Synthesis, Characterization and Antidepressant Activity of 2-Thioxo-5H-pyrrolo[3,4-d]pyrimidin-5-one Derivatives

A dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600 032.

in partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

Submitted by

Reg. No. 26108338

Under the guidance of

Prof. Dr.A.Jerad Suresh, M.Pharm.,Ph.D.,

Professor and Head,

DEPARTMENT OF PHARMCEUTICAL CHEMISTRY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003.

APRIL- 2012

CERTIFICATE

This is to certify that the dissertation entitled **"Synthesis, Characterization and Antidepressant Activity of 2-Thioxo-5H-pyrrolo[3,4-d]pyrimidin-5-one Derivatives"** submitted by the candidate bearing register no.26108338 in partial fulfillment of the requirements for the award of the degree of Master of Pharmacy in Pharmaceutical Chemistry by The Tamil Nadu Dr.M.G.R Medical University is a bonafide work done by her during the academic year 2011-2012 at the Department of Pharmaceutical chemistry, College of Pharmacy, Madras Medical College,Chennai-3.

Dr. A. JERAD SURESH, M.Pharm., Ph.D.,
Professor & Head, Dept. of Pharm. Chem,
Principal, College of pharmacy,
Madras Medical College,
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Dr. A. JERAD SURESH, M.Pharm., Ph.D.,
Project Advisor,
Department of Pharmaceutical Chemistry,
College of pharmacy,
Madras Medical College,
Chennai- 03.

ACKNOWLEDGEMENT

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I immensely express my sincere thanks to Dr. V. Kanagasabai, M.D, Dean, Madras Medical College, Chennai-3 for providing all facilities and support during the period of my academic study.

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I would like to convey my “forlorn mind” for the soul of innocent mice.

I express my heartiest thanks to my family members for their cooperation and encouragement.

ABBREVIATIONS

DALYs	-	Disability adjusted life year
5-HT	-	5-Hydroxy tryptamine
NE	-	Norepinephrine
MHPG	-	3-methoxy-4-hydroxy phenyl glycol
5-HIAA	-	5-hydroxy indole acetic acid
CRH	-	Corticotrophin releasing hormone
ACTH	-	Adrenocorticotrophic hormone
Ar	-	Aryl
TLC	-	Thin layer chromatography
IR	-	Infrared spectroscopy
NMR	-	Nuclear magnetic resonance
KBr	-	Potassium bromide
Ppm	-	Parts per million
OECD	-	Organisation for economic co-operation and development
po	-	Per oral
FST	-	Forced swim test
CMC	-	Carboxymethylcellulose
ANOVA	-	Analysis of variance

CONTENTS

CERTIFICATE

This is to certify that SELVINTHANUJA C, Post Graduate student, Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03 had submitted her protocol (PartBApplication) vide 6/243-CPCSEA for the dissertation programme to the Animal Ethical Committee, Madras Medical College, Chennai-03.

TITLE

"SYNTHESIS, CHARACTERIZATION AND ANTIDEPRESSANT ACTIVITY OF 2-THIOXO-5H-PYRROLO[3,4-d]PYRIMIDIN-5-ONE DERIVATIVES"

The Animal Ethical Committee experts screened her proposal vide 6/243-CPCSEA and have given clearance in the meeting held on 21.1.2012 at Dean's Chamber in Madras Medical College.

Signature

(Dr. JOSEPH DIXON)

1. INTRODUCTION

Depression is the leading cause of disability[1] and affecting about 121 million people world wide. Depression can lead to suicide, a tragic fatality associated with the loss of about 850000 lives every year. According to World Health Organization, by the year 2020, depression is projected to reach 2nd place of the ranking of Disability Adjusted Life Year (DALY) calculated for all ages, both sexes. Today depression is already the 2nd cause of DALYs in the age category 15-44 years for both sexes combined.

1.1 DEPRESSION

Depression[2] is the most common of the affective disorders defined as disorders of mood rather than disturbances of thought or cognition. It may range from a very mild condition, bordering on normality to severe (psychotic) depression accompanied by hallucination and delusions.

The most common mood disorders are major depression (unipolar depression) and manic depression (bipolar depression). Major depression is the most common psychiatric disorder that continues to result in considerable mortality and morbidity despite their major advances in treatment. The life time prevalence is estimated to be about 15%. It is twice as common in women than men.

Clinical depression[3] may be related to an imbalance in endogeneous amines 5-hydroxy tryptamine (5-HT) or norepinephrine (NE) in CNS led to an amine hypothesis of etiology and spurred efforts to enhance synaptic action of these amines. Most of the antidepressants primarily enhance the action of endogeneous amine neurotransmitters, they act directly, not binding to 5-HT or NE receptors but enhancing neurotransmitter action by inhibiting amine metabolism or removing neurotransmitter from synapses. Increased synaptic 5-HT or NE level then counteract the abnormally low level that produce depression.

a. Symptoms

The most common symptoms[4] of depression are the following

-depressed mood most of the day
-loss of interest in pleasurable activities
-decreased or increased appetite
-weight loss or gain
-difficulty concentrating
-insomnia or hypersomnia
-feeling of worthlessness or guilt
-psychomotor retardation or agitation fatigue
-recurrent thought of death, suicide and or a suicide attempt.

b. Causes

There are a number of factors that may increase the chance of depression including the following:

Abuse: Past physical, sexual, or emotional abuse can cause depression later in life.

Certain medications: For example, some drugs used to treat high blood pressure, such as beta-blockers or reserpine, can increase your risk of depression.

Conflict: Depression may result from personal conflicts or disputes with family members or friends.

Death or a loss: Sadness or grief from the death or loss of a loved one, though natural, can also increase the risk of depression.

Genetics: A family history of depression may increase the risk. It's thought that depression is passed genetically from one generation to the next. The exact way this happens, though, is not known.

Major events: Even good events such as starting a new job, graduating, or getting married can lead to depression. So can moving, losing a job or income, getting divorced, or retiring.

Other personal problems: Problems such as social isolation due to other mental illnesses or being cast out of a family or social group can lead to depression.

Serious illnesses: Sometimes depression co-exists with a major illness or is a reaction to the illness.

Substance abuse: Nearly 30% of people with substance abuse problems also have major or clinical depression.

c. Theory of depression (The monoamone theory)

The main biochemical theory of depression is the monoamine hypothesis, proposed by Schildkraut in 1965, which states that depression is caused by a functional deficit of monoamine transmitters at certain sites in the brain, while mania results from a functional excess. Initially, the hypothesis was formulated in terms of noradrenaline (norepinephrine), but the subsequent work showed that most of the observations were equally consistent with 5-hydroxytryptamine (5-HT) being the key mediator. Attempts to obtain more direct evidence, by studying monoamine metabolism in depressed patients or by measuring changes in the number of monoamine receptors in post-mortem brain tissue, have tended to give inconsistent and equivocal results and the interpretation of these studies is often problematic, because the changes described are not specific to depression. Many studies have sought to the amine hypothesis by looking for biochemical abnormalities in cerebrospinal fluid, blood or urine or in post-mortem brain tissue, from depressed or manic patients. They have included the studies of monoamine metabolites, receptors, enzymes and transporters, largely with negative results. The major metabolites of noradrenaline and 5-HT respectively are 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-hydroxyindoleaceticacid (5-HIAA). These appear in the cerebrospinal fluid, blood and urine.

Various attempts have been made to test for a functional deficit of monoamine pathways in depression. Hypothalamic neurons controlling pituitary function receive noradrenergic and 5-HT inputs, which control the discharge of these cells. Hypothalamic cells release corticotrophin-releasing hormone (CRH), which stimulates pituitary cells to secrete adrenocorticotrophic hormone (ACTH), leading in turn to cortisol secretion. The plasma cortisol concentration usually high in depressed patients. CRH concentration in the brain and cerebrospinal fluid of depressed patients

are increased. Therefore CRH hyperfunction as well as monoamine hypofunction, may be associated with depression.

d. Treatment of depression

The three most commonly indicated treatments for depression are psychotherapy, psychiatric medication, and (in severe cases) electroconvulsive therapy. Psychiatric medication is the primary therapy for major depression. An antidepressant is a pshchiatric medication used to alleviate mood disorders such as major depression and dysthymia and anxiety disorders. Various antidepressants are monoamine oxidase inhibitor (MAOs)selective serotonin reuptake inhibitor (SSRIs), serotonin-norepinephrine inhibitor (SNRIs), norepinephrine reuptake inhibitor (NRIs), norepinephrine-dopamine reuptake inhibitor (NDRIs), serotonin reuptake transporter inhibitor (SERT). Recent studies suggest that combination therapy, a Dopamine transporter inhibitor (DAT) and an SSRI or SNRI offers improved efficacy in the treatment of depression.

In an effort to expand beyond classical monoamine based strategies, recent medication development activities have focused on neurotropic factors, glutamatergic system and hypothalamic pituitary axis as well as number of other well characterized novel targets.

1.2. MEDICINAL CHEMISTRY

The discipline of medicinal chemistry[5] is devoted to the discovery and development of new agents for treating diseases. Most of this activity is directed to new natural or synthetic organic compounds. Medicinal chemistry was defined by IUPAC specified commission as “it concerns the discovery, the development, the identification and interpretation of the mode of action of biologically active compounds at the molecular level”.

The medicinal chemistry covers the following stages

- In the first stage new active substances or drugs are identified and prepared from natural sources, organic chemical reactions or biotechnological processes. They are known as lead molecules.
- The second stage is optimization of lead structure to improve potency, selectivity and to reduce toxicity
- Third stage is development stage, which involves optimization of synthetic route for bulk production and modification of pharmacokinetic and pharmaceutical properties of active substances to render it clinically useful.

The focus on development of new synthetic drug compounds has resulted in the incorporation of many other disciplines such as biochemistry and molecular biology into medicinal chemistry.

1.3 HETEROCYCLIC COMPOUNDS

The organic compounds have an enormous diversity of structure. Many of these structures contain ring systems. If the ring system is made up of atoms of carbon and atleast one other element, the compound can be classed as heterocyclic. The element that occur most commonly together with carbon in ring systems are nitrogen, oxygen and sulphur.

a) Therapeutic uses of heterocyclic compounds

Heterocyclic compounds used as pharmaceuticals, as agrochemicals and as veterinary products. They are used as optical brightening agents, as antoxidants, as corrosion inhibitors and as additives with a variety of other functions. Many dyestuffs and pigments have heterocyclic structures.

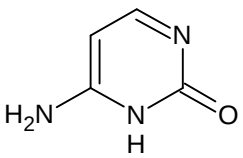
b) Reasons for wide spread uses of heterocyclic compounds

- Their structures can be subtly manipulated to achieve a required modification in function.

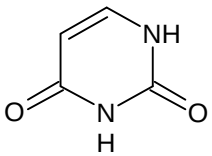
- It is possible to incorporate functional groups either as substituents or as part of the ring system itself.
- As intermediate in organic synthesis.
- Widely distributed in nature, importance to living systems and a key component in biological processes.
- Many of the pharmaceuticals and most of other heterocyclic compounds with practical application are not extracted from natural sources but are manufactured.

1.4 PYRIMIDINE

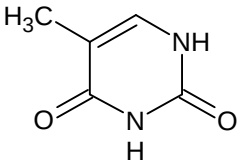
Pyrimidine[6] is a heterocyclic aromatic organic compound in which the two nitrogen atoms have the same positional relationship to each other as in amidines and in the imidazoles. It occur in many compounds vital to living systems. A substantial number of synthetic pyrimidines are valuable chemotherapeutic agents. Pyrimidines are important group of compounds since it occurs in purines, nucleic acids and synthetic barbiturates. The pyrimidines uracil, thymine and cytosine occur very widely in nature since they are components of nucleic acids in the form of N-substituted sugar derivatives (nucleosides).



Cytosine



Thymine



Uracil

a. Chemical properties

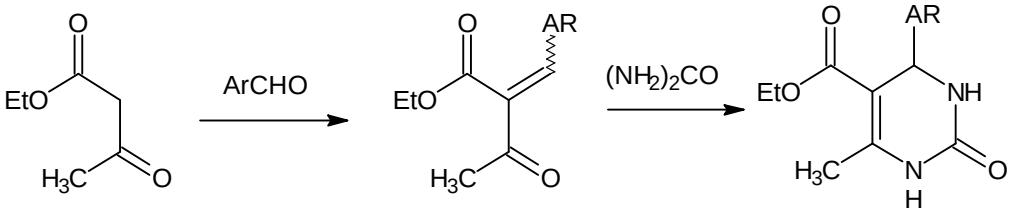
- . Water soluble hygroscopic compound, melting point 22°C
- Pyrimidines can be considered best as derivatives of pyridine and to a lesser extent as cyclic amidines
- Much weaker base than pyridine, imidazole, amidine
- Only one of the nitrogen atom is alkylated

- As the number of nitrogen atoms in the ring increases the ring pi electrons become less energetic and electrophillic substitution gets more difficult while nucleophillic substitution gets easier
- Reduction in resonance stabilization of pyrimidines may lead to addition and ring cleavage reactions rather than substitutions. This manifestation is observed in Dimroth rearrangement

b. Synthesis of pyrimidines

The most general and widely used route to pyrimidines involves the combination of reagent containing the N-C-N skeleton with one containing C-C-C unit. Urea, thiourea and guanidine are commonly used as N-C-N reagents(nucleophiles) and α,β-unsaturated ketones or acid derivatives as C-C-C fragments. Synthesis can be readily achieved by reaction is carriedout in HCl or sodiumethoxide in ethanol under reflux.

A method that is closely related to general route to pyrimidine is the Biginelli dihydropyrimidine synthesis. In its classical form it is a three component condensation of a β-ketoester, an aromatic aldehyde and urea. This method becomes a variant of the standard approach to pyrimidines.



Nowadays, Multicomponent reactions are of increasing importance in organic and medicinal chemistry. In times where a premium is put on speed, diversity and efficiency in drug discovery process, MCR strategies offer significant advantages over conventional linear type synthesis. The Biginelli three component protocol is particularly attractive since the resulting dihydropyrimidone (DHPM) scaffold displays a wide range of biological activities, which has led to the development of a number of lead compounds based on that structural core. Although a large number of

functionalized DHPMs can be potentially be prepared and the synthesized heterocyclic scaffold remains a structurally relatively simple dihydropyrimidone derivative.

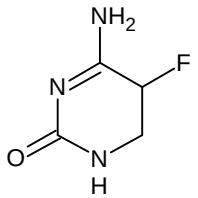
In classical Biginelli approach, we have considered the use of 4-chloroacetoacetate building block in Biginelli type condensation. The resulting functionalized DHPM appeared to be an ideal chemical template for the generation of a interesting bicyclic scaffold pyrrolo[3,4-d]pyrimines. The present study describe the synthesis of heterobicyclic scaffold from common chemical template employing conventional method.

c. Biological importance of pyrimidines[7]

The pyrimidine skeleton is of great importance as it is available in a large variety of naturally occurring compounds and also in clinically useful molecules having diverse biological activities. Because of their involvement as bases in DNA and RNA, they become very important in the world of synthetic chemistry. They include anticancer, antiviral, antibacterial, anti-inflammatory, cardiovascular, antiepileptic, antifungal activities.

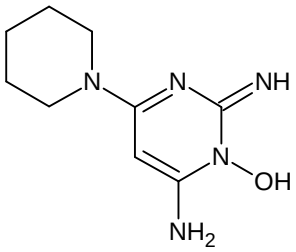
The following are some important biological and medicinal compounds possess substituted pyrimidine nucleus

Flucytosine



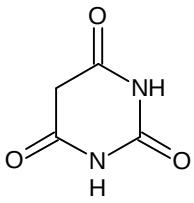
Category: antifungal

Minoxidil



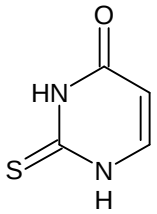
Category: potassium channel opener (vasodilator)

Barbitone



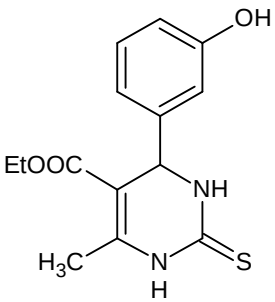
Category: Hypnotic, Sedative and Anticonvulsant

2-Thiouracil



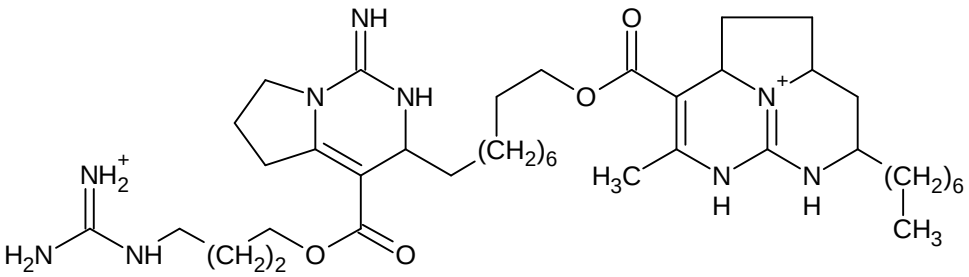
Category: Antithyroid

Monastrol



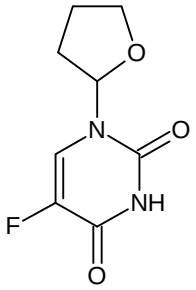
Category: Mitotic kinesin Eg5 inhibitor

Batzelladine B



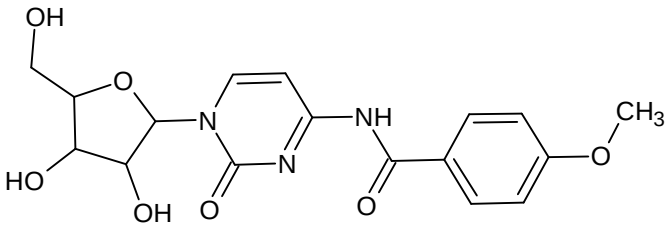
Category: Inhibitor of HIV envelope protein gp-120

Tegafur



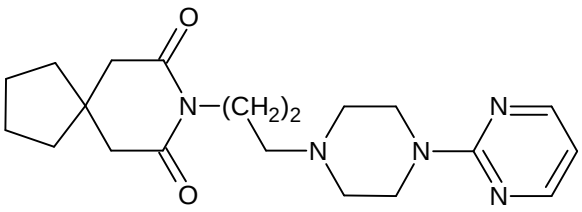
Category: Antineoplastic

Amicetin



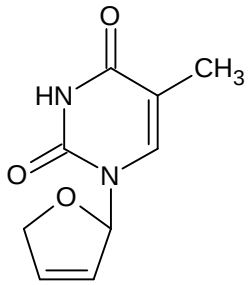
Category: Antibiotic

Buspirone



Category: 5-HT_{1A} antagonist, Anxiolytic, Antidepressant.

