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The effect of newly synthesized heterosteroids on miRNA34a, 98, and 214 expression levels in MCF-7 breast cancer cells.

Research Article

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Summary

Hybrid anticancer drugs have emerged as great therapeutic captions that can effectively overcome most obstacles facing conventional anticancer drugs. miRNAs are considered as class of non-coding RNAs that can negatively regulate protein coding gene expression. miRNA expression is commonly altered in cancer cells. The current work aimed to test the effect of new pro-apoptotic heterosteroids on some drug resistance related miRNAs expression levels (miRNA34a, 98, and 214) in MCF-7 breast cancer cells. After cell treatment with these compounds 4, 6, 7, 13, 18, 21, 22 and 24, miRNAs were extracted and subjected to reverse transcription and subsequent PCR amplification using Real Time-PCR technique. The expression levels of miR-34a, miR-98 and miR-214 were quantitatively determined. The study revealed that the expression levels of miR-34a, miR-98 and miR-214 were up-regulated upon treatment with tamoxifen, which was used as a positive control drug , as compared to control cells,. Strikingly, the levels of miR-34a, miR-98 and miR-214 expression significantly down-regulated when treated with most of the new heterosteroids as compared to control cells. These results could indicate the promising effects of these new heterosteroids on reducing drug resistance as compared to tamoxifen drug. As well established, cells develop drug resistance to tamoxifen.

I. Introduction

Breast cancer is the second most common cancer type and the most prevalent among women (Ferlay J et al., 2010). Studies confirmed that alteration in different gene expressions is involved in breast cancer development (GLOBOCAN database (2008), 2012). Consequently, alterations in proliferation and apoptosis pathways have been used as targets for treatment (Brazilian National Cancer Institute, 2012). Hybrid anti-cancer agents were recently used to diminish the toxicity and enhance specificity (Trafalis DTP et al., 2006). It is developed by the addition of heterocyclic rings to steroids. This combination leads to a change of their physiological activity and the appearance of new promising pharmacological and biological properties (Mohamed NR et al., 2012). The change in the expression of certain genes dramatically affects the development and progression of breast cancers. This change could be mediated through many factors from which miRNAs are very potent regulators of gene expression in cells (Morris KV and Mattick JS, 2014). Many miRNAs were figured out as key players in cancer progression, metastasis, chemotherapeutic multidrug resistance and endocrine resistance in breast cancer (Calin GA et al., 2002). Consequently, miRNAs have been shown to have both diagnostic and prognostic significance, in addition to, being important targets for cancer treatment (Blenkiron C et al., 2007; Tricoli JV and Jacobson JW, 2007]. Drug resistance is considered as a major obstacle facing cancer chemotherapy and accounts for the failure of chemotherapy and finally mortality (Kutanzi KR et al., 2011; Speirs CK et al., 2011). Despite the well known role of miRNAs in cancer, the exact mechanism of miRNA role in drug resistance still not fully clarified. In normal cells, there is balance between pro-apoptotic and anti-apoptotic programs, however, in cancerous cells this balance is shifted toward cell survival (Kutanzi KR et al., 2011). miRNA-34a, miRNA-98 and miRNA-214 have a well established positive role in regulating apoptosis and reducing cell proliferation (Li N et al., 2009; Cole KA et al., 2008). Moreover, miRNA-34a has been established to be associated with drug resistance in cancer (Fujita Y et al., 2008; Kojima K et al., 2010). However, less is known about the role played by miRNA-98 and miRNA-214 in modulating drug resistance. miRNA-34a is one of miRNA-34 family members which comprises three processed miRNAs that are encoded by two different genes. miRNA-34a is encoded by its own transcript while miRNA-34b and miRNA-34c share a common transcript.

miRNA-98 is a member of let-7 family, which comprises 13 members (let7-a-1, a-2, a-3, b, c, d, e, f-1, f-2 g, i, miR-98, and miR-202). Let-7 family members were found to target similar genes and play similar roles. Let-7 was found to negatively regulate other oncogenes and cell cycle regulators leading to cell cycle arrest by promoting the transition from G1 to S phase (Johnson SM et al., 2005; Kumar MS et al., 2008; Mayr C et al., 2007; Nadiminty N et al., 2012). In breast cancer, miRNA-214 was found to be reduced, however, the functional significant relevance of this finding remains non-understood (Volinia S et al., 2006; Derfoul A et al., 2011]. miRNA-214 was reported to have a tumor suppressor effect (Feinberg AP et al., 2006). The present study was conducted to test the effect of new promising heterosteroids on miRNA34a, 98, and 214 expression levels in MCF-7 breast cancer cells. The role played by miR-34a in drug resistance was tested. Moreover, we tried to investigate whether miR-98 and miR-214 have a similar role in drug resistance.

