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Biological Activity of Dihydropyrimidinone (DHPM) Derivatives: A Systematic Review

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Abstract: Dihydropyrimidinones are heterocycles with a pyrimidine moiety in the ring nucleus, which, in recent decades, have aroused interest in medicinal chemistry due to alleged versatile biological activity. In this systematic review, we describe the currently published activities of dihydropyrimidinone derivatives. Between 1990 and December 31\textsuperscript{st}, 2016, 115 articles outlined biological activities or toxicity of DHPM derivatives, 12 of those involved \textit{in vivo} experiments. The main activities associated with this class of compounds are antitumoral (43 articles), anti-inflammatory (12 articles), antibacterial (20 articles) and calcium channel antagonism/inhibition (14 articles). Antitumoral activity is the main biological property evaluated, since the main representative compound of this class (monastrol) is a known Eg5 kinesin inhibitor. This review depicts a variety of other pharmacological activities associated with DHPM derivatives, but the main findings are essentially \textit{in vitro} characteristics of the substances. This review presents the current state of the art of DHPM biological activities and demonstrates that there is still a need for further \textit{in vivo} studies to better delineate the pharmacological potential of this class of substances.

Graphical Abstract

Keywords: Dihydropyrimidinones, monastrol, toxicity, cytotoxicity, biological activity, systematic review.
Highlights:

- A systematic review on biological activities linked to DHPM derivatives was carried out
- A total of 115 articles were included after exclusion criteria
- Among 20 different biological activities, antitumoral properties were the most studied for this class of molecules
- Only 10 percent of selected articles evaluated the in vivo profiles of DHPM derivatives

INTRODUCTION

Dihydropyrimidinones (DHPM’s) and their derivatives are heterocyclic compounds synthesized by classic multi-component reactions such as the Biginelli reaction [1, 2] and by variants of this synthesis methodology [3-5]. This class of compounds became important to the field of medicinal chemistry due to the study of the biological activities of monastrol [6], a Biginelli adduct. Other DHPMs have been synthesized since then, revealing several other pharmacological properties [7].

Monastrol is a protagonist of the DHPM class. Several studies have revealed that its inhibitory action on human kinesin Eg5 leads to mitotic arrest and consequently to apoptosis, since this motor protein is involved in the formation of the mitotic spindle [8-11]. At first, this was the main action described for this class, but some studies have shown other possible targets for these molecules, such as centrin [12], calcium channels [13] and topoisomerase I [14].

Pharmacological properties described over the last twenty-five years for these compounds are reported in this review and include evaluations of anticancer [15] anti-inflammatory [16], antihypertensive [17], antibacterial [18], antifungal [19], antiviral [20], antiparasitic [21], antithyroidic [22], antimuscarinic [23], antidiabetic [24], and hypolipidemic [25] activities. We also found reports on the antagonistic/inhibitory action on acetylcholinesterase [26], urease [27], calcium channel modulation [28, 29] and GABAa agonism [30].

The general structure of these compounds is depicted in Figure 1 [31].

![Figure 1](https://example.com/fig1.png)

**Fig. (1).** Basic structure of dihydropyrimidinones / thiones

The literature has revealed that the introduction of specific clusters in heterocyclic regions may change their biological activities, and, thus, medicinal chemistry has been advanced with the synthesis of increasingly specific compounds with low cytotoxicity [18, 32, 33].
Several works [33-38] have cited pyrimidinones or tetrahydropyrimidinones as DHPM derivatives. We understand that the compounds studied by these authors may have similar synthetic origins or pharmacologically investigated purposes as DHPM. However, even a small change in the nucleus of the molecule is enough to result in a difference in activity. Interactions between ligands and biological structures are tightly associated with electrostatic mechanisms [39]. To investigate the differences between this pharmacophore site based on the locations and presence or absence of double bonds (π electrons), we performed a simulation using ArgusLab® (electrostatic surface potential – ESP and HOMO/LUMO) and ChemAxon Marvin® software (logP and pKa) (Table 1). ESP shows differences that may be relevant to biological interactions, depending on the target. The HOMO/LUMO projection characterizes the electronic structure of each molecule, providing information about the electron donating or accepting capabilities. The predicted logP and pKa values are also different among these groups of molecules. This information contributed to our decision to only include studies on molecules with a 3,4-dihydro-2(1H)-pyrimidinone/thione/amine nucleus in this review. Recently, Kaur et al. [40] reviewed the synthesis and medicinal application of DHPM compounds. Their focus was on the chemical preparation of DHPM derivatives and their biological activities, and more specifically on radicals inserted to the basic DHPM structure. Our present review, on the other hand, evaluates derivatives that possess a 3,4-dihydro-2(1H)-pyrimidinone/thione/amine nucleus, and we have used a systematic methodology to identify data sources.
Table 1. Chemical characteristics of pyrimidinone derivatives

