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Pharmacophore Analysis and Molecular Docking Simulations to Design G Protein-coupled Receptors Antagonists with Ligand Selectivity

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Abstract

Cancer development is closely associated with immunosuppressive tumor microenvironment (TME) that attenuates antitumor immune responses and promotes tumor cell immunologic escape. The sequential conversion of extracellular ATP into adenosine by two important cell-surface ectonucleosides CD39 and CD73 plays a critical role in reshaping immunosuppressive TME. The accumulated extracellular adenosine mediates its regulatory functions by binding to one of four adenosine receptors (A_1R, A_2AR, A_2BR and A_3R). Adenosine emerges as a promising target for cancer therapy down to its protumor activities by inducing tumor cell proliferation, angiogenesis, chemo-resistance, and migration/invasion. Inhibition of the adenosine pathway alone or in combination with classic immunotherapies offers a potentially effective therapeutic strategy in cancer. Herein, computer-aided drug design approaches including pharmacophore analysis and molecular docking studies were adopted to examine the predicted binding modes for suggested A_2A receptor antagonists to obtain a greater insight in SAR analyses.

Keywords: Adenosine, A_2A receptors, pharmacophore, docking.

1. Introduction:

Cancer, recognized as one of the most challenging life-threatening diseases, poses treatment obstacles due to factors such as drug resistance, side effects of therapies, tumor diversity, and the limitations of animal models. Following radiotherapy/chemotherapy and targeted drug therapy, cancer immunotherapy has emerged as the third groundbreaking approach to fight neoplastic diseases (Global Cancer Burden Growing: World Health Organization (1 February 2024) | Communitymedicine4all, n.d.). The human immune system is activated to combat pathogens while simultaneously employing various mechanisms to prevent excessive inflammatory responses and autoimmunity (Pardoll, et al., 2012). Unfortunately, tumours can exploit these mechanisms, resulting in immune evasion. These mechanisms include inhibitory receptors such as PD-1, CTLA-4, TIM-3, and A_2AR, along with their associated signalling networks, collectively referred to as "immune checkpoint pathways" (Sitkovsky et al., 2014, Cekic et al., 2016).

Currently, the blockade of immune checkpoint pathways represents one of the most promising therapeutic modalities for cancer treatment, revolutionizing cancer therapy over the past 15 years. Strategies aimed at targeting and modulating the adenosine A_2A receptor (A_2AR), which is emerging as an alternative immune checkpoint, showed significant potential in enhancing antitumor responses. Pathologically, an elevated level of extracellular adenosine (~10 μM vs <1 μM in normal tissues) (Kumar et al., 2013) is specifically present in the TME and is available for tumor cells, cancer-related fibroblasts (Turcotte et al., 2015) and some immune cells. Therefore, strategies aimed at targeting A_2AR could potentially restore the anticancer functions of various immune cell subsets. Computational chemistry played a crucial role in designing novel pharmaceutical drugs (Wang et al., 2020, Cardoso et al., 2021).

These computational methods were used to predict the best drug by docking interaction with protein and determining the physical properties (Earlia et al., 2019). This leads to finding and choosing the preferable lead compounds for treating the cancer disease (Eliaa et al., 2020, Corsello et al., 2017).

2. Computational Methods:

Structures of A_2AR were downloaded from the protein data bank website (PDB), code: 3eml (Jaakola et al., 2008). The protein was removed from its attachment to interstitial water molecules and alternative ions of crystallization such as chloride. Later, hydrogen atoms were adjusted, and the protein was ionized according to the physiological pH automatically.

Marvin was used for drawing, displaying and characterizing chemical structures and substructures, Marvin 17.21.0, Chemaxon (https://www.chemaxon.com). Docking studies of the suggested compounds were performed using Autodock vina software (Eberhardt et al., 2021, Trott et al., 2010).

