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Research Article

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 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-5h-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-4H-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 POCl3 and then refluxed with dioxane, Triehtylamine and aminopropanol to give 3-[(2-methyl-6,7,8,9-tetrahydro-5H-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, 1H 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: Anti cancer activity, Synthesis.

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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

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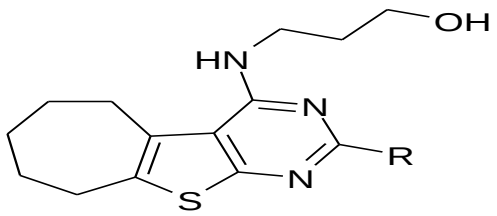
proved their therapeutic ability against cancer in the previous literature. Thienopyrimidines are reported for their antibacterial [2], antimicrobial [3], anti-inflammatory, analgesic and ulcerogenic activity[4]. Many thienopyrimidines are also reported as anticancer agents and this has laid base for 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.

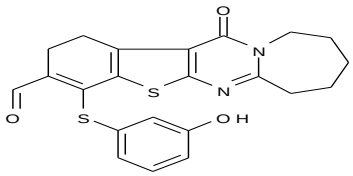


(4a-4d)

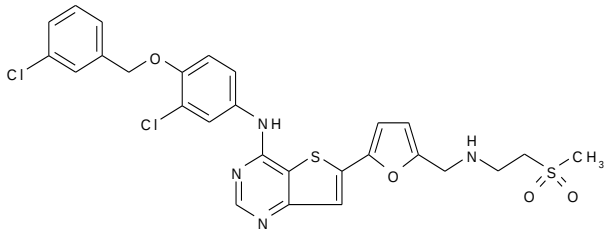
BASIS OF PROPOSAL

The chemistry of pyrimidines and its derivatives has been studied for over a century due to their diverse biological activities. They possess antibacterial[5], antiviral[6], antitumor[7], antihypertensive[8] and anti-inflammatory[9] pharmacological activities. Thienopyrimidines formed by the fusion of thiophene moiety with pyrimidine ring, have been reported to be chemotherapeutically active[10].

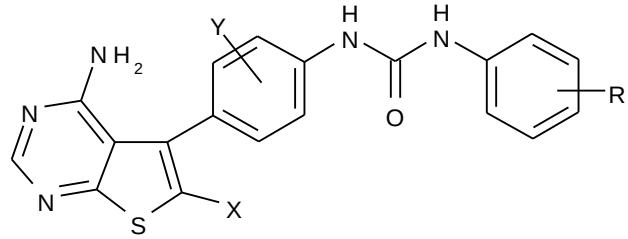
Many thienopyrimidines were reported for their antimicrobial and antifungal activities. Many compounds were screened for their anticancer activity too. Literature survey showed that the 2,4-diaminothieno[2,3-*d*]pyrimidines have the property of inhibition of dihydrofolatereductase[11]. Thieno[2,3-*d*]pyrimidin-4(3*H*)-one (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[12].



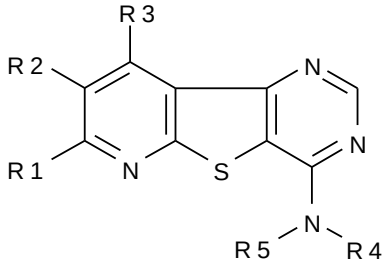
1,6-disubstituted thieno[3,2-*d*]pyrimidines(2) are reported toexhibit anticancer activity by inhibitingEGFR/ErbB-2 kinase enzymes at IC_{50} values less than 1 μM against human tumor cells in vitro[13].



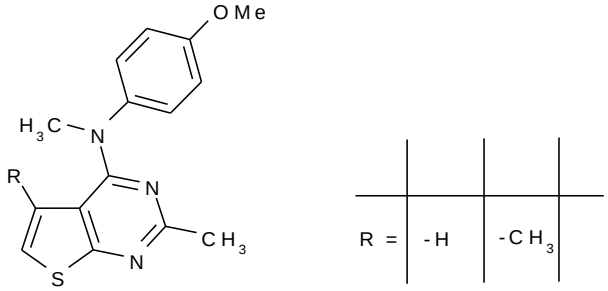
A series of 4-amino-5-(*N, N'*-diaryl urea)-6-substituted thieno[2,3-*d*]pyrimidines(3) were reported for their antitumor activity by inhibiting vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) tyrosine kinase enzyme[14].



