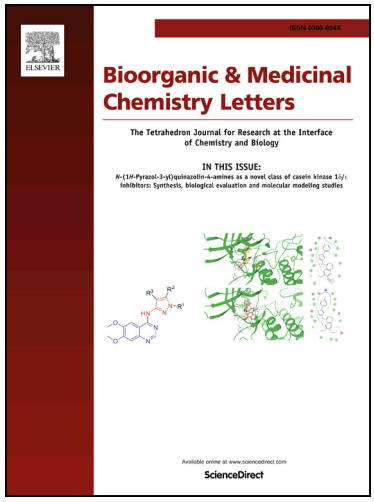


Synthesis and evaluation of modified chalcone based p53 stabilizing agents

Sunniya Iftikhar, Sardraz Khan, Aishah Bilal, Safia Manzoor, Muhammad Abdullah, Abdel-Hamid Emwas, Salim Sioud, Xin Gao, Ghayoor Abbas Chotana, Amir Faisal, Rahman Shah Zaib Saleem

PII: S0960-894X(17)30738-2
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.07.042
Reference: BMCL 25155

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 17 March 2017
Revised Date: 11 July 2017
Accepted Date: 14 July 2017

Please cite this article as: Iftikhar, S., Khan, S., Bilal, A., Manzoor, S., Abdullah, M., Emwas, A-H., Sioud, S., Gao, X., Chotana, G.A., Faisal, A., Saleem, R.S.Z., Synthesis and evaluation of modified chalcone based p53 stabilizing agents, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.07.042

Synthesis and evaluation of modified chalcone based p53 stabilizing agents

Sunniya Iftikhar,[a] Sardraz Khan,[a] Aishah Bilal,[b] Safia Manzoor,[a] Muhammad Abdullah,[a] Abdel-Hamid Emwas,[c] Salim Sioud,[c] Xin Gao,[c] Ghayoor Abbas Chotana,[a] Amir Faisal,[b]* Rahman Shah Zaib Saleem[a]*

[a] Department of Chemistry, SBASSE, Lahore University of Management Sciences, Lahore, Pakistan, 54792

[b] Department of Biology, SBASSE, Lahore University of Management Sciences, Lahore, Pakistan, 54792

[c] King Abdullah University of Science and Technology, Thawal, Saudi Arabia.

Abstract

Tumor suppressor protein p53 induces cell cycle arrest and apoptotic cell death in response to various cellular stresses thereby preventing cancer development. Activation and stabilization of p53 through small organic molecules is, therefore, an attractive approach for the treatment of cancers retaining wild-type p53. In this context, a series of nineteen chalcones with various substitution patterns of functional groups including chloro, fluoro, methoxy, nitro, benzyloxy, 4-methyl benzyloxy was prepared using Claisen-Schmidt condensation. The compounds were characterized using NMR, HRMS, IR and melting points. Evaluation of synthesized compounds against human colorectal (HCT116) and breast (Cal-51) cancer cell lines revealed potent antiproliferative activities. Nine compounds displayed GI_{50} values in the low micromolar to submicromolar range; for example (*E*)-1-phenyl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**SSE14108**) showed GI_{50} of 0.473 ± 0.043 µM against HCT116 cells. Further analysis of these compounds revealed that (*E*)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (**SSE14105**) and (*E*)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (**SSE14106**) caused rapid (4 and 8-hour post-treatment) accumulation of p53 in HCT116 cells similar to its induction by positive control, Nutlin-3. Such activities were absent in 3-(4-methoxyphenyl)propiophenone (**SSE14106H2**) demonstrating the importance of conjugated ketone for antiproliferative and p53 stabilizing activity of the chalcones. We further evaluated p53 levels in the presence of cycloheximide (CHX) and the results showed that the p53 stabilization was regulated at post-translational level through blockage of its degradation. These chalcones can, therefore, act as fragment leads for further structure optimization to obtain more potent p53 stabilizing agents with enhanced anti-proliferative activities.

Keywords: Chalcones, Antitumor, Drug discovery,

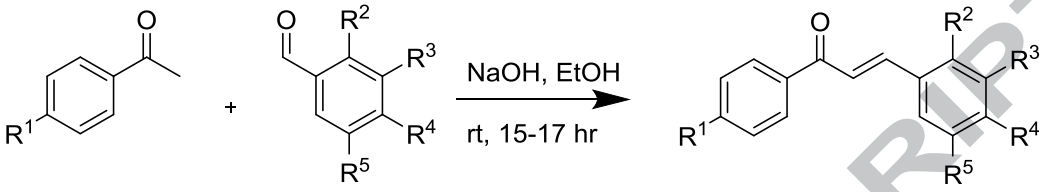
* Corresponding authors: Rahman Shah Zaib Saleem (Rahman.Saleem@lums.edu.pk) and Amir Faisal (Amir.Faisal@lums.edu.pk),

Cancer cells manipulate various key signaling pathways that allow them to continue dividing by overcoming tight controls that regulate cell death and proliferation in normal cells, and to acquire resistance towards chemotherapy.[1] *TP53* tumor suppressor gene encodes for a transcription factor that is responsible for maintaining genomic integrity.[2, 3] When activated due to cellular stress, p53 migrates from the cytoplasm into the nucleus where it binds to DNA and transcribes its target genes leading to cell cycle arrest or apoptosis.[4, 5] p53 has also been shown to induce apoptosis independent of its transcriptional activity by directly interacting with the members of Bcl-2 family.[6-8] Stabilization and activation of p53 can lead to p53 dependent cell cycle arrest, apoptosis or both. The strategy of activating p53–has been an attractive area of research with example of many compounds that stabilize p53 by inhibiting its binding to MDM2, an E3 ubiquitin ligase responsible for ubiquitination mediated degradation of p53.[9-13]