1. Materials and methods

1.1. Cell propagation, maintenance and treatment

Breast cancer MCF-7 cells were purchased from ATCC (American Type Culture Collection) and maintained in the proper conditions. The cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) (Lonza, Beligium) supplemented with 10 % fetal bovine serum (FBS), 4 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin sulfate at 37 ˚C in a humidified incubator with 5 % CO_2. The cells harvested after trypsinization (0.025 % trypsin and 0.02 % EDTA) and washed twice with Dulbecco's phosphate-buffered saline (DPBS). When the cell density reached approximately 80%, cells were split for further culture. The experiments were made up when the cells were in the logarithmic growth phase. Breast cancer MCF-7 cells were seeded in 24 well plate at a density of 30,000 cells/well. The second day after seeding, cells were treated with compounds **4**, **6**, **7**, **13**, **18**, **21**, **22** and **24** at a concentration equivalent to IC50 values. These compounds were synthesized and subject to cytotoxicity analysis in a previous work (Elmegeed GA et al., 2016). The structures of these compounds are illustrated in **fig. 1** and their IC50 values are listed in **table 1**.

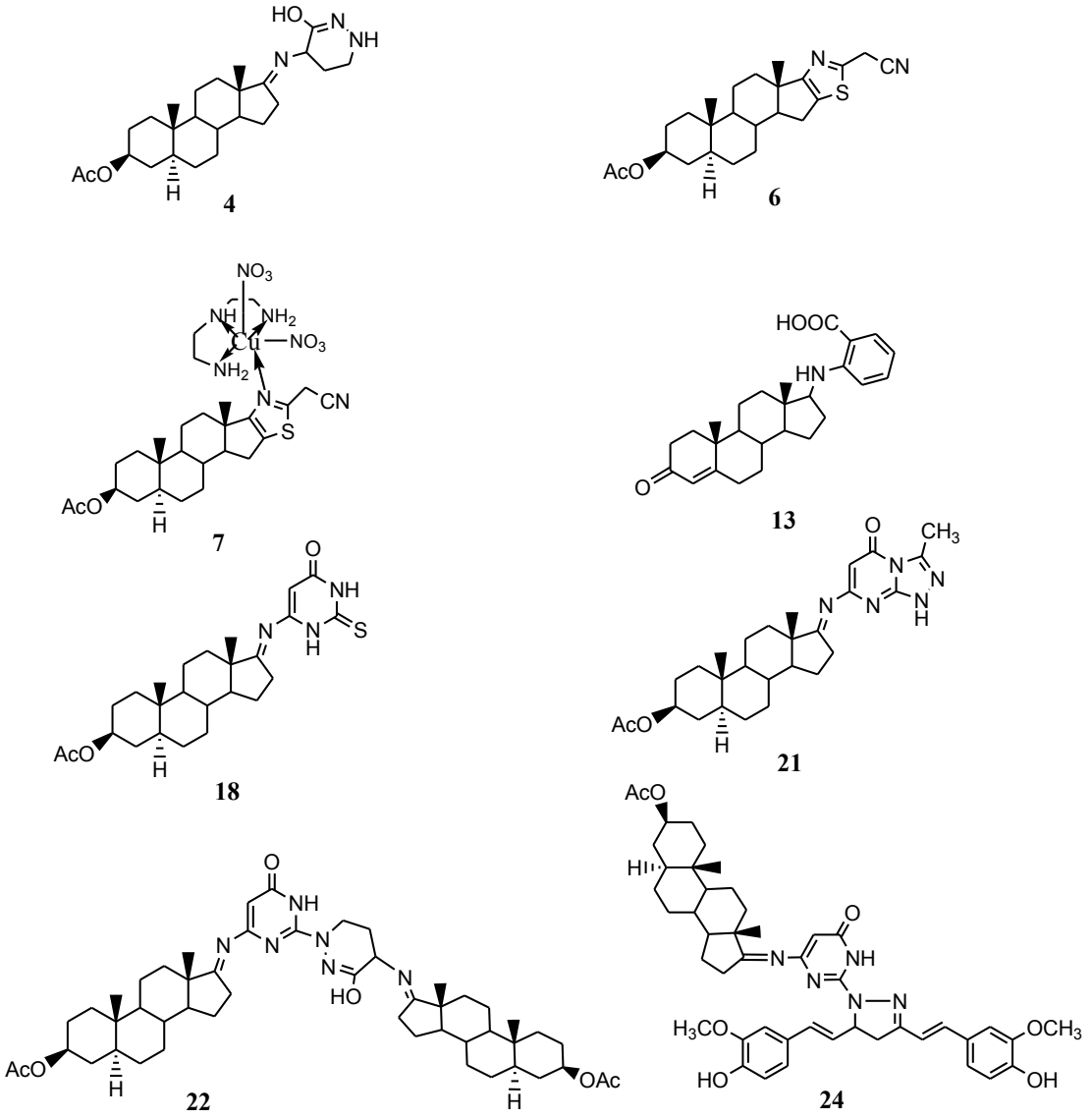


Fig. 1

Fig (1): Chemical structure of the newly synthesized heterosteroids.