Some pyrido-thienopyrimidines(4) were reported to exhibit anticancer activity by selective inhibition of Cdc7 kinase[15].

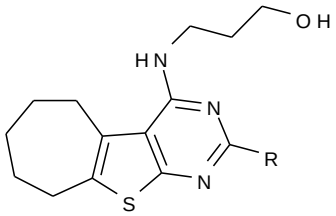


In a recent publication we found that some 4-anilino-*N*-methylthieno[2,3-*d*]pyrimidines(5) proved to be potent inducers of apoptosis at micro molar concentration [15].



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*-

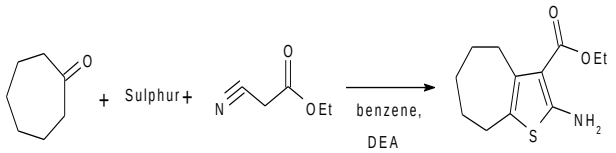
cyclohepta[b]thieno[2,3-*d*]pyrimidin-4-yl)amino]propan-1-ol derivatives and screen them for anticancer activity.



SYNTHETIC

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

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 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, 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[16]

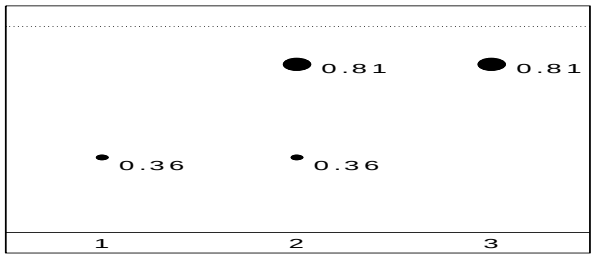
Recrystallisation solvent: Ethanol

Yield: 71.2%

M.P of crude product: 85^{0}C

M.P of pure product: 84^{0}C

TLC Studies



solvent system

Benzene:Methanol = 9:1

1: cyclopentanone

2: co-spot of 1 & 2

3: compound 1

Rf values

Cyclopentanone = 0.30

Compound 1= 0.81

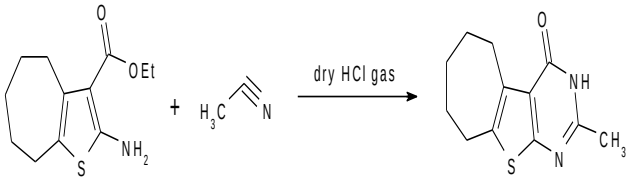
Molecular Weight: 239.34

Molecular Formula: $C_{12}H_{17}NO_2S$

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

Reaction:

(1)



(1)

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^{0}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[17]

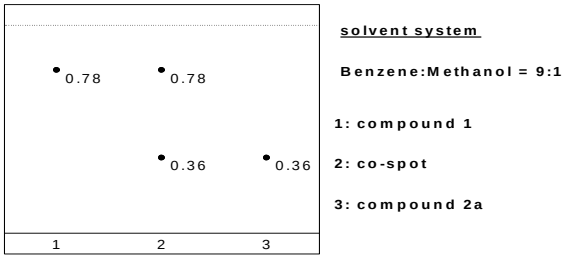
Recrystallisation solvent: Ethyl Acetate.

Yield: 87.2 %

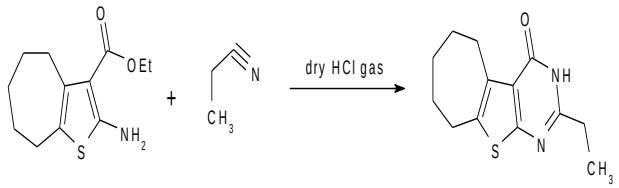
M.P of crude product: 229^{0}C

M.P of pure product: 227^{0}C

TLC Studies



Rf values:
Compound **2a** = 0.36
Molecular Weight: 234.3
Molecular Formula: $C_{12}H_{14}N_2OS$
Synthesis of 2-ethyl-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-one
Reaction

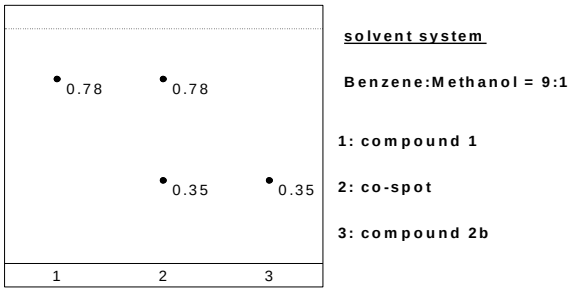


(1)

