Synthesis of (2 and 3-bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid and derivatives and study their biological activity

In this chapter, we have reported synthesis of 2-(2-bromo-4-oxothieno[3,2-c]pyridin-5(4H)-yl)acetic acid, 2-(3-bromo-4-oxothieno[3,2-c]pyridin-5(4H)-yl)acetic acid and their derivatives. These new compounds have been evaluated for antimicrobial activity (i.e. antibacterial and antifungal activities) against various Gram positive, Gram negative bacteria and fungi.

2.1 Introduction

Thio organic compounds and their derivatives are important class of compounds. Thiophenes and their fused derivatives have shown diverse pharmacological activities including antibacterial, antifungal, immunomodulatory, antiviral, anticancer, antifungal and antitubercular activity. The literature survey revealed that thiophenes in different forms like thienopyrimidine [1-11], benzothiophene [12-29], bithienyl [30-32], thienopyridine [33-50], possesses a wide spectrum of biological activities.

2.1.1 Importance of thiophene fused heterocycles in medicinal chemistry:

Thiophene enhanced biological activity when fused with various heterocyclic systems giving rise to various new compounds. Thienopyrimidines occupy a special position among these compounds. Pyrimidine fused systems containing an aneled five-membered heteroaromatic ring; thienopyrimidines are structural analogs of biogenic purines and can be considered as potential nucleic acid antimetabolites. Certain thienopyrimidine derivatives were exhibit antiallergic [51], antibacterial [52],
antidepressant [53], antidiabetic, analgesic and anti-inflammatory [54] activities. Substituted thieno[2,3-\(d\)]pyrimidines are considered to be an universal molecules in a structure based drug design [55]. Thieno[2,3-\(d\)]pyrimidine derivatives showed pronounced anti-inflammatory [56], anti-tumor [57], radioprotective and anti-convulsing activity [58], depressant or sedative properties [59] and compounds used for therapy of malaria [60], tuberculosis [61], Parkinson’s disease [62] and other diseases were designed [63].

4-Oxo-4,7-dihydrothieno[2,3-\(b\)]pyridine-5-carbonitriles I are important intermediates in the synthesis of thieno[2,3-\(b\)]pyridine-5-carbonitrile used as kinase inhibitors [64, 65].

\[
\text{I} \\
\begin{array}{c}
\text{R}^1, \text{R}^2: \text{Aryl, Alkyl} \\
\end{array}
\]

A series of thieno[2,3-\(d\)][1,3]oxazin-4-ones II was synthesized and evaluated in vitro for inhibitory activity toward Human Leukocyte Elastase (HLE) [66,67]

\[
\text{II} \\
\begin{array}{c}
a: R = \text{Me}, R^1 = R^2 = (\text{CH}_2)_4, \\ b: R = \text{Me} R^1 = R^2 = \text{Me}, c: R = \text{Et}, R^1 = R^2 = (\text{CH}_2)_4, \\ d: R = \text{Et}, R^1 = R^2 = \text{Me}, e: R = \text{CH}_2\text{Ph}, R^1 = R^2 = (\text{CH}_2)_4, f: R = \text{CH}_2\text{Ph}, R^1 = R^2 = \text{Me}, g: R = \text{CH}_2\text{CO}_2\text{Me}, R^1 = R^2 = (\text{CH}_2)_4, h: R = \text{CH}_2^2\text{CO}_2\text{Me}, R^1 = R^2 = \text{Me} \\
\end{array}
\]
Adenosine is an important endogenous tissue-protective compound released during ischemia, hypoxia or inflammation. Four receptor subtypes (A1, A2A, A2B, A3) have been defined based on pharmacological properties [67, 68]. Considerable effort has been directed towards developing therapeutic agents targeting these receptors. Cycloalkyl substituents at C-4 and C-5 position \textbf{III} and aroyl substituent in C-3 position \textbf{IV}, maintained the best allosteric enhancer activity [69, 70].

