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## DISCOVERY OF PYRAZOLOPYRIDINE DERIVATIVES DUALY TARGETING INFLAMMATION AND PROLIFERATION IN COLORECTAL CANCER CELL LINES: IN-SILICO DRUG DESIGN APPROACH

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## DISCOVERY OF PYRAZOLOPYRIDINE DERIVATIVES DUALY TARGETING INFLAMMATION AND PROLIFERATION IN COLORECTAL CANCER CELL LINES: IN-SILICO DRUG DESIGN APPROACH

### Abstract

Elimination of inflammation represents a promising strategy for cancer prevention and treatment since cancer and inflammation are related. The combined use of anti-inflammatory agents and cancer therapy is a focal point. In this frame, pyrazolopyridine derivatives DZ-BAU2021-6 and DZ-BAU2021-14, developed in BAU Labs, having promising anti-proliferative activity on colon cancer cells HCT-116 and HT-29 with notable IC<sub>50</sub> values and remarkable CDK2 inhibitory effect, were *in-silico* tested. As an approach to dual anti-inflammatory anticancer potential, their binding modes and energies on the active site of crystalline structure of CDK2 and COX2, (1HCK and 3LN1), respectively were explored. Their physicochemical and pharmacokinetic properties as well as their “drug-likeness” were studied. Computational results declared that DZ-BAU2021-6 and DZ-BAU2021-14 exhibited high binding affinities to CDK2 and COX2 receptors. DZ-BAU2021-14 exhibited lower levels of estimated binding energies with COX2 receptor compared to Celecoxib. It demonstrated high GI absorption, low interference with P-glycoprotein and cytochrome P450 isoforms.

### Keywords

In-silico drug design, colorectal cancer, anti-inflammatory, antiproliferative

## 1. INTRODUCTION

Cancer is a major public health problem and the second leading cause of death worldwide after cardiovascular diseases. Cancer is a group of malignant neoplasms that can affect any part of the body; its liability is rising globally with an estimated 18 million new cases and 10 million deaths every year. Cancer incidence is rapidly rising in all countries, and projected to increase to 30 million by 2040 (World Health Organization, 2019). There are still too many cancer affected deaths that can be prevented by early diagnosis and effective treatment (Shamseddine *et al.*, 2014). Colorectal cancer (CRC) is the fourth world's deadly cancer showing more than 900,000 deaths yearly (Siegel *et al.*, 2019).

Protein kinases represent a large family of enzymes essential for the regulation of diverse cell functions through phosphorylation of structural proteins and enzymes regulating cell division. Cyclin-dependent kinases (CDKs) are members of serine/threonine protein kinase family that have been implicated as contributing factor in cancer development. Cell cycle progression is mainly controlled by CDKs, which successively act in G1 to initiate S phase and in G2 to initiate Mitosis (Whittaker *et al.*, 2017). CDKs phosphorylate cellular substrates required for progression into different cell cycle phases (Swaffer *et al.*, 2016). Therefore, CDKs are key enzymes in cell cycle control; they are promising targets for design and discovery of antiproliferative drugs. The majority of CDK inhibitors are competitive that can bind to ATP pocket and inhibit CDKs activity (Martin MP, 2012). Cyclin dependent kinase 2 (CDK2), belonging to kinase group, acts as a mediator for cell progression from G1 to S phases in association with cyclin A and E. In this respect, CDK2 has been actively regarded as a promising drug target for anticancer therapies (Tarfah Al-Warhi, 2020). Great majority of clinically used CDK2 inhibitors, although acting by ATP-competition, their structures are quite diverse and they are generally comprising or derived from various heterocyclic families such as purines namely Roscovitine and Olomoucine, pyrimidines and indoles as in case of Meridianin, as well as pyrazoles and thiazoles for Crisotinib and Dasatinib (Bailon-Moscoso *et al.*, 2017; Kim *et al.*, 2020; Kolodziej *et al.*, 2015; Wu & Fu, 2018). CDK2 expression was proven remarkably upregulated in colorectum tumorigenesis. Its overexpression promotes the progression of early cancer and correlates with prognosis in colorectal carcinoma (Li *et al.*, 2001; Yamamoto *et al.*, 1998). Roscovitine, the potent CDK2 inhibitor, succeeded to treat different cancer types, either as single therapy or in combination with other agents. It has a noticeable antiproliferative effect on colon cancer cells HCT-116, HT-29 and other cell lines. Orally administered, Roscovitine caused a reduction in tumor growth in HCT-116 and HT-29 human colon cancer xenograft model in nude mice of 79% and 80% respectively (Cicenas *et al.*, 2015).

