Bioorganic & Medicinal Chemistry Letters 22 (2012) 1860–1863

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

New aromatase inhibitors from the 3-pyridyl arylether and 1-aryl pyrrolo[2,3-*c*]pyridine series

Frédéric Stauffer *, Pascal Furet, Andreas Floersheimer, Marc Lang

Novartis Institutes for BioMedical Research, Global Discovery Chemistry Oncology, Postfach, CH-4002 Basel, Switzerland

ARTICLE INFO

Article history:
Received 8 December 2011
Revised 20 January 2012
Accepted 21 January 2012
Available online 28 January 2012

Keywords:
Aromatase
CYP19
Aromatase inhibition

ABSTRACT

Aromatase inhibition is the new standard of care for estrogen receptor positive breast cancer and has also potential for treatment of other diseases such as endometriosis. Simple and readily available 3-pyridyl arylethers and 1-aryl pyrrolo[2,3-*c*]pyridines recapitulating the key pharmacophore elements of Letrozole (**1**) are described and their structure–activity relationships are discussed. Potent and ligand efficient leads such as compound **23** (IC_{50} = 59 nM on aromatase) have been identified.

All the endogenous estrogens (estradiol and estrone) are originating from the conversion of androgens (testosterone and androstenedione, respectively) by the aromatase enzyme complex, a cytochrome P450 dependant enzyme. The aromatase enzyme complex is comprised of two polypeptides. The first of these is a specific cytochrome P450, named aromatase cytochrome P450 (CYP450arom) and is the product of a single gene, the CYP19 gene. The second is a flavoprotein, NADPH-cytochrome P450 reductase and is ubiquitously distributed in most cells. Ovary, testis, adipose tissue, skin, hypothalamus and placenta express aromatase normally, whereas breast and endometrial cancers, endometriosis, and uterine fibroids overexpress aromatase and produce local estrogens that exert paracrine and intracrine effects. Three mechanisms are responsible for aromatase overexpression in a pathological tissue versus its normal counterpart: a cellular component is altered to increase aromatase-expressing cell types; molecular alterations in stromal cells favor binding of transcriptional enhancers versus inhibitors to a normally quiescent aromatase promoter and initiate transcription; or heterozygous mutations, which cause the aromatase coding region to lie adjacent to constitutively active cryptic promoters that normally transcribe other genes, result in excessive estrogen formation.[1]

Selective aromatase inhibition, as displayed for instance by the third generation non-steroidal aromatase inhibitor Letrozole (**1**), has become the standard of care for estrogen receptor positive breast cancer and allows reduction of total body estrogens. The abrogation of estrogen receptors activation is obtained by depletion of the ligand as compared to the former strategy using the antagonistic effect of Tamoxifen on the receptor.

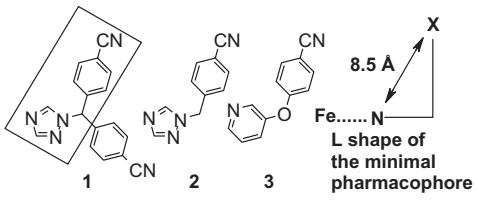


Figure 1. Schematic analysis of the minimum requirement for the aromatase pharmacophore as identified in compounds **1** (box), **2** and **3**.

Letrozole (**1**) is a potent non-steroidal aromatase inhibitor registered for clinical use. Its reported synthesis is only two steps away from commercially available starting material.[2] It has been recognized from analysis of the SAR of known non-steroidal aromatase inhibitors that the key pharmacophore necessary for activity has a L shape displaying at one end a nitrogen electron donor atom to interact with the iron of the heme and at the other end a hydrogen bond acceptor or a halogen distant of around 8.5 Å. The minimal efficient pharmacophore of the low nanomolar aromatase inhibitor Letrozole (**1**; IC_{50} = 1–11 nM)[3] is its synthetic intermediate 4-([1,2,4]triazol-1-ylmethyl)-benzonitrile (**2**; 88% inhibition at 1 μM) (Fig. 1).

In an effort to identify new aromatase inhibitor leads, we have investigated derivatives using 3-pyridyl as a replacement for the 5-membered ring triazole heme binding moiety. Such a modification had already been shown to be adequate in the 4-(aminoheteroaryl)-benzonitrile series.[4] The simple commercially available 4-(3-pyridyloxy)benzonitrile (**3**) is fulfilling the minimal

* Corresponding author. Tel.: +41 61 696 8749; fax: +41 61 696 6246.
E-mail address: frederic.stauffer@novartis.com (F. Stauffer).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.01.076

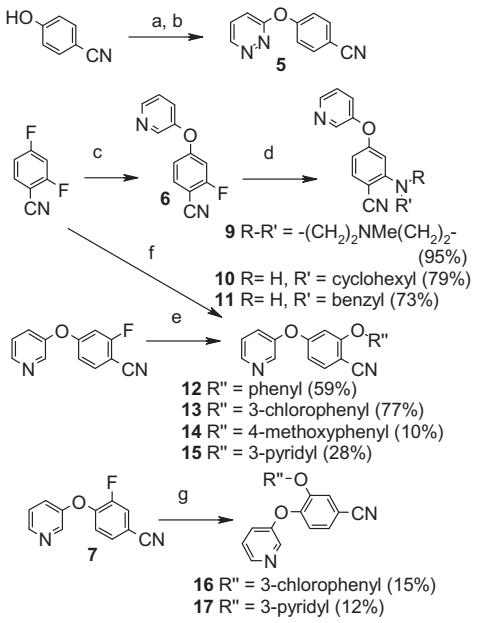
Table 1
Inhibition of aromatase at 1 μM concentration for 4-substituted benzonitrile ether compounds

Compds	Substituent on position 4 oxygen	Inhibition at 1 μM (%)	Aromatase IC_{50}[a] (μM)
3	3-Pyridyl	70	0.289 ± 0.010
4	4-Pyridyl	53	
5	3-Pyridazine	1	

[a] Average of three measurements on aromatase from human.[13]

pharmacophore requirements for efficient aromatase inhibition and shows IC_{50} = 0.289 μM (Table 1), corresponding to a ligand efficiency of 0.60 kcal × mol^{-1} per heavy atom based on IC_{50}.[5] The known isomer 4-(4-pyridyloxy)-benzonitrile (**4**)[6] shows reduced inhibitory activity (53% inhibition at 1 μM) as compared to compound **3**. Despite being isosteric to compound **3**, 4-(3-pyridazinyloxy)benzonitrile (**5**) is inactive (1% inhibition at 1 μM). Compound **5** was obtained by deprotonation of 4-hydroxybenzonitrile in DMF with 55% NaH in oil, followed by adjunction of 3-chloropyridazine and heating with microwave irradiation for 1 h at 170 °C. (Scheme 1)

The presence of the second benzonitrile group in Letrozole (**1**) is key to achieve selectivity against the other enzymes involved in the steroidogenesis. Using a divalent oxygen linker between the two aromatic moieties in **3** prevents to achieve selectivity following the same vector as seen for the third substituent of the carbon linker in Letrozole (**1**). In order to design new aromatase inhibitors, we have used a ligand based pharmacophore model built by the alignment of a diverse set of potent ligands challenging the space around the L shape minimal pharmacophore (Fig. 2).[7] This approach has still been considered valid despite the recent release of the first X-ray structure of aromatase in co-crystal with androstenedione.[8] The enzyme pocket in this structure appears to be not large enough to accommodate compound **1** suggesting some level of plasticity of the active site.



Scheme 1. Reagents and conditions: (a) NaH, DMF, 30 min at rt. (b) 3-Chloropyridazine, DMF, 1 h at 170 °C (μw) (38%). (c) 3-Hydroxypyridine, sodium salt, NMP, 2 h from 0 °C to rt (16%). d) Amine (2.5 equiv), NMP, 15–25 min at 150–170 °C (μw). (e) Sodium phenolate, NMP, 10 min at 110–130 °C (μw). (f) 3-Hydroxypyridine, sodium salt, NMP, 15 min at 100 °C (μw). (g) Sodium phenolate, NMP, 40-60 min at 150 °C (μw).

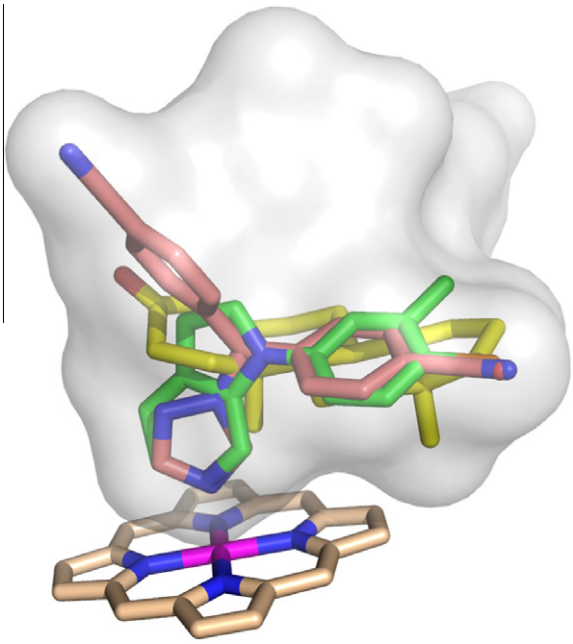


Figure 2. Represented on top of the heme (beige), the volume accessible to ligands (white) in the aromatase pocket as defined by the superimposition of diverse sterically challenging potent aromatase inhibitors. The ligands are aligned based on their geometry of interaction with the heme. Compound **23** docked in the pharmacophore model appears in green. For reference, Letrozole is shown in pink and the enzyme substrate androstenedione in yellow.

We have investigated, as an alternative vector for the elaboration of compound **3**, the substitution at position 2 (Table 2) or position 3 (Table 3) of the benzonitrile of **3**. The introduction of a fluoro substituent is unfavorable; compound **6** with fluoro at position 2 is less active than the commercially available compound **7** with fluoro at position 3 (15% and 52% inhibition at 1 μM, respectively). The commercially available 4-(3-pyridyloxy)-phthalonitrile (**8**) confirms the negative impact of a small electron withdrawing group with H-bonding acceptor capability at position 2 of the benzonitrile. Compounds **6** and **7** were further processed under microwave irradiation to displace the fluoro group with phenolates or amines in NMP (Scheme 1). Introduction at position 2 of an amine is unfavorable in the three cases exemplified with the introduction of *N*-methylpiperazine (compound **9**) being less preferred than aminocyclohexyl (compound **10**) and benzylamine (compound **11**), with an IC_{50} = 0.989 μM, being the best tolerated. The introduction at position 2 of a phenolate (compound **12**) is tolerated (78% inhibition at 1 μM) and modulation of the activity seems to be achievable with variation of the phenyl ether substitution pattern; *m*-chloro (compound **13**) is significantly weaker than *p*-methoxyphenoxy (compound **14**) (25% and 84% inhibition at 1 μM, respectively). The 2,4-bis-(3-pyridyloxy)benzonitrile (**15**) was obtained in one synthetic step from 2,4-difluorobenzonitrile and displays a similar potency as compared to **3** (Table 2).

The 3,4-substitution pattern is less easily accessible due to the reduced reactivity of the position 3 fluoro group, as compared with position 2, in a S_NAr reaction and moderate yields of isolated products (15% and 12% for compounds **16** and **17**, respectively) were obtained for the reaction of **7** with phenolates (Scheme 1). Substitution at position 3 is well tolerated when introducing a *m*-chlorophenoxy or a 3-pyridyloxy group (89% and 82% inhibition at 1 μM). (Table 3)

Using 4-pyrrolo[2,3-*c*]pyridin-1-yl-benzonitrile (compound **18**), the required conformation to inhibit the aromatase enzyme was rigidified and led to a potent inhibitor (IC_{50} = 0.093 μM). Compound

Table 2
Inhibition of aromatase at 1 μM concentration for 2-substituted 4-(pyridyloxy)benzonitrile compounds

Compds	Substituent on position 2	Inhibition at 1 μM (%)	Aromatase IC_{50}[a] (μM)
6	F	15	
8	CN	27	5.03 ± 0.33
9	4-Methypiperazinyl	8	
10	Cyclohexylamino	33	
11	Benzylamino	58	0.986 ± 0.066
12	Phenyloxy	78	
13	3-Chlorophenyloxy	25	
14	4-Methoxyphenyloxy	84	
15	3-Pyridyloxy	81	0.304 ± 0.023

[a] Average of three measurements.

Table 3
Inhibition of aromatase at 1 μM concentration for 3-substituted 4-(pyridyloxy) benzonitrile compounds

Compds	Substituent on position 3	Inhibition at 1 μM (%)	Aromatase IC_{50}[a] (μM)
7	F	52	
16	3-Chlorophenyloxy	89	0.229 ± 0.045
17	3-Pyridyloxy	82	

[a] Average of three measurements.

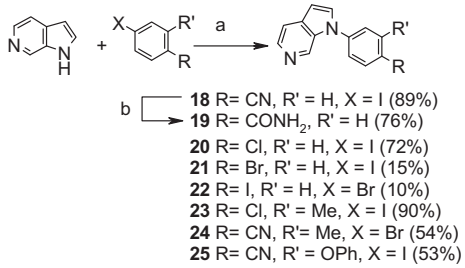
Table 4
Inhibition of aromatase at 1 μM concentration for substituted 1-aryl-1H-pyrrolo[2,3-*c*]pyridine compounds

Compds	Substituted aryl	Inhibition at 1 μM (%)	Aromatase IC_{50}[a] (μM)
18	4-Cyanophenyl	94	0.093 ± 0.005
19	4-Aminocarbonyl	60	
20	4-Chlorophenyl	85	0.523 ± 0.064
21	4-Bromophenyl	56	
22	4-Iodophenyl	26	
23	4-Chloro-3-methyl	96	0.059 ± 0.011
24	4-Cyano-3-methyl	>99	
25	4-Cyano-3-phenoxyphenyl	97	0.114 ± 0.032

[a] Average of three measurements.

18 shows good selectivity against CYP 3A4, 2C9 and 2D6 (>200×). Hydrolysis of the cyano substituent to the amide (compound **19**) reduces the activity and replacement of the cyano with a chloro (compound **20**) is tolerated with half a log unit decrease in inhibitory activity. When increasing the size of the halogeno substituent (Br compound **21** and I compound **22**) the potency of the inhibitors is decreased. The introduction of an extra methyl positioned *ortho* to the chloro substituent in compound **23** increases the potency by one log unit as compared to compound **20**. The beneficial effect of this methyl substituent is confirmed by comparing compound **19** with compound **24** (94% and >99% inhibition at 1 μM, respectively). Similarly to what as been discussed previously comparing compounds **3** and **12**, the introduction of a phenoxy substituent at the position *ortho* to the cyano group is well-tolerated giving rise to a similar potency though the ligand efficiency is notably decreased (0.57 and 0.40 kcal × mol^{-1} per heavy atom for compound **18** and **25**, respectively, based on IC_{50}). (Table 4)

Taking up the challenge of competing with the synthetic conciseness of Letrozole (**1**), compounds **18** to **25** were synthesized with poor to good yields (10–90%) in one step from the commercially available 6-azaindole and the suitable halogenoaryl



Scheme 2. Reagents and conditions: (a) Halogenoaryl (1.1 equiv), K_3PO_4 (2.1 equiv), tran-*N*,*N*′-dimethyl-1,2-cycloheanediamine (0.1 eq.), CuI (0.05 equiv), toluene, 4–36 h at 110 °C. (b) NaOH, 1,4-dioxane/water 2:1, 40 min at 100 °C.

using a non-optimized modified Ullmann copper (I) catalyzed coupling reaction (Scheme 2).[9] Compound **20** has been described as an intermediate in the synthesis of semicarbazide-sensitive amine oxidase inhibitors and was obtained in a similar manner.[10]

In summary, the simple 3-pyridyloxybenzonitrile (**3**) has been found to be a submicromolar inhibitor of aromatase. We have used a ligand based pharmacophore to guide our SAR investigation as an alternative to the claimed X-ray structure based[11] or homology model based[12] approaches. Some readily accessible derivatives of compound **3** have been synthesized in one or two steps allowing a preliminary assessment of the structure–activity relationship for aromatase inhibition. The rigidified 4-pyrrolo[2,3-*c*]pyridin-1-yl-benzonitrile (**18**) represents a new efficient aromatase inhibitor fulfilling the pharmacophore requirements as defined for fragment **2**. Derivatisation at several positions is allowed in agreement with our pharmacophore model. In addition, compound **18** shows a good ligand efficiency and straightforward accessibility making it an attractive lead for further optimization towards more specific aromatase inhibitors.