Stauvidine



Category: Anti HIV

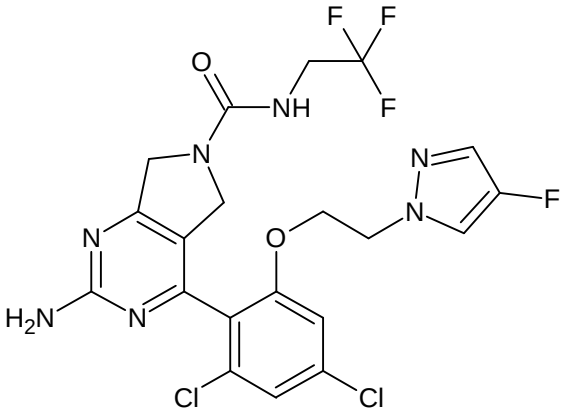
Above information clearly explains the need of an ideal antidepressant agents in treating the patients with depression.

Though there are wide range of antidepressant agents available for clinical use, a novel approach to identify a series of antidepressant agents was inspired by the fact of limited exploitation of fused pyrimidine derivatives in this segment. Pyrrole and its analogues exhibiting various pharmacological activities such as antimicrobial, analgesic. Similarly pyrimidine and its derivatives exhibiting diverse pharmacological activities such as anticancer, antiviral, antimicrobial activities. Though pyrimidine and analogues have known pharmacological activities, surprisingly pyrimidine derivatives were not much evaluated for their antidepressant activities. Hence, the above discussed information about both pyrimidine and pyrrole motivated towards synthesizing a novel series of pyrimidine fused with pyrrole.

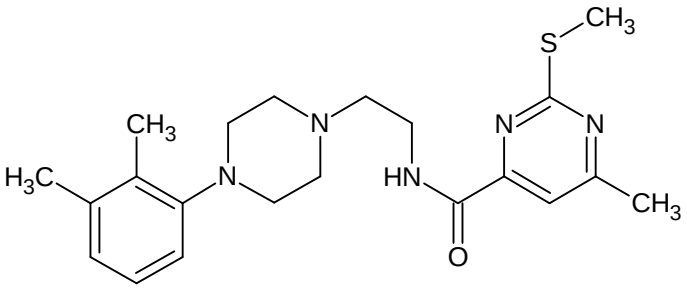
2. REVIEW OF LITERATURE

Antidepressant is a psychiatric medication used to alleviate mood disorders such as major depression, dysthymia and anxiety disorder. Many drugs produce an antidepressant effect but restriction on their use. Our current understanding of its pathogenesis is limited and existing treatments are inadequate. The present review of literature suggest that the heterocyclic moieties and their derivatives showing antidepressant activity.

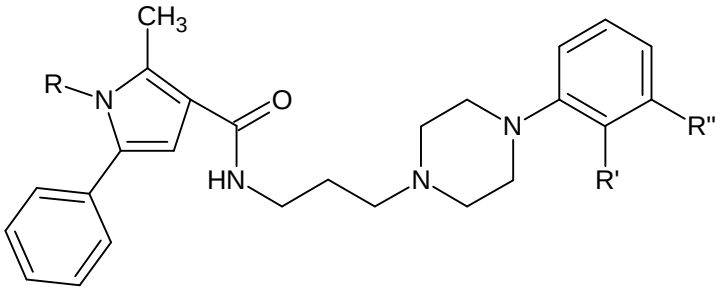
Pei-Pei Kung., *et al*[8]., (2011) studied the optimization of potent, selective and orally bioavailable Pyrrolodinopyrimidine-containing inhibitors of heat shock protein 90. Identification of development candidate 2-Amino-4-{4-chloro-2-[2-(4-fluoro-1*H*-pyrazol-1-yl)ethoxy]-6-methylphenyl}-*N*-(2,2-difluoropropyl)-5,7-dihydro-6*H*-pyrrolo[3,4-*d*]pyrimidine-6-carboxamide.



Kim JY., *et al*[9]., (2010) reported the arylpiperazine-containing pyrimidine 4-carboxamide derivatives targeting serotonin 5-HT_{2A}, 5-HT_{2C} and the serotonin transporter as a potential antidepressant.

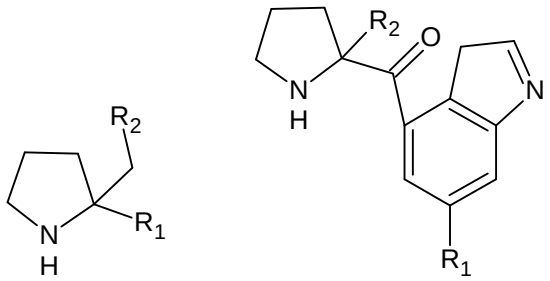


Kang., *et al*[10]., (2010) reported that the aryl piperazine containing pyrrole-3-carboxamide derivatives targeting serotonin 5-HT_{2A}, 5-HT_{2C} and the serotonin transporter as a potential antidepressant.



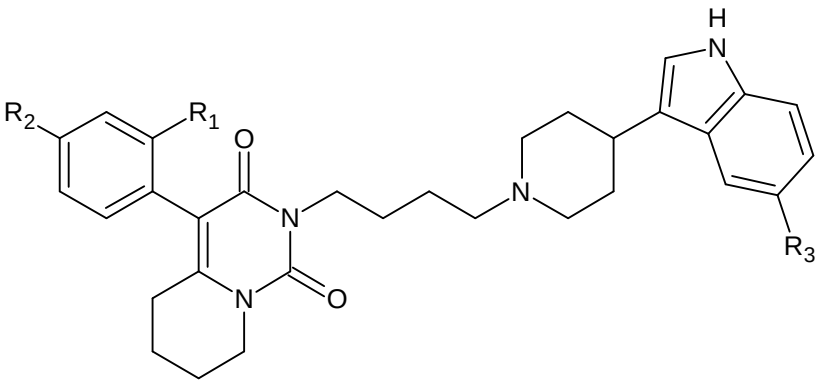
R' & R" = Cl , R = Me

Lucas., *et al*[11]., (2010) synthesized a new series of monoamine triple uptake inhibitors through scaffold homologation of recently reported series of 3,3-disubstituted pyrrolidine TRIS.



R_1 = H , R_2 = $C_6H_5CH_2CH_3$

Herhold F., *et al*[12]., (2009) reported the novel 4-aryl-pyrido[1,2-c]pyrimidines with dual SSRI and 5-HT_{1A} activity.



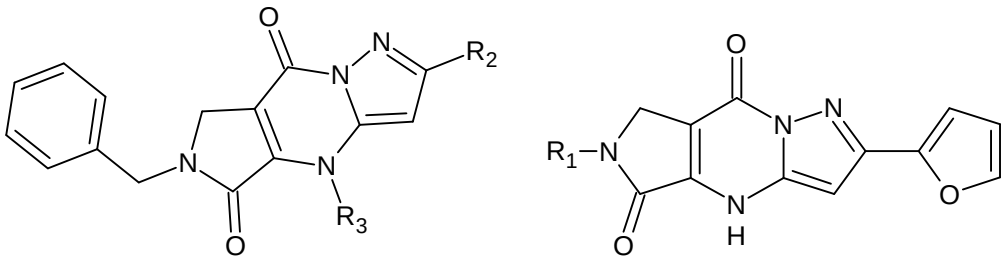
R_1 =OCH_3 , R_2& R_3 =H

R_1 = F , R_2 = H , R_3 = OCH_3

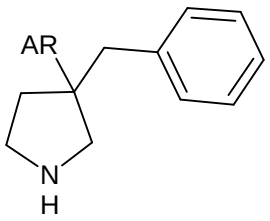
R_1& R_3 = H , R_2 = CH_3

R_1 = F , R_2& R_3= H

Su-Ying Wu., *et al*[13]., (2008) reported the identification, SAR exploration and molecular modeling of 6,7-dihydro-4H-pyrazolo-[1,5-a]pyrrolo[3,4-d]pyrimidine-5,8-dione scaffold as aurora kinase A inhibitors.

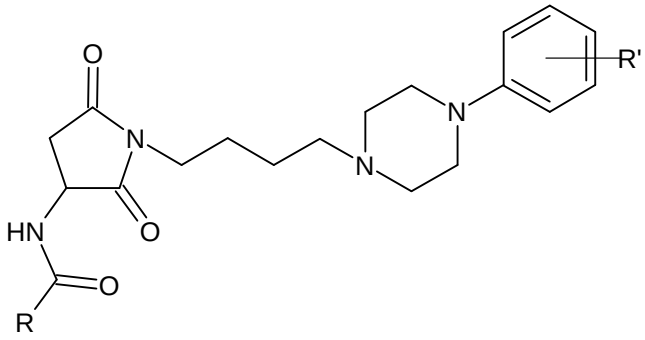


Bannwart *et al*[14]., (2008) designed a novel 3,3-disubstituted pyrrolidines as selective triple serotonin, norepinephrine, dopamine reuptake inhibitors.



AR = 5-indaonyl

Zajdel *et al*[15]., (2007) synthesized a novel class of aryl piperazine containing N-acylated aminoacids:Their synthesis, $5HT_{1A}$, $5\text{-}HT_{2A}$ receptor affinity and invivo pharmacological evaluation.

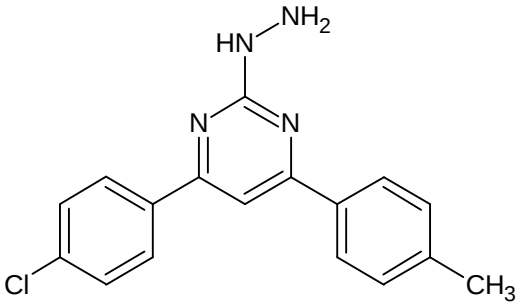


R=admantyl, R' =3-Cl

R=C_2H_4 , R'= 2-OCH_3

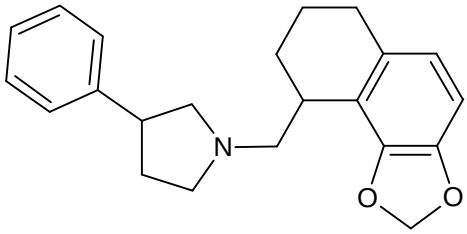
R=C_6H_{11},R'=2-OCH_3

Rodrigues ALS.*, et al*[16]., (2005) reported the antidepressant-like antinociceptive-like action of 4-(4 ' -cholorophenyl)-6-(4"-methylphenyl)-2-hydrazinepyrimidine Mannich base in mice.



Darias V.*, et al*[17]., (1999) studied the antidepressant activity of 4-phenyl-2-thioxo-benzo[4,5]thieno[2,3-d]pyrimidine derivatives.

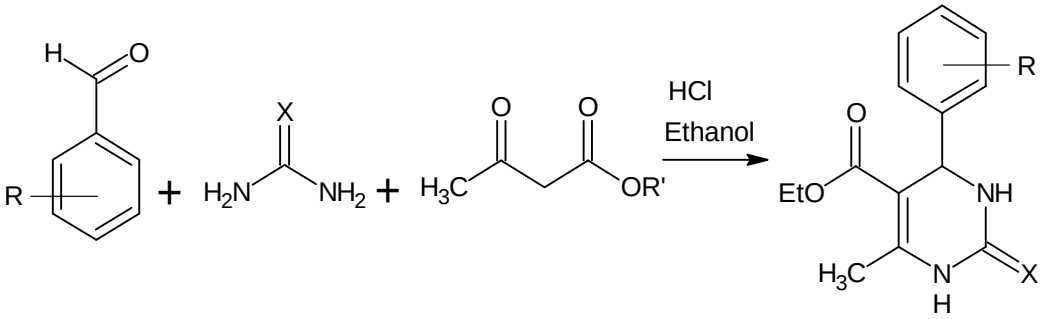
Zelle *et al*[18]., (1994) carried out the synthesis and pharmacological characterization of ABT:200:A putative novel antidepressant combining potent α_2 antagonism with moderate NE uptake inhibition.



Bernier JL., *et al*[19]., (1980) reported the synthesis and structure-activity relationship of a pyrimido[4,5-d]pyrimidine derivative with antidepressant activity.

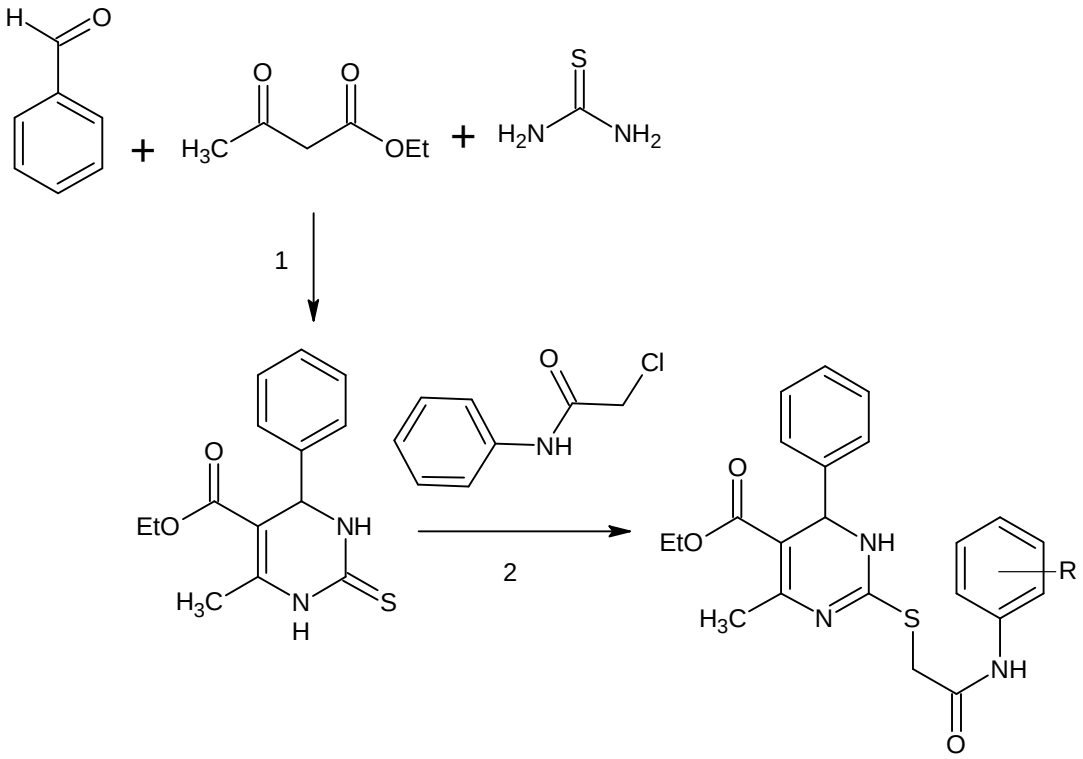
To generate, a new antidepressant agent, the heterobicyclic scaffold pyrrolo[3,4-d] pyrimidinone derivatives have been selected for synthesis. Synthesis of the above said scaffold carried out by multicomponent reaction followed by cyclization. The following literatures have been reviewed based on synthesis.

Ujwala B.Belge., *et al*[20]., (2011) synthesised and evaluated the preliminary pharmacological screening of 4-aryl substituted-3,4-dihydropyrimidin-2(1H)one/thione derivatives as calcium channel blockers.



R=4-$N0_2$, 4-OH, 4-CH_3, 4-Br

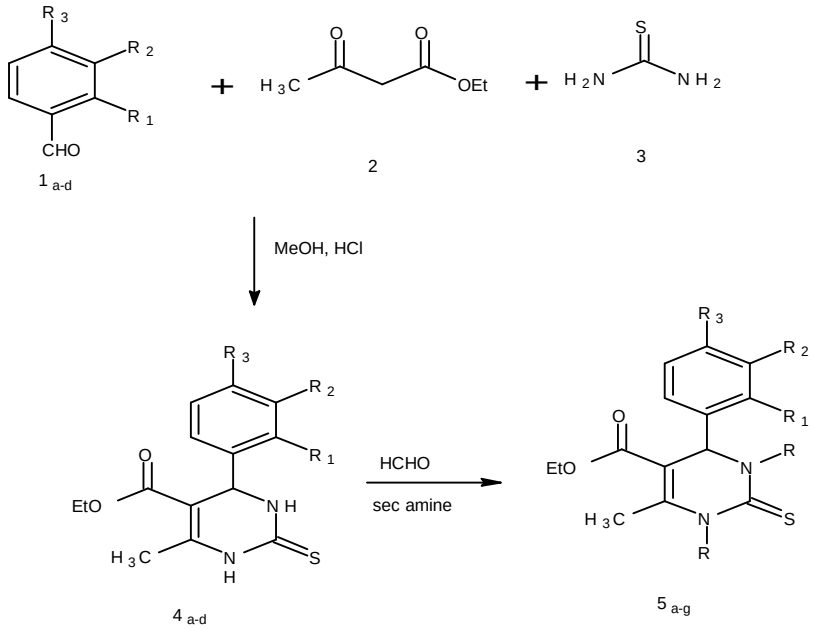
Rajasekaran S., *et al*[21]., (2011) carried out the design, synthesis and biological activity of substituted dihydropyrimidin-2-(1H)-thiones.



1.ethanol/HCl 2.pyridine

R=H, 2-CH_3, 4-CH_3, 4-Cl, 3-NO_2

Shah T.B.*, et al*[22]., (2010) conducted the synthesis and invitro study of biological activity of heterocyclic N-Manich bases of 3,4-dihydropyrimidin-2(1H)-thiones.



