Journal of Computational Methods in Molecular Design, 2013, 3 (3):26-33

Scholars Research Library
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231- 3176
CODEN (USA): JCMMDA

In silico studies of NF-κB protein as anti-cancer and anti-inflammatory target

Ranganatha S[1,3], Shruthi SD[1,2], Govindappa M[3] and Ramachandra YL[1]*

[1]*Department of P.G. Studies and Research in Biotechnology, Kuvempu University, Shankaraghatta, Karnataka, India*
[2]*P.G. Department of Biotechnology, The Oxford College of Science, Bangalore, Karnataka, India*
[3]*Sri Shridevi Research Centre, Sira Road, Tumkur, Karnataka*

ABSTRACT

Many companies have developed novel agents acting on the NF-κB pathway: some of these agents are supposed to be NF-κB specific (i.e. IKK inhibitors) while others have wide-range biological activities (i.e. proteasome inhibitors). The study was conducted to find out the interactions of NF-κB with standard anti-cancer and anti-inflammatory drug such as daunorubicin and dexamethasone respectively. The drugs were tested for interactions with NF-κB protein from different mammals, with special reference to NF-κB from Homo sapiens. The results were analysed based on number of hydrogen bonds formed for interation and energy required. At the same time the structural similarity study was done to find out the similarities of human NF-κB with that of other mammals.

Keywords: daunorubicin, dexamethasone, *Homo sapiens*, NF-κB

INTRODUCTION

Since the discovery of the NF-kB transcription factor in 1986 and the cloning of the genes coding for NF-κB and IkB proteins, many studies demonstrated that this transcription factor can, in most cases, protect transformed cells from apoptosis and therefore participate in the onset or progression of many human cancers. Molecular studies demonstrated that ancient widely used drugs, known for their chemopreventive or therapeutic activities against human cancers, inhibit NF-κB, usually among other biological effects. It is therefore considered that the anti-cancer activities of NSAIDs (non-steroidal anti-inflammatory drugs) or glucocorticoids are probably partially related to the inhibition of NF-κB and new clinical trials are being initiated [1]. A constitutive NF-κB activity is observed in many lymphoid or myeloid tumors, including multiple myeloma, Hodgkin diseases and some non-Hodgkin lymphomas.

Moreover, various solid tumors, such as for instance breast cancers, glioblastomas and many others, are also characterized by a constitutive and continuous NF-κB nuclear activity [2,3,4,5]. In both situations, constitutive or treatment-induced activity, NF-κB functions mainly as an inhibitor of apoptosis. Indeed, the inhibition of NF-κB by genetic or chemical inhibitors induces the apoptosis of various tumor cells and/or restores the apoptotic response after treatment with ionizing radiations or chemotherapeutic agents, thus reversing NF-κB linked radio- or chemoresistance in many models [2,6,7,8,9]. Therefore, a precise knowledge of the signalling pathways controlling NF-κB as well as of the NF-κB target genes is essential in order to define precisely the field of application of anti-NF-κB drugs for the treatment of human cancers. Because epidemiological and genetic studies have shown that chronic inflammation predisposes to some cancers, nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, have been extensively studied, mostly as chemopreventive agents.

The protective effects of these drugs in colorectal cancer are particularly marked. Indeed, NSAIDs inhibit the growth of colorectal cancer cells *in vitro* and *in vivo* [10] and regular aspirin uptake is associated with a reduced risk

of colorectal cancer [11,12,13]. Aspirin and other NSAIDs prevent prostaglandin synthesis through the inhibition of the cycloxygenase (COX) activity and consequently, reduced inflammation-associated cancer and exert COX-2-related anti-tumor activities.

Glucocorticoids (GCs) such as dexamethasone or prednisolone are another class of widely prescribed drugs with well established anti-inflammatory and immunosuppressive activities associated to their inhibitory effects on AP-1 and NF-κB pathways [14]. These drugs are also commonly administered for the treatment of lymphoid leukemias, Hodgkin or non-Hodgkin lymphomas and multiple myeloma. Interestingly, these glucocorticoid-responsive cancers are characterized by a constitutive NF-κB activity, thus suggesting a direct link between the NF-κB inhibition and GC therapeutic efficacy in such tumors.

The present work was conducted to find out the interactions between the NF-κB protein from different mammals namely**,** *Homo sapiens* (human), *Mus musculus* (mouse), *Bos Taurus* (bovine), *Ailuropoda melanoleuca* (giant panda), *Sus scrofa* (pig), *ovis aries* (sheep), *Pongo abelii* (Sumatran orangutan), *Macaca mulatta* (Rhesus macaque), *Rattus norvegicus* (Rat) and *Canis familiaris* (Dog) with standard anti-cancer and anti-inflammatory drug such as daunorubicin and dexamethasone respectively. Study was also conducted to find out the structural similarity between NF-κB of human with structure of other organisms.

