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A Sustainable Synthesis of Asymmetric Phenazines and Phenoxazinones Mediated by CotA-Laccase

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Abstract. An efficient and sustainable one-step procedure for the synthesis of new asymmetric phenazines and phenoxazinones from commercially available *ortho*-substituted diamines and *ortho*-substituted hydroxyamines is reported. In this study we have expanded the substrate scope of CotA-laccase-catalyzed aerobic oxidations through the use of aromatic amines presenting variable functional groups, including *N*-substitution, contributing to the rational synthesis of different heterocyclic scaffolds. The transformations proceed smoothly through a cascade of oxidative reactions to the benzoquinonediimine intermediates followed by nucleophilic addition, intramolecular cyclization and aromatization, all performed in mild conditions.

Keywords: Biocatalysis; Homocoupling reactions; Laccase; Nitrogen heterocycles; Oxidation

Introduction

Phenazines are multifunctional versatile compounds with antioxidant, antibiotic, anticancer and antimicrobial activities,[1-5] which is mainly associated with their redox activity and their capacity to intercalate between DNA subunits. Furthermore, the heteroaromatic phenazine π-core is present in a vast array of heterocyclic compounds such as pharmaceuticals,[6] sensors and biosensors,[7] OLEDS,[8] chemical switches,[9] photovoltaic cells for renewable energy and in nanotechnology.[10] The phenoxazinone core is also an important building block present in compounds displaying significant biological activities and redox properties.[11-13] Considering their broad applications, there is a growing interest in finding alternative chemical routes for the formation of new phenazines or the modification of those obtained from natural biologic sources.[14-16] The nuclear phenazine core can be substituted at different positions leading to symmetric or asymmetric derivatives and these modifications affect several properties such as the redox potential and solubility with consequences in their biological activity and thus potential applications.[17-19]

The most traditional reported methods for the preparation of phenazines[15,18] are based in the assembly of the central heterocyclic ring and showed several disadvantages like harsh reaction conditions, in the presence of organic solvents and aggressive oxidants, and limitation in the substrate scope related with the localization and electronic character of the ring substituents.[15]

The formation of asymmetric phenazines can be reached by cross-coupling reactions of aromatic amines or in substitution reactions from symmetric cores. Despite the efforts made in the development of new sustainable methods,[18,20,21] the regioselective synthesis of derivatives substituted in both rings is still considered a challenge.

Laccases (EC 1.10.3.2) are copper-containing metalloenzymes that have received increasing attention over the past few decades in the field of organic synthesis for being environmentally benign catalysts.[22] They oxidize, in aqueous medium, a

range of different substrates including aromatic amines but the vast majority of synthetic reactions reported involved radical-coupling reactions of phenolic monomers and cross-coupling reactions of substituted catechols and hydroquinones with nitrogen based[23] and carbon derived nucleophiles[24] via *in situ* generated *ortho*- and *para*-quinones. Concerning the laccase oxidation of aromatic amines and further radical-coupling reactions involving the generated *ortho* and *para*-benzodiimine or benzoquinoneimine intermediates, few reports were found.[25-28]

Our previous studies[25b,c] showed the potential of the bacterial CotA-laccase to mediate the formation of heterocyclic cores through homocoupling reactions involving substituted aromatic amines. The formation of symmetric phenazines as well as asymmetric phenoxazinones was observed[25b] and this have encouraged the extension of our studies to the use of additional *ortho*-substituted aromatic amines looking forward the formation of new asymmetric phenazines. In this study we report the enzymatic preparation of asymmetric phenazines by oxidation of aromatic amines with different substitution patterns. This process generated the corresponding *ortho*-benzodiimine intermediates followed by in situ intramolecular electrophilic aromatic substitution and aromatization. This approach also prompted us to synthetize *N*-substituted phenazines, extending the scope of the enzymatically obtained heterocyclic cores.

The dimerization of aromatic *o*-hydroxyamines with subsequent formation of phenoxazinones was also achieved, highlighting the inclusion of heteroaromatics rings in the scope of aromatic substrates used by CotA-laccase.

Results and Discussion

Our previous studies[25b] using CotA-laccase and the model substrates 1,2-phenylenediamine (1,2-PDA) and 2-aminophenol (2-AP) in aqueous medium resulted in the formation of the 2,3-diaminephenazine and 2-amino-3-phenoxazinone with 66 % and 83 % yields, respectively. Following a similar strategy under optimized reaction conditions,[25b] in this study a set of substituted aromatic *ortho*-diamines with different electron donor groups (EDG) and electron withdrawing groups (EWG) (***1a-e, 2*** Figure **1**) was used to perform the enzymatic oxidative reactions.

Two aromatic *ortho*-hydroxyamines (***3*** and ***4***) (Figure **1**) were also used to generate oxidative coupling products at good to excellent yields.