<table>
<thead>
<tr>
<th>IUPAC name</th>
<th>2(1H)-pyrimidinone</th>
<th>Tetrahydro-2(1H)-pyrimidinone</th>
<th>3,4-dihydro-2(1H)-pyrimidinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="image1" alt="Structure" /></td>
<td><img src="image2" alt="Structure" /></td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>Surface(a)</td>
<td><img src="image4" alt="Surface" /></td>
<td><img src="image5" alt="Surface" /></td>
<td><img src="image6" alt="Surface" /></td>
</tr>
<tr>
<td>HOMO(b)</td>
<td><img src="image7" alt="HOMO" /></td>
<td><img src="image8" alt="HOMO" /></td>
<td><img src="image9" alt="HOMO" /></td>
</tr>
<tr>
<td>LUMO(c)</td>
<td><img src="image10" alt="LUMO" /></td>
<td><img src="image11" alt="LUMO" /></td>
<td><img src="image12" alt="LUMO" /></td>
</tr>
<tr>
<td>LogP(d)</td>
<td>-0.65</td>
<td>-1.03</td>
<td>-0.79</td>
</tr>
<tr>
<td>pKa(d)</td>
<td>10.09</td>
<td>14.02</td>
<td>13.18</td>
</tr>
</tbody>
</table>

Legend: A – Electrostatic surface potential (ESP); B – Highest occupied molecular orbital, blue areas are positive, red areas are negative; C – Lowest unoccupied molecular orbital, blue areas are positive, red areas are negative; D – Calculated in ChemAxon Marvin Software
METHODS

Background definitions

The search method employed in this systematic review aimed to include studies that evaluated the biological activity and toxicity (both in vivo and in vitro) of DHPM (Fig. 1) derivatives.

Data Sources and Searches


Search terms were chosen based on our need to find everything published about DHPMs so we could later apply further exclusion criteria. Search terms “monastrol OR DHPM OR dihydropyrimidinone”, “inhibitor OR mechanism OR activity OR toxicity OR cytotoxicity” were used, varying the Boolean operators according to the rules of each specific database. It was necessary to exclude the term ”Dynamic High-Pressure Microfluidization” since it has the same abbreviation as dihydropyrimidinones. Searches were conducted using the final limit date of December 31st, 2016.

Results were exported to reference management software (EndNote™, Thomson Reuters), where all selections and analyses were performed.

Study selection

The review was performed in two main steps. The first step involved the evaluation of articles’ titles and abstracts, according to the eligibility criteria (Table 2). In the second step, the authors read each selected full text and withdrew articles that matched the exclusion criteria. In case of disagreement, the authors discussed the particularities of each article to decide if it fit or did not fit the inclusion criteria. In this step, we added articles found in the reference lists of selected manuscripts that had not been listed under the search terms in the databases.
Table 2. Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inclusion</th>
<th>Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Language</td>
<td>English, Spanish, Portuguese or Italian</td>
<td>Any other language</td>
</tr>
<tr>
<td>2 Duplicate articles</td>
<td>Automatically selected/excluded using “Find Duplicate” in reference manager software (EndNote)</td>
<td></td>
</tr>
<tr>
<td>3 Type of study</td>
<td>Biological activity, toxicity (systemic or cellular). <em>In vitro</em> and/or <em>in vivo</em> studies</td>
<td>Exclusively in silico, articles that focus only on synthesis or other purely chemical parameters</td>
</tr>
<tr>
<td>4 Type of publication</td>
<td>Original manuscripts</td>
<td>Reviews, book chapters, posters, contents, personal opinions, index, conference abstracts, letters</td>
</tr>
<tr>
<td>5 Search terms</td>
<td>Just citation of keywords in text</td>
<td></td>
</tr>
<tr>
<td>6 Monastrol</td>
<td>Articles use monastrol, a DHPM, as a positive control or as a tool to evaluate cell division stages.</td>
<td>Articles that only describe the mechanism of action of the compounds DHPMs</td>
</tr>
<tr>
<td>7 Mechanism of action</td>
<td>Articles that evaluate the biological activity of DHPMs</td>
<td></td>
</tr>
<tr>
<td>8 Confounders</td>
<td>Compounds named as dihydropyrimidinone, but not fit in the structure of the 3,4-dihydro-2(1H)-pyrimidinone/thione/amine class.</td>
<td></td>
</tr>
</tbody>
</table>

