MedChemComm

Cite this: *Med. Chem. Commun.*, 2012, **3**, 663

www.rsc.org/medchemcomm

CONCISE ARTICLE

Synthesis and biological evaluation of imidazolylmethylacridones as cytochrome P-450 enzymes inhibitors†

Ashraf H. Abadi,*[ab] Sahar M. Abou-Seri,[b] Qingzhong Hu,[c] Matthias Negri[d] and Rolf W. Hartmann[cd]

Received 16th March 2012, Accepted 10th April 2012
DOI: 10.1039/c2md20072d

Two positional isomers with the general formula 1*H*-imidazol-1-ylmethylacridin-9(10*H*)-one were synthesized (**5**, **6**) and evaluated for their inhibitory activity *versus* CYP11B1, CYP11B2, CYP17 and CYP19. Compound **5** was more effective than letrozole in inhibiting CYP19 (aromatase). Interestingly, compound **5** also inhibited CYP11B1 with an IC_{50} of 21.2 nM. On the other hand compound **6** was almost completely inactive against all CYPs; this indicates that the positioning and spatial orientation of the imidazolylmethyl moiety is of paramount importance to activity. Sequence alignment of the four steroidogenic CYP enzymes and docking studies with **5**, **6** and letrozole supported this finding and suggested Ser478 to be an essential residue for both inhibition and selectivity. These novel compounds may have benefits for the treatment of Cushing's syndrome, hypertension, congestive heart failure and myocardial fibrosis and breast cancer.

Introduction

Cytochromes (CYPs) are metalloenzymes involved in the metabolism of xenobiotics and biosynthesis of hormones. Among the prominent CYPs are CYP11B1, which is involved in the biosynthesis of cortisol; CYP11B2, involved in the biosynthesis of aldosterone; CYP17, which catalyzes 2 steps in the biosynthesis of testosterone and CYP19 (aromatase), responsible for ring A aromatization and formation of estradiol from the androgen testosterone. Potential applications of these CYP inhibitors are for the treatment of Cushing's syndrome, hypertension, congestive heart failure and myocardial fibrosis, prostate cancer and breast cancer.[1–4]

Among the CYP11B1 and CYP19 inhibitors available on the market are metyrapone (**1**) and letrozole (**2**), respectively. It is believed that the lone pair of electrons of the pyridine and triazole moieties of the above two drugs are responsible for chelation of the iron atom of the respective CYPs, while the remaining part of the molecule is responsible for modulating the selectivity through appropriate interaction with the residues of the apo-protein.[5,6]

In two previous reports from our group we described the CYP inhibitory profile of imidazolylmethylxanthone and imidazolylmethylthiaxanthone derivatives, and the best derivatives (**3**, **4**) were found to be potent aromatase inhibitors with an IC_{50} of 40 nM and 16.5 nM, respectively. To explore the scope and limitations of this class of compounds as CYP inhibitors and to minimize their potential toxicity, herein we are reporting the activity of the imidazolylmethylacridone derivatives as tricyclic planar systems with an NH group, the latter moiety can behave as a hydrogen bond donor as compared to the acceptor functionality of the oxygen and sulfur atoms in **3** and **4**, respectively. The nitro group present in **3** (Fig. 1) was removed to avoid its potential toxicity. The positioning of the imidazolylmethyl moiety relative to the carbonyl and NH groups was explored by designing two isomers in which the imidazolylmethyl is at position 4 (**5**) or position 2 (**6**) of the acridone ring. The selectivity profile to inhibit different CYPs was determined for both compounds **5** and **6**.[7,8] For the semi-empirical (AM1) geometry-optimized three-dimensional structures of **2**, **5** and **6**, the distances between the sp^2 hybridized N of the imidazole/triazole and the carbonyl O (for **2** the nitrile N was considered) ($d_{O=C}$), and between the sp^2 N and the NH (d_{NH}), as well as the angle between the sp^2 N, methylene carbon between the two rings, and carbonyl O/nitrile N (a) were measured. Finally, **2**, **5** and **6** were docked into the CYP19 crystal structure, and their activity and selectivity profiles discussed based on binding mode, sequence comparison (different CYPs), and geometric differences (Fig. 2). The role of Ser478, an essential residue important to be targeted for dual inhibition (in this case CYP19–CYP11B1), is explored.