MATERIALS AND METHODS

PUBCHEM (http://pubchem.ncbi.nlm.nih.gov) – PubChem is organized as three linked databases within the NCBI's Entrez information retrieval system. The drug structures are downloaded from this database.

CHEM DRAW ULTRA 6.0 - ChemDraw Ultra is a chemical structure drawing software designed for drawing stereochemically correct structures from chemical names, to get accurate IUPAC names for structures and to estimate NMR spectra from a ChemDraw structure with direct atom to spectral correlation.

KEGG (http://www.genome.jp/kegg/) - It is a collection of online databases dealing with genomes, enzymatic pathways, and biological chemicals. The PATHWAY database records networks of molecular interactions in the cells, and variants of them specific to particular organisms. Inflammation and cancer pathways along with NF-κB interactions were studied using this.

PDB (www.rcsb.org) - The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The 3D structure of NF-κB protein from different mammals were retrieved using this.

Swiss PDB Viewer - Swiss-PdbViewer is tightly linked to SWISS-MODEL, an automated homology modeling server and an application that provides a user friendly interface allowing analyzing several proteins at the same time. The PDB file is edited by removing the heteroatoms, adding C terminal oxygen.

Cast P (http://cast.engr.uic.edu.) - Computed Atlas of Surface Topography of proteins provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins. These include pockets located on protein surfaces and voids buried in the interior of proteins.

Ligplot (http://www.ebi.ac.uk) - The LIGPLOT program automatically generates schematic 2-D representations of protein-ligand complexes from standard Protein Data Bank file input. Interacting amino acids with ligands on the surface of protein are predicted.

PRODRG (http://davapc1.bioch.dundee.ac.uk/prodrg/) - PRODRG will take a description of a small molecule and from it generate a variety of topologies for use with GROMACS, PRODRG takes input from existing coordinates or various two-dimensional formats and automatically generates coordinates and molecular topologies suitable for X-ray refinement of protein-ligand complexes.

Autodock - AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoGrid calculates the energy of the non-covalent interactions between the protein and probe atoms that are located in the different grid points of a lattice that defines the area of interest. As a result of these calculations the output file of the protein-ligand complex with flexible residues and the ligand located within the binding pocket is obtained. Each structure was scored and ranked by the program by the calculated interaction energy.

TopMatch (topmatch.services.came.sbg.ac.at/) - It is a web service for the alignment and superposition of protein structures and the instant visualization of structural similarities. The tool was used to find out the structural similarities of NF-κB protein from different mammals with that of *Homo sapiens*.

RESULTS AND DISCUSSION

In the study conducted daunorubicin was taken as standard anti-cancer drug which acts on NF-κB. The drug was tested for interactions with NF-κB protein from different mammals. The result shows that drug binds with all NF-κB proteins with almost same efficacy. The PDB ID of NF-κB taken for study is shown in **table 1**. **Table 2** shows the docking efficiency of drug with different NF-κB proteins, which are analysed based on energy acquired for binding and number of hydrogen bonds formed. The pictorial orientations of which are shown in **fig 1** and **fig 2**. Another part of the study was focused towards anti-inflammatory activity. The standard drug dexamethasone was taken which is proved to be acting on NF-κB in inflammatory pathway. The drug was tested for interactions with NF-κB protein from different mammals. The result shows that drug binds with all NF-κB proteins with almost same efficacy. **Table 3** shows the docking efficiency of drug with different NF-κB proteins, which are analysed based on energy acquired for binding and number of hydrogen bonds formed. The pictorial orientations of which are shown in **fig 3** and **fig 4**. **Fig 5** and **Fig 6** shows the three dimensional structural similarity of NF-κB from *Homo sapiens* with that of other organisms taken for the study.

The regulation of NF-κB by the peroxisome proliferatoractivated receptor g (PPAR-g) agonist is a promising effect that might represent a new strategy in inflammatory diseases and cancer. Several molecular mechanisms were reported for NF-κB inhibition by GCs. Dexamethasone could induce, through the glucorticoid receptor (GR), the transcription of the IkBa gene in lymphocytic and monocytic cells, therefore increasing IkBa protein levels and NF-kB cytoplasmic sequestration [15,16]. However, this mechanism seems to be cell type and even possibly target gene dependent. Indeed, the upregulation of IkBa expression is not involved in dexamethasone effect on NF-κB-dependent gene expression in endothelial cells. It suggested that the decrease of NF-κB transcriptional activity in response to dexamethasone might be due to a direct interaction between GR and p65 leading to histone deacetylation or methylation. This interaction can also cause a decreased phosphorylation of RNA polymerase II, thus reducing its transcriptional activity. This effect was demonstrated on the IL-8 and ICAM- 1 promoters but not at the IkBa promoter, confirming that the mechanism of GR-repressed NF-κB activity is promoterspecific [17,18].

