European Journal of Pharmaceutical Sciences 206 (2025) 107013

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Combining *in vitro, in vivo,* and *in silico* approaches to evaluate the effect of serotonergic-based topical therapies on mild to moderate psoriasis

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ARTICLE INFO

Keywords:
Psoriasis
Inflammation
Specific serotonin reuptake inhibitors
In vitro
In vivo
In silico

ABSTRACT

Psoriasis, a chronic inflammatory skin disease, poses a significant burden on patients' quality of life and healthcare systems. While mild-to-moderate cases are treated topically, usually combined with phototherapy, severe cases require systemic treatment with immunosuppressants, retinoids or biologics. However, all available treatments have drawbacks in terms of efficiency and side effects. Drawing from studies linking depression treatment to psoriasis improvement, we investigated whether topical formulations of selective serotonin reuptake inhibitors (SSRIs) could offer a viable therapy for psoriasis. Five SSRIs (sertraline, fluoxetine, paroxetine, escitalopram, fluvoxamine) were evaluated for their *in vitro* cytotoxicity, in human keratinocytes and THP-1 monocytes. Their anti-inflammatory action was tested using cell differentiation assays and immunoassays of pro-inflammatory cytokines in THP-1 monocytes. The results obtained with sertraline, escitalopram, and fluvoxamine suggested further evaluation *in vivo*. Anti-inflammatory effects were evaluated by skin parameter monitoring and histopathology, in an imiquimod-induced psoriasis-like inflammation mice model, and the best results were obtained for fluvoxamine. These findings were further supported by *in silico* molecular docking studies of the structural interaction between the serotonergic receptors and the drugs. Future research will focus on developing and characterizing of topical fluvoxamine formulations, like emulsions and penetration-enhancer vesicles, which offer advantages over the gels used herein.

1. Introduction

Psoriasis is a chronic, noncommunicable, immune-mediated, inflammatory skin disease affecting roughly 2 to 3% of the world's population (Eberle et al., 2016; Luger and Loser, 2018; Martins et al., 2020a; Rendon and Schakel, 2019). Psoriasis pathogenesis involves a complex interplay of genetic and environmental factors, driven by the interaction among innate and adaptive immune cells, and keratinocytes. This intricate process, mediated by signaling molecules such as cytokines and chemokines, results in inflammation, marked by an increase of rapid keratinocyte proliferation, neoangiogenesis, and white blood cells infiltration into the skin, ultimately leading to the formation of characteristic psoriasis plaques (Martins et al., 2020a). The most common form, *psoriasis vulgaris*, presents as red, scaly, well-demarcated plaques that can merge to cover extensive skin areas (Martins et al., 2020a; Rendon and Schakel, 2019). These plaques exhibit abnormal keratinocyte differentiation and proliferation, resulting in epidermal hyperplasia, dermal inflammatory immune cell infiltration, and neovascularization (Benhadou et al., 2019; Hugh and Weinberg, 2018).

While the pathogenesis of psoriasis has been studied since the 1800s (Fasanella, 2020), the involvement of the well-known neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has emerged more recently. Serotonin is a mediator between the neuroendocrine system and the skin, playing roles in inflammation, vasodilation, immunomodulation and pruritogenesis (Martins et al., 2020a; Menezes et al., 2016). Furthermore, research indicates that 5-HT can be produced within the

Abbreviations: 5-HT, 5-hydroxytriptamine (serotonin); 5-HTR, serotonin receptor; PASI, Psoriasis Area and Severity Index; PMA, phorbol 12-myristate 13-acetate; SERT, Serotonin Transporter; SSRIs, specific serotonin reuptake inhibitors.

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https://doi.org/10.1016/j.ejps.2025.107013
Received 22 September 2024; Received in revised form 11 January 2025; Accepted 12 January 2025
Available online 13 January 2025

skin by various cell types, including immunocytes, and that it exerts its effects on skin cells through serotonin receptors (5-HTR) and the serotonin transporter (SERT or 5-HTT) (Nordlind et al., 2008; Nordlind, 2006; Thorslund, 2012, 2009) (Fig. 1).

Immunochemical studies have also highlighted significant differences in the serotonergic system between normal and psoriatic skin. Serotonin seems to be expressed in psoriatic lesions, but not in normal skin cells (Huang, 2004; Younes and Bakry, 2016.). The expression of 5-HT in psoriasis varies depending on the disease stage and the involved cells. Furthermore, the expression of 5-HTRs is significantly altered in psoriatic skin. For example, Nordlind et al. (2006) noted lower expression of 5-HT1AR and higher expression of 5-HT2AR in psoriatic dermis, while 5-HT3R was expressed in non-involved psoriatic skin. Variations in the expression of SERT in normal and psoriatic skin have also been observed. Psoriatic skin exhibits an increased number of epidermal inflammatory cells immunostained for SERT, suggesting a potential role of this transporter in regulating the apoptosis of inflammatory cells (Thorslund, 2009).

The involvement of 5-HT in psoriasis is further supported by its connection to the brain-skin axis, as psoriasis is influenced by psychological stress and can lead to depression and anxiety which, in turn, can exacerbate psoriasis symptoms (Martins et al., 2020a). Furthermore, recent research suggests that inflammation, a hallmark of psoriasis, may contribute to the pathophysiology of depression. The 'cytokine theory of depression' proposes that inflammatory processes, with associated hormonal and biochemical changes, may underlie depression and other mental disorders (Galecki et al., 2018). Studies have shown that mRNA levels of pro-inflammatory cytokines are increased in the blood of depressed patients compared to healthy individuals (Tsao et al., 2006). Additionally, specific serotonin reuptake inhibitors (SSRIs), well-known antidepressants, have been observed to impact psoriasis symptoms (D'Erme et al., 2014; Thorslund and Nordlind, 2007; Thorslund et al., 2013). For example, a population-based cohort study revealed that patients with mild-to-severe psoriasis experienced reduced need for systemic psoriasis treatment, after six months of exposure to SSRIs (Thorslund et al., 2013). This could be due to a direct effect of the SSRIs on the treatment of depression and/or due to an indirect effect explained by the anti-inflammatory effects of SSRIs. Nevertheless, conflicting findings exist regarding the effects of the systemic use of antidepressants in psoriasis, with some studies reporting exacerbation of symptoms following treatment with certain SSRIs like fluoxetine (Hemlock et al., 1992; Tamer et al., 2009; Tan Pei Lin and Kwek, 2010) and paroxetine (Osborne et al., 2002).

