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Toluene Dioxygenase-Catalyzed cis-Dihydroxylation of Quinolines: A Molecular Docking Study and Chemoenzymatic Synthesis of Quinoline Arene Oxides

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Molecular docking studies of quinoline and 2-chloroquinoline substrates at the active site of toluene dioxygenase (TDO), were conducted using Autodock Vina, to identify novel edge-to-face interactions and to rationalize the observed stereoselective cis-dihydroxylation of carbocyclic rings and formation of isolable cis-dihydriodiol metabolites. These in silico docking results of quinoline and pyridine substrates, with TDO, also provided support for the postulated cis-dihydroxylation of electron-deficient pyridyl rings, to give transient cis-dihydriodiol intermediates and the derived hydroxyquinolines. 2-Chloroquinoline cis-dihydriodiol metabolites were used as precursors in the chemoenzymatic synthesis of enantiopure arene oxide and arene dioxide derivatives of quinoline, in the context of its possible mammalian metabolism and carcionogenicity.

Keywords: arene oxides, cis-dihydriodols, dioxygenase, docking, biocatalysis

INTRODUCTION

Quinoline and substituted quinolines are widely distributed in the environment as urban particulates, resulting from partial combustion of fossil fuels and tobacco. Quinoline 1 is a mammalian hepatocarcinogen and a bacterial mutagen, with its metabolites binding covalently to DNA. Possible pathways responsible for these biological effects continue to be of interest (Hollstein et al., 1978; Tada et al., 1980, 1982; LaVoie et al., 1983; Agarwal et al., 1986, 1990; Willems et al., 1992; Saeki et al., 1993; Reigh et al., 1996; Suzuki et al., 2000; Dowers et al., 2004; Hakura et al., 2007; Diaz Duran et al., 2015; Matsumoto et al., 2018). Cytochrome P-450 (CYP-450) monooxygenases have been identified as responsible for catalyzing epoxidation and dearomatization of quinolines, during mammalian liver metabolism.

Bacterial cell metabolism can also involve an initial dearomatization step of quinolines, via arene dioxygenase-catalyzed cis-dihydroxylation, resulting in the isolation of stable, and postulation of transient, azaarene metabolites. This is exemplified by the metabolism of quinoline 1 (X = H), and substituted quinolines, which were studied earlier using different bacterial strains and arene dioxygenase enzyme types (Boyd et al., 1987, 1993, 1998, 2002; Bott et al., 1990; Fetzner et al., 1993; Kaiser et al., 1996; Zia et al., 2016).
Toluene dioxygenase (TDO), naphthalene dioxygenase (NDO), and biphenyl dioxygenase (BPDO) were among those bacterial enzymes found to catalyze the formation of cis-dihydrdiol metabolites and monohydroxylated derivatives of quinoline substrates (Boyd et al., 2002). The UV4 mutant strain of *Pseudomonas putida*, expressing TDO, was used in the biotransformation of quinoline 1 (X = H) to give cis-dihydridiols 2 (33% relative yield) and the less stable cis-dihydridiols 3 (1% relative yield, **Figure 1**) (Boyd et al., 1987, 1993). As the latter metabolite readily decomposed to 8-hydroxyquinoline 4 (27% relative yield), similar proportions of the cis-diols 2 and 3 were assumed to be initially formed from dihydroxylation of the carbocyclic ring of quinoline 1.

No direct evidence for cis-dihydroxylation of the electron-poor pyridyl ring was found during analysis of the biotransformation products (Boyd et al., 1987, 1993). The combined relative yields of the isolated achiral metabolites 3-hydroxyquinoline 5 (13%) and anthranilic acid 6 (27%), were however consistent with the initial formation of the transient cis-dihydridiols 7 as a further major initial metabolite (**Figure 1**). Formation of 3-hydroxyquinoline 5 could result from spontaneous dehydration of intermediate 7 and of anthranilic acid 6 from enzymatic ring-cleavage (ERC) of 3-hydroxyquinoline 5. If anthranilic acid 6 and phenol 5 were derived from intermediate cis-dihydridiol 7, the relative ratio of initially formed cis-dihydridiols diols 2, 3, and 7 would be estimated as ca. 33:27:40. Further in silico support for the formation of transient cis-dihydridiol 7 - the major metabolite of quinoline 1 (TDO as biocatalyst), was obtained from a joint study with a collaborating laboratory using the GOLD molecular docking program (unpublished data). This prompted our interest in employing the Autodock Vina program for the current study.

Indirect evidence for the undetected heterocyclic cis-dihydridiol 7 was acquired from the results of an earlier biotransformation (**Figure 1**) of 3-deuteroquinoline 1 (X = D; H) yielding 4-deuterioquinolin-3-ol 8 (23% D) along with other metabolites (**Figure 1**) (Barr et al., 1998). This could be accounted for by the migration and partial retention of deuterium, from the aromatization of the intermediate cis-dihydridiol 7 (X = D, **Figure 1**) via an NIH shift mechanism, as observed during aromatization of the isolated carbocyclic cis-dihydridiols of naphthalene and quinoline 2 and 3.

*Pseudomonas* strains are among the most common quinoline-degrading bacteria. Monohydroxylation at the C-2 position of quinoline often occurs during *Pseudomonad* biotransformations, to yield 2-hydroxyquinoline, which prefers to exist as the 2-quinolone tautomer (Kaiser et al., 1996). Substitution at the C-2 position could thus, in principle, improve the isolated yields of cis-dihydriodiol metabolites resulting from dioxygenase-catalyzed biotransformations of quinoline substrates. It may be a factor in: (i) the excellent isolated yield (80%) of the corresponding 7,8-cis-dihydridiol obtained from NDO-catalyzed biotransformation of 2-cyanoquinoline (Zia et al., 2016) and (ii) the improved isolated yields (35–45%) of cis-dihydridiol metabolites of 2-chloroquinoline (with TDO) and 2-methoxyquinoline (with BPDO) (Boyd et al., 2002), relative to the very low isolated yields of cis-dihydridiols 2 and 3 obtained from quinoline 1 (X = H, <5% using TDO, **Figure 1**) (Boyd et al., 1993).

Biotransformations of 2-chloroquinoline 8, conducted using *P. putida* UV4 whole cells resulted in the isolation of multigram quantities of cis-diol metabolites 9, 10, and 11, possibly via intermediate 12, and traces of 2-quinolone 13 (**Figure 2**) (Boyd et al., 1998, 2002). Since the enantiopure cis-dihydriodiol 9, 10, and 11 are more stable, and are available in much higher yields compared with quinoline cis-dihydriodiol 2 and 3, they are herein used in the chemoenzymatic synthesis of enantiopure mammalian metabolites of quinoline 1.

