EAS Journal of Pharmacy and Pharmacology
Abbreviated Key Title: EAS J Pharm Pharmacol
ISSN: 2663-0990 (Print) & ISSN: 2663-6719 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-2 | Issue-2 | Feb-2020 | DOI: 10.36349/easjpp.2020.v02i01.004

Research Article

Insilico Docking of Camptothecin with the Pathological Mediators of Rheumatoid Arthritis

P. Anjali[*1], R. Vimalavathini[1] and S. Kavimani[1]

[1]Department of Pharmacology, College of Pharmacy, MTPG & RIHS, Pondicherry 605 006, India

Article History
Received: 04.02.2020
Accepted: 13.02.2020
Published: 29.02.2020

Journal homepage:
https://www.easpublisher.com/easjpp

Quick Response Code

Abstract: ***Background:*** Rheumatoid arthritis (RA) is an autoimmune disease of complex etiology and two main principal substances such as matrix metalloproteinases and cathepsins play a crucial role in the loss of cartilage and joint destruction in RA. Camptothecin a topoisomerase 1 inhibitor has been found to inhibit angiogenesis, synoviocyte proliferation and metalloproteinases (gelatinases). ***Aim:*** To carry out insilico docking of camptothecin with various pathological mediators of RA using Autodock 4.2. ***Materials and Methods:*** camptothecin ligand was drawn in chemsketch and converted in to pdb. Various target proteins was obtained from RCSB PDB. Gpf and dpf parameters were created and autogrid and autodock was carried out. Binding energy was assessed based on their ranking order and the interactions of ligand and protein was determined from root mean square deviation table. ***Results:*** Our study results showed that camptothecin exhibited negative binding energy for proteins such as mitogen activated protein kinase (MAPK), nuclear factor kappa B (NFκB), tumor necrosis factor alpha (TNF-α), matrix metalloproteinases (collagenases,stromelysins, matrilysins), cathepsins and with enzyme involved in inflammatory pathway such as cyclooxygenase 1 and 2. ***Conclusion:*** By inhibiting MAPK, NFκB, TNF-α, metalloproteinases and cathepsins camptothecin might have an anti-arthritic and also anti-inflammatory activity. However this study warrants further in-vitro and in-vivo studies to establish the anti-arthritic activity.

Keywords: Camptothecin, rheumatoid arthritis, matrix metalloproteinases.

INTRODUCTION:

Rheumatoid arthritis (RA) is an autoimmune disease that causes persistent inflammation and joint destruction. RA occurs due to the formation of anti-citrullinated protein antibodies (ACPA) which is formed by the post translational modification of amino acid arginine in presence of peptidyl arginine deaminase enzyme (Suwannalai, P. *et al.,* 2012). It stimulates expression of nuclear factor kappa B (NFκB) leading to the release of inflammatory cytokines such as tumor necrosis factor (TNF) alpha, interleukin 1 beta and interleukin 6 (Bugatti, S. *et al.,* 2018). It also produces bone loss by binding to the osteocloast precursors ($CD68^{+}$) in the bone marrow (Guo, Q *et al.,* 2018; Harre, U. *et al.,* 2012; Krishnamurthy, A. *et al.,* 2016; & Wigerblad, G. *et al.,* 2015). Matrix metalloproteinases (MMP's) commonly called as "metzincins" is divided into 5 sub families such as collagenases (MMP-1, 8 and 13), gelatinases (MMP-2 and 9), stromelysins (MMP-3, 10 and 11) matrilysins (MMP-7 and 26) and membrane type metalloproteinases (MMP-14, 15, 17, 24 and 25) (Burrage, P. S. *et al.,* 2006). MMP's is activated by the mitogen activated protein kinase (MAPK) and NFκB which persuades its expression and degrades the components of extracellular matrix such as collagens, proteoglycans, elastin, and cell-binding glycoprotein. Cysteine proteases such as cathepsin S and K also activates synovial macrophages during inflammation and releases inflammatory cytokines which erodes collagen and elastin (Yasuda, Y. *et al.,* 2005). Cell signalling enzymes such as cyclooxygenase-1 (cox-1) and cyclooxygenase-2 (cox-2) are present in the inflamed synovium (Lee, Y. H. *et al.,* 2000). All the factors put forth to the development of "pannus" on the synovial surface causing stiffness of connective tissue leading to RA.

