SYNTHESIS OF TAMOXIFEN DERIVATIVES AND THEIR BIOLOGICAL ACTIVITIES

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ABSTRACT

Tamoxifen has remained the gold standard for the treatment of the breast cancer and extensive research is going on for the synthesis of the various novel tamoxifen analogs for their evaluation as anticancer agents against various human cancer cell lines. The main aim of this review is to summarize the various synthetic methods for the preparation of the tamoxifen analogs and their biological properties.

Keywords: Tamoxifen, Breast cancer, Anticancer activity, Suzuki coupling

Introduction

Breast cancer is the second leading cause of cancer related deaths among the women (Siegel et al., 2017) with more than 1 million cases per year (McPherson et al., 2000). This disease is associated with the risk of 1 death per 35 patients (Lynch et al., 1990) and is the primary cause of mortality of women between 45-55 years in age (Jemal et al., 2009). Almost 1 in 8 women are at higher risk of breast cancer which requires complete tissue removal, hormone therapy, chemotherapy or radiotherapy (Heravi et al., 2013). According to WHO data, an estimated of 627000 death s were recorded during 2018 because of this deadly disease which was 15% of the total deaths associated with cancer among women (https://www.who.int/ cancer/prevention/diagnosis-screening/breast-cancer/en/). There are commonly two types of breast cancer. Non-invasive breast cancer is confined to the duct and do not penetrate to the connective or fatty tissues of the breast. Ductal carcinoma in situ (DCIS) is an example of the most commonly occurred non-invasive form of breast cancer whereas Lobular carcinoma in situ (LCIS) is the less common form of non-invasive breast cancer. On the other hand, invasive breast cancer involves the penetration of the cancer cells through lobular and duct wall of the breast thereby penetrating to the connective or fatty tissue of the breast (Sharma et al., 2010). Infiltrating ductal carcinoma (IDC) is an example of invasive breast cancer. The initial symptoms of breast cancer are the lumps found in armpit or breast. In advanced stage, additional symptoms like bone pain, shortness of breath, headache, neurological pain etc. are also observed (http://breastcancer.about.com/ od/whatisbreastcancer/a/bc_symptoms.htm). There are many techniques available for the screening of the breast cancer like mammography, magnetic resonance imaging (MRI) and ultrasound (Warner et al., 2008; Krieger et al., 2004; Kelly et al., 2010). Many targeted therapies are available for the treatment of breast cancer like aromatase inhibitors (Petra et al., 2013), antibody treatment (Slamon et al., 2001; Pegram et al., 1998; Higgins et al., 2011), pertuzumab and lapatinib (Swain et al., 2015; Maximiano et al., 2016) and inhibitors of downstream pathways like RAS/MEK/ERK and PI3K/AKT/mTOR but all are associated with the one or other limitations such as development of resistance mechanism in the body. Among the present therapies, tamoxifen has remained the gold standard for the treatment of the breast cancer. It is a first generation estrogen receptor modulator which can be used at early or advanced stage for the treatment of breast cancer for pre- and post-menopausal women (Rochefort et al., 1983; Jordan et al., 2003; Brauch et al., 2009). Tamoxifen has been reported to show long lasting benefits for high risk women (Davies et al., 2013) and it could lead to 31% reduction in the annual death rates due to breast cancer (Early Breast Cancer Trialists’ Collaborative Group et al., 2005). However, its use is associated with side effects like increase in the venous thromboembolic event (Lin et al., 2018), thermoregulatory dysfunction (Heery et al., 2018), decrease in the bone density (Cohen et al., 2008) and the intrinsic resistance developed with its use (Chang et al., 2012). Therefore, lot many studies have been done for the design and synthesis of novel derivatives of tamoxifen which can give desired therapeutic activity with minimal side effects. Various review articles have been reported in literature specifically for tamoxifen derivatives but either they have not reported the anticancer activities of the synthesized derivatives or they are not updated till date (Tandon et al., 2020; Shagufa et al., 2018; Kasiotis et al., 2012). The main aim of this review article is to compile the latest data pertaining to synthesis of the tamoxifen derivatives along with their anticancer activities.