Cancer development is a multi-step process promoted by exposure to chemical irritants or inflammatory mediators that induce cell proliferation (O'Neill *et al.*, 2018). Cancer and inflammation are related to each other, where great correlation between formation of precancerous lesions and inflammation were observed (Matkowskyj *et al.*, 2013). Many reports related overexpression of different inflammatory mediators in chronic inflammation to cancer risk, increased cell proliferation, metastasis and angiogenesis. Therefore targeting inflammation could develop good strategy for cancer therapy and prevention since many epidemiological studies have demonstrated that inflammation can predispose to tumors (Silvia Zappavigna & Caraglia, 2020). Many reviews traced cyclooxygenase-2 (COX2), studied the overexpression of its biological products prostaglandin E2 and COX2 mediated factor IL-11 and described their role in regulating angiogenesis, tumorigenesis and metastasis (Howe, 2007; Singh-Ranger *et al.*, 2008; Singh *et al.*, 2006). In parallel, clinical studies have indicated that long term use of nonsteroidal anti-inflammatory drugs (NSAIDs) decreased the incidence of cancer and were believed to play a role in cancer prevention and treatment (Rayburn *et al.*, 2009; Thun *et al.*, 2002; Wong, 2019).

Sheng *et al.* described specifically the role of COX2 and related mediators in colorectal carcinogenesis (Sheng *et al.*, 2020). COX2 signaling and promotion of pathogenesis, angiogenesis and metastasis of colorectal cancer were comprehensively detailed in near literature (Liu *et al.*, 2017; Masferrer *et al.*, 2000; Zhou *et al.*, 2017). Numerous epidemiologic studies have found that long-term users of NSAIDs have a lower risk of colorectal adenomatous polyps and colorectal cancer than nonusers (Grau *et al.*, 2009; Mayor, 2015; S. Friis, 2015). NSAIDs whether non-selective or selective COX2 inhibitors were proven curative for some adenomatous colorectal cancer stages where Sulindac and Celecoxib have been described in a randomized clinical study to reduce the number of polyps and their size up to 65%. The uses of COX inhibitors for treatment of colorectal cancer have been

raised since colorectal tumor tissue showed higher concentration of prostaglandin E2 than in surrounding normal mucosa, randomized clinical trials have confirmed that the prodrug Sulindac and the selective COX2 inhibitor Celecoxib inhibited the growth of adenomatous polyps in colorectal cancer (Kemp Bohan *et al.*, 2020; Sheng *et al.*, 2020; Steinbach G & Med, 2000). Combining Celecoxib and Cetuximab improved the antiproliferative activity by 50% in cetuximab resistant colon HT-29 cells (Valverde *et al.*, 2017).

Colorectal cancer, that still needs proper and effective treatment, represents a therapeutic target for drug discovery and development. Co-administration of combined anti-inflammatory anticancer treatments might be a respectable approach nevertheless their metabolic and excretory burden. Having acknowledged antiproliferative effect on colon cancer cells HCT-116 and HT-29, the purine CDK2 inhibitor, Roscovitine was adopted as a lead compound, and directed researchers in the current work to select isosteric pyrazolopyridine congeners DZ-BAU2021-6 and DZ-BAU2021-14 for further study. The chosen compounds for investigation are reported to have promising CDK2 inhibitory effect and encouraging antiproliferative activity on colon cancer cells HCT-116 and HT-29 (Kassem *et al.*, 2021). DZ-BAU2021-6 and DZ-BAU2021-14 comprise fused pyrazolo core which is pharmacophoric for COX2 inhibition. The previous stated facts directed the research group to explore the anti-inflammatory potential of DZ-BAU2021-6 and DZ-BAU2021-14. This might promote the discovery of individual inhibitors possessing both CDK2 and COX2 activities which adds highlights towards a twin scope activity saving pharmacokinetic burden of combination treatment.

