ISSN 2091-2889 (Online)
ISSN 2091-2412 (Paper)

Journal of Chitwan Medical College 2013, 3(3): 37-41
Available online at: www.jcmc.cmc.edu.np

ORIGINAL RESEARCH ARTICLE

SYNTHESIS OF INTERMEDIATES OF POTENTIAL INHIBITORS OF ENZYME P450 AROMATASE IN THE TREATMENT OF BREAST CANCER

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ABSTRACT

We report the synthesis, analysis and optimization of the reaction conditions (e.g. best solvent, temperature and time) to enhance the yield of number of intermediates (i.e. para-substituted phenoxy propanols) of the final azole based inhibitors (i.e. para-substituted phenoxy alkyl azoles) which is considered to inhibit the production of estrogens by binding to the iron atom in the haem ring of aromatase P450 enzyme thus leading to the potential treatment of hormone dependant breast cancer. Starting from phenol and range of substituted phenols various para-substituted phenoxy alkyl alcohols [i.e. compounds (22-26)] were synthesized. GC-MS, IR, NMR (13C & 1H) and TLC analysis of these compounds were also performed. Comparatively, a very good yield for compound 25 [3-(4-fluorophenoxy)propan-1-ol, 59.29 % yield] and compound 26 [3-(4-iodophenoxy)propan-1-ol, 55.49 %yield] were reported. In addition, to optimize the reaction condition for the synthesis of compounds (22-26), initially reaction was performed using dimethylformamide (DMF) as reaction solvent at various time intervals (i.e. 6, 12 and 24 hours) and various temperature (i.e. with heat and without heat). However, due to many laboratory based problems encountered with the use of this solvent especially the evaporation of this solvent; it was later on replaced with tetrahydrofuran (THF).Future work will involve multiple reaction steps for the synthesis of para-substituted phenoxy alkyl azoles from synthesized compounds (22-26) involving the removal of alcoholic group (OH) from the structure and attachment of azole group (i.e. triazole or imidazole).

Key Words: *Estrogens, TLC analysis and NMR*

INTRODUCTION

Breast cancer is the second largest cause of cancer deaths in women in the Western world. High level of estrogens (i.e. estrone, estradiol and estriol) production in body is considered to contribute to the development of hormone dependant breast tumors. Enzyme aromatase plays a significant role in the biosynthesis of estrogens, as it catalyses the conversion of androstenedione to estrone and testosterone to estradiol in the steroidal cascade (Figure 1). [1]

Therefore, this project is focused on the synthesis of the intermediates of azole based inhibitors (i.e. para-substituted phenoxyalkyl azoles) which are proposed to inhibit the production of estrogen by binding to iron atom in the heam ring of aromatase, hence leading to potential treatment of breast cancer. [2] The key aim of the project, therefore, is to optimize the reaction conditions to enhance the yield of these intermediates.

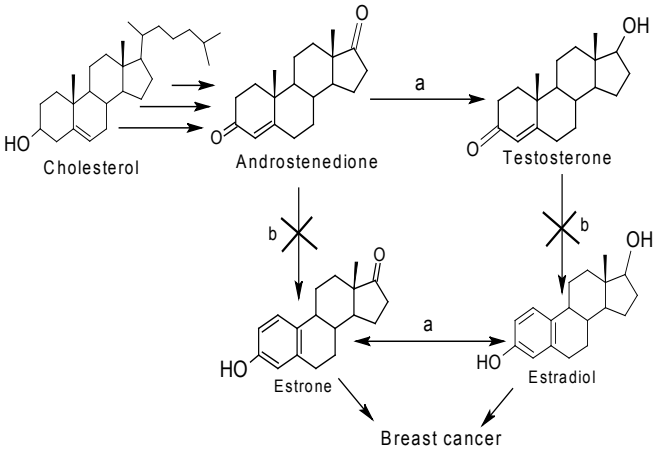
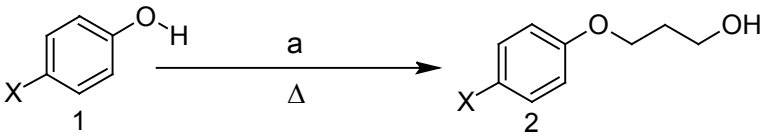


Figure 1. Showing the role of aromatase in the biosynthesis of estrogen hormones and how its inhibition (as shown by crosses) leads to the reduction in estrogens production(as shown by the crosses) thus leading to the potential reduction in the severity of hormone dependant breast cancer. [where,a=17β hydroxysteroid dehydrogenase (17b-HSD), b= enzyme aromatase.