Docking for ligand and receptor was determined using a preparing file step followed by docking run via a command line

```
vina --receptor receptor.pdbqt --ligand ligand.pdbqt \
    --center_x 10 --center_y 15 --center_z 12 \
    --size_x 20 --size_y 20 --size_z 20 \
    --out output.pdbqt --log log.txt
```

After automating docking using a Python script (e.g., looping through ligands), results were represented as CSV ===

```
with open(csv_file, "w", newline="") as f:
    writer = csv.writer(f)
    writer.writerow(csv_headers)
    writer.writerows(results)
```

3. Results and Discussion:

The docking study was applied to estimate the intermolecular binding interactions of several hit compounds, fig. (1). these hits were suggested as modifications of our previously synthesized and biologically evaluated compounds (Manar et al., 2021).

G protein-coupled receptors (GPCRs), fig (2) represent a large and diverse family of proteins involved in a wide range of physiological functions, including autocrine, paracrine, and endocrine signaling. Despite their functional diversity, GPCRs exhibit significant sequence variation, allowing them to be classified into distinct groups. These groups collectively referred to as a clan, comprise families that share evolutionary links, even though they may lack statistically significant sequence similarity.

Currently, the recognized GPCR clan includes several classes: rhodopsin-like receptors (Class A, GPCRA), secretin-like receptors (Class B, GPCRB), metabotropic glutamate receptors (Class C, GPCRC), fungal mating pheromone receptors (Class D, GPCRD), cAMP receptors (Class E, GPCRE), and frizzled/smoothened receptors (Class F, GPCRF).

Due to their critical roles in various biological processes, GPCRs are major targets for drug development and have attracted significant research interest. In humans and mice, the GPCR repertoire responsive to endogenous ligands comprises approximately 400 receptors. Most of these receptors are identified based on their DNA sequences rather than the ligands they bind. Those for which no endogenous ligand has been identified are referred to as orphan or unclassified GPCRs.

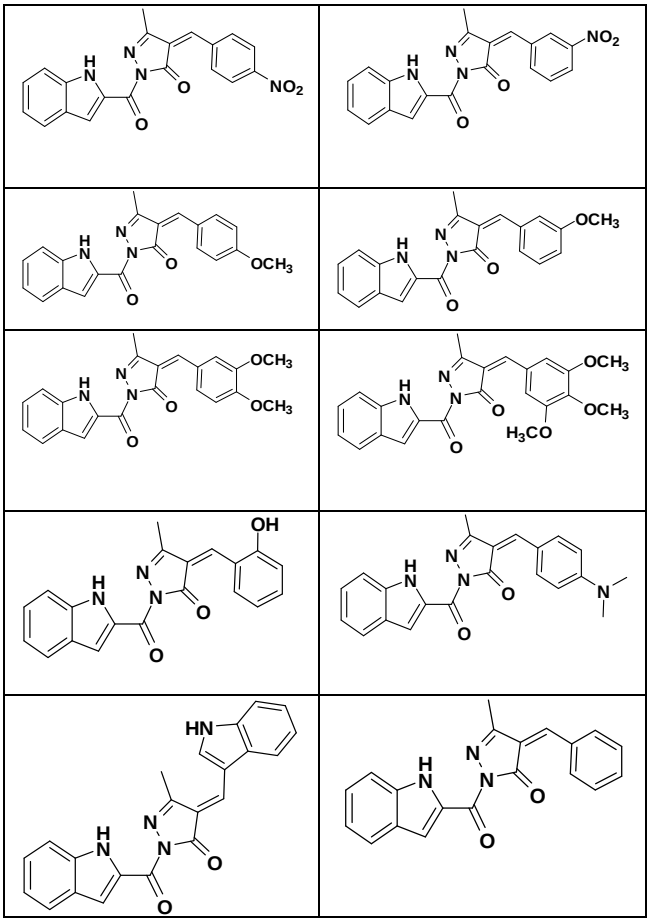


Fig.1: Molecular structure of proposed hit

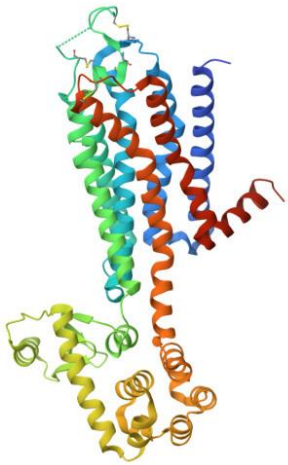


Fig.2: X-ray crystal structure of A_2AR (PDB): (3eml)

