Design, Synthesis, Docking and 2D QSAR studies of novel 3,5-diaryl Pyrazole Derivatives and their evaluation as Antioxidants and as Immunomodulators, inhibitors of TNF-α, IL-2, IL-6

Dr Dalia Soliman

Assoc. Proff. Of Pharmaceutical Chem.

Egyptain Russian University/AlAzhar University

Cairo, Egypt

Introduction

- **Orally active small molecules** that modify the pro-inflammatory cytokine release associated with many auto-immune disorders such as rheumatoid arthritis (RA) have generated considerable interest in the pharmaceutical industry. They offer a cost-effective and convenient alternative to biologics such as Enbrel, Remicade, Humira and Kineret

- These agents are **expensive, parenterally** administered. They are also under review for increased risk of **cancer**, **infection**, **multiple sclerosis**, and for the potential to induce **neutralizing antibodies** over the long term

- **Tumor necrosis factor-α (TNF-α),** one of the major pro-inflammatory cytokines, has been proven to be a potential target for these agents. TNF-α has been called a sentinel cytokine or “the body's fire alarm”
- The overexpression of TNF-α has been implicated in a number of serious inflammatory disorders such as **rheumatoid arthritis**, **multiple sclerosis**, **inflammatory bowel disease**, **graft-versus-host disease**, and **adult respiratory distress syndrome**.
- TNF-α is a strong inducer of other pro-inflammatory cytokines such as interleukins IL-1, IL-6 and IL-8

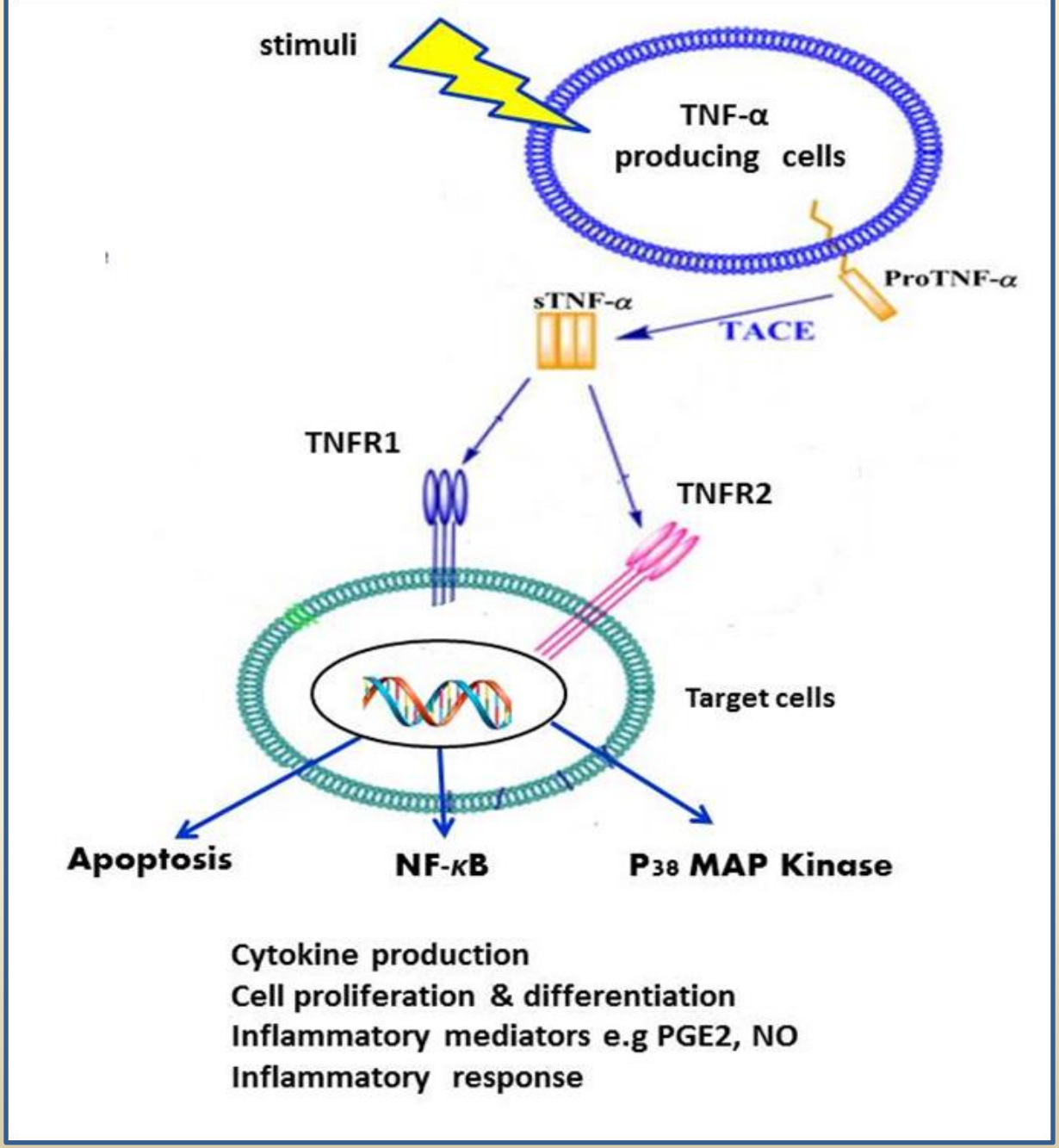


Figure (1): Receptor binding and biological actions of TNF-α ***(Chem Biol Drug Des 2010)***

- **IL-6** is a potent pro-inflammatory agent that plays a crucial role in the pathogenesis of **systemic** inflammatory disease. Targeting this pathway in rheumatoid arthritis (RA) seems an attractive route as IL-6 is important for both **joint destruction** and **systemic manifestations**.
- It promotes inflammatory events through the expansion and activation of **T cells** and the differentiation of **B cells**.
- IL-6 blockade is a major advancement in the treatment of RA as it targets a unique molecule.

- **IL-2** proved to play a pivotal role in regulating immune response, its suppression has been widely used to prevent **allograft rejection** in organ transplantion.
- IL-2 inducible T-cell kinase (ITK) has been found to play an important role in T-cell activation and proliferation, where it is primarily expressed.
- Therefore, ITK represents a novel potential target for anti-inflammatory therapy in a variety of indications such as psoriasis and allergic asthma.

- **P38-α** also known as cytokine-suppressive anti-inflammatory drug binding protein (CSBP), is a member of the **mitogen activated protein** (MAP) kinase family that is involved in stress and inflammatory response signal transduction pathways.

- It is critical for the production and activity of multiple pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, and IL-8, in cells such as macrophages, monocytes, synovial cells, and endothelial cells.

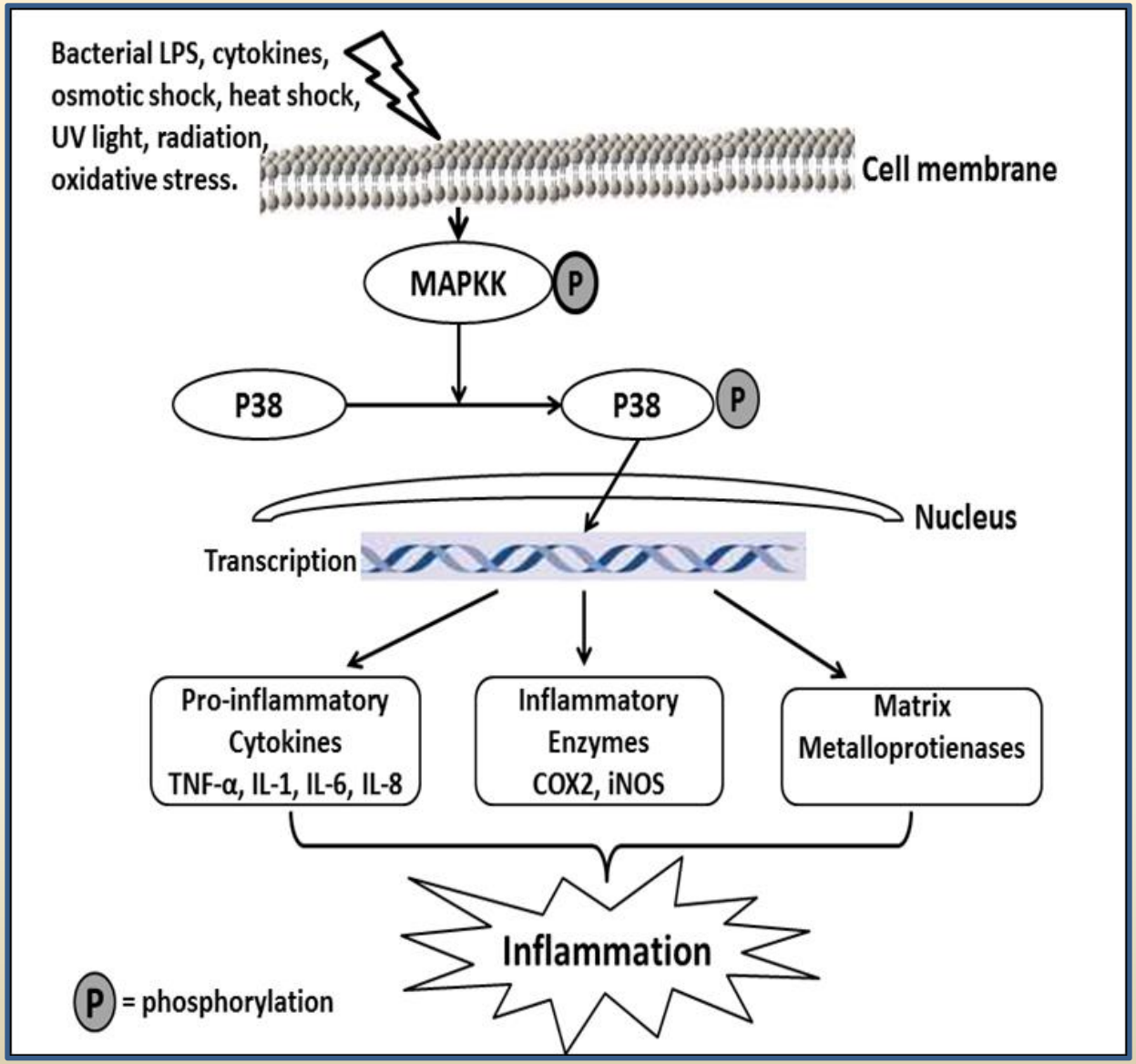
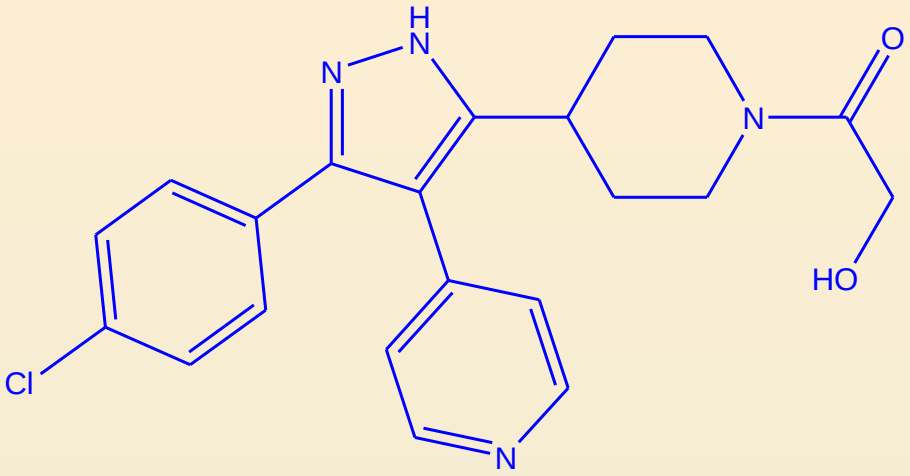


Figure (2): P38 MAPK regulation of inflammation ***(Pharmacol Ther 1999)***

- Therefore, inhibition of these targets has become a major focus of current drug discovery and development in treatment of severe inflammatory disorders.

