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A Study Based on In Silico and Optimization Characterization and In Vitro Studies of Novel Molecules

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Abstract- In current work designed and docked the novel berberine and berberine hydrochloride also after suggestive root mean square value and good scoring functioned, we did its optimization like nuclear magnetic resonance study. In which we find the overlapping result with standard molecule. We also did the physiochemical parameters and we find the good in range results when compared with standard results. We also go for the cell cytotoxicity study and very better results we find with the molecules the results were very suggestive and optimum.

Keywords: *Docked, Berberine, Resonance, Cytotoxicity, Physiochemical*

1. Introduction

Apoptosis is a highly regulated mechanism by which cells undergo cell death in an active way. As one of the most challenging tasks concerning cancer is to induce apoptosis in malignant cells, researchers increasingly focus on natural products to modulate apoptotic signaling pathways. Several cellular pathways cumulate in the activation of apoptosis. Overly simplified, the two main apoptosis pathways are the extrinsic and the intrinsic pathway. A third apoptotic pathway, the "endoplasmic reticulum (ER) stress" pathway has recently been described [1]. Different compounds induce apoptosis in cancer cells via different pathways as for example diallyl sulphide through intrinsic pathway, longilactone through extrinsic pathway and tocotrienols through ER stress pathway [2]. Excitingly, one of the natural compounds curcumins obtained from Curcuma longa is known to induce apoptosis via all the three pathways (Reuter et al., 2008). However, aberrations in p53 regulation

and/or defective signaling in the p53 pathway in many cases results in inadequate tumor suppression. One important cause of p53 function deficiency involves the murine double minute-2 gene product (MDM2, or HDM2 for the human congener), the negative regulator of the activity and stability of p53 [3] The MDM2 genes are found to be upregulated abnormally in many human tumors [4] (about 13% of human esophageal carcinomas, 16% of osteogenic sarcomas, and 20% of soft tissue sarcomas) by amplified.

2. Materials

Docking using a search algorithm; d) Analysis of the binding conformation using a scoring function.

Subsequently, after preparing the receptor. pdbqt file and ligand .pdbqt file as in earlier the grid parameters file (.gpf) and docking parameters file (.dpf) were prepared. Based on previously reported structural information, grid across the active-site regions for the comparative ADT simulations of the compounds under study with the respective apoptotic markers were constructed. The grid was sketched as such that the ligand was allowed to rotate freely inside the grid. The genetic algorithm parameters in ADT were set to default values, by which the program itself determines the optimal run parameters depending on the nature of the ligand and the receptor active site. “Number of genetic algorithms runs”, “Crossover frequency”, and “Mutation rates” parameters were thus automatically adjusted by the ADT. The experiments were repeated three times with 20 generations in each run to improve the precision level of result. The docking results generated the glg and .dlg files of which the .dlg file was loaded along with the receptor file and was analyzed for the receptor-ligand interactions. The results were clustered using a root mean square deviation (RMSD) cutoff value and the best scoring conformation in each cluster was selected. The generated docked structures were furthermore minimized in the end. The interactions were studied in terms of binding energy (kcal/mol) and inhibition constant (μM). The figures of the best docked solutions of all the compounds with apoptotic markers were generated using the Accelrys Discovery Studio Visualizer.

Melting point determination

Thin- walled capillary melting point tubes were used to hold melting point samples. This tube needs to be sealed at one end by using the bunsen flame to pack the tube, the open end was pressed gently into a small amount of the sample of the crystalline materials. To transfer the crystals from the open end to the bottom of the tube [5-6]. A densely pack of coloumn of crystals about 3mm high in mercury thermometer was shown to the left. Melting point of drugs was determined by capillary tube method in melting point determination apparatus.

