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SYNTHESIS AND AROMATASE INHIBITORY ACTIVITY OF TETRAHYDROQUINOLINE DERIVATIVES

Prafulla M. Sabale*, Priyanka Walke and Umang Solanki[1]
Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440033
[1]Parul Institute of Pharmacy, Limda-391 760
E-mail : prafullasable@yahoo.com

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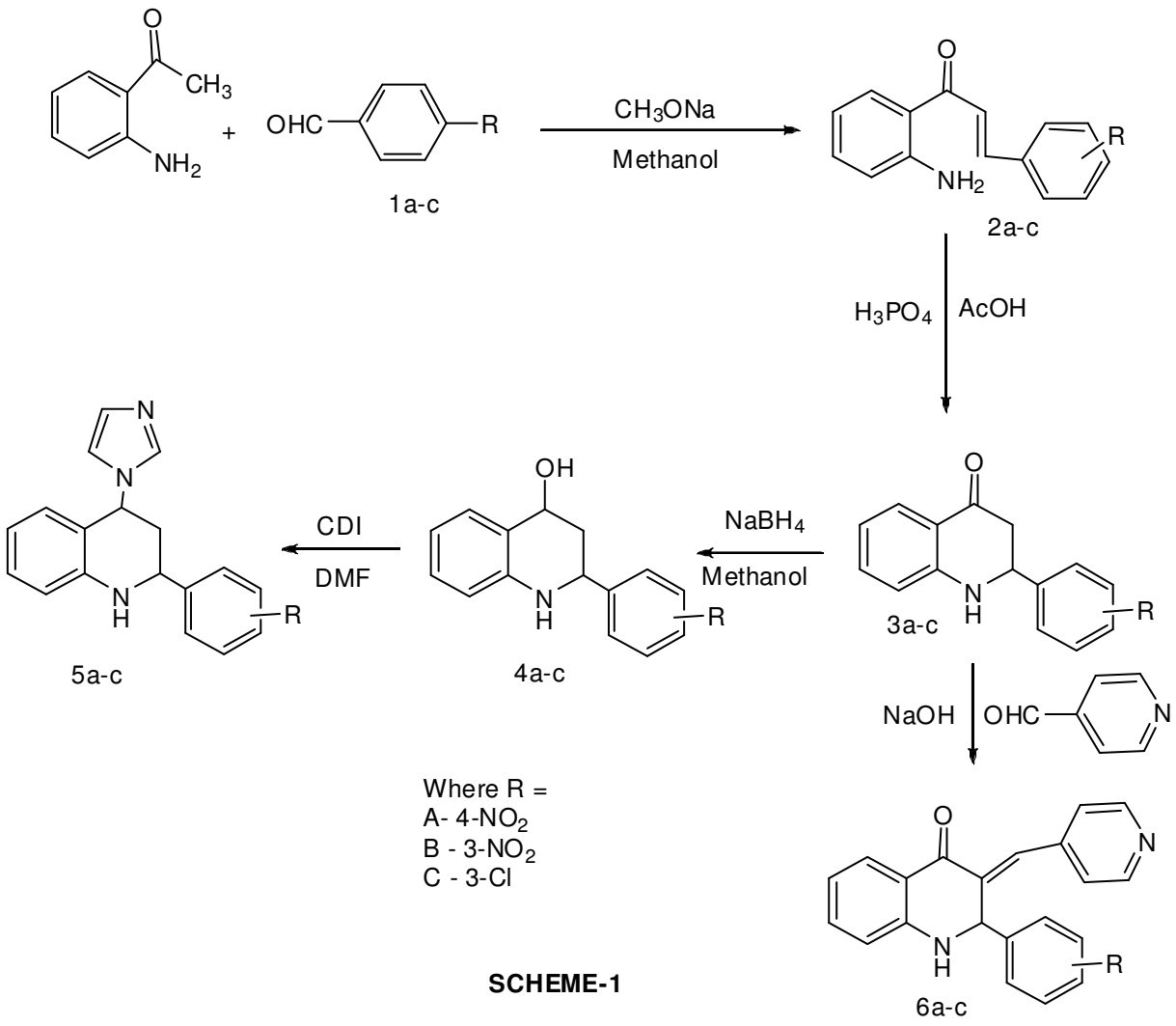
Aromatase, a cytochrome P450 hemo-protein which is responsible for estrogen biosynthesis, by the conversion of androgens to estrogens, has been an attractive target in the treatment of hormone-dependent breast cancer. As a result, a number of synthetic steroidal or non-steroidal aromatase inhibitors have been successfully developed. Inhibition of aromatase is an efficient approach for the prevention and treatment of breast cancer. The present study involve the synthesis of 1,2,3,4-tetrahydroquinoline derivatives as non-steroidal aromatase inhibitors. The 1,2,3,4-tetrahydroquinoline derivatives were synthesized as per described schemes and evaluated for aromatase inhibitory activity. Different inhibitory profile could be observed, the compound **5**c was found to have optimal inhibitory activity for aromatase inhibition.