While the dioxygenase-catalyzed cis-dihydroxylation of electron-rich furan and thiophene rings has been reported, to give heterocyclic dihydriodiol metabolites (Boyd et al., 2012; Lewis, 2016), little evidence is available for the deamoratization of electron-poor pyridine rings by a similar mechanism. The possibility that TDO-catalyzed cis-dihydroxylation of a pyridyl ring was proposed as a result of *P. putida* UV4 biotransformations of: (i) quinoline 1, to yield 3-hydroxyquinoline 5 via unstable intermediate 7 (**Figure 1**) (Boyd et al., 1987, 1993), (ii) 2-chloroquinoline 8, to yield cis-diol 11 via transient intermediate 12 (**Figure 2**) (Boyd et al.,...
1998, 2002), and (iii) 2-chloropyridine 14, to yield 2-chloro-3-hydroxyquinoline 16 via undetected intermediate 15 (Figure 3) (Garrett et al., 2006). Since none of the cis-dihydriodiol 7, 12, and 15 could be detected directly, evidence for their intermediacy is sought through the current in silico molecular docking studies of TDO with the corresponding substrates, quinoline 1, 2-chloroquinoline 8 and 2-chloropyridine 14 (Results section).

Major objectives of this study were to: (i) review proposed metabolic pathways of quinoline 1 and 2-chloroquinoline 8 by P. putida UV 4 cells, and to compare with TDO docking results (ii) use the isolated cis-dihydriodiol metabolites 9 and 10 and their derivatives, in the quest for improved chemoenzymatic synthetic routes to enantiopure arene oxide and dioxide derivatives of quinoline 1.

### METHODS

#### Laboratory Studies

$^1$H and $^{13}$C NMR spectra were recorded on Bruker Avance DPX-300 and DPX-500 instruments. Chemical shifts ($\delta$) are reported in ppm relative to SiMe$_4$ and coupling constants ($J$) are given in Hertz (Hz). Mass spectra were run at 70 eV, on an AEI-MS90 mass spectrometer updated by VG Autospec, using a heated inlet system. Accurate molecular weights were determined by the peak matching method, with perfluorokerosene as the standard. Elemental microanalyses were carried out on a Perkin-Elmer 240 CHN microanalyser and IR spectra were recorded in KBr disc or in thin film, using a Perkin-Elmer Spectrum RX1 FT-IR spectrometer. ECD spectra were obtained using a Jasco J-720 instrument and MeCN as solvent. Optical rotations ($[\alpha]_D$) measurements ($10^{-1}$ deg cm$^2$ g$^{-1}$) were carried out at ambient temperature on a Perkin-Elmer 214 polarimeter and specified solvent concentration (g/100 ml) at sodium D-line (589 nm). Melting points were recorded in degrees Celsius using a Stuart SMP10 melting point apparatus. Column chromatography and preparative layer chromatography (PLC) were performed on Merck Kieselgel type 60 (250–400 mesh) and PF$_{254}$/366, respectively. Merck Kieselgel type 60F$_{254}$ analytical plates were used for TLC.

The structures discussed in this section are found in Figures 8, 9, 11. Compounds 1, 4, 5, 6, 8, 13, 14, 16, 17, 19, 22, 23, 24 were purchased commercially and cis-dihydriodiol (+)-9 and (+)-10, arene oxides (+)-20 and (+)-25, were available from earlier studies (Agarwal et al., 1990; Boyd et al., 1991a, 1994, 2002).

(--)(5R,6S)-2-Chloro-5,6-dihydroquinoline-5,6-diol 9 (Boyd et al., 1998, 2002)

mp 120–122°C (EtOAc/hexane); $[\alpha]_D$ +140 ($c 0.40$, MeOH); $^1$H NMR $\delta$H (500 MHz, CDCl$_3$) 2.05 (1H, br s, OH), 2.71 (1H, br s, OH), 4.38 (1H, dd, $J$ 5.0, 5.0, H-6), 4.77 (1H, d, $J$ 5.0, H-5), 6.45 (1H, dd, $J$ 5.0, 9.9, H-7), 6.68 (1H, d, $J$ 9.9, H-8), 7.21 (1H, d, $J$ 8.0, H-3), 7.85 (1H, d, $J$ 8.0, H-4).

(+)-(7S,8R)-2-Chloro-7,8-dihydroquinoline-7,8-diol 10 (Boyd et al., 1998, 2002)

Colorless crystalline solid, mp 112°C (from EtOAc/hexane); $[\alpha]_D$ +148 ($c 0.5$, MeOH); $^1$H NMR $\delta$H (500 MHz, CDCl$_3$) 2.61 (1H, br s, OH), 4.32 (1H, br s, OH), 4.46 (1H, dd, $J$ 5.0, 5.0, H-6), 4.75 (1H, d, $J$ 5.0, H-7), 6.30 (1H, dd, $J$ 9.6, 5.0, H-6), 6.63 (1H, d, $J$ 9.6, H-5), 7.25 (1H, d, $J$ 8.0, H-3), 7.42 (1H, d, $J$ 8.0, H-4).

(--)-(5R,6S)-5,6-Epoxy-5,6-dihydroquinoline 20 (Agarwal et al., 1990; Boyd et al., 1991a, 1994)

mp 68–71°C (pentane) [lit.rac. (Agarwal et al., 1990) 33–36°C]; $[\alpha]_D$ +23 ($c 0.9$, CHCl$_3$); $^1$H NMR $\delta$H (300 MHz, CDCl$_3$) 4.16 (1H, m, H-6), 4.49 (1H, d, $J$ 3.7, H-5), 6.73 (1H, dd, $J$ 9.9, 3.8, H-7), 6.98 (1H, d, $J$ 9.9, H-8), 7.24 (1H, d, H-7), 7.90 (1H, dd, $J$ 7.60, 1.4 H-4), 8.62 (1H, dd, $J$ 4.9, 6.0, 1.4 H-2).

(+)-(7S,8R)-7,8-Epoxy-7,8-dihydroquinoline 25 (Agarwal et al., 1990; Boyd et al., 1994)

mp 54–56°C (pentane) [lit.rac. (Agarwal et al., 1990) 44–46°C]; $[\alpha]_D$ +55 ($c 1.1$, CHCl$_3$); $^1$H NMR $\delta$H (300 MHz, CDCl$_3$) 4.19
Hydrogenation/Hydrogenolysis of cis-dihydrodiols (+)-9 and (+)-10 to Yield Tetrahydrodiols (–)39 and (–)-40
(5R,6S)-5,6,7,8-Tetrahydroquinoline-5,6-diol 39 (Boyd et al., 1993)

cis-Dihydrodiol metabolite 9 (30 mg, 0.15 mmol) was dissolved in MeOH (8 cm³) and 10% Pd/C (0.5 mg) was added. The mixture was stirred under a H₂ atmosphere at reduced pressure. Aqueous Na₂CO₃ solution (5%) was added to the residue, the mixture saturated with NaCl and then extracted into EtOAc (3 × 10 cm³). The combined organic layers were dried (Na₂SO₄) and the solvent evaporated to furnish (5R,6S)-diol 39 (19 mg, 81%); mp 138–140°C (EtOAc) [lit. (Boyd et al., 1993) 138–140°C]; [α]D = −7.0 (c 0.5, MeOH); ¹H NMR (300 MHz, CDCl₃) δH 1.96 (1H, m, H-7), 2.16 (1H, m, H-7), 2.85 (1H, m, H-8), 3.09 (1H, m, H-8), 4.1 (1H, m, H-6), 4.35 (2H, bs, 2 × OH) 4.67 (1H, d, J 3.4, H-5), 7.13 (1H, dd, J 4.8, 7.7, H-3), 7.79 (1H, dd, J 1.1, 7.2, H-4), 8.35 (1H, dd, J 1.5, 4.7, H-2).