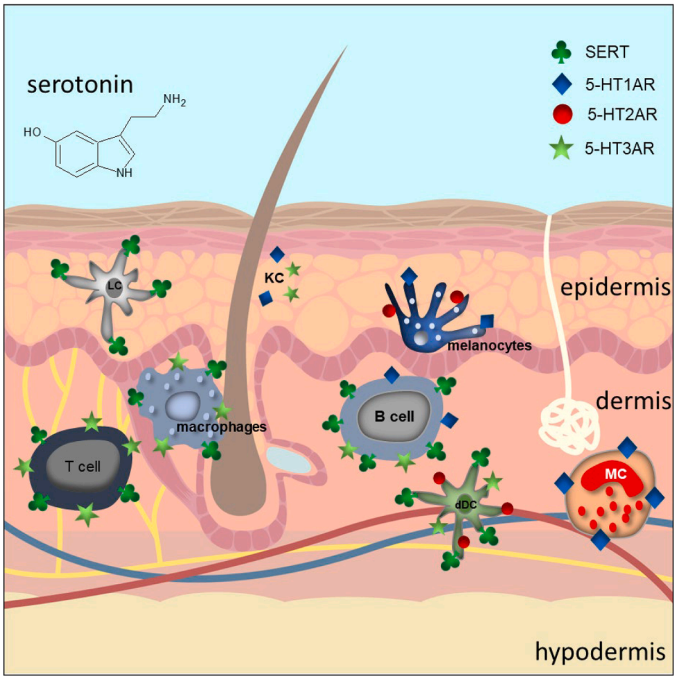

Fig. 1. The serotonergic system in skin. Serotonin (5-HT), serotonin transporter (SERT) and serotonin receptors (5-HTRs) in human skin immunocytes (LCs, Langerhans cells; MCs, mast cells; dDCs, dermal dendritic cells; T cells; B cells; macrophages) and non-immunocytes (KC, keratinocytes; melanocytes). The figure shows all the membrane proteins that may be present at any given time. The receptors (5-HTR) shown are only the ones used for *in silico* studies.

The involvement of the serotonergic system in psoriasis and the findings from SSRI systemic administration studies on psoriasis symptoms have led to the hypothesis that these compounds could be effective in topical formulations for localized treatment. Psoriasis treatments, recently reviewed by our group (Martins et al., 2020b), typically involve topical drugs (corticosteroids, vitamin D analogues, immunosuppressants), phototherapy or a combination of both for mild-to-moderate cases, or systemic treatment with small molecules or biologics for more severe cases.

The aim of this study was to assess the efficacy of topical formulations of SSRIs - sertraline, fluoxetine, paroxetine, escitalopram, and fluvoxamine - in treating psoriasis symptoms. This was achieved through a combination of *in vitro* cytotoxicity and anti-inflammation studies, and *in vivo* experiments using an imiquimod (IMQ)-induced psoriasis-like inflammation mice model. Furthermore, this experimental approach was complemented by *in silico* molecular docking studies that analyzed the interaction between the drugs and 5-HT transporter (SERT) and receptors (5-HT1A, 5-HT2A and 5-HT3A) present in skin. These studies, employing a structural-based virtual screening, evaluate ligand conformations adopted within the designated binding sites of macromolecular targets and determine ligand-receptor affinities using scoring functions available within docking programs. Structural properties of the binding regions, flexibility, the type of interactions and molecular properties are fundamental to the rational development of a molecular docking campaign. Validated methods allow for the assessment of small molecule affinities and the selection of candidates for further biological research.

2. Materials and methods

2.1. Materials

Sertraline hydrochloride, escitalopram oxalate, fluvoxamine maleate, fluoxetine hydrochloride and paroxetine hydrochloride were a generous gift from Generis Farmacêutica (Portugal). Dimethyl-β-cyclodextrin was provided by Wacker (Portugal). Transcutol® P (Diethylene Glycol Monoethyl Ether) was a kind gift from Gattefossé (Lyon, France). The parabens Nipagin™ M Sodium (Sodium Methylparaben) and Nipasol M Sodium™ (Sodium Propylparaben) were purchased from Clariant (Muttenz, Switzerland). Hydroxypropylmethylcellulose (HPMC) was obtained from Sigma Aldrich (Germany). The Aldara® cream (5% imiquimod) was obtained from Meda AB (Solna, Sweden) and Dermovate® (0.5 mg/g clobetasol propionate) was obtained from GlaxoSmithKline. Ultrapure water was obtained by inverse osmosis in a MILLI-Q System Elix 3, from EMD Millipore. All other reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of the SSRIs gels

First, 12% (w/w) of di-methyl-β-CyD was solubilized in purified water, followed by the addition of the drug (fluvoxamine maleate, escitalopram oxalate, sertraline hydrochloride) to final concentrations of 5% (w/w), under magnetic stirring (300 rpm). In the second step 0.18% (w/w) methylparaben and 0.02% (w/w) propylparaben were added together with 5% (w/w) Transcutol® P. The HPMC polymer was added in the end and the mixture was stirred at 7500 rpm for about 4

min, using the Ultra Turrax Homogenizer IKA T25 Digital with a Dispersion Tool T25 S25N-10G probe, until the gelled appearance was observed.

2.2.2. *In vitro studies*

2.2.2.1. Cytotoxicity. The five SSRIs (sertraline, fluoxetine, paroxetine, escitalopram, fluvoxamine) were tested *in vitro* in the human keratinocyte HaCaT cell line (CLS, Germany) and in the human monocytic THP-1 cell line (ATCC TIB-202™). THP-1, THP-1-derived macrophages (differentiation induced by 100 ng/mL *E. coli* lipopolysaccharide (LPS) and 100 nM phorbol 12-myristate 13-acetate (PMA) for 24 h, as described by Lund et al. (2016)) and HaCaT cells were exposed to medium containing different drug concentrations (1.5–100 μM for sertraline, fluoxetine and paroxetine; 3–200 μM for escitalopram and fluvoxamine) for 24 h. Cytotoxicity was assessed using the resazurin assay, which quantifies metabolically active cells (Nociari et al., 1998). Inhibitory concentrations (IC50) were calculated using the GraphPad Prism software v5.0.

2.2.2.2. Anti-inflammatory studies. The anti-inflammatory action of the SSRIs was tested by cell differentiation assays and immunoassay determination of cytokines. THP-1 monocytes were treated with different concentrations of each drug for 2 h (1.5–100 μM for sertraline, fluoxetine and paroxetine; 3–200 μM for escitalopram and fluvoxamine) prior to inducing their differentiation with LPS and PMA (24 h). Supernatant aliquots were then retrieved and stored at −80 °C until cytokine quantification. The non-adherent non-differentiated cells were separated from the adherent differentiated ones and, in both cases, viable cells were quantified using the resazurin assay (Nociari et al., 1998). Untreated cells exposed to LPS and PMA were used as 100 % differentiation controls, while unstimulated cells were used as blank controls. The pro-inflammatory cytokines IL1-β and TNF-α were determined with the DuoSet Elisa Kits (R&D Systems), according to the manufacturer's protocols.

2.2.3. *In vivo studies using a mice IMQ-induced psoriasis-like inflammation model*

The three SSRI candidates (sertraline, fluvoxamine, and escitalopram) selected in the previously described *in vitro* cytotoxicity and anti-inflammatory assays, were evaluated for their antipsoriasis activity *in* vivo. The drugs were formulated as gels, as described in 2.2.1. The studies were performed using a (IMQ)-induced psoriasis-like inflammation mice model, as described previously (Nunes et al., 2020; Pivetta et al., 2018).

This study used 36 female BALB/c mice aged 6 weeks (Charles River Laboratories, Saint Germain Nuelles, France) and all experiments were carried out in accordance with the animal welfare body of the Faculty of Pharmacy, University of Lisbon, approved by the competent national authority (Direção Geral de Alimentação e Veterinária - DGAV) under the project "Ser4Skin - Serotonergic-based therapies to decrease the impact of moderate to severe psoriasis: translation of fundamental research into industry" and in accordance with the EU Directive (2010/63/EU), Portuguese laws (DL 113/2013, 2880/2015, 260/2016 and 1/2019) and all relevant legislation. The animal strain was selected according to published data where BALB/c mice were used as a model of human psoriasis (Nunes et al., 2020; Pivetta et al., 2018). The animals were housed in individual polypropylene cages at room temperature (20–24 °C), relative humidity (55 ± 5%), light/dark cycle for 12 h, and received a standard feed and water *ad libitum*. No anesthesia was required for the experiments and euthanasia was achieved by CO_2 exposure.