**Data extraction process**

The following information was extracted from all included studies: type of study (*in vitro* or *in vivo*), the biological matrix used, compound structure and nomenclature, and main conclusions.

**RESULTS AND DISCUSSION**

The database search identified 1455 records. Using a duplicate removal tool (EndNote software), 227 repeated files were discarded, leaving 1228 citations. After the first evaluation phase (title/abstract), 1015 records were excluded. To the remaining 213 articles, 14 papers were added from the reference lists of the identified studies (which had not been found in the initial search). Phase 2 was therefore conducted with a total of 227 articles.

After the full-text reading, 112 articles were excluded (Appendix 1). At the end, 115 records were included in this review. This process is illustrated by a flow diagram in Figure 2.

After critical reading, articles were divided according to the pharmacological property assessed in each study (Table 3). Manuscripts were possibly assigned to more than one category, since the same study may have evaluated more than one type of activity.
Table 3. Activities associated with DHPM derivatives.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antitumoral</td>
<td>[5, 14, 15, 33, 41-79]</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>[16, 56, 80-89]</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>[80, 82]</td>
</tr>
<tr>
<td>Antibacterial</td>
<td>[18, 58, 61, 79, 83, 85, 90-103]</td>
</tr>
<tr>
<td>Antifungal</td>
<td>[18, 19, 61, 83, 91, 94, 95, 101, 102, 104]</td>
</tr>
<tr>
<td>Antiviral</td>
<td>[20, 105-108]</td>
</tr>
<tr>
<td>Calcium channel antagonism/inhibition</td>
<td>[13, 28, 29, 109-119]</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>[50, 56, 73, 90, 120-124]</td>
</tr>
<tr>
<td>Antimuscarinic</td>
<td>[23]</td>
</tr>
<tr>
<td>Acetylcholinesterase inhibition</td>
<td>[26, 120, 125]</td>
</tr>
<tr>
<td>Antithyroid</td>
<td>[22]</td>
</tr>
<tr>
<td>Hypolipidemic</td>
<td>[25]</td>
</tr>
<tr>
<td>Antiparasitic</td>
<td>[21, 126-128]</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>[24, 129]</td>
</tr>
<tr>
<td>Urease Inhibition</td>
<td>[27]</td>
</tr>
<tr>
<td>GABA_A agonism</td>
<td>[30]</td>
</tr>
<tr>
<td>Tyrosinase inhibition</td>
<td>[130]</td>
</tr>
<tr>
<td>α1 adrenoceptor antagonism</td>
<td>[131, 132]</td>
</tr>
<tr>
<td>Carbonic anhydrase inhibition</td>
<td>[133, 134]</td>
</tr>
<tr>
<td>Cardiac effects</td>
<td>[17, 135]</td>
</tr>
</tbody>
</table>

Study characteristics

Selected studies were published between 1990 and 2016 and were all written in English. Twelve articles described in vivo assays. In vitro tests were performed in all other publications to justify the proposed DHPM activities.

Biological activities

Antitumoral activity

Antitumoral properties are the most studied and described activity of DHPM compounds. In 1983, ethylene 4-(3-hydroxyphenyl)-6-methyl-2-sulfanylidene-3,4-dihydro-1H-pyrimidine-5 carboxylate was synthesized through a Pietro Biginelli multi-component reaction, and its activity was tested in 1999 by Mayer et al [6].

Mayer was searching for small, cell-permeable compounds that could disturb cell division without affecting tubulin function. Using a small-molecule library, Mayer found Biginelli’s synthesized compound and through immunocytochemistry experiments observed mono-astral spindles in treated cells [6].

In the same decade, other studies were published to evaluate the function of kinesin Eg5 in cell division [136, 137]. Based on these studies, Mayer hypothesized that the motor protein Eg5 was a target of the small molecule that he was studying, named
monastrol. Additional studies later confirmed that Mayer’s predictions were correct [9, 49, 138, 139].