Solubility studies

The solubility is the maximum quantity of a solute that can be dissolved in certain quantity of a solute that can be dissolved in certain quantity of solution at a specified temperature. Drugs (10mg) was dissolved in a 10ml of different solvents (i.e., chloroform.0.1N NaOH. Ethanol, ether, 1-2 dichloromethane, water, PBS (7.4)) and phosphate buffer (6.0) at room temperature and kept for 3 days for equilibrium in separating funnel. Funnel was regularly shaken. Equilibrium solubility was determined by taking supernatant and analyzing it on the U.V. spectrophotometer (Thermoscientific Evolution 201) [7].

Characterization of synthesized material through NMR

NMR samples, 2-5 mg of the compounds (berberine and berberine chloride) were dissolved in both 500 pL DMSO and 90% H_2O/10% D_2O solution. NMR spectra were acquired at 298 K with a Bruker DRX 500 spectrometer equipped with triple axis gradients. Data were collected with a 7002Hz spectral width, 2048 complex points in t2 and 128 increments in t1 domain. Two-dimensional double quantumfiltered (DQF)

COSY,19,20 rotating frame nuclear Overhauser enhancement spectroscopy and 13C-1H-heteronuclear multiple-bond-correlated (HMBC) experiments were performed. The two-dimensional ROESY with mixing times of 150 ms and DQF-COSY experiments were served to obtain inter-proton distance and dihedral angle constraints for structural information.

Partition Coefficient

The partition coefficient is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium. Behavior of drug was examined in n-octanol: PBS (7.4) system. It was determined by shaking 10 mg drug sample (Drugs) in 2 seperating funnels. One containing 10 ml of n-octanol and 10ml water and other containing, 10ml portion of n-octanol and 10ml of PBS (7.4 pH) respectively. The separating funnels were shaken for 2hrs using wrist action shaker equilibration. Two phases were separated and the amount of drug in aqueous phases was analyzed spectrophotometrically at 300nm after appropriate dilution [8]. The PC of the drug was calculated by using the following formula and result.

PC (K) = Amount of drug in organic layer/ Amount of drug in aqueous layer

UV Visible Spectroscopy (UV Method)

0.1N sodium hydroxide is prepared by according to the method given in I.P. (1996). Drugs (100mg) was taken and dissolved in 100 ml of 0.1N sodium hydroxide to make the standard stock solution of 1000 µg/ml. 10 ml of standard stock solution taken and further diluted with 10ml of 0.1N sodium hydroxide in 100ml volumetric flasks. Various aliquots of different concentration ranging from 2 to 20µg/ml were prepared by transferring 0.2, 0.4, 0.6, 0.8, 1.0… up to 2.0ml volume of stock solution (100µg/ml) in 10ml volumetric flask and make up the

volume up to 10ml with 0.1N sodium hydroxide [9]. The samples were analyzed by UV Visible Spectrometry (Thermo scientific). The UV Visible Spectrometry determination was performed and results.

***In-vitro* cell cytotoxicity study**

Sh-sy-5y cell were cultured in RPMI 1640 medium at a cell density of 4×10^4 per well. The cells were supplemented with a medium containing 10% FBS and 1% penstrep, and maintained at 37°C in a humidified 5% CO_2/95% air atmosphere [10]. After 24 h and 48 h of incubation of untreated cells, the best fit molecule was treated at 37°C, a final dilution of 1/10 per cell volume of alamar Blue reagent was added to the treated cells, followed by incubation for 4 h prior to analysis**.**

3. Result and Discussion

Molecular docking

The docked complexes obtained in the process further revealed the aglycones docked in the same binding pocket as used by the inhibitor in the original PDB ID: 3LKB, surrounded by Gln72, Val92, Tyr47, Met62, Gly57 and Ile61 key amino acid residues as used by the nutlins. Relative binding affinities of aglycones and nutlins