Procedure

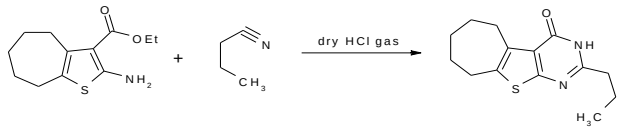
Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**1**, 9.4 mmol, 2.25 g), propionitrile (18.0 mmol, 1.25 mL) and 10 mL of dioxane were taken in a conical flask and hydrochloric acid gas was passed through it for 6 hrs. This reaction mixture was kept aside for 12 hrs at room temperature. It was then poured into a beaker containing crushed ice and neutralized using ammonium hydroxide (10%). The resultant precipitate was filtered and dried[17]
Recrystallisation solvent: Ethyl Acetate
Yield: 80.4 %
M.P of crude product: 217^0C
M.P of pure product: 216^0C

TLC Studies



Rf values
Compound **2b** = 0.35
Molecular Weight:248.34
Molecular Formula: $C_{13}H_{16}N_2OS$

Synthesis of 2-propyl-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-one
Reaction:

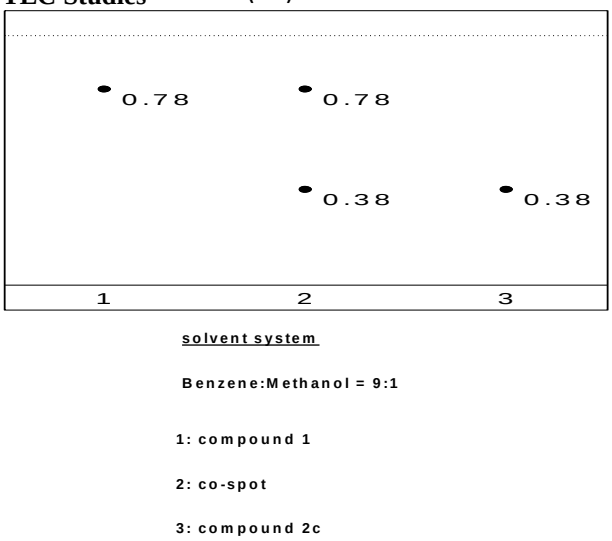


(1)

Procedure

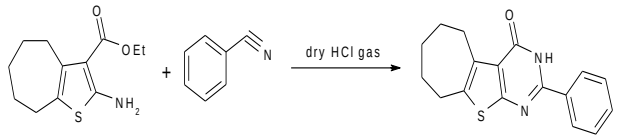
Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**1**, 9.4 mmol, 2.25 g), butyronitrile (18.0 mmol, 1.56 mL) and 10 mL of dioxane were taken in a conical flask and hydrochloric acid gas was passed through it for 6 hrs. This reaction mixture was kept aside for 12 hrs at room temperature. It was then poured into a beaker containing crushed ice and neutralized using ammonium hydroxide (10%). The resultant precipitate was filtered and dried[17].
Recrystallisation solvent: Ethyl Acetate
Yield: 73.5%
M.P of crude product: 211^0C
M.P of pure product: 210^0C

TLC Studies (2b)



Rf values:
Compound **2c** = 0.38
Molecular Weight:262.37
Molecular Formula: $C_{14}H_{18}N_2OS$

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



(1)

Procedure

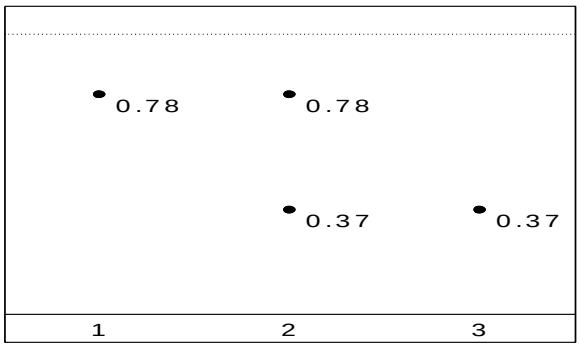
Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**1**, 9.4 mmol, 2.25 g), butyronitrile (18.0 mmol, 1.85 mL) and 10 mL of dioxane were taken in a conical flask and hydrochloric acid gas was passed through it for 6 hrs. This reaction

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[17].