Drugs used in the treatment of RA include anti-inflammatory drugs, glucocorticoids, disease modifying antirheumatic drugs (DMARDS) and biological agents. On chronic use these drugs modify immune system but are associated with alarming unfavorable effects. Hence the utmost aim of the treatment is to alleviate pain, inflammation and prevent further structural damage with minimal adverse effects.

***Corresponding Author:** P. Anjali Email:

Autodock 4.2 is a molecular modeling simulation mainly used in predicting the binding energy and interaction of ligand with the biomolecular target. Camptothecin a topoisomerase 1 inhibitor has been found to inhibit synoviocyte proliferation, angiogenesis and metalloproteinases (Jackson, J. K. *et al.,* 2008). So insilico docking of camptothecin was carried out with various mediators of RA such as MAPK, NFκB, TNF alpha, MMP-3, MMP-8, MMP-10, and MMP-13, cox-1, cox-2 and cathepsin K was carried out in order to predict whether camptothecin had interaction on pathological mediators of RA.

MATERIALS AND METHODS:

Preparation of Ligand:

Camptothecin (ligand) was prepared using chemsketch ACD 2019 version 1.2 and converted into pdb using open babel GUI software version 2.3.1.

Preparation of Protein:

The various target proteins for camptothecin are fetched from RCSB protein data bank. Proteins with method x-ray crystallography and resolution less than 2Å was selected for docking process. Hetero atoms present in the proteins were removed.

Generating Grid and Docking Parameters:

The coordinate parameters for ligand and protein were prepared in pdbqt format. Grid parameters were generated by changing numbers in X, Y and Z dimension to 60 (Jackson, J. K. *et al.,* 2008). The spacing was fixed in the range of 0.3-0.4Å and the gpf files were created. Docking parameters was prepared by using Lamarckian genetic algorithm (4.2) conformation. The number of evaluations was set to 25 and dpf were also created.

Running Autodock and Autogrid:

Autogrid and autodock was carried out such that the glg and dpf files were obtained. Analysis of results was performed based on their ranking order and the interaction between ligand and protein was predicted from the root mean square deviation (RMSD) table 1(Selvaraj, G. *et al.,* 2016; Wu, J. *et al.,* 2017; Mann, S. *et al.,* 2015; Biswajit, D. *et al.,* 2012; Sowmya, H. 2019; Zaka, M. *et al.,* 2017; Chen, Q. *et al.,* 2017; Aparna, H.P.K. *et al.,* 2019; Shruthi, S.D., & Ramachandra, Y.L. 2011).

RESULTS:

The binding energy of camptothecin with various proteins has been docked and tabularized. The present study exhibited good binding energy with proteins such as MAPK, NFκB, TNF-α, matrix metalloproteinases (collagenases, stromelysins, and matrilysins), cathepsins, cox-1 and cox-2. The inhibition constant was found to be high for enzymes such as MMP-3, MMP-13 and cell signalling enzyme cox-1 and cox-2.

TABLE 1: Root Mean Square Deviation

PROTEIN	CODE	BINDING ENERGY (kcal mol-1)	INHIBITION CONSTANT (mm)=ki	H-BONDS	BONDING
MAPK	5mtx	-6.1	34.05	1	1::LIG1:O 1
NF-κB	6qci	-6.0	40.28	1	6qci_3:C:GLN74:HE21
TNF-alpha	5m2j	-7.94	1.51	2	5m2j:A:GLN47:HE22 5m2j:A:LYS90:HZ3
MMP-3	4dpe	-8.52	567.31	1	4dpe_3:B:GLN243:HE22
MMP-7	2y6c	-7.11	6.11	1	2y6c:A:ALA186:HN
MMP-8	2oy4	-7.52	3.06	1	2oy4_3:A:SER225: O
MMP-10	3v96	-1.63	64.21	3	3v96_3:A:GLU156:OE2 3v96_3:A:THR98: O 3v96_3:A:SER100: O
MMP-13	1xuc	-8.65	458.78	1	1xuc_3:B:ARG109:HH221
Cox-1	3n8y	-9.03	241.96	5	3n8y_3:B:ARG376:HH21 3ny8_3:B:ASN375:HN 3ny8_3:B:ARG374:HH21 3ny8_3B:ASN375 3ny8_3:B:ARG374:HE
Cox-2	5ikt	-9.02	244.1	2	5ikt_3:A:ASN375:HN 5ikt_3:B:ARG376:HH21
Cathepsin K	5z5o	-7.12	6.03	1	5zo5:A:ARG:107:HH12

