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# 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 DERIVATIVES.

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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 antibacterial, antiviral, antitumor, antihypertensive and antiinflammatory pharmacological activities. Thienopyrimidines formed by the fusion of thiophene moiety with pyrimidine ring, have been reported to be chemotherapeutically active. In view of various biological activities and its enormous importance of thienopyrimidines, we have made an attempt to synthesize and characterize some new 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 evaluate them for anticancer activity. Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate was treated with acetonitrile in presence of hydrochloric acid gas to give 2-methyl-3,5,6,7,8,9-hexahydro-4H-cyclohepta[*b*]thieno[2,3-d]pyrimidin-4-one, which was reacted with excess $POCl_3$ and then refluxed with dioxane, Triehtylamine and aminopropanol to give 3-[(2-methyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-yl)amino]propan-1-ol. All the intermediate and final compounds were purified and their chemical structures have been confirmed by IR, $^1$H NMR and Mass spectral data. All the newly synthesized compounds were screened for their anticancer activity by MTT assay and analyzed statistically. Compounds showed considerable anticancer activity when compared with cyclophosphamide.

**KEYWORDS:** Pyrimidines, thienopyrimidines, thiophene, anticancer activity, cyclophosphamide.

## 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.[1] 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.[2,3] In the past few years lots of compounds were screened for anticancer activity due to the availability of various cell lines and screening methods.[4]

In this process of investigation, many pyrimidine derivatives including thienopyrimidines proved their therapeutic ability against cancer in the previous literature.[5] Thienopyrimidines are reported for their antibacterial[6], antimicrobial[7], anti-inflammatory, analgesic and ulcerogenic activity.[8] 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.

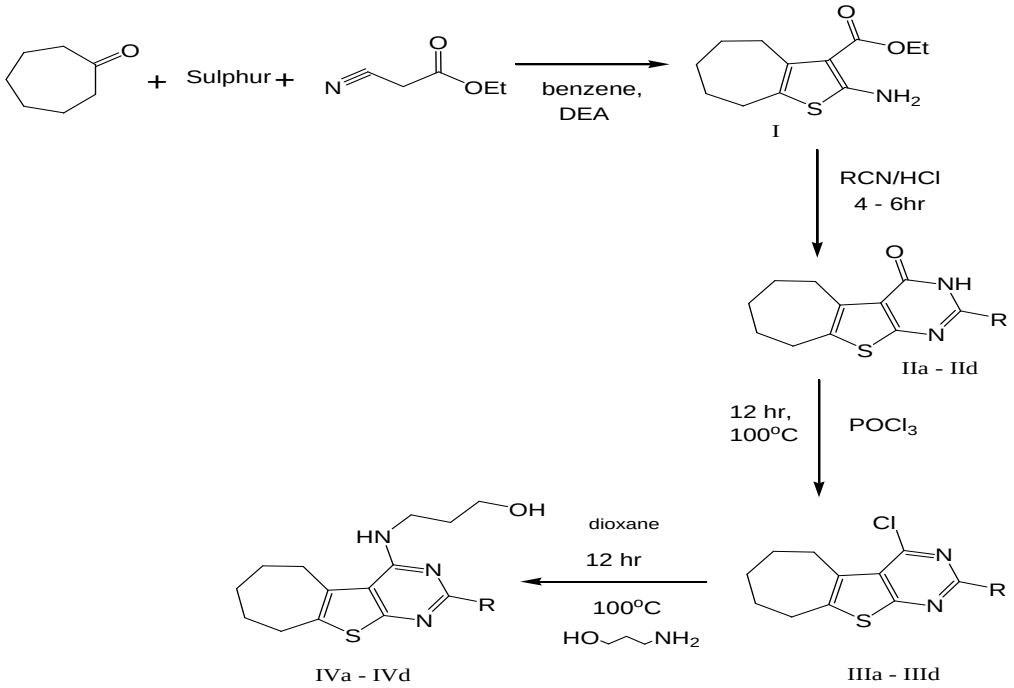
## MATERIALS AND METHODS

The solvents and chemicals used for the experimental work were commercially procured from E. Merck, India, S.D. Fine Chemicals, India and Qualigens, India. The Silica gel G used for analytical chromatography (TLC) was obtained from S.D. Fine Chemicals, India. Melting points were determined in an open glass capillary using a Kjeldahl flask with liquid paraffin and are uncorrected. The proton magnetic resonance spectra ($^1$H NMR) were recorded on Bruker 300 MHz instrument (Bruker, Germany) in DMSO/CDCl3 using TMS as internal standard. Chemical shifts (δ) are expressed in ppm. The infrared spectra of compounds were recorded in KBr on a FTIR- -8400S, Fourier Transform (Shimadzu), Japan infrared spectrophotometer. Mass spectra were recorded

on LC-MS/MS (API-4000 TM), Applied BioSystems, MDS SCIEX (Canada).

**Experimental**

**Scheme**




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

Cycloheptanone (40.0 mmol), ethylcyanoacetate (40.0 mmol), 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 sodiumcarbonate 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) and stirred for one hour, with constant slow addition of diethylamine [DEA] (40.0 mmol). The resultant solution was kept in the deep freezer for 12 hrs. The precipitate obtained was filtered and dried.[9] **Recrystallisation solvent:** Ethanol, **Yield:** 71.2%, **M.P:** 84°C.

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

Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**I**), (9.4 mmol) and acetonitrile (18.0 mmol) 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.[10] **Recrystallisation solvent:** Ethyl Acetate, **Yield**: 87.2%, **M.P:** 227°C.