In order to appraise the redox requirements for the enzymatic oxidation, we have determined the redox potential of the substrates by cyclic voltammetry using a platinum wire working electrode in 20 mM phosphate buffer pH 6.0 (the pH used in the enzymatic reactions). All substrates showed a very similar electrochemical behavior generating one or two well-defined irreversible oxidation waves (E_{pa}) in

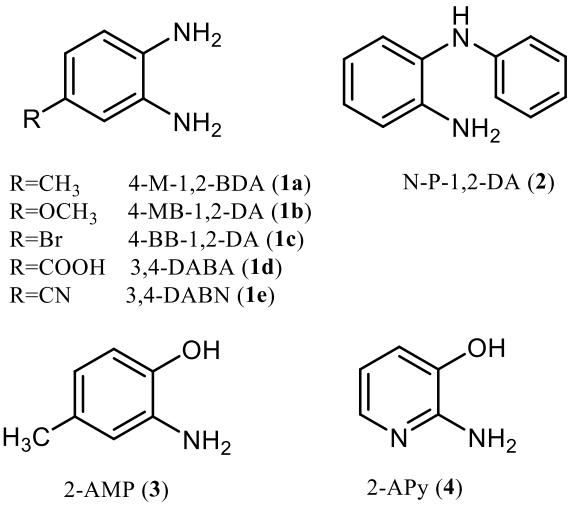


Figure 1. Chemical structures of the aromatic *ortho*-diamines and *ortho*-hydroxyamines used as CotA-laccase substrates. (4-methyl-1,2-benzenediamine (***1a***), 4-methoxybenzene-1,2-diamine (***1b***), 4-bromobenzene-1,2-diamine (***1c***), 3,4-diaminobenzoic acid (***1d***), 3,4-diaminobenzonitrile (***1e***), 2-*N*-phenylbenzene-1,2-diamine (***2***), 2-amino-4-methylphenol (***3***) and 2-aminopyridin-3-ol (***4***))

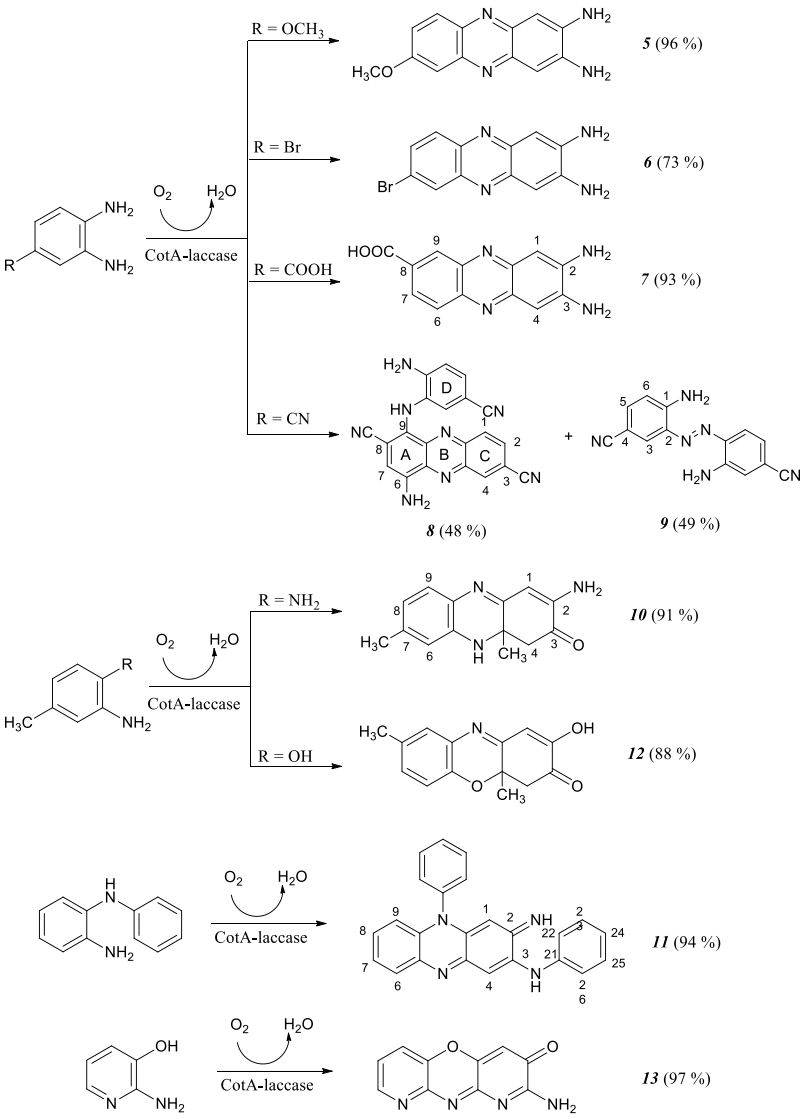
the range between 0.57 V (for 4-MB-1,2-DA, ***1b***) and 1.02 V (for 4-BB-1,2-DA, ***1c***) (see table S1, Supplementary material). The absence of reductive waves indicated that the oxidation process most probably generated highly reactive quinoneimine or quinone-diimine species which undergoes further spontaneous reactions. The oxidation potential was found to be influenced by the presence of the substituent group (R) (see Figure **1** and Table S1): lower oxidation potentials for EDG groups which is expected to favour the enzymatic oxidation and higher oxidation potentials for the EWG groups. Considering that the redox potential for CotA-laccase is only 0.535 V *vs* NHE,[29] slightly lower than most of the selected substrates, the enzymatic reactions were carried out in the absence of exogeneous redox mediators.