Recrystallisation solvent: Ethyl Acetate
Yield: 68.9 %

M.P of crude product: 227^0C
M.P of pure product: 225^0C

TLC Studies



solvent system

Benzene:Methanol = 9:1

1: compound 1

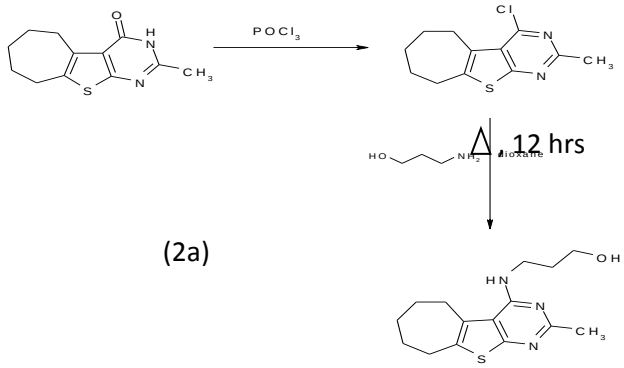
2: co-spot

3: compound 2d

Rf values:
Compound **2d** = 0.37
Molecular Weight:296.38
Molecular Formula: $C_{17}H_{16}N_2OS$

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
Conventional method

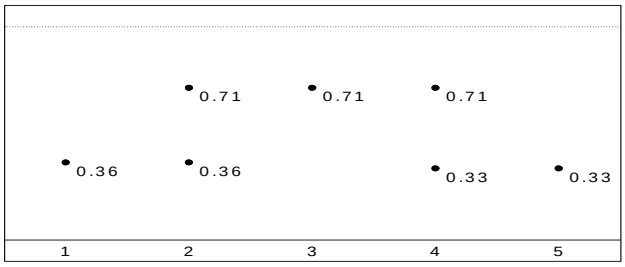
Reaction:



Procedure

2-methyl-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-one (2a, 8.0 mmol, 1.88 g) and 20 mL of $POCl_3$ were taken in a round bottom flask and refluxed for 12 hrs at 100ºC 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, (3a) was filtered off and air dried. The obtained dried product (3a, 4.0 mmol, 1.01 g) was dissolved in 10 mL of dioxane and added Triehtylamine (4.0 mmol, 0.56 mL), aminopropanol (6.0 mmol, 0.45 mL). This reaction mixture was refluxed for 8 hrs at 100ºC 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[17].
Recrystallisation solvent: $CHCl_3$
Yield: 54.1 %
M.P of crude product: 292^0C
M.P of pure product: 290^0C

TLC Studies

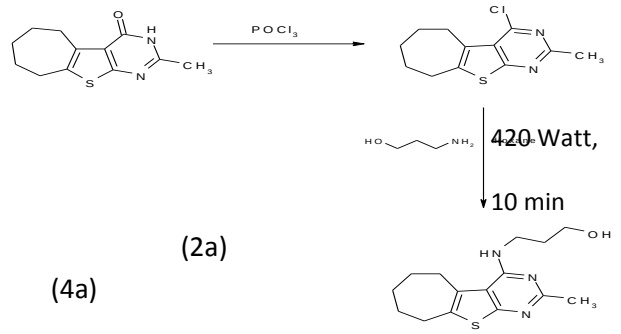


Solvent system:
benzene:methanol=9:1

1: compound 2a
2: co-spot of 2a and 3a
3: compound 3a
4: co-spot of 3a and 4a
5: compound 4a

Rf values:
Compound **4a** = 0.33
Molecular Weight:291.42
Molecular Formula: $C_{15}H_{21}N_3OS$.
^{1}H NMR (400 MHz, $CDCl_3$) δ=1.79-1.89 (m, 10H, C<u>H</u>$_2$ at 5,6,7,8,9), 2.54 (s, 3H, C<u>H</u>$_3$), 2.84-2.87 (m, 2H, *J*=5.4 Hz, –NHCH$_2$C<u>H</u>$_2$CH$_2$OH), 3.62-3.65 (t, 2H, *J*=5.4 Hz, –NHC<u>H</u>$_2$CH$_2$ CH$_2$OH), 3.75-3.79 (t, 2H, *J*=5.4 Hz, –NHCH$_2$CH$_2$C<u>H</u>$_2$OH).
MS (m/z): 292.3(M^++1), 293.3(M^++2), 294.2(M^++3).