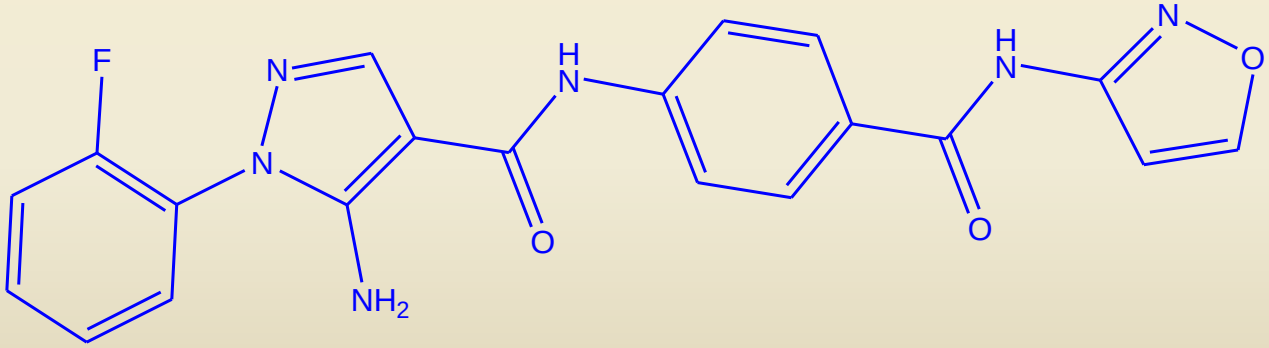
Examples of pyrazole based scaffolds as immunomodulators



Clinical candidate SD0006

Burnette *et al*., Pharmacology (2009)

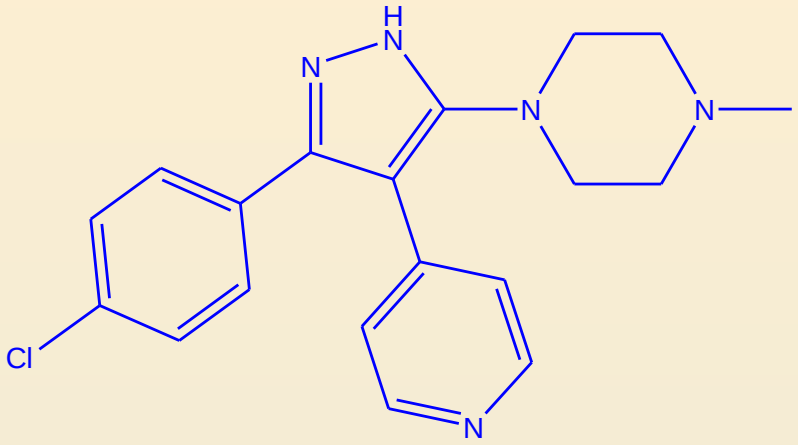
TNF-α = 0.016 µmol/L

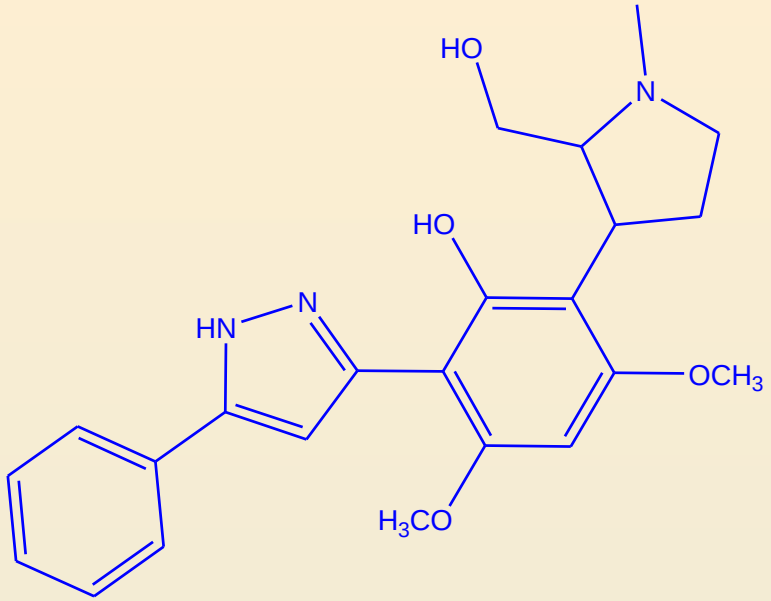


Das *et al*., Bioorg Med Chem (2010)

p38α IC50 = 2 nM

TNF α = 75 %





Pfizer (SC 806) 2003

p38α IC50 = 50 Nm

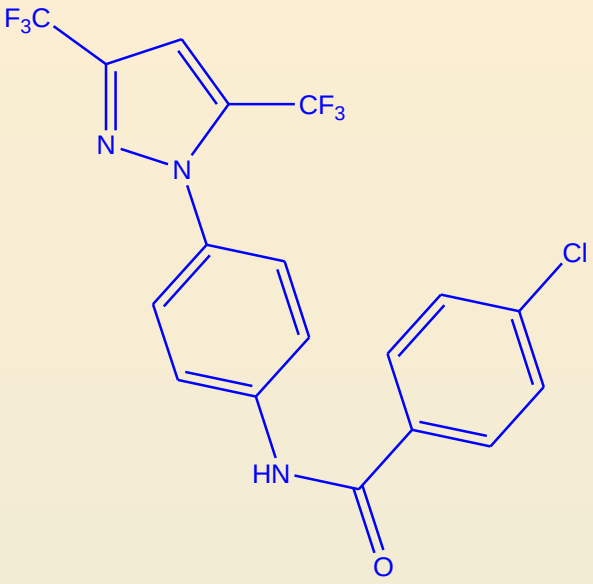
TNF α = 98 %, at 5mg/kg

B. P. Bandgar *et al.* Bioorg. Med. Chem. (2010)

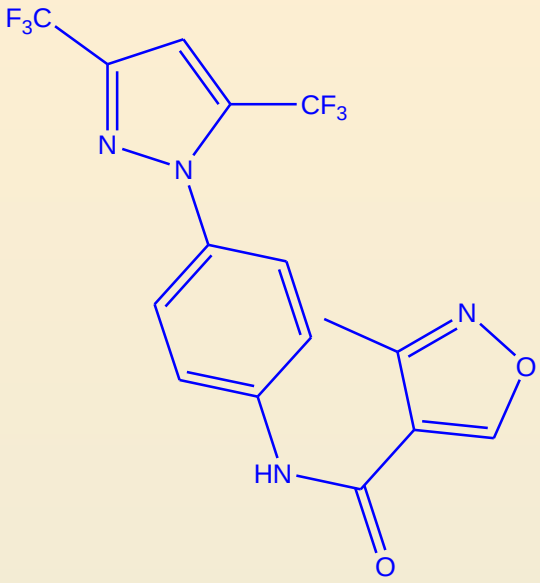
IL-6 = 47%

TNF- α = 24% inhibition at 10μM

IL-2



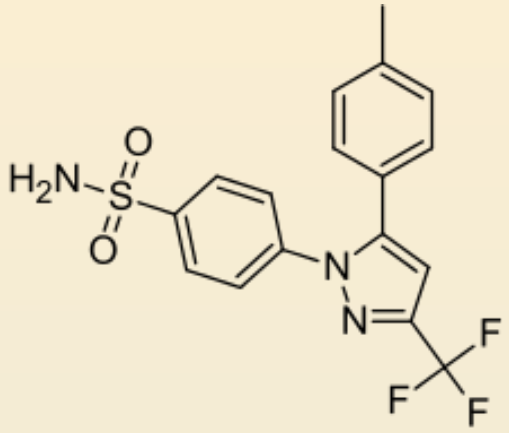
BTP-1= 417 nM



BTP-3 = 314 nM

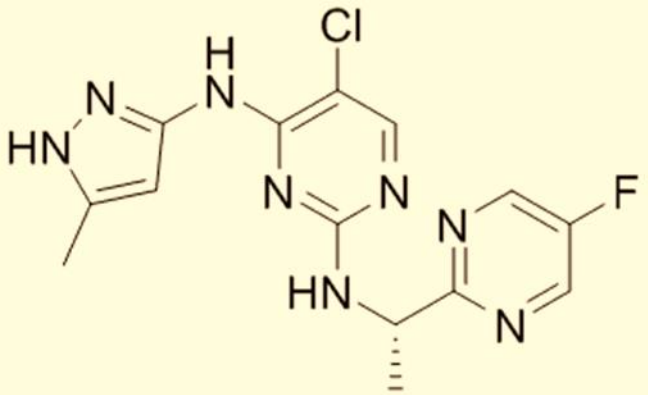
Wu Chen *et al*., Cellular Immunology (2002)

IL-6



Celecoxib

Liu Y *et al.,* Cancer Prev Res (Phil 2011) Wang *et al.,* Oncol Rep (2014)



Scuto et al., Leukemia (2011)

Aim of The Work

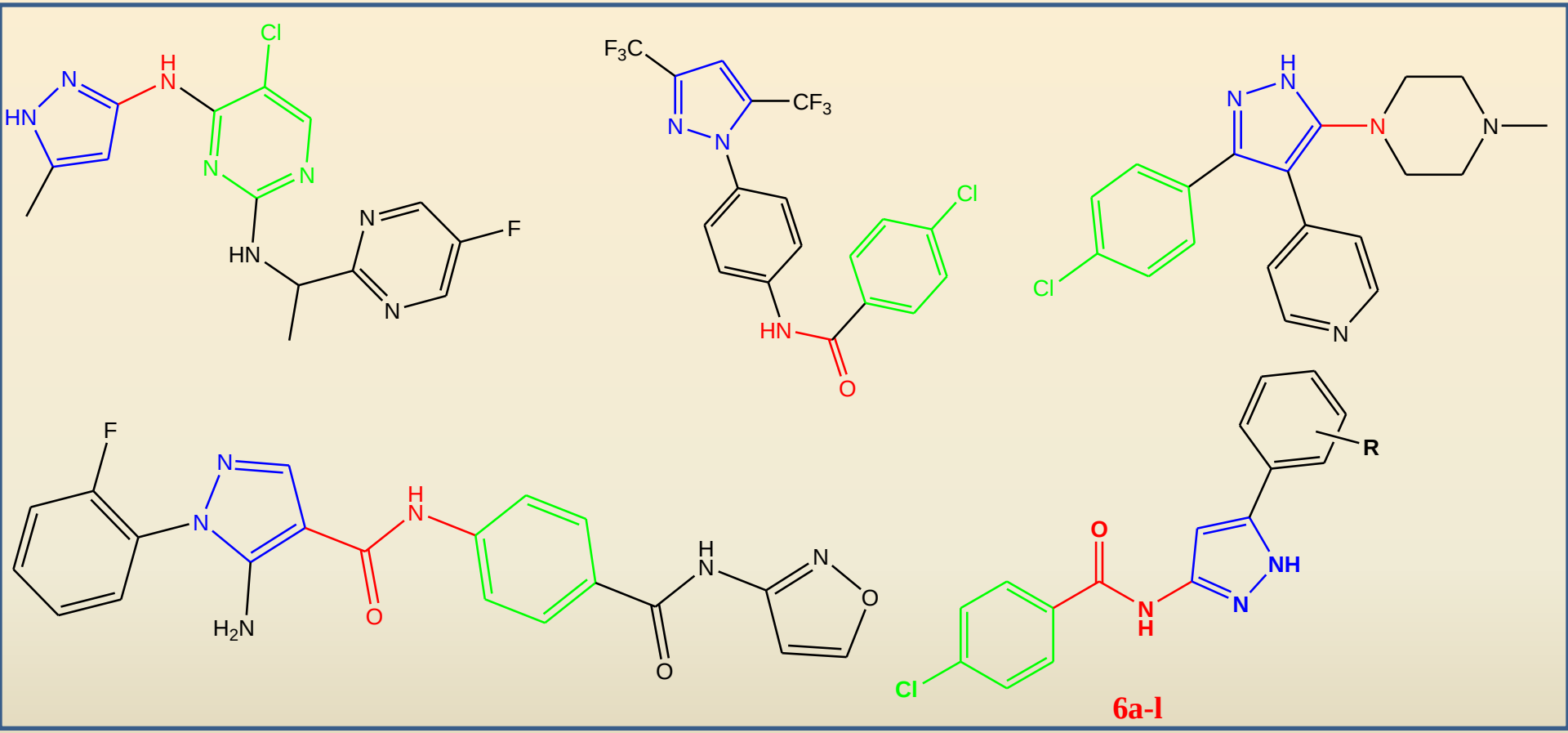
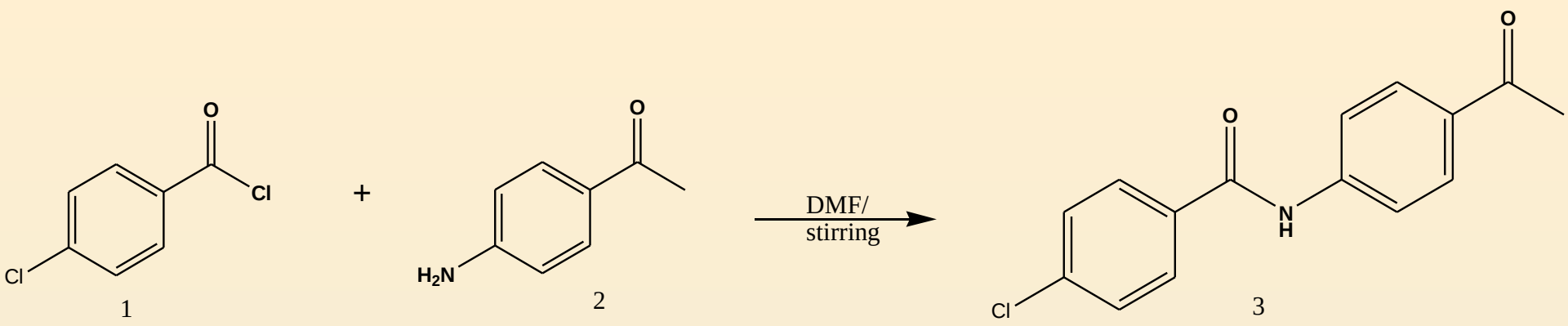
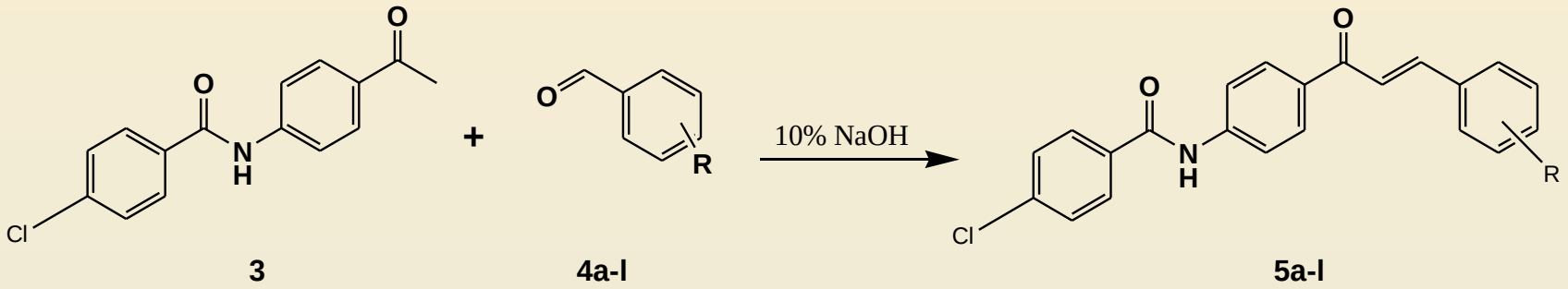


