Synthesis of Tamoxifen Derivatives as Anticancer Agents

A

Thesis submitted

In the partial fulfillment of the requirement for the degree of

MASTERS OF SCIENCE

IN

CHEMISTRY

Submitted By

MANDEEP KAUR

(301502016)

UNDER THE SUPERVISION

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SCHOOL OF CHEMISTRY AND BIOCHEMISTRY,

THAPAR UNIVERSITY,

PATIALA-147004

2017

CERTIFICATE

This is to certify that the thesis entitled **"Synthesis of Tamoxifen Derivatives as Anticancer Agents"** submitted by **Mandeep Kaur** in the partial fulfillment of the requirements for the degree of **Master of Science in Chemistry** from **Thapar University, Patiala** is a bonafied piece of work carried out under the guidance and supervision of **Dr. Kamaldeep Paul**, Associate Professor, School of Chemistry and Biochemistry, Thapar University, Patiala and no part of this project has been submitted for award of any other degree in this or any other university.

Mandeep Kaur

(MANDEEP KAUR)

This is to certify the above statement made by student concerned is correct and true to the best of my knowledge.

Kamaldeep Paul.

(Dr. Kamaldeep Paul)
Associate Professor (Supervisor),
School of Chemistry and Biochemistry,
Thapar University, Patiala

SELF DECLARATION

The work embodied in the project entitled **"Synthesis of Tamoxifen Derivatives as Anticancer Agents"** has been done by me in the partial fulfillment of requirement for the award of degree of **Masters of Science in Chemistry,** submitted in the **School of Chemistry and Biochemistry, Thapar University, Patiala,** is an authentic record of my own carried out under the supervision and guidance of **Dr. Kamaldeep Paul** Associate Professor, School of Chemistry and Biochemistry, Thapar University, Patiala. All the ideas and references have been duly acknowledged.

Date: 17 July 2017

Place: Patiala

Mandeep Kaur

Mandeep Kaur

This is to certify the above statement made by student concerned is correct and true to the best of my knowledge.

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(Dr. Kamaldeep Paul)

Associate Professor (Supervisor),

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Words are not enough to express my feelings about my immense gratitude that I owe to my dear

Parents for their endless love, blessings and moral support throughout my life. Last but not the least I am thankful to all the persons who helped me directly or indirectly during the tenure of my project work.

Mandeep Kaur

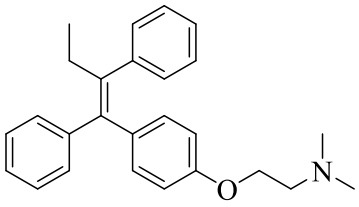
Mandeep Kaur

Place: Patiala

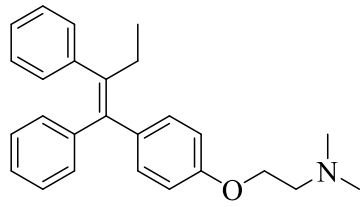
TABLE OF CONTENT

1. INTRODUCTION

Cancer[1] is the major health problem which has increased mortality rate worldwide by 8.8 million deaths in 2015. Breast cancer is the one cause of death among women.[2] To cure cancer, there are certain drugs under clinical trials. Among them, tamoxifen[3] has attracted researchers over many years which have brand name Nolvadex used against breast cancer as a first line therapy. In 1962, ICI (Imperial Chemical Industries) synthesized tamoxifen which is the first Selective Estrogen Receptor Modulator (SERM) belonged to a class of triphenylethylene.[4] It is effective against estrogen receptor (ER) positive cancerous cells.



Z-tamoxifen



E-tamoxifen

Tamoxifen is a prodrug,[5] which is metabolized by cytochrome P450-CYP2D6 and CYP3A4 in liver into its active forms like 4-hydroxytamoxifen (also known as afimoxifene) and *N*-desmethyl-4-hydroxytamoxifen (endoxifen).[6] These metabolites[7] of tamoxifen have about 30-100 times enhanced affinity for binding with the estrogen receptor than tamoxifen. 4-Hydroxytamoxifen exhibits higher binding affinity than tamoxifen and endoxifen. When 4-hydroxytamoxifen binds with estrogen receptor, forms complex which gathers co-repressors namely NCoR (Nuclear receptor co-repressor) and SMRT (Silencing mediator for retinoid of thyroid hormone receptors) to modulate gene expression by binding to DNA.[8] Tamoxifen for exerting its full anticancer effect needs a protein PAX2 which helps in the reduction of pro-proliferative protein ERBB2. If the expression of AIB-1 is higher than PZX2,[9] the complex formed by the estrogen receptor and the tamoxifen irregulates the ERBB2 's expression leads to stimulation of growth of breast cancer.[10] 4-Hydroxytamoxifen binds competitively to estrogen receptor in cancerous cells and other target cells, thereby generates a complex that suppress the synthesis of DNA and estrogen effects. Tamoxifen also prevents pre-cancerous cells to grow or divide the cells in the G_0 and G_1phases of cell cycle.[11]

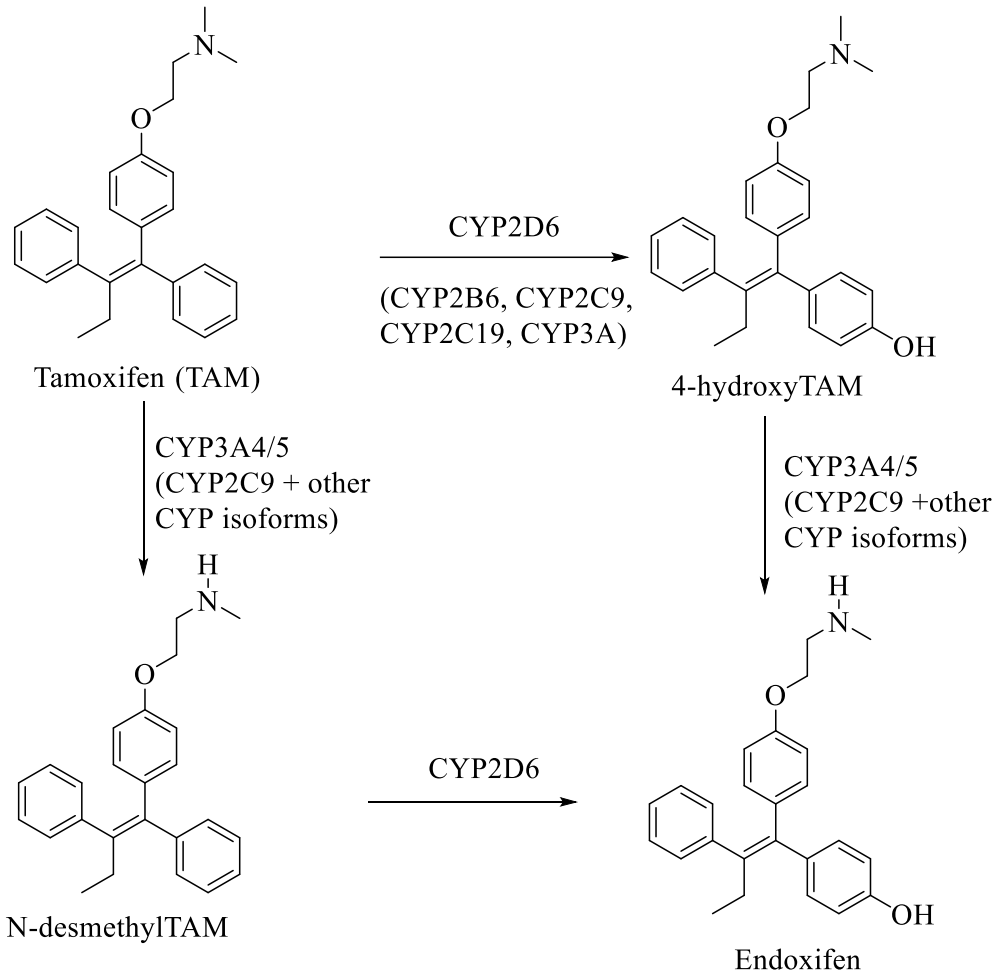
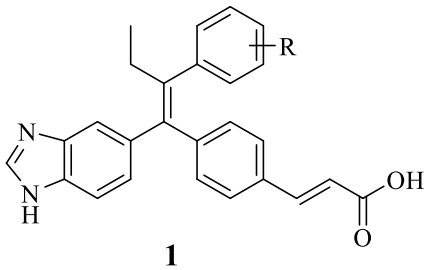


Figure 1 Mechanism showing metabolism of tamoxifen into its active metabolites

Tamoxifen exhibits both agonist as well as the antagonist effects depending on the estradiol concentration. By keeping in mind for advantages of tamoxifen, we have planned to synthesised hetero aryl substituted tri/tetraphenyl olefins.