Among GPCRs, the rhodopsin-like group (GPCRA) is one of the most prevalent and includes receptors for hormones, neurotransmitters, and light. These receptors function by transmitting extracellular signals via interactions with guanine nucleotide-binding (G) proteins. Although their ligands differ greatly in structure and function, these receptors share highly conserved amino acid sequences and a common structural motif consisting of seven transmembrane (TM) helices. Purines, particularly adenosine and adenine nucleotides, play additional roles beyond energy metabolism by eliciting

diverse pharmacological effects through activation of specific cell surface receptors. Several distinct receptors exist for adenosine. In peripheral tissues, adenosine induces vasodilation, bronchoconstriction, immunosuppression, inhibition of platelet aggregation, cardiac depression, stimulation of pain receptors, and inhibition of neurotransmitter and hormone release. Within the central nervous system (CNS), adenosine has both pre- and post-synaptic depressant effects, leading to reduced motor activity, respiratory depression, sleep induction, and anxiolysis. These actions help regulate energy use according to oxygen availability. Many of the clinical effects of methylxanthines are attributed to their antagonistic activity at adenosine receptors. There are four known subtypes of adenosine receptors: A_1, A_2A, A_2B, and A_3. A_2A receptors are primarily localized in specific brain regions such as the striatum, olfactory tubercle, and nucleus accumbens. Peripherally, A_2 receptors are involved in vasodilation, immune suppression, inhibition of platelet aggregation, and stimulation of gluconeogenesis. These receptors activate adenylyl cyclase via G protein coupling.

The A_2A receptor (UniProt code: P29274) comprises 314 amino acids in its sequence, >AAP36402.1 *Homo sapiens* adenosine A_2A receptor,

MPIMGSSVYITVELAIAVLAILGNVLVCWAV
WLNSNLQNVTNYFVVSLAAADIAVGVLAIPF
AITISTGFCAACHGCLFIACFVLVLTQSSIFSLL
AIAIDRYIAIRIPLRYNGLVTGTRAKGIIAICWV
LSFAIGLTPMLGWNNCGQPKEGKNHSQGCGE
GQVACLFEDVVPMNYMVYFNFFACVLVPLLL
MLGVYLRIFLAARRQLKQMESQPLPGERARS
TLQKEVHAAKSLAIIVGLFALCWLPLHIINCFT
FFCPDCSHAPLWLMYLAIVLSHTNSVVNPFIY
AYRIREFRQTFRKIIRSHVLRQQEPFKAAGTSA
RVLAAHGSDGEQVSLRLNGHPPGVWANGSA
PHPERRPNGYALGLVSGGSAQESQGNTGLPD
VELLSHELKGVCPEPPGLDDPLAQDGAGVSL.

In this signaling pathway, the signal is propagated through the activation of adenylyl cyclase, leading to an increase in intracellular levels of cyclic AMP (cAMP). This pathway is negatively regulated by phosphodiesterase, an enzyme that degrades cAMP and thereby terminates the signal. The biological response begins when adenosine binds to its receptor, triggering signal transduction across the cell membrane by activating an associated G protein.

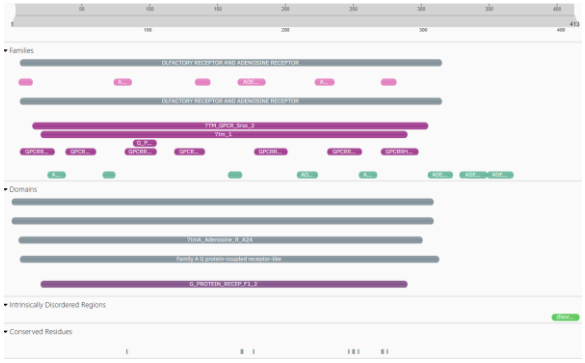


Fig. 3: GPCR families, domains, and conserved residues.