Chalcones, the open chain flavonoids, constitute an important class of naturally occurring and synthetic compounds.[14] Various chalcones have been reported with different biological activities[15, 16] including anti-infective[17], anti-inflammatory,[18] antifungal,[19] anti-bacterial,[20] antimalarial,[21] anti-oxidant[22] and anti-protozoal activities.[23] Modified chalcones have also been studied for potential anti-cancer properties in various cancers including ovarian cancer,[24] non-small cell lung carcinoma,[25] skin carcinogenesis, [26] prostate cancer,[27, 28] and pulmonary and mammary carcinogenesis.[15, 29, 30] Structure of some of the chalcones has been decorated with various functional groups and heterocyclic scaffolds to make them potent against various targets.[31-33] Chalcones have also been implicated in inhibition of MDM2-p53 interaction. Often however, these studies suggest that in addition to disruption of MDM2-p53 interaction, alternative mechanisms may also be responsible for the activities of many of these compounds. Our analysis of these reports reveals a pattern of three-atom long chain on either one of the phenyl rings in these compounds (shown in Figure S1 of supplementary information). In one instance, this chain carries a carboxylic acid terminus,[34] in another a boronic acid terminus[35] and in the third report, it is simply a propyl chain.[36] A couple of other publications have discussed the activity of chalcones with tri-substituted phenyl rings.[37, 38] Intrigued with these reports, we have been interested in finding out if the increase in carbon count on such chains would be favorable and can a simple chalcone with only one substituent cause p53 stabilization and act as a lead for future structure optimization. In this regard, we have prepared a library of 19 chalcones with various electron donating and electron with-drawing functional groups (for example, chloro, fluoro, methoxy, nitro, benzyloxy, and 4-methyl benzyloxy) on the two phenyl rings offering various levels of electron richness in the rings and lengths of the molecules. Eight of these compounds are either new or do not have any reported biological activity in Reaxys (Table S1 in the supplementary information), furthermore, this is the first report of the cytotoxicity of ten of the remaining eleven compounds against HCT116 and Cal-51 cells line or for p53 stabilization. The Claisen-Schmidt condensation routes used for the syntheses of chalcones are presented in scheme 1. Compounds **SSE14101**- **SSE14109** and **SSE14115** were prepared by reacting 4-substituted acetophenone with appropriate aldehyde in ethanol using 15 N ethanolic solution of sodium ethoxide (as shown in scheme 1a). For compounds **SSE14110**- **SSE14114** and **SSE14116**- **SSE14119**, 4-hydroxyacetophenone (1 eq) was first reacted with appropriate benzyl bromide (1.3 eq) in the presence of K_2CO_3 (1.3 mmol). The mixture was stirred for 2 hours at room temperature. The product was extracted using ethyl acetate and dried over magnesium sulfate. Removal of solvent yielded the desired ketone, which was then reacted with appropriate aldehyde (1 eq) in ethanol using 15 N ethanolic solution of sodium ethoxide (as shown in scheme 1b). The yields were in the range of 61-98% and the structures were established using ^{1}H NMR,

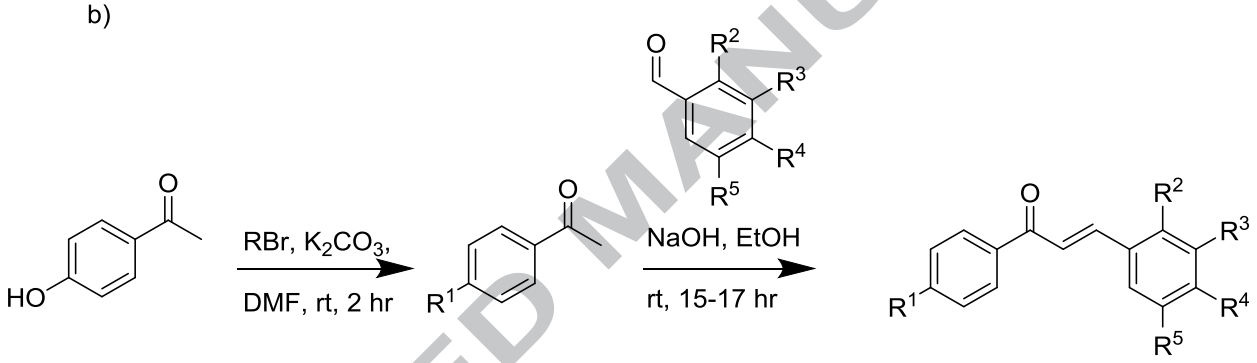
^{13}C NMR, IR and mass spectrometry. The values of coupling constants revealed formation of E-isomers.

a)