The enzymatic reactions were performed in a phosphate buffer (20 mM) or in a mixture containing 90 % of buffer and 10 % of ethanol, to guarantee the complete solubility of aqueous-insoluble substrates, in the presence of catalytic amounts of CotA-laccase ($1U.mL^{-1}$) at 37 °C with stirring. The time course of reactions was followed by the formation of color in the reaction mixtures and by TLC. The oxidative processes occurred in aqueous media and in the presence of air; oxygen is converted to water by the enzyme and no toxic byproducts are formed. The time course of reactions was reduced to 2h, a significantly shorter period compared to our earlier studies in the oxidation of aromatic amines mediated by laccases,[25b,c] where the products were recovered after 24h of reaction. Furthermore, the possibility of recyclability of the enzymatic catalyst was also examined using substrate ***4*** as an example. After completion of the homocoupling reaction in

approximately 2h, the reaction was recharged with an additional amount of ***4*** (5 mM). This procedure was repeated for three cycles without significant changes on the final product yields.

The products ***5-13*** were isolated at good to excellent yields (Scheme **1**), identified and fully characterized by spectroscopic methods. Structural assignments were made based on the observed ^{1}H-^{13}C HSQC direct and HMBC 3-bond correlations (see the details in the Supplementary data).

The enzymatic oxidation of *ortho*-diamines ***1b-d*** originated the respective water soluble colored phenazines ***5-7*** (Scheme ***1***), as single products. These compounds were isolated as brown solids with conversion yields of 96%, 93% and 73%, respectively. Their ^{1}H-NMR spectra showed a similar pattern with resonances for the aromatic protons in the range 6.03-8.64 ppm, typical for the heteroaromatic phenazine core. For compound ***5***, the singlet at 3.90 ppm was indicative of the presence of only one methoxy group in the molecule. Thus, the formation of the phenazine core was accompanied by the loss of the methoxy, bromo and carboxyl groups in one of the substrate molecules, which is consistent with similar observations previously reported for the formation of phenoxazine cores.[25b,30] The elimination of the methoxy, bromo and carboxyl groups was also supported by the ESI- MS results. The first-order full



Scheme 1 – Asymmetric heterocyclic cores obtained from the oxidation of aromatic *ortho*-diamines and *ortho*-hydroxyamines mediated by CotA-laccase

mass spectrum of compound ***5*** shows the protonated molecule $[M+H]^{+}$ at *m/z* 241 and its fragmentation yielded fragment ions with *m/z* 226 and *m/z* 198, due to the characteristic loss of the methyl radical and the

subsequent loss of CO. For compound ***6***, the proposed structure was corroborated by a group of peaks at *m/z* 289/291 present in the ESI-mass spectrum in positive mode, that exhibits the isotopic distribution expected for the presence of one bromine in the structure. For compound ***7***, the $[M+H]^+$ and $[M-H]^-$ ions at *m/z* 255 and 253 respectively, were found in the full mass spectra. The MS^2 spectrum of the deprotonated molecule (*m/z* 253) was characterized by the loss of CO_2 resulting in an abundant fragment ion with *m/z* 209.

CotA-laccase mediated oxidation of 3,4-DABN (***1e***), containing the electron acceptor cyano group, under the same reaction conditions, led to the correspondent phenazine product ***8*** (48 %) and to a second product (***9***) (49 %) (Scheme **1**). Furthermore, the isolated phenazine ***8*** is not a dimeric structure, but resulted from the coupling of three substrate molecules as supported by the NMR and ESI-MS data. The ^{1}H-NMR spectrum of compound ***8*** clearly showed the presence of more resonances than the expected for a basic dimeric phenazine core. Indeed, three additional resonances at 7.52, 7.07 and 6.28 ppm with the same multiplicity as the substrate, suggested a very similar spin system pattern (D ring). The presence of one singlet for proton H7 described the A ring, while the C ring was outlined by two duplets and one singlet for protons H1, H2 and H4, respectively. In the ^{13}C spectrum, all resonances were identified as expected in the range 160 to 95 ppm. The ESI-MS spectrum, in negative-mode shows the $[M-H]^-$ species at *m/z* 375 while its sequential fragmentation resulted in the rearrangement ion at *m/z* 348 due to the loss of HCN.

For compound ***9***, the ^{1}H-NMR spectrum showed a similar pattern of the precursor molecule, with one singlet at 8.17 ppm and two doublets at 7.03 and 7.45 ppm. This observation together with the deshielding effect observed for all aromatic resonances is consistent with the presence of a symmetrical structure connected by a N=N bond, as previously observed.[25a,b] Moreover, the respective MS spectrum, in negative mode shows a peak at *m/z* 261 ($[M-H]^-$), which generated in the MS^2 analysis a fragment with *m/z* 233 that results from the loss of N_2 from the azo group.

Overall these results indicate that the reaction course, leading to different type of products, can be affected by the electronic character of the R substituent. While the presence of an electron donor group or a medium electron acceptor group (COOH) in the *meta(para)* position allowed the formation of asymmetric phenazines with excellent yields, the presence of the stronger acceptor CN group originated two different products, a trimer based on the phenazine core and an azo compound.

The enzymatic oxidation of 4-M-1,2-BDA (***1a***) resulted in the water soluble dark red phenazine ***10***, obtained with an excellent yield (91 %). Based on the ^{1}H NMR spectrum, it was evident the presence of two resonances at 1.24 and 2.25 ppm for the aliphatic methyl groups, which indicates the presence of two different groups in the final product. This observation was the first evidence of the occurrence of a cyclization without demethylation. The presence of two doublets at 3.09 and 3.27 ppm attributed to the methylene protons, prompted us to suggest the structure ***10***, similar to the one previously proposed in the literature for the oxidation of *ortho*-aminophenols by human hemoglobin.[31] The proposed structure was also corroborated by the ESI mass spectrum which showed in positive mode the protonated molecule (*m/z* 242). MS^2 fragmentation of the precursor *m/z* 242 produced two major product ions with *m/z* 227 and 214, due to the losses of the methyl radical and CO, respectively.