Table 1: The PDB ID's of NF-κB proteins taken for the interaction studies

Sl.No.	Organisms	PDB ID	Experimental method	PubMed entry	Resolution[Å]	Polymer-length
1	*Homo sapiens*	2V2T	X-RAY DIFFRACTION	17869269	3.05	RELB-288 P105-326 DNA-11
2	*Mus musculus*	1SVC	X-RAY DIFFRACTION	7830764	2.60	DNA-19 Protein- 365
3	*Bos taurus*	1MSZ	SOLUTION NMR	12547203	--	Protein- 86
4	*Ailuropoda melanoleuca*	3T6P	X-RAY DIFFRACTION	22021857	1.90	Protein- 345
5	*Sus scrofa*	1NFK	X-RAY DIFFRACTION	7530332	2.30	DNA- 11 Protein-325
6	*Ovis aries*	3JV5	X-RAY DIFFRACTION	--	2.65	p100- 104
7	*Pongo abelii*	3EB5	X-RAY DIFFRACTION	18784070	2.00	Protein-74
8	*Macaca mulatta*	3JWE	X-RAY DIFFRACTION	19962385	2.70	Protein- 320
9	*Rattus norvegicus*	2DBF	SOLUTION NMR	--	--	p105- 100
10	*Canis familiaris*	1OOA	X-RAY DIFFRACTION	12886018	2.45	RNA- 29 p105- 326

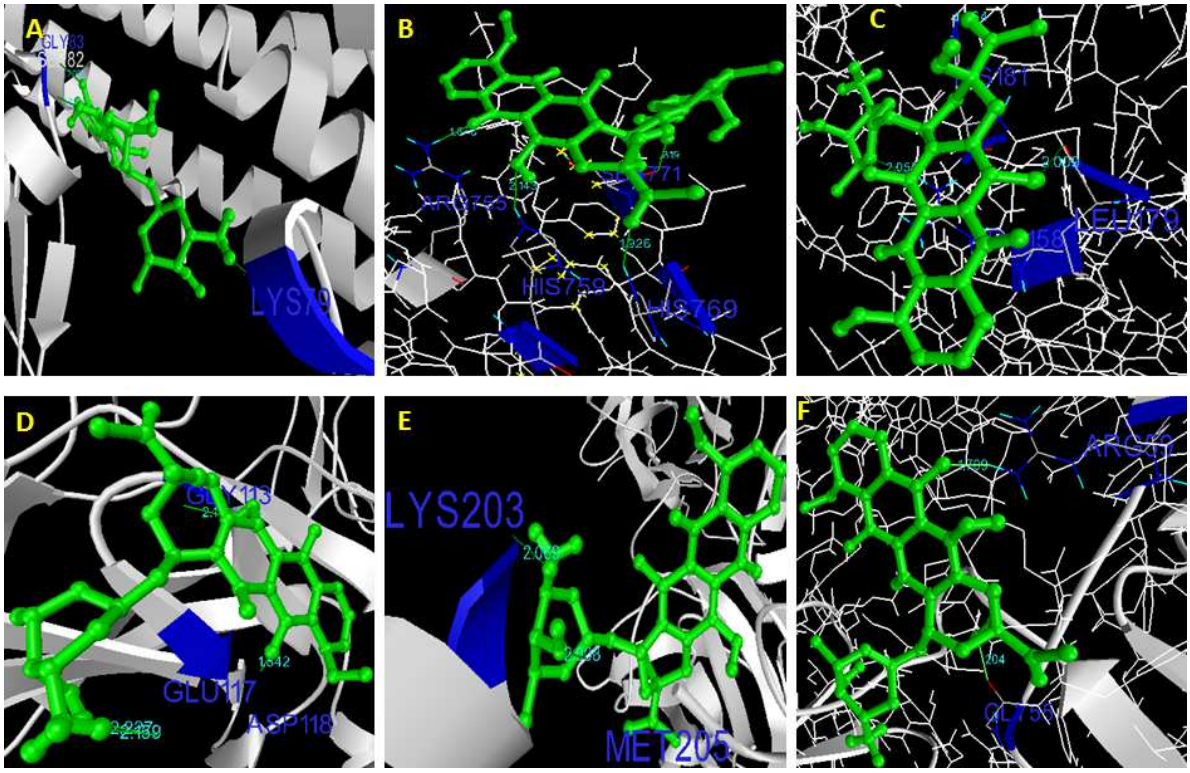

Fig 1: Orientation of daunorubicin with NF-κB of (A) *Ailuropoda melanoleuca*, (B) *Bos taurus*, (C) *Homo sapiens*, (D) *Sus scrofa*, (E) *Canis familiaris* and (F) *Mus musculus*.