2. REVIEW OF LITERATURE

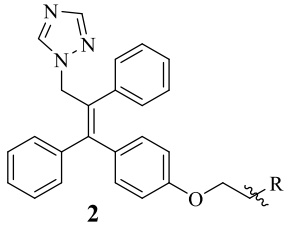
Lai *et al.*[12] synthesized tamoxifen analogues by replacing side basic chain with acrylic acid group, substituting one of the phenyl rings with benzimidazole ring and another ring with various electron withdrawing groups (**1a-i**) and evaluated their efficacies as estrogen receptor (ER)-degraders. Compounds substituted at *ortho*-position showed high ER- degradation activity as compared to compounds substituted at *meta*- or *para*- position. Di-substituted compouds such as **1g** and **1h** exhibited substantial 30 folds increase in potency over the compounds having mono-substituted phenyl ring (**1a-f)**.



1

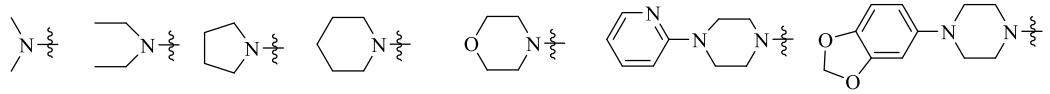
a; R = *o*-Cl
b; R = *m*-Cl
c; R = *p*-Cl
d; R = *o*-CN
e; R = *m*-CN
f; R = *p*-CN
g; R = *o*-Me, *p*-CN
h; R = *o*-Cl, *p*-CN
i; R = *o*-Cl, *p*-F

Murty and coworkers synthesized tamoxifen-1,2,4-triazole conjugates by replacing methyl group of tamoxifen with triazole.[13]Compound **2g(**GI_{50}= 0.23μM) showed enhanced antiproliferative activity as compared to tamoxifen (GI_{50} = 0.24 μM), against breast cancer cell lines MDA-MB-231, while thecompound **2f (**GI_{50}= 0.12μM)displayed similar activity with tamoxifen (GI_{50} = 0.12μM) against SiHa cell lines.



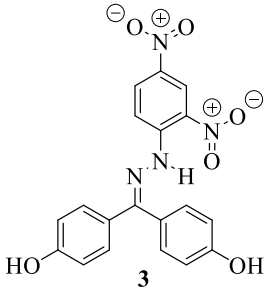
2

R =

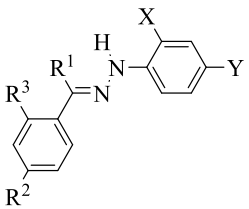


a **b** **c** **d** **e** **f** **g**

Morgan and co-workers have been synthesized a novel series of 4,4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone analogues (**3** and **4**).[14]These compounds showed anticancer activity with minimal toxicity. Compound **4d** showed improved activity as compared to compounds **3** and **4e**. While compounds **4f** and **4g** displayed reduced activity as compared tocompound **3**.Introduction of different functional groups and increase in polarity in compound **3** led to enhance the anticancer activity.



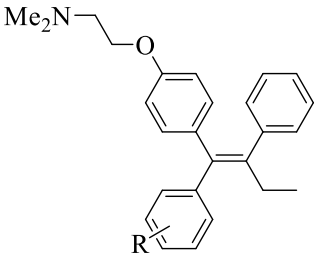
3



4

a; R^1 = Ph, R^2 = R^3 = H, X = Y = NO_2
b; R^1 = Ph, R^2 = OH, R^3 = H, X = Y = NO_2
c; R^1 = 4-HO-Ph, R^2 = OH, R^3 = Y = H, X = NO_2
d; R^1 = 4-NH_2-Ph, R^2 = NH_2, R^3 = H, X = Y = NO_2
e; R^1 = 4-Me_2N-Ph, R^2 = NMe_2, R^3 = H, X =Y= NO_2
f; R^1 = 4-Et_2N-Ph, R^2 = Et_2N, R^3 = H, X = Y = NO_2
g; R^1 = 4-AcHN-Ph, R^2 = AcHN, R^3 = H, X = Y = NO_2

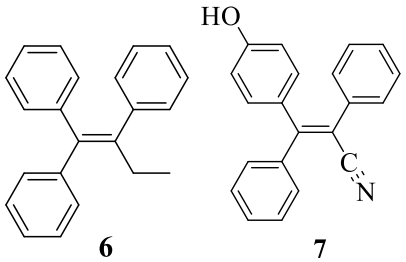
Shani and co-workers synthesized a potent series of tamoxifen derivatives by substituting various electron withdrawing and electron donating groups on one of the α - phenyl rings of tamoxifen.[15] Compounds containing halogens (**5b-e**), thiol (**5f**) and thio methyl **(5g)** groups displayed high receptor binding affinity than tamoxifen. The receptor binding affinity of compounds **5g** and **5j** is higher than **5h**. High binding affinity at the *para* position of the α-phenyl ring is due to the presence of hydrophilic functional group.



5

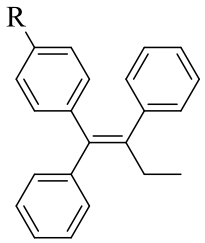
a; R = H
b; R = 4-I
c; R = 4-Br
d; R = 4-F
e; R = 4-Cl
f ; R = 4-SH
g; R = 4-SMe
h; R = 4-CHO
i; R = 4-CH_2OH
j; R = 4-NH_2

Bignon and co-workers have been synthesized 4-hydroxytamoxifen analogue by replacing ethyl group of substituted triphenyl olefin with cyanide.[16] Compound **6** without any substitution on rings displayed 1000 times lesser affinity while the compound **7** with cyano group showed 3-folds increase in affinity than tamoxifen.



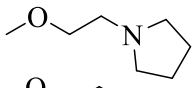
6 **7**

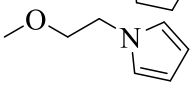
Jarman*et al*. synthesized tamoxifen analogues by replacing side chain with various basic substitutents.[17] Compound **8b** with pyrrolidine moiety showed 3.6 folds more binding affinity while compound **8c** with pyrrole ring exhibited 20 folds lesser affinity as compared to tamoxifen. Estrogen receptors affinity of the compounds **8d-f** was same as that of tamoxifen.



8

a; R = $-OCH_2CH_2NMe_2$

b; R = 

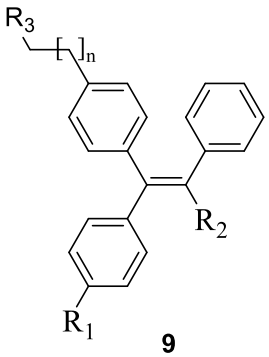
c; R = 

d; R = $-OCH_2CH_2OH$

e; R = $-OCH_2CH_2\overset{+}{N}Me_3$

f; R = -CH=CHCOOH

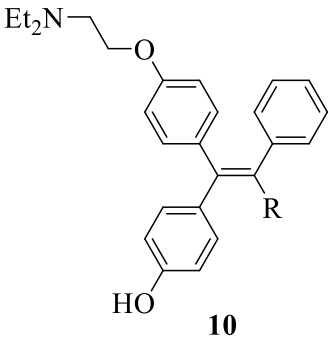
Hardcastle *et al.* synthesized tamoxifen derivatives by varying the length of basic side chain, substituting iodo group on phenyl ring and replacing ethyl group of tamoxifen with halide group.[18] Compounds **9a-e** did not show any drastic change of binding affinity for estrogen receptors. Compound **9f** with side chain of 10 methylene groups displayed 22-folds decrease in affinity for estrogen receptors.



9

	R_1	R_2	R_3	n
a;	-H	-Et	$-NMe_2$	2
b;	-I	-Et	$-NMe_2$	2
c;	-I	-Et	$-NMe_2$	3
d;	-I	-Et	$-NMe_2$	4
e;	-H	-Cl	$-NEt_2$	4
f;	-H	-Cl	$-NEt_2$	10

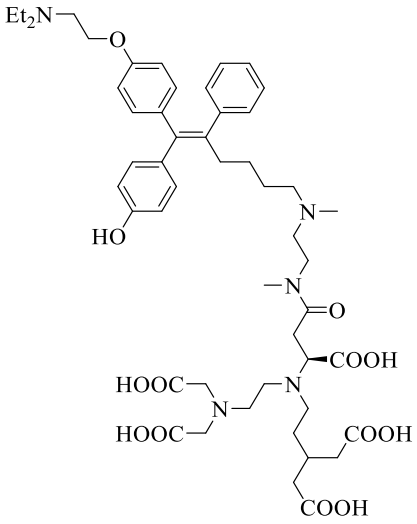
Ruenitz*et al.* synthesized 4-hydroxytamoxifen analogues by replacing ethyl group of substituted olefin with different electron withdrawing groups.[19] Compound **10e**, a chloro derivative of 4-hydroxy tamoxifen was 10 times more potent towards estrogen receptors than compounds **10b, 10c** and **10d**. Compound **10e** showed more potency for binding of estrogen receptors than compound **10d** while compounds **10b** and **10c** showed lowest binding affinity for estrogen receptors.