Fig (3): Some representative examples of pyrazole-based cytokine inhibitors and the novel compounds

Synthesis

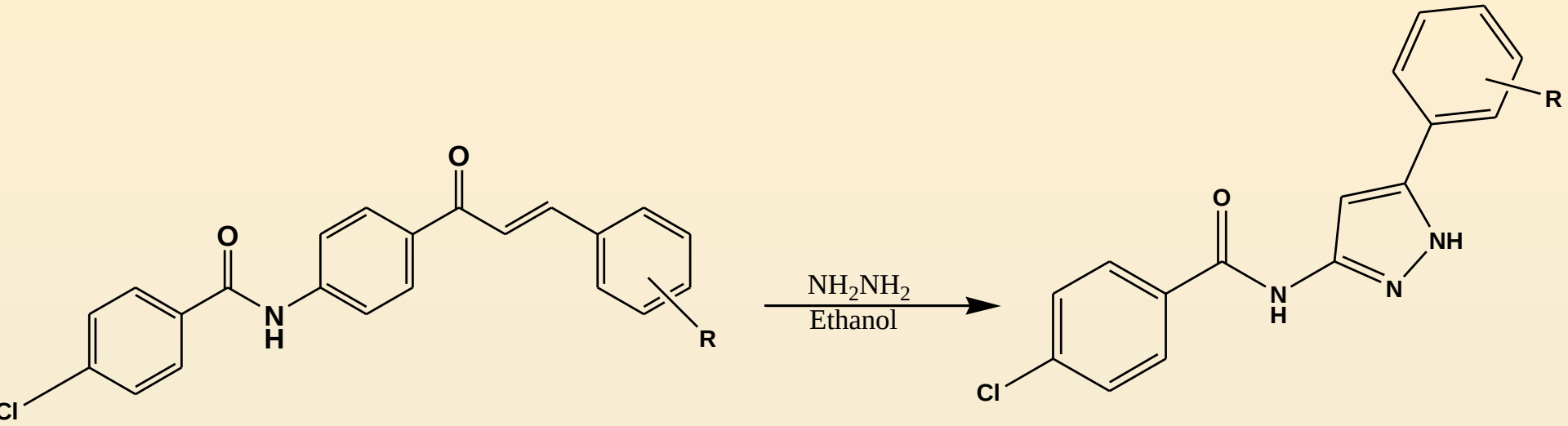


Scheme 1: Synthesis of N-(4-acetyl phenyl) benzamide derivatives



5a R =H
5b R = 4-F
5c R = 2-OH
5d R = 4-OCH_3
5e R =4-CH_3
5f R =3,4,5-tri-OCH_3
5g R =2-OH-3-OCH_3
5h R = Furyl
5i R = 4-Cl
5j R = 2,4-Cl
5k R = 2-Cl
5l R = 2-CH_2CH_3

Scheme 2: Synthesis of N-(4-3-(pheny)-1-prop-2-en-1-one phenyl)benzamide derivatives



5a-l **6a-l**

a R =H
b R = 4-F
c R = 2-OH
d R = 4-OCH_3
e R =4-CH_3
f R =3,4,5-tri-OCH_3
g R =2-OH-3-OCH_3
h R = Furyl
i R = 4-Cl
j R = 2,4-Cl
k R = 2-Cl
l R = 2-CH_2CH_3

Scheme 3: Synthesis of 4-chloro-N-(subs. phenyl-1H-pyrazol-3-)benzamide derivatives

BIOLOGICAL EVALUATION

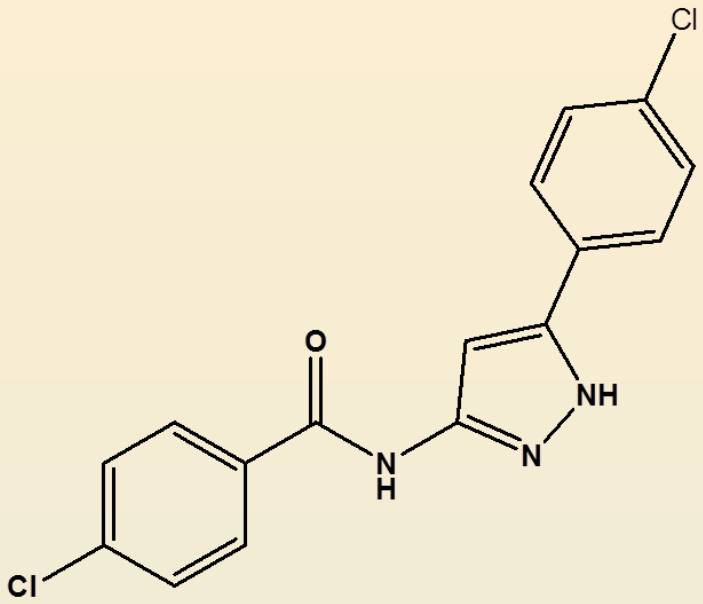
In vivo TNF-α, IL-2, IL-6 Assay In Rat (Acute LPS Model)

- The novel compounds were evaluated for their ability to inhibit LPS-induced production of TNF-α, IL-2 and IL-6 in rat at **30 mg/kg p.o.**

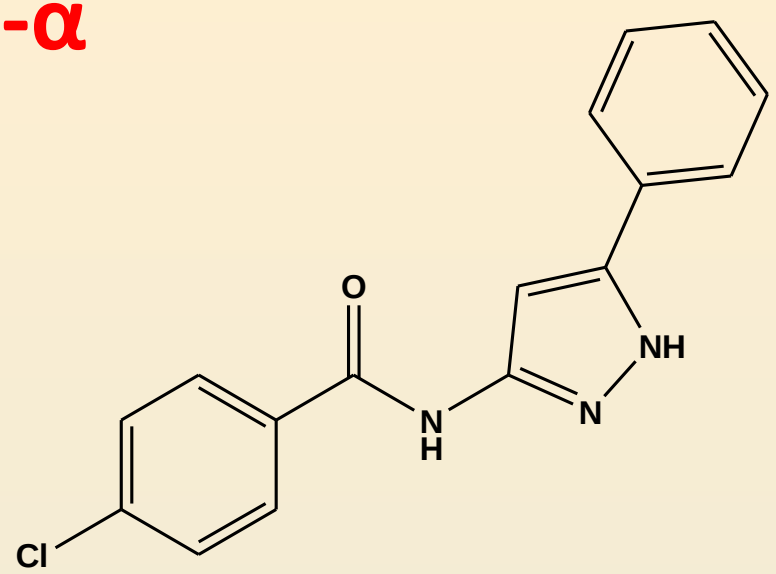
- Enzyme-linked immunosorbent assay kit, life science inc. (E90133Ra), (E90073Ra), (E90079Ra).

- **Dexamethasone** was used as a reference drug.