\begin{center}
\begin{align*}
\text{III} & \quad \text{IV} \\
\begin{array}{c}
\begin{tikzpicture}
\draw[thick] (0,0) -- (1,0) -- (1,1) -- (0,1) -- cycle;
\draw[thick] (0,0) -- (1,1);
\draw[thick] (0,1) -- (1,0);
\draw[thick] (0.5,0) -- (0.5,1);
\draw[thick] (0,0.5) -- (1,0.5);
\draw[thick] (0,0) .. controls (0.5,0.5) and (0.5,0.5) .. (1,0);
\draw[thick] (0,0) .. controls (0.5,0.5) and (0.5,0.5) .. (0,1);
\end{tikzpicture}
\end{array}
\end{align*}
\end{center}

The synthesis and antitumor activity of thieno[2,3-\textit{b}]azepin-4-one \textbf{V} and \textbf{VI} based antineoplastic agents was reported [71].

\begin{center}
\begin{align*}
\text{V} & \quad \text{VI} \\
\begin{array}{c}
\begin{tikzpicture}
\draw[thick] (0,0) -- (1,0) -- (1,1) -- (0,1) -- cycle;
\draw[thick] (0,0) -- (1,1);
\draw[thick] (0,1) -- (1,0);
\draw[thick] (0.5,0) -- (0.5,1);
\draw[thick] (0,0.5) -- (1,0.5);
\draw[thick] (0,0) .. controls (0.5,0.5) and (0.5,0.5) .. (1,0);
\draw[thick] (0,0) .. controls (0.5,0.5) and (0.5,0.5) .. (0,1);
\end{tikzpicture}
\end{array}
\end{align*}
\end{center}

\begin{align*}
R^1 = R^2 = \text{Me}, & \quad R^1 = \text{Ph}, \quad R^2 = \text{Me}, \quad R = \text{tosyl or benzoyl}
\end{align*}

Thieno[2,3-\textit{d}]pyrimidine-4-hydrazide derivatives and related structures were discovered as a moderately potent inhibitors of TGase-2 (tissue transglutaminase) [72]. Number of novel fused thiophene derivative \textbf{VII} have been prepared and identified as potent inhibitors of MEK [73]
A thienodiazepine \textbf{VIII} is a heterocyclic compound containing diazepine ring fused to a thiophene ring. Thienodiazepine forms the central core of several pharmaceutical drugs including Brotizolam \textbf{IX}, Clotiazepam \textbf{X}, Etizolam \textbf{XI}, Bentazepam \textbf{XII}. Since thienodiazepines interact with the benzodiazepine receptor site and similar effects as benzodiazepines [74-75].
A thienobenzodiazepine XIII is a heterocyclic compound containing a diazepine ring fused to a thiophene ring and a benzene ring. Thienobenzodiazepine forms the central core of some pharmaceutical drugs including olanzapine. Thienobenzodiazepines seem to act relatively selectively at the $\alpha$-2 subunit of the GABA-A receptor.

Prasugrel XIV, Ticlopidine XV, Clopidogrel XVI [76-78] thienopyridines are a class of selective irreversible ADP receptor/ P2Y12 inhibitors used for their ant-platelet activity.

With the discovery of antibiotics, people were convinced that infectious diseases might someday be wiped out. However, the emergence pathogenic bacteria that resist the effects of most powerful antibiotics available today are posing a great challenge to medicines. Hence there are many studies focused on antibacterial and antifungal compounds [79-81]. It is well known that thienopyridine derivatives are of great biological interest, especially as antiviral, antitumor and antimicrobial agents. In this chapter, we have describe the synthesis of 2-(2-bromo-4-oxothieno[3,2-c]pyridin-5(4H)-yl)acetic acid, 2-(3-bromo-4-oxothieno[3,2-c]pyridin-5(4H)-yl)acetic acid as a very crucial synthetic precursors; and further explore their chemistry to prepare several derivatives. These derivatives have
been evaluated for antimicrobial activity (i.e. antibacterial and antifungal activities) against various Gram positive, Gram negative bacteria and fungi.