## 2. MATERIAL AND METHODS

### 2.1. Molecular Docking Studies

*In-silico* pharmacodynamic studies of DZ-BAU2021-6 and DZ-BAU2021-14<sup>1</sup> were carried out to gain a perception of their binding forces and energies to the active sites of target proteins. Mol files of docked compounds were generated by drawing their structures using ChemSketch (ACD/Labs Chemistry Software) and transformed into PDB format via Open Babel (O'Boyle *et al.*, 2011). The PDB files of X-ray crystal structures of selected receptors CDK2 (PDB ID: 1HCK) (Schulze-Gahmen *et al.*, 1996) and COX2 (PDB ID: 3ln1) (Wang *et al.*, 2010) were retrieved from the online protein database (Berman *et al.*, 2000; RCSB-Protein Data Bank). The crystal structure of target protein 1HCK was treated by removing the co-crystallized ligand ATP, water and magnesium. Similarly, 3ln1 was freed from Celecoxib and water molecules. Optimization and energy minimization were achieved using Swiss PDB viewer V.4.1.0 software (Johansson *et al.*, 2012). AutoDock4.0 software was used to perform Molecular docking based on Lamarckian Genetic Algorithm (Morris *et al.*, 2009). Polar hydrogens addition to protein and Kollman united atomic charges were computed using AutoDock hydrogen module. Using Auto Grid function, grid maps were calculated, Grid size was set to 40\*40\*40 points and grid spacing of 0.375 Å. Grid box was assigned to include the active residue in center. Default docking algorithms were specified in agreement with standard docking protocol. Ten runs were executed for each ligand using Cygwin (Cygwin project). Lowest binding energies were clustered according to 1.0 Å RMSD tolerance criteria and estimated inhibition constant Ki and docking energies were calculated. UCSF Chimera was used for H-bond analysis (Pettersen *et al.*, 2004). MOE2014.0901 (MOE, 2014) was used to analyze pi bonds, lipophilic, hydrogen donor-acceptor interactions and Van der Waals forces of attraction with different amino acids.

### 2.2. Validation of Molecular Docking

Validation of prepared protein receptor models 1HCK and 3LN1 was achieved by experimental docking of ATP and Celecoxib to their binding sites respectively. They exhibited docking patterns equivalent to the original electronic crystal structures of 1HCK and 3LN1.

<sup>1</sup> Two derivatives developed in Faculty of Pharmacy - Beirut Arab University, as apart of pharmacist Zahra Kassem PhD thesis.



residue interactions. The estimated docking energies and 3D interaction models are illustrated in Table 1 and Fig. 2-5.

Table 1: Ligand-protein docking energies in kcal/mol at the active site of 1HCK

Compound	Estimated Free Binding Energy	Estimated Inhibition Constant, $K_i$ ( $\mu\text{M}$ ) <sup>a</sup>	Final Inter-molecular Energy	VdW +Hbond +desolv. Energy	Electro-static Energy	Final Total Internal Energy	Torsional Free Energy	Unbound System's Energy
DZ-BAU2021-6	-7.35	4.11	-8.35	-8.03	+0.01	-0.58	+0.30	-0.69
DZ-BAU2021-14	-8.84	0.33	-10.12	-9.88	-0.24	-0.79	+1.19	-0.87
ATP	-6.21	28.25	-10.70	-9.09	-1.61	-5.46	+4.18	-5.77
Roscovitine	-7.88	1.68	-9.58	-9.15	-0.43	-1.65	+2.68	-0.66

<sup>a</sup> Estimated Inhibition Constant,  $K_i$  is characterized in Micro-molar concentration at Temperature = 298.15 K.

The estimated ligand-receptor free binding energy, upon redocking of ATP, was -6.21 kcal/mol (table 1). At its docking active site against prepared 1HCK, at the level of ribose, ATP exhibited three H-bond donors with the side chain of Asp145 of 2.57, 2.95 and 3.20 Å, four H-bond acceptors of 2.74, 3.08, 3.17 and 3.59 Å bond distances with amino group of Lys33. The phosphate residue dominated many hydrogen bonds; three H-bond acceptors with Lys129 and three H-bonds involving the hydroxyl group of Thr14, the carboxamide side chain of Asn132 and the back bone of Gly13 of 2.72, 3.00 and 3.14 Å respectively. Purine and ribose skeleton were hosted by the hydrophobic region formed by Gln131, Gly13, Leu83, Leu134, Ile10, Lys129 and Val18 (Fig. 2).