The 36 mice were randomly assigned to six different groups: Group 1 ($n = 6$) – non-treated animals, naïve group; Group 2 - animals treated with 60 mg of Dermovate® (containing 0.5 mg/g clobetasol propionate) as a positive control of inflammation inhibition; Group 3 ($n = 6$) - animals treated with 60 mg of placebo gel; Group 4 ($n = 6$) - animals treated with 60 mg of 5 % fluvoxamine maleate (50 mg/g gel); Group 5 ($n = 6$) - animals treated with 60 mg of 5 % escitalopram oxalate (50 mg/g gel); Group 6 ($n = 6$) – animals treated with 60 mg of 5 % sertraline hydrochloride (50 mg/g gel).

The study was carried out for 6 consecutive days (days 0 to 5), with the treatments applied every day. First, all mice were shaved on the dorsal area and kept in individual cages. The gels to be tested were spread on the shaved areas with a spatula, at an average 26.7 mg/cm^2. Five hours post-treatment, 60 mg of Aldara® cream containing 5% IMQ was applied on the treated areas. After the first day, all mice were visually examined daily (from day 1 to day 5), after the treatment, and skin thickness and body weight were measured. The skin thickness of the treated zone was determined with a digital caliper (Fisherbrand, Leicestershire, UK). The psoriasis-inflammation evaluation criteria were assessed according to the clinical Psoriasis Area and Severity Index (PASI) score: erythema, scaling, and thickening were scored from 0 to 4 (0: none; 1: slight; 2: moderate; 3: marked; 4: very marked). The animals were euthanized on the sixth day and the back-skin tissue was fixed with a 10% formalin solution, to be used in histopathological analysis. Tissues were embedded in paraffin, cut into 4 μm longitudinal sections and then stained with haematoxylin and eosin (HE). The evaluation was performed by a pathologist blinded to experimental groups. Lesions were characterized by extensive inflammation expanding to the dermis, and infiltrate related to granulation tissue with a marked neutrophilic component. Measurements were performed in slides digitally scanned in the NanoZoomerSQ with NDP.view2 software (Hamamatsu, Japan) corresponding to the mean value obtained from 7 to 12 different points for epidermis and dermis layers.

2.2.4. *In silico molecular docking studies*

2.2.4.1. Selection and preparation of the ligands and proteins structures. The initial three-dimensional structures of the fluvoxamine, sertraline and escitalopram molecules were obtained from the PubChem database; the structures were energy minimized. The crystal structures of SERT and 5-HT1R, 5-HT2R, and 5-HT3R were selected considering their quality (resolution and the absence of errors) and retrieved from Protein Data Bank (PDB, PDBID: 6VRH for SERT, PDBID: 7E2Y for 5-HT1AR, PDBID: 6A94 for 5-HT2AR and PDBID 6Y5A for 5-HT3AR). All these proteins have available X-ray crystal structures where they are complexed with ligands, which facilitated the mapping and selection of the binding site to be used in docking calculations. The 3D structures were individually prepared for each protein using the Molecular Operating Environment program (MOE v2020.09). Initially, all crystallographic elements other than the protein and drug (water, ions, etc.) were discarded. Afterwards, the protein and ligand were separated, and hydrogens were added to the protein molecule. The protonation states at pH 7.4 and 300 K were assigned using the protonation tool (Protonate 3D) implemented in MOE.

2.2.4.2. Docking strategy (preparation and protocol). Once the structures of proteins and ligands were ready, they had to be validated, to establish their suitability for docking studies. For this purpose, self-docking calculations were performed for the four protein structures prepared by redocking their respective X-ray ligand and comparing the obtained docking poses with the experimental ones (RMSD<2 Å).

For validation, the four scoring functions that are implemented in the GOLD 2020.001 program were tested. All scoring functions and systems were analyzed for score, performance, and ligand interactions. All calculations performed had full flexibility for the ligands while the protein structure coordinates remained rigid.

The centre of the search space was defined by a single atom at the binding site (previously selected from the X-ray structures) for each of the proteins with a radius of 15 Å

2.2.5. Statistical analysis

The results were reported as mean ± standard deviation. The results of *in vitro* studies were statistically analyzed by one-way ANOVA, followed by multiple comparisons using Turkey's test, in SigmaPlot 11.0 software®. For *in vivo* assays, a two-way ANOVA followed by Bonferroni's post hoc test was used. The differences were considered statistically significant (*) when $p < 0.05$.

3. Results and discussion

3.1. In vitro cytotoxicity and anti-inflammatory studies

The serotonergic drugs underwent initial testing for their *in vitro* cytotoxicity using two different cell lines: HaCaT human keratinocytes and human leukemia monocytes (THP-1 cells). The first cell line represents the main epidermal skin cells, while THP-1 cells were further used in anti-inflammatory assays. The cytotoxicity results for the various cell types and tested SSRIs are summarized in Table 1. Based on the IC_{50} results obtained, it can be observed that, in general, sertraline exhibited the highest cytotoxicity, while fluvoxamine and escitalopram showed the lowest cytotoxicity. Regarding HaCaT cells, there were no significant differences between the IC_{50} values obtained for sertraline, fluoxetine and paroxetine. For THP-1 cells, differentiated and undifferentiated, the IC_{50} for fluoxetine was significantly higher than for sertraline ($p < 0.05$ for undifferentiated cells, $p < 0.001$ for differentiated cells) and paroxetine (($p < 0.01$ for undifferentiated cells, $p < 0.001$ for differentiated cells).

These findings guided the selection of an appropriate concentration range for subsequent assays.

Subsequently, the anti-inflammatory action of the five SSRIs was assessed in THP-1 monocytes, which serve as an *in vitro* model of macrophages engaged in anti-inflammatory responses (Chanput et al., 2014). These cells undergo differentiation upon exposure to PMA and are activated by LPS, leading to cytokine production and enhanced differentiation. Pre-exposure of the cells to the different SSRIs prior to differentiation and activation had an anti-inflammatory effect, as depicted in Fig. 2.

The analysis of pro-inflammatory cytokines produced by activated THP-1 cells pre-treated with the different SSRIs revealed that, even at low concentrations, all drugs led to a decreased production of IL-1β, with fluvoxamine being the most effective. However, paroxetine and escitalopram had a significantly lower anti-inflammatory effect than the other SSRIs, as shown by the higher concentrations required to decrease the concentration of TNF-α by 50 %. Overall, fluvoxamine emerged as the most effective drug in decreasing cytokine production by THP-1 cells (Fig. 2).

In summary, the *in vitro* results led to the selection of sertraline, fluvoxamine, and escitalopram as the SSRIs for subsequent *in vivo* studies and further evaluation through *in silico* studies. Escitalopram and fluvoxamine showed low cytotoxicity, while sertraline, fluoxetine, and paroxetine exhibited the highest cytotoxicity across the tested cells. Nonetheless, given sertraline's favorable *in vitro* anti-inflammatory effect, this SSRI was included in the *in vivo* studies.