2.2 Literature review

In literature 3-Bromo-5H-thieno[3,2-c]pyridin-4-one (18) were synthesized by following routes:

Compound 17 was converted into 3-Bromo-5H-thieno[3,2-c]pyridin-4-one 18 by heating in diphenyl ether in presence of catalytic amount of I₂ at 150°C for 2 hours (Scheme 1). First acyl azide converts into corresponding isocynate and after isomerisation cyclized to thieno[3,2-c]pyridin-4-one.

![Scheme 1](image)

The 2-bromo-5H-thieno[3,2-c]pyridin-4-one 12 is synthesized in similar way, detail synthesis of 12 was given in chapter 1.

2.3 Present work

We have further explored chemistry on 2-bromo-5H-thieno[3,2-c]pyridin-4-one 12 and 3-bromo-5H-thieno[3,2-c]pyridin-4-one 18. The 2-bromo-5H-thieno[3,2-c]pyridin-4-one 12 was N-alkylated using ethylbromoacetate, K₂CO₃ as base and acetone as solvent at reflux temperature for 6 hrs. The compound 19 was obtained in 92% yield. The ester 19 was further hydrolyze to corresponding acid 20 using NaOH in THF : Water (1:1) as solvent at room temperature for 16 hours in 98% yield (Scheme 2)
The (2-bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid 20 was arylated using Suzuki-Miyaura coupling reaction. Compounds 21(a-e) were synthesized by using corresponding arylboronic acids, Pd(PPh3)4 (tetrakistriphenylphosphine palladium) as catalyst, K2CO3 as base and using water as solvent under reflux condition for 4-6 hour. Compounds 21(a-e) obtained in moderate to good yield from 75-85% (Scheme 3).

Similarly to 2-bromo-5H-thieno[3,2-c]pyridin-4-one 12, 3-bromo-5H-thieno[3,2-c]pyridin-4-one 18 was N-alkylated using ethylbromoacetate, K2CO3 as base and acetone as solvent at reflux temperature for 6hrs. The compound 22 was obtained in 90% yield. The ester compound 22 was further hydrolyze to corresponding acid 23 using NaOH in THF: water (1:1) as solvent at room temperature for 16 hours in 97% yield (Scheme 4).
The (3-bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid 23 was arylated using Suzuki-Miyaura coupling reaction. Compounds 24(a-e) were synthesized by using corresponding arylboronic acids, Pd(PPh₃)₄ (tetrakistriphenylphosphine palladium) as catalyst, K₂CO₃ as base and using water as solvent under reflux condition for 4-6 hour. Compounds 24(a-e) obtained in 70 -78% yield (Scheme 5).

All synthesized compounds are characterized by 1H-NMR, 13C-NMR and LCMS analysis.
Fig 1: IR Spectra of \((2\text{-Bromo}-4\text{-oxo}-4\text{-H-thieno}[3,2-c]\text{pyridin}-5\text{-yl})\text{-acetic acid ethyl ester}\) (19)

Molecular Weight: 316.18
Molecular Formula: \(\text{C}_{11}\text{H}_{10}\text{BrNO}_{3}\text{S}\)

Fig 1: \(^1\text{H NMR (DMSO-}d_6\text{)}\) (2-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester (19)
Fig 2: $^{13}$C NMR (DMSO-$d_6$) (2-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester (19)

Fig 3: APT (DMSO-$d_6$) (2-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester (19)

