CONTROLLABLE SYNTHESIS OF N-ARYLHYDROXYLAMINES FROM NITROARENES BY HIGHLY CHEMO-SELECTIVE TWO-STEP TANDEM REDUCTION USING A NOVEL BACTERIAL NITROREDUCTASE

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ABSTRACT

N-Arylhydroxylamines serve as versatile intermediate organic molecules for the synthesis of industrially valuable fine chemicals, bioactive drugs, and polymerization inhibitors. Due to the active electron group of -NHOH in arylhydroxylamines, it is an extremely unstable and easily further reduced to corresponding amines. In addition, the synthesis of arylhydroxylamines by conventional chemical is usually performed using toxic heavy metals and chemical additives under high pressure and high temperature conditions. Thus, a novel eco-method is urgently needed. In this study, we developed a continuous flow method to synthesize arylhydroxylamines by a novel nitroreductase from Geodermatophilus obscurus, which shows high activity and chemo-selectivity in converting nitroarenes to N-Arylhydroxylamines (>99%) under mild condition. This method therefore provides a novel avenue to synthesis N-hydroxylamine.

1. INTRODUCTION

N-Arylhydroxylamines are an important class of compounds frequently used as key precursors for the synthesis of fine chemicals (Ahmad & Hughes, 2002; Ho & Lau, 2000; Lamar & Nicholas, 2009; Rios et al., 2006; Spence et al., 2003; Sridharan et al., 2006; Wang et al., 2011) and biologically active substances (Johansson et al., 2003; R. J. Knox, 1991; Smith et al., 1999; Svensson, 2003; Vyas et al., 2005). Therefore, the synthesis of arylhydroxylamines has been an attractive topic for synthetic chemists. Several methods have been developed for the preparation of arylhydroxylamines, including stoichiometric reduction (using zinc, tin or sulfide) (Bartra et al., 1990; Gassman & Grandrud, 1984; Haworth & Lapworth, 1925; Kamm, 1941; Liu et al., 2009; Marvel & Kamm, 1919), electrochemical reduction (H. A. Cyr, 1989; Seshadri & Kelber, 1999), catalytic hydrogen transfer (Beaudoin & Wuest, 2011; Davis, 1988; K.Taya, 1966; Takenaka, 2008; Takenaka et al., 2010; Takenaka et al., 2011**)** and selective catalytic hydrogenation of nitro compounds (Ayyangar et al., 1984; Entwistle et al., 1978; Karwa & Rajadhyaksha, 1987; Rondestvedt

et al., 1977). However, arylhydroxylamines, as intermediates formed during the reduction of nitroaromatics, are frequently further reduced to amines, meanwhile some side-reaction products such as azoxy-, azo-, and hydrazo compounds are often formed simultaneously (Corma et al., 2007; Makosch et al., 2012). Thus, it is challenging to obtain arylhydroxylamines in high chemo-selectivity by these chemical catalytic routes.

Recently, efforts have focused on the direct synthesis of arylhydroxylamines through selective catalytic hydrogenation of nitroarenes. (Rong et al., 2010; Takenaka et al., 2009) However, these processes are not environmentally benign due to using of toxic heavy metals and chemical additives. Therefore, the development of more efficient and greener methods for highly chemoselective production of arylhydroxylamines remains highly desirable. The selective bioreduction of nitro compounds may an ideal choice to produce arylhydroxylamines because biocatalysts inherently exhibit high chemoselectivity, and the reduction frequently occurs under mild reaction conditions with theoretical yield of 100%, making processes are environmentally benign (Bornscheuer et al., 2012). Early biocatalytic methods used baker's yeast (Li et al., 2004) and grape cells (Li et al., 2005) for preparing arylhydroxylamines. In the baker's yeast system, the amount of biocatalyst and reaction time must be precisely controlled; otherwise the formed hydroxylamines would be further converted to amines. In the grape cell system, due to the poor activity of the plant cells, the reduction has the shortcoming of low efficiency with long reaction time, using a large quantity of biocatalyst and low concentration of substrate.