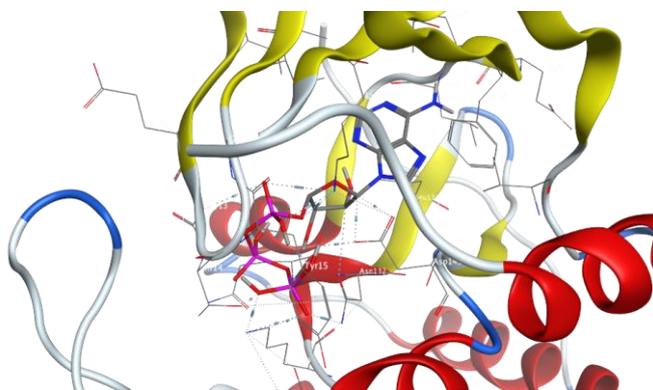


Fig.2: 3D interaction model of ATP with CDK2 kinase (1HCK) upon redocking

DZ-BAU2021-6 revealed estimated free binding energy of -7.35 kcal/mol (table 1). Its 3D interaction binding model with CDK2 kinase (1HCK) illustrated two H-bond acceptors at the level of pyrazolo N6 and pyridino N7 with amino group of Lys33 of 3.68 and 2.83 Å respectively. The carbon skeleton was seen lodged in the lipophilic pocket formed by non-polar residues of Ileu10, Leu134 and Val18, with non-coplanar Van der Walls forces of attraction involving benzene rings of Phe80 and Phe82 (Fig. 3).

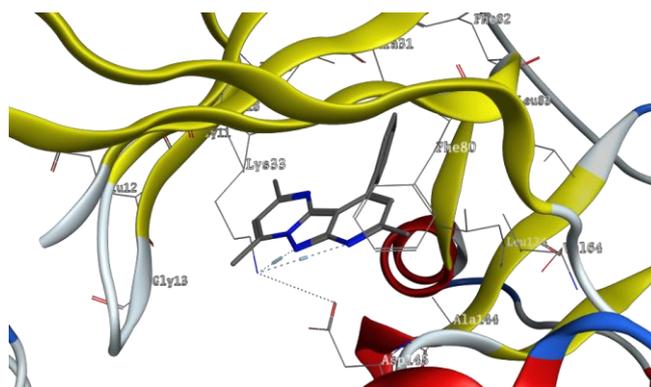


Fig.3: 3D interaction model of DZ-BAU2021-6 with CDK2 kinase (1HCK)

Compound DZ-BAU2021-14 showed estimated free binding energy of -8.84 kcal/mol (table 1). It displayed many H-bond interactions with various amino acid residues at the active pocket of CDK2 kinase (1HCK); triazino N1 and N2 were stabilized as two H-bond acceptors with Lys33 amino group of 2.98 and 2.75 Å respectively. Two H-bond acceptors were depicted between the ligand ethanoate carbonyl and NH groups of Lys129 and Thr14 with a bond distance 3.29 and 3.22 Å respectively. In addition, the ethanoate oxygen was involved in H-bond acceptor of 3.18 Å length with NH group of Tyr15 while the methyl group elaborated a H-bond donor 3.14 Å with carbonyl side chain of Asp86. The exposed skeleton was accommodated in a hydrophobic pocket formed by Gln131, Ile10, Leu134 and Val18 amino acid residues (Fig. 4).

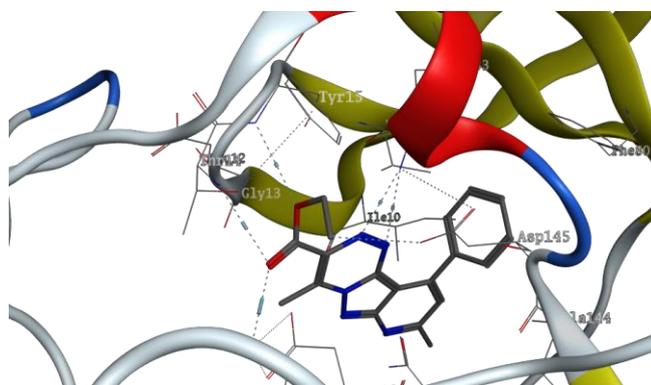


Fig.4: 3D interaction model of DZ-BAU2021-14 with CDK2 kinase (1HCK)

Docking of Roscovitine to CDK2 active pocket depicted a binding energy of -7.88 kcal/mol (table 1). Ligand-receptor interaction displayed H-bond acceptor of 3.21 Å between purine N3 and amino group of Lys33. In addition, butanol amino NH established a H-bond donor of 3.11 Å with the carbonyl side chain of Asp86. The hydroxyl group showed H-bond acceptor of 3.18 Å with amino group of Lys89 and H-bond donor of 2.67 Å with carbonyl side chain of Asp86. The carbon chains were interacting hydrophobically with Gln131, Ile10 and Leu134 (Fig. 5).

