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Research

Synthesis and anticancer activity of some novel 3-[(2-substituted-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]thieno [2,3-*d*]pyrimidin-4-YL)amino]propan-1-OL]

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Abstract

The chemistry of pyrimidines and its derivatives has been studied for over a century due to their diverse biological activities. They possess Anticancer Activity. 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-olwas compared with microwave assisted method with respect to their reaction time and yield. All the compounds synthesized were characterized by physical (Rf values, M.P., Molecular weight, molecular formula), spectral data (1H NMR, 13C 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.

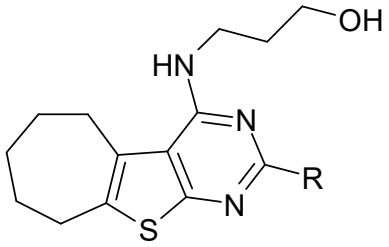
Keywords: Anticancer Activity. PYRIMIDIN-4-YL)AMINO]PROPAN-1-OL

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 treatment[1]. 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 antibacterial[2], antimicrobial[3], anti-inflammatory, analgesic and ulcerogenic activity[4]. 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-5*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-yl)amino]propan-1-ol and to test their ability as anticancer agents.



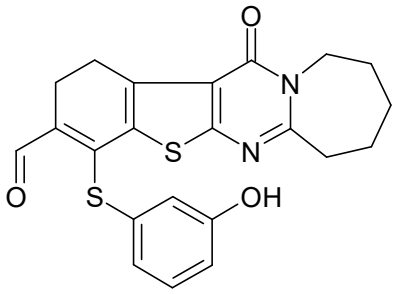
(4a-4d)

Aim And Objectives

1. To prepare thienopyrimidines from the ortho amino ester of thiophene.
2. To compare synthetic procedures and yields between the preparations of thienopyrimidines by conventional acid catalyzed method and microwave-assisted base catalyzed method.
3. To synthesize the title compounds viz. 3-[(2-substituted-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[2,3-d]pyrimidin-4-yl)amino]propan-1-ol.
4. To evaluate the title compounds for anticancer activity
5. To characterize all the synthesized compounds by physical (molecular weight, molecular formula, melting point, Recrystallisation, Rf value) and spectral data (1H NMR, 13C NMR and MASS spectra).

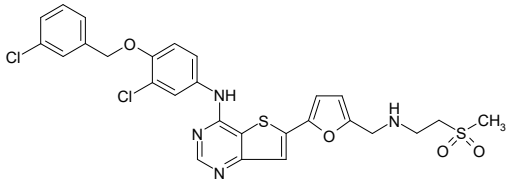
BASIS OF PROPOSAL

The chemistry of pyrimidines and its derivatives has been studied for over a century due to their diverse biological activities. They possess antibacterial[32], antiviral[33], antitumor[34-36], antihypertensive[37] and antiinflammatory[38,39] pharmacological activities. Thienopyrimidines formed by the fusion of thiophene moiety with pyrimidine ring, have been reported to be chemotherapeutically active[40]. Many thienopyrimidines were reported for their antimicrobial and antifungal activities. Many compounds were screened for their anticancer activity too. Literature survey showed that the 2,4-diaminothieno[2,3-*d*]pyrimidines have the property of inhibition of dihydrofolate reductase[41]. Thieno[2,3-*d*]pyrimidin-4(3*H*)-one **[1]** compounds have been reported to be effective inhibitors of 17β-HSD1, which results in inhibition of the E_2 dependent tumor growth and hence these compounds are useful for the treatment and prevention of breast cancer and other hormone dependent disorders[1].



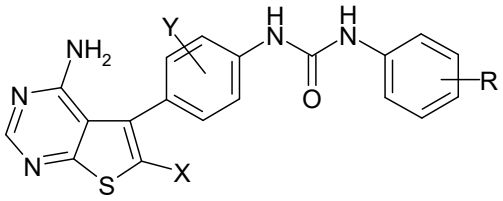
[1]

1,6-disubstituted thieno[3,2-*d*]pyrimidines **[2]** are reported to exhibit anticancer activity by inhibiting EGFR/ErbB-2 kinase enzymes at IC_{50} values less than 1 μM against human tumor cells in vitro[28].