Nitroreductase (NTR) is a famlily of reduction enzyme that could reduce the nitro-group in the nitroaromatic compounds through a ping-pong bi-bi kinetic mechanism (Roldan et al., 2008). NTR could be futher devided into two subclass: oxygen-insensitive (type 1) and oxygen-sensitive (type 2). Type 1 NTR could be further divided into major and minor protein groups. The major group could utilise both nicotinamide adenine dinucleotide (NAPH) or nicotinamide adenine dinucleotide phosphate (NADPH) as the electron donor while minor group can only utilise NADPH as the electron donor (Hollmann et al., 2011; Nyanhongo et al., 2005; Roldan et al., 2008; Symons & Bruce, 2006). The reduction of nitro-group by nitroreductase is a dynamic process in which many intermediates compounds could be produced, including nitroso, hydroxylamine and amine under mild condition (Thomas & Gwenin, 2021). Recently, many researcher groups tried to used NTR from bacteria to degrade nitroaromatic compounds, and could detect the N-Arylhydroxylamines in the mixture of reaction (Cenas et al., 2021). These findings imply the possibility of obstaining N- Arylhydroxylamines from nitroaromatic compounds by NTRs. In this report, by using bioinformatic tools, we identified NTR from Geodermatophilus obscurus exhibits oustanding reduction activity and chemo-selectivity for N-Arylhydroxylamines synthesis.

2. MATERIALS AND METHODS

Screening for potential NTRs

Two nitroreductases sequence from Bacillus licheniformis DSM 13 (yfkO and NfrA; SwissProt, Q65MG6 and Q65DM9, respectively) was inputted into the Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information (NCBI) as a probe. Candidate NTRs was selected between 75%-50% similarity citeria on the result of the pBLAST searching with these two probes.

Chemical and reagent

All nitroarenes were purchased from Aladdin Chemicals Co. Ltd. (Shanghai, China) with >97% purity. T4 DNA Ligase, Restriction endonuclease BamH I, EcoR I, Xho I, Nde I and Hind III were purchased from Takara Bio USA, Inc.

NTRs activity definition

Nitroreductase activity was determined in 1 ml final volume of 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM nitroarene and 0.1 mM NADPH. One unit of the enzyme activity (IU) was defined as the amount of enzyme required to oxidize 1 μmol NADPH per minute at 30°C and pH 7.0, and was measured by following the initial and linear decrease in absorbance at 340 nm (Kim & Song, 2005).

Protein purification

The recombinant nitroreductase with an *N*-terminal His-tag was rapidly purified to electrophoretic homogeneity by nickel affinity chromatography (Hochuli et al., 1988; Sun et al., 2023). The samples of cell-free extract and purified target protein were analyzed by SDS-PAGE.

Production detection

The conversion of substrates, chemoselectivity and yield of products were determined by HPLC (Shimazu, China) equipped with a reverse-phase C18 column (Inerstill ODS-4, Φ 4.6 mm × 250 mm × 5 μm). The samples were eluated by a mobile phase of methanol-water-citric acid: 60/40/1 (v/v/g) in flow rate of 0.8 ml/min and monitored at 254 nm with an UV detector. ^{1}H NMR and ^{13}C NMR were measured on a Bruker Avance 400 MHz and 500 MHz spectrometer with chemical shifts reported as ppm (TMS as internal standard) (Willoughby et al., 2014; Zhang et al., 2016).

3. RESUTLS

NTR from Geodermatophilus obscurus exhibit highest nitrorecutase activity

In the attempt finding novel enzyme that could be used in N-Arylhydroxylamines synthesis, we first used BLAST function of NCBI with two published NTRs coding-sequence as a probe. Nine candidate NTRs were selected which show the similarity with probe sequence between 75%-50% (Table 1).

Table 1. List of primers used in this study for amplifying coding-sequence of potential NTRs.