This activation promotes the exchange of GDP for GTP on the alpha subunit of the heterotrimeric G protein complex, initiating downstream signaling events, shown in fig. (4).

From previous illustrations, we can note that there are many amino acids such as (His), (Phe), and (Glu) in the active site.

Docking of proposed hits with A_2AR (PDB): (3eml)

The results for binding energy (binding affinity) were determined. Binding energy represents the predicted free energy of binding between the ligand (small molecule) and the receptor (protein) and is expressed in kilocalories per mole (kcal/mol).

A more negative value indicates stronger binding (i.e., more favorable interaction), where Vina uses a scoring function that approximates the binding energy based on: Steric interactions (van der Waals forces), hydrogen bonding, hydrophobic effects, electrostatics, and torsional entropy (penalty for ligand flexibility). Binding Energies (kcal/mol) from 0 to –4indicates weak or non-binding, while from –5 to –7 indicates moderate binding and –7 and below indicates strong binding.

As shown in table (1), the values observed for the proposed hits (A-4, A-1, A-8, A-9, A-10, A-6, A-7, A-7, A-5, A-2 and A-3) were -7.6426, -7.4006, -7.2696, -7.0345, -6.8653, -6.7395, -6.6913, -6.5491, -6.4279 and -5.7599 respectively. Subsequently, all the proposed hits showed a higher binding affinity than the co-crystallized ligand.

Table (1) confirmed that compounds with 3-methoxy indole had a more stable value (binding affinity = -7.6426), while 4-methoxy indole derivatives were less stable with a value of -5.7599. In 3-methoxy indole derivatives, hydrogen bonds with Glu169 and Asn253 and Van der Waals

interactions with Phe168 were noticed as shown in fig. (5), while 4-methoxy indole derivative was in contact with the amino acid Phe168 by aromatic system only. Hence, 3-methoxyindoles could be considered as lead compounds.