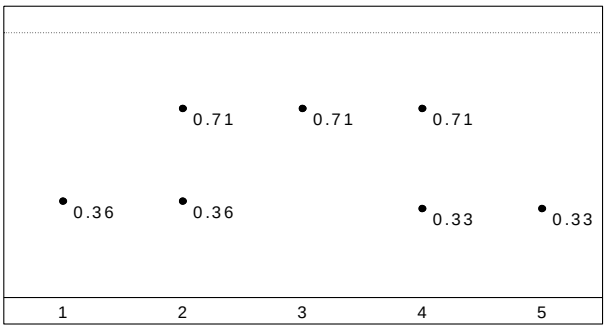
Microwave assisted method



Procedure

2-methyl-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-one (2a, 8.0 mmol, 1.98 g) and $POCl_3$ (8.0 mmol, 0.90 mL) were taken in a small reaction flask and irradiated in CATA-R scientific microwave systems at 420 Watt for 10 min.The resultant reaction mixture was poured into crushed ice and neutralizedwithdil.$NaHCO_3$ solution. The resulting precipitate was filtered off and air dried[18]. The obtained dried product (3a, 5.0 mmol, 1.26 g) and aminopropanol (5.0 mmol, 0.38 mL) 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 chloroform[18].
Recrystallisation solvent: $CHCl_3$
Yield: 91.2 %
M.P of crude product: 292^{0}C
M.P of pure product: 290^{0}C

TLC Studies



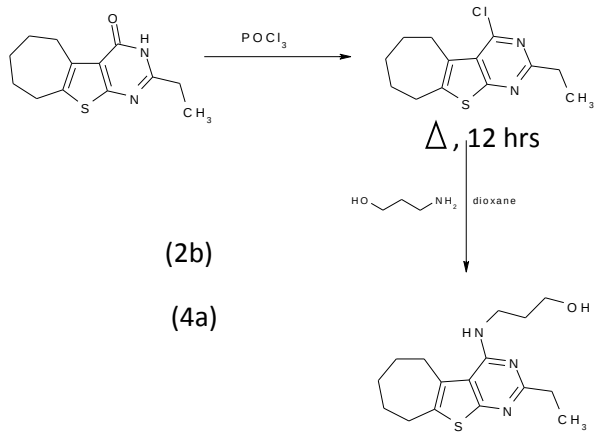
Solvent system:
benzene:methanol=9:1

1: compound 2a
2: co-spot of 2a and 3a
3: compound 3a
4: co-spot of 3a and 4a
5: compound 4a

(3a)

Rf values
Compound **4a** = 0.33
Molecular Weight:291.42
Molecular Formula: $C_{15}H_{21}N_3OS$.

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



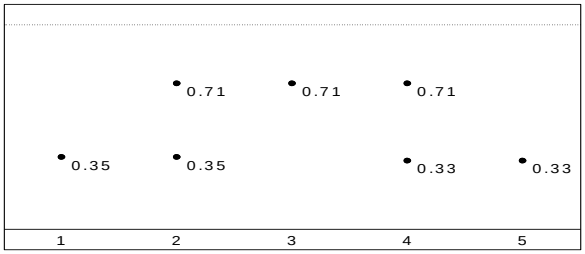
Procedure

2-ethyl-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-one (2b, 8.0 mmol, 1.98 g) and 20 mL of $POCl_3$ were taken in a round bottom flask and refluxed for 12 hrs at 100ºC 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, (3b) was filtered off and air dried[45]. The obtained dried product (3b, 4.0 mmol, 1.07 g) was dissolved in 10 mL of dioxane and added Triehtylamine (4.0 mmol, 0.56 mL), aminopropanol (6.0 mmol,

0.45mL). This reaction mixture was refluxed for 8 hrs at 100ºC 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[19]

Recrystallisation solvent: $CHCl_3$
Yield: 70.6 %

M.P of crude product: 271^0C
M.P of pure product: 269^0C
TLC Studies

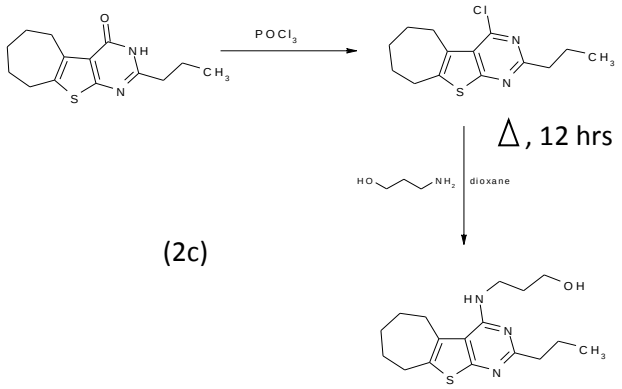


Solvent system:
benzene:methanol=9:1

1: compound 2b
2: co-spot of 2b and 3b
3: compound 3b
4: co-spot of 3b and 4b
5: compound 4b