Genbank No.	Source	aa	Primer (5'->3')
D5DW24	*Bacillus megaterium*	249	CCGGAATTCATGAACTCAG TTATTGAAAC CCGCTCGAGTTATTTCTTGTTAAATCCTT
B4AGS2	*Bacillus pumilus*	224	CGCGGATCCATGCGAGACA AAGAGCAATT CCGCTCGAGTTAACGAATC CAAACGGCTA
Q6FRQ1	*Candida glabrata*	205	CCGGAATTCATGACCAAGC ATTCACCTAC CCGCTCGAGTTACTCATAC ACTTTGACTA
Q6CUX4	*Kluyveromyces lactis*	196	CCGGAATTCATGTCTGCTG CTGCCACTAA CCGCTCGAGCTAGTTCAAG ATTTTGACAG
DAA07459	Baker's yeast	193	CCGGAATTCATGTCCCCAA CTGGAAACTA CCGCTCGAGTCAGTGATAA ACGTTGATTA
A5W253	*Pseudomonas putida*	242	CCGGAATTCATGCTTATTG ATCGCTTGCG CCGCTCGAGTTTATTCTGAG TGAAAGGTCG
Q0K1B9	*Cupriavidus necator*	223	CCGGAATTCATGGAGGCAA TCCAGATGAC CCGCTCGAGTCACCGGTCG ACGAACGAGA
ABX34950	*Delftia acidovorans*	209	CCGGAATTCATGAGCACCA ACGACAGATC CCGCTCGAGTTCAGCCTTCG TCGGCCGCGC
ADB77573	*Geodermatophilus obscurus*	222	CGCGGATCCATGACCGGCA GCGAGTGGCC CCCAAGCTTTCAGGAGTCG ACGCGGGCGG

These coding sequences were then inserted into pET-28a (+) plasmid. Constructed plasmids were then transformed into E. coli BL21 for heterogeneous expression. We used 4-cyanonitrobenzene as substrate for investigating NTR activity. E. coli cells that express NTRs were homogenized by sonication for releasing intracellular contexts. The liquid was then centrifuged to remove cell debris. The supernatant was used for enzyme activity measuring. As shown in Figure 1, NTR from Geodermatophilus obscurus (GoNTR) shows the highest nitroreductase activity. Note that the measuring was performed under mild conditions implying the possibility for synthesis N-hydroxylamine by biocatalytic.

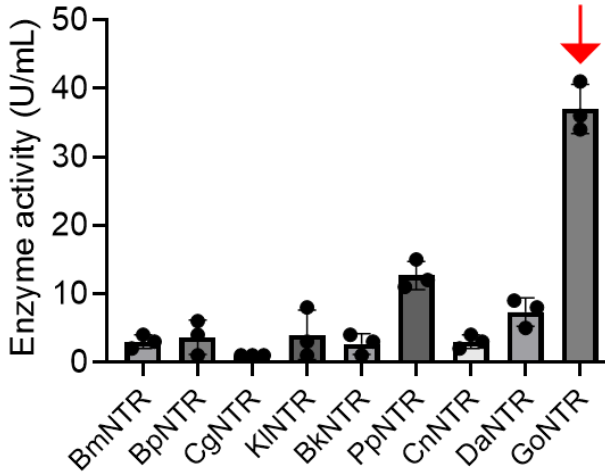

Figure 1. The activity of candidate NTRs toward 4-cyanonitrobenzene. The NTRs from Geodermatophilus obscurus (GoNTR) exhibited the highest nitroreductase activity (labelled by red arrow).

GoNTR shows high chemo-selectivity to N-arylhydroxylamine

As mentioned above, the reduction of nitroaromatic compounds could produce nitroso, hydroxylamine, and amine. The chemo-selectivity of N-hydroxylamine is defined as the percentage of N-hydroxylamine per nitroso and amine. To test the chemo-selectivity of N-hydroxylamine, we extracted the reaction mixture, detected, and quantified the concentration of N-hydroxylamine. As shown in Figure 2A, GoNTR shows excellent chemo-selectivity (N-(4-cyanophenyl)hydroxylamine≥99%). We did not detect the existence of 4-cyanophenyl amine in the reaction mixture. The production of reaction