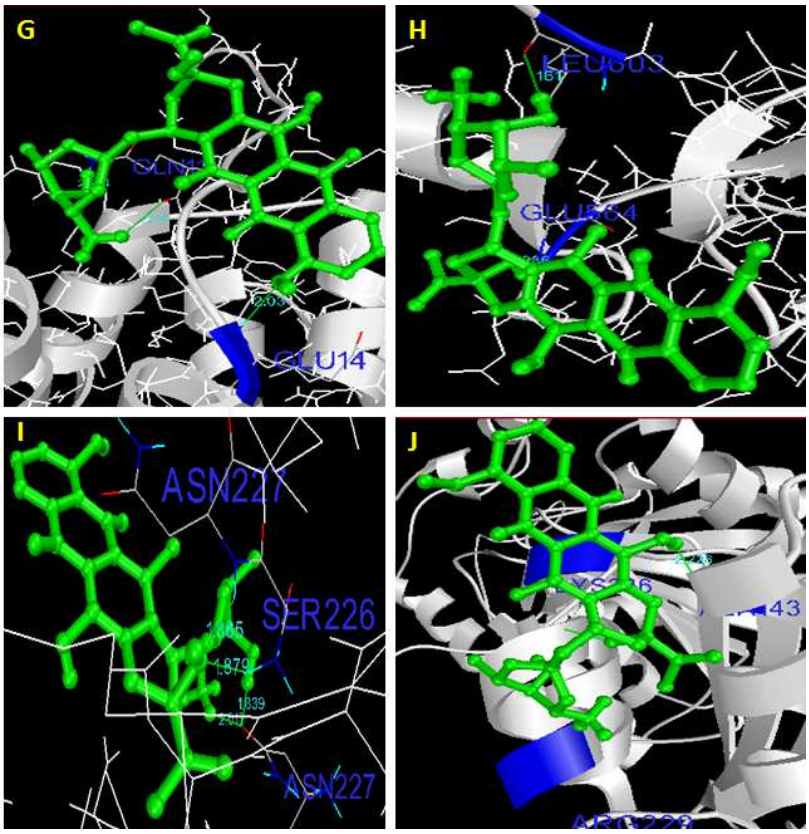

Fig 2: Orientation of daunorubicin with NF-κB of (G) *Rattus norvegicus*, (H) *Pongo abelii*, (I) *Ovis aries* and (J) *Macaca mulatta*.

Table 2: Docking results of NF-κB proteins against anti-cancer drug daunorubicin

Sl.No.	NFkB Structures	Binding energy	Docking energy	Inhibitory constant	No. of H bonds	H-bond formation
1	2RAX (3T6P)	-5.36	-6.33	0.000118	3	DRG1:OAS::2RAX:A:SER82:HN DRG1:OAV::2RAX:A:GLY83:HN DRG1:HBP::2RAX:E:LYS79:O
2	1MSZ	-5.38	-6.22	0.000114	4	DRG1:HBO::1MSZ:A:SER771:O DRG1:OAV::1MSZ:A:ARG755:HH21 DRG1:OAZ::1MSZ:A:HIS769:HD1 DRG1:OAS::1MSZ:A:HIS759:HE2
3	1NFI (2V2T)	-6.15	-7.49	3.1 e-005	3	DRG1:HAS::1NFI:A:LEU179:O DRG1:OB1::1NFI:A:ARG158:HH22 DRG1:OAZ::1NFI:A:HIS181:HE2
4	1NFK	-5.05	-5.91	0.000198	5	DRG1:HBN::1NFK:B:GLU117:OE2 DRG1:HBQ::1NFK:B:GLU117:OE2 DRG1:OAU::1NFK:B:ASP118:HN DRG1:OAS::1NFK:B:GLY113:HN DRG1:HAS::1NFK:B:GLY113:O
5	1OOA	-5.14	-6.34	0.00017	2	DRG1:HBP::1OOA:A:LYS203:O DRG1:OBK::1OOA:A:MET205:HN
6	1SVC	-3.36	-4.79	0.0	2	DRG1:HBM::1SVC:P:GLY55:O DRG1:OAV::1SVC:P:ARG59:HH22
7	2DBF	-4.39	-5.44	0.000605	3	DRG1:HBO:: 2DBF:A:GLN11:O DRG1:OBK:: 2DBF:A:GLN11:HE22 DRG1:OAW:: 2DBF:A:GLU14:HN
8	3EB5	-4.68	-6.02	0.000373	2	DRG1:HBN:: 3EB5:A:LEU603:O DRG1:OBB:: 3EB5:A:GLU564:HN
9	3JV5	-6.46	-7.14	1.84 e-005	4	DRG1:HBN:: 3JV5:C:ASN227:OD1 DRG1:HBP:: 3JV5:C:ASN227:OD1 DRG1:OBB:: 3JV5:B:ASN227:HN DRG1:OBB:: 3JV5:B:SER226:HN3
10	3JWE	-3.03	-4.05	0.01	3	DRG1:HBP:: 3JWE:A:ARG229:O DRG1:HAS:: 3JWE:A:ALA143:O DRG1:OBB:: 3JWE:A:LYS236:HZ1