Table 1
Concentration of SSRIs that reduced the cell viability to 50 % after 24 h of incubation. THP-1 und. – THP-1 undifferentiated cells, THP-1 dif. – THP-1 cells differentiated with 100 ng/mL LPS and 100 nM PMA. Results are mean ± standard deviation, $n = 6$. Statistical significance, *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, ǂ $p < 0.001$.

Drug	IC_{50} (μM)		
	HaCaT	*THP-1 und.*	*THP-1 dif.*
Sertraline	36 ± 3	34 ± 5*	28 ± 6***
Fluoxetine	48 ± 10	50 ± 2*, **	53 ± 1***, ǂ
Fluvoxamine	> 200	> 200	> 200
Paroxetine	48 ± 8	28 ± 6**	31 ± 2ǂ
Escitalopram	> 200	> 200	> 200

3.2. In vivo studies using a mice IMQ-induced psoriasis-like inflammation model

The anti-inflammatory effectiveness of the selected SSRIs - sertraline, fluvoxamine and escitalopram - was then assessed *in vivo* using a mouse model of psoriasis-like inflammation induced by IMQ. To ensure simplicity and ease of application, gel formulations were chosen for the study. All gels were transparent and had shear-thinning behavior (Fig. S1, Supplementary Data). Therapeutic efficacy was evaluated through PASI scores, calculated from days 1 to 6 during treatment. Subsequently, after euthanasia, histopathological analysis of the treated skin was conducted. The animals were treated with a fixed concentration of each SSRI (50 mg/g gel), while a non-medicated gel served as placebo. The control groups included non-treated animals (normal skin, naïve group), and animals treated with a commercially available anti-psoriatic cream containing 0.5 mg/g clobetasol propionate (Dermovate®) as the positive control.

The PASI score results, summarized in Fig. 3, depict the assessed criteria (erythema, scaling and thickness) along with the cumulative scores (Fig. 3d). Sertraline and fluvoxamine demonstrate outcomes closely resembling those of Dermovate®, the positive control, in terms of erythema and scaling. While sertraline and fluvoxamine exhibit similar results concerning thickness, the PASI score exceeds that of Dermovate®. Overall, these findings suggest the preferential use of fluvoxamine due to its superior anti-inflammatory efficacy and lower cytotoxicity compared to sertraline, as evidenced in the previous *in vitro* studies. The results obtained for the control non-treated animals (normal skin, naïve group) were not plotted in the graph shown in Fig. 3 for clarity, as they overlapped with data from the Dermovate® group. As expected, no skin changes were observed.

Following euthanasia, histopathological analysis of skin samples was conducted (Fig. 4). Psoriatic skin typically exhibits hyperkeratosis, a prominent feature of chronic epidermal diseases and skin irritation. Hyperkeratosis is characterized by thickening of the *stratum corneum* due to increased turnover of epidermal cells and/or reduced desquamation of corneocytes (Farci and Mahabal, 2022). This condition is often accompanied by or secondary to epidermal hyperplasia, manifested as increased thickness of the non-keratinized layers of the epidermis. Most often, hyperkeratosis primarily manifests as orthokeratotic, wherein the *stratum corneum* thickens without retained nuclei. Conversely, parakeratotic hyperkeratosis is characterized by the presence of nuclei in the *stratum corneum*, is less common and coincides with increased epithelial hyperplasia (van der Fits et al., 2009).

As depicted in Fig. 4, the administration of IMQ resulted in alterations across all groups, namely epidermal hyperplasia, and both orthokeratotic and parakeratotic hyperkeratosis. All treated groups, including placebo, showed re-epithelization indicative of a significant skin reparation mechanism. These findings align with previous reported results (Pivetta et al., 2018). Mice skin treated with escitalopram gel presented multifocal areas of epidermal erosion, covered by serocellular fibrinous crust with necrotic debris infiltrated with neutrophils (Fig. 4 e2). Similarly, animals treated with sertraline gel exhibited comparable epidermal erosion (Fig. 4 f2) along with parakeratotic hyperkeratosis marked by the presence of nuclei within the cornified layer (Fig. 4 f3). In contrast, the positive control group presented minimal hyperplasia without cellular infiltration (Fig. 4b) while normal skin (no IMQ challenge) presented typical cell infiltration or hyperplasia (Fig. 4a). Compared to placebo, fluvoxamine notably reduced epidermal thickness and attenuated IMQ-induced conditions, including epidermal thickening and parakeratosis.

Overall, the *in vivo* studies using an (IMQ)-induced psoriasis-like inflammation model in mice demonstrated that fluvoxamine effectively ameliorated symptoms induced by IMQ application, resulting in

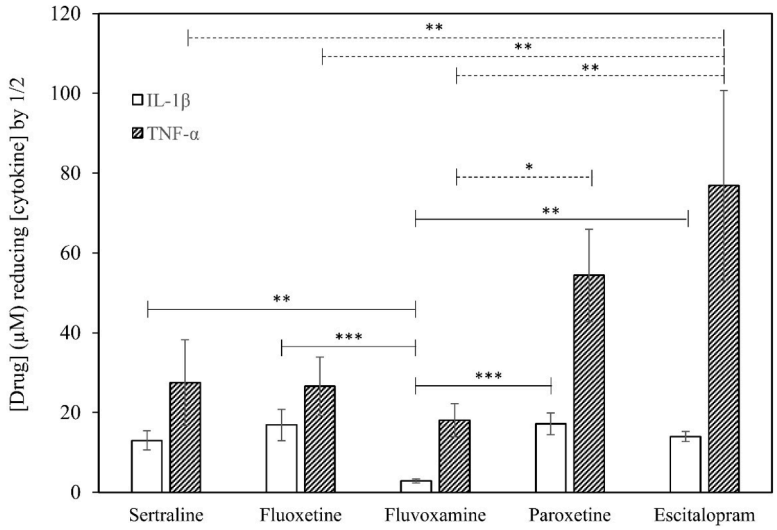

Fig. 2. *In vitro* anti-inflammatory effect of the evaluated SSRIs. The graph shows the concentrations of each SSRI solution (sertraline hydrochloride, fluoxetine hydrochloride, fluvoxamine maleate, paroxetine hydrochloride and escitalopram oxalate) which can reduce the concentration of pro-inflammatory cytokines (IL-1β and TNF-α) produced by THP-1 cells by 50 %. Results are mean ± standard deviation, $n = 6$. Statistical significance, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

