Recent Advance in the Pharmacology of Dihydropyrimidinone

J.-P. Wan[1,2] and Y. Pan*[,1]

[1]*Department of Chemistry, Zhejiang University, Hangzhou 310027, P.R. China*

[2]*College of Chemistry and Chemical Engineering, Jiangxi Normal University, Nanchang 330022, P.R. China*

Abstract: Dihydropyrimidinones (DHPMs) are a series of highly valuable small molecules possessing versatile pharmaceutical properties. Although the first one-pot synthesis of DHPMs had been reported more than 100 years ago, the fascinating achievement in DHPMs-based pharmacology during the past century promoted durative interests to the pharmacological and related studies of the scaffold, which lead to the discovery of many new biological functions of DHPMs. Recent pharmacological development on DHPMs-based molecules have been summarized in this review.

Keywords: Dihydropyrimidinone, pharmacology, enzyme/mitotic kinesin inhibitor, receptor antagonist, calcium channel blocker.

1. INTRODUCTION

Dihydropyrimidinones, also known as Biginelli compounds, representing the heterocyclic system of partially reduced pyrimidine moiety. The original structure of this scaffold was obtained in the form of 4-aryl-dihydropyrimidin -1(1*H*)-ones (**1**) (Fig. **1**) from the three-component reaction of aryl aldehyde, β-keto ester and urea (or thiourea) as reported by the Italian chemist P. Biginelli [1]. Since the discovery of the pharmacological properties embedded in DHPMs early last century, significant interests have been devoted to these heterocyclic compounds both by the synthetic and medicinal communities. In terms of the synthesis of DHPMs, there are now a variety of different methodologies available for the synthesis of structurally diversified products of the type in addition to classical Biginelli reaction [2]. For example, the Atwal modification is especially practical in synthesizing *N3* substituted DHPMs [3-5]. The synthetic application of Biginelli reaction has also been recently expanded to the solid phase, fluorous phase as well as microwave chemistry to ensure availability of much more functionalized DHPMs for the sake of high throughput screening (HTS) [6-11]. Since the development of DHPMs synthesis has already been comprehensively reviewed in several review papers, the details on DHPMs synthesis will therefore not be introduced herein [12-14]. The pharmacological research of DHPMs, as a broadly concerned topic, has been previously reviewed with focus on their functions as calcium channel modulators and selective α_{1a} adrenoceptor antagonists [15]. An even earlier review has summarized the pharmacological advance on some DHPM-based marine natural products [16]. Since DHPM unit has been originally noticed due to its structural resemblance with the well-known pharmacophore dihydropyridines (DHPs), the early studies on the pharmacological functions of DHPMs were consequently performed following those results obtained from DHPs, such as the function of serving as calcium channel modulator [17-18] and selective α_{1a} adrenoceptor antagonist [19]. However, DHPMs are now making their way as pharmacophore far more than the aza analog of DHPs. Thus, as an update account, both the new progress in those previously referred pharmacological properties as well as the new discoveries on pharmacological functions associated with DHPMs will be outlined in this review. According to the difference on the function patterns of DHPMs, the content mainly consists of three independent categories: enzyme and mitotic kinesin inhibitors, receptor antagonists as well as calcium channel blockers.

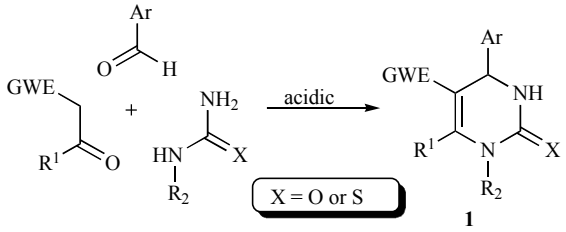

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Fig. (1).

2. DHPMS AS ENZYME AND MITOTIC KINESIN INHIBITORS

2.1. DHPM Inhibitors of ROCK1

Compared to other known biological functions, serving as enzyme inhibitors is a relatively new subject for DHPMs. Even though the DHPM monastrol (see below introduction in Fig. **6**) has been found as an excellent inhibitor of motor protein Eg5 early in 1999 [20], the exploration on DHPMs enzyme inhibitors is just in its infancy. The recent discovery of ROCK1 inhibitor **2**, for instance, exemplified the great potential of DHPMs as enzyme inhibitors [21]. The serine-threonine kinase ROCK1 (or ROKβ, p160ROCK) is one of the two known isoforms of Rho-associated kinases [22]; it had been recognized that ROCK1 is crucial in mediating many cellular functions such as smoothing muscle

*Address correspondence to this author at the Department of Chemistry, Zhejiang University, Hangzhou 310027, PR China; Tel: 86 571 87951264; Fax: 86 571 87951629; E-mail: panyuanjiang@zju.edu.cn

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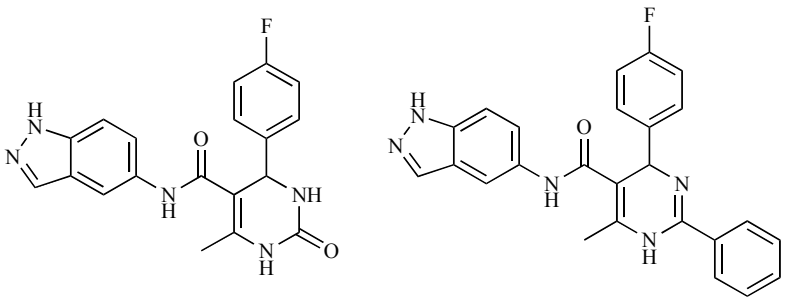


2 **3**

Fig. (2).

contraction, cytoskeletal reorganization, cell proliferation and mobility, gene expression, focal adhesion as well as the membrane ruffling *etc.* [23-25]. ROCK1 has also been proven to be in close relationship with various cardiovascular process and is therefore regarded as a potential target for the treatment of cardiovascular diseases [26-27]. The indazole amide functionalized DHPM **2** (Fig. **2**) was firstly discovered as the lead compound by Goodman and coworkers, in the screening against a panel of 33 different kinases; DHPM **2** exhibited potent inhibitory effect against ROCK1 (IC_{50} = 14 nM) and appealing selectivity (> 30 fold). However, compound **2** was determined with remarkably lower activity in rat aortic ring dilation assay as well as low oral bioavailability and high clearance. The structural modification of methylation on *N*1 of the indazole fragment led to a dramatic loss of *in vitro* activity (ROCK1 IC_{50} > 2500 nM). Combined with the results displayed in the corresponding homology model, the indazole unit was therefore hypothesized to be important, while the DHMP fragment allows for structural modification [21].

