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Ethynyl and Propynylpyrene Inhibitors of Cytochrome P450

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Abstract

The single-crystal X-ray structures and in vivo activities of three aryl acetylenic inhibitors of cytochromes P450 1A1, 1A2, 2A6, and 2B1 have been determined and are reported herein. These are 1-ethynylpyrene, 1-propynylpyrene, and 4-propynylpyrene. To investigate electronic influences on the mechanism of enzyme inhibition, the experimental electron density distribution of 1-ethynylpyrene has been determined using low-temperature X-ray diffraction measurements, and the resulting net atomic charges compared with various theoretical calculations. A total of 82,390 reflections were measured with Mo K α radiation to a $(\sin\theta/\lambda)_{\max} = 0.985 \text{ \AA}^{-1}$. Averaging symmetry equivalent reflections yielded 8,889 unique reflections. A least squares refinement procedure was used in which multipole parameters were added to describe the distortions of the atomic electron distributions from spherical symmetry. A map of the model electron density distribution of 1-ethynylpyrene was obtained. Net atomic charges calculated from refined monopole population parameters yielded charges that showed that the terminal acetylenic carbon atom (C18) is more negative than the internal carbon (C17). Net atomic charges calculated by ab initio, density functional theory, and semi-empirical methods are consistent with this trend suggesting that the terminal acetylenic carbon atom is more likely to be the site of oxidation. This is consistent with the inhibition mechanism pathway that results in the formation of a reactive ketene intermediate. This is also consistent with assay results that determined that 1-ethynylpyrene acts as a mechanism-based inhibitor of P450s 1A1 and 1A2 and as a reversible inhibitor of P450 2B1. Crystallographic data: 1-ethynylpyrene, C₁₈H₁₀, P2₁/c, $a = 14.571(2) \text{ \AA}$, $b = 3.9094(5) \text{ \AA}$, $c = 20.242(3) \text{ \AA}$, $\beta = 105.042(2)^\circ$, $V = 1,113.5(2) \text{ \AA}^3$; 1-propynylpyrene, C₁₉H₁₂, P2₁/n, $a = 8.970(2) \text{ \AA}$, $b = 10.136(1) \text{ \AA}$, $c = 14.080(3) \text{ \AA}$, $\beta = 99.77(2)^\circ$, $V = 1,261.5(4) \text{ \AA}^3$; 4-propynylpyrene, C₁₉H₁₂, Pbca, $a = 9.904(1) \text{ \AA}$, $b = 13.174(2) \text{ \AA}$, $c = 19.401(1) \text{ \AA}$, $V = 2,531.4(5) \text{ \AA}^3$.

Keywords

X-ray structure; Mechanism-based inhibitor; Ethynylpyrene; Propynylpyrene; Electron density distribution; Theoretical calculations

Introduction

Cytochrome P450 enzymes protect the body from foreign substances through a mechanism that involves oxidation of the substance. These enzymes oxidize both endogenous compounds (steroids, fatty acids, and lipophilic vitamins) and xenobiotic compounds (drugs,

environmental pollutants, and procarcinogens). Although the metabolism of both endogenous and xenobiotic substances by P450 enzymes is intended to increase the water solubility and allow for easy elimination of the compound, some of the oxidized products have been shown to be responsible for cancer initiation and promotion. Specifically, the P450 enzymes known as P450s 1A1, 1A2, 2B1, and 2A6 have been shown to oxidize polycyclic aromatic hydrocarbons to produce cytotoxic, mutagenic, or carcinogenic metabolites [1–7]. Certain aryl and arylalkyl acetylenes have been shown to act as inhibitors of these enzymes [8–10]. When these compounds fit into the active sites of the P450 enzymes with the correct orientation, they can deactivate the enzyme by irreversibly and covalently binding to the protein (suicide inhibition). The degree and type of inhibition, as well as the selectivity towards different P450 enzymes, greatly depend on the size and shape of the ring system and the placement of the triple bond. Suicide inhibitors can be used to inhibit the metabolism of specific procarcinogens into the ultimate carcinogenic forms. These compounds can also be used as probes into the active sites of the target enzymes, as models for anticancer drugs, or in protein labeling studies.

The inhibition mechanism of acetylenic compounds involves two possible pathways. Pathway **a** involves the oxidation of the internal carbon of the triple bond which leads to heme destruction and enzyme deactivation through the formation of an $\text{Fe}-\text{O}-\text{CR}=\text{C}(\bullet/+)-\text{H}$ complex where a radical or positive charge is localized on the terminal carbon [11]. Pathway **b** involves the oxidation of the terminal carbon of the triple bond which results in the formation of a reactive ketene intermediate. This ketene intermediate can covalently bind to a nucleophilic amino acid residue in the enzyme's active site leading to irreversible inhibition without destruction of the heme. If an appropriate nucleophilic residue is not available, the ketene will be hydrolyzed to the corresponding carboxylic acid [12].