Synthesis of tamoxifen derivatives

Elena Catanzaro et al. have discussed the synthesis of xanthene and enyne hybrid of tamoxifen which were further investigated for the anticancer activity against MCF-7 and MDA-MB-231 cell lines (Scheme 1) (Catanzaro et al., 2019). The synthesis of the targeted derivatives started with the reaction of 3-Hydroxy-9H-xanthan-9-one (1) with octafluotoluene by using phase transfer catalyst to afford compound 2 which was reacted with propiophenone in second step under Mcmurry reaction conditions to give
isomer mixture of compound 3 (isomeric mixture of E and Z isomers). The next step involved the reaction of compound 3 with NaOMe and in presence of perfluorotoly protecting group to afford compound 4. Further, compound 4 was reacted with 1,2-dibromoethane by using acetone as solvent and a base at room temperature to give compound 5. Finally, compound 5 was further treated with pyridoline at room temperature (to avoid cis trans isomerisation) to afford target compound 6.

These synthesized derivatives were further examined for the anticancer activities against MDA-MB-231 and MCF-7 cancer cell lines. The data suggested that compound 6 showed better activities with IC$_{50}$ = 12.4 ± 0.54 micromolar towards MCF-7 cancer cell lines and has IC$_{50}$ = 25.4 ± 0.40 on ER-negative MDA-MB-231 as compared to Tamoxifen with 12.4 micromolar towards MCF-7. However, the Compound 5 showed comparatively lower activity towards MCF-7 cell lines.

Scheme 1: Synthesis of xanthine based derivatives of tamoxifen

On the other hand, synthesis of enyne derivatives started with the reaction of compound 7,8 and 9 in presence of 3-bromoprop-1-yne in acetone to afford compounds 10-12. The final target molecules 13-15 were synthesized by reacting 10-12 with CuSO$_4$ and selected amine in presence of formaldehyde. These synthesized derivatives were explored towards ER-positive MCF-7 and ER-negative MDA-MB-231. The data showed that compound 13 showed IC$_{50}$ value of 20.7 ± 4.05 micromolar towards MCF-7 as compared tamoxifen with IC$_{50}$ value of 10.5 ± 0.76 micromolar. Further, compounds 14 and 15 showed lesser activity as compared to 13.

Scheme 2: Synthesis of enyne based derivatives of tamoxifen
Ashraf H. Abadi et al. reported the synthesis of novel tamoxifen analogues which were further analysed for their anticancer activity against MCF-7 cell line (Abadi et al., 2016). The synthesis of E or Z form of tamoxifen analogues is presented in Scheme 3. The synthesis started with the reaction of compound 16 with acetophenone to afford compound 17 which was further reacted with different amine derivatives to give compound 18. Then finally, Compound 18 was reacted with RCOCl to give the compound 19. These synthesized derivatives were studied for their anticancer activities against MCF-7 and MOLT-4 cell lines. The data suggested that derivatives 19 showed higher activity for MCF-7 cell line with GI<sub>50</sub> = <0.005 micromolar as compared to tamoxifen which has GI<sub>50</sub> = 1.58 micromolar. However, derivatives 18 showed comparatively lower activity against MCF-7 cell line with GI<sub>50</sub> value < 0.01 micromolar as compared to tamoxifen.

Carpenter et al. have reported that the synthesis of triarylacrylonitrile analogues of tamoxifen which have better binding selectivity for protein Kinase C (Carpenter et al., 2016). The synthesis of these analogues of tamoxifen is presented in Scheme 4. The synthesis started with Compound 20 which reacted with Et<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>Cl·HCl in the presence of Cs<sub>2</sub>CO<sub>3</sub> to afford compound 21 which was further reacted with PhCHLICN in THF to give the compound 22.