Table 3: Docking results of NF-κB proteins against anti-inflammatory drug dexamethasone

Sl.No.	NFkB Structures	Binding energy	Docking energy	Inhibitory constant	No. of H bonds	H-bond formation
1	2RAX (3T6P)	-6.2	-6.65	2.87 e-005	2	DRG1:HBE:: 2RAX:A:ASN119:O DRG1:OAR:: 2RAX:E:LYS115:HZ2
2	1MSZ	-6.94	-7.25	8.2 e-006	3	DRG1:OAW:: 1MSZ:A:ARG755:HE DRG1:OAW:: 1MSZ:A:HIS759:HE2 DRG1:OAU:: 1MSZ:A: HIS769:HD1
3	1NFI (2V2T)	-7.6	-7.94	2.7 e-006	2	DRG1:HBC:: 1NFI:A:GLU49:O DRG1:OAW:: 1NFI:A:LYS28:HZ2
4	1NFK	-5.06	-5.72	0.000168	2	DRG1:HBN::1NFK:B:GLU117:OE2 DRG1:HAS::1NFK:B:GLY113:O
5	1OOA	-7.74	-7.98	2.12 E-006	3	DRG1:HBE:: 1OOA:A:SER208:OG DRG1:HBD:: 1OOA:A:MET205:O DRG1:OAW:: 1OOA:A:LEU207:HN
6	1SVC	-3.34	-4.80	0.0	2	DRG1:HBM::1SVC:P:GLY55:O DRG1:OAV::1SVC:P:ARG59:HH22
7	2DBF	-5.12	-5.53	0.000176	3	DRG1:HBE:: 2DBF:A:ASP61:OD1 DRG1:HBD:: 2DBF:A:ASP61:OD2 DRG1:OBB:: 2DBF:A:TYR21:HH
8	3EB5	-6.54	-6.82	1.62 e-005	4	DRG1:HBC:: 3EB5:A:LEU575:O DRG1:HBE:: 3EB5:A:GLU554:O DRG1:OAU:: 3EB5:A:LYS558:HZ1 DRG1:HBD:: 3EB5:A:GLU554:O
9	3JV5	-7.49	-7.8	3.22 E-006	2	DRG1:HBE:: 3JV5:C:ARG313:O DRG1:OAU:: 3JV5:B:GLN271:HE21
10	3JWE	-7.28	-7.62	4.61 e-006	-	--