The $^1$H-NMR spectrum of 19 showed doublet at $\delta$ 7.58 ppm with $J = 7.2$ Hz region for C$_6$-H proton and singlet at $\delta$ 7.57 ppm for thiophene proton. The doublet at $\delta$ 6.92 region
with $J = 7.2$ Hz is due to C$_7$-H proton on C$_7$ carbon. The singlet in the 4.77 ppm region corresponds to CH$_2$ group between nitrogen and ester. The quartet at 4.15 ppm region with $J = 7.2$ Hz and triplet at 1.2 ppm region with $J = 7.2$ Hz corresponds to CH$_2$ and CH$_3$ protons of ethyl ester respectively. The $^{13}$C NMR spectrum of this solid showed the ester carbonyl carbon at 168.64 ppm and pyridone carbonyl carbon at 157.09 ppm. All remaining six aromatic carbons appeared between 101.32-149.63 ppm. The CH$_2$ carbon appeared at 61.51 region and CH$_2$ and CH$_3$ carbon of ethyl ester appeared at 50.28, 14.51 ppm region respectively. Attach proton test (APT) analysis showed that carbon at 149.63, 130.27 112.76 ppm are quaternary carbon and are deshielded carbon at 149.63 due to carbon attached to bromine. The carbon at 101.32, 127.34, 135.1 ppm are due to aromatic CH carbon and shiled carbon at 101.32 is C$_7$ carbon. The mass spectrum showed isotopic peaks with equal intensity at 314.32, 316.33 (M-H)$^-$ due to Br. On the basis of above spectral and analytical data structure 19 was assigned to this compound i.e. (2-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester 19.
The 1H-NMR spectrum of 20 showed broad singlet in the δ 13.0 ppm region is due to carboxylic acid proton. The doublet at the δ 7.57 ppm with $J = 7.2$ Hz region due to the
C₆-H proton and singlet at δ 7.56 ppm for thiophene proton. The doublet in the δ 6.89 region with $J = 7.2$ Hz due to C₇-H proton. The singlet at the δ 4.69 ppm region is due to the CH₂ group between nitrogen and acid (N-CH₂-COOH). The $^{13}$C NMR spectrum of this solid showed the carboxylic acid carbonyl carbon at δ170.05 ppm and pyridone carbonyl carbon at δ157.14 ppm. All remaining six aromatic carbons appeared between δ 101.13-149.54 ppm. The CH₂ carbon appeared at δ 50.21 ppm region. Attach proton test (APT) analysis showed that carbon at δ 149.54, 130.32, 112.58 ppm are quaternary carbons and deshielded carbon at δ 149.54 due to carbon attached to bromine. The carbon at δ 101.13, 127.37, 135.33 ppm are aromatic CH carbon and shielded carbon at δ 101.13 is C7 carbon. The mass spectrum showed isotopic peaks with equal intensity at 286, 288.14 (M-H)⁻ due to Br. On the basis of above spectral and analytical data structure 20 was assigned to this compound i.e (2-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid 20.

IR Spectra of (3-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester (22)
Fig 7: $^1$H NMR (DMSO-$d_6$) (3-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester (22)

Molecular Weight : 316.18  
Molecular Formula : C$_{11}$H$_{10}$BrNO$_3$S

Fig 8: $^{13}$C NMR (DMSO-$d_6$) (3-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester (22)
The $^1$H-NMR spectrum of 22 showed singlet at $\delta$ 7.74 due to thiophene proton and doublet in the $\delta$ 7.61 ppm with $J$ = 7.2 Hz region due to the C$_6$-H proton. The doublet at $\delta$ 6.98 region with $J$ = 7.2 Hz due to proton on C$_7$ carbon. The singlet in the $\delta$ 4.75 ppm is due to the CH$_2$ group between nitrogen and ester. The quartet at $\delta$ 4.13 ppm with $J$ = 7.2 Hz and triplet at $\delta$ 1.22 ppm region with $J$ = 7.2 Hz is due O-CH$_2$-CH$_3$ group respectively. The $^{13}$C NMR spectrum of this solid showed the ester carbonyl carbon at $\delta$ 168.74 ppm and pyridone carbonyl carbon at $\delta$ 157.47 ppm. All remaining six aromatic carbons appeared between $\delta$ 101.71-149.19 ppm. The CH$_2$ carbon appeared at $\delta$ 61.50 region and CH$_2$ and CH$_3$ carbon of ethyl ester appeared at $\delta$ 50.21, 14.53 ppm region respectively. Attach proton test (APT) analysis showed that carbon at $\delta$ 149.19, 124.72, 107.6 ppm are quaternary carbon and deshield carbon at $\delta$ 149.19 due to carbon attached to bromine. The carbon at $\delta$ 101.71, 124.41, 135.71 ppm are aromatic CH carbon and
shileded carban at δ 101.71 is C₇ carbon. The mass spectrum showed isotopic peaks at 314.22, 316.08 (M-H⁻). On the basis of above spectral and analytical data structure 22 was assigned to this compound i.e. (3-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester 22.