10

a; R = NO_2
b; R = CN
c; R = H
d; R = Br
e; R = Cl

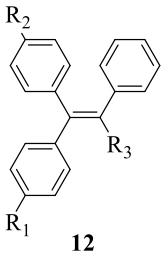
Lashley*et al*. synthesized 4-hydroxytamoxifen analogues by replacing ethyl group of substituted olefin with a bulky group. Compound **11** showed 10 folds weaker potency than tamoxifen due to replacement of the ethyl side chain with a bulky group.[20]



11

Bignon and co-workers synthesized derivatives of tamoxifen substituting various groups on phenyl rings and replacing ethyl group with electron donating and electron withdrawing groups.[16,21] Compound **12a** with acetylene group displayed increase in potency to inhibit Protein Kinase C (PKC). Compound **12b** was 3 times more effective than the compound **12c** in the inhibition of the PKC activation due to addition of second

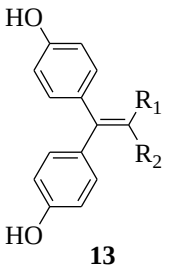
aminoethoxy side chain at the *para* position of the α-ring. In the inhibition of the protein kinase C, compounds **12d** and **12e** were more effective than tamoxifen. Compounds **12c**, **12g** and **12h** showed increase in PKC inhibition activity due to increase in size of chain. Substitution of aminoethoxy side chain in compounds **12i** and **12j** by hydroxyl group, resulted decrease in affinity for protein kinase C.



12

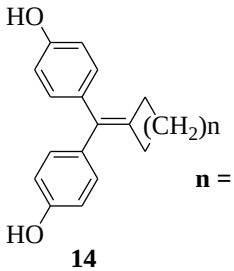
	R_1	R_2	R_3
a;	-C≡CH	$-OCH_2CH_2NMe_2$	-Et
b;	$-OCH_2CH_2NEt_2$	$-OCH_2CH_2NMe_2$	-CN
c;	-OH	$-OCH_2CH_2NMe_2$	-CN
d;	-H	$-OCH_2CH_2NHMe$	-Et
e;	-H	$-OCH_2CH_2$-c-NC_4H_8NH	-Et
f;	$-NMe_2$	$-NMe_2$	-CN
g;	-I	$-O(CH_2)_4NMe_2$	-Et
h;	-I	$-O(CH_2)_8NMe_2$	-Et
i;	-OH	-OH	-CN
j;	-OH	-OH	$-CH_2NH_2$

Bignon*et al.* also synthesized compounds by replacing phenyl rings of tamoxifen with various groups such as halide, alkyl etc. Compounds **13** and **14** showed enhanced PKC activity than tamoxifen.



13

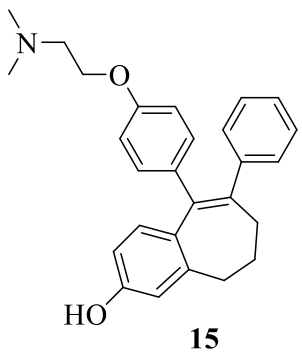
a; $\mathbf{R_1}$ = -H, $\mathbf{R_2}$ = -*i*Pr
b; $\mathbf{R_1}$ = -Cl, $\mathbf{R_2}$ = -*i*Pr



n = 2, 3, 4

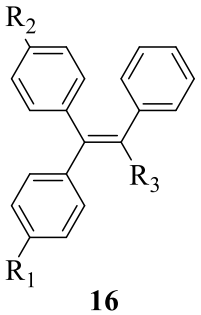
14

Rowlands *et al.* synthesized another tamoxifen's analogue, in which double bond is incorporated into cyclic structure which displayed decrease in inhibition activity on Calmodulin (CaM).[22]



15

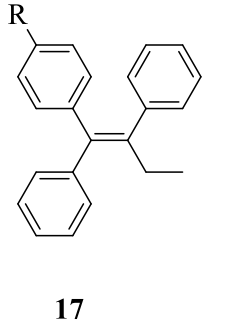
Hardcastle and co-workers have also been synthesized tamoxifen analogues by replacing various groups at the phenyl rings for inhibition of CaM (a calcium binding protein found to be involved in control of cell proliferation) activity.[18] Compounds **16b** and **16c** inhibited the CaM activity much more than compound **16a**. Increase in length of the aminoalkoxy side chain as in compound **16d,** showed higher CaM inhibitory potency than compounds **16a-c**. In compound **16e**, the enhanced activity for antiestrogen binding site (AEBS) was due to presence of CF_3 at the *meta* position of the α-ring.



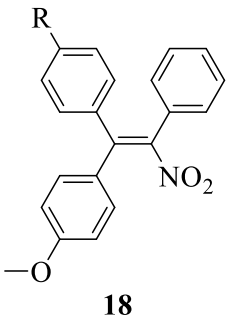
16

	R_1	R_2	R_3
a;	-I	$-OCH_2CH_2NMe_2$	-Et
b;	-I	$-O(CH_2)_3NMe_2$	-Et
c;	-I	$-O(CH_2)_4NMe_2$	-Et
d;	-I	$-O(CH_2)_8NMe_2$	-Et
e;	-3-CF_3	$-OCH_2CH_2NEt_2$	$-NO_2$

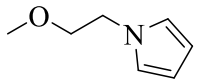
Sudo*et al*. reported tamoxifen analogues by replacing ethyl group with nitro group for antiestrogen binding site (AEBS).[23] Compounds **17a** and **17b** showed increase in affinity than tamoxifen due to presence of the cyclic amines. For the nitromiphene analogues; compound **18b** displayed 12 times more affinity than compound **18a**.

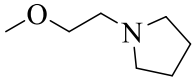


17



18

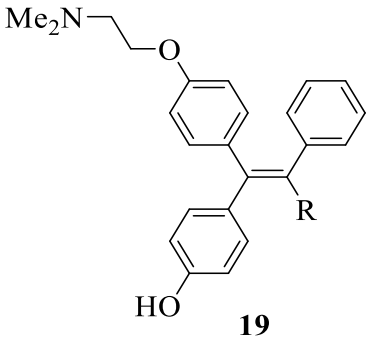
a; R = 

b; R = 

a; R = $-OCH_2CH_2NMe_2$

b; R = $-OCH_2CH_2CH_2NMe_2$

Ruenitz*et al.* synthesized 4-hydroxytamoxifen analogues by replacing ethyl group of substituted olefin with different electron withdrawing groups.[19] Replacement with nitro in case of compound **19a**, cyano in case of **19b** and bromo in case of **19c** have no influence on the antiestrogen binding site.



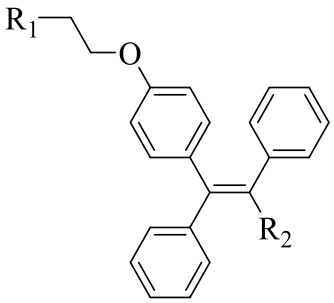
19

a; R = -NO_2

b; R = -CN

c; R = -Br

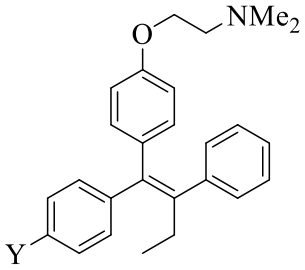
Koedijk and co-workers have been synthesized tamoxifen analogue by varying side chain and replacing ethyl group with various electron withdrawing groups.[24] In compound **20c,** substitution of ethyl group on C-2 position by chlorine group led to increase in affinity by 2-folds while in compound **20b,** substitution by chloroethyl group led to 4-folds diminished binding affinity to the antiestrogen binding site than compound **20c**. Compound **20a**, resulted in 33-folds loss of affinity as compared to tamoxifen due to presence of electron withdrawing cyano group.



20

	R_1	R_2
a;	-NMe_2	CN
b;	-NMe_2	CH_2CH_2Cl
c;	-NEt_2	Cl

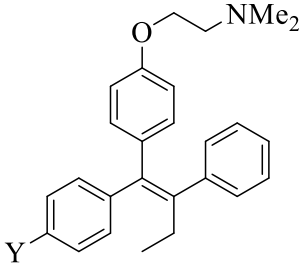
McCague and co-workers have been synthesized a series of tamoxifen derivatives by substituting halides (**21**) and methyl sulphonyl (**22**) groups at the 4^{th} position at one of phenyl rings.[25] Compounds **22** exhibited greater affinity for estrogen receptors than tamoxifen against MCF-7 breast cancer cell line.