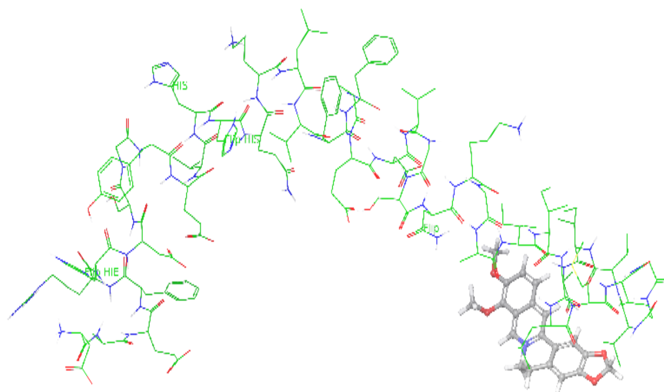

Fig 1. Docked result with susceptible receptor

Melting point

The melting point was determined by the capillary fused method. The observed melting point was matched with literature, which confirms that the drug used in the present study was in its pure form.

Table 1 Results of melting point study

Method applied	Observed	Reported
Capillary fusion method	294 °F	292 to 295 °F

Quantitative and qualitative estimation of solubility of Berberine and berberine chloride

Solubility of drug was determined results were presented in Table 2 having better solubility in phosphate buffer (pH 6.8) and it does not have good solubility in distilled water and 0.1N HCl (pH 1.2).

Table 2. Results of solubility study of drugs

Solvent	Solubility (mg/ml) Berberine	Berberine chloride
Distilled water	0.187	0.210
0.1N HCl (pH 1.2)	0.283	0.339
Phosphate buffer pH 6.8	0.841	1.080

NMR of Berberine and berberine chloride

The proton signals for berberine (Fig. 2) were assigned based on a report published by Tripathi et al. Figure 3 demonstrates the 1HNMR spectra of standard berberine hydrochloride the chemical shifts of both berberine and berberine hydrochloride shows the prominent results as per standard and confirms the material with purity by overlapping all the delta values with standard drugs

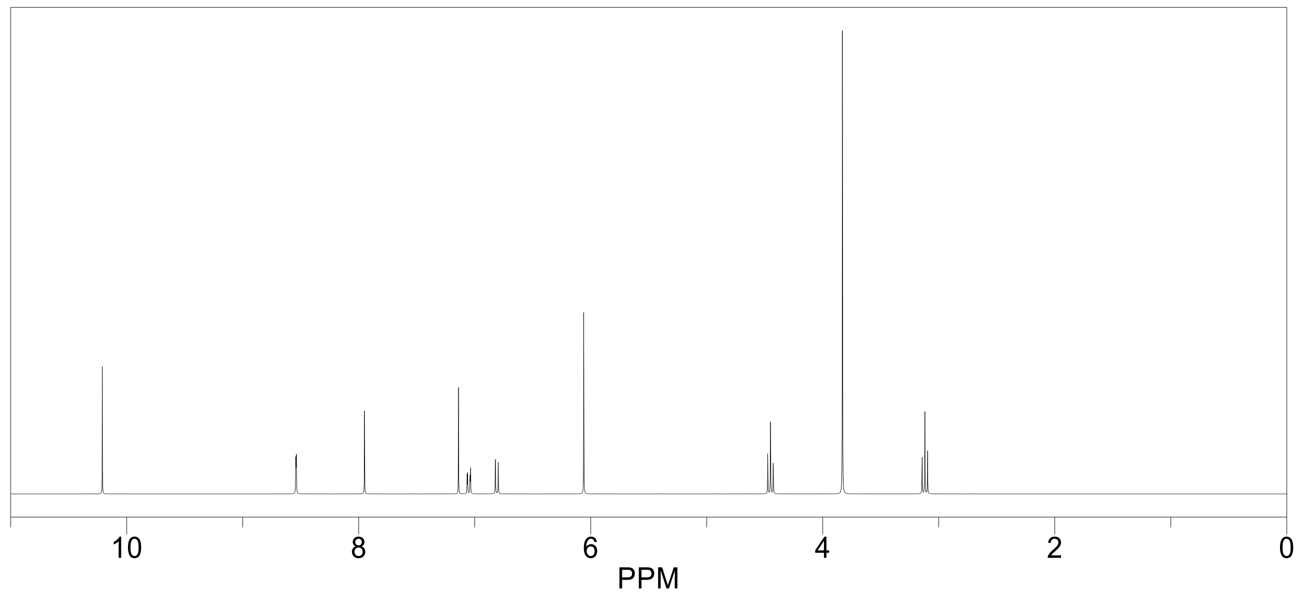

Fig 1.

