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Virtual Screening-based Molecular Analysis of Marine Bioactive Molecules as Inhibitors for Janus Kinase 3

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ABSTRACT

Rheumatoid arthritis (RA), a chronic autoimmune disorder, can cause joint deformity and disability. The Janus kinases (JAKs), intracellular tyrosine kinases family (includes JAK1, JAK2, and JAK3), play an essential role in the signaling of various cytokines and are implicated in the pathogenesis of inflammatory diseases, including RA. Consequently, JAKs have attracted significant attention in recent years as therapeutic targets of RA. In the current study, we explored the role of a set of biomolecules from marine sources that could be used as specific inhibitors of JAKs and treat arthritis. The binding affinity of these molecules including astaxanthin (ATX), fucoxanthin (FX), fuscoside E (FsE), fucosterol (Fs), and phlorofucofuroeckol (PFFE) JAK3 has been analyzed. In addition, the details of relative structural interactions have been compared to those of the recently Food and Drug Administration-approved inhibitor, tofacitinib. Interestingly, some of these marine biomolecules showed a higher binding energy (b.e.) and specific binding to JAK3 active/potential sites when compared to the approved inhibitors. For instance, FsE binds to two key regulator residues of JAK3 required for its activity and for inhibitor stability, CYS909 and LYS905, with higher b.e. (-9.6) than the approved inhibitors. Thus, FsE may have a potential inhibitory action on JAKs and especially on JAK3. Additionally, PFFE can bind to several kinase critical regulators of JAK3 and the b.e. may reach -10.7. Based on the evaluation of oral availability, drug-likeness, pharmacokinetics, and medicinal chemistry friendliness, FsE seems to be the most appropriate potential inhibitor for JAK3.

KEYWORDS

marine biomolecules, rheumatoid arthritis, visual screening, molecular docking, Janus kinase 3

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune inflammatory disorder with complex etiological features. Pathophysiologically, RA is manifested as infiltrated immune cells, synovial lining hyperplasia leading to the destruction of articular cartilage and bone (Lee and Weinblatt, 2001; Singh et al., 2015; Ahmed and Alzahrani, 2023). Fortunately, studies in the past few years have enabled a better understanding of the pathophysiology of RA and have allowed significant progress in the treatment protocols and strategies (Hu et al., 2021; Huang et al., 2021a, 2021b). In this regard, a significant number of preclinical and clinical trials were conducted to test the chemotherapeutic effect of various agents in RA (George et al., 2020; McLornan et al., 2021; Huang et al., 2021a, 2021b). The treatment strategies for RA rely on supplementing drugs that can counteract the active immune system and reduce the chronic inflammatory state. These therapeutic agents include anti-inflammatory and analgesic drugs, immunosuppressive agents, glucocorticoids, and disease-modifying antirheumatic drugs (DMARDs). Of these, the inhibitors of the intracellular tyrosine kinases Janus kinases (JAKs) are the latest class of DMARDs to emerge in treating RA (Ferrao and Lupardus, 2017; Burja et al., 2020). The JAKs are a family of nonreceptor protein kinases that catalyze tyrosine residues adenosine triphosphate (ATP)-dependent phosphorylation on substrates and involve in various cytokine and growth factor signaling pathways; hence they regulate the function of immune cells and oncogenesis. JAK proteins include four members, JAK1, JAK2, JAK3, and tyrosine kinase 2. Structurally, each JAK protein contains seven distinct domains, JH1–JH7, of which JH1 is the kinase domain and JH2 is the pseudokinase domain, while the SH2 domain is composed of part of JH3 and JH4 and JH5, the JH6 and part of the JH4 domain form the FERM

domain (Williams et al., 2009; Ferrao and Lupardus, 2017). Generally, the recently discovered or developed drugs aim to inhibit JAK enzymes by obstructing ATP binding. Thus, significant attention has been given to JAK inhibition after being identified as a promising target for anti-RA drugs (Sk et al., 2022).

Tofacitinib, a pan-JAK inhibitor, was the first studied JAK inhibitor in humans and the first to be approved by Food and Drug Administration (FDA) for treating RA and other autoimmune diseases, as it can efficiently inhibit JAK1 and JAK3, but to a lower extent JAK2 (Ferrao and Lupardus, 2017; Burja et al., 2020). Upon oral administration, tofacitinib binds to JAK proteins and subsequently inhibits the activation of JAK-signal transducers and activators of transcription (STAT) signaling pathway. The conserved residues of the amino acids glutamate and leucine, located in the hinge region of JAKs, were found to stabilize the binding of tofacitinib and other inhibitors *via* the formation of strong hydrogen bonds. These include GLU957 and LEU959 of JAK1, GLU930 and LEU932 of JAK2, and GLU903 and LEU905 of JAK3 (Sanachai et al., 2020). Importantly, Cys909 amino acid residue on JAK3 protein was considered a potential target for the covalent attachment of inhibitors (Tan et al., 2015).