The efforts on structural modification of DHPM **2** led to the discovery of dihydropyrimidine **3** (Fig. **2**), In contrast to compound **2**, **3** showed remarkably enhanced pharmacokinetics (PK) with a sound ROCK1 activity (IC_{50} = 105 nM). The oral bioavailability (oral F) of **3** has been observed as 16 %, while the clearance decreased to 15 ± 2.8 mL/min/kg compared to the 49 ± 5 of **2**, and the good inhibitory selectivity (> 30 fold) against 33 kinases panel was maintained. During further structure activity relationship (SAR) studies, optimization of 2-aryl ring disclosed that 4-methoxyphenyl derivative **4** (Fig. **3**) possesses promising PK with the oral bioavailability of 35 % and an improved ROCK1 activity (IC_{50} = 46 nM). Consequently, based on the SAR implication on **4**, further SAR by varying the substitution on 4-position proved that the 2-fluoro-4-chlorophenyl derivative (**6**) has > 100 fold selectivity with the cellular activity of IC_{50} = 5 nM against ROCK1. In addition, the bioavailability of **6** has been determined as 58 %. However, dramatic P450 inhibition against CYP2D6 has been observed (IC_{50} = 0.20 μM). Analysis on the crystal structure of ROCK1−2-pyridyl dihydropyrimidine **5** (ROCK1 IC_{50} = 8 nM) complex suggested the presence of additional binding affinity caused by 4-pyridine nitrogen-Lys200 interaction. Upon the screening on the analogous of **5**, compound **7** with 2-Cl substitution in the pyridyl has been found bearing good ROCK1 activity (IC_{50} = 4 nM), but the oral bioavailability of 7 ± 2.7 % is not ideal. On the other hand, analogs **8** and **9** (Fig. **3**) have been found with significantly better bioavailabilities of 49 ± 10 % and 53 ± 12 %, respectively. What is more, **8** and **9** exhibited substantially diminished P450 inhibiting activity as compared with **6**. Finally, **9** has also exhibited good *in vivo* activity in lowering blood pressure in a spontaneously hypertensive rat model [28].

2.2. DHPMs Inhibitors of Hsp70 ATPase

The heat shock proteins family of Hsp70 is ATPase with molecular chaperons and cytoprotective activity. These proteins play an important role in mediating diversified cellular processes, the degradation of incorrectly folded polypeptides, protein transport as well as the reorganization of multi-protein complexes, to name but a few, [29-30]. Therefore, Hsp70 ATPase is a therapeutic target for many human diseases, such as cystic fibrosis [31]. In addition, the overexpression of Hsp70 is known to lead to tumorigenesis, which makes it possible to develop modulators of Hsp70 and related ATPase as anticancer drugs [32]. The highly functionalized DHPM **10**, termed MAL3-101 (Fig. **4**), has been observed with inducing effect on breast cancer cell apoptosis [33]. Its structural resemblance with the known compounds NSC-630668-R/1 (inhibitor of endogenous and Hsp40-stimulated ATPase) [34] and the clinically used Hsp70 modulator 15-deoxysperagulin [35] promoted the search of potential DHPM modulators of Hsp70 ATPase. Biochemical screening on these products denoted that some of these DHPMs possess the activity of enhancing the rate of Hsp70 ATP hydrolysis. Among the representative compounds outlined in (Fig. **4**), MAL3-38 (**11**) and MAL3-90 (**15**), respectively, led to the increase of Hsp70 ATP turnover rate in 3.1 and 5.2-fold at the concentration of 0.3 mM. MAL3-55 (**14**) displayed the concentration-dependent effect on Hsc70-mediated ATP hydrolysis with the fold change of -1.5, 1.4 and 2.8-fold correspondingly in 0.1 mM, 0.3 mM and 0.6 mM. The examination on the large tumor antigen (TAg)-stimulated Hsp70 ATPase activity revealed that **11** and **15** enhanced the TAg-stimulated ATP turnover by 2 and 4.2-fold, but simultaneously reduced the amplitude of the reaction in the TAg-stimulated assay. Notably, MAL3-101 (**10**), MAL3-39 (**12**) and MAL3-54 (**13**) exhibited selective inhibition to the TAg-stimulated Hsp70 ATP hydrolysis, while showing no significant effect on the endogenous rate of ATP turnover. In addition, **10** has been

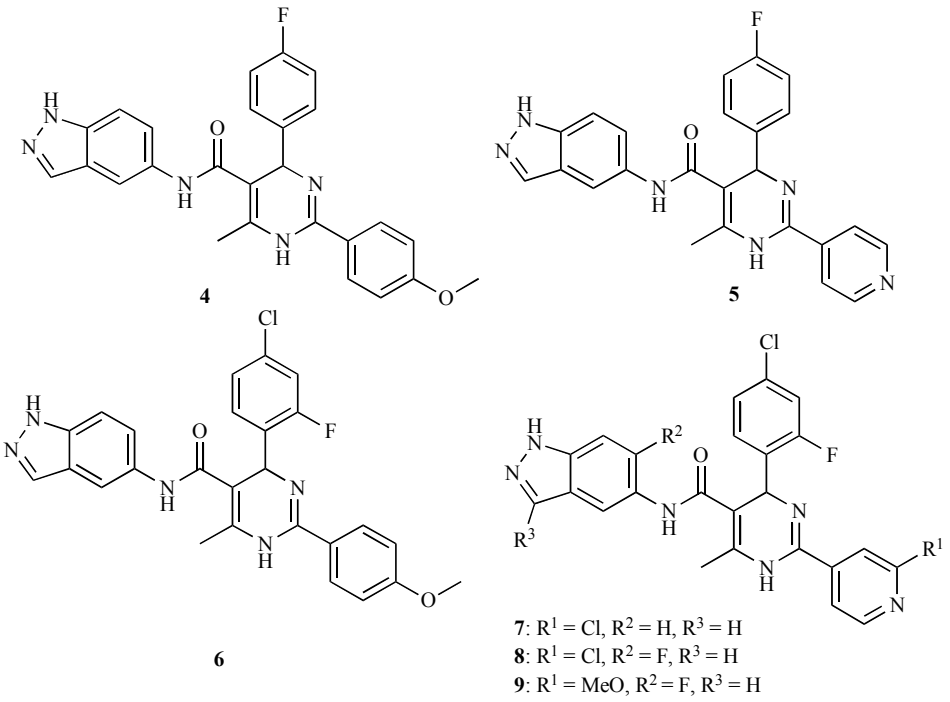


Fig. (3).