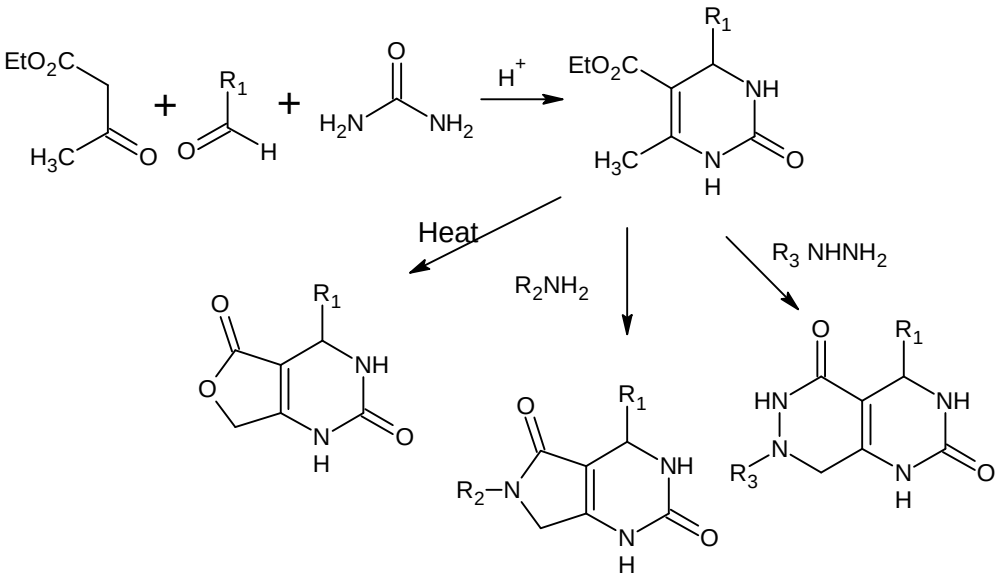
4a-d : R1=R2=R3=H	5a-g: R=benzimidazole, benztriazole
R1=OH, R2&R3=H	phthalimide, 2-ph bezimidazole
R1=H,R2=OMe,R3=OH	2-Me benzimidazole,morpholine
R1&R2=H,R3=OMe	tetrahydrocarbazole

Mithun Ashok., *et al*[23]., (2007) carried out the convenient one pot synthesis of some novel derivatives of thiazolo[2,3-b]dihydropyrimidinone possessing 4-methylthiophenyl moiety and evaluation of their antibacterial and antifungal activities.

Selma Sarac., *et al*[24]., (2006) conducted the synthesis of 4-aryl-3,4-dihydropyrimidin -2(1H)-thione derivatives as potential calcium channel blockers.

Abdel Momen A EI Khamry., *et al*[25]., (2006) demonstrated the synthesis of 4-aryl-isoxazolo[5,4-d]pyrimidin-6-one(thione) and 4-aryl-pyrazolo-[3,4-d]-pyrimidin-6-one derivatives of potential antihypertensive activity.

Oliver Kappe. C., *et al*[26]., (2002) conducted the traceless solid-phase synthesis of bicyclic dihydropyrimidones using multidirectional cyclization cleavage.

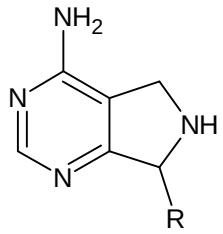


Mirzaei. Y.R., *et al*[27]., (2001) investigated the chemical reactivity of positions N-3, C-5 and C_6-methyl group in biginelli type compounds and synthesis of new dihydropyrimidine derivatives.

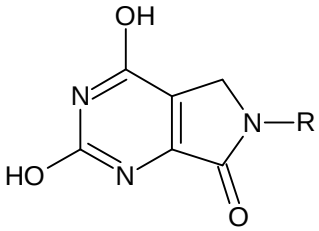
Tarzia.G., *et al*[28].,(1979) performed the synthesis and anti-inflammatory properties of some pyrrolo(1H,3H)[3,4-d]pyrimidin-2-ones and pyrrolo(1H,6H)[3,4-d]pyrimidin-2-ones.

Philip L. Southwick., *et al*[29]., (1974) reported the synthesis of antifols related to 2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*] pyrimidine. Enhancement of antiparasitic selectivity by nitrogen-linked mono- and dichlorobenzoyl groups or the 3,4–dichlorophenylthiocarbamoyl group.

Tuvia Sheradsky., *et al*[30]., (1964) reported the 6,7-dihydro-5H-pyrrolo[3,4-d]pyrimidines. Syntheses based on 3-amino- and 3-methoxy-1-acyl-4-cyano-3-pyrrolines.



Cavalla. J.F[31]., (1964) reported the 3-amino-4-cyano-3-pyrrolines: Their conversion to pyrrolo[3,4-d]pyrimidines.



Philip L. Southwick., *et al*[32]., (1963) reported the compounds in the Pyrrolo[3,4-d]pyrimidine series. Syntheses Based on 2,3-Dioxopyrrolidines.

From the exclusive literature survey conducted from the available sources, it is clear that so far no derivatives of pyrimidine fused with pyrrole has been screened for antidepressant activity.

3. AIM AND OBJECTIVE

Depression affects up to 20% of population world wide and is the fourth leading cause of disease or disability. Most antidepressants include delayed clinical benefit, serious side effects and response in less than 50% of patients. Neither the pathophysiology of depression nor the mechanism of action of various antidepressants is fully understood. Consequently, there is still a greater need or fast acting, safer and more effective treatments for depression. Hence the search for newer antidepressant agents is made an attempt to synthesis a series of compounds.

Nitrogen containing heterocyclic compounds featured predominantly in early studies of chemistry and they were closely associated with the development of organic chemistry. Pyrimidines are the most important one among them because of their wide range of pharmacological uses. Pyrimidine fused with pyrrole derivatives are synthesized and evaluated for antidepressant activity.

The main objective of the present study is the following:

1. Synthesis of new series of 2-thioxo-5H-pyrrolo[3,4-d]pyrimidin-5-one derivatives
2. Characterization of the synthesized compounds by spectral methods viz.infrared (IR Spectra), Nuclear magnetic resonance spectra (^{1}H NMR) and Mass spectra.
3. Evaluation of acute toxicity study.
4. Evaluation of antidepressant activity by behavioural despair test.

4. MATERIALS AND METHODS

The methods employed in this research included the following:

1. Introduction
2. Synthesis
3. Characterization
4. Evaluation of biological activity

4.1 INTRODUCTION

One of the things that makes chemistry unique among the science is synthesis. Synthetic organic chemistry is focused largely on compounds of pharmaceutical importance and covers novel molecule design, natural product analogues and derivatives of natural products.

Lipinski Rule of Five

Christopher Lipinski's rule-of-five analysis helped to raise awareness about properties and structural features that make molecules more or less drug-like. The guidelines were quickly adopted by the pharmaceutical industry as it helped apply ADME considerations early in preclinical development and could help avoid costly late-stage preclinical and clinical failures. The guidelines predict that poor absorption or permeation of a orally administered compound are more likely if the compound meets the following criteria:

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms).
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms).
- A molecular mass not greater than 500 daltons.
- An octanol-water partition coefficient log *P* not greater than 5.
- No of rotatable bonds ≤ 10.

The physical properties of the synthesized molecules were calculated using a Software called Molinspiration on line database. The values are given in Table 1 Lipinski rule of the synthesized compounds

Table 1. Lipinski rule of synthesized compounds.

S.No	miLogP	M.Wt	nON	nOHNH	n rot b	TPSA	Molar volume	n violation
IIa	1.635	315.39	5	3	4	64.59	276.87	0
IIb	0.878	289.36	5	3	3	64.59	249.46	0
IIc	0.98	332.42	6	3	4	67.82	295.36	0
IId	1.556	323.80	5	3	3	64.59	262.99	0
IIe	0.819	305.35	6	4	3	84.81	257.47	0
IIf	0.525	349.41	7	3	5	83.05	300.55	0
IIg	0.217	335.38	7	4	4	94.05	283.02	0

miLogP	-	LogP
M.Wt	-	Molecular weight
nON	-	No of hydrogen bond acceptor
nOHNH	-	No of hydrogen bond donar
n rot b	-	No of rotatable bonds
TPSA	-	Total polar surface area

From the above data, the synthesized compounds have satisfied the Lipinski rule of Five.

4.2 SYNTHESIS

The synthesis of pyrrolo[3,4-d]pyrimidine[33,34] involves both pyrimidine and pyrrole precursors almost evenly. The presence of a carboxylic ester at position 5 of a pyrimidine ring contributes to a series of fused pyrroles containing a carbonyl

group. The pyrimidine containing a carboxylic ester at position 5 is heated at reflux conditions with primary amine providing pyrrolo[3,4-d]pyrimidine.

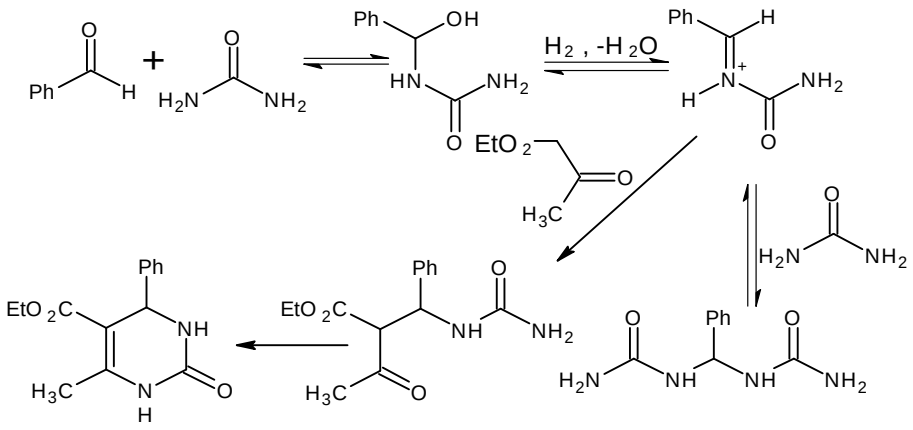
Step 1:

The synthesis is based on "Biginelli dihydropyrimidine synthesis" which involves acid catalyzed three component cyclocondensation of β-ketoester, an aromatic aldehydes and urea or thiourea.

Reaction mechanism:

The first mechanism for the synthesis of dihydropyrimidine (Biginelli reaction) were conducted by Folkers and Johnson in 1933. In 1973, a second mechanistic proposal was suggested by Sweet and Fissekis. In 1997, the mechanism was provided by Kappe whose proposal is currently the accepted mechanism for the Biginelli reaction.

Kappe[35](1997)



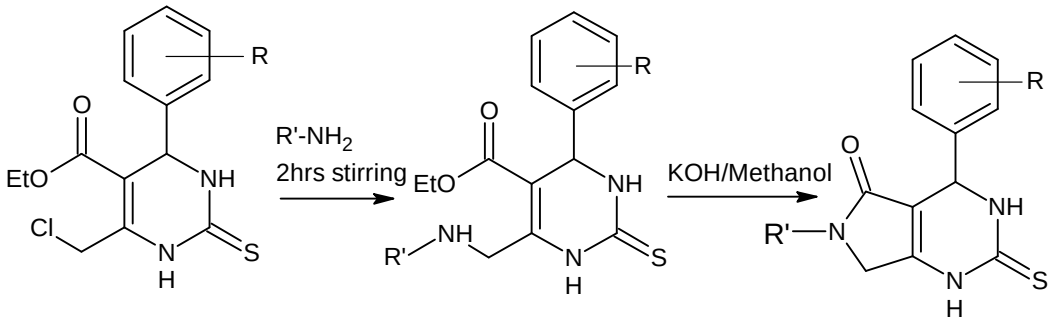
Step 2:

6-Chloromethyl functionalised dihydropyrimidine intermediates on treatment with an excess of primary amine in methanol provided the pyrrolo[3,4-d]pyrimidine. The corresponding amine was allowed to react with the appropriate chloromethyl dihydropyrimidine at ambient or at a slightly elevated temperature for

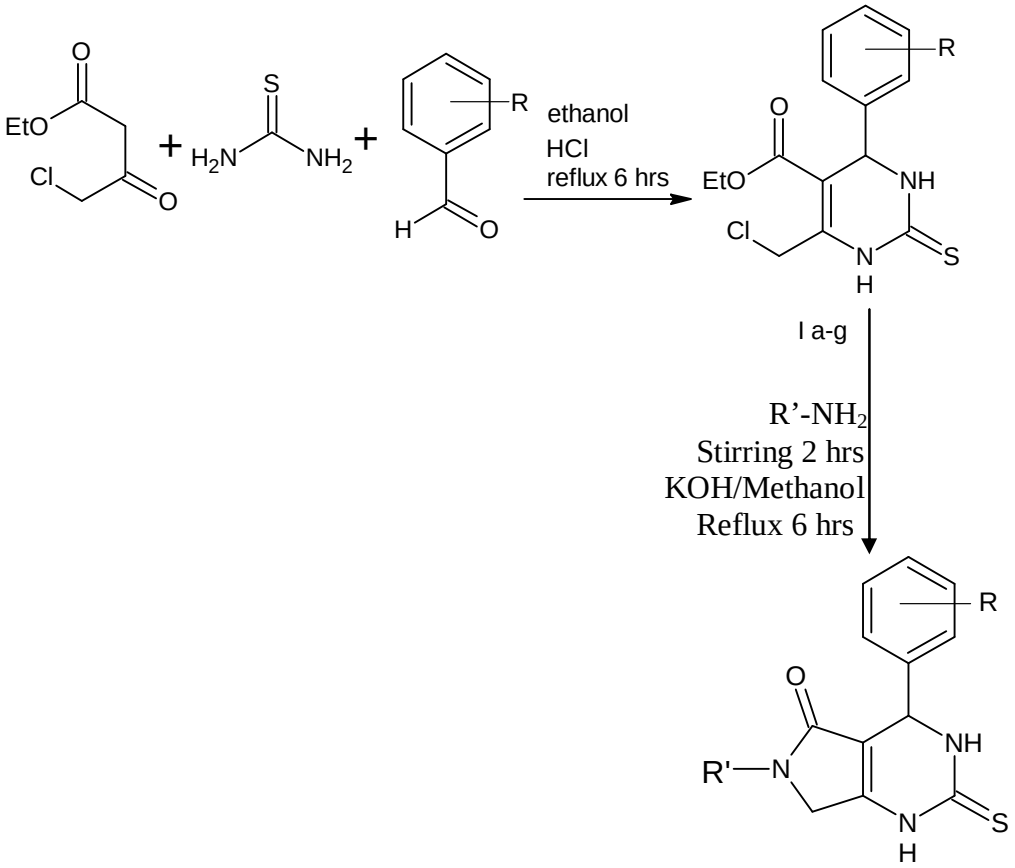
1-2 hours followed by heating under reflux for 5-6 hours. The solid pyrrolo[3,4-d]pyrimidine was directly precipitated from the solution upon cooling.

Reaction mechanism:

The reaction takesplace in two steps. In the first step, the chlorogroup was displaced by excess amine nucleophile. In the second step, the cyclative nucleophillic cleavage (Lactam formation) by cyclization of ethyl ester with the resulting secondary amine. On the basis of different reactivity of the primary amines, the nucleophillic displacement was carried out at room temperature for alkylamines and phenylethylamines or at 50°C for benzylamines and sterically hindered amines.



a. Synthetic scheme



R = (i) -CH=CH- (ii) –H (iii) -$N(CH_3)_2$

(iv) -4-Cl (v) -2-OH (vi)-3,4-di-OCH_3

(vii) -3-OCH_3, -4-OH

R' = -CH_2-CH_2-OH

b. Reactant profile[36]

The synthesis was carried out in room temperature. Solvents and reagents used were of laboratory grade. All the reactions were conducted in thoroughly dried apparatus. The reactants and solvents used for the synthesis were given in Table 2.

Table 2. Reactant profile

Reagent	Mol. Formula	Mol. weight	Description	mp
Ethyl-4-chloroacetoacetate	$C_6H_9ClO_3$	164.59	pale reddish-yellow clear liquid	115°C
Thiourea	CH_4N_2S	76.12	White solid	182°C
Benzaldehyde	C_7H_60	106.12	Colourless liquid	178.1°C
Cinnamaldehyde	C_9H_8O	132.15	Yellow oily liquid	252°C
P-dimethyl aminobenzaldehyde	$C_9H_{11}NO$	149.19	yellowish solid	74°C
p-chloro benzaldehyde	C_7H_5ClO	140.57	White crystalline solid	46°C
Salicylaldehyde	$C_7H_6O_2$	122.13	pale yellow oily liquid	97°C
Veratraldehyde	$C_9H_{10}O_3$	166.17	White to offwhite crystalline fused mass	43°C
Vanillin	$C_8H_8O_3$	152.15	White crystals with floral pleasant odour	81°C

c. Synthetic procedure:

Step 1:

Synthesis of 6-chloromethyl derivative of dihydropyrimidine-2-thione (Ia-g):

A mixture of Ethyl-4-chloroacetoacetate (3.0mmol), appropriate aldehydes (3.0mmol), thiourea (3.5mmol) were refluxed in 30ml ethanol containing 0.5ml hydrochloric acid for 5-6 hrs. The reaction mixture was cooled and poured into ice-cold water. The solid product which was obtained was filtered, dried and recrystallized from ethanol.

Step 2:

Synthesis of 2-thioxo-5H-pyrrolo[3,4-d]pyrimidin-5-one (IIa-g):

To the product obtained in first step, solution of ethanolamine in methanol was added dropwise with stirring at 25-30°C. Stirring was continued for 1-2 hrs. Then methanolic sodium hydroxide solution (5ml) was then added. The reaction mixture was refluxed for 6 hrs and the excess of solvent was distilled. The reaction mixture was cooled to room temperature. The solid product which was obtained was washed with cold methanol, dried and recrystallized from suitable solvent.

