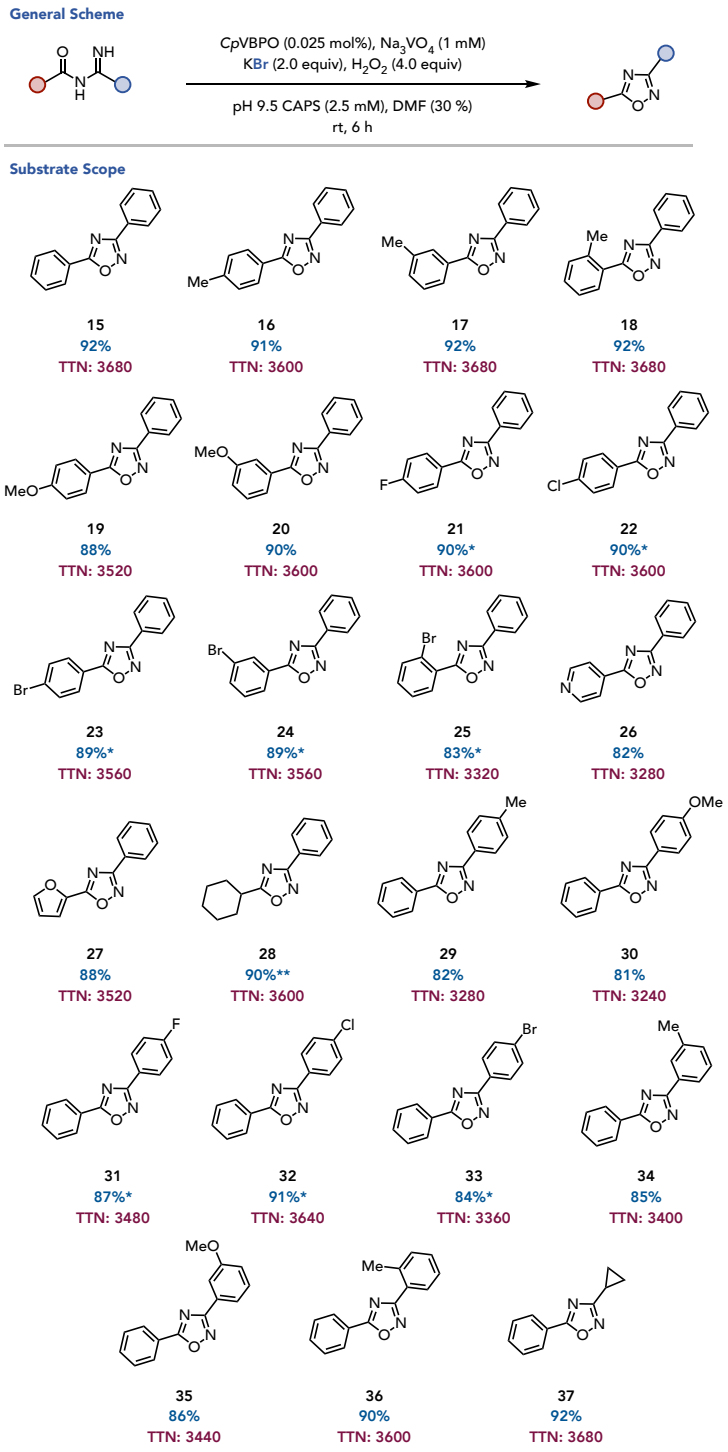
14 **15**

entry	enzyme and loading	KBr	H_2O_2	yield (%)
1	0.025 mol% *Ci*VCPO	1.0 equiv	2.0 equiv	17
2	0.025 mol% *Co*VBPO	1.0 equiv	2.0 equiv	63
3	0.025 mol% *Cp*VBPO	1.0 equiv	2.0 equiv	71
4	0.025 mol% *Am*VBPO	1.0 equiv	2.0 equiv	25
5	0.025 mol% *Cp*VBPO	2.0 equiv	4.0 equiv	95
6	no enzyme (w/ Na_3VO_4)	2.0 equiv	4.0 equiv	0
7	0.025 mol% *Cp*VBPO (no Na_3VO_4)	2.0 equiv	4.0 equiv	0
8	0.025 mol% *Cp*VBPO	0.0 equiv	4.0 equiv	0
9	0.025 mol% *Cp*VBPO	2.0 equiv	0.0 equiv	0