NMR study of Berberine

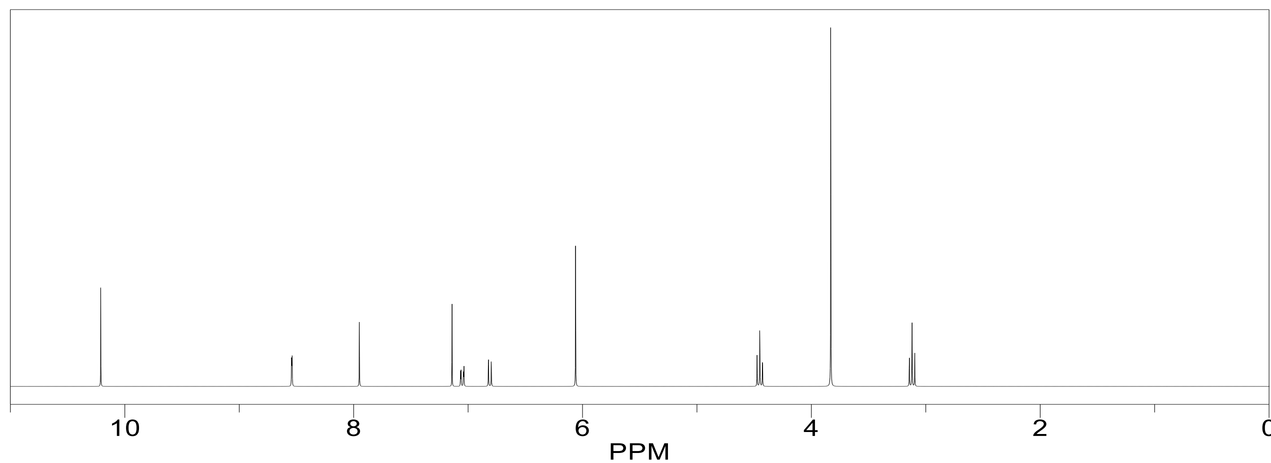

Fig 3.

NMR study of berberine hydrochloride

NMR of Berberine

```
Protocol of the H-1 NMR Prediction (Lib=SU Solvent=DMSO 300 MHz):

Node    Shift    Base + Inc.   Comment (ppm rel. to TMS)

CH2 6.06           5.90     1,3-dioxole
                   0.16     general corrections
CH  10.21          9.23     pyridinium
                  -0.03     1 -1:C*C*C*C*C*C*1 from 2-pyridine
                   0.15     1 -C
                   0.86     general corrections
CH2 4.45           1.37     methylene
                   3.70     1 alpha -Np(*C)*C
                   0.29     1 beta -1:C*C*C*C*C*C*1
                  -0.91     general corrections
CH  7.95           7.26     1-benzene
                  -0.38     1 -O-C
                   0.00     1 -O-C
                   0.34     1 -C*R
                   0.00     1 -CC
                   0.73     general corrections
CH  7.14           7.26     1-benzene
                   0.00     1 -O-C
                  -0.38     1 -O-C
                   0.19     1 -C*R
                  -0.08     1 -CC
                   0.15     general corrections
CH  6.81           7.26     1-benzene
                   0.00     1 -O-C
                  -0.38     1 -O-C
                  -0.07     general corrections
CH  8.54           8.50     pyridinium
                   0.16     1 -1:C*C*C*C*C*C*1 from 2-pyridine
                  -0.28     1 -C
                   0.16     general corrections
CH  7.05           7.26     1-benzene
                  -0.32     1 -O-C
                   0.00     1 -O-C
                   0.11     general corrections
CH2 3.12           1.37     methylene
                   1.22     1 alpha -1:C*C*C*C*C*C*1
                   0.70     1 beta -Np(*C)*C
                  -0.17     general corrections
CH3 3.83           0.86     methyl
                   2.87     1 alpha -O-1:C*C*C*C*C*C*1
                   0.10     general corrections
CH3 3.83           0.86     methyl
                   2.87     1 alpha -O-1:C*C*C*C*C*C*1
                   0.10     general corrections

1H NMR Coupling Constant Prediction

shift   atom index  coupling partner, constant and vector

6.06      20
10.21      7
4.45      11
             12     7.1     H-CH-CH-H
7.95      18
7.14      15
6.81       1
              6     7.5     H-C*C-H
8.54      10
              6     1.5     H-C*C*C-H
7.05       6
              1     7.5     H-C*C-H
             10     1.5     H-C*C*C-H
3.12      12
             11     7.1     H-CH-CH-H
3.83      25
3.83      23
```