On the other hand, more recent attention has been paid to the molecular treasure of natural biomolecules of antioxidant and anti-inflammatory agents that can be obtained from seawater-living organisms. However, several potential anti-inflammatory agents can still be tested preclinically and clinically to treat arthritis. For instance, the natural xanthophyll carotenoid, astaxanthin (ATX), has been proven to have significant antioxidant and anti-inflammatory properties but its potential preclinical role in treating arthritis has not been yet clarified (Donoso et al., 2021). ATX, one of the most important carotenoids, can be obtained from marine organisms such as green algae, bacteria, yeast, sea snails and urchin, and other organisms (Alves et al., 2020). Another natural brown seaweed carotenoid, fucoxanthin (FX), known for its therapeutic potential against several inflammatory diseases, including bone disease, has still not yet been tested in RA patients or models. Moreover, the potent and long-lasting murine anti-inflammatory drug, fuscoside E (FsE), an algae-derived unique phytosterol, was suggested to be an effective local therapeutic agent for managing autoimmune diseases due to its inhibitory effect on 5-lipoxygenase inhibitors (Ha et al., 2021; Lee et al., 2021). Fucosterol (Fs, 24-ethylidene cholesterol) is a biologically active molecule belonging to the sterol group that is isolated from marine algae. Fs was found to exhibit many biological activities, including anticancer, antiosteoarthritic, anti-inflammatory, antiphotoaging, hepatoprotective, antineurological, immunomodulatory, antioxidant, algicidal, antiobesity, and antimicrobial (Hannan et al., 2020; Meinita et al., 2021). Additionally, the brown algae derivative, phlorofucofuroeckol (PFFE), has been shown to exhibit anti-inflammatory activity but remains to be tested as a possible therapeutic molecule for RA (Shibata et al., 2003; Sugiura et al., 2021).

In the present study, we have carefully selected a set of marine biomolecules that can be used as inhibitors for RA signaling molecules. Then, besides reviewing the values of the studied marine biomolecules, the binding affinity of these molecules including ATX, FX, FsE, Fs, and PFFE to JAK3 (active and ATP-binding sites) has been investigated visually using molecular docking analysis. In addition, the relative structural interaction has been compared to that of the recently FDA-approved inhibitor, tofacitinib. Interestingly, molecules such as FX, FsE, and PFFE may have a potential inhibitory action on JAK3. However, prospective preclinical and clinical studies are needed to clarify the inhibitory effect of these marine biomolecules on JAK proteins.

MATERIALS AND METHODS

Analysis of ADME properties using SwissADME

An ADME online free tool was used to evaluate the ADME properties such as physiochemical properties, lipophilicity, water solubility, drug-likeness, pharmacokinetics, and medicinal and chemical properties of the compounds (Daina et al., 2017; http://www.swissadme.ch).

Preparation of the three-dimensional protein structure

The three-dimensional (3D) structure of JAK3 proteins was obtained from the Protein Data Bank (https://www.rcsb.org). A schematic representation of JAK protein domains showing the active sites and the ATP-binding sites is shown in Figure 1. CASTp 3.0 Details of the JAK proteins' topography, structures, pockets, and functional sites were retrieved from the CASTp 3.0 web server that is freely accessible at http://sts.bioe.uic.edu/castp/.

Preparation of the ligand structure

Chemical structures of the ligands were retrieved from the PubChem compounds database, (http://www.ncbi.nlm.nih.gov/search). The retrieved ligand structures in the sdf format were used directly for ligand–protein interaction at the cb dock2 website.