21

a; Y = -I

b; Y = -Br

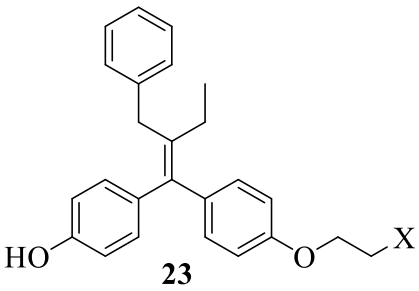


22

a; Y = -SOMe

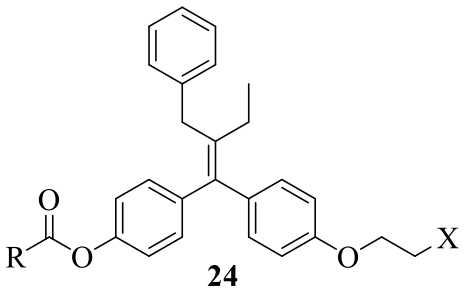
b; Y = -$SOMe_2$

Elghazawy and coworkers have been synthesized analogues of tamoxifen by blocking the site for *para*-hydroxylation by using ester groups.[26] The IC_{50} values of compounds **23a (**IC_{50} = 2.12 μM) and **23b (**IC_{50} = 2.51 μM) were found to be higher than compounds **24a (**IC_{50} = 1.88 μM), **24b (**IC_{50} = 0.83 μM), **24c (**IC_{50} = 0.93 μM) and **24d (**IC_{50} = 2.26 μM) on breast cancer MCF-7 cell lines. Compound **23c (**GI_{50} = 0.02 μM) displayed 80 times more estrogen receptor binding affinity than tamoxifen (GI_{50} = 1.25 μM) against breast cancer cell lines because of more basic nitrogen in pyrrolidine ring.



23

a; X = -$N(Me)_2$
b; X = Piperidine
c; X = Pyrrolidine

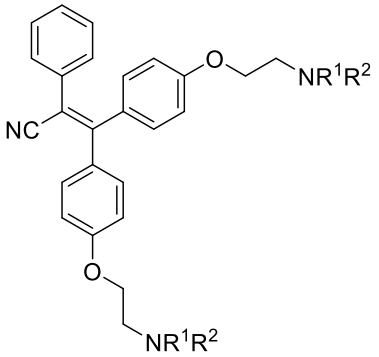


24

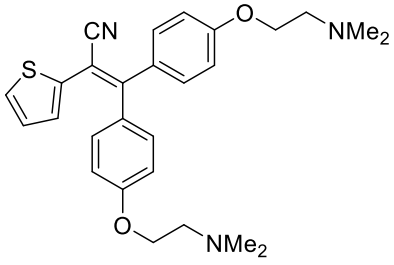
a; X = $N(Me)_2$, **R** = C_9H_{11}
b; X = Piperidine, **R** = CH_3
c; X = Piperidine, **R** = C_3H_7
d; X = Piperidine, **R** = C_9H_{19}

Carpenter and coworkers synthesized tamoxifen's triarylacrylonitrile derivatives.[27] Compound **25a** with the ethoxy side chain showed the same potency as tamoxifen for PKC inhibition. Amongst compounds **25a-c,** compound **25c** with 4-methylpiperazin-1-yl showed the highest potency and selectivity for protein kinase C inhibition due to presence of more basic side chain. Replacement of β-phenyl ring with

a thiophen-2-yl ring in compound **26** led to lose in potency for protein kinase C inhibition but with similar selectivity pattern to compound **25c**.



25



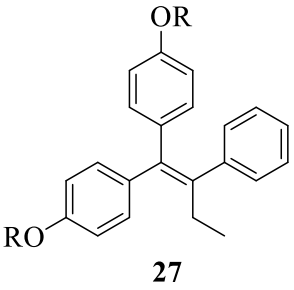
26

a; $NR^1R^2 = NEt_2$

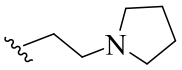
b; NR^1R^2 = morpholino

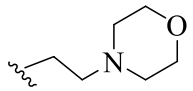
c; NR^1R^2 = 4-methylpiperazin-1-yl

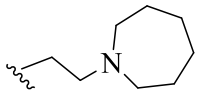
Hasegawa and co-workers have been reported a novel series of tamoxifen derivatives by substituting side chain with various cyclic alkyl amines (**27**).[28] Compound **27c** with IC_{50} value of 0.64 μM, was found to be the most potent to inhibit cell division or growth than tamoxifenagainst CT-L cell line.

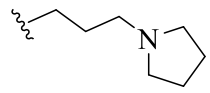


27

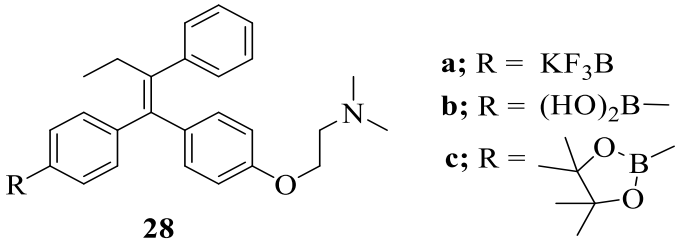
a; R = 

b; R = 

c; R = 

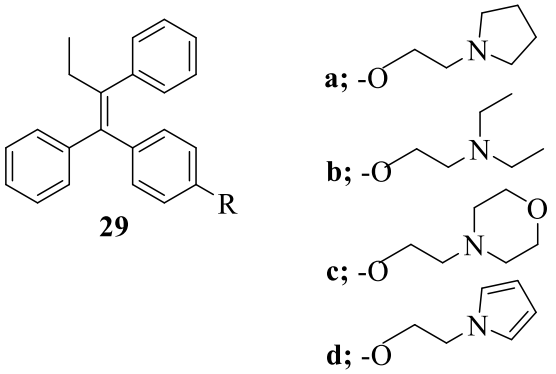
d; R = 

Jiang and coworkers synthesized boron based 4-hydroxytamoxifen bioisoteres derivatives[29] which showed anticancer activity against MCF-7 and T47D breast cancer cell lines. Compound **28a** (IC_{50} = 0.03 μM) showed five times higher anticancer potency than 4-hydroxytamoxifen (IC_{50} = 0.15 μM).



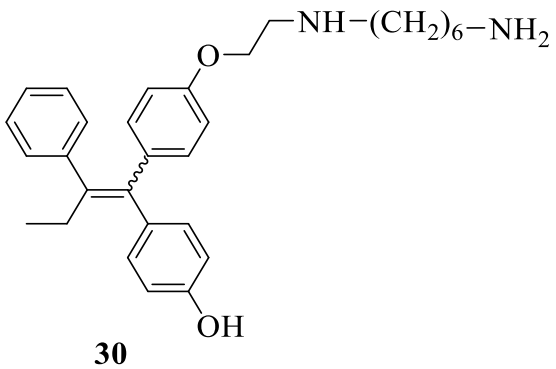
28

Dayan and co-workers have been reported a series of derivatives of tamoxifen by substituting various amines in side chains.[30] Compound **29d**, with aromatic amine displayed 4.5-folds enhancement of IC_{50} value. While compound **29c** with morpholine showed 2.5-folds increase in IC_{50} value. Compound **29a** having pyrrolidine ring displayed lower potency than 4-hydroxytamoxifen.



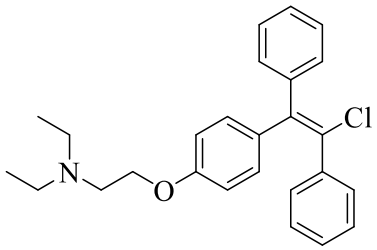
29

Rickert and co-workers have been synthesized a series of 4-hydroxytamoxifen analogue which was conjugated to a diaminoalkyl linker and then conjugated to activated esters of a poly(methacrylic acid) polymer.[31] Compound **30** showed high affinity for estrogen receptors α and β. Compound **30** with IC_{50} value of 15 ± 5 nM for estrogen receptor α and IC_{50} value of 9± 5 nM for estrogen receptor β. But compound **30** showed less potency than 4-hydroxytamoxifen having IC_{50} value of 1 ± 0.3nM for estrogen receptor α and IC_{50} value of 8± 5 nM for estrogen receptor β.