NMR of Berberine hydrochloride

```
Protocol of the H-1 NMR Prediction (Lib=SU Solvent=DMSO 300 MHz):

Node     Shift    Base + Inc.   Comment (ppm rel. to TMS)

ClH ?              n.a.      not estimated
OH2 ?              n.a.      not estimated
CH2 6.06           5.90      1,3-dioxole
                   0.16      general corrections
CH  10.21          9.23      pyridinium
                  -0.03      1 -1:C*C*C*C*C*C*1 from 2-pyridine
                   0.15      1 -C
                   0.86      general corrections
CH2 4.45           1.37      methylene
                   3.70      1 alpha -Np(*C)*C
                   0.29      1 beta -1:C*C*C*C*C*C*1
                  -0.91      general corrections
CH  7.95           7.26      1-benzene
                  -0.38      1 -O-C
                   0.00      1 -O-C
                   0.34      1 -C*R
                   0.00      1 -CC
                   0.73      general corrections
CH  7.14           7.26      1-benzene
                   0.00      1 -O-C
                  -0.38      1 -O-C
                   0.19      1 -C*R
                  -0.08      1 -CC
                   0.15      general corrections
CH  6.81           7.26      1-benzene
                   0.00      1 -O-C
                  -0.38      1 -O-C
                  -0.07      general corrections
CH  8.54           8.50      pyridinium
                   0.16      1 -1:C*C*C*C*C*C*1 from 2-pyridine
                  -0.28      1 -C
                   0.16      general corrections
CH  7.05           7.26      1-benzene
                  -0.32      1 -O-C
                   0.00      1 -O-C
                   0.11      general corrections
CH2 3.12           1.37      methylene
                   1.22      1 alpha -1:C*C*C*C*C*C*1
                   0.70      1 beta -Np(*C)*C
                  -0.17      general corrections
CH3 3.83           0.86      methyl
                   2.87      1 alpha -O-1:C*C*C*C*C*C*1
                   0.10      general corrections
CH3 3.83           0.86      methyl
                   2.87      1 alpha -O-1:C*C*C*C*C*C*1
                   0.10      general corrections

1H NMR Coupling Constant Prediction

shift   atom index  coupling partner, constant and vector

0        27
0        26
6.06        20
10.21        7
4.45        11
              12     7.1      H-CH-CH-H
7.95        18
7.14        15
6.81        1
              6     7.5      H-C*C-H
8.54        10
              6     1.5      H-C*C*C-H
7.05        6
              1     7.5      H-C*C-H
             10     1.5      H-C*C*C-H
3.12        12
             11     7.1      H-CH-CH-H
3.83        25
3.83        23
```