Our interest is in determining the structural and electronic characteristics of these molecules that make them optimal candidates for use as P450s 1A1, 1A2 and 2B1 inhibitors. Using X-ray crystallographic techniques, we have determined the crystal structures of a series of aryl acetylenes. These include 1-ethynylpyrene (1-EP), 1-propynylpyrene (1-PP), and 4-propynylpyrene (4-PP). We have found that all three of these compounds act as suicide inhibitors of P450 1A1 while both 1-PP and 4-PP act as reversible inhibitors of P450 1A2. All three compounds only act as reversible inhibitors of P450 2B1. In addition, we have mapped the experimental electron density distribution of 1-ethynylpyrene, a selective suicide inhibitor that may be used to discriminate between members of the P450 1A and 1B families in humans [9]. This electron density distribution (EDD) was measured in an effort to understand the distribution of electron density that could have an effect on the pathway through which the compound is metabolized. Using net atomic charges calculated from the EDD as well as calculated net atomic charges (ab initio, density functional theory, semi-empirical, and molecular modeling), we hope to be able to predict the deactivation pathway of these compounds.

Experimental

Conventional X-ray Structure Determination

Samples were recrystallized by slow evaporation of solvent mixtures listed in Table 1. The structures were determined by X-ray crystallographic techniques described below. Data for 1-propynylpyrene and 4-propynylpyrene were measured at room temperature.

Single crystals of 1-propynylpyrene and 4-propynylpyrene were mounted separately on an Enraf–Nonius CAD4 diffractometer with a normal focus molybdenum target X-ray tube, a 1.3 mm collimator, and a graphite crystal monochromator. All three dimensional intensity data were collected in the $\omega:2\theta$ scan mode. The data were corrected for Lorentz and polarization effects. Absorption as a function of ψ was minimal and not corrected. Three standard reflections measured every 2 h showed no significant change in intensity. Symmetry equivalent reflections were averaged. Structures were solved using SIR [13]. The hydrogen atom positions for the 1-propynylpyrene and 4-propynylpyrene were calculated based on sp and sp^2 geometry and a C–H bond length of 0.95 Å. Reflections with $I > 3\sigma(I)$ were considered observed and were included in the refinements. Full-matrix least-squares refinements were on F with non-hydrogen atoms refined anisotropically. Hydrogen atom positions and isotropic temperature factors in 1-propynylpyrene and 4-propynylpyrene were fixed [14].^{1,2} Refinement results are summarized in Table 1.

The structure of 1-ethynylpyrene was determined using a subset of the data collected for the determination of the electron density distribution described below. The subset consisted of 53,611 X-ray reflections (5,784 unique) with $\sin\theta/\lambda < 0.857 \text{ \AA}^{-1}$. Data were collected on a Bruker 3-circle diffractometer equipped with a SMART 1 K CCD detector and a normal focus molybdenum target X-ray tube with a 0.8 mm collimator measured at 130 K with a stream of cold N_2 gas generated with a Bruker LT-2 low temperature device. Data were collected using the Bruker SMART software package and the intensities were processed using the Bruker SAINT integration program.³ A conventional independent atom model (IAM) least-squares refinement on F^2 was performed with SHELX97 [15]. Non-hydrogen atoms were refined anisotropically, and hydrogen atom positions and isotropic thermal parameters were included in the refinement. Results of the IAM refinement of 1-ethynylpyrene are also included in Table 1.

Electron Density Distribution of 1-Ethynylpyrene

In order to determine the experimental electron density distribution of 1-ethynylpyrene, multiple X-ray intensity measurements were collected at low temperature to a maximum

¹Conventional reliability indices: $R = \sum ||F_O| - |F_C|| / \sum |F_O|$ and $R_w = (\sum (|F_O| - |F_C|)^2 / \sum w |F_O|^2)^{1/2}$, where F_O and F_C are observed and calculated structure factors, $w = 1/\sigma(F)^2$, and where $\sigma(F)$ is the estimated standard deviation in F_O .

²The supplementary crystallographic data for (a) 1-ethynylpyrene (1-EP) (CCDC 731480), (b) 1-propynylpyrene (1-PP) (CCDC 731481), and (c) 4-propynylpyrene (4-PP) (CCDC 731482), have been deposited. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

³SMART Software, Bruker-AXS, 6300 Enterprise Dr., Madison, WI 53719-1173, (1994); SAINT Software, Bruker-AXS, 6300 Enterprise Dr., Madison, WI 53719-1173, (1995).

resolution of 0.51 Å ($\sin \theta_{\max}/\lambda = 0.985 \text{ \AA}^{-1}$). A single crystal of 1-ethynylpyrene was mounted on the Bruker diffractometer described above. A set of ω -scans was collected with seven different ϕ values with the detector 3.50 cm from the crystal at $2\theta = 33.0^\circ$. Each scan consisted of 606 frames collected for 90 s per frame with a width of 0.30° in ω . Five more scans were collected with the detector positioned at $2\theta = 45.0^\circ$. The raw data from the combined scans were integrated, and Lorentz, polarization, and empirical corrections applied yielding a final data set of 82,390 reflections of which 8,889 were unique.