TNF-α

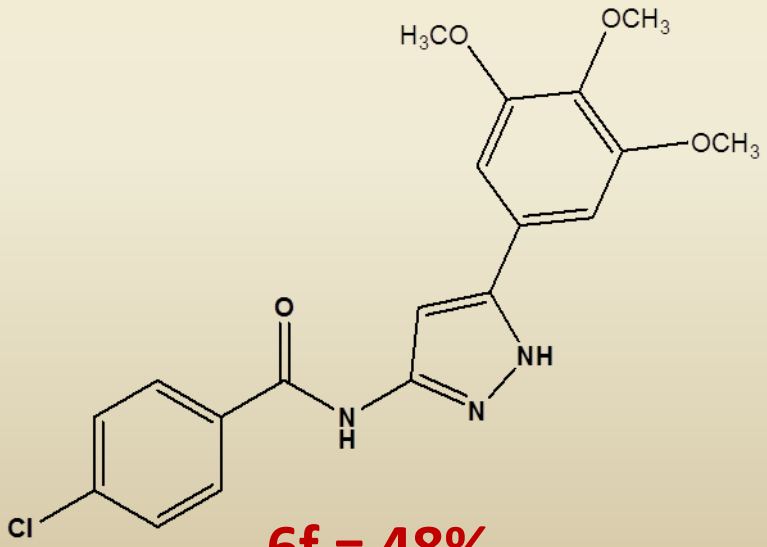


6i = 50%



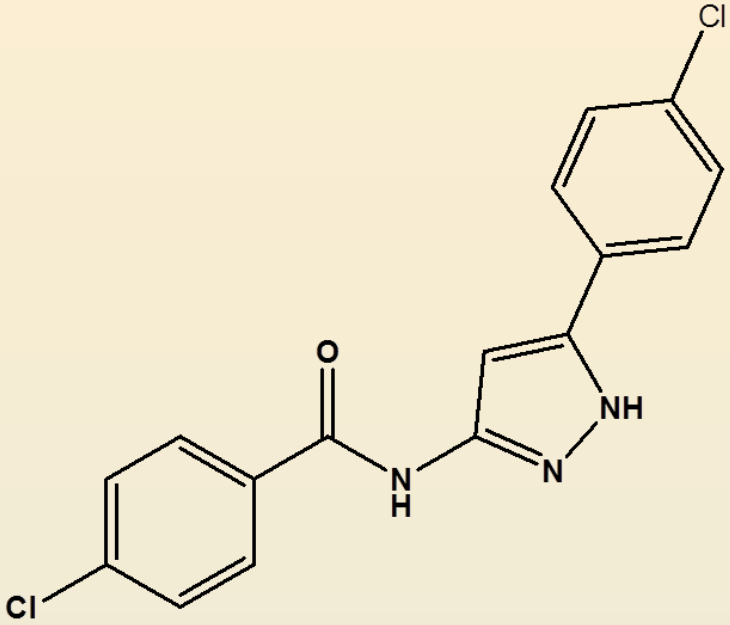
6a = 47%

Dexamethasone = 63%

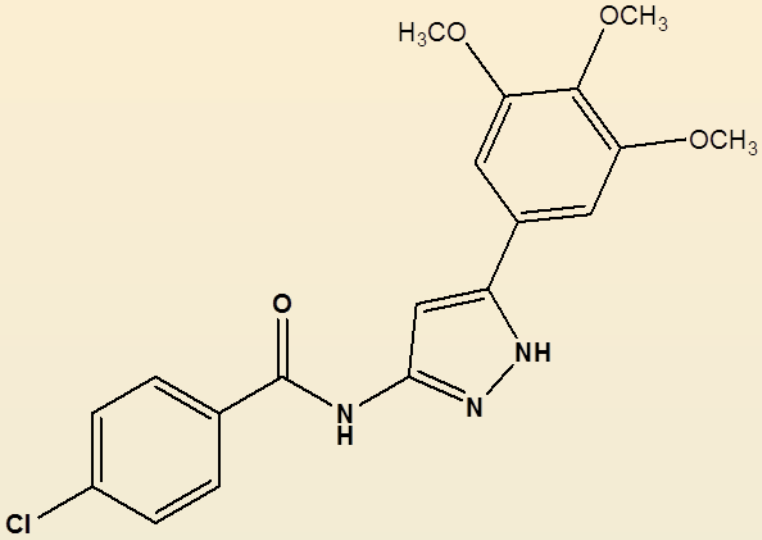


6f = 48%

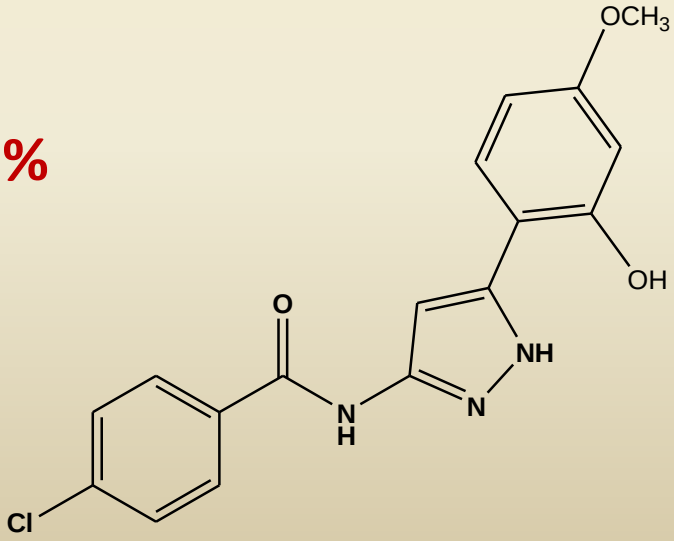
IL-2



6i = 60 %



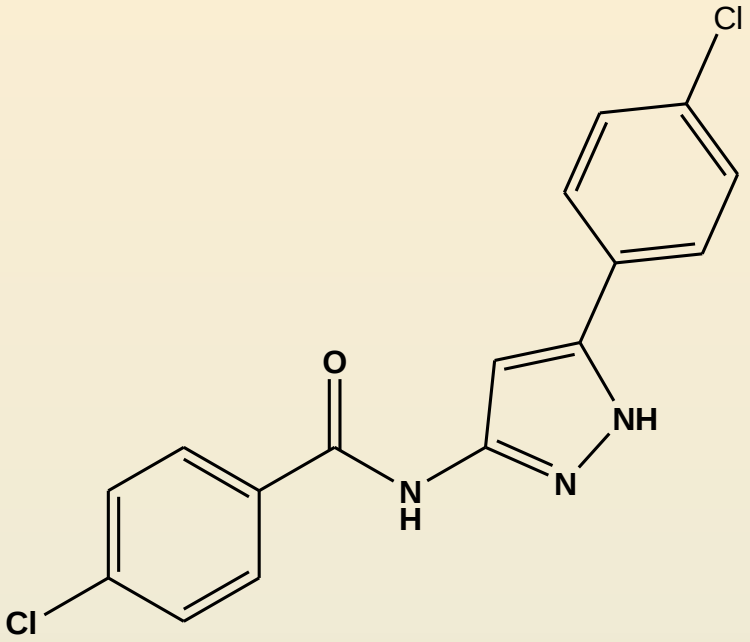
6f = 58 %.



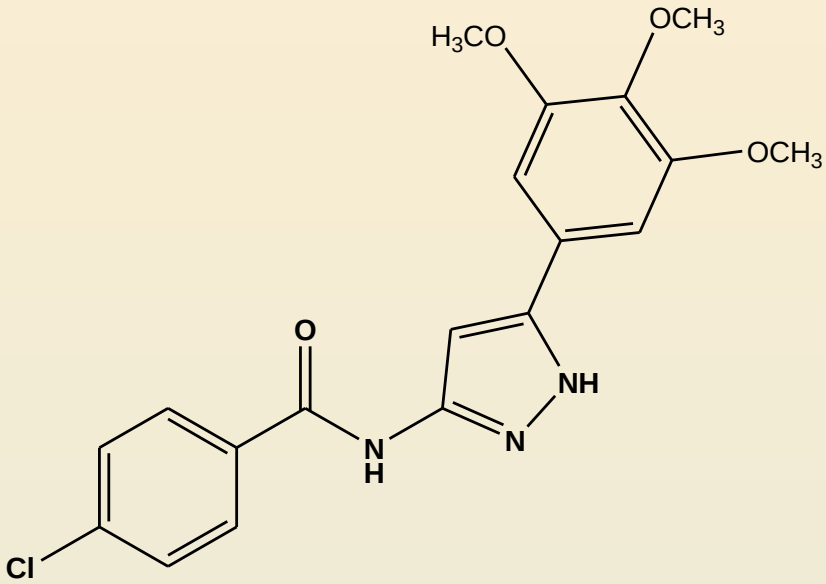
6g = 62 %

Dexamethasone = 66%

IL-6



6i = 45 %

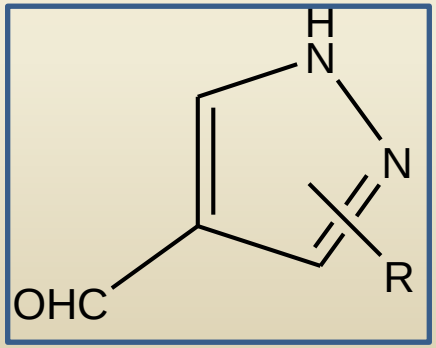


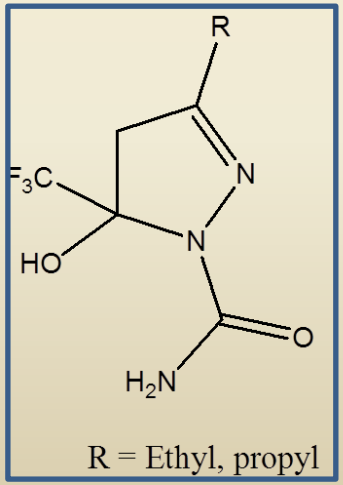
6f = 42 %

Dexamethasone = 57 %

Antioxidant Activities

- Increased generation of reactive oxygen species (ROS) has been observed in **degenerative** diseases. It has been reported that the pyrazole core possesses radical-scavenging ability and even its modulation in inflammatory response was sometimes related to its considerable antioxidant activity.





Tarun *et al.*, International journal of Research in Pharmacy and Science (2012)

- Moreover, it has been reported that certain **antioxidants** reduce LPS-induced inflammation and fever.
- **Glutathione peroxidase** is an important enzyme in cellular antioxidant defense systems, detoxifying peroxides and hydroperoxides.
- If GPX activity is decreased, more hydrogen peroxide is present, which leads to direct tissue damage and activation of nuclear factor-κB–related inflammatory pathways.

- **Superoxide Dismutase (SOD)** is one of the most important antioxidative enzymes.
- It catalyzes the dismutation of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2).

Antioxidant Activities

- **Glutathione Peroxidase** Cellular activity Assay Kit was used to measure GPX.
- **LPS** reduced it by 52%
- **Dexamethasone** 51 %
- **All the tested compounds** reduced GPX by 45-47 %.
- **Superoxide Dismutase** Activity Assay KIT
- **LPS** reduced it by 64%
- **Dexamethasone** 62 %
- **All the tested compounds** reduced SOD in the range 57-52 %
- **Finally, Compound 6i** reduced the enzyme by 57%.

2D QSAR Studies

- **Development of QSAR Models**
- QSAR analyses for inhibitory activities of the synthesized pyrazole derivatives against **TNF-α, IL-2** and **IL-6** were performed in order to determine the crucial factors governing this activity. The analysis was run by means of the DS 2.5 software (Discovery Studio 2.5, Accelrys, Co., Ltd., San Diego, CA, USA).
- **Training set** was prepared from the synthesized compounds with their measured pIC50s
- "**Calculate Molecular Properties**" module was used for calculating different molecular properties for the training set compounds

- **Genetic function approximation** (GFA) was utilized to search for the **best** possible QSAR regression equation capable of correlating the variations in the biological activities of the training compounds with variations in the generated descriptors
- **multiple linear regression modeling** (MLR)
- **QSAR model was validated** employing leave **one-out cross-validation**, r_2 (squared correlation coefficient value) and r_2 prediction (predictive squared correlation coefficient value), **residuals** between the predicted and experimental activity of the **test** set and **training** set

TNF-α

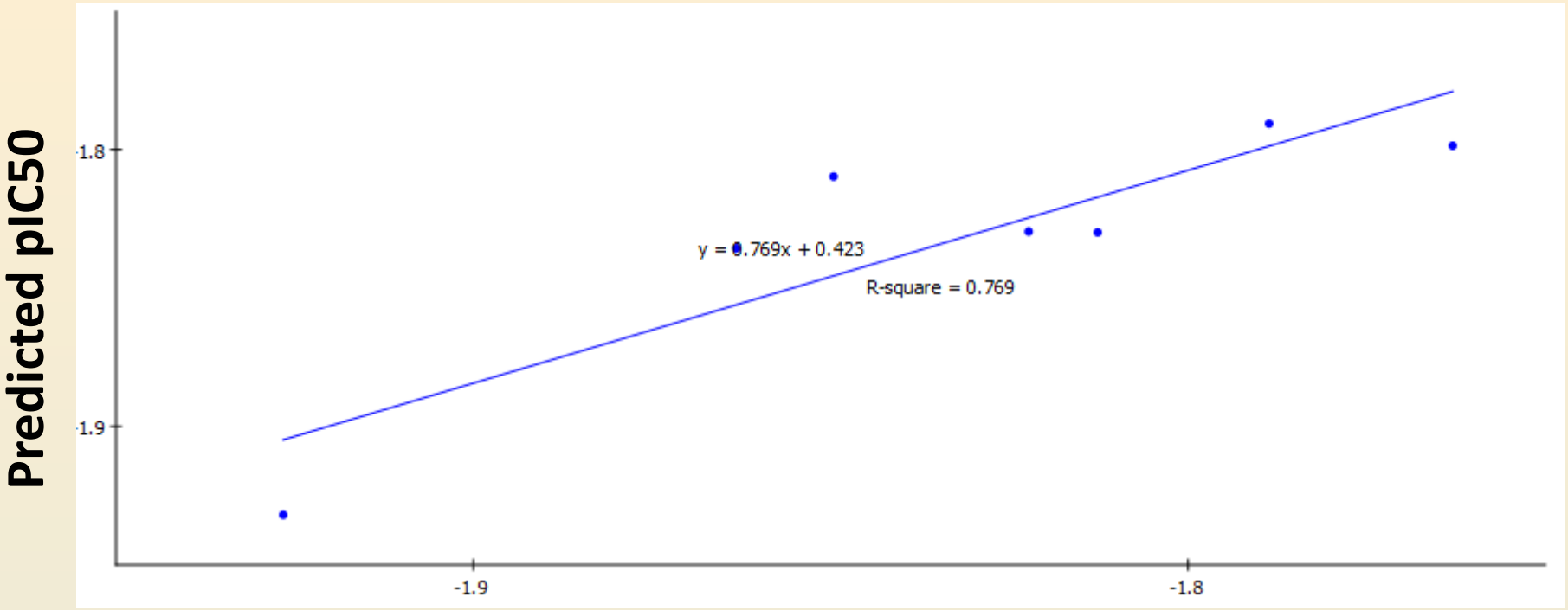


Fig (4) Predicted versus experimental PIC50 of the tested compounds against TNF-α according to equation **1** r^2 = 0.769, r^2 **(prediction)** = 0.654, **Least square error** = 0.000572

Equation (1) representing the best performing QSAR model for the activity against TNF-α:

-logIC50 = -1.42953 – 0.16598 **SC_3_C** – 0.0033134 **Jurs_WNSA_2**.