Table 1: The in vitro cytotoxic activity of the newly synthesized compounds on MCF-7 cancer cell line

Compd. No	IC_{50} (μM)	Compd. No	IC_{50} (μM)
4	9.6	**18**	26
6	4.8	**21**	29
7	23.3	**22**	32
13	23.6	**24**	18
Tamoxifen	4	**Tamoxifen**	4

IC_{50}: Concentration required to inhibit cell viability by 50%.

1.2. **Quantitation and QRT-PCR measurement of miRNA-34a, miRNA-214 and miRNA-98**

miRNA was extracted from total RNA using a miRNeasy kit (Qiagen). The miScript PCR system enables sensitive, specific miRNA quantification and profiling using SYBR Green real-time PCR. The miScript PCR system covers all the steps involved in conversion of RNA to cDNA and subsequent real-time PCR detection of miRNAs. MiRNA profiling of low-quantity RNA samples requires the following 3 steps: reverse transcription using the miScript II RT kit, preamplification using the miScript PreAMP PCR kit and miScript PreAMP primer mix and real-time PCR using the miScript SYBR Green PCR kit and specific primers for miRNA-34a, miRNA-214 and miRNA-98 that were provided from Qiagen. The cycling conditions were as follows: Denaturation for 15s at 94ºC, Annealing for 30s at 55ºC, Extension for 30s at 70ºC. Then fluorescence data using MiniOpticonTM Bio-Rad Real Time Thermal Cycler Collection was performed.

1.3. **Statistical analysis**

The data were analyzed using student t\-test to detect the significant difference among the studied compounds. All the data are expressed as Mean± standard error mean. A level of $P< 0.05$ was defined as statistically significant.

2. Results and discussion

The mechanisms of resistance to chemotherapeutic agents are complex and not fully understood. Our previous study (Elmegeed GA et al., 2016) revealed the promising effect of newly synthesized heterosteroids (**4**, **7**, **18**, **22**, **21** and **24**) on apoptotic pathway and cell cycles. This study figured out the promising effects of these compounds is mediated through their effects on CCND1, Survivin, BCL-2, CDC2, P21 and P53 genes. Compounds **4**, **7**, **18**, **24** down-regulated CCND1, Survivin, BCL-2 and CDC2 significantly, while compounds **21**, **22** significantly up-regulated P21 and P53 genes **(Fig. 2).** Upon establishing these promising effects, we set out this study to investigate the drug resistance triggered by these new heterosteroids through studying their effects on miR-34a, miR-98 and miR-214 expression **(Fig. 2).**