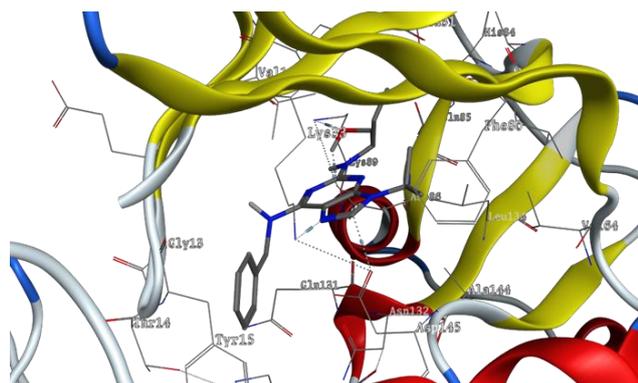


Fig.5: 3D interaction model of Roscovitine with CDK2 kinase (1HCK)

Both compounds DZ-BAU2021-6 and DZ-BAU2021-14 showed low levels of estimated binding energies. DZ-BAU2021-14 illustrated the lowest binding energies and the lowest estimated inhibition constant. It exhibited better binding criteria than Roscovitine; -8.84, -10.12, and -9.88 Kcal/mol compared to -7.88, -9.58 and -9.15 Kcal/mol assigned to estimated free binding energy, final intermolecular energy and total sum of Van der Waals + hydrogen bonding + desolvation energy, respectively. DZ-BAU2021-14 demonstrated one-fourth the estimated inhibition constant ( $K_i$ ) of Roscovitine (0.33 to 1.68  $\mu\text{M}$ ). The endogenous substrate ATP exhibited the best final intermolecular energy (table 1). The final intermolecular energy value -10.12 Kcal/mol assigned for DZ-BAU2021-14 compared to -1.70 Kcal/mol for ATP indicates the stability of DZ-BAU2021-14 in its ligand-receptor complex pocket.

### 3.1.2. Binding Mode of Study Compounds at COX2 Active Site

The anti-inflammatory effect of DZ-BAU2021-6 and DZ-BAU2021-14 was tested by inspecting their COX2 inhibitory activity *in-silico*; the anticipated binding interactions and affinities to COX2 receptor were studied by docking them to the active site of COX2 using the prepared crystalline protein structure 3ln1. In parallel, the binding mode of Celecoxib to COX2 receptor was explored to recognize the assembly of amino acids-ligand setting.

The crystalline structure 3ln1 depicted the interaction of the embedded celecoxib through H-bonds at the level of Arg499, Gln178, His75, Leu338, Phe504 and Val50 as well as an assembly of pi-C, pi-H and hydrophobic interactions with Ala513, Gln178, Gly513, Leu338, Phe504, Ser339, Tyr341, Val335 and Val509. Trifluoromethyl group was logged in the lipophilic pocket created by Leu517, Tyr341 and Val335 (Fig. 6).

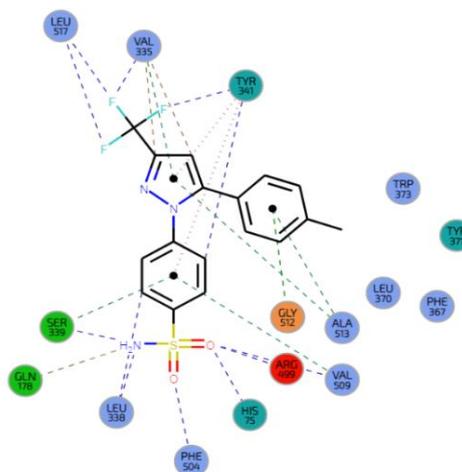


Fig.6: 2D view of Celecoxib interaction with COX2 (pdb entry: 3LN1) (Protein Data Bank in Europe; Wang et al., 2010)

Molecular docking of DZ-BAU2021-6 and DZ-BAU2021-14 was carried out against the treated 3ln1 active binding site. Celecoxib was redocked for validation, it proved analogous ligand-receptor interactions compared to the untreated 3ln1 crystallized ones, involving same amino acid residues. The estimated docking energies and 3D interaction models are demonstrated in Table 2 and Fig. 7-9.