(–)-(7S,8R)-5,6,7,8-Tetrahydroquinoline-7,8-diol 40 (Boyd et al., 1993)

Similar treatment of cis-dihydrodiol 10 (30 mg, 0.15 mmol) yielded (7S,8R)-diol 40 (21 mg, 81%), mp 137–139°C (EtOAc) [lit. (Boyd et al., 1993) 138–140°C]; [α]D = −72.0 (c 0.47, MeOH); ¹H NMR (300 MHz, CDCl₃) δH 1.95 (1H, m, H-7), 2.22 (1H, m, H-6), 2.75 (1H, m, H-5), 3.20 (1H, m, H-5), 4.32 (1H, m, H-7), 4.92 (1H, d, J H-8), 7.17 (1H, dd, J 4.7, 7.7, H-3), 7.46 (1H, d, J 7.7, H-4), 8.40 (1H, J 4.7, H-2).

Synthesis of (–)-(5R,6R,7R,8R)-quinoline-5,6,7,8-dioxide 28 (Boyd et al., 1991b)

Treatment of (–)-(5R,6S)-5,6-epoxy-5,6-dihydroquinoline 20 (120 mg, 0.8 mmol) in CH₂Cl₂ with aqueous sodium hypochlorite in the presence of phase transfer reagent (t-Bu₄NHSO₄) and workup, under identical conditions earlier in the synthesis of racemic anti-quinoline dioxide 29, produced (–)-29 (51mg, 38%). White crystalline solid (ether-hexane); mp 85–86°C [lit.rac. (Boyd et al., 1991b), 44–46°C]; [α]D = −100 (c 0.11, CHCl₃); HRMS calcld for C₁₁H₁₀NO₂, 161.0477, M⁺; found, 161.0472; ¹H NMR (300 MHz, CDCl₃) δH 3.73 (1H, d, J 3.9, H-6b), 3.91 (1H, d, J 4.0, H-2a), 4.05 (1H, m, H-6), 4.08 (1H, m, H-1b), 7.28 (1H, dd, J 7.7, 4.8, H-5), 7.73 (1H, dd, J 7.5, 1.5, H-6), 8.53 (1H, dd, J 4.8, 1.5, H-4); ¹³C NMR (125 MHz, CDCl₃) δC 50.9, 53.2, 54.7, 55.5, 123.9, 127.7, 138.7, 149.8, 152.4; m/z 161 (M⁺, 15%), 132 (100). Electronic CD data: 269 nm (Δε = −3.299), 226 nm (Δε = −14.97), 197 nm (Δε = 11.46).

Synthesis of (+)-(5S,6S,7R,8R)-quinoline-5,6,7,8-dioxide 28 (Boyd et al., 1991b)
(+)-(7S,8R)-7,8-Epoxy-7,8-dihydroquinoline 25 (120 mg, 0.8 mmol), it was reacted with N-bromosaccharide (130 mg, 1.0 mmol) in THF: water (2:1), under identical reaction conditions reported for a racemic arene oxide 25 (Vila et al., 2017). After extraction (CH₂Cl₂) drying and concentrating, the crude product, the correct NMR spectrum for racemic 6-bromo-5-hydroxy-7,8-epoxy-5,6,7,8-tetrahydroquinoline 41, was then treated directly with sodium methoxide in THF to yield (+)-(5S,6S,7R,8R)-quinoline-5,6,7,8-dioxide 28. White solid mp 153–156°C [lit.rac. (Boyd et al., 1991b) 138–152°C]; [α]D = +25 (c 0.2, CHCl₃); HRMS calcld for C₁₁H₁₀NO₂, 161.0477, M⁺; found, 161.0472; ¹H NMR (500 MHz, CDCl₃) δH 3.95–3.99 (2H, m, H-1a and H-1b), 4.05 (1H, d, J 3.5, H-6b), 4.18 (1H, d, J 3.4, H-2a), 7.38 (1H, m, H-5), 7.95 (1H, dd, J 7.7, 1.6, H-6), 8.65 (1H, dd, J 4.9, 1.6, H-4); m/z 161 (M⁺, 15%), 132 (100). Electronic CD data: 274 nm (Δε = 15.93), 268 nm (Δε = 14.31), 246 nm (Δε = −3.425), 192 nm (Δε = −16.08).

Cis-dihydroxylation of (+)-cis-diol 9 [and 10] and Acetylation to Yield Tetrahydroacetates, (++)-44, (−)-44 [(+)-42 and (−)-45]

To a stirred solution of (+)-cis-dihydrodiol 9 (200 mg, 1.02 mmol) and trimethylamine-N-oxide dihydrate (150 mg, 1.34 mmol) in CH₂Cl₂ (50 ml) was added a catalytic amount of OsO₄ and the mixture stirred overnight at room temperature. After the addition of a 10% solution of sodium metabisulfite (2 ml), the reaction mixture was allowed to stir for another 0.5 h and then concentrated under reduced pressure to give a crude mixture of inseparable syn- and anti-tetraols. The mixture was acetylated by heating (75–80°C), overnight, with excess of acetic anhydride (ca. 2 ml) in pyridine (2 ml) solution. Concentration of the reaction mixture in vacuo, yielded a crude mixture (3:1) of anti-45 and syn-44 tetraacetates. After initial purification of the mixture, using flash chromatography (silica gel, 3% MeOH in CHCl₃), followed by multiple elution PLC (20% EtOAc in hexane) separation gave major anti-tetraacetate (−)-45 and minor syn-tetraacetate (−)-44.

(+)-(5R,6R,7R,8R)-5,6,7,8-Tetraacetoxy-2-chloro-5,6,7,8-tetrahydroquinoline 45

White crystalline solid (121 mg, 30%); (R) 0.3, 30% EtOAc in hexane; mp 87–88°C (from CHCl₃); [α]D = +14.6 (c 0.83, CHCl₃); (Found: C, 50.6; H, 4.7; N, 3.3. C₁₇H₁₄Cl₂NO₆ requires C, 51.1; H, 4.5; N, 3.5%); ¹H NMR (500 MHz, CDCl₃) δH 2.07 (3H, s, OMe), 2.08 (1H, s, OMe), 2.11 (1H, s, OMe), 2.13 (1H, s, OMe), 5.63 (1H, dd, J 10.1, 3.9, H-6), 5.73 (1H, dd, J 10.1, J 4.1, H-7), 6.34 (1H, d, J 3.9, H-5), 6.39 (1H, d, J 4.1, H-8), 7.35 (1H, d, J 8.3, H-3), 7.63 (1H, d, J 8.3, H-4); ¹³C NMR (CDCl₃, 125 MHz) δC 20.5, 20.6, 20.7, 20.8, 66.7, 67.1, 67.3, 67.6, 124.9, 127.1, 139.1, 151.5, 152.3, 169.7, 169.8, 169.9, 170.2, m/z 339 (M⁺, 15%, 35Cl), 43 (100); vmax/cm⁻¹: 1,741 (C=O).
Hydrolysis of Chlorotetraacetates (−)-(−)-44, (+)-44, and (−)-45 to Yield Tetrols (−)-(−)-46, (+)-46, and (−)-48

A solution of tetrol (−)-(−)-42 (200 mg, 0.86 mmol) in MeOH (20 ml) containing 10% Pd/C (50 mg) was stirred overnight at room temperature, under H₂ atmosphere at 1 atm. pressure. The reaction mixture was basified (NH₄ solution), the catalyst was filtered off and the filtrate concentrated under reduced pressure to yield tetrol (−)-(−)-46 (120 mg, 71%), white solid (MeOH); mp 164–165 °C (MeOH); [α]D +25 (c 0.5, MeOH); (Found: C, 53.3; H, 5.3; N, 6.6. CaH₂NO₃ requires C, 54.8; H, 5.6; N, 7.1%); ¹H NMR (500 MHz, D₂O) δH 4.28 (1 H, d, J 4.3, 1.4, H-7), 4.34 (1 H, dd, J 4.0, 1.4, H-6), 4.88 (1 H, d, J 4.3, H-8), 4.93 (1 H, d, J 4.0, H-5), 5.27 (1 H, dd, J 7.9, 4.5, H-3), 7.75 (1 H, d, J 7.9, H-4), 8.16 (1 H, d, J 7.9, H-4), 8.62 (1 H, d, J 4.5, H-2).