To obtain the experimental electron density distribution, the X-ray data were refined using the multipole refinement program XD [16]. XD is a least squares refinement program that attempts to fit the observed X-ray structure factors with a model that includes a set of atom-centered deformation density functions with spherical harmonic angular dependence. In addition to the 3 positional parameters and 6 thermal parameters for each atom, XD allows refinement of an additional 25 deformation parameters per atom when spherical harmonic functions up to the hexadecapole ($l = 4$) level are included. Monopole terms used in the multipole expansion were taken to be the valence electron distributions of neutral, spherical atoms calculated from Hartree–Fock wavefunctions and modified with a refineable population and radial expansion/contraction parameter, κ .

In order to reduce the number of deformation parameters, chemical symmetry can be imposed. For 1-ethynylpyrene, mirror plane symmetry was imposed on the multipole deformation parameters of the carbon atoms in the pyrene ring. In addition, the pyrene ring carbon atoms were divided into two groups, those with hydrogen atoms attached, and those without hydrogen atoms, and the deformation parameters were constrained to be equal for all carbon atoms in each group. The deformation density of both carbon atoms of the ethynyl group were constrained to have cylindrical symmetry. However, since the difference in net atomic charges of the carbon atoms of the ethynyl group is relevant to the mechanism of enzymatic activation, the remaining parameters were refined independently, and not constrained.

Since hydrogen atoms lack core electrons, positional and thermal parameters free of bias from the valence electron density distribution cannot be reliably determined from high-resolution X-ray scattering measurements of multipole refinements. Hydrogen atom positions were therefore determined from the IAM refinement, and then extended along the C–H bond direction to a final C–H bond length of 1.07 Å. In the XD refinement, the hydrogen atoms were fixed at the extended positions, and the isotropic thermal parameters were fixed at the values determined in the IAM refinement. The hydrogen multipole deformation parameters were limited to cylindrically symmetric terms up to the quadrupole ($l = 2$) level. Results of the multipole refinement are summarized in Table 2, and net atomic charges calculated from the monopole populations are included in Table 3.

Electron Density Maps

Distortions of the atomic electron density in a molecule as a result of chemical bonding are revealed in a plot of the deformation density. The deformation density, $\Delta\rho$, is the difference between the total density of the molecule and the density calculated for a superposition of neutral, spherical atoms (the IAM). The deformation density maps can be calculated using the refined multipole parameters from XD for the molecular density, and subtracting the IAM density calculated with the same atomic positions. The maps presented here are referred to as static model deformation density maps calculated as

$$\Delta\rho = \rho_{\text{mult}} - \rho_{\text{IAM}}$$

where ρ_{mult} is the density calculated for the molecule from the multipole parameters, and ρ_{IAM} is the density calculated for a superposition of neutral, spherical atoms. The thermal parameters are not included in the calculation, and thus the density maps do not include the effects of smearing due to atomic thermal motion.

Theoretical Net Atomic Charges

Several methods were used to calculate the net atomic charges of the ethynyl substituent including semi-empirical (MOPAC and MMFF94), density functional theory, and ab initio calculations in an effort to differentiate the internal and external carbon atoms. Two methods of calculating the charges were considered: Mulliken populations and Natural Bond Order. To explore the dependence of the charges on the computational method, charges were calculated with different standard basis sets as implemented in Gaussian 98 W [17], both with and without electron correlation. The geometry was optimized at each level of theory and charges were obtained at the optimized geometries. For comparison, semi-empirical charges for the ethynyl carbons and aromatic ring carbons were also obtained from molecules with optimized geometries using MMFF94 [18] and MOPAC [19]. Both MMFF94 and MOPAC are available as part of the Tripos SYBYL package [20]. All charges are tabulated in Table 3.

Assays of P450-Dependent 7-Alkoxyresorufin Dealkylation and Coumarin 7-Hydroxylation

Rat CYP2B1 supersomes (rat CYP2B1 + P450 reductase + cytochrome b₅, P450 content 1,000 pmol/mL, b₅ content 230 pmol/mg protein), and human CYP1A1, 1A2, and 2A6 supersomes (human CYP enzymes + P450 reductase, P450 content 1,000 pmol/mL for 1A1 and 1A2, and 2,000 pmol/mL for 2A6) were purchased from B.D. Biosciences Corporation (Woburn, MA, USA). All other chemicals were purchased from Sigma–Aldrich Company (Milwaukee, WI).