IL-2

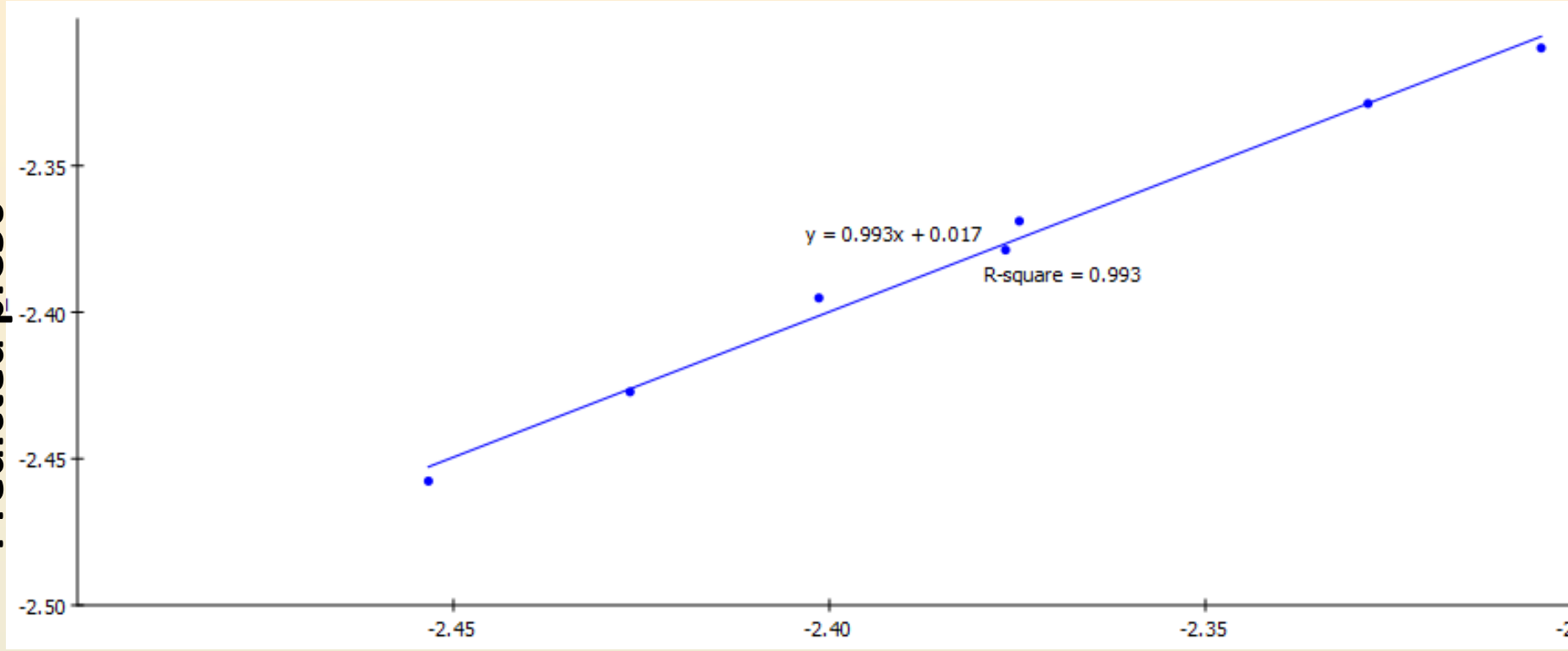


Fig (5): Predicted versus experimental pIC50 of the tested compounds against IL-2 according to equation 2 $r^2 = 0.993$, r^2 (prediction) = 0.892, Least square error = 0.0054

Equation (2) = -2.2525 + 0.00049194 **PMI_Y** − 0.0039522 **Molecular_Volume**

IL-6

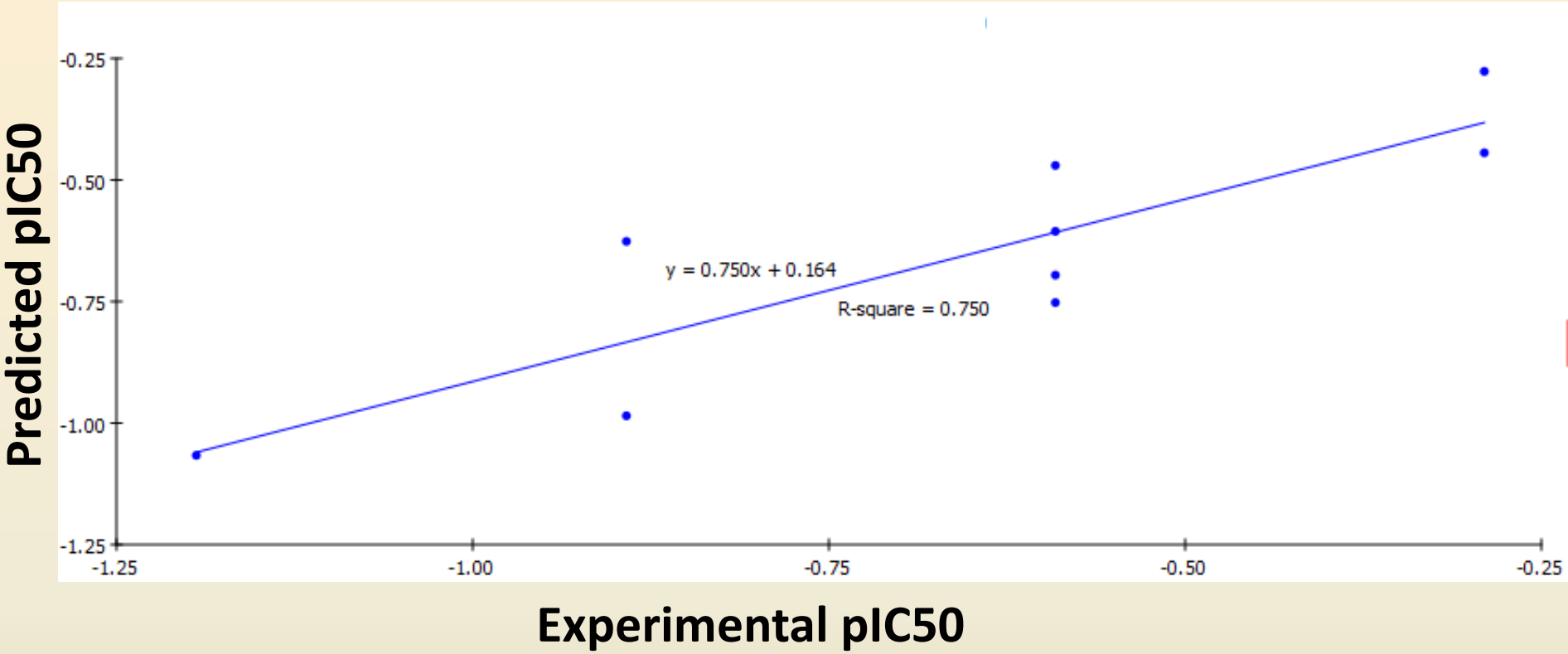


Fig (6) Predicted versus experimental pIC50 of the tested compounds against IL-6 according to equation 3 $\mathbf{r^2}$ = 0.750, r^2 (prediction) = 60, Least square error = 0.019

Equation (3) = -2.0117 − 0.36899 **Kappa_3_AM** + 0.00066893 **PMI_Y**

Docking Studies

IL-2

Schematic representation of important interactions between SB 203580 and ITK.