Recrystallization

All the synthesised compounds were purified by recrystallization. Recrystalization of the synthesised compounds were done using suitable solvents such as acetone or ethanol.

Identification methods

The synthesised compounds were identified by following methods

Melting point

The melting points of the synthesised compounds were determined by open capillary tube method and are presented uncorrected.

Thinlayer chromatography

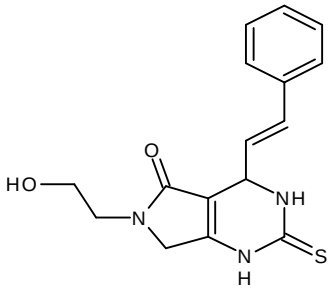
Precoated silicagel GF plates were used as a stationary phase, benzene and ethylacetate as mobile phase for the synthesised compounds. The spots were identified by iodine vapour or UV chamber.

A single spot with no secondary spots and absence of spot for parent compounds confirmed the purity of the compounds.

d. Product profile

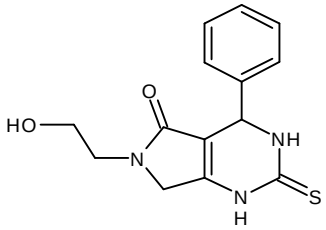
The synthesized compounds and their structure are given below.

IIa



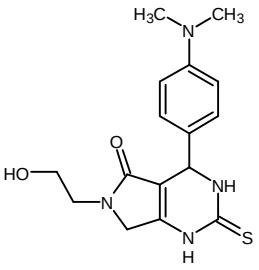
6-(2-hydroxyethyl)-4-[(*E*)-2-phenylethenyl]-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one.

IIb



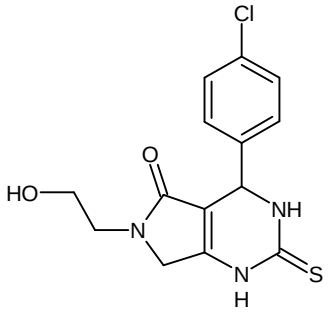
6-(2-hydroxyethyl)-4-phenyl-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one

IIc



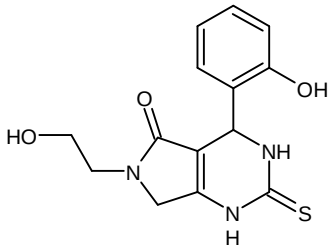
4-[4-(dimethylamino)phenyl]-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one

IId



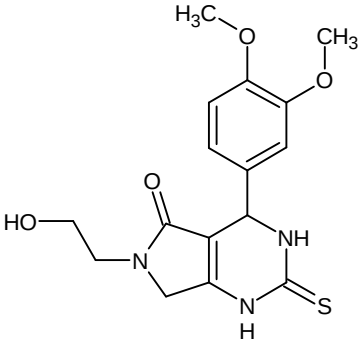
4-(4-chlorophenyl)-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one

IIe



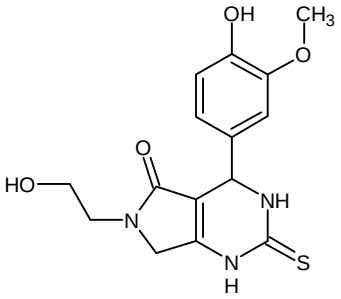
6-(2-hydroxyethyl)-4-(2-hydroxyphenyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one

IIf



4-(3,4-dimethoxyphenyl)-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one

IIg



6-(2-hydroxyethyl)-4-(4-hydroxy-3-methoxyphenyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one

4.3 CHARACTERIZATION[37,38,39]

The synthesised compounds were characterized by using the following methods

IR Spectra

IR Spectra was recorded on ABB Spectrum spectrometer in the range of 4000-400cm^{-1} using KBr pellet technique and expressed in cm^{-1}.

^{1}H NMR Spectra

Proton NMR Spectra was recorded on BRUKER Advance 500 NMR Spectrometer using the solvent Deuterated Dimethyl sulphoxide. Chemical shifts are reported in parts per million, relative to TMS as an internal standard.

Mass spectra

The Mass Spectra recorded on a JEOL GC Mate II Mass Spectrophotometer operating as direct probe using Electron Ionization technique

Table 3. Physical data of the synthesised compounds

S.No	Description	Mol formula	Mol weight	Solubility	mp (°C)	% Yield
IIa	Brown solid	$C_{16}H_{17}N_3O_2S$	305.39	DMSO, ethanol	118	66.67
IIb	Yellow solid	$C_{14}H_{15}N_3O_2S$	289.35	DMSO, ethanol, acetone	220	50.25
IIc	Red solid	$C_{16}H_{20}N_4O_2S$	332.42	DMSO, ethanol, acetone	120	47.72
IId	Yellow solid	$C_{14}H_{14}ClN_3O_2S$	323.79	DMSO, ethanol, acetone	210	67.4
IIe	Yellow solid	$C_{14}H_{15}N_3O_3S$	305.35	DMSO, ethanol	235	56.09
IIf	Yellow solid	$C_{16}H_{19}N_3O_4S$	349.40	DMSO, ethanol	157	54.11
IIg	Brown solid	$C_{15}H_{17}N_3O_4S$	335.37	DMSO, ethanol	102	53.69

Table 4. Spectral data of the synthesised compounds

S.No	IR ν cm^{-1} (KBr)	^{1}H NMR δ ppm	Mass(m/z value)
IIa	3425(OH str) 2970(CH str methylene) 2932(NH str)1662(C=O str) 1450(C=C str aromatic)	0.9-1.3(m,4H,CH_2) 2.9(s,2H,CH_2)5.2(s,1H,CH) 6.3(s,1H,CH) 6.7(s,1H,CH) 7.8(m,1H,OH) 8-8.5(m,5H,Ar-CH),9.5(s,1H,NH) 9.8(s,1H,NH)	315.24(M^+ion peak)
IIb	3317(OH str)3202(NH str) 2854(CH str methylene)2368(C=S str) 1520(C=O str)1450(C=C str aromatic)	0.9-1.3(m,4H,CH_2)2.9(s,2H,CH_2)4.0(s,1H,CH) 7.7(m,1H,OH)7.8-8.5(m,5H,ArCH)9.6(s,1H,NH) 9.8(s,1H,NH)	289.54(M^+ion peak)
IIc	3317(OH str)3194(NH str) 2924(CH str aromatic)1065(C=O str) 1443(C=C str aromatic)	0.8-1.3(m,4H,CH_2)2.9(s,1H,CH_2)3.0(s,6H,CH_3) 4.0(s,1H,CH)6.5-7.0(m,4H,Ar-CH) 7.8(m,1H,OH)9.2(s,1H,NH)9.8(s,1H,NH)	332.03(M^+ion peak)
IId	3379(OH str)2924(CH str methylene) 2361(C=S str)1589(C=0 str) 1489(C=C str aromatic)	0.9-1.3(m,4H,CH_2)3.9(s,2H,CH_2)4.1(s,1H,CH) 6.5-7.7(m,4H,Ar-CH)7.9(m,1H,OH) 8.3(s,1H,NH)9.2(s,1H,NH)	323.14(M^+ion peak)
IIe	3418(OH str)2955(CH str methylene) 2368(C=S str)1566(C=O str) 1458(C=C str aromatic)	1.4(m,2H,CH_2)1.9(m,2H,CH_2)2.6(s,2H,CH_2) 3.8(s,1H,CH)5.1(s,1H,Ar-OH) 7.2-7.5(m,4H,Ar-CH)7.9(m,1H,OH) 8.5(s,1H,NH)9.2(s,1H,NH)	305.36(M^+ion peak)
IIf	3410(OH str)2939(CH str aromatic) 1636(C=O str) 1466(C=C str)	0.8-1.2(m,4H,CH_2)2.6(s,2H,CH_2)2.9(s,6H,CH_3) 4.2(s,1H,CH)6.7-7.2(m,3H,Ar-CH) 7.3(m,1H,OH)8.2(s,1H,NH)8.5(s,1H,NH)	349.25(M^+ion peak)
IIg	3418(OH str) 2932(CH str methyl) 1636(C=O str) 1381(C=C str aromatic)	0.8-1.2(m,4H,CH_2)2.9(s,2H,CH_2)3.2(s,3H,CH_2) 4.1(s,1H,CH)5.3(s,1H,Ar-OH) 6.5-7.0(m,3H,Ar-CH)8.0(s,1H,NH) 9.2(s,1H,NH)	335.43(M^+ion peak)

Spectroscopic study of synthesised compounds

Figure 1 shows the IR spectrum of compound IIa

[6-(2-hydroxyethyl)-4-[(*E*)-2-phenylethenyl]-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

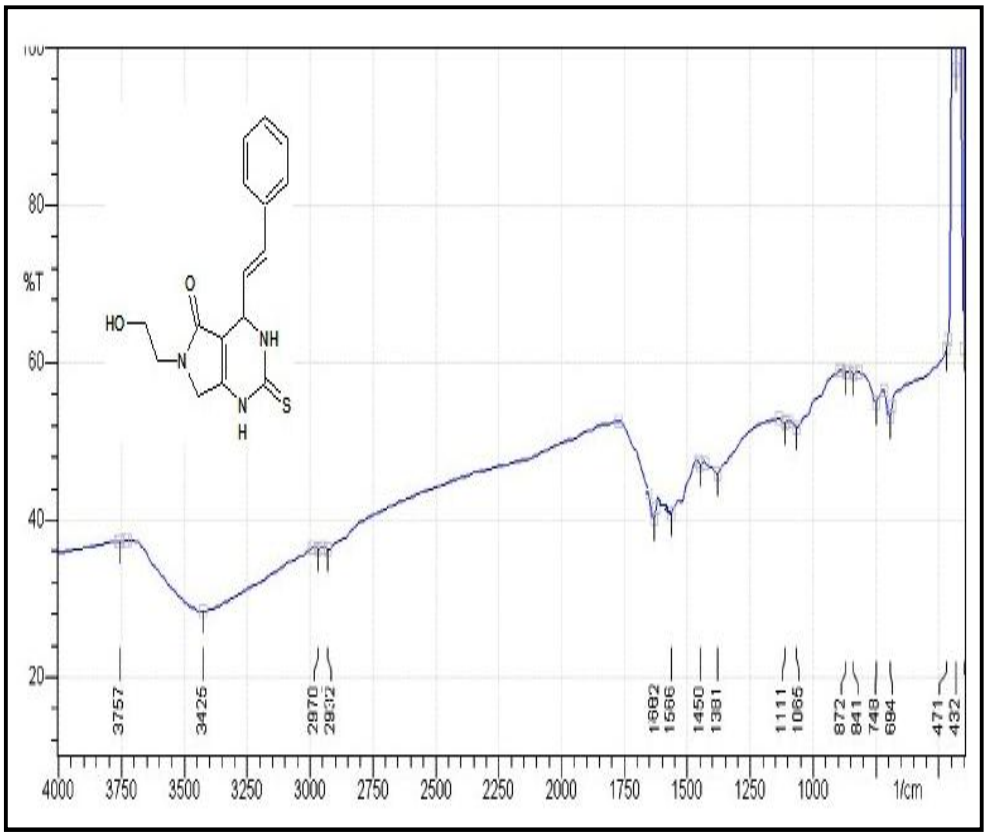


Fig. 1 3425(OH str), 2970(CH str methylene), 2932(NH str), 1662(C=O str), 1450(C=C str aromatic)

Figure 2 shows the ^{1}H NMR spectrum of compound IIa

[6-(2-hydroxyethyl)-4-[(*E*)-2-phenylethenyl]-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

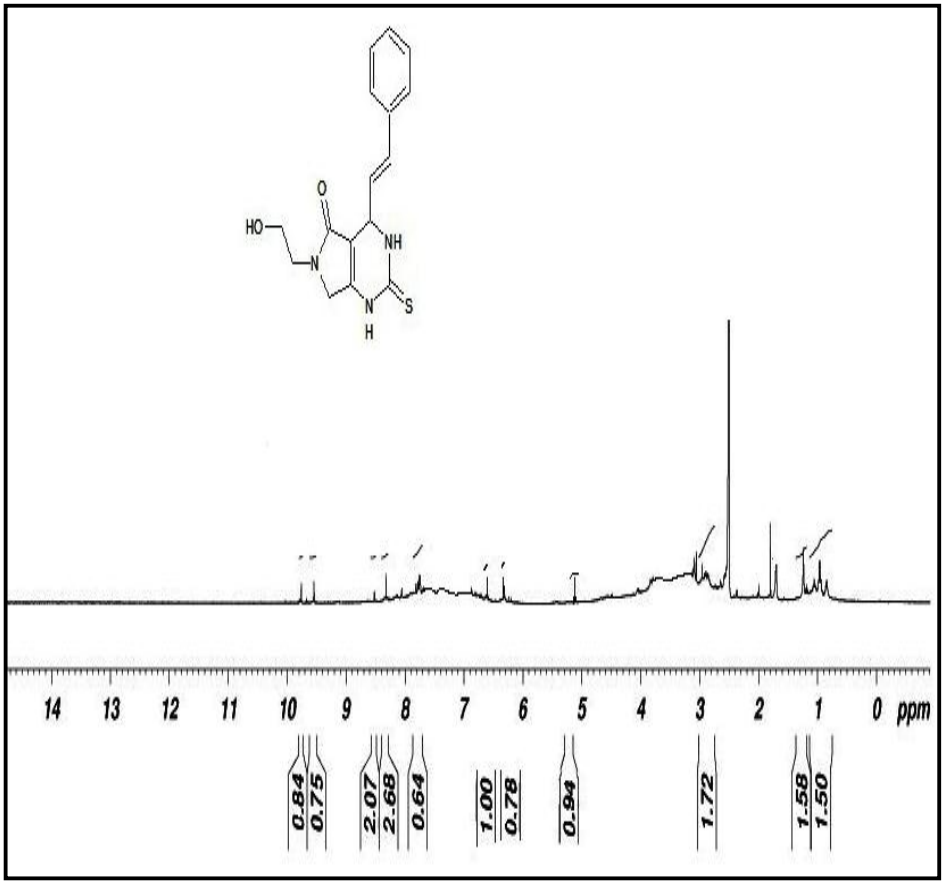


Fig. 2 0.9-1.3(m,4H,CH_2), 2.9(s,2H,CH_2), 5.2(s,1H,CH), 6.3(s,1H,CH), 6.7(s,1H,CH) 7.8(m,1H,OH), 8-8.5(m,5H,Ar-CH), 9.5(s,1H,NH), 9.8(s,1H,NH).

Figure 3 shows Mass spectrum of compound IIa

[6-(2-hydroxyethyl)-4-[(*E*)-2-phenylethenyl]-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

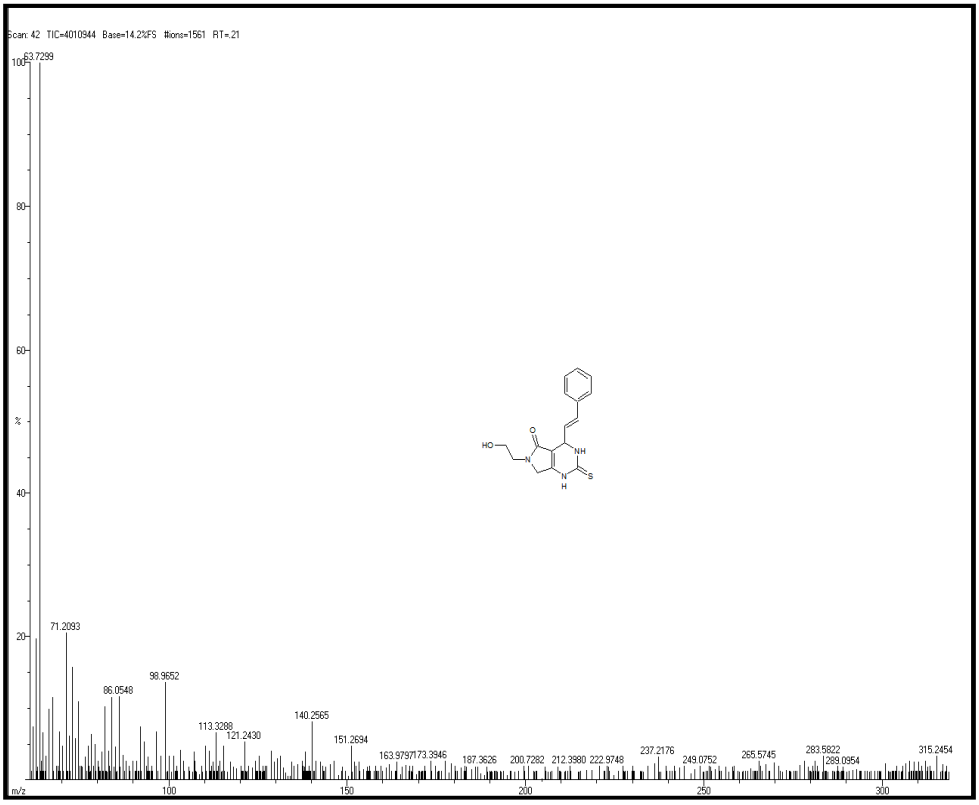


Fig 3 315.24 (M^+), 237.21, 173.39, 63.7.