Hydrolysis of Chlorotetrols (−)-(−)-42, (+)-42, and (−)-43 to Yield Tetros (−)-(−)-46, (+)-46, and (−)-48

A solution of chlorotetrol (−)-(−)-42 (200 mg, 0.86 mmol) in MeOH (20 ml) containing 10% Pd/C (50 mg) was stirred overnight at room temperature, under H₂ atmosphere at 1 atm. pressure. The reaction mixture was basified (NH₄ solution), the catalyst was filtered off and the filtrate concentrated under reduced pressure to yield tetrool (−)-(−)-46 (120 mg, 71%), white solid (MeOH); mp 164–165 °C (MeOH); [α]D +25 (c 0.5, MeOH); (Found: C, 53.3; H, 5.3; N, 6.6. CaH₂NO₃ requires C, 54.8; H, 5.6; N, 7.1%); ¹H NMR (500 MHz, D₂O) δH 4.28 (1 H, d, J 4.3, 1.4, H-7), 4.34 (1 H, dd, J 4.0, 1.4, H-6), 4.88 (1 H, d, J 4.3, H-8), 4.93 (1 H, d, J 4.0, H-5), 5.27 (1 H, dd, J 7.9, 4.5, H-3), 7.75 (1 H, d, J 7.9, H-4), 8.16 (1 H, d, J 7.9, H-4), 8.62 (1 H, d, J 4.5, H-2).

Synthesis of Dibromohydroxyacetates (−)-(−)-47, (+)-47 and Dibromodiacetate 49

To a stirred solution of tetrol (−)-(−)-46 (100 mg, 0.51 mmol) in dry CH₃CN (5 ml) maintained at ice temperature, was added 1-bromocarbonyl-1-methyl ester acetate (0.15 ml, 1.04 mmol) and the solution continued (2.5 h) at room temperature. The reaction mixture was concentrated in vacuo, the concentrate taken up into ether (20 ml) and washed repeatedly with 2.5% NaHCO₃ solution until the aqueous layer was basic. The combined aqueous layer was back extracted with ether and the combined ether extract dried (Na₂SO₄) and concentrated under reduced pressure. Purification of the crude product by PLC yielded compound (−)-(−)-47 (80 mg, 65%), pale yellow oil; (R₉ 0.25, 55% EtOAc-hexane); [α]D −31 (c 0.54, CHCl₃); HRMS calcld for C₁₁H₁₀NO₃Br, 282.9844, (M – HBr)⁺; found,
282.9839; 1H NMR (500 MHz, CDCl3) δH 2.08 (3 H, s, COCH3), 4.90 (1 H, dd, J 8.3, 2.3, H-6), 5.30 (1 H, d, J 4.0, H-8), 5.40 (1 H, d, J 8.3, H-5), 5.75 (1 H, dd, J 4.1, J 2.3, H-7), 7.32 (1 H, d, J 8.9, H-4), 8.00 (1 H, d, J 8.9, H-4), 8.59 (1 H, d, J 5.6, H-2); 13C NMR (125 MHz, CDCl3) δC 20.9, 46.3, 51.5, 70.3, 74.3, 123.9, 130.2, 139.6, 150.3, 151.2, 169.9; m/z 285 (M-HBr)+, 79Br, 15%), 287 (M+-HBr, 81Br, 15%), 242 (100).

(+)-(5R,6R,7S,8S)-5,8-Dibromo-6-hydroxy-5,6,7,8-tetrahydro-7-quinolinyl acetate 47
A similar treatment procedure on tetrrol (--)46 yielded compound (+)-47 [α]D +30 (c 0.4, CHCl3).

(5S,6S,7S,8S)-6,7-(Diacetoxy)-5,6,7,8-tetrahydroquinoline 49
Similar treatment of (--)46 (0.15 g, 0.78 mmol) with sodium methoxide gave a mixture of two products. PLC separation (50% EtOAc–hexane) yielded quinoline dioxide (--)29 (46 mg, 60%) and bromoquinoline oxide (--)50 (16 mg, 15%).

Synthesis of (5R,6R,7R,8R)-Quinoline-5,6:7,8-dioxide 29 and (5R,6S)-8-bromoquinoline-5,6-oxide 50
From diacetoxy-5,6-dibromo-5,6,7,8-tetrahydroquinoline 49
Similar treatment of dibromodiacetate 49 (200 mg, 0.48 mmol) with sodium methoxide gave a mixture of two products. PLC separation (50% EtOAc–hexane) yielded quinoline dioxide (--)29 (46 mg, 60%) and bromoquinoline oxide (--)50 (16 mg, 15%).

Docking Process
All small molecule structures were created in.pdb format using UCSF Chimera (University of California). The crystal structures of TDO (PDB ID: 3en1) and NDO (1o7M) were obtained from the RCSB Protein Data Bank. The toluene contained in the TDO crystal structure was removed using UCSF Chimera. The dioxygen molecule was added to the iron prosthetic group of TDO (Fe, His222, His228, Asp376) from the iron prosthetic group (Fe, dioxygen, His-208, His-213, and Asp-362) of the TDO crystal structure was removed using UCSF Chimera. The crystal structures in.pdb format were then stripped of water molecules and copy the dioxygen atoms to the 3en1 model. All structures in.pdb format were then stripped of water molecules and converted to .pdbqt format using AutoDock Tools 1.5.6 (Scripps Research Institute).

The docking was performed using AutoDock Vina 1.1.2 (Scripps Research Institute) with the following configuration:

```plaintext
center_x = 46.46
center_y = 120.49
center_z = 201.877
size_x = 16
```
This configuration includes the amino acids within 6 Å of the toluene present in the 3en1 crystal structure: Gln215, Phe216, Met220, His222, Ala223, His228, Leu 272, Ile276, Ile232, Val309, His311, Leu321, Ile324, Phe366, Phe372, and Asp376. Only Met220 is not shown in the docking structures as it blocked the view on the docking orientation and was considered irrelevant for catalytic purposes.

Based on earlier publications and current understanding of the binding site and catalytic mechanism, His 222 is involved in edge-to-face binding of the substrate to direct a planar orientation, presenting a face to the active site. The probability for the methyl group of the toluene substrate to be oriented toward Phe216 is reduced by this residue, thus resulting in the observed enantiomeric excess. His311 and Asn215 are considered to be essential for the catalytic mechanism, as His311 is a H-acceptor and Phe216 is reduced by this residue, thus resulting in the observed enantiomeric excess. His311 and Asn215 are considered to be part of a water channel.