found to be concentration -dependent in inhibiting TAg stimulation to Hsp70 ATPase. It is interesting that compounds **10** and **15** displayed good TAg-stimulated Hsp70 ATPase inhibitory activity and endogenous Hsp70 ATPase rate enhancing activity, respectively. **10** And **12** have been also disclosed to have a sound modulating effect on protein post-translational translocation [36]. The salient activities to Hsp70 ATPase of these functionalized DHPMs implied their potential utility in antitumor treatment since Hsp70 ATPase contributes to the related cell functions *via* interaction with specific J domain proteins [37]. Although MLA3-101 (**10**) exhibited reasonable activity in inducing the apoptosis of SK-BR-3 breast cancer cell, it suffered from the disadvantages of poor solubility, high molecular weight and high lipophilicity. In order to overcome these problems, a series of peptoid functionalized DHPMs has been prepared (Fig. **5**). Upon the improvement in physicochemical parameters such as molecular weight, solubility and lipophilicity in contrast to MAL3-101, These new compounds have been screened against SK-BR-3 breast cancer cell growth, the results disclosed that most of these MAL3-101 derivatives bear inhibitory activity with 6.0 ± 0.4 to 42.6 ± 6 μM GI_{50} to the positive control of paclitaxel. Among those prominent compounds (Table **1**), GI_{50} of **18**, **20**, **21** were measured as 8.8 ± 0.3 μM, 6.2 ± 0.4 μM and 6.5 ± 0.7 μM respectively, they simultaneously showed impressive inhibition on MCF7 breast cancer and HT29 colon cancer cells (GI_{50} 2.4-7.8μM). The variation of R^1 in the DHPM unit in MAL3-101 turned out to make significant difference in the anti-proliferation activity as exemplified by the activities of **17** (6.0 μM), **19** (6.9 μM), **21** (6.2 μM) and **23** (7.1 μM). Notably, the 4-aliphatic derivative in DHPM exhibited good anti-proliferative effect, too (**20**, GI_{50} = 6.2 μM). In the subsequent activity assay on Hsp70, of ATPase function, many of these compounds displayed inhibition against the J domain stimulation to Hsp70 and no effect on ATPase endogenous activity. Typically, the fold changes of TAg-stimulated ATP hydrolysis at the presence of **25** was -3.5-fold without showing endogenous Hsp70 ATPase activity. However, **25** (Fig. **5**) and its homologs exhibited no breast cancer cell anti-proliferation activity. On the other hand, some compounds displayed both inhibitory activity to breast cancer cell proliferation and the TAg-stimulated Hsp70 ATP hydrolysis, for example, compound **16** inhibited the TAg stimulation by 5.2-fold and has the SK-BR-3 GI_{50} of 29 ± 0 μM while compound **22** inhibit TAg-stimulated Hsp70 ATP hydrolysis by 1.9-fold, and has the SK-BR-3 GI_{50} of 14 ± 2 μM. SAR analysis indicated that the pyrimidinone unit is sufficient for the inhibition of J protein stimulation of Hsp70. The peptoid, on the other hand, is necessary for anti-proliferation activity. In addition, the substitution in 4-position of DHPM center remarkably affect the inhibition activity of co-chaperon stimulation based on the results of **16** (-5.2-fold), **25** (-3.5-fold) and **19** (-1.1-fold). No direct relationship between the breast cancer cell proliferation activity and regulation activity of Hsp70 ATPase chaperon has been found. Finally, the analogous DHPM **24** was discovered with a dramatic modulatory effect on the Ssa1p (a yeast mitochondrial Hsp70) ATP hydrolysis and TAg stimulated Ssa1p ATP hydrolysis [38].

2.3. DHPMs Inhibitors of Mitotic Kinesin

The discovery of DHPM **26** (Fig. **6**), now known as monastrol is one of the landmark discoveries in the pharmacology of DHPMs [14, 19]. During their pioneering studies, Mayer and coworkers found that monastrol **26** specifically inhibit the activity of mitotic kinesin Eg5 as the first cell permeable small molecule [20]. Since mitotic kinesin plays an indispensable role during the formation of mitotic spindle, inhibition against these mitotic kinesin is

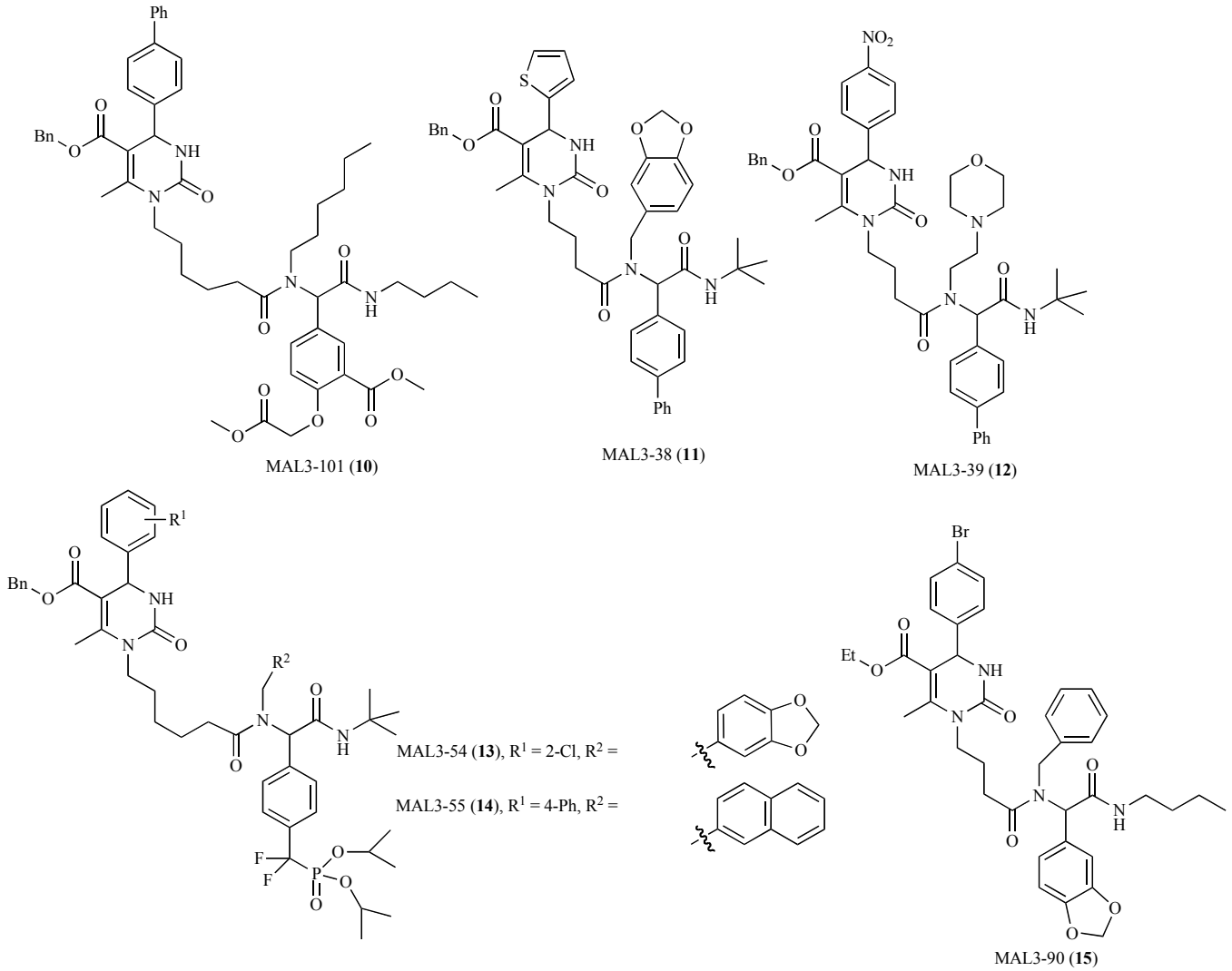


Fig. (4).