SSE14101= R^1=R^2=R^3=R^4=R^5=H 74% **SSE14102**= R^1=H, R^2=NO_2, R^3=H, R^4=H, R^5=H 81%
SSE14103= R^1=R^2=H, R^3=NO_2, R^4=R^5=H 89% **SSE14104**= R^1=R^2=R^3=H, R^4=NO_2, R^5=H 82%
SSE14105= R^1=R^2=R^3=H, R^4=Cl, R^5=H 96% **SSE14106**= R^1=R^2=R^3=H, R^4=OMe, R^5=H 88%
SSE14107= R^1=R^2=R^3=H, R^4=F, R^5=H 83% **SSE14108**= R^1=H, R^2=H, R^3=R^4=R^5=OMe 91%
SSE14109= R^1=OMe, R^2=R^3=R^4=R^5=H 61% **SSE14115**= R^1=R^2=R^3=H, R^4=OBn, R^5=H 85%

b)



SSE14110= R^1=OBn, R^2=R^3=R^4=OMe, R^5=H 67% **SSE14111**= R^1=OBn, R^2=R^3=H, R^4=Cl, R^5=H 68%
SSE14112= R^1=OBn, R^2=H, R^3=OMe, R^4=R^5=H 76% **SSE14113**= R^1=OBn, R^2=R^3=R^4=R^5=H 95%
SSE14114= R^1=OBn, R^2=R^3=H, R^4=F, R^5=H 92% **SSE14116**= R^1=OBn, R^2=Cl, R^3=R^4=R^5=H 98%
SSE14117= R^1=$OCH_2C_6H_4Me$, R^2=R^3=R^4=R^5=H 93% **SSE14118**= R^1=$OCH_2C_6H_4Me$, R^2=R^3=H, R^4=F, R^5=H 83% **SSE14119**= R^1=$OCH_2C_6H_4Me$, R^2=R^3=H, R^4=Cl, R^5=H 69%

Scheme 1: General procedure for the synthesis of compounds **SSE14101- SSE14119**

A 3 day Sulforhodamine B (SRB) proliferation assay was carried out to identify the compounds with significant antiproliferative activity against p53 competent HCT116 colon cancer cells. Cells were treated in a 96-well plate with two different concentrations, 25µM and 50µM, of each of the nineteen compounds; treatment with DMSO alone was used as a solvent control. The experiments were performed in duplicates and percentage inhibition compared to DMSO control was calculated as shown in figure 1 (and table S2 in supplementary information). The results revealed that nine compounds (**SSE14101-SSE14109**) with smaller substituents displayed excellent anti-proliferative activity. One the other hand, the compounds with longer benzyloxy or 4-methyl benzyloxy at R^1 or R^4 had lower or no activity at these concentrations suggesting that the linear extension of the structure in this dimension beyond three-atoms may be making the

molecule sterically challenged to fit inside the cavity of its target protein and adding additional cost of solvent interaction for the protruding aromatic ring.

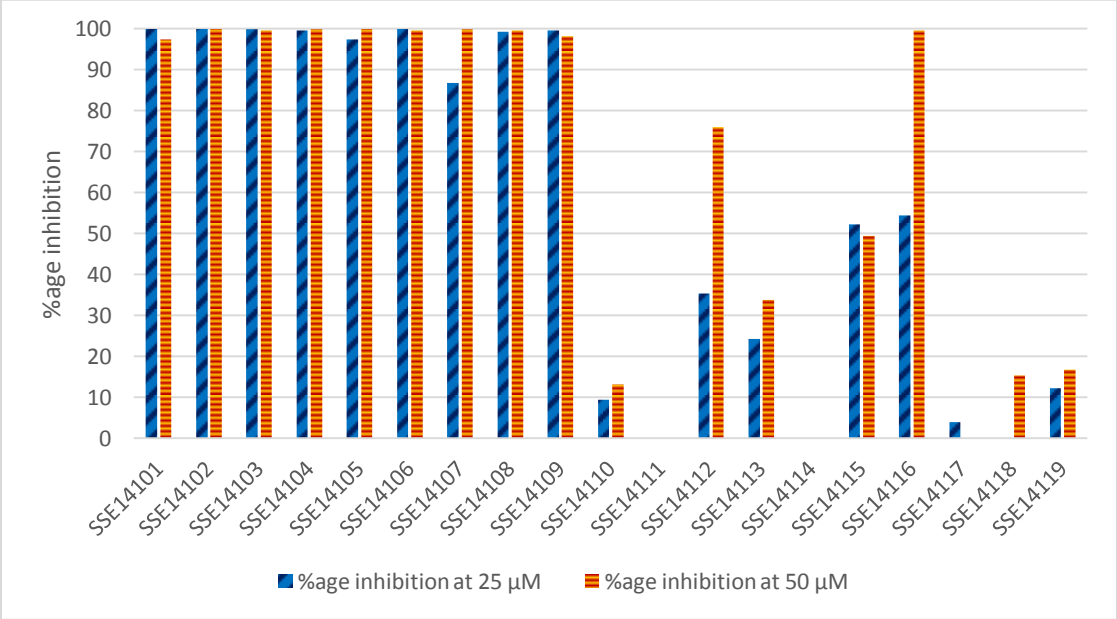


Figure 1: Antiproliferative activities of chalcone derivatives at 25 µM and 50 µM against HCT116 human colon cancer cell line

