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Synthesis And Anticancer Activity Of Some Novel 3-[(2-Substituted-6,7,8,9-Tetrahydro-5H-Cyclohepta[b]Thieno [2,3-d] Pyrimidin-4-YL) Amino] Propan-1-OL

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Abstract

Synthesis of novel heterocyclic compounds has gained considerable attention in medicinal chemistry because of their potential therapeutic applications, particularly in cancer research. The present study focuses on the synthesis and evaluation of the anticancer activity of novel 3-[(2-substituted-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-yl)amino]propan-1-ol derivatives. The designed compounds incorporate a fused thienopyrimidine scaffold with a substituted cycloheptane ring and a propanol side chain, providing a framework for investigating structure–activity relationships. The synthesized derivatives are intended to be characterized using appropriate analytical and spectroscopic techniques to confirm their chemical structures and purity. Their anticancer potential can be evaluated using suitable in vitro cytotoxicity assays against selected cancer cell lines, with appropriate controls for comparison. The relationship between structural modifications and biological activity may provide insights into the influence of substituents on anticancer effects. This study aims to contribute to the development of novel heterocyclic compounds as potential candidates for further anticancer drug discovery and preclinical investigation.

Keywords

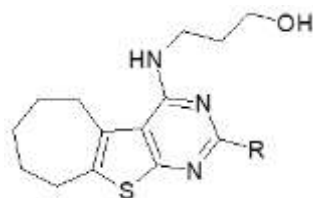
Thienopyrimidine derivatives; Organic synthesis; Anticancer activity; Heterocyclic compounds; Spectral characterization; Cytotoxicity; MTT assay; Structure–activity relationship.

INTRODUCTION

The research of anticancer drugs in the past several decades has shown significant progress and has cured substantial number of patients. Still it is the intense area of investigation due to the complex physiological changes in the cell functionality, metastasis and apoptotic mechanisms. Hence it has a multiple ways of therapeutic strategies ranging from chemotherapy (nitrogen mustard), anti-metabolites to irradiation of cancerous tissues, recently developed targeted therapy.

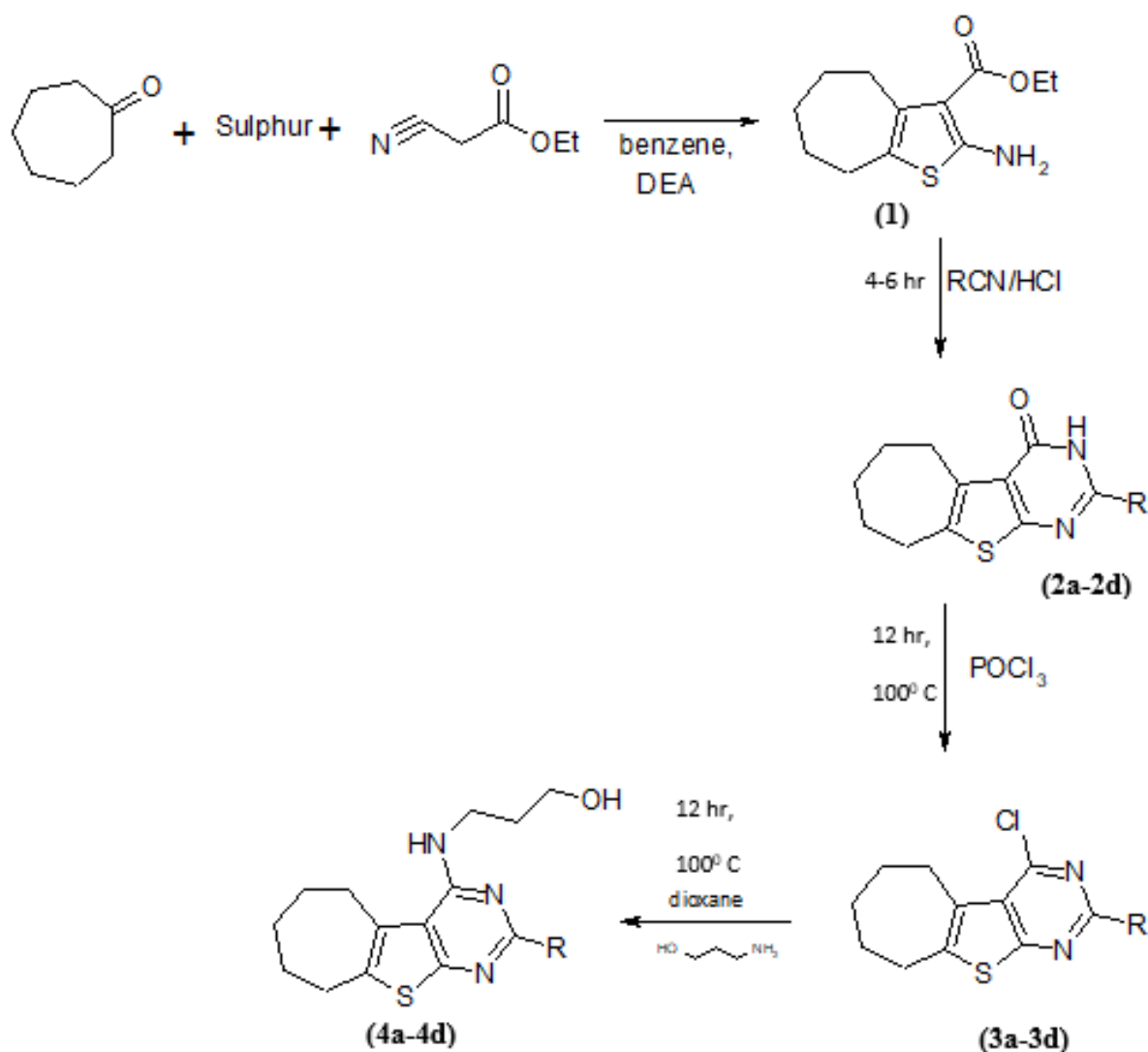
The overall cancer incidence rates were stable from 1995 through 1999, while cancer death rates decreased steadily from 1993 through 1999, which reflects the combined impact of improved screening, prevention, and treatment¹. In the past few years lots of compounds were screened for anticancer activity due to the availability of various cell lines and screening methods.