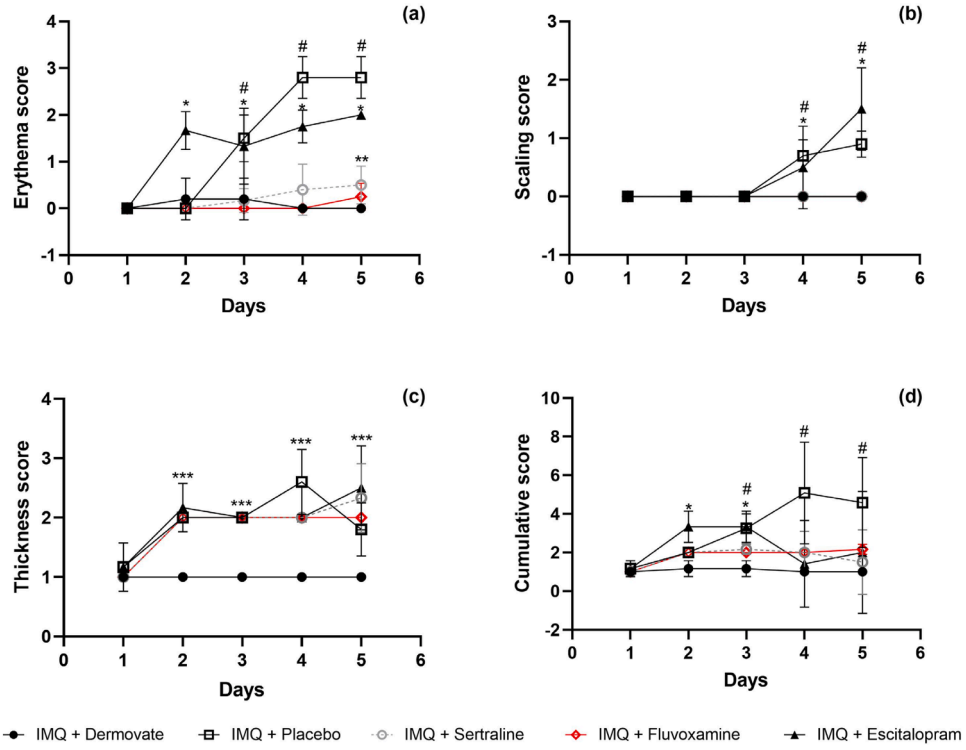

Fig. 3. Anti-inflammatory effects of fluvoxamine, escitalopram and sertraline gels on an IMQ-induced psoriasis-like inflammation model in mice. The graphs show the PASI scores obtained for: (a) Erythema; (b) Scaling; (c) Thickness; (d) Cumulative. The scores are from 0 to 4, as follows: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked. Results are mean ± standard deviation, $n = 6$. The negative control is the placebo, *i.e.*, the vehicle of the drug formulations, while the positive control is Dermovate® (clobetasol propionate), an anti-inflammatory drug. Statistical analysis was performed *versus* the Dermovate® group and the results are indicated in the figure: $^{\#}p < 0.05$ Placebo; $^{*}p < 0.05$ Escitalopram; $^{**}p < 0.05$ Sertraline; $^{***}p < 0.05$ for all tested groups *versus* Dermovate®.

milder skin symptoms (Fig. 2) and fewer structural changes (Fig. 3) in the epidermis compared to other treatments. Furthermore, statistical analysis suggests that treatment with fluvoxamine maleate yielded results closer to the positive control than the placebo. Although the IMQ model is a subacute model, where prolonged treatment is not possible, it is extremely useful since it allows for the comparison between different drug candidates using a reduced number of animals.

3.3. *In silico molecular docking studies*

For the docking campaigns, the 3D structures of both the proteins (transporter and receptors) and the ligands (fluvoxamine, sertraline and escitalopram) were selected and validated. The calculations carried out to validate the docking protocol used the four scoring functions within the GOLD software: Chemscore (Eldridge et al., 1997), ChemPLP (Korb et al., 2009), GoldScore (Jones et al., 1995), and ASP (Mooij and

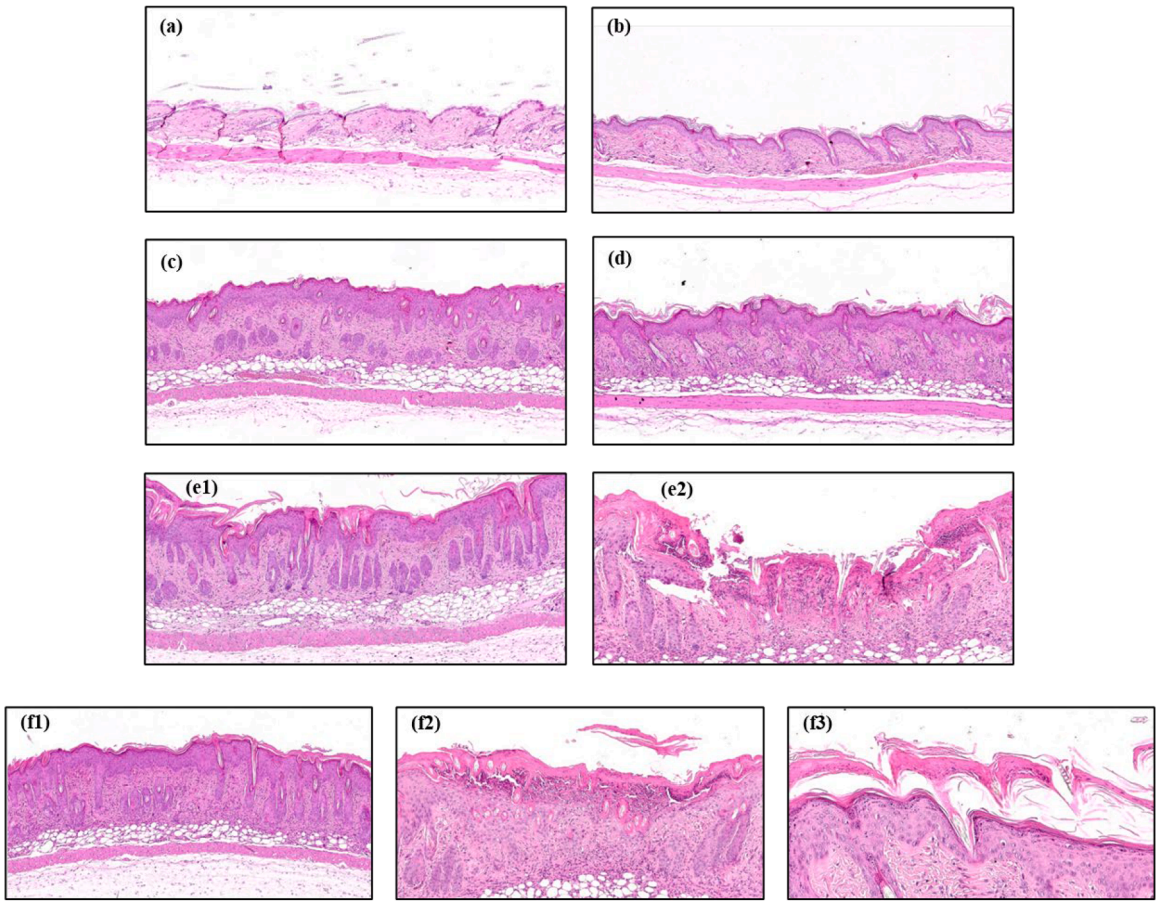

Fig. 4. *In vivo* studies of the anti-inflammatory effect of fluvoxamine, escitalopram and sertraline on IMQ-induced psoriasis-like inflammation model: histological images of mice skin (stained with HE). (a) Normal skin (magnification 8×); (b) Skin treated with Dermovate® (magnification 8×); (c) Skin treated with placebo (magnification 8×); (d) Skin treated with fluvoxamine gel (magnification 8×); (e1) Skin treated with escitalopram gel (magnification 8×); (e2) Focal area of skin treated with escitalopram gel (magnification 10x); (f1) Skin treated with sertraline gel (magnification 8×); (f2) Focal area of skin treated with sertraline gel (magnification 10x); (f3) Focal area of skin treated with sertraline gel (magnification 20×). The placebo (vehicle of the drug formulations) is the negative control while Dermovate® is the positive control (anti-inflammatory).