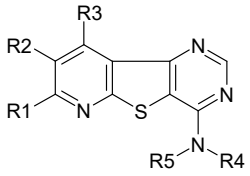
[2]

A series of 4-amino-5-(*N, N'*-diaryl urea)-6-substituted thieno[2,3-*d*]pyrimidines **[3]** were reported for their antitumor activity by inhibiting vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) tyrosine kinase enzyme[2].



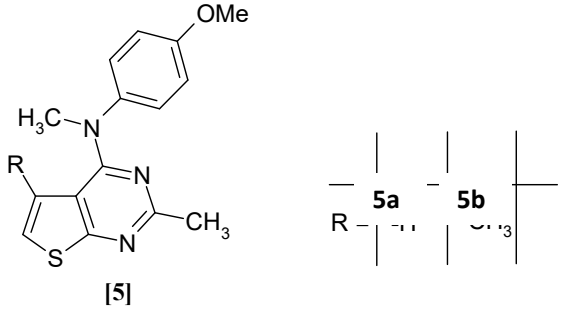
[3]

Some pyrido-thienopyrimidines **[4]** were reported to exhibit anticancer activity by selective inhibition of Cdc7 kinase[30].



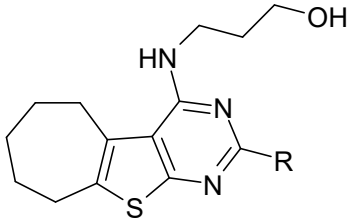
[4]

In a recent publication we found that some 4-anilino-*N*-methylthieno[2,3-*d*]pyrimidines **[5]** proved to be potent inducers of apoptosis at micro molar concentrations[3].



[5]

The literature survey reveals that the potency of thienopyrimidine derivatives as anticancer agents in multiple ways, along with their antimicrobial and other activities. All of these interesting facts gave us an impetus to make an attempt to synthesize a novel series of 3-[(2-substituted-6,7,8,9-tetrahydro-5*H*-cyclohepta[b]thieno[2,3-*d*]pyrimidin-4-yl)amino]propan-1-ol derivatives and screen them for anticancer activity.



(4a-4d)

EXPERIMENTAL

Anticancer activity testing

Cell lines used: HC 29-Colorectal adenoma cell line, MDA 231-Adenocarcinoma breast cancer cell line.

Procedure:

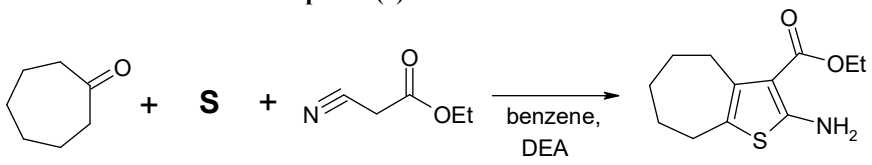
1. The monolayer cell culture was trypsinized and the cell count was adjusted to 3.0 x 105 cells /mL using medium containing 10% new born calf serum.
2. To each well microtitre plate, 0.1 mL of the diluted suspension (approx. 10,000 cells) was added and kept for 24 hrs in incubator at 370C in 5% CO2 atmosphere for cell monolayer formation.

3. After 24 hrs, when a partial monolayer was formed at the bottom of the well, the supernatant was flicked off, washed the monolayer once and 100 µL of different drug concentrations (10, 20 and 50 µg) i.e. title compounds (4a-4d) were added to the cells in microtitre plates.
4. The plates were then incubated at 37 °C for 3 days in 5% CO atmosphere and microscopic examination was carried out and observations recorded every 24 hrs.
5. After 72 hrs, the sample solution in the wells was flicked off and 50 mL of MTT dye was added to each well, plates were gently shaken and incubated for 4hrs at 37°C in 5% CO2 incubator.
6. The supernatant was removed and 50 µL of propanol was added and the plates were gently shaken to solubilize the formed formazan.
7. The absorbance was measured using a microplate reader at a wavelength of 490 nm.