Binding pocket and molecular docking

For drug discovery using computer-aided technology, protein and ligand 3D structures were used to predict their binding sites and affinity (Liu et al., 2022; Yang et al., 2022). The established binding pockets, the binding length and involved amino acid sequences at the ligand binding regions of the protein surface, besides the binding energy

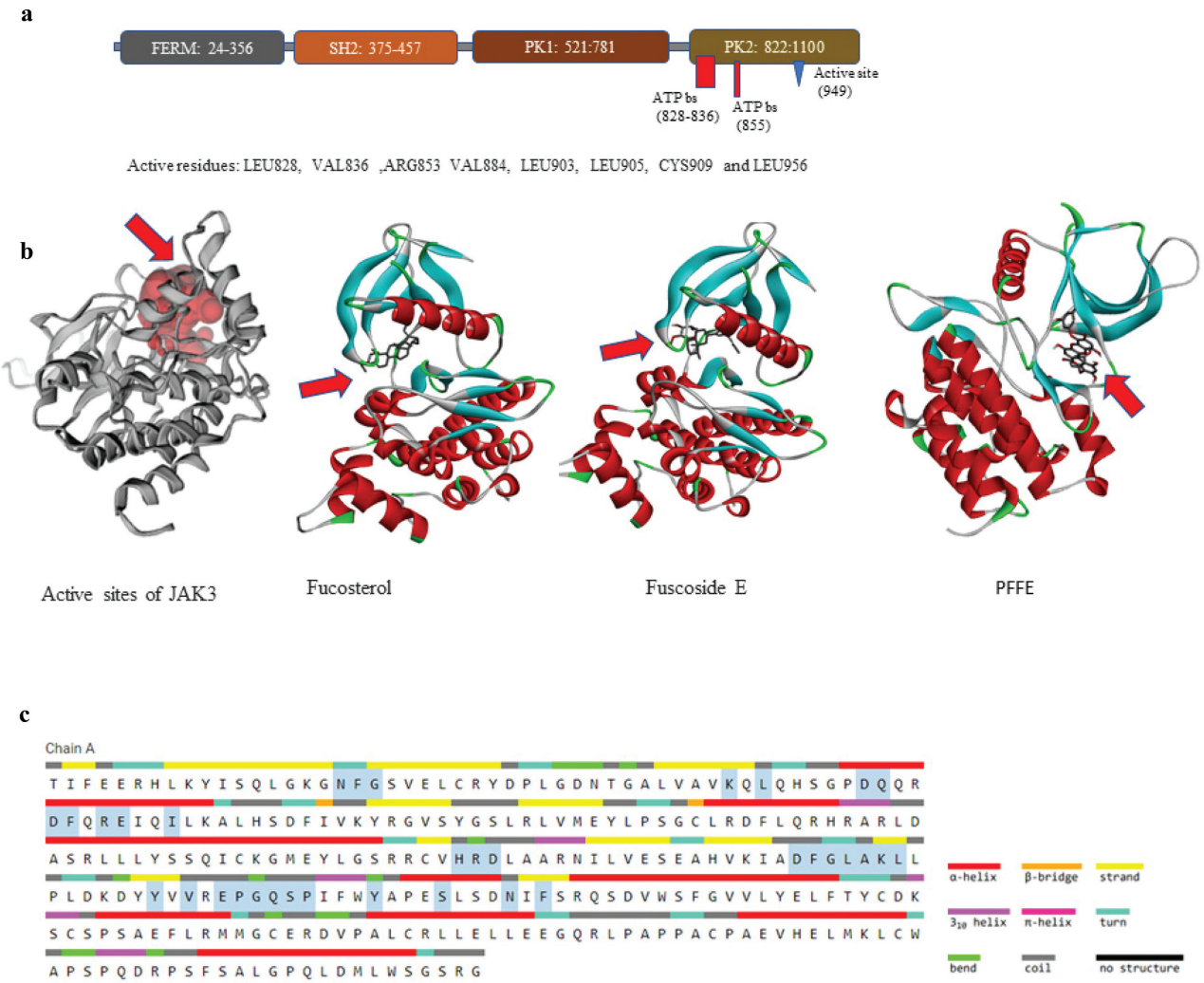

Figure 1: JAK3 structure, pockets, and functional sites. Representative diagram for the active and the conserved (ATP binding) phosphorylation sites of the JAK3 protein (a). The binding sites (docked, red arrows) of the currently studied molecules located at the active area (red area) of JAK3 as compared to the known JAK3 binding sites obtained using CASTp 3.0 the dipected are JAK3 domains including the N-terminal FERM domain, The SH2 (Src Homology 2) domain, and the psedukinase domains PK1 and PK2 (b). Details of the JAK3 topography, structures, pockets, and functional sites as retrieved from the CASTp 3.0 web server, colored areas' names are indicated at the lower left side of the image (c). Abbreviations: ATP, adenosine triphosphate; JAK, Janus kinase; PFFE, phlorofucofuroeckol.