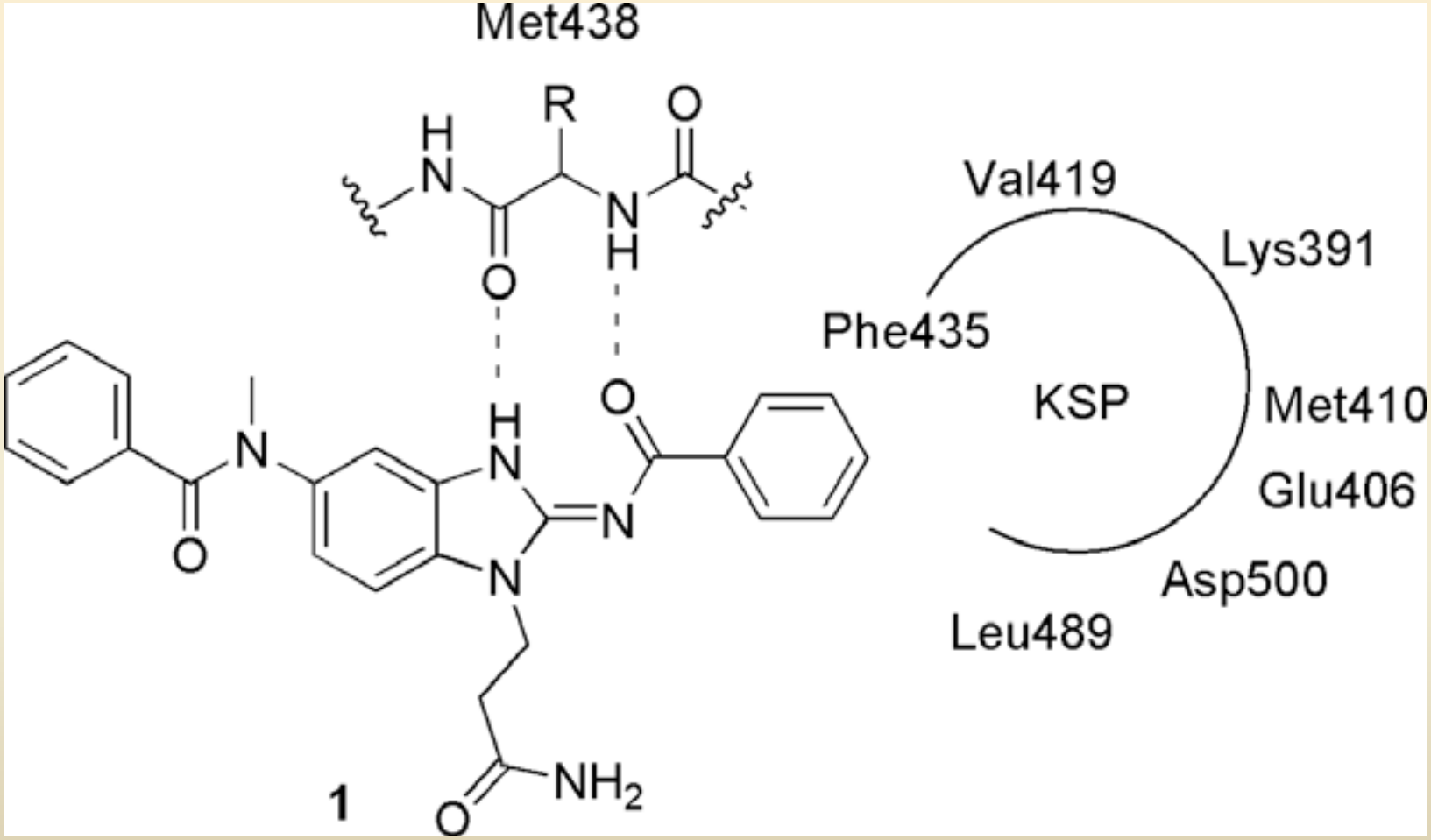


Fig (7). The structure and binding mode into the active site of ITK

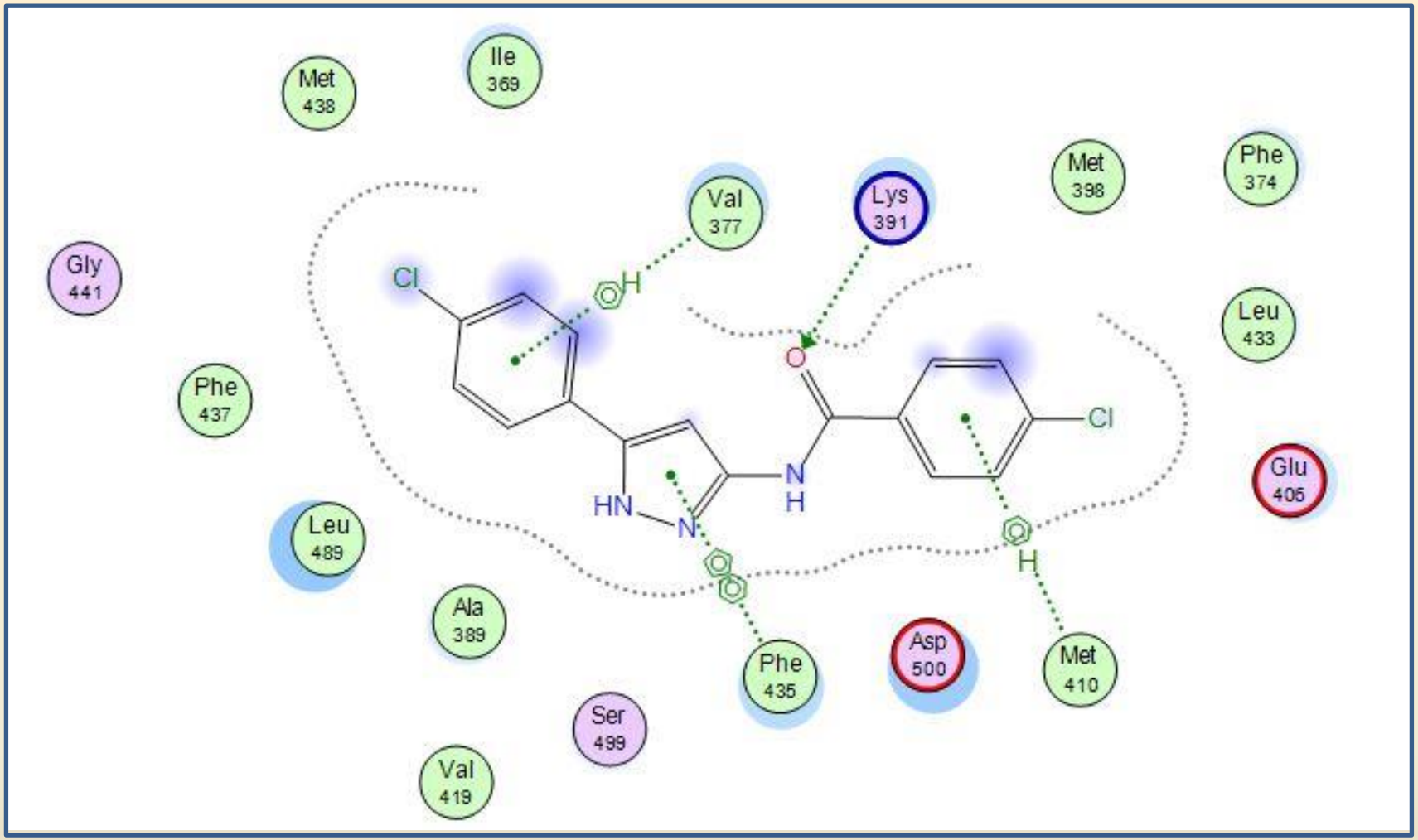


Fig (8) : Binding interactions of **6i** into the active site of ITK (PDB ID: 1SM2).The important amino acid residues are shown together with their respective number.

S =-11.721, E-Conf = -6.627 Kcal/mol, RMSD = 1.014

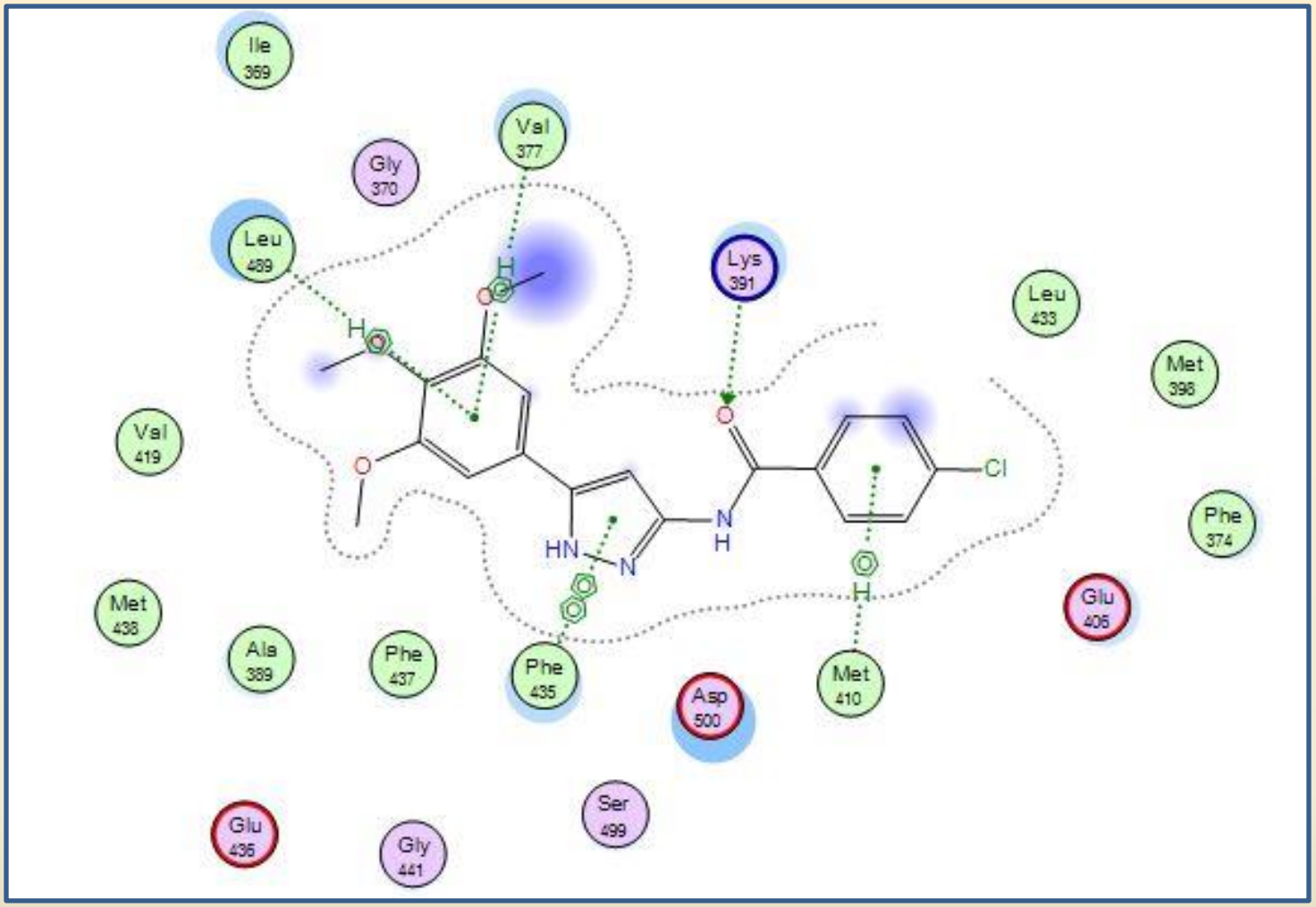


Fig (9). Binding mode of **6f** into the active site of ITK (PDB ID: 1SM2). The important amino acid residues are shown together with their respective number.

S = -13.221, E-Conf = -3.626 Kcal/mol, RMSD = 1.26

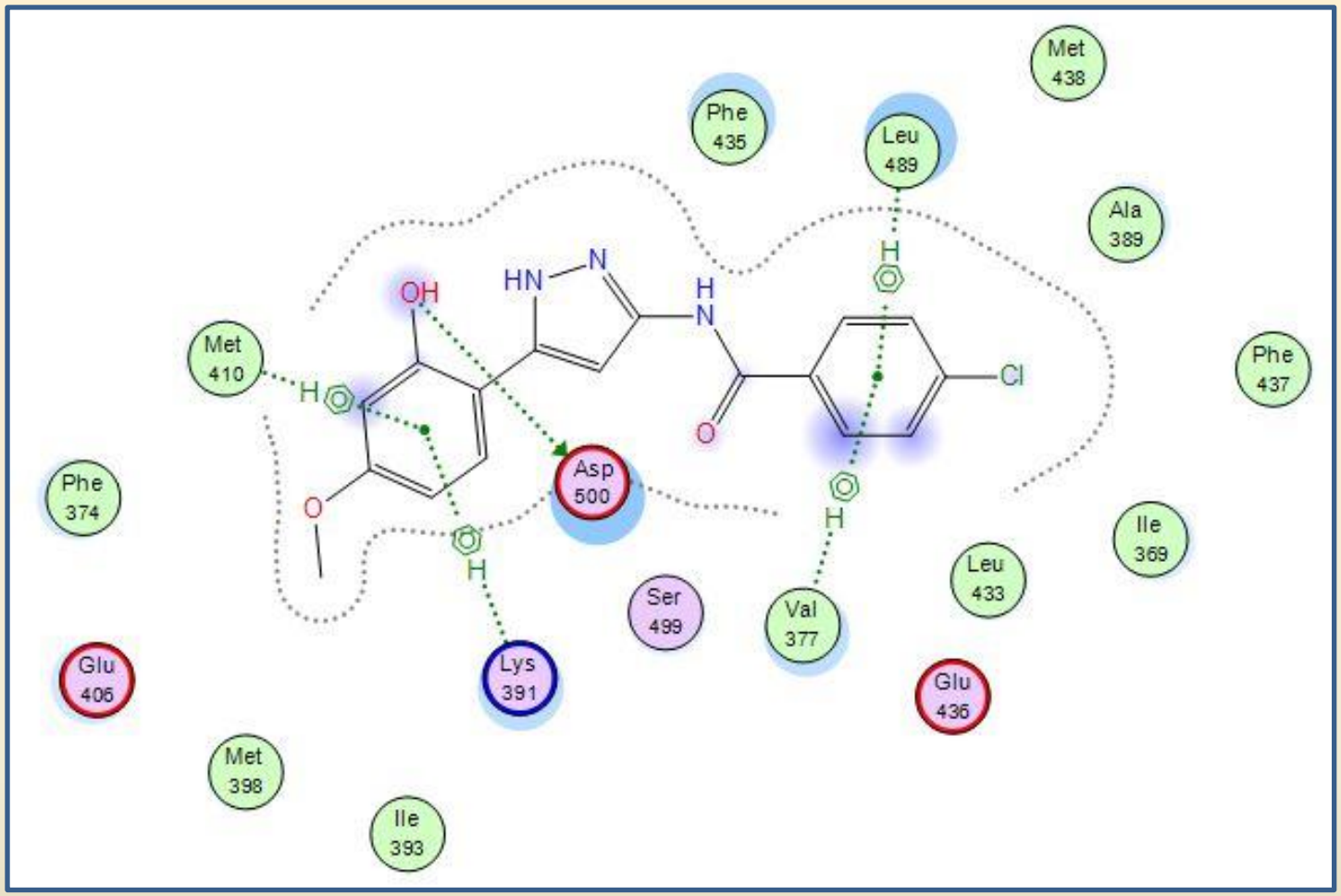
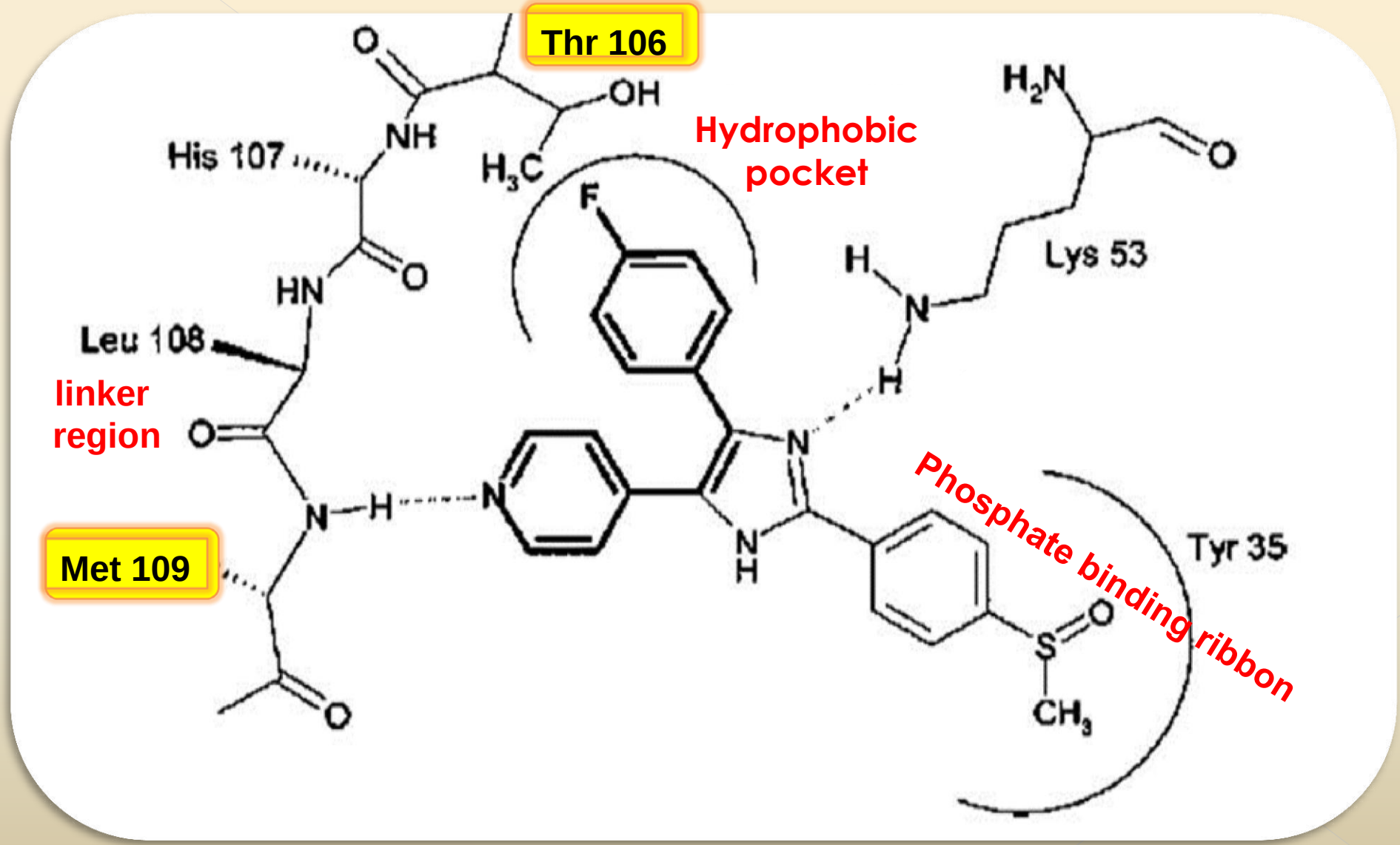