These synthesized derivatives of tamoxifen were analysed for anticancer activities against estrogen receptor alpha. From the data, it can be concluded that compound 21 (iii) showed good activity against estrogen receptor alpha with IC<sub>50</sub> value of 80 nM as compared to tamoxifen with IC<sub>50</sub> = 222 nM. Further in general, the compound 22 showed lesser activity as compared to compound 21.
Forest et al. have reported the synthesis of fluorinated derivatives of tamoxifen (Forest et al., 2013). These derivatives were explored for anticancer activities against MDA-MB-231 and MCF-7 cell lines (Scheme 5). Compound 23 was reacted with TBSOCH(CH₂)₂Br in the presence of base to afford compound 24 which was further reacted with CF₃CH₂I in the presence of LDA and ZnCl₂ to give compound 25 with E/Z ratio of 97/3. Next step involved the reaction of compound 25 with TMSCl in the presence of n-BuLi to give compound 26. Compound 16 reacted with aryl lithium to give compound 27 in which was further reacted with Bu₄NBr₃ in presence of MeOH to afford 28. Finally, compound 28 reacted under Suzuki conditions to afford the target compound 29(i-iv) in presence of amine, TBAI, MeOH. These derivatives which were synthesized were also analysed for their anticancer activities against MCF-7, HT-27, M21 and MDA-MB-231 cell lines. The data suggested that compound 29(i) showed better activities against MCF-7 and MDA-MB-231 cell lines with GI₅₀ value of 3.6 micromolar as compared to tamoxifen with GI₅₀ value of 7.3 micromolar against MDA-MB-231 cell line. Further, 29(i) showed GI₅₀ = 8.7 micromolar for HT-29 and 4.4 micromolar for M21 cell lines. Compound 29(ii) showed GI₅₀ value of 5.6 micromolar against MCF-7 and 10.6 micromolar against MDA-MB-231 cell line. Compound 29(iii) showed GI₅₀ = 7.4 micromolar towards MCF-7 and 12.6 micromolar against MDA-MB-231 cell lines.

Isamu shiina et al. have reported that the synthesis of heterocyclic derivatives of tamoxifen which were further taken for anticancer activities (Shiina et al., 2008). The synthesis of the targeted derivatives of tamoxifen is presented in Scheme 6. The starting compound 30 was reacted with anisole in the presence of HfCl₄ to afford Compound 31 which was further heated in the presence of t-BuOK in DMSO at 90°C to give compound 32. Next step of reaction involved the reaction of compound 32 with different chloroamine derivatives to give the compounds 34-36.

These derivatives were analysed for their anticancer activities against HL-60 cancer cell lines. Compound 34 showed better activity but compound 35 showed medium activity and 36 showed no effect on cell viability.
Takuji Shoda et al. reported synthesis of tamoxifen derivatives with long alkyl side chain which were further analysed for their anticancer activities against MCF-7 cell lines (Shoda et al., 2015). The synthesis of derivatives is shown in Scheme 7. The starting compound 37 was reacted with CH₂(CH₂)ₙCH₂ in the presence of triethylamine in methanol to give 38-42.

Scheme 6: Synthesis of heterocyclic derivatives of tamoxifen

Compound 39 showed IC₅₀ value of 3.6 nM towards MCF-7 cell line as compared to the Compound 42 which was least active with IC₅₀ value of 210 nM. Compound 42 showed lesser activity among other compounds which was synthesized in this Scheme.

Scheme 7: Synthesis of tamoxifen derivatives with long alkyl side chain

38: n = 7, R = H, (80%)
39: n = 9, R = H, (67%)
40: n = 13, R = H, (36%)
41: n = 15, R = H, (43%)
42: n = 9, R = OH, (43%)
The synthesis of derivative \(47\) is presented in the Scheme 8. The synthesis started with reaction of compound\(43\) with (Boc)\(_2\)O in dichloromethane to afford compound\(44\) which was further reacted with TBAF to give compound\(45\). The next step of the synthesis involved the reaction of compound\(45\) with HCl in presence of dioxane at room temperature to give compound\(46\) in efficient yield. Finally, reaction of compound\(46\) with compound \(37\) in the presence of DEIA afforded compound\(47\).