Looking forward to extend the scope of the present enzymatic approach to *N*-substituted phenazines, the oxidation of the 2-*N*-phenylbenzene-1,2-diamine (***2***) was performed and the phenazine ***11*** was isolated as a water soluble brown solid. The ^{1}H NMR spectrum showed diverse signals in the aromatic region and the ^{13}C spectrum was consistent with the presence of twenty four carbons in the proposed structure, with two resonances at low field (159.1 and 149.5 ppm) characteristic of a diimine structure. The structure was corroborated by the ESI-mass spectrum in positive mode that exhibits a main peak at *m/z* 363 ($[M+H]^+$).

The substitution of the *ortho*-amino group by a hydroxyl group led to the formation of phenoxazinone derivatives, which confirms the use of CotA-laccase for the preparation of compounds based on structural variations of the 2-aminophenoxazin-3-one scaffold. Moreover, the introduction of a substituted pyridine ring as substrate extends the scope of the type of substrates used by this enzyme.

The enzymatic oxidation of 2-amino-4-methylphenol (***3***) resulted in the formation of a brownish yellow compound 3-amino-1,4*a*-dihydro-4*a*,8-dimethyl-2*H*-phenoxazin-2-one (***12***) (Scheme **1**), with a very good yield (88 %) in a short time (*ca* 2h). In accordance with the structure proposed to compound ***10***, the ^{1}H NMR spectrum of the compound exhibits two singlet methyl signals at 1.05 and 1.26 ppm and two isolated methylene signals at 3.4 and 3.6 ppm (J = 15.6 Hz). All other proton resonances were consistent with the proposed structure, which resulted from deamination by an hydrolysis process during the reaction, in agreement with the observed with other substrates.[25b]

The enzymatic oxidation of 2-APy (***4***), a pyridine derivative, resulted in the formation of the pyridyloxazinone ***13*** (Scheme **1**), a planar electron rich heterocyclic aromatic compound, for which the ^{1}H NMR spectrum showed resonances in the 6.55–8.54 ppm region correspondent to the four aromatic protons of the asymmetrical tricyclic pyridyloxazinone core. The structure was also confirmed by the ESI-MS spectrum in the positive mode that exhibited a major peak at *m/z* 215 assigned to the protonated molecule of ***13***.

The proposed molecular pathway (Scheme **2**)[25b, 28e,32] that led to the formation of asymmetric phenazines in CotA-laccase mediated reactions, using aromatic

amines as substrates, initiates with the abstraction of one electron from the more liable amino group in the substrate molecule, generating the *ortho*-quinonediimine key intermediate (**A**). Under the reaction conditions, this electrophilic species (**A**) suffer rapid nucleophilic addition by other substrate molecule in the *para*-position to an imine group forming the (**B**) intermediate. The substrate's amino group responsible for the nucleophilic addition to the intermediate is dependent on the electronic nature of the other substituent group on the *ortho*-diamine ring (EDG or EWG). Therefore, for substrates with EDG (4-M-1,2-BDA, 4-MB-1,2-DA and 4-BB-1,2-DA), the nucleophilic attack is performed by the *para* amino group, while for substrates with EWG (3,4-DABA and 3,4-DABN) the nucleophilic attack is attributed to the *meta* amino group. After a second oxidation step, also expected to be mediated by the laccase, the process continues by an intramolecular cyclization with the concomitant displacement of the R group (OCH_3, COOH and Br), followed by hydrogen shift giving rise to the asymmetric phenazines. The loss of R group in one of the two substrate molecules is consistent with similar observations on the formation of the phenoxazine cores. [25b,30a,33]

The tricyclic phenazine nuclear core of compound (***10***) is also obtained by the intramolecular Michael addition, but no further demethylation occurs and the presence of the methyl group at C12 blocks the final dehydrogenation, as already suggested by Tomoda *et al*.[31a]

For the diamine substituted with a cyano group (3,4-DABN), the final phenazine ***8*** is obtained from the intermediate (**B'**) following a different pattern. The structural characterization of the product ***8***, showed a trimeric structure with the presence of three cyano groups on the molecule, which is not compatible with the loss of the R group as mentioned above. In this case, a third precursor molecule is inserted by nucleophilic attack before intramolecular cyclization. Several positions on the diimine (**B'**) are susceptible to nucleophilic attack and the selected site depends on the nucleophile and the structure of the diimine. For electronic and steric reasons, the nucleophilic attack occurs on the C1 position rather than C4, followed by an intramolecular cyclization involving the imino group and the carbon bearing the amino group. The displacement of the NH_2 group and rearomatization yields the final product. The formation of *N*-substituted phenazine ***11*** from the precursor ***2*** follow the pathway presented for the formation of phenazines (**5-7**).

The dihydrophenozaxinone ***12*** and the pyridyloxazinone ***13*** can also be obtained by a similar pathway to the one reported for the formation of products ***5-7*** and ***10*** respectively and in agreement with previously reported results.[25b]

Conclusion

In this work the formation of asymmetric phenazines by a one-step sustainable and environmental friendly synthesis from commercially available starting materials was described. The CotA-laccase enzymatic system was shown to be selective towards the synthesis of phenazine derivatives using *ortho*-diamine derivatives with electron-rich and electron-poor substituents, as substrates. Importantly, the enzymatic process showed attractive advantages including mild reaction conditions and short reaction times with excellent yields when compared to the established chemical oxidative methods.