The P450 1A1, 1A2, and 2B1 dependent activities were assayed using ethoxyresorufin, methoxyresorufin, and pentoxyresorufin fluorescent substrates, respectively [21]. P450 2A6 dependent 7-hydroxylation of coumarin was used in a similar assay with minor differences as described below for measuring 2A6 activity [22,23]. 7-Ethoxyresorufin O-deethylation (EROD), 7-methoxyresorufin O-demethylation (MROD), 7-pentoxyresorufin O-depentylation (PROD), and coumarin 7-hydroxylation assays were performed as follows.

Potassium phosphate buffer (1,750 μL of a 0.1 M solution, pH 7.6) was placed in a 1.0 cm quartz cuvette, and 10 μL of a 1.0 M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ solution, 15 μL of a 1.0 mM corresponding resorufin or coumarin substrate solution in Me_2SO (DMSO), 10 μL of the microsomal P450 protein, and 15 μL of an inhibitor in DMSO were added. For the controls, 15 μL of pure DMSO was added in place of the inhibitor solution. The reaction was initiated by the addition of 200 μL of a NADPH regenerating solution. The regenerating solution was prepared by combining 797 μL of a 0.10 M potassium phosphate buffer solution (pH 7.6), 67 μL of a 15 mM NADP^+ solution in buffer, 67 μL of a 67.5 mM glucose 6-phosphate solution in buffer, and 67 μL of a 45 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ solution, and incubating the mixture for 5 min at 37 °C before the addition of 3 units of glucose 6-phosphate dehydrogenase/mL and a final 5 min incubation at 37 °C. The final assay volume was 2.0 mL. The production of 7-hydroxyresorufin anion was monitored by a spectrofluorimeter (OLIS DM 45 Spectrofluorimetry System) at 530 nm excitation and 585 nm emission, with a slit width of 2 nm. The production of 7-hydroxycoumarin was monitored at 338 nm excitation and 458 nm emission, with a slit width of 2 nm. The reactions were performed at 37 °C. For each inhibitor, a number of assay runs were performed using varying inhibitor concentrations.

Assay Data Analysis [24]

The data obtained from these assays were analyzed by a computer analysis method of the reaction progress curve in the presence of various inhibitor concentrations and in the absence

of the inhibitor as the control run. Results are tabulated in Table 4. An equation describing product formation with respect to reaction time in seconds was obtained for each inhibitor concentration and the control. The Microsoft Excel program was used to curve fit the data obtaining the parameters of the best-fit second order curves. Using the parameters obtained from the above, activities were calculated using first order derivatives. The log of the percent enzymatic activities in the presence of the inhibitor relative to that in the absence of the inhibitor were calculated and plotted versus time. For mechanism-based (suicide) inhibitors, these plots of the change in enzymatic activity versus time manifest pseudo-first-order time-dependent losses of enzymatic activity. The linear portions of these plots were used to determine $t_{1/2}$ ($0.693/k_{\text{inactivation}}$) values at various concentrations for the observed time-dependent losses of activity. For these suicide inhibitors, values of $1/k_{\text{inactivation}}$ were plotted versus reciprocals of the inhibitor concentrations in Kitz–Wilson plots. The limiting $k_{\text{inactivation}}$ values were obtained from the abscissa intercepts of the plots, and the K_I values (apparent dissociation constants of the enzyme–inhibitor complexes leading to enzyme deactivation) were calculated from the ordinate intercepts ($-1/K_I$). In cases when no time-dependent loss of activity was observed, Dixon plots were used ($1/v$ vs. $[I]$) in order to determine apparent dissociation constants for reversible enzyme–inhibitor complexes.

Pre-Incubation Assays in the Presence and Absence of NADPH

For compounds that showed mechanism-based inhibition in the above assays, pre-incubation assays were performed as follows in order to confirm the results. All assay solution components had the same concentrations as in the above assays. For pre-incubation assays in the presence of NADPH, potassium phosphate buffer (1,550 μL of a 0.1 M solution, pH 7.6) was placed in a 1.0 cm quartz cuvette followed by 10 μL of a 1.0 M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ solution, 10 μL of the microsomal P450 protein, 15 μL of an inhibitor in DMSO (for the control, 15 μL of pure DMSO was added in the place of the inhibitor solution), and 200 μL of a NADPH regenerating solution. The assay mixture was incubated for 5 min at 37 °C, before reaction initiation by the addition of 200 μL of buffer and 15 μL of the corresponding substrate solution. The final assay volume was 2.0 mL. The production of 7-hydroxyresorufin anion was monitored at 530 nm excitation and 585 nm emission. The production of 7-hydroxycoumarin was monitored at 338 nm excitation and 458 nm emission. The reactions were performed at 37 °C. For each inhibitor, a number of assay runs were performed using varying inhibitor concentrations. For the pre-incubation assays in the absence of NADPH, potassium phosphate buffer (1,750 μL of a 0.1 M solution, pH 7.6) was placed in a 1.0 cm quartz cuvette followed by 10 μL of a 1.0 M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ solution, 10 μL of the microsomal P450 protein, and 15 μL of an inhibitor in DMSO (for the control, 15 μL of pure DMSO was added in the place of the inhibitor solution). The assay mixture was incubated for 5 min at 37 °C, before reaction initiation by the addition of 200 μL of the NADPH regenerating solution and 15 μL of the corresponding substrate solution. The final assay volume was 2.0 mL. The production of P450-dependent reaction products were monitored as previously described. The reactions were performed at 37 °C. For each inhibitor, a number of assay runs were performed using varying inhibitor concentrations.