Figure 4 shows IR spectrum of compound IIb

[6-(2-hydroxyethyl)-4-phenyl-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

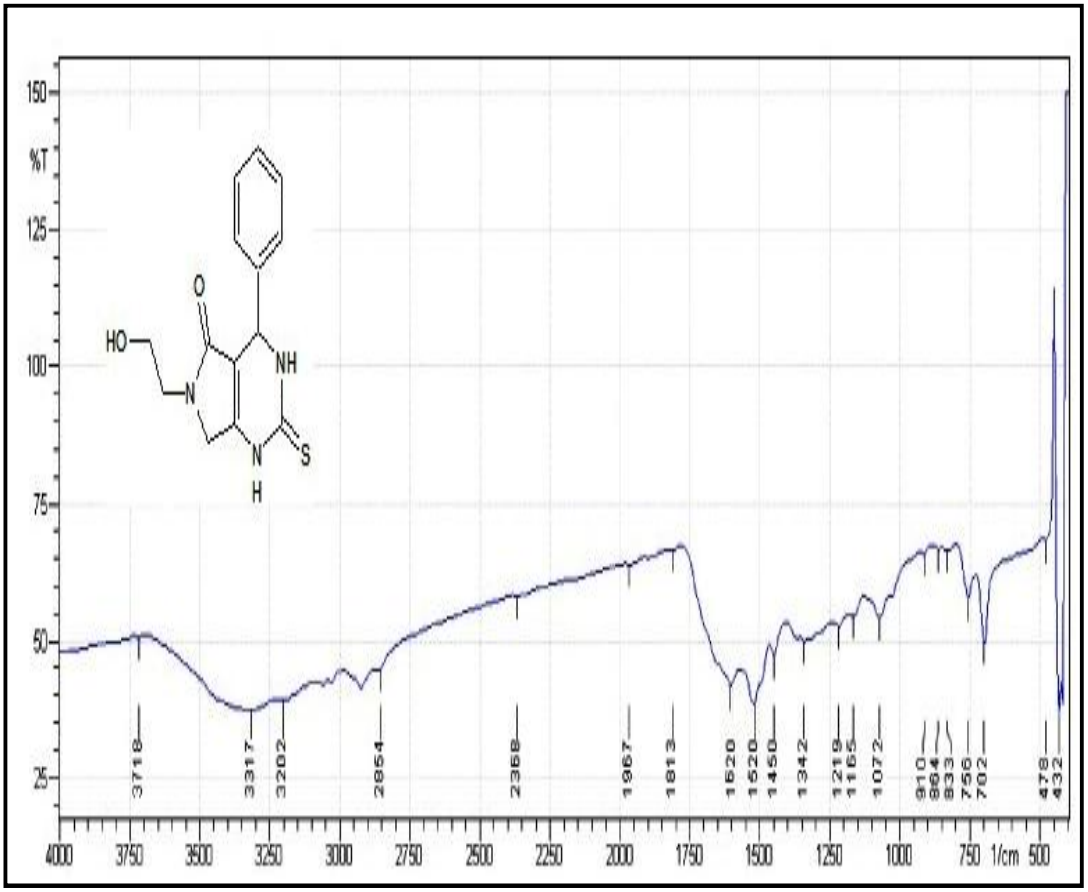


Fig 4 3317(OH str), 3202(NH str), 2854(CH str methylene), 2368(C=S str), 1520(C=O str), 1450(C=C str aromatic)

Figure 5 shows ^{1}H NMR Spectrum of compound IIb

[6-(2-hydroxyethyl)-4-phenyl-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4 *d*]pyrimidin-5-one].

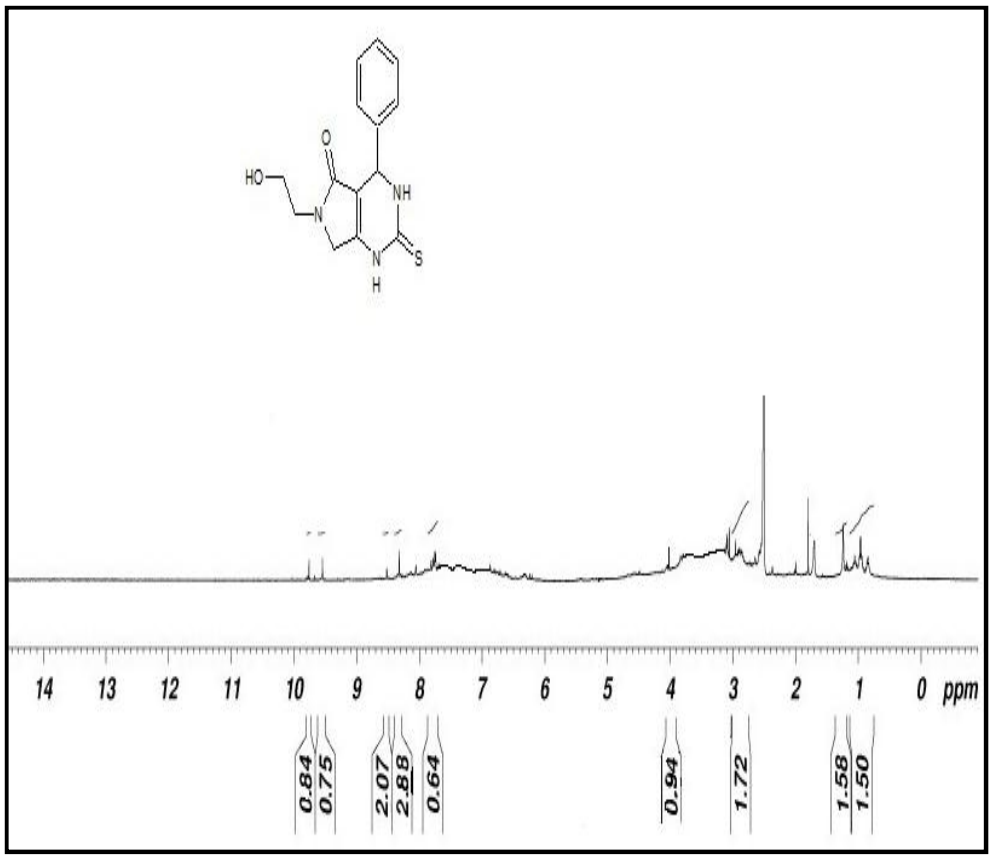


Fig 5 0.9-1.3(m,4H,CH_2), 2.9(s,2H,CH_2), 4.0(s,1H,CH), 7.7(m,1H,OH), 7.8- 8.5(m,5H,ArCH), 9.6(s,1H,NH), 9.8(s,1H,NH)

Figure 6 shows Mass spectrum of compound IIb

[6-(2-hydroxyethyl)-4-phenyl-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one]

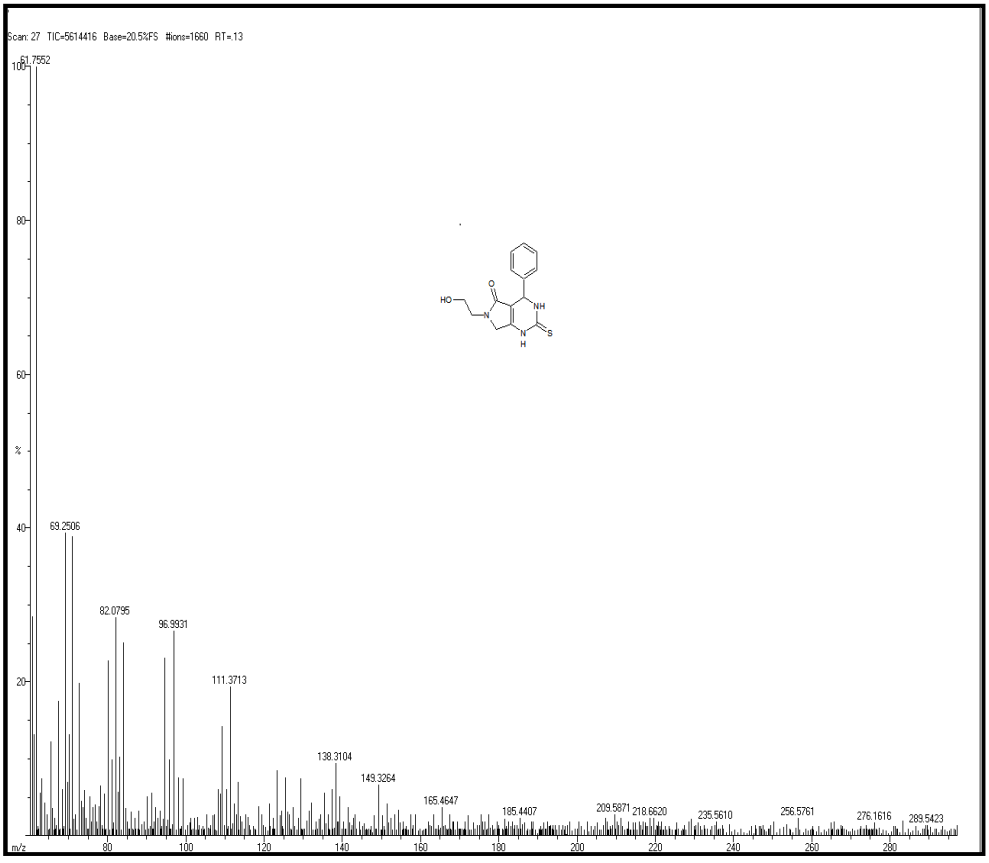


Fig 6 289.54 (M^+), 256.57, 149.32, 61.7.

Figure 7 shows IR spectrum of compound IIc

[4-[4-(dimethylamino)phenyl]-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

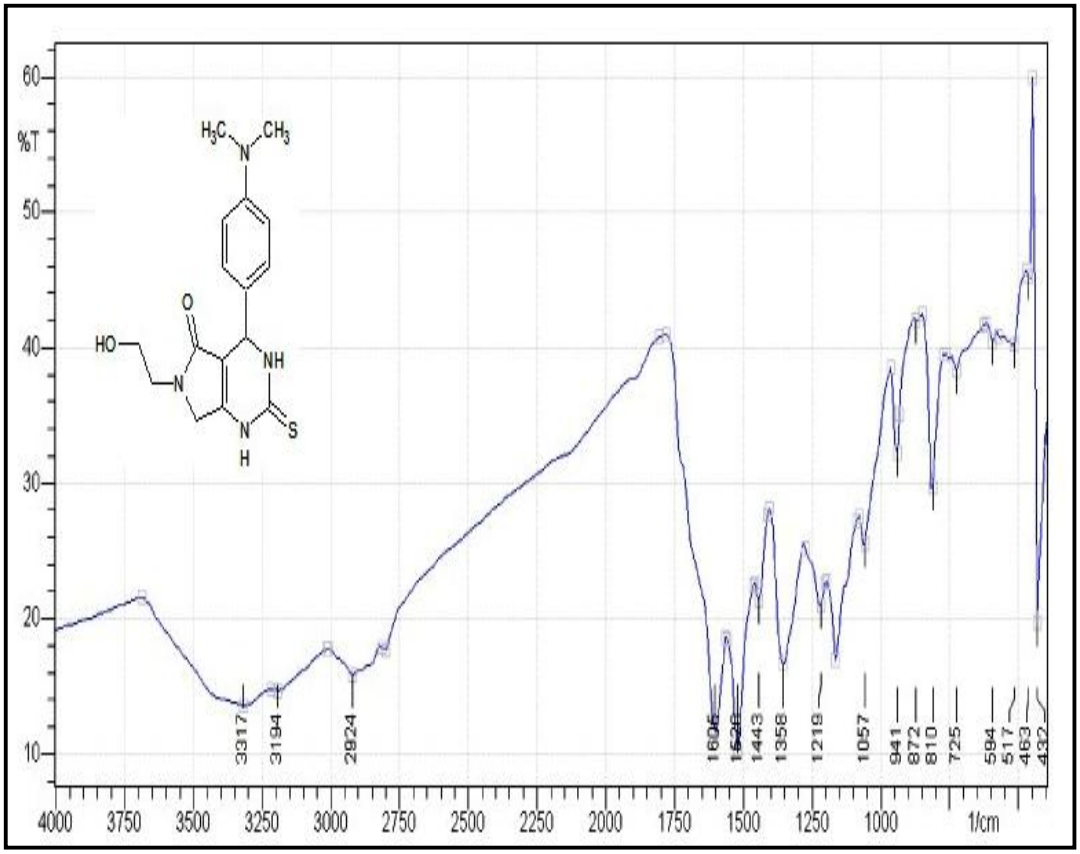


Fig 7 3317(OH str), 3194(NH str), 2924(CH str aromatic), 1065(C=O str), 1443(C=C str aromatic)

Figure 8 shows ^{1}H NMR spectrum of compound IIc

[4-[4-(dimethylamino)phenyl]-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*- pyrrolo[3,4-*d*]pyrimidin-5-one].

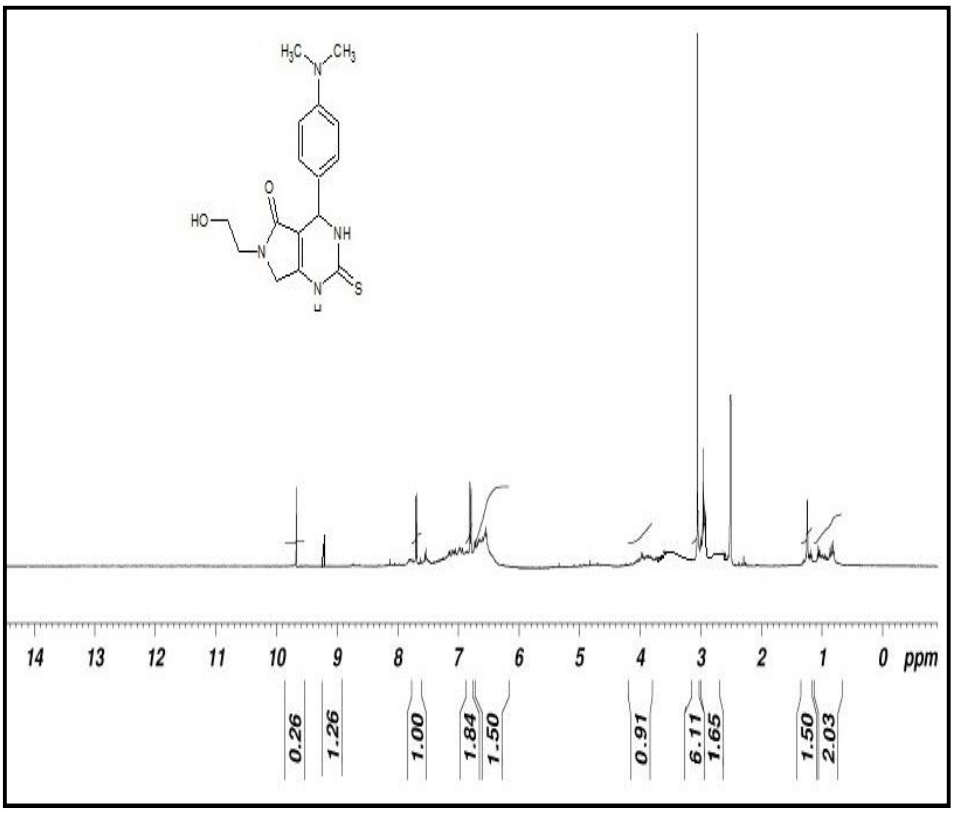


Fig 8 0.8-1.3(m,4H,CH_2), 2.9(s,1H,CH_2), 3.0(s,6H,CH_3), 4.0(s,1H,CH), 6.5-7.0(m,4H,Ar-CH)7.8(m,1H,OH), 9.2(s,1H,NH), 9.8(s,1H,NH)

Figure 9 shows Mass spectrum of compound IIc

[4-[4-(dimethylamino)phenyl]-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

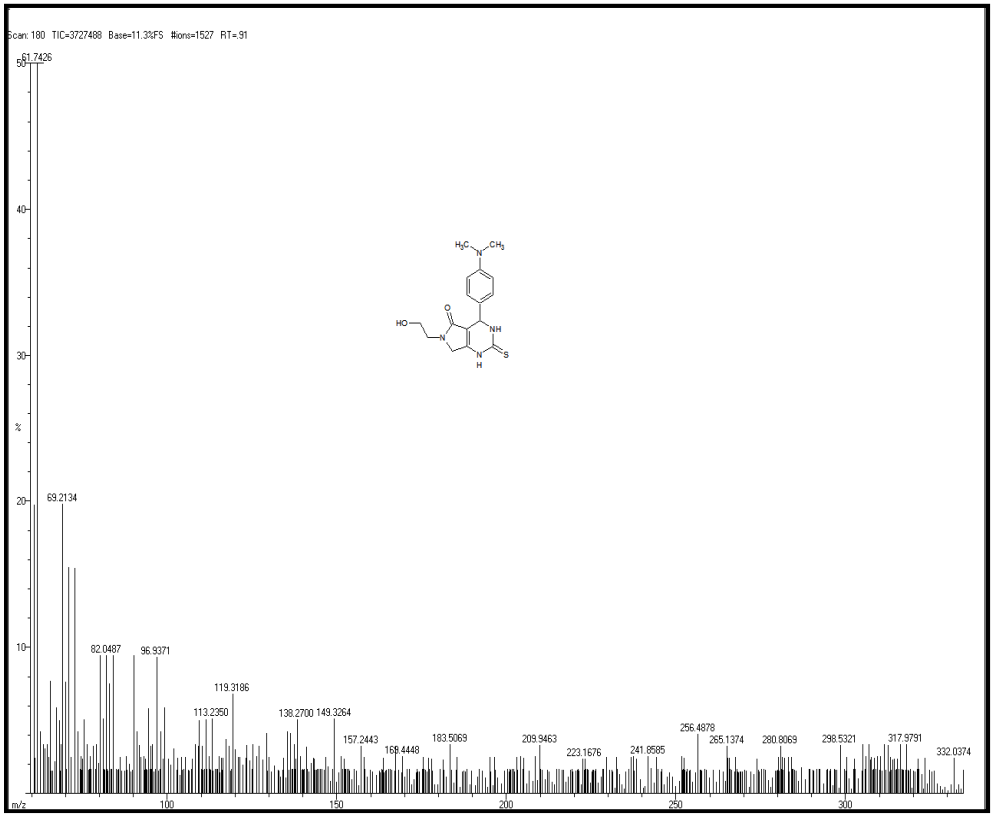


Fig 9 332.03(M^+), 280.8, 119.31, 61.7.