In this process of investigation, many pyrimidine derivatives including thienopyrimidines proved their therapeutic ability against cancer in the previous literature. Thienopyrimidines are reported for their antibacterial², antimicrobial³, anti-inflammatory, analgesic and ulcerogenic activity⁴. Many thienopyrimidines are also reported as anticancer agents and this has laid base for our intention to synthesize some novel 3-[(2-substituted-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-yl)amino]propan-1-ol and to test their ability as anticancer agents.



(4a-4d)

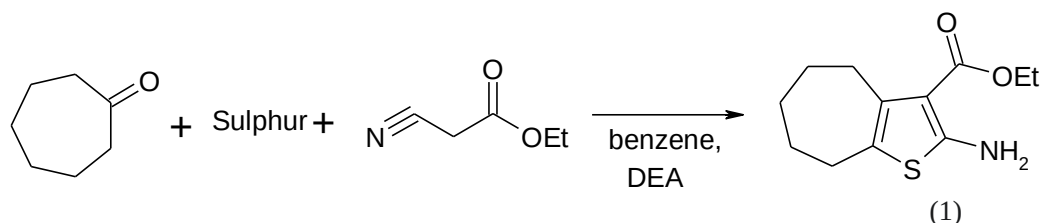
SCHEME



SYNTHETIC

I. Synthesis of ethyl 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate: (1)

Reaction:



Procedure:

Cycloheptanone (40.0 mmol, 3.2 mL), ethylcyanoacetate (40.0 mmol, 4.52 mL), ammonium acetate (500 mg), glacial acetic acid (2 mL) and benzene were taken in round bottom flask and heated to reflux using Dean Stark Apparatus for 10 hrs with constant removal of water. The solution was washed with sodium carbonate solution (10%) and dried using anhydrous sodium sulphate. The excess benzene was distilled off until 5 mL of solution is left. This solution was added to hot alcoholic solution of sulphur (40.0 mmol, 1.28 g) and stirred for one hour, with constant slow addition of diethylamine [DEA] (40.0 mmol, 2.92 mL). The resultant solution was kept in the deep freezer for 12 hr. The precipitate obtained was filtered and dried^{2,40}.

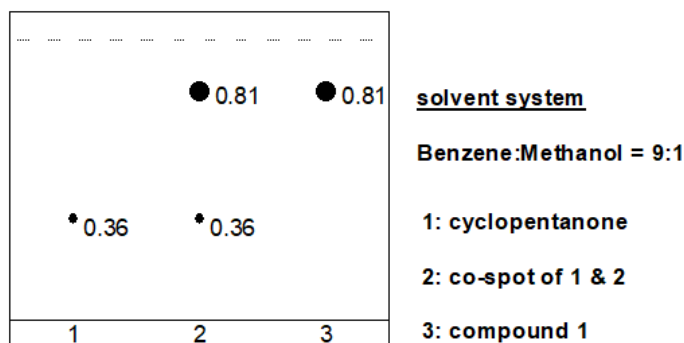
Recrystallisation solvent: Ethanol

Yield: 71.2%

M.P of crude product: 85°C

M.P of pure product: 84°C

TLC Studies



Rf values:

Cyclopentanone = 0.30

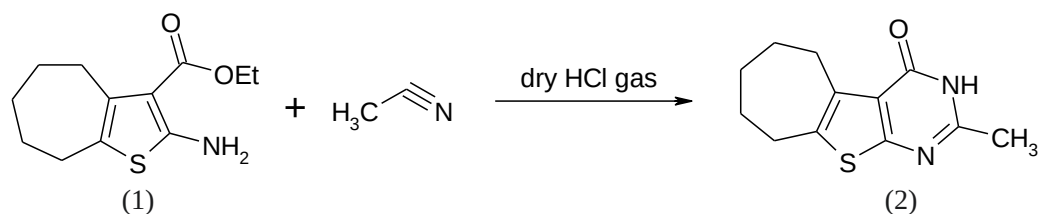
Compound 1 = 0.81

Molecular Weight: 239.34

Molecular Formula: C₁₂H₁₇NO₂S

II. Synthesis of 2-methyl-3,5,6,7,8,9-hexahydro-4H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-one: (2a)

Reaction:



Procedure:

Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**1**, 9.4 mmol, 2.25 g) and acetonitrile (18.0 mmol, 0.94 mL) were taken in a conical flask and hydrochloric acid gas was passed through it for 4 hrs. The reaction mixture was heated at around 50°C for an hour and kept aside for 12 hrs at room temperature. The mixture was poured into a beaker containing crushed ice and neutralized using 10% ammonium hydroxide. The resulting precipitate was filtered and dried^{2,18}.

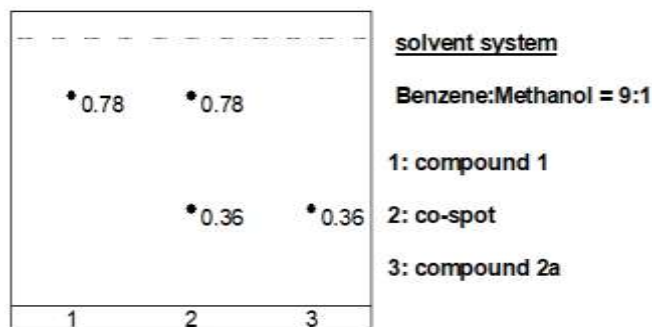
Recrystallisation solvent: Ethyl Acetate.

Yield: 87.2 %

M.P of crude product: 229°C

M.P of pure product: 227°C

TLC Studies



Rf values:

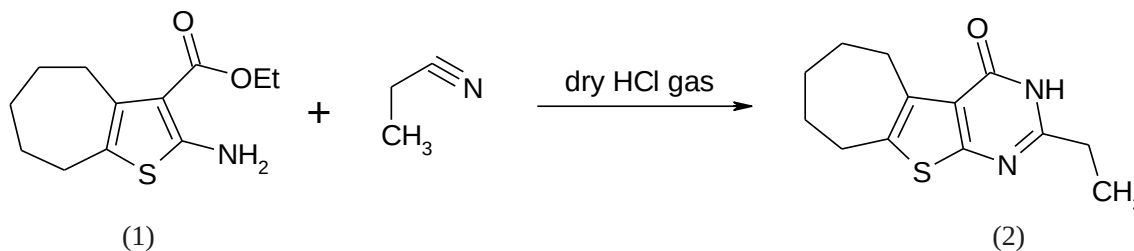
Compound 2a = 0.36

Molecular Weight: 234.3

Molecular Formula: C₁₂H₁₄N₂OS

III. Synthesis of 2-ethyl-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-one: (2b)

Reaction:



Procedure:

Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**1**, 9.4 mmol, 2.25 g), propionitrile (18.0 mmol, 1.25 mL) and 10 mL of dioxane were taken in a conical flask and hydrochloric acid gas was passed through it for 6 hrs. This reaction mixture was kept aside for 12 hrs at room temperature. It was then poured into a beaker containing crushed ice and neutralized using ammonium hydroxide (10%). The resultant precipitate was filtered and dried^{2,18}.

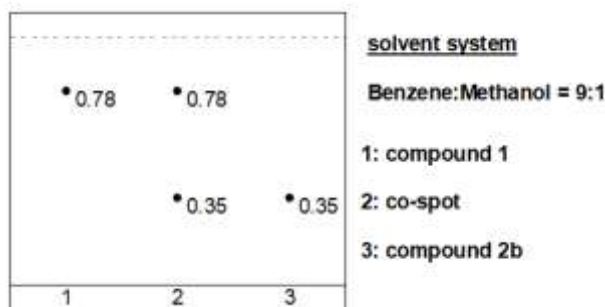
Recrystallisation solvent: Ethyl Acetate

Yield: 80.4 %

M.P of crude product: 217°C

M.P of pure product: 216°C

TLC Studies:



Rf values:

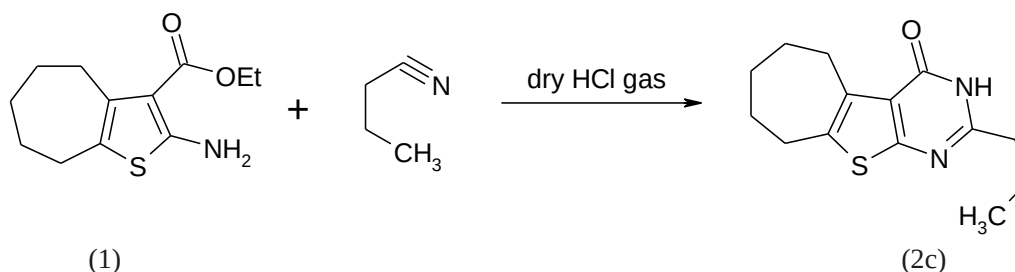
Compound **2b** = 0.35

Molecular Weight: 248.34

Molecular Formula: C₁₃H₁₆N₂OS

IV. Synthesis of 2-propyl-3,5,6,7,8,9-hexahydro-4H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-one: (2c)

Reaction:



Procedure:

Ethyl 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate (**1**, 9.4 mmol, 2.25 g), butyronitrile (18.0 mmol, 1.56 mL) and 10 mL of dioxane were taken in a conical flask and hydrochloric acid gas was passed through it for 6 hrs. This reaction mixture was kept aside for 12 hrs at room temperature. It was then poured into a beaker containing crushed ice and neutralized using ammonium hydroxide (10%). The resultant precipitate was filtered and dried^{2,18}.

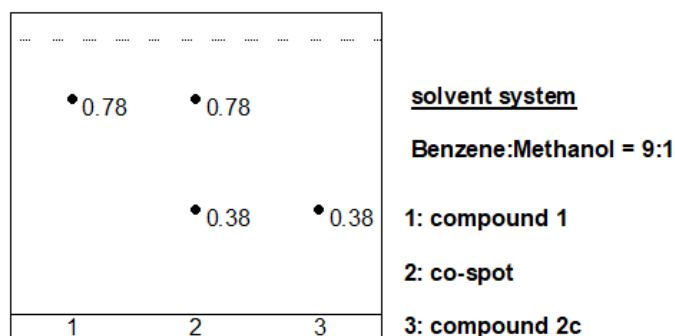
Recrystallisation solvent: Ethyl Acetate

Yield: 73.5%

M.P of crude product: 211⁰C

M.P of pure product: 210⁰C

TLC Studies:



Rf values:

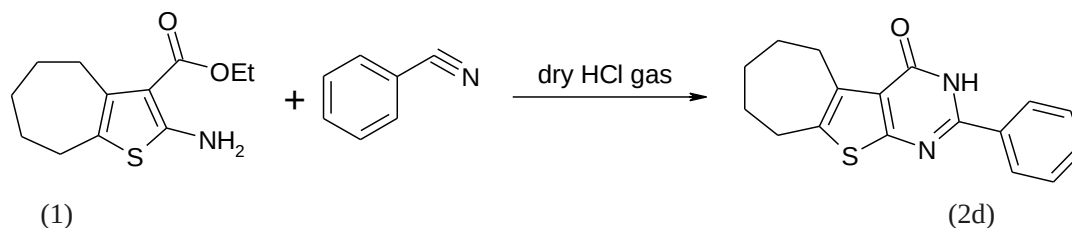
Compound 2c = 0.38

Molecular Weight: 262.37

Molecular Formula: C₁₄H₁₈N₂OS

V. Synthesis of 2-phenyl-3,5,6,7,8,9-hexahydro-4H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-one: (2d)

Reaction:



Procedure:

Ethyl 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate (**1**, 9.4 mmol, 2.25 g), butyronitrile (18.0 mmol, 1.85 mL) and 10 mL of dioxane were taken in a conical flask and hydrochloric acid gas was passed through it for 6 hrs. This reaction mixture was kept aside for 12 hrs at room temperature. It was then poured into a beaker containing crushed ice and neutralized using ammonium hydroxide (10%). The resultant precipitate was filtered and dried^{2,18}.