The identification and characterization of products provided a basis for the proposed oxidative enzymatic pathway of substituted aromatic amines. The first oxidative step generates the electrophilic *ortho*-quinonediimine (or *ortho*-quinoneimine) intermedia-te, which further reacts with a nucleophilic partner (a precursor molecule that has not been oxidized) followed by intramolecular cyclization processes.

The scope of aromatic substrates that can be transformed by this enzymatic approach was further extended with the introduction of *N*-substituted *ortho*-diamines and the heterocyclic pyridine derivative, allowing the formation of *N*-substituted phenazine and a pyridyloxazinone. The formation of these products also contributes to increase the variety of heterocyclic cores that can be obtained by this methodology establishing this enzymatic route as a valuable alternative and a promising approach for the rational synthesis of different heterocyclic scaffolds.

Experimental Section

All reagents were from analytical grade, acquired from commercial suppliers and used without further purification. 1D (^1H and ^{13}C) and 2D (COSY, HSQC and HMBC) NMR spectra were recorded on a Bruker Avance (400 MHz) spectrometer in CD_3OD-d_4, $(CD_3)_2CO$-d_6 and CD_2Cl_2-d_2 as solvents. Chemical shifts are reported in ppm relative to the solvent peaks and coupling constants (*J*) are reported in hertz. Resonance and structural assignments were based on the analysis of coupling patterns, including the $^{13}C-^1H$ coupling profiles obtained in bidimensional heteronuclear multiple bond correlation (HMBC) and heteronuclear single quantum coherence (HSQC) experiments, performed with standard pulse programs. Low resolution mass spectra were recorded on a LCQ Fleet, Thermo Scientific Ion Trap mass spectrometer, operated in the electrospray ionization (ESI) positive/negative ion modes. The optimized parameters were as following: ion spray voltage, ±4.5 kV; capillary voltage, +16 and -20 V; tube lens offset, -63 and +82 V; sheath gas (N_2), 80 arbitrary units; auxiliary gas, 5 arbitrary units; capillary temperature,

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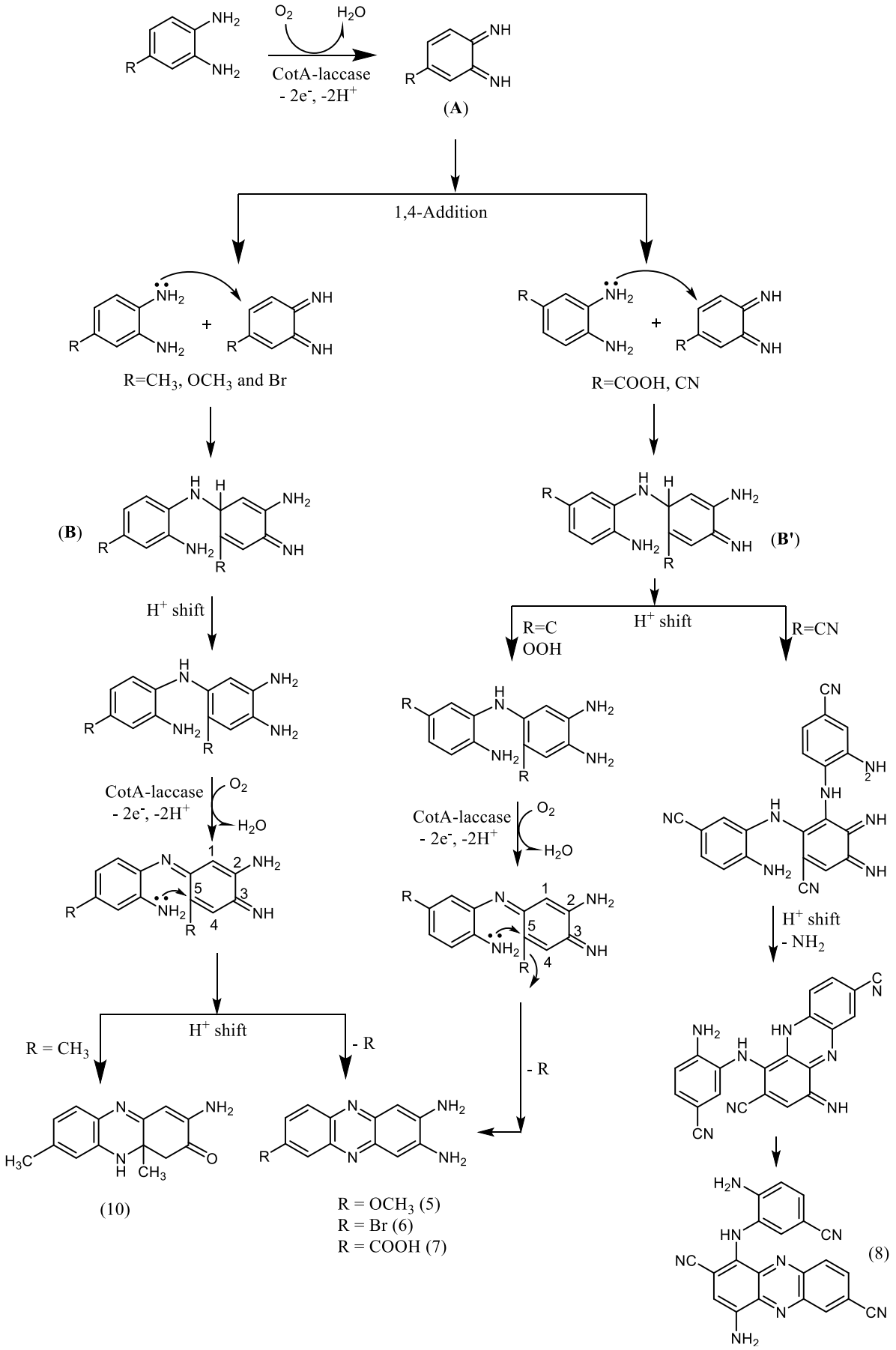