Verdonk, 2005). The results underscored that the ChemPLP scoring function most accurately reproduced the experimental X-ray structures, so all the subsequent calculations and analyses exclusively used this function. The following sections present the outcomes concerning binding regions, the flexibility and positioning of the ligands in the pocket, interaction types, and protein-ligand affinities. These characteristics, fundamental for rational drug development, were studied in the analysis.

3.3.1. *SERT*

In the X-ray structures available for SERT, the protein is co-crystallized in the presence of a drug, which allowed the easy identification of the drug binding site and the interactions it establishes with the transporter protein. Coleman et al. (2020) and Plenge et al. (2021) previously studied the SERT protein and guided the selection of the binding site and the residues important for the binding of the ligand. In the 6VRH structure, SERT is complexed with paroxetine, establishing key interactions with Phe335 and Asp98 residues. The interactions established between the Asp98 residue and paroxetine in the 6VRH structure are also present in the 7LWD structure, where SERT is complexed with vilazodone, which reveals a unique binding pocket in relation to this drug but maintaining key receptor-ligand molecular interactions (Plenge et al., 2021).

The similarity of the binding mode to other antidepressant drugs (Coleman et al., 2020) and the conservation of the interaction with Asp98 led to the selection of the 6VRH structure to be used for SERT docking calculations. The analysis of the docking calculations confirmed a highest score for fluvoxamine, meaning that, from the three evaluated drugs, fluvoxamine has the highest affinity for SERT, followed by sertraline (Table 2).

The three drugs penetrated the pocket but a better occupancy was observed for fluvoxamine, which anchored from the initial region of the pocket (Figs. 5 and 6). Sertraline and escitalopram were placed in the back top of the pocket, exposing its frontal region. Sertraline was placed in the internal region of the pocket, sharing the same region of the CF_3 group of the aromatic ring of the fluvoxamine, but the rest of the molecule was perpendicular. Escitalopram was placed in the same direction as fluvoxamine. It was not observed a pattern formed by the protein-ligand interactions defined by the drugs (Table 2). However, the position of the molecules was shared in the top direction of the binding region. Escitalopram presented an interaction with Asp98. Although fluvoxamine does not interact with Asp98, the molecule is oriented and in the interface with this amino acid residue, reinforcing the good

Table 2
Compilation of the docking scores results for each system and the SERT amino acids of the protein-ligand binding pocket interactions.

Drug	Sertraline	Fluvoxamine	Escitalopram
Score	68.23	78.46	65.48
protein-ligand interactions (aa)	Thr439	Glu493, Glu494	Asp98, Phe335, Arg104, Tyr176

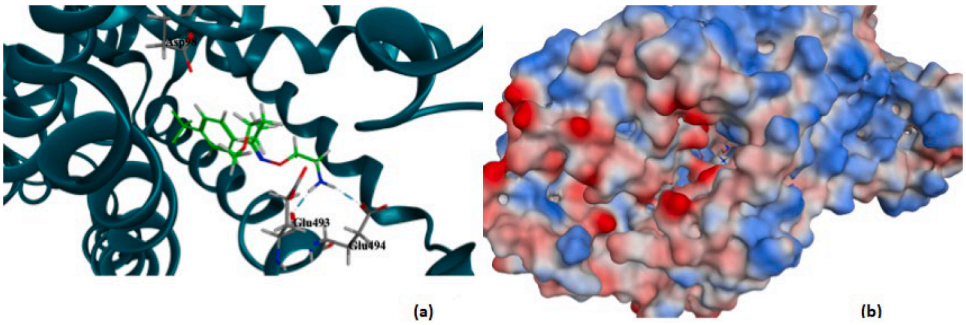

Fig. 5. Docking of fluvoxamine: binding pocket and protein surface. (a) The fluvoxamine in the binding region with identification of the residues Asp98, Glu493 and Glu494. (b) Surface of the protein that identifies the binding pocket of the ligands with fluvoxamine. In blue is the hydrophilic contribution and in red the lipophilic contribution of the surface.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

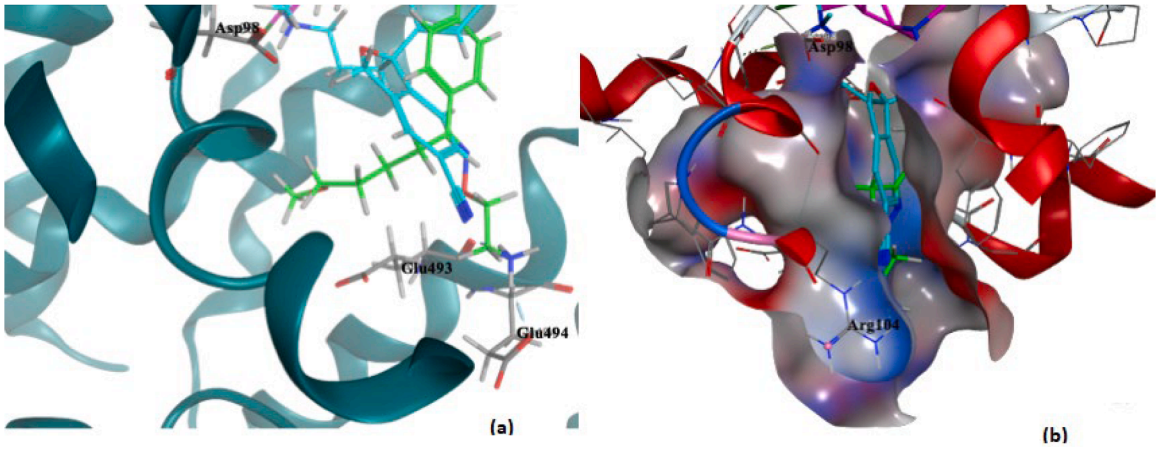

Fig. 6. Docking of SERT: binding pocket and receptor surface. (a) The overlap of fluvoxamine (green), sertraline (purple) and escitalopram (cyan) in the binding region with identification of the residues. (b) Surface of the receptor that identifies the binding pocket of the ligands. In blue is the hydrophilic contribution and in red the lipophilic contribution of the surface.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

molecular docking performance of the structure.

3.3.2. 5-HT1A

Xu et al. (2021) resolved the crystal structure of the 5-HT1A receptor free and complexed with different drugs: the apo state (7E2X), bonded to 5-HT (7E2Y) or bonded to the antipsychotic drug aripiprazole (7E2Z). From X-ray examination, we observed that 5-HT and aripiprazole shared the same binding site region, and both interacted with Asp116. The occupancy of the binding pocket by 5-HT and aripiprazole is smaller due to the small size of both molecules. However, both molecules interact with the residue mentioned and the aromatic moiety are in the same region. Analyzing the exposition of the amino acids, it was selected Asp116 of the 7E2Y to use in the molecular docking simulation.