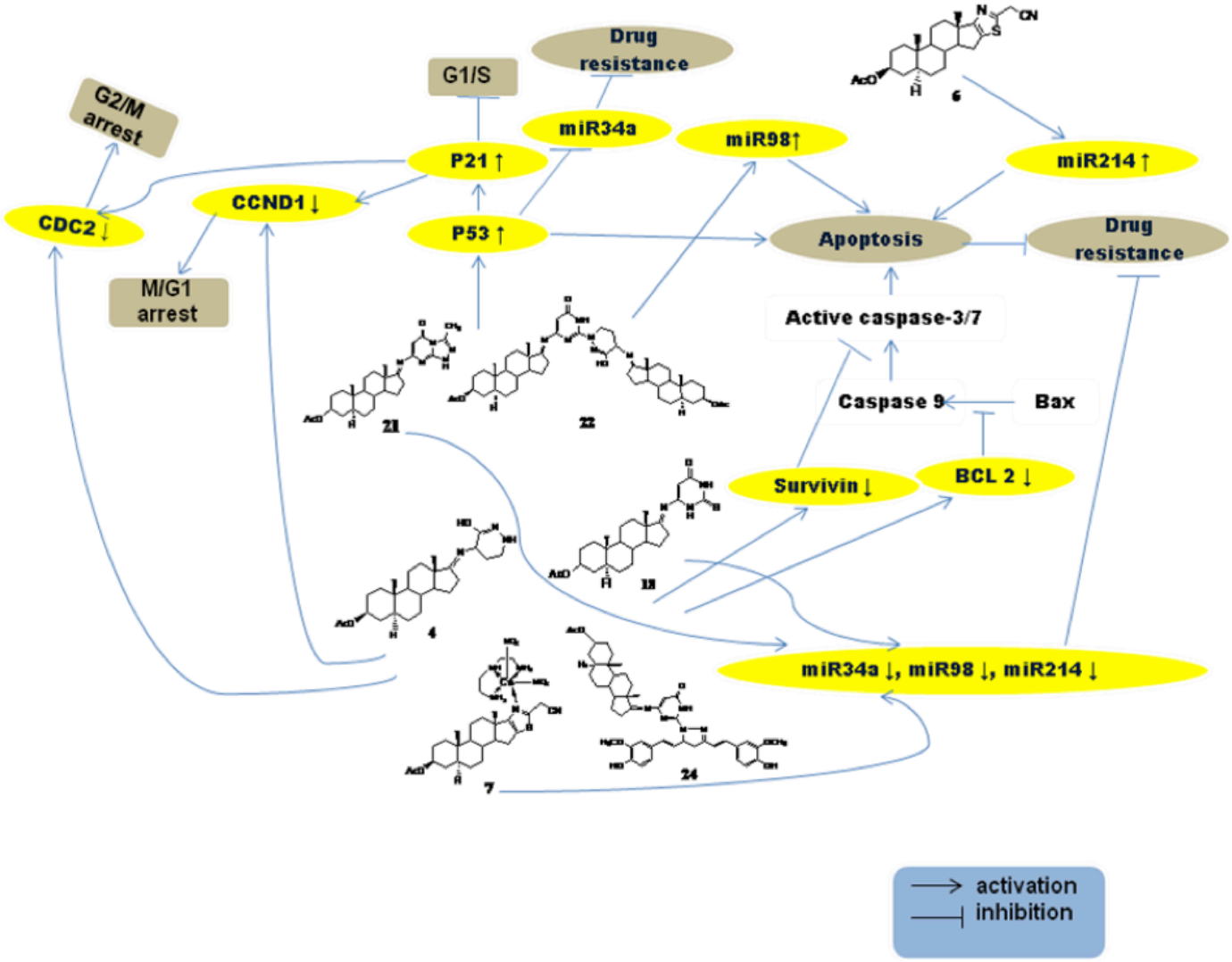


Fig (2): Molecular regulation of new heterosteroids on apoptotic genes and miRNAs. A part of this figure was quoted from Elmallah and Micheau , (2015).

Recently, miRNAs were figured out as a key regulator of drug resistance to different chemotherapeutic agents. From these miRNAs, miR-34a family, which gained great interest because its participation with a functional role in tumor suppressor pathway. This was due to it is directly down-regulated by P53 (Raver-Shapira N et al., 2007; Chang TC et al., 2007; He L et al., 2007; Bommer GT et al., 2007; Tarasov V et al., 2007; Corney DC et al., 2007). In addition to, miR-34a targets many mRNA, from which MET, the proto-oncogenic hepatocyte growth factor. Besides, BCL-2, SIRT1, CDK and CCND1 were also reported as targets for miR-34a (Fujita Y et al., 2008; Bommer GT et al., 2007; Yamakuchi M et al., 2008). Surprisingly, high levels of miR-34a were associated with increased resistance in MCF-7 breast cancer cells to docetaxel drug (Kastl L et al., 2012). In the current study, miR-34a expression level was insignificantly increased in tamoxifen treated MCF-7 cells as compared to control cells. Building on the previous work, this finding indicates increased resistance in MCF-7 against tamoxifen drug. However, cells treated with compounds 4, 6, 7, 13, 18, 21 and 24 showed a significant reduction in miR-34a expression levels **(Fig. 3).** Compound 4, 7, 18 and 24 reduced the expression levels of CCND1, Survivin, BCL-2 and CDC2 significantly. In previous study, both BCL-2 and CCND1 expression levels were reduced in MCF-7 docetaxel-resistant cells, parallel to miR-34a increase (Kastl L et al., 2012). Hence the decrease in miR34a expression levels in this study could be attributed to the reduction in drug resistance to the newly synthesized compounds. Regarding compound 21 which was previously established to up-regulates P53 and P21 expression levels (Elmegeed GA et al., 2016), it caused significant reduction in miR-34a expression levels. As mentioned previously, P53 has a down-regulating effect on miR-34a transcription ((Raver-Shapira N et al., 2007; Chang TC et al., 2007; He L et al., 2007; Bommer GT et al., 2007; Tarasov V et al., 2007; Corney DC et al., 2007; Hermeking H, 2007; He X et al., 2007). Consequently, we could attribute the decrease in miR-34a expression levels in MCF-7 treated cells with compound 21 to its up-regulationby P53 levels. Like miR-34a, both miR-98 and miR-214 have a positive role in inducing apoptosis and reduction of proliferation (Tang J et al., 2012; Feinberg AP et al., 2006). Similarly, like miR34a, tamoxifen treated cells showed increased expression levels of both miR98 and miR-214, however, this increase was