Fig (10): Binding mode of **6f** into the active site of ITK (PDB ID: 1SM2). The important amino acid residues are shown together with their respective number.

S = -12.991, E-Conf = -5.442 Kcal/mol, RMSD = 1.454

TNF-α

Schematic representation of important interactions between SB 203580 and p38a.



Med Res Rev. 2006, 26, 1-62.

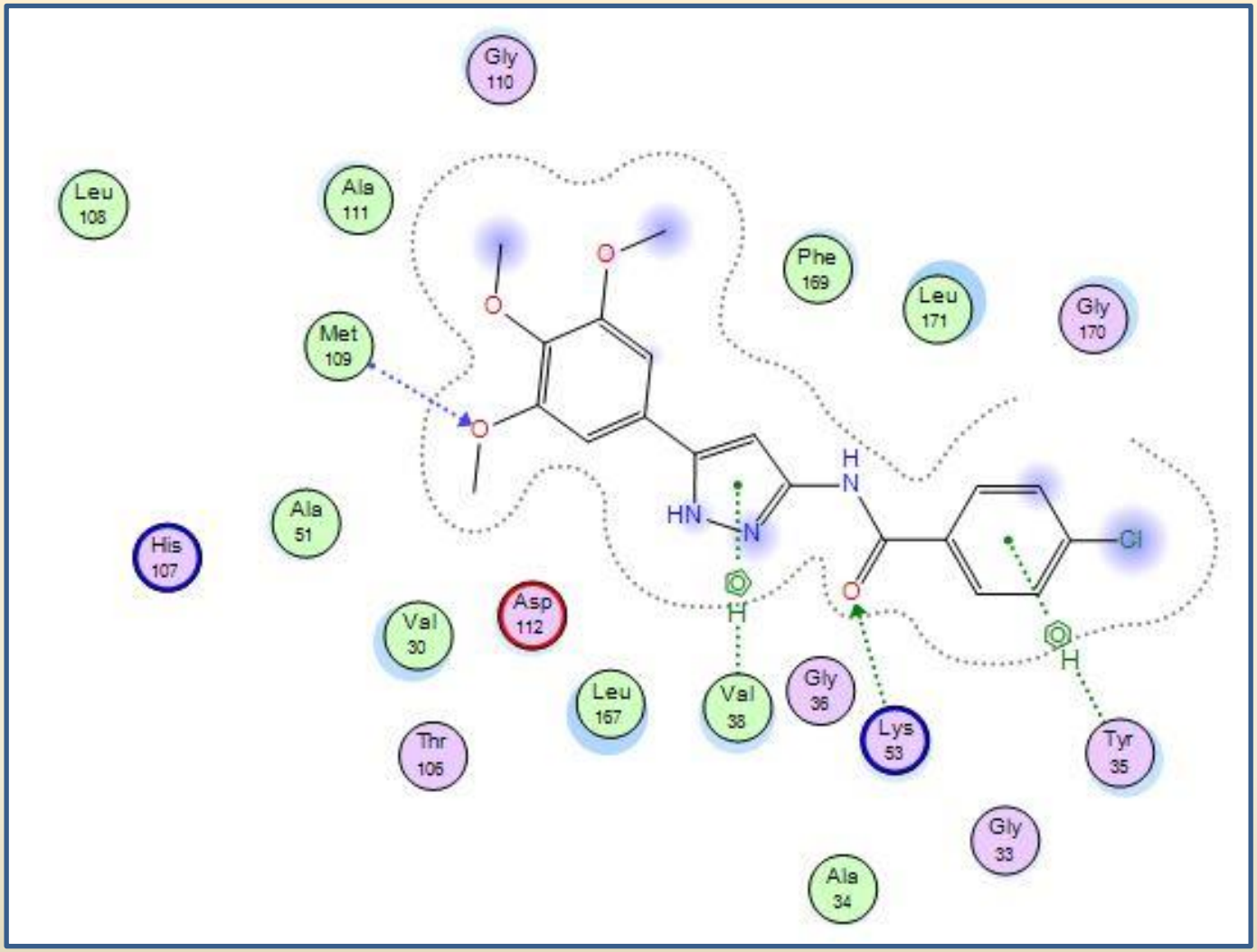


Fig (11): Binding interactions of **6f** into the active site of p38α (PDB ID: 1GM2). The important amino acid residues are shown together with their respective number.

S = -13.59, E-Conf = -4.842 Kcal/mol, RMSD = 0.843

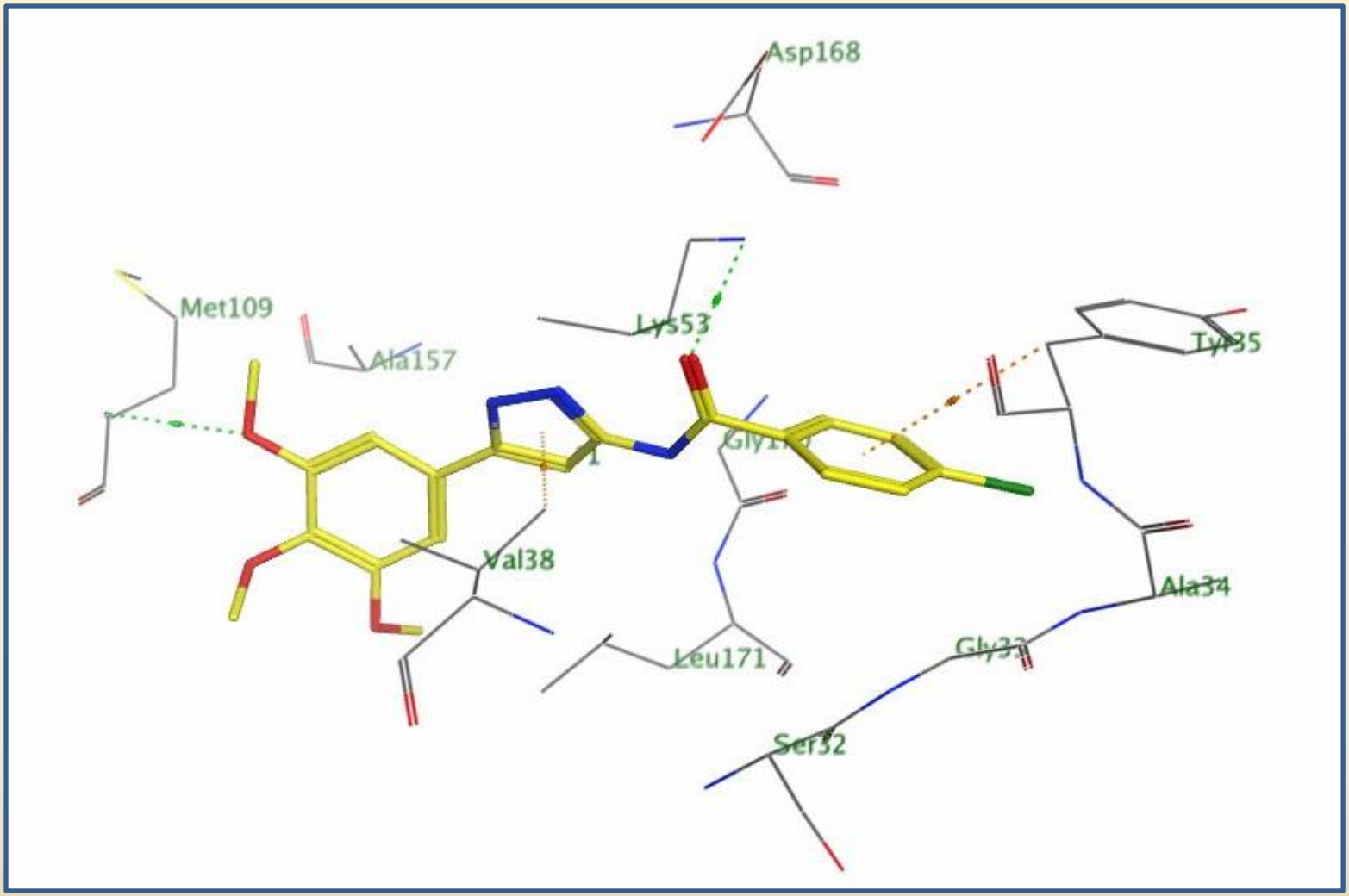


Fig (12): 3D representation of binding interactions of **6f** within the active site of p38α (PDB ID: 1GM2). The important amino acid residues are shown together with their respective number.