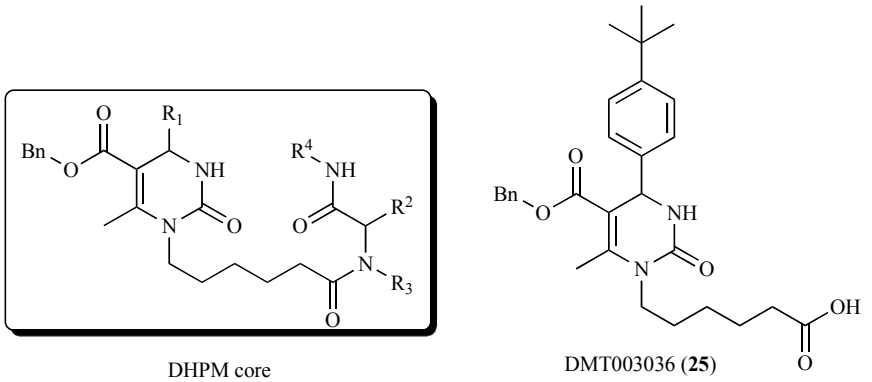
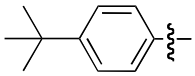
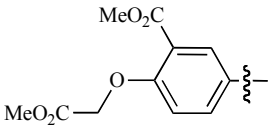
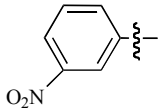
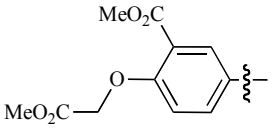
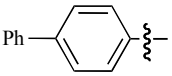
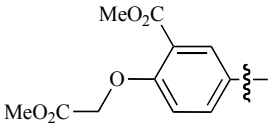
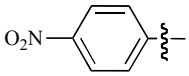
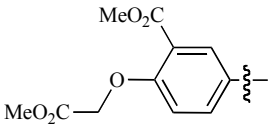
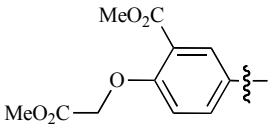
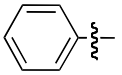
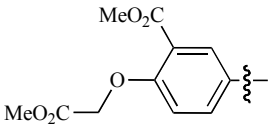
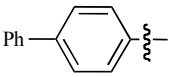
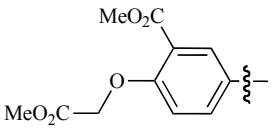
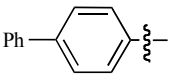
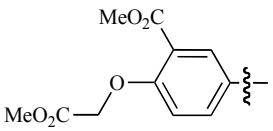
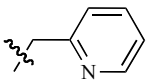
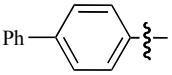
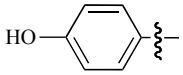


Fig. (5).

therefore an efficient tactic for th cancer treatment [39-40]. The motor protein Eg5 or *Xenopus laevis* homolog of human kinesin spindle protein (KSP) is a key medium for the separation and formation of bipolar spindles [41, 46]. Eg5 Inhibitor **26** is able to block the microtubule- stimulated ATP hydrolysis and inhibit cell proliferation *via* the arrest of mitosis. Notably, the interests derived from monastrol have been substantially expanded in recently years.

In the studies on the anti-tumor mechanism of monastrol, Maliga *et al.* found that monastrol doesn't compete with ATP or microtubule binding as many other Eg5 inhibitors

Table 1. Representative DHPMs with Anti-Proliferation Activity

Name	No.	R^1	R^2	R^3	R^4
DMT003084	**16**			*n*-Hexyl	*n*-Butyl
DMT003086	**17**			*n*-Hexyl	*n*-Butyl
DMT003052	**18**			$CH_3O(CH_2)_2$	*n*-Butyl
DMT003092	**19**			*n*-Hexyl	*n*-Butyl
DMT003132	**20**			*n*-Hexyl	*n*-Butyl
DMT003088	**21**			*n*-Hexyl	*n*-Butyl
DMT003102	**22**			Benzyl	*n*-Butyl
DMT003106	**23**				*n*-Butyl
DMT002220	**24**			*n*-Hexyl	*n*-Butyl

do, instead, monastrol inhibits the microtubule- stimulated ATPase activity by binding to a novel allosteric site on the motor domain of Eg5. The authors have also discovered that *S*-monastrol is the more potent Eg5 inhibitor *in vitro* and *in vivo*. The derivative *p*-monastrol **28** displayed only weak or no activity in these assays [42]. Similar results have been reported by Kozielski and coworkers. Based on the enzymatic technique, monastrol had been found directly binding to the motor domain of Eg5 without competing with microtubules and ATP. The enzymatic experiments and *in vivo* assays also indicated the higher activity of *S*-monastrol than that of *R*-monastrol [43]. The *S*-enantiomer's superior activity had been further confirmed by Kuo and coworkers based on X-ray crystallography analysis on monastrol-bound motor protein [44]. The comparison of the crystal models of Eg5: ADP in the presence and absence of inhibitor [44, 45] promoted extensive examinations on the drug mechanisms. The detailed kinetic analysis on monastrol's inhibition against KSP demonstrated that monastrol favorably binds to the KSP-ADP complex and the inhibition of microtubule-stimulated KSP ATP hydrolysis is sensitively influenced by the ionic strength [46]. Kim and coworkers studied interactions between Eg5 and monastrol with different FT-IR technique; the molecular information observed in the experiment suggested a clear disparity in allosteric interaction in the presence of ADP and ATP [47].

Based on the steady-state and presteady-state kinetics combined equilibrium binding analysis, Gilbert and colleagues observed the reversal of Eg5 ATP hydrolysis and proposed that *S*-monastrol stabilizes the conformation of ATP hydrolysis to prevent Eg5 from generating the required force for the formation of bipolar spindle [48], similar tactic had also been employed to examine the ATPase mechanism of monastrol inhibition to Eg5 in the absence of microtubule. The results demonstrated the formation of KSP-ADP-monastrol ternary complex, which excluded the binding to microtubules [49]. By using spectroscopic probe to monitor the pathway of drug's interaction with Eg5, Maliga *et al.* confirmed the formation of Eg5-ADP-monastrol ternary complex in solution phase [50]. Finally, the mutational study based on SAR with different monastrol analog by the same group interpreted the specificity of monastrol's inhibition to Eg5, which also supported the conclusion deduced from the X-ray crystallographic binding model [51]. A recent study on the relationship between the Eg5 spindle checkpoint and monastrol disclosed that the cell apoptosis induced by monastrol is independent of the spindle checkpoint [52], which had been previously proposed to be necessary for the Eg5 inhibitors to induce apoptosis [53].

Besides the research on the inhibitory mechanism of monastrol to Eg5, the effects of monastrol on other related proteins, cells as well as neurons, have been the other important issue. Mailhes *et al.* observed occurrence of syntelic orientation of chromosomes and aneuploidy of mouse oocytes at the transient exposure to monastrol [54]. Gheber *et al.* examined the long-term (> 20 h) effect of monastrol on two human cell lines, AGS from gastric carcinoma and HT29 from colon carcinoma and discovered that 24 h treatment of monastrol led to the reversible arrest in both cell lines whereas the arrest of AGS was not irreversible when the treatment time was prolonged to 48 h. Different inhibition capacity of monastrol against two cell lines, were also described [55]. The debilitating neuropathy is one of the major side effects resulting from typical anticancer agents targeting on microtubule such as the taxanes [56], this kind of neurotoxicity, however, was not evidently observed with monastrol in the assay of cultured postmitotic neurons [57]. Later on, Kim and coworkers observed weak deleterious effects of monastrol on the axonal microtubule organization based on the primary rat cortical neuron cultures [58]. The research on the interaction of monastrol with P-glycoprotein (Pgp), a protein in close association with the multidrug resistance, suggested that monastrol is a weak inhibitor of Pgp in viro and is not transported by Pgp. The results provided interesting clues for developing more potent Eg5 inhibitors [59]. Gilbert and colleagues compared the interaction of monomeric and dimeric Eg5 motors with monastrol; the kinetic and binding equilibrium analyses combined with cryo-EM 3-D structural modeling indicated that monastrol plausibly stabilizes the conformation in which the neck linker of all motor domains locked onto the catalytic core, which manifests as the inhibition of microtubule binding [60]. The molecular modeling analysis on the inhibition of monastrol against different kinesin motor proteins confirmed monastrol's specific effect on Eg5 [61].