MATERIALS AND METHODS

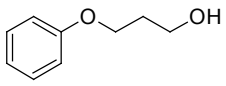
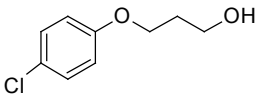
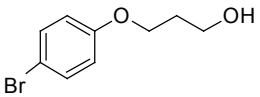
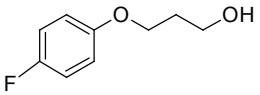
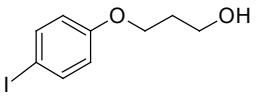
A range of phenoxy alkyl alcohols were synthesized by reacting various phenol based starting materials with 3-bromo-1-propanol, using potassium hydroxide (KOH) as the base and tetrahydrofuran (THF) as the solvent. The reactions, generally, were found to proceed without any major problems, however extensive use of column chromatography was utilized to purify each of the compounds (Scheme 1).



Scheme 1. Showing the synthesis of phenoxyalkyl alcohols (2) from phenol based compounds (1), where X= H, Cl, Br, I and F; a= KOH, 3-bromo-1-propanol and THF, reflux/24 hours.

Initially dimethylformamide (DMF) was used as the solvent, however due to problems in removing DMF, it was decided to use THF, due to its lower boiling point and aprotic nature. This resulted in successful synthesis of desired compounds with yields ranging from 27%-59% (Table 1).

Table 1: Showing various synthesized phenoxyalkyl propanols.

Compound No.	Synthesized compounds using reaction 1.	% Yield	Data Interpretation
22.	 3-phenoxypropanol	53.47% (0.87 g)	^{1}H NMR (400 MHz, CDCl3) δ ppm 6.97-6.90 (Ar H, 5 H), 4.12-2.04 (Al H, 6 H), 1.92 (Alc H, 1 H); 13C NMR (100 MHz, CDCl3) δ ppm 158.73-114.49 (Ar C, 6 C), 65.67-32 (Al C, 3 C); GC-MS: tR 9.57 min, m/z 152 (M+), m/z 94 (B+).
23.	 3-phenoxypropanol	29.62% (0.58 g)	^{1}H NMR (400 MHz, CDCl3) δ ppm 6.97-6.90 (Ar H, 5 H), 4.12-2.04 (Al H, 6 H), 1.92 (Alc H, 1 H); 13C NMR (100 MHz, CDCl3) δ ppm 158.73-114.49 (Ar C, 6 C), 65.67-32 (Al C, 3 C); GC-MS: tR 9.57 min, m/z 152 (M+), m/z 94 (B+).
24.	 3-(4-bromophenoxy)propan-1-ol	27% (0.54 g)	^{1}H NMR (400 MHz, CDCl3) δ ppm 7.24-6.84 (Ar H, 4 H), 4.08-2.03 (Al H, 6 H), 1.80 (Alc H, 1 H); 13C NMR (100 MHz, CDCl3) δ ppm 157.39-115.76 (Ar C, 6 C), 65.87-31.92 (Al C, 3 C); GC-MS: tR 12.52 min, m/z 233 (M+2)+, m/z 231 (M)+, m/z 173 (B+).
25.	 3-(4-bromophenoxy)propan-1-ol	59.29% (1.35 g)	^{1}H NMR (400 MHz, CDCl3) δ ppm 6.97-6.84 (Ar H, 4 H), 4.08-2.03 (Al H, 6 H), 1.91 (Alc H, 1 H); 13C NMR (100 MHz, CDCl3) δ ppm 158.50-115.70 (Ar C, 6 C), 66.32-32 (Al C, 3 C); GC-MS: tR 9.62 min, m/z 170 (M)+, m/z 112 (B+).
26.	 3-(4-iodophenoxy)propan-1-ol	55.49% (1.10 g)	^{1}H NMR (400 MHz, CDCl3) δ ppm 7.56-6.67 (Ar H, 4 H), 4.08-2.03 (Al H, 6 H), 1.71 (Alc H, 1 H); 13C NMR (100 MHz, CDCl3) δ ppm 158.91-116.88 (Ar C, 6 C), 82.87-31.88 (Al C, 3 C); GC-MS: tR 13.55 min, m/z 278 (M)+, m/z 220 (B+).