significant in this case **(Fig. 3-5).** On the contrary, most of our newly synthesized heterosteroids showed a significant reduction in expression levels of miR-98 and miR-214. miR-98 is one of let7 members. These members of let7 family target similar genes and have similar functions. They have a well established role as a tumor suppressor miRNAs (Xing Z et al., 2014). The expression levels of both miR-98 and miR-214 were reported to be reduced in cancerous cells (Siragam Vet al., 2012; Wang ET et al., 2008). MCF-7 treated with compounds 6, 7, 13, 18, 21 and 24 showed a significant decrease of miR98 expression levels. Nevertheless, this increase did not reach significance in cells treated with compound 13. On the contrary, cells treated with compound 22 showed a significant elevation in miR-98 expression levels as compared to control cells. This elevation could be a compensatory mechanism by which the cells promote apoptosis, due to the positive role played by miR-98 in promoting apoptosis and reducing cell proliferation (Tang J et al., 2012). Likewise, MCF-7 cells treated with compounds 4, 7, 13, 18, 21 and 22 showed significant reduction in miR-214 expression levels **(Fig. 4, 5).** However cells treated with compound 6 showed a significant elevation in miR-214 expression levels as compared to control cells. miR-214 has a positive role in promoting apoptosis (Feinberg AP et al., 2006). Building on this will established effect; we could consider the elevation in miR-214 expression levels as a cofactor in accelerating apoptosis. These results suggest that miR98 and miR-214 levels are reduced, similar to miR-34a, in MCF-7 treated with the new heterosteroids. Building on this finding, we could postulate that both miR-98 and miR-214 are similar to miR-34a, which was found to be up-regulated in drug resistant cells and decreased upon the reduction of drug resistance. Our results are in line with the study of Kastl et al. (2012) who stated that miR-34a expression was increased in MCF-7 docetaxel-resistant breast cancer cells and docetaxel response could be altered upon miR-34a modulation.

Conclusion

This study clarified that the newly synthesized heterosteroids have a lowered drug resistance response on MCF-7 as compared to tamoxifen. This effect was monitored through the reduced levels of miR-34a, miR-98 and miR-214 expression levels. These results provide evidence on the promising effects of these new heterosteroids as a potent anticancer drug against MCF-7 breast cancer cells.

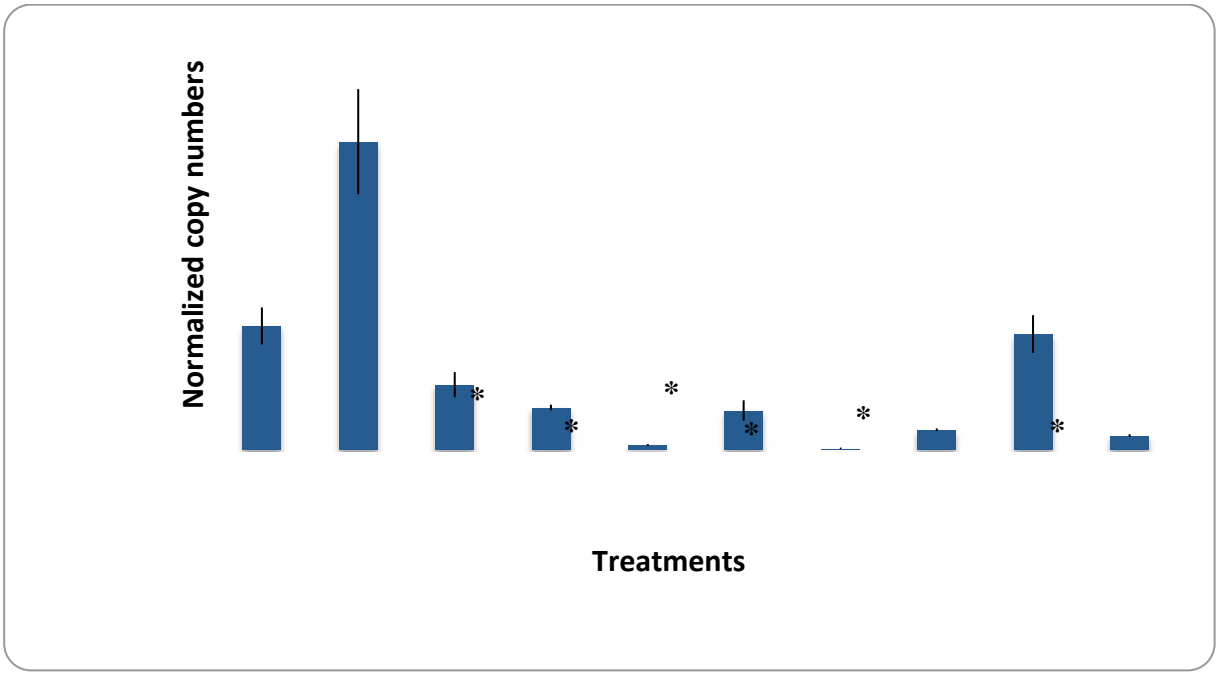


Fig (3): The effect of new heterosteroids on miR-34a expression in MCF-7 cells, * P< 0.05.

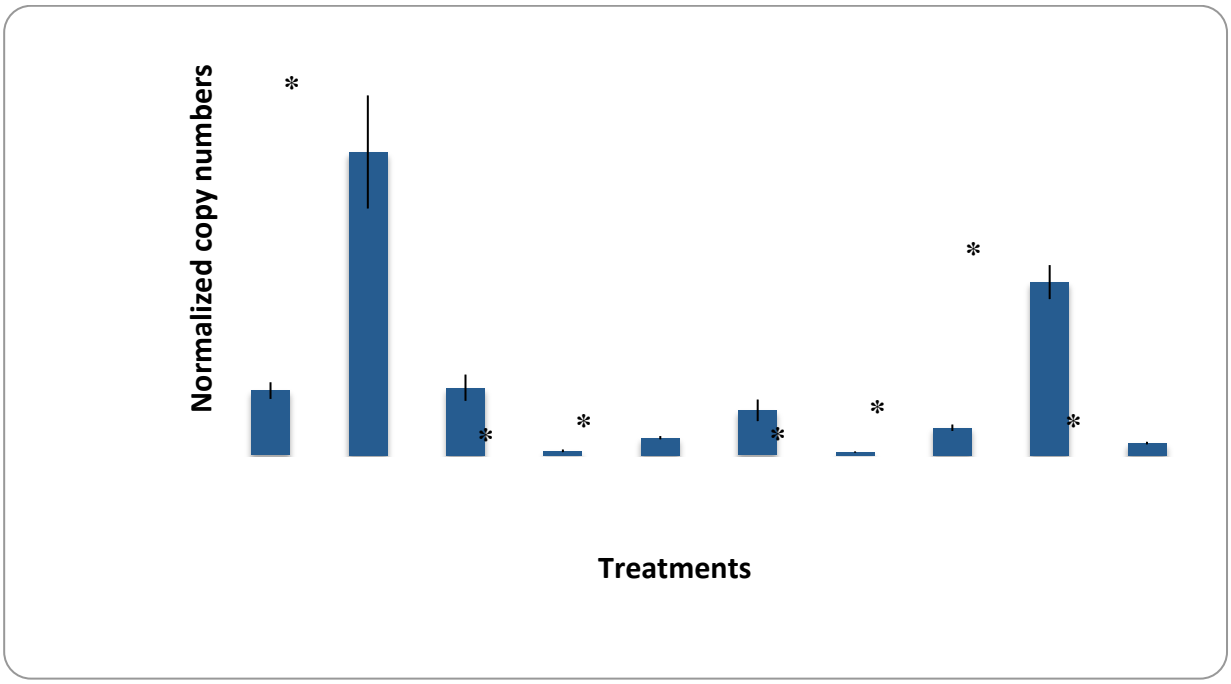


Fig (4): The effect of new heterosteroids on miR-98 expression in MCF-7 cells, * P< 0.05.

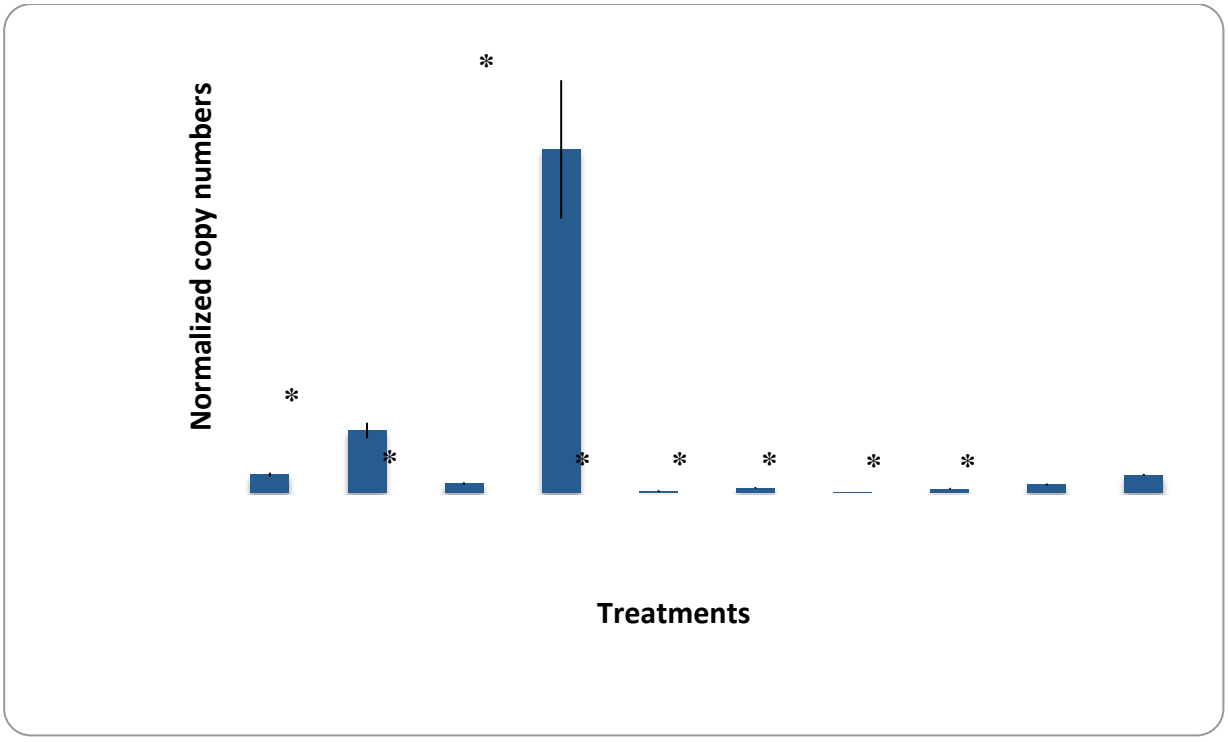
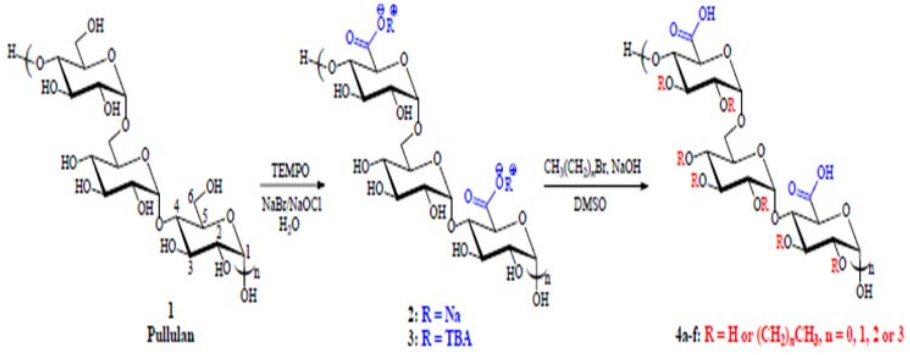


Fig (5): The effect of new heterosteroids on miR-214 expression in MCF-7 cells, * P< 0.05.



References

Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, Barbosa- Morais NL, Teschendorff AE, Green AR, Ellis IO, Tavaré S, Caldas C, Miska EA. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. **Genome Biol. 2007**; 8: R214.

Bommer GT, Gerin I, Feng Y, Kaczorowski AJ, Kuick R, Love RE, Zhai Y, Giordano TJ, Qin ZS, Moore BB, MacDougald OA, Cho KR, Fearon ER. p53- mediated activation of miRNA34 candidate tumor-suppressor genes. **Curr Biol. 2007**;17:1298-307.

Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16at 13q14 in chronic lymphocytic leukemia. **Proc Natl Acad Sci. (USA) 2002**; 99:15524-9.

Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, Feldmann G, Yamakuchi M, Ferlito M, Lowenstein CJ, Arking DE, Beer MA, Maitra A, Mendell JT. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. **Mol Cell. 2007**;26:745-52.

Cole KA, Attiyeh EF, Mosse YP, Laquaglia MJ, Diskin SJ, Brodeur GM, Maris JM. A functional screen identifies miR-34a as a candidate neuroblastoma tumor suppressor gene. **Mol Cancer Res. 2008**; 6: 735-42.

Corney DC, Flesken-Nikitin A, Godwin AK, Wang W, Nikitin AY. MicroRNA- 34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. **Cancer Res. 2007**; 67:8433-8.

Derfoul A, Juan AH, Difilippantonio MJ, Palanisamy N, Ried T, Sartorelli V. Decreased microRNA-214 levels in breast cancer cells coincides with increased cell proliferation, invasion and accumulation of the Polycomb Ezh2 methyltransferase. **Carcinogenesis. 2011**;32(11):1607-14.

Elmallah MI, Micheau O. Marine Drugs Regulating Apoptosis Induced by Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL). **Mar Drugs. 2015;**13(11):6884-909.

Elmegeed GA, Yahya SMM, Abd-Elhalim MM, Mohamed MS, Mohareb RN, Elsayed GH. Evaluation of heterocyclic steroids and curcumin derivatives as antibreast cancer agents: Studying the effect on apoptosis in MCF-7 breast cancer cells. **Steroids. 2016**; 115: 80–89.

Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. **Nat Rev Genet. 2006**;7:21-33.

Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. **Int J Cancer. 2010;**127:2893–917.

Fujita Y, Kojima K, Hamada N, Ohhashi R, Akao Y, Nozawa Y, Deguchi T, Ito M. Effects of miR-34a on cell growth and chemoresistance in prostate cancer PC3 cells. **Biochem Biophys Res Commun. 2008**;377:114-9.