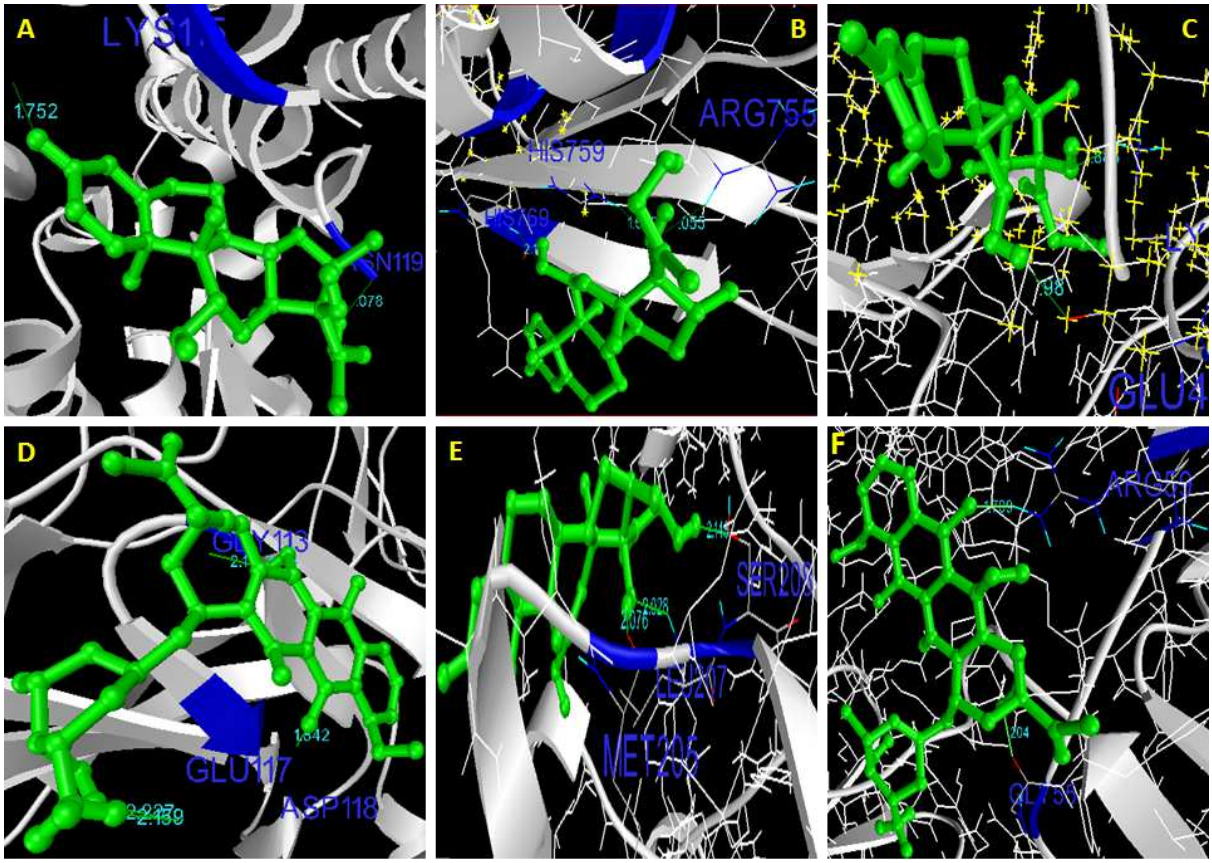

Fig 3: Orientation of dexamethasone with NF-κB of (A) *Ailuropoda melanoleuca*, (B) *Bos taurus*, (C) *Homo sapiens*, (D) *Sus scrofa*, (E) *Canis familiaris* and (F) *Mus musculus*.

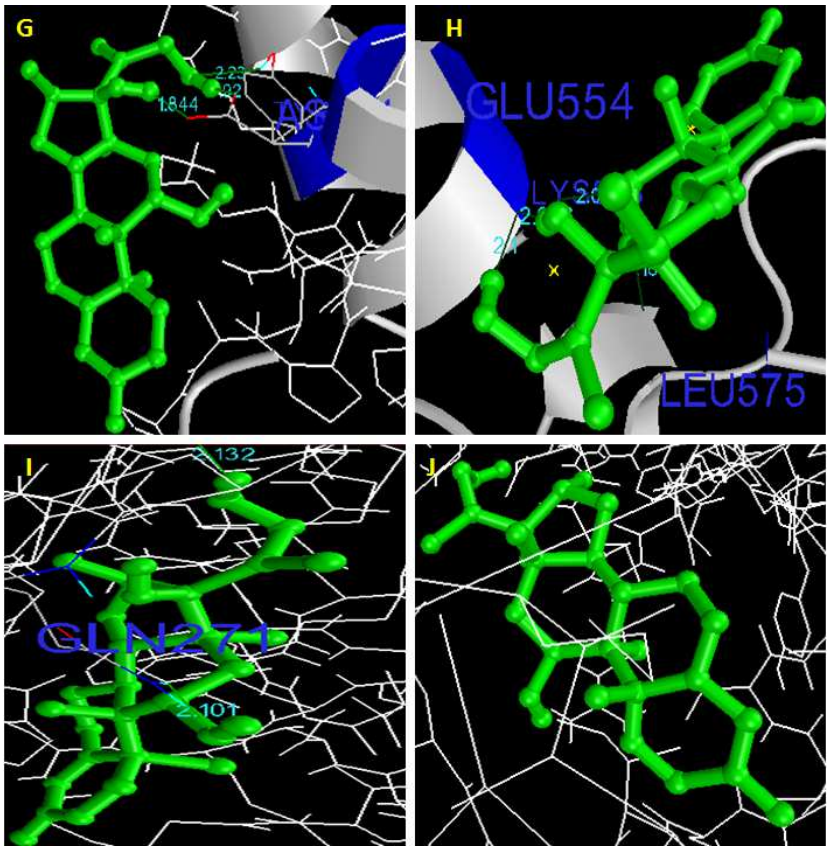

Fig 4: Orientation of dexamethasone with NF-κB of (G) *Rattus norvegicus*, (H) *Pongo abelii*, (I) *Ovis aries* and (J) *Macaca mulatta.*