We next determined the GI_{50} values of these compounds in same 3-day SRB proliferation assay in order to rank these compounds according to their potency. Cells were treated in duplicates in 96-well plates with three-fold dilutions of the compounds starting from 50µM using DMSO as a solvent control and Nutlin-3 as a positive control. Compounds **SSE14101-SSE14109** had GI_{50} values in the range of 0.473-16.54 µM in HCT116 and 0.78-5.85 µM in Cal-51 cell lines (Figure 2 and table S3 in supplementary information). Nutlin-3, a positive control for p53 induction, had GI_{50} value of 1.52 µM in HCT116 cells, indicating that we had good hits in our compounds. The activities of compounds **SSE14110-SSE14119** ranged from 14.86 to >50 µM (Table S3 in the supplementary information). A closer look at the GI_{50} values suggested that the chalcones with electron-donating methoxy group displayed superior activities, for example **SSE14108** with three methoxy groups had GI_{50} values of 0.473 µM in HCT116 and 0.78 µM against Cal-51 cell lines while the **SSE14106** with one methoxy group had GI_{50} values of 3.86 µM in HCT116 and 2.69 µM in Cal-51 cell lines. On the other hand, compounds with electron-withdrawing groups had GI_{50} values on the higher side. Since the chalcones are smaller in size with potential to bind to various cellular targets, these biological activities could be a result of their cumulative effect. In order to identify the mechanism of action of these chalcones, we evaluated their ability to stabilize p53.

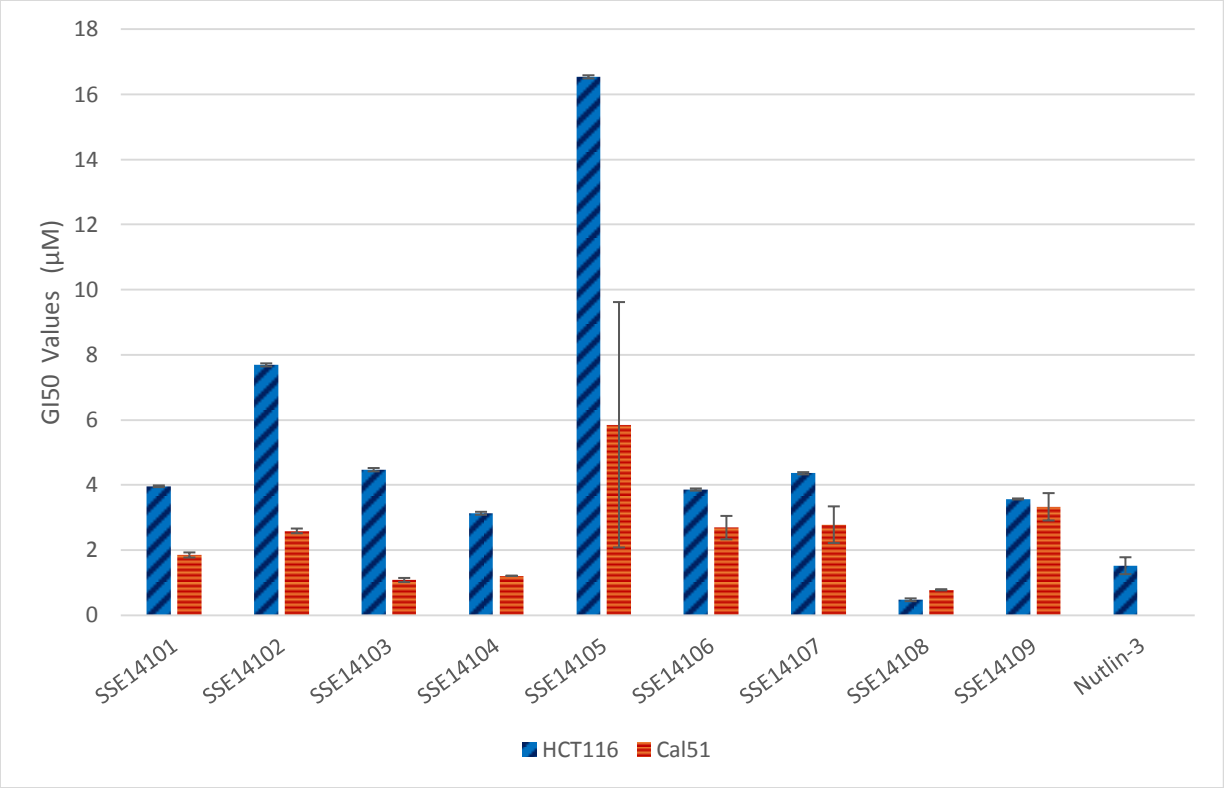
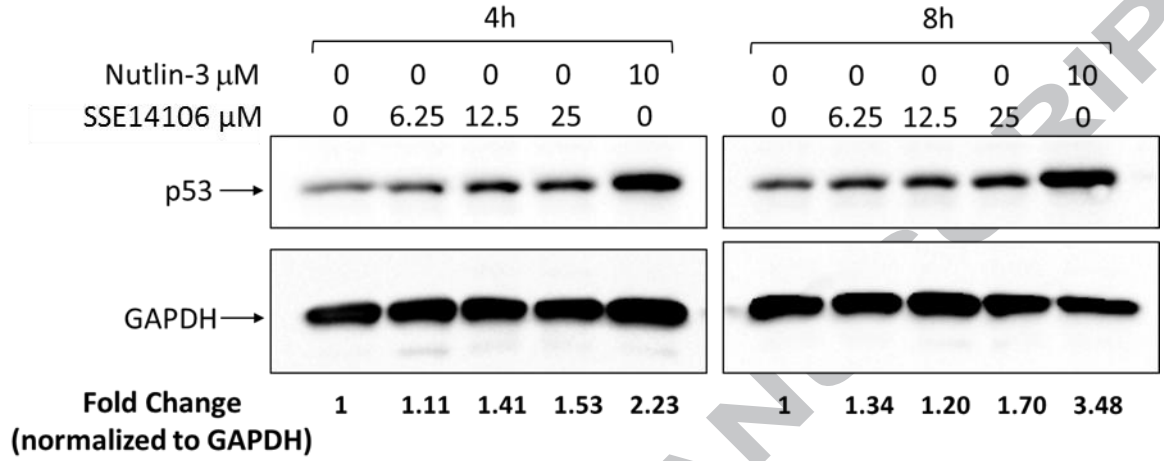