(b.e.) have been conducted using curvature-based cavity detection methods and the molecular docking (Auto-Dock Vina-based procedure) in CB-Dock serve (https://cadd.labshare.cn/cb-dock2/php/index.php) was used in January and February 2023.

Discovery studio and scoring the binding affinity, distance, and sites

Molecular docking results of ligand–protein interactions, binding sites, and hydrophobic interactions between H–H and H–C as well as other types of interactions were analyzed using the discovery studio software, Discovery Studio 2021 Client. The length of these bonds (distance between H–H and H–C) was measured in Angstrom. Vander Waals attractions were also analyzed and are provided in Figures 1-5, Tables 1 and 2, and in the Supplementary materials.

RESULTS

JAK inhibitor, tofacitinib: its virtual interaction with JAK3 protein

The structure, the active sites, and the conserved (ATP binding) phosphorylation sites of the JAK3 protein are shown in Figure 1. As a reference to our proposed inhibitors of JAK proteins, we have evaluated the binding affinity and the interaction of tofacitinib with these proteins. Molecular docking data indicated that tofacitinib can bind to the ATP-binding sites *via* conventional hydrogen (H–H) or carbon–hydrogen (C–H) interaction. Virtual binding sites of tofacitinib to JAK3 protein are shown in Table 1. In JAK3 protein, the ATP-binding residues are located at LEU828, VAL836, ALA853, VAL884, GLU903, LEU905, CYS909, and LEU956 (Fig. 1). In addition, residues GLU903 and

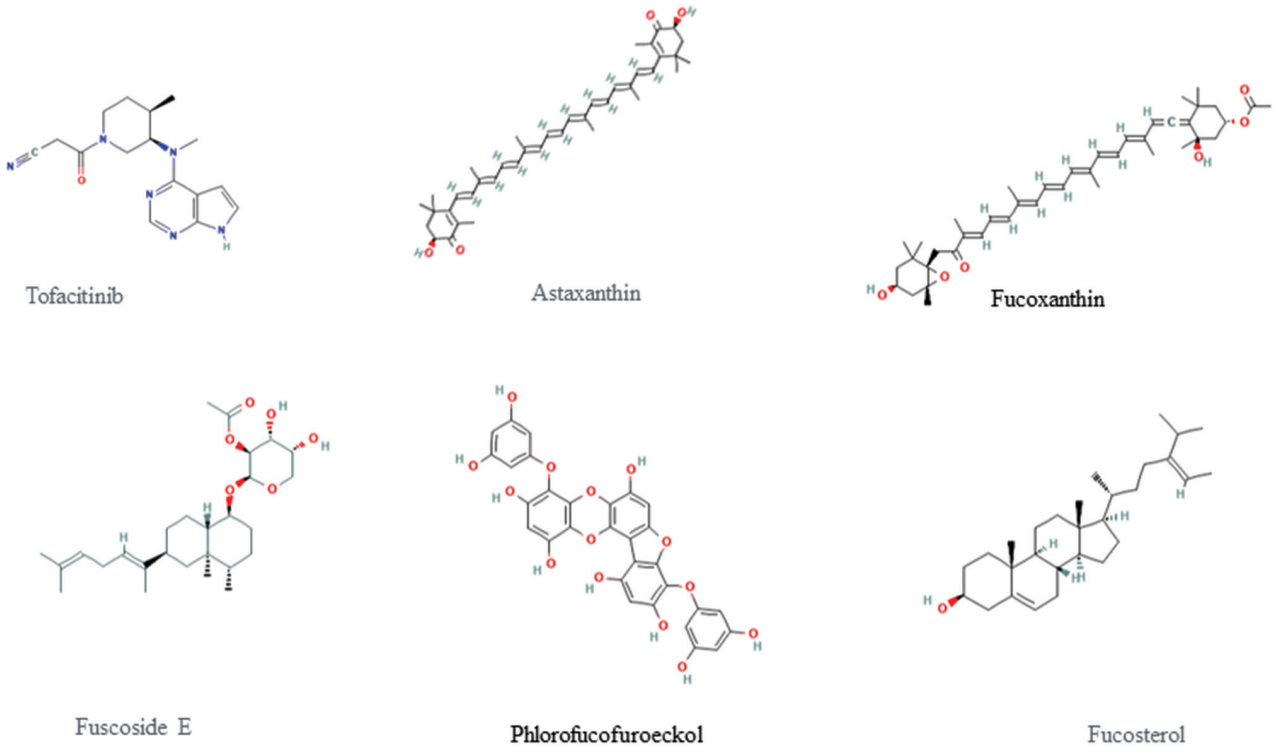

Figure 2: The structures of the proposed molecules as retrieved from PubChem website.