Rf values:
Compound **4b** = 0.33
Molecular Weight: 305.45
Molecular Formula: $C_{16}H_{23}N_3OS$.
^{1}H NMR (400 MHz, CDCl3) δ=1.31-1.35 (t, 3H, *J=7.6 Hz*, -$CH_2C\underline{H}_3$), 1.74-1.92 (m, 10H, $C\underline{H}_2$ at 5,6,7,8,9), 2.77 (*quartet*, 2H, *J=7.6 Hz*, -$C\underline{H}_2CH_3$), 2.98-3.00 (t, 2H, , *J*=4.8 Hz, –NH CH_2 $C\underline{H}_2CH_2OH$), 3.63-3.66 (t, 2H, *J*=4.8 Hz, –$NHC\underline{H}_2CH_2$ CH_2OH), 3.76-3.81 (m, 2H, –$NHCH_2CH_2\underline{C}H_2OH$).
^{13}C NMR (100 MHz, CDCl3) δ= 12.80 (CH_3), 26.32 (–$NHCH_2\underline{C}H_2CH_2OH$), 29.79 (C-7), 30.16 (C-6), 30.40 (C-8), 32.12 (C-5), 32.46 (C-9), 32.73 (-$\underline{C}H_2CH_3$), 37.87 (–$NH\underline{C}H_2CH_2$ CH_2OH), 59.07 (–$NHCH_2CH_2\underline{C}H_2OH$), 114.85 (C-2′), 135.82 (C-3′), 136.85 (C-4′), 157.85 (C-1′), 164.85 (C-4), 165.68 (C-2).
MS (m/z): 306.4(M^++1), 307.4(M^++2), 308.4(M^++3).

Synthesis of 3-[(2-propyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-yl)amino]propan-1-ol
Reaction:

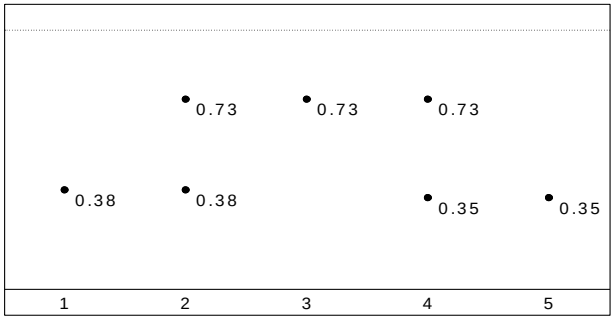


Procedure

2-propyl-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-one (2c, 8.0 mmol, 2.10 g) and 20 mL of $POCl_3$ were taken in a round bottom flask and refluxed for 12 hrs at 100ºC 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, (3c) was filtered off and air dried. The obtained dried product (**3c**, 4.0 mmol, 1.12 g) was dissolved in 10 mL of dioxane and added Triehtylamine (4.0 mmol, 0.56 mL), aminopropanol (6.0 mmol, 0.45 mL). This reaction mixture was refluxed for 8 hrs at 100ºC 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[19].

Recrystallisation solvent: $CHCl_3$
Yield: 52.3 %
M.P of crude product: 259^0C
M.P of pure product: 257^0C

TLC Studies

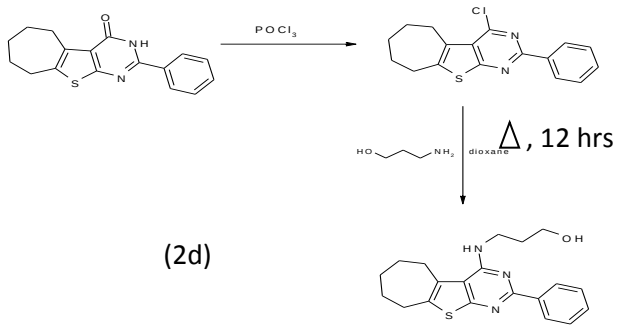


Solvent system:
benzene:methanol=9:1

1: compound 2c
2: co-spot of 2c and 3c
3: compound 3c
4: co-spot of 3c and 4c
5: compound 4c