Figure 2: GI_{50} values of chalcone derivatives in a 3 day SRB proliferation assay in HCT116 and Cal-51 cells.

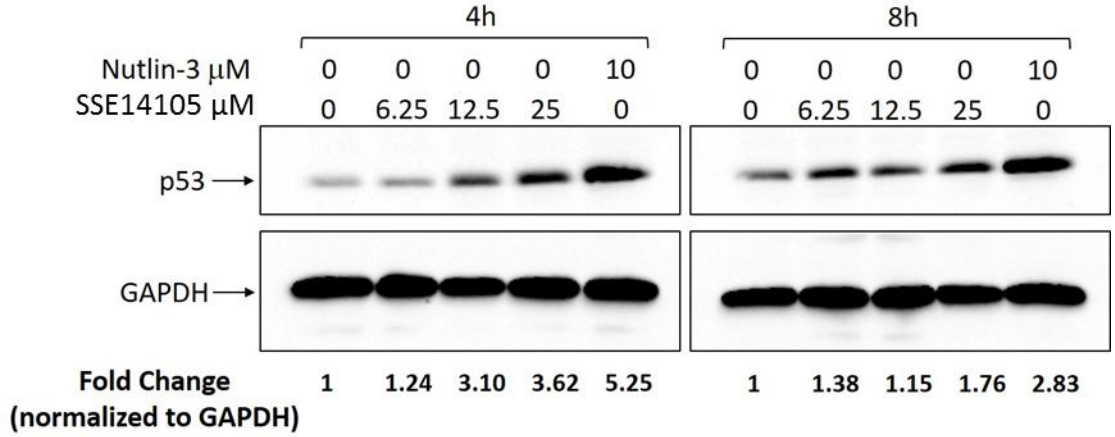
In the next phase, all the nineteen chalcones were investigated for their ability to stabilize and accumulate p53 in HCT116 cells. Cells were treated with two concentrations (25 µM and 50 µM) of the compounds for 8 hours and analyzed for p53 levels by immunoblotting. Nutlin-3 (a known inhibitor of p53-MDM2 interaction)[39] was used as a positive control whereas DMSO was used as a solvent control. Treatment of cells with two of these compounds, **SSE14105** and **SSE14016** for 8 hours, resulted inp53 accumulation. We then analyzed these two compounds for rapid p53 accumulation at different concentrations (6.25 µM, 12.5 µM and 25 µM) following 4 and 8 hour treatment of cells. Both compounds increased p53 expression at 4 and 8 hours in a dose dependent manner compared to the DMSO control (Figure 3 A and B). The levels of house-keeping protein Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as loading control. Such rapid accumulation at 4 and 8 hours could possibly be a result of inhibition of p53 degradation pathways. Presence of more electron withdrawing fluoro group at this position, a nitro-group at any position or presence of 3,4,5-trimethoxy substitution led to no p53 accumulation suggesting electronic and steric limitations of substituents. The antiproliferative activity of some of these compounds without stabilization of p53 suggests alternative targets responsible for their effect on proliferation. In order to determine the importance of double bond in the structure of chalcone,we evaluated compound **SSE14106H2** (Figure 3C) for p53 stabilization . As shown in Figure 3D, the p53 stabilizing activity was completely lost when the

double bond was removed. The compound **SSE14106H2**, with no double bond at 2-position had a GI_{50} value of >100 µM (n=2) and was unable to induce p53 stabilization following 4h and 8h treatment in HCT116 cells. This shows that the presence of conjugated ketone structure is vital for the activity of the chalcone.