Recrystallisation solvent: Ethyl Acetate

Yield: 68.9 %

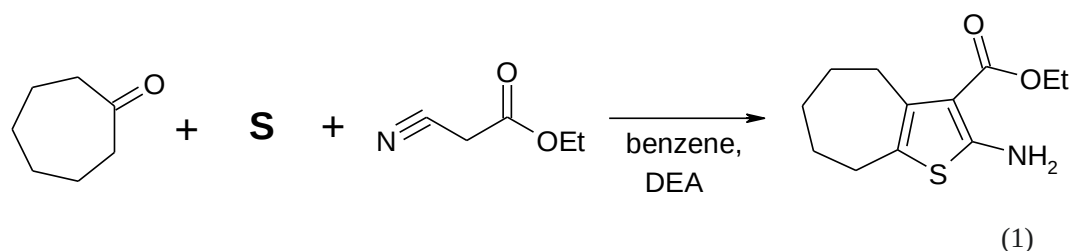
M.P of crude product: 227⁰C

M.P of pure product: 225⁰C

DISCUSSION-SYNTHETIC

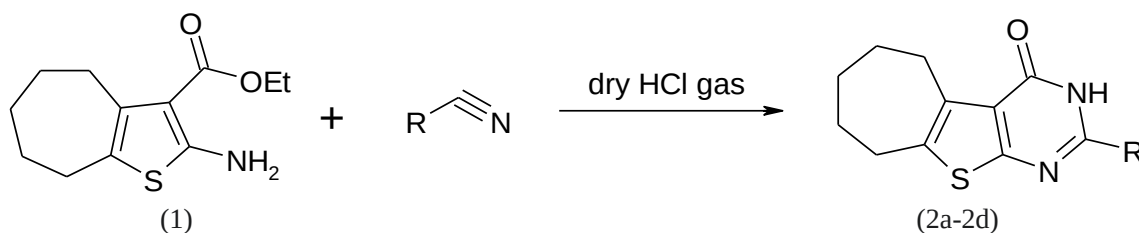
A series of 3-[(2-substituted-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d] pyrimidin-4-yl)amino]propan-1-ol (4a – 4d) has been synthesized using the appropriate synthetic procedures.

STEP I: Synthesis of ortho aminoester of thiophene (1)

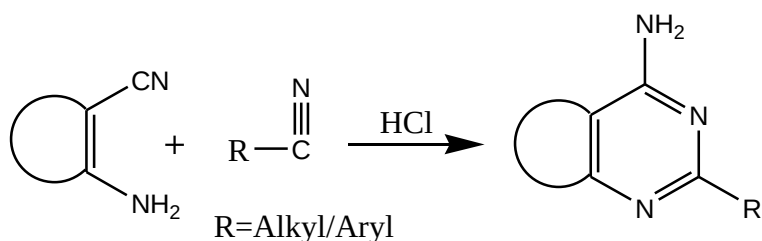


The synthesis of ortho aminoester of thiophene (**1**) has done by using Dean Stark's apparatus⁴⁰. The molar quantities of cycloheptanone, ethylcyanoacetate, ammonium acetate, glacial acetic acid and benzene were taken in a round bottom flask and heated to reflux using Dean Stark Apparatus for 6 hrs with constant removal of water. The solution was washed with sodium carbonate solution (10%) and dried using anhydrous sodium sulphate. The excess benzene was distilled off until 5 mL of solution is left. This solution was added to hot alcoholic solution of sulphur and stirred for one hr; with constant slow addition of diethylamine (DEA). The resultant solution was kept in the deep freezer for 12 hr. The precipitate obtained was filtered and dried. This method gave us substantial quantity of compound (1) in pure form and was used for next step after further purification².

STEP II: Synthesis of Thienopyrimidines (2a-2d)

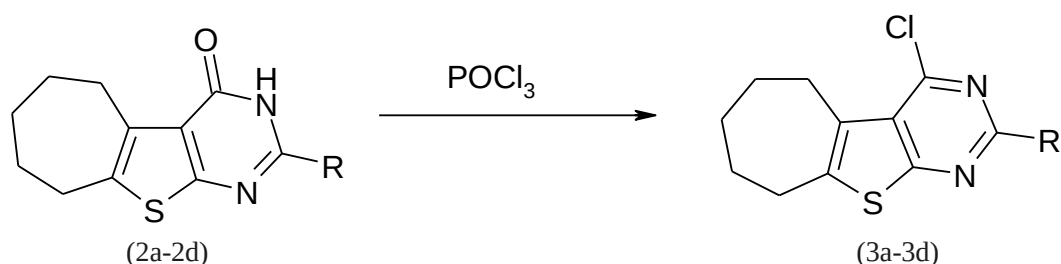


C.J. Shishoo *et al.*¹⁸ have reported that the condensation of a nitrile with orthoaminoester of thiophene in the presence of dry hydrogen chloride yields 4-aminopyrimidines in varying proportions depending upon the nature of nitrile.



Following the above reported method, compound (1) was treated with various aryl and alkyl nitriles in the presence of hydrogen chloride gas to obtain compounds 2-substituted-3,5,6,7,8,9-hexahydro-4H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-one (2a-2d). The compound (1) and the required nitrile were dissolved in dioxane and hydrogen chloride gas was passed through it. Then the reaction mixture was heated at around 50°C for an hour and kept aside for 12 hrs at room temperature to remove excess HCl from the reaction mixture. Then the product (2a-2d) was precipitated by neutralizing the reaction mixture using dilute ammonium hydroxide solution. The resulting precipitate was filtered and dried. The dried compound was recrystallised from ethylacetate to purify. This was used for the synthesis of compounds 3a-3d.

STEP III: Chlorination of Thienopyrimidinones (3a-3d)



The chlorination of thienopyrimidines was done by using $POCl_3$ as it is the mostly used method for the chlorination⁴⁵.