Results and Discussion

Assay Results

Based on the assay data, 1-EP is a good mechanism-based inhibitor of both P450s 1A1 and 1A2, and a good reversible inhibitor of P450 2B1, while showing no significant inhibition of 2A6-dependent reactions. The MROD assay data point to this compound being a reversible type inhibitor of P450 1A2, however, the pre-incubation data with varying inhibitor concentrations and incubation times all point to NADPH-dependence of this inhibition. We propose that this compound inhibits P450 1A2 both directly in a reversible manner and indirectly by one of its metabolites in a mechanism-based manner.

1-PP is a much stronger mechanism-based inactivator of P450 1A1, a good reversible inhibitor of P450 1A2, and a weak reversible inhibitor of P450 2B1. It does not cause any significant loss of activity in P450 2A6-dependent reactions.

4-PP shows weak mechanism-based inhibition of P450 1A1. It acts as a reversible inhibitor of P450s 1A2 and 2B1, and does not cause any significant loss of activity in P450 2A6-dependent reactions.

Based on the pre-incubation assays run in the presence and absence of NADPH, it was confirmed that 1-EP is a good mechanism-based inhibitor of P450 1A1, 1-PP is a great mechanism-based inhibitor of P450 1A1, and 4-PP is a reversible inhibitor of all of the enzymes studied.

Computation Results

In all cases, it was found that C18, the external carbon on the alkyne, was more negative than C17, the internal alkyne carbon.

X-ray Crystallography Results

The numbering systems may be found in Fig. 1 for 1-ethynylpyrene, Fig. 2 for 1-propynylpyrene, and Fig. 3 for 4-propynylpyrene. Stereographic projections have been drawn at the 50% probability level. Figure 1 shows 1-ethynylpyrene at 130 K while Figs. 2 and 3 show 1- and 4-propynylpyrene at room temperature. Fractional coordinates, isotropic thermal parameters, bond lengths, and bond angles have been deposited. There are no hydrogen bonding interactions or unusual close intermolecular contacts in the crystal structures of these compounds. As expected, all three compounds are planar with the ethynyl and propynyl substituents in the plane of the aromatic rings.

Charge Density Results

The small crystal size and relatively large thermal parameters of 1-ethynylpyrene, even at 130 K, results in a limited number of observable intensities at high scattering angles, even when a relatively slow scan of 90 s per data frame was used. However, since the structure of 1-ethynylpyrene does not contain sharp features in the deformation density such as lone-pairs, which are known to contribute to high-order scattering, but contains only covalent bonding peaks which contribute significantly only to reflections with $\sin\theta/\lambda < 0.65 \text{ \AA}^{-1}$, the high-order data are only necessary to determine atomic positions and thermal parameters which are free from the bias due to the aspherical features in the valence electron distribution. In this study, the result is a multipole refinement with relatively high *R*-factors, due to the inclusion of weak high order data, which yields multipole deformation parameters which accurately fit the low-angle X-ray scattering, and provide a very reasonable description of the valence electron distribution. This can be confirmed by calculation of the *R*-factors for the fit of the multipole model structure factors to low-angle X-ray data ($\sin\theta/\lambda < 0.65 \text{ \AA}^{-1}$), which are significantly lower than the *R*-factors for the neutral, spherical atom (IAM) model (see Table 2).

Figure 4 shows a static model deformation density map in the plane of the aromatic ring system of 1-ethynylpyrene. Separate deformation density maps were calculated for each aromatic ring plane and the plane of the ethynyl substituent. These maps were then joined together to give the composite map. Dotted contours correspond to areas of depleted electron density, and solid contours correspond to areas with a buildup of electron density relative to the density of neutral, spherical atoms. From the estimated errors in the low-angle X-ray structure factors, the estimated standard deviation in the deformation density is calculated to be $0.035 \text{ e\AA}^{-3}$, except near the atomic nuclei where it is higher. The deformation density map shows a buildup of electron density in all of the covalent bonds in the molecule. In the pyrene ring, the sp^2

hybridization of the carbon atoms results in large deformation density peaks which are directed towards neighboring atoms, and is reflected in the large magnitude of the $Y_{3,3+}$ octapolar deformation functions. These deformation peaks are also elongated perpendicular to the plane of the molecule, consistent with significant π -bonding interactions in the aromatic rings. As expected, there is also an accumulation of excess electron density in the $C \equiv C$ triple bond of the ethynyl group that is larger than accumulation of excess density found in bonds of the aromatic rings.