Table 2. Ligand-protein docking energies in kcal/mol at the active site of 3ln1

Compound	Estimated Free Binding Energy	Estimated Inhibition Constant, $K_i$ ( $\mu\text{M}$ ) <sup>a</sup>	Final Inter-molecular Energy	VdW +Hbond +desolv. Energy	Electro-static Energy	Final Total Internal Energy	Torsional Free Energy	Unbound System's Energy
DZ-BAU2021-6	-7.50	3.20	-7.86	-7.85	-0.01	-0.62	+0.30	-0.69
DZ-BAU2021-14	-9.66	0.08	-10.80	-10.63	-0.17	-0.93	+1.19	-0.87
Celecoxib	-9.47	0.11	-10.38	-10.36	-0.02	-0.13	+0.89	-0.14

<sup>a</sup> Estimated Inhibition Constant,  $K_i$  is characterized in Micro-molar concentration at Temperature = 298.15 K.

DZ-BAU2021-6 showed low estimated free binding energy of -7.50 Kcal/mol. In its 3D interaction model with COX2 crystallized structure (3ln1), it displayed a H-bond donor of 3.62 Å length between methyl group and carbonyl side chain of Gln178 while the exposed groups underwent hydrophobic contacts with Ala513, Leu338, Tyr341, Val509 and Val335 (Fig. 7).

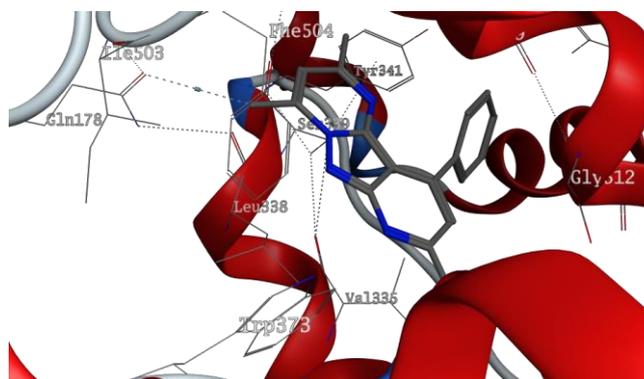


Fig.7: 3D interaction model of DZ-BAU2021-6 with COX-2 (3ln1)

DZ-BAU2021-14 demonstrated estimated free binding energy of -9.66 Kcal/mol. Its binding interaction against COX2 crystallized structure (3ln1) displayed many H-bonds with different amino acid residues. Triazino methyl and ethanoate methylene groups were stabilized by establishing H-bond donors with carbonyl side chain of Gln178 and carbonyl backbone of Leu338 with bond lengths 3.79 and 3.04 Å respectively. The ethanoate carbonyl group exhibited a H-bond acceptor of 3.14 Å with the basic amino acid Arg499. In addition, the triazino ring interacted with Val509 through two pi-H bonds of 3.63 and 3.91 Å respectively. The exposed carbon skeleton was lodged in the hydrophobic pocket formed by six amino acid residues Ala513, Leu338, Ser339, Tyr341, Val335 and Val509 (Fig. 8).

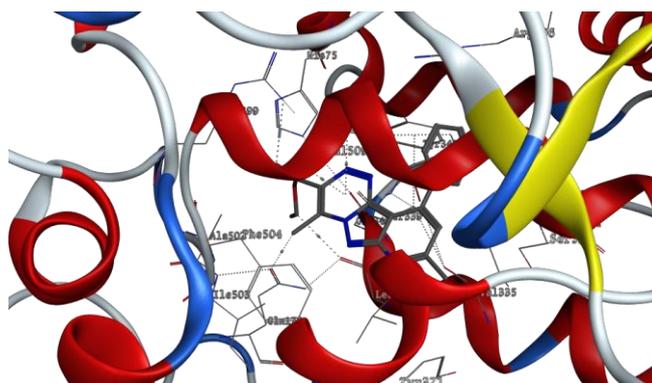


Fig.8: 3D interaction model of DZ-BAU2021-14 with COX-2 (3ln1)

