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Structure-activity relationships and docking studies of synthetic 2-arylindole derivatives determined with aromatase and quinone reductase 1

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Abstract

In our ongoing effort of discovering anticancer and chemopreventive agents, a series of 2-arylindole derivatives were synthesized and evaluated toward aromatase and quinone reductase 1 (QR1). Biological evaluation revealed that several compounds (e.g., **2d**, IC₅₀ = 1.61 μM; **21**, IC₅₀ = 3.05 μM; and **27**, IC₅₀ = 3.34 μM) showed aromatase inhibitory activity with half maximal inhibitory concentration (IC₅₀) values in the low micromolar concentrations. With regard to the QR1 induction activity, **11** exhibited the highest QR1 induction ratio (IR) with a low concentration to double activity (CD) value (IR = 8.34, CD = 2.75 μM), while **7** showed the most potent CD value of 1.12 μM. A dual acting compound **24** showed aromatase inhibition (IC₅₀ = 9.00 μM) as well as QR1 induction (CD = 5.76 μM) activities. Computational docking studies using CDOCKER (Discovery Studio 3.5) provided insight in regard to the potential binding modes of 2-arylindoles within the aromatase active site. Predominantly, the 2-arylindoles preferred binding with the 2-aryl group toward a small hydrophobic pocket within the active site. The C-5 electron withdrawing group on indole was predicted to have an important role and formed a hydrogen bond with Ser478 (OH). Alternatively, *meta*-pyridyl analogs may orient with the pyridyl 3'-nitrogen coordinating with the heme group.

Graphical Abstract

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Supplementary data

Supplementary data (synthetic procedures, compound characterization, ¹H and ¹³C NMR spectra, HPLC chromatographs, computational docking protocol, aromatase inhibition and QR1 induction assays) associated with this article can be found, in the online version, at .

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Keywords

2-Arylindole; Anticancer; Aromatase; Docking; Estrogen; Quinone reductase 1

Breast cancer is the most common type of cancer affecting women worldwide.¹ According to the World Health Organization (WHO), breast cancer caused more than half a million deaths in 2015.² Postmenopausal woman are most susceptible to develop breast cancers and these are often associated with increased serum estrogen levels which are purported to be a prominent driving factor of tumor growth and proliferation.^{1, 3} Approximately 70–80% of breast tumors in postmenopausal woman are found to be estrogen or progesterone dependent.¹ In these cases, unusually high levels of estrogen are found in breast tissue due to the biosynthesis of estrogen in adipose, breast, and other peripheral tissues by cytochrome P450 aromatase.³ Aromatase is a microsomal enzyme complex that plays a crucial role in the conversion of androgens into estrogens in peripheral tissues by aromatization of the steroid A ring.^{4, 5} Specifically, it catalyzes the conversion of androstenedione, testosterone, and 16 α -hydroxytestosterone into estrone, 17 β -estradiol, and 17 β ,16 α -estriol, respectively.⁶ Clinically, estrogen lowering aromatase inhibitors are used as first line therapy to treat estrogen receptor-positive breast cancer in menopausal woman today.⁶ Their effectiveness can be attributed to the fact that aromatase is the only known enzyme in vertebrates that can catalyze the aromatization of a six-membered ring,⁶ as such, aromatase represents an attractive druggable target to block estrogen biosynthesis. A representative list of landmark and/or clinically used aromatase inhibitors is shown in Figure 1 and several of them have been approved for the treatment of estrogen-dependent breast cancer.^{4, 6} Among them, anastrozole, letrozole, and exemestane are clinically used in the US.⁷ A review article that describes the history of aromatase inhibitor development was recently published.⁶

Although aromatase inhibitors have shown success in the clinic in treating and/or preventing breast cancer, currently available drugs exhibit treatment limiting side effects including nausea, fatigue, insomnia, headaches and arthralgia.^{6, 8, 9} Moreover, mounting drug resistance is another concern commonly associated with anti-aromatase drugs.¹⁰ Therefore, continued search and development of novel and alternative aromatase inhibitors is highly warranted.