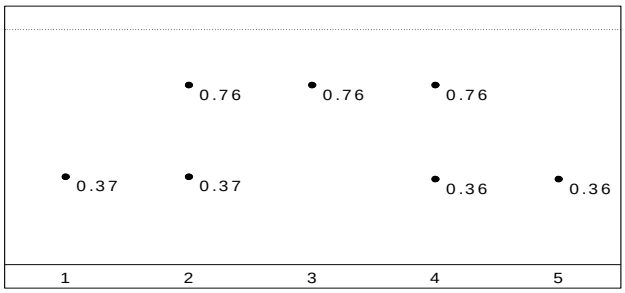
Rf values:
Compound **4c** = 0.35 (3c)
Molecular Weight:319.47
Molecular Formula: $C_{17}H_{25}N_3OS$.
^{1}H NMR (400 MHz, CDCl3) δ=0.96-1.05 (m, 2H, -$CH_2C\underline{H}_2CH_3$), 1.46-1.50 (t, *J=7.4 Hz*, 3H, -$CH_2CH_2C\underline{H}_3$), 1.70-1.86 (m, 10H, $C\underline{H}_2$ at 5,6,7,8,9), 2.69-2.76 (m, 2H, -$NHCH_2C\underline{H}_2CH_2OH$), 3.63-3.65 (t, 2H, *J=4.8 Hz*, -$NHC\underline{H}_2CH_2CH_2OH$), 3.77-3.78 (t, 2H, *J=4.8 Hz*, -$NHCH_2CH_2C\underline{H}_2OH$), 4.03-4.07 (t, 2H, *J=7.4 Hz*, -$C\underline{H}_2CH_2CH_3$).
MS (m/z): 320.4(M^++1), 321.4(M^++2), 322.4(M^++3).

Synthesis of 3-[(2-phenyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-yl)amino]propan-1-ol
Reaction:



Procedure
2-phenyl-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[*b*]thieno[2,3-*d*]pyrimidin-4-one (2d, 8.0 mmol, 2.36 g) and 20 mL of $POCl_3$ were taken in a round bottom flask and refluxed for 12 hrs at 100ºC 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, (3d) was filtered off and air dried[20]. The obtained dried product (3d, 4.0 mmol, 1.26 g) was dissolved in 10 mL of dioxane and added Triehtylamine (4.0 mmol, 0.56 mL), aminopropanol (6.0 mmol, 0.45 mL). This reaction mixture was refluxed for 8 hrs at 100ºC 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[19]
Recrystallisation solvent: $CHCl_3$
Yield: 61.5%
M.P of crude product: 268^{0}C
M.P of pure product: 266^{0}C

TLC Studies



Solvent system:
benzene:methanol=9:1

1: compound 2d
2: co-spot of 2d and 3d
3: compound 3d
4: co-spot of 3d and 4d
5: compound 4d

Rf values:
Compound **4d** = 0.36
Molecular Weight:353.49
Molecular Formula: $C_{20}H_{23}N_3OS$.
^{1}H NMR (400 MHz, CDCl3) δ=1.79-1.93 (m, 10H, $C\underline{H}_2$ at 5,6,7,8,9), 2.86-2.90 (m, 2H, -$NHCH_2C\underline{H}_2CH_2OH$), 3.71-3.74 (t, 2H, -$NHC\underline{H}_2CH_2CH_2OH$), 3.90-3.95 (m, 2H, -$NHCH_2CH_2C\underline{H}_2OH$), 7.38-7.48 (m, 3H, Ar$\underline{H}$ at 2',3' and 4'), 8.35-8.37 (dd, 2H, Ar$\underline{H}$ at 1' and 6').
MS (m/z): 354.4(M^++1), 355.4(M^++2), 356.4(M^++3).

Δ, 8 hrs

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

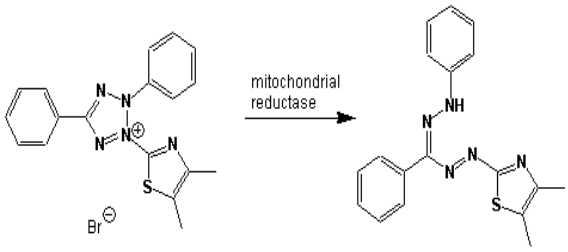
Procedure
1. The monolayer cell culture was trypsinized and the cell count was adjusted to 3.0 x 10^5 cells /mL using medium containing 10% new born calf serum.
2. To each well microtitre plate, 0.1 mL of the diluted suspension (approx. 10,000 cells) was added and kept for 24 hrs in incubator at 37^{0}C in 5% CO_2 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.