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt. E-mail: ashraf.abadi@guc.edu.eg; Fax: +20 2-27581041; Tel: +20 2-27590716*
[b]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt*
[c]*Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C2.3, 66123 Saarbrücken, Germany*
[d]*Department of Drug Design and Optimization, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus C2.3, 66123 Saarbrücken, Germany*
† Electronic supplementary information (ESI) available: See DOI: 10.1039/c2md20072d

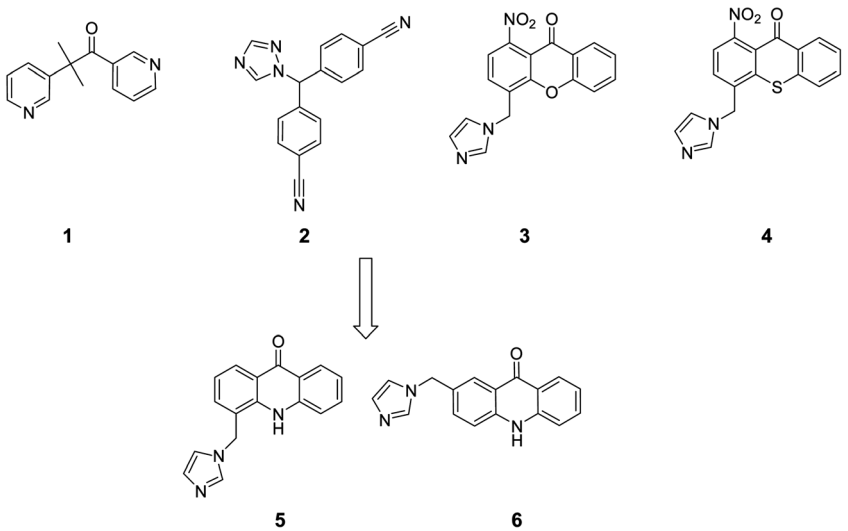


Fig. 1 Chemical structures of metyrapone (**1**) and letrozole (**2**), xanthone derivative (**3**) and thiaxanthone derivative (**4**), and the newly reported acridones **5** and **6**.

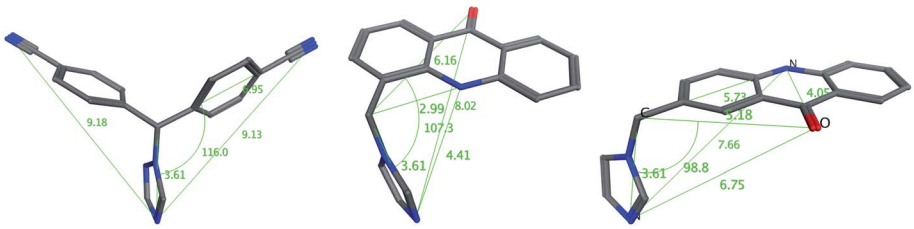


Fig. 2 Energy minimized forms of compounds **2**, **5** and **6**, shown left to right, showing the orientation and distance of the imidazole function relative to the aryl part.

Results and discussion

Chemistry

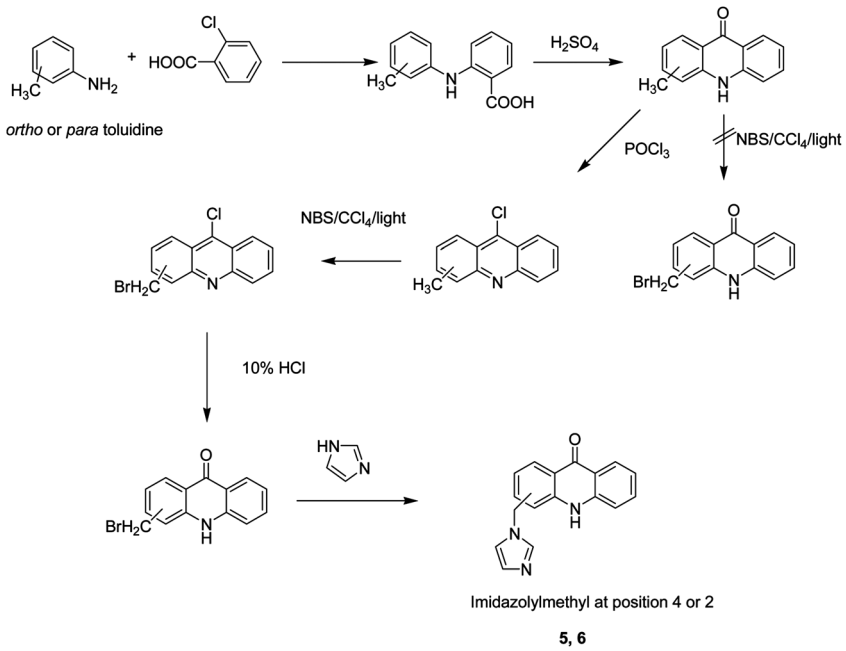
The synthesis of **5** and **6** is depicted in Scheme 1. In brief, Ullman copper-catalyzed condensation of 2-chlorobenzoic acid and the appropriate toluidine yielded the diphenylamine derivative.[9] This was followed by cyclization to the methylacridone derivative with H_2SO_4. Our attempts to synthesize the bromomethyl derivative from the methylacridone using *N*-bromosuccinimide were unsuccessful. We modified our scheme by reaction of the methylacridone with $POCl_3$ to give the chloroacridine derivative, which is then reacted with NBS/CCl_4 to yield the bromomethyl derivative, followed by heating with 10% HCl to yield the bromomethylacridone. The last step involved condensation of the latter with imidazole to obtain the final product.