The results obtained show that, similarly to SERT, fluvoxamine is the drug showing a better docking score in the simulations (Table 3). When comparing the best poses obtained by docking the three drugs in this study with the 5-HT position in the crystal structure 7E2Y, it can be noted that the aromatic moiety (with the CF_3 substituent) of fluvoxamine overlaps the inner ring of 5-HT, with a better fit to the pocket surface (Fig. 7a). Fluvoxamine migrates deeper into the inner region of the pocket, as does 5-HT in the crystal structure. Both sertraline and escitalopram are further away from this region, and more distant from the Asp116 residue (Fig. 7b). The two latter drugs are located near the entrance region of the pocket (Fig. 7c). A pattern of protein-ligand interactions between the 5HT1A receptor and the three studied drugs was not observed.

Table 3
Compilation of the docking scores for each drug and the 5HT1A amino acids involved in the protein-ligand interactions.

Drug	Sertraline	Fluvoxamine	Escitalopram
Score	56.81	68.29	58.77
Protein-ligand interactions (aa)	Asn386	Ser190, Thr196, Asn386	Lys191

3.3.3. 5-HT2A

Kimura et al. (2019) resolved the complete structure of the 5-HT2A receptor complexed with the antipsychotic drugs risperidone and zotepine, which stabilize the protein by forming molecular interactions with the residues at the bottom of the binding pocket. The crystal structures 6A94 and 6A93 are very similar (RMSD=0.58 Å) having good quality resolution (3 Å). Both drugs interact with the Asp155 residue that was selected from the 6A94 structure to define the binding site for the docking calculations with 5-HT2A receptor. From the molecular docking simulation, we found that the poses obtained for the three drugs evaluated are similar by placing them in the binding site superimposed on the ligands of the crystallized structures with the receptor (Fig. 8). The best docked poses drug placed at the beginning of the region where the drugs zotepine and risperidone (6A94 and 6A93 crystal structures) place a fluorobenzisoxazole and a benzene rings, an important region that Kimura et al. identified as relevant for interaction (Kimura et al., 2019). Fluvoxamine and escitalopram occupy the entrance into the pocket, particularly the latter drug, which may explain the better score obtained in this simulation (Table 4). This placement obtained for the drugs at the pocket entrance may hinder access to the binding pocket to other

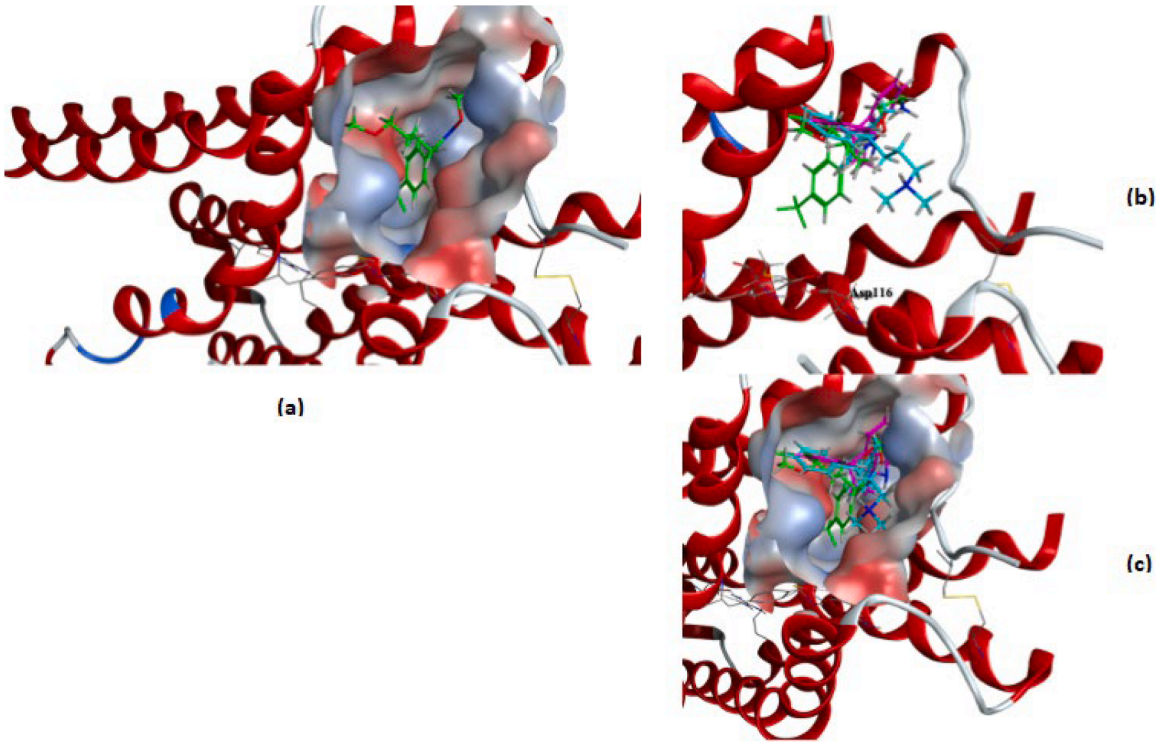

Fig. 7. Docking of 5HT1A: binding pocket and receptor surface. (a) Fluvoxamine in the binding pocket represented by the receptor surface. The color code of the surface representation: blue is for hydrophilic contribution and red for the lipophilic contribution of the surface. (b) Surface of the receptor that identifies the binding pocket of the ligands fluvoxamine (green), sertraline (purple) and escitalopram (cyan). (c) The overlap of the drugs in the binding region with identification of the residues.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

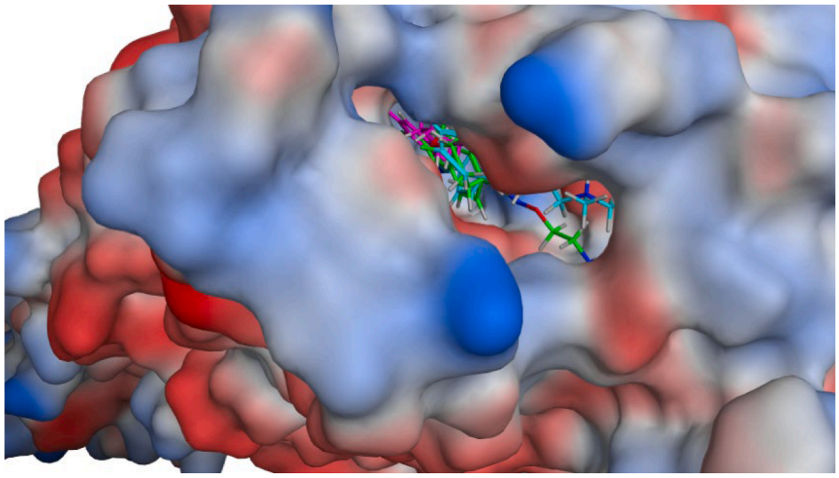

Fig. 8. Docking of 5HT2A: binding pocket and receptor surface. Surface of the receptor 5HT2A that identifies the binding pocket of the ligands fluvoxamine (green), sertraline (purple) and escitalopram (cyan) reinforcing its overlapping. The color code of the surface representation: blue is for hydrophilic contribution and red for the lipophilic contribution of the surface.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Compilation of the docking scores for each drug and the 5HT2A amino acids involved in the protein-ligand interactions.

Drug	Sertraline	Fluvoxamine	Escitalopram
Score	63.85	70.63	72.21
Protein-ligand interactions (aa)	Val156	Ser131, Asn343	Cys227

potential structures.