Figure 2. Optimization experiments for VHPO-catalyzed cyclization of N-(imino(phenyl)methyl)benzamidine (**14**). Reaction conditions: **14** (4.0 μmol, 0.9 mg), VHPO (0.025 mol%), Na_3VO_4 (1 mM), KBr (1.0–2.0 equiv), H_2O_2 (2.0–4.0 equiv), CAPS buffer (2.5 mM, pH = 9.5, 5 μL), DMF (300 μL), 1 mL total reaction volume. Yields determined by HPLC based on a calibration curve. See the Supporting Information for details.

Having identified the optimal conditions for biocatalytic N-halogenation-induced cyclization, the substrate scope of the reaction was explored starting with substituents on the N-aroyl ring of

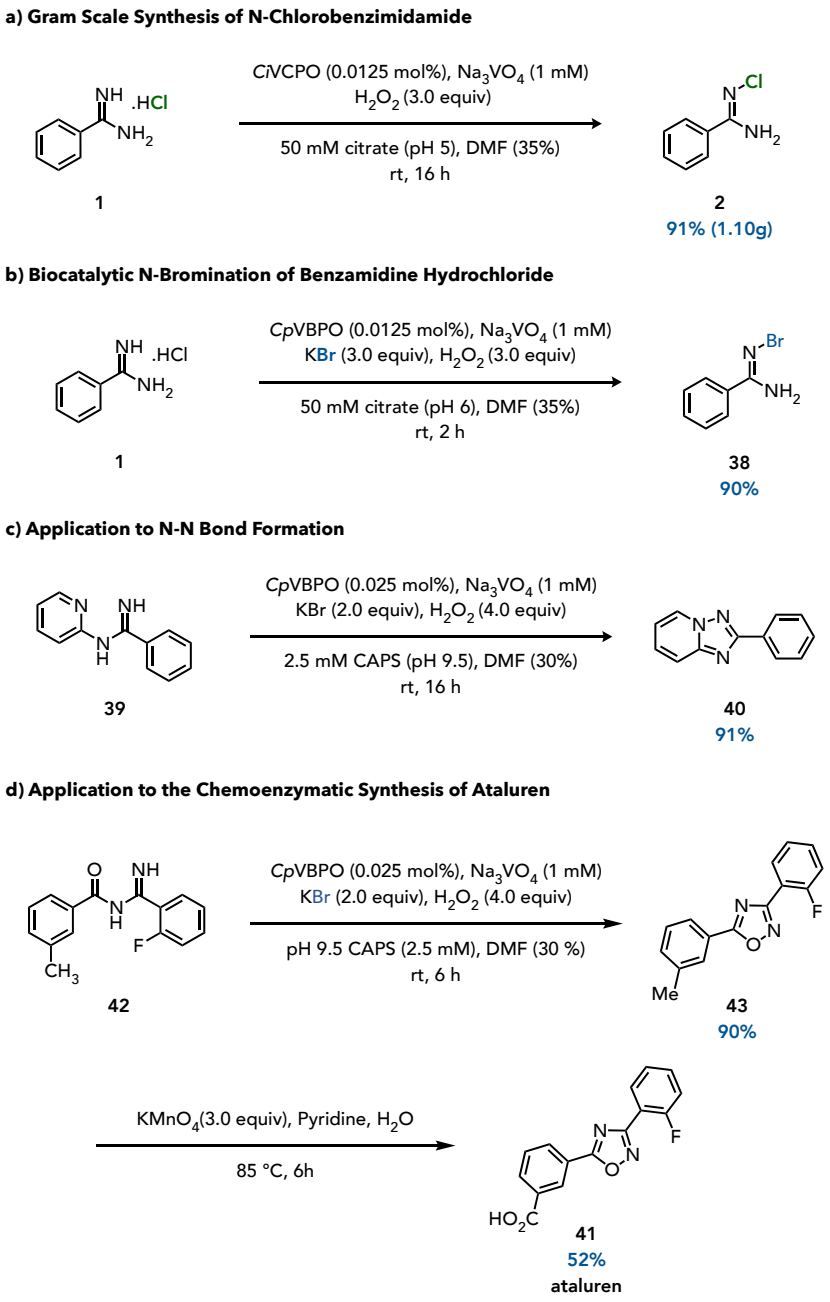
the starting N-acylamidine. Methyl substitution was well tolerated on the *ortho*-, *meta*-, and *para*-positions, providing the desired 1,2,4-oxadiazoles in 91-92% yield and with 3600-3680 TTN (Scheme 3, **16-18**). Electron-donating methoxy-substitution in the *para*- and *meta*-position was accommodated in 88% yield (3520 TTN) and 90% yield (3600 TTN), respectively (Scheme 3, **19-20**). A notable decrease in reaction rate was observed for N-acylamidines bearing a halogen substituent, but extension to a 10 h reaction time allowed for their production in 83-90% yield and 3320-3600 TTN (Scheme 3, **21-25**). The optimized conditions were compatible with pyridyl- and furanyl-substitution in 82% yield (3280 TTN) and 88% yield (3520 TTN), respectively (Scheme 3, **26-27**). Notably, when a cyclohexyl-containing substrate was used, hydrolysis of the starting N-acylamidine was observed. This challenge was effectively overcome by merely increasing the amount of co-solvent (DMF) to 50% (v/v), resulting in 90% yield (3600 TTN) of the desired product (Scheme 3, **28**). After exploration of the N-aroyl substitution was complete, attention was turned to probing the functional group tolerance on the benzamidine moiety. Substrates containing *para*-methyl- and methoxy- substituents provided the corresponding desired 1,2,4-oxadiazoles in 82% yield (3280 TTN) and 81% yield (3240 TTN), respectively (Scheme 3, **29-30**). Like previous halogen-bearing substrates used in the study, halogen atoms on the *para* position performed in appreciable yield and TTNs (84-91% yield, 3360-3640 TTN), but required longer run times for reaction completion (Scheme 3, **31-33**). Substrates with *meta*-substitution, including methyl- and methoxy- groups, were also tolerated in 85-86% yield and 3400-3440 TTN (Scheme 3, **34-35**). Finally, the reaction conditions were effective for a substrate with *ortho*-methyl substitution as well as an aliphatic cyclopropyl-substituted N-benzoyl amidine, producing the corresponding 1,2,4-oxadiazoles in 90-92% yield and 3600-3680 TTN (Scheme 3, **36-37**).



Scheme 3. Substrate scope for biocatalytic synthesis of 1,2,4-oxadiazoles. Standard reaction conditions: **substrate** (0.4 mmol), *Cp*VBPO (0.025 mol%), Na_3VO_4 (1 mM final concentration), KBr (2.0 equiv), H_2O_2 (4.0 equiv), CAPS buffer (2.5 mM, pH = 9.5), DMF (30%), 6h, rt. *reaction time = 10 h, **DMF (50%)

used. Yields determined by isolation. TTNs were determined by dividing the quantity of the resulting product by the concentration of the enzyme used. See the Supporting Information for more details.