Figure 10 shows IR spectrum of compound IId

[4-(4-chlorophenyl)-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

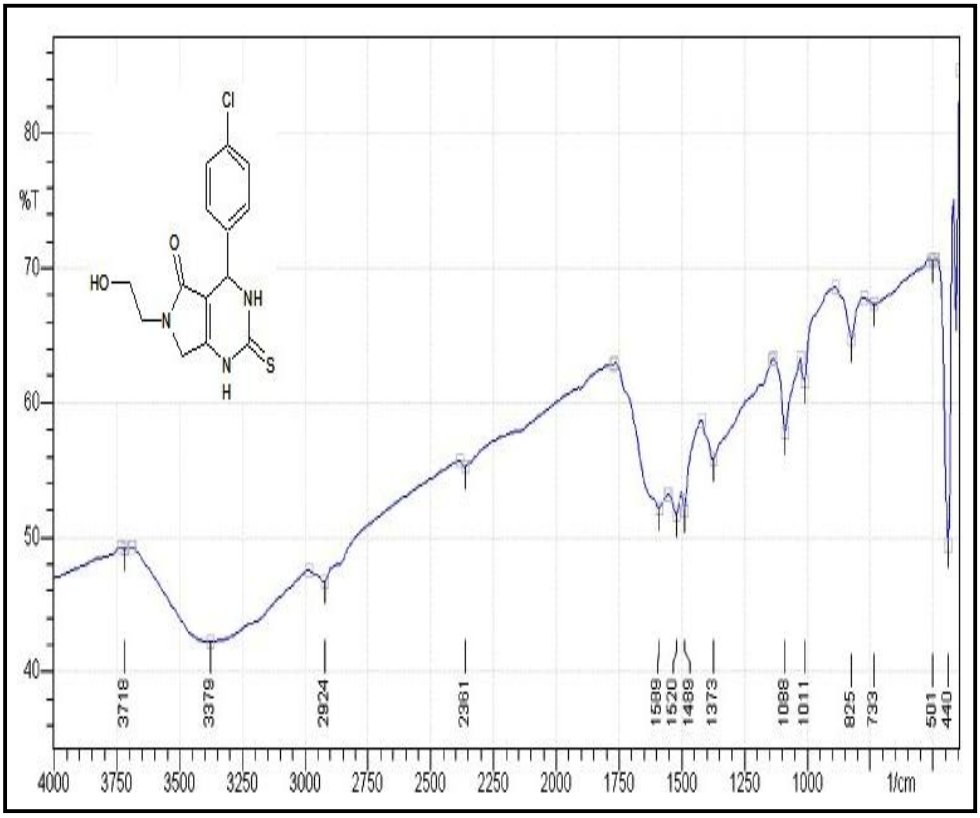


Fig 10 3379(OH str), 2924(CH str methylene), 2361(C=S str), 1589(C=0 str), 1489(C=C str aromatic)

Figure 11 shows ^{1}H NMR Spectrum of compound IId

[4-(4-chlorophenyl)-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

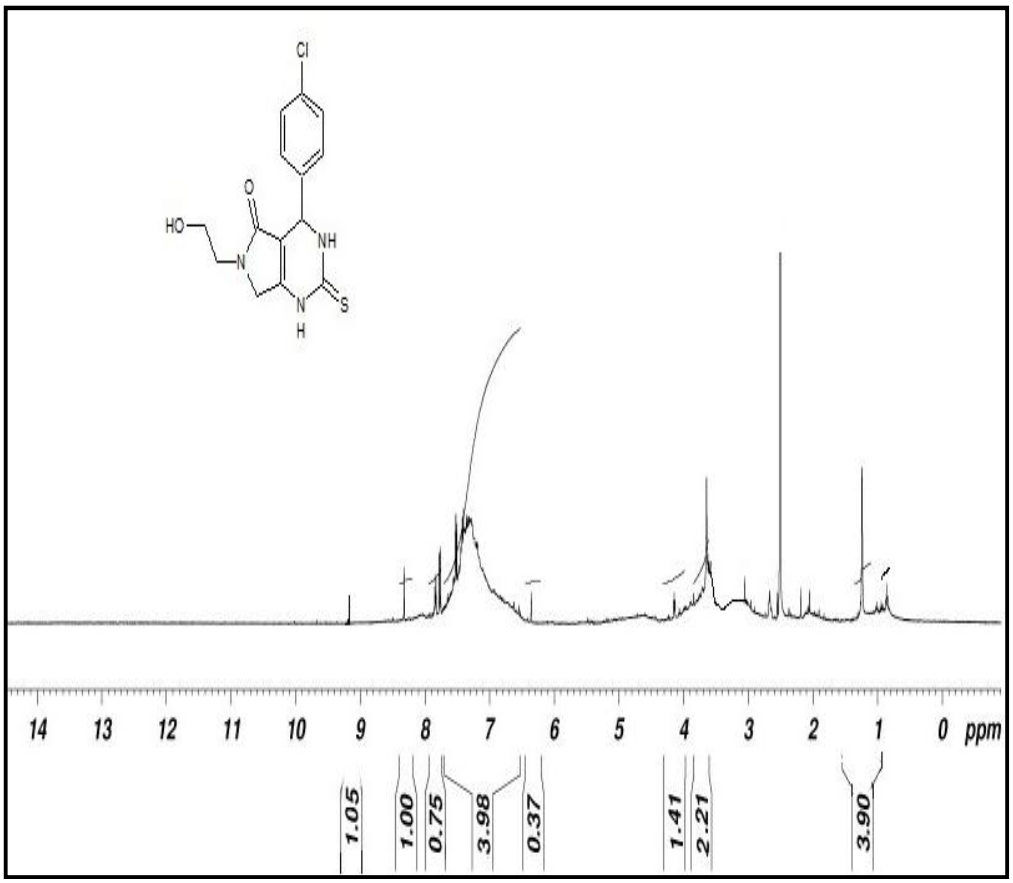


Fig 11 0.9-1.3(m,4H,CH_2), 3.9(s,2H,CH_2), 4.1(s,1H,CH), 6.5-7.7(m,4H,Ar-CH), 7.9(m,1H,OH), 8.3(s,1H,NH), 9.2(s,1H,NH)

Figure 12 shows Mass spectrum of compound IId

[4-(4-chlorophenyl)-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4- *d*]pyrimidin-5-one].

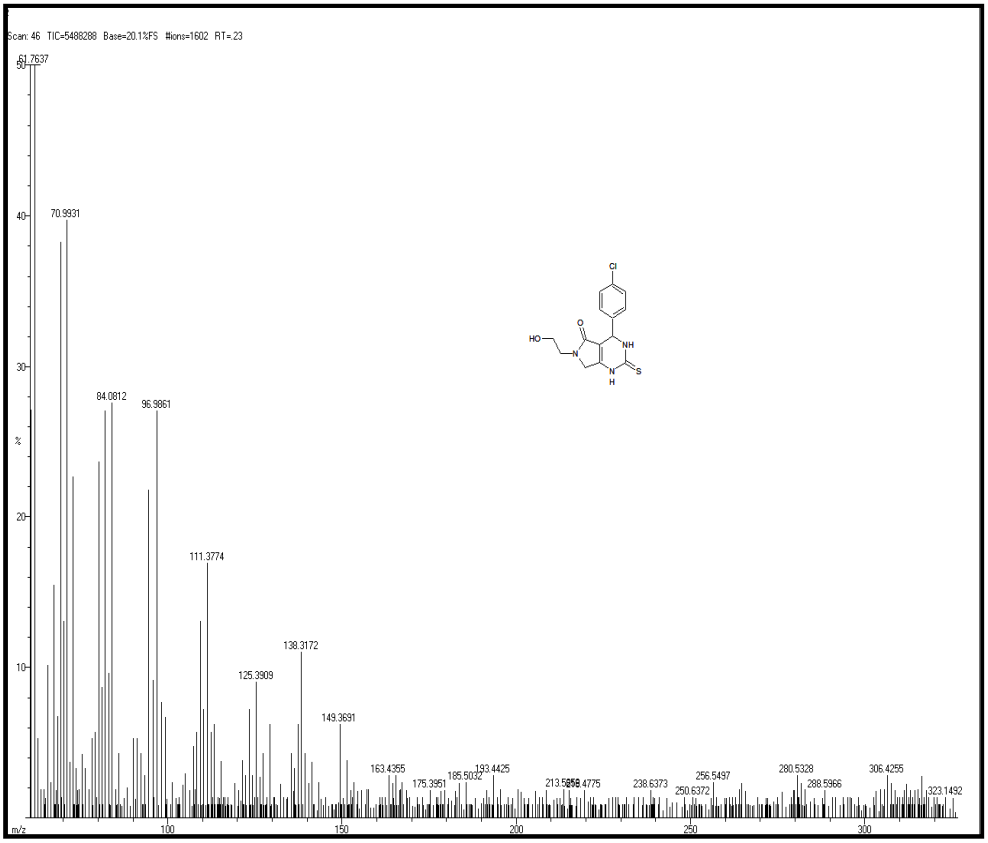


Fig 12 323.14 (M^+), 238.6, 125.39, 61.7

Figure 13 shows IR spectrum of compound IIe

[6-(2-hydroxyethyl)-4-(2-hydroxyphenyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

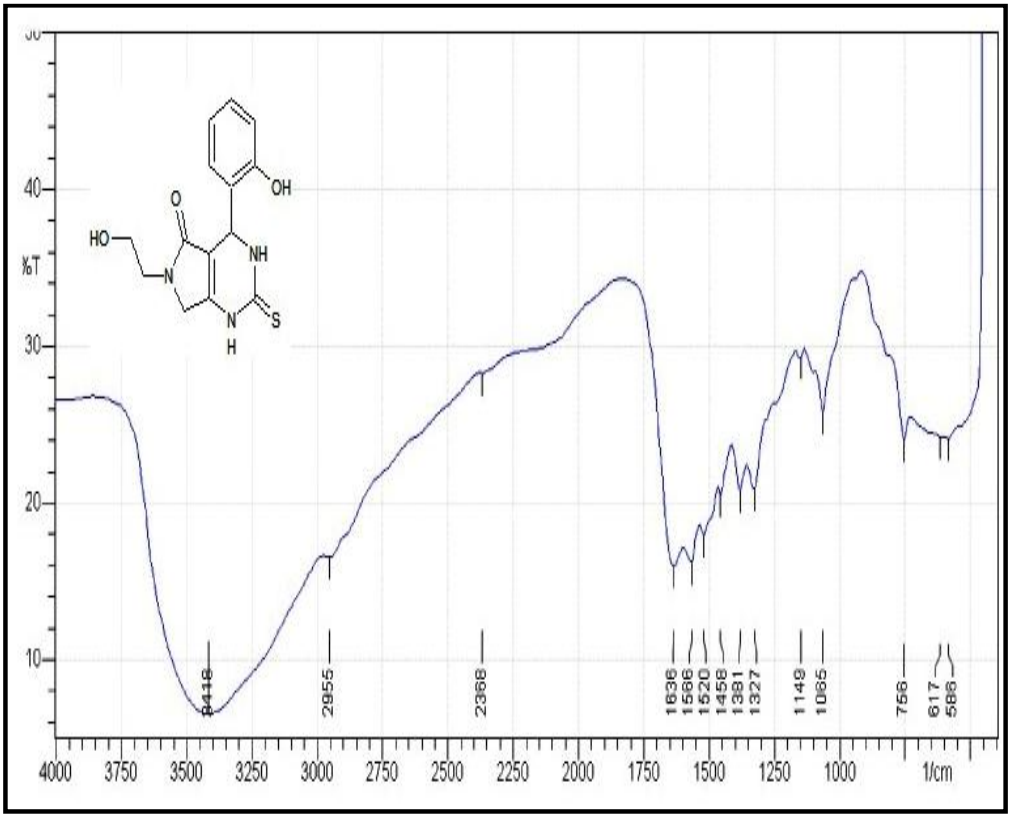


Fig 13 3418(OH str), 2955(CH str methylene), 2368(C=S str), 1566(C=O str), 1458(C=C str aromatic)

Figure 14 shows ^{1}H NMR spectrum of compound IIe

[6-(2-hydroxyethyl)-4-(2-hydroxyphenyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

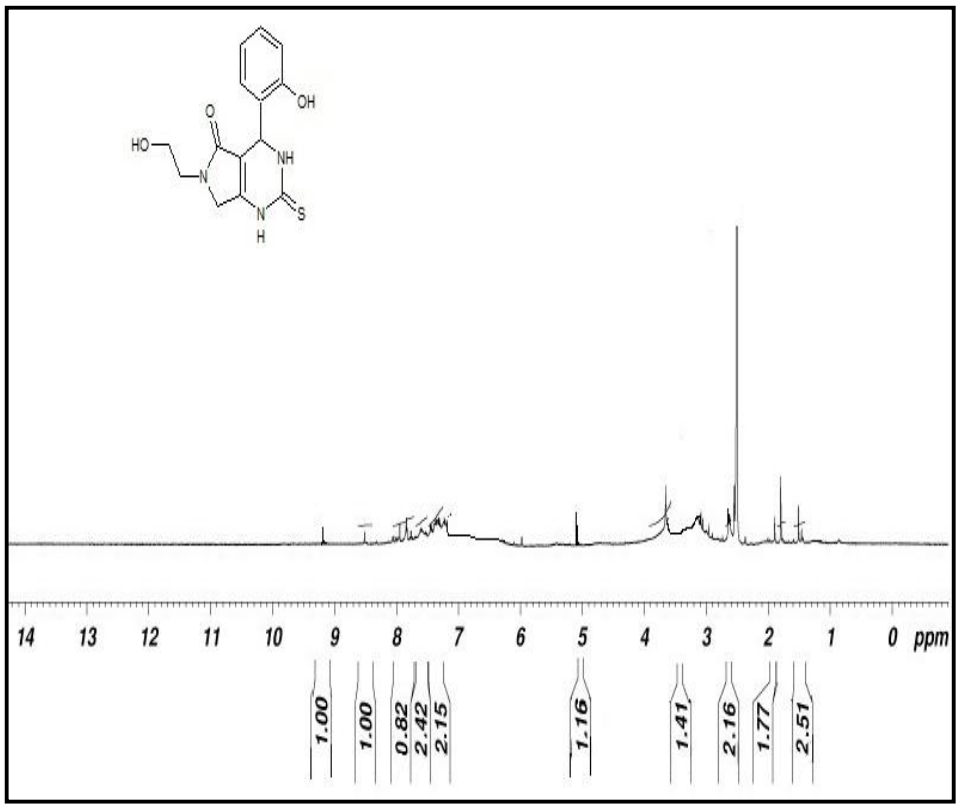


Fig 14 1.4(m,2H,CH_2), 1.9(m,2H,CH_2), 2.6(s,2H,CH_2), 3.8(s,1H,CH), 5.1(s,1H,Ar-OH), 7.2-7.5(m,4H,Ar-CH), 7.9(m,1H,OH), 8.5(s,1H,NH), 9.2(s,1H,NH)

Figure 15 shows Mass spectrum of compound IIe

[6-(2-hydroxyethyl)-4-(2-hydroxyphenyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

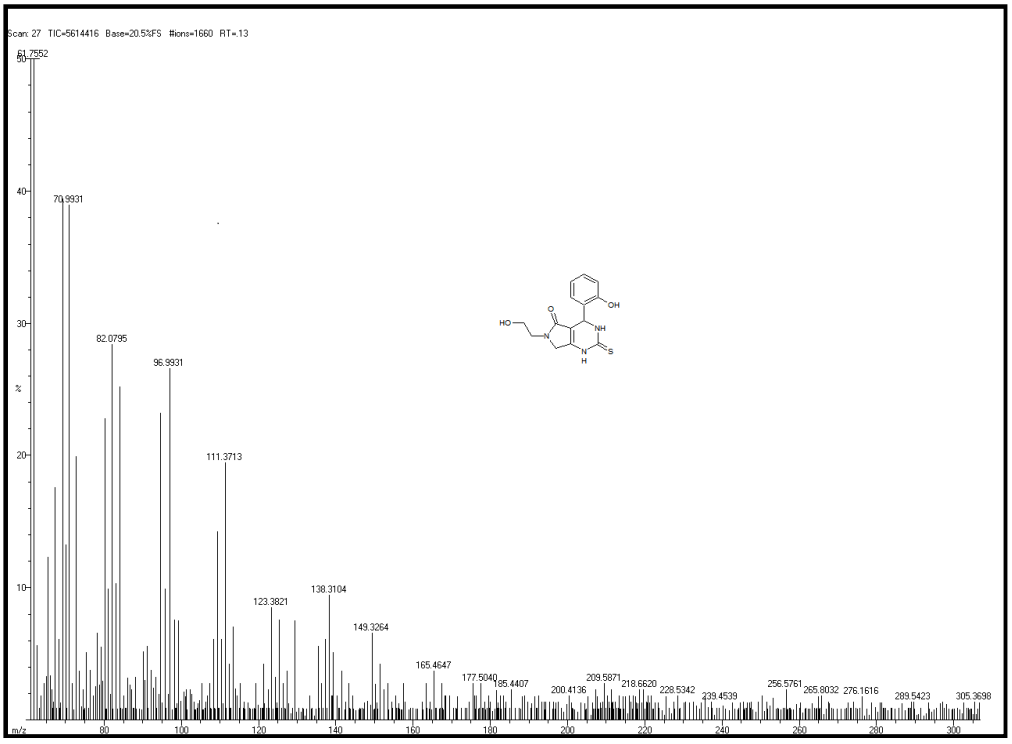


Fig 15 305.3 (M^+), 276.16, 218.68, 177.5, 61.7

Figure 16 shows IR spectrum of compound IIf

[4-(3,4-dimethoxyphenyl)-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

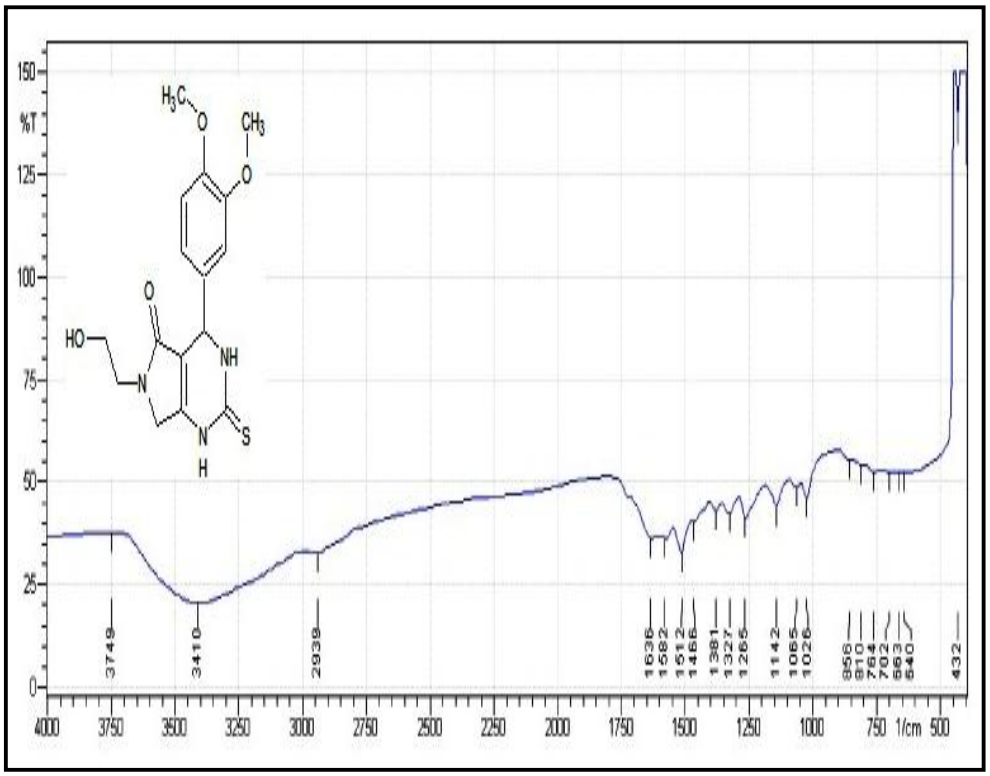


Fig 16 3410(OH str), 2939(CH str aromatic), 1636(C=O str), 1466(C=C str)

