Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

Synthesis and Biological Evaluation of Imidazolylmethylacridones as Cytochrome P-450 Enzymes Inhibitors

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Experimental

A. Chemistry

All reactions were performed with commercially available reagents and they were used without further purification. Solvents were dried by standard methods and stored over molecular sieves. All reactions were monitored by thin-layer chromatography (TLC) carried on fluorescent precoated plates and detection of the components was made by short UV light. Melting points were determined in open capillaries using MEL-TEMP II and Buchi B-540 Melting Point apparatus and are uncorrected. FTIR spectra were recorded on Nicolet Avatar 380 spectrometer. ^{1}H- and ^{13}C-NMR spectra were mad in DMSO-d_6 recorded on Bruker spectrometer at 500 MHz and at 125 MHz, respectively. Mass spectra were obtained with Hewlett Packard GC-MS, model 5890, series II at an ionization potential of 70 ev. Elemental analyses were performed by the Microanalytical Unit, Faculty of Science, Cairo University; found values were within $\pm$ 0.4% of the theoretical ones, unless otherwise indicated. Yields were not optimized.

General procedure for the preparation of 1*H*-imidazol-1-ylmethylacridin-9(10*H*)-one (5, 6)

A mixture of the appropriate bromomethyl derivative (0.001mol) and imidazole (0.003 mol) in 50 mL of acetonitrile was refluxed for 7 h under nitrogen. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography.

4-((1*H*-imidazol-1-yl)methyl)acridin-9(10*H*)-one (5)

Synthesized from 4-(bromomethyl)acridin-9(10*H*)-one and imidazole.
Yield: 63% buff yellow powder; m.p. 286-88 ^{o}C; IR (cm^{-1}) 3340, 1705; ^{1}H-NMR: δ 5.96 (s, 2H, CH_2-imidazole) , 7.25-7.31 (m, 3H, aromatic), 7.65-7.77 (m, 3H, aromatic), 8.08-8.34 (m, 4H, aromatic), 11.37 (brs, 1H, NH); ^{13}C-NMR: δ 47.1, 118.2, 120.4, 120.9, 121.3, 121.7, 123.3, 125.7, 126.9, 133.5, 134.0, 135.4,

136.4, 138.5, 141.0, ,176.7; MS (EI-70 eV) m/z 276 (M^++1), m/z 208 (100%); Anal. ($C_{17}H_{13}N_3O$) calcd; C: 74.17, H: 4.76, N: 15.26; found: C: 74.10, H: 4.70, N: 15.31

2-((1*H*-imidazol-1-yl)methyl)acridin-9(10*H*)-one (6)

Synthesized from 2-(bromomethyl)acridin-9(10*H*)-one and imidazole.

Yield: 58% buff yellow powder; m.p. 294-96 ^oC; IR (cm^{-1}) 3328, 1696; ; ^1H-NMR: δ 6.05 (s, 2H, CH_2-imidazole) , 7.66 (s, 1H, aromatic), 7.93-8.01 (m, 2H, aromatic), 8.26-8.57 (m, 5H, aromatic), 8.87-8.95 (m, 2H, aromatic), 10.78 (brs, 1H, NH); ^{13}C-NMR: δ 54.34, 122.60, 123.18, 125.43, 125.72, 126.36, 130.05, 131.23, 134.00, 135.72, 138.19, 138.72, 145.57, 146.03, 181.82; MS (EI-70 eV) m/z 276 (M^++1), m/z 208 (100%); Anal. ($C_{17}H_{13}N_3O$) calcd; C: 74.17, H: 4.76, N: 15.26; found: C: 73.95, H: 4.65, N: 15.30

B. Biology

Inhibition of aldosterone synthase (CYP11B2) and 11β-hydroxylase (CYP11B1) expressed in V79MZ cells

Method

Determination of % inhibition for CYP11B1 and CYP11B2 in stably transfected V79MZ cells.

Inhibitory activities were determined as described before [1] and with some modifications. In short, V79MZh11B1 and V79MZh11B2 cells (8 x 10^5 cells/well) were grown on 24-well culture plates until confluence. Before testing, the DMEM culture medium was removed and 450 µl of fresh DMEM, containing the inhibitor (or 1 % ethanol for control) was added to each well. After a preincubation step of 60 min at 37°C, the reaction was started by the addition of 50 µl of DMEM in which the substrate deoxycorticosterone (containing 0.15 µCi of [1,2-^{3}H]-deoxycorticosterone in ethanol, final test concentration 100 nM) was dissolved. Incubation times were 25 min for V79MZh11B1 and 50 min for V79MZh11B2 cells, respectively. Enzyme reactions were stopped by extracting the supernatant

with ethyl acetate. Samples were centrifuged (10 000g, 10 min) and the solvent was pipetted into fresh cups. When the solvent was evaporated, the steroids were redissolved in 40 µl acetonitrile/water (50:50, v/v) and analyzed by HPLC. Detection and quantification of the steroids were performed using a radioflow detector.

Inhibition of aromatase (CYP19)

Method

Enzyme preparation:

The enzyme was obtained from the microsomal fraction of freshly delivered human term placental tissue according to the procedure of Thompson and Siiteri [2]. The isolated microsomes were suspended in a minimum volume of phosphate buffer (0.05 M, pH 7.4, 20 % glycerol). Additionally, DTT (dithiothreitol, 10 mM) and EDTA (1mM) were added to protect the enzyme from degradation.The enzyme preparation was stored at -70°C.

Inhibition of aromatase:

The assay was performed monitoring enzyme activity by measuring the $^{3}H_2O$ formed from [1β-^{3}H] androstenedione during aromatization. Each incubation tube contained 15 nM [1β-^{3}H]androstenedione (0.08 µCi), 485 nM unlabeled androstenedione, 2 mM NADP, 20 mM glucose-6-phosphate, 0.4 units of glucose-6-phosphate-dehydrogenase and inhibitor in phosphate buffer (0.05 M, pH 7.4). The test compounds were dissolved in DMSO and diluted with buffer. The final DMSO concentration in the control and inhibitor incubation was 2 %. Each tube was preincubated for 5 min at 30°C in a water bath. Microsomal protein was added to start the reaction (0.1 mg). The total volume for each incubation was 0.2 ml. The reaction was terminated by the addition of 200 µl of a cold 1 mM $HgCl_2$ solution. After addition of 200 µl of an aqueous dextran-coated charcoal (DCC) suspension (2 %), the vials were shaken for 20 min and centrifuged at 1500 x g for 5 min to separate the charcoal-absorbed steroids. The supernatant

was assayed for $^{3}H_2O$ by counting in a scintillation mixture using a PerkinElmer-Wallac β-counter.

Inhibition of 17α-hydroxylase-C17/20-lyase (CYP17)

Method

Enzyme preparation:

Recombinant *E.coli* pJL17/OR co-expressing human CYP17 and rat NADPH-P450-reductase were grown and stored as described by Ehmer et al [3]. For isolation of membrane fractions, 5 ml of bacterial suspension with an OD_{578} of 50 were washed using phosphate buffer (0.05 M, pH 7.4, 1 mM $MgCl_2$, 0.1 mM EDTA and 0.1 mM dithiothreitol). Bacteria were harvested by centrifugation (2000 x g) and the pellet was resuspended in 10 ml ice-cold TES buffer (0.1 M Tris-acetate, pH 7.8, 0.5 mM EDTA, 0.5 M sucrose). Lysozyme was added with 10 ml of ice-cold water resulting in a concentration of 0.2 mg/ml followed by incubation for 30 min on ice with continuous shaking. Spheroplasts were harvested by centrifugation (12000 x g, 10 min), and resuspended in 4 ml of ice-cold phosphate buffer (the same as described above plus 0.5 mM phenylmethylsulfonylfluoride (PMSF).

After that spheroplasts were sonicated on ice (pulse 20 s on, 30 s off, five times), using a sonicator Sonopuls HD60 (Bandelin, Berlin, Germany) at maximum power. Unbroken cells and debris were pelleted at 3000 x g for 7 min, and the supernatant was centrifuged at 50000 x g for 20 min at 4°C. The membrane pellet was resuspended in 2 ml of phosphate buffer (the same as described above) with 20 % glycerol using an ultra-turrax T25 (IKA-Labortechnik, Staufen, Germany). Protein concentration was determined by the method of Lowry. Aliquots of this preparation, which generally had a content of about 5 mg protein per ml, were stored at -70°C until used.

Inhibition of CYP17:

The assay was performed as follows: a solution of 6.25 nmol progesterone (in 5 µl methanol) in 140 µl phosphate buffer (0.05 M, pH 7.4, 1 mM $MgCl_2$, 0.1mM EDTA and 0.1 mM dithiothreitol), 50 µl NADPH generating system (in phosphat buffer with 10 mM NADP, 100 mM glucose-6-phosphate and 2.5 units of glucose-6-phosphate-dehydrogenase) and inhibitor (in 5 µl DMSO) was preincubated at 37°C for 5 min. Control cups were supplemented with 5 µl DMSO without inhibitor. The reaction was started by adding 50 µl of a 1:5 diluted membrane suspension in phosphate buffer (0.8-1.0 mg protein per ml). The maximum DMSO concentration in each sample was 2 %. After mixing, incubation was performed for 30 min at 37°C. Subsequently the reaction was stopped with 50 µl 1 N HCl.

Extraction of the steroids was performed by addition of 1.0 ml ethyl acetate and vigorous shaking for 1 min. After a centrifugation step (5 min, 2500 x g) the organic phase (0.9 ml) was transferred into a fresh cup containing 0.25 ml of incubation buffer and 50 µl 1 N HCl and mixed again. After centrifugation, 0.8 ml ethyl acetate solution was evaporated to dryness in a fresh cup. After that the steroids were redissolved in solvent for HPLC analysis.

C. Molecular modeling

Compound **2**, **5** and **6** were built and semi-empirical (AM1) energy minimized, their partial charges calculated. Chain A of the crystal structure of human CYP19 (placental aromatase cytochrome P450 in complex with androstenedione; PDB-id 3EQM) was retrieved from the PDB database. Androstenedione, all water molecules and ions were removed. Hydrogens were added and then the stripped CYP19 structure was energy optimized with the backbone atoms constraint with 1 kcal/mol using the LigX module of MOE2010.

Compounds **2, 5 and 6** were then docked using GOLD v5.01 [4, 5] with the CYP dedicated scoring function *goldscore.p450_pdb.params.* The active site was defined as all residues within 12 Å of the formerly present androstenedione. Each ligand was docked 50 times. Default parameters were used otherwise.

References

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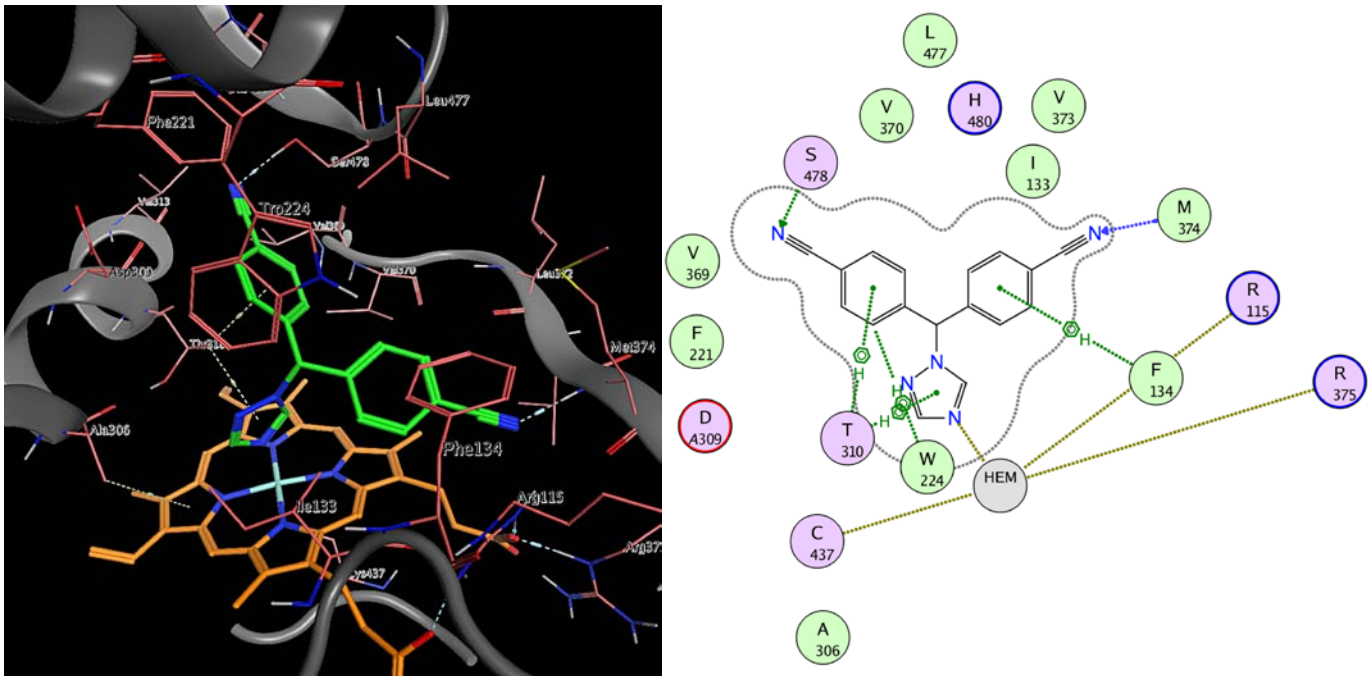

Figure 1-SI: Docking pose of Letrozole (**2**) to the crystal structure of human aromatase (PDB 3EQM), showing similar interaction to compound **5**