The data showed that compound\(47\) showed better anticancer activity for MCF-7 cancer cell line with IC\(_{50}\) of value 3.4 nM. The data also suggested that the fluoro group which is present at terminal position of alkyl chain increases the down-regulation without decreasing the binding activity of the compounds.

Nermin S. Ahmed et al. have reported the synthesis of novel flexible tamoxifen analogues which were further examined for their anticancer activities against cell lines (Scheme 9) (Ahmed et al., 2020). The starting compound\(48\) reacted with \(49\) in presence of presence of Zn / TiCl\(_4\) to give compound \(50\) which was further reacted with ClCH\(_2\)CH\(_3\)NR\(_1\)R\(_2\) in DMF at 80\(^\circ\)C to give Compound\(51\). Finally, \(51\) was reacted with RCOCl in presence of dichloromethane at room temperature to afford the target Compound\(52\) and \(53\).

These synthesized derivatives were analysed for their anticancer activities against cell lines. compound\(51\) with R\(_1\) and R\(_2\) are methyl group and R=C\(_2\)H\(_5\) showed IC\(_{50}\) value of 167 nM against MCF – 7 BUS. The compound\(50\) showed lesser anticancer activity as compared to \(51\) against MCF-7 BUS.

Nermin S. Ahmed et al. have reported the synthesis of novel flexible tamoxifen analogues which were further examined for their anticancer activities against cell lines (Scheme 9) (Ahmed et al., 2020). The starting compound 48 reacted with 49 in presence of presence of Zn / TiCl\(_4\) to give compound 50 which was further reacted with ClCH\(_2\)CH\(_3\)NR\(_1\)R\(_2\) in DMF at 80\(^\circ\)C to give Compound 51. Finally, 51 was reacted with RCOCl in presence of dichloromethane at room temperature to afford the target Compound 52 and 53.

These synthesized derivatives were analysed for their anticancer activities against cell lines. Compound 51 with R\(_1\) and R\(_2\) are methyl group and R=C\(_2\)H\(_5\) showed IC\(_{50}\) value of 167 nM against MCF – 7 BUS. The compound 50 showed lesser anticancer activity as compared to 51 against MCF-7 BUS.

Scheme 8: Synthesis of tamoxifen derivative

Scheme 9: Synthesis of flexible tamoxifen analogues
Schwarze et al. have reported the 2,2'-Bipyridine modified tamoxifen derivative which was further analysed for its anticancer activities against MCF-7 cancer cell lines (Scheme 10) (Schwarze et al., 2019). In the synthesis, the starting compound 2-Haloisonicotinic acid (54) was reduced to 1-(2-halopyridin-4-yl)propan-1-one which was further reacted with ethyl magnesium bromide in THF at low temperature to give 55 which was further reacted with Pd(PPh₃)₄ to give 56 with good yield. Then, Horner Wadsworth Emmons modification of 56 lead to the formation of 57 in good yield. Compound 57 under Suzuki coupling reaction conditions gave 58. Finally, demethylation of aryl-methyl ether of 58 with BBr₃ in DCM yielded final compound 59.

Compound 59 showed better activity with IC₅₀ value of 1.8 ± 0.4 in MTT assay and 2.1 ± 0.6 in CV assay against MCF-7 cell line. Compound 57 showed lesser activity of IC₅₀ value of 43.0 ± 2.9 in MTT assay and in CV assay 41.3 ± 2.2 against MCF-7 cell line.

Scheme 10: Synthesis of 2,2'-Bipyridine modified tamoxifen derivative

Ahmed et al. have reported the synthesis of tamoxifen derivatives with different substitutions at amine group which were further analysed for their anticancer activities (Ahmed et al., 2016). The synthesis of derivatives of tamoxifen is presented in Scheme 11. The starting compound dihydroxybenzophenone (60) reacted with acetophenone to give 61. Then, Intermediate compound 61 was reacted with dialkylamine ethylenechloride in the presence of K₂CO₃ in DMF to give compound 62. Finally, E/Z Tamoxifen analogue 63 was obtained by esterification.