Breast cancer is one of the leading causes of morbidity and mortality world-wide. Despite advances in its treatment, remains the second most common cause of death in women in the western world[1]. Generally considered to be the disease of older women, 22% of the cases occurs in females below the age of 50[2]. Worldwide, more than one million women develop breast cancer each year with nearly half of these diagnoses occur in the Unites States and Europe. Moreover, nearly 40% of these women die of their diseases[3]. Approximately, two-thirds of postmenopausal breast cancer patients have estrogen-dependent breast cancer, which contains estrogen receptors (ERs) and requires estrogen for tumor growth[4]. In a high percentage of cases, it proves to be hormone-dependent, because tumor progression is dependent on high levels of circulating estrogens, which play a critical role in cancer cell proliferation[5]. A series of enzymes are involved in this intratumoral or in situ production of estrogens in breast carcinoma tissues, but aromatase (AR) (CYP19a), a member of the cytochromes P450 family, is the key enzyme involved in their synthesis, promoting the aromatization of the A ring of androgen precursors[6]. AR is localized in the endoplasmic reticulum of cells, and expressed as a multienzymatic complex consisting of two main components, a form of cytochrome P450 (CYP19) and NADPH-cytochrome P450 flavoprotein reductase. It catalyzes conversion of androgens to estrogens through transformation of the steroid enone A-ring to the aromatic phenolic ring with the concomitant loss of the C19 methyl group in the form of formic acid.

Different strategies have been devised to control or block the progression of hormone-dependent breast cancer, and one of the main approach involve is the reduction of estrogen levels by the inhibition of CYP19. The first clinically used AR inhibitor has been Aminoglutethimide (AG), marketed in the late 1970s. Unfortunately, AG was far from being an ideal drug since it showed several drawbacks, such as high toxicity and lack of selectivity, which have limited its use and induced its ultimate withdrawal. Third generation AR inhibitors have shown improved efficacy and lower toxicity when compared with the estrogen antagonist Tamoxifen in both the advanced and the early breast cancer. For this reason, the third generations of AR inhibitors, particularly, Anastrozole and Letrozole, have been recommended by the FDA as the first line drugs in the therapy of breast carcinoma. Although third generation aromatase



SCHEME-1

inhibitors (AIs), such as Letrozole, Anastrozole, and Exemestane are now considered a valid alternative to Tamoxifen as first line treatment of advanced breast cancer, the search for potent and selective AIs still remains an attractive subject. Moreover, alternative strategies such as development of multipotent compounds are being evaluated from different research groups to deal with the complexity and multiplicity of factors involved in the development of hormone-dependent breast cancer[7,8].

In this context we began a systematic, long term study aiming at the synthesis of new AR inhibitor capable with better pharmacological and toxicological profiles. In the first paper of this series, we reported on some molecules featuring different 1,2,3,4-tetrahydroquinoline containing imidazole and pyridine as aromatase inhibitors. Then, with the aim to increase potency and selectivity versus CYP enzymes, mainly, different structural modifications on this scaffold were introduced and very potent and selective AIs were synthesized.

The synthesis of compounds has been carried out as outlined in Scheme-1. 1-(2-Aminophenyl)-3-aryl-pro-2-en-1-ones, (**2**a-c) were prepared by Claisen-Schmidt condensation of 2-amino acetophenone with substituted benzaldehydes in the presence of base. Acid catalyzed cyclization was carried out in presence of ortho-phosphoric acid and acetic acid to obtain