In addition, activation and/or upregulation of anticarcinogenic enzymes have also been considered as an alternative strategy to achieve cancer chemoprevention. Among these anticarcinogenic enzymes is the cytoprotective enzyme quinone reductase 1 (QR1). QR1 induction was shown to protect cells against chemical-induced carcinogenesis in animal models.¹¹ Mechanistically, this detoxification and cytoprotective effect was achieved via the catalyzing step of transforming excessive quinones into the corresponding hydroquinones by

QR1.^{12, 13} In this context, it is therefore of great importance to develop potent and efficient QR1 inducers as potential chemopreventive and anticancer agents.

The indole scaffold is ubiquitous in natural products and pharmaceutical molecules.¹⁴ Some indole derivatives such as azolylmethyl-1*H*-indoles,^{15–18} azolylbenzyl-1*H*-indoles,^{15, 19, 20} 5-aryl(imidazol-1-yl)methyl-1*H*-indoles,²¹ 6- and 4-arylindoles,²² indole-3-carbinols,²³ indole-imidazoles,²⁴ and 2- and 3-aryl(azolyl)methylindoles²⁵ have been previously synthesized and evaluated as potential anti-aromatase agents. Recently, Cushman and coworkers developed a series of resveratrol analogs by replacing the *trans* double bond with a thiadiazole moiety and discovered a promising new compound with dual activity against aromatase and QR1 (Figure 2).^{26–28} Lang and coworkers identified 1-arylpyrrolo[2,3-*c*]pyridine as a potent aromatase inhibitor with an IC₅₀ value of 59 nM.²⁹ Due to the structural similarity of these compounds to 2-arylindole scaffold (Figure 2), we became interested in investigating our series of 2-arylindoles as potential dual acting small molecules that inhibit aromatase while simultaneously activate QR1. We hypothesized that the more rigid 2-arylindole skeleton could mimic the resveratrol backbone whilst at the same time reducing the entropic penalty of binding creating a more favorable binder.³⁰

The 2-arylindole compound library was synthesized according to Schemes 1 and 2. To start, a preliminary 2-arylindole set was initially synthesized based on chemical transformations of commercially available 2-phenylindole (**1**) and 2-phenylindole-3-carboxaldehyde (**2**) to afford *N*-alkyl derivatives **1a–e**, the reduction product **1f**, the oxime derivatives **2a–c**, and 3-cyano-2-phenylindole **2d**, respectively (Scheme 1).³¹ Further modifications of the 2-arylindole library were done by varying the substituents R¹, R², and R³ around the 2-arylindole scaffold. Synthesis of these derivatives was achieved using a one pot process via initial Sonogashira coupling of 2-iodoanilines with terminal alkynes followed by base-assisted cyclization (Scheme 2).³¹

Most of the reactions proceeded smoothly to generate the expanded 2-arylindole library in good yields. Further chemical derivatizations were achieved by introducing the *N*-Me functionality (**8a**, **24a**, **31a**, and **31c**) or reducing the nitro into amino group (**24b** and **31b–c**). All the synthesized compounds were tested in aromatase inhibition and QR1 induction assays, and their biological activities are shown in Table 1.

Anti-aromatase activity

A brief SAR summary of our 2-arylindole series on aromatase inhibition is highlighted in Figure 3. In general, small, polar electron withdrawing groups (EWGs) on the C-5 indole ring resulted in favorable anti-aromatase activity and increased in the order of Br < Cl < NO₂ < CN with IC₅₀ values of 39.7, 22.8, 9.0, and 3.3 μM, respectively. Changing the position of the EWG (CN) from C-5 to C-3 resulted in a two-fold improvement of anti-aromatase activity (Table 1, entry 11 vs 39) while moving the EWG (Cl) from C-5 to C-6 resulted in a five-fold decrease in activity (Table 1, entry 38 vs 40). Although the prototype 2-phenylindole (**1**) showed only slight activity against aromatase, switching the C-3' carbon to nitrogen on the 2-phenyl ring resulted in dramatic increase in activity, providing **21** with an IC₅₀ of 3.05 μM (Table 1, entry 1 vs 31). Subsequent docking studies suggest that the 3'-