In the conventional synthetic method, 2-substituted-3,5,6,7,8,9-hexahydro-4H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-ones (2a-2d) and $POCl_3$ in approximately 1:25 ratio were used. For every 4 mmol of 2-substituted-3,5,6,7,8,9-hexahydro-4H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-one, 10 mL of $POCl_3$ (107.3 mmol) was used. Hence it is necessary to distill off the excess $POCl_3$ after completion of reaction, which makes the work up process tedious and it occasionally leads to formation of sticky mass. In case of formation of sticky mass, the compound was extracted in ethyl acetate (50 mL X 3) and the extract was dried by distillation and was directly used for the next step without any purification.

As this method required long hours of heating and had tedious work up procedures, the synthesis of 4-chloro-2-methyl-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d]pyrimidines (3a) was carried out by using microwave assisted method.

Since 1986, when Gedye and Giguere published their first articles in Tetrahedron Letters on microwave-assisted syntheses in household microwave ovens, there has been a steadily growing interest in this research field. Since the use of microwaves comprises more than the simple application of a goal-oriented, innovative tool, it is crucial to be aware of the fundamentals of chemistry in the microwave field before investigating challenging reaction mechanisms.

Green Chemistry:

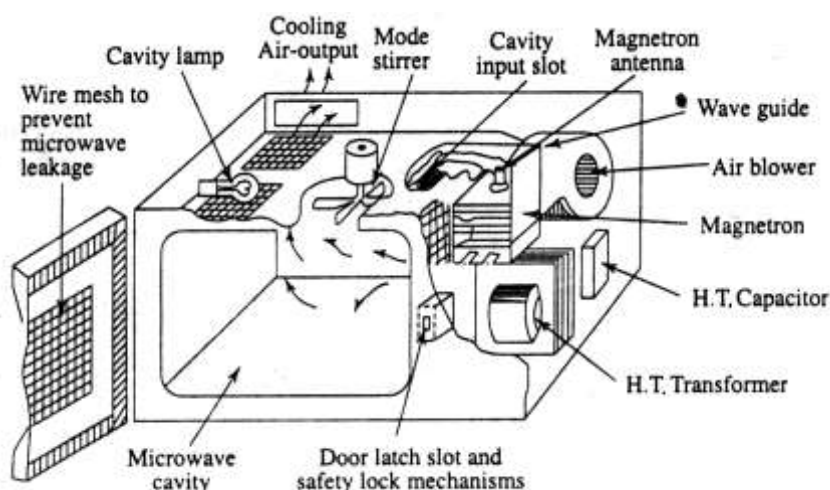
Introduced in the early 1990's, green chemistry is defined as the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products. The concept of green chemistry is enshrined in particular principles, such as the prevention of waste, the design of energy efficient processes, the use of safe, environmentally benign solvents where possible, the use of renewable feed stocks *etc.*



Microwaves have the capacity to alter, because of the fact that the energy is directly transferred and concentrated in the reaction species.⁵ In recent years, reagents impregnated on mineral solid support and assisted by microwaves has gained popularity in the synthesis of various heterocyclic compounds like quinoxalinones,⁶ triazoles,⁷ quinolines,^{8,9} benzofurans,¹⁰ quinazolines,¹¹ thienopyrimidines¹² *etc.* This could be because of their enhanced selectivity, improved reaction rates, and associated ease of manipulation and above all eco friendliness.

The Microwave Oven:

The microwave region of the electromagnetic spectrum lies between infrared radiation and radiofrequencies and corresponding wavelength of 1cm to 1m (frequencies of 30 GHz to 300 MHz respectively) Domestic and industrial microwave heaters operates at 12.2 cm (2450 MHz) or 33.3 cm (900 MHz).



A microwave oven consists of a high power source, a waveguide feed and the oven cavity. The source is generally a magnetron in which the microwaves are generated. A magnetron is a thermodynamic diode having an anode and a directly heated cathode. As the cathode is heated, electrons are released and are attracted towards the anode. The anode is made up of an even number of small cavities, each of which acts as a tuned circuit. The gap across the end of each cavity behaves as a capacitance.

A very strong magnetic field is induced axially through the anode assembly, and has the effect of bending the path of the electrons as they travel from the cathode to the anode. As the deflected electrons pass through the cavity gaps, they induce a small charge into the tuned circuit, resulting in the oscillation of the cavity. Alternate cavities are linked by two small wire straps which ensure the correct phase relationship. This process of oscillation continues until the oscillation has achieved a sufficiently high amplitude. It is then taken off the anode via an antenna. Of the 1200W of electric line power used by the magnetron, around 600W is converted into electromagnetic energy. The remainder is converted into heat that must be dissipated through air or water cooling.