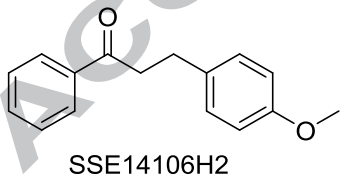
A



B



C



D

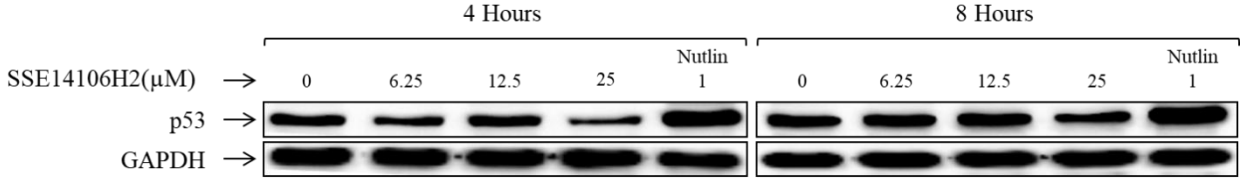


Figure 3: Compounds **SSE14105** and **SSE14106** induce p53 levels in HCT116 cells. Cells were exposed to various concentrations of **SSE14106** (A) or **SSE14105** (B) for 4 and 8 hours. Samples were analyzed by immunoblotting using p53 and GAPDH antibodies. Treatment with 10μM Nutlin-3 was used as positive control, while treatment with DMSO alone was used as a solvent control. Blots were quantified using Image J and levels of p53 were normalized with GAPDH. Fold change compared to DMSO control is shown at the bottom of A and B (C) Structure of **SSE14106H2** (D) Compound **SSE14106H2** is unable to induce p53 levels in HCT116 cells. Cells were exposed to various concentrations of **SSE14106H2** for 4 and 8 hours. Samples were analysed by immunoblotting using p53 and GAPDH antibodies. Treatment with 10μM Nutlin-3 was used as positive control, while treatment with DMSO alone was used as a solvent control.

The accumulation of p53 could be a result of transcriptional activation or inhibition of its degradation pathways. In order to determine whether p53 induction in response to these compounds was regulated at the transcriptional or post-translational level, we evaluated their effect on the p53 levels in the presence or absence of cycloheximide (CHX), which is an inhibitor of protein translation. As shown in figure 4A, treatment with 12.5μM **SSE14106** resulted in increased stability of p53 compared to the DMSO control in the presence of cycloheximide. Quantification of the blots in Figure 4B shows that the relative p53 levels in **SSE14106** treated cells remain stable for longer compared to the DMSO control in the presence of cycloheximide. This clearly demonstrates that these compounds upregulate p53 levels by increasing its stability independent of transcriptional or translational activation. This also point to them being inhibitors of a cellular pathway responsible for negative regulation and subsequent degradation of p53.

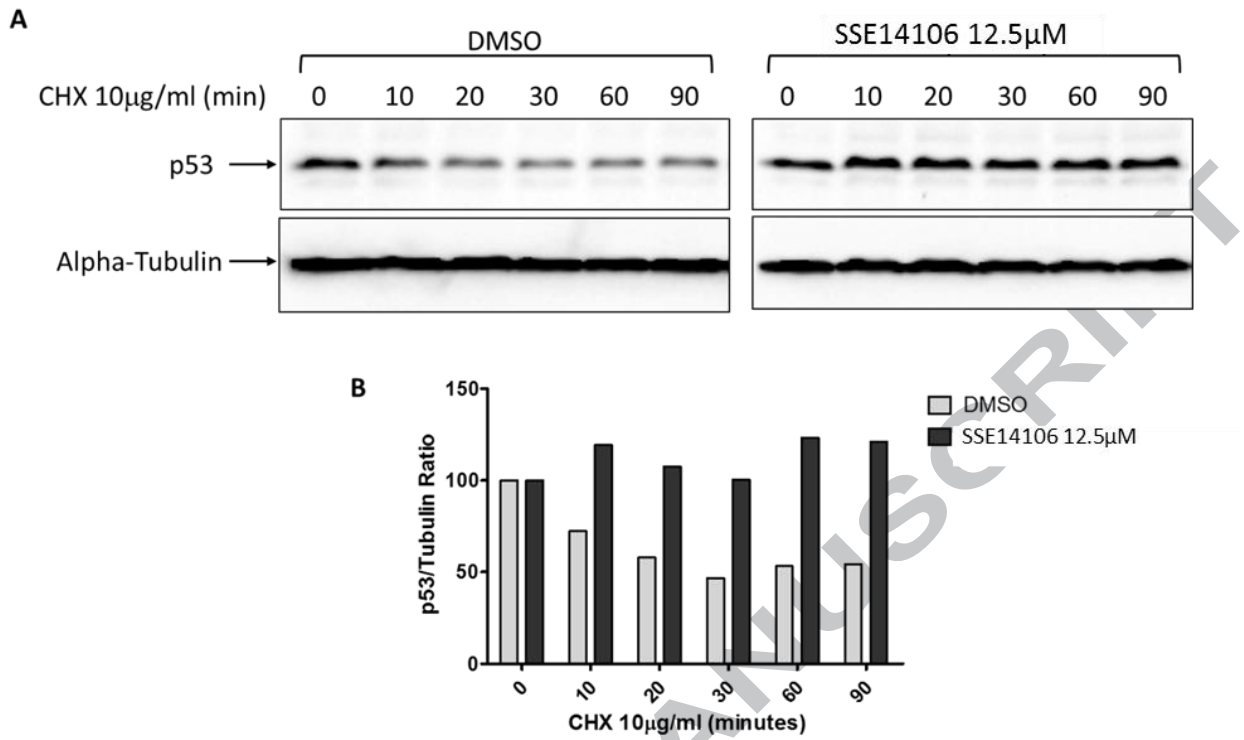


Figure 4: Compound **SSE14106** stabilized p53 through post-translational mechanism. (A) HCT116 cells were treated with cycloheximide in the presence or absence of 12.5μM **SSE14106** and samples were collected at times indicated. Samples were analyzed by immunoblotting using p53 and alpha-tubulin antibodies. (B) Blots were quantified and p53 levels normalized to alpha-tubulin.