Scheme 2 – Proposed pathways to asymmetric phenazines through the homocoupling oxidative process of *ortho* substituted diamines mediated by CotA-laccase.

250 °C. The spectra were recorded in the range 100 – 1000 Da. Spectrum typically corresponds to the average of 20–35 scans. Sequential mass spectra was obtained with an isolation window of 2 Da, a 25-30% relative collision energy and with an activation energy of 30 msec. High Resolution ESI(+/-) mass spectra were obtained on a QTOF Impact II™ mass spectrometer (Bruker Daltonics, GMBH; Germany), operating in the high resolution ion mode. Calibration of the TOF analyser was performed with a 10 mM sodium formate calibrant solution. Data was processed using Data Analysis 4.2 software (Bruker Daltonics, Bremen, Germany).

The redox potentials were measured by cyclic voltammetry using an EG&G Princeton Applied Research (PAR) Model 273A potentiostat/galvanostat monitored with the Electrochemistry PowerSuite v2.51 software from PAR. Cyclic voltammograms were obtained using 1 mM of substrates in phosphate buffer (pH 6, 20 mM):ethanol (9:1) using a three-electrode configuration cell with an home-made platinum disk working electrode (1.0 mm diameter), a platinum wire counter electrode and an Ag/AgCl reference electrode (purchased from Radiometer analytical, SAS, France). The potential was scanned from -0.7 to 1.2 V at a scan rate of 100 $mV.s^{-1}$. All measurements were done at room temperature and the solutions were deaerated with dinitrogen. The measured potentials were corrected by +0.197 V to the normal hydrogen electrode (NHE).

General procedure for CotA laccase mediated reactions

Recombinant CotA-laccase from *Bacillus subtilis* was produced and purified as previously described.[34-36]

Reactions were performed in 10 mL of phosphate buffer (pH 6, 20 mM) in the presence or absence of 10% ethanol with 5 mM of the precursor molecules (***1a-1e, 2-4***) (4-M-1,2-BDA, 4-MB-1,2-DA, 4-BB-1,2-DA, 3,4-DABA, 3,4-DABN, N-P-1,2-DA, 2-AMP and 2-APy), at room temperature with stirring and started by adding $1U.mL^{-1}$ of purified CotA-laccase. 1U is defined as the amount of enzyme that transformed 1µmol of ABTS per min 37°C. The oxidation of ABTS was followed by an absorbance increase at 420 nm (ε= 3.6 x104 $M^{-1}.cm^{-1}$).[34] The protein concentration was measured using the Bradford assay.[37]

Reactions were monitored by thin layer chromatography (TLC) on aluminum-backed Merck silica gel 60 F254. The insoluble products (***8*** and ***9***) were separated by filtration and dried under vacuum. For the soluble products (***6***, ***7*** and ***11***) the solvent was evaporated under reduced pressure and the products were isolated by solid-phase extraction with ethanol and solvent evaporation. For the other soluble products (***5***, ***10***, ***12*** and ***13***), the aqueous solution was extracted with ethyl acetate and the combined organic phases were collected, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The product mixtures were purified by silica preparative or column chromatography. Control reactions in the absence of enzyme were also performed to check for the auto-oxidation of substrates.

Oxidation of 4-methoxybenzene-1,2-diamine (4-MB-1,2-DA):

After 2h of reaction, the brown solution was extracted with acetyl acetate (3 x 10 mL) and after solvent evaporation, a pure brown solid (***5***) was obtained.

5: yield: 5.8 mg (0.006 mmol, 96 %); ^{1}H NMR (CD_3OD-d_4) δ(ppm) = 7.84 (d, 1H, *J* = 9.2 Hz, H9), 7.29 (dd, 1H, *J* = 2.4 Hz and *J* = 9.2 Hz, H8), 7.22 (dd, 1H, *J* = 2.4 Hz, H6), 7.02 (s, 1H, H4), 6.97 (s, 1H, H1), 3.97 (s, 3H, CH_3).^{13}C{H} NMR (CD_3OD-d_4) δ(ppm) = 161.4 (C7), 147.7 (C2, C11), 144.9 (C3, C12), 141.8 (C13), 136.9 (C14), 129.3 (C9), 122.6 (C8), 104.1 (C4), 103.8 (C6), 101.2 (C1) and 56.3 (CH_3). ESI-MS positive mode: *m/z* 241 $[M+H]^+$; MS^2 *m/z* 226 $[M+H\text{-}CH_3^{\bullet}]^{\bullet+}$: MS^3 *m/z* 198 $[M+H\text{-}CH_3^{\bullet}\text{-}CO]^{\bullet+}$. ESI-HRMS: *m/z* calcd. for $[C_{13}H_{12}N_4O+H]^+$: 241.1084; found 241.1077.