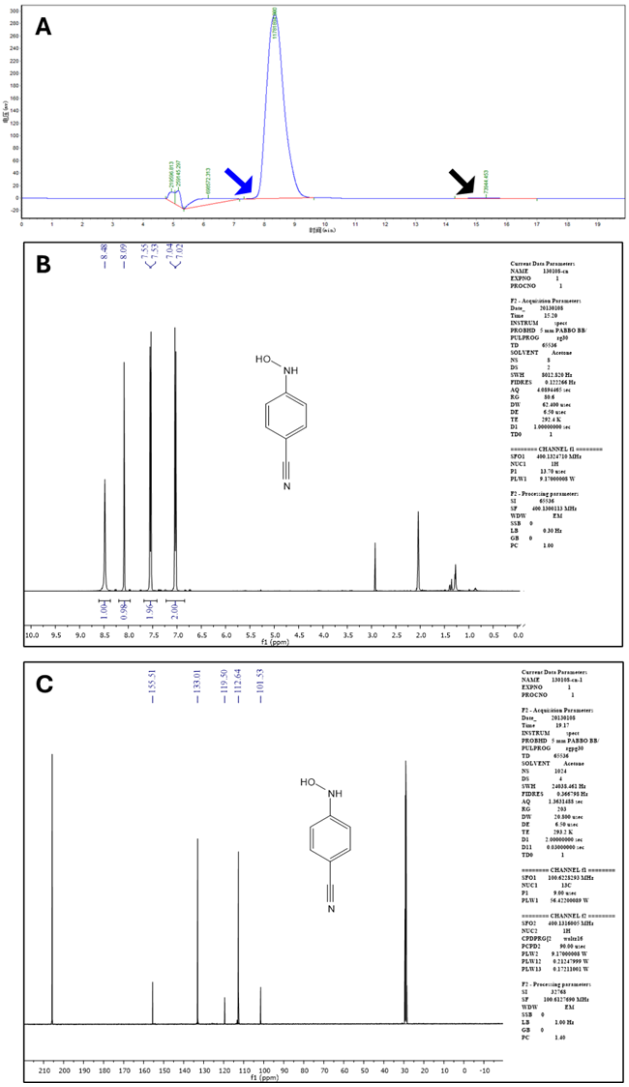

Figure 2. GoNTR exhibits high chemo-selectivity to arylhydroxylamine. (A) High-performance liquid chromatography (HPLC) of the end-point reaction mixture shows a unique peak of product (blue arrow). The substrate (nitro aromatic compound) in reaction mixture is below detectable level (black arrow). (B) ^{1}H NMR spectra and (C) ^{13}C NMR spectra of N-(4-cyanophenyl)-hydroxylamine.

was further performed Nuclear Magnetic Resonance (NMR) of 1H and 13C determining the content and purity of a sample as well as its molecular structure (Figure 2B&C).

The characteristic of GoNTR

For precisely investigating the characteristic of GoNTR, we purified GoNTR by immobilized metal ion affinity chromatography (IMAC). The supernatant mixture before and after purification of GoNTR shows in Figure 3A. We used purified protein to investigate the characteristics of GoNTR, including the effect of pH and temperature on its activity.

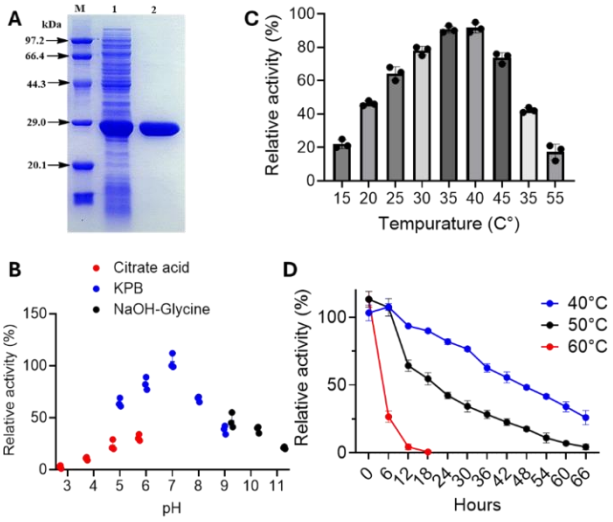

Figure 3. The characteristic of GoNTR. (A) SDS-PAGE analysis of the purified GoNTR protein. Lane M: protein markers; Lane 1: cell-free extract; Lane 2: purified protein. (B) Effects of temperature on activity of the purified nitroreductase GoNTR. (C) Effects of temperature on activity of the purified nitroreductase GoNTR. (D) Thermostability of the purified nitroreductase GoNTR in different temperatures.