All the compounds were analyzed using various analytical techniques, such as fourier transform (FT)-NMR, FT-IR and GC-MS. The spectra showed successful synthesis of all the target intermediates with good purity (Table 1). All the para-substituted intermediates [for example 3-(4-iodophenoxy)propan-1-ol (Figure 2)] indicated the presence of 4 protons in the aromatic region and 6 protons in the aliphatic region of the 1H-FTNMR, while the 13C-FTNMR showed the presence of 7 non-equivalent carbon atoms (i.e. 4 in the aromatic and 3 in the aliphatic region).

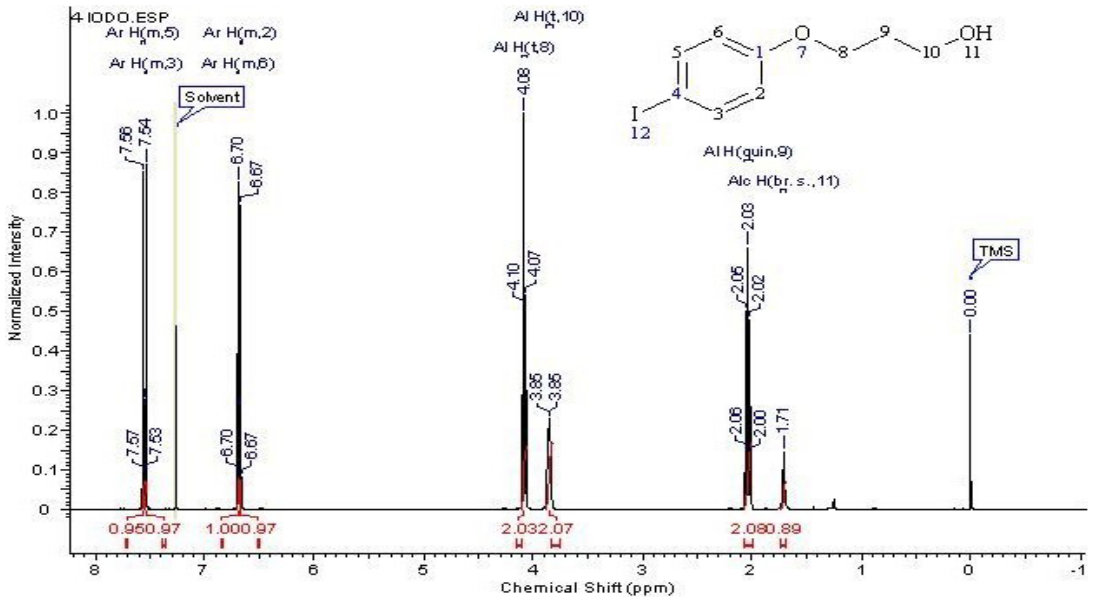


Figure (2a)