Since the electron density of an atom is a real, measurable physical property, the relative amount of electron density at any point in a molecule can be determined by evaluating the experimental deformation density functions at that point. An approximate value of the electron density can also be obtained simply by comparing the number of contours on the model deformation maps in different regions. Another measure of the electron distribution widely used in chemistry is the net atomic charge. Experimental net atomic charges can be obtained directly from monopole populations or by integration of the deformation density over the volume of each atom. In either case, the continuous electron density distribution must be partitioned into atoms, and the results are thus dependent on how atoms are defined in the model. The same model dependence also applies to net atomic charges obtained from theoretical calculations. Interpretation of net atomic charges should therefore focus on consistent trends, rather than on the exact values of the atomic charges.

Inspection of the net atomic charges obtained from the experiment and various theoretical methods shows considerable variation between methods. In all cases, however, the terminal acetylene carbon is found to be more negative than the internal acetylene carbon atom. Based on the experimental and theoretical charge distribution of 1-ethynylpyrene, the terminal carbon is the stronger electron donor which when bonding with oxygen leads to the formation of the more stable reactive intermediate. This leads to a hydrogen rearrangement resulting in the formation of a reactive ketene intermediate which is consistent with inhibition mechanism pathway **b**. This is also consistent with the assay results which determined that 1-EP acts as a mechanism-based inhibitor of P450s 1A1 and 1A2 and as a reversible inhibitor of P450 2B1.

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References

1. Ortiz de Montellano, PR., editor. Cytochrome P450 structure, mechanism, and biochemistry. 2. Plenum Press; New York: 1995. p. 1-652.
2. Estabrook RW. The remarkable P450s: a historical overview of these versatile hemeprotein catalysts. FASEB J 1996;10:202-204. [PubMed: 8641552]
3. Masters BSS. Introduction: cytochrome P450. FASEB J 1996;10(2):205. [PubMed: 8641553]
4. Bhattacharyya KK, Brake PB, Eltom SE, Otto SA, Jefcoate CR. Identification of a rat adrenal cytochrome P450 active in polycyclic hydrocarbon metabolism as rat CYP1B1. Demonstration of a unique tissue-specific pattern of hormonal and aryl hydrocarbon receptor-linked regulation. J Biol Chem 1995;270:11595-11602. [PubMed: 7744798]
5. Schenkman, JB.; Greim, H., editors. Cytochrome P450 handbook of experimental pharmacology. Vol. 105. Springer; Berlin: 1993. p. 1-739.
6. Hasemann CA, Kurumbail RG, Boddupalli SS, Peterson JA, Deisenhofer J. Structure and function of cytochromes P450: a comparative analysis of three crystal structures. Structure 1995;3(1):41-62. [PubMed: 7743131]
7. Alexander DL, Jefcoate CR. Proc Am Assoc Cancer Res 1995;36:152. abstract 905.