As shown in Figure 3B, the activity of GoNTR gradually increases with pH value and reaches its peak at pH =7.0 in potassium phosphate buffers (KBP) buffer. We also note that KBP buffer exhibits higher activity than citrate acid in the same pH value (Figure 4B, pH=5.0 and pH =6.0). GoNTR shows highest activity around 35°C and 40°C (Figure 3C). In large-scale biosynthesis, the thermostability of enzyme is one of the most important parameters. GoNTR shows excellent thermo-resistance. The activity of GoNTR still remains around 50% after 48h at 40°C condition. The activity of GoNTR rapidly reduces to 25% after 6h and loses it activity after 12h at 60°C condition (Figure 3D). Taken all together, the optimal reaction condition of GoNTR is in pH=7.0 KBP buffer at 35°C.

Synthesis N-arylhydroxylamine by GoNTR

NTRs is a reduction enzyme which used NADPH as the electron donor and produce NADP+ (Ferri et al., 2011). Glucose dehydrogenase (GDH) is an oxidoreductase that participates in glucose metabolism. GDH catalyzes the oxidation of glucose in the presence of cofactors like NADP+ to produce NADPH. Therefore, GDH is a general tool for driving nicotinamide (NADPH) regeneration in synthetic biochemistry (Qian et al., 2020). To establish a continuous flow bioreaction, we coexpressed GoNTR and BmGDH in E. coli BL21 for cell self-regeneration of the coenzyme **(Figure 4A).** We applied the coexpressed system with the optimal reaction condition, shown in Figure 3, to synthesis 4-(Cyanomethanyl)phenyl)hydroxylamine from 4-cyanonitrobenzene.

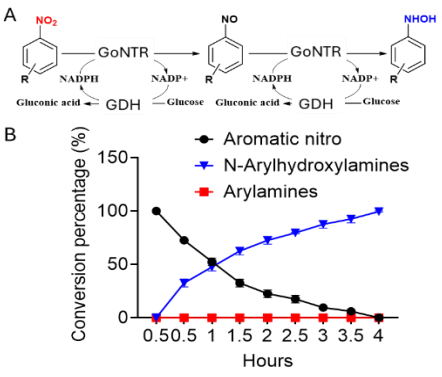

Figure 4. The continuous flow bioreaction of N-arylhydroxylamine. (A) The schematic shows how the system works. (B) Real-time conversion percentages of aromatic nitro, N-arylahydroxylamines, and arylamines.

Fermentation of coexpressed GoNTR and BmGDH in E. coli BL21 cells was harvested, weighted, and directly added to the reaction. In a 1-L reaction scale, 4-cyanonitrobenzene was converted to 4-(Cyanomethanyl)phenyl)hydroxylamine in 4 h with 100% conversion and >99% chemo-selectivity by 10 g/L coexpressed cells without any external addition of NADP+. A two-phase system was applied to increase the substrate loading. In toluene/water (v/v, 1/1), 30 g/L coexpressed cells, 300 mM p-cyanonitrobenzene was efficiently converted to hydroxylamine in 8 h with 100% conversion and >99% selectivity. We noted that no amine was detected during the reaction (Figure 4B).

4. CONCLUSION

Traditionally, N-aryhydroxylamine compounds are synthesized though the conventional chemical methods, which often requires extremely high temperatures and pressures condition. These processes generate environmentally harmful waste that requires additional treatment. Moreover, achieving high purity of N-arylhydroxylamine compounds is challenging due to their tendency to reduce to the corresponding amine. Although several biocatalytic approaches have been explored, they often suffer from poor selectivity (<80%) (Li et al., 2004) or require prolonged reaction times (~2 days) (Li et al., 2005). In this study, we report a green, highly selective, and substantiable system for N-aryhydroxylamine synthesis utilizing a co-expression system nitroreducatse (GoNTR) and glucose dehydrogenase (BmGDH). This system enables the synthesis of 4-(cyanomethyl)phenylhydroxylamine at room temperature within 4 hours, achieving >99% conversion and >99% selectivity without the formation of by-products or the need for additional compounds. Our results demonstrate that this co-expression system is a promising tool for the synthesis of arylhydroxylamines. Future work will focus on expanding the substrate spectrum of GoNTR and exploring its potential application in biodegradation.