Upon completion of reaction development, the synthetic utility of this biotechnology was explored. The N-chlorination protocol was readily scalable, producing over a gram of N'-chlorobenzimidamide (**2**) in a single run (Scheme 4a). Notably, the catalyst loading could be decreased to 0.0025 mol% without diminishing the yield (91%, 36400 TTN). It was also discovered that *Cp*VBPO was a viable catalyst for N-bromination of benzamidine hydrochloride (**1**) to furnish the corresponding N'-bromobenzimidamide (**38**)[19] in 90% yield (Scheme 4b). Structures for both representative N'-halobenzimidamides (**2** and **38**) were confirmed by X-ray crystallography (see SI for details). To highlight the synthetic versatility of the catalyst system, it was applied to an unnatural biocatalytic nitrogen-nitrogen (N-N) bond formation.[20] In this context, biocatalytic halogenation-induced cyclization of N-pyridylamidine **39** provided 2-phenyl-1,2,4-triazolo[1,5-a]pyridine **40** in 91% yield (Scheme 4c). This serves as a comparatively mild procedure in comparison to other halogenation-induced processes of this reaction type that typically require elevated temperatures.[21] Finally, the synthetic applicability of the protocol was further demonstrated in a chemoenzymatic synthesis of the Duchenne muscular dystrophy drug, ataluren (**41**). The optimized protocol for biocatalytic synthesis of 1,2,4-oxadiazoles was used to synthesize 5-(2-fluorophenyl)-3-*m*-tolyl-1,2,4-oxadiazole (**43**) in 90% yield. This was followed by benzylic oxidation with potassium permanganate ($KMnO_4$) to give ataluren (**41**) in a 52% yield (Scheme 4d).



Scheme 4. Synthetic utility of VHPO-catalyzed N-halogenation. (a) Gram-Scale Synthesis of N'-Chlorobenzimidamide. Reaction conditions: **1** (8.0 mmol) *Ci*VCPO (0.0025 mol%), Na_3VO_4 (1 mM), H_2O_2 (3.0 equiv), citrate buffer (50 mM, pH = 5), DMF (35%), 16 h. (b) Biocatalytic N-Bromination of Benzamidine Hydrochloride. Reaction conditions: **1** (0.8 mmol), *Cp*VBPO (0.0125 mol%), Na_3VO_4 (1 mM), KBr (3.0 equiv), H_2O_2 (3.0 equiv), citrate buffer (50 mM, pH = 6), DMF (35%), 2 h, rt. (c) Application to N-N Bond Formation. Reaction conditions: **39** (0.4 mmol), *Cp*VBPO (0.025 mol%), Na_3VO_4 (1 mM),

KBr (2.0 equiv), H_2O_2 (4.0 equiv), CAPS buffer (2.5 mM, pH = 9.5), DMF (30%), 16 h, rt. (d) Application to the Chemoenzymatic Synthesis of Ataluren. TTNs were determined by dividing the quantity of the resulting product by the concentration of the enzyme used. See the Supporting Information for more details.

Conclusion:

In conclusion, we have discovered that the VHPO class of enzymes are effective biocatalysts for N-X bond formation. This catalyst system is effective for performing N-halogenation-induced cyclization of N-acylamidines to produce 1,2,4-oxadiazoles across a broad range of substrates and its versatility has been demonstrated in N-N bond formation and a representative chemoenzymatic synthesis. These studies expand the synthetic repertoire of VHPOs in organic chemistry and provide a biocompatible platform for N-X bond-mediated chemoenzymatic synthesis.

Supporting Information:

The authors have cited additional references within the Supporting Information.[22-25]

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Keywords:

biocatalysis • halogenation • vanadium haloperoxidase

References:

[1] For recent reviews, *see*: a) N. Kerru, L. Gummidi, S. Maddila, K. K. Gangu, S. B. Jonnalagadda, *Molecules* **2020**, *25*, 1909-1951; b) M. M. Heravi, V. Zadsirjan, *RSC Adv.* **2020**, *10*, 44247-44311.