Upon redocking of Celecoxib in its target binding site of COX2 crystallized structure (3ln1), it showed estimated free binding energy of -9.47 Kcal/mol. Celecoxib amino group was stabilized by establishing two interactions; H-bond donor with back bone carbonyl of Val102 of 3.09 Å and H-bond acceptor with side chain NH of Arg106 of 2.69 Å. A pi-H bond of 3.96 Å length was depicted between the methyl substituted phenyl and Val509. The exposed carbon backbone was hosted by the hydrophobic parts of Ala513, Leu338, Leu517, Phe504, Ser339, Tyr341, Val335 and Val509 (Fig. 9).

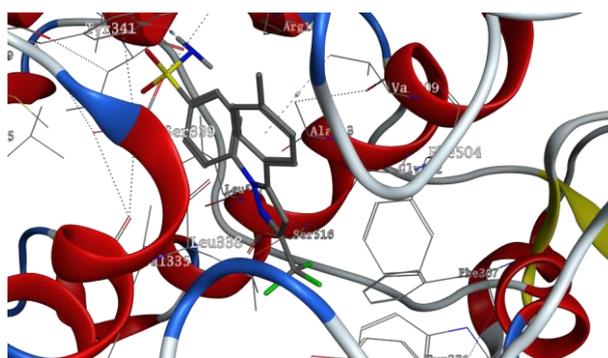


Fig.9: 3D interaction model of Celecoxib with COX-2 (3ln1) upon redocking

The binding modes attributed to Celecoxib (Dhanjal *et al.*, 2015), Valdecoxib (Di Fiore *et al.*, 2006) and Rofecoxib (Orlando & Malkowski, 2016) with COX2 receptor were quite similar. They pointed a particular active pocket and same amino acid residues that are shared with the study compounds DZ-BAU2021-6 and DZ-BAU2021-14. This raised the presumption hits of their anti-inflammatory activity.

Estimated binding energies for DZ-BAU2021-6 and DZ-BAU2021-14 indicated binding affinities to COX2 receptor (3ln1) (table 2). According to the obtained values DZ-BAU2021-14 showed the lowest binding energies and estimated inhibition constant (Ki). Compared to Celecoxib, DZ-BAU2021-14 showed -9.66, -10.80 and -10.63 Kcal/mol to -9.47, -10.38 and -10.36 Kcal/mol for Celecoxib specifying their estimated free binding energy, final intermolecular energy and total sum of Van der Waals + hydrogen bonding + desolvation energy, respectively. DZ-BAU2021-14 demonstrated three-fourth the estimated inhibition constant (Ki) of Celecoxib (0.08 to 0.11µM). From the previous data, DZ-BAU2021-14 revealed superior binding interactions with COX2 crystalline structure (3ln1) compared to Celecoxib. This can be proved considering not only its lower values at the level of binding energies and estimated inhibition constant, but also possessing higher number of hydrogen bonding and additional pi-H interactions at the active pocket of COX2 involving more amino acid residues than Celecoxib does.

### 3.2. Prediction of Pharmacokinetic Properties

As the penetration of DZ-BAU2021-14 to colorectal cell lines HCT-116 and HT-29 was proven by its antiproliferative CDK2 inhibitory activity as concluded from its biological *in-vitro* results (Kassem *et al.*, 2021), tracing its physicochemical and pharmacokinetic properties is fundamental to develop an idea about its bioavailability and different tissue concentration. In this respect Swiss ADME web tool (Daina *et al.*, 2017; Swiss Institute of Bioinformatics, 2021) was used to evaluate the pharmacokinetics and drug-likeness of DZ-BAU2021-6 and DZ-BAU2021-14.

From the first glance the Bioavailability Radar presentation of compound DZ-BAU2021-6 reflected high lipophilicity and low polarity levels with notably limited flexibility and high unsaturation level (Fig.10). The estimated n-octanol/water partition coefficients expressed as Log  $P_{o/w}$  demonstrated a range of values between 2.94-3.87 with a consensus Log  $P_{o/w}$  of 3.60 predicting high lipophilicity. Water solubility was read as poor to moderate. Compound DZ-BAU2021-6 exhibited a high estimated GI absorption, BBB penetration. It was detected as a substrate for permeability glycoprotein (P-gp) and showed inhibitory potential to CYP1A2, CYP2C19, CYP2C9 and CYP3A4 while it had no effect on CYP2D6. Swiss ADME described compound DZ-BAU2021-6 as a drug-like having bioavailability score of 0.55. No violation was depicted.