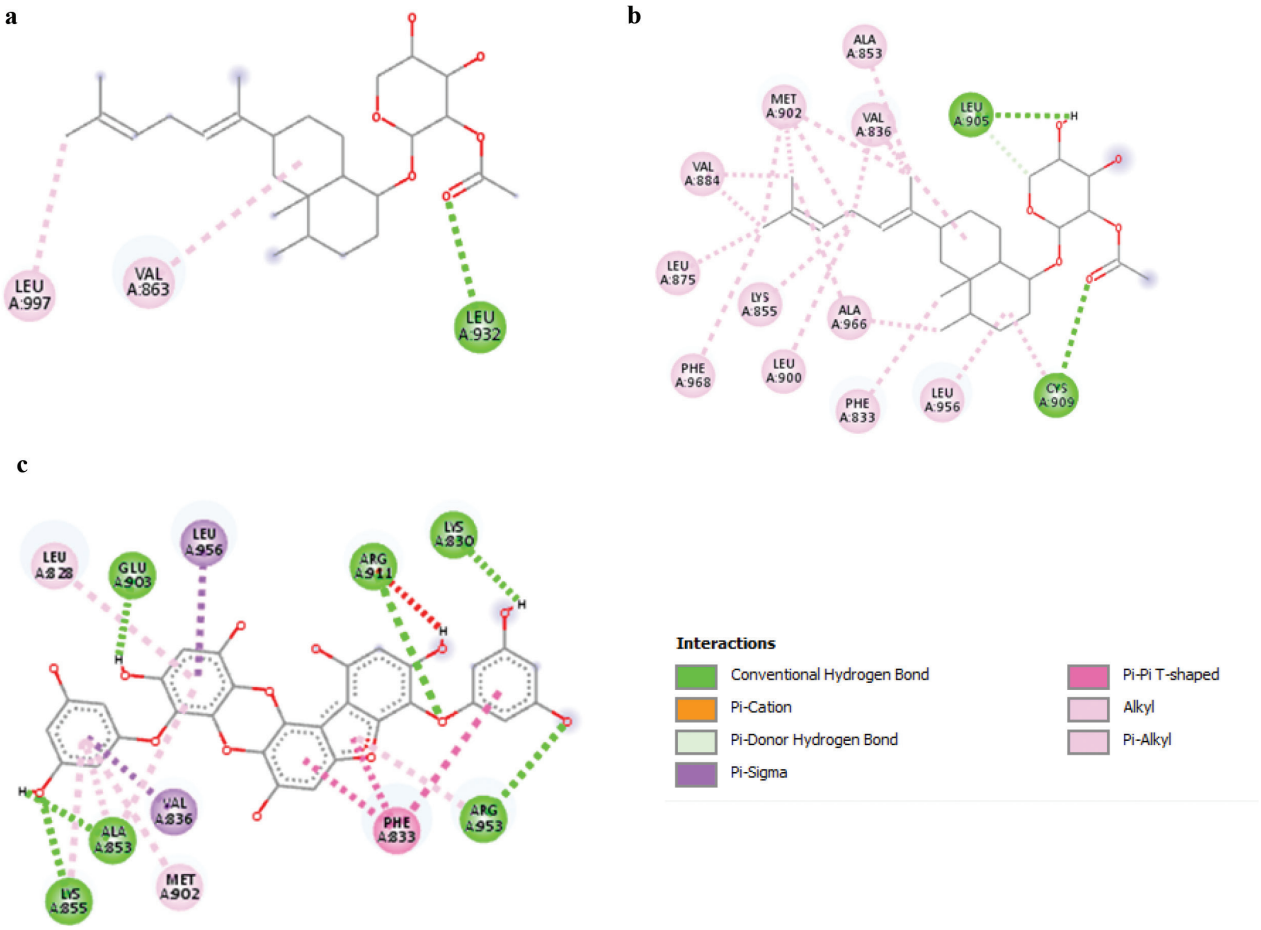

Figure 3: Representative in silico images of the two-dimensional binding sites of fucosterol, fuscoside E, and phlorofucofuroeckol to JAK3. Abbreviation: JAK, Janus kinase. Conventional hydrogen binding and other interactions are shown in diffrent grades of color.