Besides the research on the antitumor property and mechanism of monastrol, developing new DHPMs bearing more potent activity is obviously another important direction since monastrol is actually a moderate Eg5 inhibitor with the IC_{50} of 30 μM [42]. In general principle, derivatives without the *meta*-hydroxyl substitution in the 4-phenyl group of DHPM center such as **27** and **28** (Fig. **6**), displayed weak or no inhibitory effect in Eg5 [15, 42]. Thanks to the facility of classical Biginelli reaction, various monastrol analogs could now be easily prepared for the sake of structural modification. The derivatives enastron (**30**), enastrol (**31**) and dimethylenastron (**32**), for example, have been discovered as remarkably more potent Eg5 inhibitors than monastrol. The IC_{50} of **30**, **31** and **32** have been tested as 2μM, 2μM and 200 nM respectively in the *in vitro* steady-state ATPase assay. The SAR analysis on these screened compounds implied that the variation of the 4-aryl substitution, the alteration of 2-thiocarbonyl to carbonyl in the heterocyclic moiety generally resulted in evident lose in activity. Similar drop in activity occurred at replacement of 6-methyl with phenyl. It is noteworthy that the specific property of low or non-neurotoxic property remained in these compounds whilst showing much stronger activity [62]. The same group subsequently developed the DHPMs bearing the structural skeleton of 3,4-dihydroquinazoline-1-(1*H*)-thione, termed vasatrol (VS), which also exhibited significantly improved Eg5 activity in contrast to monastrol. The most potent ones are VS-83 (**33**) and VS-54 (**34**) (Fig. **6**). The IC_{50} from the *in vitro* malachite green ATPase assay and EC_{50} (the concentration at which 50 % of mitotic cells showed monoasters) of these two compounds were measured as 1.17 ± 0.31/7.27 ± 0.83 and 1.81 ± 0.15/8.51 ± 0.22 while corresponding values of monastrol are 20.73 ± 0.75/58.74 ± 2.60. The SAR analysis on the obtained products corroborated that *m*-hydroxyl in the 4-phenyl of the DHPM unit is necessary for desirable activity. The fluorine substitution in position 6 and 8 gave the most potent activity as exemplified by **33** and **34**. The specific inhibitory effect of these compounds to Eg5 has been demonstrated by measuring on different motor proteins [63]. Further study on the antiproliferative activity of the **30**, **32** and **33** against human glioblastoma cells revealed that these new compounds bear at least one order of magnitude higher activity than monastrol against U-87 MG, U-118 MG and U-373 MG. More importantly, these compounds didn't show effect on the cytoskeleton of resting cells [64]. The novel derivative **29** has been found to bear substantially stronger cytotoxic effect than monastrol against melanoma UACC.62, Kidney 786-0, breast MCF-7, ovarian OVCAR03 and colon HT-29 cancer cells respectively with IC_{50} of 6.0, 2.0, 1.9, 6.6 and 2.5 μM [65]. Upon screening a class of sterically demanding derivative of monastrol, compound **35** has also been found with far more potent inhibitory ability than monastrol (IC_{50} = 9.2 μM) [66]. Compound **36** represents an interesting case as reported by Garcia-Saez and coworkers, while previous studies generally illustrated the superior activity of *S*-monastrol over *R*- and racemate monastrol, the *R*-enantiomer of compound **36**, a DHPM sorted out from the investigation on 140 monastrol analogues, has been found to bear evidently more potent activity than its *S*- counterpart. Quantificationally, the IC_{50} from basal ATPase assays and

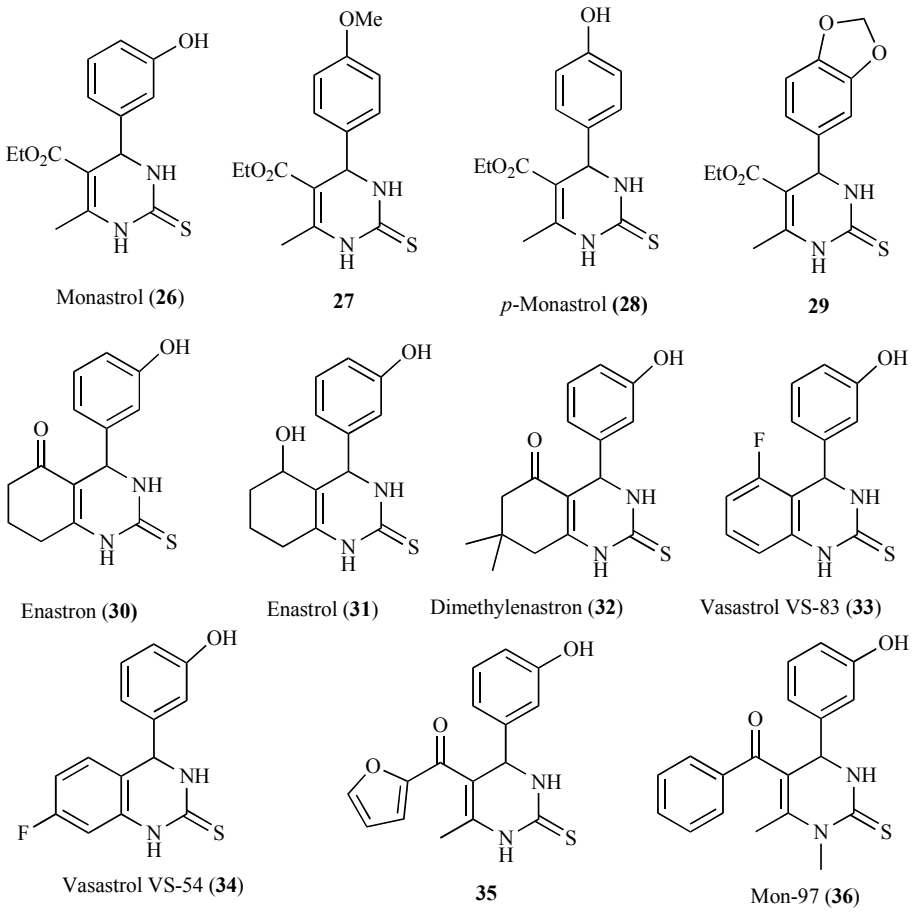


Fig. (6).

microtubule-stimulated ATPase assays are 110/150 nM for *R*-enantiomer and 520/650 nM for *S*-enantiomer, respectively. The crystal structure of human Eg5-*R*-mon-97 complex showed that the *R*-mon-97 (**36**) bainds to Eg5 at the same site as *S*-monastrol does, it also disclosed that drug-binding pocket and the overall Eg5 structure is rather similar with the results observed in *S*-monastrol-Eg5 complex [67-68].