8. Foroozesh M, Primrose G, Guo Z, Bell LC, Alworth WL, Guengerich FP. Aryl acetylenes as mechanism-based inhibitors of cytochrome P450-dependent monooxygenase enzymes. *Chem Res Toxicol* 1997;10(1):91–102. [PubMed: 9074808]
9. Shimada T, Yamazaki H, Foroozesh M, Hopkins NE, Alworth WA, Guengerich FP. Selectivity of polycyclic inhibitors for human cytochrome P450 1A1, 1A2, and 1B1. *Chem Res Toxicol* 1998;11(9):1048–1056. [PubMed: 9760279]
10. Strobel SM, Szklarz GD, He YQ, Foroozesh M, Alworth WL, Roberts ES, Hollenberg PF, Halpert JR. Identification of selective mechanism-based inactivators of cytochrome P450 2B4 and 2B5, and determination of the molecular basis for differential susceptibility. *J Pharmacol Exp Ther* 1999;290:445–451. [PubMed: 10381811]
11. Komives EA, Ortiz de Montellano PR. Mechanism of oxidation of π bonds by cytochrome P-450: electronic requirements of the transition state in the turnover of phenylacetylenes. *J Biol Chem* 1987;262:9793–9802. [PubMed: 3597438]
12. CaJacob CA, Chan WK, Shephard E, Ortiz de Montellano PR. The catalytic site of rat hepatic lauric acid ω -hydroxylase: protein versus prosthetic heme alkylation in the ω -hydroxylation of acetylenic fatty acids. *J Biol Chem* 1988;263:18640–18649. [PubMed: 3198593]
13. Mackay, S.; Gilmore, C.J.; Edwards, C.; Stewart, N.; Shankland, K. MaXus computer program for the solution and refinement of crystal structures. Bruker Nonius; The Netherlands: Mac-Science; Japan: The University of Glasgow; 1999.
14. International tables for X-ray crystallography. Vol. IV. Kynoch Press; Birmingham, England: 1974. p. 71-151.
15. Sheldrick, GM. Program for the refinement of crystal structures. University of Gottingen; Germany: 1997. SHELXL9.
16. Koritsanszky, T.; Howard, ST.; Su, Z.; Mallinson, PR.; Richter, T.; Hansen, NK. XD: a computer program package for multipole refinement and analysis of electron densities from diffraction data. Free University of Berlin; Germany: 1995.
17. Frisch, MJ.; Trucks, GW.; Schlegel, HB.; Scuseria, GE.; Robb, MA.; Cheeseman, JR.; Zakrzewski, VG.; Montgomery, JA., Jr; Stratmann, RE.; Burant, JC.; Dapprich, S.; Millam, JM.; Daniels, AD.; Kudin, KN.; Strain, MC.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, GA.; Ayala, PY.; Cui, Q.; Morokuma, K.; Rega, N.; Salvador, P.; Dannenberg, JJ.; Malick, DK.; Rabuck, AD.; Raghavachari, K.; Foresman, JB.; Cioslowski, J.; Ortiz, JV.; Baboul, AG.; Stefanov, BB.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, RL.; Fox, DJ.; Keith, T.; Al-Laham, MA.; Peng, CY.; Nanayakkara, A.; Challacombe, M.; Gill, PMW.; Johnson, B.; Chen, W.; Wong, MW.; Andres, JL.; Gonzalez, C.; Head-Gordon, M.; Replogle, ES.; Pople, JA. Gaussian 98, revision A.11.4. Gaussian Inc; Pittsburgh: 2002.
18. Halgren TA. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J Comput Chem* 1996;17(5–6):490–519.
19. Stewart, JJP. MOPAC 6.0. Fujitsu Limited; Tokyo, Japan: 1994.
20. SYBYL molecular modeling software, versions 7.2. Tripos Associates Inc; St. Louis, Missouri 63144: 2004.
21. Burke MD, Thompson S, Weaver RJ, Wolf CR, Mayer RT. Cytochrome P450 specificities of alkoxyresorufin O-dealkylation in human and rat liver. *Biochem Pharmacol* 1994;48:923–936. [PubMed: 8093105]
22. Buters JT, Schiller CD, Chou RC. A highly sensitive tool for the assay of cytochrome P450 enzyme activity in rat, dog, and man. *Biochem Pharmacol* 1993;46:1577–1584. [PubMed: 8240414]
23. Deluca JG, Dysaft GR, Rasnick D, Bradley MO. A direct, highly sensitive assay for cytochrome P-450 catalyzed O-deethylation using a novel coumarin analog. *Biochem Pharmacol* 1989;37:1731–1739. [PubMed: 3259881]
24. Hopkins NE, Foroozesh MK, Alworth WL. Suicide inhibitors of cytochrome P450 1A1 and P450 2B1. *Biochem Pharmacol* 1992;44:787–796. [PubMed: 1510726]

Fig. 1.
Stereographic projection of 1-ethynylpyrene plotted at 130 K and 50% probability

Fig. 2.
Stereographic projection of 1-propynylpyrene plotted at room temperature and 50% probability

Fig. 3.
Stereographic projection of 4-propynylpyrene plotted at room temperature and 50% probability

Fig. 4. Experimental electron deformation density map of 1-ethynylpyrene. Contours are calculated at 0.10 eÅ⁻³ intervals with zero and negative contours broken

Table 1

Crystal and refinement data of 1-ethynylpyrene, 1-propynylpyrene, and 4-propynylpyrene

Compound	1-EP	1-PP	4-PP
Dimensions/color (mm)	$0.80 \times 0.12 \times 0.08$	$1.10 \times 0.88 \times 0.43$	$0.95 \times 0.58 \times 0.15$
Chemical formula	$C_{18}H_{10}$	$C_{19}H_{12}$	$C_{19}H_{12}$
Crystal system	$P2_1/c$	$P2_1/n$	Pbca
Recrystallization solvents	Ethylene glycol/acetone	Hexane/acetone	Cyclohexane
Temperature (K)	130	298	298
Unit cell dimensions			
a (Å)	14.571(2)	8.970(2)	9.904(1)
b (Å)	3.9094(5)	10.136(1)	13.174(2)
c (Å)	20.242(3)	14.080(3)	19.401(2)
α (°)	90	90	90
β (°)	105.042(2)	99.77(2)	90
γ (°)	90	90	90
Volume (Å ³)	1,113.5(2)	1,261.5(4)	2,531.4(5)
Z	4	4	8
Density (calculated) (Mg/m ³)	1.350	1.265	1.261
Absorption coefficient (cm ⁻¹)	0.77	0.72	0.71
θ range for data collection (°)	$2.0 \leq \theta \leq 37.5$	$0.5 \leq \theta \leq 30$	$0.5 \leq \theta \leq 30$
Reflections measured	53,611	4,148	4,266
Independent reflections	5,784	3,794	3,672
Number of observed reflections	3,508 ($I > 2\sigma(I)$)	2,483 ($I > 3\sigma(I)$)	1,251 ($I > 3\sigma(I)$)
Number of parameters	203	172	172
R	0.054	0.052	0.058
R_w	0.108	0.116	0.109
Goodness of fit	1.021	0.837	1.500
CCDC deposit number	731,480	731,481	731,482