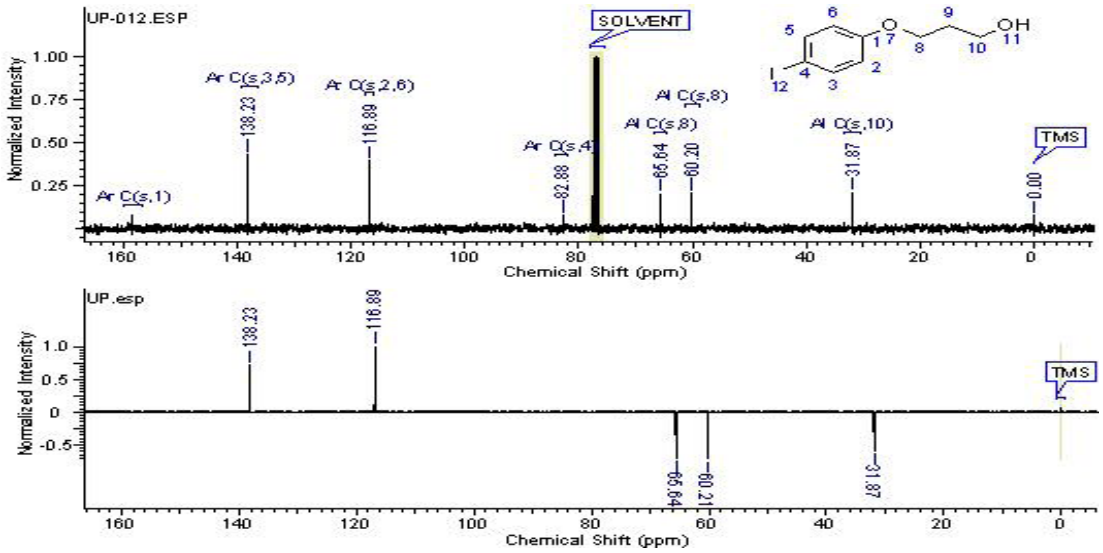


Figure (2b)

Figure 2: Showing the 1H-FTNMR (2a) and 13C-FTNMR compared with the DEPT-135 (2b) of 3-(4-iodophenoxy) propan-1-ol.