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REFERENCES

Ahmad, F., & Hughes, J. B. (2002). Reactivity of partially reduced arylhydroxylamine and nitrosoarene metabolites of 2,4,6-trinitrotoluene (TNT) toward biomass and humic acids. Environmental Science & Technology, 36(20), 4370-4381. <Go to ISI>://WOS:000178581300023

Ayyangar, N. R., Brahme, K. C., Kalkote, U. R., & Srinivasan, K. V. (1984). Synthesis, 938-941.

Bartra, M., Romea, P., Urpí, F., & Vilarrasa, J. (1990). A fast procedure for the reduction of azides and nitro compounds based on the reducing ability of Sn(SR)3-species. Tetrahedron, 46(2), 587-594. https://doi.org/http://dx.doi.org/10.1016/S0040-4020(01)85439-9

Beaudoin, D., & Wuest, J. D. (2011). Synthesis of N-arylhydroxylamines by Pd-catalyzed coupling. Tetrahedron Letters, 52(17), 2221-2223.

https://doi.org/10.1016/j.tetlet.2010.12.034

Bornscheuer, U. T., Huisman, G. W., Kazlauskas, R. J., Lutz, S., Moore, J. C., & Robins, K. (2012). Engineering the third wave of biocatalysis. Nature, 485(7397), 185-194. https://doi.org/10.1038/nature11117

Cenas, N., Nemeikaite-Ceniene, A., & Kosychova, L. (2021). Single- and Two-Electron Reduction of Nitroaromatic Compounds by Flavoenzymes: Mechanisms and Implications for Cytotoxicity. Int J Mol Sci, 22(16). https://doi.org/10.3390/ijms22168534

Corma, A., Concepcion, P., & Serna, P. (2007). A different reaction pathway for the reduction of aromatic nitro compounds on gold catalysts. Angew Chem Int Ed Engl, 46(38), 7266-7269. https://doi.org/10.1002/anie.200700823

Davis, G. C. (1988). US Pat, 4, 723 030.

Entwistle, I. D., Gilkerson, T., Johnstone, R. A. W., & Telford, R. P. (1978). Tetrahedron, 34, 213-215.

Ferri, S., Kojima, K., & Sode, K. (2011). Review of glucose oxidases and glucose dehydrogenases: a bird's eye view of glucose sensing enzymes. J Diabetes Sci Technol, 5(5), 1068-1076. https://doi.org/10.1177/193229681100500507

Gassman, P. G., & Grandrud, J. E. (1984). J. Am. Chem. Soc, 106, 1498.

H. A. Cyr, J. F. M., G. Belot, E. Laviron and J. Lessard. (1989). Electrochimica Acta, 439-445.

Haworth, R. D., & Lapworth, A. (1925). J. Chem. Soc., Trans, 127, 2970.

Ho, C. M., & Lau, T. C. (2000). Copper-catalyzed amination of alkenes and ketones by phenylhydroxylamine. New Journal Of Chemistry, 24(11), 859-863. <Go to ISI>://WOS:000165067900008

Hochuli, E., Bannwarth, W., Döbeli, H., Gentz, R., & Stüber, D. (1988). Genetic Approach to Facilitate Purification of Recombinant Proteins with a Novel Metal Chelate Adsorbent. Bio/Technology, 6(11), 1321-1325. https://doi.org/10.1038/nbt1188-1321

Hollmann, F., Arends, I. W. C. E., & Holtmann, D. (2011). Enzymatic reductions for the chemist. Green Chemistry, 13(9), 2285-2314. https://doi.org/Doi 10.1039/C1gc15424a

Johansson, E., Parkinson, G. N., Denny, W. A., & Neidle, S. (2003). Studies on the nitroreductase prodrug-activating system. Crystal structures of complexes with the inhibitor dicoumarol and dinitrobenzamide prodrugs and of the enzyme active form. Journal Of Medicinal Chemistry, 46(19), 4009-4020. <Go to ISI>://WOS:000185232700008

K.Taya. (1966). Chem. Commun, 465.

Kamm, O. (1941). β-PHENYLHYDROXYLAMINE. Organic Syntheses, 1, 445.

Karwa, S. L., & Rajadhyaksha, R. A. (1987). Selective Catalytic Hydrogenation of Nitrobenzene to Phenylhydroxylamine. Ind. Eng. Chem. Res, 26, 1746-1750.