pyridyl nitrogen can form a favorable N-Fe interaction with the heme of aromatase which may account for this notable increase in anti-aromatase activity. The 2-thiophenyl and 2-naphthyl derivatives, **22** and **23** (Table 1, entries 32–33), did not show encouraging activity. Attaching various alkyl groups to the nitrogen atom of the indole motif in general had no improvement in anti-aromatase activity (Table 1, entries 2–6) except for *N*-methylation of **24** to give **24a** which effected a two fold increase in activity (Table 1, entry 34 vs 35). The reduced 2-phenylindole variant, indoline **1f** did not show significant activity (entry 7). Similarly, the C-3 oxime derivatives **2a–c** were largely inactive against aromatase (Table 1, entries 8–10). A variety of 2-arylindole derivatives bearing electron donating groups (NMe₂, NH₂, OMe, alkyl) or electron withdrawing groups (F, Cl, Br, OCF₃) on C-2', C-3' or C-4' position of the 2-aryl ring were tested against aromatase. However, no significant increase in anti-aromatase activity was observed for these derivatives compared to **1**. In fact, incorporation of 2-aryl substituents on **24** to give **31** totally destroyed aromatase activity (Table 1, entry 34 vs 43), suggesting that substituents on the 2-aryl moiety are not well tolerated. On the basis of excellent anti-aromatase activities of **21**, **24**, and **27**, two hybrid derivatives **32** and **33** were subsequently designed and synthesized in an effort to identify more potent anti-aromatase agents. Nonetheless, no improvements were achieved and only **33** showed activity in low micromolar range (IC₅₀ = 11.5 μM) (Table 1, entries 47 and 48). Moreover, the commercially available 2-phenylbenzimidazole (**34**) with structural similarity to 2-phenylindole, was not active (Table 1, entry 49).

QR1 induction activity

A brief SAR summary for our 2-arylindole library on QR1 induction is shown in Figure 4. The prototype 2-phenylindole **1** having a free indole NH showed very good QR1 induction activity with a CD value of 2.65 μM (Table 1, entry 1) and was used as a benchmark to compare activities of other analogs. *N*-Alkylation of the indole nitrogen destroyed QR1 induction activity as seen in derivatives **1a–e** (Table 1, entries 2–6). This suggested that the free indole NH functionality is important for QR1 induction. Relative to **1**, 2-phenylindoline **1f** only exhibited weak activity (IR = 1.8), indicating a flat, aromatic indole scaffold is superior to indoline for QR1 induction (Table 1, entry 7). Among the oxime derivatives **2a–c**, only **2a** having a free hydroxyl group showed reasonable QR1 activity with an IR value of 3.10 and CD value of 11.70 μM (Table 1, entries 8–10). Introducing a NO₂, Cl, or Br function on the indole moiety at C-5 generally led to a two-fold loss in activity relative to **1** with CD values of 5.76 μM, 5.50 μM, and 6.76 μM, respectively (Table 1, entries 34, 38, and 41). Interestingly, the introduction of a cyano group at C-3 or C-5 of the indole moiety totally destroyed QR1 activity (Table 1, entry 11 and 39). In general, substituents on the 2-aryl ring of **1** weakened QR1 activity, except for the 3',5'-dimethoxy derivative **7** (CD = 1.12 μM, entry 16) which displayed a 2-fold increase in QR1 induction activity over **1** (CD = 2.65 μM, entry 1). The activity of *para*-substituted 2-arylindoles increased in the order of OCF₃ < F < Br < Cl with CD values of 29.10, 17.80, 5.01, and 2.75 μM, respectively (Table 1, entries 21–22, 24, 26). However, the *para* substituted analogs **17** (*n*-pentyl), **18** (amino), and **20** (*N,N*-diMe) were all inactive as QR1 inducing agents (Table 1, entries 27, 28 and 30). The *meta* derivatives **6** (3'-OCH₃) and **10** (3'-Cl) had comparable activity to their corresponding *para* isomers **5** and **11** (Table 1, entries 14, 20 vs 15, 21), however, the *meta*