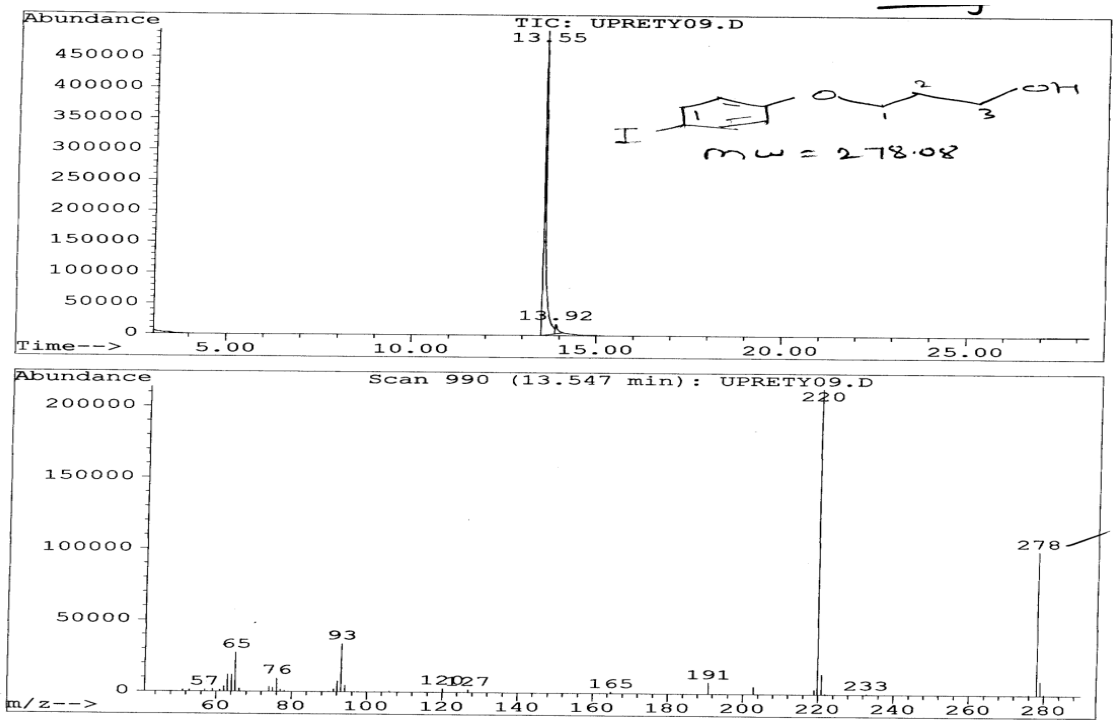


Figure 3: Showing GC-MS of 3-(4-iodophenoxy)propan-1-ol

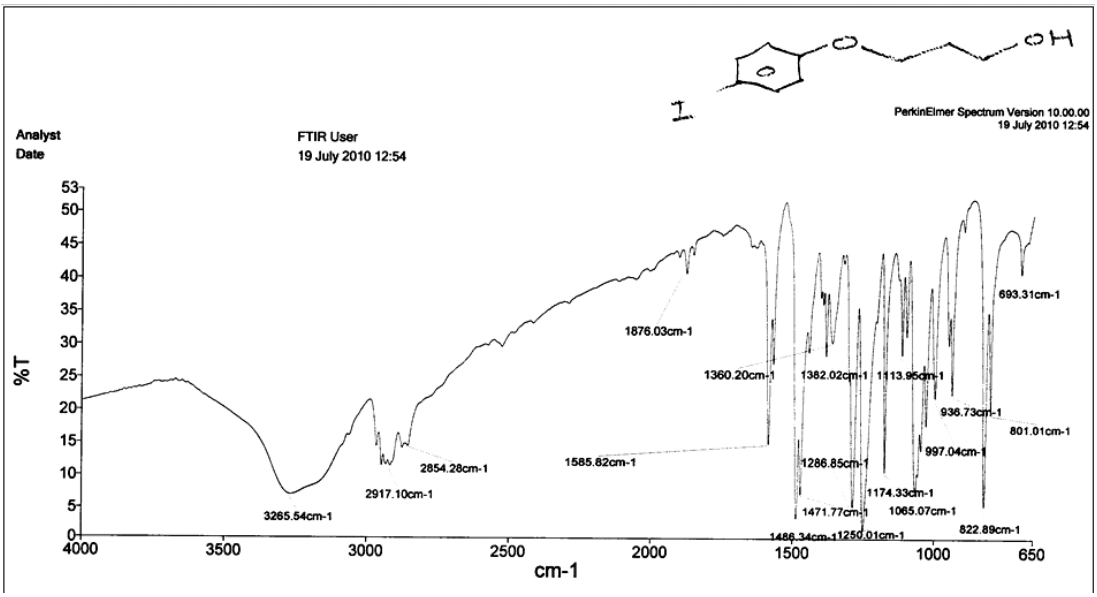


Figure 4: Showing IR spectrum of 3-(4-iodophenoxy)propan-1-ol

RESULT AND DISCUSSION

ν(max)(Film)cm-1: 3265.54 (O-H stretch), 3020 (Ar, sp2 C-H stretch), 2917.10 (Alk, sp3 C-H stretch), 1471.77 & 1585.82 (Ar, C=C ring stretch), 1250.01 & 1065.07 (Eth, C-O stretch), 1065.07 (Alc, C-O stretch; ArI, C-I stretch), 822.89 (Ar, strong C-H bend, para-substituted Ring); 1H NMR (400 MHz, CDCl3) d ppm 7.56 [Ar H, (C-I)=(C-H), m, 1 H], 7.54 [Ar H, (C-I)-(C-H), m, 1 H], 6.70 [Ar H, (C-H)=(C-O), m, 1 H], 6.67 [Ar H, (C-H)-(C-O), m, 1 H], 4.08 [Al H, (C-O)-(C-H2), t, J=5.9 Hz, 2 H], 3.85 [Al H, (C-H2)-(O-H), t, J=2.0 Hz, 2 H], 2.03 [Al H, (C-H2)-(C-H2)-(C-H2), quin, J=6.0 Hz, 2 H], 1.71 [Alc H, (O-H), br. s., 1 H]; 13C NMR (100 MHz, CDCl3) d ppm 158.91 [Ar C, (C-O), s, 1 C], 138.23 [Ar C, (C-I)=(C-H) and (C-H)-(C-I), s, 2 C], 116.88 [Ar C, (C-H)=(C-O) and (C-O)-(C-H), s, 2 C], 82.87 [Ar C, (C-I), s, 1 C], 65.87 [Al C, (C-O)-(C-H2), s, 1 C], 60.22 [Al C, (O-H)-(C-H), s, 1 C], 31.88 [Al C, (C-H2)-(C-H2)-(C-H2), s, 1 C]; GC-MS: tR 13.55 min, m/z 278 [molecular ion peak (M+), C9H11IO2], m/z 220 [base peak (B+), C6H5IO].