Many effective anticancer drugs act on cell division, so compounds that interfere with the cell cycle are promising antineoplastics. Monastrol was tested in many different cell lines [43, 49, 59, 60, 66, 140], and some DHPM derivatives were shown to be more efficient than vinca alkaloids, taxanes and epothilones because of their lack of effect on microtubules, thus reducing neurotoxic activity [48, 64].

Based on these findings for monastrol, other DHPMs were synthesized and their antitumoral activity was evaluated. More than 300 derivatives have been tested for growth inhibition in several cancer cell lines (Table 4).

In addition to in vitro studies, Bhat and colleagues in 2016 confirmed antitumoral activity of a DHPM derivative, called DHP-5, in nude mice. They observed growth reduction of LOVO tumors (colon cancer xenografts) in intraperitoneally treated animals after 14 days using a daily dose of 50 mg/kg [46].

In 2014, Guido et al. verified angiogenesis inhibition in vitro in HUVECs and in vivo with a fertilized chicken egg chorioallantoic membrane (CAM) model, using the compounds dimethylenastron and 4p, another DHPM derivative [55].
Fig. (2). Flow diagram of study selection adapted from Moher, 2009 [141]
Table 4. Antitumoral activity associated with DHPM derivatives

<table>
<thead>
<tr>
<th>Type of antitumoral activity (cell line)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>[5, 14, 15, 33, 44, 47, 54-56, 58, 62, 63, 66, 67, 69, 78, 79]</td>
</tr>
<tr>
<td>Liver</td>
<td>[43, 63, 67, 68, 70, 73, 77]</td>
</tr>
<tr>
<td>Ovarian</td>
<td>[15, 33, 44, 50, 66, 69]</td>
</tr>
<tr>
<td>Gastric</td>
<td>[43, 56, 59]</td>
</tr>
<tr>
<td>Lung</td>
<td>[14, 15, 33, 50, 56-58, 65, 67, 68, 72]</td>
</tr>
<tr>
<td>Kidney</td>
<td>[15, 33, 50, 61, 66, 68]</td>
</tr>
<tr>
<td>Skin</td>
<td>[15, 33, 58, 66, 69, 74]</td>
</tr>
<tr>
<td>Colorectal</td>
<td>[59, 63, 68]</td>
</tr>
<tr>
<td>Prostate</td>
<td>[15, 33, 43, 50, 51, 56, 63, 66, 67]</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>[15, 33, 48, 50, 52, 64, 71, 76]</td>
</tr>
<tr>
<td>Cervical</td>
<td>[14, 15, 33, 41-44, 46, 49-51, 53, 57, 58, 65, 66, 70, 78]</td>
</tr>
<tr>
<td>Endothelial</td>
<td>[70]</td>
</tr>
<tr>
<td>Pancreas</td>
<td>[51, 57, 60, 69, 72]</td>
</tr>
<tr>
<td>Blood</td>
<td>[15, 33, 57, 65, 74]</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>[44, 45] [65]</td>
</tr>
<tr>
<td>Myeloma</td>
<td>[60]</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>[77]</td>
</tr>
</tbody>
</table>

**Antimicrobial activity**

The first study in this field was performed by Chhillar in 2006, which determined that diethyl 4 - (4 - methoxyphenyl) - 2,6 –dimethyl - 1,4 -dihydropyridin - 3,5 - dicarboxylate showed significant activity against *Aspergillus fumigatus* in disk diffusion experiments [19].

After Chhillar's study, other DHPM derivative molecules were synthesized and their antibacterial properties were evaluated against different strains, showing moderate to good activity (Table 5).

Following these positive antibacterial results, other microbial targets were addressed, including fungi. Despite the limited quantity of studies on this activity, some effective antifungal compounds have been described in the literature [18, 19, 61, 83, 94, 95].

In 2008, Duguay confirmed the inhibitory action of other DHPMs on *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces cerevisiae* strains using amphotericin B as a control [104].

Kim et al. demonstrated the antiviral potential of DHPMs in 2012, after successfully inhibiting replication of the HIV-1 virus in an *in vitro* model [20, 105]. This activity was proposed after demonstrating that marine alkaloids batzelladine A and B inhibited HIV gp-12 binding to CD4 cells. These compounds possess pyrimidine rings similar to DHPM derivatives [142].