Kim, H. Y., & Song, H. G. (2005). Purification and characterization of NAD(P)H-dependent nitroreductase I from Klebsiella sp. C1 and enzymatic transformation of 2,4,6-trinitrotoluene. Appl Microbiol Biotechnol, 68(6), 766-773. https://doi.org/10.1007/s00253-005-1950-1

Lamar, A. A., & Nicholas, K. M. (2009). Direct synthesis of 3-arylindoles via annulation of aryl hydroxylamines with alkynes. Tetrahedron, 65(19), 3829-3833. <Go to ISI>://WOS:000265756800002

Li, F., Cui, J. N., Qian, X. H., Rong, Z. A., & Xiao, Y. (2005). Highly chemoselective reduction of aromatic nitro compounds to the corresponding hydroxylamines catalysed by plant cells from a grape (Vitis vinifera L.). Chemical Communications(14), 1901-1903. https://doi.org/Doi 10.1039/B418675c

Li, F., Cui, J. N., Qian, X. H., & Zhang, R. (2004). A novel strategy for the preparation of arylhydroxylamines: chemoselective reduction of aromatic nitro compounds using bakers' yeast. Chemical Communications(20), 2338-2339. https://doi.org/Doi 10.1039/B408566c

Liu, S., Wang, Y., Jiang, J., & Jin, Z. (2009). The selective reduction of nitroarenes to N-arylhydroxylamines using Zn in a CO2/H2O system. Green Chemistry, 11(9), 1397. https://doi.org/10.1039/b906283a

Makosch, M., Sá, J., Kartusch, C., Richner, G., van Bokhoven, J. A., & Hungerbühler, K. (2012). Hydrogenation of Nitrobenzene Over Au/MeOx Catalysts-A Matter of the Support. ChemCatChem, 4(1), 59-63. https://doi.org/10.1002/cctc.201100299

Marvel, C. S., & Kamm, O. (1919). J. Am. Chem. Soc, 41, 282.

Nyanhongo, G. S., Schroeder, M., Steiner, W., & Gübitz, G. M. (2005). Biodegradation of 2,4,6-trinitrotoluene (TNT): An enzymatic perspective. Biocatalysis And Biotransformation, 23(2), 53-69. https://doi.org/10.1080/10242420500090169

Qian, W. Z., Ou, L., Li, C. X., Pan, J., Xu, J. H., Chen, Q., & Zheng, G. W. (2020). Evolution of Glucose Dehydrogenase for Cofactor Regeneration in Bioredox Processes with Denaturing Agents. Chembiochem, 21(18), 2680-2688. https://doi.org/10.1002/cbic.202000196

R. J. Knox, F. F., T. Marchbank and J. J. Roberts. (1991). Bioactivation of CB 1954: Reaction of the active 4-hydroxylamino derivative with thioesters to form the ultimate DNA-DNA interstrand crosslinking species. Biochemical Pharmacology, 42(9), 1691-1697.

Rios, R., Sunden, H., Ibrahem, I., Zhao, G. L., & Cordova, A. (2006). A one-pot organocatalytic asymmetric entry to

tetrahydrothioxanthenones. Tetrahedron Letters, 47(49), 8679-8682. <Go to ISI>://WOS:000242424600009

Roldan, M. D., Perez-Reinado, E., Castillo, F., & Moreno-Vivian, C. (2008). Reduction of polynitroaromatic compounds: the bacterial nitroreductases. FEMS Microbiol Rev, 32(3), 474-500. https://doi.org/10.1111/j.1574-6976.2008.00107.x

Rondestvedt, C. S., Jr., & Johnson, T. A. (1977). Synthesis, 850-851.

Rong, Z., Du, W., Wang, Y., & Lu, L. (2010). Carbon supported Pt colloid as effective catalyst for selective hydrogenation of nitroarenes to arylhydroxylamines. Chemical Communications, 46(9), 1559-1561. https://doi.org/10.1039/b916686f

Seshadri, G., & Kelber, J. A. (1999). A study of the electrochemical reduction of nitrobenzene at molybdenum electrodes. Journal Of The Electrochemical Society, 146(10), 3762-3764. <Go to ISI>://WOS:000083126400036