amino derivative **19** showed improved activity (CD = 18.40 μ M) over its *para* isomer **18** (Table 1, entry 28 vs 29). The *ortho* substituted derivatives **3**, **9**, and **25** displayed minimal QR1 activity with IR values less than 1.5 (Table 1, entries 12, 19 and 37). The loss of QR1 induction activity is likely due to steric effects and subsequent conformational change resulting from having an *ortho* substituent on the 2-aryl ring. Notably, increasing the number of halogen atoms on the 2-aryl ring was detrimental to the QR1 inductive activity as seen in **8** (3',4'-diCl) and **15** (3',4'-diF) that were inactive in comparison to their corresponding mono substituted analogs (Table 1, entries 17, 25 vs 20, 21, and 24). The pyridyl (**21**), 2-thiophenyl (**22**), and 2-naphthyl (**23**) analogs (Table 1, entries 31–33) were also inactive. Collectively, the SAR studies of this 2-arylindole library from both aromatase and QR1 assays identified four compounds (**24**, **26**, **29** and **33**) that showed dual activity in both assays. The most promising dual acting compound, **24**, exhibited an IC₅₀ value of 9.00 μ M and CD value of 5.76 μ M for anti-aromatase and QR1 induction, respectively.

Computational docking studies

Computational docking has become a powerful virtual screening tool for discovering novel potential drug leads and advanced computer based drug design.³² Recently, Del Rio and coworkers identified highly active aromatase inhibitors with a new core through high-throughput docking protocol.³³ The currently accepted binding modes of non-steroidal aromatase inhibitors (e.g., letrozole and anastrozole) to aromatase have been learned from computational^{34, 35} and site directed mutagenesis studies^{35, 36} because the X-ray cocrystal structures of these compounds bound to aromatase are lacking. In 2011, Wood *et al.*³⁴ and Hong *et al.*³⁵ reported the proposed binding modes of letrozole with aromatase using computational docking. Both studies agreed that the triazole moiety of letrozole binds to the heme prosthetic group of aromatase. In the model reported by the Chen group, the distance between the triazole of letrozole and iron of heme was reported to be 3.7 Å.³⁵ The Potter group reported more clearly the role of the benzonitrile groups of letrozole.³⁴ One of the nitrile groups forms a hydrogen bond to Met374 (NH), the same residue that androstenedione hydrogen bonds with, while the other nitrile group forms a hydrogen bond with Ser478 (OH).³⁴ This is supported by earlier mutagenesis experimental studies that demonstrated a S478A mutation destroys letrozole activity.³⁶

To gain further insight into the potential binding modes of our active 2-phenylindole inhibitors with human aromatase, a similar computational docking approach was undertaken using CDOCKER (Discovery Studio 3.5).³⁷ The protein X-ray crystal structure of human aromatase (PDB code: 3EQM) was recovered from the protein data bank (PDB) and the bound natural substrate ligand (androstenedione) was removed prior to docking. All ligands for docking were initially constructed in ChemDraw and exported into Discovery Studio (DS). The ligands were prepared for docking using the prepare ligands protocol in DS followed by energy minimization. Before docking our 2-arylindole library, the reliability of our docking protocol (CDOCKER) was verified by docking androstenedione and letrozole back into the active site of aromatase. The CDOCKER energy scoring function (–CDOCKER energy) that takes into account ligand-receptor interaction energy and internal ligand strain energy was used to rank docked poses. Encouragingly, the top scoring pose for docked androstenedione overlaid favorably with that in the protein bound X-ray cocrystal

structure (Figure 5a and 5b). Key hydrogen bond interactions were predicted between the C-17 carbonyl of androstenedione and the protein at Met374 (NH, backbone) and Arg115 (NH, side chain). Similarly, the top scoring pose for letrozole agreed favorably with that in previously published letrozole-aromatase docking studies (Figure 5b).^{34, 35} In our model, the distance between the triazole function of letrozole and the heme of aromatase was found to be 2.7 Å. Both benzonitrile groups of letrozole were predicted to form hydrogen bonds to the protein within the active site, one to Met374 (NH) and the other to Ser478 (OH) with measured distances of 2.0 Å and 2.2 Å, respectively. An additional hydrogen bond was observed between a benzonitrile group of letrozole and Arg115 (NH side chain) with a distance of 2.2 Å.