Table 3 Partition coefficient of Berberine

S. No.	**Aqueous Phase**	**Oily phase**	**Berberine Aqueous**	**Berberine Oily**	**Partition coefficient**
1.	Distilled water	n-octanol	1.87	1.12	0.28
2.	PBS (7.4)	n-octanol	1.74	1.18	0.31

Table 4 Partition coefficient of Berberine Chloride

S. No.	**Aqueous Phase**	**Oily phase**	**Berberine Hydrochloride Aqueous**	**Berberine Hydrochloride Oily**	**Partition coefficient**
1.	Distilled water	n-octanol	2.97	1.08	0.31
2.	PBS (7.4)	n-octanol	2.71	1.09	0.42

The log *P* was determined experimentally following the conventional shake flask method at 25°C and compared with the predicted log *P* values obtained from various other computational software's. The solvents (octanol and water) were pre-saturated for a period of 24 h prior to the experiment to ensure equilibrium.

UV Visible Spectroscopy (UV Method)

Calibration curve of (A) Berberine and (B) Berberine Hydrochloride

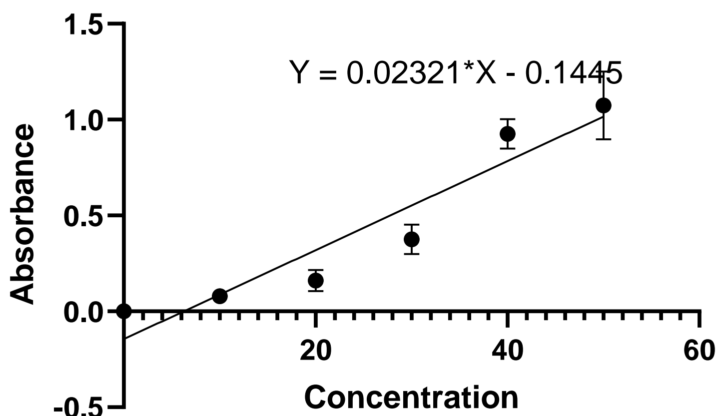

Fig 4 A

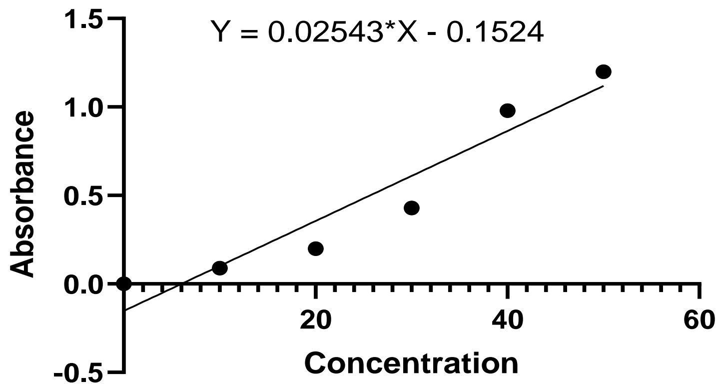

Fig 5 B

Cell viability assay

Cytotoxicity is preferred as a pilot project test and an important indicator for toxicity evaluation of drugs samples as it is simple, fast, has a high sensitivity and can save animals from toxicity.