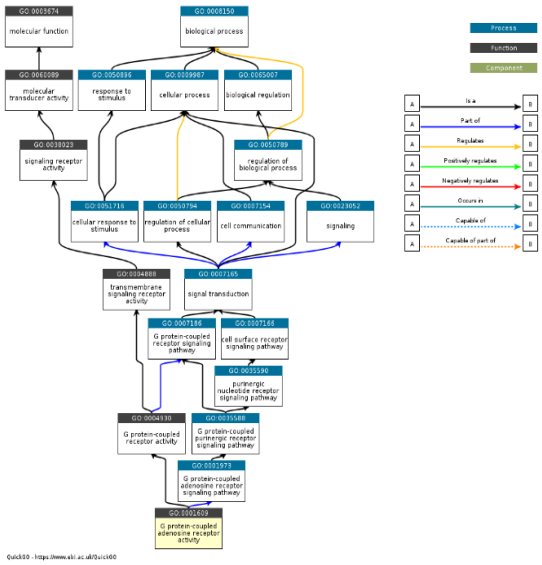
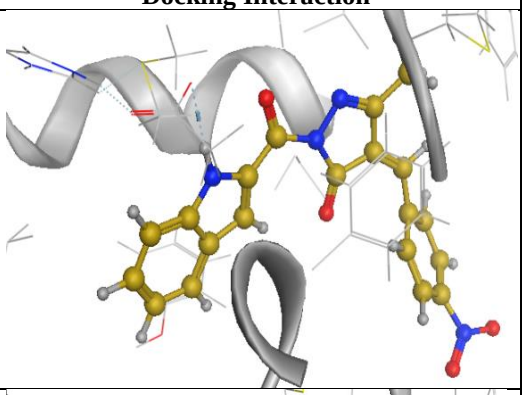
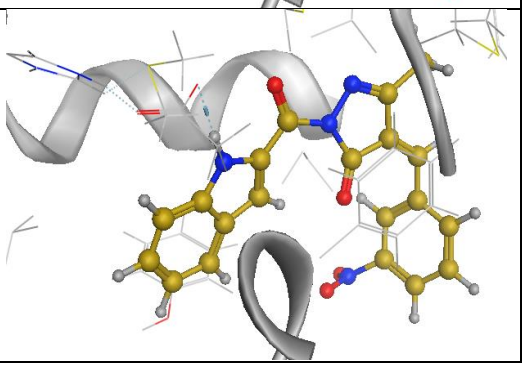
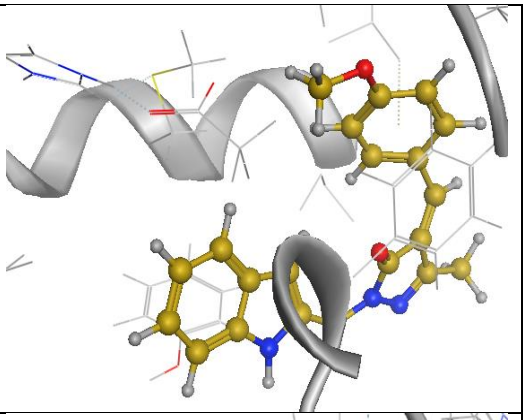
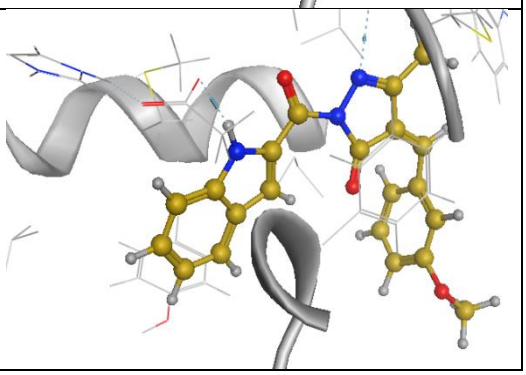


Fig. 4 Ancestor chart for A_2AR

Table 1. Binding affinities of the proposed hits after docking

Comp. No.	Proposed hits	Binding affinity
A-1	4-Nitro derivative	-7.4006
A-2	3-Nitro derivative	-6.4279
A-3	4-Methoxy derivative	-5.7599
A-4	3-Methoxy derivative	-7.6426
A-5	3.4-Dimethoxy derivative	-6.5491
A-6	3,4,5-Trimethoxy derivative	-6.7395
A-7	2-Hydroxy derivative	-6.6913
A-8	4-Dimethylamino derivative	-7.2696
A-9	Indole derivative	-7.0345
A-10	Phenyl derivative	-6.8653