After validation of the docking protocol, the binding poses for our 2-arylindole scaffolds were predicted using analogous docking experiments. The –CDOCKER energy scores for the docked library ranged from –9.5 to 26 where a more positive value means more favorable binding prediction. Compound **21** was the best scoring ligand and returned a –CDOCKER energy score of 26. This was the second most active aromatase inhibitors in our series. Compound **2d**, the most active aromatase inhibitor in our series returned a favorable –CDOCKER energy score of 17. Overall, aromatase inhibitors gave favorable –CDOCKER energy scores with values greater than 10. In general, compounds that were bulky and/or had substituents on the 2-aryl ring, especially on the *para* C-4' position, scored poorly and is consistent with SAR data. The lowest scoring ligand was **31a** which showed a –CDOCKER energy score of –9.5 and was not active in the aromatase assay. The docking results were intriguing as more than one favorable pose were predicted depending on the substitution pattern around the 2-arylindole scaffold. Generally, the 2-arylindole scaffold preferred to orient within the active site in such that the aromatic 2-aryl group positioned towards a well-known hydrophobic pocket comprising residues Phe134, Val370, Leu372, Val373, Met374, and Leu477 (Figures 5c and 5d). In **2d** (Figure 5c), the 2-aryl group made close contact to Phe134 (2.7 Å), Leu372 (3.0 Å), Met374 (3.1 Å), and Leu477 (3.1 Å) whereas in Figure 5d, **27** made closest contact with Phe134 (2.9 Å), Leu372 (3.4 Å), Val373 (3.2 Å), Met374 (3.5 Å), and Leu477 (3.5 Å). The indole NH of **2d** (Figure 5c) oriented away from the heme and toward residues Leu477, Phe221, and Ser478. The distance between the indole NH and Ser478 side chain hydroxyl was measured to be 4.6 Å, suggesting a hydrogen bond interaction is unlikely. A slight variation in binding was predicted for compounds having a C-5 EWG such as **25**, **26**, **27**, **29**, and **33**. In these cases, the 2-aryl group occupied the same hydrophobic pocket as **2d**, however the orientation of the indole moiety was flipped in such that the indole NH pointed toward the back of the pocket and Trp224 (Figure 5d). The flipped docking pose of these derivatives is not surprising given the relatively symmetrical nature of the 2-arylindole scaffold. This may also highlight the limitation associated with molecular modelling. Additionally the 2-arylindole scaffold was rotated by approx. 35° relative to **2d**, which brought the C-5 indole substituents in close proximity (< 2.5 Å) to Ser478 side chain hydroxyl group (Figure 5d). This suggested a favorable hydrogen bond may be possible in these cases. Notably, the six membered indole ring of C-5 substituted analogs (e.g., **27**) was positioned toward a small gap in-between Thr310 and Ser478, the same space normally occupied by one of the benzonitrile groups of letrozole. The six membered indole ring of **2d** oriented towards residues Ala306 and Thr310

with close contacts of 3.6 Å and 3.1 Å, respectively. The C-2 nitrile group of **2d** positioned towards the side chain of Arg115 and had a close contact of 3.2 Å. Very interestingly, **21** having a 3'-pyridyl nitrogen in the 2-aryl ring, docked in a completely opposite fashion to the other 2-arylindoles. Instead, the 3'-pyridyl nitrogen preferred to coordinate to the iron of heme in all top 10 scoring poses with a measured nitrogen-heme distance of approximately 2.4 Å (Figure 5e). The 2-aryl substituent oriented towards Ala306 and Thr310 while the indole backbone formed close contacts with Phe134, Val370, Leu372, Met374, and Leu477 all within a distance of 4.1 Å.

Collectively, the predicted binding modes for 2-arylindoles within the active side of aromatase provided some valuable insights that may help rationalize SAR data for these compounds. The active site of aromatase is relatively small being less than 400 Å³.³⁵ The 2-aryl ring predominantly docked into a small hydrophobic pocket. This suggests that substituents on the 2-aryl ring are not generally well tolerated and is consistent with activity data. Active compounds **25**, **26**, **27**, **29**, and **33** docked in an orientation that put the C-5 EWG close to Ser478 (OH), strongly suggesting favorable hydrogen bonding or dipole-dipole interactions may be possible in these cases. Given the mostly hydrophobic nature of the active site of aromatase, the 2-fold increase in activity of *N*-methyl derivative **24a** over free NH derivative **24** may be a result of favorable van der Waals (VDW) interaction between the small hydrophobic methyl group and the active site. Moreover, the free indole NH function was not found to play a significant role in any of our predicted binding models.