Table-1
Physicochemical data of all syntheized compounds

Comp	R	M.P. (^{0}C)	Yield (%)	R_f	Solvent System
2a	4-NO_2	106-107	70	0.55	A
2b	3-NO_2	91-93	45	0.59	A
2c	3-Cl	78-80	55	0.49	A
3a	4-NO_2	150-152	45	0.42	A
3b	3-NO_2	134-135	23	0.37	B
3c	3-Cl	154-155	46	0.41	B
4a	4-NO_2	121-124	78	0.57	B
4b	3-NO_2	109-112	88	0.53	C
4c	3-Cl	114-116	51	0.49	C
5a	4-NO_2	177-178	46	0.30	C
5b	3-NO_2	149-151	36	0.26	C
5c	3-Cl	168-171	29	0.22	C
6a	4-NO_2	211-213	37	0.25	D
6b	3-NO_2	198-201	42	0.22	D
6c	3-Cl	212-214	39	0.27	D

A. Chloroform, B. Hexane: Ethyl acetate (8.5:1.5), C. Chloroform : Acetone (8:2), D. Hexane: Ethyl acetate (8:2).

compounds (**3**a-c) in good yield. Then treatment of quinolon-4-ones (**3**a-c) with sodium borohydride gives reduced products (**4**a-c) in quantitative yield. Finally, (**5**a-c) were prepared by condensation of imidazole at C-4 atom of carbinol by stirring (**4**a-c) with carbonyl diimidazole for 4-8 hr. These compounds were purified by column chromatography. The treatment of

Table-2
FT-IR spectroscopic data of compounds (**2**a-c, **3**a-c & **4**a-c)

Compd	IR (cm)
2a	3439, 3335 (-NH_2), 3137 (-CH), 1645 (C=O), 1339 (-NO_2).
2b	3401, 3241 (-NH_2), 3109 (-CH), 1643 (C=O) (-NO_2).
2c	3315,3133 (-NH_2), 2926 (-CH), 1640 (C=O), 721 (C-Cl).
3a	3312 (-NH_2), 1661 (C=O), 1342 (-NO_2).
3b	3112 (-NH), 1660 (C=O), 1349 (-NO_2)
3c	3132 (-NH), 1666 (C=O), 798 (C-Cl).
4a	3378 (-NH), 3132 (-CH), 1347 (-NO_2).
4b	3418 (-NH), 3111 (-CH), 1344 (-NO_2).
4c	3405 (-NH), 3150 (-CH), 815 (C-Cl).

compounds **3**a-c with pyridine-4-carboxaldehyde in presence of base gave 2-aryl-3-(pyridine-4-yl methyl) quinolo-4 (1*H*)-ones (**6**a-c).

Biological activity

In screening studies, substituted tetrahydroquinolin-4-ols (**4**a-c), imidazol-1-yl tetrahydroquinolines (**5**a-c) and (pyridine-4-yl-methyl) quinolo-4(1*H*)-ones (**6**a-c) were studied for aromatase inhibitory activity using human placental microsomal aromatase assay. Compounds **4**a-c, analogues of tetrahydroquinolin-4-ol does not show any inhibiton, confirms that nitrogen containing heme coordinating moiety is essential for aromatase inhibition. The imidazole containing tetrahydroquinoline derivative **5**c showed optimal inhibitory activity, due to presence of electron withdrawing group at position **3**. The imidazole containing tetrahydroquinoline derivatives **5**a and **5**b showed minimal inhibitory activity, due to presence of bulky group attached to phenyl nucleus. In tetrahydroquinoline series the nitro containing derivative **5**a showed good inhibitory activity than other derivatives due to bulkiness of group. The compounds **6**a-c showed negligible inhibitory activity due to presence of methylene group between hydrophobic

Table-3
Biological activity (% inhibition of aromatase enzyme) of compounds

Comp	Conc. μM	% Inhibition ± SD
4a	5.0	0.5±1.0
4b	5.0	0.3±0.9
4c	5.0	1.3±0.7
5a	5.0	16 ± 0.8
5b	5.0	25±1.05
5c	5.0	57±1.4
6a	5.0	4.9±3.3
6b	5.0	2.0±2.8
6c	5.0	11.0±1.5

SD = Standard Deviation, Mean of three experiments.

spacer and heme coordinating moiety. All the results were determined by mean of three experiments, as tabulated in Table-3.

Experimental

Moisture sensitive reactions were carried out under a dry nitrogen atmosphere in vacuum oven-dried glassware. Thin-layer chromatography was performed on the precoated silica gel F_{254} plates. Silica gel column chromatography was performed using silica gel 60A. Melting points were determined in open glass capillaries using a VEEGO make microprocessor based melting point apparatus having silicone oil bath and are uncorrected. IR spectra (wave numbers in cm^{-1}) were recorded on a BRUKER ALPHA-T FT-IR spectrophotometer using potassium bromide discs. ^1H NMR spectra were recorded on BRUKER AVANCE II 400 MHz instrument in $CDCl_3$ with TMS as internal standard. Chemical shift values are mentioned in δ ppm. Mass spectra were recorded on Shimadzu LC MS 2010 spectrometer.