No.	Docking Interaction
A-1	
A-2	

A-3	
A-4	

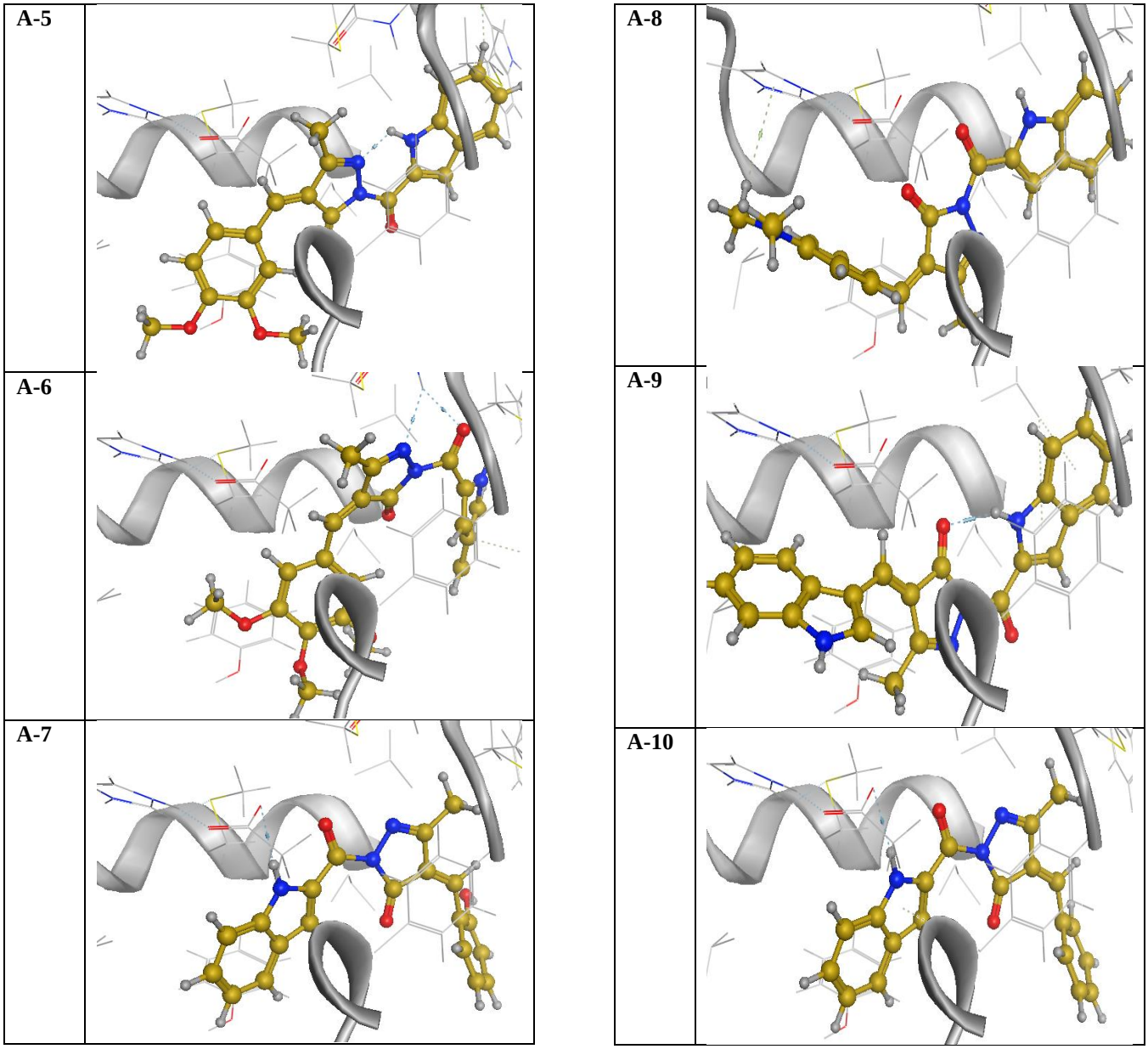


Fig.5: Docking of the proposed hits with A_2AR

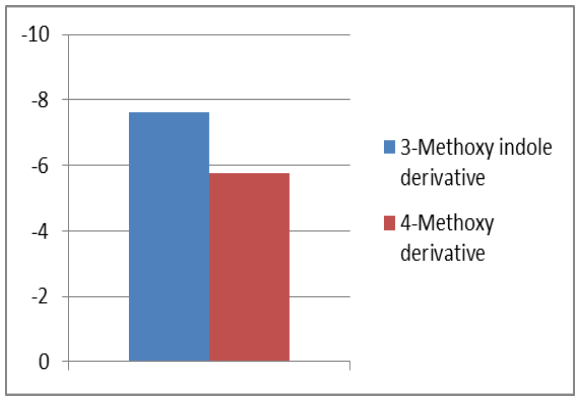


Fig.6: Comparison score between medicines with (3eml) protein.

Fig. (6) demonstrated the comparison between 3-methoxy indole and 4-methoxy indole with A_2AR.

4. Conclusion:

The binding affinities conferred the interaction between the lead compound and the acceptor. The docking results showed that the active moiety 3-methoxy indole was the most active compared with others against the protein under investigation.

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