30

Mikelmanand coworkers reported analogue of tamoxifen by replacing ethyl group of substituted olefin with chloro group. Compound **31** exhibited same antitumor activity as tamoxifen.[32]



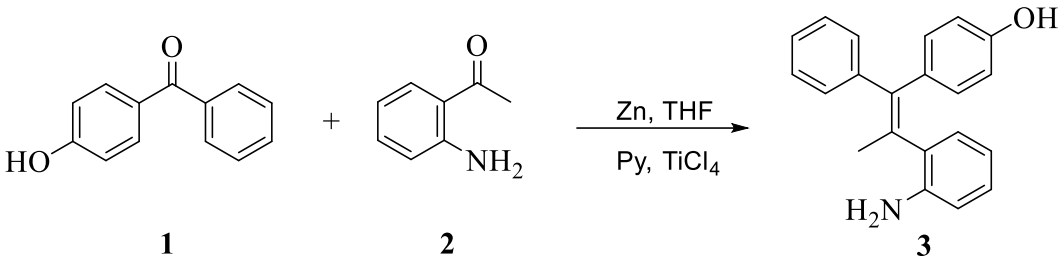
31

3. RESEARCH GAPS AND OBJECTIVES

Literature survey suggests that individual moiety such as tamoxifen showed potent anticancer properties but lack an efficient activity values against the human cancer cell lines and also leads to endometrial cancer. Few reports have been given in literature using the combination of the biological active pharmacophores for molecular hybridized compounds. Thus, on the basis of literature, we have tried to use technique of drug hybridisation to synthesize a new hybridized tamoxifen derivativethat will be used for anticancer activity against suitable cancer cell lines.

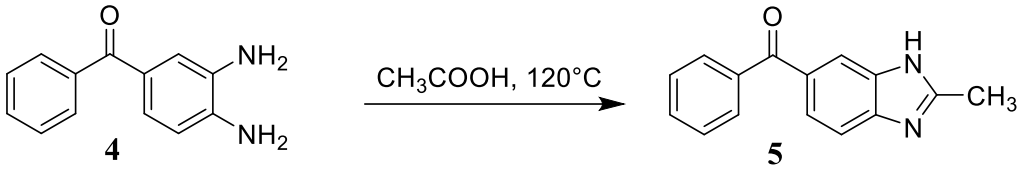
4. RESULTS AND DISCUSSION

For the synthesis of tamoxifen derivatives, we have used commercially available 4-hydroxybenzophenone **(1)** and 2-aminoacetophenone **(2)** for the synthesis of 4-(2-(2-aminophenyl)-1-phenylprop-1-en-1-yl)phenol**(3)** and reaction was done with McMurry reaction. The reaction was monitored by TLC. The crude material was purified by column chromatography to give the desired brown coloured compound **3** with low yield (**Scheme-1**). Due to low yield, compound 3 was not used for further reactions.



Scheme 1. Synthesis of 4-(2-(2-aminophenyl)-1-phenylprop-1-en-1-yl)phenol**(3)**

So, another series of compounds were tried for tamoxifen derivatives. In the another series of compounds, (2-methyl-1*H*-benzo[*d*]imidazol-6-yl)(phenyl)methan -one (**5**) was obtained by refluxing of 3,4-diaminobenzophenone (**4)** in 20 mL of acetic acid for 24 hours. The confirmation of the reaction was checked by TLC. The yield obtained was 89% and the product was brownish orange in colour, showing a melting point of 222-224 ˚C (**Scheme-2**). ^{1}H NMR spectrum of the compound **5** showed the signals of singlet of one proton at δ 8.04, multiplet of three protons at δ 7.81-7.79, multiplet of two protons at δ 7.59-7.57 and multiplet of two protons at δ 7.50-7.48 of aromatic rings, broad singlet of one proton at δ 3.80 due to NH and singlet of three protons at 2.69 corresponding to CH_3 (**Figure 2**).



Scheme 2. Synthesis of (2-methyl-1*H*-benzo[*d]*imidazol-6-yl)(phenyl)methanone**(5)**

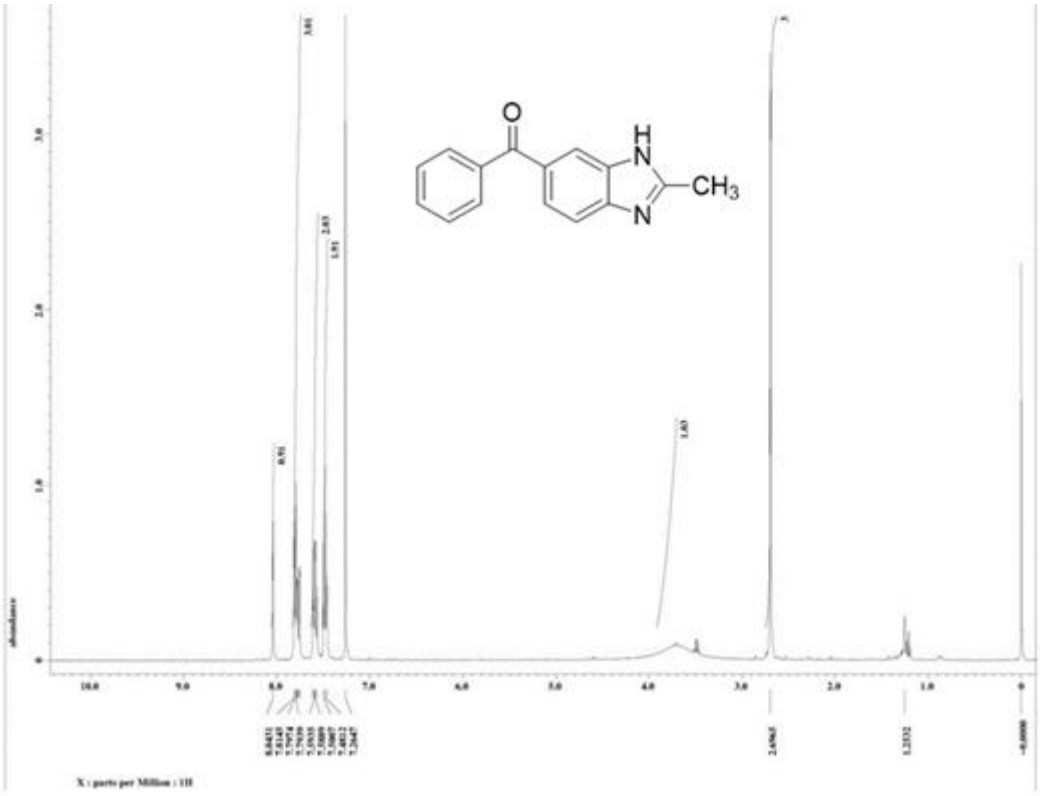
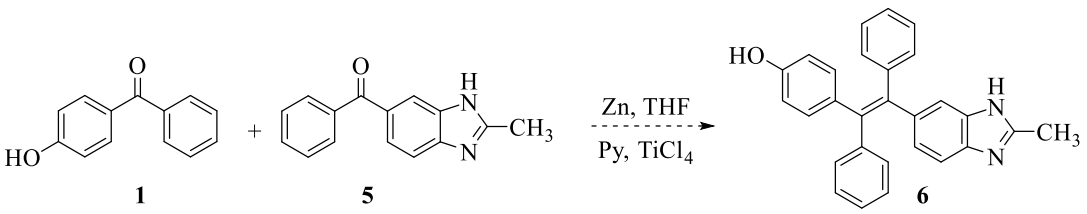


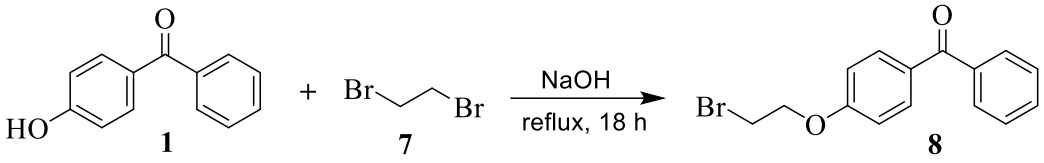
Figure 2.[1]H NMR spectrum of(2-methyl-1*H*-benzo[*d*]imidazol-6-yl)(phenyl)methan -one **(5)**

Compound **5** was used further for the synthesis of tamoxifen derivatives. So, various reactions were tried with different reaction substrates and reaction conditions. Compound **6** was tried to synthesize by reacting compound **1** and **5** by McMurry reaction. Compound **5** was treated with 4-hydroxybenzophenone (**1**) in the presence of Zn and $TiCl_4$ and pyridine. The reaction was monitored by TLC showing that carbonyl compounds were not consumed. The reaction was again rechecked and after 48 hours there was no new spot found on TLC (**Scheme-3**). The low reactivity of 4-hydroxybenzophenone may be due to the electron donating hydroxy group.