1-(2-Aminophenyl)-3-(substituted phenyl) prop-2-en-1-ones (2a-c)

A solution of 2-aminoacetophenone (0.1 mol) in dry methanol (15 ml) was stirred with a solution of substituted benzaldehyde (0.1 mol) in ethanol containing sodium methoxide at 0-5^0 for 10 hr. The completion of reaction was monitored by TLC, after completion of reaction, further it was sirred for 1 hr at room temp. Reaction mixture was poured on crushed ice, a crude product filtered off, washed with cold water and recrystallized from methanol. The compounds (**2**a-c) were confirmed by physico-chemical parameter mentioned in Table-1 and by matching FT-IR frequencies of compounds mentioned in Table-2.

2-(Substituted phenyl)-2,3-dihydroquinoline-4(1*H*)-ones (3a-c)

A mixture of 1-(2-aminophenyl)-3-(substituted phenyl) prop-2-en-1-ones (**2**a-c) (0.1 mol), *ortho*-phosphoric acid (10 ml) and acetic acid (10 ml) was heated in microwave oven at low energy level for 10-12 minutes. The reaction mixture was cooled to room temp and poured into water (50 ml) and precipitate was filtered and washed with sodium bicarbonate solution and then with water. The crude product was recrystallized from ethyl acetate. The compounds (**3**a-c) were confirmed by physico-chemical parameter mentioned in Table-1 and by matching FT-IR frequencies of compounds mentioned in Table-2.

2-(Substituted phenyl)-1,2,3,4-tetrahydroquinolin-4-ols (4a-c)

A solution of sodium borohydride (1.5 mmol) in dry methanol (10 ml) was added slowly with constant stirring at specific interval to a solution of 2-(substituted phenyl)-2,3-dihydroquinoline-4(1*H*)-ones (**3**a-c) (1.0 mmol) in dry methanol (10 ml) at 0-5^0 over a period of 3 hr. The reaction mixture was further stirred for 30 minutes and excess of solvent was recovered under reduced pressure. pH of the solution was attenuated to 6 by aqueous hydrochloric acid. This solution was extracted with ethyl acetate, dried over sodium sulphate and concentrated under reduced pressure to obtain crude product. The crude product was passed through column chromatography on silica gel using hexane: ethyl acetate (90:10v/v) as eluent.

2-(Substituted phenyl)-4-(1*H*-imidazol-1-yl)-1,2,3,4-tetrahydroquinolines (5a-c)

Excess quantity of 1,1′-carbonyldiimidazole (4 mmol) was stirred with 2-(substituted phenyl)-1,2,3,4-tetrahydroquinolin-4-ols (1 mmol) (**4**a-c) in dry dimethylformamide (5 ml) under inert atmosphere at room temp for 10 hr. The reaction mixture was poured on brine solution. The brine solution was extracted with chloroform, the organic layer was washed with water dried over sodium sulphate and concentrated under reduced pressure. The crude product was recrystalized from hexane.

2-(4-Nitrophenyl)-4-(1*H*-imidazol-1-yl)-1,2,3,4-tetrahydrquinoline (5a)

Molecular ion peak, M^+ peak appeared at 320.9 in mass spectrum. The ^{1}H NMR gives signals at 2.26-2.24 (q, 2H, 3-CH_2), 3.36 (m, 1H, 2-CH), 4.32-4.36 (d, 1H, 1-NH), 5.23-5.61 (d, 1H, 4-CH), 6.54-6.71 (m, 3H, imidazole H-2′′, H-4′′, H-5′′), 7.05-7.46 (m, 6H, ArH), 8.13-8.14 (d, 2H, ArH).

2-(3-Nitrophenyl)-4-(1*H*-imidazol-1-yl)-1,2,3,4-tetrahydroquinoline (5b)

Molecular ion peak, M^+ peak appeared at 320.5 in Mass spectrum. Its ^{1}H NMR gaves signals at 2.22-2.36 (q, 2H, 3-CH_2), 3.37 (t, 1H, 2-H), 4.61-4.69 (d, 1H, 1-NH), 5.08-5.16 (d, 1H, 4-H), 6.45-6.83 (H-2′′, H-4′′, H-5′′), 7.72-7.91 (m, 7H, ArH), 8.12-8.14 (d, 1H, ArH).