[2] For recent reviews, *see*: a) C. Lamberth, *Pest Manag. Sci.* **2013**, *69*, 1106-1114; b) S. Jeanmart, A. J. F. Edmunds, C. Lamberth, M. Pouliot, J. A. Morris, *Bioorg. Med. Chem. Lett.* **2021**, *39*, 116162; c) S. Jeanmart, A. J. F. Edmunds, C. Lamberth, M. Pouliot, J. A. Morris, *Synthesis* **2024**, *56*, 357-367.

[3] For recent reviews, *see*: a) J. O. Akindoyo, M. D. H. Beg, S. Ghazali, M. R. Islam, N. Jeyaratnam, A. R. Yuvaraj, *RSC Adv.* **2016**, *6*, 114453-114482. b) A. Nichugovskiy, G. C. Tron, M. Maslov, *Molecules* **2021**, *26*, 6579; c) X. Shi, X. Ye, H. Zhong, T. Wang, F. Jin, *Mol. Catal.* **2021**, *505*, 111517.

[4] For recent reviews, *see*: a) X. Dai, F. Shi, *Org. Biomol. Chem.* **2019**, *17*, 2044-2054; b) A. Trowbridge, S. M. Walton, M. J. Gaunt, *Chem. Rev.* **2020**, *120*, 2613-2692.

[5] For recent reviews, *see*: a) O. I. Afanasyev, E. Kuchuk, D. L. Usanov, D. Chusov, *Chem. Rev.* **2019**, *119*, 11857-11911; b) K. Murugesan, T. Senthamarai, V. G. Chandrashekhar, K. Natte, P. C. J. Kamer, M. Beller, R. V. Jagadeesh, *Chem. Soc. Rev.* **2020**, *49*, 6273-6328; c) T. Irrgang, R. Kempe, *Chem. Rev.* **2020**, *120*, 9583-9674.

[6] For recent reviews, *see*: a) P. Ruiz-Castillo, S. Buchwald, *Chem. Rev.* **2016**, *116*, 12564-12649; b) N. Diyali, S. Rasaily, B. Biswas, *Coord. Chem. Rev*. **2022**, *469*, 214667; c) Y. Zhong, H. Xiong, J. Low, R. Long, Y. Xiong, *eScience* **2023**, *3*, 100086.

[7] For recent reviews, *see*: a) L. G. O'Neil, J. F. Bower, *Angew. Chem. Int. Ed.* **2021**, *60*, 25640-25666; b) C. Pratley, S. Fenner, J. A. Murphy, *Chem. Rev.* **2022**, *122*, 8181-8260; c) K. Kwon, R. T. Simons, M. Nandakumar, J. L. Roizen, *Chem. Rev.* **2022**, *122*, 2353-2428.

[8] For recent reviews, *see*: a) E. L. Bell, W. Finnigan, S. P. France, A. P. Green, M. A. Hayes, L. J. Hepworth, S. L. Lovelock, H. Niikura, S. Osuna, E. Romero, K. S. Ryan, N. J. Turner, S. L. Flitsch, *Nat. Rev. Methods Primers* **2021**, *46*, 1-21; b) J. B. Pyser, S. Chakrabarty, E. O. Romero, A. R. H. Narayan, *ACS Cent. Sci.* **2021**, *7*, 1105-1116; c) R. Buller, S. Lutz, R. J. Kazlauskas, R. Snajdrova, J. C. Moore, U. T. Bornscheuer, *Science* **2023**, *382*, eadh8615.

[9] a) U. Hanefeld, F. Hollmann, C. E. Paul, *Chem. Soc. Rev.* **2022**, *51*, 594-627.

[10] For a biocatalytic reaction proposed to proceed through bromination at nitrogen, *see*: a) A. But, J. L. Nôtre, E. L. Scott, R. Wever, J. P. M. Sanders, *ChemSusChem* **2012**, *5*, 1199-1202; b) A. But, A. van Noord, F. Poletto, J. P. M. Sanders, M. C. R. Franssen, E. L. Scott, *Mol. Catal.* **2017**, *443*, 92-100.