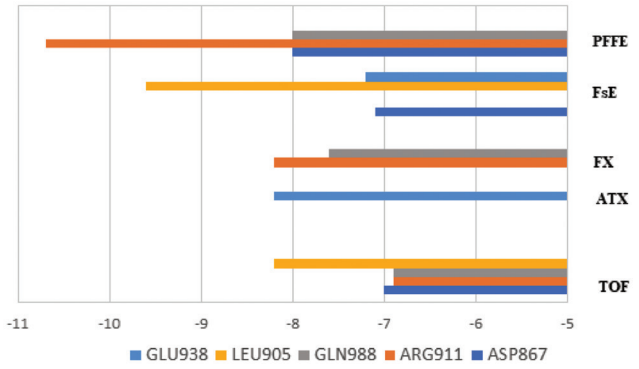

Figure 4: The estimated binding score of tofacitinib to the potential JAK protein residues relative to the currently proposed biomolecules. Abbreviations: ATX, astaxanthin; FsE, fuscoside E; FX, fucoxanthin; PFFE, phlorofucofuroeckol; TOF, tofacitinib.

LEU905 of JAK3 can strongly stabilize tofacitinib binding. Here, we found that tofacitinib is able to bind virtually to JAK3 at ARG948, ASP867, and TRY994 (b.e. −7). However, tofacitinib can bind to the critical site for JAK3 activity LEU905 with a b.e. of −8.2.

ATX and possible inhibition of JAK3 proteins

Cancer biology data indicated that AST is involved in the cellular inhibition of the JAK1/STAT3 signaling pathway (Song et al., 2012; Wu et al., 2016). Then, we checked the possible virtual interaction between AST and JAK3. Data showed an *in silico* H–H interaction at amino acids residues ASP1096, THR815, SER1089, GLU938, and SER1102, with b.e. between −6.4 and −7. Some of these residues seem to be required for the JAK3 activity, such as GLU938.

The natural brown seaweed FX as a potential inhibitor of JAK3 proteins

Without referring to the involvement of the JAK pathway, so far two preclinical studies reported that FX could inhibit osteoclastogenesis and arthritis progression (Gong et al., 2014; Lv et al., 2023). Here, our *in silico* data showed that FX was able to form H–H interaction with ARG911, ASP967, GLN988, ALA952, SER1087, ARG1085, ASP1025, ARG1059, and GLN1094 (b.e. between −6.3 and −8.2); some of these residues such as ARG911 and ASP967 are located at the active pocket of JAK3 kinase.

The brown algae derivative Fs could virtually interact with JAK protein active sites

Fs is another marine bioactive compound from the sterol group and can be extracted from marine algae. It was reported to exhibit several biological activities, such as anti-inflammatory, antioxidant, antiosteoporosis, and anticancer (Meinita et al., 2021). However, similarly to what we have seen in the proposed molecules, a preclinical or clinical study investigating its role in RA or JAK inhibition is not