Oxidation of 4-bromobenzene-1,2-diamine (4-BB-1,2-DA):

After 2h of reaction, the green olive solution was evaporated under reduced pressure and the crude extracted with ethanol to yield a pure olive green solid (***6***).

6: yield: 5.2 mg (0.018 mmol, 73 %); ^{1}H NMR (Acetone-d_6) δ(ppm) = 7.36 (s, 1H, H1), 6.97 (d, 1H, *J* = 2.1Hz, H6), 6.73 (dd, 1H, *J* = 8.4 Hz and 2.1 Hz, H8), 6.57 (d, 1H, *J* = 8.4 Hz, H9), 6.03 (s, 1H, H4), 5.89 (bs, 2H, NH_2) and 4.71 (bs, 2H, NH_2). ESI-MS positive mode: *m/z* 289/291 $[M+H]^+$. ESI-HRMS: *m/z* calcd. for $[C_{12}H_9BrN_4+H]^+$: 289.0083; found 289.0075.

Oxidation of 3,4-diaminobenzoic acid (3,4-DABA):

After 2h of reaction, the brown solution was evaporated under reduced pressure and crude residue extracted with ethanol to afford a pure brown solid (***7***).

7: yield: 5.9 mg (0.023 mmol, 93 %); ^{1}H NMR (CD_3OD-d_4) δ(ppm) = 8.64 (s, 1H, H9), 8.15 (d, 1H, *J* = 9.2 Hz, H7), 7.95 (d, 1H, *J* = 9.2 Hz, H6), 7.03 (s, 1H, H4), 7.01 (s, 1H, H1). ^{1}H NMR (D_2O^a) δ(ppm) = 7.46 (dd, 1H, *J* = 2.0 Hz, H9), 7.44 (dd, 1H, *J* = 2.0 Hz and *J* = 8.4 Hz, H7), 7.44 (d, 1H, *J* = 8.4 Hz, H6), 6.89 (s, 1H, H4), 6.87 (s, 1H, H1). ^{13}C{H} NMR (D_2O^a) δ(ppm) = 173.7 (COOH), 139.3 (C11, C12), 129.6 (C13, C14), 125.4 (C7), 124.3 (C6), 119.9 (C9), 116.3 (C1, C4); ESI-MS positive mode: *m/z* 255 $[M+H]^+$; MS^2 *m/z* 211 $[M+H\text{-}CO_2]^+$. ESI-HRMS: *m/z* calcd. for $[C_{13}H_{10}N_4O_2+H]^+$: 255.0877; found 255.0873.

[a] referenced to $(CD_3)_2CO$-d_6 solvent.

Oxidation of 3,4-diaminobenzonitrile (3,4-DABN):

After 3h of reaction, a dark orange solid was isolated by filtration and purified by preparative chromatography (ethyl acetate:hexane 2:1 v/v) affording two isolated fractions. The first eluted yellow fraction and the second eluted orange fraction were concentrated under vacuum to afford compounds ***9*** and ***8*** respectively.

8: dark orange solid ; yield: 3.0 mg (0.008 mmol, 48 %); ^{1}H NMR (Acetone-d_6) δ(ppm) = 8.57 (s, 1H, H4), 8.26 (d, 1H, *J* = 8.8 Hz, H2), 7.92 (d, 1H, *J* = 8.8 Hz, H1), 7.62 (s, 1H, H7), 7.52 (d, 1H, *J* = 9.2 Hz, H18), 7.07 (d, 1H, *J* = 9.2 Hz, H17), 6.91 (bs, 2H, NH_2), 6.28 (s, 1H, H20), 5.96 (bs, 2H, NH_2). ^{13}C{H} NMR (Acetone-d_6) δ(ppm) = 158.3 (C12), 157.8 (C11), 151.1, 150.4, 143,9 (C16), 140.5 (C6), 136.2, 135.2 (C4), 134.3 (C18), 133.5 (C7), 132.2 (C2),

128.6, 127.2 (C1), 123.7, 120.0, 118.8, 117.4, 116.6, 115.2 (C17), 98.9 (C8), 95.8 (C20). ESI-MS negative mode: *m/z* 375 $[M-H]^-$; MS^2 *m/z* 348 $[M-H-HCN]^-$. ESI-HRMS: *m/z* calcd. for $[C_{21}H_{12}N_8-H]^-$: 375.1112; found 375.1123.

9: dark yellow solid ; yield: 3.2 mg (0.012 mmol, 49 %); ^{1}H NMR (Acetone-d_6) δ(ppm) = 8.17 (s, 2H, H3, H3'), 7.45 (d, 2H, *J* = 8.8 Hz, H5, H5'), 7.07 (bs, 4H, NH_2), 7.02 (d, 2H, *J* = 8.8 Hz, H6, H6'). ^{13}C{H} NMR (Acetone-d_6) δ(ppm) = 150.2 (C2, C2'), 136.7 (C1, C1'), 135.2 (C5, C5'), 127.0 (C3, C3'), 120.2 (C2, C2'), 118.4 (C7, C7'), 98.9 (C4, C4'). ESI-MS negative mode: *m/z* 261 $[M-H]^-$; MS^2 *m/z* 233 $[M-H-N_2]^-$. ESI-HRMS: *m/z* calcd. for $[C_{14}H_{10}N_6-H]^-$: 261.0894; found 261.0898.