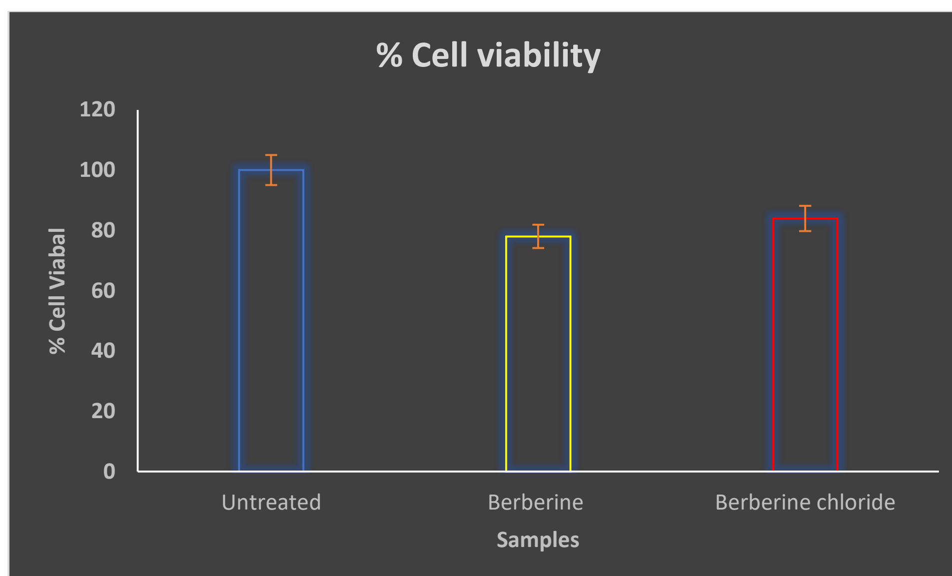

Fig 6 Cell viability or cytotoxicity assay

4. Conclusion

The above results are very suggestive and upto the mark. It suggestive toward further studies. We find the result which having very good impact on the sh-sy-5y cell lines so we can say that the above molecule is very effective in the cancer cells and they are also fisible to synthesize in the laboratory there is no any tedious task. We also support the molecules for its further studies.

5. References.

1. Arriola, E.L.; Rodriguez-Lopez, A.M.; Hickman, J.A. and Chresta, C.M. (1999), „Bcl-2 overexpression results in reciprocal downregulation of Bcl-X(L) and sensitizes human testicular germ cell tumours to chemotherapy-induced apoptosis.' Oncogene, 18, 1457–1464.

2. Bouchet, B.P.; de Fromentel, C.C.; Puisieux, A. and Galmarini, C.M. (2006), 'p53 as a target for anti-cancer drug development.' Crit Rev Oncol Hemat., 58, 190–207.
3. Oliner, J. D., Kinzler, K. W., Meltzer, P. S., George, D. L., & Vogelstein, B. (1992). Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature, 358(6381), 80-83.
4. Bullock, A.N. and Fersht, A.R. (2001), 'Rescuing the function of mutant p53.' Nat Rev Cancer., 1(1), 68-76.
5. Menter A, Gottlieb A, Feldman SR, Van Voorhees AS, Leonardi CL, Gordon KB, Lebwohl M, Koo JY, Elmets CA, Korman NJ, Beutner KR, Bhushan R "Guidelines of care for the management of psoriasis and psoriatic arthritis: Overview of psoriasis and guidelines of care for the treatment of psoriasis with biologics". *J Am Acad Dermatol*. **58** (5): 826–50. doi:10.1016/j.jaad.2008.02.039. PMID 18423260.
6. Palfreeman AC, McNamee KE, and McCann FE "New developments in the management of psoriasis and psoriatic arthritis: a focus on apremilast". *Drug DesDevel Ther*. **7**: (March 2013). 201 210. doi:10.2147/DDDT.S32713. PMC 3615921 . PMID 23569359.
7. Weigle N, McBane S (May 2013). "Psoriasis". *Am Fam Physician*. **87** (9): 626–33. PMID 23668525
8. Fry, L; Baker, BS (2007). "Triggering psoriasis: the role of infections and medications". Clinii indermatology. 25 (6): 606 15. doi:10.1016/j.clindermatol.2007.08.015 . PMID 18021899
9. ES, Chong WS, Tey HL (December 2012). "Nail psoriasis: a review". Am J Clin Dermatol. **13** (6):375–88. doi:10.2165/11597000-0000000 00000. PMID 22784035.
10. *Menter A, Griffiths CE "Current and future management of psoriasis". Lancet.* ***370*** *(9583) (July 2007).: 272–84. doi:10.1016/S0140-6736(07)61129-5. PMID 17658398*