Overall this study shows that simple chalcone derivatives exhibit significant antiproliferative activities accompanied by rapid p53 induction in both colon and breast cancer cell lines. The presence of an electron donating group was common in both these compounds. Presence of more electron with-drawing fluoro group at this position, a nitro-group at any position or presence of 3,4,5-trimethoxy substitution leads to no p53 accumulation suggesting electronic and steric limitations of substituents on this ring. Similarly, presence of a bigger substituent at positions R^1 or R^4 (compound **SSE14110- SSE14119**) or lack of conjugation (**SSE14106H2**) also leads to significant decrease or complete loss of the activity. This decrease in the activity may be a consequence of the elongation of the molecule making it sterically challenging to fit inside the cavity of its target as the protruding aromatic ring would have to pay the cost of solvent interaction making it unfit to bind or lack of structural rigidity in case of last. It has been demonstrated that boronic acid derivatives and carboxylic acid derivatives can stabilize p53. In the current work, we show that chalocnes with simpler substituents like chloro (**SSE14105**) and methoxy (**SSE14106**) can impart p53 stabilizing features to the chalcones with better or comparable GI_{50} values. Chalcones are small in size and can probably fit into the binding cavities of various cellular targets, so their measured activity could be a result of a combination of

different interactions. The fact that these compounds are small in size (mol. wt. of **SSE14106**: 238) and stabilize p53 make them good leads for fragment based approach to develop novel drug candidates through further structure optimization.

In summary, we have presented the synthesis of a series of chalcones and have evaluated them for their biological activities. The *in vitro* effects on HCT116 colon cancer and Cal-51 breast cancer cell lines led to identification of nine compounds with very good GI_{50} values in the low micromolar to submicromolar range for example (*E*)-1-phenyl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**SSE14108**) showed GI_{50} of 0.473 ± 0.043 µM against HCT116. We have demonstrated the antiproliferative effects of (*E*)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (**SSE14105**) and (*E*)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (**SSE14106**) and their ability to accumulate p53. Evaluation of p53 accumulation by these compounds in the presence of cycloheximide (CHX) rules out any transcriptional or translational activation of p53. The chalcones synthesized in this report suggest that extension of structure to cause linear expansion of the molecule in the form of extra-phenyl ring deteriorates their activity and the simpler compounds with one substituent (in our study chloro or methoxy) could serve as leads for fragment based discovery projects to develop novel and potent p53 stabilizing agents.

Acknowledgement

Authors acknowledge the support by LUMS in the form of faculty initiative fund to enable this research work and extend our acknowledgement to KAUST for NMR and HRMS analysis.

Reference

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144(5): 646-674.
2. Teodoro JG, Evans SK, Green MR. Inhibition of tumor angiogenesis by p53: a new role for the guardian of the genome. *J Mol Med (Berl).* 2007;85(11): 1175-1186.
3. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell.* 1997;88(3): 323-331.
4. Chène P, Fuchs J, Bohn J, García-Echeverría C, Furet P, Fabbro D. A small synthetic peptide, which inhibits the p53-hdm2 interaction, stimulates the p53 pathway in tumour cell lines. *J Mol Bio.* 2000;299(1): 245-253.
5. Yee KS, Vousden KH. Complicating the complexity of p53. *Carcinogenesis.* 2005;26(8): 1317-1322.
6. Mihara M, Erster S, Zaika A, et al. p53 has a direct apoptogenic role at the mitochondria. *Mol Cell.* 2003;11(3): 577-590.
7. Chipuk JE, Kuwana T, Bouchier-Hayes L, et al. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science.* 2004;303(5660): 1010-1014.
8. Leu JI, Dumont P, Hafey M, Murphy ME, George DL. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat Cell Biol.* 2004;6(5): 443-450.
9. Vassilev LT. MDM2 inhibitors for cancer therapy. *Trends Mol Med.* 2007;13(1): 23-31.
10. Zhao Y, Aguilar A, Bernard D, Wang S. Small-molecule inhibitors of the MDM2-p53 protein-protein interaction (MDM2 Inhibitors) in clinical trials for cancer treatment. *J Med Chem.* 2015;58(3): 1038-1052.
11. Ted R, David P. Strategies for manipulating the p53 pathway in the treatment of human cancer. *Biochem J.* 2000;352: 1-17.