In summary, a series of 2-arylindole derivatives were designed, synthesized, and evaluated for aromatase inhibition and QR1 induction. Biological evaluation revealed that **2d** was the most potent anti-aromatase inhibitor (IC₅₀ = 1.60 μM), but **21** and **27** also displayed good aromatase inhibitory activity with IC₅₀ values of 3.05 and 3.34 μM, respectively. Computational docking studies were utilized to predict the binding modes of 2-arylindoles with aromatase active site. Despite not being definitive, most 2-arylindoles preferred binding with the 2-aryl group towards a small hydrophobic pocket within the active site. The C-5 EWG on indole was predicted to form hydrogen bonding with Ser478 (OH) which may explain the increased potency for these analogs. Alternatively, 3'-N (pyridyl) analogs preferred to orient with the pyridyl nitrogen coordinating with heme. In terms of QR1 induction, the 3',5'-dimethoxy derivative **7** displayed the best activity with a CD of 1.12 μM. The 2-phenylindole **1** also showed promising QR1 induction activity with a CD of 2.65 μM. Together, a promising dual acting compound, **24**, was uncovered which exhibited an IC₅₀ value of 9.00 μM and CD value of 5.76 μM in anti-aromatase and QR1 induction assays, respectively. These results provide promising leads for further optimization of chemical synthesis and expand potential applications for the development of new chemopreventive and chemotherapeutic agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Landmark and/or clinically used aromatase inhibitors.

resveratrol

 $IC_{50} = 25.0 \mu M$ (aromatase) $CD = 21.0 \mu M$ (QR1) $IC_{50} = 0.24 \mu M$ (aromatase) $CD = 53.1 \mu M$ (QR1) $IC_{50} = 59 \text{ nM}$ (aromatase) **2-arylindole****Figure 2.**

New anti-aromatase and QR1 activating agents as well as design rationale for 2-arylindoles.

Figure 3.
Brief SAR of aromatase inhibitory activity based on modifications of 2-arylidole.

Figure 4.
SAR for QR1 induction of 2-phenylindole derivatives.

Figure 5.

Docking models of natural substrate androstenedione, known aromatase inhibitor letrozole, **2d**, **21**, and **27** with aromatase (3EQM). a) An overview of superimposed binding poses of androstenedione (pink), letrozole (yellow), and **2d** (orange) with aromatase as well as the zoom-in view of binding site. Crystal structure position of bound androstenedione is shown in blue. b) Superimposed binding poses of androstenedione (pink) and letrozole (yellow) to aromatase active site with the crystal structure of bound androstenedione in blue showing hydrogen bonding interactions. c) Predicted binding pose of **2d** with aromatase and representative binding pose for 2-arylindoles. d) Predicted binding mode for C-5 substituted 2-arylindoles **25**, **26**, **27**, **29**, and **33**. For clarity, only **27** is shown as a representative. e)

Alternative binding mode for 3'-pyridyl derivative **21** showing a predicted N-Fe interaction of 2.7 Å. Phe134, Val370, Leu372 and Val373 are removed from Figures 5b–e for clarity.

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Scheme 1.

Synthesis of **1a–f** and **2a–d**. Reagents and conditions: (i) NaBH_3CN , AcOH , rt, 20 h; (ii) 1. NaH , DMF or DMF/THF (1/2), $0\text{ }^\circ\text{C}$; 2. R^1X ($\text{X} = \text{I, Br, Cl}$), $0\text{ }^\circ\text{C}$ –rt. For **1e**: DMAP , Boc_2O , CH_3CN . (iii) 1. $\text{NH}_2\text{OH}\cdot\text{HCl}$, NaOH , MeOH , rt; 2. $\text{Cu}(\text{OAc})_2$ (5 mol%), CH_3CN , rt, ultrasound; (iv) $\text{R}^2\text{ONH}_2\cdot\text{HCl}$, pyridine, EtOH , rt.

