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Recent Progress in Biological Activities of Dihydropyrimidine Derivatives: An Updated Mini-Review

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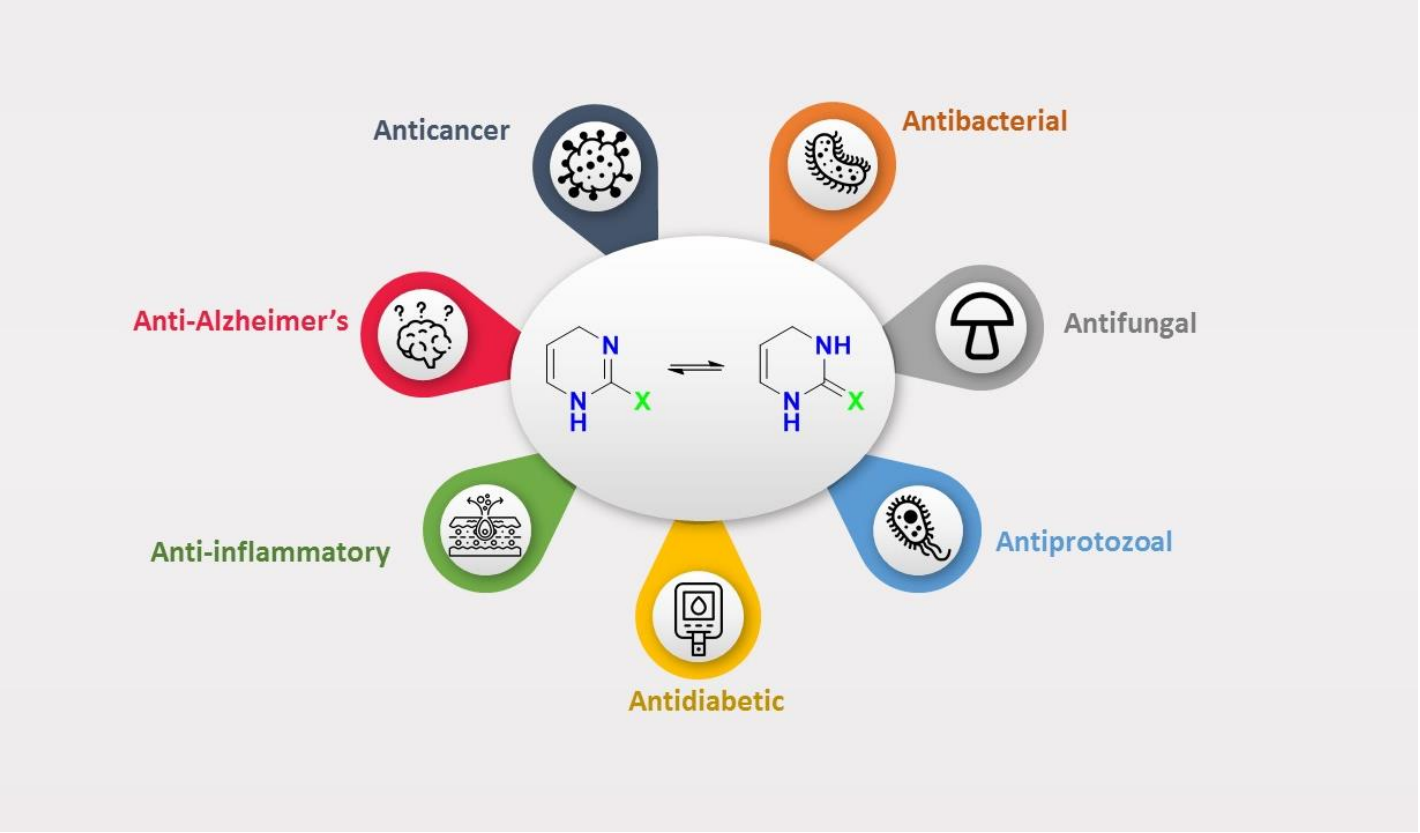
Abstract

Dihydropyrimidines (DHPMs) are a class of nitrogen-containing heterocycles that have attracted considerable interest due to their great significance in organic and medicinal chemistry. In recent years, many biologically active molecules featuring DHPM moiety have been synthesized and biologically evaluated, some of which have served as potential leads for critical unmet pathological conditions such as the antimitotic Monastrol and the antiviral GLS4, which entered clinical trials a few years ago. This review discusses briefly the recent advances in DHPM-based bioactive compounds, their biological activities, and their mechanisms of action, which could provide references for future developments of more potent and selective leads.

Keywords
Dihydropyrimidine, Biginelli, Medicinal impact, anticancer, antibacterial, anti-Alzheimer's.



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1.Introduction

Nitrogen-based heterocyclic compounds have drawn interest of researchers over the years, due to their biological importance[1–5]. The dihydropyrimidine nucleus, a doubly unsaturated six-membered ring possessing two nitrogen atoms at positions 1 and 3, is one of the most important nitrogen-containing heterocycles which was first synthesized by Pietro Biginelli in 1891 in the multicomponent Biginelli reaction[6]. Soon after it became an important scaffold in medicinal chemistry due to its broad spectrum of diversified biological activities such as anticancer[7–9], antibacterial[10–12], antifungal[13], antiviral[14–17], antiprotozoal[18–20], antioxidant[21], anti-inflammatory[22–24], anti-Alzheimer's[25,26] and antihypertensive activities[27]. It gained even more popularity with the discovery of a number important leads incorporating the dihydropyrimidine skeleton (**Fig. 1**) such as monastrol, a kinesin-5 inhibitor which disrupts the normal spindle bipolarity in mitosis and leads to cancer cell apoptosis[28,29], and GLS4 which is a capsid assembly modulator used in the treatment of Hepatitis B infection and is currently in phase 1 clinical trials[30] together with Bay 41-4109, another dihydropyrimidine derivative[31]. This mini-review aims to explore and summarize the biological significance of a variety of dihydropyrimidine derivatives reported in the past five years (2018-2022). We hope this mini-review will help medicinal chemists explore this potentially privileged scaffold and become a starting point for rationally designing more efficient dihydropyrimidine-containing drug candidates.

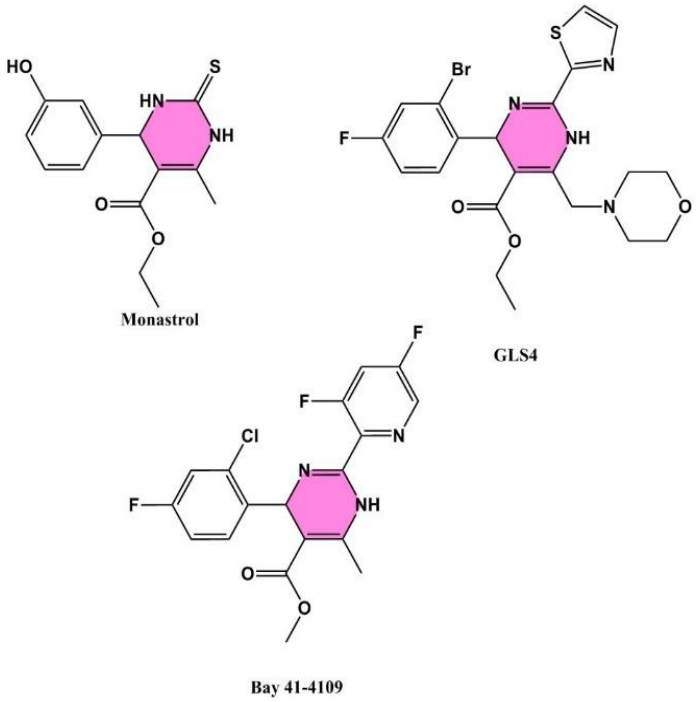

Fig. 1 Lead compounds containing the dihydropyrimidine core

2. Biological Activities

2. 1. Dihydropyrimidines as anticancer agents

2. 1. 1. Dihydropyrimidines as alkaline phosphatase inhibitors

Alkaline phosphatase is highly expressed in many disorders such as cancer. Also, elevated levels of alkaline phosphatase have been considered as a non-specific tumor marker for a long time[32–35]. Among some novel dihydropyrimidinone derivatives synthesized and screened by Altaf *et al.* (**Fig. 2**) as potential alkaline phosphatase inhibitors using the calf alkaline phosphatase assay, Compound **1** showed the most promising activity demonstrating an IC_{50} value of 1.271 µM at 0.1 µM concentration against the standard KH_2PO_4 which had an IC_{50} value of 2.80 µM. Furthermore, compounds **2**, **3**, and **4** also exhibited potent inhibition of the enzyme with IC_{50} values of 2.502, 2.943, and 2.132 µM, at the same concentration, respectively. When screened for their potential antioxidant activity, compound **2** showed the best free radical scavenging activity with an IC_{50} of 0.48 at 100 µg/mL concentration[36].

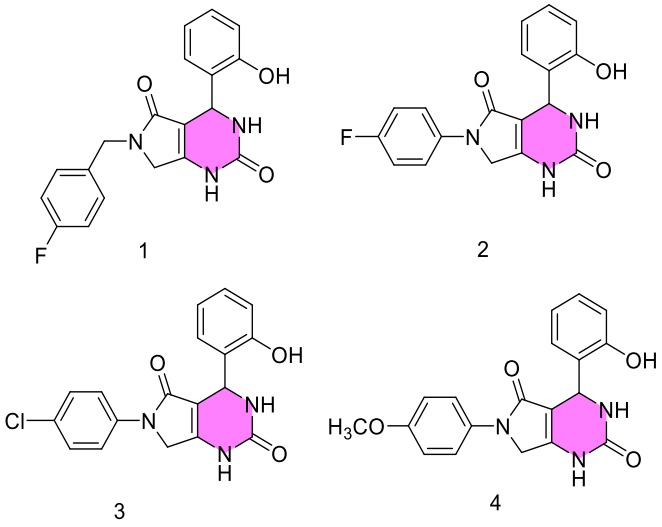

Fig. 2 Dihydropyrimidinones as alkaline phosphatase inhibitors having anticancer activities

2. 1. 2. Dihydropyrimidines as EGFR inhibitors

Another important target for dihydropyrimidines as anticancer agents is Epidermal Growth Factor Receptor (EGFR). Ahmed *et al.*, in 2021, reported 18 novel indolyl-dihydropyrimidinethione derivatives as potential antitumor agents (**Fig. 3**). They were screened against three human cancer cell lines (MCF-7, HepG2 and HCT-116) in addition to the lung fibroblast normal cell line (WI83) and their IC_{50} values were obtained using the Resazurin cell viability assay. Compound 5 showed the most potent inhibitory profile against cancer cell lines when compared to normal cells with IC_{50} values of (5.1, 5.02, 6.6, and 16.32 µM) against the cell lines MCF-7, HepG2, HCT-116 and WI83 respectively. Structure-activity relationship studies revealed that presence of the hydrazone moiety on the C5 of the dihydropyrimidine ring increased the inhibitory activity of the compounds when compared to their ester or free hydrazide counterparts. Moreover, condensation of the hydrazone to form thiazolidinone ring further improved the potency of these derivatives with the highest potency found when the phenyl ring is substituted with lipophilic electron withdrawing groups such as chlorine. This explains why compound **5** which has two chlorine atoms shows the best inhibitory potential against all three cancer cell lines. Additionally, Compounds were also evaluated for their *in vivo* antitumor efficacy against Ehrlich ascites carcinoma (EAC) in mice using 5-FU as a standard. It was found that compound **5** exhibited the highest % increase in lifespan (% ILS) of mice, as well as results showed that treatment with compound **5** brought about the lowest increase in tumor

volume and also the lowest increase in viable tumor cell count. Compounds were also assessed for their EGFR inhibitory activity against erlotinib as a standard because most of the compounds had a higher inhibitory activity against MCF-7 and HCT-116 cancer cell lines when compared to HepG2 cancer cells which can be attributed to the significant EGFR expression in MCF-7 and HCT-116 cells when compared to the undetectable expression in HepG2 cells. Compound **5** also showed the highest % inhibition with 79% inhibition of EGFR at 10 µM concentration and the lowest IC_{50} value of 0.25 ± 0.01 µM which is lower than erlotinib (IC_{50} = 0.30 ± 0.01 µM). Molecular docking studies revealed that the presence of the dihydropyrimidine nucleus resulted in extra binding with additional amino acids in the EFGR active site such as Phe699 when compared to the reference erlotinib. These results further corroborate their mechanism as EGFR inhibitors[37].

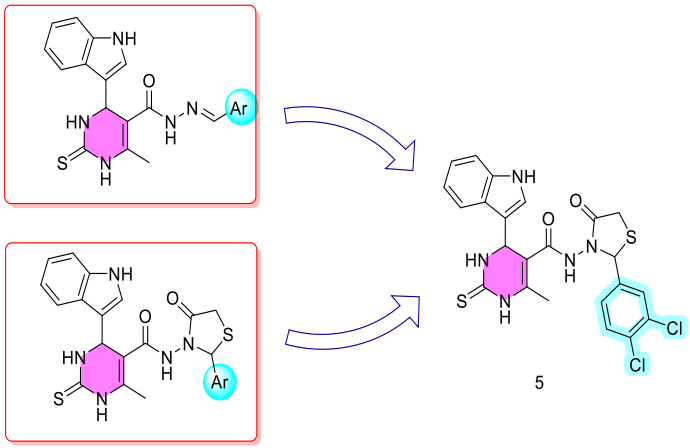

Fig. 3 Indolyl-dihydropyrimidinethione hybrids as EGFR inhibitors

2. 1. 3. Dihydropyrimidines as estrogen receptor modulators

Breast cancer is the most commonly diagnosed type of cancer amongst women. It's categorized into two major classes, hormone receptor positive and negative. Hormone receptor positive cancers constitute about 70% of all breast cancer cases worldwide and are easier to treat and grow slower than their negative counterparts[38]. Many DHPMs in literature have been proven effective in targeting estrogen receptors as a strategy to treat breast cancer. In an attempt to synthesize novel anti-breast cancer compounds, Ejaz *et al.* developed two new series of indenopyrimidine-2,5-dione derivatives with potential antiproliferative activity targeting estrogen receptors (**Fig. 4**). The anti-breast cancer activities of the synthesized compounds were assessed against the estrogen-positive MCF-7 cell lines as well as the estrogen-negative MDA-MB-231 cell lines and their activities were compared with tamoxifen as the reference drug. Among these compounds, compounds **6** and **7** were the most active against MCF-7 cells with IC_{50} values in the nanomolar region (IC_{50} = 81 nM and 77 nM) respectively compared to the reference drug (IC_{50} = 8.53 µM). Most of the compounds showed IC_{50} values > 50 µM against Estrogen-negative cell line (MDA-MB-231) and were selective against cancer cell lines as evidenced by their low cytotoxicity when screened against normal human HEK-293 cell lines. The binding affinity of these compounds were also assessed against estrogen receptors ERα and Erβ using fluorescence polarization assay and relative binding affinities (RBA %) were also calculated against ERα receptors by comparing them with estradiol (IC_{50} ERα = 0.0045 µM). Results revealed that compound **6** had 77.5-fold better binding affinity towards ERα (IC_{50} = 0.004 µM) as compared to ERβ (IC_{50} = 0.31 µM) and had RBA% value of 112.5 for Erα which indicated a better binding with the estrogen receptor α than estradiol. Furthermore, compound **7** had 74.5-fold stronger binding to ERα than Erβ with RBA% value for Erα of 63.38. Also the most potent compounds were evaluated as potential inhibitors of angiogenesis using chicken embryo chorioallantoic membrane (CAM) assay on the fertilized eggs against methotrexate as the positive control. Results showed that the tested compounds had significant anti-angiogenic properties with compound **6** being the most potent with IC_{50} value in the submicromolar region (IC_{50} = 0.86 µM) compared to methotrexate (IC_{50} = 3.65 µM)[39].

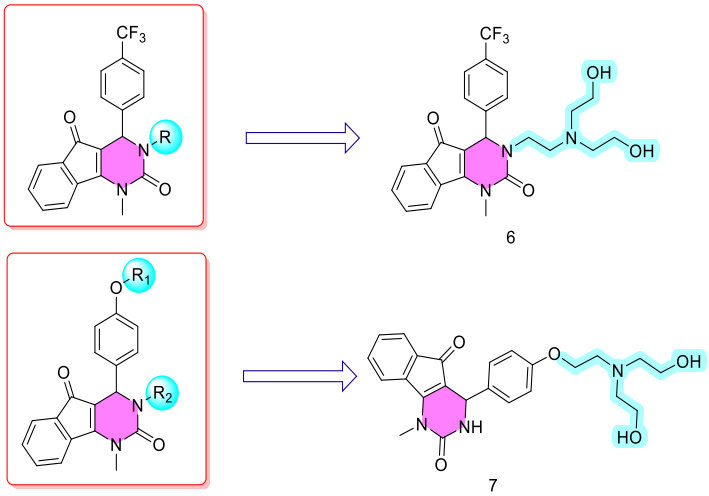

Fig. 4 Indenopyrimidine-2,5-dione derivatives as estrogen receptor antagonists

2. 1. 4. Dihydropyrimidines as mTOR & VEGFR-2 antagonists

mTOR & VEGFR-2 are members of the tyrosine kinase family of enzymes. These enzymes are normally responsible for many signalling pathways inside the living cells. Mutations in the cellular DNA can cause these enzymes to be hyperactive which can lead to uncontrolled cell growth and malignancy[40,41]. With this in mind, a series of Biginelli hybrids bearing different heterocyclic moieties were designed and synthesized by Mostafa *et al.* (**Fig. 5**) and twelve of these compounds were submitted to be screened against the NCI-60 cancer cell line panel at 10 µM concentration and % growth inhibition was calculated for each compound against each cell line. Results showed that compounds **8** and **9** inhibited the growth of all cell lines and were broad in spectrum with compound **8** being the most cytotoxic among the 12 compounds having % inhibition of 85, 88 and 86% against HL-60, NCI-H460 and SK-MEL-5 cell lines respectively. Compound **10** was the most potent against K-562 leukemia cancer cells where their growth was inhibited by 83%. It should be emphasized that all three compounds contained dithiocarbamate bridge at C6 of the dihydropyrimidine scaffold. Compound **11** which has the same dicyclohexylamino group as compound **8** but without its dithiocarbamate bridge was much less cytotoxic against all 60 cell lines. Other compounds of the series bearing heterocyclic rings attached to the dihydropyrimidine nucleus at C6 through $-CH_2-$ or $-SCH_2-$ bridges showed weak to moderate activity against cell lines. The four compounds that were not selected by NCI were evaluated for their anticancer activity against breast cancer MCF-7, prostate cancer PC-3 and colon cancer HCT-116 cells and their IC_{50} was determined compared with doxorubicin as the positive control. Compound **12** which has 5-nitro benzimidazole attached to the

C6 through -SCH_2- bridge was the most potent among the group with IC_{50} values of 9.39 , 9.18 and 7.29 µM against the aforementioned three cell lines respectively. To determine whether the synthesized compounds were more cytotoxic to cancer cells than normal cells, Compounds **12** and **8** were evaluated against three normal cell lines MRC-5 lung cells, BJ skin cells and WPE1-NA22 prostate cells and compared with doxorubicin as a reference drug. Both compounds were less cytotoxic on MRC-5 cells (IC_{50} = 44.16 and 32.04 µM) compared to doxorubicin (IC_{50} = 14.76 µM). In case of skin BJ cell lines, compound **12** was less cytotoxic than compound **8** and doxorubicin while compound **8** produced less cytotoxicity than compound **12** and doxorubicin on prostate WPE1-NA22 cell lines. When evaluated for its potential to increase active caspases 3 & 9 expression levels (protease enzymes involved in apoptosis), compound **8** increased caspase-3 level by 10 fold while caspase-9 level was increased by about 100 fold. It also increased the percentage of A549 cells undergoing early apoptosis by 3.27% and cells undergoing late apoptosis by 3.31% as determined from the Annexin v and propidium iodide (PI) assay. Compounds **12** and **8** being the most potent amongst the series were evaluated as potential inhibitors of the tyrosine kinases VEGFR-2 and mTOR. They exhibited potent inhibitory activity with IC_{50} values of 1.20 µM for **12** and 1.97 µM for **8** and inhibited VEGFR-2 by 75.99% and 68.52% respectively when compared to the positive control, sorafinib, which had an IC_{50} of 0.32 µM with 91.49% VEGFR-2 inhibition. Likewise, both compounds demonstrated strong inhibition against mTOR with IC_{50} values of 0.72 µM for compound **12** and 0.64 µM for compound **8** with % inhibition of 84.58% and 86.57%, respectively when compared to the standard, rapamycin, which had an IC_{50} of 0.43 µM, and inhibited mTOR by 94.88%[42].

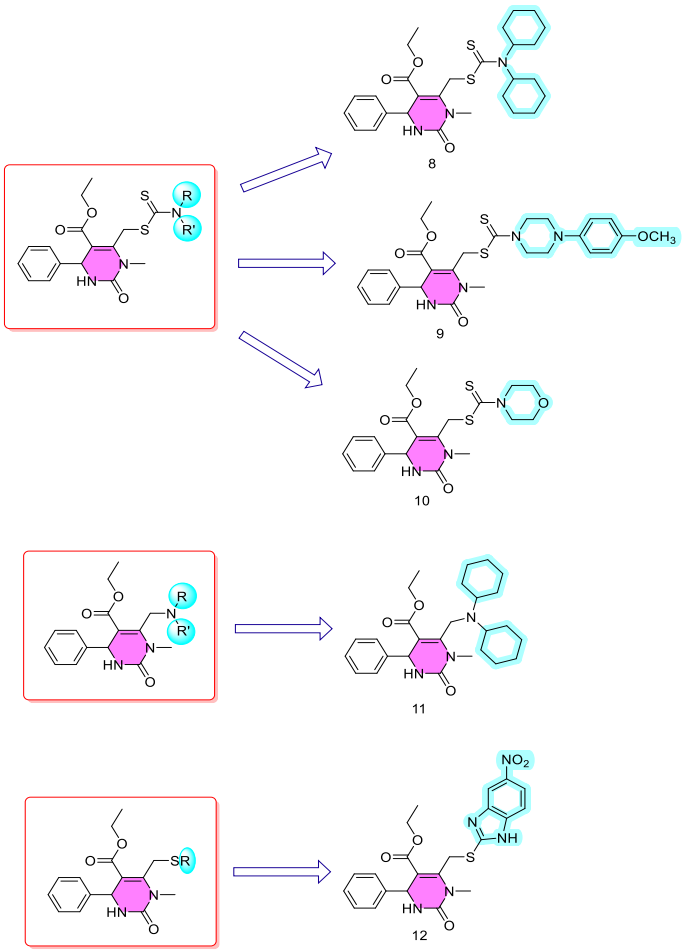

Fig. 5 Biginelli hybrids bearing different heterocyclic moieties as antagonists of kinases mTOR & VEGFR-2

2. 1. 5. Dihydropyrimidines as Thymidine Phosphorylase inhibitors

Thymidine phosphorylase (TP) plays a key part in angiogenesis and tumor metastasis and is overexpressed in many different types of cancers[43]. In order to investigate the ability of dihydropyrimidines to inhibit TP, Iftikhar *et al.*, in 2018 synthesized a series of eleven compounds (**Fig. 6**) from which only compound **13** had a significant inhibitory activity of more than 70% with an IC_{50} of 150.5 ± 0.005 µM using 7-Deazaxanthine (7-DX) as the positive control. In an attempt to further optimize this hit, a series of novel 18 structurally related derivatives of **13** were synthesized by inserting different substitutions on the 6-methyl moiety of the dihydropyrimidine ring. All compounds were evaluated for their TP inhibitory activity and the results showed that the strongest inhibition was exhibited by compounds containing an oxadiazole ring in the C6 side chain and all five compounds were more potent than the reference 7-DX with **14** having the lowest IC_{50} value (IC_{50} = 1.09 ± 0.004 µM) which is about 35 fold more potent than 7-Deazaxanthine. The synthesized compounds were assessed for their ability to inhibit angiogenesis using the *in vivo* chick chorioallantoic membrane (CAM) model on fertilized eggs by calculating the average number of blood vessels at five different doses of each compound and comparing them to 7-DX, dexamethasone and methotrexate as the reference drugs. Likewise, compounds bearing an oxadiazole ring at the C6 side chain showed the highest inhibition of neovascularization with IC_{50} values ranging from 11.34 to 27.6 µM with the strongest anti-angiogenic activity exhibited by compound **14** (IC_{50} = 11.34 ± 0.71 µM) which was about 2.5 fold more potent than dexamethasone (30.0 µM) but less potent than both the reference drugs 7-DX (9.92 µM) and methotrexate (0.85 µM)[44].

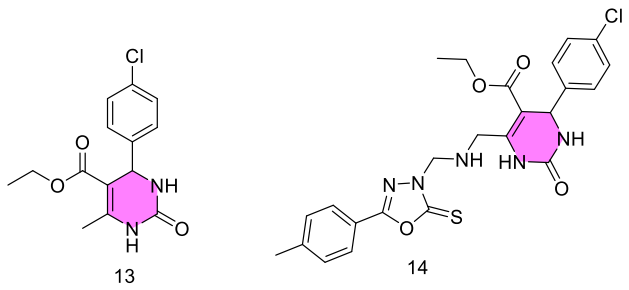

Fig. 6 Structures of anti-TP dihydropyrimidinones with antiangiogenic activities

2. 1. 6. Dihydropyrimidines as Eg5 inhibitors

Monastrol is a dihydropyrimidine analogue which was the first reported small molecule inhibitor of the Eg5 motor protein (a member of the kinesin-5 subclass). Kinesins are a family of proteins responsible for many motor functions like the transport of nutrients, chemical transmitters and even organelles across the cells acting like a train on a railroad track, or in this case the microtubules. Eg5 plays an important rule in mitosis maintaining a bipolar mitotic spindle. Its inhibition leads to formation of anomalous monopolar spindles which can activate spindle assembly checkpoint (SAC) and arrest the cell cycle which eventually leads to apoptosis. This represents a promising strategy to design novel anticancer compounds by structural optimization of this lead compound[45]. An attempt to enhance the anticancer potential of monastrol was performed by Hernández *et al.* who managed to synthesize dual acting

anticancer agents inhibiting both Eg5 and L-type calcium channels (**Fig. 7**). Calcium channels are often found overexpressed in many tumors and play a role in different cancer hallmarks such as angiogenesis, metastasis and evading apoptosis. They synthesized a series of eight compounds and evaluated them as L-type calcium channel blockers. Compounds **15**, **16** and **17** were the most potent vasorelaxants against KCl-pretreated aortic rings and had IC_{50} values in the range of 1.2 to 16 µM. Compound **16** produced a maximum vasodilator effect (E_{max}) of 95.4% compared to the reference nifedipine (98.7%) and decreased contractions induced by CaCl2 after being pre-incubated with the aortic rings in calcium-free Krebs solution. However, it didn't remarkably affect the contractions induced by phenyl ephrine (18.1%) unlike compounds **15** and **17** which decreased the response by 81.8 and 41.8 % respectively. This proves that compound **16** affects mainly influx of calcium ions through the channels rather than its intracellular release from the sarcoplasmic reticulum. This is further confirmed by the compound **16** significantly decreasing the contractions induced by the Ca channel agonist Bay K8644 at concentrations 10^{-4} and 10^{-5} M. All compounds were assessed for their potential cytotoxicity against different cancer cell lines. Compound **16** was more potent than the reference monastrol against A-549 and MCF-7 cell lines with IC_{50} values of 44.9 and 32.2 µM respectively. When evaluated for their potential to inhibit Eg5 protein, both compounds **16** and **17** increased the number of monopolar spindle mitosis with compound **16** increasing it by more than 2.5 fold compared to the negative control[46].

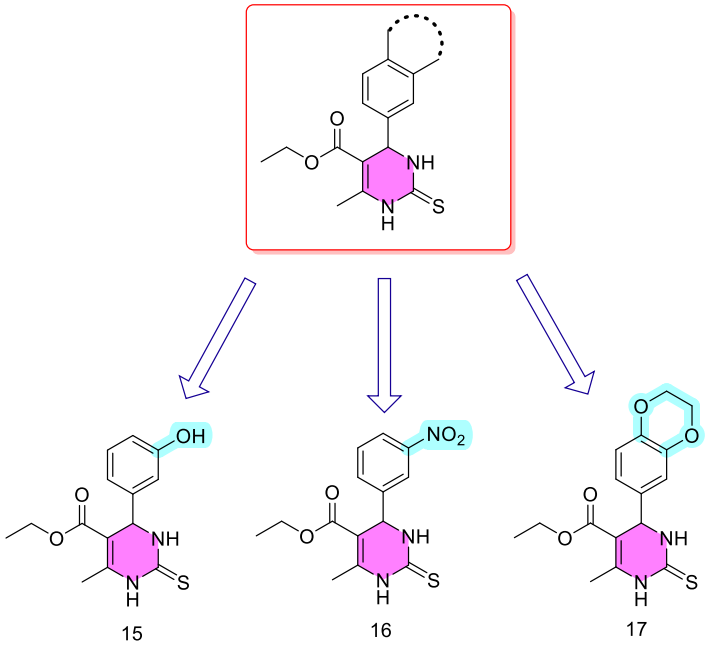

Fig. 7 Structures of dihydropyrimidine derivatives as Eg5 inhibitors

2. 2. Dihydropyrimidines with antibacterial activity

Shamim and coworkers described the activity of a series of 43 dihydropyrimidinone derivatives as promising urease inhibitors (**Fig. 8**). Nearly all compounds were more potent or had comparable activity to the reference thiourea (IC_{50} = 21.25 ± 0.15 µM). The most potent compound of the series was **18** having p.trifluoromethyl phenyl substitution at C4 of the dihydropyrimidone ring. It was about 6 times more potent than thiourea with an IC_{50} value of 3.70 ± 0.5 µM. Structure-activity relationship studies showed that substitution at the phenyl ring greatly affected the anti-urease activity. Compounds with hydroxy substituents generally showed potent activity against urease especially compound **19** which has 3-bromo 4-hydroxy substituents on the phenyl ring (IC_{50} = 4.95 ± 0.7). Methoxy-substituted derivatives had varying activities with di- and tri-methoxy derivatives showing little to no activity. Introduction of both halogens and methoxy groups remarkably enhanced the inhibitory potential. Absence of any substitutions completely abolished the activity. Presence of fluoro substituents provided highly potent compounds with the most potent having a trifluoromethyl moiety especially at ortho or para positions. Introduction of heterocyclic rings instead of phenyl produced derivatives with significant to comparable activities with the reference thiourea[47].

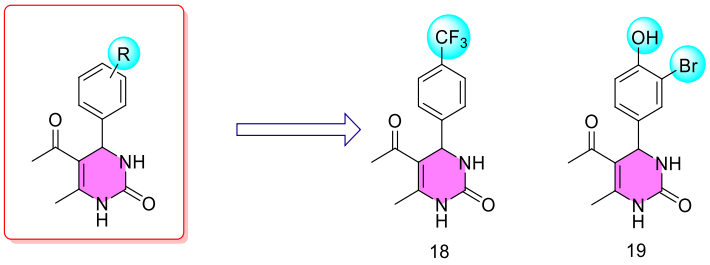

Fig. 8 Dihydropyrimidines showing anti-urease activity

Tiwari *et al.* designed and synthesized a series of Chromone-dihydropyrimidine hybrids bearing ester and hydrazide substituents at C5 position of the ring (**Fig. 9**). All compounds were screened for their antibacterial activity against Staph. Aureus and two E.coli strains 1411 and SM1411 using D-cycloserine as the positive control. Results showed that Compound **20** was the most potent of the series and had comparable activity with D-cycloserine against S. aureus (MIC_{100} = 32 µg/ml) but was slightly more potent against E. coli 1411 (MIC_{100} = 14 µg/ml) and E. coli SM1411 (MIC_{100} = 14 µg/ml). Mechanistic studies revealed that compound **20** inhibited the D-alanine-D-alanine ligase enzyme which is important for the normal peptidoglycan biosynthesis in bacterial cells. It was more potent than the reference D-cycloserine as evidenced by its lower IC_{50} value. The synthesized compounds were also evaluated for their potential antifungal activities against a number of candida, Aspergillus and Fusarium species and it was revealed that all compounds showed modest activities with compound **21** being the most potent of the series. It was equipotent with the reference drug miconazole with MIC_{100} values ranging from 15 µg/ml to 33 µg/ml. Compound **22** was the second most potent with MIC_{100} values ranging from 16 µg/ml to 32 µg/ml. It's worth noting that both compounds had the electron-donating methoxy group at position 6 of the chromone ring. Compound **21** was found to exert its mechanism through inhibition of lanosterol 14α-demethylase which is a key enzyme in fungal ergosterol biosynthesis. This mechanism was confirmed by the decrease in the UV absorption of ergosterol extracted from fungal cell cultures at wavelength 281.5 nm which is accompanied by increasing the concentration of compound **21**[48].

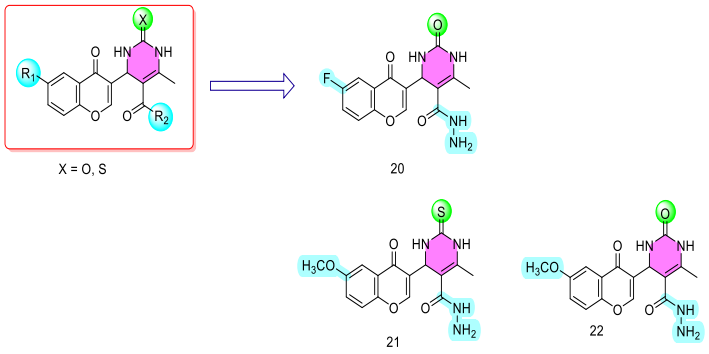

Fig. 9 Chromone-dihydropyrimidine hybrids with antibacterial & antifungal activities

Yang *et al.* investigated the potential antibacterial properties of new dihydropyrimidinone-imidazole hybrids against different gram-negative and gram-positive bacteria. Some compounds displayed significant antibacterial activities with the most potent of the series , compound **23** (**Fig. 10**), which contains a sulfamethoxazole moiety showing exceptional potency toward all the tested strains with MIC range of (0.5 – 16 µg/ml). It was several folds more potent than the reference compounds norfloxacin, clinafloxacin and sulfamethoxazole against all tested strains especially the gram-negative K. pneumonia and A. baumanii with MIC value of 0.5 µg/ml against both bacteria. It produced a much rapid bactericidal activity in time-kill assay and took only four hours to significantly reduce viable K. pneumonia and A. baumanii at MIC and two hours when used at 2 µg/ml which was much better than norfloxacin and clinafloxacin when tested at similar concentrations. When evaluated for its ability to induce drug resistance, the two bacteria didn't develop noticeable resistance to the drug after 16 continuous treatments which was comparable to clinafloxacin unlike norfloxacin whose MIC value increased by 32 fold over the course of the treatment. Potential antibiofilm activity of Compound **23** was also assessed and it showed a concentration-dependant inhibition of formation of biofilm by K. pneumonia and A. baumanii by 30 and 21% respectively at MIC, a percentage that increased significantly by increasing drug concentration which peaked at 89% and 84% respectively. It also decreased the viability of pre-formed biofilm in a similar dose-dependent manner to a minimum viability % of 36% and 41% for K. pneumonia and A. baumanii respectively. Compound **23** also showed selectivity toward bacterial cells and did not produce any noticeable hemolysis toward human RBCs at concentrations as high as 1024 times its MIC value. Similarly, it exhibited very low cytotoxicity against human HepG2 and endothelial cells when used several folds its MIC value. Further studies using the fluorescent probes diSC35, propidium iodide and 1-N-phenylnaphthylamine revealed that Compound **23** caused damage to both outer and inner bacterial cell membranes, increased membrane permeability and caused cell lysis which was confirmed by electron microscopy. Furthermore, this compound was evaluated for its ability to facilitate intracellular accumulation of reactive oxygen species (ROS) in bacterial cells using dichlorofluorescin diacetate dye. It was revealed that compound **23** produced dose-dependant increase in ROS accumulation which helps in disruption of bacterial cell membrane and increasing its permeability. This oxidative stress was further confirmed by the gradual decrease in intracellular glutathione (GSH) activity by increasing the concentration of compound **23** by folds of MIC. Compound **23** also displayed ability to bind with calf thymus DNA and replace the known DNA intercalator, acridine orange (AO) from DNA when the bacterial cells were pretreated with it as evidenced by the gradual decrease in the fluorescence of AO-DNA complex by increasing the concentration of compound **23**[49].

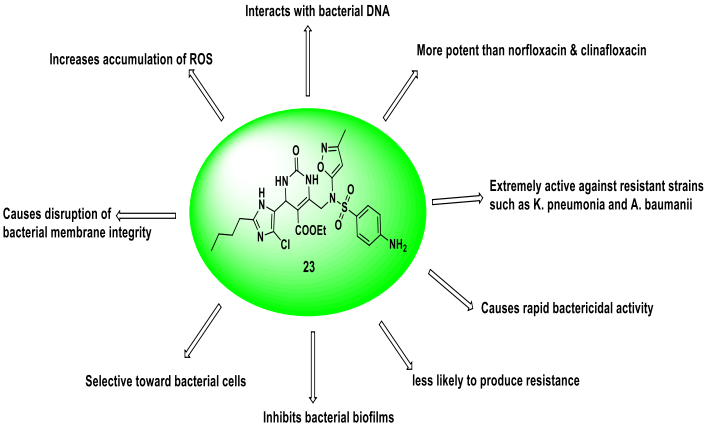

Fig. 10 Different antibacterial effects of sulfamethoxazole-dihydropyrimidine hybrid

2. 3. Dihydropyrimidines with antiprotozoal activity

Bibi *et al.* explored the antileishmanial activity of a series of biginelli hybrids (**Fig. 11**) against Dihydrofolate reductase enzyme (DHFR) from Leishmania major. The activities of the tested compounds were evaluated based on their IC_{50} values and it was found that compounds **24**, **25** and **26** showed excellent inhibition in submicromolar range with IC_{50} values of 0.38, 0.25 and 0.19 µM, respectively against the reference trimethoprim (TMP) which had an IC_{50} value of 20.5 µM. The selectivity of these compounds to inhibit l.major DHFR enzyme over human DHFR was assessed and it appeared that compounds **24**, **25** and **26** showed good selectivity for the parasitic enzyme with IC_{50} values of 0.84, 0.81 and 0.53 µM respectively with the selectivity index in the range of 2.2-3.2. When evaluated for their in-vitro antileishmanial activity against Leishmania major and Leishmania donovani, all compounds showed significant activity with IC_{50} values in the range of 1.23 – 15.1 µM against L. major compared to the reference Amphotericin B (0.60 µM) and IC_{50} values ranging from 0.94 to 17.4 µM against L. donovani in comparison with Sodium stibogluconate (IC_{50} = 3.27 µM) as the reference drug. Compound **25** was the most potent of the group against the two parasitic species with activity about 3.5 times stronger than the positive control drug[50].

2. 4. Dihydropyrimidines with antidiabetic activity

α-glucosidase inhibitors are one of the well known treatment strategies in combatting type 2 diabetes. These inhibitors such as acarbose and miglitol increase the time required for total carbohydrate digestion which in turn delay its absorption and prevent postprandial hyperglycemia[51]. The possible α-glucosidase inhibitory activity of two series of novel aminoalcohol biginelli hybrids (**Fig. 12**) was studied by Sujayev *et al.* and it was found that all compounds of the first series exhibited significant α-glucosidase inhibition having IC_{50} values

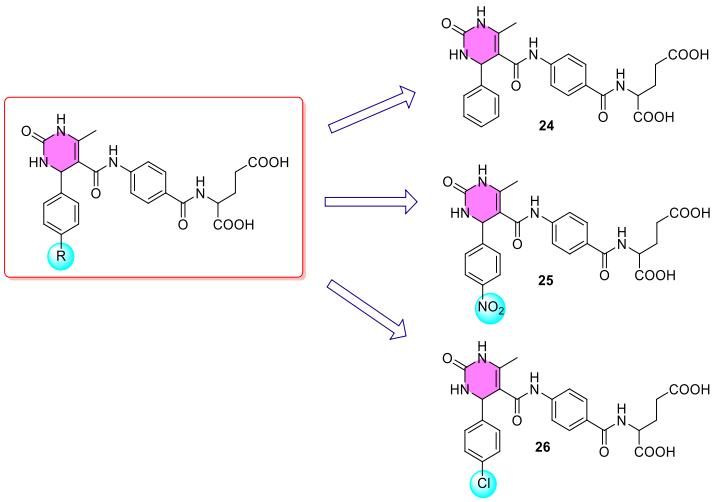

Fig. 11 Chemical structures of some antileishmanial dihydropyrimidines

ranging from 8.35 µM for compound **27** which is the most potent of the series to 43.28 µM for compound **28** against acarbose (IC_{50} = 2.05 µM) as the positive control. Compound **27** also showed the lowest Ki value amongst the series (Ki = 10.43 µM). All compounds of the second series also showed notable inhibition but lower than that of the first series. The strongest inhibitor of the series, compound **29**, inhibited the enzyme with IC_{50} value of 57.26 µM. Also this compound had the lowest Ki value of the series (68.93 µM). The synthesized compounds were also evaluated for their potential to inhibit α-amylase enzyme and similarly compounds **27** and **29** were the most potent in their respective groups with IC_{50} values of 14.38 µM and 49.36 µM respectively compared to the reference acarbose (IC_{50} = 11.48 µM)[52].

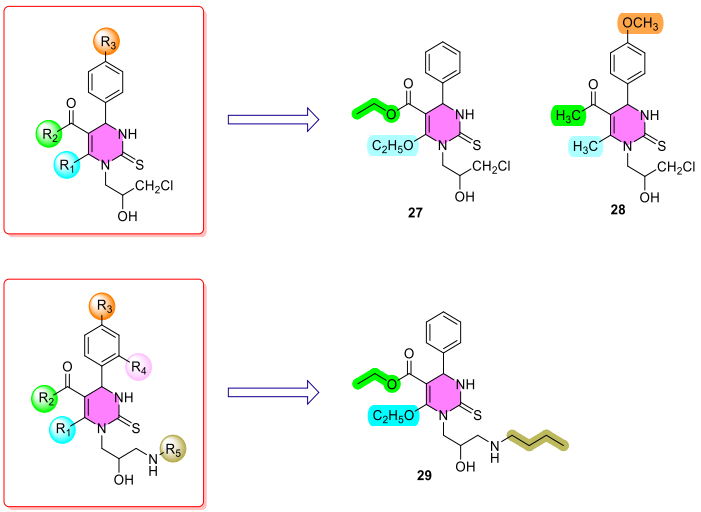

Fig. 12 Examples of some dihydropyrimidinethiones as α-glucosidase inhibitors

2. 5. Dihydropyrimidines with anti-inflammatory activity

Two series of novel compounds incorporating dihydropyrimidine scaffold (**Fig. 13**) were synthesized by Alfayomy *et al.* and evaluated for their inhibition against ovine COX-1 and human recombinant COX-2 and their IC_{50} values and selectivity indices were calculated. All the tested compounds showed marked COX-2 inhibition with 6 of these compounds (**30**, **31**, **32**, **33**, **34** and **35**) having IC_{50} values in the low submicromolar region ranging from (0.041 – 0.081 µM). All seven compounds showed remarkable selectivity toward COX-2 with selectivity indices ranging from (170 – 321) with only mild inhibition to COX-1 enzyme. Compound **32** showed the highest potency against COX-2 with an IC_{50} value of 0.041 µM and exhibited excellent selectivity (SI = 321.95). SAR studies showed presence of chloro substituents on the phenyl ring of the dihydropyrimidine moiety generally increased potency and selectivity toward COX-2 enzyme in the majority of the series while replacement with phenyl or 4-fluorophenyl substituents proved to be detrimental to the activity. *In vivo* studies using intraplantar injection of carrageenan in rats pretreated with the synthesized compounds showed that compounds **30**, **33** and **34** inhibited edema in the right rat paw with the maximum inhibition observed after 4 hours of treatment with 78.99, 89.51 and 79.23 % inhibition respectively which was stronger than that observed for celecoxib (65.44 %). When tested for its ulcerogenic effects on rat gastric mucosa, compound **33** exhibited 2.5 fold lower ulcerogenic liability (ulcer index = 8.25) than the reference indomethacin (ulcer index = 20.40) and comparable to celecoxib (ulcer index = 9). Compounds **30** and **34** showed slightly higher ulceration than celecoxib with ulcer indices of 11.75 and 12.17 respectively. These findings were corroborated by histopathological examination which showed mild lesions produced by these compounds comparable to celecoxib which were much less than the severe ulceration following indomethacin treatment[53].

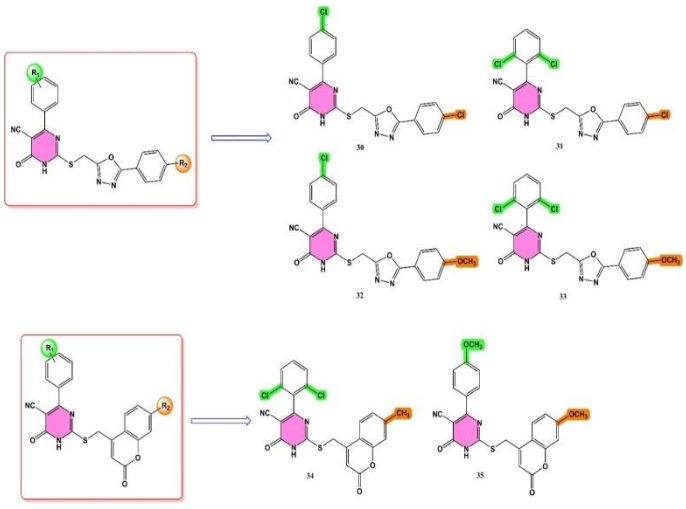

Fig. 13 Structures of dihydropyrimidine hybrids showing anti-inflammatory activities

2. 6. Dihydropyrimidines with anti-Alzheimer's activity

Alzheimer's disease is a neurodegenerative disease and the most common cause of dementia in elderly people. It's caused by a combination of genetic, lifestyle and environmental factors that

affect the brain over time although the causes of Alzheimer's aren't yet fully understood. It's thought to be caused by the formation of abnormal deposits of protein in the brain. These deposits are either amyloid β plaques that aggregate between neurons or neurofibrillary tangles that result from abnormal accumulation of tau protein inside the neurons[54]. Current treatment strategies don't eradicate the disease but rather ameliorate the symptoms. The most commonly used medications are cholinesterase inhibitors, NMDA receptor antagonists and MAO inhibitors[55]. In light of this information, two series of (methyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylates) and (ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylates) were synthesized by Khan *et al.* and evaluated for their potential to inhibit monoamine oxidases (MAO A and B) and cholinesterases (AChE and BChE) (**Fig. 14**). Among these inhibitors, compound **36** showed the highest inhibitory against MAO-A with an IC_{50} value of 0.31 ± 0.11 μM. Similarly, compound **37** exhibited an IC_{50} value of 0.86 ± 0.06 μM. Interestingly, both these inhibitors contained 2 and 5 substituents on the phenyl ring. Furthermore, compounds **38** and **39** demonstrated remarkable selectivity as MAO-A inhibitors and showed only 28 % and 22 % inhibition for MAO-B. All compounds of the second series didn't exhibit noticeable inhibition against MAO-A. On the other hand, compounds **40**, **37**, **36** and **41** showed significant activity against MAO-B with IC_{50} values in the range of 0.34 – 12.76 μM. Compound **40** showed the highest inhibitory activity against MAO-B (IC_{50} = 0.34 ± 0.04 μM), followed by compound **41** with an IC_{50} value of 1.53 ± 0.04 μM. It is important to note that only compound **41** displayed selective inhibition of MAO-B and only very slightly inhibited MAO-A (6 % inhibition). Additionally, potential inhibitory activities of both series against Acetyl- and Butyryl-cholinesterase were also investigated. Among first series, compound **42** showed the highest inhibition for AChE with an IC_{50} value of 0.13 ± 0.09 μM. All compounds in this series were highly selective AChE inhibitors with only negligible inhibition of BChE (< 29 % inhibition). On the other hand, only compound **43**, **44** and **45** from the second series showed highly selective inhibition for AChE and only < 12% inhibition for BChE. Compound **45** was the most potent AChE inhibitor of the series with an IC_{50} of 4.50 ± 0.87 μM[56].

Mahgoub *et al.* developed a series of thiazolo[3,2-a]pyrimidine bromide salts (**Fig. 15**) and evaluated them for their acetyl cholinesterase inhibition at different micromolar concentrations using a standard Ellman-based colorimetric protocol by measuring the decrease in enzyme activity monitored by the decrease in yellow color produced from thiocholine when it reacts with dithiobisnitrobenzate ion. The results were compared with the anti-cholinesterase methyl paroxon as the reference drug. Results showed that all compounds displayed remarkable acetyl cholinesterase inhibition at the low micromolar region ranging from 73 – 77.5 % at 10 μM to 59 – 74 % at 5 μM compared to the 100 & inhibition of the standard drug exhibited at the same concentrations. Compound **46** was found to be the most potent inhibitor of the series with IC_{50} value equal to 1 μM compared to the standard methyl paroxon (IC_{50} = 4.9 nm)[57].

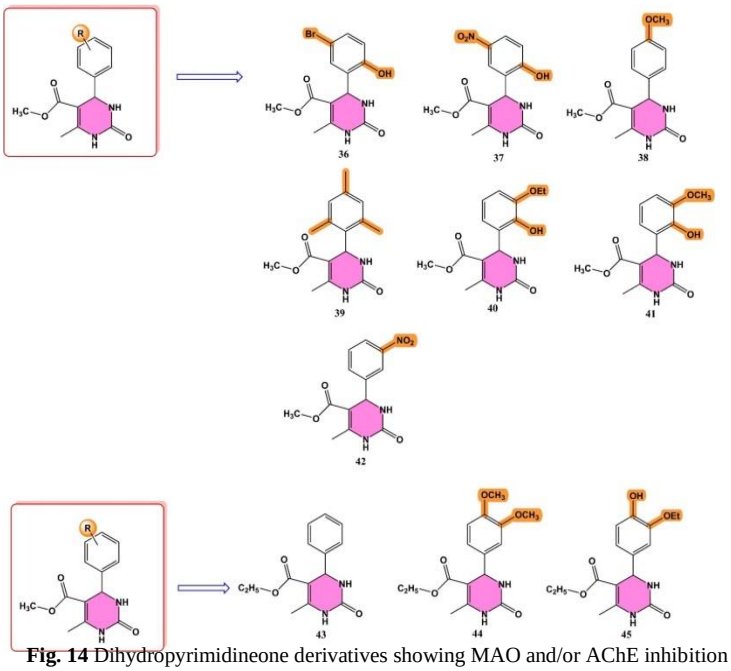

Fig. 14 Dihydropyrimidineone derivatives showing MAO and/or AChE inhibition

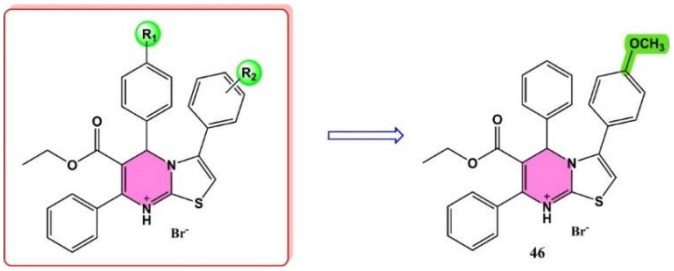

Fig. 15 Thiazolopyrimidine derivatives showing anti-cholinesterase activity

Another important target in battling Alzheimer's disease is β-secretase 1 (BACE-1). It's a protease enzyme which is responsible for the degradation of a transmembrane protein called amyloid precursor protein (APP) to form amyloid-β peptide (A), which tends to form interneuronal plaques which ultimately results in neurodegeneration, dementia and memory loss associated with the disease[58]. Based on this information, a series of 3,4-dihydropyrimidin-2(1H)-one/thione/imine derivatives were synthesized by Bais *et al.* and investigated as potential BACE-1 inhibitors (**Fig. 16**). It was found that substitution at R1 with aliphatic or aromatic groups retained the activity. Derivatives with larger aromatic groups such as benzothienyl and naphthyl retained the activity but orientation of the ring seemed to drastically affect the activity especially in the case of 4-benzothienyl substituted derivatives where compound **47** containing benzothien-2-yl group was 52 fold more potent than its benzothien-3-yl counterpart (compound **48**). On he other hand, substitution at R2 led to more noticeable changes with, in most cases, the methyl derivatives being more potent than the

corresponding ethyl or amide ones. Substitution of ester with a free carboxylic group in compound **49** markedly decreased the inhibitory activity. Imine derivatives in most cases demonstrated superior inhibitory activity than their oxa and thia counterparts with compound **50** exhibiting the most potent inhibition overall with an IC_{50} value of 0.2 ± 0.1 μM. This can be attributed to the better interactions of these imine derivatives (or their amino tautomers) with the enzyme's active site than oxa or thia analogs. At the optimal pH of the enzyme, protonation of the more basic guanidine system occurs, which interacts with the active site via extended hydrogen bonding and charge complementarity as corroborated by molecular docking[59].

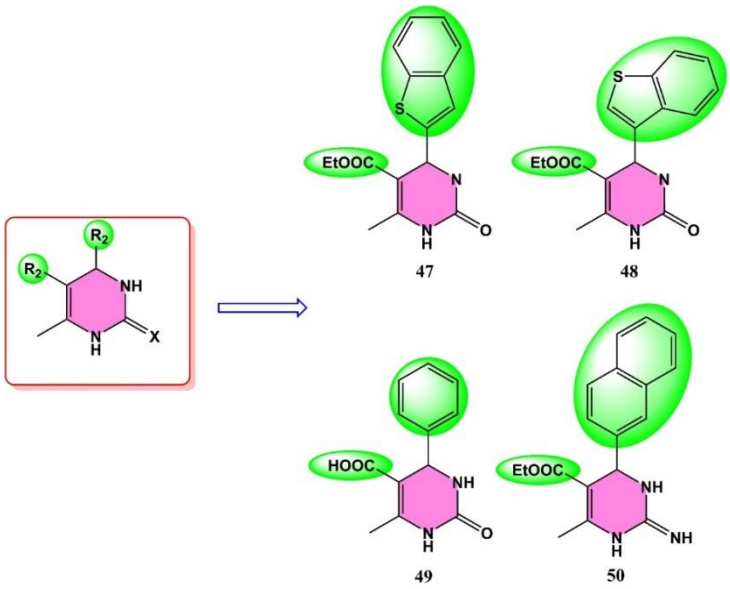

Fig. 16 Examples of dihydropyrimidines exhibiting BACE-1 inhibition

3. Conclusion

In summary, the present review focuses on the recently reported heterocyclic dihydropyrimidine derivatives which exhibit a wide spectrum of biological potential in medicinal chemistry such as anticancer, antibacterial, antiprotozoal, antidiabetic, anti-inflammatory and neuroprotective in Alzheimer's disease. All these have strongly emphasized the infinite potentiality of these derivatives in medicinal field. Several mechanisms that involve inhibition of different enzymatic pathways by this scaffold are highlighted in this work. It's expected that the information discussed in this work could provide a valuable guidance for medicinal chemists for the development and optimization of better molecules with enhanced biological activities and higher selectivity in their future research.

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