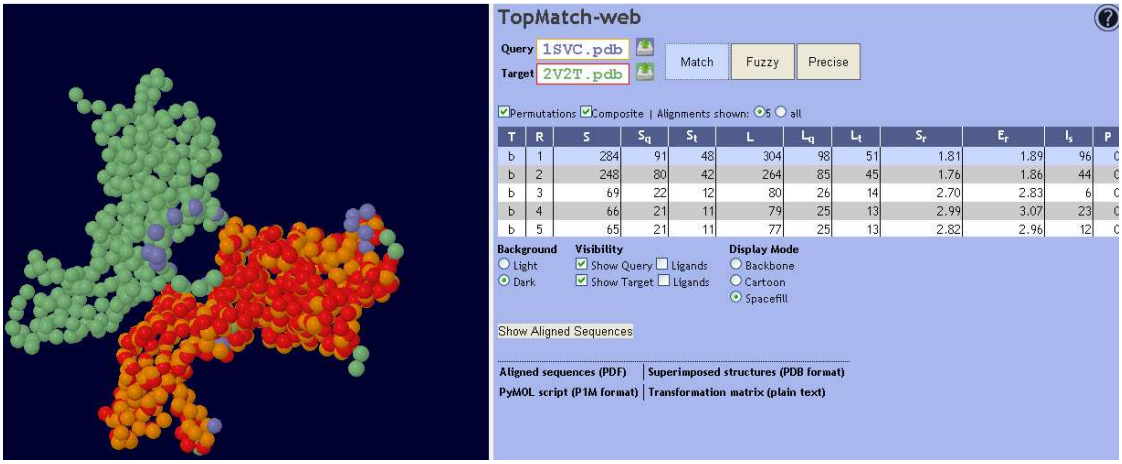

Fig5: Structural comparison of Nf-κb of *Homo sapiens* with that of *Mus musculus* as obtained in TopMatch

