Review article

Recent synthetic and medicinal perspectives of dihydropyrimidinones: A review

Ramandeep Kaur [a], Sandeep Chaudhary [a], Kapil Kumar [a], Manish K. Gupta [b], Ravindra K. Rawal [a, *]

[a] Department of Pharmaceutical Chemistry, Indo-Soviet Friendship College of Pharmacy (ISFCP), Moga 142001, India
[b] School of Pharmacy, Lloyd Institute of Management and Technology, Greater Noida 203207, India

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ABSTRACT

Dihydropyrimidines are the most important heterocyclic ring systems which play an important role in the synthesis of DNA and RNA. Synthetically they were synthesized using Multi-component reactions like Biginelli reaction and Hantzschdihydropyridine. In the past decades, such Biginelli type dihydropyrimidones have received a considerable amount of attention due to the interesting pharmacological properties associated with this heterocyclic scaffold. In this review, we highlight recent developments in this area, with a focus on the DHPMs, recently developed as anti-inflammatory, anti-HIV, anti-tubercular, antifungal anticancer, antibacterial, antifilarial, antihyperglycemic, antihypertensive, analgesic, anticonvulsant, antioxidant, anti-TRPA1, anti-SARS, and anti-cancer activity and α_{1a} binding affinity.

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* Corresponding author.
  E-mail address: rawal.ravindra@gmail.com (R.K. Rawal).

1. Introduction

Heterocyclic chemistry is an important branch of organic chemistry accounting for nearly one-third of modern publications [1]. Heterocyclic compounds have vital role in our biological system. They are an integral part of many pharmacologically active molecules, natural products and nucleic acids. The base pair of DNA& RNA (guanine, cytosine, adenine and thymine) are also made up of heterocyclic compounds like purine, pyrimidine etc. Heterocyclic compound are also present in large variety of drug candidate like antitumor, antibiotic, anti-inflammatory, antidepressant, anti-malarial, anti-HIV, antimicrobial, antibacterial, antifungal, antiviral, antidiabetic, herbicidal, fungicidal and insecticidal agents [2]. Some naturally occurring molecules are discovered and having a good biological activity against the many diseases e.g. quinine is used as an antimalarial drug, vinblastine and vincristine are also used as anticancer agent. In this review, our focus will be on the 3,4-dihydropyrimidine (DHPM) ring. Basically it is a selective review on dihydropyrimidinones. Literature of last two decades is incorporated in this review. The pyrimidine is the most important heterocyclic moiety. Pyrimidine derivatives have various therapeutic applications in medicinal chemistry. One anticipated reason for their activity is presence of a pyrimidine base in thymine, cytosine and uracil, which are essential building blocks of nucleic acids, DNA & RNA [3]. Number of chemical compounds consisting of pyrimidine as core nucleus were synthesized and evaluated for antihypertensive [4], anticancer [5], antimicrobial [6], antihyperglycemic [7], antiarrhythmic, anti-inflammatory [8], analgesic [9], antibacterial [10], anti-HIV [11] and antitubercular activity [12]. Due to the wide range of therapeutic properties scientist have attracted towards developing new dihydropyrimidine molecules. Recently in 2013, Dragovich et al. synthesized substituted 2-thio-6-oxo-1,6-dihydropyrimidines inhibitors of human lactate dehydrogenase [5]. The DHPMs are synthesized with the help of Multi-component reactions (MCR). MCR are special types of theoretically useful organic reactions in which three or more starting materials react to give a desired product [13]. Combine synthesis pathways generally show advantages over continuous or atypical approaches with respect to time, speed, yield and reproducibility. Among organic reactions, MCR with more than two starting materials are allowed to form a complex product. Therefore, they constitute a superior tool for diversity oriented and complexity-generating synthesis for drug discovery [14]. Commonly used multi component reaction for heterocyclic synthesis are Biginelli reaction and Hantzsch dihydropyridine synthesis [15] etc. Few pharmacologically active dihydropyrimidinones are shown in Fig. 1.

Dihydroprymidinone nucleus is also found in marine natural alkaloids Batzelladine A and B which are known to inhibit the binding of HIV gp-120 to CD4 cells [16,17]. Various other synthetic analogs such as monastrol [18], L-771,688 [19], SQ 32926 [20], have been developed (Fig. 2). Monastrol is the most important anticancer compound and has an ability to cross the cell membrane. It causes mitosis by reversible and specific inhibition of Eg5 myosin kinase. Various other analogs of monstraol such as oxo-monastrol, thio and 3,4-methylenedioxy derivatives of manostrol have been developed and tested against HT-29 colon cancer cell lines. 3,4-methylenedioxy analog was found to be 30 times more potent than monastrol [18].

Various drug interactions and side effects have been reported for some of the drugs containing dihyropyrimidinone nucleus. Monastrol is well known as mitotic kinesin inhibitors [21] but it is found to have neurotoxicity as one of the major side effect. Interaction of aminophylline and topiramate has been studied carefully which revealed that aminophylline containing the dihyroprymidinone nucleus markedly attenuated the anticonvulsant potential of topiramate in the mouse maximal-electroshock-induced seizure model [22]. Seizure prolonging action of aminophylline has also been observed which is mainly attributed by blocking adenosine receptors [23]. However, seizure prolonging action was of aminophylline was antagonized by RO 15-1788, which is partial benzodiazepine agonist [23,24]. Interaction of 5-flourouracil with misonidazole has revealed that the clearance of the former was significantly reduced. Misonidazole is a radio-sensitiser of hypoxic cells which has been shown to enhance antitumor activity of several chemotherapy drugs [25].

2. Synthetic strategies

Research and development in the past years have effectively accomplished the purpose of introduction of various synthetic strategies. Numerous synthetic strategies have been outlined for the synthesis of DHPMs illustrated in Scheme 1. In 1893, Pietro Bignelli's had reported the synthesis of dihydropyrimidine (monastrol) 4 by condensation of ethyl acetoacetate 3, 3-hydroxybenzaldehyde 1 and thiourea 2 under slightly acidic condition using concentrated hydrochloric acid as catalyst in appropriate solvent such as ethanol [26]. Matthews et al. treated β-keto ester 5 with aldehyde 1 and urea 2 for the synthesis of DHPMs 6 [27]. In 2004, Holla et al. published one pot synthesis of thiazolo-dihydropyrimidinones 8 by condensing benzaldehyde1 with 2,4-dichloro-5-fluoroacetophenones 7 and thiourea 2 in the presence of sodium hydroxide and ethanolic potassium hydroxide under the Claisen-Schmidt reaction conditions [28]. Yadlapalli et al. were synthesized DHPMs with the help of 1-(piperidin-1-yl)butane-1,3-dione 9, benzaldehyde 1, an excess of thiourea 2 in ethanol under mild acidic condition provided the expected DHPMs in good to excellent yield [29]. In 2010, Shaabni et al. described the synthesis of a new class of 3,4-dihydropyrimidine-2(1H) one derivatives in a one-pot process by a four-component condensation reaction of an aliphatic or aromatic amine 11, diketene 12, an aromatic aldehyde 1 and urea/thiourea 2 in the presence of p-toluenesulfonic acid (p-TsOH.H$_2$O) as a catalyst in dichloromethane at ambient temperature. Since the number of possible combinations in four-component reactions is greater than the three-component reactions, the diversity of Biginelli reaction is more explored under four-component reaction strategy [30].
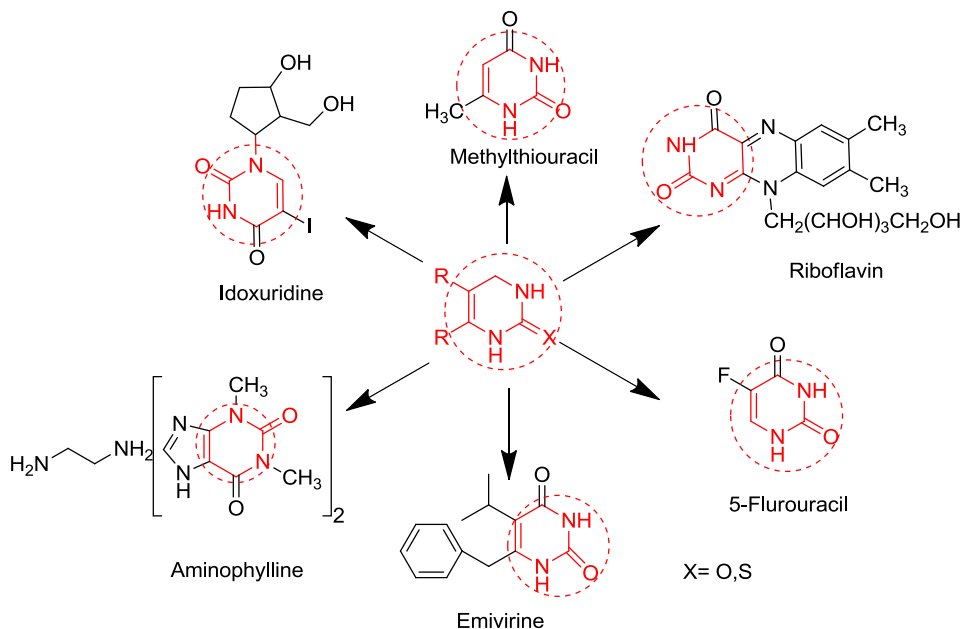
Fig. 1. Dihydropyrimidinone skeleton containing drugs.
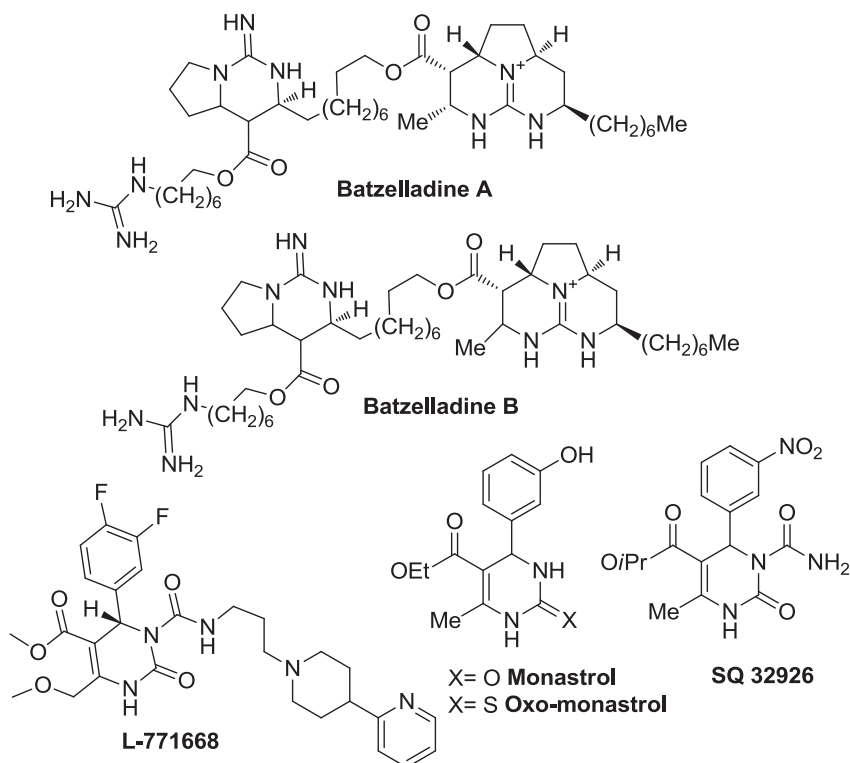
Fig. 2. DHPM containing various natural and synthetic analogs.

In 2000, Kappe had developed alternative synthetic strategy for the synthesis of DHPMs apart from the traditional Biginelli condensation. The enone **14** was condensed with protected urea or thiourea derivative **15** under the neutral conditions. Deprotection of intermediate with hydrochloric acid has led to the emergence of dihydropyrimidines **18** [31]. Sondhi et al. synthesized 2-thiopyrimidine derivatives with 3-isothiocyanatobutanal **17** with commericially available functionalized amines **16** in absolute methanol for the synthesis of **19**. Kappe and Sondhi's reaction conditions for DHPMs synthesis are illustrated in Scheme 2.

Various efficient methods on the synthesis of dihydropyrimidines have been developed in the past decade which is very well described in Scheme 1 and 2. But still, various recent alternative approaches have been reported for the synthesis of this attractive molecule as described in Scheme 3. Multi-component reactions have been very successful in generating complex molecules in a
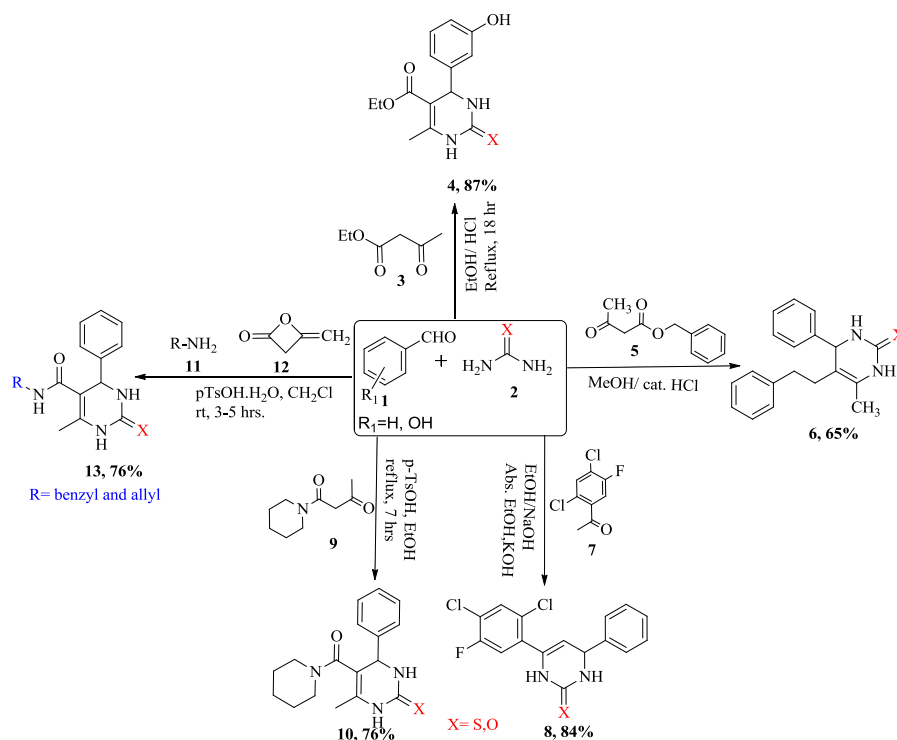
Scheme 1. Various approaches for synthesis of 3,4-dihydropyrimidinones.
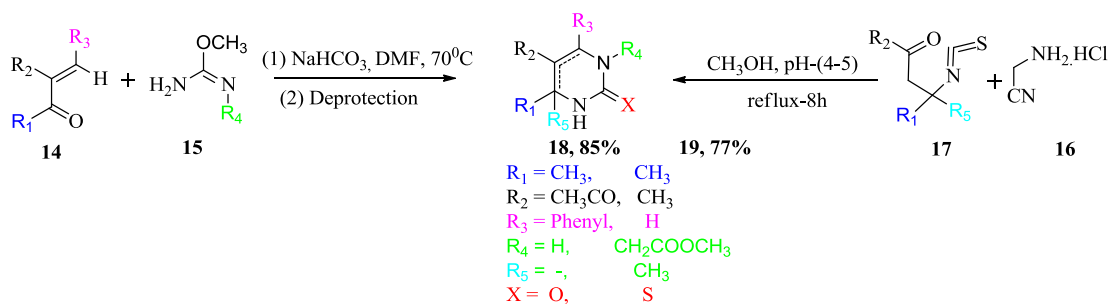
Scheme 2. Kappe and Sondhi's strategies for synthesis of 3,4-dihydropyrimidine.

single and simple synthetic procedure [32,33]. In 2005, Gong et al. reported the use of β-cyclodextrin-propyl sulfonic acid (2 mol %) as a catalyst in the synthesis of 3,4-dihydropyrimidinones **20** through a one-pot-multicomponent reaction of benzaldehydes **1**, urea/thiourea **2** and ethyl acetoacetate **3** under solvent-free conditions at 80 °C [2]. Cyclodextrins are macrocyclic oligosaccharides, possessing hydrophobic cavities which bind to the substrate *via* noncovalent interactions. This property of cyclodextrin is utilized in various applications [33,34]. Benazzouz et al. described a simple and efficient synthetic startegy for a new series of 4-aryl-6-methyl-5-(2-oxo-2*H*chromene-3-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-ones/thiones **22** or **23** [35]. The reaction was initiated using 3-(acetoacetyl) coumarin derivatives as key synthon **21**, *para*-substituted benzaldehyde **1** and urea/thiourea **3** in the presence of sulfuric acid, acting as a catalyst, under refluxing acetonitrile.

In 2016, Saher et al. synsesized 3,4-dihydropyrimidones using Keggin and Dawson type polyoxometalates as an acid catalysts. The keggin type polyoxometalates have acidic and oxidizing properties which are mainly dependent upon the nature of components and composition that can be easily modified according to the reaction needs. The Bignelli reaction was carried out using two solvent EtOH

(polar protic) and MeCN (polar aprotic) under reflux conditions. The condensation of ethyl/methyl acetoacetate **3**, benzaldehyde **1**, and urea **2** in the presence of $H_4SiMo_{12}O_4$ as catalyst, using ethanol and MeCN as solvent for 1.5 h afforded 3,4-dihydropyrimidin-2(1*H*)-ones **20** in a moderate to good yields (52–82%) [36]. In 2015, Treptow et al. reported the fatty acid synthesis of 3,4-dihydropyrimidinones **20** by utilizing the bignelli multicomponent reaction. The reaction was carried out by reacting aromatic aldehyde **1**, urea or thiourea **2**, β-ketoesters **3**, in the presence of catalytic amount of $InCl_3$ (10mol %) using acetonitrile as solvent, thereby affording DHPM-fatty acid synthesis in good to excellent yields [37]. Qiu et al. described cerium (III) trislaurylsulfonate $(Ce(LS)_3)$ as an efficient, stable catalyst which is a combination of lewis acid with surfactant. This catalyst is being ultilised to catalyse one-pot bignelli as well as solvent free esterification reactions. 3,4-dihyropyrimidinone were obtained in 83% yield by employing a reaction of benzaldehydes **1**, ethylacetoacetate **3**, and urea **2** in presence of catalytic amount of $Ce(C_{12}H_{25}SO_3)_3$, and one drop of conc. HCl in ethanol at 80 °C for 6 h as described in Scheme 3 [38].

In 2014, Kour et al., reported the use of green heterogeneous
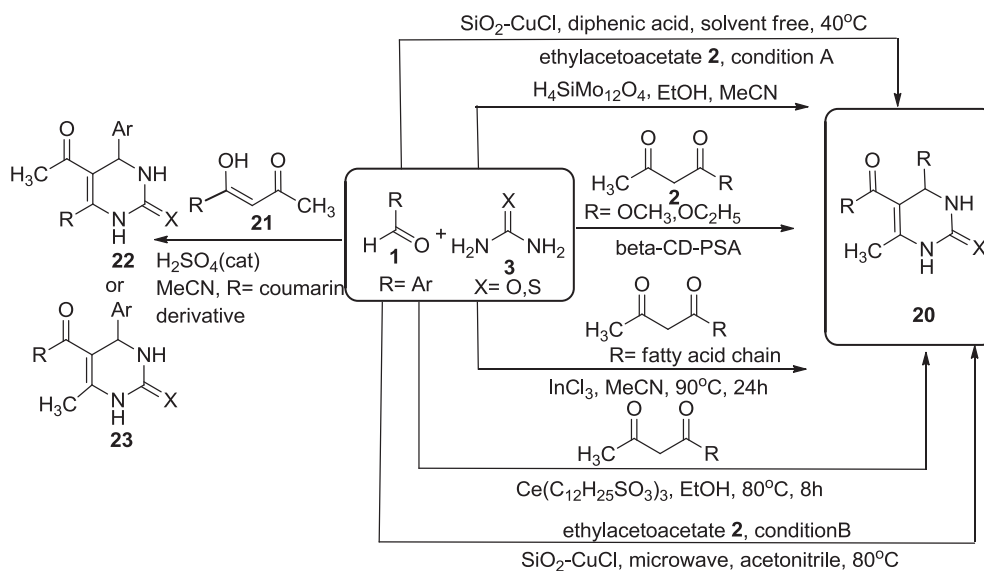
Scheme 3. Alternative synthetic approaches utilized for the synthesis of 3,4-dihydropyrmidin-2(1H)-ones.

catalyst, SiO$_2$-CuCl$_2$, silica supported copper (II) chloride for the one-pot synthesis of DHPM under microwave and thermal conditions. The reaction was carried out with similar substrates, as benzaldehyde **1**, urea **2**, and ethylacetoacetate **3**. A comparative study was undertaken using solvent-free conditions at 40 °C using diphenic acid under thermal conditions (condition A) and under microwave exposure at 80 °C in acetonitrile without the use of diphenic acid (condition B) as described in Scheme 3. Diphenic acid is used to provide polar conditions to the reaction and to make the carbonyl group of aldehyde more polar for the nucleophile-electrophile attack. It was found that reaction carried out in microwave radiation was fast, clean, and produce excellent yields. The structure-activity relationship study has also been conducted for both the conditions and it was found that in case of aromatic aldehyde substituted with electron-donating groups afforded 80–85% yield. While aromatic aldehydes substituted with electron-withdrawing group afforded 80–95% yield and heteroaromatic aldehydes end up with 90–95% yields. In case of aliphatic aldehyde, like formaldehyde produce a yield of 92% whereas acetaldehyde and butanal afforded the product in trace amounts [39].

Many synthetic approaches have been conducted under solvent-free conditions for the synthesis of 3,4-dihyrdopyrimidin-2(1H)-one using Bignelli reaction conditions as described in Scheme 4. The nature of solvent play a crucial role in the reaction, as higher values of dielectric constant induces higher reaction yields. In 2011, Kumaran et al. reported an efficient synthesis of 3,4-dihydropyrimidinone **20** and corresponding thione with 98% yield using lanthanium oxide (La$_2$O$_3$) as catalyst from aromatic

aldehydes **1**, urea/thiourea **2**, and β-ketoesters **3** without using any solvent under the effect of microwave radiation at 320 W for 20–60 s [40]. Slimi et al. synthesized 3,4-dihydropyrimidinone **20** or thione and their derivatives via bignelli reaction involving aromatic aldehydes **1**, urea/thiourea **2**, and β-ketoesters **3** in the presence of bismuth nitrate in acetonitrile or PPh$_3$ without solvent at room temperature [41]. Good yields were obtained when the reaction was carried out using aliphatic aldehydes and bismuth nitrate as catalyst. However, when bignelli reaction was carried out using bismuth nitrate in acetonitrile under the effect of microwave radiation in the absence of solvent, product was obtained in quanitative yield [41,42]. In 2011, Mansoor et al. described the synthesis of DHPM using triphenylphoshphine (10 mol%) as catalyst under solvent free conditions at 100 °C for 8 h from the use of similar three component substrates aromatic aldehydes **1**, urea/thiourea **2**, and β-ketoesters **3** [43]. Triphenyphosphine act as a lewis base by interacting with the electrophilic carbon of the aldehyde. The enolate of β-ketoester is formed by coordinating the aldehyde with PPh$_3$, which helps in the deprotonation of β-ketoester. In 2014, Attri et al. utilized triethylammoniumacetae (TEAA) as a catalyst and reaction medium for the synthesis of 3,4-dihydropyrimidinone **20** and its derivatives under solvent free conditions using aldehydes **1**, urea/thiourea **2**, and β-ketoesters **3** as subtrates [44]. The reaction yield was obtained upto 92%. TEAA is relatively inexpensive, thermostable, non-toxic, recyclable and has vast applications as catalyst and reaction medium.

Apart from the classical methods of bignelli reaction, focus has also been shifted to the catalytic asymmetric bignelli reaction in
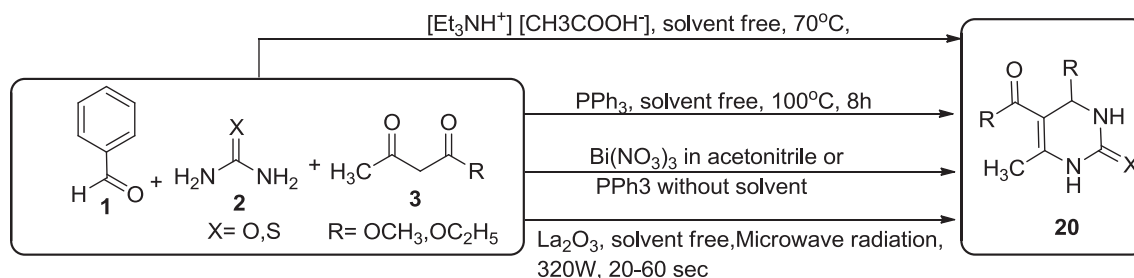
Scheme 4. Synthesis of 3,4-dihydropyrimidin-2(1H)-ones using different catalyst under solvent free conditions.

order to obtain enantiomerically pure DHPM [45]. It has been subdivided in two categories: metal-catalyzed enantioselective bignelli reaction and organocatalytic enantioselective bignelli reaction. In case of metal-catalyzed reaction, Muñoz-Muñiz and Juaristi reported enantioselective synthesis of DHPM **20**, by utilsing $CeCl_3$ or $InCl_3$-catalysed bignelli reaction in the presence of chiral ligand. Different chiral ligands were employed with $CeCl_3$ or $InCl_3$ to achieve the best enantioselective synthesis. It was found that ligand (*R,R*)-**24** showed best results in inducing enantioselectivity whereas other ligands as benzylamine (*S*)-**25** did not revealed any stereoselective synthesis because it directly incorporates to the aldehyde component. When the reaction occurred between the intermediate **26** and ethylacetoacetate **3** at −78 °C to 0 °C, highest enantioselectivity was obtained upto 40% ee as described in Scheme 5 [45,46]. In 2006, Chen et al. and co workers were able to synthesize a series of chiral-phosphoric acids which act as catalyst for the enantioselective synthesis of bignelli reaction. Chiral phosphoric acid **27**, termed as H_8-bino-based phosphoric acid was found to be effective in providing product **20** in yield upto 40–86% and 88–97% enantioselectivity was observed using three-component substrate as aldehydes **1**, urea/thiourea **2**, and β-ketoesters **3** [45,47].

Thorat and co-workers in 2014, have reported the metal free *O*-arylation of 4-aryl-6-methyl-pyrimidin-2(1*H*)-one sacffold of bignelli type to produce 2-aryloxy pyrimidine derivatives in appropriate yields using diaryliodonium salts as catalyst under metal free conditions [48]. The *O*-arylation of 4-aryl-6-methyl-pyrimidin-2(1*H*)-one **20** was carried out in presence of diphenyliodonium triflate **28** with K_2CO_3 as base in the presence of toluene and afforded **29** in 65% yield as described in Scheme 6.

Karami et al. described the one-pot reaction of aryl gloxals **30**, urea **2** and ethylacetoacetate **3** using molybdate sulfuric acid as catalyst to furnish novel 5-acetyl-4-(aryloyl)-3,4-dihydropyrimidinones **31** in 70–85% yield under solvent free conditions [49]. The reaction is in accordance with green chemistry

principles and **31** undergoes knorr-condensation with hydrazines to produce pyrimido[4,5-*d*]pyrazines. In 2014, Ábrányi-Balogh et al. have published 1-aryl-β-carboline-3-carbaldehydes **32** as versatile building blocks and is found to be useful starting material for bignelli type reaction [50]. Compound **32** was successfully reacted with urea **2** and four different β-dioxocompounds [pentane-2,4-dione **3a**, ethyl acetoacetate **3b**, ethyl isobutyrylacetate **3c**, and ethyl benzoylacetate **3d**]. The reaction was catalyzed with $NiCl_2.6H_2O$ with ethanol containing catalytic amount of conc. HCl as solvent afforded the product as 2-oxo-1,2,3,4-tetrahydropyrimidine derivatives **33** in 36–90% yield as described in Scheme 7.

3. Biological activities

The biological investigation of dihydropyrimidines involve various mechanism like enzymatic action, receptor mediated mechanism and activity against the ion channel etc. The biological investigations have revealed that substitution of various groups on the ring imparts different activity. An outline of different activities of DHPMs is shown in Fig. 3.

3.1. Anti-inflammatory activity

In 2004, Kumar et al. synthesize and evaluated novel [4,6-(4-substituted aryl)-2-thioxo-1,2,3,4-tetrahydroyrimidine-5-yl]-acetic acid derivatives as potential anti-inflammatory agents. The anti-inflammatory activity of all the compounds had been recorded on the basis of reference standard drug diclofenac sodium. All the compounds showed tendency to prevent edema and showed anti-inflammatory activity. The compounds are tested for the anti-inflammatory activity according to the method of Winter et al. [51] in albino rats employing the carageenan induced rat paw edema test. Percentage reduction in the inflammation after 3 h of administration of carageenan was recorded and test compounds
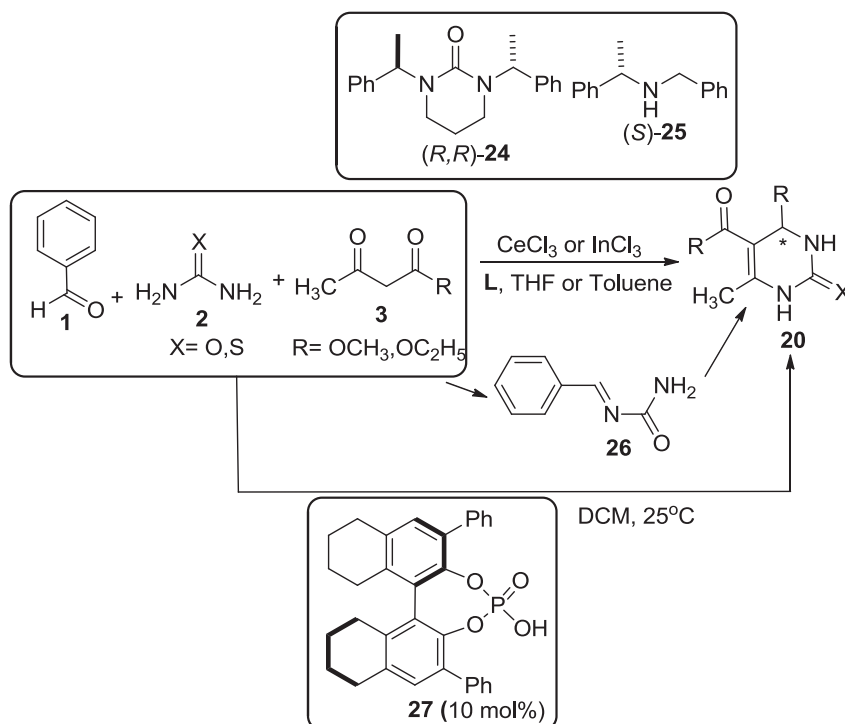
Scheme 5. Enantioselective synthesis of 3,4-dihydropyrimidinones using chiral ligands.
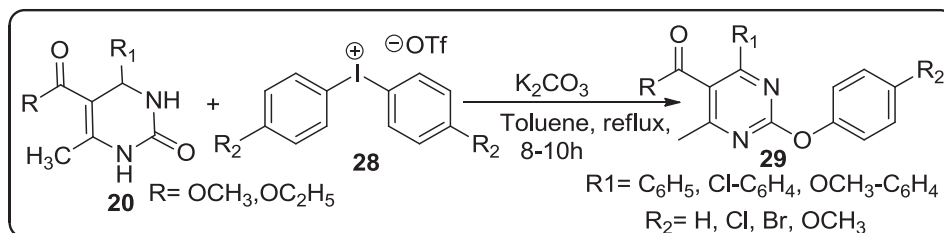
Scheme 6. O-arylation of 4-aryl-6-methyl-pyrimidin-2(1H)-one.

R1= Me, OMe, OEt
R2= Me, i-Pr, Ph; Ar= Ph, 4-MeO-C6H4 , 4-Cl-C6H4, 4-NO2-C6H4
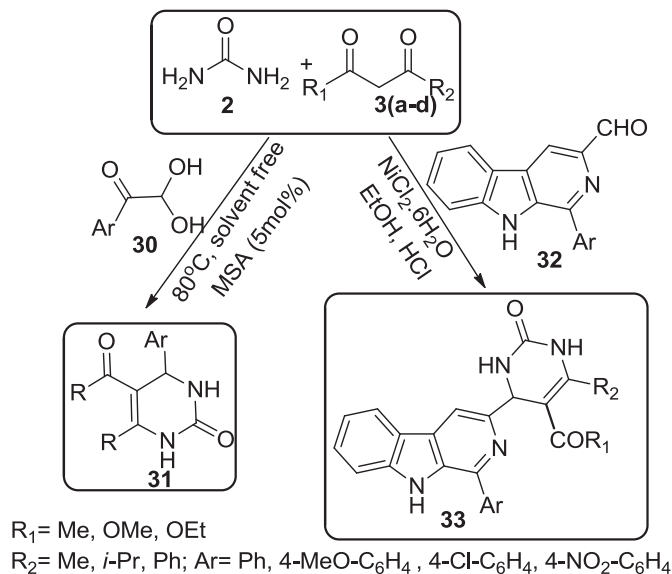
Scheme 7. Synthesis of 3,4-dihyropyrimidinone derivatives.

were compared with that of the animals administrated with carangeenan using the reference standard diclofenac sodium. They synthesized three series of molecules with different functional groups which are mentioned by R & R1 in Fig. 4. We observed different molecules which have shown anti-inflammatory activity. Compounds **36, 40 & 44** were found the most potent among the all molecules. The presence of 4-methoxy group at C-4 plays an important role in the activity of compounds. It was observed that 4-methoxy phenyl increases the activity of the compounds. On the other hand, C-6 phenyl group reduces the activity of the compounds but when the phenyl group was replaced by the p-chlorophenyl at C-6 & 4-methoxy phenyl at C-4 then the activity of the compound was found to be increased. When the methoxy group at C-4 was replaced by any other group then the activity decreased [52]. The structure and inhibitory percentage 50 value (IP$_{50}$) of the compound are given in Fig. 4.

3.2. Anti-HIV activity

In 2015, Sari et al. synthesized dihydropyrimidine α,γ-diketobutanoic acid derivatives to tartgeting HIV integrase (3′-P and ST). They synthesized two series of new compounds. The compounds are shown in Fig. 5. They synthesized twenty new molecules and
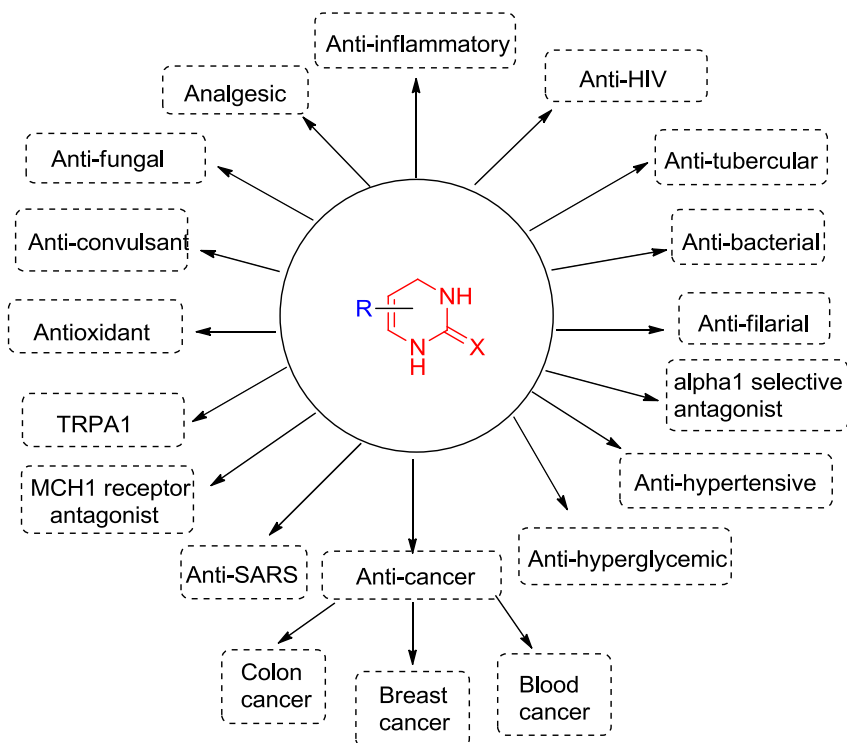

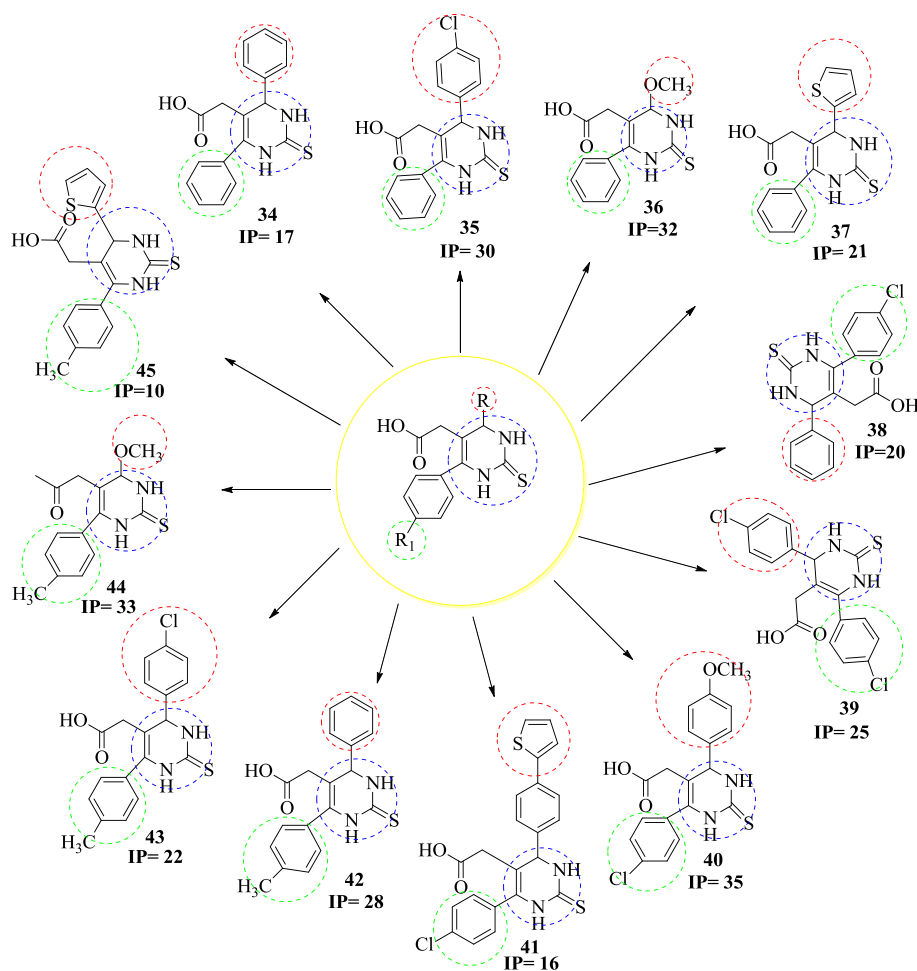**Fig. 3.** Biological activities of dihydropyrimidines.

Fig. 4. The structure of potent DHPMs having anti-inflammatory activity.

evaluated for their enzymatic activity against HIV integrase [11]. Compound **46, 48, 53** & **55** inhibited strand transfer and submicromolar activities. They observed that C-6 substitution play an important role in the potency of the compounds. When the R_2 was substituted with methyl or ethyl groups, an enhancement of activity was observed. On the other hand, when R_2 was substituted with phenyl or isopropyl groups, a decremental effect in activity was observed. This proves that steric hindrance at C-6 was not tolerated. The structures of the potent compounds are given in Figs. 5 and 6 and their IC_{50} values are shown in Table 1, respectively. The different substitution R_1, R_2 and R_3 are differentiated by the help of different color.

3.3. Antitubercular activity

In 2010, Trivedi et al. synthesized novel dihydropyrimidines as a potential new class of antitubercular agents. They synthesized thirty new molecules by the multi-component Biginelli reaction. Initially nine compounds were screened with the help of *Mycobacterium tuberculosis* strain. They all were inhibited MTB upto 90−100%. In the secondary level, two compounds (**58** and **59**) inhibited MTB with MIC of 1 μg/mL and three compounds (**61, 65** & **66**) with MIC of 2 μg/mL. Among all the compounds, compounds **58** & **59** were found to be most potent compounds (MIC of 0.02 μg/mL and SI > 500) of the series. The compounds were found to be much better than existing drug INH (MIC: 0.03 μg/mL) in the *in vitro* studies. The compounds [53] gave a direction to develop an

excellent lead as novel antitubercular active molecules. At fourth position of C-3 phenyl ring of pyrazolyl play an important role in the activity of the newly developed molecules. At C-3 position of phenyl ring of pyrazolyl substituted with the different electronegative element instead of methyl group, the compounds found to be more active [12]. The structures of potent compounds for tuberculosis are shown in Fig. 7 and their summarized data is given in Table 2, respectively.

3.4. Antifungal activity

In 2012, Aly and Kamal developed a novel fused chromeno [2,3-*d*] pyrimidine and pyrano [2,3-*d*] pyrimidine derivatives. Screening of newly prepared compounds was done separately *in vitro* for their antifungal activity. The compounds were tested against the two fungal species, namely, Fungus, *Aspergillus flavus* and one yeast fungus *Candida albicans* on sabouraund dextrose agar plates. The antifungal activity measured by agar well diffusion method. Nine compounds were tested for their antifungal activity. The test was performed three times for each fungus. The tested compounds were compared with standard Amphotericin B to evaluate their potency. Zone of inhibition were determined for synthesized compounds and the result were summarized in Table 3 [53]. From the results obtained it have been concluded that the compound **72** was more potent than the standard drug Amphotericin B against the *Aspergillus flavus* fungus. On the other hand, it was found to be inactive against the *Candida albicans* fungus. The structures of
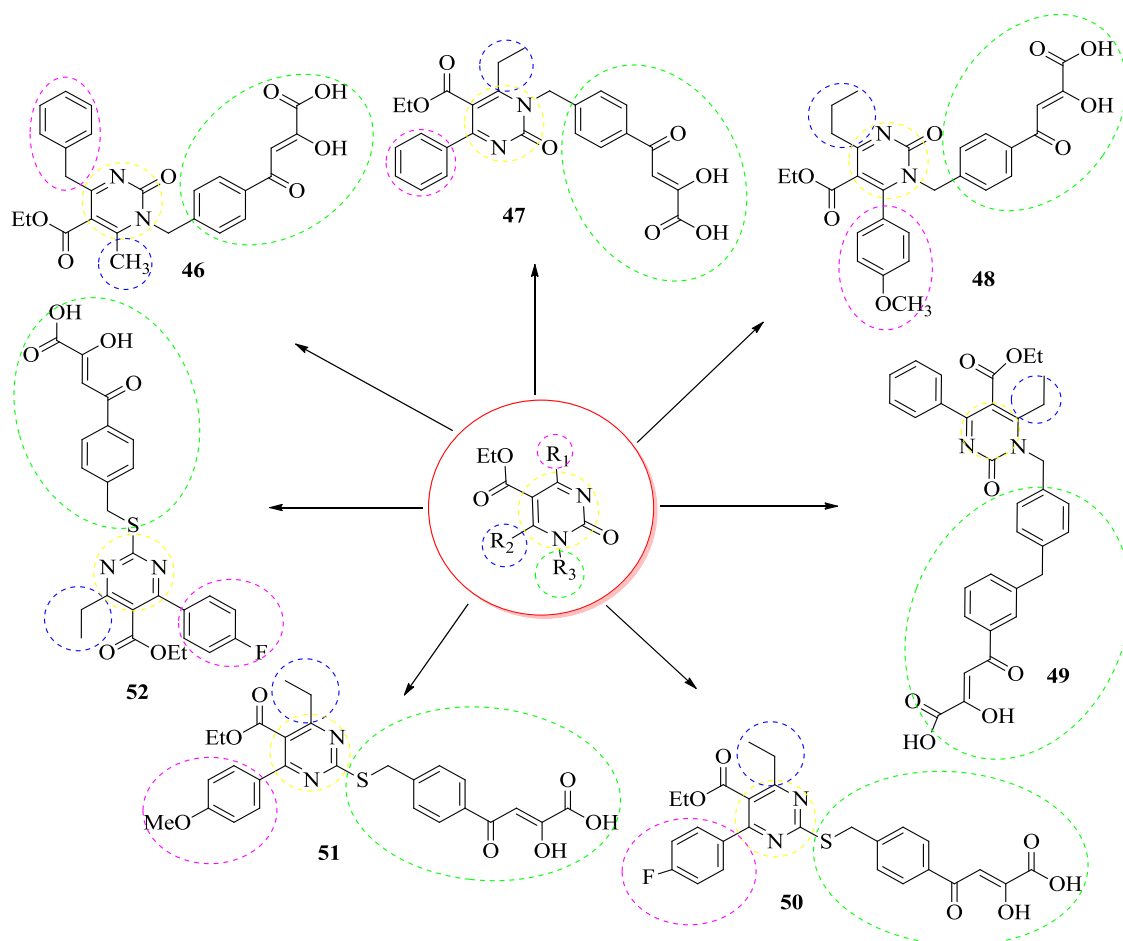
Fig. 5. The structure of first generation anti-HIV compounds.

3.5. Antibacterial activity

Rajanarendar et al. synthesized the antibacterial novel piperazine and morpholine linked substituted pyrimidine derivatives as antimicrobial agents. The newly synthesized compounds were estimated for *in vitro* antibacterial against the different types of Gram-positive, Gram-negative bacterial using broth dilution method. The results are shown in Table 4. Ciprofloxacin was used as standard drug for comparison. The bacterial strains used in the present study are *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 511), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656). The results showed that compounds have average to good antibacterial activity and are more active than standard drug Ciprofloxacin. The activity was shown in terms of minimum inhibitory concentration (MIC). The SAR study disclosed that when the benzene ring is substituted by the electron withdrawing group like chloro and bromo it enhances the activity of compounds. Compound **76, 77,** and **78** were found to be most potent compounds. They exhibit the same antibacterial activity compared to standard drug ciprofloxacin [54]. The structures of potent compounds are shown in Fig. 9 and their summarized data is given in Table 4 respectively (see Fig. 10).

Attri et al. also reported the antibacterial activity of the synthesized compounds (**84−96**) as shown in Fig. 10. These compounds were evaluated using disk diffusion assay and zone of inhibition was measured [44]. The assay method involves the pouring of nutrient agar (25 mL) into the petri dishes under aseptic conditions in a laminar flow hood. The plates were then kept for solidification, after solidification 100 μL of the fresh culture was spread onto the surface of the solidified medium. The plates were then left for drying in a laminar fashion. After drying, five plain sterile disks were placed in the plate and 5 μL of test solution of different concentrations (*250.000−15.625 ppm) was loaded onto the individual disks. Commercially available ampicillin (10μg/disk) was used in the control plate. The plates were then kept for incubation at 37 °C for 24 h. The plates were then taken out from incubator and zone of inhibition was recorded for all the test compounds and control. The experiments were conducted in triplicate for each treatment against each bacterium.

The MIC (Minimum inhibitory concentration) is defined as the lowest possible concentration of the test compound that is able to inhibit the organism and was measured using micro-dilution assay. It was determined on the basis of the serial dilution method by varying the concentration of test compounds ranging from 200 to 15.625 μg/mL. Requisite concentrations of test compounds were added to all the sterile Erlenmeyer flask, each containing 10 mL nutrient broth and were sonicated for 10 min. The flasks were inoculated with 1 mL of the freshly prepared bacterial suspension (*E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumonia*) in order to maintain the initial bacterial concentration of 103−104 CFU/mL. The flasks
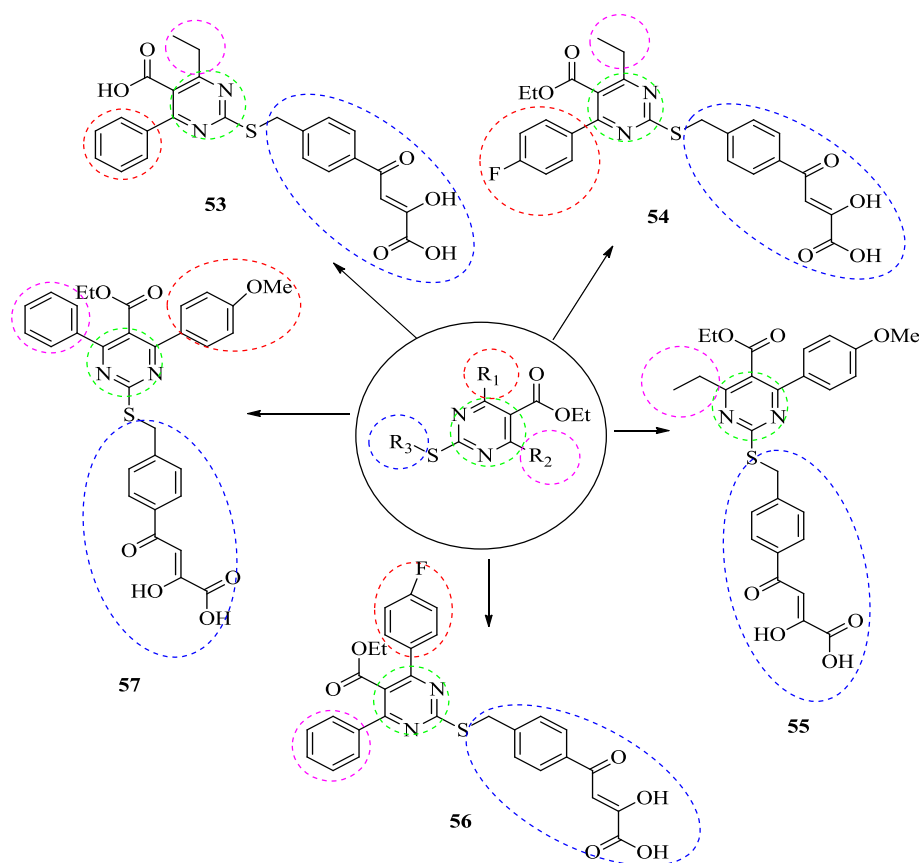
Fig. 6. The structure of second generation Anti-HIV compounds.

Table 1
Inhibition of integrase activities (3′-processing and strand transfer) of dihydropyrimidine α,γ-diketobutanoic acid derivatives.

Compound No.	IC$_{50}$ (μM) $\pm$ S.D.	
	3′- processing	Strand transfer (ST)
46	16.9 ± 3.4	0.19 ± 0.12
47	17.4 ± 2.1	1.3 ± 0.1
48	19.1 ± 4.0	0.64 ± 0.16
49	35 ± 8	1.1 ± 0.4
50	72 ± 4.5	5.6 ± 4.6
51	16.3 ± 3.5	1.3 ± 0.5
52	16.6 ± 2.4	1.3 ± 0.5
53	22.5 ± 2.0	0.85 ± 0.12
54	21.1 ± 1.2	2.23 ± 0.27
55	29.3 ± 3.5	0.92 ± 0.14
56	30.7 ± 2.7	2.27 ± 0.28
57	22 ± 4	2.8 ± 0.9

were then incubated in an orbital shaker at 200 rpm at 37 °C. Bacterial growth was then monitored using spectrophotometer by using an increase in absorbance at 600 nm. The experiments included positive control (flask containing test compound, nutrient media and absence of inoculum) as well as negative control (flask with inoculums, test compound and no test compounds). All the experiments were performed in triplicate.

All the compounds possessed moderate to good inhibitory activity. Compounds **86**, **87**, **88** and **89** were found to have MIC in the range of 31,250−15,625 ppm against *E. coli* which is comparable to the standard. Compounds **87**, **88** and **92** were active against *S. aureus* with MIC values comparable to the standard while the compounds **94**, **95** and **96** were completely inactive with MIC

values of 250 ppm, whereas the remaining compounds were moderately active with MIC value ranging from 62.5 to 125.0 ppm. Compounds **84**, **85**, **86** and **88** showed good antibacterial activity with MIC value in the range of 15,625 to 31,250 ppm, while the compounds **87** and **93** showed moderate activities with MIC value of 62.5 ppm and rest of the compounds were inactive with MIC value of 250 ppm against *P. aeruginosa*. Similarly for *K. pneumonia*, compounds **86**, **87**, **88**, **90**, **91**, **92** and **95** showed moderate antibacterial activities with MIC 31.25−62.50 ppm and the rest of the compounds were found to be inactive. Overall compounds **86**, **87** and **88** showed the good antibacterial activity against all bacteria which is possibly attributed due to the presence of halogen atom in them. While compound **88** was found to be effective against bacteria with lower MIC in comparison to compound **86** and **87**. This is due to the presence of two halogen atoms in compound **88** which tend to enhance the antibacterial activity.

3.6. Antifilarial activity

In 2008, Singh et al. Synthesized 2-sulfanyl-6-methyl-1,4-dihydropyrimidines as a antifilarial agents. All the synthesized compounds were evaluated *in vitro* for their macrofilaricidal activity against *Brugia malayi* according to the method of Murthy and Chatterjee [55]. Micro- and macro-filaricidal activities were evaluated by the method described by Lammler, Wolf, Chatterjee and Gaur [56]. It was concluded that all compounds showed in complete loss of motility of adult worms of *B. malayi* at 100 μM concentrations and they had shown 15.4−68.61% inhibition in MTT reduction assays while compound **103** do not show any inhibition in MTT reduction assay. Compound **98, 101** and **102** were affected
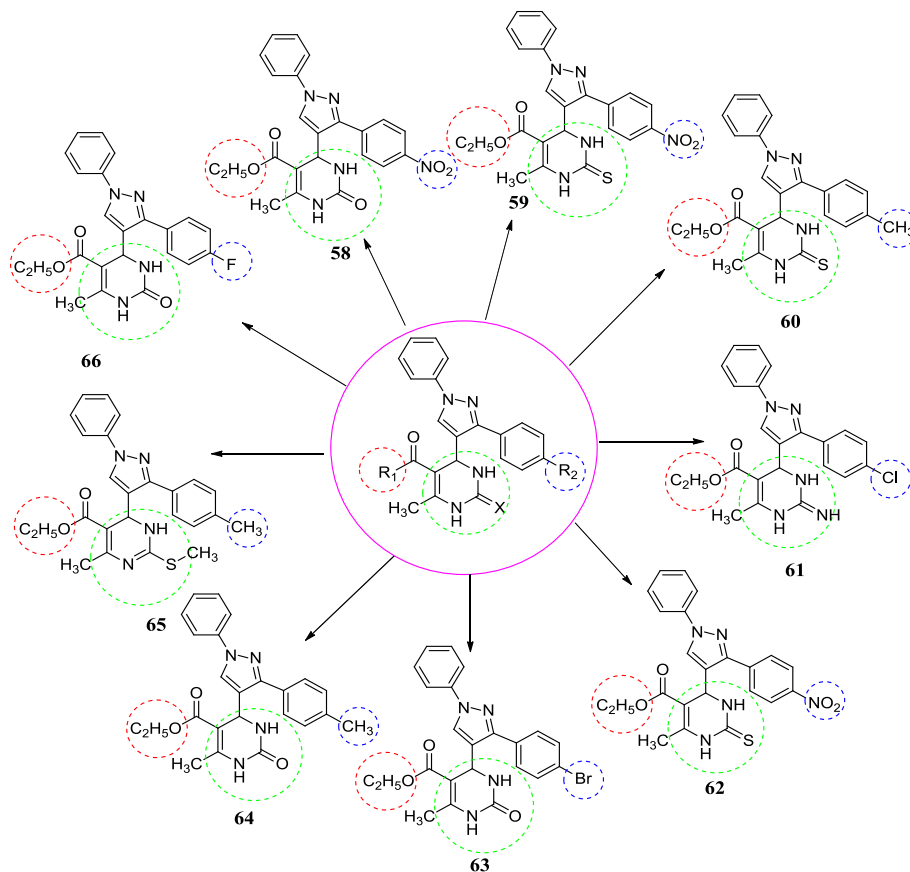
Fig. 7. The structure of potent antitubercular compounds.

Table 2
In vitro antitubercular screening data of dihydropyrimidines.

Compound No.	% Inhibition	MIC µg/mL	S.I.
58	100	0.02	>500
59	100	0.02	>500
60	92	3.13	>3.2
61	96	1.56	5.7
62	94	3.13	>3.2
63	91	3.13	3.0
64	90	6.25	>1.6
65	98	1.56	>6.4
66	97	1.56	4.7
Isoniazid	–	0.03	–

MIC: Microbial inhibitory concentration.

Table 3
Antifungal activity of chemical substances tested.

Compond No.	Inhibition zone diameter(mm)	
	Aspergillusflavus	*Candida albicans*
67	0	0
68	0	10
69	0	13
70	14	14
71	0	12
72	36	0
73	0	10
74	0	12
75	0	11
Amphotericin B	16	19

both motility (irreversible loss) and MTT reduction (50% inhibition or more) and compounds **97, 99, 100, 101** and **103** either affected motility with <50% MTT reduction or only motility. When the compounds were screened at their 50% concentration also show the positive result. Compound **98** at 50 µM concentration resulted in complete loss of motility of filarial worms with no inhibition in MTT reduction assay, while at 25 µM concentration it showed only slack motility of filarial worm and displayed approximately 30% inhibition in MTT reduction assay. Compound **101** on the other hand displayed complete loss of motility in filarial worms at 50 µM concentration with 19% inhibition in MTT reduction assay. The most potent compound of the series was found to be compound **101** which resulted in complete loss of motility at two lower concentrations (50 and 25 µM) screened and good inhibition (around 70%) in MTT reduction assay. SAR study showed that when the 4-aryl-1,4-dihydropyridines substituted by less bulky electron-withdrawing group at the fourth position of phenyl ring (4-F-phenyl) compound **102** was the best compound of the series. Only three compounds **98, 101** and **102** were found to be most potent *in vitro* antiflarial activity [57]. The structures of synthesized compound are shown in Fig. 11. All the data of potent compounds are summarized in Table 5.

3.7. Antihyperglycaemic activity

In 2015, Bhosle et al. synthesized thiazolyl methoxyphenyl pyrimidine and evaluated antihyperglycaemic activity. Anti-hyperglycaemic activity was evaluated in male albino rats of Sprague Dawley strain. Present data showed that in normoglycemic rats, compound exhibit glycaemic control by decreasing the peak
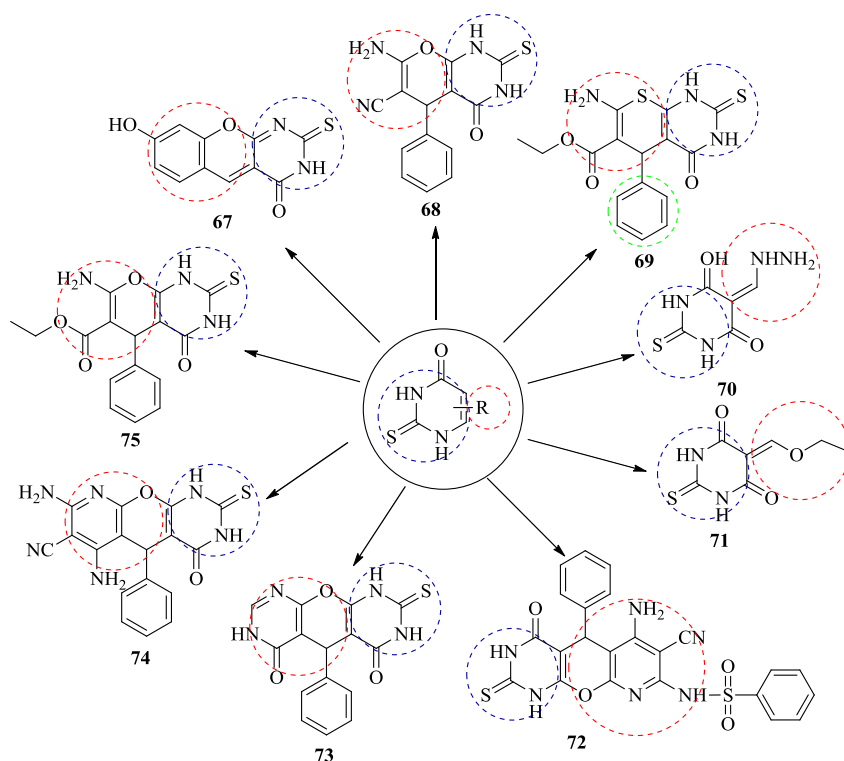
Fig. 8. The structure of potent antifungal compounds.

Table 4
Antibacterial activity data (MIC (in μg/mL) values) of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10b-tetrahydro-1H-pyrimido [5,4-c]quinolin-5-ones.

Compound No.	Gram positive			Gram negative		
	B. subtilis	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C.violaceum*
76	19	15	15	20	13	18
77	16	11	10	10	08	06
78	20	15	10	15	14	18
79	06	08	09	09	07	05
80	22	18	25	20	15	18
81	16	15	15	20	15	16
82	18	18	12	08	13	11
83	08	10	09	05	08	06
Ciprofloxacin	20	20	25	30	25	25

Negative control (acetone)-no activity. Values are indicated in lg/mL.

blood glucose and the area under oral sucrose tolerance (OSTT) curve. After 2 h of sucrose load which was indicative of an enhanced glucose utilization which may be provoke either by insulin production from the pancreatic β cells or by inhibiting the intestinal α-glucosidase enzyme responsible for breakdown polysaccharides into monomeric form and thus inhibit the postprandial hyperglycaemia. Amongst screened compounds **76, 77, 78** and **79** had reduced the blood glucose level in normal rats. The activity of the tested compounds was compared with standard drug metformin. Compounds number **78** and **79** have notably reluctance in the postprandial rise in blood glucose level of sucrose loaded rats. The main role for the activity of these compounds having functional group like nitro, fluro, methoxy on the benzenoid ring may be governing acidic behavior through mesomeric effect of the respective heterocyclic ring, which probably assisting for the activity [7]. The structures of potent compounds are shown in Fig. 12 and their summarized data is given in Table 6 respectively.

3.8. Antihypertensive activity

In 2010, Alam et al. synthesized 1,4-dihydro-5-pyrimidine carboxamides as antihypertensive agents. They synthesized thirty molecules and all the molecules were screened for their antihypertensive activity by using tail-cuff method that measures the systolic blood pressure. The activity of the synthesized compounds was compared with standard drug nifedipine. Out of thirty compounds five compounds found to be most potent molecules. Structure activity relationship (SAR) study reveal that the different substitution at phenyl ring attached to the pyrimidine moiety played an important role in governing the antihypertensive activity. When the electron releasing groups like -OCH₃ were substituted at position 3 and 4. Then there is increase in the antihypertensive activity. Substitutions at other phenyl rings were also important as the substituted benzyl derivatives were more active in the series. The *para* chloro-substituted derivatives were found to be more active than the other halogen. The phenyl ring attached to the amide linkage does not seem to have any significant effect on
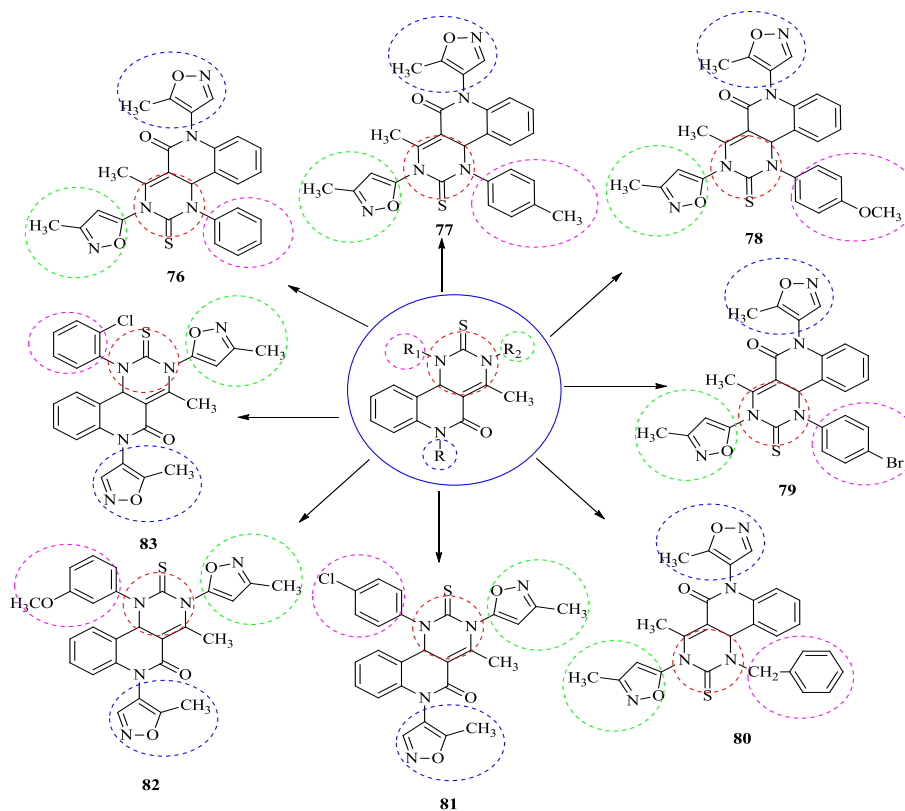
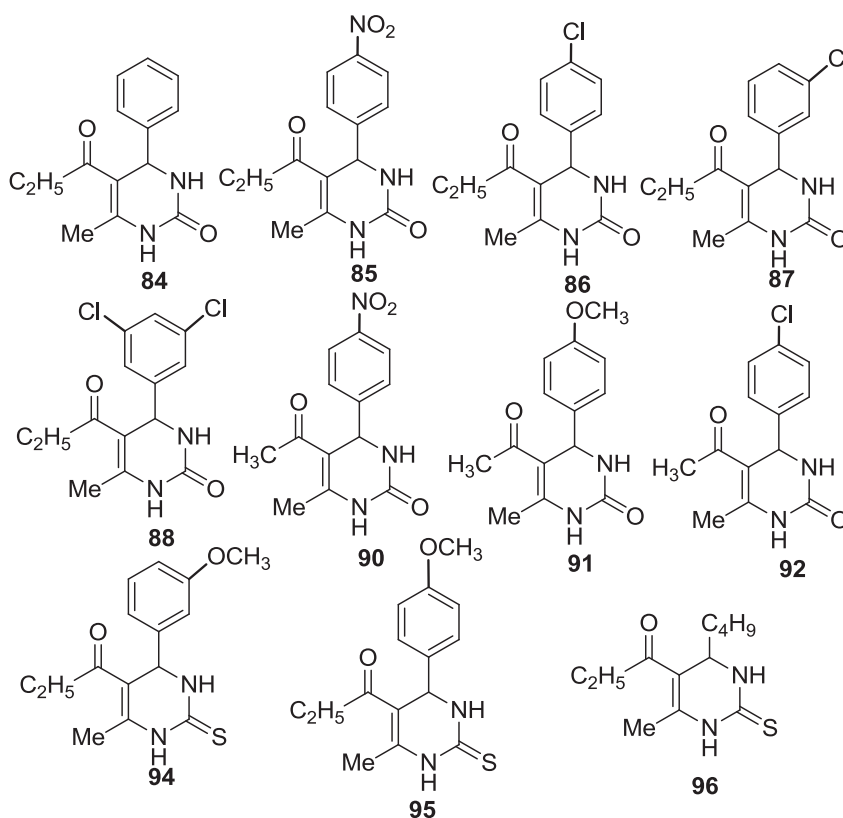**Fig. 9.** The structure of potent anti-bacterial compounds.

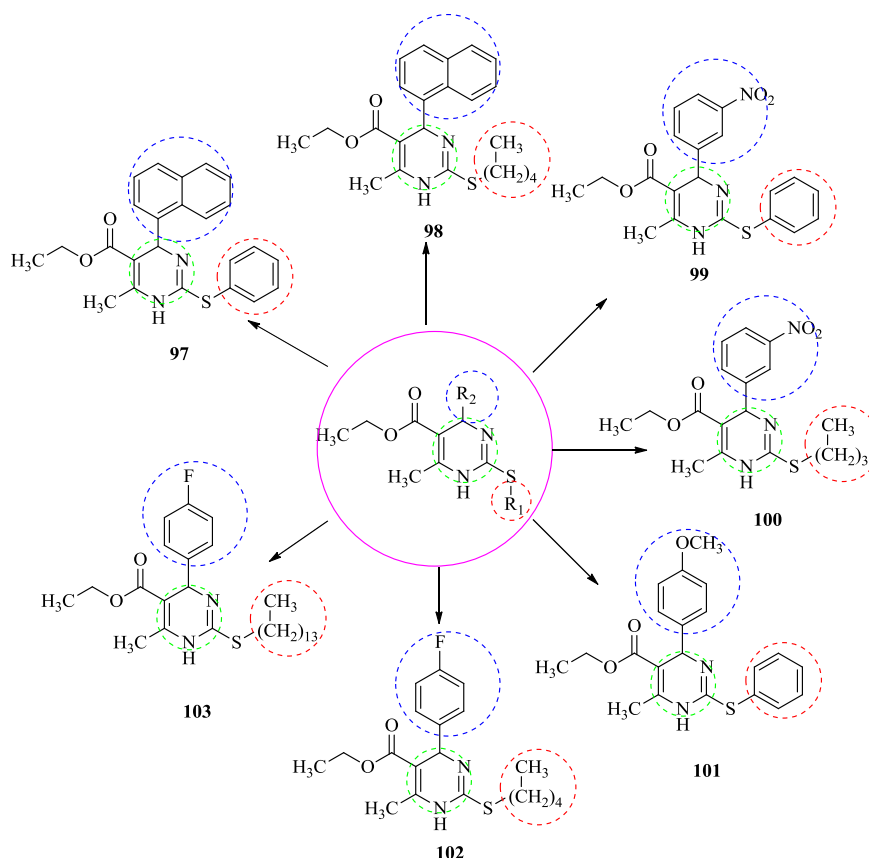**Fig. 10.** The structure of compounds possessing antibacterial activity.

Fig. 11. The structure of potent anti-filarial compounds.

Table 5
In vitro macrofilaricidal activity of 2-sulfanyl-6-methyl-1,4-dihydropyrimidines against *Brugia malayi*.

Compound No.	Concentration (μM)	Motility[a]	Percent inhibition in MTT reduction over control (means ± SD)
97	100	0	18.97 ± 1.77
98	100	0	49.0 ± 11.50
	50	0	NI
99	100	0	43.14 ± 5.11
100	100	0	25.74 ± 3.44
101	100	0	52.11 ± 26.92
	50	0	19.29 ± 9.49
102	100	0	68.61 ± 5.13
	50	0	68.4692
103	100	0	NI
DEC (citrate)	100	3	NI
	50	3	NI

Motility is expressed as highly motile (3), low (2), sluggish (1, irreversible) and dead (0); NI = no inhibition.

antihypertensive activity [58]. The structures of potent molecules are shown in Fig. 13 and their summarized data is given in Table 7.

3.9. Analgesic activity

In 2005, Sondhi et al. synthesized some mono, bi- and tricyclic pyrimidine derivatives and evaluated for the analgesic activity. They synthesized ten molecules and screened by using phenyl quinone writhing assay [59]. Ibuprofen was taken as standard drug for comparison of tested compounds for analgesic activity. The synthesized compounds **113, 114** and **115** exhibited 100, 70 and 75% activity at 100 mg/kg dose respectively [60]. The structure of potent compounds are shown in Fig. 14.

Fig. 12. The structure of potent anti-hyperglycaemic compounds.

Table 6
Effect of compounds and standard antidiabetic drug metformin on oral sucrose tolerance (OSTT) in post sucrose loaded normal rats.

Compound No.	Dose (mg/kg)	%Improvement on OSSTT	Significance
104	100	0.07	–
105	100	1.05	–
106	100	8.22	p<0.05
107	100	7.04	p<0.05
Metformin	100	14.1	p<0.01

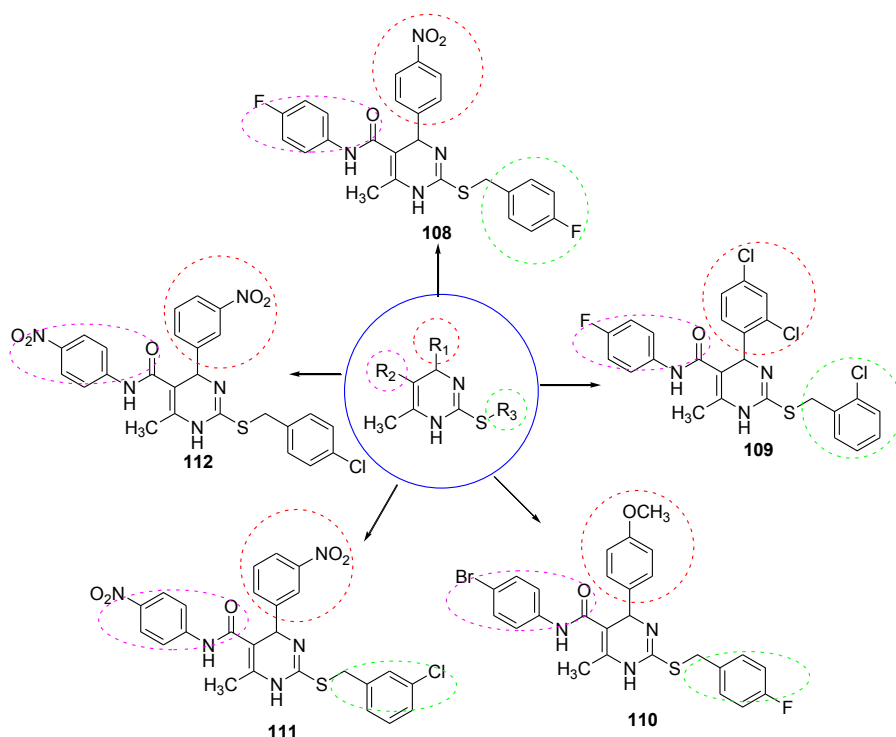**Fig. 13.** The structure of potent antihypertensive compounds.

Table 7
Antihypertensive activity data shown by compounds at 10 mg/kg dose.

Compound No.	Average systolic blood pressure (mmHg) at time (min)											
	0	15	30	60	120	180	240	300	360	600	720	900
108	193 ± 3	189 ± 2	183 ± 1	174 ± 2	161 ± 2	155 ± 1	145 ± 2	136 ± 1	130 ± 2	145 ± 1	156 ± 3	168 ± 4
109	194 ± 1	189 ± 1	183 ± 2	172 ± 1	163 ± 3	151 ± 2	139 ± 1	124 ± 2	120 ± 3	147 ± 2	154 ± 4	163 ± 2
110	193 ± 3	188 ± 1	182 ± 2	178 ± 1	169 ± 2	161 ± 3	152 ± 3	144 ± 2	129 ± 3	148 ± 2	157 ± 1	165 ± 2
111	194 ± 2	190 ± 1	186 ± 2	180 ± 1	174 ± 2	162 ± 1	154 ± 2	145 ± 1	131 ± 2	142 ± 1	160 ± 2	166 ± 3
112	195 ± 1	191 ± 2	188 ± 3	182 ± 3	170 ± 1	165 ± 2	156 ± 2	148 ± 1	130 ± 3	144 ± 1	164 ± 2	171 ± 2
Nifedipine	195 ± 3	192 ± 1	188 ± 2	180 ± 1	172 ± 1	160 ± 2	145 ± 1	132 ± 3	120 ± 2	148 ± 1	158 ± 2	162 ± 1

3.10. Anticonvulsant activity

In 2010, Khanage et al. synthesized and evaluated some new pyrimidine derivatives containing 1,2,4-triazole. The synthesized compounds were screened for their anticonvulsant activity by maximal electroshock seizure method (MES). The albino mice were used in the study. The animals were stimulated by corneal electrodes to 50 mA current at a pulse of 60 Hz applied for 0.2 s. The test drug was given by the intraperitoneally. Destruction of the hind limb tonic extension spasm was recorded as the anticonvulsant activity. The test compounds were suspended in a 0.5% methyl
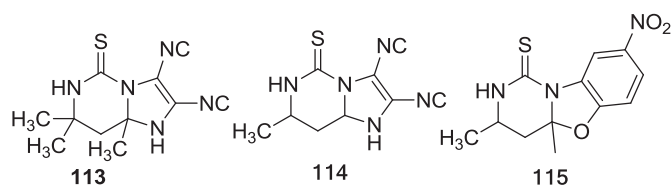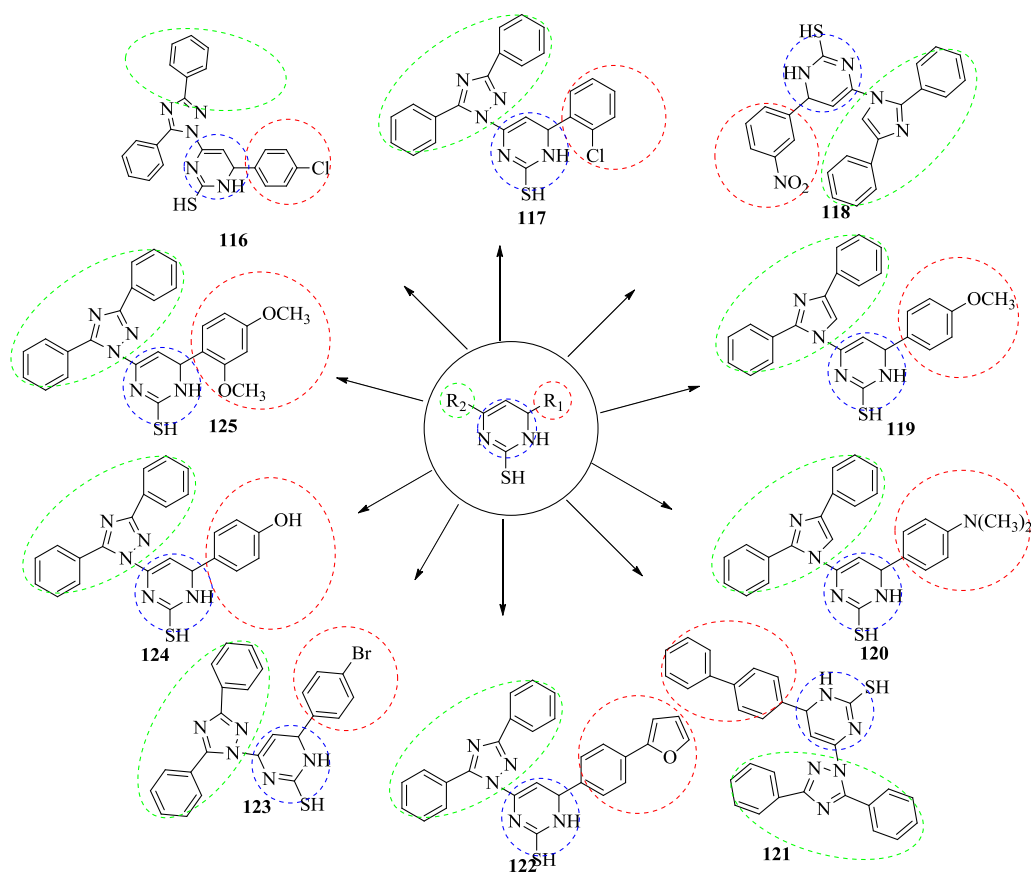

Fig. 14. The structure of potent analgesic compounds.

Table 8
Anticonvulsant screening of compounds **116**–**125**.

Compound No.	MES screen a	
	0.5 h	4 h
116	30	300
117	30	300
118	30	300
119	100	300
120	100	300
121	100	300
122	30	300
123	100	300
124	30	300
125	30	300
Phenytoin	30	30
Carbamazepine		100

Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of mice. The animals were examined 0.5 and 4 h after injections were given.

Fig. 15. The structure of potent anticonvulsant compounds.

cellulose-water mixture. In preparatory screening, each compound was administered through an intraperitoneally injection at three dose levels (30, 100 and 300 mg/kg) and the anticonvulsant activity was assessed after 0.5 h and 4 h intervals of administration. The anticonvulsant efficacy was evaluated by the maximal electroshock-induced seizure (MES) and screening data of compounds **116**–**125** are presented in Table 8 and their structures are given in Fig. 15. All the screened compounds were compared with standard drugs phenytoin and carbamazepine. Compound **116**, **117**,

118, **123** and **125** were found to be most potent molecules among the series. The electron withdrawing groups substituted phenyl ring at sixth position of dihydropyrimidine shows marked increase in their anticonvulsant activity [61].

3.11. Antioxidant activity

In 2009, Kumar et al. synthesized thirty two compounds and all the synthesized molecules were screened for their antioxidant
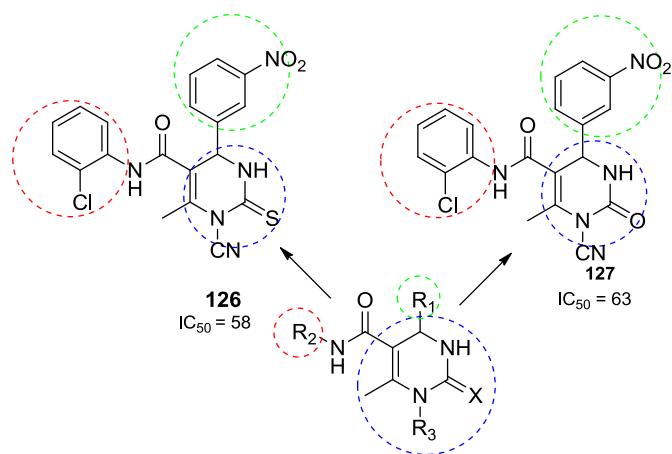
Fig. 16. The structure of potent antioxidant compounds.

dark colored DPPH radical solution in the presence of antioxidant compounds change to yellow colored diphenylpicrylhydrazine, the absorbance of the solution generally decreases. Different concentration of test solutions were prepared such as 100 μg/mL, 50 μg/mL, 25 μg/mL and 12.5 μg/mL and were pipetted to test tube and volume was adjusted to 3 mL using methanol. 1 mL of DPHH solution (0.1 mM) was mixed with 1 mL of the sample and standard separately. The samples were vortexed and incubated in dark for 30 min. Change in absorbance was measured at 517 nm using spectrophotometer. The absorbance was measured and radical scavenging activity was expressed as percent inhibition of DPPH racdical. It was calculated by the use of following equation:

$$\% \text{ inhibition} = (\text{Absorbance of control} - \text{Absorbance of sample}/\text{Absorbance of control}) \times 100$$

A noticeable decline in the concentration of DPPH radical in terms of % inhibition was revealed due to the scavenging ability of test compounds. The percent inhibition of the test compound range from 64.4% to 17.3%. At 100 ppm, the standard gallic acid and quercitin exhibited inhibition percentage of 92.4% and 82.3% respectively, whereas compound **89** was found to show highest percent inhibition of 64.4% in comparison to all the remaining test compounds. Whereas at 12.5 ppm, gallic acid and quercitin possessed inhibition percent of 62.4% and 55.3% respectively, but all the test compounds showed a decreased percent inhibition. Compound **88** was found to posses the lowest inhibition percentage of 17.3%. Thus, finally all the compounds revealed moderate antioxidant activity in comparison to the standard compound. Compound **91** found to be the most promising antioxidant as described in Fig. 17.

CUPRAC assay is most widely used to determine the total antioxidant capacity of the antioxidant compound. The basic priniciple employed in this assay is the redox reduction of Cu (II) to Cu (I) which is monitored by change in absorbance at 450 nm. This redox reaction is a result of the CUPRAC reagent, Cu(II)-neocuproine $(Cu(Nc)_2^{2+})$, with an antioxidant (A-OH), to form the CUPRAC chromophore, Cu(I)-neocuproine $(Cu(Nc)^{2+}$ chelate [44,63]. CUPRAC reagent solution was prepared by mixing 1 mL of 1.0×10^{-2} M copper (II) chloride, 1 mL of 7.5×10^{-3} M neocuprine solution and 1 mL of ammonium acetate buffer at pH 7.0. Sample solution and distilled water were added and mixed to obtain a total volume of 4 mL. The reduction capability is assessed by increased absorbance of the reaction mixture.

The results obtained from CUPRAC assay can easily be extended possibly to *in vivo* reactions of antioxidants. The assay is basically performed at physiological pH closer to 7. The absorbance of Cu (I)-chelate formed as a result of redox reaction with reducing antioxidant was measured at 450 nm. The color is possibly due to the formation of Cu (I)-Nc chelate. The increase in absorption indicates
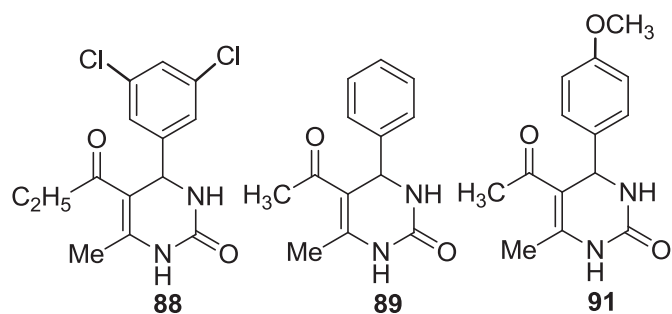

Fig. 17. The structure of potent antioxidant compounds using DPPH assay.

activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Among the thirty two title compounds, compounds **126 & 127** found to be most potent molecules. The 3-nitro phenyl moiety at the fourth position of the dihydropyrimidine showed good antioxidant activity with IC$_{50}$ value 58 and 63 μg concentrations, respectively. The DPPH free radicals formed in this assay will be reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donors of the antioxidant changes to yellow colour. It is a discolouration assay, which is evaluated by the addition of the antioxidant or test compound to a DPPH solution in ethanol and the decrease in absorbance was measured. The assay was carried out in a 96-well microtitre plate. To 200 μl of each of DPPH ethanolic solution, 10 ml of each of the test compound (100 μg) or standard (ascorbic acid, 10 μg) solution was added separately to wells of the microtitreplate. The plates were incubated at 37 °C for 30 min and the absorbance of each solution was measured at 490 nm, using ELISA reader. The IC$_{50}$ values (concentration which inhibits 50% of free radicals) for the compounds **126 and 127** were determined by serial dilution method at the concentrations below 100 μg. The structures of potent compounds (**126 and 127**) are shown in Fig. 16 with their IC$_{50}$ value [62].

Attri et al. and coworkers synthesized 3,4-dihydropyrimidinone derivatives (**84–96**) and were evaluated for their antioxidant and antibacterial activity. The antioxidant activity was determined using DPPH (1,1-dipheny-2-picrylhydrazyl) free radical scavenging and (cupric reducing antioxidant capacity) CUPRAC assays. DPPH assay is basically used to determine the free radical scavenging property of antioxidant compounds [44]. Gallic acid and quercitin were used as standard compounds and the working solutions of test extracts and standards were prepared in methanol. When the
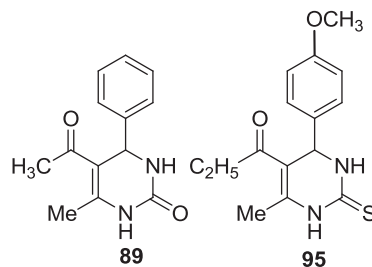

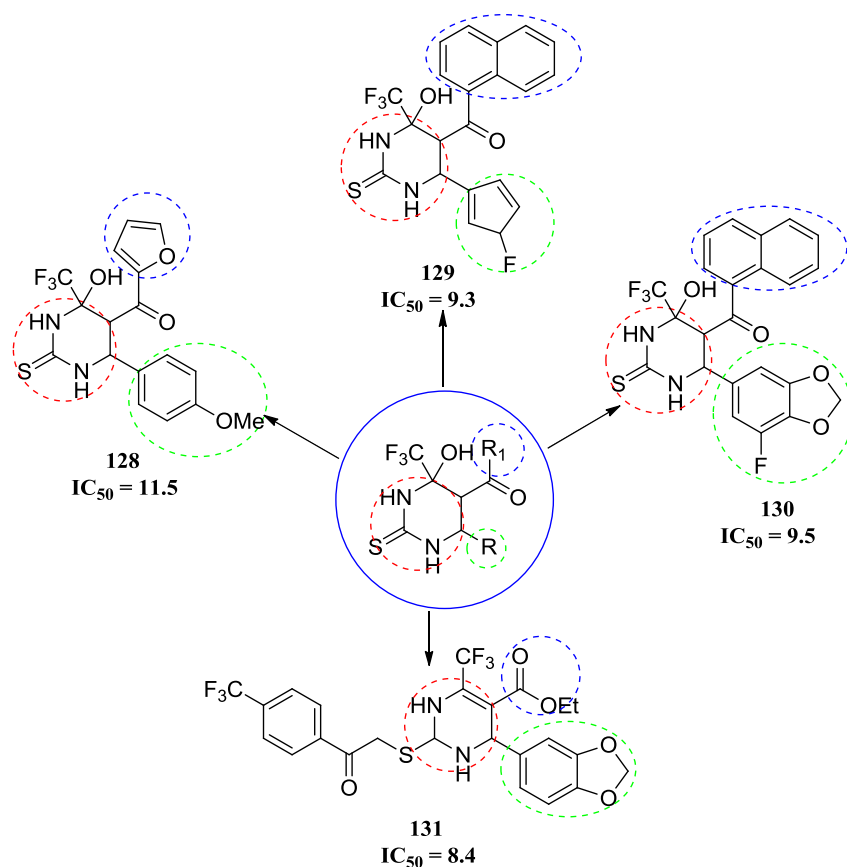Fig. 18. The structure of potent antioxidant compounds using CUPRAC assay.

Fig. 19. The structure of potent compounds against colon cancer.

the higher antioxidant activity. All the compounds show moderate activity in comparison to the standard compounds. All the compounds were found to reduce copper ions from Cu (II) to Cu (I) in a concentration-dependent manner. At 100 ppm, compound **89** was found to have highest absorbance value of 0.87 in comparison to the standard gallic acid and quercitin having absorbance value of 1.4 and 1.2 respectively as described in Fig. 18. Whereas at the lowest concentration 12.5 ppm, absorbance of test compounds range from 0.21 to 0.05 with compound **95** possessing the lowest absorbance value of 0.05. Thus in conclusion, it is stated that at lower concentration compounds do not show antioxidant activity.

3.12. Anticancer activity

The pyrimidines and their derivatives exhibit anticancer activity *via* interaction with different enzymes like tyrosinase, cytochrome P450, glutathione assisted and receptors like somaostatin, estrogen and progesterone receptors.

3.12.1. Colon cancer

In 2011, Agbaje et al. synthesized hexahydropyrimidine derivatives. They synthesized a series of seventeen molecules and the synthesized compounds were screened for the cytotoxicity against the COLO 320 cell line. Among the series of seventeen molecules only four (**128, 129, 130 and 131**) compounds found to be potent analogs. The compound **128, 129** and **130** showed highest activity with IC_{50} values 11.5, 9.3 and 9.9 μM respectively. Compound **130** was found to be most potent molecule with IC_{50} = 8.1 μM. The anticancer studies showed that compounds **128, 129, 130** and **131** represent novel leads for further development [64]. The structures

of potent molecules are shown in Fig. 19 with their IC_{50} values. The different substitutions on the basic ring are differentiated by the different colour.

3.12.2. Breast cancer

In 2012, Yadlapalli et al. synthesized diarylpyrazole ligated dihydropyrimidines and synthesized compounds were screened for *in vitro* anticancer activity against the MCF-7 human breast cancer cell line. They synthesized twenty three compounds, in which six compounds found to be active. The compounds were screened by using SRB assay protocol [65]. All compounds were tested at four dose levels (1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M and 1×10^{-4} M) and each experiment was repeated thrice. Six compounds **132, 133, 134, 135, 136** and **137** were found to inhibit growth of MCF-7 cell line at GI_{50} (concentration of compound causing 50% inhibition of cell growth) exhibited excellent growth inhibition of MCF-7 cell lines. Compound **135** and **136** were found to be the most potent molecules among the six compounds. Compound **136** show the more affinity to inhibition of cell growth with GI_{50} of 33.2 μM and compound **136** also show a good dose response. Structure activity relationship (SAR) study suggests that the compounds containing thio-group are more potent than the compounds containing oxo-group. The SAR study showed that presence of thio-urea functional group in DHPMs enhances the anticancer activity of these types of scaffolds [66]. The structures of potent compound are given in Fig. 20 with their IC_{50} values.

3.12.3. Blood cancer

In 2004, Holla et al. synthesized thiazolodihydropyrimidinones as an anticancer agent. They screened the synthesized compounds
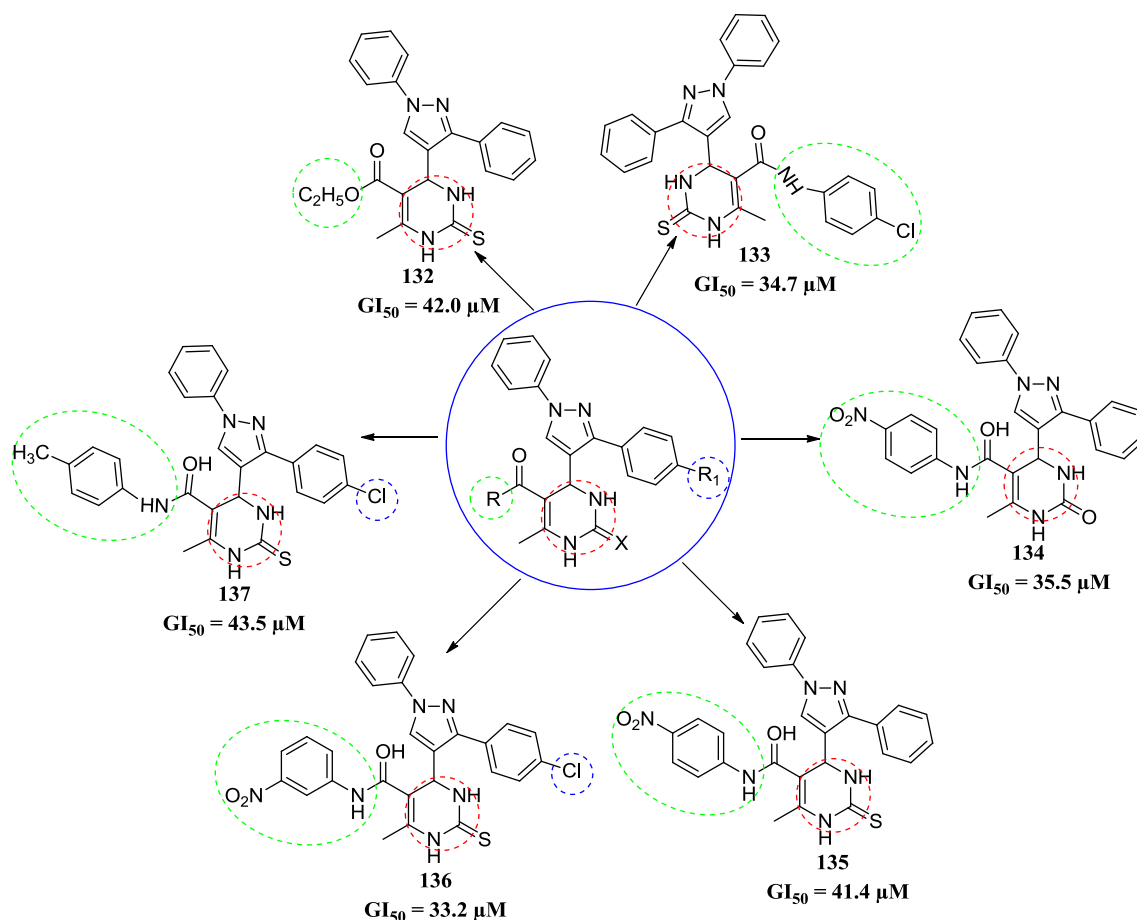
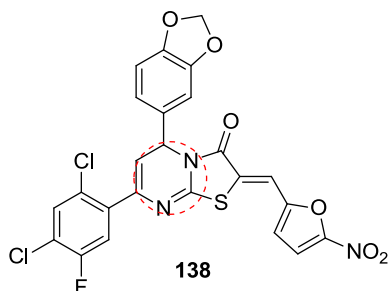Fig. 20. The structure of potent molecules against breast cancer.

Fig. 21. The structure of potent compound for blood cancer.
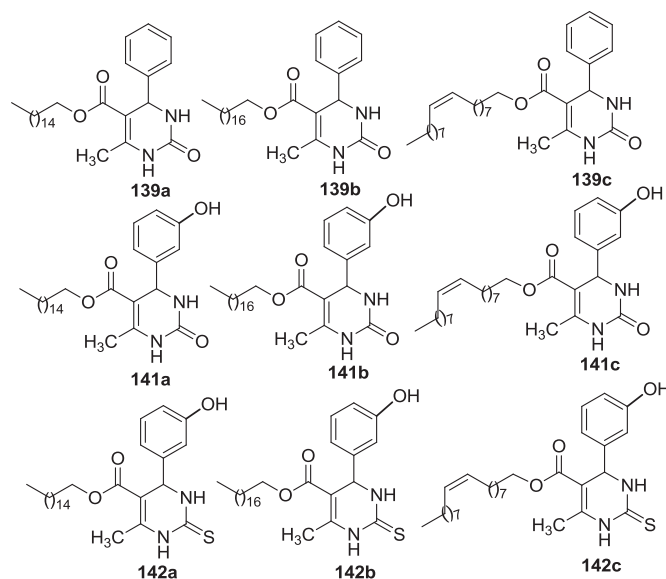
Fig. 22. DHPM fatty acid compounds active against glioma cells.

against the sixty human cancer cell lines. But they found that the synthesized compound show maximum activity against the leukemia cancer cell line. They were used different leukemia cancer cell lines like CCRF-CEM, HL-60[TB], K-562, MOLTA-4, RPMI-8226 and SR. The compound 138 displayed maximum inhibitory action against the SR and CCRF-CEM cancer cell line. Among the synthesized compound only one molecule showed the excellent potency against both cell lines. The Growth inhibition of 50% (GI50) value was found to be 3.34 μM and 3.24 μM respectively. The structure of the potent compound is shown in Fig. 21. The SAR study suggests that the presence of 5-nitro-2-furfuryl moiety and 4-chlorophenyl groups as substituent tend to increase the anticancer activity [28].

3.12.4. Anticancer activity against glioma cells

Treptow et al. have synthesized a new hybrid series of fatty acid

dihyropyrimidinones which were further investigated for antitumor activity against two glioma cell lines (C6-rat and U-138-MG

Fig. 23. The structure of potent TRPA1 antagonists.

Table 9
Anti-TRPA1screening of compound **128**–**139** in human and rat.

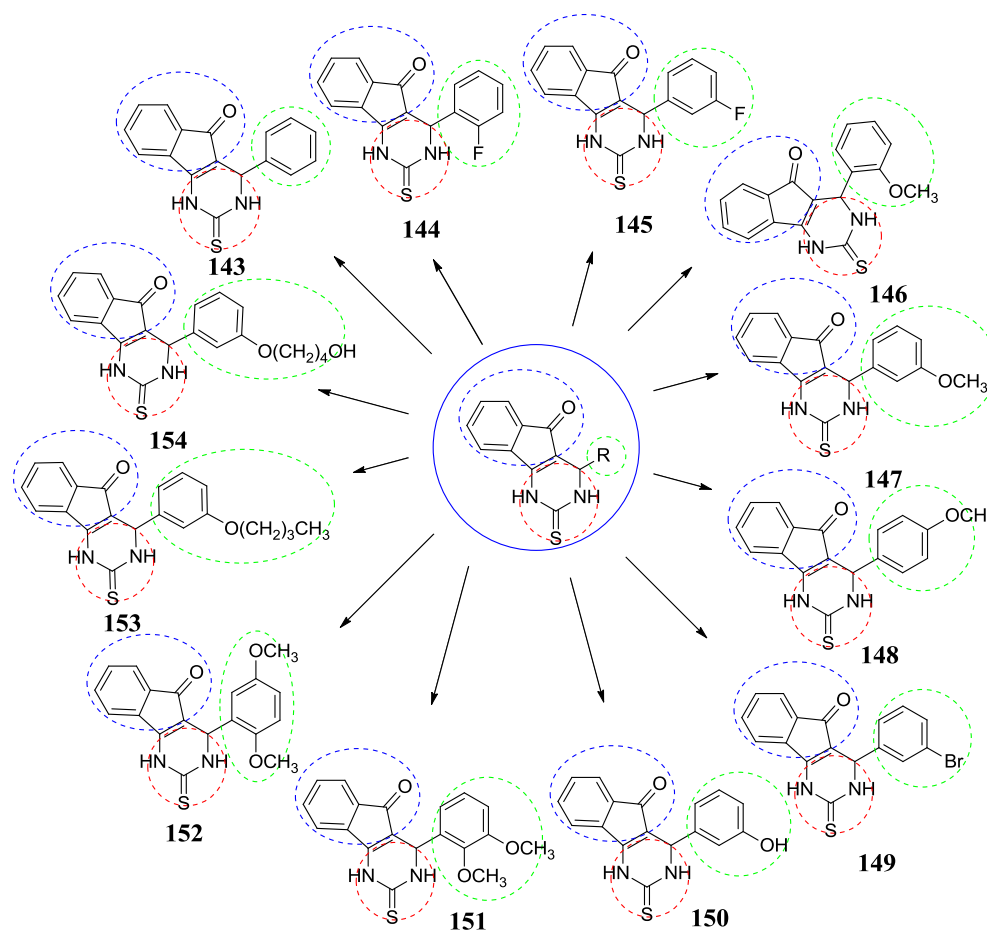| Compound No.	hTRPA1 IC$_{50}$ (µM)	rTRPA1 IC$_{50}$ (µM)
143	1.8	1.9
144	2.07	3.39
145	1.1	0.39
146	>10	9.4
147	0.13	0.02
148	3.1	3.5
149	0.5	0.071
150	0.71	0.044
151	>10	2.7
152	>10	0.8
153	0.05	0.011
154	0.07	0.010

human) [37]. The relationship between antitumor activity and lipophilicity, unsaturation, number of carbon atoms, and functional group was also determined. A set of compounds derived from palmitic acid (set a), stearic (set b) and oleic acid (set c) was synthesized with the DHPM nucleus. Oxo-monastrol **141a-c** and monastrol analogs **142a-c**, as well as compounds **139a-c**, **140a-c** which lacked aromatic ring substitution were screened for cell viability in the C6 rat glioma cell line. The screening was conducted after 24 h of treatment with test compound at concentration ranging from 0 to 200 µM. Compound **139c** was the only compound to reduce the C6 cell viability in a concentration dependent manner. Out of the compounds **141a-c**, **142a-c** only few were able to reduce the cell viability at 50 µM except compound **142b**. However,

compounds **139a-c**, **141a-c**, **142a-c** were also screened in human glioma cell lines (UG-138-MG human) as described in Fig. 22. The hybrid DHPM-fatty acid **141a-c** and **142a-c** were able to reduce the cell viability at 50 µM with **141a** (hybrid oxo-monastrol-palmitic acid) being the most potent at 10 µM. Compound **139c** again was found to decrease the cell viability in a concentration dependent manner. The result described that the hydroxyl group present in the aromatic ring are essential for the antitumor activity in hybrid DHPM-fatty acid against gliomas. It was also observed that all novel oxo-monastrol and monastrol fatty acid analogs were found to be more potent than monastrol itself. These fatty acid analogs tend to impart lipophilic character which is able to cross the blood brain barrier. This may be considered as a promising approach for overcoming resistance to chemotherapy and help in the development of new anti-tumor drugs.

The cytotoxic activity of the compounds was evaluated using organotypic hippocampal slice cultures as models. This culture is composed of different neural cells and were prepared from 6 to 8 day old male Wistar rat using a McWallin tissue chopper and separated in ice-cold HBSS with pH maintained at 7.2. The slices were then placed into Millicell Culture insertsand further transferred to 6-well culture plate. The medium usually consists of 50% MEM, 25% HBSS, 25% horse serum which was changed after every three days and experiment was conducted after every 14 days. Cells were then treated with 200 µM concentrations of **139c**, **141a**, **141c**, **142a** and **142c for** 24 h. Cellular death in organotypic hippocampal cultures was determined by flouroscent imaging analysis of PI uptake. 5 µM of PI was added to the cultures after 24 h of treatment
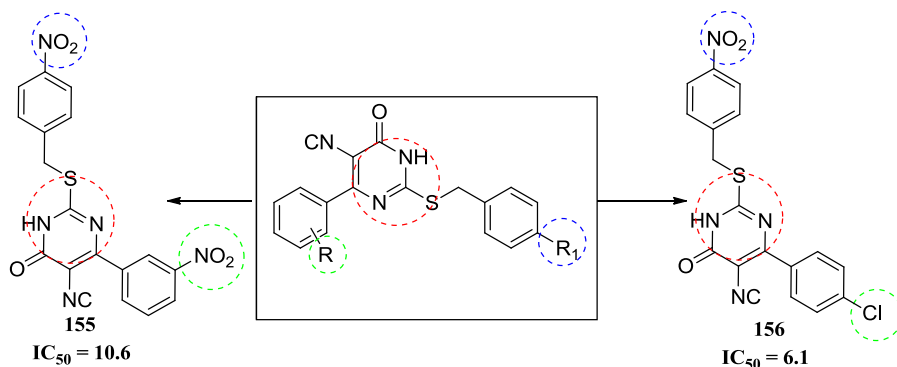
Fig. 24. The structure of potent anti SARS compounds.

and incubated for another 1 h. The most active compounds **139c**, **141a**, **141c**, **142a** and **142c** did not showed any significant signs of neural cell death against glioma cell at 200 μM concentrations after 24 h. It was found that DHPM fatty acids **139c**, **141c**, **142a** did not cause neural cell damage. Whereas, the compound **141a** and **142c** did not increase the neural cell death by at least 20-times and 4-times the active glioma concentration at10 μM and 50 μM respectively.

3.13. Transient receptor potential A1 (TRPA1) antagonist activity

In 2012, Gijsen et al. synthesized tricyclic 3,4-dihydropyrimidine-2-thione derivatives as potent TRPA1 antagonists. TRPA1 is believed to function as a mechanical and chemical stress sensor. The specific function of this protein may involve a role in signal transduction and growth control. All the synthesized compounds were tested on both the human and rat TRPA1 channel. In general, a similar activity was monitored for the human and rat channel, with most of the compounds, a slightly higher potency for rat TRPA1 was observed. At all concentrations the tested compounds behaved as true antagonists, in competition with the applied agonists. This is unlike many electrophilic ligands, which can display functional antagonism *via* desensitization of the channel, depending on their concentration. Introduction of various substituents on the aryl substituent of **143** had a strong effect on the potency of the tricyclic DHPMs. *Ortho*- and *para*-substituted compounds **144, 146** and **148** led to a drop in activity compared to unsubsited compound **128**. In variance, *meta*-substitution such as in **145, 147, 149, 150** and **151** increased the potency relative to compound **143**. Especially *meta*-methoxy substituted compound **147** displayed a more than 10-fold enhanced potency and additional analogs around compound **147** were investigated. Combination of a *meta*-methoxy substituent with additional methoxy substituents as in **151**–**152** led to a loss in activity. Increase in the length of methoxy group proved to be better tolerated and compound **153** and **154** being the most potent compounds identified [67]. The structure and IC$_{50}$ value of the compound are given in Fig. 23 and Table 9 respectively.

3.14. Anti-SARS activity

In 2010, Ramajayam et al. synthesized a series of pyrimidines and evaluated for the Severe acute respiratory syndrome (SARS) activity. The target compounds were tested for anti-SARS activity against SARS-CoV 3CL^{pro}, by using previously developed assay method containing 0.05 μM SARS 3CL^{pro}, 6 μM fluorogenic

substrate Dabcyl-KTSAVLQSGFRKME-Edans and 50 μM of test compounds. Enhanced fluorescence of the reactions in the buffer of 20 μM Bis-Tris at pH 7.0 was monitored at 538 nm with excitation at 355 nm using a fluorescence plate reader. The compounds which inhibited more than 50% of the protease activity at 50 μM were selected for the next assay run at 10 μM for IC$_{50}$ calculation. Only two compounds were found to be active against SARS-CoV 3CL^{pro} among the fourteen molecules. The IC$_{50}$ values of the active (**155 &156**) compounds are given 10.6 and 6.1 μM respectively. Compound **155** was found to be the most potent molecule. The SAR study showed that when the R′ position of the C-4 atom substituted by the nitro group and also R was replaced by Cl group. The potency of the molecule was increased markedly. When the both R′ position was replaced with the nitro group. The activity of the compound was decreased markedly [68]. Structure of molecules is shown in Fig. 24 with their IC$_{50}$ values (see Fig. 25).

3.15. DHPM as melanin-concentrating hormone (MCH 1) receptor antagonists

Melanin-concentrating hormone is a cyclic 19-amino acid neuropeptide found mainly in fish and mammalian brain [69]. The biological functions of MCH are mediated by two receptors, MCH$_1$ and MCH$_2$. It plays vital role in regulating food intake, water balance, energy metabolism, attention, memory and psychiatric disorders. It mostly controls the food intake and stress in rodents. Mice generally lacks MCH gene encoding which is found to be lean, hypophagic with elevated metabolic rate. The overexpression of MCH gene in mice is suspectible to obesity and insulin resistance. Most of the *in vitro* and *in vivo* pharmacology of MCH$_1$ receptor has been published which make it as an attractive target for the development of antagonist. Various MCH$_1$ receptor antagonists have been developed out of which (+) SNAP-7941(**157**) has been found to have high affinity and selectivity towards MCH$_1$ receptor (Fig. 25) [70].

The data for affinity of mammalian cell lines which express MCHR$_1$ was found as K$_b$ = 0.57 nM and more than 1000 times selectivity was observed in MCH (K$_i$ = 15 ± 0.11 nM). When *in vivo* assays were performed on rats, the anorectic acid of **157** was observed by decreasing the palatable food intake of rats without working as malaise agent. Moreover, Diet-induced obese study (DIO) was done in rats, which revealed that **157** exhibited continous weight reduction in rats and also lead to 26% less weight than the vehicle-treated counterpart in 4 weeks period which is almost 2 times than the data obtained from the standard clinical drug fenfluramine.
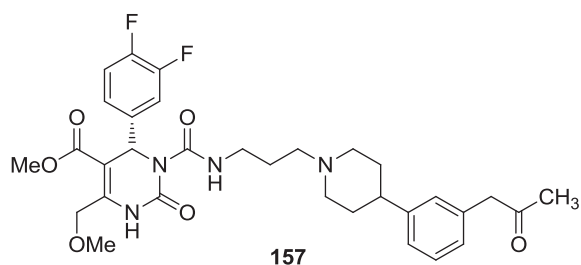
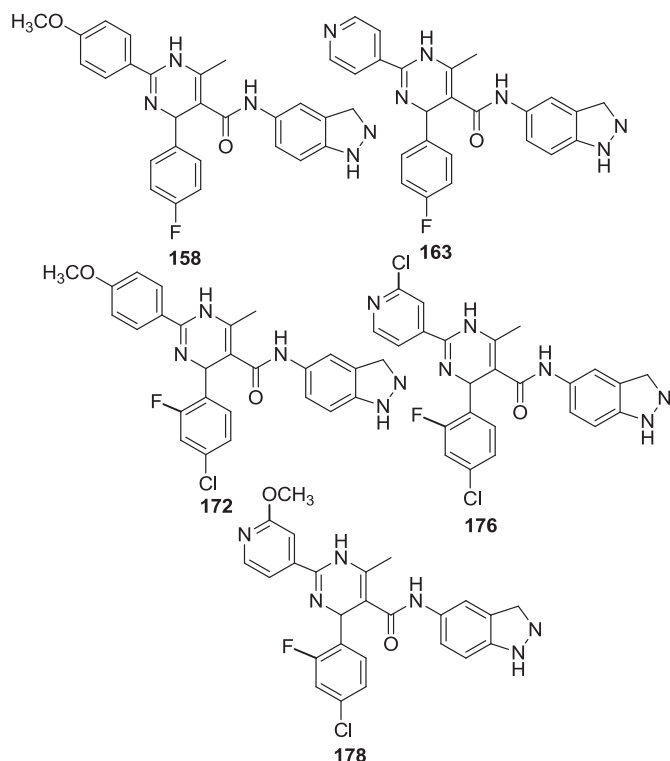**Fig. 25.** Structure of MCH1 receptor antagonist.

Fig. 26. Structure of active inhibitors against ROCK1.

Furthermore interestingly, **157** have also been found to have moderate antidepressant and antianxiety activity in different paradigms However, different statement and results have been made by different researchers on their antidepressant and anxiolytic activity. But recently Millan et al. have published that MCH1 antagonist tends to increase social recognition paradigm of rats and also enhances the level of acetylcholine in frontal cortex of rats by carrying out dialysis study at reasonable doses.

3.16. DHPM inhibitors of ROCK1 as potential therapeutic agent for cardiovascular disease

Rho-associated kinase isoform 1 (ROCK1) is a protein serine/threonine kinase enzyme associated with diverse cellular signaling functions such as smooth muscle contraction, cytoskeleton rearrangement, cell migration and cell proliferation. ROCK 1 is considered as a potential therapeutic target for the treatment of cardiovascular diseases and hypertension. Sehon et al. reported a new class of dihyropyrimidinone derieved amides (**158**–**180**) which were then evaluated for their activity against ROCK 1 [71]. The 4-OMe group present in the compound **158** was found to have
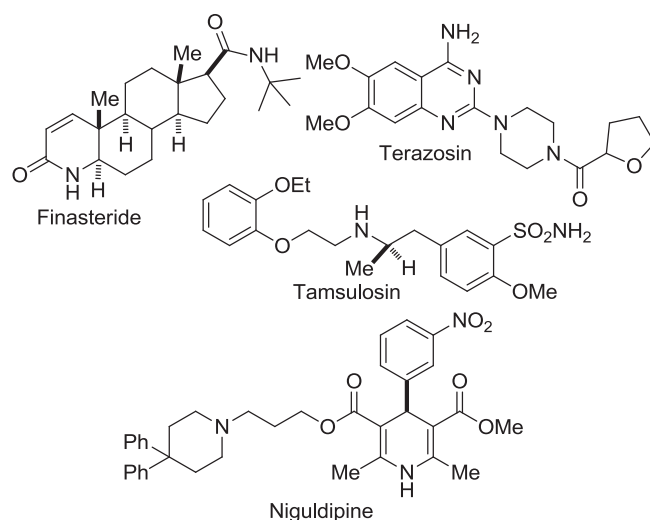

Fig. 27. Currently available α_1 antagonists for prostate cancer.

better oral bioavailablity (35%) compared to compound **163** (0%) and therefore it was further studied. Compound **172** substituted with 2-F, 4-Cl group which are found to be optimal for the activity against ROCK1. It was found to possess good oral bioavailability at 58% and is 100 times more selective than other selected kinases. It revealed a remarkable IC_{50} value of 200 nM when the rat aortic contraction assay was performed. However, IC_{50} value against RSK1 and p70S6K was 510 and 2100 respectively. But it possessed significant P450 inhibition against CYP2D6 which appears to be general for the 4-OMe series and is insurmountable. Because of these issues, the focus was shifted to compound **163** which was substituted with pyridyl ring in which ROCK1 enzyme activity was maintained and CYP2D6 was substantially diminished but the oral bioavailability was decreased. Compound **176** and **178** were found to have improved oral bioavailability of 49% and 53% respectively as shown in Fig. 26. Compound **176** consist of chloro group which showed improved half-life in rats (1.8 h), good selectivity upto 100 folds against a panel of 31 kinases, IC_{50} of 256 nM and has improved P450 profile. It was therefore selected for *in vivo* efficacy study and was used in a spontaneously hypertensive rat (SHR) model for hypertension. This revealed a drop in blood pressure of 25 mm of Hg at a single dose of 30 mg/kg.

3.17. α_{1a} receptor antagonist activity

Benign prostatic hyperplasia (BPH) is the progressive enlargement of the prostate gland. It has been estimated that it may affect atleast 50% of the male population in the United States by 50 years of age. However, the incidence rate may increase to 85% by the age of 80 [72,73]. BPH is a result of two mechanistic components: mechanical and dynamic component. Mechanical component is a result of increased prostatic mass which is mainly attributed due to the 5α-dihydrotestosterone. 5α-reductase is the enzyme responsible for the conversion of testosterone to 5α-dihydrotestosterone. Various inhibitors of this enzyme such as finasteride have been clinically proven to reduce the size of the prostate gland and decrease the symptoms of BPH. The dynamic component of BPH is attributed to the endogeneous adrenergic tone which restricts the flow through urethra. Adrenergic receptor α_1 antagonists such as terazosin, relieves symptoms of BPH by relaxing lower urinary tract tissue and finally reducing prostatic and uretheral tone. Tamsulosin is considered as the 'prostate selective' α_1 antagonists and is currently marketed due to better therapeutic index when
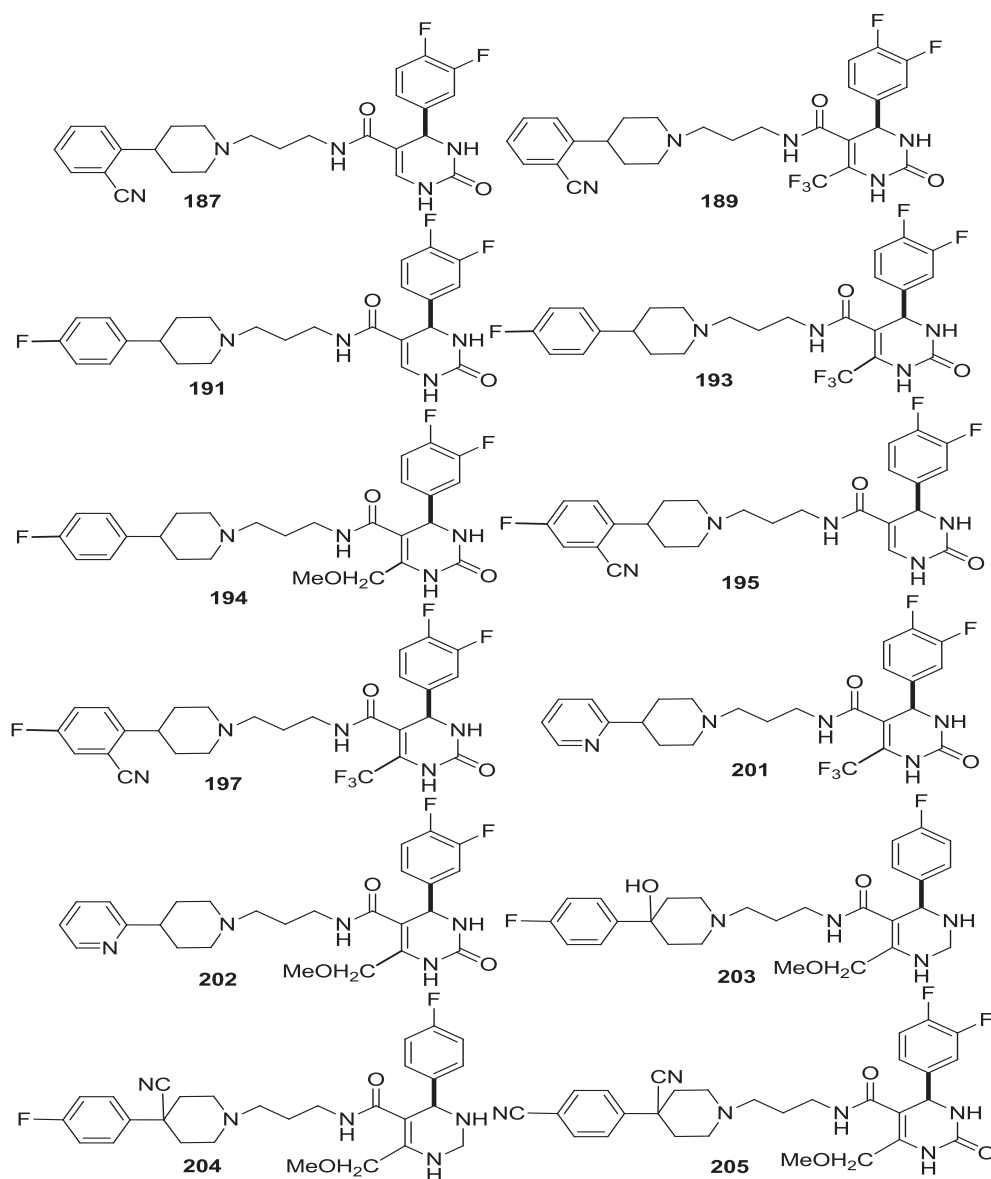
Fig. 28. Compounds tested for α_{1a} binding affinity.

compared to terazosin as shown in Fig. 27. Recently it has been found that niguldipine, a calcium channel blocker was found to be potent α_{1a} receptor subtype antagonists with 100 times greater selectivity than other α_1 receptor subtypes (see Fig. 28).

Several modifications have been done over niguldipine skeleton and thereby led to the formation of dihydropyrimidinone moiety with two logical sites of attachment of the piperidine containing side chain with most important attachment being at the *N*-3 position of DHP. It was found that the exact structure of central moiety is not important but the mode of attachment of the piperidine containing side-chain *via* amide bond formation of the DHP C-5 carboxylate is crucial to produce potent and selective compounds. Thus, 4-aryldihydropyrimidinone heterocycle derivatives attached to aminopropyl-4-arylpiperidine *via* C-5 amide were synthesized and were then investigated for their *in vitro* and *in vivo* binding affinity of α_1 receptor antagonist. The binding affinity is expressed as $K_i \pm SEM$ (nM).

It was found that among 4-arylpiperidines derivative (**187**–**214**), 2-cyanophenyl **187** (0.13 ± 0.015 nM) was found to be as potent as

4-fluorophenyl **191** (0.17 ± 0.030 nM) but has greater selectivity. However, combination of two, slightly increases the potency to similar level as that observed for **195** (0.07 ± 0.020 nM) which possess extra ordinary binding affinity to the α_1 receptor subtype. 2-pyridyl substituent was found to be highly selective but 5−100 times less potent than others except when combined with the 6-methoxymethyl DHP **202** (0.45 ± 0.16 nM). The 4-position of the piperidine was also examined for the receptor binding affinity. It revealed that hydroxy substituent leads to slight decrease in the binding affinity with modest increase in the selectivity when relative to the unsubstituted part (**203** vs **191**). However, cyano substitution possessed additional selectivity against the α_{1b} and α_{1d} receptor subtypes along with the α_{1a} binding affinity **204** (0.17 ± 0.040 nM) and **205** (0.16 ± 0.070 nM). The triflouromethyl group at the 6-position of DHP was not essential for binding (**189**, **193**, **197** and **201**) but hydrogen, methyl, methoxymethyl described excellent potency and 100 times greater selectivity relative to other α_{1a} subtype.

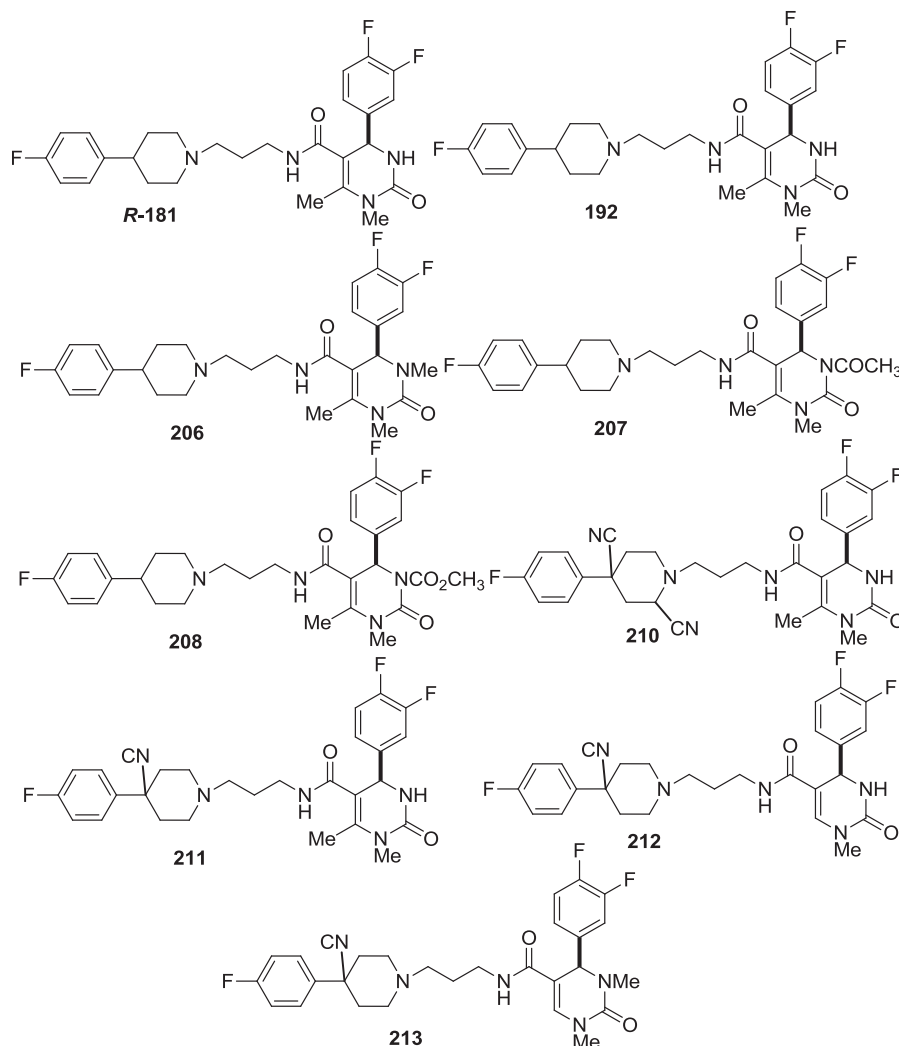The polarity of substituents attached may limit absorption

Fig. 29. Compounds tested for α_{1a} binding affinity.

therefore, alkylation and acylation of the DHP nitrogens was examined for the effect on the binding affinity. Methylation of *N*-1 improves selectivity while methylation at *N*-3 decreased but both of them possessed similar potencies [**181**(0.18 ± 0.025 nM), and **206**(0.14 ± 0.040 nM) vs **192**(0.39 ± 0.05 nM)]. On combining *N*-1 methylated DHP with 4-cyanopiperdines, extraordinary selective compounds were obtained (**201**–**213**). However on acylation of *N*-3 with acetyl **207**(2.2 ± 0.10 nM) and carbomethoxy **208**(0.75 ± 0.010 nM), a decrease in both selectivity and potency was observed. Compound **194** was the only compound to exhibit 1000 time greater selectivity and α_{1a} receptor binding affinity (0.24 ± 0.050 nM) than any other compound synthesized.(see Fig. 29).

Many of the compounds were also investigated for *in vivo* pharmacodynamic and pharmacokinetic parameters. The compounds were tested on rat models where the prostate of anesthetized rat was exposed. Compounds were tested for their inhibiton (AD$_{50}$ (µg/kg) value) of contractile response induced by the α_{1a} selective agoinst A61063 [26,29]. Compounds containing flouro and cyano substituent on the 4-arylpiperidine were found to be quiet potent with AD$_{50}$ value below 2 µg/kg whereas the piperidine X = substituent followed a trend as OH < H < CN (**203** vs **191** vs **204**). The pharmacodynamic parameter *i.e.* duration of action for majority of compounds which was measured at agonist

concentration and is 4 times greater AD$_{50}$ and was found to be short and is unexplainable. Compounds with marked plasma-half lives were also found to have short duration of action as others. Methylation at *N*-1 nitrogen of DHP was found to have increased duration of action (**181**, **185**, **209** and **211**–**214**). The *in vitro* metabolism of most of the compounds (**187**, **188**, **189**, **194**–**196**, **198**, **209**) was examined using human liver microsomal preparations (10–50 µM substrate, microsomal protein 2 mg/mL, for 1 h, LC-MS/MS detection). These experiments revealed rapid *N*-dealkylation of the piperidine and *N*-3 methyl of the DHP as well as the desaturation of DHP nucleus to the pyrimidine nucleus as primary metabolic pathways.(see Fig. 30).

Compound **194** and **209** were able to pass first-pass metabolism in rats and therefore their pharmacodynamic and pharmacokinetic parameters were evaluated in dog. Compound **194** was found to have long half-life in dogs (7–8 h) thus once a daily dosing is appropriate in humans. Pharmacodynamic efficacy and selectivity was done by canine intraurethral pressure (IUP) assay for compound **194**. This leads to the simultaneous monitoring of the heart rate and blood pressure. When determined for inhibition of phenylepherine-induced changes in IUP, Compound **194** was found to be more potent than terazosin. It was also found to have longer duration of action of more than 6 h.
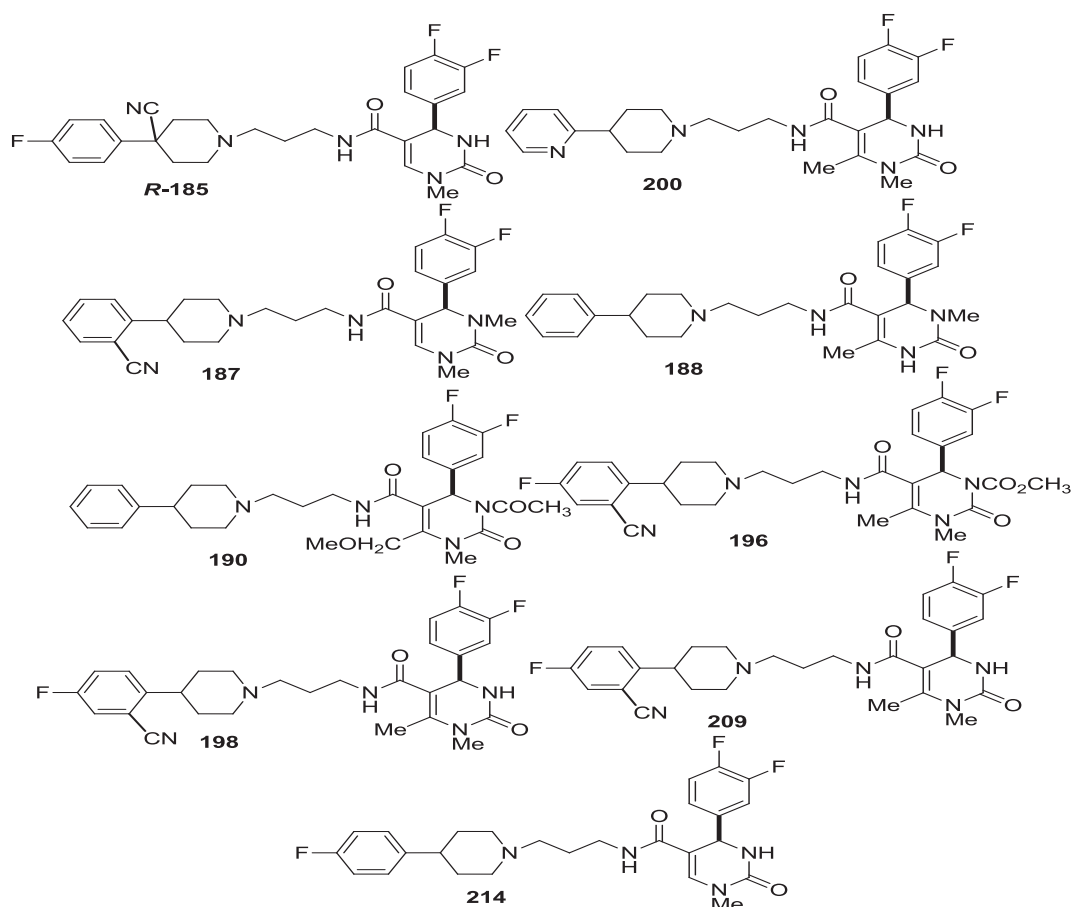
Fig. 30. The structure of compounds investigated for the *in vitro* and *in vivo* study.

4. Conclusion

Dihydropyrimidinones are prototype acting *via* various mechanism that emerge out to be potent in several diseases. Dihydropyrimidinones revolutionized the chemistry of purines as well as pyrimidines by their different biological activities which make them a advantageous scaffold. Their antagonistic nature towards the natural pyrimidines makes them powerful candidates for the synthesis of various effective and successful molecules. A number of drugs like 5-fluorouracil, idoxuridine, methythiouracil, emivirin, aminophylline containing dihydropyrimidinones are already discovered and effectively used in the prevention or treatment of multiple diseases. The DPHMS and their derivatives are the versatile molecules, which have been explored for the various diseases as antimicrobial, antihypertensive, anti-HIV, anticancer etc. The researchers explored their SAR, as well as binding mode through molecular modeling studies. In addition, recognition of a rational picture towards the appropriate substitutions accountable for its effectiveness and toxicity may be a future skeleton so that the toxicity problems related with the dihydropyrimidinones can be recognized and overcome.

Conflict of interest

There is no conflict of interest.

Acknowledgements

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