GLOBOCAN database (2008) Available: http://globocan.iarc.fr. Accessed 03 October 2012. Brazilian National Cancer Institute – INCA (2012) **Available: http://www.inca.gov.br/estimativa/2012/index.asp?ID=2 Accessed 28 August 2012.**

He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA, Hannon GJ. A microRNA component of the p53 tumour suppressor network. **Nature. 2007**;447:1130-4.

Hermeking H. p53 enters the microRNA world. Cancer Cell. 2007;12:414-8. 3 He X, He L, Hannon GJ. The guardian's little helper: microRNAs in the p53 tumor suppressor network. **Cancer Res 2007**;67:11099-101.

Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. **Cell. 2005**;120:635-47.

Kastl L, Brown I, Schofield AC. miRNA-34a is associated with docetaxel resistance in human breast cancer cells. **Breast Cancer Res Treat. 2012**; 131:445- 54.

Kojima K, Fujita Y, Nozawa Y, Deguchi T, Ito M. MiR-34a attenuates paclitaxelresistance of hormone-refractory prostate cancer PC3 cells through direct and indirect mechanisms. **Prostate. 2010**;70:1501-12.

Kumar MS, Erkeland SJ, Pester RE, Chen CY, Ebert MS, Sharp PA, Jacks T. Suppression of non-small cell lung tumor development by the let-7 microRNA family. **Proc Natl Acad Sci. (USA) 2008**;105:3903-8.

Kutanzi KR, Yurchenko OV, Beland FA, Checkhun VF, Pogribny IP. MicroRNAmediated drug resistance in breast cancer. **Clin Epigenetics. 2011**;2(2):171-185.

Li N, Fu H, Tie Y, Hu Z, Kong W, Wu Y, Zheng X. miR-34a inhibits migration and invasion by down-regulation of c-Met expression in human hepatocellular carcinoma cells. **Cancer Lett. 2009**; 275: 44-53.

Mayr C Hemann MT, Bartel DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. **Science. 2007;**315:1576-9.

Mohamed NR, Abdelhalim MM, Khadrawy YA, Elmegeed GA, Abdel-Salam OME. One-pot three-component synthesis of novel heterocyclic steroids as a central antioxidant and anti-inflammatory agents. Steroids. 2012;77:1469–76.

Morris KV, Mattick JS. The rise of regulatory RNA. **Nat Rev Genet. 2014;**15:423- 37.

Nadiminty N, Tummala R, Lou W, Zhu Y, Shi XB, Zou JX, Chen H, Zhang J, Chen X, Luo J, deVere White RW, Kung HJ, Evans CP, Gao AC. MicroRNA let-7c is downregulated in prostate cancer and suppresses prostate cancer growth. **PLoS One. 2012**;7:e32832.

Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, Bentwich Z, Oren M. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. **Mol Cell. 2007**;26:731-43.

Siragam V, Rutnam ZJ, Yang W, Fang L, Luo L, Yang X, Li M, Deng Z, Qian J, Peng C, Yang BB. MicroRNA miR-98 inhibits tumor angiogenesis and invasion by targeting activin receptor-like kinase-4 and matrix metalloproteinase-11. **Oncotarget. 2012**;3(11):1370-85.

Speirs CK, Hwang M, Kim S, Li W, Chang S, Varki V, Mitchell L, Schleicher S, Lu B. Harnessing the cell death pathway for targeted cancer treatment. **Am J Cancer Res. 2011**;1(1):43-61.

Tang J, Ahmad A, Sarkar FH. The role of microRNAs in breast cancer migration, invasion and metastasis. **Int J Mol Sci. 2012**; 13:13414-37.

Tarasov V, Jung P, Verdoodt B, Lodygin D, Epanchintsev A, Menssen A, Meister G, Hermeking H. Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. **Cell Cycle. 2007**;6:1586-93.

Trafalis DTP, Geromichalos GD, Koukoulitsa C, Papageorgiou A, Karamanakos P, Camoutsis C. Lactandrate: a D-homo-aza-androsterone alkylator in the treatment of breast cancer. **Breast Cancer Res Treat. 2006**;97:17–31.

Tricoli JV, Jacobson JW. MicroRNA: Potential for Cancer Detection, Diagnosis, and Prognosis. **Cancer Res. 2007**;67:4553-5.

Volinia S, Calin G, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. **Proc Natl Acad Sci. (USA) 2006**;103:2257-61.

Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. **Nature. 2008**;456(7221):470-6.

Xing Z, Li D, Yang L, Xi Y, Su X. MicroRNAs and anticancer drugs. **Acta Biochim Biophys Sin. (Shanghai) 2014**;46(3):233-9.

Yamakuchi M, Ferlito M, Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis Proc. **Natl Acad Sci. (USA) 2008;**105:13421-6.