**3. Synthesis of 3-[(2-methyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]thieno[2,3-*d*] pyrimidin-4-yl)amino]propan-1-ol (IIIa & IVa)**

2-methyl-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-one (**IIa**, 8.0 mmol) and 20 ml of $POCl_3$ were taken in a round bottom flask and refluxed for 12 hrs at 100°C under anhydrous conditions. The excess $POCl_3$ was distilled off from the reaction medium and was added to crushed ice. Then it was neutralized with dil.$NH_3$ solution. The resultant precipitate, (**IIIa**) was filtered off and air dried. The obtained dried product (**IIIa**, 4.0 mmol) was dissolved in 10 ml of dioxane and added Triehtylamine (4.0 mmol), aminopropanol (6.0 mmol). This reaction mixture was refluxed for 8 hrs at 100°C under anhydrous conditions. The excess dioxane was distilled off from the reaction mixture and added to the beaker containing crushed ice. Then it was neutralized with Dil.$NaHCO_3$ solution. The resulting precipitate was filtered off, dried and crystallized from chloroform.[11,12] **Recrystallisation solvent:** $CHCl_3$, Yield: 54.1%, **M.P:** 290°C.

**$^1$H NMR** (400 MHz, $CDCl_3$) δ = 1.79-1.89 (m, 10H, $CH_2$ at 5,6,7,8,9), 2.54 (s, 3H, $CH_3$), 2.84-2.87 (m, 2H, *J*=5.4 Hz, –$NHCH_2CH_2CH_2OH$), 3.62-3.65 (t, 2H, *J*=5.4 Hz, –$NHCH_2CH_2$ $CH_2OH$), 3.75-3.79 (t, 2H, *J*=5.4 Hz, –$NHCH_2CH_2CH_2OH$). **MS (m/z):** 292.3($M^+$+1), 293.3($M^+$+2), 294.2($M^+$+3).

**Table 1 Physical data of the title compounds (IVa - IVd)**

| Sl.No. | Compound | Molecular Formula | Molecular Weight | Melting Point $^{o}$C | Yield % |
|---|---|---|---|---|---|
| 1 | IVa | $C_{15}H_{21}N_3OS$ | 291.42 | 290 | 54.1 |
| 2 | IVb | $C_{16}H_{23}N_3OS$ | 305.45 | 269 | 70.6 |
| 3 | IVc | $C_{17}H_{25}N_3OS$ | 319.47 | 257 | 52.3 |
| 4 | IVd | $C_{20}H_{23}N_3OS$ | 353.49 | 266 | 61.5 |

## ANTICANCER ACTIVITY

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

The monolayer cell culture was trypsinized and the cell count was adjusted to 3.0 x $10^5$ cells /ml using medium containing 10% new born calf serum. 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 37$^{o}$C in 5% $CO_2$ atmosphere for cell monolayer formation. 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 (IVa-IVd) were added to the cells in microtitre plates. 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. 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% $CO_2$ incubator. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm. The same procedure[13,14,15] was followed for the two cell lines.

**Table 2 Anticancer activity of the title compounds (IVa - IVd)**

| Compound code | Concentration (µmol) | Percentage Inhibition of cell growth | |
|---|---|---|---|
| | | HC 29-Colorectal adenoma cell line | MDA 231-Adenocarcinoma breast cancer cell line |
| IVa | 0.03 | 32.92 % | 31.18 % |
| | 0.07 | 31.43 % | 29.35 % |
| | 0.17 | 27.96 % | 24.47 % |
| IVb | 0.03 | 30.73 % | 24.94 % |
| | 0.07 | 27.94 % | 25.79 % |
| | 0.17 | 25.27 % | 26.41 % |
| IVc | 0.03 | 30.11 % | 23.48 % |
| | 0.07 | 28.16 % | 25.21 % |
| | 0.17 | 24.32 % | 24.94 % |
| IVd | 0.03 | 34.52 % | 33.68 % |
| | 0.07 | 32.83 % | 30.72 % |
| | 0.17 | 28.45 % | 25.35 % |

## RESULTS AND DISCUSSION

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 (IVa – IVd) have been synthesized using the appropriate synthetic procedures.

The purity and homogeneity of all the newly synthesized compounds were confirmed by their sharp melting points and TLC. All the compounds synthesized have been obtained in solid state and the yields varied from minimum 45% to maximum 71%. The structures of these compounds were confirmed by C, H and N analytical data, IR, $^1$HNMR and Mass spectral data. The m/z values of mass spectrum of compounds **(IVa - IVd)** showed the ($M^+$ +1) as base peak and ($M^+$ +2), ($M^+$ +3) peaks in common due to isotopic sulfur. The presence of significant ($M^+$ +2) peak is the characteristic feature of mass spectra of sulfur containing compounds. From the anticancer activity it was observed that all the compounds exhibited activity against both the cell lines employed as indicated in Table 2. The compound **IVd** had shown better anticancer activity at all concentrations on both the cell lines (HC 29-Colorectal adenoma cell line and MDA 231-Adenocarcinoma breast cancer cell line) followed by compound **IVa** and then **IVb**. The compound **IVc** has shown least activity among all the newly synthesized compounds.

## CONCLUSION

The present study suggested that 3-[(2-substituted-6,7,8,9-tetrahydro-5*h*-cyclohepta[*b*]thieno [2,3-*d*]pyrimidin-4-yl)amino]propan-1-ol analogues have been synthesized successfully as per the scheme and all the newly synthesized compounds have shown anticancer activity against both the cell lines (HC 29-Colorectal adenoma cell line and MDA 231-Adenocarcinoma breast cancer cell line). The compound

with phenyl substitution **(IVd)** has shown better anticancer activity.

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