Revendra et al. tested three different DHPMs *in vivo* on Newcastle disease virus-infected chickens. DHPM treatments resulted in improvement of animal survival [107].

A recent study performed by Manos-Turvey and colleagues (2016) demonstrated activity of dihydropyrimidinones and thiones against polyomavirus BK and JC, with two different proposed mechanisms of action. The first inhibits the cellular chaperone Hsp70,
while the second inhibits the ATPase activity of T antigen, both of which are necessary for virus replication [106].

Table 5. Antibacterial activity associated with DHPM derivatives

<table>
<thead>
<tr>
<th>Antibacterial activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>[18, 58, 61, 83, 85, 90-95]</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>[58, 61, 83, 90, 91, 94-97, 101, 102]</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>[58]</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>[83, 91, 95, 97]</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>[83, 96]</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>[18, 58, 61, 83, 90, 91, 95, 96, 101, 102]</td>
</tr>
<tr>
<td><em>Pseudomonas pseudomallei</em></td>
<td>[95]</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>[83]</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>[18, 58, 61, 83, 92-94, 102]</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>[18, 58, 83, 90, 91]</td>
</tr>
<tr>
<td><em>Klebsiella aerogenes</em></td>
<td>[102]</td>
</tr>
<tr>
<td><em>Shigella boydii</em></td>
<td>[94]</td>
</tr>
<tr>
<td><em>Aeromonas ssp</em></td>
<td>[94]</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>[79, 98-100, 103]</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>[96]</td>
</tr>
<tr>
<td><em>Agrobacterium tumefaciens</em></td>
<td>[96]</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>[96]</td>
</tr>
</tbody>
</table>

**Antiparasite activity**

Malaria is an infectious disease caused by a parasitic protozoan of the genus *Plasmodium*, of which its four species, *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* are all infectious, with the first one being the most lethal [143]. There is no effective vaccine against malaria, and current drugs are facing drug resistance [144].

Chiang et al. (2009) evaluated 157 Biginelli adducts against *P. falciparum* and proposed a mechanism of action in which chaperone Hsp 70 is inhibited by ATPase inactivation. The results include seven promising antimalarial DHPM compounds [126].

Leishmaniasis is a chronic infectious disease caused by parasites of the genus *Leishmania* which affects phagocytic human cells. The most aggressive manifestation of this disease (visceral Leishmania) is provoked by the species *L. donovani* [144, 145].

One study assessed in vivo antileishmanial activity using *Mesocricetus auratus* hamsters infected intracardially with *L. donovani* amastigotes. Animals received the compound (4 – fluoro - phenyl) – 6 – methyl – 2 – thioxo – 1,2,3,4 tetrahydropyrimidine – 5 – carboxylic acid ethyl ester for five days. The results indicated a potent inhibitory effect on leishmanial proliferation without major signs of toxicity at doses of 50, 75 and 100 mg/kg [21].

Another study with a similar experimental methodology evaluated antiparasitic activity against *L. donovani*, but in this case, hamsters were treated orally with two doses of monastrol (25 mg/kg and 50 mg/kg). The authors observed significant reduction of parasitic load with both doses, without evident toxicity to the animals [127].
Leishmania major was evaluated in another study, where DHPM molecules presented potent activity in vitro against promastigotes [128].

**Calcium channel modulators**

Nifedipine, a dihydropyridine, was developed in 1969. In 1972, it was shown to reduce high blood pressure, angina and cardiac arrhythmia by inhibiting calcium channels [146-148]. Based on this finding, the molecular structure of nifedipine has been used as an inspiration for the synthesis of new compounds including DHPMs.

Atwal et al. heavily contributed to the current knowledge of DHPM interactions with calcium channels. They evaluated vasorelaxant potency by determining DHPM concentrations required for relaxation of K⁺ depolarized rabbit aortic strips. Relaxation of K⁺ depolarized strips is predictive of calcium channel-blocking activity [28, 117-119]. Using a similar methodology, Zorkun et al. demonstrated blockage of calcium channels by 4 – aryl – 3,4 – dihydropyrimidin – 2(1H) – thione derivatives [115]. Putatunda et al. (2012) evaluated the same activity with DHPMs with or without an N1-alkyl substitution, revealing that this substitution abolishes calcium channel inhibition [113].