Oxidation of 4-methyl-1,2-benzenediamine (4-M-1,2-BDA):
After 1h30m of reaction, the red solution was extracted with ethyl acetate (3 x 10 mL) and after solvent evaporation, a dark red solid (***10***) was obtained.
10: Dark red solid; yied: 5.4 mg (0.022 mmol, 91 %); ^{1}H NMR (CD_3OD-d^4) δ(ppm) = 7.39 (d, 1H, *J* = 8.4 Hz, H9), 6.92 (d, 1H, *J* = 7.6 Hz, H8), 6.76 (s, 1H, H6), 6.66 (s, 1H, H1), 3.27 (d, 1H, *J* = 14.4 Hz, H4a), 3.09 (d, 1H, *J* = 16.4 Hz, H4b), 2.25 (s, 3H, CH_3) and 1.24 (s, 3H, CH_3). ESI-MS positive mode: *m/z* 242 $[M+H]^+$; MS^2 *m/z* 227 $[M+H-CH_3^{\bullet}]^{\bullet+}$, 214 $[M+H-CO]^+$. ESI-HRMS: *m/z* calcd. for $[C_{14}H_{15}N_3O+H]^+$: 242.1288; found 242.1287.

Oxidation of 2-*N*-phenylbenzene-1,2-diamine (N-P-1,2-DA):
After 2h of reaction, the brown solution was evaporated under reduced pressure and the crude extracted with ethanol to yield a pure brown solid (***11***).
11: yield: 8.5 mg (0.003 mmol, 94 %); ^{1}H NMR (Acetone-d_6) δ(ppm) = 7.43 (t, 4H, *J* = 10.4 Hz, H7, H8, H23, H25), 7.30-7.18 (m, 5H, H6, H9, H22, H24, H26), 6.91 (t, 3H, *J* = 10.8 Hz H17, H18, H19), 6.77 (dd, 2H, *J* = 8.0 Hz, *J* = 2.8 Hz, H16, H20), 6.70 (s, 1H, H1), 6.15 (s, 1H, H4) and 5.88 (bs, 1H, NH). ^{13}C{H} NMR (Acetone-d_6) δ(ppm) = 159.1 (C2), 149.5 (C12), 147.7 (C15), 143.1 (C13), 138.1, 129.1 (C7, C8, C23, C25), 128.6, 128.5, 125.1 (C22, C26), 124.6 (C6, C9), 121.6 (C1), 120.9 (C16), 120.4 (C20), 120.2 (C17, C18, C19), 119.1, 118.7 (C24), 99.8 (C4). ESI-MS positive mode: *m/z* 363 $[M+H]^+$. ESI-HRMS: *m/z* calcd. for $[C_{24}H_{18}N_4+H]^+$: 363.1604; found 363.1606.

Oxidation of 2-amino-4-methylphenol (2-AMP):
After 2h of reaction, a yellow solution, with a slight precipitate, was obtained. The product was extracted with ethyl acetate (3 x 10 mL) and after solvent evaporation, the residue purified by silica gel column chromatography (ethyl acetate:hexane 3:1 v/v) to afford the yellow solid ***12***.
12: Yellow brownish solid; yield: 5.1 mg (0.021 mmol, 88 %); ^{1}H NMR (CD_2Cl_2) δ(ppm) = 6.78 (s, 1H, H9), 6.66 (d, 1H, *J* = 7.6 Hz, H6), 6.61 (d, 1H, *J* = 7.6 Hz, H7), 5.32 (s, 1H, H1), 3.60 (d, 1H, *J* = 15.6 Hz, H4a), 3.40 (d, 1H, *J* = 15.6 Hz, H4b), 1.26 (s, 3H, CH_3) and 1.05 (s, 3H, CH_3). ^{13}C{H} NMR (CD_2Cl_2) δ(ppm) = 181.4 (C3), 163.7 (C2), 141.1 (C13), 132.8 (C6), 126.4 (C7), 117.2 (C8, C9), 106.6 (C1), 72.1 (C12), 51.7 (C4), 23.7 (CH_3) and 20.9 (CH_3). ESI-MS positive mode: *m/z* 244 $[M+H]^+$; MS^2 *m/z* 226 $[M+H-H_2O]^+$, 216 $[M+H-CO]^+$. ESI-HRMS: *m/z* calcd. for $[C_{14}H_{13}NO_3+H]^+$: 244.0968; found 244.0961.

Oxidation of 2-aminopyridin-3-ol (2-APy):
After 2h, the reaction was completed and the yellow solution was extracted with ethyl acetate (3 x 10 mL) and after solvent evaporation, afforded the pure yellow solid (***13***).
13: yield: 5.2 mg (0.024 mmol, 97 %); ^{1}H NMR (MeOD-d_4) δ(ppm) = 8.54 (d, 1H, *J* = 8.4 Hz, H7), 7.93 (d, 1H, *J* = 8.4 Hz, H9), 7.51-7.47 (m, 1H, H8), 6.55 (s, 1H, H1).^{13}C{H} NMR (MeOD-d_4) δ(ppm) = 147.6 (C7), 121.7 (C9), 125.6 (C8), 106.6 (C1). ESI-MS positive mode: *m/z* 215 $[M+H]^+$; ESI-HRMS: *m/z* calcd. for $[C_{10}H_6N_4O_2+H]^+$: 215.0564; found 215.0548.

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