Scheme 3 Synthesis of 4-(2-(2-methyl-1*H*-benzo[*d*]imidazol-6-yl)-1,2-diphenylvinyl) phenol **(6)**

So, we tried the McMurry reaction by first substituting the hydroxy group with alkoxy group. Using commercially available 4-hydroxybenzophenone (**1)** and 1,2-dibromoethylene (**7)** as initial substrates, compound (4-(2-bromoethoxy)phenyl) (phenyl)methanone **(8)** was synthesized using aqueous solution of NaOH at reflux temperature for 18 hours. The confirmation of the reaction was done by TLC (**Scheme-4**). The crude was white in colour and having a yield of 63% showing melting point of 68-69 ˚C. The ^{1}H NMR spectrum showed the signals at δ 7.82-7.71 multiplet of four protons, δ 7.55 triplet of one proton, δ 7.45 triplet of two protons and δ 6.96 doublet of two protons corresponding to aromatic ring. Two triplets of two protons each at δ 4.34 and 3.65 corresponding to ethyl bromide were also observed **(Figure-3).**



Scheme 4 Synthesis of (4-(2-bromoethoxy)phenyl)(phenyl)methanone **(8)**

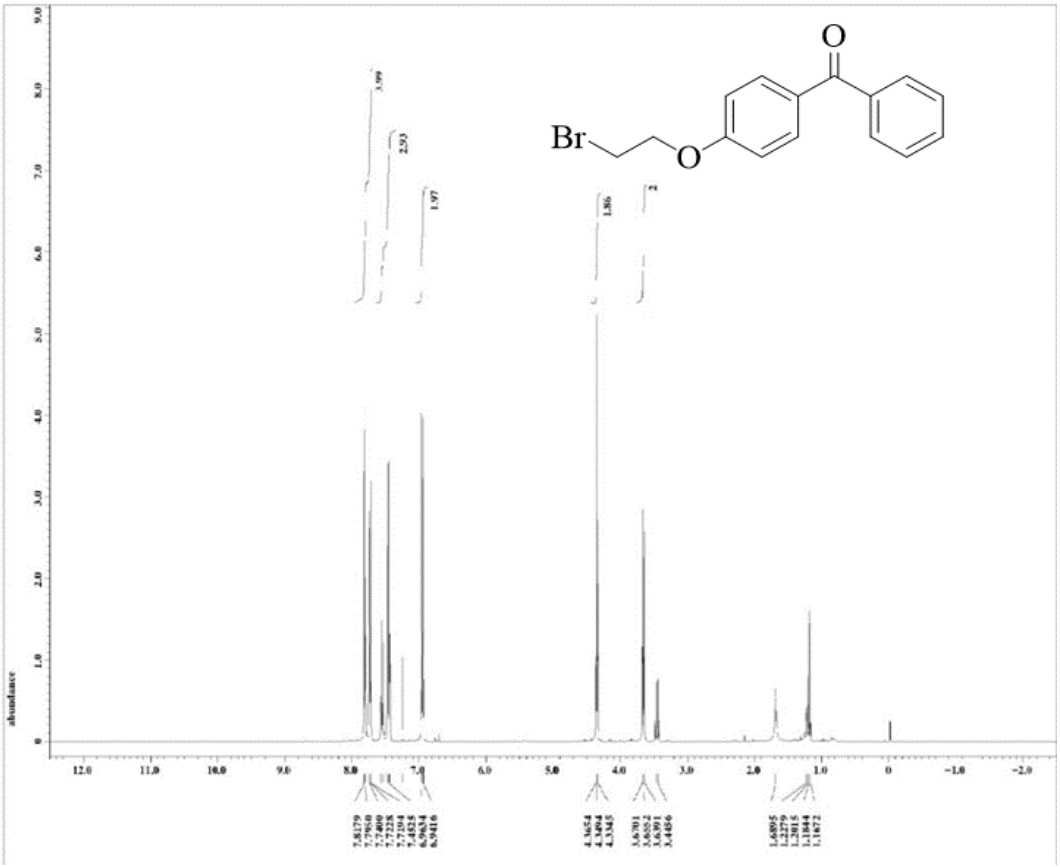
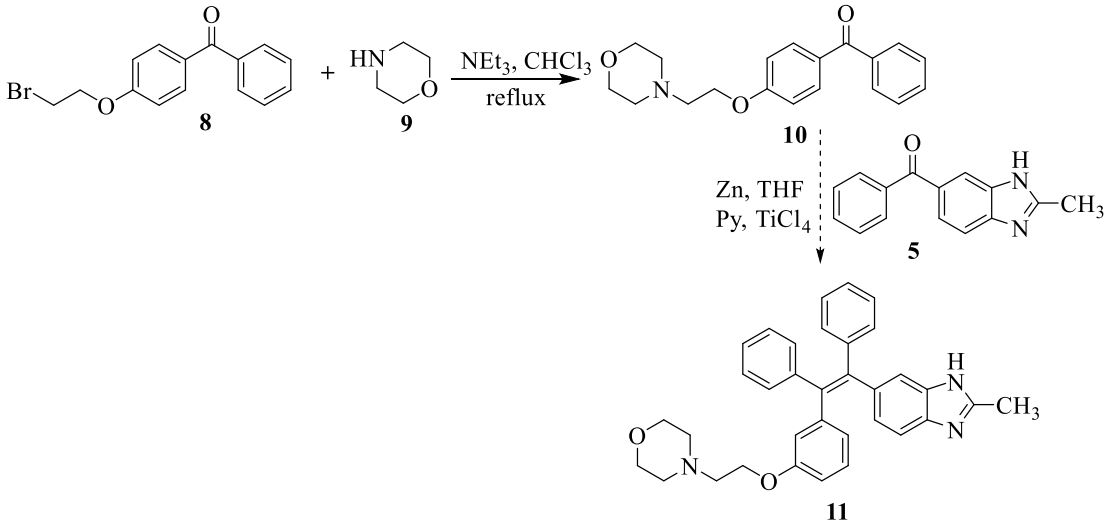


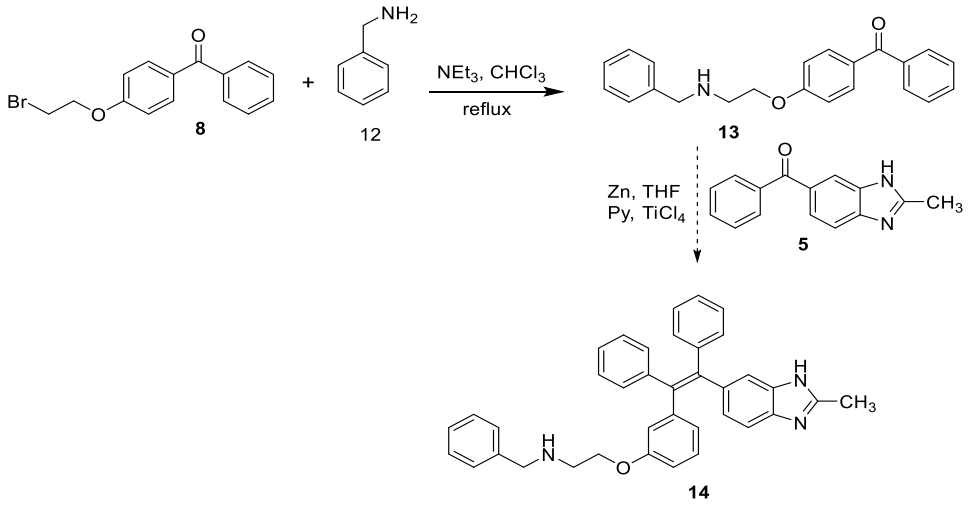
Figure-3 ^{1}H NMR spectrum of (4-(2-bromoethoxy)phenyl)(phenyl)methanone**(8)**

For the synthesis of target compound **11** through McMurry reaction, compound **8** was further used for nucleophilic substitution reaction with secondary amine, morpholine in the presence of triethylamine in chloroform but the desired product was not obtained (**Scheme-5**).



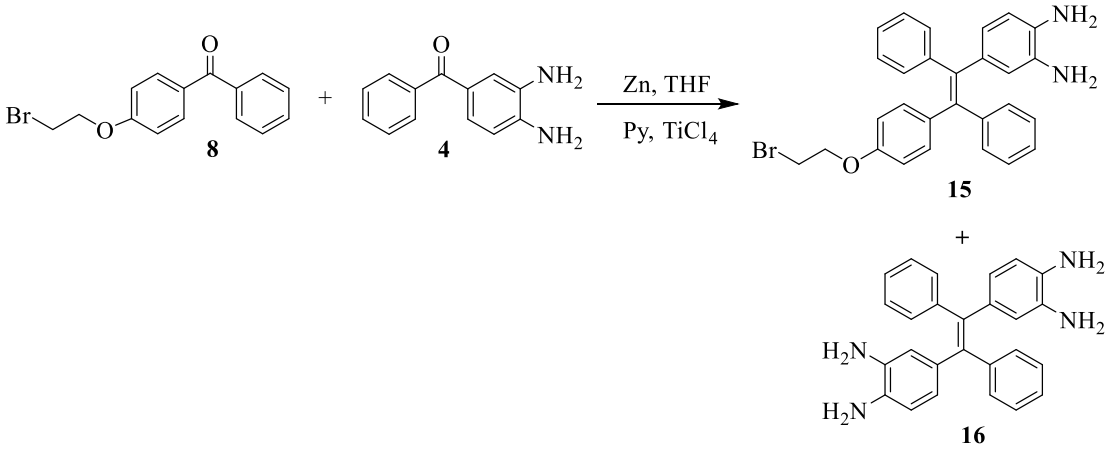
Scheme 5 Synthesis of tamoxifen intermediate (**11**)

Similarly, compound **8** was also treated with primary amine, benzyl amine in the presence of triethylamine in chloroform for the final McMurry compound **14** but again, the desired product was not obtained (**Scheme-6**). Different bases and solvent systems were used for the synthesis of compounds **10** and **13**, in all the cases, reactions were not successful.