Monastrol has shown an ability to block calcium channels, but its activity is weak compared to nifedipine. Calcium uptake through L-type calcium channels was evaluated in HEK293 cells, and results showed a significant blockage of cation absorption (approximately 70%) to a final concentration of 100 µM [13].

Singh et al. (2009) synthesized several DHPM derivatives and assessed them as calcium channel modulators based on their capacity to relax smooth muscle in swine carotid arteries. However, the effects of these compounds were less effective than nifedipine [111, 112].

Based on these results, Singh et al. (2012) produced other DHPM derivatives containing diaminophosphinyl heterocycles, phosphonate, and phosphorus. After testing using the same methodology as their previous study, they concluded that these new compounds can block calcium channels, but still less effectively than nifedipine [114].

In 2003, Yarim et al. evaluated 25 DHPM derivatives in studies on isolated rat ilea and lamb carotid arteries. Their results showed that 24 of 25 compounds induced vasorelaxation (compared to the reference drug nicardipine) associated with calcium channel blockage. While one of the tested molecules did not exert an effect on vasorelaxation, it did show antispasmodic action [116].

There is a relationship between intracellular calcium concentrations and the mechanism of chronic obstructive pulmonary disease (COPD) development [149]. Manral et al. evaluated the inhibition of COPD mediated by ethyl 4 - (4’ - heptanoyloxyphenyl) – 6 – methyl – 3,4 – dihydropyrimidin – 2 – one – 5 – carboxylate (H-DHPM) by measuring intracellular calcium in COPD patient lymphocytes using spectrofluorimetry. The results indicated that there was an increase of 95% in the viability of H-DHPM treated lymphocytes [109].

Calcium channel blockage by H-DHPMs was further evaluated by Priya et al. through electron microscopy, revealing that Ca²⁺ is blocked in the cytosol of human platelets. H-DHPM also appeared to inhibit platelet aggregation comparably to amlodipine, showing a better antithrombotic effect than aspirin in treated rats treated with a single dose of 33 µmol/kg [110].
Chikhale et al. attempted to compare DHPM derivatives for inhibition of angiotensin-converting enzyme, but their results showed that these molecules exerted antihypertensive activity due to calcium channel inhibition, and they also exhibited ulcerogenic activity [29].

Based on its well-described calcium channel blockage property, a study evaluated 4,6-di(het)aryl-5-nitro-3,4-dihydropyrimidin-(1H)-2-ones treatment for two types of experimental arrhythmia in rats, demonstrating a high antiarrhythmic activity without an influence on blood pressure [17]. Another study has demonstrated potential cardiotonic activity of several DHPMs [135].

**α1-adrenoceptor antagonism**

Drugs that block α1-adrenergic receptors are used for treating benign prostatic hyperplasia (BPH) [150] and, according to the FDA, they are the most prescribed class of medications for this disease [151]. Among these drugs is niguldipine (a dihydropyridine derivative), initially described as a calcium channel inhibitor and later as an α1-receptor antagonist [152]. Following this finding, many changes in niguldipine’s chemical skeleton have been made, giving rise to several DHPMs with similar biological action [131, 132].

Various in vitro experiments were performed evaluating the selective link of these niguldipine-derived DHPMs to α1-receptors and the molecules exhibiting positive results were evaluated in vivo. Several compounds showed efficacy in prostate tissue from rats, dogs, and humans [132]. Many DHPM derivatives exhibited affinity to the α1-adrenoceptor. Due to this observation, Barrow et al. suggested that the receptor binding domain is flexible, allowing many compounds to fit [131].

**Anti-inflammatory activity**

Some DHPM derivatives have been identified as promising anti-inflammatory drugs by carrageenan-induced paw edema assays in rat and mouse [16, 56, 80, 88, 89].

The anti-inflammatory action of DHPMs involves inhibiting the expression of chemical mediators, including TNFα [81, 83], interleukin [81, 83], prostaglandin, iNOS [81, 86], hyaluronidase [87] and COX-2 [81].

Transient receptor potential ankyrin 1 (TRPA1) antagonism reduces pain perception due to inflammation in animal models, and the derivative 4-phenyl-2-thioxo-1,2,3,4-tetrahydro-indeno[1,2-d]pyrimidin-5-one was identified as a potent antagonist of TRPA1 [84].