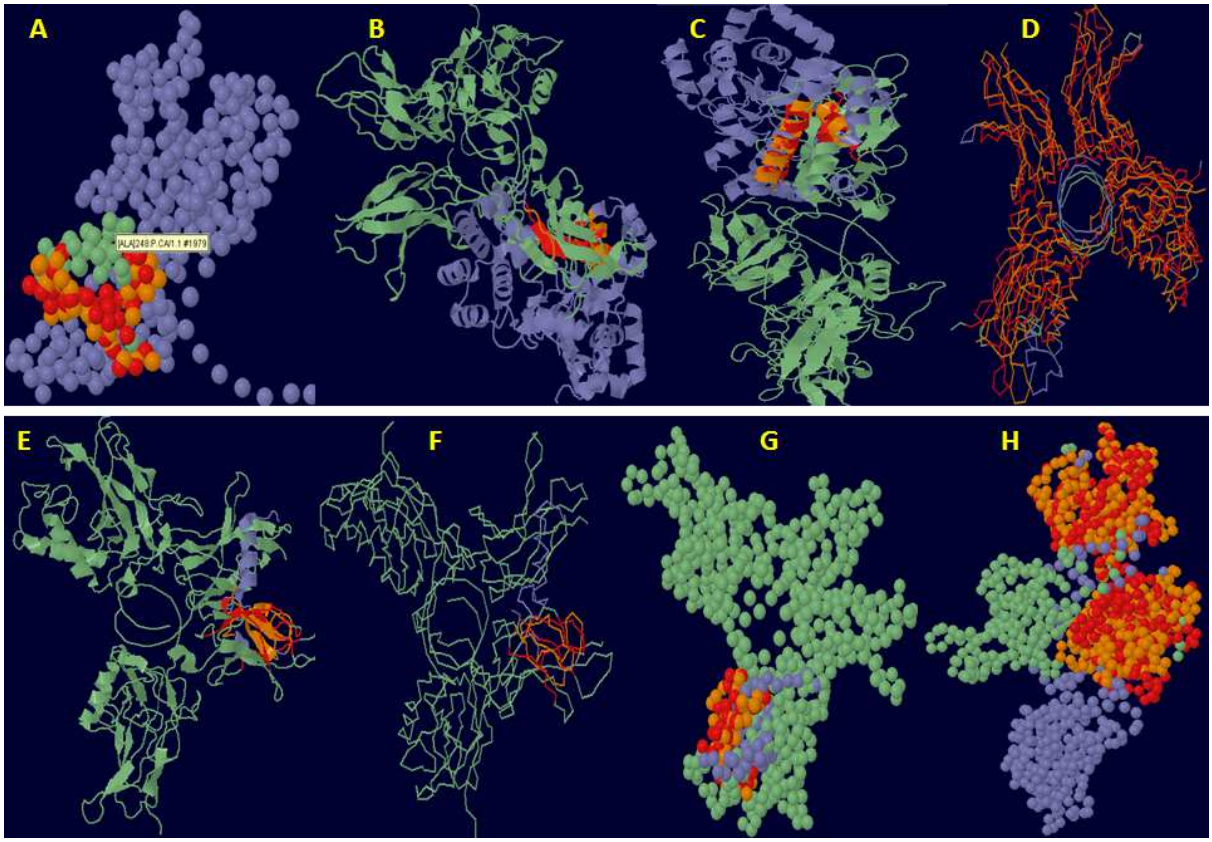

Fig6: Structural comparison of NfKb of *Homo sapiens* with (A) *Bos taurus*; (B) *Ailuropoda melanoleuca*; (C) *Sus scrofa*; (D) *Ovis aries*; (E) *Pongo abelii*; (F) *Macaca mulatta*; (G) *Rattus norvegicus* and (H) *Canis familiaris.*

CONCLUSION

A very large number of experimental data accumulated during the last two decades clearly indicate that NF-kB plays an important role in the development or progression of several human cancers. Researchers established that well-known and widely used agents, such as glucocorticoids, exert their antitumoral activities at least partially through NF-kB inhibition. Meanwhile, clinical trials are being performed with several novel drugs that block the NF-kB activity. In the future, it would be essential to pursue the precise identification of the mechanisms controlling NF-kB target gene expression. Possibly, it will thus be possible to act downstream of the general NF-kB switch, such as the IKK complexes, to target specific groups of regulated genes, hoping that the gain is specificity will not be associated with a loss in efficacy.

REFERENCES

[1] Sabine Olivier; Pierre Robe; Vincent Bours. *Biochemical pharmacology*, **2006**, 72, 1054–1068.
[2] MA Sovak; RE Bellas; DW Kim; GJ Zanieski; AE Rogers; AM Traish. *J Clin Invest*, **1997**,100, 2952–60.
[3] M Karin; Y Cao; FR Greten; ZW Li. *Nat Rev Cancer*, **2002**, 2, 301–10.
[4] A Loercher TL Lee; JL Ricker; A Howard; J Geoghegen; Z Chen. *Cancer Res*, **2004**, 64, 6511–23.
[5] PA Robe; M Bentires-Alj; M Bonif; B Rogister; M Deprez; H Haddada. *Clin Cancer Res*, **2004**, 10, 5595–603.
[6] CY Wang; MW Mayo; AS Baldwin Jr. *Science*, **1996**, 274, 784–7.
[7] CY Wang; JC Cusack Jr; R Liu; AS Baldwin Jr. *Nat Med*, **1999**, 5, 412–7.
[8] M Bentires-Alj; V Barbu; M Fillet; A Chariot; B Relic; N Jacobs. *Oncogene*, **2003**, 22, 90–7.
[9] C Frelin; V Imbert; E Griessinger; AC Peyron; N Rochet; P Philip. *Blood*, **2005**, 105, 804–11.
[10] LA Stark; FV Din; RM Zwacka; MG Dunlop. *Faseb J.* **2001**, 15, 1273–5.
[11] JP Collet; C Sharpe; E Belzile; JF Boivin; J Hanley; L Abenhaim. *Br J Cancer*, **1999**, 81, 62–8.
[12] RS Sandler; S Halabi; JA Baron; S Budinger; E Paskett; R Keresztes. *N Engl J Med*, **2003**, 348, 883–90.
[13] AT Chan; EL Giovannucci; ES Schernhammer; GA Colditz; DJ Hunter; WC Willett. *Ann Intern Med*, **2004**, 140, 157–66.
[14] K De Bosscher; W Vanden Berghe; G Haegeman. *Endocr Rev*, **2003**, 24, 488–522.
[15] N Auphan; JA DiDonato; C Rosette; A Helmberg; M Karin. *Science*, **1995**, 270, 286–90.
[16] RI Scheinman; PC Cogswell; AK Lofquist; AS Baldwin Jr. *Science*, **1995**, 270, 283–6.
[17] RM Nissen; KR Yamamoto. *Genes Dev*, **2000**, 14, 2314–29.
[18] M Kagoshima; T Wilcke; K Ito; L Tsaprouni; PJ Barnes; N Punchard. *Eur J Pharmacol*, **2001**, 429, 327–34.