3. DHPMs AS RECEPTOR ANTAGONISTS

3.1. DHPMs As Melanin-Concentrating Hormone Receptor 1 Antagonists

Melanin-concentrating Hormone Receptor (MCH) has been found in both fish and mammalian brain [69-70], the MCH in mammalian has been identified as a cyclic 19-amino acid polypeptide which is expressed in the lateral hypothalamus and zona incerta of the central nervous system. Studies on MCH implied its vital role in regulating the energy balance, appetite or food intake and mood [71-74]. Rats lack of MCH was found to be lean and hypophagic with elevated metabolic rate, while the overexpression of MCH led to the susceptible obesity and insulin resistance in rats [75-76]. Thus, the MCHR1 has been recognized as a promising therapeutic target of obesity [71, 77]. A number of antagonists of MCHR1 have been developed during the past decade, the DHPM-based antagonist (+)SNAP-7491 **37** (Fig. **7**) has been discovered with high-affinity to MCHR1. The data of affinity in the mammalian cell line expressing MCHR1 was obtained as K_b = 0.57 nM and > 1000-fold selectivity in MCH (K_i = 15 ± 0.11 nM). The *in vivo* assay on rats clearly indicated the anorectic activity of SNAP-749 by decreasing palatable food intake of rats without working as a malaise agent. More interestingly, in the study on diet induced obese (DIO) rats, **37** exhibited continuous effect on rats' weight reduction and led to 26 % less weight than the vehicle-treated counterpart in 4 weeks period, which is almost 2-fold than the date obtained from clinical drug D-fenfluramine. The toxic effect of **37** on losing weight was excluded by the separate study of longer treatment of **37**, whereas full reversibility in gaining weight and food intake of rats have been observed after two weeks termination of **37**. Additionally, **37** has been found to possess sound activity against depression/anxiety in different behavioral paradigms [78]. The potential antidepressant and anxiolytic properties of **37** have been supported by the studies from Millan *et al.* based on evaluating the restriction of immobility and marble-burying/aggressive behavior inhibition [79]. However, different results have been claimed by Natermet and coworkers. According to the activity examination performed by the authors, both racemate and the optically pure (+) SNAP-7941 did not exhibit the expected antidepressant/anxiolytic activity [80]. Interestingly, a rather recent publication from Millan *et al.* declared that the MCHR1 antagonist **37** increases social recognition in the

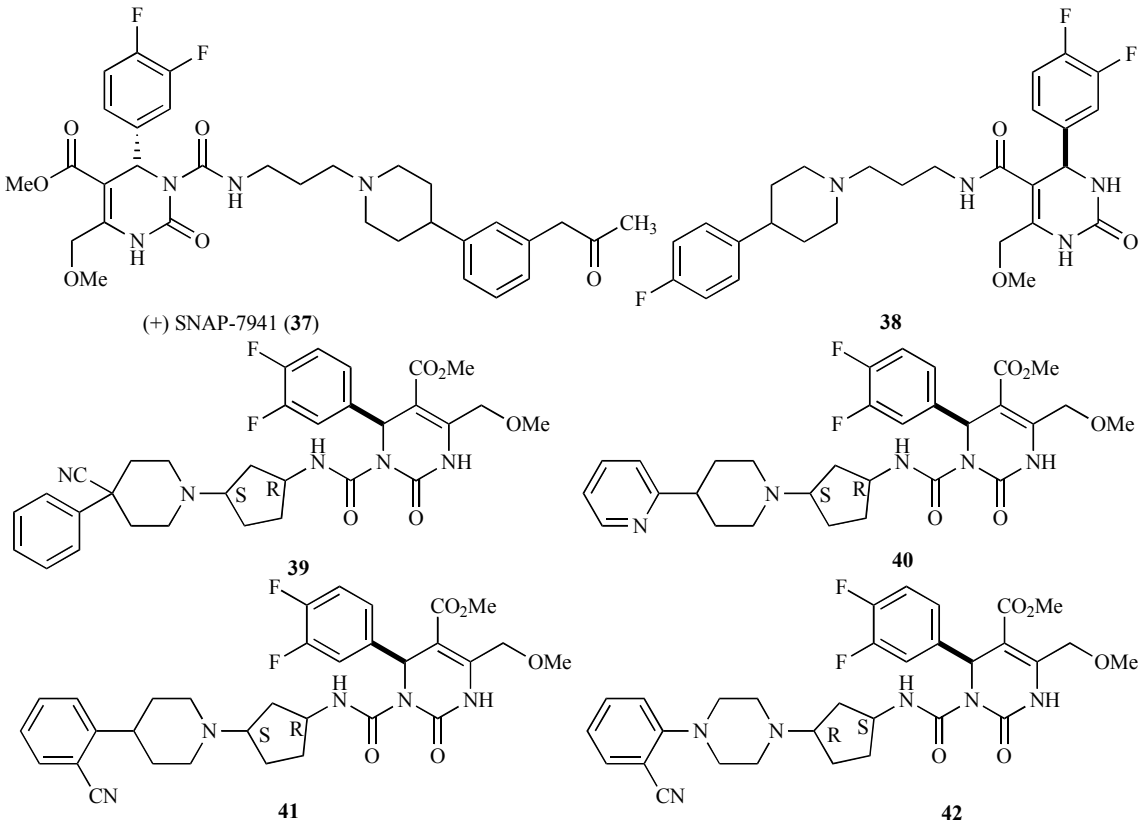


Fig. (7).

social recognition paradigm of rats and enhances the level of acetylcholine specifically in the frontal cortex of rats in dialysis study at reasonable dose. In contrast to the decrease of social recognition and non-elevated acetylcholine level induced by conventional antidepressant /anxiolytic agents, **37** is probably a mechanistically novel MCHR1 antagonist [81]. According to these reported results, the general effects displayed by those DHPMs of type **37** is worth of further investigation as novel and promising MCHR1 antagonists [82].

3.2. DHPMs As Selective α_{1a} Adrenergic Receptor Antagonists

Benign prostatic hyperplasia (BHP) is one of the major male diseases which troubles the life quality with obstructive and irritative symptoms. A proportion of over 85 % male suffers from BHP symptom after the age of 80 in the United States [83]. Although the population with BHP diagnosis differs based on the age and countries [84], it is out of question that BHP is a prevalent health disturber. The agents serving as selective α_{1a} adrenergic receptor antagonists provide the major treatment of BHP. In corresponding biological research, the DHPMs linked with piperidine, e.g. **38** (Fig. **7**) have been discovered with excellent binding affinity as well as the receptor selectivity; these compounds were therefore regarded as ideal replacers of corresponding DHPs as there are more resistant against oxidation [15, 85]. To further enhance the binding affinity and examine the effect of stereochemistry on the DHPMs antagonists, a class of piperidine functionalized DHPMs with stereochemical mutation was synthesized and screened for binding affinity towards α_{1a}-,α_{1b}- and α_{1d}- receptors. Among the 16 tested analogous compounds bearing different functional units of **39**, **40**, **41** and **42**, generally high affinity have been observed (0.24 ± 0.45 nM ~ 8.3 ± 2.8 nM). The noteworthy point suggested in the study is that the stereochemistry on these compounds makes substantial difference in terms of both the affinity and the receptor selectivity. In particular, compound **39** with *S*, *R* configuration present a good binding affinity (1.0 ± 0.15 nM) and an excellent α_{1a} receptor selectivity (> 600-fold) while its *R*, *R* diastereoisomer exhibited higher affinity (0.37 ± 0.12 nM) but poorer selectivity (> 500-fold). **40** as well as its *R*,*R* diastereoisomer have also been observed with impressive antagonist potency based on the corresponding data of 0.40 ± 0.15 nM/> 800-fold selectivity and 0.38 ± 0.01 nM/> 900-fold selectivity. Other isomers and compounds with a slight structural distinction (**41**, **42** and related isomers) were found with either remarkably lower binding affinity or poorer selectivity [86].