Figure 17 shows ^{1}H NMR spectrum of compound IIf

[4-(3,4-dimethoxyphenyl)-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

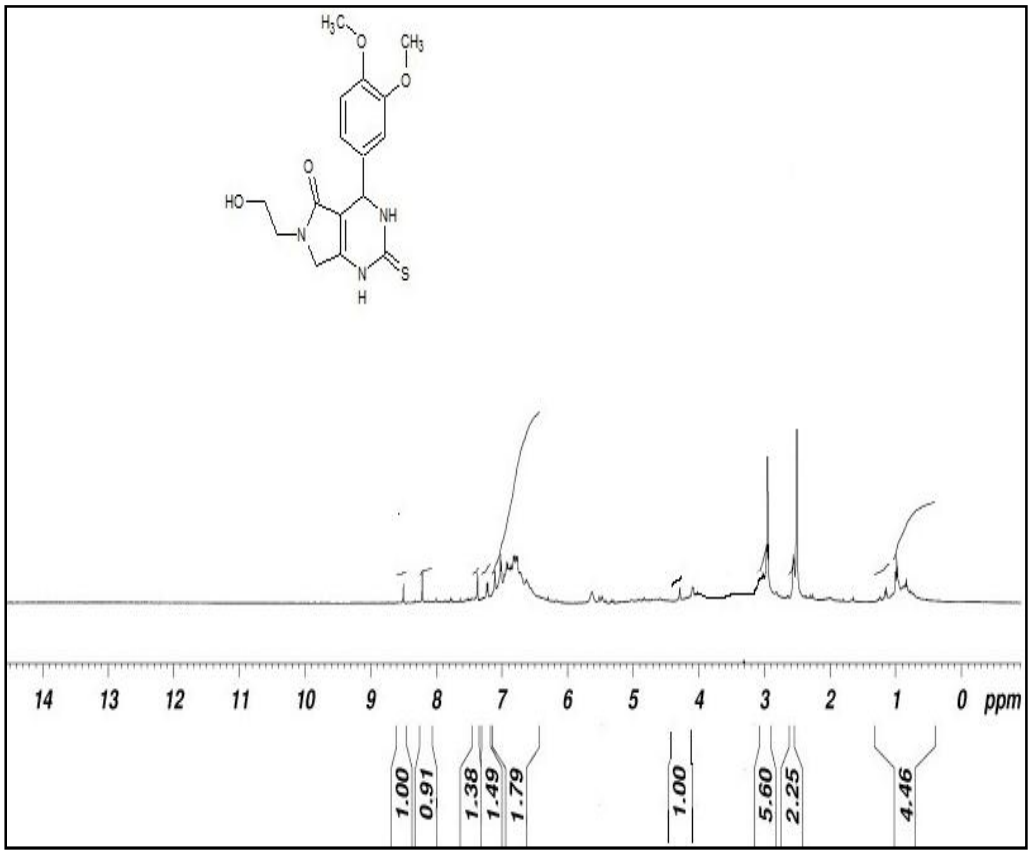


Fig 17 0.8-1.2(m,4H,CH_2), 2.6(s,2H,CH_2), 2.9(s,6H,CH_3), 4.2(s,1H,CH), 6.7-7.2(m,3H,Ar-CH), 7.3(m,1H,OH), 8.2(s,1H,NH), 8.5(s,1H,NH)

Figure 18 shows Mass spectrum of compound IIf

[4-(3,4-dimethoxyphenyl)-6-(2-hydroxyethyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

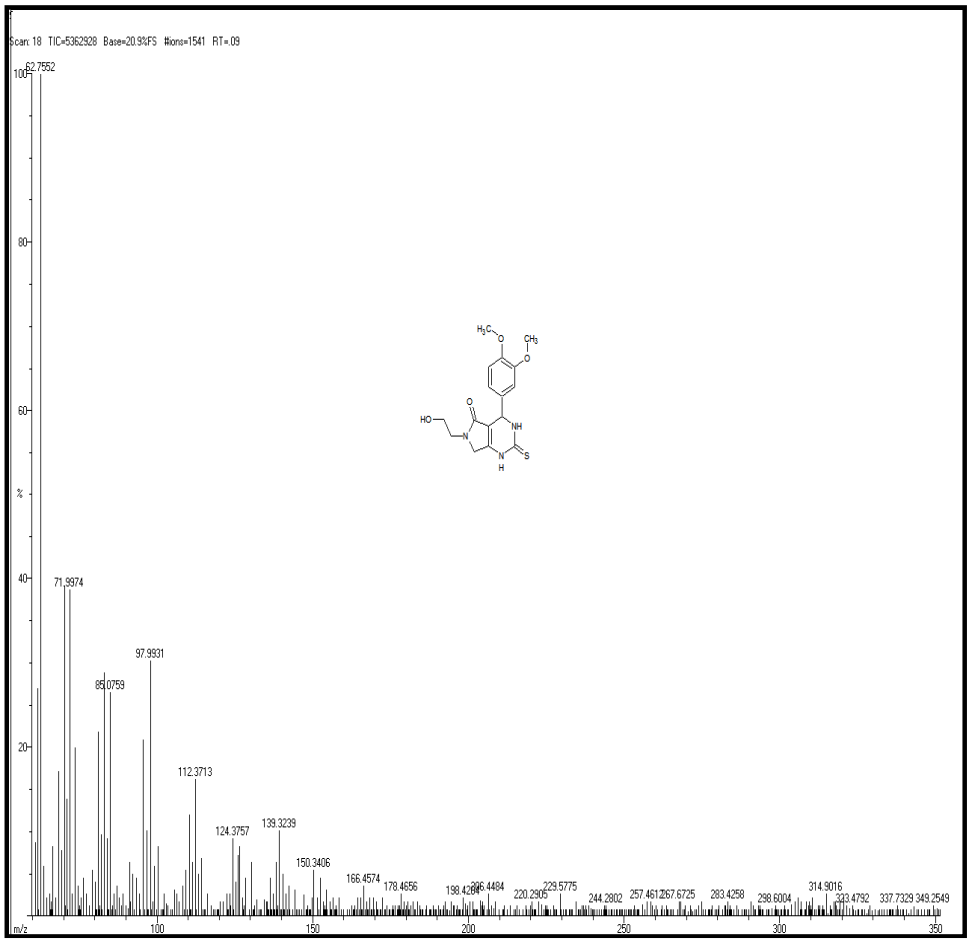


Fig 18 349.2(M^+), 220.2, 150.3, 62.7.

Figure 19 shows IR spectrum of compound IIg

[6-(2-hydroxyethyl)-4-(4-hydroxy-3-methoxyphenyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

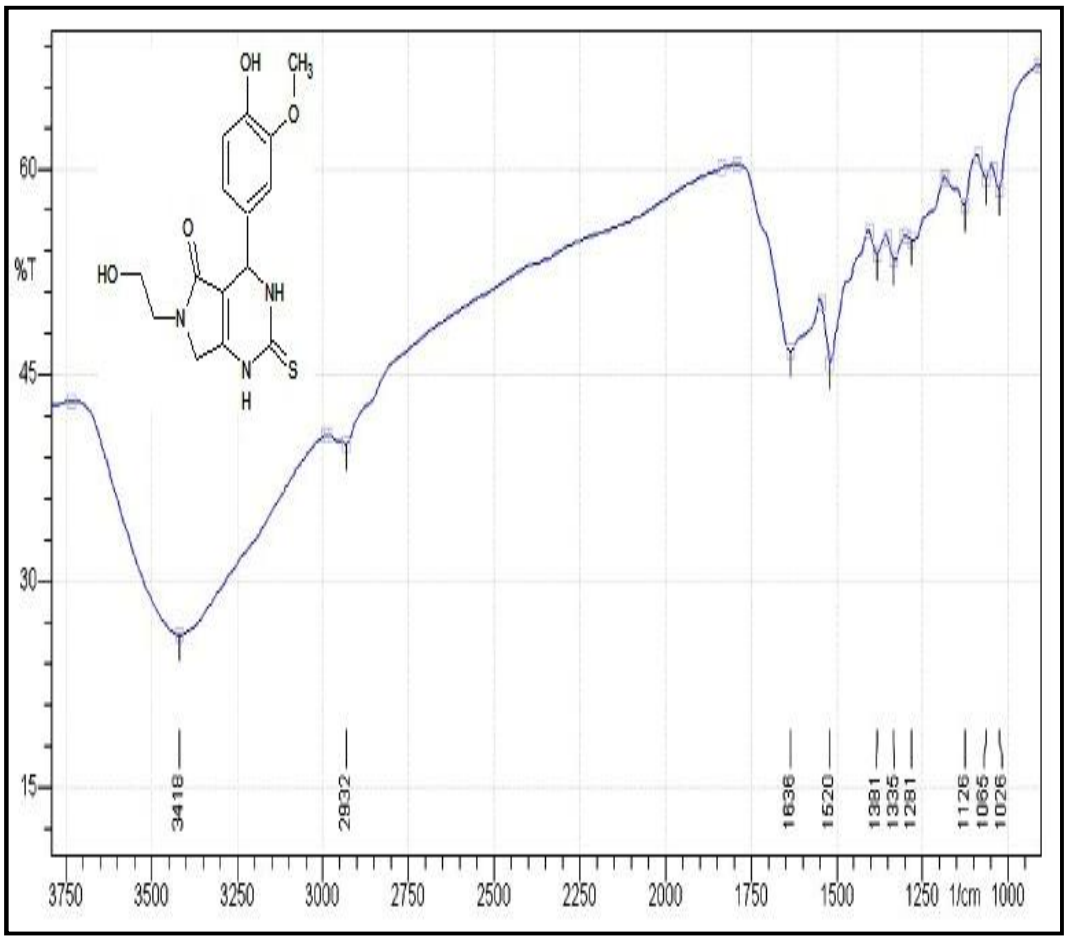


Fig 19 3418(OH str), 2932(CH str methyl), 1636(C=O str), 1381(C=C str aromatic)

Figure 20 shows ^{1}H NMR spectrum of compound IIg

[6-(2-hydroxyethyl)-4-(4-hydroxy-3-methoxyphenyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

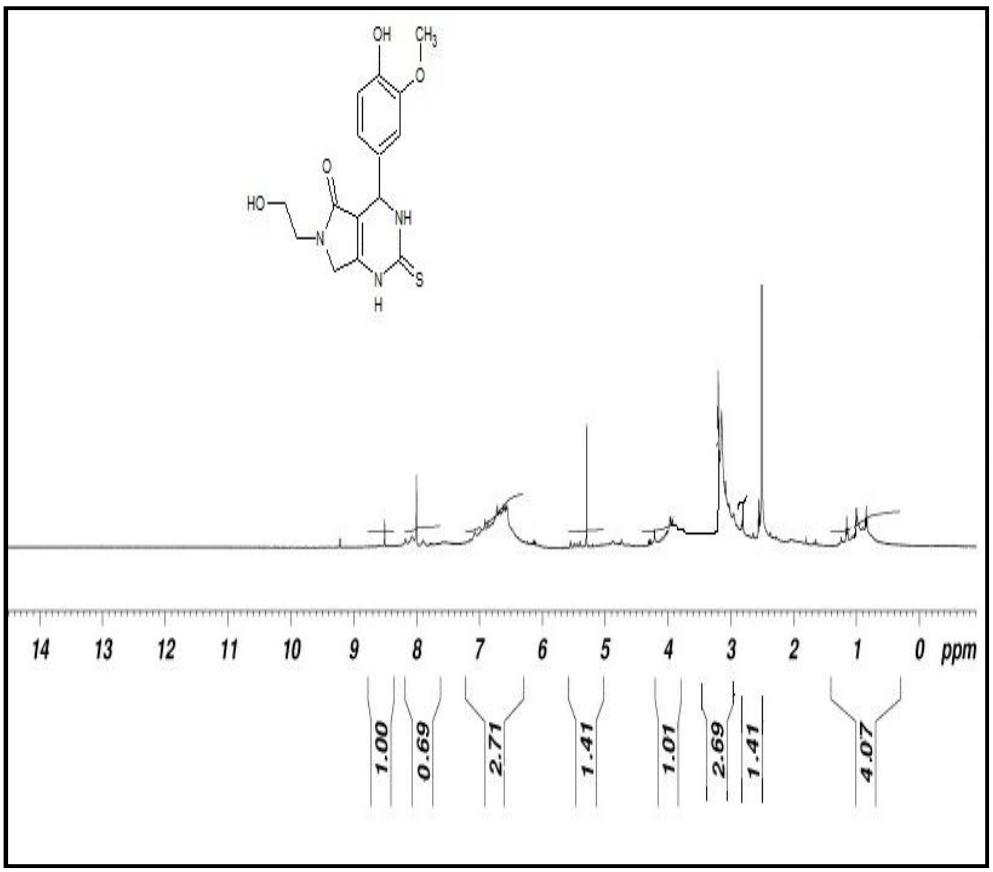


Fig 20 0.8-1.2(m,4H,CH_2), 2.9(s,2H,CH_2), 3.2(s,3H,CH_2), 4.1(s,1H,CH), 5.3(s,1H,Ar-OH), 6.5-7.0(m,3H,Ar-CH), 8.0(s,1H,NH), 9.2(s,1H,NH).

Figure 21 shows Mass spectrum of compound IIg

[6-(2-hydroxyethyl)-4-(4-hydroxy-3-methoxyphenyl)-2-thioxo-1,2,3,4,6,7-hexahydro-5*H*-pyrrolo[3,4-*d*]pyrimidin-5-one].

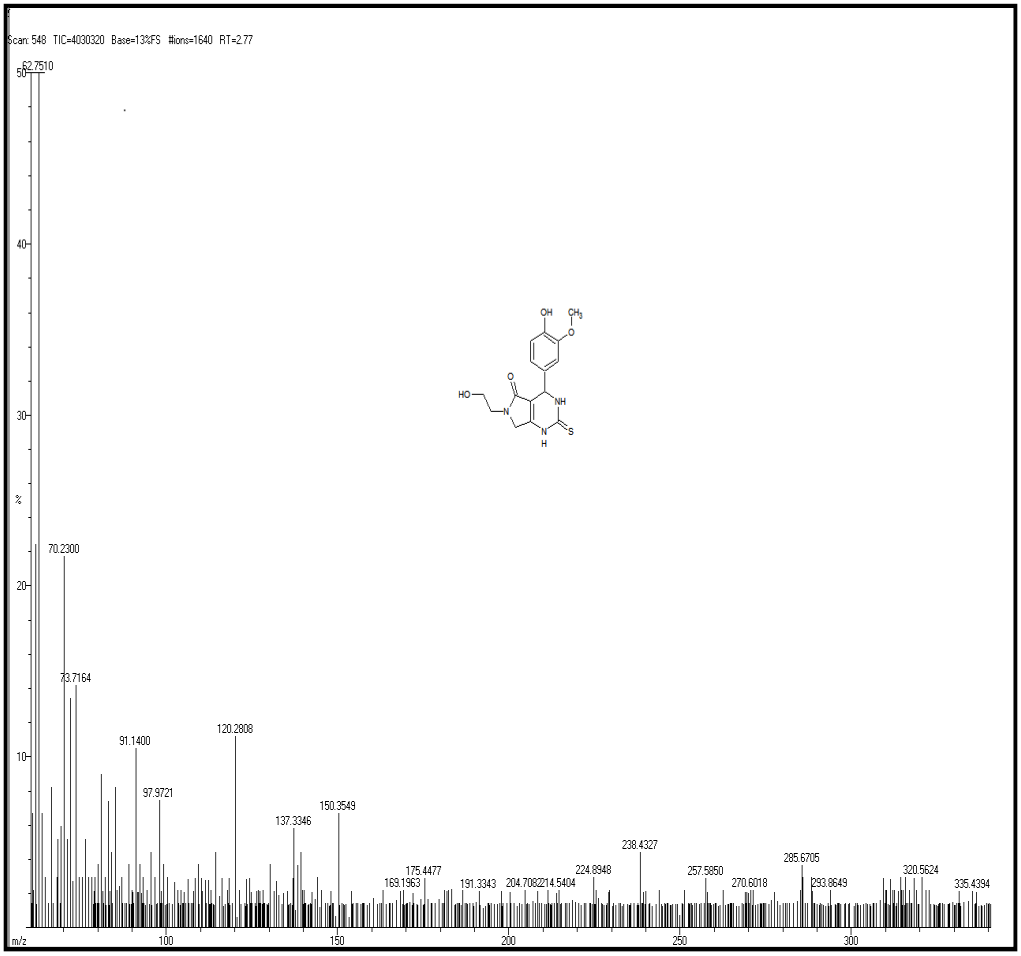


Fig 21 335.4 (M^+), 191.3, 120.2, 62.75.