Biology

Compounds **5**, **6**, letrozole **2** and metyrapone **1** were evaluated for their inhibitory effect on CYP11B1, CYP11B2, CYP17 and CYP19. Screening was carried out in two stages, single dose screening at a relatively high concentration, followed by exact IC_{50} determination for the active compounds. The results are shown in Table 1.

Compound **5** showed dual activity by inhibiting both CYP19 and CYP11B1, but with no significant inhibition of CYP11B2 and CYP17. Compound **5** inhibited CYP19 more than the reference letrozole, while it was less active than metyrapone *versus* CYP11B1. As seen from the docking pose (Fig. 3) **5** was stabilized by π-stacking with Phe134, Phe221 and Trp224 and by a hydrogen bond between its carbonyl moiety and Ser478. Notably, the potent antiestrogenic letrozole **2** was also found to interact with these very same residues,[15] it formed a hydrogen bond to the backbone of Met374 (Fig. 1, ESI†). Compound **6** had no significant inhibitory effect on the four CYPs, highlighting the importance of a precise spatial orientation between *e.g.* the imidazolylmethyl function and the carbonyl (Fig. 2 and Table 2). The inactivity of **6** can be explained by the dissimilarity in angle and distances compared to the active compounds **2** and **5**, as reported in Table 2 (compare $d_{O=C}$, d_{NH} and angle *a* between compounds **2**, **5** and **6**).

Previous studies proposed the circadian cortisol rhythm as a biomarker for the prognosis of breast cancer. Moreover, women with advanced breast cancer have significant elevations in levels of basal cortisol, which is released in response to stress. Such individuals are significantly more likely to die than patients with normal levels of the hormone.[10–12] In addition, elevated cortisol concentration down-regulates the tumor suppressor gene BRCA1A7 and therefore accelerates the progress of breast cancer.[13] Recent studies also revealed that excessive cortisol mediates the resistance to paclitaxel by a CDK-1-dependent pathway.[13,14] Thus, in this case, the dual inhibition reported for



Scheme 1

Table 1 Biological results for CYP inhibition by compounds **5**, **6** and the reference compounds letrozole **2** and metyrapone **1**[a]

	CYP11B1		CYP11B2		CYP17		CYP19	
Cpd	%inhibition at 500 nM	IC_{50} (nM)	%inhibition at 500 nM	IC_{50} (nM)	%inhibition at 2 μM	IC_{50} (nM)	%inhibition at 2 μM	IC_{50} (nM)
5	97.5 ± 1.2	21.2 ± 1.1	40.8 ± 1.4	ND	1.4 ± 0.3	ND	95.5 ± 1.7	14.4 ± 1.1
6	40.7 ± 4.7	ND	3.4 ± 1.3	ND	0.0 ± 0.0	ND	0.0 ± 0.0	ND
2	16.4 ± 2.6	ND	25.6 ± 1.6	ND	6.8 ± 1.0	ND	99.0 ± 0.5	36.2 ± 2.0
1	98.0 ± 0.8	14.6 ± 1.6	95.0 ± 1.9	71.8 ± 2.1	0.0 ± 0.0	ND	0.0 ± 0.0	ND

[a] Results are the average of 3 experiments, each done in duplicate, the results are expressed as the mean ± SD. ND = not determined.