The same procedure[44] was followed for the two cell lines. The *in vitro* testing for anticancer activity was done by Indian institute of chemical technology (IICT), Hyderabad.

DISCUSSION ACTIVITY

The cytotoxic activity of target compounds was tested by using MicrocultureTetrazolium (MTT) assay. In the mitochondria of living cells yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is reduced to purple formazan. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. This reduction takes place only when mitochondrial reductase enzymes are active and therefore conversion can be directly related to the number of viable cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced through the production of a dose-response curve. The absorption max is dependent on the solvent employed to solubilize the crystals of formazan, which are insoluble in aqueous medium [18-19].



[MTT] (yellow) [Formazan] (purple)

This was done using two cell lines namely HC 29-Colorectal adenoma cell line, MDA 231-Adenocarcinoma breast cancer cell line. Here the absorption max. used was 490 nm and the solvent used for solubilization of formazan crystals was propanol[17].

SCHEME

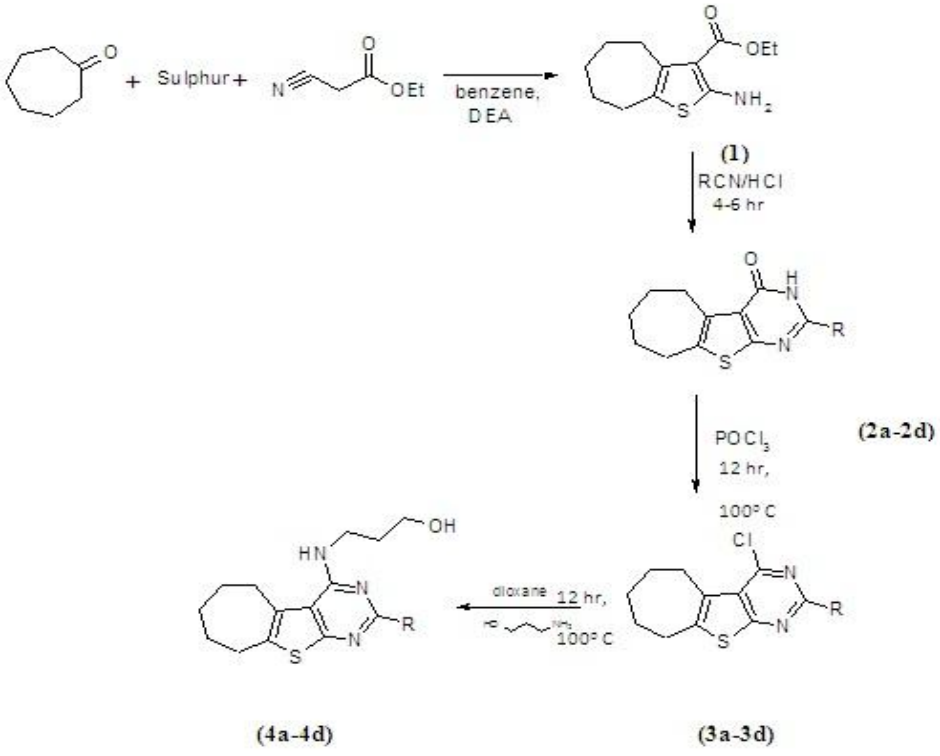


Table 1. Percentage inhibition of cell growth

Compound code	Concentration (µmol)	Percentage Inhibition of cell growth	
		HC 29-Colorectal adenoma cell line	MDA 231-Adenocarcinoma breast cancer cell line
4a	0.03	32.92 %	31.18 %
	0.07	31.43 %	29.35 %
	0.17	27.96 %	24.47 %
4b	0.03	30.73 %	24.94 %
	0.07	27.94 %	25.79 %
	0.17	25.27 %	26.41 %
4c	0.03	26.81%	22.45%
	0.07	25.54%	23.65%
	0.17	24.65%	23.18%
4d	0.03	25.45%	23.56%
	0.07	24.56%	21.45%
	0.17	22.34%	22.45%

The afore mentioned results showed that the compounds 4a, 4b ,4c and 4d have considerable anticancer activity and comparatively 4a has shown little better activity.

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-5*H*-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 (^{1}H 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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CONFLICT OF INTEREST

No interest

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