The docking model was assessed by docking toluene and comparing obtained orientations to the toluene position in the crystal structure. A total of nine orientations were obtained when comparing obtained orientations to the toluene position in the crystal structure. A total of nine orientations were obtained ranging from −5.3 to −4.2 kcal/mol. Three orientations emerged that present the 2,3-double bond to the docking with dioxygen, as the crystal structure does not contain dioxygen, allowing the toluene to be closer to the iron prosthetic group. Two identical orientations emerged (see Supplementary Figures S-13A–C) which would result in the opposite enantiomer being formed. Given the enantiomeric excess of >98% of the dihydroxylated product of toluene, it appears unlikely that this orientation is readily dihydroxylated.

RESULTS

Molecular Docking of TDO With Quinoline 1, 2-Chloroquinoline 8, 2-Chloropyridine 14 and Chlorobenzene 17

Molecular docking studies of TDO-catalyzed cis-dihydroxylation of mono- and di-substituted benzene substrates were conducted in other laboratories, using the GOLD program (Vila et al., 2016a,b, 2017). AutoDock and AutoDock Vina programs have similarly been used in our laboratories, to study TDO-catalyzed cis-dihydroxylation of other types of monocyclic arenes, e.g., substituted phenols and anilines and tricyclic heteroarenes, e.g., dibenzofuran and dibenzothiophene (Hoering et al., 2016; Boyd et al., 2017, 2019). Relative free binding energies (kcal/mol) and relative proximity of arene bonds to dioxygen, within the TDO active site (Å), were determined by applying the AutoDock Vina program to monocyclic arenes (toluene, chlorobenzene) and mono- and bicyclic- azarenes in the current study.

Using the AutoDock Vina docking model, to indicate binding energy (kcal/mol) and bond proximity (Å) values of TDO with quinoline substrate 1 (X = H), cis-dihydroxylation of the carbocyclic ring was predicted to occur preferentially at the 5,6- position (Figure 4A, −6.4 kcal/mol, 3.6–3.7 Å) and 7,8- bonds (Figure 4B, −6.0 kcal/mol, 3.6 Å). This would be consistent with formation of similar quantities of the 5R,6S and 7S,8R cis-dihydrodiols 2 and 3, respectively. When the isolated yields of cis-dihydrodiol 3 and the derived 8-hydroxyquinoline 4 are combined, the relative proportions of originally formed cis-dihydrodiol regiosomers 2:3 were similar and 5R,6S and 7S,8R configurations were firmly established (Boyd et al., 1987, 1993).

Alternative in silico orientations of quinoline 1 within the TDO active site (Figure 4C, −6.5 kcal/mol, 3.7–4.2 Å, and (Supplementary Figure 4D, −5.8 kcal/mol, 3.8–4.2 Å), would lead to the incorrect 5S,6R and 7R,8S absolute configurations for isolated metabolites 2 and 3, respectively. Although these orientations had similar binding energies, compared with Figures 4A,B it was presumed that the larger distances between the relevant bonds and proximate dioxygen atom were the determining factors.

Support for the intermediacy of the undetected heterocyclic ring cis-dihydrodiol 7 was provided by the AutoDock Vina-derived prediction that TDO-catalyzed cis-dihydroxylation could occur at the 3,4-bond of the pyridine ring to yield transient intermediate (3S,4R)-7 (Figure 4C, −6.5 kcal/mol, 3.6–3.7 Å). The opposite (3R,4S) enantiomer 7 might also be expected from the orientation of quinoline 1 in Figure 4A. While a similar binding energy (−6.4 kcal/mol) is shown in Figure 4A, this orientation may be less favored, due to a larger distance from the nearest dioxygen atom (3.8–4.3 Å).

In silico data collected using the AutoDock Vina TDO-docking program with 2-chloroquinoline 8 as substrate, similarly led to the prediction that cis-dihydroxylation should occur at the 5,6-position, to give (5R,6S)-dihydrodiol 9 (Figure 5A, −5.8 kcal/mol, 3.7–3.8 Å) and at the 7,8-bond to form (7S,8R)-dihydrodiol 10 (Figure 5B, −6.0 kcal/mol, 3.5–3.6 Å). Alternative docking orientations could also result in cis-dihydroxylation at the 5,6 bond (Figure 5C, −5.1 kcal/mol, 4.1–4.5 Å) and the 7,8 bond (Supplementary Figure 5D, −6.2 kcal/mol, 4.1–4.5 Å), but the resulting cis-diol metabolites 9 and 10 would have the opposite absolute configurations to the isolated metabolites (5R,6S and 7S,8R, respectively). The orientations in Figure 5C and Supplementary Figure 5D were again assumed to be less favored, due to the larger distances between substrate and nearby dioxygen atoms. Thus, the absolute configurations of the quinoline cis-dihydridiols (2, 3, 9, 10) predicted by in silico results were identical to those found earlier for metabolites isolated from P. putida UV4 biotransformations (Boyd et al., 1987, 1993, 1998, 2002).

More evidence for the TDO-catalyzed cis-dihydroxylation of a pyridyl ring was sought by docking studies of 2-chloropyridine 14 and comparison with chlorobenzene 17. Substrates 14 (Figure 6, −4.8 kcal/mol, 3.0–3.6 Å) and 17 (Figure 7, −4.9 kcal/mol, 3.0–3.6 Å) were found to adopt similar orientations, within the
TDO active site, leading to the prediction that the derived (S, S) enantiomers of cis-dihydrodiols 15 and 18, would be formed preferentially. The (2S,3S) configuration for isolated metabolite 18, and its dehydration to yield phenol 16, was already established (Ziffer et al., 1977). The undetected intermediate 15, derived from 2-chloropyridine 14, was assumed to dehydrate more readily to yield the isolated hydroxypyridine 16 (Garrett et al., 2006). These docking results thus support the mechanism shown in Figure 3 (14 → 15 → 16).
Based on the AutoDock Vina results, the orientations within the TDO active site, favored by heterocyclic arene substrates, e.g., 1 (X = H), 8, and 14, as well as substituted benzene substrates e.g., toluene and chlorobenzene 17, all revealed evidence of edge-to-face (T-bonding) interactions with Phe-216 and His-222. The simultaneous edge-to-face interactions, of amino acid residues Phe-216 and His-222, with both the phenyl and pyridyl rings of substrates 1, 8, and 14 in the present context, were noteworthy. Docking distances between the proximate T-bonding H atoms and arene faces of quinoline 1 (Supplementary Figures 4A1-A3) were estimated to be within the range 2.6–2.8 Å, assuming a C-H bond length of 1.08 Å. These inter-ring distances are in accord with the calculated interacting H-to-ring center perpendicular distances, of ca. 2.6–2.8 Å and observed distances ca. 2.70–2.86 Å, from X-ray crystallographic analysis, using a range of model systems (Hoering et al., 2016; Boyd et al., 2019). Keyhole pictures, looking through the rear face of the component aromatic ring, showed both edge to face-T (Supplementary Figures 4A3,B3,C3), and face tilted-T dockings (Supplementary Figures 4A2,B2,C2) of quinoline 1 with TDO. This was consistent with the attractive interactions between phenyl rings (Supplementary Figures 4A1,B1), phenyl/pyridyl rings (Supplementary Figure 4C1), phenyl/imidazoyl rings (Supplementary Figures 4A1,B1), and pyridyl/imidazoyl rings (Supplementary Figure 4C1).