Table 2

Summary of the XD multipole refinement of 1-ethynylpyrene

Compound	1-EP
Chemical formula	C ₁₈ H ₁₀
Crystal system	P2 ₁ /c
Temperature (K)	130
θ range for data collection (°)	$2.0 \leq \theta \leq 44.5$
Reflections measured (all data)	82,390
Independent reflections	8,889
Number of observed reflections ($I > 2 \sigma(I)$)	4,773
Number of parameters	190
R_{int}	0.127
R	0.182
R_w	0.101
Goodness of fit	1.251
Independent reflections ($\sin\theta/\lambda < 0.650 \text{ \AA}^{-1}$)	2,561
Number of observed reflections ($I > 2 \sigma(I)$ and $\sin\theta/\lambda < 0.650 \text{ \AA}^{-1}$)	1,294
R_{int} ($\sin\theta/\lambda < 0.650 \text{ \AA}^{-1}$)	0.025
R ($\sin\theta/\lambda < 0.650 \text{ \AA}^{-1}$)	0.035
R_w ($\sin\theta/\lambda < 0.650 \text{ \AA}^{-1}$)	0.054
Goodness of fit ($\sin\theta/\lambda < 0.650 \text{ \AA}^{-1}$)	1.085

Table 3

Net atomic charges in 1-ethynylpyrene

Method	C17	C18	H18	C1	C2	H2
X-ray experiment	-0.29(2)	-0.33(3)	0.45(4)	-0.24(1)	-0.17(1)	0.38(3)
Mulliken						
RHF/3-21G	-0.077	-0.350	0.348	-0.058	-0.195	0.250
B3LYP/6-31G*	0.378	-0.638	0.201	0.081	-0.192	0.135
B3LYP/6-31 + G**	0.212	-0.526	0.211	-0.026	-0.100	0.132
Natural bond order						
RHF/3-21G	-0.033	-0.224	0.249	-0.045	-0.208	0.244
B3LYP/6-31G*	-0.039	-0.208	0.242	-0.042	-0.207	0.240
MMFF94	-0.073	-0.177	0.177			
MOPAC	-0.130	-0.199	0.227			

Estimated standard deviations in the experimental charges are given in parentheses. Theoretical charges for C1, C2, and H2 are averages over the carbon atoms without hydrogen atoms bonded, the carbon atoms with hydrogen atoms bonded, and the hydrogen atoms in the pyrene ring

Table 4

Results of inhibition studies of 1A1, 1A2, 2B1, and 2A6 dependent activities by 1-ethynylpyrene, 1-propynylpyrene, and 4-propynylpyrene

Compound	1-ethynylpyrene	1-propynylpyrene	4-propynylpyrene
1A1			
K_I (uM)	0.04	0.01	0.14
Limiting	0.15	0.34	0.10
$K_{inactivation}$ (min ⁻¹)			
K_i (uM)			
IC50 (uM)	0.18	0.02	0.37
1A2			
K_I (uM)			
Limiting			
$K_{inactivation}$ (min ⁻¹)			
K_i (uM)	0.32	0.06	0.45
IC50 (uM)	0.32	0.06	0.45
2B1			
K_I (uM)			
Limiting			
$K_{inactivation}$ (min ⁻¹)			
K_i (uM)	0.04	0.83	2.26
IC50 (uM)	0.04	0.83	2.26
2A6			
K_I (uM)			
Limiting			
$K_{inactivation}$ (min ⁻¹)			
K_i (uM)			
IC50 (uM)	> 10	> 10	> 10

Limiting $K_{inactivation}$ (min⁻¹): Apparent rate constant for time-dependent loss of P450-dependent activity in the presence of inhibitor and NADPH as determined by Kitz–Wilson plots

K_I (uM): Apparent dissociation constants of enzyme–inhibitor complexes leading to time-dependent loss of P450 dependent activity in the presence of inhibitor and NADPH as determined by Kitz–Wilson plots

K_i (uM): Apparent dissociation constants of enzyme–inhibitor complexes for reversible type inhibition in the presence of inhibitor and NADPH as determined by Dixon plots