Compared to DZ-BAU2021-6, compound DZ-BAU2021-14 showed in its Bioavailability Radar presentation slight lower lipophilicity and less polarity. Higher flexibility and saturation levels were observed (Fig. 10). Compound DZ-BAU2021-14 exhibited lower order of lipophilicity; its estimated n-octanol/water partition coefficients were ranging between 2.90-3.43 with a consensus Log  $P_{o/w}$  value of 3.16, anticipating optimum lipophilic behavior with poor to moderate water solubility scores. The predicted pharmacokinetic properties illustrated high GI absorption, and no BBB penetration. It was not assigned as a P-gp substrate which boosts its intracellular concentration and limits its efflux. Compound DZ-BAU2021-14 demonstrated inhibitory activity for CYP1A2, CYP2C19 and CYP2C9 while it had no effect on CYP2D6 and CYP3A4. It was defined as a drug-like exhibiting bioavailability score of 0.55 with no violation.

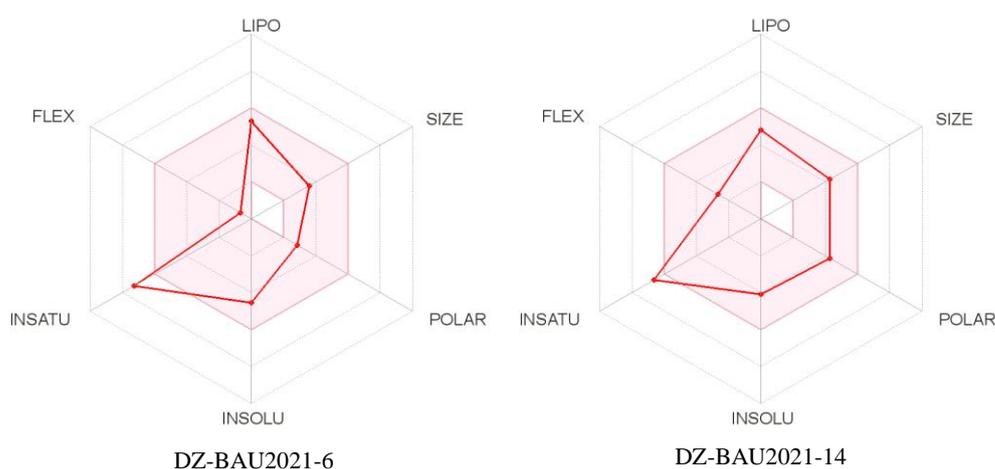


Fig.10: Bioavailability Radar describing lipophilicity, size, polarity, insolubility, unsaturation and flexibility

The estimated high levels of lipophilicity and low levels of polarity and water solubility in addition to GI absorption indicated potential oral bioavailability for both candidates DZ-BAU2021-6 and DZ-BAU2021-14 and postulated them for drug-likeness. Superior pattern was observed for DZ-BAU2021-14; it was not determined P-gp substrate which would reflect its intracellular concentration. In addition, it revealed lower potential for drug-drug interaction at

the level of CYP inhibition in comparison to DZ-BAU2021-6. Moreover, having no BBB penetration, it is hypothesized to have no central side effect.

#### 4. CONCLUSION

Understanding the complementary response associated to COX2 and CDK2 inhibition in controlling cell proliferation and prognosis of colorectal cancer, the current work affords the computational studies of DZ-BAU2021-6 and DZ-BAU2021-14 depicting the superior *in-silico* activity of the CDK2 inhibitor DZ-BAU2021-14 against COX2 receptor model compared to Celecoxib. DZ-BAU2021-14 exhibited high GI absorption along with absence of P-gp interference and lower CYP interaction which promotes its nomination as an individual candidate assembling CDK2-COX2 inhibitory effects against colorectal cancer cells with designated oral activity and low drug-drug interaction possibilities. This might inaugurate the discovery of discrete CDK2-COX2 inhibitors and add exponential highlights towards a dual scope activity saving pharmacokinetic loads of individual drug combination.

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