It was concluded that the observed absolute configurations of the isolated carbocyclic ring cis-dihydrodiol metabolites 2, 3, 9, and 10 were in agreement with preferred in silico docking orientations of the corresponding substrates (Figures 4A,B, 5A,B). The preferred orientations of quinoline 1 (Figure 4C), 2-chloroquinoline 8 (Figure 5C), and 2-chloropyridine 14 (Figure 6), would also be supportive of TDO-catalyzed cis-dihydroxylation occurring at the pyridine rings to give the undetected cis-dihydrodiol intermediates 7, 12, and 15.

**Chemoenzymatic Synthesis of Isolated and Potential Arene Oxide Metabolites of Quinoline**

The docking studies described in Figures 4A,B, 5A,B enabled us to develop novel chemoenzymatic approaches for the preparation of different types of chiral azaheteroarene derivatives. The availability of the cis-diol 10 as the major metabolite from TDO-catalyzed cis-dihydroxylation of 2-chloroquinoline 8, resulted in its first use in chemoenzymatic synthesis, when chiral 2,2'-bipyridines and 2,2'-bipyridine N-oxides were produced (Boyd et al., 2008, 2010). These compounds were found to be useful ligands for asymmetric oxidation (→ 97% ee) (Boyd et al., 2008), cyclopropanation (→ 95% ee) (Boyd et al., 2008), aminolysis (→ 84% ee) (Boyd et al., 2010), and allylation reactions (→ 86% ee) (Boyd et al., 2010). Further possible applications of both cis-diol metabolites 9 and 10, to the chemoenzymatic synthesis of detected and possible mammalian metabolites of quinolines, were also developed.

The identification of a 1,2-epoxide, a mammalian metabolite of naphthalene (Jerina et al., 1968), resulting from cytochrome P-450 (CYP-450)-catalyzed epoxidation, led to a quest for other examples of arene oxide metabolites. A similar type of monooxygenase-catalyzed dearomatization was later found to yield epoxide metabolites of both monocyclic arenes (e.g., benzene 1,2-oxide) and polycyclic azaarenes (e.g., quinoline 5,6-oxide) (Agarwal et al., 1986). Arene dioxides, trans-dihydrodiols, trans-tetrahydrodiol epoxides, and tetrahydroarene tetrols were later identified as other mammalian metabolites of naphthalene (Stillwell et al., 1978, 1982; Horning et al., 1980). Further studies established that liver metabolism of larger (tetracyclic and pentacyclic) bay-region arene and heteroarene substrates often involved epoxide intermediates associated with their mutagenicity and tumorigenicity. The metabolic sequence: arenes → arene oxides → trans-dihydrodiols → trans-tetrahydrodiol epoxides → DNA-adducts, became known as the Bay-region Mechanism (Bueining et al., 1978; Karle et al., 2004; Chang et al., 2013).

Identification of mammalian metabolites of quinoline 1 and their role in its mutagenicity and tumorigenicity, has been of interest for more than 40 years (Hallstein et al., 1978; Tada et al., 1980, 1982; LaVoie et al., 1983; Agarwal et al., 1986, 1990; Willems et al., 1992; Saeki et al., 1993; Reigh et al., 1996; Suzuki et al., 2000; Dowers et al., 2004; Hakura et al., 2007; Diaz Duran et al., 2015; Matsumoto et al., 2018). Several quinoline arene oxides, e.g., 20 and 27, were initially postulated as possible liver microsomal metabolites of quinoline 1 (Hallstein et al., 1978; Tada et al., 1980, 1982; LaVoie et al., 1983), before 5,6-arene oxide 20 was finally confirmed as a major metabolite (Figure 8) (Agarwal et al., 1986). Epoxide hydrolyase-catalyzed hydrolysis of the identified 5,6-arene oxide 20 would also account for the isolated trans-dihydrodiol metabolite 21, and aromatization of the undetected 3,4-arene oxide 27 could yield the isolated 3-hydroxyquinoline 5. Transient arene oxides, including compound 27, resulting from CYP-450-catalyzed epoxidation of quinoline 1, were initially postulated to be responsible for its mutagenicity and carcinogenicity (Hallstein et al., 1978; Tada et al., 1980, 1982; LaVoie et al., 1983). Liver microsomal metabolism of quinoline 1 also yielded DNA adducts, which on hydrolysis, under acidic or basic conditions, gave 3-hydroxyquinoline 5. Efforts to identify mammalian metabolic pathways responsible for the mutagenicity (and carcinogenicity) of quinoline 1 (Willems et al., 1992; Suzuki et al., 2000), required the chemical synthesis of identified, and possible, metabolites of similar structures to those derived earlier from naphthalene (e.g., phenols, arene oxides, arene dioxides, trans-dihydrodiols, and trans-tetrahydrodiol epoxides) (Jerina et al., 1968; Stillwell et al., 1978, 1982; Horning et al., 1980).

The syntheses of racemic quinoline arene oxides (20, 25), trans-dihydrodiols (21, 26), syn- and anti- arene dioxides (28 and 29), and trans-diol epoxides (30 and 31), were previously reported (Agarwal et al., 1986; Boyd et al., 1989, 1994; Bushman et al., 1989; Willems et al., 1992) (Figures 8, 10). These chiral compounds, along with achiral metabolites including N-oxide 24 and phenols 4, 5, 22, and 23, were tested for mutagenicity, using the Ames/Salmonella microsome method (Willems et al., 1992; Suzuki et al., 2000). As none of these metabolites or possible metabolites, were found to be more mutagenic than quinoline 1, it was assumed that a metabolic sequence similar to that in the Bay-region Mechanism, involving racemic arene oxides,
FIGURE 8 | Mammalian metabolites of quinoline 1, both isolated (4, 5, 20–24) and postulated (25–27).

e.g., 20 → trans-dihydrodiols 21 → trans-tetrahydrodiol epoxides 31, was unlikely to be involved in its mutagenicity. It was later established that the particular absolute configurations of polycyclic arene metabolites, associated with the Bay-region Mechanism, had a marked effect on their carcinogenicity (Buening et al., 1978; Karle et al., 2004; Chang et al., 2013). The mutagenicity of the enantiopure derivatives of quinoline 20, 21, 25, 26, 28, 29, 30, and 31 was not investigated.

An alternative metabolic pathway for quinoline 1 in mammals was proposed involving dearomatization of the pyridyl ring and formation of the undetected quinoline hydrate 36 (Saeki et al., 1993, 1996, 1997a,b; Suzuki et al., 2000) (Figure 9). While relatively stable arene hydrate derivatives of quinoline were isolated earlier as metabolites of 5,6- and 7,8-dihydroquinolines (Agarwal et al., 1994), compound 36, being both an enamine and an arene hydrate, would be expected to be less stable. The CYP-450-catalyzed epoxidation of enamine intermediate 36 was postulated to give the undetected epoxide 37 and to react with DNA to yield unstable adduct 38, which rearomatized under both acidic and basic conditions to yield 3-hydroxyquinoline 5 (Saeki et al., 1993, 1996, 1997a,b; Suzuki et al., 2000). The metabolic pathway 1 → 36 → 37 → 38 (Enamine Epoxide Theory, Figure 9) was thus proposed to account for the mutagenicity and carcinogenicity of quinoline 1. It is noteworthy that while some substituted quinolines were found to be carcinogenic, 2-chloroquinoline 8 was not (Hirao et al., 1976; Hakura et al., 2007).