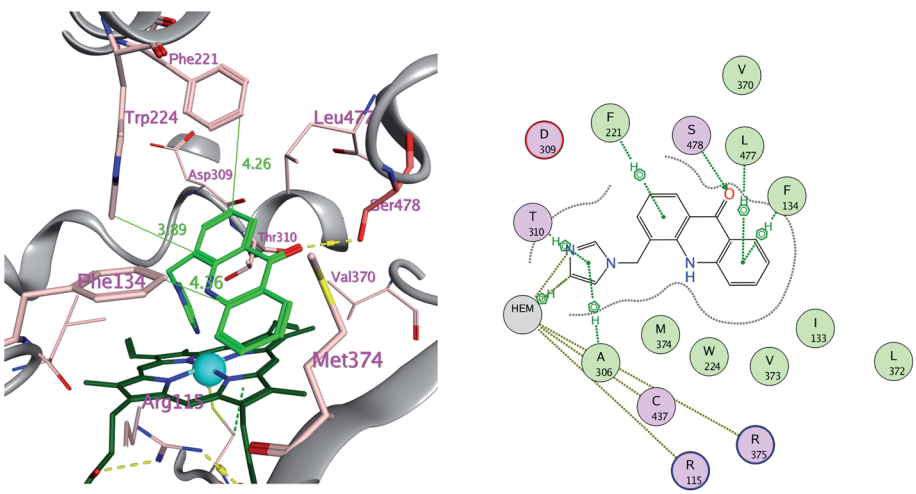


Fig. 3 Binding mode of compound **5** in the crystal structure of human aromatase (PDB 3EQM). Compound **5** is stabilized by a hydrogen bond to Ser478 (with the carbonyl), by heme-complexation (the imidazolyl function faces the porphyrin iron with a distance of 1.8 Å), and by π–π stacking with F134, F221 and W224. Additional hydrophobic interactions occur with Val370, Leu477, Met374 and Ile133.

Table 2 Spatial orientation of the imidazolylmethyl group of compound **5** and **6** relative to the carbonyl and NH groups. $d_{O=C}$ is the distance between the sp^2 hybridized nitrogen of the imidazole/triazole and the carbonyl oxygen or the nitrile nitrogen, d_{NH} the distance between the sp^2 N and the NH of the acridone scaffold, and *a* is the angle between the sp^2 N, the bridge-methylene group and the carbonyl oxygen or the nitrile nitrogen

	$d_{O=C}$	d_{NH}	*a*
2	9.18 Å	—	116°
5	8.02 Å	4.41 Å	107.3°
6	6.75 Å	7.66 Å	98.8°

the first time has to be seen as a positive addition and not as an unwanted side-effect. Since there are no pure CYP11B1 inhibitors in clinical use, it might be beneficial to develop selective dual inhibitors of CYP19 and CYP11B1. According to the docking results as well as the sequence alignment we might come to the identification of Ser478 as an essential residue both in terms of inhibition of CYP19, and selectivity *versus* other CYP enzymes, which have to be targeted whenever a dual-inhibition is wanted.

Molecular modeling

Docking. Compounds **2**, **5** and **6** were docked into the CYP19 crystal structure PDB-ID 3EQM by means of the docking software GOLD v5.01 and the CYP dedicated GOLDSCORE function *goldscore.p450_pdb.params*.

In its main pose compound **5** was found to dock with the imidazolyl plane perpendicular to the heme with the sp^2 N at 1.8–2.1 Å from the iron. The carbonyl oxygen pointed toward the C-terminal loop of CYP19 forming a hydrogen bond with the –OH of Ser478. The tricyclic core (~7.75 Å length) was oriented almost perpendicular to the longitudinal axis of the I-helix occupying the whole width of the active site (distance $C\alpha_{Met374}$ to $C\alpha_{Thr310} \approx 16$ Å), with the imidazolyl moiety-bearing ring facing the I-helix residues Ile305–Thr310. Thereby, **5** was stabilized by π-stacking with Phe134, Phe221 and Trp224 and by hydrophobic interactions with Ile133, Val370, Met374 and Leu477.