These derivatives were explored for their anticancer activities against MCF-7 cancer cell lines. Compound 63 showed better activity with IC₅₀ value of against cell line. The data suggested that all the synthesized derivatives exhibited higher activity for MCF-7 cancer cell line with IC₅₀ 1-3.7 µM as compared to IC₅₀ equal to 4.4 µM in case of tamoxifen which supported the fact that placement of hydroxyl or ester group at 4-position of tamoxifen moiety led to enhancement of anticancer activity for MCF-7 cancer cell line.
Takushi Shoda et al. have reported the synthesis of tamoxifen derivatives as a selective estrogen receptor down-regulator which were further explored for their anticancer activities (Scheme 12) (Shoda et al., 2014). Compound 64 was reacted with SOCl₂ in presence of lewis acid to afford 65 which was further reacted with BrMg-Ph-OTH and conc. HCl to produce 66 in efficient yield. Finally, compound 66 reacted with different amine derivatives in presence of methanol gave 67.

Further, all the synthesized derivatives showed better activity against MCF-7 cell lines as compared to tamoxifen.

Makoto Hasegawa et al. have reported the synthesis of novel tamoxifen derivatives which were further analysed for their anticancer activity against HEK293 and HL-60 human cell lines (Hasegawa et al., 2014). The synthesis of the derivatives is shown in Scheme 13. The starting compound 68 reacted with [CH₂]₃N(CH₂)₂Cl.HCl in presence of NaH to afford target compound 69 (RID-F). Then for synthesis of another active compound took place.
when Bis(4-hydroxyphenyl)methane (70) reacted with [(CH₂)₃N(CH₂)₂Cl.HCl in presence of NaH to afford final compound RID-F (71).

These derivatives showed better activity on HEK293 and HL-60 human cells. Compound 69 showed better activity with IC₅₀ value of 0.64 on HEK293 and HL-60 human cells and compound 71 showed activity with 0.67 on HEK 293 human cells.

![Chemical structure of compound 68](image)

**Scheme 13:** Synthesis of novel tamoxifen derivatives

Abdellatif et al. have reported the synthesis of six novel tamoxifen analogues of tamoxifen 75(a-f) and explored their anticancer activity against MCF-7 and MDA-MB-231 cancer cell lines (Scheme 14) (Abdellatif et al., 2013). The starting compound, benzophenone derivatives (73), underwent reductive cross coupling with 72 under the McMurry reagents to afford compound 74 in low yields. At last, compound 74 reacted with piperidine, piperazine or N-methylpiperazine in presence of ethanol at high temperature to give 75(a-f).

These synthesized compounds 75(a-f) were explored for their anticancer activity for MCF-7 and MDA-MB-231 cancer cell lines. The data supported that compound 75a and 75f possessed almost similar activity as tamoxifen against MCF-7 and MDA-MB-231 cell lines whereas compounds 75c and 75e showed two-fold activity in comparison with tamoxifen which was due to the replacement of dimethylamino group of tamoxifen with more basic groups likepiperazino or N-methylpiperazino moieties.

![Chemical structure of compound 72](image)

**Scheme 14:** Synthesis of six novel tamoxifen analogues of tamoxifen

**Conclusion**

From the past few years, much of the attention has been paid for the synthesis of tamoxifen derivatives by utilizing various functional group transformation or substitutions at different rings as well as side chain of the tamoxifen framework which has led to number of important tamoxifen analogues which possess better in vitro anticancer activity as compared to tamoxifen. Further, exploration of these molecules for in vivo studies and investigation of the new
molecules can pave a way for a drug candidate with desired therapeutic outcome.

References


Pegram, M.D., A. Lipton, D.F. Hayes, B.L. Weber, J.M. Baselga, D. Tripathy, D. Baly, S.A. Baughman, T. Twaddell, J.A. Glaspy and D.J. Salmon, Phase II study of receptor-enhanced chemosensitivity using...