4. DHPMs AS CALCIUM CHANNEL BLOCKERS

Calcium channel blockers (CCBs) selectively block Ca^{2+} influx to enter the cell through the voltage-dependent calcium channels and modulate the intracellular calcium concentration. CCBs also slow down the recovery of calcium channel to reduce the intracellular calcium lever, which improves the vascular condition and protect the cardiovascular function from disorder. The attention paid to the calcium channel blocking activity on DHPMs was originally derived from their structural resemblance with

those of clinical dihydropyridine CCBs, e.g. nifedipine (**43**), felodipine (**44**) and nicardipine (**45**) (Fig. **8**). However, during the research process of DHPMs-based CCBs, some compounds have been discovered with not only more potent and longer lasting effects on vasodilation than DHPs, but also novel hypertensive activity.

Due to the great potential of Biginelli and related new multicomponent reactions in affording various DHPMs libraries, it is highly expectable to develop much more effective DHPMs agents as CCBs following know discoveries [15]. By employing 2-nitro-1-phenylethanone as the active methylene donor in Biginelli reaction, Bryzgalov *et al.* prepared a class of 4,6-di(hetero)aryl-5-nitro-3,4 – dihydro pyrimidin-(1*H*)-2-ones (**46-50**) (Fig. **8**). The antiarrhythmic activity evaluated in calcium chloride and epinephrine hydrochloride induced arrhythmia models revealed that **46-48** bear generally higher antiarrhythmic activity than lidocaine and quinidine [87]. The intravenous injection assay on rats indicated that **48** and **49** have the highest arrhythmia protection effect while the 2-thiocarbonyl derivative **51** was identified to have the lowest activity, suggesting necessity of the 2-carbonyl for desired activity. More interestingly, unlike the case in CCBs of DHPs, the DHPMs **40-50** displayed no influence on arterial pressure [88]. The DHPMs with mimic structure with aforementioned dimethylenatron, e.g. **52** and **53**, have also been disclosed with promising calcium channel blocking property. Among the 25 examined analogs, **52** and **53** exhibited the best activity. At the concentration of 10^{-4} M, **52** and **53** displayed the relaxant effect on isolated rat ileum in 68.67 ± 4.50 % and 62.33 ± 6.77 %, respectively. More interestingly, **52** showed 38.83 ± 5.84 % relaxant effect on lamb carotid artery, which is more potent than the reference agent nicardipine (35.50 ± 4.16 %) [89]. Recently, the same group reported the synthesis and calcium channel blocking activity of some new DHPMs. Representatively, **54** and **55** were found to have the most notable effect. At the control result of nicardipine 100 %, C = 10^{-5} M), the inhibition data of **54** and **55** to $BaCl_2$ contraction in rat ileum are respectively measured as 95.50 ± 2.07 % and 87.50 ± 1.50 %. The DHPM **55** simultaneously exhibited 19.33 ± 6.59 % inhibition activity to KCl contractions in rat thoracic aorta, which is exactly comparable with date of 20.5 ± 2.89 % from nicardipine [90]. The screening study performed by Sujatha and coworkers have also declared novel DHPMs with good cardiac effect. In the tested DHPMs with main variation in the 4-ary group, compound **56** with 4-(4-hydroxyphenyl) in the dihydropyrimidinone core turned out to have more potent cardiotonic activity than the digoxin [91]. Singh and coworkers [92] synthesized a class of racemic and eantiopure DHPMs with aliphatic chain in C4-position and evaluated their calcium channel blocking activities based on their abilities to relax a membrane -depolarization-induced

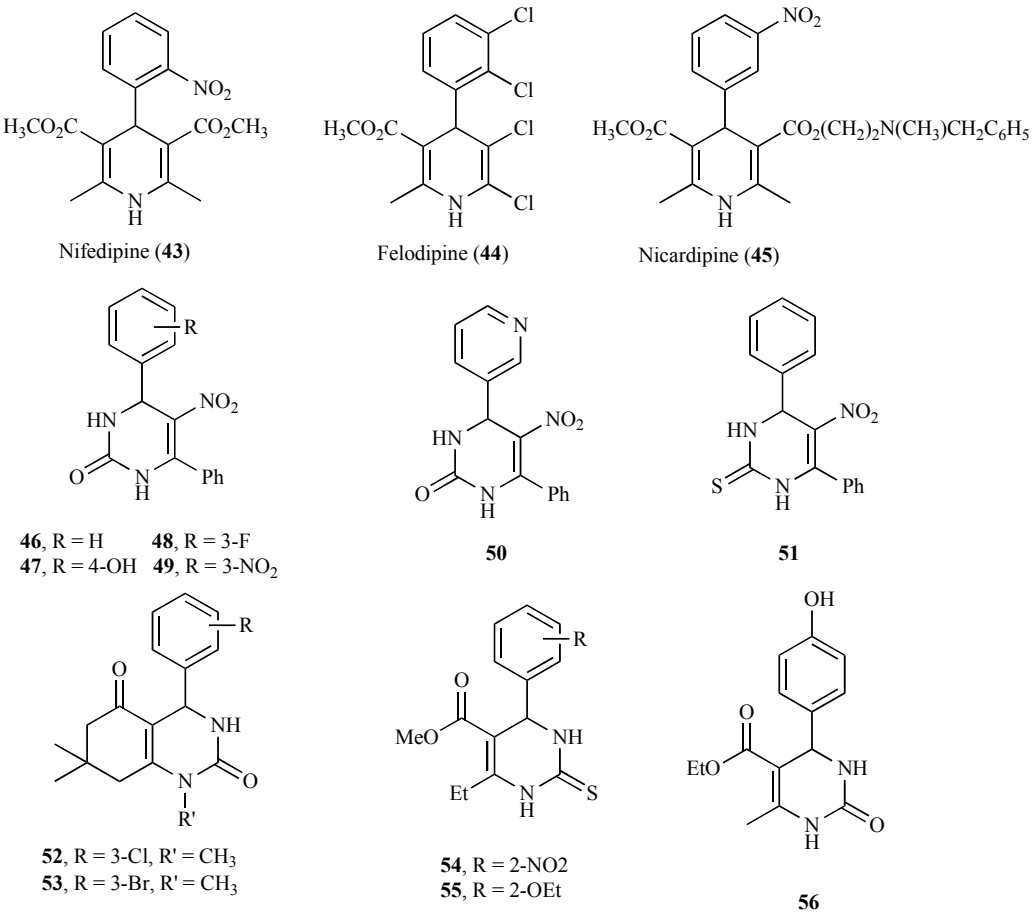


Fig. (8).