Also, letrozole (**2**) was found to have the triazole sp^2 N pointing perpendicular to the heme iron and π-stacked with Phe134, Phe221 and Trp224. One of the *p*-CN-phenyl rings pointed towards the hydrophobic loop opposite to the I-helix, while the other *p*-CN-phenyl ring was placed on the left of the C-terminal loop. Contrary to **5**, a hydrogen bond was formed between the nitrile N and the backbone NH of Met374 and not with the –OH of Ser478. However, changing the crystallographic rotamer of Ser478 resulted in the formation of a second hydrogen bond, this time between the nitrile of the second *p*-CN-phenyl and Ser478.

Compound **6** was also docked into the CYP19 active site. However, no pose was found with the imidazolyl nitrogen pointing to the heme, which would be expected for potent inhibitors.

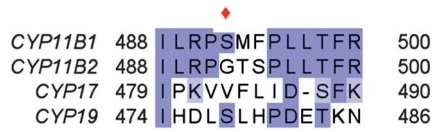


Fig. 4 Sequence alignment of CYP19, CYP17, CYP11B1 and CYP11B2. Zoomed view of the C-terminal turn region with Ser478, an important interaction partner for CYP19-inhibitors, marked with the red sign.

Sequence alignment. In order to understand the selectivity seen for compound **5** between the four CYP enzymes we aligned their sequences (for CYP17 and CYP19 also a superimposition was done) using T-coffee and manual refinement. According to the crucial polar interaction with Ser478 (missing for 6 and partially also for 2) we focused on the C-terminal turn regions (Fig. 4). Notably, while for CYP11B1 the counterpart to Ser478 (of CYP19) was also a Ser (Ser492), for CYP17 and CYP11B2 a hydrophobic (Val483) and an apolar (Gly492) residue was found, both incapable of hydrogen bond formation with their side-chains.

Acknowledgements

The authors are grateful for the STDF-Egypt and DAAD for a mobility grant to Ashraf Abadi in Rolf Hartmann's lab.

References

1 L. M. Tompkins and A. D. Wallace, *J. Biochem. Mol. Toxicol.*, 2007, **21**, 176–181.
2 D. F. Lewis and P. Lee-Robichaud, *J. Steroid Biochem. Mol. Biol.*, 1998, **66**, 217–233.
3 Q. Hu, C. Jagusch and U. E. Hille, *J. Med. Chem.*, 2010, **53**, 5749–5758.
4 C. Zimmer, M. Hafner, M. Zender, D. Ammann, R. W. Hartmann and C. A. Vock, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 186–190.
5 M. J. Murphy, Jr, *Oncologist*, 1998, **3**, 129–130.
6 M. Ekroos and T. Sjögren, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **203**, 13682–13687.
7 M. Recanatini, A. Bisi, A. Cavalli, F. Belluti, S. Gobbi, A. Rampa, P. Valenti, M. Palzer, A. Palusczak and R. W. Hartmann, *J. Med. Chem.*, 2001, **44**, 672–680.
8 S. Gobbi, C. Zimmer, F. Belluti, A. Rampa and R. W. Hartmann, *J. Med. Chem.*, 2010, **53**, 5347–5351.
9 V. Sourdon, S. Mazoyer, V. Pique and J. P. Galy, *Molecules*, 2001, **6**, 673–682.
10 G. van der Pompe, M. H. Antoni and C. J. Heijnen, *Psychoneuroendocrinology*, 1996, **21**, 361–374.
11 S. E. Sephton, F. S. Dhabhar, A. S. Keuroghlian, J. Giese-Davis, B. S. McEwen, A. C. Ionan and D. Spiegel, *Brain, Behavior, and Immunity*, 2009, **23**, 1148–1155.
12 S. E. Sephton, R. M. Sapolsky, H. C. Kraemer and D. Spiegel, *J. Natl. Cancer Inst.*, 2000, **92**, 994–1000.
13 L. Antonova and C. R. Mueller, *Genes, Chromosomes Cancer*, 2008, **47**, 341–352.
14 M. S. Flint, G. Kim, B. L. Hood, N. W. Bateman, N. A. Stewart and T. P. Conrads, *Psychoneuroendocrinology*, 2009, **34**, 1533–1541.
15 P. M. Wood, L. W. L. Woo, M. P. Thomas, M. F. Mahon, A. Purohit and B. V. L. Potter, *ChemMedChem*, 2011, **6**, 1423–1438.