12. Bullock AN, Fersht AR. Rescuing the function of mutant p53. *Nat Rev Cancer.* 2001;1(1): 68-76.
13. McVean M, Xiao H, Isobe K, Pelling JC. Increase in wild-type p53 stability and transactivational activity by the chemopreventive agent apigenin in keratinocytes. *Carcinogenesis.* 2000;21(4): 633-639.
14. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci.* 1999;65(4): 337-353.
15. Singh P, Anand A, Kumar V. Recent developments in biological activities of chalcones: a mini review. *Eur J Med Chem.* 2014;85: 758-777.
16. K Sahu N, S Balbhadra S, Choudhary J, V Kohli D. Exploring pharmacological significance of chalcone scaffold: a review. *Current medicinal chemistry.* 2012;19(2): 209-225.
17. Gupta D, Jain D, Trivedi P. Recent advances in chalcones as antiinfective agents. *Int J Chem Sci.* 2010;8(1): 649-654.
18. Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. *Eur J Med Chem.* 2007;42(2): 125-137.
19. Lahtchev K, Batovska D, St P P, Ubiyvovk V, Sibirny A. Antifungal activity of chalcones: a mechanistic study using various yeast strains. *Eur J Med Chem.* 2008;43(10): 2220-2228.
20. Sivakumar PM, Ganesan S, Veluchamy P, Doble M. Novel chalcones and 1,3,5-triphenyl-2-pyrazoline derivatives as antibacterial agents. *Chem Biol Drug Des.* 2010;76(5): 407-411.
21. Dominguez JN, Leon C, Rodrigues J, Gamboa de Dominguez N, Gut J, Rosenthal PJ. Synthesis and evaluation of new antimalarial phenylurenyl chalcone derivatives. *J Med Chem.* 2005;48(10): 3654-3658.
22. Anto RJ, Sukumaran K, Kuttan G, Rao MN, Subbaraju V, Kuttan R. Anticancer and antioxidant activity of synthetic chalcones and related compounds. *Cancer Lett.* 1995;97(1): 33-37.
23. Salem MM, Werbovetz KA. Antiprotozoal compounds from Psorothamnus polydenius. *J Nat Prod.* 2005;68(1): 108-111.
24. De Vincenzo R, Scambia G, Benedetti Panici P, et al. Effect of synthetic and naturally occurring chalcones on ovarian cancer cell growth: structure-activity relationships. *Anticancer Drug Des.* 1995;10(6): 481-490.
25. Hsu YL, Kuo PL, Chiang LC, Lin CC. Isoliquiritigenin inhibits the proliferation and induces the apoptosis of human non-small cell lung cancer a549 cells. *Clin Exp Pharmacol Physiol.* 2004;31(7): 414-418.
26. Satomi Y. Inhibitory effects of 3'-methyl-3-hydroxy-chalcone on proliferation of human malignant tumor cells and on skin carcinogenesis. *Int J Cancer.* 1993;55(3): 506-514.
27. Szliszka E, Czuba ZP, Mazur B, Paradysz A, Krol W. Chalcones and dihydrochalcones augment TRAIL-mediated apoptosis in prostate cancer cells. *Molecules.* 2010;15(8): 5336-5353.
28. Tang Y, Li X, Liu Z, Simoneau AR, Xie J, Zi X. Flavokawain B, a kava chalcone, induces apoptosis via up-regulation of death-receptor 5 and Bim expression in androgen receptor negative, hormonal refractory prostate cancer cell lines and reduces tumor growth. *Int J Cancer.* 2010;127(8): 1758-1768.
29. Shibata S. Anti-tumorigenic chalcones. *Stem Cells.* 1994;12(1): 44-52.
30. Wattenberg LW, Coccia JB, Galbraith AR. Inhibition of carcinogen-induced pulmonary and mammary carcinogenesis by chalcone administered subsequent to carcinogen exposure. *Cancer Lett.* 1994;83(1-2): 165-169.
31. Das M, Manna K. Chalcone Scaffold in Anticancer Armamentarium: A Molecular Insight. *J Toxicol.* 2016;2016: 7651047.
32. Mahapatra DK, Bharti SK, Asati V. Chalcone scaffolds as anti-infective agents: structural and molecular target perspectives. *Eur J Med Chem.* 2015;101: 496-524.
33. Mahapatra DK, Bharti SK, Asati V. Anti-cancer chalcones: Structural and molecular target perspectives. *Eur J Med Chem.* 2015;98: 69-114.
34. Stoll R, Renner C, Hansen S, et al. Chalcone Derivatives Antagonize Interactions between the Human Oncoprotein MDM2 and p53†. *Biochemistry.* 2001;40(2): 336-344.

35. Achanta G, Modzelewska A, Feng L, Khan SR, Huang P. A boronic-chalcone derivative exhibits potent anticancer activity through inhibition of the proteasome. *Mol Pharmacol.* 2006;70(1): 426-433.
36. Pereira D, Lima RT, Palmeira A, et al. Design and synthesis of new inhibitors of p53–MDM2 interaction with a chalcone scaffold. *Arabian J Chem.* 2016.
37. Silva G, Marins M, Fachin AL, Lee SH, Baek SJ. Anti-cancer activity of trans-chalcone in osteosarcoma: Involvement of Sp1 and p53. *Mol Carcinog.* 2016;55(10): 1438-1448.
38. Zhang Y, Srinivasan B, Xing C, Lu J. A new chalcone derivative (E)-3-(4-methoxyphenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one suppresses prostate cancer involving p53-mediated cell cycle arrests and apoptosis. *Anticancer Res.* 2012;32(9): 3689-3698.
39. Vassilev LT, Vu BT, Graves B, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science.* 2004;303(5659): 844-848.

Synthesis and evaluation of modified chalcone based p53 stabilizing agents

Sunniya Iftikhar,[a] Sardraz Khan,[a] Aishah Bilal,[b] Safia Manzoor,[a] Muhammad Abdullah,[a] Abdel-Hamid Emwas,[c] Salim Sioud,[c] Xin Gao,[c] Ghayoor Abbas Chotana,[a] Amir Faisal,[b]* Rahman Shah Zaib Saleem[a]*

[a] Department of Chemistry, SBASSE, Lahore University of Management Sciences, Lahore, Pakistan, 54792

[b] Department of Biology, SBASSE, Lahore University of Management Sciences, Lahore, Pakistan, 54792

[c] King Abdullah University of Science and Technology, Thawal, Saudi Arabia.

Graphical Abstract: