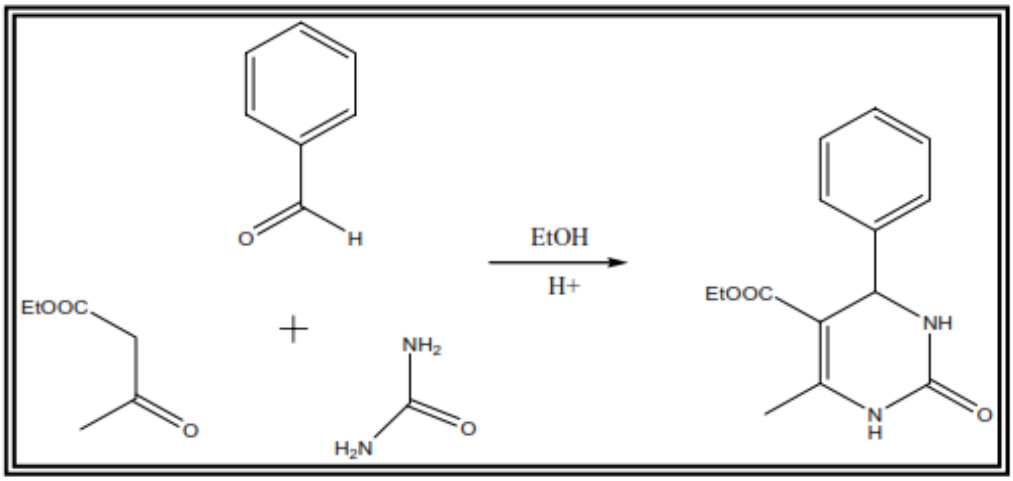
Chapter-3
Part-A

Synthesis, characterization and biological activity of pyrimidine-2-thione and pyrimidin-2-one derivatives by Biginelli reaction

3.1 Biginelli Reaction

Multicomponent condensation reaction was reported by **P. Biginelli** for the synthesis of functionalized 3,4-dihydropyrimidin-(1*H*)-ones (DHPMs) via three-component condensation reaction of an aromatic aldehyde ethyl acetoacetate and urea. In the last few years, this multicomponent reaction has been found a notable revival, primarily due to the excellent pharmacological properties related with this dihydropyrimidine moiety[1].



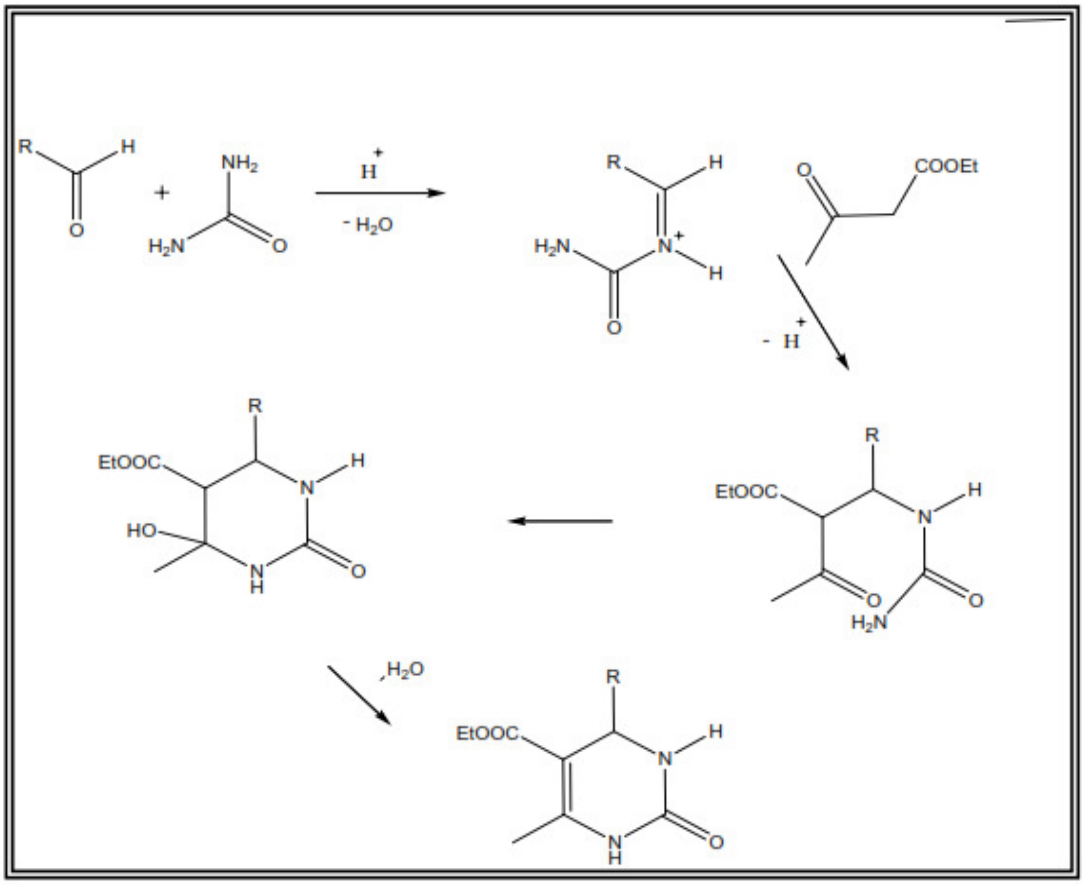
Biginelli Dihydropyrimidine Synthesis

The reaction was carried out in the presence of catalytic amount of hydrochloric acid. The reaction is carried out in alcoholic solvent like ethanol by simply refluxing a mixture of the three components. The isolation of this product of novel one-pot, three-component synthesis that on cooling of the reaction mixture it is crystallize. Biginelli has identified correctly this compound as 3,4-dihydropyrimidin-2(1*H*)-one. Even though the late **Karl Folkers** has made a series of publications in the mid 1930s, the "Biginelli reaction" or "Biginelli condensation" as it was henceforth called was largely ignored in the early part of the 20th century. For quite some time the synthetic potential of this new heterocycle synthesis therefore remained new. Interest slowly increased in the 1970s and 1980s, and the possibility of the original cyclo-condensation reaction was slowly stretched by

changing substitution of all three building blocks, to allow a large number of multi-functionalized dihydropyrimidines.[2-4]

3.2 Mechanistic Studies of Biginelli Reaction[5-9]

The mechanism is debated over past few decades for Beginelli reaction. Early work by **Folkers and Johnson**[3] suggested that first intermediate of this reaction was bisureide, i.e. the primary bimolecular condensation product of benzaldehyde and urea.



Sweet and Fissekis[5] in 1973 had given a pathway that suggest that carbenium ion is produced by an acid-catalyzed aldol reaction of ethyl acetoacetate with benzaldehyde which is formed in the first and limiting step of the Biginelli condensation. **Kappe et al**[6] in 1997 reinvestigated the mechanism with the help of Nuclear Magnetic Resonance

spectroscopy and trapping experiments and have established that the prime step in this sequence involves the acid-catalyzed formation of an *N*-acyliminium ion intermediate from the urea and aldehyde precursors. Further reaction of the *N*-acyliminium ion by ethylacetoacetate, primarily through its enol tautomer, generates an open-chain ureide which consequently cyclizes to hexahydropyrimidine. Elimination of water by acid-catalyzed elimination leads to the final DHPM moiety. The reaction mechanism can therefore be classified as an α-amidoalkylation, or more specifically as an α-ureido alkylation. The other "carbenium ion mechanism" does not establish a major pathway; however, a little amount of enoneis sometimes found as by-product. Isolation of the extremely reactive *N*-acyliminium ion could not be possible further evidence for the proposed mechanism was obtained by isolation of intermediates, employing sterically bulky or electron-deficient acetoacetates respectively. The relative stereochemistry in hexahydropyrimidine was established by an X-ray analysis. By using perfluorinated α-keto esters or 1,3-dicarbonyl compounds as a building blocks in the Biginelli condensation a number of hexahydropyrimidines could be synthesized.

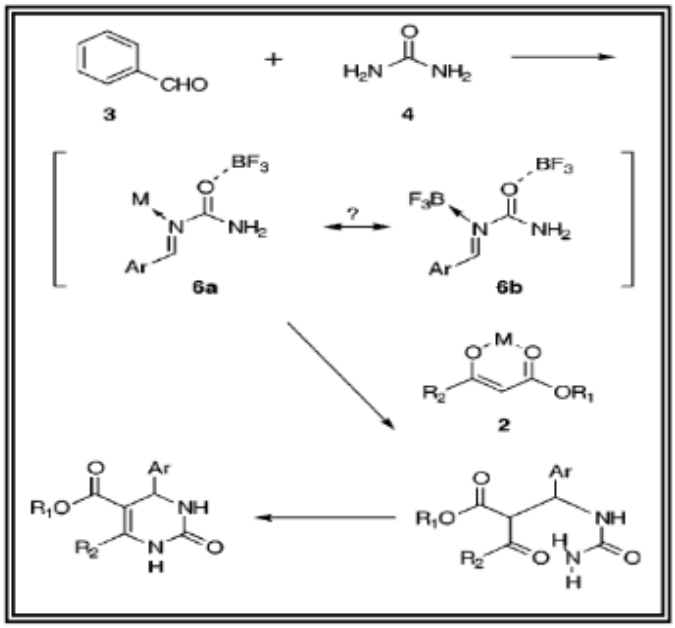
3.3 Alternative synthetic routes for better yield, shorter reaction time and to synthesize new analogues

Several alterations have been made to Biginelli reaction to obtained better yield and to synthesize biologically active moieties. Different catalysts have been reported to improve the yield of the reaction. To shorten the reaction time microwave synthesis strategies are also applied. Combinatorial chemistry and solid phase synthesis has made possible to generate library of DHPM analogues.

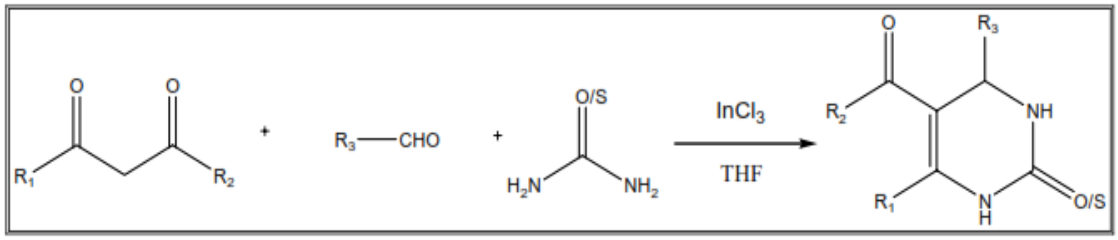
3.3.1 Catalysts

Essa H. Hu et al[10] described Biginelli reaction using trifluoroborane etherate catalyst with transition metal salt and proton source to yield about 80-90% Biginelli product. **Essa H. Hu et al** proposed a mechanism similar to that of **Folkers and Johnson**[3] and to that of

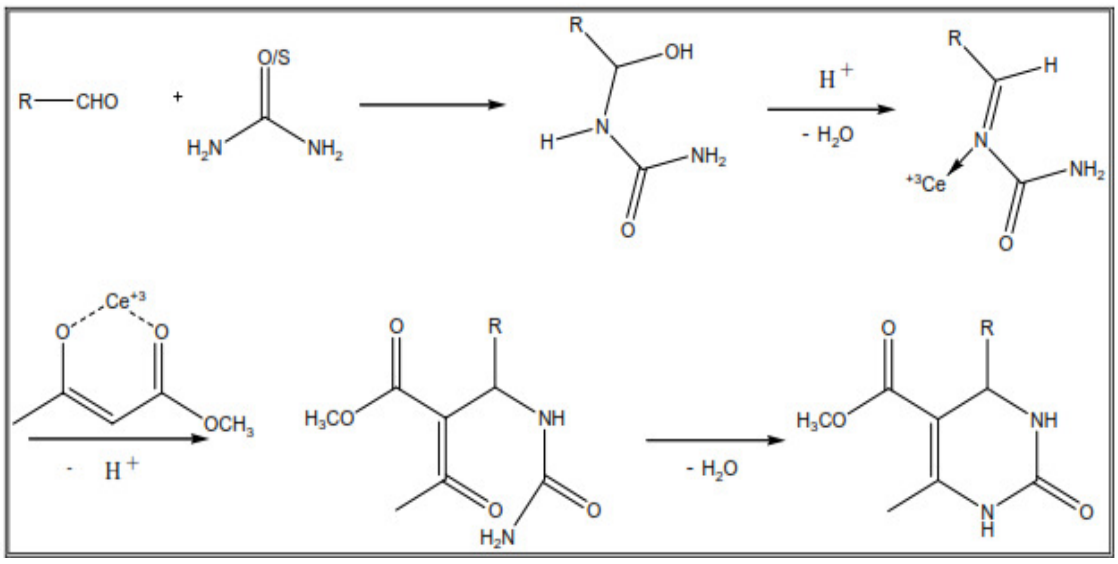
Kappe[6] for the Biginelli reaction. Acyl imine intermediate generates by the reaction of the aldehyde with urea which was stabilized by either trifluoroborane or the transition metal, is the key, rate limiting step. Addition of the α-keto esterenolate subsequently, followed by cyclization and water elimination (dehydration), would give the dihydropyrimidinone.



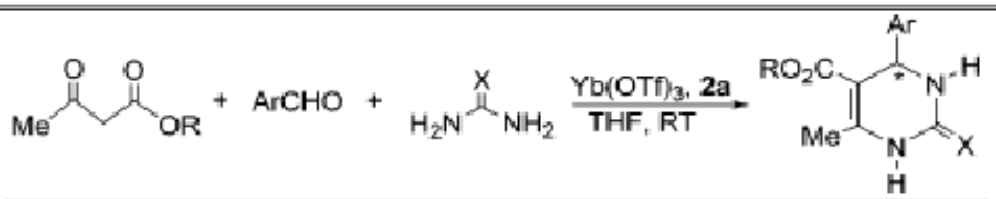
Brindaban C. Ranu et al[11] reported Indium (III) Chloride as an effective catalyst for Biginelli reaction. Different substituted α-dicarbonyl compound, aldehyde and urea were coupled together by this process to produce the analogous dihydropyrimidinones moieties. Differently substituted aldehydes (aromatic, aliphatic and heterocyclic) have been subjected to this condensation very efficiently to generate substituted DHPMs. Dihydropyrimidin-2(1H)-thiones can be prepared by using thiourea with similar success.



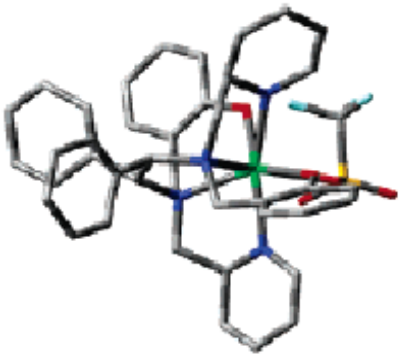
Bose et al[12] used cerium (III) chloride as a catalyst by using solvent free process of Biginelli compounds. By using this procedure yield is higher; reaction time is shorter and simple work up methods. By using this catalyst under solvent free reaction condition gave about 70% yield and reaction time was about 10 hours. About 90% yield was obtained if ethanol is used as solvent with this catalyst. To push the reaction forward about 25 mol % amount of cerium (III) chloride heptahydrate was found to be sufficient as catalyst. No further advantage of the increased amount of the catalyst was used.

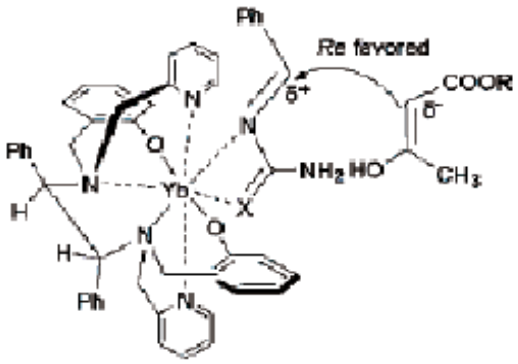


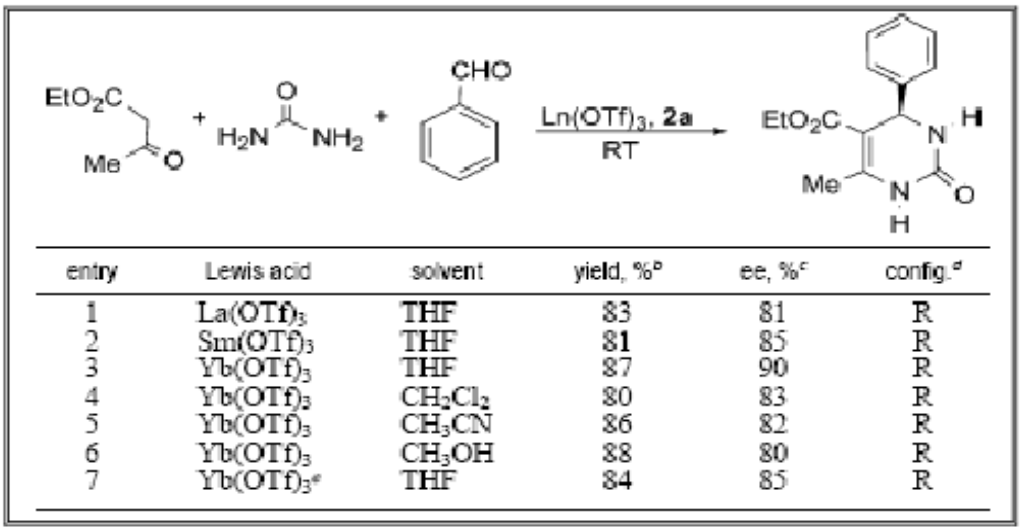
Some of the enantio-selective catalysts are also used for the one pot synthesis of Biginelli reaction. Enantio-selective catalysts are reported like lanthanide triflates/ytterbium triflates. These catalyst leads to highly enantio-selectivity and up to 99% enantio-selective Biginelli reaction can be carried out.[13]



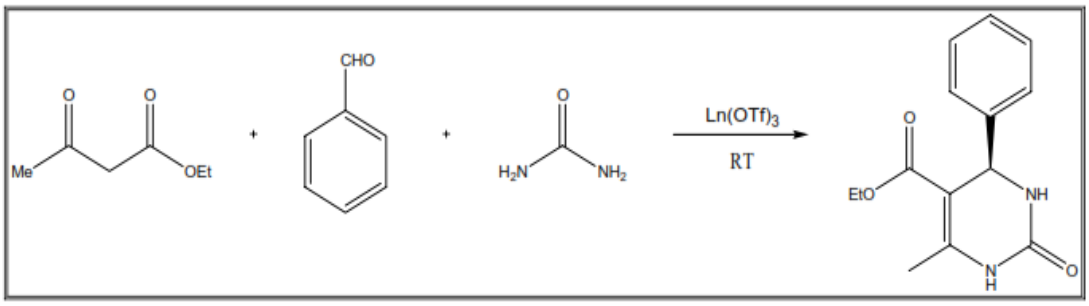
entry	Ar	R	X	yield, %[b]	ee, %[c]	config.
1	C_6H_5	Et	O	87	90	*R*[e]
2	C_6H_5	Et	S	81	99	*R*[e]
3	3-(NO_2)-C_6H_4	^{i}Pr	O	90	>99	*R*[d]
4	3-(NO_2)-C_6H_4	^{i}Pr	S	88	87	*R*[e]
5	3-(F)-C_6H_4	Et	O	80	97	*R*[e]
6	2-(Cl)-C_6H_4	Et	S	73	98	*R*[e]
7	2-(Cl)-C_6H_4	Et	O	78	89	*R*[e]
8	4-(Br)-C_6H_4	Et	O	82	95	*R*[e]
9	3-(OH)-C_6H_4	Et	O	81	91	*R*[e]
10	3-(OH)-C_6H_4	Et	S	80	99	*R*[d]
11	2-(OH)-C_6H_4	Et	O	86	98	*R*[e]
12		Et	O	81	80	*R*[e]
13		Et	O	82	82	*R*[e]
14		Et	O	87	93	*R*[e]





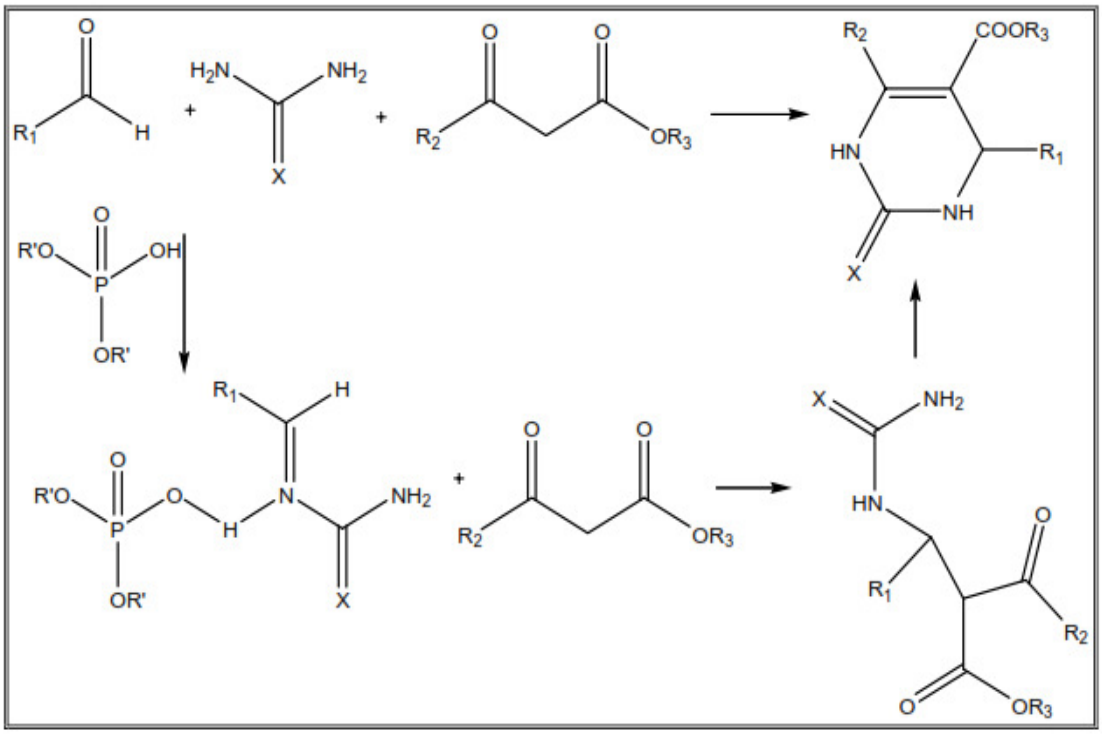


entry	Lewis acid	solvent	yield, %[b]	ee, %[c]	config.[d]
1	$La(OTf)_3$	THF	83	81	R
2	$Sm(OTf)_3$	THF	81	85	R
3	$Yb(OTf)_3$	THF	87	90	R
4	$Yb(OTf)_3$	CH_2Cl_2	80	83	R
5	$Yb(OTf)_3$	CH_3CN	86	82	R
6	$Yb(OTf)_3$	CH_3OH	88	80	R
7	$Yb(OTf)_3$[e]	THF	84	85	R

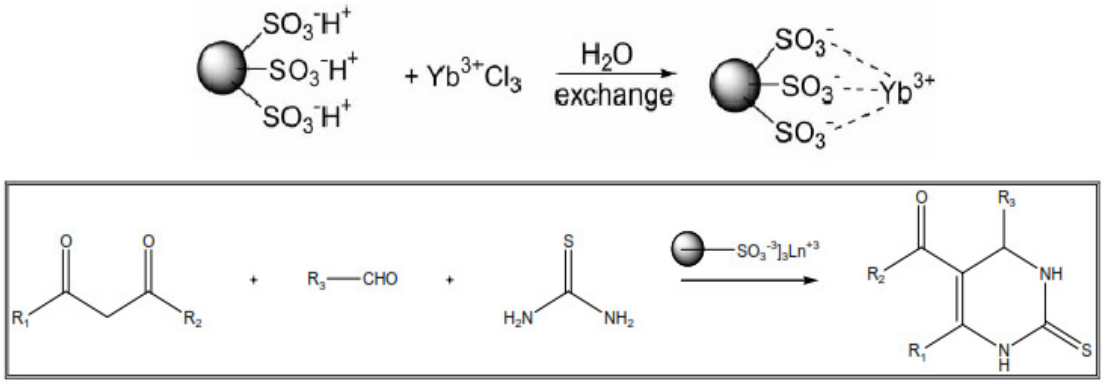


In the recent development highly enantioselective catalyst like chiral phosphoric acid is reported for Biginelli reaction. To obtain the desired enantioselective product reaction is carried out in the presence of 10 mol % of chiral phosphoric acid. The first organocatalytic asymmetric Biginelli reaction with high enantioselectivity can be obtained. The primary requirement is the optimum chiral phosphoric acid afforded the

reaction in good to high yields with high enantioselectivities of up to 97% ee. An extensive variety of compounds, including different keto esters and aldehydes could be tolerated. The main advantage of this reaction has to avoid the impurities of transition metals in the production of the pharmaceutically relevant chiral 3,4-dihydropyrimidin-2-(1*H*)-ones.[14]



Under solvent free condition scientists **Jiang and Chen**[15] used Yb(III)-Resin catalyst in Biginelli reaction.



In recent scenario, 12-molybdophosphoric acid as catalyst in refluxing acetic acid, **Heravi et al**[16] reported synthesis of dihydropyrimidones. The catalyst is used to this multi-component condensation reaction to generate the corresponding pyrimidinones with good yield.

An upgraded method has been found to execute the Biginelli reaction for the preparation of 3,4- dihydropyrimidin-2(1H)-one products. The presence of hydrochloric acid and β-cyclodextrin in ethanol solution is used in this improved process. The main advantage of this improved process or new approach compared with the Biginelli reaction conditions, is good to excellent yields and the reaction time is also reducesignificantly.[17]

An good method for the preparation 3,4-dihydropyrimidinones from the aldehyde, β-keto ester and urea with nickel chloride hexahydrate and ferric chloride hexahydrate is used as catalyst in ethanol as a solvent is described.[18] Good to excellent yields is obtained compared with the classical Biginelli reaction conditions. The yield reported is around (53.0-97.0%) in this new method. Another advantage is the shorten reaction time (Approx. 240 minutes to 300 minutes).

Synthesis of 5-Alkoxycarbonyl-4-aryl-3,4-dihydropyrimidin-2-ones can be produced by the one-pot reactions of aldehydes, ß-ketoesters and urea with the catalytic amount of

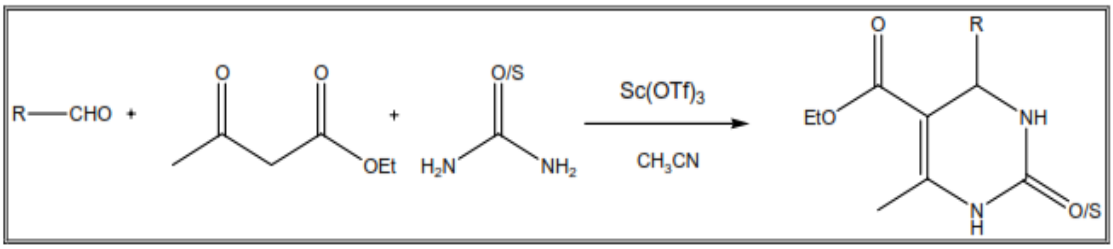
phosphor-tungstic acid (PTA) insolvent a like ethanol. The improved process of Biginelli cyclo-condensation not only shortens the reaction cycle and simplifies the operation, but also increases the yields.[19]

Ruthenium catalyst like Ruthenium (III) chloride is used efficiently to improve the yield of the three-component Biginelli reaction of analdehyde, a β-keto ester, and urea or thiourea under solvent-free conditions to afford the corresponding 3,4-dihydropyrimidine-2-(1*H*)-ones. The yields are good to excellent.[20]

In the Biginelli reaction, a one-pot cyclocondensation of aldehydes, urea or thiourea and dicarbonyl compounds is efficiently catalyzed by samariumdiiodide. The biologically active dihydropyrimidinones are easily synthesized in moderate to excellent yields under solvent-free conditions.[21]

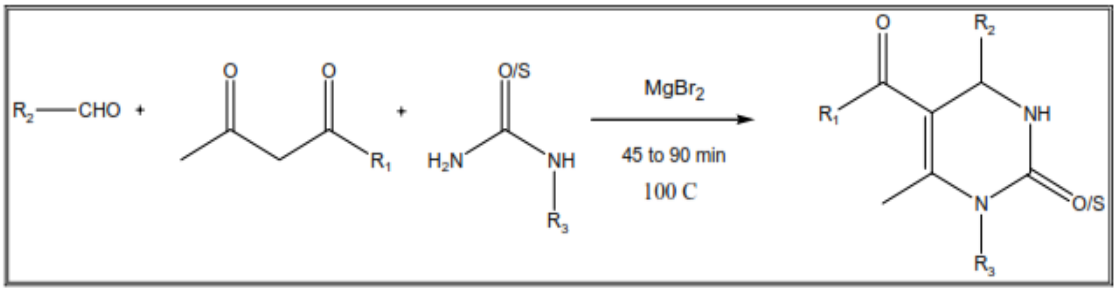
Dihydropyrimidinones in high yields can be prepared by using Hydroxyapatite doped with $ZnCl_2$, $CuCl_2$, $NiCl_2$ and $CoCl_2$ as a catalyst efficiently. The one pot three components Biginelli reaction between an aldehyde, ethyl acetoacetate and urea is performed in refluxing toluene to afford the corresponding DHPMs.[22]

An environmentally acceptable process by using Scandium (III)triflate efficiently catalyzes the one pot three-component condensation reaction of an aldehyde, a β-ketoester, and urea at reflux temperature using acetonitrile as a solvent to afford the corresponding 3,4-dihydropyrimidin-2(1H)-ones in outstanding yields. The re-usability and recovery of the catalyst provides the eco-friendly and environmentally acceptable method.[23]

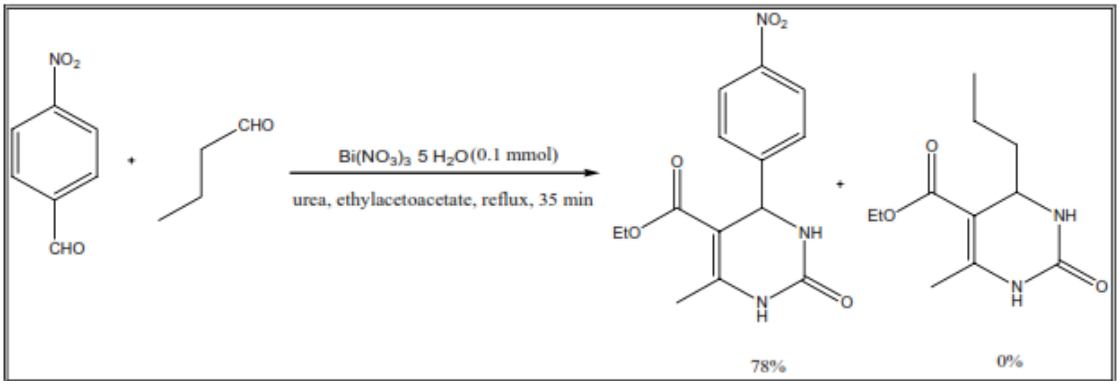


A straightforward and a very simple approach have been demonstrated by **Shailaja et al**[24] by the combination system of SnCl–LiCl which provides dihydropyrimidin-2-one system in excellent yield with high purity while retaining the simplicity of the Biginelli concept.

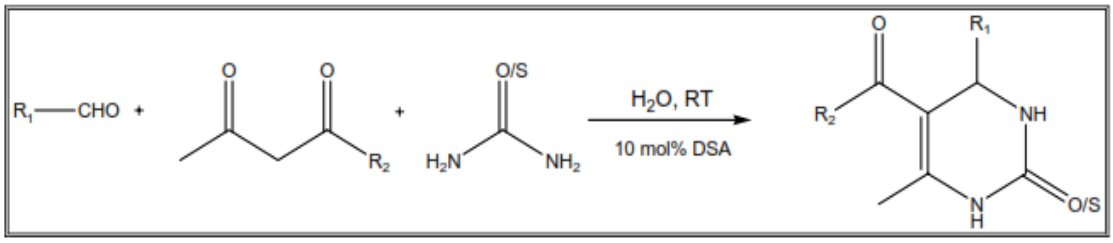
Under solvent free conditions $MgBr_2$ can be used for the three-component condensation reaction of aldehyde, β-diketone and urea/thiourea. The corresponding dihydropyrimidinones is obtained in high yields and the reaction time is also short i.e. around 45 to 90 minutes.[25]



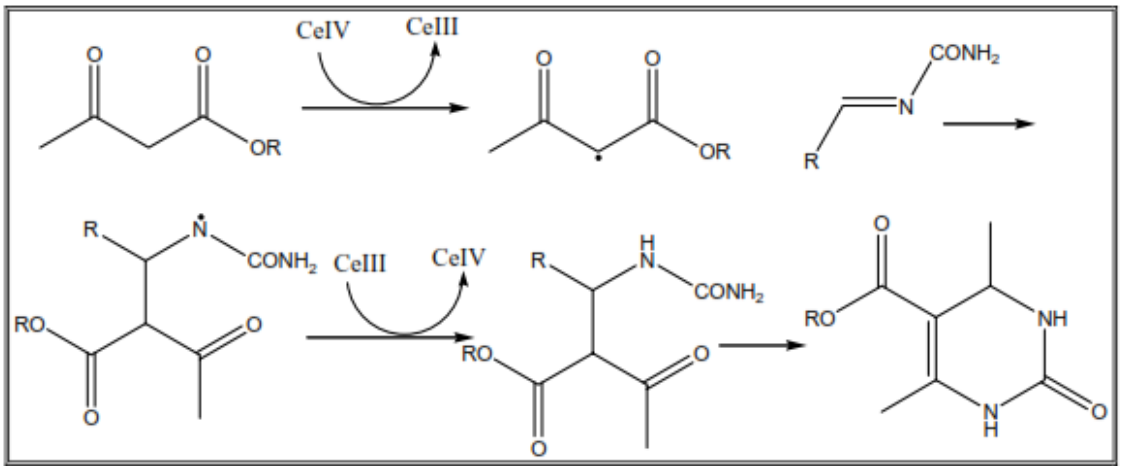
Under the solvent free conditions Bismuth nitrate pentahydrate catalyzes the three component condensation reaction of an aromatic aldehyde, urea and a β-ketoester or a β-diketone. The corresponding dihydropyrimidinones (DHPMs) is obtained in high yields. The method for the preparation is also effective for the selective condensation of aryl aldehydes in the presence of aliphatic aldehydes.[26]



Sharma et al[27] had developed a green, mild, and effective method for the synthesis of 3,4-dihydropyrimidin-2-ones employing dodecyl sulfonic acid (DSA) as a good surfactant-type Bronsted acid catalyst in an aqueous media. The temperature is also ambient.

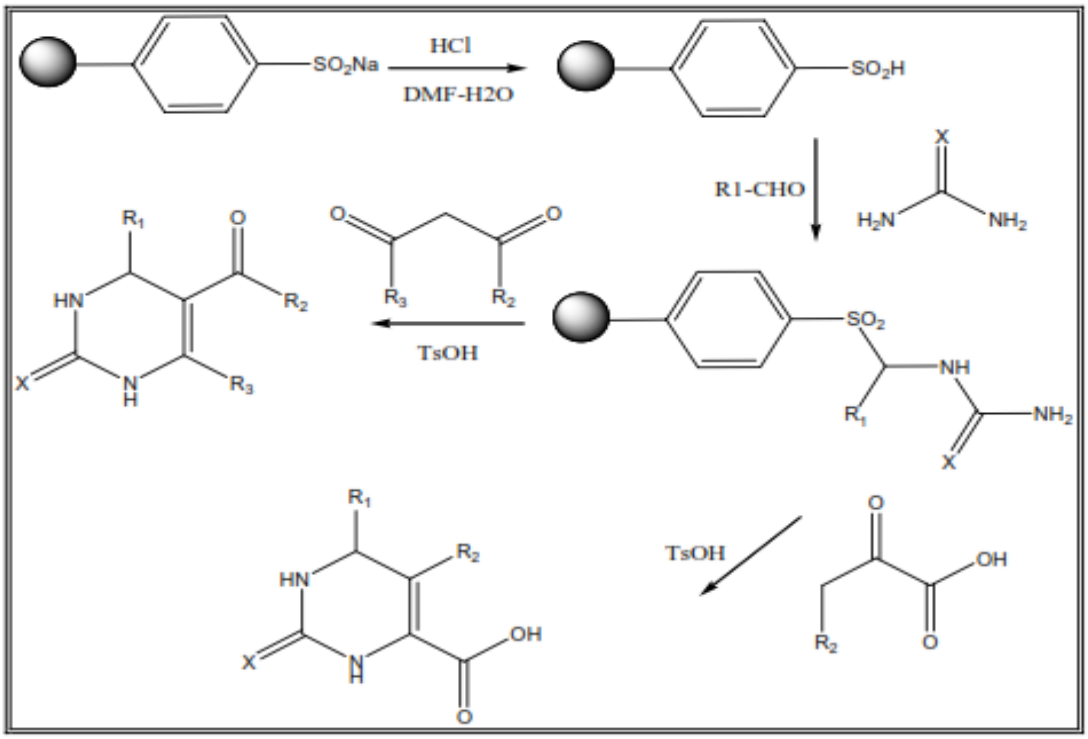


Yadav et al[28] reported that Ceric ammonium nitrate efficiently catalyzes the three component Biginelli reaction in methanol to afford the corresponding dihydropyrimidinones in excellent yields under sonication.

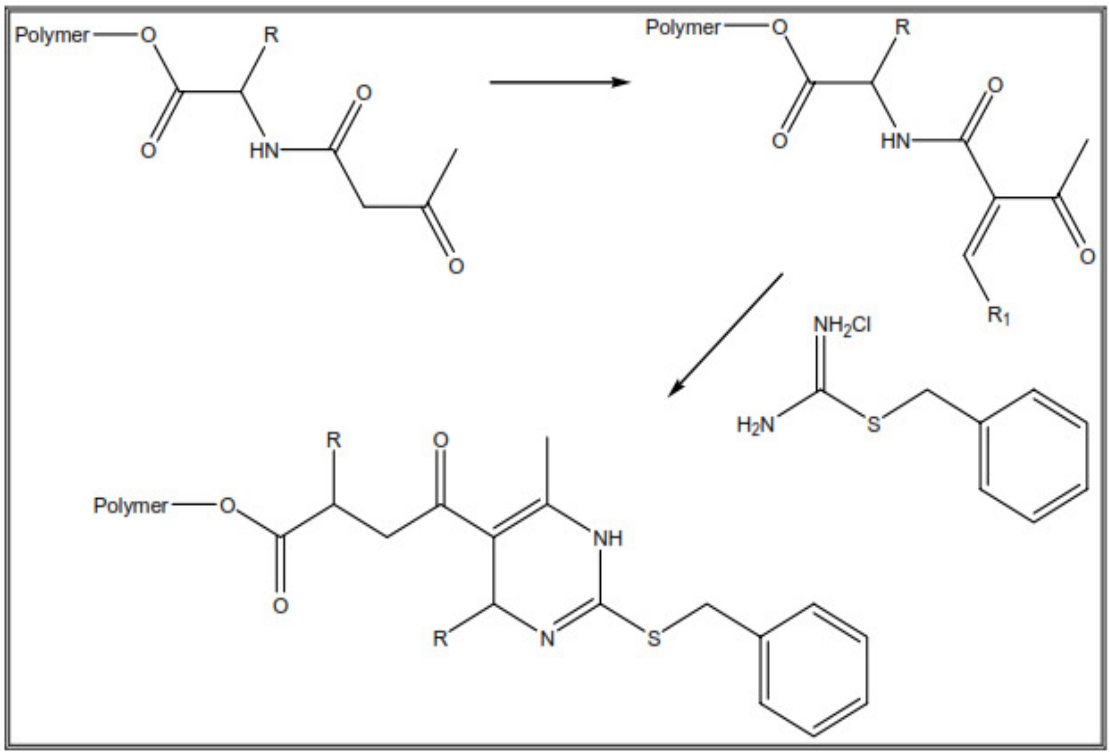


3.3.2 Solid Phase Synthesis

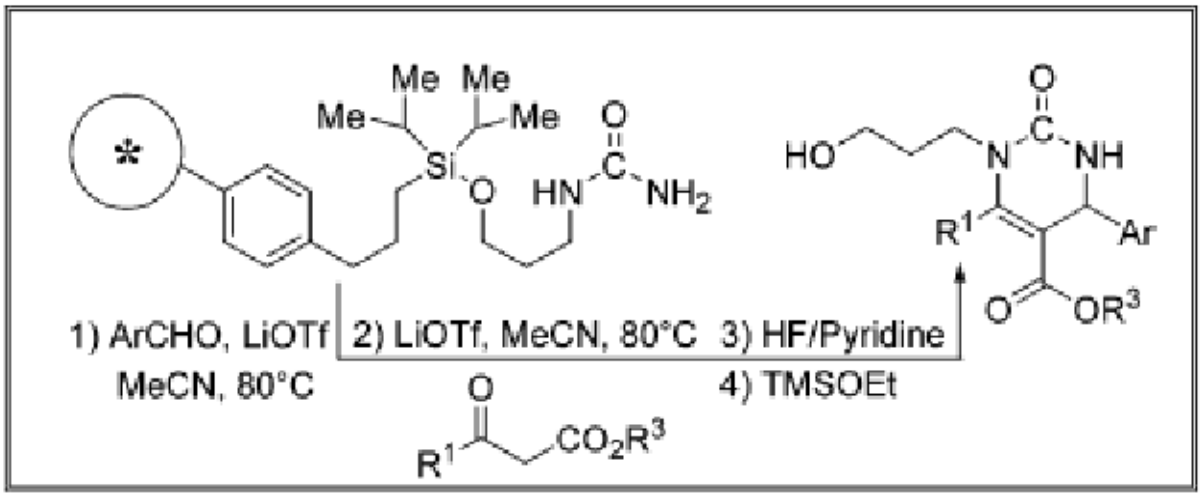
In recent years, the solid phase synthesis takes prime role in the synthesis of DHPM. Many methods have been studied for the synthesis of DHPM on solid phase. **Li and Lam**[29] demonstrated an appropriate solid-phase method to synthesize 3,4-dihydropyrimidine-2-ones with traceless product release. Primary steps in the synthesis are (i) sulfinate acidification, (ii) condensation of urea or thiourea with aldehydes and sulfinic acid and (iii) traceless product release by a one-pot cyclization-dehydration process. Since a variety of reagents can be used in steps (ii) and (iii), the overall strategy seems to be valid to library generation.



Very recently, **Gross et al**[30] established a protocol based on immobilized α-ketoamides to increase the diversity of DHPM. A small library using different building blocks can be generated using the resulting synthetic protocol. The expected DHPM derivatives with good quality and yield were found if aromatic aldehyde and α-ketoamide building blocks were used. The aliphatic aldehyde leads to mixture of DHPM (isomeric mixture of DHPM). There is no impact on purities and yields if thiourea was used in place of urea.

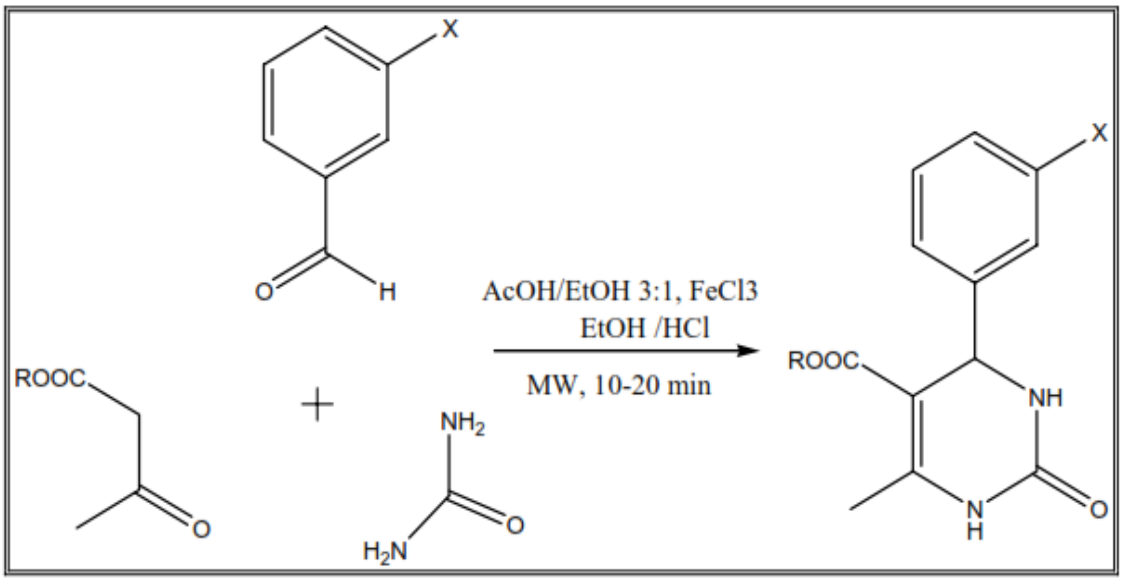


Lusch and Tallarico[31] developed a direct, Lewis acid-catalyzed Biginelli synthesis of 3,4-dihydropyrimidinoneson high-capacity polystyrene macro beads with a polymer O-silyl attached N-(3-hydroxypropyl)urea. They have prepared separately resin-urea by the reaction of either 4-bromo or 4-chlorobenzaldehyde or lithium trifluoro methanesulfonate in solvent like ACN at 80°C temperature. The beads were washed, pooled and reacted with ethyl acetoacetate. The process for the formation of only one kind of Biginelli product per bead established the feasibility of a solid-phase non-Atwal two-step split and pool synthesis of 3,4-dihydropyrimidinones.



3.3.3 Microwave assisted synthesis

In general, the Biginelli condensation involves the standard procedure like in solvent ethanol using strongly acidic catalyst like hydrochloric acid; one-pot condensation of the three building blocks generates DHPM. The classical reaction involves longer reaction time even after at reflux temperature that are the major drawbacks. Even at reflux temperature the moderate yields frequently observed when using more complex building blocks. Kappe et al by utilizing controlled single-mode microwave irradiation described a high yielding and rapid microwave-assisted protocol that allows the synthesis of gram quantities of DHPMs. They have performed the first model reaction for scale-up experiments; they selected the standard Biginelli cyclocondensation, where in a one-pot process equimolar amounts of benzaldehyde, ethyl acetoacetate, and urea react under Lewis acid (FeCl3) catalysis to the corresponding dihydropyrimidine. But by utilizing single-mode microwave irradiation, the reaction can be carried out on a 4.0 mmol scale in a mixture of acetic acid and ethanol (3:1) at 120˚C temperature within 10 minutes, compared to3 to 4 hours using conventional thermal heating, providing DHPM in 88% isolated yield and high purity (>98%).



One pot cyclocondensation reaction which provide high yield of dihydropyrimidines using various acid catalysts like Amberlyst 15, Nafion-H, KSF clay and dry acetic acid under microwave irradiation are reported.[33]

The Antimony (III) chloride on alumina efficiently catalyses a one-pot, three-component condensation reaction among an aldehyde, a β-ketoester, and urea or thioureato afford the corresponding dihydropyrimidinones in good to excellent yields. The reactions are probed in microwave (MW), ultrasonic, and thermal conditions and the best results are found using MW under solvent-free conditions.[34]

Cupric chloride dehydrate catalyzes the three-component Biginelli condensation between an aldehyde, an acetoacetic esters and urea or thiourea under microwave irradiation in the absence of solvent to yield various substituted 3,4-dihydropyrimidin-2(1*H*)-ones. The reaction is also effective when performed at room temperature in acetonitrile or at 100°C in a solvent free approach, without any side reactions as observed by Biginelli and others.[35]

The publications by **Gupta**[36] and **Dandia**[37] demonstrated 26 examples of microwave-enhanced solution-phase Biginelli reactions by using ethyl acetoacetate, (thio) ureas, and substituted or un-substituted aromatic aldehydes as building blocks. In an open glass beaker inside the cavity of a domestic microwave oven, upon irradiation of the individual reaction mixtures (ethanol, catalytic HCl) the reaction times were reduced from 2 to 24 hours of conventional heating (80ºC, reflux) to 3 to 11 minutes under microwave activation (*ca.*200–300 W).At the same time the yields of DHPMs obtained by the authors were markedly improved compared to those reported earlier using conventional conditions.

Kappe[38] reinvestigated above reactions using a purpose-built commercial microwave reactor with on-line temperature, pressure, and microwave power control. Transformations carried out under microwave heating at atmospheric pressure in ethanol solution show no rate or yield increase when the temperature is identical to conventional thermal heating. In the case of superheating by microwave irradiation at atmospheric pressure the observed yield and rate increase phenomenon are rationalized as a consequence of a thermal (kinetic) effect. Under sealed vessel conditions (20 bar, 180ºC) the yield of products is decreased and formation of various by-products observed. The only significant rate and yield enhancements are found when the reaction is performed under "open system" conditions where the solvent is allowed to rapidly evaporate during microwave irradiation. However, the observed rate and yield enhancements in these experiments are a consequence of the solvent-free conditions rather than caused specifically by microwave irradiation. This was confirmed by control experiments of the solvent less Biginelli reaction under microwave and thermal heating.

3.3.4 Ionic liquid

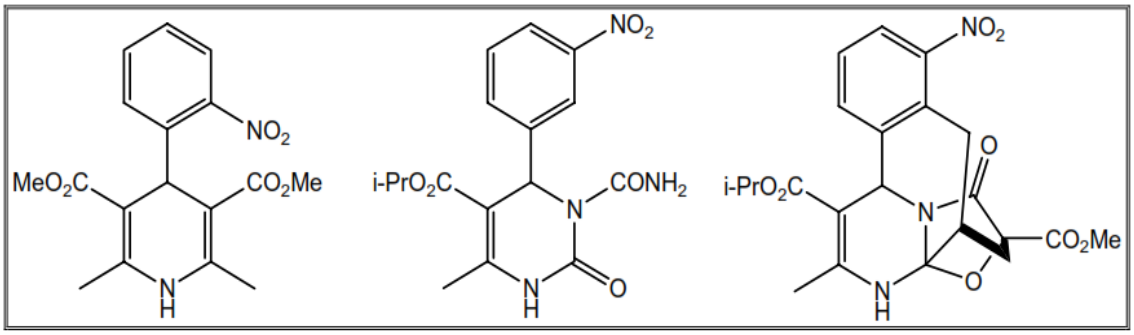
Wang et al[39] reported the Biginelli reaction between an aromatic aldehyde, ethyl acetoacetate and urea catalyzed by polymer-supported, re-usable, room temperature ionic liquids (RTIL) - was shown to efficiently proceed in glacial acetic acid at 100°C to afford

the corresponding pyrimidine-5-carboxylates in yields up to 99% within 2 hours. The catalyst(s) could be reused at least five times, basically without loss of activity, which makes this transformation not only straight-forward, but also considerably less expensive compared to methods involving classical RTIL catalysts.

3.4 Biologically active dihydropyrimidones

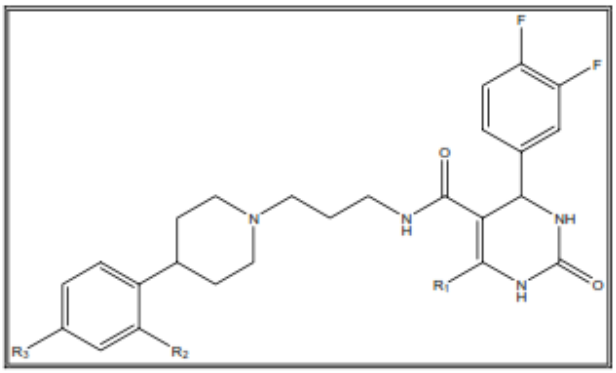
4-Aryl-1,4-dihydropyridines (DHPs, e.g nifedipine,) are the most studied class of organic calcium channel modulators. More than 30 years after the introduction of nifedipine, many DHP analogs have now been synthesized and numerous second-generation commercial products have appeared on the market.[40, 41]

In recent years, interest has also focused on aza-analogs such as dihydropyrimidines (DHPMs) which show a very similar pharmacological profile to classical dihydropyridine calcium channel modulators.[42-49] Over the past few years several lead-compounds were developed that are superior in potency and duration of antihypertensive activity to classical DHP drugs, and compare favorable with second-generation analogs such as amlodipine and nicardipine.[50]



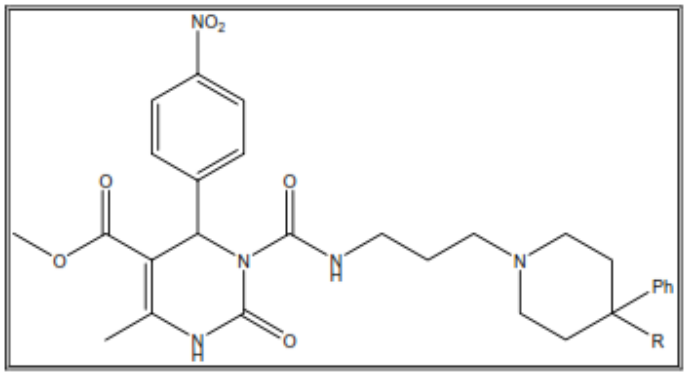
Barrow et al[55] reported *in vitro* and *in vivo* evaluation of dihydropyrimidinone C-5 amides as potent and selective α_{1a} Receptor Antagonists for the treatment of benign prostatic hyperplasia.

α_1 adrenergic receptors mediate both vascular and lower urinary tract tone, and a receptor antagonists such as terazosin are used to treat both hypertension and benign prostatic hyperplasia (BPH). Recently, three different subtypes of this receptor have been identified, with the α_{1a} receptor being most prevalent in lower urinary tract tissue. 4-aryl-dihydropyrimidinones attached to an aminopropyl-4-arylpiperidine via a C-5 amide as selective α_{1a}receptor subtype antagonists. In receptor binding assays, these types of compounds generally display *K*i values for the α_{1A} receptor subtype <1 nM while being greater than 100-fold selective versus the α_{1b} and α_{1d}receptor subtypes. Many of these compounds were also evaluated *in vivo* and found to be more potent than terazosin in both a rat model of prostate tone and a dog model of intra-urethral pressure without significantly affecting blood pressure. While many of the compounds tested displayed poor pharmacokinetics, one compound was found to have adequate bioavailability (>20%) and half-life (>6 h) in both rats and dogs. Due to its selectivity for the α_{1a}over the α_{1b} and α_{1d} receptors as well as its favorable pharmacokinetic profile, it has the potential to relieve the symptoms of BPH without eliciting effects on the cardiovascular system.[51-55]

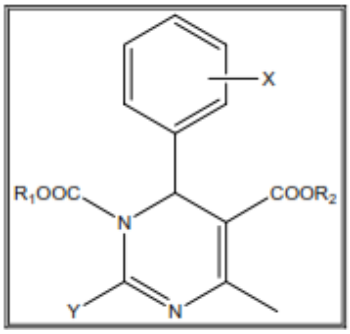


The 4-aryldihydropyrimidinone heterocycle attached to an aminopropyl-4-arylpiperidine via a C-5 amide has proved to be an excellent template for selective α_{1a} receptor subtype antagonists. These types of compounds are exceptionally potent in both cloned receptor binding studies as well as in *in vivo* pharmacodynamic models of prostatic tone.

Compounds exhibited high binding affinity and subtype selectivity for the cloned human α_{1A} receptor. Systematic modifications led to identification of highly potent and sub type selective compounds with high binding affinity (*K*i =0.2 nM) for α_{1a} receptor and greater than 1500-fold selectivity over α_{1b} and α_{1d} adrenoceptors. The compounds were found to be functional antagonists in human, rat, and dog prostate tissues. They exhibited excellent selectively to inhibit intraurethral pressure (IUP) as compared to lowering diastolic blood pressure (DBP) in mongrel dogs (*K*b(DBP)/*K*b(IUP) suggesting uroselectivity for a selective compounds.[56]



Cho et al[57] reported 3-N-substituted-3,4-dihydropyrimidine and 3-N-substituted dihydropyrimidin-2(1H)-ones as calcium channel antagonist. Compounds [especially [R_1=$(CH_2)_2$N(benzyl)(2-naphthylmethyl), R_2=i-Pr, X=2-NO_2] and [R'= $(CH_2)_2$N(benzyl)(3,4-dichlorobenzyl), R_2=i-Pr, X=2-NO]] exhibited not only more potent and longer lasting vasodilative action but also a hypertensive activity with slow onset as compared with dihydropyridines. Moreover, some dihydropyrimidines [R'= $(CH_2)_2$N(benzyl)(3-phenylpropyl), R_2=CH_2(cyclopropyl), X=2-NO_2] were weaker in blocking atrioventricular conduction in anesthetized open-chest dogs and less toxic than the dihydropyridines.



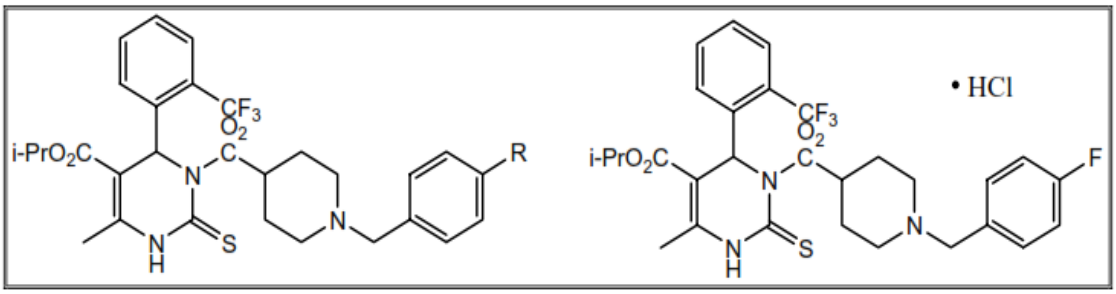
Atwal et al[58] examined a series of novel dihydropyrimidine calcium channel blockers that contain a basic group attached to either C_5 or N_3 of the heterocyclic ring. Structure activity studies show that l-(phenylmethyl)-4-piperidinyl carbamate moiety at N_3 and sulfur at C_2 are optimal for vmrelaxant activity in vitro and impart potent and long-acting antihypertensive activity *in vivo*. One of the compounds was identified as a lead, and the individual enantiomers were synthesized.

Two key steps of the synthesis were:

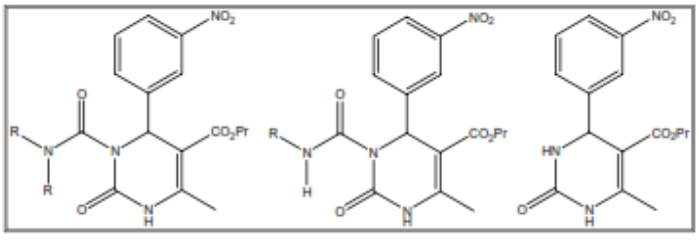
(1) The efficient separation of the diastereomeric ureido derivatives and

(2) The high-yield transformation of 2-methoxy intermediate to the (p-methoxybenzy1)thio intermediates.

Chirality was demonstrated to be a significant determinant of biological activity, with the dihydropyridine receptor recognizing the enamino ester moiety but not the carbamate moiety. Dihydropyrimidine is equipotent to nifidepine and amlodipine *in vitro*. In the spontaneously hypertensive rat, dihydropyrimidine is both more potent and longer acting than nifidepine and compares most favorably with the long-acting dihydropyridine derivative amlodipine. Dihydropyrimidine has the potential advantage of being a single enantiomer.

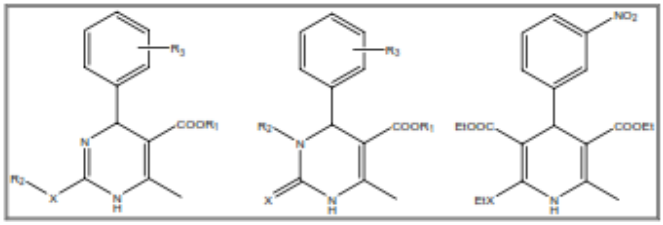


In order to explain the potent antihypertensive activity of the modestly active (ICw = 3.2pM) dihydropyrimidine calcium channel blocker**, Atwal et al**[59] carried out drug metabolism studies in the rat and found itis metabolized. Two of the metabolites (ICw =16 nM) and (ICw = 12 nM), were found to be responsible for the antihypertensive activity of compound**.** Potential metabolism *in vivo* precluded interest in pursuing compounds related to it. Structure-activity studies aimed at identifying additional aryl-substituted analogues led to comparable potential *in vivo*, though these compounds were less potent *in vitro*. To investigate the effects of absolute stereochemistry on potency, authors resolved via diastereomericureas, prepared by treatment with (R)-α-methylbenzylamine. The results demonstrate that the active R-(-)-enantiomer is both more potent and longer acting than nifedipine as an antihypertensive agent in the SHR. The *in vivo* potency and duration is comparable to the long-acting dihydropyridine amlodipine. The superior oral antihypertensive activity compared to that of previously described carbamates could be explained by its improved oral bioavailability, possibly resulting from increased stability of the urea functionality.

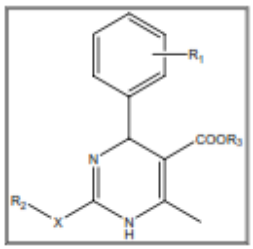


Atwal et al[60] modified the structure of previously described dihydropyrimidine i.e. 3-substituted 1,4-dihydropyrimidines**.** Structure activity studies using potassium

depolarized rabbit aorta show that ortho, meta-disubstituted aryl derivatives are more potent than either ortho- or meta- monosubstituted compounds. While vasorelaxant activity was critically dependent on the size of the C_5ester group, isopropyl ester being the best, a variety of substituents (carbamate, acyl, sulfonyl, alkyl) were tolerated at N_3. The results show dihydropyrimidines are significantly more potent than corresponding 2-heteroalkyl-l,4-dihydropyrimidinesand only slightly less potent than similarly substituted 2-heteroalkyl-1-4-dihydropyridines. Whereas dihydropyridine enantiomer usually show 10-15-fold difference in activity, the enantiomers of dihydropyrimidine show more than a 1000-fold difference in activity. These results strengthen the requirement of an enamino ester for binding to the dihydropyridine receptor and indicate a nonspecific role for the N_3-substituent.



2-Heterosubstituted-4-aryl-l,4-dihydro-6-methyl-5-pyrimidinecarboxylicdiesters, which lack the potential symmetry of dihydropyridine calcium channel blockers, were prepared and evaluated for biological activity. Biological assays using potassium-depolarized rabbit aorta and radio ligand binding techniques showed that some of these compounds are potent mimics of dihydropyridine calcium channel blockers.[60]



3.5 Current work

Pyrimidine-2-thione and pyrimidin-2-one have been synthesized by using indanone derivatives[61]. Substituted indanone derivatives are used to synthesize pyrimidine derivatives. Multicomponent condensation reactions (MCRs) have been discovered to be a powerful method for the synthesis of organic compounds, since the products are formed in a single step and diversity can be achieved by simply varying each component. The original Biginelli protocol for the preparation of the DHMPs consisted of heating a mixture of the three components (aldehyde, β-keto-ester, and urea) in ethanol containing a catalytic amount of HCl. This procedure leads in one step-one pot to the desired DHPM. The major drawback associated with this protocol is the low yields, particularly for substituted aromatic and aliphatic aldehydes.

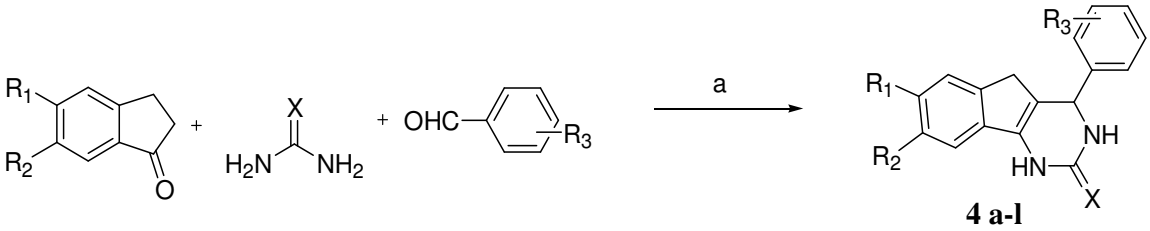
We have used antimony(III)chloride ($SbCl_3$) in the synthesis of pyrimidine-2-thione and pyrimidin-2-one derivatives to provide better results with more sterically hindered substrates with high yield. $SbCl_3$ is inexpensive, easy to handle on large scale. In view of the above observation, we wish to report herein biologically active heterocyclic systems containing pyrimidine-2-thione and pyrimidin-2-one derivatives. Herein antimony (III) chloride ($SbCl_3$) catalyst was significantly more effective than other acid catalyst in the Biginelli reaction of cyclic ketones and it provides better results with more sterically hindered substrates with high yields.

Current work describes the synthesis of pyrimidine-2-thione and pyrimidin-2-one derivatives by using substituted or un-substituted indanone derivatives using antimony(III)chloride ($SbCl_3$). In the Section 3.6 reaction scheme shows the synthesis of pyrimidine-2-thione and pyrimidin-2-one by reacting substituted or un-substituted indanone, urea/thiourea and aromatic aldehyde. The reaction time was observed 8-12 hours depending on the substitution on substitutions on 4-phenyl ring. The synthesized compounds are characterized by IR, NMR and Massspectral analysis and are screening against antibacterial activity against *Escherichia coli (E.coli),Pseudomonas aeruginosa (P.aeruginosa)*, *Staphylococcus aureus (S.aureus), Streptococcus pyogenes (S.pyogenes)*

as well as antifungal activity against and *Candida albicans (C.albicans)*. Physical data of synthesized compounds is reported in Section 3.8, spectral data is discussed in Section 3.9 and biological activity of synthesized compounds is reported in Section 3.10.

3.6 Reaction Schemes

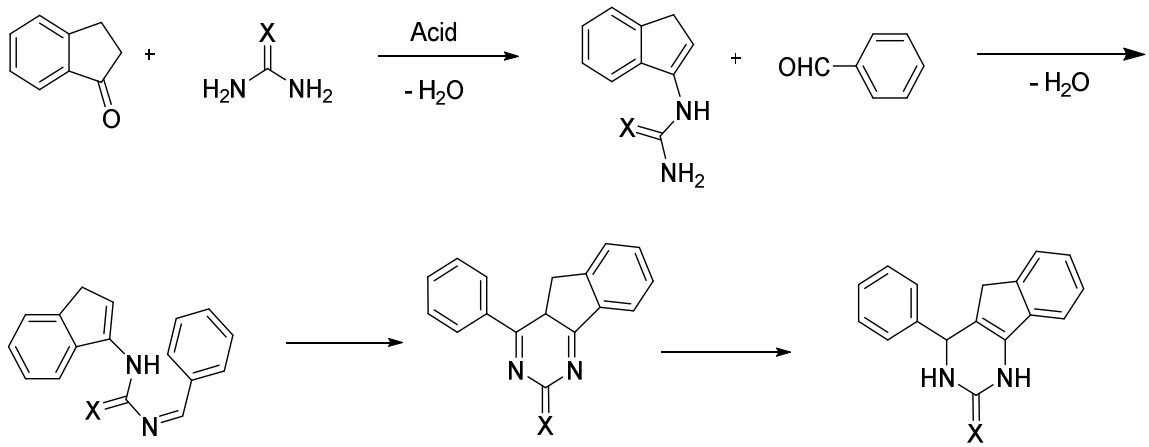
3.6.1 Synthesis of pyrimidine-2-thione and pyrimidin-2-one



4 a-l

Reagent and conditions: a = Acetonitrile, Antimony(III)chloride (20 mol %), reflux.

Reaction Mechanism



3.7 Experimental

To a mixture of cyclic ketone (1mmol), urea or thiourea (1.5 mmol) and aldehyde (1mmol) in acetonitrile, antimony(III)chloride (20 mol %) was added and content was refluxed for 8-12 hours. After completion of the reaction as monitored by thin layer

chromatography, the reaction mixture is poured into ice-cold water and stirred for 10-15 minutes. The content of the flask were then filtered and washed with cold water (20 ml) to remove excess urea or thiourea. The solid so obtained was the corresponding pyrimidine-2-thione and pyrimidin-2-one. It was then recrystallized by hot ethanol to get the pure product. (Physical data is given in Table 3.8.1)

3.8 Physical data

3.8.1 Physical data of 4-(substitutedphenyl)-1,3,4,5-tetrahydro-substitutedindeno[1,2-d]pyrimidine-2-thione or pyrimidin-2-one [compounds 4a-l].

Compound	R_1	R_2	R_3	X	m.p. °C	Yield %	M.F. (M.Wt.)	Elemental analysis (calcd./ found)		
								%C	%H	%N
4a	H	H	H	S	220-223	75	$C_{17}H_{14}N_2S$ 278.37	73.35 73.30	5.07 5.15	10.06 10.17
4b	H	H	H	O	256-259	82	$C_{17}H_{14}N_2O$ 262.31	77.84 77.75	5.38 5.25	10.68 10.74
4c	H	NO_2	4-Br	S	290-293	85	$C_{17}H_{12}BrN_3O_2S$ 402.27	50.76 50.65	3.01 3.00	10.45 10.52
4d	H	NO_2	4-Cl	S	>300	86	$C_{17}H_{12}ClN_3O_2S$ 357.81	57.06 57.14	3.38 3.25	11.74 11.62
4e	OCH_3	OCH_3	H	S	>300	80	$C_{19}H_{18}N_2O_2S$ 338.42	67.43 67.36	5.36 5.47	8.28 8.32
4f	OCH_3	OCH_3	4-OCH_3	S	>300	90	$C_{20}H_{20}N_2O_3S$ 368.45	65.20 65.34	5.47 5.35	7.60 7.72
4g	OCH_3	OCH_3	2-Cl	S	>300	81	$C_{19}H_{17}ClN_2O_2S$ 372.87	61.20 61.34	4.60 4.50	7.51 7.61

4h	OCH_3	OCH_3	3-OCH_3	S	>300	79	$C_{20}H_{20}N_2O_3S$ 368.45	65.20 65.11	5.47 5.60	7.60 7.68
4i	OCH_3	OCH_3	H	O	244-246	72	$C_{19}H_{18}N_2O_3$ 322.36	70.79 70.81	5.63 5.68	8.69 8.65
4j	OCH_3	OCH_3	4-OCH_3	O	256-257	69	$C_{20}H_{20}N_2O_4$ 352.38	68.17 68.22	5.72 5.74	7.95 7.93
4k	H	H	4-Cl	O	265-266	71	$C_{17}H_{13}ClN_2O$ 296.75	68.81 68.79	4.42 4.45	9.44 9.45
4l	OCH_3	OCH_3	3-OCH_3	O	236-237	74	$C_{20}H_{20}N_2O_4$ 352.38	68.17 68.20	5.72 5.69	7.95 7.97

TLC solvent system: Compound 4a-l (EA:Hexane) 3:7

MPs were taken in open capillary and are not corrected

3.9 Spectral discussion

3.9.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. The DHPMs having chlorine atom showed this characteristic peak. Fragmentation pattern can be observed to be particular for this kind of compounds and the characteristic peaks obtained for each compound. Various characteristic peaks obtained for each compound in this series can be discussed as below.

3.9.2 IR spectral study

Various functional groups present in molecule were identified by characteristic frequency obtained for them. Presence of carbonyl group can be confirmed by IR spectra because carbonyl stretching frequency was observed for carbonyl group present in the moiety. C=O group (NH-CO-) was observed between 1655-1700 cm^{-1} and C=S groups was observed between 1150-1200$cm^{-1}$for (thioketone group). Two N-H groups gave peaks between 3210-3380 cm^{-1}. Peaks were identified for aromatic and alkyl group as per their characteristics. In case of compounds having different substations on aromatic ring, characteristic frequencies were observed depending on the functional group present i.e. nitro, hydroxyl, chloro, methoxy etc.

3.9.3 ^{1}H NMR spectral study

Numbers of proton identified from NMR spectrum and their chemical shift (ppm) wereinagreement of structure of molecule. Methoxy protons were observed at 3.8δ ppm.Proton on C-4 carbon atom gave singlet at 5.4-6.2 δ ppm. Aromatic protons were observed between 6.9-7.9 δ ppm. J values were calculated to identify ortho and metacoupling. In some cases, aromatic protons were obtained as multiplet.

3.9.4 Elemental analysis

Elemental analysis showed calculated and found percentage values of carbon, hydrogen and nitrogen in support of structure of synthesized compounds. The spectral and elemental analysis data are given below for individual compounds.

3.9.5 Spectral data of synthesized compounds (4a-l)

4-Phenyl-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidine-2-thione (4a). White solid, (75 %), m.p. 220-223 °C, Anal.Calcd for $C_{17}H_{14}N_2S$: C 73.35, H 5.07, N 10.06% Found: C

73.30, H 5.15, N 10.17%. IR (KBr, cm^{-1}): 3311, 3229 (2NH), 3010, 3024 (ArC-H), 1611 (C=C), 1188 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.73-2.80 (d, 1H, CH), 3.21-3.29 (d, 1H, CH), 5.58 (s, 1H, CH), 7.10-7.59 (m, 9H, Ar-H), 7.75 (s, 1H, NH), 9.83 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-*d_6*) δ: 26.9 (CH_2), 60.4 (CH), 103.7, 122.1, 123.1, 124.0, 127.4, 128.2, 128.8, 135.2, 135.9, 139.5, 141.7, 142.4 (Ar-C), 179.4 (C=S), MS: (M+1) 279.09.

4-Phenyl-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidine-2-one (4b). Off-white solid, (82 %), m.p. 256-259 °C, Anal.Calcd for $C_{17}H_{14}N_2O$: C 77.84, H 5.38, N 10.68% Found: C 77.75, H 5.25, N 10.74%. IR (KBr, cm^{-1}): 3312, 3230 (2NH), 3010, 3024 (ArC-H), 1611 (C=C), 1688 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.76-2.83 (d, 1H, CH), 3.19-3.26 (d, 1H, CH), 5.50 (s, 1H, CH), 7.0-7.55 (m, 9H, Ar-H), 7.82 (s, 1H, NH), 9.74 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-*d_6*) δ: 28.2 (CH_2), 62.4 (CH), 105.6, 125.1, 126.3, 126.8, 127.0, 127.4, 128.5, 128.8, 129.3, 137.3, 137.5, 141.7 (Ar-C), 162.5 (C=O), MS: (M-1) 261.25.

4-(4-Bromo-phenyl)-8-nitro-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidine-2-thione (4c). Yellowsolid, (85 %), m.p. 290-293 °C, Anal.Calcd for $C_{17}H_{12}BrN_3O_2S$: C 50.76, H 3.01, N 10.45% Found: C 50.65, H 3.00, N 10.52%. IR (KBr, cm^{-1}): 3323, 3260 (2NH), 2951 (ArC-H), 1614 (C=C), 1530, 1350 (N-O), 1183 (C=S), 592 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.73-2.8 (d, 1H, CH), 3.21-3.28 (d, 1H, CH), 5.43 (s, 1H, CH), 7.1 (dd, 2H, Ar-H), 7.27 (d, 1H, Ar-H), 7.4 (dd, 2H, Ar-H), 7.7 (s, 1H, NH),7.9 (dd, 1H, Ar-H), 8.1 (dd, 1H, Ar-H), 9.78 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-d_6) δ: 27.3 (CH_2), 59.7 (CH), 104.2, 122.1, 122.3, 124.5, 128.2, 128.6, 129.9, 135.4, 139.5, 141.7, 142.1, 146.4 (Ar-C), 175.4 (C=S), MS: (M+1) 403.98.

4-(4-Chloro-phenyl)-8-nitro-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidine-2-thione (4d). Off-white solid, (86 %), m.p. >300 °C, Anal.Calcd for $C_{17}H_{12}ClN_3O_2S$: C 57.06, H 3.38, N 11.74% Found: C 57.14, H 3.25, N 11.62%. IR (KBr, cm^{-1}): 3310 (NH), 2959 (ArC-H), 1618 (C=C), 1527, 1344 (N-O), 775 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.70-2.82 (d, 1H, CH), 3.18-3.26 (m, 1H, CH), 5.39 (s, 1H, CH), 7.14-7.20 (m, 2H, Ar-H), 7.42-7.48 (m, 2H, Ar-H), 7.70 (s, 1H, CH), 7.89 (d, 2H, Ar-H), 9.72 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-d_6) δ: 27.1 (CH_2), 58.2 (CH), 103.9, 121.1, 121.3, 124.7,

128.6, 128.8, 130.3, 137.4, 139.6, 141.9, 142.3, 145.9 (Ar-C), 177.6 (C=S), MS: (M+1) 357.6.

7,8-Dimethoxy-4-phenyl-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidine-2-thione (4e). White solid, (80 %), m.p. >300 °C, Anal.Calcd for $C_{19}H_{18}N_2O_2S$: C 67.43, H 5.36, N 8.28% Found: C 67.36, H 5.47, N 8.32%. IR (KBr, cm^{-1}): 3312, 3249 (2NH), 2965 (ArC-H), 1622 (C=C), 1238 & 1046 (OCH_3), 1182 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.71-2.83 (d, 1H, CH), 3.16-3.26 (d, 1H, CH), 3.90 (s, 6H, OCH_3), 5.38 (s, 1H, CH), 6.89 (s, 1H, Ar-H), 7.09 (s, 1H, Ar-H), 7.23-7.61 (m, 5H, Ar-H), 7.79 (s, 1H, NH), 9.78 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-d_6) δ: 27.8 (CH_2), 56.5 (OCH_3), 60.9 (CH), 103.8, 123.7, 116.4, 125.7, 127.3, 128.4, 128.7, 128.9, 139.6, 141.8, 144.6, 146.4 (Ar-C), 175.9 (C=S), MS: (M+1) 339.11.

7,8-Dimethoxy-4-(4-methoxy-phenyl)-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidine-2-thione (4f). White solid, (90 %), m.p. >300 °C, Anal.Calcd for $C_{20}H_{20}N_2O_3S$: C 65.20, H 5.47, N 7.60% Found: C 65.34, H 5.35, N 7.72%. IR (KBr, cm^{-1}): 3312, 3249 (2NH), 2965 (ArC-H), 1622 (C=C), 1238 & 1046 (OCH_3), 1182 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.71-2.83 (d, 1H, CH), 3.16-3.26 (d, 1H, CH), 3.80 (s, 3H, OCH_3), 3.90 (s, 6H, OCH_3), 5.38 (s, 1H, CH), 6.89 (s, 1H, Ar-H), 7.09 (s, 1H, Ar-H), 7.33-7.62 (m, 4H, Ar-H), 7.79 (s, 1H, NH), 9.78 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-d_6) δ: 27.3 (CH_2), 55.7 (OCH_3), 56.5 (OCH_3), 60.8 (CH), 103.8, 113.4, 114.2, 116.4, 128.6, 128.7, 128.9, 135.6, 141.8, 143.6, 146.4, 159.8 (Ar-C), 175.9 (C=S).

4-(2-Chloro-phenyl)-7,8-dimethoxy-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidine-2-thione (4g). Off-white solid, (81 %), m.p. >300 °C, Anal.Calcd for $C_{19}H_{17}ClN_2O_2S$: C 61.20, H 4.60, N 7.51% Found: C 61.34, H 4.50, N 7.61%. IR (KBr, cm^{-1}): 3325, 3252 (2NH), 2965 (ArC-H), 1620 (C=C), 1239 & 1056 (OCH_3), 1152 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.68-2.79 (d, 1H, CH), 3.11-3.19 (d, 1H, CH), 3.90 (s, 6H, OCH_3), 5.38 (s, 1H, CH), 6.79 (s, 1H, Ar-H), 7.02 (s, 1H, Ar-H), 7.29-7.65 (m, 4H, Ar-H), 7.89 (s, 1H, NH), 9.78 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-d_6) δ: 27.3 (CH_2), 56.3 (OCH_3), 59.8 (CH), 104.4, 114.2, 116.4, 126.5, 127.2, 128.3, 128.7, 128.9, 131.4, 139.6, 141.8, 145.2, 146.9 (Ar-C), 177.9 (C=S).

7,8-Dimethoxy-4-(3-methoxy-phenyl)-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidine-2-thione (4h). White solid, (79 %), m.p. >300 °C, Anal.Calcd for $C_{20}H_{20}N_2O_3S$: C 65.20, H 5.47, N 7.60% Found: C 65.11, H 5.60, N 7.68%. IR (KBr, cm^{-1}): 3334, 3241 (2NH), 2955 (Ar-H), 1630 (C=C), 1240 & 1037 (OCH_3), 1183 (C=S). ^1H NMR (400 MHz, DMSO-d_6): δ 2.77-2.8 (d, 1H, CH), 3.11-3.23 (d, 1H, CH), 3.73 (s, 3H, OCH_3), 3.88 (s, 6H, OCH_3), 5.29 (s, 1H, CH), 6.77 (s, 1H, Ar-H), 6.97 (s, 1H, Ar-H), 7.3-7.52 (m, 4H, Ar-H), 7.81 (s, 1H, NH), 9.0 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-d_6) δ: 27.6 (CH_2), 55.7 (OCH_3), 56.4 (OCH_3), 58.8 (CH), 104.2, 113.2, 114.2, 115.4, 120.7, 128.7, 128.9, 129.2, 141.8, 142.3, 146.2, 146.4, 160.8 (Ar-C), 177.1 (C=S).

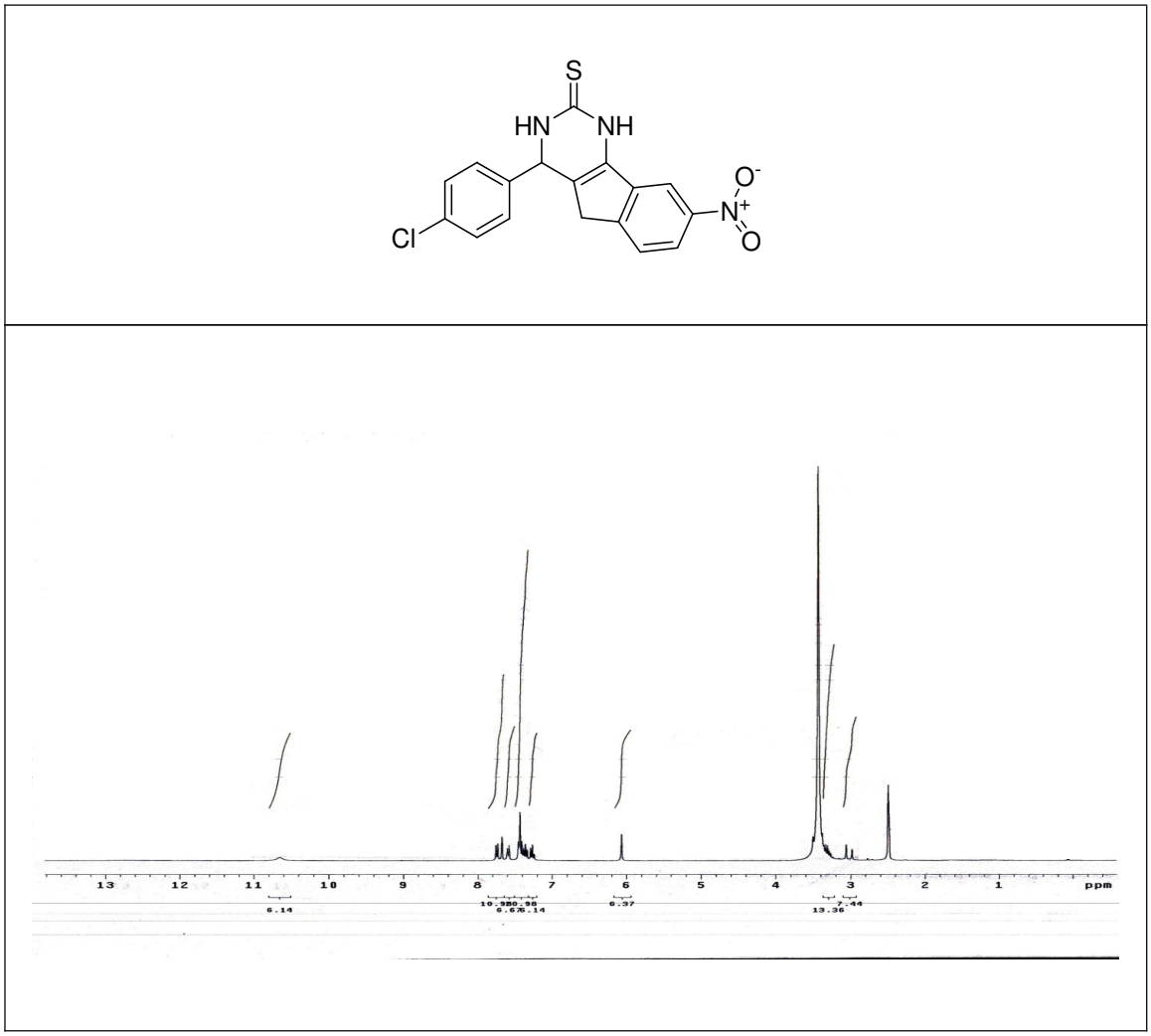
7,8-Dimethoxy-4-phenyl-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidin-2-one (4i). Creamish solid, (72 %), m.p. 244-246 °C, Anal.Calcd for $C_{19}H_{18}N_2O_3$ C 70.79, H 5.63, N 8.69% Found: C 70.81, H 5.68, N 8.65%.IR (KBr, cm^{-1}): 3419, 3241 (-NH-), 3012 (Ar-H), 1690 (C=O), 1244& 1039 (OCH_3).

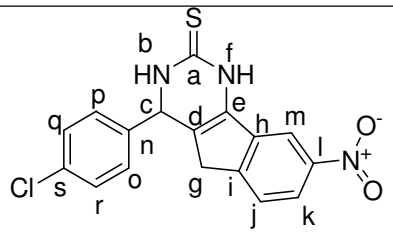
7,8-Dimethoxy-4-(4-methoxy-phenyl)-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidin-2-one (4j). Off-white solid, (69 %), m.p. 256-257 °C, Anal.Calcd for $C_{20}H_{20}N_2O_4$ C 68.17, H 5.72, N 7.95% Found: C 68.22, H 5.74, N 7.93%.IR (KBr, cm^{-1}): 3390, 3231 (-NH-),3009 (Ar-H), 1685 (C=O), 1247& 1034 (OCH_3).

4-(4-Chloro-phenyl)-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidin-2-one(4k). Off-white solid, (71 %), m.p. 265-266 °C, Anal.Calcd for $C_{17}H_{13}ClN_2OC$ 68.81, H 4.42, N 9.44% Found: C 68.79, H 4.45, N 9.45%.IR (KBr, cm^{-1}): 3395, 3234 (-NH-),3013 (Ar-H), 1683 (C=O), MS: (M+1) 296.8.

7,8-Dimethoxy-4-(3-methoxy-phenyl)-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidin-2-one (4l). Off-white solid, (74 %), m.p. 236-237 °C, Anal.Calcd for $C_{20}H_{20}N_2O_4$ C 68.17, H 5.72, N 7.95% Found: C 68.20, H 5.69, N 7.97%.IR (KBr, cm^{-1}): 3392, 3235 (-NH-), 3011 (Ar-H), 1692 (C=O), 1245& 1034 (OCH_3).

3.9.5.1 ^{1}H NMR spectrum of 4d



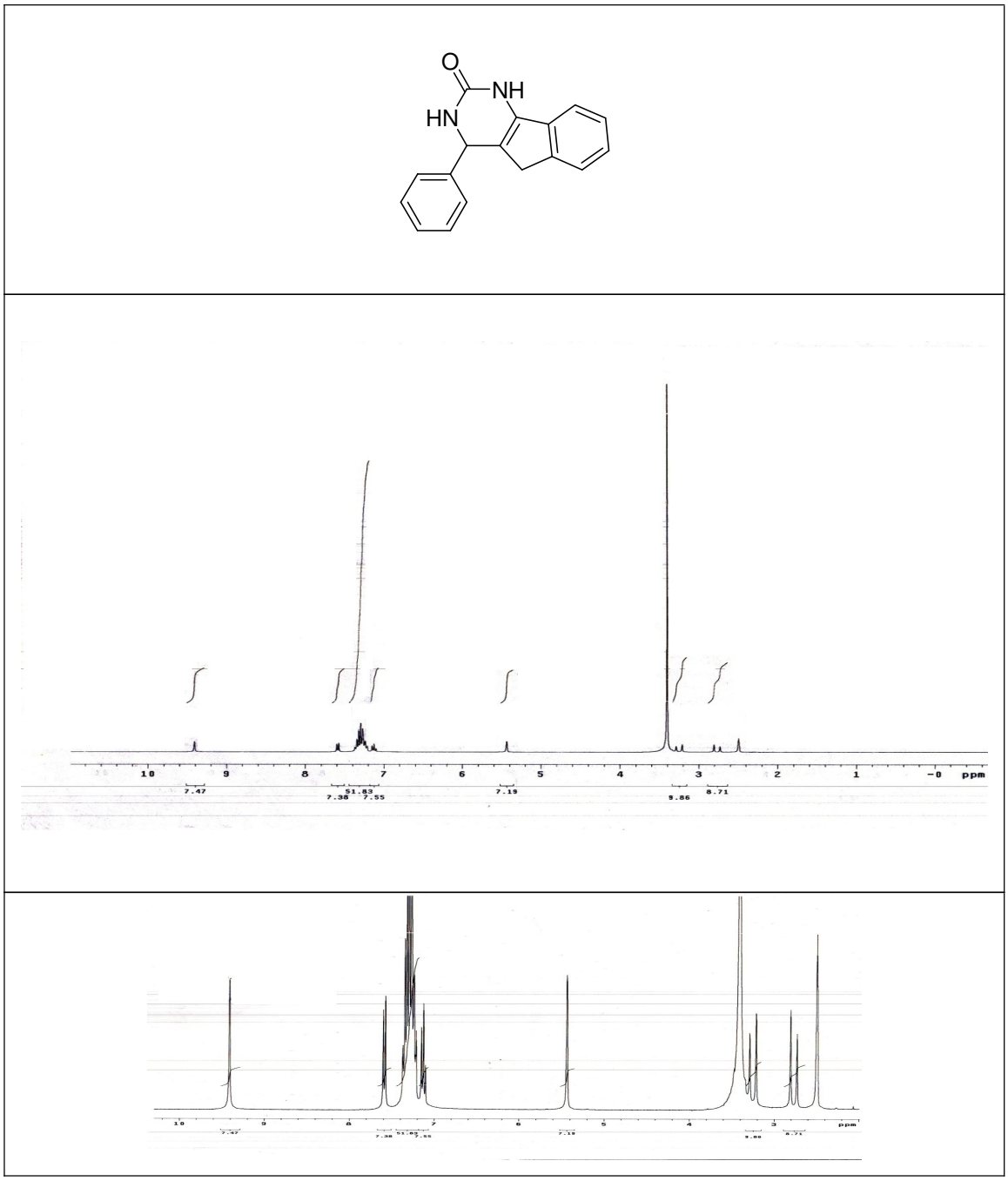


4-(4-Chloro-phenyl)-8-nitro-1,3,4,5-tetrahydro-indeno[1,2-d]pyrimidine-2-thione
Compound-4d

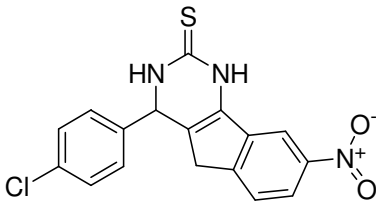
Assignment of ^{1}H NMR:

Sr.No.	Signal (δppm)	No. of Protons	Multiplicity	Assignment
1	2.70-2.82 3.18-3.26	2H	doublet	g
2	5.39	1H	singlet	c
3	7.14-7.20	2H	multiplet	o,p
4	7.42-7.48	2H	multiplet	q, r
5	7.70	1H	singlet	m
6	7.89	2H	doublet	j, k
7	9.72	1H	singlet	f

3.9.5.2 ^{1}H NMR spectrum of 4b

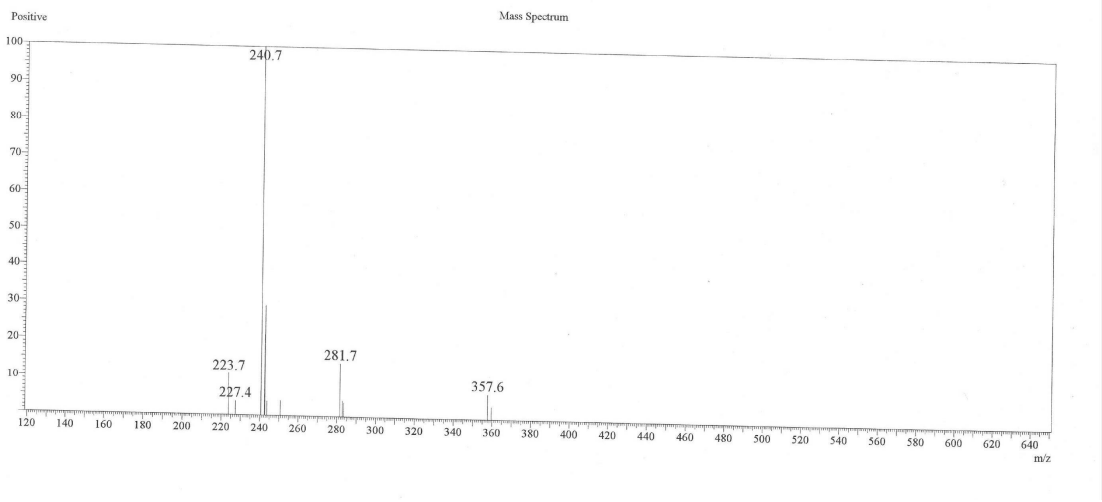


3.9.5.3 Mass Spectrum of 4d

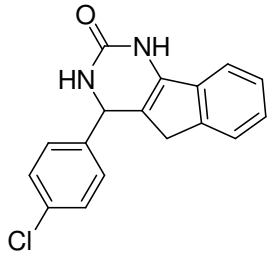


$C_{17}H_{12}ClN_3O_2S$
m/e.: 357.03

Mass (m/z): 357.6 (M+1)

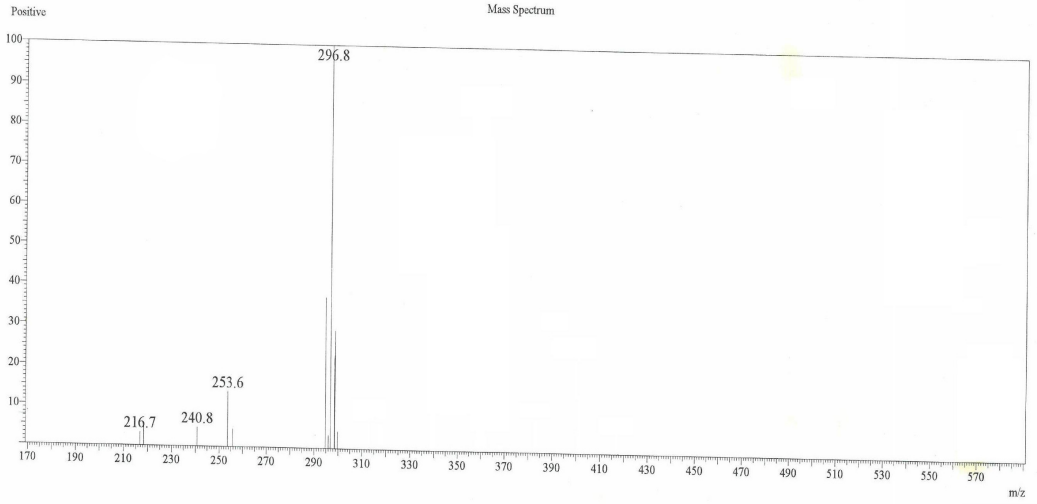


3.9.5.4 Mass spectrum of 4k

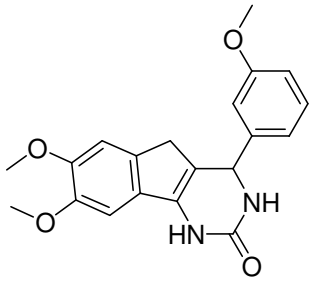


$C_{17}H_{13}ClN_2O$
m/e.: 296.07

Mass (m/z): 296.8 (M+1)

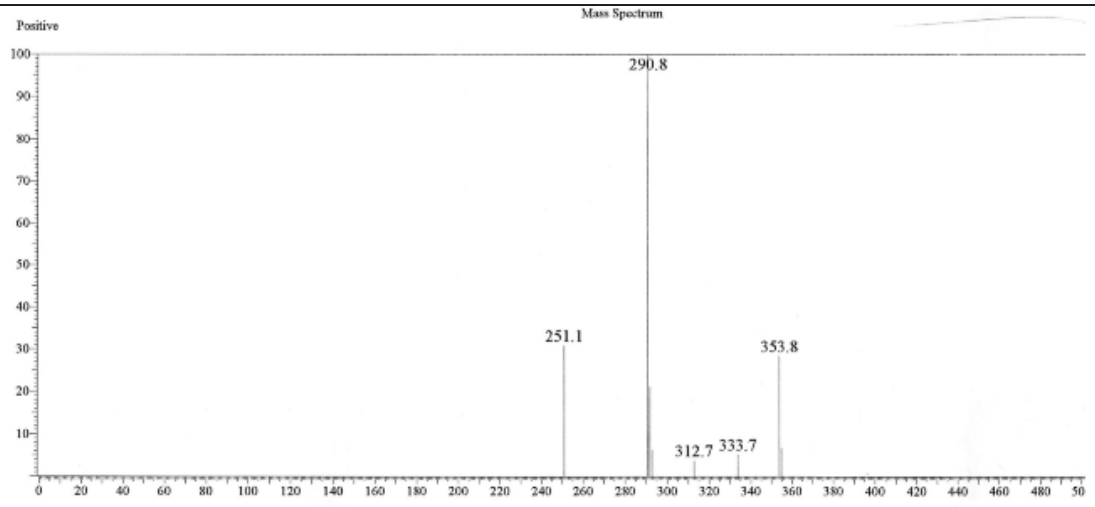


3.9.5.5 Mass spectrum of 4l

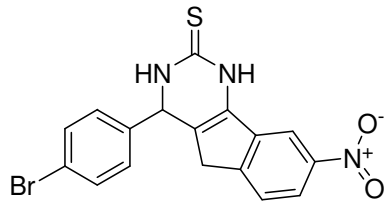


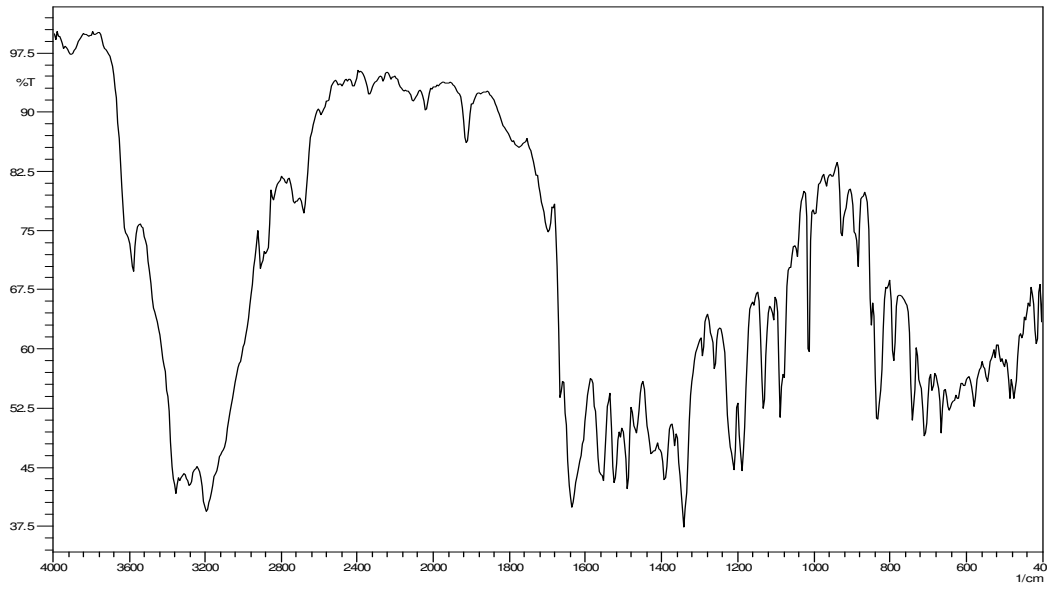
$C_{20}H_{20}N_2O_4$
m/e.: 352.38

Mass (m/z): 353.8 (M+1)



3.9.5.6 IR spectrum of 4c



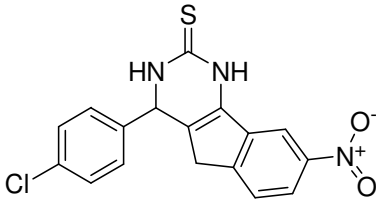


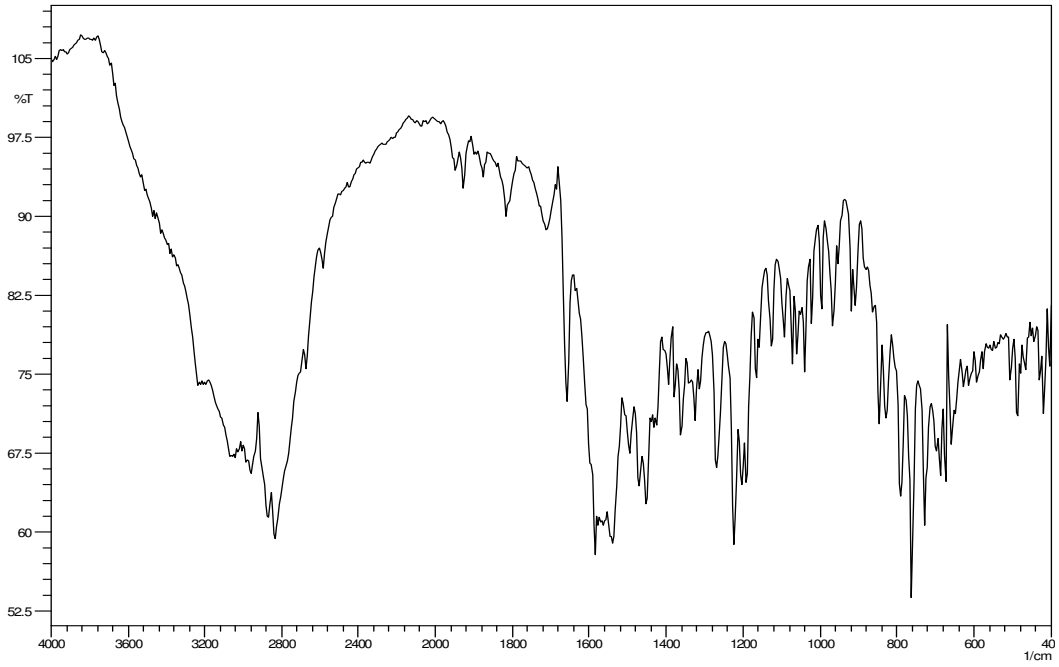
Instrument: SHIMADZU FTIR 8400 spectrophotometer,

Frequence range: 4000-400 cm^{-1} (KBr disc)

IR (KBr, cm^{-1}): 3323, 3260 (NH str.), 2951 (C-H str.), 1614 (C=C), 1530, 1350 (N-O), 1183 (C=S), 592 (C-Br)

3.9.5.7 IR spectrum of 4d





Instrument: SHIMADZU FTIR 8400 spectrophotometer,

Frequence range: 4000-400 cm^{-1} (KBr disc)

IR (KBr, cm^{-1}): 3310 (NH), 2959 (C-H), 1618 (C=C), 1527, 1344 (N-O), 775 (C-Cl)

3.10 Biological activity

Antimicrobial activity of the synthesized compounds:

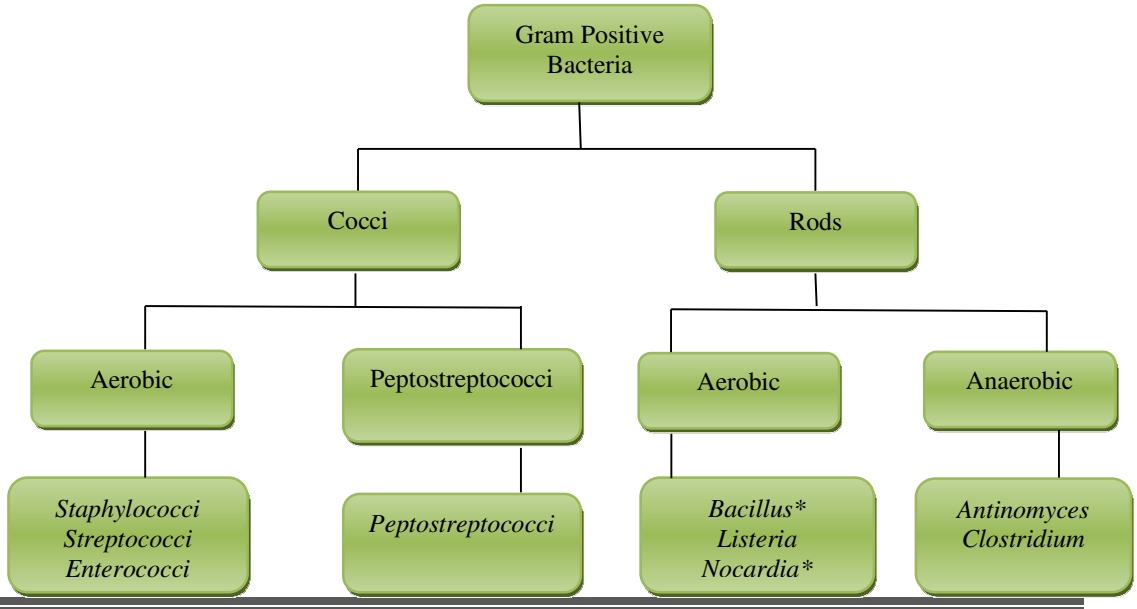
The *in-vitro* antimicrobial activity of the synthesized compounds against two Gram (+ve) [*Staphylococcus aureus (S. aureus), Staphylococcus pyogenus (S. pyogenus)*] and two Gram (-ve) [*Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa)*] microorganism using broth dilution method.

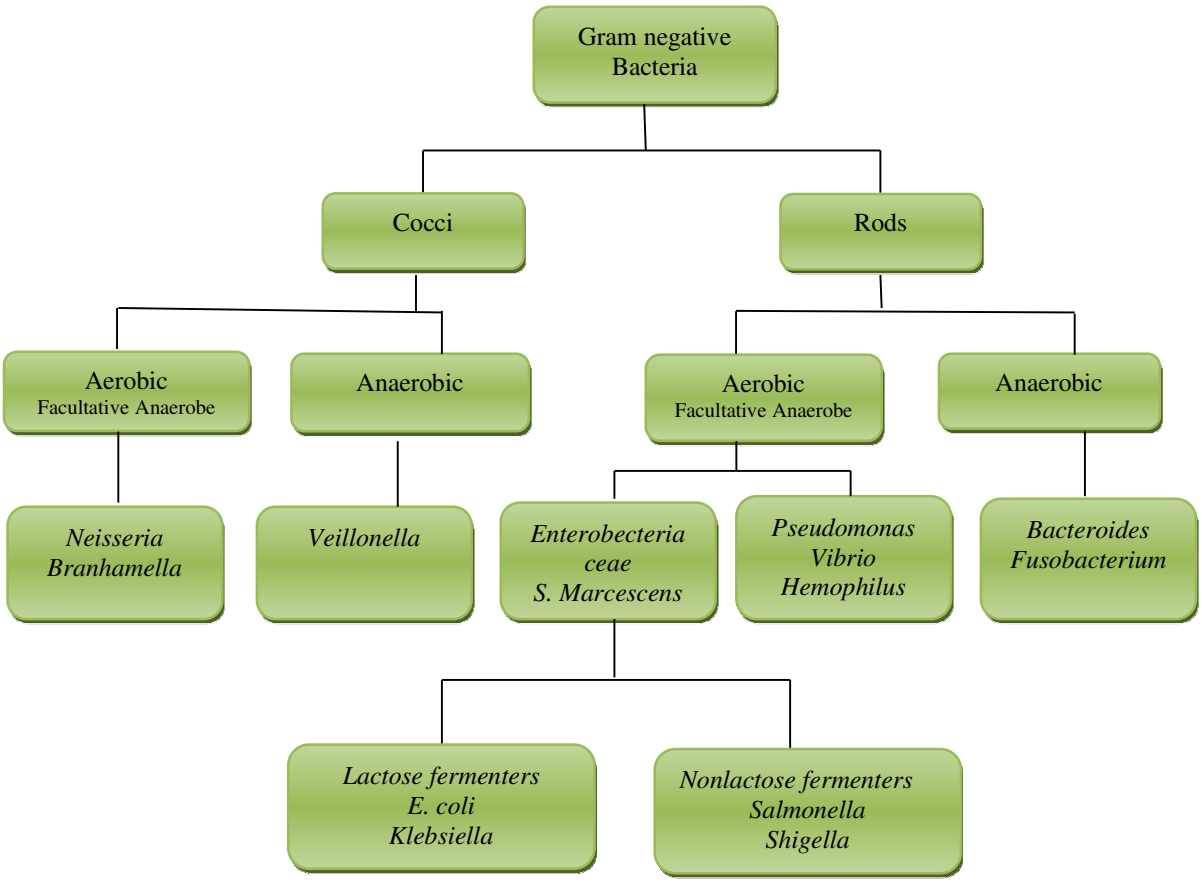
Bacteria were among one of the first life forms appear on the earth. It is present in most habitats on the planet. Bacteria were present in soil, water, deep in earth, live bodies of plants and animals etc. MICs are used for the *in vitro* activity of antimicrobials and such an information obtained from the studies have been utilized to measure MIC of the compounds, which give a definitive answer when a borderline result is obtained by other methods of testing or incase of disc diffusion methods are inappropriate.

Classification of bacteria:

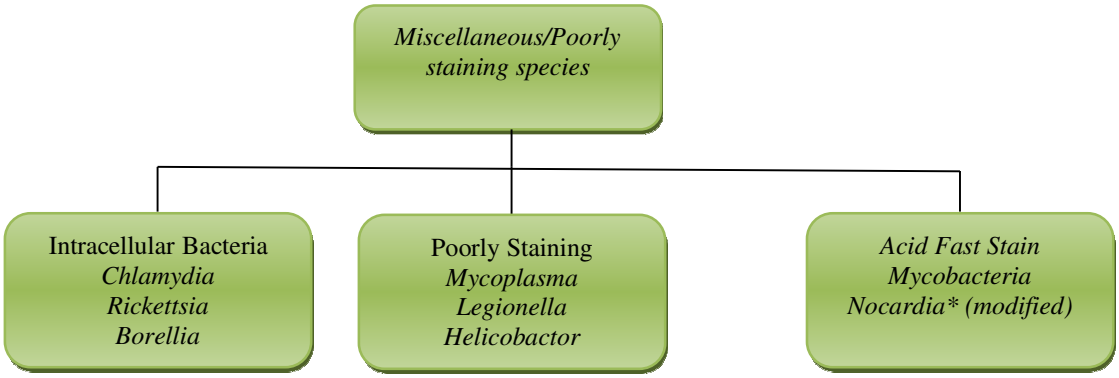
- Bacterial morphology (rod and cocci shape)
- Staining properties of the organism (Gram positive and negative)
- O_2 growth requirement of the species utilized (aerobic and anaerobic)

Based on the staining properties the chart is given below:





Miscellaneous bacteria:



Bacteria used for study of antibacterial activity:

Some of the bacteria which have been used for antibacterial activity along with their shapes, occurance and diseases spread by them are list below:

Bacteria	Shape	Occurrences	Disease
Serratia marcescens	Rod shaped	• Respiratory and urinary tracts of hospitalized adults • Gastrointestional system of child	• Pneumonia and other respiratory disease • Urinary tract infections • Bloodstream infections, including endocarditis • Septicarthritis, osteomyelitis and endocarditis
Escherichia coli	Rod shaped	• Lower intestine of warm-blooded organisms	• Mild to severe and bloody diarrhea, mostly without fever
Pseudomonas aeruginosa	Rod shaped	• Soil • Water • Skin	• Blood stream, urinary track infection • Surgical site infection • Lung infection
Staphylococcus aureus	Round shaped	• Skin • Nose • Respiratory track	• Wound, skin and deep tissue infections • Pneumonia, septicaemia and endocarditis • Staphylococcal scalded skin syndrome (SSSS) • Toxic shock syndrome • Food poisoning

Bacillus subtilis	Rod shaped	• Upper layers of the soil • Human faeces	• Food poisoning • Nausea • Vomiting • Diarrhoea

The synthesized compounds were screened for their antibacterial and antifungal activities using ampicillin, chloramphenicol and griseofulvin as standard drugs.

Experimental:

Equipment and chemicals:

Sterilized pipettes, Erlenmeyer flask, sterilized sugar tubes, control microorganisms, sterilized double distilled water, Nutrient Broth (NB), cotton plug and test tube stand.

Stock solutions: Stock solution were prepared in DMSO

Microorganism cultures:

The microorganisms were grown in nutrient broth dissolved in 50 ml double distilled water followed by incubating them for 24 hours at 35˚C.

Broth dilution method:

Appropriate volume of 2% Nutrient Broth was transferred to the sugar tubes for sterilization purpose using autoclave. Then, the desired concentration of the compound (μM) was achieved in each sugar tubes by adding appropriate volume of the stock solution. Now, each of the microorganism culture (10 μL) was added to each of the previously prepared sugar tubes of the test compounds.

Interpretation:

If the dilution inhibits the growth, a whole experiment was repeated with the next dilution i.e. half the concentration of the test compound than the early one. This procedure was repeated till the faint turbidity by the inoculums itself was observed and the said concentration is termed as Minimum Inhibitory Concentration (MIC).

The *in vitro* antimicrobial activity of all the synthesized compounds was carried out by broth microdilution method. Mueller Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose broth used for fungal nutrition. Inoculum size for test strain was adjusted to 10^8 CFU [Colony Forming Unit] per milliliter by comparing the turbidity. The strains employed for the activity were procured from [MTCC – Micro Type Culture Collection] Institute of Microbial Technology, Chandigarh.

Results and discussion:

The compounds **4a-l** were screened for their antibacterial activity against *Escherichia coli (E.coli),Pseudomonas aeruginosa (P.aeruginosa)*, *Staphylococcus aureus (S.aureus), Streptococcus pyogenes (S.pyogenes)* as well as antifungal activity against and *Candida albicans (C.albicans)*. DMSO was used as vehicle to get desired concentration of compounds to test upon microbial strains. The lowest concentration, which showed no visible growth after spot subculture was considered as MIC for each compound. The standard antibiotics used for comparison in the present study were ampicillin, chloramphenicol for evaluating antibacterial activity as well as griseofulvin for antifungal activity. The protocols are summarized in 3.10.1.

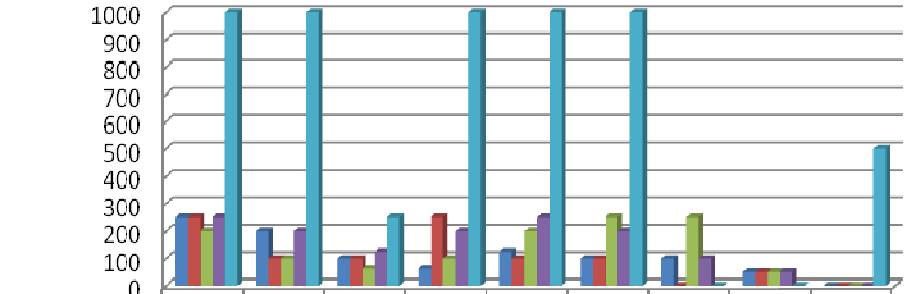
3.10.1: Antimicrobial activity of compounds **4a-l**

Comp.No.	Minimal inhibitory concentration µg/ml				
	Gram-negative bacteria		Gram-positive bacteria		Fungi
	E.coli	***P.aeruginosa***	***S.aureus***	***S.pyogenus***	***C.albicans***
4a	250	250	200	250	1000
4b	200	100	100	200	>1000
4c	100	100	62.5	125	**250**
4d	62.5	250	100	200	1000
4e	125	100	200	250	>1000
4f	100	100	250	200	1000
4g	125	62.5	100	125	500
4h	200	125	125	200	**250**
4i	62.5	100	200	250	1000
4j	62.5	100	100	100	**200**
4k	62.5	100	100	100	**250**
4l	250	250	200	250	1000
Ampicillin	100	--	250	100	--
Chloramphenicol	50	50	50	50	--
Griseofulvin	--	--	--	--	500

(--) No inhibition zone.

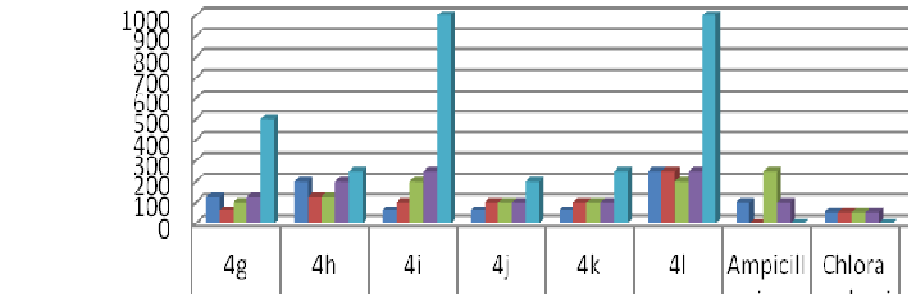
For better understanding of comparative biological activity of the synthesized compounds the graphical chart for the compounds (4a-l) is given below.

Graphical chart of antimicrobial activity of compounds (4a-f):



	4a	4b	4c	4d	4e	4f	Ampicillin	Chloramphenicol	Griseofulvin
E.coli	250	200	100	62.5	125	100	100	50	0
P.aeruginosa	250	100	100	250	100	100	0	50	0
S.aureus	200	100	62.5	100	200	250	250	50	0
S.pyogenus	250	200	125	200	250	200	100	50	0
C.albicans	1000	1000	250	1000	1000	1000	0	0	500

Graphical chart of antimicrobial activity of compounds (4g-l):



	4g	4h	4i	4j	4k	4l	Ampicillin	Chloramphenicol	Griseofulvin
E.coli	125	200	62.5	62.5	62.5	250	100	50	0
P.aeruginosa	62.5	125	100	100	100	250	0	50	0
S.aureus	100	125	200	100	100	200	250	50	0
S.pyogenus	125	200	250	100	100	250	100	50	0
C.albicans	500	250	1000	200	250	1000	0	0	500

It can be concluded from Table-3.10.1 that compound **4c** is active against Gram positive bacteria *Staphylococcus aureus (S.aureus)*. Compounds **4d, 4i, 4j** and **4k** are found to be active against Gram negative bacteria *Escherichia coli* as compared to standard antibiotic ampicillin.

Antifungal study revealed that compounds **4c, 4h, 4j** and **4k** are are more potent as compared to standard fungicidal griseofulvin against *Candida albicans* (*C.albicans*).

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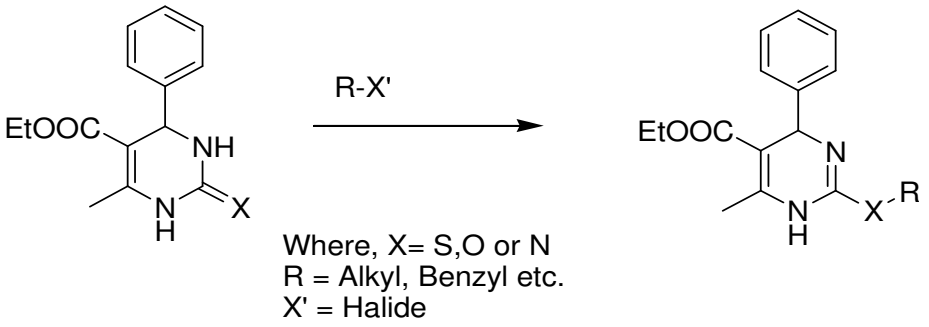
Chapter-3
Part-B

Synthesis, characterization and biological activity of dihydro-1H-indeno[1,2-d]pyrimidine derivatives

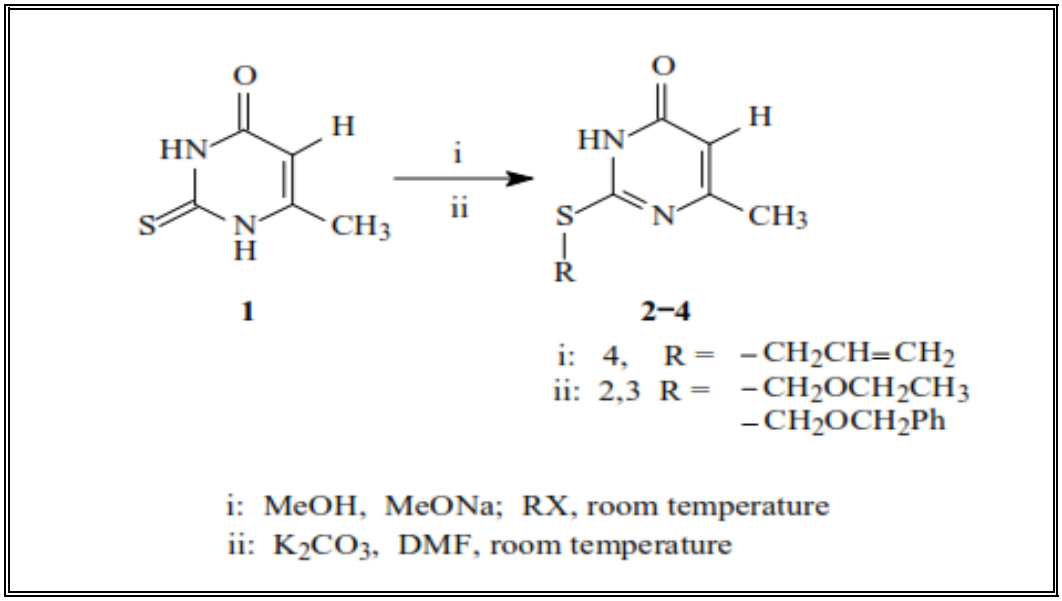
3.12 S-Alkylation Reaction

Different alkylation reactions are reported which are O-alkylation, N-alkylation and S-alkylation in DHPMs. In our previous discussion (Part-A) the detailed synthesis of DHPMs are reported via Biginelli reaction. DHPMs are synthesized via MCRs in a quantitative yield. DHPMs are further alkylated using different alkylating agents. The alkylation reaction is carried out by simple experimental conditions. Structure-activity relationship suggested that the alkylthio chains were more potent than the corresponding oxygen-substituted molecules. Hence selective S-alkylation of DHPMs to obtain the S-alkylated pyrimidine is the key step. However, little or no information is available about general S-alkylation methods. Therefore, we decided to find efficient ways to synthesize S-alkylated derivatives for developing new and more effective agents.

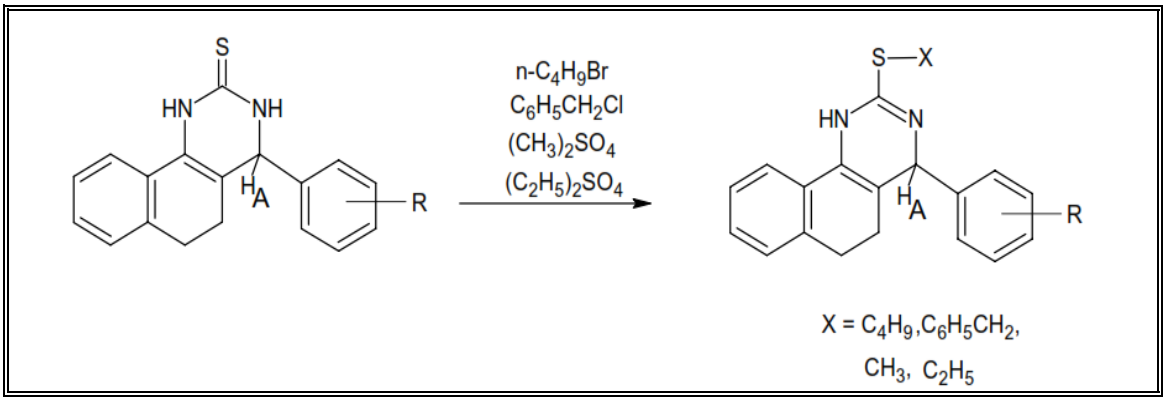


The reaction is carried out by using DHPMs in alkylating agents like Ethyl halide, Benzyl halide, n-propyl halide etc. or in the presence of solvent with/without base.

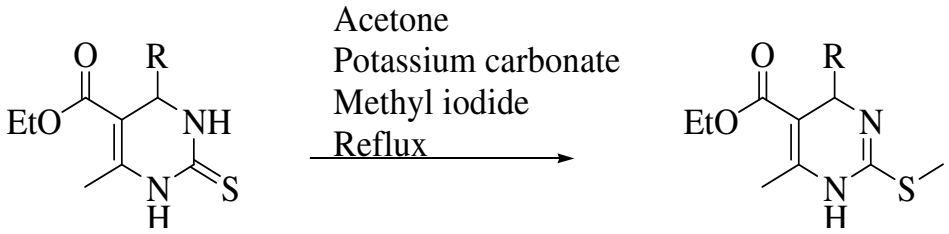
Y. Xu et al[1] investigated the regioselective alkylation of 2-thiopyrimidine. Very useful method to obtain region-selective thio-alkyl pyrimidines starting from the 2-thiopyrimidine via a procedure of potassium carbonate in Dimethyl formamide or CH_3ONa-CH_3OH at room temperature.



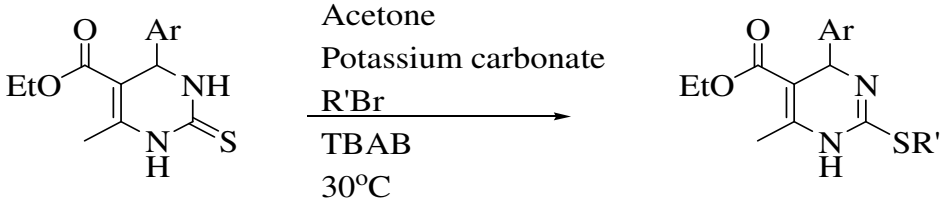
Balbir kaur et al[2] reported S-alkylation using alkylating agent and ethanol under reflux condition for 3 to 5 hours.



N.N. Karade et al[3a] reported alkylation using acetone and potassium carbonate under reflux condition to prepare 1,4-dihydropyrimidine. **Atwal et al**[3b] also reported alkylation using potassium carbonate in acetone at ambient temperature.



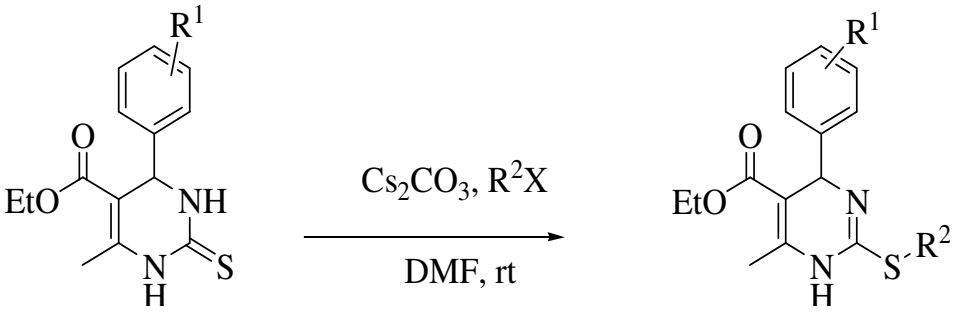
B.K. Singh et al[4] reported alkylation using acetone and potassium carbonate by using tetra butyl ammonium bromide as phase transfer catalyst at ambient condition to prepare 1,4-dihydropyrimidine. Use of catalytic amount of tetra butyl ammonium bromide as phase transfer catalyst not only improves the yield of sulfanyl derivatives but the reaction time is also considerably reduced.



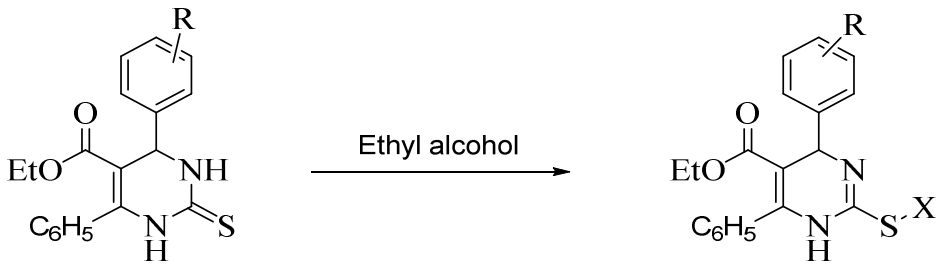
Ar= Napthyl, 3-nitrophenyl, 4-methoxy phenyl, 4-fluorophenyl,4-chlorophenyl, thiophen

R' = Benzyl, Pentyl, Butyl, Tetradecyl

S. Putatunda et al[5] reported alkyl derivative in good to excellent yields in the presence of Cs_2CO_3 which is mild base. Selective S-alkylation of thio-DHPM using Cs_2CO_3 in an anhydrous condition using DMF as solvent gives better regioselectivity.Cesium ion due to its large cationic redius is less solvated in polar aprotic solvens, and thus Cs^+ ion is more 'nacked' and highly reactive.

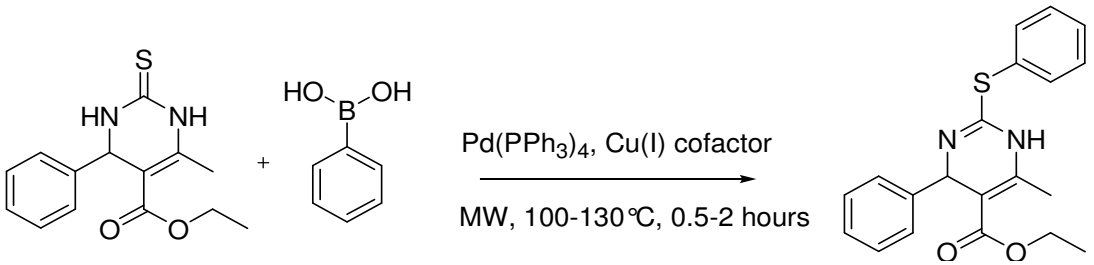


Priyanka Pathak et al[6] reported alkylation using ethyl alcohol as a solvent and benzyl bromide and butyl bromide as alkylating agents under reflux condition. S-methyl and S-ethyl derivative can be prepared by using sodium hydroxide and methanol.

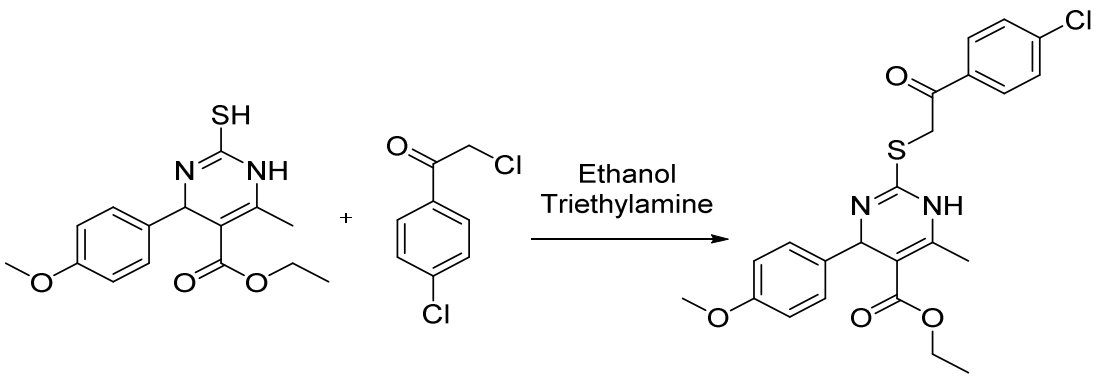


X= $(CH_3)_2SO_4$,$(C_2H_5)_2SO_4$, C_4H_9Br, $C_6H_5CH_2Br$

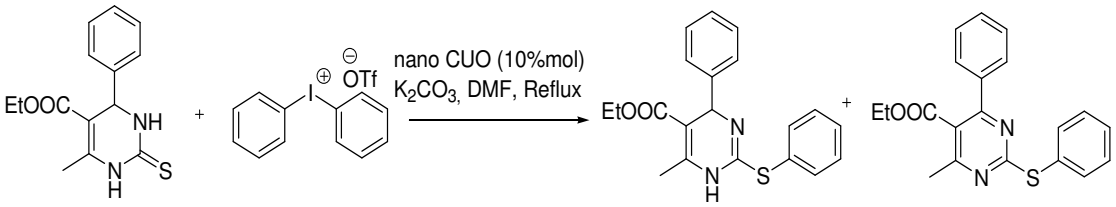
Kappe et al[7a] reported palladium (0)-catalyzed, copper (I)-mediated coupling of Boronic acids with cyclic thiomides. The desulfitative carbon-carboncross coupling protocol is performed under neutral condition and can be applied to a range of heterocyclic structures with embedded thiomide fragments. **Lengar A.** and **Kappe C. Oliver**[7b] also reported direct microwave-assisted Pd(0)-catalyzed/Cu(I)-mediated carbon-carbon cross-coupling of 3,4-dihydropyrimidine-2-thiones and boronic acids under Liebeskind-Srogl conditions leads to 2-aryl-1,4-dihydropyrimidines in moderate to high yield. In contrast, Cu(II)-mediated reaction ofthe same substrates leads to carbon-sulfur cross-coupling.



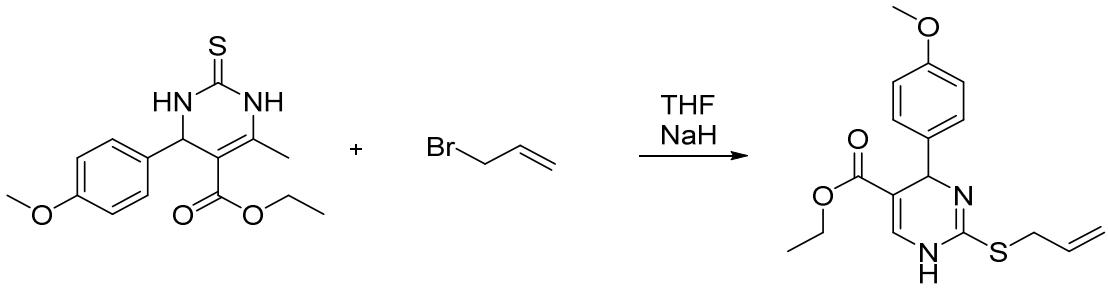
Nadia Y. Megally Abdo et al[8a] reported the alkylation using ω-bromo-4-chloroacetophenone of DHPM using absolute ethanol containing triethylamine as base under reflux conditions. **Sukhdeep Singh et al**[8b] also reported alkylation of DHPM using α-bromoketones.



Nandkishor N. Karade et al[9] reported nano copper oxide mediated ligand-free C-S cross-coupling and concomitant oxidative aromatization of 4-aryl-3,4-dihydropyrimidin-2(1H)-thione with diaryliodonium salts. A wide range of Biginelli 4-aryl-3,4-dihydropyrimidin-2(1H)-thiones undergo ligand-free C-S cross coupling with diaryliodoniumtriflates in the presence of CuO nanoparticles with the concomitant oxidative aromatization to form highly substituted 2-(thioaryl)pyrimidine. The nano CuO catalyst can be recycled and reused three times without any significant loss of catalytic activity.



Rasheeth et al[10] reported the alkylation of DHPM in sodium hydride in Tetrahydrofuran (THF) at around 20°C under inert atmosphere.

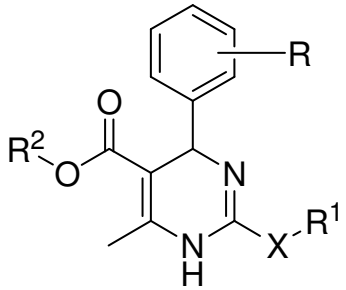


3.13 Biologically active S-alkylated dihydropyrimidones

Structure-activity relationship suggested that the alkylthio chains were more potent than the corresponding oxygen-substituted molecules. Hence selective S-alkylation of DHPMs to obtain the S-alkylated pyrimidine is the key step. However, little or no information is available about general S-alkylation methods. Therefore, we decided to find efficient ways to synthesize S-alkylated derivatives for developing new and more effective agents.

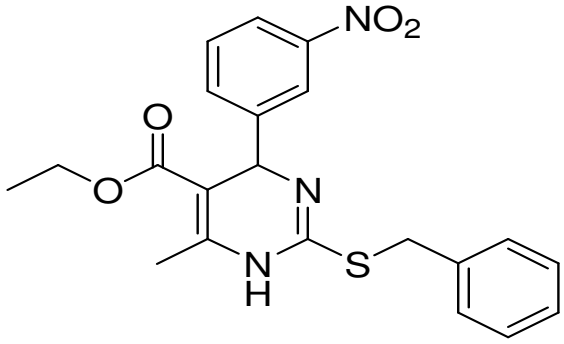
3.13.1 Antihypertensive agents

Hetero-substituted DHPMs with a branched ester (e.g. isopropyl, sec-butyl) and an alkylthio group (e.g. SMe) was found to be optimal for biological activity.



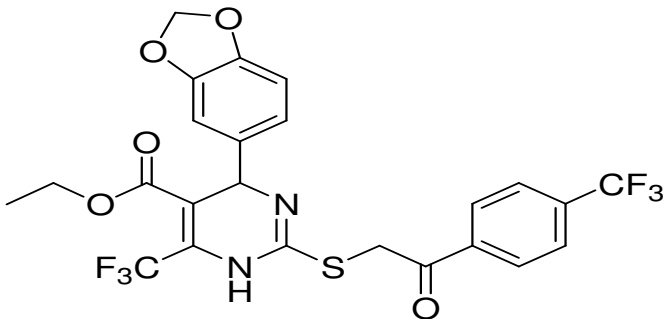
R = 2-NO_2, 3-NO_2, 2-CF_3, 2,3-Cl
R^1 = Me, $CH_2CH{=}CH_2$, $CH_2(CH_2)_3CH_3$,
$CH_2C_6H_5$, $CH_2CH_2N(Me)Bn$, $CH_2CH_2NMe_2$
R^2 = Et, I-Pr,Me, SBu, $CH_2CH_2N(Me)Bn$
X= O,S

The compound shown below is potent mimic of dihydropyridine calcium channel blockers.[11]



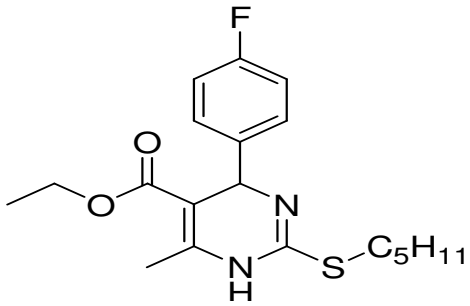
3.13.2 Antitumor activity

The compound shown below represent promising new leads for the development of highly potent and selective anticancer compound and also their in vitro cytotoxic activities were determined in colon cancer cell line.



3.13.3 Miscellaneous activities

The compound shown below is Anti-filarial agents[12].



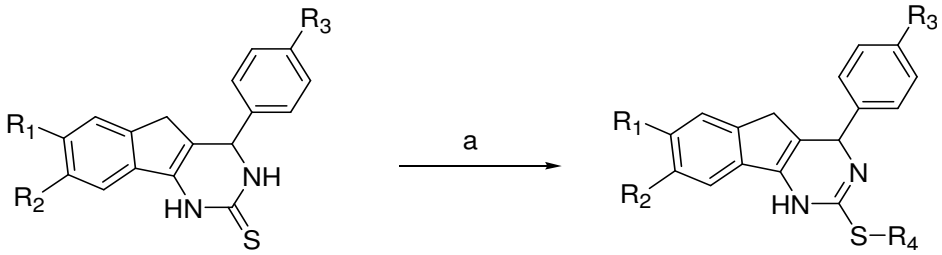
3.14 Current work

There was a very limited information is available about general S-alkylation methods along with s-alkyl compounds of DHPMs. Therefore, we decided to find efficient ways to synthesize S-alkylated derivatives for developing new and more effective agents. Alkylations of DHPMs have been carried out in refluxing ethanol using different alkyl halides. In our previous discussion in this chapter i.e. Part A, we have shown synthesis of different tetrahydro-indeno-[1,2-d]pyrimidinone (pyrimidine-2-thione) derivatives[61]. Further the synthesized compounds from the series were alkylated using different alkylating agents to generate dihydro-1H-indeno[1,2-d]pyrimidine derivatives[61]. Section 3.15 shows general scheme for the synthesis of dihydro-1H-indeno[1,2-d]pyrimidine derivatives.

The synthesized compounds are characterized by IR, NMR and Mass spectral analysis and are screening against antibacterial activity against *Escherichia coli (E.coli),Pseudomonas aeruginosa (P.aeruginosa)*, *Staphylococcus aureus (S.aureus), Streptococcus pyogenes (S.pyogenes)* as well as antifungal activity against and *Candida albicans (C.albicans)*. Physical data of synthesized compounds is reported in Section 3.17, spectral data is discussed in Section 3.18 and biological activity of synthesized compounds is reported in Section 3.19.

3.15 Reaction Scheme

3.15.1 Synthesis of dihydro-1H-indeno[1,2-d]pyrimidine derivatives



Reagent and conditions: a = Ethanol, alkyl halide (1.5 mmol), reflux.

3.16 Experimental

To a mixture of corresponding tetrahydro-indeno-[1,2-d]pyrimidinone (pyrimidine-2-thione) derivatives (1 mmol) and alkyl halide (1.5 mmol), ethanol (10ml) was added, content was refluxed for 20 hours. After completion of the reaction as monitored by TLC, the reaction mixture is poured into water and stirred for 10-15 minutes. The content of the flask were then filtered and washed with water (20 ml). The solid so obtained was the corresponding (5a-j). It was then recrystallized by hot isopropyl alcohol to get the pure product.

3.17 Physical data

3.17.1 Physical data of dihydro-1H-indeno[1,2-d]pyrimidine derivatives [compounds 5a-j].

Compound	R_1	R_2	R_3	R_4	m.p. (^oC)	Yield (%)
5a	H	NO_2	Br	C_2H_5	>300	86
5b	H	NO_2	Br	CH_3	>300	84
5c	H	H	H	cyclopentyl	>300	88
5d	H	H	H	n-hexyl	261-262	87
5e	H	H	$-OCH_3$	$-CH_2Ph$	233-234	81
5f	H	NO_2	H	n-butyl	244-245	85
5g	H	NO_2	H	$-CH_2Ph$	222-223	88
5h	H	H	H	$-CH(CH_3)_2$	249-250	83
5i	H	H	H	Trifluoro benzyl	270-271	81
5j	H	H	H	Biphenyl benzyl	266-267	89

TLC solvent system: (EA:Hexane) 3:7

MPs were taken in open capillary and are not corrected

3.18 Spectral discussion

3.18.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. The sulfanyl dihydro-1H-indeno[1,2-d]pyrimidine (DHPMs) having chlorine atom showed this characteristic peak. Fragmentation pattern can be observed to be particular for this kind of compounds and the characteristic peaks obtained for each compound. Various characteristic peaks obtained for each compound in this series.

3.18.2 IR spectral study

Various functional groups present in molecule were identified by characteristic frequency obtained for them. Presence of C-S groups was observed between 800-600 cm^{-1}. N-H group present in the moiety gave peak between 3210-3380 cm^{-1}. Peaks were identified for aromatic and alkyl group as per their characteristics. In case of compounds having different substations on aromatic ring, characteristic frequencies were observed depending on the functional group present i.e. nitro, methoxy etc.

3.18.3 ^{1}H NMR spectral study

Numbers of proton identified from NMR spectrum and their chemical shift (ppm) were in agreement of structure of molecule. Methoxy protons were observed at 3.8 δ ppm. Proton on C-4 carbon atom gave singlet at 5.4-6.2 δ ppm. Aromatic protons were observed between 6.9-7.9 δ ppm. J values were calculated to identify ortho and meta coupling. In some cases, aromatic protons were obtained as multiplet.

3.18.4 Elemental analysis

Elemental analysis showed calculated and found percentage values of carbon, hydrogen and nitrogen in support of structure of synthesized compounds. The spectral and elemental analysis data are given below for individual compounds.

3.18.5 Spectral data of synthesized compounds (5 a-j)

4-(4-Bromo-phenyl)-2-ethylsulfanyl-8-nitro-4,5-dihydro-1H-indeno[1,2-d]pyrimidine (5a). Off-white solid, (86 %), m.p. >300 °C, Anal.Calcd for $C_{19}H_{16}BrN_3O_2S$: C 53.03, H 3.75, N 9.76% Found: C 53.11, H 3.69, N 9.88%. IR (KBr, cm^{-1}): 3260 (NH), 1654 (C=C), 1591 & 1359 (N-O), 788 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.24-1.29 (t, 3H, CH3), 2.98-3.06 (d, 1H, CH), 3.23-3.28 (d, 1H, CH), 3.30-3.37 (q, 2H, CH_2 merged with DMSOd_6), 6.06 (s, 1H, CH), 7.24-7.75 (m, 7H, Ar-H), 10.65 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-*d_6*) δ: 15.5 (CH_3), 17.9 (CH_2), 27.9 (CH_2), 58.8 (CH), 103.2, 120.2, 121.3, 122.8, 130.7, 131.7, 131.9, 136.5, 136.9, 141.8, 142.9, 146.2 (Ar-C), 162.1 (C-S), MS: (M+1) 431.01.

4-(4-Bromo-phenyl)-2-methylsulfanyl-8-nitro-4,5-dihydro-1H-indeno[1,2-d]pyrimidine (5b). White solid, (84 %), m.p. >300 °C, Anal.Calcd for $C_{18}H_{14}BrN_3O_2S$: C 51.93, H 3.39, N 10.09% Found: C 52.02, H 3.45, N 9.98%. IR (KBr, cm^{-1}): 3363 (NH), 1630 (C=C), 1535 & 1339 (N-O), 825 (C-S). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97 (s, 3H, CH_3), 2.99-3.08 (d, 2H, CH_2), 5.95 (s, 1H, CH), 7.21-7.73 (m, 7H, Ar-H), 10.65 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-*d_6*) δ: 12.5 (CH_3), 27.9 (CH_2), 58.8 (CH), 103.9, 120.7, 121.0, 122.9, 129.7, 131.3, 131.7, 135.1, 137.9, 142.8, 142.9, 147.2 (Ar-C), 164.1 (C-S).

2-Cyclopentylsulfanyl-4-phenyl-4,5-dihydro-1H-indeno[1,2-d]pyrimidine (5c). White solid, (88 %), m.p. >300 °C, Anal.Calcd for $C_{22}H_{22}BrN_2S$: C 76.26, H 6.40, N 8.08% Found: C 76.39, H 6.30, N 7.99%. IR (KBr, cm^{-1}): 3251 (NH), 2964 (ArC-H), 1622 (C=C). ^{1}H NMR (400 MHz, DMSO-*d_6*): δ 1.44 (m, 4H, CH_2), 2.3-2.5 (m, 5H, CH & CH_2), 3.21-3.25 (d, 2H, CH_2), 5.62 (s, 1H, CH), 7.21-7.43 (m, 8H, Ar-H), 10.4 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO-*d_6*) δ: 21.5 (CH_2), 27.7 (CH_2), 32.4 (CH_2), 32.9 (CH),

58.9 (CH), 103.2, 125.2, 125.7, 126.3, 127.8, 128.1, 129.4, 129.9, 135.9, 136.5, 136.9, 141.8 (Ar-C), 163.1 (C-S), MS: (M+1) 346.9.

2-Hexylsulfanyl-4-phenyl-4,5-dihydro-1H-indeno[1,2-d]pyrimidine (5d). Off-white solid, (87%), m.p. 261-262, Anal.Calcd for $C_{23}H_{26}N_2S$: C 76.20, H 7.23, N 7.73% Found: C 76.29, H 7.30, N 7.79%. IR (KBr, cm^{-1}): 3262 (NH).

2-Benzylsulfanyl-4-(4-methoxy-phenyl)-4,5-dihydro-1H-indeno[1,2-d]pyrimidine (5e). Off-white solid, (81%), m.p. 233-234, Anal.Calcd for $C_{25}H_{22}N_2S$: C 75.35, H 5.56, N 7.03 % Found: C 75.42, H 5.50, N 7.09%. MS: (M+1) 398.7.

2-Butylsulfanyl-8-nitro-4-phenyl-4,5-dihydro-1H-indeno[1,2-d]pyrimidine (5f). Light yellow solid, (85%), m.p. 244-245, Anal.Calcd for $C_{21}H_{21}N_3O_2S$: C 66.47, H 5.58, N 11.07 % Found: C 66.42, H 5.65, N 11.15%.

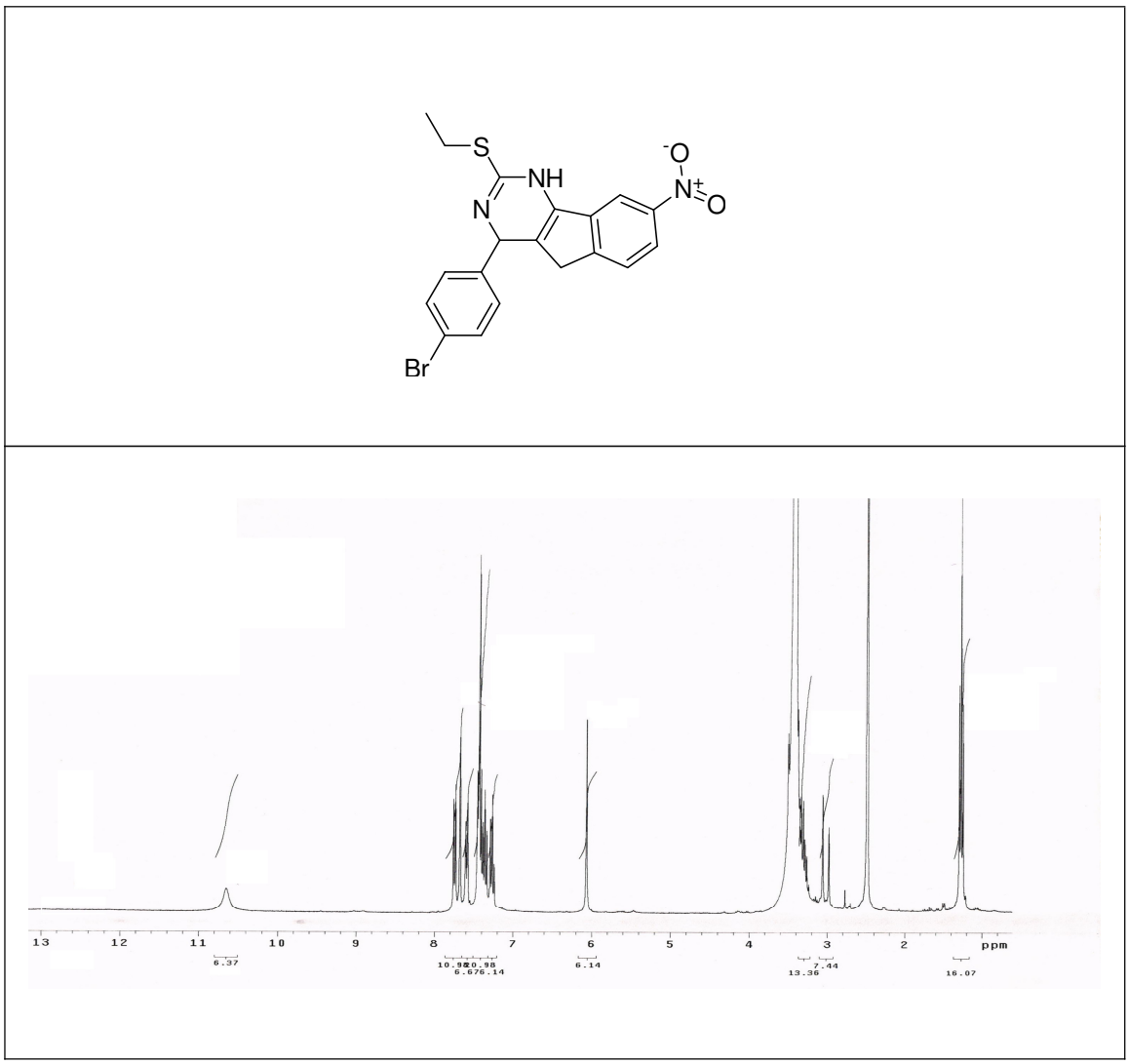
2-Benzylsulfanyl-8-nitro-4-phenyl-4,5-dihydro-1H-indeno[1,2-d]pyrimidine (5g). Light yellow solid, (88%), m.p. 222-223, Anal.Calcd for $C_{24}H_{19}N_3O_2S$: C 69.71, H 4.63, N 10.16 % Found: C 69.79, H 4.67, N 10.10%.

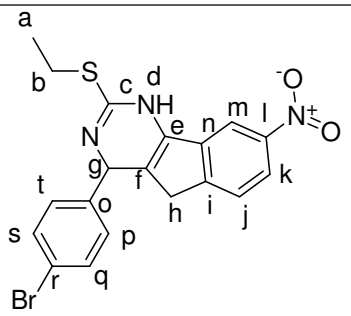
2-Isopropylsulfanyl-4-phenyl-4,5-dihydro-1H-indeno[1,2-d]pyrimidine (5h). Off-white solid, (83%), m.p. 249-250, Anal.Calcd for $C_{20}H_{20}N_2S$: C 74.96, H 6.29, N 8.74 % Found: C 74.88, H 6.35, N 8.77%.

4-Phenyl-2-(2,4,5-trifluoro-benzylsulfanyl)-4,5-dihydro-3H-indeno[1,2-d]pyrimidine (5i). Off-white solid, (81%), m.p. 270-271, Anal.Calcd for $C_{24}H_{17}F_3N_2S$: C 68.23, H 4.06, N 6.63 % Found: C 68.30, H 4.01, N 6.70%.

2-(Biphenyl-2-ylmethylsulfanyl)-4-phenyl-4,5-dihydro-3H-indeno[1,2-d]pyrimidine (5j). Off-white solid, (89%), m.p. 266-267, Anal.Calcd for $C_{30}H_{24}N_2S$: C 81.05, H 5.44, N 6.30 % Found: C 81.01, H 5.41, N 6.35%, MS: (M+1) 444.9.

3.18.5.1 ^{1}H NMR spectrum of 5a



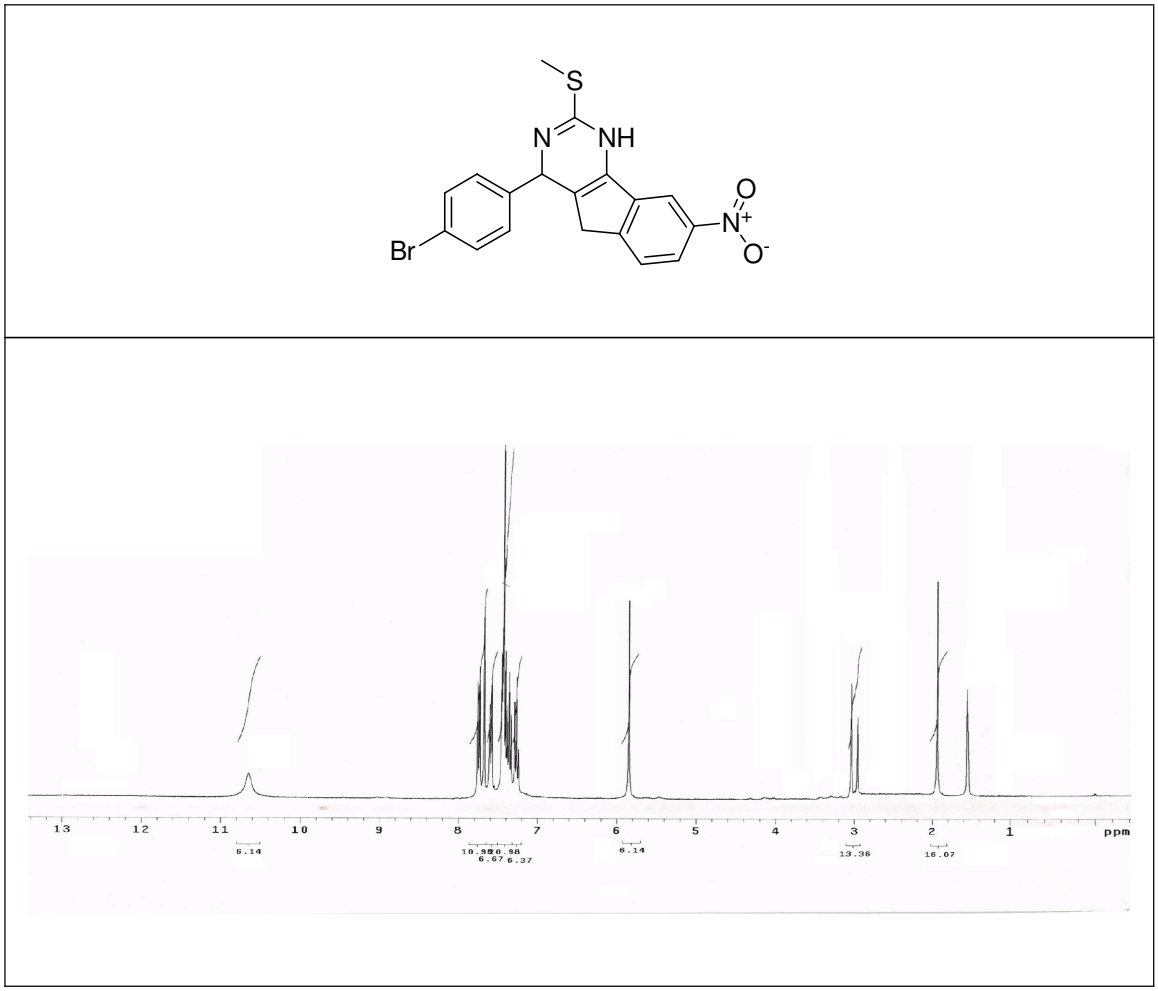


4-(4-Bromo-phenyl)-2-ethylsulfanyl-8-nitro-4,5-dihydro-1H-indeno[1,2-d]pyrimidine
Compound 5a

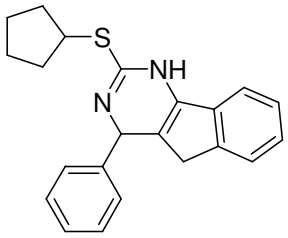
Assignment of ^{1}H NMR:

Sr.No.	Signal (δppm)	No. of Protons	Multiplicity	Assignment
1	1.24-1.29	3H	triplet	a
2	2.98-3.06 3.23-3.28	2H	doublet	h
3	3.30-3.37	2H	quartet	b
4	6.06	1H	singlet	g
5	7.24-7.75	7H	multiplet	j,k,m,p,q,s,t
6	10.65	1H	broad singlet	d

3.18.5.2 ^{1}H NMR spectrum of 5b

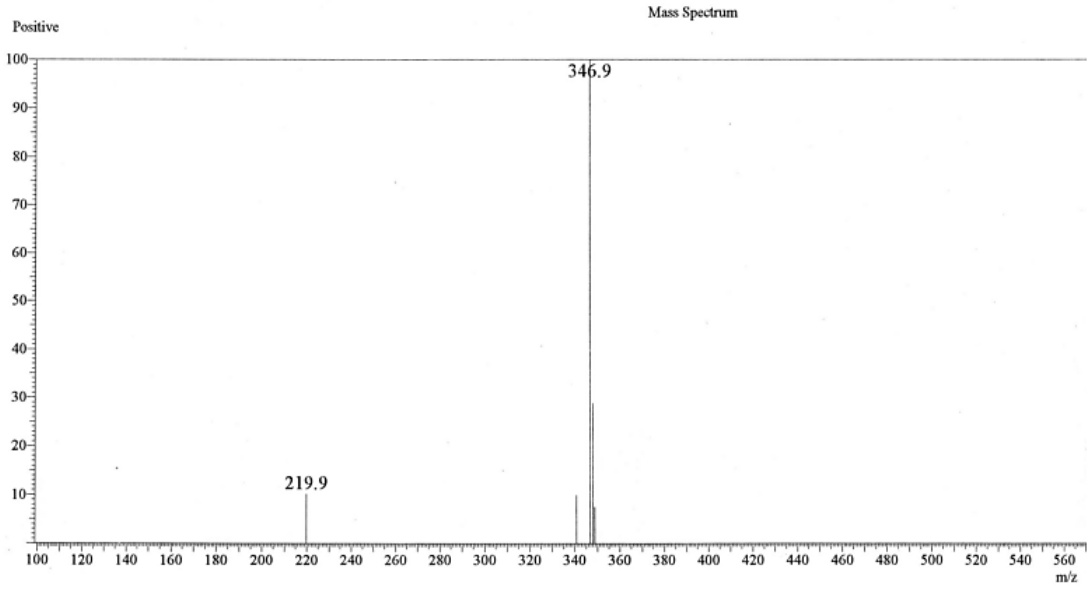


3.18.5.3 Mass Spectrum of 5c

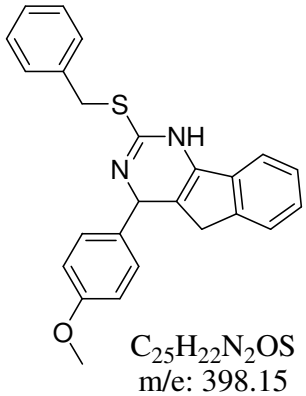


$C_{22}H_{22}N_2S$
m/e.: 346.15

Mass (m/z): 346.9 (M+1)

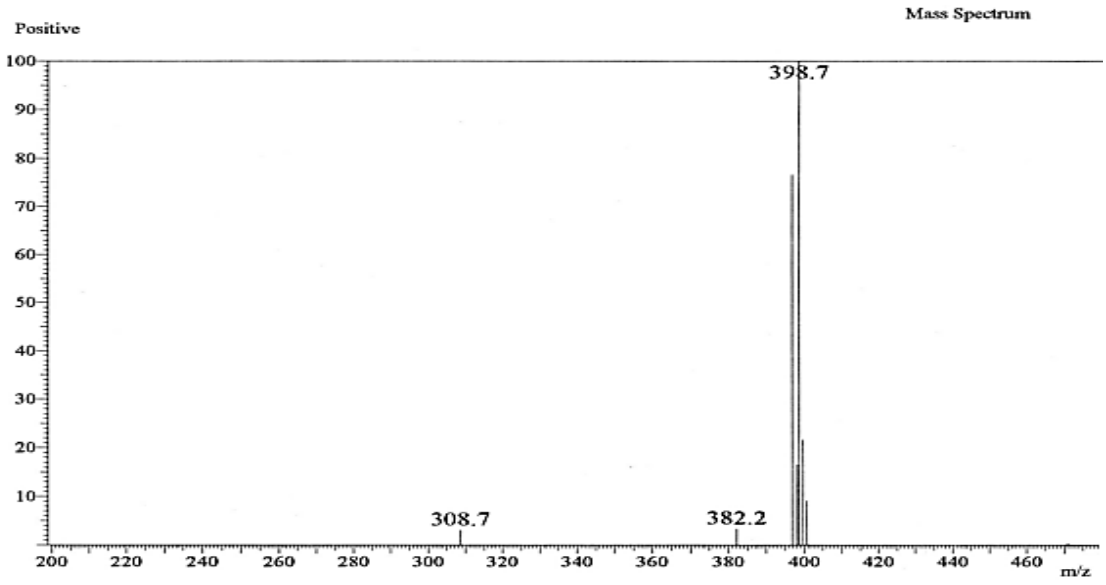


3.18.5.4 Mass Spectrum of 5e

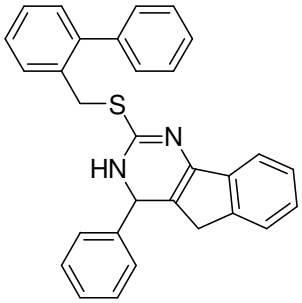


$C_{25}H_{22}N_2OS$
m/e: 398.15

Mass (m/z): 398.7 (M+1)

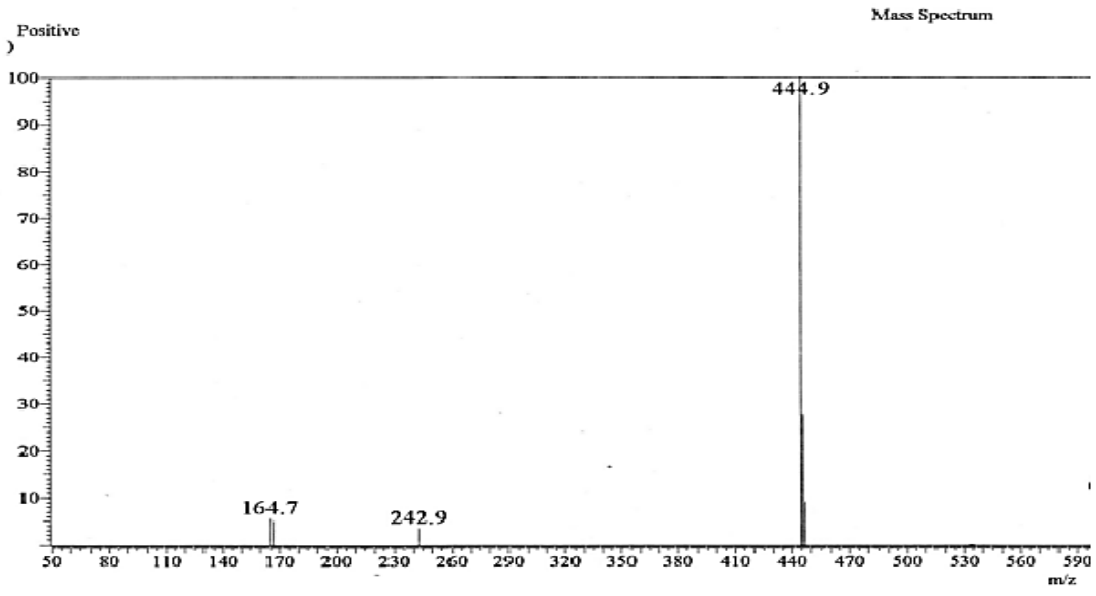


3.18.5.5 Mass Spectrum of 5j

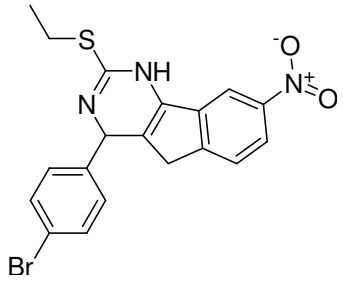


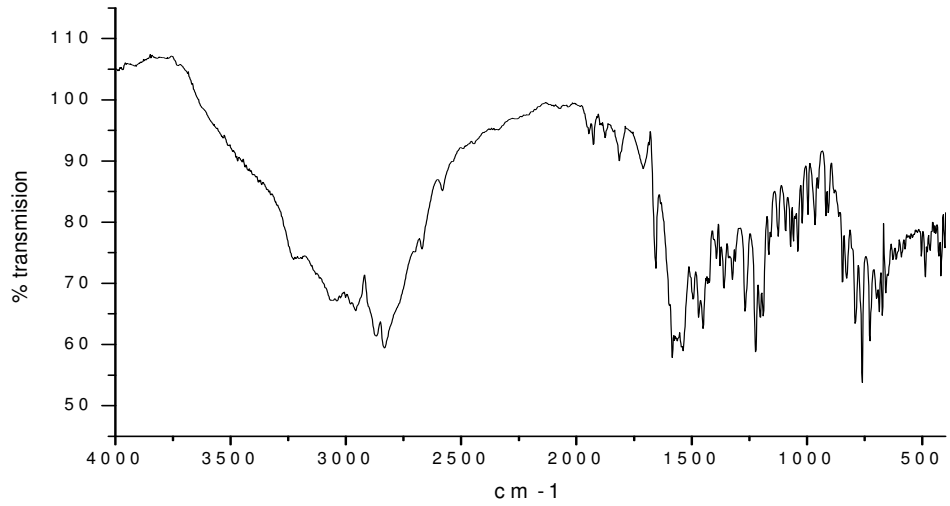
$C_{30}H_{24}N_2S$
m/e: 444.17

Mass (m/z): 444.9 (M+1)



3.18.5.6 IR spectrum of 5a



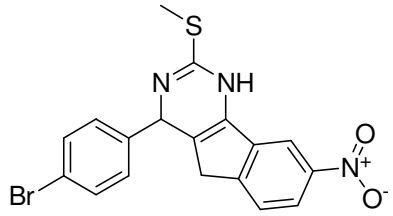


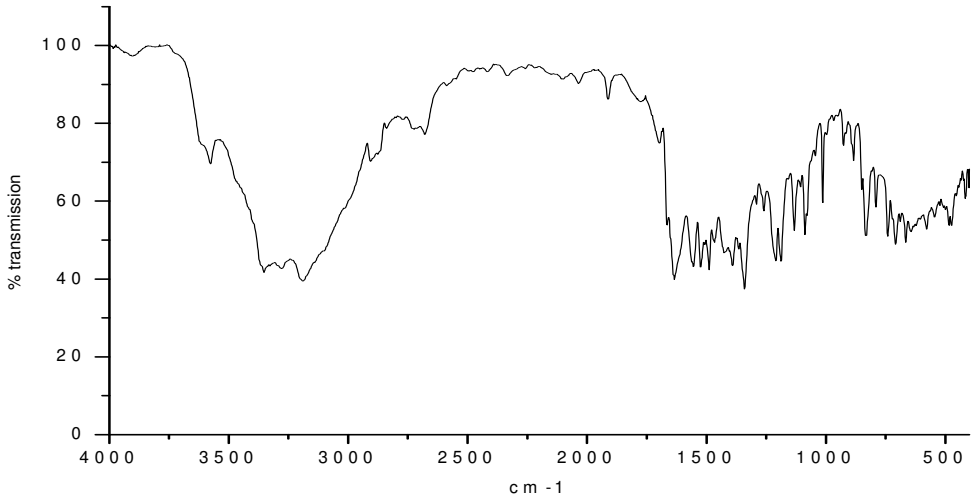
Instrument: SHIMADZU FTIR 8400 spectrophotometer,

Frequence range: 4000-400 cm^{-1} (KBr disc)

IR (KBr, cm^{-1}): 3260 (NH), 1654 (C=C), 1591 & 1359 (N-O), 788 (C-S)

3.18.5.7 IR spectrum of 5b





Instrument: SHIMADZU FTIR 8400 spectrophotometer,

Frequence range: 4000-400 cm^{-1} (KBr disc)

IR (KBr, cm^{-1}): 3363 (NH), 1630 (C=C), 1535 & 1339 (N-O), 825 (C-S)

3.19 Biological activity

Antimicrobial activity of the synthesized compounds:

The *in-vitro* antimicrobial activity of the synthesized compounds against two Gram (+ve) [*Staphylococcus aureus (S. aureus), Staphylococcus pyogenus (S. pyogenus)*] and two Gram (-ve) [*Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa)*] microorganism using broth dilution method.

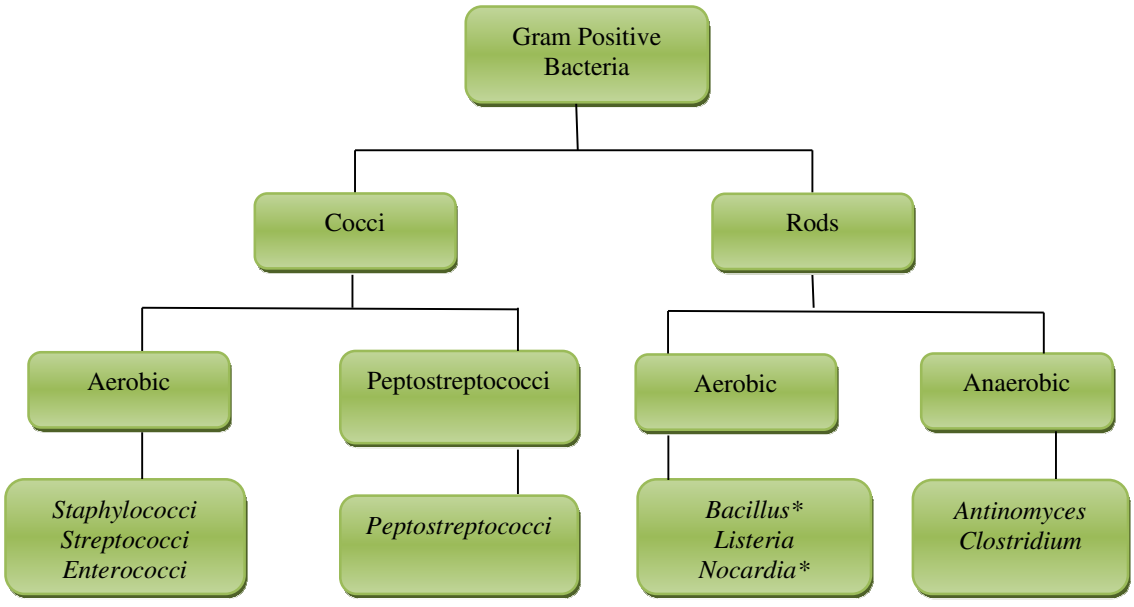
Bacteria were among one of the first life forms appear on the earth. It is present in most habitats on the planet. Bacteria were present in soil, water, deep in earth, live bodies of plants and animals etc. MICs are used for the *in vitro* activity of antimicrobials and such an information obtained from the studies have been utilized to measure MIC of the compounds, which give a definitive answer when a borderline result is obtained by other methods of testing or incase of disc diffusion methods are inappropriate.

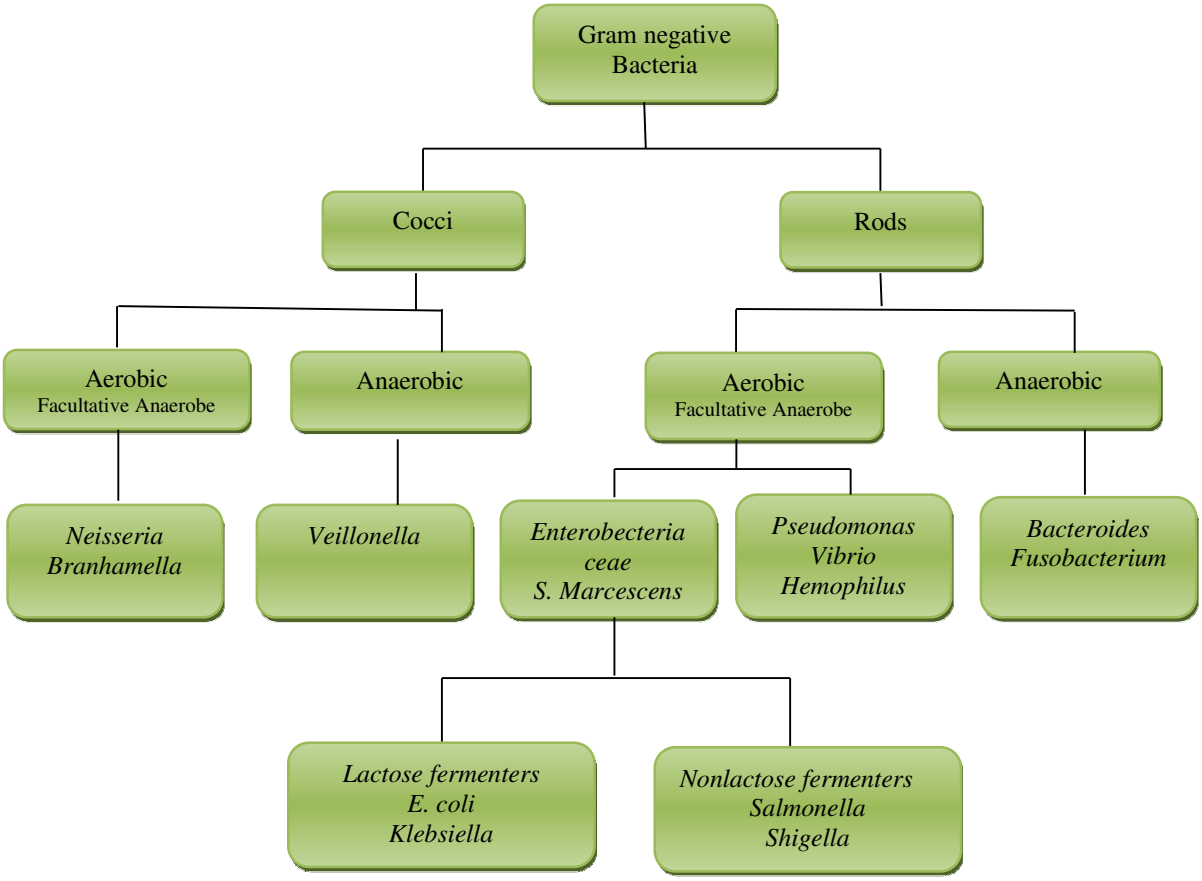
Classification of bacteria:

- Bacterial morphology (rod and cocci shape)
- Staining properties of the organism (Gram positive and negative)
- O_2 growth requirement of the species utilized (aerobic and anaerobic)

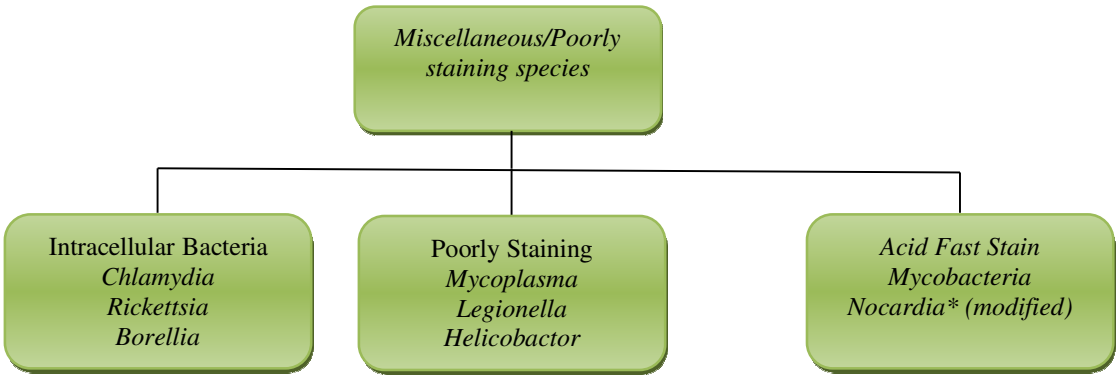
Based on the staining properties the chart is given below

:





Miscellaneous bacteria:



Bacteria used for study of antibacterial activity:

Some of the bacteria which have been used for antibacterial activity along with their shapes, occurance and diseases spread by them are list below:

Bacteria	Shape	Occurrences	Disease
Serratia marcescens	Rod shaped	• Respiratory and urinary tracts of hospitalized adults • Gastrointestional system of child	• Pneumonia and other respiratory disease • Urinary tract infections • Bloodstream infections, including endocarditis • Septicarthritis, osteomyelitis and endocarditis
Escherichia coli	Rod shaped	• Lower intestine of warm-blooded organisms	• Mild to severe and bloody diarrhea, mostly without fever
Pseudomonas aeruginosa	Rod shaped	• Soil • Water • Skin	• Blood stream, urinary track infection • Surgical site infection • Lung infection
Staphylococcus aureus	Round shaped	• Skin • Nose • Respiratory track	• Wound, skin and deep tissue infections • Pneumonia, septicaemia and endocarditis • Staphylococcal scalded skin syndrome (SSSS) • Toxic shock syndrome • Food poisoning
Bacillus subtilis	Rod shaped	• Upper layers of the soil	• Food poisoning • Nausea

		• Human faeces	• Vomiting • Diarrhoea

The synthesized compounds were screened for their antibacterial and antifungal activities using ampicillin, chloramphenicol and griseofulvin as standard drugs.

Experimental:

Equipment and chemicals:

Sterilized pipettes, Erlenmeyer flask, sterilized sugar tubes, control microorganisms, sterilized double distilled water, Nutrient Broth (NB), cotton plug and test tube stand.

Stock solutions: Stock solution were prepared in DMSO

Microorganism cultures:

The microorganisms were grown in nutrient broth dissolved in 50 ml double distilled water followed by incubating them for 24 hours at 35˚C.

Broth dilution method:

Appropriate volume of 2% Nutrient Broth was transferred to the sugar tubes for sterilization purpose using autoclave. Then, the desired concentration of the compound (μM) was achieved in each sugar tubes by adding appropriate volume of the stock solution. Now, each of the microorganism culture (10 μL) was added to each of the previously prepared sugar tubes of the test compounds.

Interpretation:

If the dilution inhibits the growth, a whole experiment was repeated with the next dilution i.e. half the concentration of the test compound than the early one. This procedure was repeated till the faint turbidity by the inoculums itself was observed and the said concentration is termed as Minimum Inhibitory Concentration (MIC).

The *in vitro* antimicrobial activity of all the synthesized compounds was carried out by broth microdilution method. Mueller Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose broth used for fungal nutrition. Inoculum size for test strain was adjusted to 10^8 CFU [Colony

Forming Unit] per milliliter by comparing the turbidity. The strains employed for the activity were procured from [MTCC – Micro Type Culture Collection] Institute of Microbial Technology, Chandigarh.

Results and discussion:

The compounds 5**a-j** were screened for their antibacterial activity against *Escherichia coli (E.coli),Pseudomonas aeruginosa (P.aeruginosa)*, *Staphylococcus aureus (S.aureus), Streptococcus pyogenes (S.pyogenes)* as well as antifungal activity against and *Candida albicans (C.albicans)*. DMSO was used as vehicle to get desired concentration of compounds to test upon microbial strains. The lowest concentration, which showed no visible growth after spot subculture was considered as MIC for each compound. The standard antibiotics used for comparison in the present study were ampicillin, chloramphenicol for evaluating antibacterial activity as well as griseofulvin for antifungal activity. The protocols are summarized in 3.19.1.

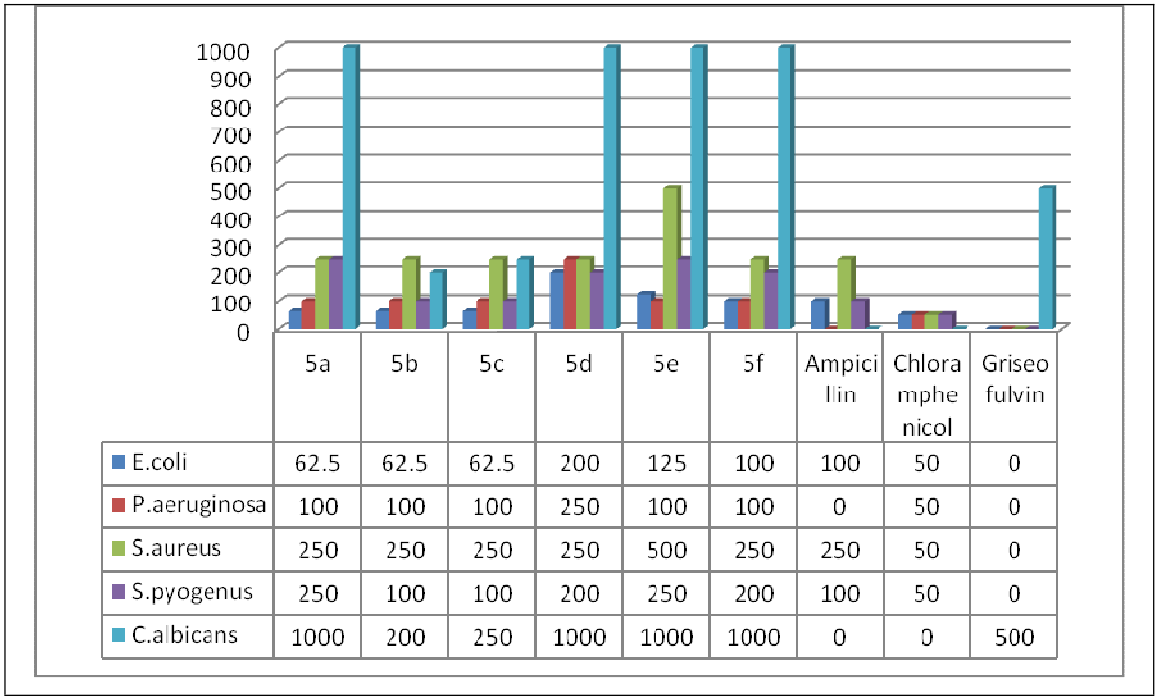
3.19.1: Antimicrobial activity of compounds 5**a-j**

Comp.No.	Minimal inhibitory concentration µg/ml				
	Gram-negative bacteria		Gram-positive bacteria		Fungi
	E.coli	***P.aeruginosa***	***S.aureus***	***S.pyogenus***	***C.albicans***
5a	62.5	100	250	250	1000
5b	62.5	100	250	100	**200**
5c	62.5	100	250	100	**250**
5d	200	250	250	200	1000
5e	125	100	500	250	>1000
5f	100	100	250	200	1000
5g	200	250	500	125	500
5h	200	100	250	200	500
5i	200	100	500	250	1000
5j	200	250	500	100	1000
Ampicillin	100	--	250	100	--
Chloramphenicol	50	50	50	50	--
Griseofulvin	--	--	--	--	500

(--) No inhibition zone.

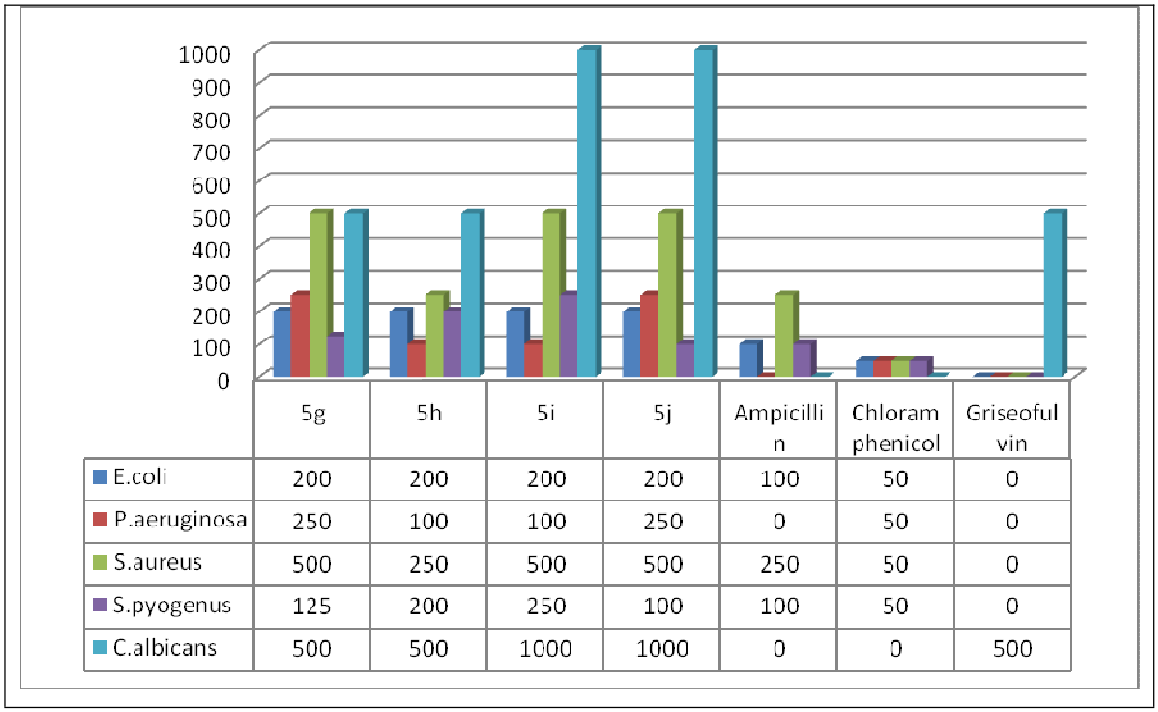
For better understanding of comparative biological activity of the synthesized compounds the graphical chart for the compounds (5a-j) is given below.

Graphical chart of antimicrobial activity of compounds (5a-f):



	5a	5b	5c	5d	5e	5f	Ampicillin	Chloramphenicol	Griseofulvin
E.coli	62.5	62.5	62.5	200	125	100	100	50	0
P.aeruginosa	100	100	100	250	100	100	0	50	0
S.aureus	250	250	250	250	500	250	250	50	0
S.pyogenus	250	100	100	200	250	200	100	50	0
C.albicans	1000	200	250	1000	1000	1000	0	0	500

Graphical chart of antimicrobial activity of compounds (5g-j):



	5g	5h	5i	5j	Ampicillin	Chloramphenicol	Griseofulvin
E.coli	200	200	200	200	100	50	0
P.aeruginosa	250	100	100	250	0	50	0
S.aureus	500	250	500	500	250	50	0
S.pyogenus	125	200	250	100	100	50	0
C.albicans	500	500	1000	1000	0	0	500

It can be concluded from section 3.19.1 that compounds **5a, 5b** and **5c** are found to be highly active against Gram negative bacteria *Escherichia coli* as compared to standard antibiotic ampicillin.

Antifungal study revealed that compounds **5b** and **5c** are more potent as compared to standard fungicidal griseofulvin against *Candida albicans* (*C.albicans*).

3.20 References

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