[11] For select reviews, *see*: a) F. H. Vaillancourt, E. Yeh, D. A. Vosberg, S. Garneau-Tsodikova, C. T. Walsh, *Chem. Rev.* **2006**, *106*, 3364-3378; b) V. Agarwal, Z. D. Miles, J. M. Winter, A. S. Eustáquio, A. A. El Gamal, B. S. Moore, *Chem. Rev.* **2017**, *117*, 5619-5674; c) J. Latham, E. Brandenburger, S. A. Shepherd, B. R. K. Menon, J. Micklefield, *Chem. Rev.* **2018**, *118*, 232-269; d) E. Hegarty, J. Büchler, R. M. Buller, *Curr. Opin. Green Sust. Chem.* **2023**, *41*, 100784.

[12] a) E. de Boer, H. Plat, M. G. M. Tromp, R. Wever, M. C. R. Franssen, H. C. van der Plas, E. M. Meijer, H. E. Shoemaker, *Biotechnol. Bioeng.* **1987**, *30*, 607-610; b) A. Frank, C. J. Seel, M. Groll, T. Gulder, *ChemBioChem* **2016**, *17*, 2028-2032; c) R. Wever, B. E. Krenn, R. Renirie, *Methods Enzymol.* **2018**, *605*, 141-201.

[13] For halogenation-induced 1,2,4-oxadizaole synthesis from N-acylamidines, *see*: E. Li, M. Wang, Z. Wang, W. Yu, J. Chang, *Tetrahedron* **2018**, *74*, 4613-4618.

[14] For biological importance of 1,2,4-oxadiazoles, *see*: a) K. Biernacki, M. Daśko, O. Ciupak, K. Kubiński, J. Rachon, S. Demkowicz, *Pharmaceuticals* **2020**, *13*, 111; b) J.-J. Wang, W. Sun, W.-D. Jia, M. Bian, L.-J. Yu. *Enzym. Inhib. Med. Chem.* **2022**, *37*, 2304-2319; c) O.M. Hendawy, *Arch. Pharm.* **2022**, *355*, e2200045.

[15] a) R. A. Moss, J. Wlostowska, W. Guo, M. Fedorynski, J. P. Springer, J. M. Hirschfield, *J. Org. Chem.* **1981**, *46*, 5048-5050; b) T. Martinu, W. P. Dailey, *J. Org. Chem.* **2006**, *71*, 5012-5015.

[16] J. W. van Schijndel, E.G. Vollenbroek, R. Wever, *Biochim. Biophys. Acta.* **1993**, *1161*, 249-256.

[17] H. Yu, J. W. Whittaker, *Biochem. Biophys. Res. Commun.* **1989**, *160*, 87-92.

[18] N. Itoh, Y. Izumi, H. Yamada, *J. Biol. Chem.* **1986**, *261*, 5194-5200.

[19] S. M. Ahmad, D. C. Braddock, G. Cansell, S. A. Hermitage, J. M. Redmond, A. J. P. White, *Tetrahedron Lett.* **2007**, *48*, 5948-5952.

[20] For examples of N-N bond formation, *see*: a) L. M. Blair, J. Sperry, *J. Nat. Prod.* **2013**, *76*, 794-812; b) L. Chen, Z. Deng, C. Zhao, *ACS Chem. Biol.* **2021**, *16*, 559-570; c) H.-Y. He, H. Niikura, Y.-L. Du, K. S. Ryan, *Chem. Soc. Rev.* **2022**, *51*, 2991-3046; d) S. Schröder, A. Maier, S. Schmidt, C. Mügge, D. Tischler, *Mol. Catal.* **2024**, *553*, 113733.

[21] L. Song, X. Tian, Z. Lv, E. Li, J. Wu, Y. Liu, W. Yu, J. Chang, *J. Org. Chem.* **2015**, *80*, 7219-7225.