The variable power available in domestic oven is produced by switching the magnetron on-and-off according to a duty cycle. When working with small quantities of material, poorly absorbing material or at high powers, a beaker of water should always be placed in the cavity along with the sample to absorb the excess energy.

A waveguide is a rectangular channel made of sheet metal. Its reflective walls allow the cavity necessary to prevent leakage of radiation and to increase the efficiency of transmission of microwaves from the magnetron to the microwave cavity. The reflective walls of the microwave oven.

In the absence of any soothing mechanism, the electric field pattern produced by the standing waves set up in the cavity may be extremely complex. For smoothing the incoming energy in the cavity, a mode stirrer (a reflective, fan-shaped paddle) is sometimes used. Most microwave ovens are also having a turntable, which ensures that the average field experienced by the sample is approximately the same in all directions¹³.

Reaction vessel and medium :

The preferred reaction vessel for microwave-induced organic reaction enhancement (MORE) chemistry, is a tall beaker, loosely covered with a capacity much greater than the volume of the reaction mixture. Alternatively, teflon and polystyrene vessels can be used. These materials are transparent to microwaves. No metallic container can be used as a reaction vessel as it gets heated soon due to preferential absorption and reflection of rays.

In MORE chemistry, the reactions are carried out in solvent medium or on solid support in which no solvent is used. For reactions in solvent medium, the choice of solvent is most important. The solvent used must have a dipole moment so as to absorb microwaves and a boiling point at least 20-30 °C higher than the desired reaction temperature^{14, 15}.

An excellent energy transfer medium for many types of reactions in a domestic microwave oven is N, N-dimethyl formamide (DMF), a good solvent with high boiling point (160 °C) and high dielectric constant ($\epsilon = 36.7$). The solvent can retain water formed in a reaction, thus obviating the need for a water separator. The temperature can be raised to about 140 °C without noticeable vapourisation. Other solvents of choice are as follows:

Formamide	(b.p 216 °C, $\epsilon = 111$)
Methanol	(b.p 65 °C, $\epsilon = 32.7$)
Ethanol	(b.p 78 °C, $\epsilon = 24.6$)
Chlorobenzene	(b.p 132 °C, $\epsilon = 5.6$)
Ethylene Glycol	(b.p 196 °C, $\epsilon = 37.7$)

The presence of salts in polar solvents can frequently enhance microwave coupling. Hydrocarbon solvents such as hexane ($\epsilon = 1.9$), benzene ($\epsilon = 2.3$), toluene ($\epsilon = 2.4$) and xylene are unsuitable because of less dipole moment, resulting in the poor absorption of microwave radiation. But the addition of small amounts of alcohol or water to these solvents can lead to dramatic coupling effects. Hence, a 1:4 ethanol: toluene mixture can be heated to boiling in few minutes in a standard microwave oven.

For solid state reactions, the reaction is to perform on a solid support (no solvent) which couples effectively with microwaves. Examples of such solid supports are : Phyllosilicate (M^{n+} - montmorillonite), silica, alumina.

Advantages and Limitations :

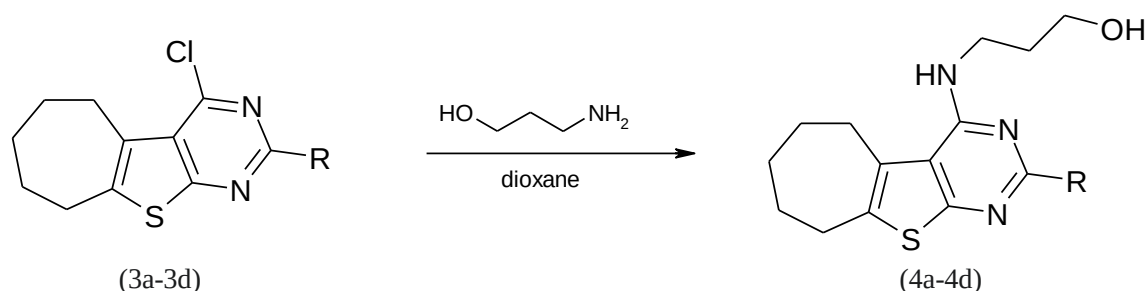
The rapid heating capability of the microwave leads to a considerable saving in dissolution or reaction time. Further, the use of microwave eliminates the need of heating baths, reaction flasks and reflux condensers with ground glass joints. The smaller volume of solvents required in this procedure reduces the cost spent on solvents and chemicals. Further it diminishes the waste disposal problem^{16,17}.

The procedures are strongly limited by the presence of solvents which reach their boiling points within very short time (<1 min) of exposure to microwaves. Consequently, high pressures are developed, thus leading to damage to vessels material or microwave oven itself and occasionally lead to explosions. Thus long heating times and reactions involving a large volume of reactants should be avoided¹⁸.