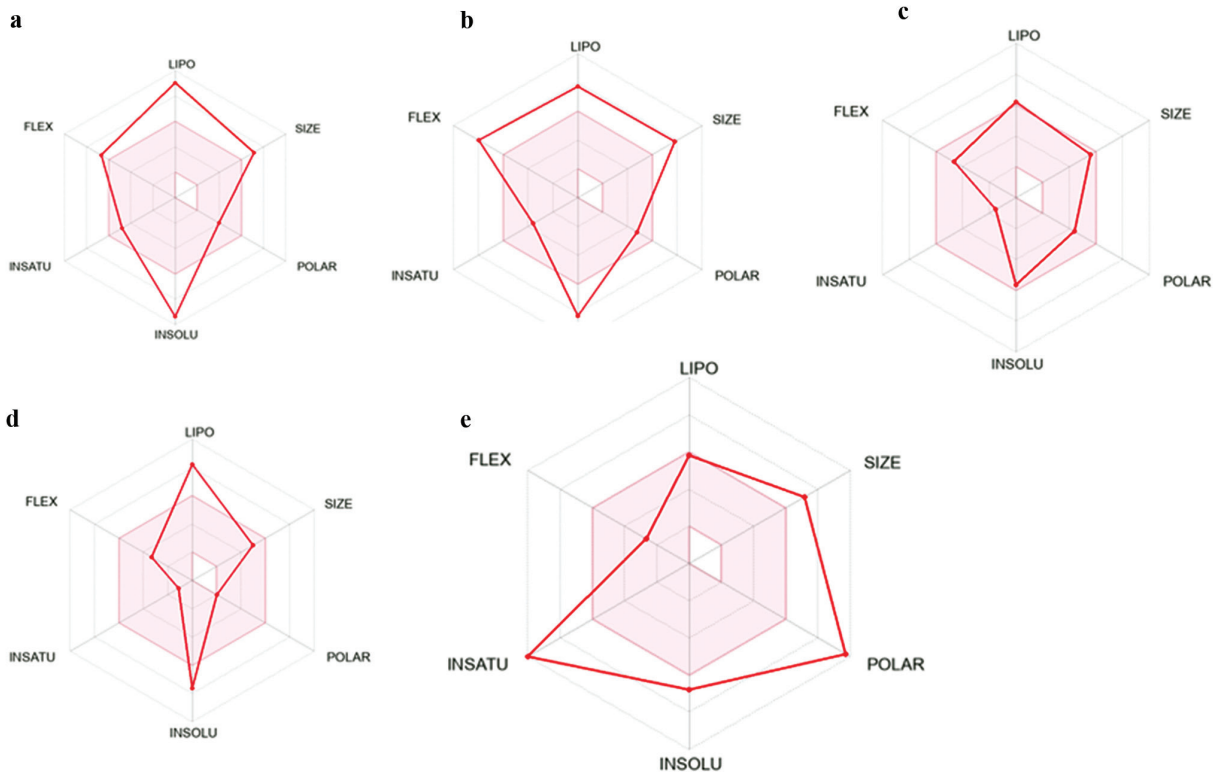

Figure 5: The oral bioavailability of the molecules astaxanthin (a), fucoxanthin (b), fuscoside E (c), fucosterol (d), and phlorofucofuroeckol (e). The colored zone is the ideal area for physicochemical space.

Table 1: Tofacitinib, astaxanthin, and fucoxanthin, and their virtual interaction sites and binding score with JAK3, through H–H and H–C binding.

Bond interaction	Docking score	Bound amino acid residues	Distance in Angstrom (A)
Tofacitinib			
H–H	–7	ARG948, ASP867, TRY994	2.99, 2.54, 2.92
H–H	–8.2	LEU905	2.25
H–H	–6.9	GLN988, ARG911	2.8, 3.16
H–H	–5.6	GLU1069, ALA923, TYR1023	2.20, 2.17, 2.83, 2.20
C–H		GLU1098	2.8
Astaxanthin			
H–H	–7	ASP1096, THR815	2.21, 2.97
H–H	8.2	SER1089, GLU938	2.65, 2.98
H–H	–6.4	SER1102	1.86
Fucoxanthin			
H–H	–8.2	ARG911	2.8
H–H	–8.1	ASP967	3, 2.7
H–H	–7.6	GLN988	2.9
H–H	–6.7	ALA952, SER1087, ARG1085, ASP1025	3.3, 3, 2.6, 3.3
H–H	–6.3	ARG1059, GLN1094	2.8, 3

Abbreviation: JAK, Janus kinase.

Table 2: Fucosterol, fuscoside E, and phlorofucofuroeckol, and their virtual interaction sites and binding score with JAK3, through H-H and H-C binding.

Bond interaction	Docking score	Bound amino acid residues	Distance in Angstrom (A)
Fucosterol			
H–H	–8	ALA1046	3.32
H–H	–7.3	SER999, AEG948	
H–H	–5.8	TRY1023	3
H–H	6.5	SER989, ARG948, SER860	2.8, 2.9, 2
H–H	–7.4	GLN1007	3.1
Fuscoside E			
H–H	–9.6	CYS909, LEU905	2.9, 2.1
H–H	–7.2	GLU938, SER1089	2.9, 2
H–H	–7.1	ARG953, GLY969, GLN864	3, 2.5, 2.7
C–H		ASP949, ASP867	3.2, 3.6
Phlorofucofuroeckol			
H–H	10.7	LYS830, LYS855, ALA853, GLU903, ARG911	2.1, 3.2, 2.5, 2.9, 2.9
H–H	–8.2	ASN832	3.4
H–H	–8	GLN988, ASP867, GLN1007	2.6, 2.5, 1.9
C–H		GLU1098	3.43

Abbreviation: JAK, Janus kinase.

yet available. Our *in silico* analysis revealed that the virtual binding between Fs and JAK3 occurs via H–H interaction at amino acids residues ALA1046, TRY1023, SER989, ARG948, and SER860 (b.e. between –5.8 and –8).