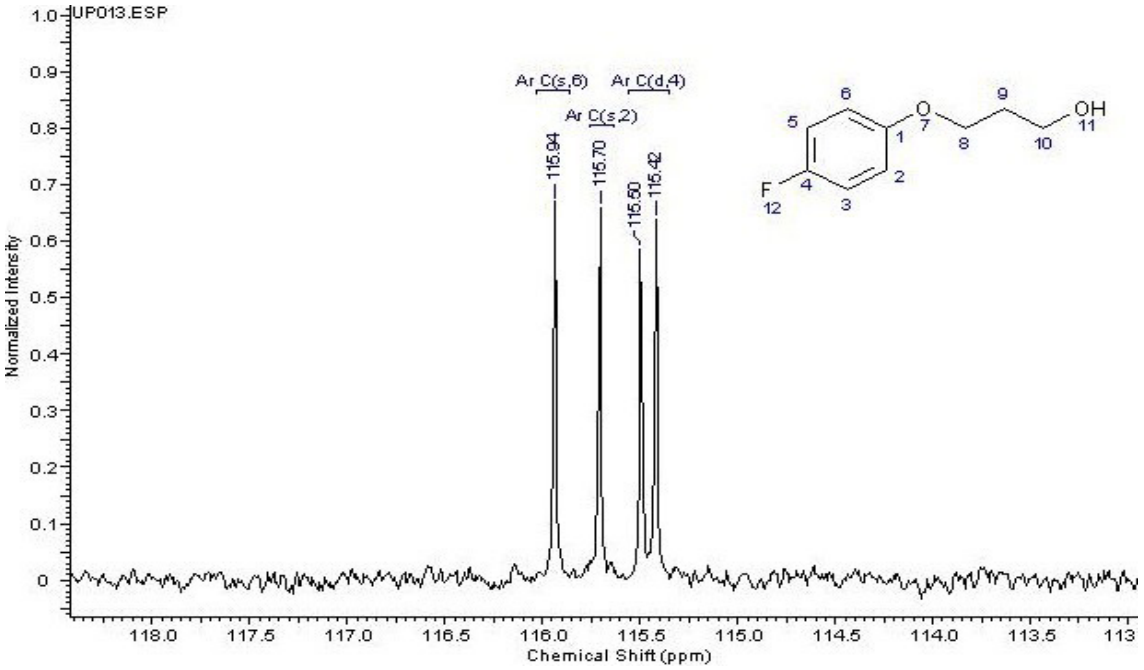


Figure 5: Showing heteronuclear coupling between fluorine and carbon (C-4) resulting in a doublet.

An interesting feature was the observation of a doublet in case of proton decoupled 13C-FTNMR of 3-(4-fluorophenoxy) propan-1-ol (Figure 3). This is due to fluorine-19 atom present in the compound which interacts with the 13C nuclei [as it also possess same number of spin state as 13C atom (i.e. 2; I = + ½ and – ½)] due to which splitting of peaks takes place giving rise to a doublet instead of typical singlet peak. [3]

CONCLUSION

In conclusion, we managed to synthesize the target intermediates in the given time with no major problems observed with the very nature of the reaction. We obtained an average to good reaction yields (27%-59%) and observed that the optimum conditions for the successful synthesis of these intermediates were found to be 24 hours (reflux) with THF as the reaction solvent. Future work will involve bromination and attaching of azoles group (i.e. imidazole or triazole) to the synthesized compounds (Table 1). These para-substituted phenoxyalkyl azole based compounds would then be checked for their potency and the ability to inhibit the enzyme aromatase as compared to other non-steroidal inhibitors.

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