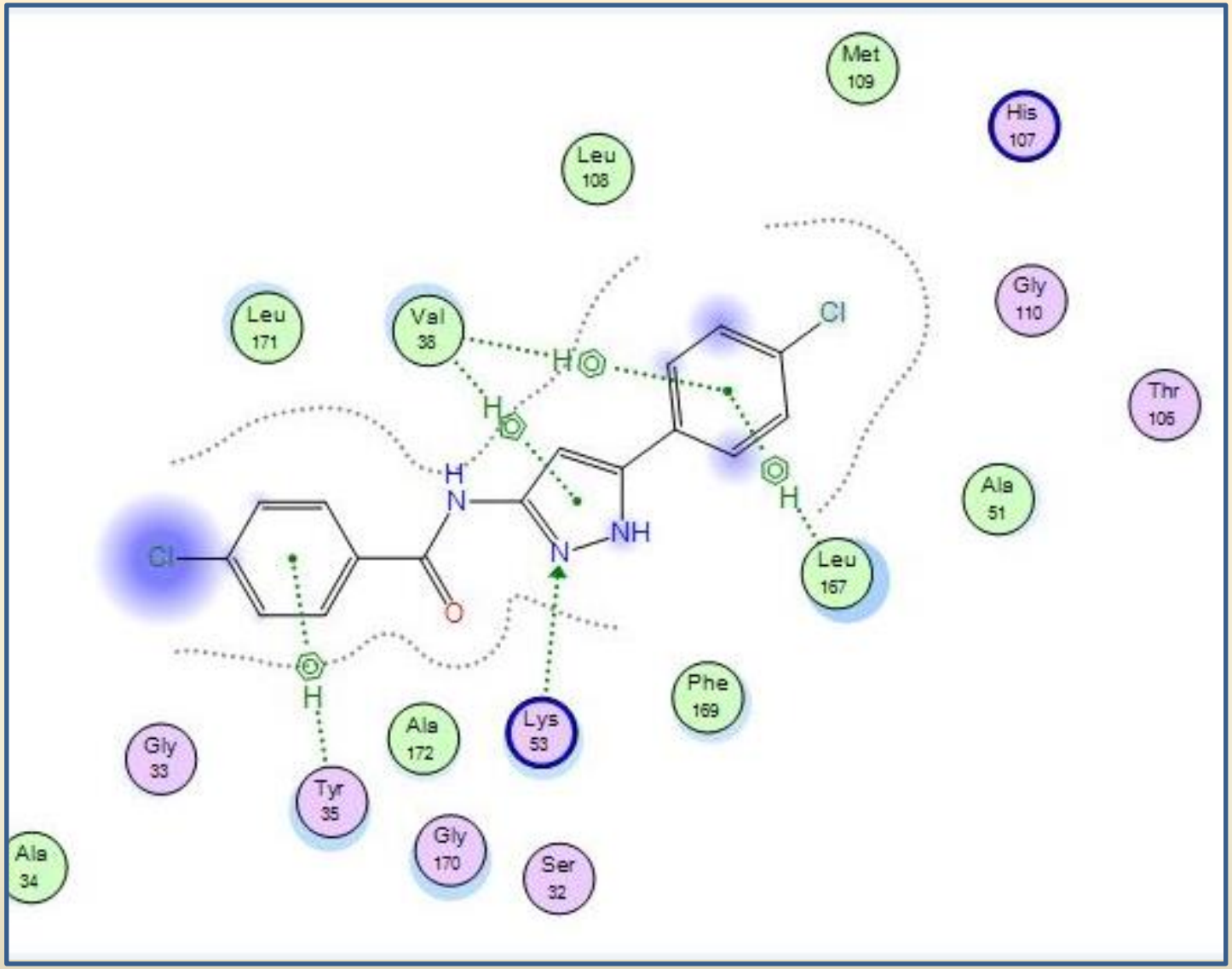


Fig (13): Binding interactions of **6i** into the active site of p38α (PDB ID: 1GM2). The important amino acid residues are shown together with their respective number.

S = -11.650, E-Conf = -3.842 Kcal/mol, RMSD = 2.23

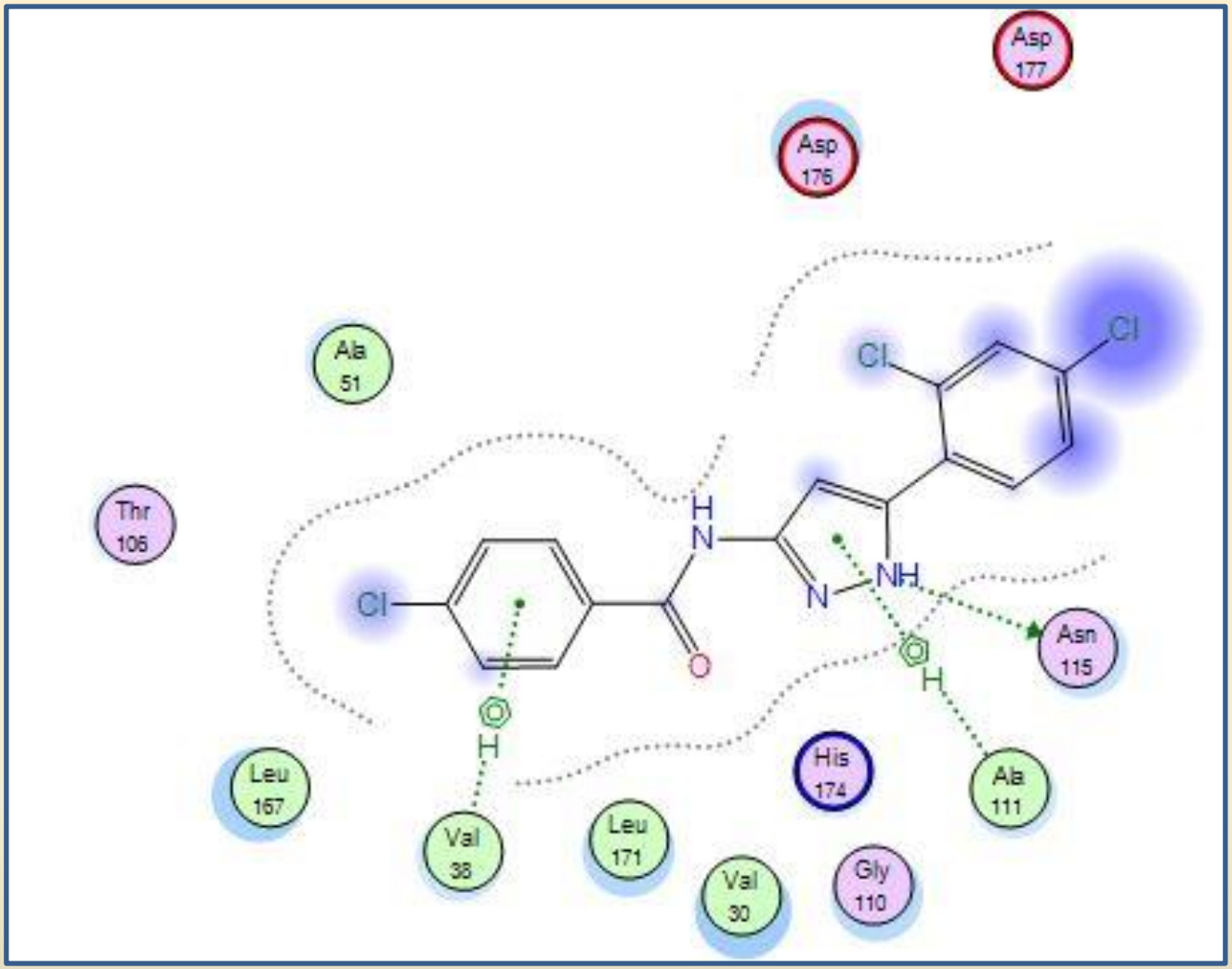


Fig (14): Binding interactions of **6j** into the active site of p38α (PDB ID: 1GM2). The important amino acid residues are shown together with their respective number.

S = -11.897, E-Conf = 1.032, Kcal/mol, RMSD = 1.762

-

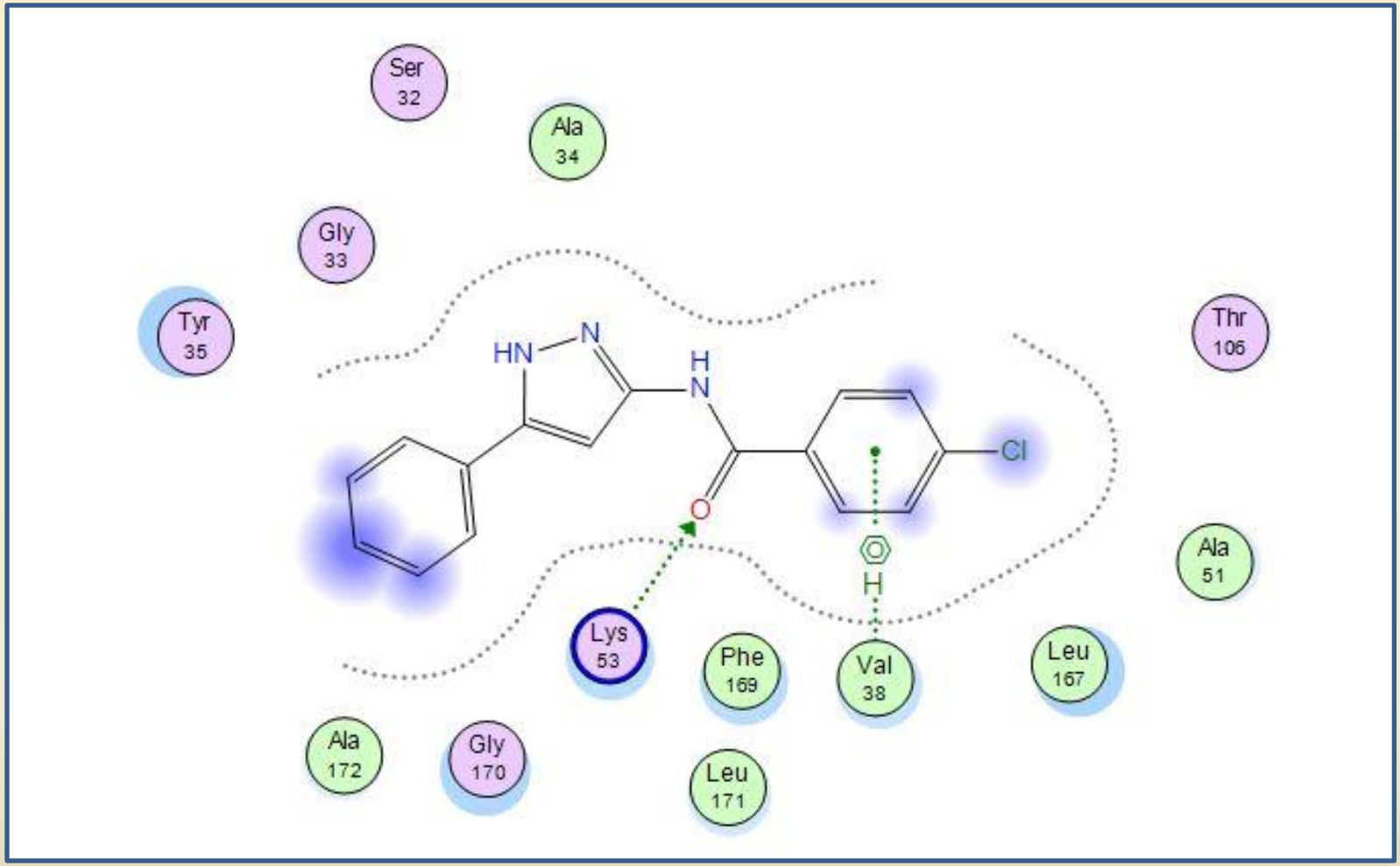


Fig (15): Binding interactions of **6a** into the active site of p38α (PDB ID: 1GM2). The important amino acid residues are shown together with their respective number.

S = -13.11, E-Conf = 1.184, Kcal/mol, RMSD = 1.184

-

Conclusions

- In an attempt to generate 3,5-diaryl pyrazoles as immunomodularors through inhibition of multiple pro-inflammatory cytokines such as TNF-α, IL-2, and IL-6 novel derivatives were synthesized and evaluated against these cytokines.
- Compounds **6i** and **6f** demonstrated significant inhibitory activities against the three cytokines compared to the reference dexamethasone.
- A 2D QSAR model was generated for each of these activities where the models were characterized by high correlation coefficient values and good predictive ability.
- The biological results were in agreement with docking scores and binding interactions into the active sites of the enzymes.

Synthesis: Al-Azhar University, Pharmaceutical Chemistry Department.

Biological evaluation: Biochemistry lab at Animal Reproduction Institue.

In Silico studies: were performed on MOE.10 and DS 2.5 softwares.

Thank you