Acknowledgments

We thank Arco Jeng and Chii-Whei Hu for their technical support in measuring aromatase inhibition.

References and notes

1. Bulun, S. E.; Lin, Z.; Imir, G.; Amin, S.; Demura, M.; Yilmaz, B.; Martin, R.; Utsunomiya, H.; Thung, S.; Gurates, B.; Tamura, M.; Langoi, D.; Deb, S. *Pharmacol. Rev.* **2005**, *57*, 359.
2. Bowman, R.M.; Steele, R.E.; Browne, L. U.S. Patent 4 978 672, **1990**.
3. Haynes, B. P.; Dowsett, M.; Miller, W. R.; Dixon, J. M.; Bhatnagar, A. S. *J. Steroid. Biochem. Mo.l. Biol.* **2003**, *87*, 35.
4. Okada, M.; Yoden, T.; Kawaminami, E.; Shimada, Y.; Kudoh, M.; Isomura, Y. *Chem. Pharm. Bull.* **1997**, *45*, 482.
5. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
6. Buchstaller, H.P.; Wiesner, M.; Schadt, O.; Amendt, C.; Zenke, F.; Sirrenberg, C.; Grell, M.; Finsinger, D. WO 2004037789, **2004**.
7. Furet, P.; Batzl, C.; Bhatnagar, A.; Francotte, E.; Rihs, G.; Lang, M. J. Med. Chem. **1993**, *36*, 1393. The molecules used to construct the model are compounds 4, 7, 8, 11, 12, 13 and 14 of this publication in addition to Letrozole, androstedione, 3-cyclohexyl-3-(4-aminophenyl)piperidine-2,6-dione (an aminoglutethimide derivative) and LY-113174 a pyrimidine inhibitor.
8. (a) Ghosh, D.; Griswold, J.; Erman, M.; Pangborn, W. *J. Steroid Biochem. Mol. Biol.* **2010**, *118*, 197; (b) Ghosh, D.; Griswold, J.; Erman, M.; Pangborn, W. *Nature* **2009**, *457*, 219.
9. Antilla, J.C.; Klapars, A.; Buchwald, S.L. J. Am. Chem. Soc. **2002**, *124*, 11684; Representative synthesis of compound **23**: In a Schlenk tube are added 6-azaindole (0.846 mmol), potassium phosphate (1.78 mmol) and CuI (0.042 mmol). The tube is twice evacuated and back-filled with Ar. Toluene (1 ml), 2-chloro-5-iodotoluene (0.846 mmol) and (trans)-*N*,*N*′-dimethyl-1,2-cyclohexanediamine (0.085 mmol) are added. The tube is sealed and the reaction mixture is stirred for 22 h at 110 °C. The reaction mixture is diluted with dichloromethane and dry loaded on silica gel to be purified by flash chromatography (hexane/EtOAc 10% to 30%) to give 1-(4-chloro-3-methylphenyl)-1*H*-pyrrolo[2,3-*c*]pyridine as a white solid (184 mg). ESI-HRMS (M+H): 243.06827, 245.06514 (cal. $C_{14}H_{12}N_{23}5Cl$: 243.06835); H NMR (DMSO-d_6, 600 MHz) δ 8.92 (s, 1H), 8.24 (d, 1H), 7.72 (br s, 1H), 7.66

(d, 1H), 7.63 (d, 1H), 7.55 (br d, 1H), 6.79 (d), 2.44 (s, 3H); C NMR δ 139.2, 137.5, 137.0, 133.7, 133.5, 132.1, 132.1, 131.5, 130.1, 126.4, 122.9, 115.4. 103.0, 19.7.
10. Evans, D.; Carley, A.; Stewart, A. et al. WO 2011113798, **2011**.
11. Ghosh, D. WO 2009061859, **2009**.
12. Karkola, S.; Höltje, H. D.; Wähälä, K. *J. Steroid Biochem. Mol. Biol.* **2007**, *105*, 63.
13. Human CYP19 + P450 reductase SUPERSOMES (#456260, BD Biosciences); fluorometric assay adapted from Stresser, D.M.; Turner S.D.; McNamara, J; Stocker, P.; Miller, V.P.; Crespi, C.L.; Patten, C. *J. Anal. Biochem.* **2000**, *284*, 427.