3.3.4. 5-HT3A

The structure of the 5-HT3A receptor was obtained complexed and in the apo state. All the structures presenting a ligand were selected for further analysis and the most recent available electron microscopy structure 6Y5A (2.8 Å resolution) was selected for the docking studies (Zhang et al., 2021). The structure was resolved complexed with 5-HT. In this structure, we can observe a hydrogen bond with Tyr64, a residue that was selected to define the binding site for docking. The pocket formed in the 5-HT binding region is exposed to the environment and the

cavity is closer to the surface of the protein. In the docking studies, the positioning of the drugs is in the binding region defined by 5-HT and a good overlap with this molecule is visible (Fig. 9). Both fluvoxamine and escitalopram exhibit a conformation adapted to the protein surface. From the experimental structures, it is known that the protein-ligand interactions of 5-HT are established with Tyr64 and Tyr126. From docking studies, it is possible to observe that the aromatic group of fluvoxamine and sertraline is close to the Tyr64 residue, with the aromatic group of escitalopram a bit further away. For the poses obtained for the drugs in this study, it can be observed that fluvoxamine and escitalopram also interact with Tyr126. This may indicate a protein-ligand interaction profile with this residue that is critical for binding activity. Again, fluvoxamine showed a better docking score, in agreement with the results obtained for the other receptors in the other systems (Table 5).

From sertraline, fluvoxamine and paroxetine docking studies on SERT, 5-HT1R, 5-HT2R and 5-HT3R receptors, it is possible to conclude that, in general, the compounds studied are well positioned in the binding site, but there is a greater affinity of fluvoxamine for these transporter/receptor proteins, as demonstrated both by the better positioning and the better interaction with residues important for ligand protein binding.

4. Conclusion

To the best of our knowledge, no previous studies have explored the topical action of SSRIs as a treatment for psoriasis. Despite extensive research, current treatments for this condition still have significant limitations in terms of efficacy and potential side effects. Whenever feasible, especially for mild-to-moderate psoriasis cases, topical treatment is preferred. Localized treatments avoid the first pass effect associated with the drug metabolism after ingestion, and usually cause fewer adverse side effects. The results obtained with the fluvoxamine gel developed in this study are promising. While fluvoxamine exhibited a less pronounced anti-inflammatory effect compared to Dermovate®, the positive control corticosteroid clobetasol propionate, it is essential to note that the drugs were formulated as basic gels. Considering the outcomes of the *in vivo* experiments, we believe that incorporating fluvoxamine into more advanced topical formulations will lead to a synergistic effect, increasing its permeation and direct delivery to the skin. In future studies, we aim to develop and characterize such advanced formulations, while simultaneously exploring various fluvoxamine concentrations.

Table 5
Compilation of the docking scores for each drug and the 5HT3A amino acids involved in the protein-ligand interactions.

Drug	Sertraline	Fluvoxamine	Escitalopram
Score	68.68	73.17	69.26
Protein-ligand interactions (aa)	Pro128	Trp63, Tyr126	Trp63, Tyr126

Funding sources

This work was supported by FCT (Fundação para a Ciência e a Tecnologia) Portugal [grant numbers PTDC/MEC-DER/30198/2017, EXPL/QUI-OUT/1288/2021, UIDB/04138/2020 and UIDP/04138/2020]. Lídia Gonçalves is financed by FCT under principal investigator grant CEECIND/03143/2017. Joana Marto is financed by FCT under the Scientific Employment Stimulus—Institutional Call (CEECINST/00145/2018).

CRediT authorship contribution statement

Ana M. Martins: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Lídia Gonçalves:** Writing – review & editing, Investigation, Formal analysis. **Sandra Simões:** Writing – review & editing, Investigation, Formal analysis. **Patrícia A. Serra:** Writing – original draft, Formal analysis. **Rita C. Guedes:** Writing – review & editing. **Helena Ribeiro:** Writing – review & editing. **Joana**

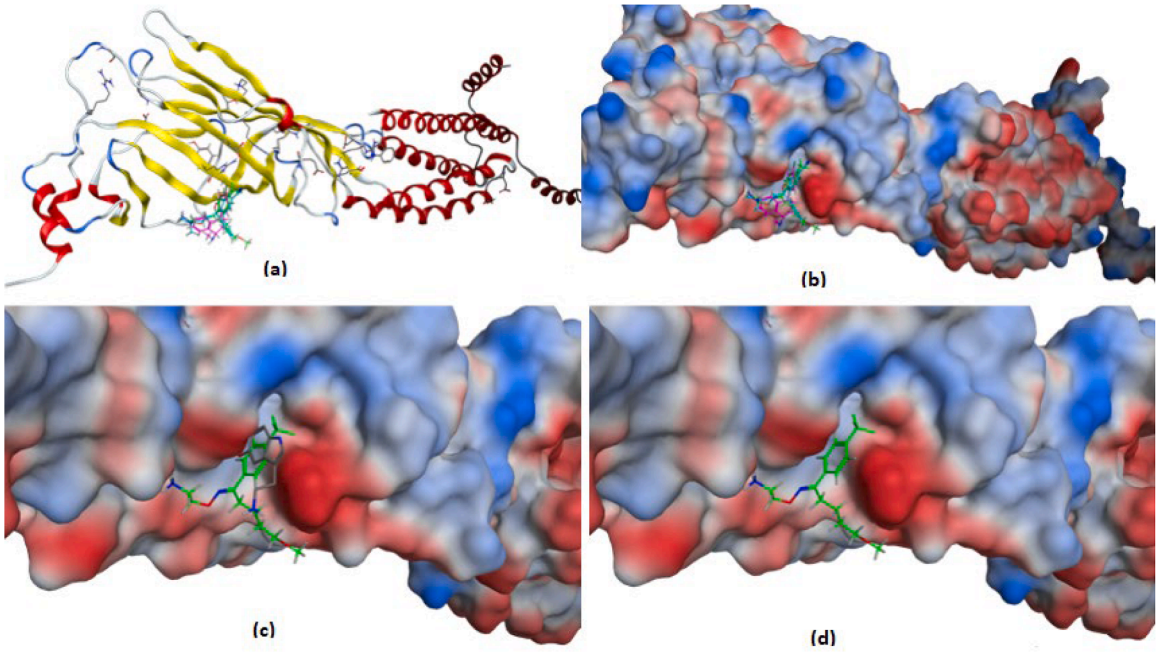

Fig. 9. Docking of 5HT3A: binding pocket and receptor surface. (a) Elongated structure of 5HT3A with the conformations resulted from the docking study. (b) Surface of the receptor 5HT3A that identifies the binding pocket of the ligands fluvoxamine (green), sertraline (purple) and escitalopram (cyan) reinforcing its position at the surface of the protein. The binding pocket is superficial. The color code of the surface representation is: blue for hydrophilic contribution and red for the lipophilic contribution of the surface. (c) Overlapping of the fluvoxamine with the crystal serotonin, with proximity to the residue Tyr64. (d) Representation of the system 5HT3A-Fluvoxamine showing a good adaptation of the ligand to the surface.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Marto: Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declarations of competing interest

None

Acknowledgments

The authors acknowledge GENERIS for supplying sertraline, fluoxetine, paroxetine, escitalopram and fluvoxamine.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2025.107013.

Data availability

Data will be made available on request.

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