In the context of mutagenesis studies of known and possible mammalian metabolites of quinoline 1 (Willems et al., 1992), our multistep chemical synthesis routes to racemic quinoline oxides (28 and 29), diol epoxides (30 and 31), tetrahydrobromohydrins (32 and 34), and derived tetrahydroepoxides (33 and 35) (Figure 10) (Agarwal et al., 1986, 1990; Boyd et al., 1989, 1991b).
While the individual enantiomers of arene oxides 20 and 25 were separable by analytical chiral stationary phase (CSP) HPLC, this analytical scale approach was unsuitable for further chemoenzymatic synthesis studies and therefore alternative preparative methods for obtaining these enantiopure compounds were developed. The chemical resolution of bromohydrin derivatives of 1,2,3,4-polycyclic tetrahydroarenes as MTPA esters provided a generally applicable route to a range of enantiopure arene oxide derivatives, e.g., naphthalene 1,2-oxide and anthracene 1,2-oxide (Akhtar et al., 1979). Further application of this MTPA ester resolution method to racemic bromohydrin 32, yielded enantiopure tetrahydroepoxides (7S,8R)-33 ([α]D +157) and (7R,8S)-33 ([α]D −157) intermediates and finally quinoline 7,8-oxide, (7S,8R)-25 ([α]D +55) and (7R,8S)-25 ([α]D −55, unpublished data). The racemic tetrahydroquinoline bromohydrin 34 was also resolved, but as dibenzo tartrate salts to give enantiomers of quinoline 5,6-oxide (5R,6S)-20 ([α]D −23) and (5S,6R)-20 ([α]D +23) via tetrahydroepoxides (5S,6S)-35 and (5S,6R)-35 (Akhtar et al., 1979). The enantiopurities (>98% ee) of arene oxides 20 and 25 were confirmed by CSPHPLC analysis; more than ten synthetic steps from quinoline 1 were required using these chemical resolution methods.

An alternative chemoenzymatic approach to the synthesis of arene oxide enantiomers 20 and 25, using the quinoline cis-dihydrodiol metabolites (5R,6S)-2 and (7S,8R)-3 (X = H) as precursors was then developed (Boyd et al., 1991a). This again produced the quinoline oxide enantiomers (−)-(5R,6S)-20 and (+)-(7S,8R)-25, in seven steps from quinoline 1, via tetrahydroepoxides (5S,6S)-35 and (7S,8R)-33. Although this chemoenzymatic approach required fewer steps than the earlier methods involving the chemical resolution of bromohydrins 32 and 34 (Boyd et al., 1994), the very low (<5%) isolated yields of the less stable cis-dihydrodiol precursors 2 and 3 obtained by P. putida UV biotransformation (Boyd et al., 1993), was a limiting factor. As the isolated yields of the more stable cis-dihydrodiol metabolites 9 (9%) and 10 (21%), obtained from of 2-chloroquinoline 8 were higher, under similar conditions, they were used as alternative starting compounds to yield cis-tetrahydrodiol intermediates (5R,6S)-39 and (7S,8R)-40 in the current study (Figure 11).

(5R,6S)-cis-Tetrahydrodiol 39 ([α]D = −7, CHCl3) and (7S,8R)-cis-tetrahydrodiol 40 ([α]D = −72, CHCl3), known precursors of arene oxides 20 and 25 (Boyd et al., 1994) were efficiently synthesized (ca. 80% yield), by catalytic hydrogenation/hydrogenolysis (H2, Pd/C, EtOH) of the (5R,6S)-cis-dihydrodiol 9 ([α]D = +140, MeOH) and (7S,8R)-cis-dihydrodiol 10 ([α]D = +148, MeOH), respectively. This new approach avoided use of the less stable cis-dihydrodiol metabolites 2 and 3 and provided an improved chemoenzymatic route the corresponding arene oxides (−)-20 and (+)-25 (Figure 11) (Boyd et al., 1994).

Attempted epoxidation of available arene oxides 20 and 25 to yield the arene dioxide isomers 28 and 29, using several oxidants, including m-chloroperoxybenzoic acid and dimethyl dioxirane, resulted in the formation of N-oxides. The epoxidation of arene oxides 20 and 25 in CH2Cl2 solution was achieved using sodium hypochlorite solution (pH 8.6) in the presence of tetrabutylammonium hydrogen sulfate and gave anti-quinoline dioxide 29 exclusively. With arene oxide (−)-20 available from earlier studies (Boyd et al., 1991a, 1994), NaOCl oxidation gave the (5R,6R,7R,8R)—arene dioxide 29 ([α]D = −100, 38% yield, Figure 11). Reaction of the (+)-(7S,8R) enantiomer of arene oxide 25 with N-bromoacetamide under reported conditions, yielded the crude trans-bromohydrin intermediate 41, which upon treatment with sodium methoxide furnished the syn-quinoline dioxide (5S,6S,7R,8R)-28 ([α]D +25, Figure 11) (Boyd et al., 1991b).

A further approach to the synthesis of the syn-quinoline dioxide enantiomers (−) and (+)-28 involved the cis-dihydroxylation of the 5,6-(−)-9 and 7,8 cis-dihydrodiol (+)-10. Catalytic cis-hydroxylation (OsO4, Me2NO) of 2-chloroquinoline cis-dihydrodiol (+)-9, produced an isomeric mixture of polar tetrols 42 and 43 that could not be separated by chromatography and were converted directly to the corresponding tetracetate derivatives 44 and 45 (1:3, Figure 12). Purification of the crude tetracetates was achieved by flash chromatography followed by multiple elution PLC.

Hydrolysis of the minor syn-tetraacetate enantiomer (−)-44, under basic conditions (NH3, MeOH) yielded the 2-chloroquinoline syn-tetrol (−)-42 which was converted to the

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**Figure 11** | Synthesis of quinoline arene oxides (−)-20, (+)-25, and arene dioxides (−)-syn-28 and (−)-anti-29 from cis-dihydrodiols (−)-10 and (−)-9.
corresponding quinoline tetrol (+)-46, by hydrogenolysis (Pd-C). Treatment of tetrol (+)-46 with acetoxyisobutryl bromide in MeCN solution yielded mainly a dibromoacetate, tentatively assigned as (–)-47; it was separated from trace amounts of the expected dibromodiacetate by PLC. Dibromoacetate 47 was formed by partial hydrolysis of the dibromodiacetate during the isolation process. Treatment of dibromomonoacetate (–)-47 with NaOMe in THF yielded the syn-arene dioxide enantiomer (–)-28 from 5,6-cis-dihydrodiol (+)-9 in the sequence (+)-9 → (–)-44 → (–)-42 → (+)-46 → (–)-47 → (–)-28 (Figure 12).