Inhibiting the epoxide hydrolase enzyme is a strategy for normalizing blood pressure and vascular inflammation. Based on this, Rezaee et al. synthesized several DHPMs and determined that all of their tested compounds shown inhibition of this enzyme [82].

**Antioxidant activity**

Reactive Oxygen Species (ROS) activity in biological systems may cause lipid peroxidation, which has an important role in cellular proliferation, especially in tumors [153]. Accordingly, many DHPMs with antitumoral properties also show antioxidant action [50, 73].
Stefani et al. synthesized several DHPM analogues, some of which showed strong activity against lipid peroxidation induced by Fe-EDTA, while others were more potent in reducing ROS levels, with activity independent from glutathione [121]. Similarly, many DHPMs have been synthesized and evaluated as antioxidants in TBARS (thiobarbituric acid reactive substances) assays for lipid peroxidation [120, 122] and as iron II chelators, with promising results [120, 123].

Using three different methodologies, the antioxidant activity of 3,4-dihydro-2(1H)-pyrimidinone derivatives was tested. This compound’s activities include elimination of DPPH free radicals [123, 124], reducing potential [124] and hydrogen peroxide elimination [124]. While the compounds obtained by Mansouri et al. had positive results in each of the three different assays, in the last one, all compounds showed a weak activity compared to the control (gallic acid).

**Miscellaneous biological effects**

In a small number of manuscripts, miscellaneous activities have been presented, such as antimuscarinic [23], acetylcholinesterase inhibition [26, 120, 125], antithyroid [22], hypolipidemic [25], antidiabetic [24, 129], urease inhibition [27], GABA\textsubscript{A} agonism [30], tyrosinase inhibition [130], carbonic anhydrase inhibition [133, 134], and cardiac effects [17, 135].

**CONCLUSION**

Since 1893, dihydropyrimidinones have aroused great interest in medicinal chemistry, a fact justified by the wide variety of biological activities described for these heterocycles. The 115 articles selected in this review confirm their therapeutic versatility.

The mechanisms of DHPMs’ antitumoral properties and inhibitory activities on calcium channels are well described in the literature. For other described activities, there are still gaps to be filled in regarding the interaction of these compounds and their pharmacological targets.

The diversity of pharmacological effects may be associated with the promiscuity of this chemical class. On the one hand, this property is acceptable to those who are investigating novel biological activities of DHPMs. On the other hand, this property may result in a wide spectrum of adverse effects.

The number of newly synthesized DHPMs has increased in the last two decades. This research is driven by hopes for improvement in the treatment of several pathologies, including neglected diseases such as malaria and leishmaniasis, for diseases with high mortality rates such as cancer and AIDS, or even as a new therapy against bacteria and fungi resistant to traditional treatments.

In this review, we found only 12 articles that used animal models for investigating pharmacological activity. More studies are needed to evaluate the \textit{in vivo} impact of this promising chemical class.

**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest in the elaboration of this article.
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1. Articles in the language other than English, Spanish, Portuguese or Italian.
3. Unaccessed articles.
4. Just citation from keywords.
5. Articles that do not evaluate biological activity.
6. Articles that only describe the mechanism of action of the compounds DHPMs.
7. Articles use monastrol, a DHPM, as positive control or as a tool to evaluate cell division stages.
8. "In silico" studies.
9. Compounds which lack the basic structure of a DHPM.
10. Article that do not have the molecular structure of the evaluated compounds.
11. Articles that focuses only in synthesis or other purely chemical parameters.


[94] K. Singh, K. Singh, B. Wan, S. Franzblau, K. Chibale, J. Balzarini, Facile transformation of Biginelli pyrimidin-2(1H)-ones to pyrimidines. In vitro evaluation as inhibitors of


**Fig. (1).** Basic structure of dihydropyrimidinones/ thiones

\[ \text{X} = \text{O, NH or S} \]

\[ \text{R1-5} = \text{H, alkyl, aryl, ester, amide, aryl, (thio)urea or an heterocycle} \]
Fig. (2). Flow diagram of studies selection adapted from Moher, 2009 [141]
Highlights:

- A Systematic Review on biological activities linked to DHPM derivatives was carried out
- 115 articles were included after exclusion criteria
- Among 20 different biological activities, antitumoral properties were the most studied to this class of molecules
- Only 10 percent of the selected articles evaluated some \textit{in vivo} profile of the DHPM derivative
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