Smith, D. G., Gribble, A. D., Haigh, D., Ife, R. J., Lavery, P., Skett, P.,…West, A. (1999). The inhibition of human cytomegalovirus (hCMV) protease by hydroxylamine derivatives. Bioorganic & Medicinal Chemistry Letters, 9(21), 3137-3142. <Go to ISI>://WOS:000083562300015

Spence, J. D., Raymond, A. E., & Norton, D. E. (2003). Condensations of N-arylhydroxylamines for the preparation of 5,5 '-di-tert-butyl-2,2 '-dihydroxydiphenylamine. Tetrahedron Letters, 44(4), 849-851. <Go to ISI>://WOS:000180464000053

Sridharan, V., Karthikeyan, K., & Muthusubramanian, S. (2006). Unexpected multicomponent reaction of 2/4-methoxyarylaldehydes with arylhydroxylamines and maleic anhydride: a novel synthesis of unsymmetrical diarylamines. Tetrahedron Letters, 47(25), 4221-4223. <Go to ISI>://WOS:000238111400028

Sun, H., Wang, H., Chen, Q., Dong, W., Gao, C., Song, H.,…Luo, H. (2023). Cation affinity purification of histidine-tagged proteins. Appl Microbiol Biotechnol, 107(7-8), 2639-2651. https://doi.org/10.1007/s00253-023-12425-3

Svensson, C. K. (2003). Do arylhydroxylamine metabolites mediate idiosyncratic reactions associated with sulfonamides? Chemical Research In Toxicology, 16(9), 1035-1043. <Go to ISI>://WOS:000185410700001

Symons, Z. C., & Bruce, N. C. (2006). Bacterial pathways for degradation of nitroaromatics. Natural Product Reports, 23(6), 845-850. https://doi.org/10.1039/b502796a

Takenaka. (2008). WO Pat, 117 844.

Takenaka, Y., Kiyosu, T., Choi, J.-C., Sakakura, T., & Yasuda, H. (2009). Selective synthesis of N-aryl hydroxylamines by the hydrogenation of nitroaromatics using supported platinum

catalysts. Green Chemistry, 11(9), 1385. https://doi.org/10.1039/b904672k

Takenaka, Y., Kiyosu, T., Choi, J. C., Sakakura, T., & Yasuda, H. (2010). Selective synthesis of N-alkyl hydroxylamines by hydrogenation of nitroalkanes using supported palladium catalysts. ChemSusChem, 3(10), 1166-1168. https://doi.org/10.1002/cssc.201000137

Takenaka, Y., Kiyosu, T., Mori, G., Choi, J.-C., Sakakura, T., & Yasuda, H. (2011). Selective hydrogenation of nitroalkane to N-alkyl hydroxylamine over supported palladium catalysts. Catalysis Today, 164(1), 580-584. https://doi.org/10.1016/j.cattod.2011.02.001

Thomas, C., & Gwenin, C. D. (2021). The Role of Nitroreductases in Resistance to Nitroimidazoles. Biology (Basel), 10(5). https://doi.org/10.3390/biology10050388

Vyas, P. A., Roychowdhury, S., Woster, P. M., & Svensson, C. K. (2005). Reactive oxygen species generation and its role in the differential cytotoxicity of the arylhydroxylamine metabolites of sulfamethoxazole and dapsone in normal human epidermal keratinocytes. Biochemical Pharmacology, 70(2), 275-286. <Go to ISI>://WOS:000230295700011

Wang, Y. Z., Ye, L. W., & Zhang, L. M. (2011). Au-catalyzed synthesis of 2-alkylindoles from N-arylhydroxylamines and terminal alkynes. Chemical Communications, 47(27), 7815-7817. <Go to ISI>://WOS:000292113700075

Willoughby, P. H., Jansma, M. J., & Hoye, T. R. (2014). A guide to small-molecule structure assignment through computation of ((1)H and (1)(3)C) NMR chemical shifts. Nat Protoc, 9(3), 643-660. https://doi.org/10.1038/nprot.2014.042

Zhang, Y. C., Qin, Y., Wang, H., Zhang, D. W., Yang, G., & Li, Z. T. (2016). Bipyridinium Polymers That Dock Tetrathiafulvalene Guests in Water Driven by Donor-Acceptor and Ion Pair Interactions. Chem Asian J, 11(7), 1065-1070. https://doi.org/10.1002/asia.201600017