In a similar manner metabolite (+)-10 was converted into the mixture of tetraacetates 44 and 45 (1:3). The minor syn-tetraacetate (+)-44, was separated by PLC; it was assigned the opposite (5S,6S,7R,8R) absolute configuration. The syn-tetraacetate (+)-44 was used in an enantiocomplementary synthetic sequence (+)-10 → (+)-44 → (+)-42 → (–)-46 → (–)-47 → (+)-28 (Figure 12).

FIGURE 12 | Synthesis of anti (–)-29 and syn-quinoline dioxides (–)-28 and (–)-28 from cis-dihydrodiols (–)-9 and (+)-10. Reagents: (i) OsO₄, Me₃NO-CH₂Cl₂; (ii) Ac₂O-pyridine; (iii) NH₃, MeOH; (iv) H₂, Pd-C, MeOH; (v) AcOMe₂COBr, MeCN; (vi) NaOMe, Et₂O.
anti-Tetraacetate (5R,6R,7R,8R) enantiomer (+)-45 obtained from 5,6-(+)-9 and 7,8-(+)-10 cis-dihydrodiol metabolites, was treated in a similar manner to the syn-tetraacetate enantiomers (+)-44 and (-)-44 to yield tetrols (+)-43, (-)-48, and dibromodiacetate -49 (Figure 12). Treatment of bromoacetate 49 with sodium methoxide yields a mixture of the expected anti-quinoline dioxide (-)-29 as the major product accompanied by a minor compound which was identified as 8-bromoquinoline-5,6-oxide 50. A competing ElE mechanism could account for the unexpected loss of acetic acid to form the minor product, arene oxide 50. These products were thus formed via the synthetic sequence (+)-9 or (+)-10 → (+)-45 → (+)-43 → (-)-48 → 49 → (-)-29 + (-)-50 (Figure 12).

DISCUSSION

Both Autodock and Gold programs proved to be very successful in matching the preferred in silico orientations of arene substrates with the experimentally confirmed regiochemistry and absolute stereochemistry of the isolated cis-diol metabolites. Previous docking studies of arene substrates with TDO indicated that the preferred orientations were controlled by: (i) attractive edge-to-face T shaped interactions with the orthogonal phenyl group of Phe-216 and imidazole ring of His 222 and (ii) Van der Waals interactions with the proximate hydrophobic amino acids Ile-276, Leu-272, Ile-324, Val-309, Leu-272, Phe-352 (Hoering et al., 2016; Vila et al., 2016a,b, 2017; Boyd et al., 2017, 2019). Theoretical, crystallographic and experimental support for phenyl-phenyl and phenyl-pyridyl edge-to-face bonding (T-bonding) interactions, between arene and heteroarene rings, has been reported (Jennings et al., 2001; Escudero et al., 2009; Gonzalez-Rosende et al., 2017; Aliev and Motherwell, 2019). Little evidence was available, from crystalline protein structures, for similar edge-to-face interactions between the imidazole ring of histidine with other aromatic residues, e.g., Phe, Tyr, Trp, His (Bhattacharyya et al., 2003). The possibility of further types of edge-to-face interactions between heteroarene substrates 1 (Figures 4A–C), 8 (Figures 5A–C), and 14 (Figure 6) with Phe-216 and His-222 in the active site of TDO (Figures 4–6), is also apparent.

Based on the docking orientations of 2-chloroquinoline 8 (Figures 5A,C), cis-dihydroxylation could also occur at the 3,4-bond of the pyridine ring. The shorter distance between this bond and dioxygen in Figure 5C (3.3–3.4 Å), would appear to favor formation of the undetected (3R,4R)-dihydridiol 12, over the (3S,4S) configuration resulting from the orientation of substrate 8 shown in Figure 5A (3.6–4.0 Å). Conversely, the higher binding energy, −5.8 kcal/mol−1, associated with Figure 5A relative to Figure 5C (−5.1 kcal/mol−1), would suggest a preference for the (3S,4S) absolute configuration, thus making a prediction of the preferred stereochemistry of cis-dihydridiol 11 less reliable.

To account for the unexpected isolation of (3S,4S)-diol 11 as a product from P. putida UV4 biotransformations of 2-chloroquinoline 8, the undetected cis-dihydridiol 12 of unknown absolute configuration, was originally proposed as a possible intermediate in the metabolic sequence 8 → 12 → 11 (Figure 2) (Boyd et al., 1998). Later GC-MS identification of 2-quinolone 13 as a very minor metabolite of substrate 8, was consistent with an alternative or additional metabolic pathway (8 → 13 → 11, Figure 2) (Boyd et al., 2002). Stronger evidence for the latter biosynthetic route was provided when 2-quinolone 13 was used as substrate and (3S,4S)-dial 11 was found to be the only identifiable metabolite. Since 2-hydroxyquinoline is a minor tautomer of 2-quinolone 13, the formation of cis-diol metabolite 11 could also be considered as a TDO-catalyzed cis-dihydroxylation within a substituted pyridyl ring. It is therefore possible that cis-diol metabolite 11 was formed from 2-chloroquinoline 8, via both 2-quinolone 13 and cis-dihydridiol 12 intermediates.

The previously reported regioselective and enantioselective cis-dihydroxylation, of the carbocyclic ring of quinoline 1, 2-chloroquinoline 8 and 2-chloropyridine 14, was rationalized by molecular docking (Autodock Vina) studies of these substrates. The in silico results revealed novel edge-to-face attractive interactions at the active site of TDO. These studies also provided support for the TDO-catalyzed cis-dihydroxylation of: (i) the pyridyl ring in substrates, 1, 8, and 14, to yield the corresponding unstable cis-dihydridiol metabolites, 7, 12, and 15, and (ii) the carbocyclic ring to give stable cis-dihydridiol metabolites 2, 3, 9, and 10 having the correct absolute configurations. Evidence for the monooxygenase-catalyzed epoxidation and dioxygenase-catalyzed cis-dihydroxylation of a pyridyl ring was discussed, in the context of both bacterial and mammalian metabolism of quinoline 1.

Our findings are consistent with the current thinking on the catalytic mechanism for these enzymes, which is not fully understood. Barry and Challis (2013) discuss an important point, i.e., whether the Fe(III)-OOH complex in the TDO catalytic cycle reacts directly with the substrate or first undergoes rearrangement to an Fe(V)-O(OH) complex. They highlight the question of which arene carbon atom first forms a bond with an oxygen atom.

In conclusion, new synthetic applications of the relatively stable cis-diol metabolites 9 and 10, derived from 2-chloroquinoline 8 were successfully investigated. These cis-dihydriodios were utilized in the chemoenzymatic synthesis of the corresponding cis-tetrahydrodiols 39 and 40, required in the synthesis of enantiopure quinoline arene oxides, (−)-20 and (+)-25. This process was greatly facilitated by molecular docking preliminary studies. The unexpected 8-bromoquinoline oxide (−)-50 was studied during the synthesis of anti-(−)-29 and syn-quinoline dioxide enantiomers (−)-28 and (+)-28 from cis-dihydriodios 9 and 10.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.
AUTHOR CONTRIBUTIONS

CA, DB, NS, and PS supervised the research, designed the research, obtained the funding, wrote the manuscript, and designed the experiments. PH conducted the modeling and docking. JC, PL, and NS conducted the laboratory synthesis/biotransformations. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2020.619175/full#supplementary-material
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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