DISCUSSIONS

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.

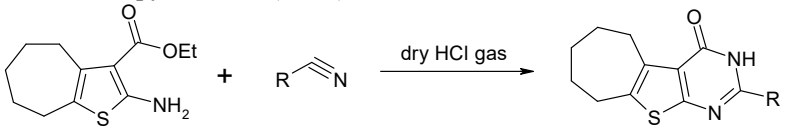
STEP I: Synthesis of ortho aminoester of thiophene (1)



(1)

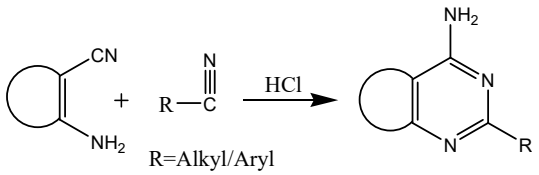
The synthesis of ortho aminoester of thiophene **(1)** has done by using Dean Stark's apparatus[40]. The molar quantities of cycloheptanone, ethylcyanoacetate, ammonium acetate, glacial acetic acid and benzene were taken in a round bottom flask and heated to reflux using Dean Stark Apparatus for 6 hrs 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 purification[4].

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



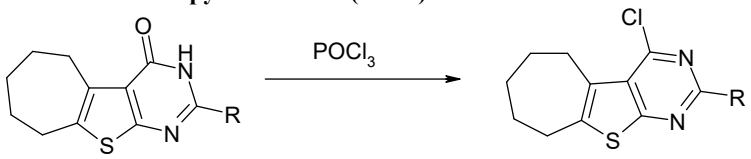
(1) (2a-2d)

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 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 compound 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 50^0C 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)



(2a-2d) (3a-3d)

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

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-5*H*-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.

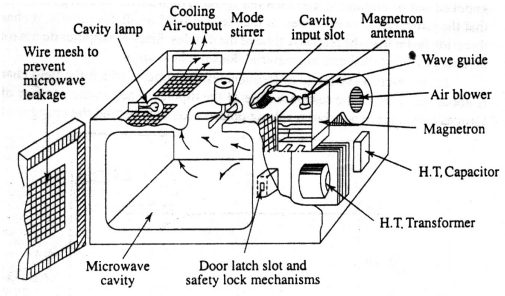
Green Chemistry

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] thienopyrimidines[12] *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 eletromagnetic 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 are necessary to prevent leakage of radiation and to increase the efficiency of transmission of microwaves from the magneron to the microwave cavity. The reflective walls of the microwave the oven. In the absence of any soothing mechanism, the electric field pattern produced by the standing waves set up in the cavity may be extremly 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 directions.

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 ^{0}C higher than the desired reaction temperature. 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 ^{0}C) and high dielectric constant (ε = 36.7). The solvent can retain water formed in a reaction, thus obviating the need for a water seperator. The temperature can be raised to about 140 ^{0}C without noticeable vapourisation. Other solvents of choice are as follows:

Formamide (b.p 216 ^{0}C, ε = 111)
Methanol (b.p 65 ^{0}C, ε = 32.7)
Ethanol (b.p 78 ^{0}C, ε = 24.6)
Chlorobenzene (b.p 132 ^{0}C, ε = 5.6)
Ethylene Glycol (b.p 196 ^{0}C, ε = 37.7)

The presence of salts in polar solvents can frequently enhance microwave coupling. Hydrocarbon solvents such as hexane (ε = 1.9), benzene (ε = 2.3), toluene (ε = 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+} - montomorillonite), silica, alumina.

SUMMARY AND 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 (Rf values, M.P., Molecular weight, molecular formula), spectral data (1H NMR, 13C 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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