2-(3-Chlorophenyl)-4-(1*H*-imidazol-1-yl)-1,2,3,4-tetrahydroquinoline (5c)

Molecular ion peak, M^+ peak appeared at 310.1 in mass spectrum. ^{1}H NMR shows signals at 2.31-2.39 (q, 2H, 2-CH_2), 3.13 (t, 1H, 2-H), 4.29-4.34 (d, 1H, 1-NH), 4.99-5.02 (d, 1H, 5-H), 6.81-7.77 (m, 10H, Ar-CH), 7.9 (s, 1H, ArH).

2-(Substituted phenyl)-3-(pyridine-4-yl-methyl) quinolo-4(1*H*)-ones (6a-c)

An aqueous sodium hydroxide solution (30%, 2 ml) was added with constant stirring to a solution of 2-(substituted phenyl)-2,3-dihydroquinoline-4(1*H*)-ones (**3**a-c) (1 mmol) in ethanol (5 ml). Pyridine-4-carboxaldehyde (0.128gm, 1.2 mmol) was then added to the reaction mixture. After complete addition, reaction mixture was stirred for 24 hr at room temperature. Then it was evaporated under reduced pressure. The residue was dissolved into chloroform and the organic layer washed with water, dried over sodium sulphate and evaporated to dryness. Crude product purified by column chromatography on silica gel using ethyl acetate: hexane (90:10 v/v) as eluent.

2-(4-Nitrophenyl)-3-(pyridine-4-ylmethyl) quinolo-4(1*H*)-one (6a)

Molecular ion peak, M^+ peak appeared at 358.7 in mass spectrum. ^{1}H NMR gave signals at 2.36 (s, 1H, Ar-CH), 3.37 (d, 1H, 2-H), 4.22-4.25 (d, 1H, 1-NH), 6.45-6.63 (m, 4H, pyridine-H), 7.12-7.14 (d, 2H, ArH), 7.22-7.97 (m, 6H, ArH).

2-(3-Nitrophenyl)-3-(pyridine-4-ylmethyl) quinolo-4(1*H*)-ones (6b)

Molecular ion peak, M^+ peak appeared at 359.3 in mass spectrum. ^{1}H NMR shows signals at 2.38 (s, 1H, Ar=CH), 3.45-3.46 (d, 1H, H-2), 4.72-4.76 (d, 1H, 1-NH), 6.45-6.62 (m, 4H, pyridine-1H), 7.12-7.72 (m, 6H, ArH), 8.59 (s, 1H, Ar-H-2′).

2-(3-Chlorophenyl)-3-(pyridine-4-ylmethyl) quinolo-4(1*H*)-ones (6c)

Molecular ion peak, M^+ peak appeared at 347.9 in mass spectrum. The ^{1}H NMR shows signals at 2.19 (s, 1H, Ar=CH), 3.16-3.23 (d, 1H, H-2), 4.91-4.94 (d, 1H, 1-NH), 6.96-7.01 (m, 4H, pyridine-H), 7.13-7.75 (m, 7H, ArH), 8.01 (s, 1H, Ar-H-2′).

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References

1. E.R. Simpson and S.R. Davis, *Endocrinology,* **142** (2001), 4589.
2. O.C. Freedman, S. Verma and M.J. Clemons, *Breast Can. Res. Treat*, **99** (2006), 241.
3. F. Janicke, *The Breast,* **13** (2004), 10.
4. R.W. Brueggemeier, J.C. Hackett and E.S. Diaz-Cruz, *Endocrine Rev.,* **26** (2005), 331.
5. R.B. Dickson and M.E. Lippman, *Endocrine Rev.,* **16** (1995), 559.
6. T. Takehiko, K. Norio, O. Takehiro, Y. Shiro and R.W. Michael, *Bio. Chem.,* **45** (2006), 5631.
7. R.J. Santen, T.J. Worgul, E. Samojlik, A. Interrante, A.E. Boucher, A. Lipton and H.A. Harvey, *N. Engl. J. Med.,* **305** (1981), 545.
8. P.V. Plourde, M. Dyroff, M. Dowsett, L. Demers, R. Yates and A. Webster, *J. Steroid Bio. Chem. Mol. Bio.,* **53** (1995), 175.

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