Scheme 2.
Synthesis of 2-arylindole derivatives.

Table 1Aromatase inhibition and QR1 induction activities of 2-arylindole derivatives^a

Entry	Compound	Aromatase % inhibition ^b	IC ₅₀ (μM)	QR1 IR ^c	CD ^d (μM)
1	1	27.75±2.87		2.30±0.12	2.65±0.51
2	1a	0.00±3.88		0.50±0.63	
3	1b	0.00±3.58		0.37±0.59	
4	1c	20.19±1.95		1.90±0.24	
5	1d	26.29±3.31		0.87±0.81	
6	1e	11.89±1.81		1.00±0.88	
7	1f	48.98±2.84		1.80±0.55	
8	2a	40.36±3.25		3.10±0.29	11.70±3.60
9	2b	24.66±1.17		1.30±1.10	
10	2c	23.25±1.34		0.50±0.65	
11	2d	88.00±1.07	1.61±0.20	0.55±0.64	
12	3	27.06±0.45		0.54±1.20	
13	4	9.33±3.39		5.20±1.50	6.40±2.90
14	5	0.00±3.84		1.56±0.53	
15	6	48.37±2.57		1.30±0.59	
16	7	9.18±3.25		3.75±0.52	1.12±0.20
17	8	22.65±5.01		0.57±0.63	
18	8a	20.00±1.78		4.40±1.23	2.10±0.80
19	9	21.51±2.73		1.40±0.81	
20	10	24.15±1.48		5.20±0.79	2.17±1.80
21	11	15.78±2.93		8.34±1.60	2.75±0.63
22	12	4.80±0.81		2.80±0.84	5.10±1.00
23	13	9.27±4.36		1.48±0.59	
24	14	21.80±2.67		2.73±0.29	17.80±4.60
25	15	11.02±3.87		0.58±1.12	
26	16	1.86±2.34		2.48±0.63	29.10±7.02
27	17	0.00±3.22		0.78±0.23	

Entry	Compound	Aromatase		QR1	
		% inhibition ^b	IC ₅₀ (μM)	IR ^c	CD ^d (μM)
28	18	46.21±1.35		0.56±0.80	
29	19	26.74±4.83		2.70±0.71	18.40±2.60
30	20	0.00±2.63		1.21±0.69	
31	21	81.55±2.09	3.05±0.61	1.14±0.33	
32	22	21.98±3.81		0.67±0.15	
33	23	0.07±3.06		0.74±0.69	
34	24	77.72±2.89	9.00±0.49	3.92±0.41	5.76±0.80
35	24a	76.99±3.52	3.88±0.38	0.89±0.49	
36	24b	41.43±3.41		0.72±0.84	
37	25	71.47±0.21	6.87±0.64	0.78±1.21	
38	26	70.53±1.39	22.81±3.39	5.10±1.03	5.50±0.94
39	27	82.27±4.98	3.34±0.71	1.06±0.23	
40	28	13.60±2.84		4.25±1.12	6.80±2.10
41	29	58.08±0.18	39.71±1.77	5.50±0.98	6.76±3.10
42	30	32.36±2.32		1.00±0.96	
43	31	7.59±3.42		1.07±0.80	
44	31a	34.04±5.10		1.56±0.90	
45	31b	21.37±2.89		4.90±1.40	10.33±2.60
46	31c	41.99±2.35		2.14±0.20	46.70±2.05
47	32	27.90±0.10		1.70±0.20	
48	33	93.70±7.00	11.50±2.40	2.10±0.50	31.20±4.00
49 ^e	34	7.64±7.82		0.86±1.40	
Standard control ^f			9.95±1.46 (nM)		0.01

^aConcentration for initial testing: 50 μM.

^bPercentage of inhibition in comparison with vehicle-treated controls.

^cIR: induction ratio at a concentration of 50 μM.

^dCD is the concentration that double the activity and is determined for compounds with IR > 2.

^e2-Phenylbenzimidazole was purchased from Sigma-Aldrich.

IC_{50} or CD values of known bioactive compounds (letrozole for aromatase and 4',-bromoflavone for QR1).