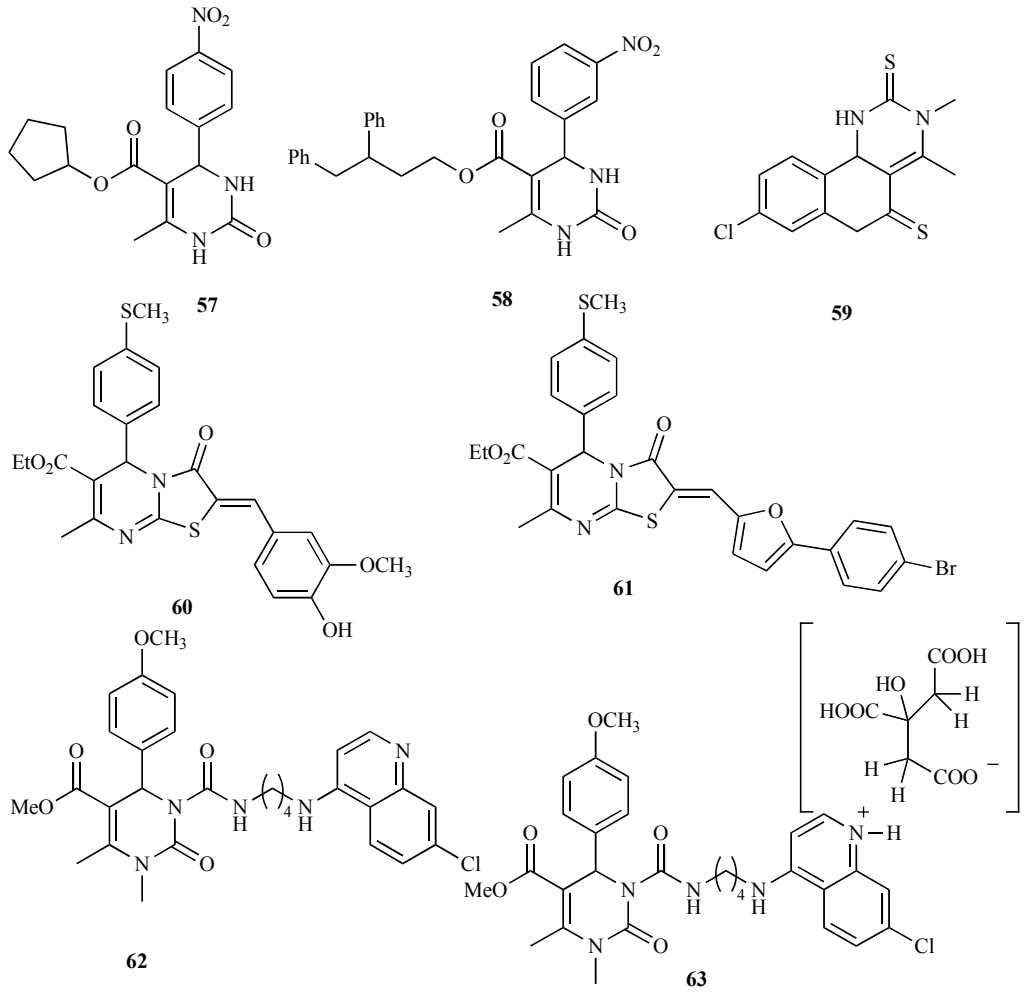
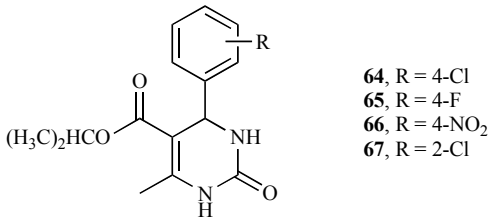


Fig. (9).

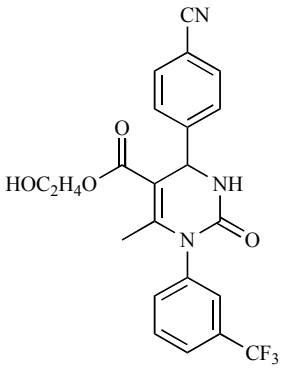
contraction of vascular smooth muscle using swine carotid arteries. 100-300 μM Of IC50 were observed with these compounds. No superior activities on the DHPMs bearing long aliphatic chains at C-4 site over the corresponding 4-aryl substituted DHPMs was observed.

5. THE MISCELLANEOUS

During the research process on DHPMs, the known results implied their fascinating pharmacological profile not only in those areas above mentioned, but also many other types of important functions. For example, the *S*-isomer of DHPMs **57** has been screened out as promising *in vitro* inhibitor of the fatty acid transporter 4 (FATP4), which disclosed its stirring foreground to treat obesity [93]. The 5-ester functionalized DHPMs, as exemplified by **58**, exhibited significance for their strong antioxidant activity against lipid peroxidation induced by Fe+EDTA and/or in reducing the reactive oxygen species (ROS) levels [94]. The fused DHPM **59** prepared from simple Biginelli compound has also been identified as a potent antioxidant based on its radical scavenging ability in DPPH and hydroxyl radical models [95]. On the other hand, the fused heterocycles of type **60** and **61** derived from the corresponding DHPMs exhibited moderate to excellent antibacterial and antifungal activities [96]. Rather recently, the DHPMs' functionalized chloroquines **62** and **63** have emerged with a quite interesting antimalaria activity, according to October and coworkers' research. In addition to the antimalaria property, the specific value of these products laid in their unique functions as reversal inhibitors, which is pivotal to overcome the drug resistance occurring in the use of conventional clinic drugs, e.g. chloroquines [97]. Meanwhile, some newly synthesized DHPMs **64**-**67** (Fig. **10**) have been discovered to possess good *in vitro* antimicrobial and antifungal activities. As reported by Pandiarajan and coworkers. By using ciprofloxacin as the standard drug, **64** and **67** both exhibited 25 μg/mL minimum inhibitory concentration (MIC) against *Staphylococcus aureus* (25 μg/mL for ciprofloxacin) and *Salmonella typhi* (50 μg/mL for ciprofloxacin), while **65** and **66** shown even better activities as they gave both MIC of 12.5 μg/mL against *Staphylococcus aureus* and *Salmonella typhi*. What's more, these compounds displayed good microbial activities against *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Analogously excellent antifungal activities have also been discovered with these products against *Candida albicans*, *Aspergillus flavus*, *Rhizopus* and *Mucor* compared to standard drug Amphotericin B [98]. Monastrol and some analogous



64, R = 4-Cl
65, R = 4-F
66, R = 4-NO_2
67, R = 2-Cl



68

Fig. (10).

DHPMs have shown interesting bioactivity of positively modulating the γ-Aminobutyric acid type A receptor subtype containing δ-subunit expressed in HEK293T cells [99]. Rather recently, DHPM **68** has been demonstrated as potent inhibitor of human neutrophil elastase, which suggested the potential therapeutic of **68** to the chronic obstructive pulmonary disease [100].

CONCLUSION

As a class of typical heterocyclic small molecules possessing versatile bioactivities, DHPMs are gaining daily increasing attention for the sake of finding new lead compounds and drugs probably because of their extraordinary binding ability to different proteins and enzymes. In the context of rapid development on modern synthetic chemistry, the availability of much more structurally diversified DHPMs makes it possible to conduct considerably more extensive investigations in the pharmacology of this scaffold. Therefore, promising results in finding excellent drugs containing central backbone of heterocyclic scaffold is reasonably expectable.

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