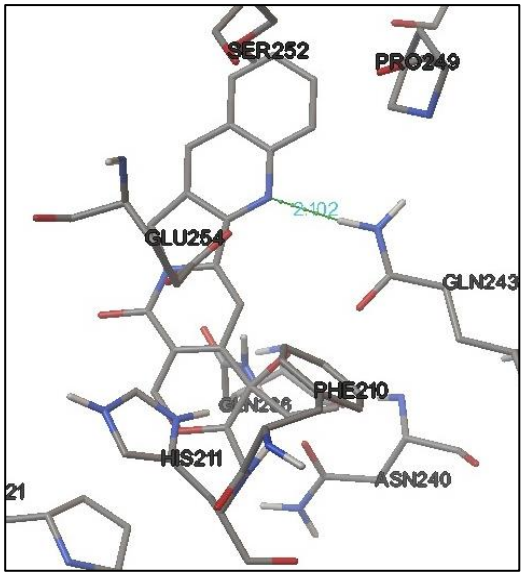

Figure 1: NFκB

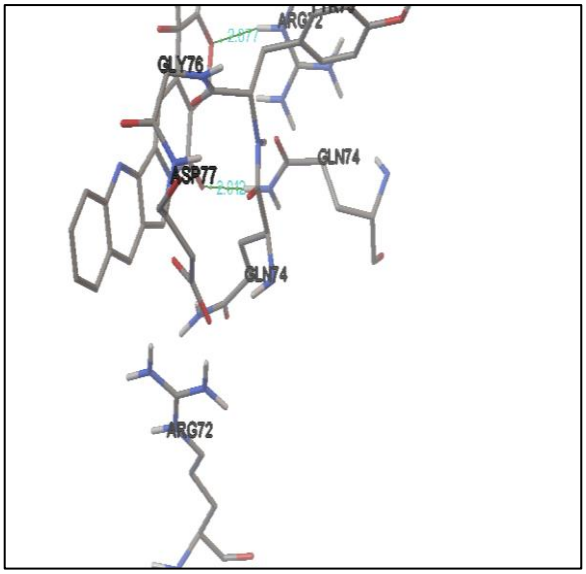

Figure 2: MMP-3

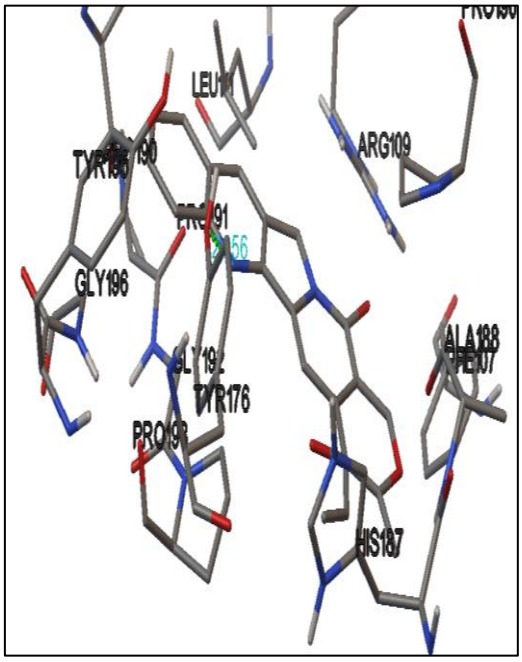

Figure 3: MMP-13

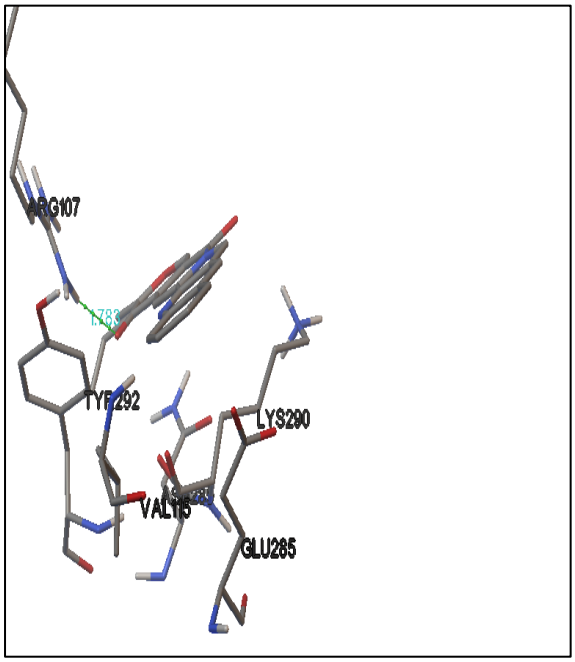

Figure 4: Cathepsin K

DISCUSSION:

RA is a chronic autoimmune disease of heterogeneous pathophysiology that predominantly degrades synovial tissues. Available synthetic metalloproteinase inhibitors such as collagen peptidomimetics, non peptidomimetics exhibited disappointing results in treating RA at clinical trials (Rengel, Y. *et al.,* 2007). Anti-inflammatory agents, DMARDS and biological agents also down regulates serum levels of MMPs, but these agents are yet to provide chronic, safe and protective role against joint destruction. Even though there are many advanced techniques involved in treating RA, the complete cure for the disease is still uncertain.

Since MMPs plays a vital role in joint destruction, they have been regarded as useful biomarkers and therapeutic targets. MMP-3, MMP-10 which is found in osteoblast activates pro MMP-1, 8 and 13 that destructs collagen (Sun, S. *et al.,* 2014). Controlling these enzymes expression can prevent further collagen destruction and targeting MMP-13 which is present in deep layers of cartilage can lower articular cartilage damage (Moore, B. A. *et al.,* 2000). Blocking of cell signalling enzymes such as cox-1 and cox-2 which are present in inflamed synoviam can overcome inflammatory status in RA.

Thus our study reveals that camptothecin not only inhibits topoisomerase 1 but also may interact with various mediators of RA such as MAPK, NFκB, TNF alpha, MMP-3, MMP-8, MMP-10, MMP-13, cox-1, cox-2 and cathepsin K suggesting that camptothecin might have anti-arthritic and anti-inflammatory effect. It is a known fact that camptothecin, a topoisomerase 1 inhibitor also reduces angiogenesis by increasing endothelial cell apoptosis by inhibiting NFκB (Legarza, K., & Yang, L. X. 2006; & Huang, T. T. *et al.*, 2000). It also attenuates the expression of MMP-2 and 9 in primary chondrocytes in a concentration dependent manner (Jackson, J. K. *et al.*, 2008). In addition to this, our in silico study results show that it may also act on cox enzymes, MAPK, cathepsin, cytokines, and matrix metalloproteinases thus inhibiting all these factors may provide better insights in the treatment of RA. But further in vivo studies are necessary to establish the results.

CONCLUSION:

Thus our in silico docking study results recommends that camptothecin may exhibit significant anti-arthritic and anti-inflammatory effect by acting on MAPK, NFκB, metalloproteinases, cathepsin, cyclooxygenases and cytokines. Futuristic in-vitro and in-vivo studies are in need to establish that camptothecin can be used as potential disease modifying and anti-inflammatory agent for the treatment of RA.

REFERENCES:

1. Aparna, H.P.K., Priyanka, P., & Samiksha, W. (2019). Association of herbal plant in Rheumatoid Arthritis studies using in silico approach. *International Journal of Advance Research, Ideas and Innovations in Technology*, *5*(3), 1648-1654.
2. Biswajit, D., Uttam, K.M., Shyamal, S., Pranab, G., & Asim, K.B. (2012). Molecular Docking of Triazine analogues. *Journal of Chemical and Pharmaceutical Research*, *4*(3), 1595-1600.
3. Bugatti, S., Manzo, A., Montecucco, C., & Caporali, R. (2018). The clinical value of autoantibodies in rheumatoid arthritis. *Frontiers in medicine*, *5*, 339.
4. Burrage, P. S., Mix, K. S., & Brinckerhoff, C. E. (2006). Matrix metalloproteinases: role in arthritis. *Front Biosci*, *11*(1), 529-543.
5. Chen, Q., Niu, X., & Li, N. (2017). Exploring the natural chemiome to target interleukin-6 receptor (IL-6R) cytokines: an atomic scale investigation for novel rheumatoid arthritis drug discovery. *Brazilian Journal of Pharmaceutical Sciences*, *53*(3), 1-7.
6. Guo, Q., Wang, Y., Xu, D., Nossent, J., Pavlos, N. J., & Xu, J. (2018). Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone research*, *6*(1), 1-14.
7. Harre, U., Georgess, D., Bang, H., Bozec, A., Axmann, R., Ossipova, E., ... & Sarmay, G. (2012). Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *The Journal of clinical investigation*, *122*(5), 1791-1802.
8. Huang, T. T., Wuerzberger-Davis, S. M., Seufzer, B. J., Shumway, S. D., Kurama, T., Boothman, D. A., & Miyamoto, S. (2000). NF-κB Activation by Camptothecin A LINKAGE BETWEEN NUCLEAR DNA DAMAGE AND CYTOPLASMIC SIGNALING EVENTS. *Journal of Biological Chemistry*, *275*(13), 9501-9509.
9. Jackson, J. K., Higo, T., Hunter, W. L., & Burt, H. M. (2008). Topoisomerase inhibitors as anti-arthritic agents. *Inflammation Research*, *57*(3), 126-134.
10. Krishnamurthy, A., Joshua, V., Hensvold, A. H., Jin, T., Sun, M., Vivar, N., ... & Magnusson, M. (2016). Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. *Annals of the rheumatic diseases*, *75*(4), 721-729.
11. Lee, Y. H., Choi, S. J., Kim, A., Kim, C. H., Ji, J. D., & Song, G. G. (2000). Expression of cyclooxygenase-1 and-2 in rheumatoid arthritis synovium. *Journal of Korean medical science*, *15*(1), 88-92.
12. Legarza, K., & Yang, L. X. (2006). New molecular mechanisms of action of camptothecin-type drugs. *Anticancer research*, *26*(5A), 3301-3305.
13. Mann, S., Sharma, A., Biswas, S., & Gupta, R. K. (2015). Identification and molecular docking analysis of active ingredients with medicinal properties from edible Baccaurea sapida. *Bioinformation*, *11*(9), 437.-443.
14. Moore, B. A., Aznavoorian, S., Engler, J. A., & Windsor, L. J. (2000). Induction of collagenase-3 (MMP-13) in rheumatoid arthritis synovial fibroblasts. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1502*(2), 307-318.
15. Rengel, Y., Ospelt, C., & Gay, S. (2007). Proteinases in the joint: Clinical relevance of proteinases in joint destruction. *Arthritis Research and Therapy*, *9*, 221-231.
16. Selvaraj, G., Kaliamurthi, S., & Thiruganasambandam, R. (2016). Molecular docking studies of rutin on matrix metalloproteinase. *Insights Biomed*, *1*(1).
17. Shruthi, S.D., & Ramachandra, Y.L. (2011). RBP-J as a therapeutic target to rheumatoid arthritis- an in silico study. *International Journal of Preclinical and Pharmaceutical Research*, *2*(1), 38-44.

18. Sowmya, H. (2019). In silico molecular docking and ADME/T analysis of plant compounds against IL17A and IL18 targets in gouty arthritis. *Journal of Applied Pharmaceutical Science*, *9* (07), 18-26.
19. Sun, S., Bay-Jensen, A. C., Karsdal, M. A., Siebuhr, A. S., Zheng, Q., Maksymowych, W. P., ... & Henriksen, K. (2014). The active form of MMP-3 is a marker of synovial inflammation and cartilage turnover in inflammatory joint diseases. *BMC musculoskeletal disorders*, *15*(1), 93.
20. Suwannalai, P., Trouw, L. A., Toes, R. E., & Huizinga, T. W. (2012). Anti-citrullinated protein antibodies (ACPA) in early rheumatoid arthritis. *Modern rheumatology*, *22*(1), 15-20.
21. Wigerblad, G., Bas, D.B., Fernades, C.C., Krishnamurthy, A., Nandakumar, K.S., Rogoz, K. et al (2015). Autoantibodies to citrullinated proteins induce joint pain independent of inflammation via a chemokine-dependent mechanism. Basic and Translational Research, 0, 1-9.
22. Wu, J., Qu, Y., Deng, J. X., Liang, W. Y., Jiang, Z. L., Lai, R., & Yu, Q. H. (2017). Molecular docking studies of kirenol a traditional Chinese medicinal compound against rheumatoid arthritis cytokine drug targets (TNF-?, IL-1 and IL-6).
23. Yasuda, Y., Kaleta, J., & Brömme, D. (2005). The role of cathepsins in osteoporosis and arthritis: rationale for the design of new therapeutics. *Advanced drug delivery reviews*, *57*(7), 973-993.
24. Zaka, M., Sehgal, S. A., Shafique, S., & Abbasi, B. H. (2017). Comparative in silico analyses of Cannabis sativa, Prunella vulgaris and Withania somnifera compounds elucidating the medicinal properties against rheumatoid arthritis. *Journal of Molecular Graphics and Modelling*, *74*, 296-304.