FsE is a potential inhibitor candidate of JAK proteins

Classical studies indicated that FsE is among the anti-inflammatory diterpenoid that can be extracted from marine resources. In the present study, FsE can bind virtually to JAK3 CYS949, LEU905, GLU938, SER1089, ARG953, GLY969, and GLN864 amino acid residues (via H–H interaction, b.e. –7.1 and –9.6) and to ASP949 and ASP867 (H–C, b.e. –7.1). Based on our analysis, FsE binds to two key regulator residues of JAK3; these two critical sites, CYS909 and LYS905, are required for JAK3 activity and for inhibitor stability. The binding occurs with a higher b.e. (–9.6) than the approved inhibitor, tofacitinib, which can bind only to the JAK3 at LEU905 with a b.e. of –8.2. Additionally, other important targets of the inhibitors at JAK3 are residues ASP867, ARG911, GLN988, and GLU938, with which other currently investigated molecules virtually interact. Of these active sites, ASP867 and ARG911 are crucial for JAK3 activity. Subsequently, FsE is probably an ideal inhibitor for JAK3 protein, to be tested preclinically.

The brown algae derivative, PFFE, can potentially interact with several JAK3 ATP-binding sites

The brown algae derivative PFFE has been reported to exhibit anti-inflammatory activity (Shibata et al., 2003; Sugiura et al., 2021); however, its possible inhibitory effect on JAK proteins remains to be investigated. Our visual analysis showed that PFFE can bind to many sites at JAK3 with high energy. PFFK seems to interact virtually with JAK3 (H–H bonds) at amino acids residues LYS830, LYS855, ALA853, GLU903, and ARG911 with a high b.e. (−10.7). Similar binding occurred at ASN832 but with a lower b.e. (−8.2). Interestingly, the binding occurs at phosphorylation sites, as at LYS830, ASN832, and LYS855. In addition, more H–H binding and H–C binding were found (with b.e. −8), the former were at GLN988, ASP867, and GLN1007; however, the latter were seen at GLU1098. Together these data suggest that PFFE could be a potential inhibitor for JAK3 proteins.

SwissADME and evaluation of the behavior such as physiochemical properties of the studied molecules

Analyzing the oral bioavailability of the molecules ATX, FX, FsE, Fs, and PFFE based on several parameters is shown in Figure 5. The colored zone in each biomolecule is the ideal space for the best physicochemical activity including behaviors such as physiochemical properties, water solubility, lipophilicity, pharmacokinetics, drug-likeness, and the medicinal chemistry properties of the molecules. Interestingly, FsE seems to be the most appropriate potential inhibitor that has ideal physiochemical properties.

DISCUSSION

In the current study, we used molecular docking and investigations of the structural analysis to explore the possible inhibitory effects of a set of novel biomolecules from marine resources on JAK3 protein. We have analyzed the binding affinity of this set of biomolecules that included ATX, Fs, FsE, and PFFE to JAK3 protein. For data validation and reliable analysis, we screened the active sites of JAK3 protein that can be specifically targeted by these inhibitors. Then, we compared the docking results of the proposed marine molecules with those of the recently FDA-approved inhibitor, tofacitinib.

To inhibit JAK enzymes, the recently approved and/or developed drugs aimed to obstruct ATP binding. The pseudokinase domain of JAKs is a major regulatory domain for the kinase activity (Ferrao and Lupardus, 2017; Sanachai et al., 2021). Based on our analysis, FsE binds to two key regulator residues of JAK3, required for its activity and for the inhibitor stability, CYS909 and LYS905, with a higher b.e. (−9.6) than that of the approved inhibitor. The molecular dynamics simulations suggested that the residues GLU903 and LEU905 of JAK3 strongly stabilize tofacitinib binding (Sanachai et al., 2020). In addition, in JAK3 protein, Cys909 serves as a potential target for the covalent attachment of inhibitors (Goedken et al., 2015; Forster et al., 2017; Xu et al., 2019). Then, selective inhibitors of JAK3 appear to bind to LYS905 and to Cys909, with which FsE interacts. Additionally, with high energy, both FsE and PFFE are bound to JAK3 at other critical sites ASP867 and ARG911 located at the active pocket of JAK3 kinase.

To this end, the marine-extracted diterpene, FsE, which has been reported to be a powerful anti-inflammatory agent (Reina et al., 2011) is a potential candidate to be tested preclinically and clinically to treat RA as it can bind to (with higher energy and short distances) and target several critical active residues in JAK3. In addition, it has ideal physiochemical and oral availability characteristics. To conclude, we provide a detailed analysis of the amino acid residues in JAK proteins with which the currently proposed set of bioactive molecules interact. The current finding has been validated through the comparative analysis with the FDA-approved inhibitor tofacitinib and the binding affinity to the ATP-binding and active sites.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest in association with the present study.

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