4.4 PHARMACOLOGICAL EVALUATION[40]

All the animal experimentation were performed according to the protocol and the recommendation of the Institutional Animal Ethics Committee.

The following activities were carried out to the synthesized compounds

(i) Acute oral toxicity by acute toxic class method in mice
(ii) Antidepressant activity by Forced swim test in mice

4.4.1. Acute Oral Toxicity Study[41]

The test in which a single dose of the drug is used in each animal on one occasion only for the determination of gross behavior and LD50 or Median lethal dose. LD50 value is determined in a 24hr test using two species, rodent(mice or rat) and nonrodent(rabbit).

Acute oral toxicity study was designed as per OECD guidelines 423 for testing chemicals.

a. Principle of the test:

The test is based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.;

- no further testing is needed,
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level.

b. Experimental protocol (Acute toxic class method)

Toxicity of the synthesized compounds were tested using a stepwise procedure, each step using three mice of a single sex. Healthy young adult Swiss albino mice of weighing 20-25g of single sex were used in the test. The animals were kept in colony cages at 22°C (±3°C), relative humidity 50-60% under 12hrs light and 12hrs dark. The dose levels to be used as the starting dose is selected from one of four fixed levels 5, 50, 300 and 2000mg/kg body weight. Test compounds administered in the form of suspension in 1% carboxymethylcellulose.

The mice were fasted prior to dosing (food but not water should be withheld for 3-4hrs). Following the period of fasting, the animals were weighed and the test substances were administered orally at a single dose of 300mg/kg body weight by intragastric tube. After the test substances administered, food withheld for further 1-2hrs.

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days.

As no mortality was observed with the above dose, the next dose 2000mg/kg body weight administered and the animals were observed for 14 days. Mortality observed at 2000mg/kg body weight, a series of doses selected below 2000mg/kg were 250mg/25kg, 500mg/25kg body weight for further pharmacological evaluation.

Figure 22 shows the test procedure with a starting dose of 300mg/kg body weight as per OECD guidelines 423.

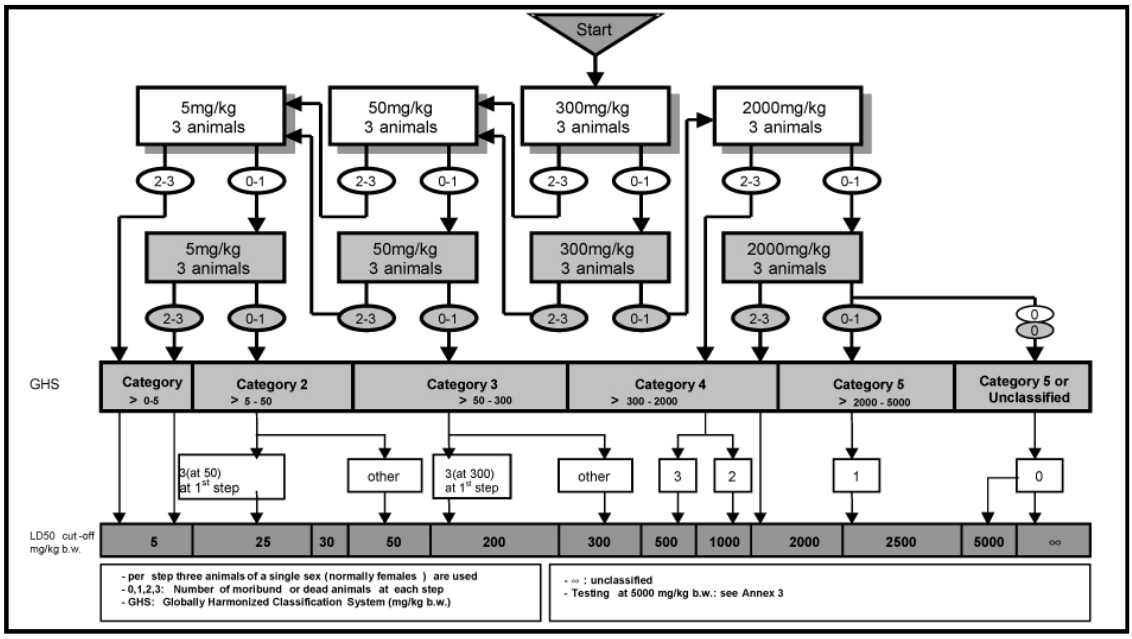


Fig 22 starting dose of 300mg/kg body weight.

4.4.2. Evaluation of Antidepressant Activity

Behavioural despair test[42,43,44] was proposed as a model test for antidepressant activity by Porsolt et al. In this method mice or rat forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can reduced by several agents which are therapeutically effective in human depression.

a. Experimental procedure:

Adult albino mice of either sex were used under standard condition with free access to food and water. The mice were housed in groups of 6 animals for each cage in a quite and temperature and humidity controlled room in which a 12hrs light and dark was maintained. On the testing day, mice were assigned into different groups (n=6 for each groups). The synthesised compounds and fluoxetine as reference antidepressant drug were suspended in a 1% aqueous solution of carboxymethyl cellulose. The drugs were given orally in a standard volume of

0.5ml/20g body weight, 1hr prior to the test. Control animals received 1% aqueous solution of carboxymethyl cellulose. Then the mice were dropped individually into the Plexiglass cylinder (25cm height, 30 cm diameter containing water to a height of 20cm at 21-23°C) and left for 6min. After the first 2min of the initial vigorous struggling the animals were immobile. A mouse was judged immobile if it floated in water in an upright position and made only slight movements in order to prevent sinking. The duration of immobility was recorded during the last 4min of the 6min test.

b. Grouping and drug treatment:

Animals were divided into four groups, each consisting of 6 animals

Group 1 : Control/vehicle treated (1% CMC)

Group 2 : Standard drug fluoxetine 20mg/kg body weight

Group 3(3a-g): Dose 1 (250mg/kg body weight)

Group 4(4a-g): Dose 2 (500mg/kg body weight)

The one way ANOVA test was performed using Graph Pad Prism software.

5. RESULTS AND DISCUSSION

5.1 SYNTHESIS AND CHARACTERIZATION

The target compounds were synthesized by Biginelli reaction followed by nucleophillic and cyclization reaction. The synthesized compounds were obtained in good yield and purities. The percentage yield and melting point of synthesized compounds were recorded.

a. IR Spectroscopy

The following characteristic stretching vibrations observed from the IR spectrum of the synthesized compounds.

3425cm^{-1}-3300cm^{-1}	-	OH stretching
3200cm^{-1}-3000cm^{-1}	-	NH stretching
2970cm^{-1}-2850cm^{-1}	-	CH stretching of methylene
1640cm^{-1}-1600cm^{-1}	-	C=O stretching of amide
1460cm^{-1}-1430cm^{-1}	-	C=C stretching of aromatic
800cm^{-1}-600cm^{-1}	-	C-S stretching

These vibrations are indicative of the new functional group that has formed.

Absence of stretching vibrations at 2720cm^{-1} and 1730cm^{-1}-1720cm^{-1} indicated that the absence of impurities like aldehydes and β-ketoester respectively.

b. ^{1}H NMR Spectroscopy.

The spectral data of the synthesized compounds shown the following peaks.

8-10ppm - singlet was observed due to the presence of NH protons

6.5-7.5ppm - aromatic protons as multiplet

6-7ppm - alcoholic proton appeared as mutiplet

0.3-1.5ppm - multiplet because of the presence of aliphatic protons

c. Mass Spectrometry

All the synthesized compounds were shown their respective molecular peak and it was observed that the fragment ion peak at m/z value of 63 which is may be due to the fragment cyclopentadienyl ion.

5.2 PHARMACOLOGICAL EVALUATION

a. Acute toxicity

Acute oral toxicity study was performed according to the OECD guideline 423. The synthesized compounds were suspended in 1% caboxymethyl cellulose solution and administered in a single dose by intragastric tube to the healthy adult mice at starting dose of 2000mg/kg body weight (po). Animal were observed after dosing for a total of 14days mortality.

No changes in skin and fur, eyes and mucous membrane, autonomic and central nervous systems, somatomotor activity, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were observed.

Since sign of toxicity was observed at 2000mg/kg body weight to the group of animals, the LD50 values of the synthesized compounds(IIa-IIg) expected to be less than the above dose.

b. Antidepressant activity

The antidepressant activity of the synthesized compounds were evaluated in adult Swiss albino mice by Porsolt behavioural despair test. In this method, the mice were forced to swim in a narrow cylinder from which they cannot escape. Score immobility during the last 4 min of 6 min session by summing the total time spent immobile.

The data obtained from antidepressant activity of the synthesized compounds and reference drug were given in Table 5. It reveal that the compounds IIe and IIg significantly reduced the duration of immobility at all dose level (250 and 500mg/kg body weight) when compared to control. Compound IId reduce the duration of immobility at both the dose level. Compounds IIc and IIf were effective at dose of 250mg/kg body weight than 500mg/kg body weight (po). Compounds IIa and IIb were effective at dose level of 500mg/kg than 250mg/kg body weight (po).

The present study demonstrated that the compounds IIe and IIg were more active than standard floxetine using despair swim test.

Duration of immobility time in seconds were recorded and given in Table 5.

Table 5. Duration of immobility time

S.No	Treatment Groups	Duration of immobility times in seconds After 1hr	
		Dose at 250mg/kg	Dose at 500mg/kg
1.	IIa	87.33	59.17
2.	IIb	78.00	65.5
3.	IIc	35.50	72.33
4.	IId	54.50	55.66
5.	IIe	19.17	23.0
6.	IIf	20.67	23.6
7.	IIg	30.17	44.5
8.	Standard	116	
9.	Control	25.67	

Figure 23 shows the effect of series of synthesized compounds on immobility in sec.

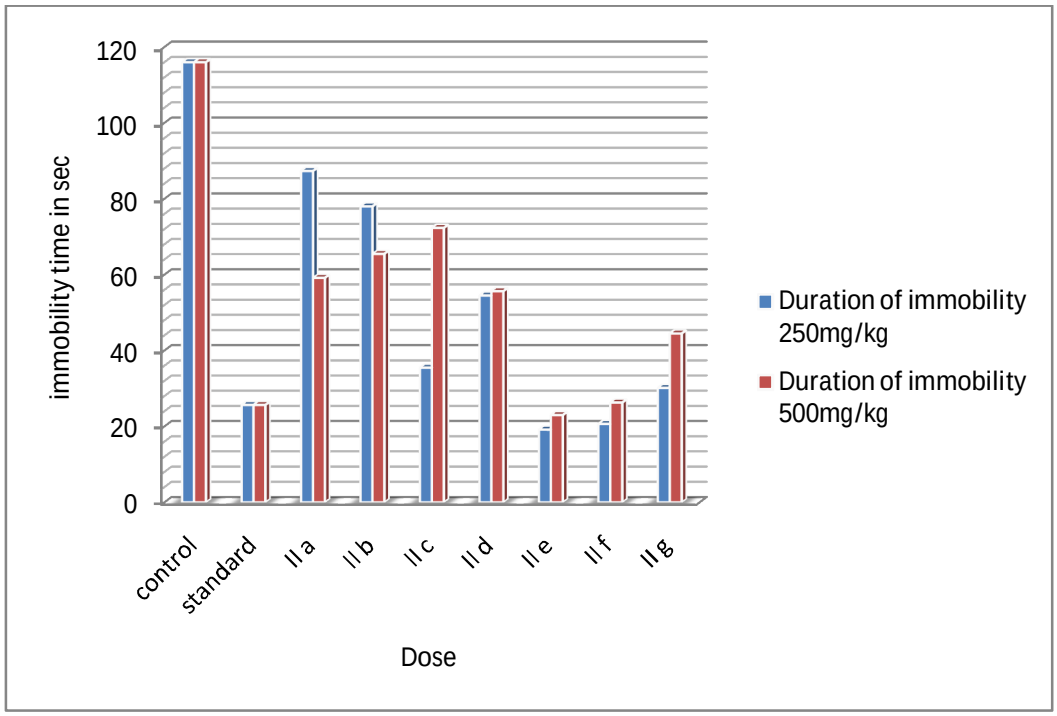


Fig 23 Effects of synthesized compounds on duration of immobility in mice using forced swim test**.** Dose 250mg/kg and 500mg/kg body weight were given and immolity time was recorded after 1 hr.

Table 6. Effect of synthesized compounds on immobility

S. No	Treatment groups	Duration of immobility in seconds (mean± SEM) After 1hr		% Change from control	
		Dose at 250mg/kg	Dose at 500mg/kg	Dose at 250mg/kg	Dose at 500mg/kg
1.	IIa	87.33±5.05	59.17±3.28	-24.71	-48.99
2.	IIb	78.0±5.12	65.5±5.65	-32.76	-43.53
3.	IIc	35.5±2.06	72.3±3.70	-69.39	-37.64
4.	IId	54.5±6.07	55.67±6.32	-53.02	-52.02
5.	IIe	19.17±2.63	23.0±2.34	-83.47	-80.17
6.	IIf	30.17±2.96	44.5±9.09	-82.18	-77.33
7.	IIg	20.67±1.34	26.33±4.47	-73.99	-61.64
8.	Standard	116.00±22.75		--	
9.	Control	25.67±6.25		-74.33	

Values are represented as mean ± SEM, (n=6)

Significant when compared to normal control (Dunnet's test, p□0.05)

All compounds i.e. IIa-IIg showed a significant decrease in duration of immobility time when compared to control.

Statistical analysis

Results are expressed as mean ± SEM, n represent the number of animals. Data obtained from pharmacological experiments were analyzed by one way analysis of variance (ANOVA) followed by Dunnet's post hoc test. A p-value of less than 0.05 was considered statistically significant.

Figure 24 shows the effect of synthesized compounds

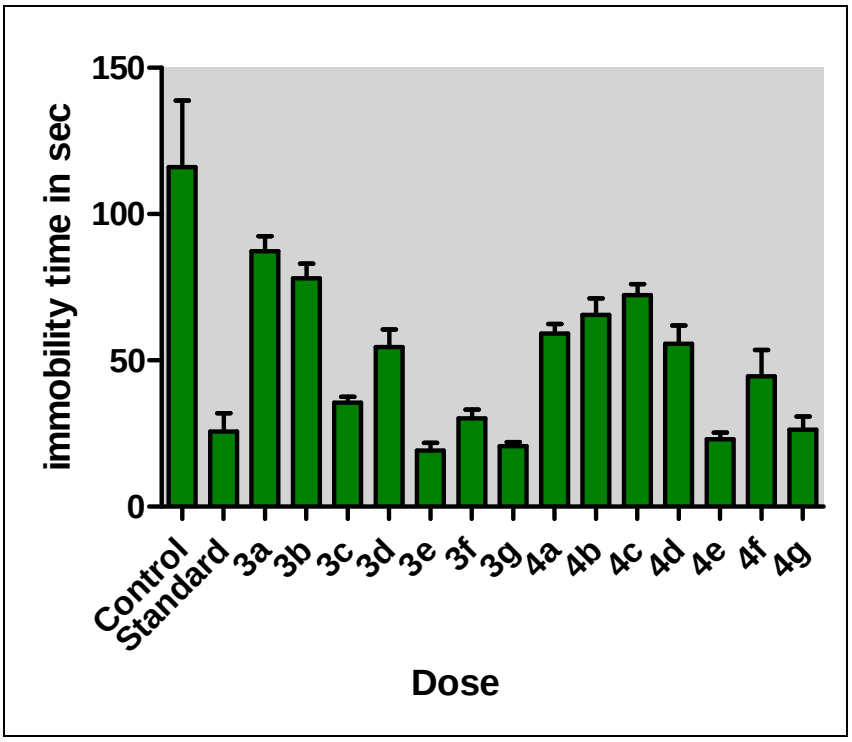


Fig 24 Effects of synthesized compounds on immobility in FST on mice. Compounds (250mg/kg & 500mg/kg) were injected orally (po) 60 min before the testing, and total duration of immobility was recorded during the last 5 min of the 6-min testing period. Values are means ± SEM.

6. SUMMARY AND CONCLUSION

The present work deals with the synthesis, characterization and pharmacological evaluation as antidepressant activity of 2-thioxo-5H-pyrrolo[3,4-d]pyrimidin-5-one derivatives.

The compounds (IIa-IIg) were synthesized by multicomponent reaction followed by nucleophillic substitution and cyclocondensation reaction. The synthesized compounds were obtained in good yield.

The melting points of the synthesized compounds were determined and have been reported uncorrected. Thin layer chromatography was performed on precoated silicagel G plates using suitable mobile phase.

The synthesized compounds were characterized by IR, Mass and NMR spectroscopic techniques.

Synthesized compounds were subjected to acute oral toxicity studies. The LD50 values of the synthesized compounds (IIa-IIg) were expected to be less than 2000mg/kg bodyweight.

All the synthesized compounds were evaluated for their antidepressant activity by forced swim test in mice. The synthesized compounds IIe and IIg were found to be more active than standard fluoxetine.

However, neurobiological and basic research as well as clinical studies have shown that the monoamines have the role in the development of depression syndrome.

Antidepressant activity is achieved through various mechanism such as Monoamine oxidase inhibition, Selective serotonin transporter inhibition, inhibition of Selective serotonin reuptake and norepinephrine reuptake, Dopamine transporter inhibition, Corticotropin releasing hormone antagonism.

In this present study it has been identified that the synthesized compounds possess antidepressant activity but their actual mechanism involved towards antidepressant activity is unknown. A focused study, in order to identify the actual mechanism may reveal several facts with respect to antidepressant activity of the synthesized compounds.

The information and knowledge gained during the course of focused study may facilitate the process of identifying the ideal antidepressant agent. Hence, an attempt to study their actual mechanism may be considered in future.

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