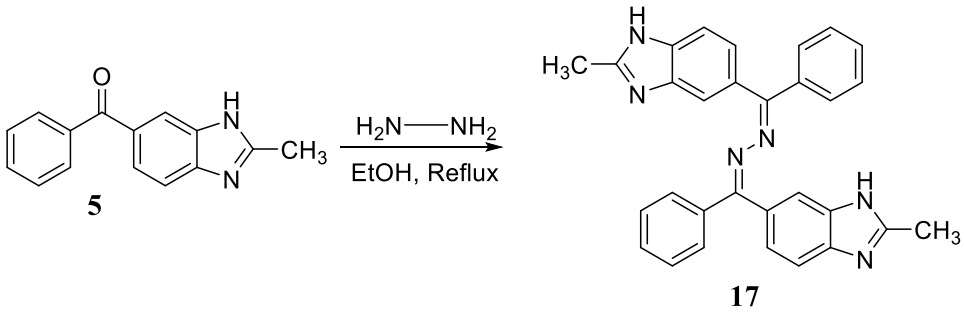
Scheme 6 Synthesis of *N*-benzyl-2-(3-(2-(2-methyl-1*H*-benzo[*d*]imidazol-6-yl)-1, 2-diphenylvinyl)phenoxy)ethan-1-amine

Compound **8** was then tried to react with 3,4-diaminobenzophenone (**4**) in Zn, $TiCl_4$ and pyridine for the synthesis of compound **15**. The reaction was monitored by TLC. The product was extracted with ethyl acetate. The crude was purified by column chromatography. But instead of **15**, compound **4** was dimerized and product **16** was obtained. Compound **16** was confirmed by ^{1}H NMR spectroscopy (**Scheme-7**)



Scheme 7 Synthesis of tamoxifen analogue (**15**)

Another derivative of tamoxifen (**17)** was synthesized by refluxing of compound **5** with hydrazine hydrate in ethanol. The reaction was monitered by TLC. After 2 hours yellow solid was separated out. The compound was confirmed by ^{1}H NMR spectrometry. ^{1}H NMR spectrum showed peaks of multiplet at δ 7.55-7.46 corresponding to eight protons, multiplet at δ 7.32-7.26 due to seven protons and doublet of one proton at δ 7.01 corresponding to aromatic rings. Two singlets at δ 5.4 and 5.3 of one proton each corresponding to two NH groups, and two singlets at δ 2.66 and 2.58 corresponding to two methyl groups were also observed (**Scheme-8**)



Scheme 8 Synthesis of 2-methyl-6-(2-methyl-1*H*-benzo[*d*]imidazole-5-yl)(phenyl) methylene)hydrazono)(phenyl)methyl)-1*H*-benzo[*d*]imidazole **(17)**

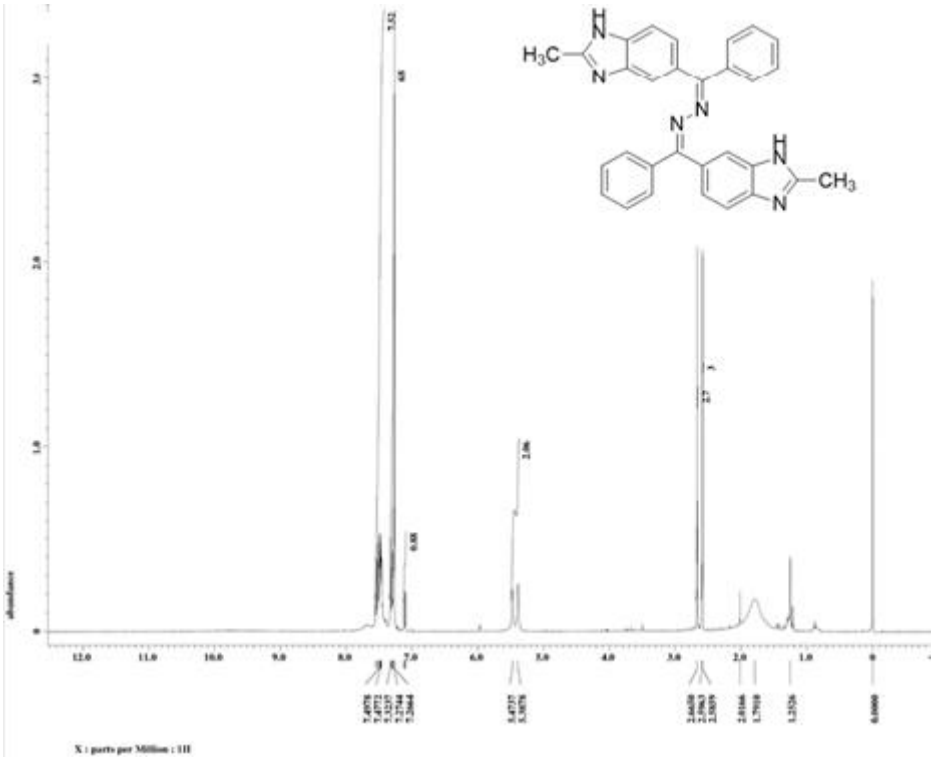
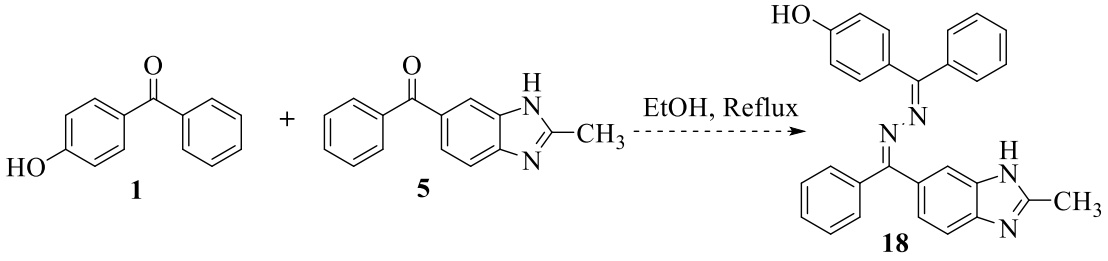


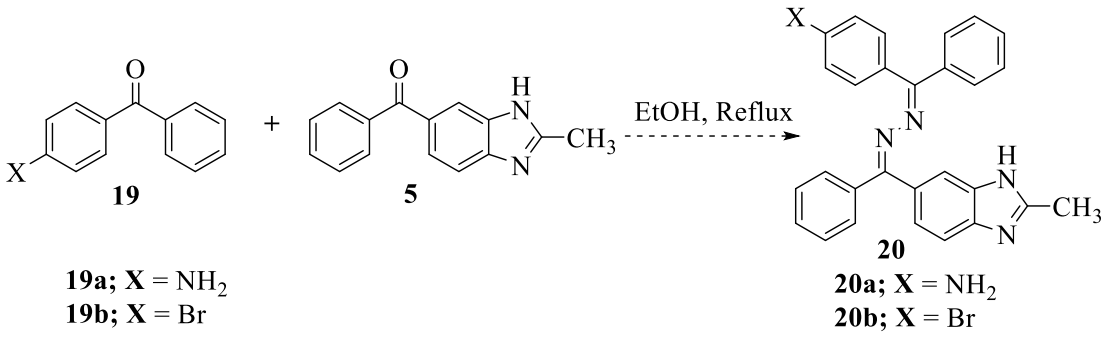
Figure 4 ^{1}H NMR spectrum of 2-methyl-6-(2-methyl-1*H*-benzo[*d*]imidazole- 5-yl) (phenyl)methylene)hydrazono)(phenyl)methyl)-1*H*-benzo[*d*]imidazole **(17)**

With the success of reaction having hydrazine between two similar moieties, we have also tried the reactions of compound **5** with different substitution on both sides of hydrazine. So, 4-hydroxybenzophenone was first tried to treat with compound **5** in presence of hydrazine hydrate in ethanol for the formation of compound **18,** but strating materials were not consumed. The reaction was also tried in the presence of base with different solvent condition but the desired product was not formed (**Scheme-9**)



Scheme 9 Synthesis of 4-(2-methyl-1*H*-benzo[*d*]imidazol-6-yl)(phenyl)methylene) hydrazono)(phenyl)methyl)phenol (**18**)

Compound **5** was also tried with 4-amino benzophenone (**19a**) and 4-bromo benzophenone (**19b**) at different reaction condition but again the strating materials were not consumed (**Scheme-10**).