We have performed the synthesis of 4-chloro-2-methyl-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d]pyrimidines (3a) in microwave by taking 2-methyl-3,5,6,7,8,9-exahydro-4H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-ones (2a) and POCl₃ in 1:1 ratio in a small reaction vessel and irradiated in CATA-R scientific microwave systems at 420 Watt for 10 min. The resultant reaction mixture was poured into crushed ice and neutralized with dilute sodium bicarbonate solution⁴⁶. The resulting precipitate was filtered off and air dried. This dried product was directly used for the next step.

This method results in convenient workup procedures and comparatively high yield with 87.4% when compared to conventional synthetic method, where the yield is 65.8%.

STEP IV: Amination of 4-chloro-thienopyrimidines (4a-4d)



The unstable chlorinated thienopyrimidines (3a-3d) were heated to reflux with aminopropanol in basic medium for 10 hr. The crystals obtained (4a-4d) were recrystallised by using chloroform.

This is performed by basing on a published procedure⁴⁶. We have followed the same by using different reaction time and temperature in conventional synthetic approach. We have used 4-chloro-2-methyl-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d] pyrimidine and aminopropanol in 1:1.5 ratio. Equimolar concentration of triethylamine was used with 4-chloro-2-methyl-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d] pyrimidine to drive the reaction forward by removing generated HCl from the reaction mixture by forming a salt with HCl (Et₃NH⁺Cl⁻). The reactants were taken in dioxane and were refluxed for 8 hrs at 100°C. The excess dioxane was distilled off from the reaction mixture and was added to crushed ice. Then it was neutralized with dil.NaHCO₃ solution. The resulting precipitate was filtered off, dried and crystallized from chloroform to obtain the target compounds.

We have synthesized the 3-[(2-methyl-6,7,8,9-tetrahydro-5H-cyclohepta [b]thieno[2,3-d]pyrimidin-4-yl)amino]propan-1-ol (4a) also by microwave assisted method, and got comparably high yield when compared with conventional synthetic method along with convenient work up procedures. In this method, each 5 mmol of 4-chloro-2-methyl-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d]pyrimidine (3a) and aminopropanol (1:1) were taken along with 0.01 mL of triethylamine (0.07 mmol) in a small reaction vessel to irradiate the reaction mixture in CATA-R scientific microwave systems at 420 Watt for 2 min. The reaction mixture was then poured into crushed ice and neutralized with dil.HCl solution. The obtained precipitate was filtered off, dried and crystallized from chloroform⁴⁶.

The obtained yield was 91.2% which was comparatively higher than that of conventional method, where the yield was 54.1%.

The ¹H NMR spectrum of the compounds (4a-4d) recorded at 400 MHz in CDCl₃ showed the multiplet for 10 hydrogens at δ 1.7 to 1.9 ppm which characterizes the cycloheptane ring⁴⁷. The presence of two triplets at δ 3.7 to 3.9 ppm and 3.6 to 3.7 ppm represents the presence of CH₂ group adjacent to –OH and –NH of aminopropanol side chain respectively⁴⁸. Multiplet at δ 2.7 to 3.0 ppm for NHCH₂CH₂CH₂OH was recorded.

The ¹³C NMR spectrum of the compounds (4b) recorded at 100 MHz in CDCl₃ showed the peaks at δ 12.80 (CH₃), 26.32 (–NH–CH₂–CH₂–CH₂–OH), 29.79 (C-7), 30.16 (C-6), 30.40 (C-8), 32.12 (C-5), 32.46 (C-9), 32.73 (–CH₂–CH₃), 37.87 (–NH–CH₂–CH₂–CH₂–OH), 59.07 (–NH–CH₂–CH₂–CH₂–OH), 114.85 (C-2'), 135.82 (C-3'), 136.85 (C-4'), 157.85 (C-1'), 164.85 (C-4), 165.68 (C-2) confirmed the presence of all carbons of 4b⁴⁹.

The m/z values of mass spectrum of compounds (4a-4d) showed the ($M^+ + 1$) as base peak and ($M^+ + 2$), ($M^+ + 3$) peaks in common due to isotopic sulfur⁵⁰. The presence of significant ($M^+ + 2$) peak is the characteristic feature of mass spectra of sulfur containing compounds⁵¹.

CONCLUSION:

- 4-Chloro thienopyrimidines were treated with aminopropanol to give corresponding aminopropanol derivatives (4a-4d), title compounds in good yields.
- The conventional method of preparation of 3-[(2-substituted-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-yl)amino]propan-1-ol was compared with microwave assisted method with respect to their reaction time and yield.
- All the compounds synthesized were characterized by physical (R_f values, M.P., Molecular weight, molecular formula), spectral data (^1H NMR, ^{13}C NMR, MASS).
- The title compounds were screened for anticancer activity by MTT assay and analyzed statistically.
- Compounds showed considerable anticancer activity when compared with cyclophosphamide.
- Further lead optimization should be carried out for the better expected anticancer activity.

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