Scheme 10. Synthesis of compound **20**

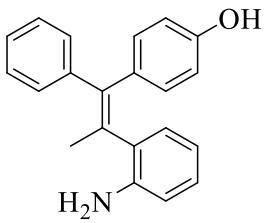
5. EXPERIMENTAL

5.1. General chemistry

All reactions were carried out in oven-dried glasswares. Commercial grade solvents were used without further purification and were supplied by Loba, Spectrochemicals and Aldrich. Melting points were determined in open capillaries and were uncorrected. Jeol-ECS 400 MHz and 100 MHz NMR spectrometer was used for recording ^{1}H and ^{13}C NMR spectra, respectively using $CDCl_3$as solvent. The chemical shifts were expressed in parts per million with TMS as an internal reference and *J* values are given in Hz. Reactions were monitored by thin layer chromatography (TLC) using plates coated with silica gel HF-254 and column chromatography was performed with silica gel 60-120 mesh. Hexane/ethyl acetate was the adopted solvent system.

5.2 Synthesis of Tamoxifen Derivatives

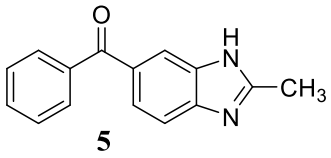
5.2.1 Synthesis of 4-(2-(2-aminophenyl)-1-phenylprop-1-en-1-yl)phenol (3)



3

Compound **3** was synthesized by McMurry reaction. Under nitrogen atmosphere, a 3 necked round bottom flask equipped with a magnetic stirrer charged with zinc powder (1.97 g, 30 mmol), and 40 mL freshly distilled THF was added. The mixture was cooled to -5 to 0 ˚C and addition of $TiCl_4$ (1.6 ml, 15 mmol) was done slowly while maintaining a temperature under 10 ˚C. The mixture was shifted to room temperature and stirred for 4.5 hours, and then heated to reflux for 2.5 hours, the mixture was again cooled to -5 to 0 ºC, a solution of 4-hydroxybenzophenone (2.0 g, 10 mmol) (**1**) and 2-aminoacetophenone (1.36 g,10 mmol) (**2**) in 20 ml THF was added slowly. After addition, the reaction mixture was heated until the carbonyl compound was consumed (monitered by TLC). The reaction was quenched with 10% aq. solution of K_2CO_3 and extracted with ethyl acetate. The organic layer was collected and concentrated. The crude material was purified by column chromatography to give the desired compound **3**. Yield: 10%; Colour: Brown; m.pt. > 300 ˚C.

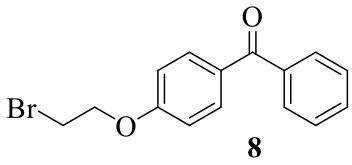
5.2.2 Synthesis of (2-methyl-1*H*-benzo[*d*]imidazol-6-yl)(phenyl)methanone (5)



5

The compound **5** was obtained by refluxing 3,4 diaminobenzophenone (**4**) (1 g, 2.3 mmol) in 20 mL of acetic acid for 24 hours. The confirmation of the reaction was checked by TLC. Then the reaction mixture was allowed to cool at room temperature followed by neutralized with aqueous solution of potassium carbonate. Then, the product formed was extracted with ethyl acetate and water. The organic layer was distilled off. Yield: 89%; Colour: Brownish orange; m.pt. 222-225˚C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.04 (s, 1H,ArH), 7.81-7.79 (m, 3H, ArH), 7.59-7.57 (m, 2H,ArH), 7.50-7.48 (m, 2H, ArH), 3.80 (bs, 1H, NH), 2.69 (s, 3H, CH_3).

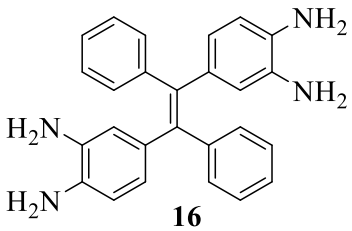
5.2.3 Synthesis of (4-(2-bromoethoxy)phenyl)(phenyl)methanone (8)



8

Compound **8** was obtained by dissolving 4-hydroxybenzophenone (**1**) (5.0 g, 0.025 mol), tetra-*n*-butylammonium bromide (0.37 g, 0.001 mol), 1,2-dibromoethane (9.4 g, 0.05 mol) and NaOH (2.0 g, 0.05 mol) in water (100 mL) and then the reaction was shifted at refluxing for 18 hours. The monitoring of the reaction was checked by TLC. Then, the product was extracted with water and ethyl acetate. The washing of the organic layer was done with water, brine, and then dried over Na_2SO_4. White solid was obtained. Yield: 63%; Colour: White solid; m.pt. 68-69 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82–7.71 (m, 4H, ArH), 7.55 (t,*J*= 7.80 Hz,1H, ArH), 7.45 (t, *J*= 7.80 Hz, 2H, ArH),6.96 (d, *J*= 8.70 Hz, 2H, ArH), 4.34 (t, *J*= 6.40 Hz, 2H, CH_2), 3.65 (t, *J*= 5.96 Hz, 2H, CH_2).

5.2.4 Synthesis of 4-(2-(4-(2-bromoethoxy)phenyl)-1,2-diphenylvinyl)benzene-1,2-diamine (16)

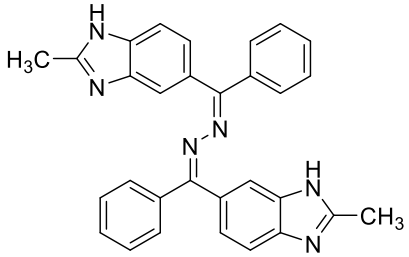


16

Compound **16** was synthesized by McMurry reaction. Under nitrogen atmosphere, a 3 necked round bottom flask equipped with a magnetic stirrer charged with zinc powder (1.97 g, 30 mmol) and 40 mL freshly distilled THF was added. The

mixture was cooled to -5 to 0 ˚C and $TiCl_4$ (1.6 ml,15 mmol) was added slowly by a syringe with the temperature kept under 10˚C. The suspending mixture was warmed to room temperature and stirred for 4.5 hours then heated to reflux for 2.5 hours, the mixture was again cooled to -5 to 0ºC, solution of 3,4-diaminobenzophenone (2.0 g,10 mmol) and 4-(2-bromoethoxy)benzophenone (2.0 g,10 mmol) in 20 ml THF was added slowly. After addition, the reaction mixture was heated until the carbonyl compound was consumed (monitored by TLC). The reaction was quenched with 10% aq. solution of K_2CO_3 and extracted with ethyl acetate. The organic layer was collected and concentrated. The crude material was purified by column chromatography to give the compound **16** instead of desired compound **15**. Yield: 53% (crude); Colour: Brown; m.pt. > 300 ˚C.

5.2.5 Synthesis of 2-methyl-6-(2-methyl-1*H*-benzo[*d*]imidazole-5-yl)(phenyl) methylene)hydrazono)(phenyl)methyl)-1*H*-benzo[*d*]imidazole (17)



17

Compound **17** was synthesized by refluxing compound **5** (200 mg, 1 mmol) with hydrazine hydratre (266 µL, 10 mmol) in ethanol. The reaction was monitored by TLC. After 2 hours, yellow solid was separated out, filtered off and dried. The compound was confirmed by ^{1}H NMR spectrum.

Yield: 73%; Colour: Brown; m.pt. 235-240 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.55 – 7.46 (m, 8H, ArH), 7.32 – 7.26 (m, 7H,ArH), 7.01(d, *J* = 4.00 Hz, 1H, ArH), 5.40 (s, 1H, NH), 5.30 (s, 1H, NH), 2.66 (s, 3H, CH_3), 2.58 (s, 3H, CH_3).

6. CONCLUSIONS

- Intermediates such as(2-methyl-1*H*-benzo[*d]*imidazol-6-yl)(phenyl)methanone(**5**), (4-(2-bromoethoxy)phenyl)(phenyl)methanone**(8)** etc. were synthesized in moderate to good yields for the formation of tamoxifen derivatives.
- 2-Methyl-6-(2-methyl-1*H*-benzo[*d*]imidazole-5-yl)(phenyl)methylene)hydrazono) (phenyl)methyl)-1*H*-benzo[*d*]imidazole(**17)** was synthesised in moderate yields.
- Synthesized intermediates will further be used for the synthesis of tamoxifen derivatives.

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