Fig 10: ¹H NMR (DMSO-d₆) (3-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (23)
The $^1$H-NMR spectrum of 23 showed broad singlet at the $\delta$ 13.05 ppm is due to carboxylic acid proton. The singlet at $\delta$ 7.73 ppm for thiophene proton and doublet in the 7.60 and 6.95 ppm with $J = 7.2$ Hz region due to the C$_6$-H and C$_7$-H proton respectively. The singlet at the $\delta$ 4.67 ppm is due to the CH$_2$ group between nitrogen and acid. The $^{13}$C NMR spectrum of this solid showed the carboxylic acid carbonyl carbon at $\delta$ 170.15 ppm and pyridone carbonyl carbon at $\delta$ 157.50 ppm. All remaining six aromatic carbons appeared between $\delta$ 101.50-149.08 ppm. The CH$_2$ carbon appeared at $\delta$ 50.15 ppm region. Attach proton test (APT) analysis showed that carbon at $\delta$ 149.08, 124.73, 107.63 ppm are quaternary carbons and deshiled carbon at $\delta$ 149.08 due to carbon attached to bromine. The carbon at $\delta$ 101.50, 124.23, 135.85 ppm are aromatic CH carbon and shiled carbon at $\delta$ 101.5 is C$_7$ carbon. The mass spectrum showed isotopic peaks at 286.34, 288.14 (M-H)$^-$. On the basis of above spectral and analytical data structure 23
was assigned to this compound i.e (3-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid.

![Fig 13: IR spectra of (4-Oxo-2-phenyl-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (21c)](image)

Molecular Weight : 285.32  
Molecular Formula : $C_{15}H_{11}NO_3S$

![Fig 13: $^1$H NMR (DMSO-$d_6$) (4-Oxo-2-phenyl-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid](image)
Fig 14: $^{13}$C NMR (DMSO-$d_6$) (4-Oxo-2-phenyl-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (21c)

The $^1$H- NMR spectrum of 21c showed broad singlet in at $\delta$ 12.90 ppm region is due to carboxylic acid proton. The singlet at $\delta$ 7.87 ppm is for thiophene proton. The doublet in the $\delta$ 7.80 ppm region with $J = 7.5$ Hz and multiplate in the $\delta$ 7.30-7.50 ppm due to the aromatic phenyl protons. The doublet in the 7.54 and 6.89 ppm with $J = 7.2$ Hz region due to the C$_6$-H and C$_7$-H proton respectively The singlet in the $\delta$ 4.67 ppm region due to the CH$_2$ group between nitrogen and acid (N-CH$_2$-COOH). The $^{13}$C NMR spectrum of this solid showed the carboxylic acid carbonyl carbon at $\delta$170.50 ppm and pyridone carbonyl carbon at $\delta$ 158.30 ppm. All aromatic carbons appeared between $\delta$ 101.28-147.23 ppm. The mass spectrum showed peaks at 284.32 (M-H)$^-$. On the basis of above spectral and analytical data structure 21c was assigned to this compound i.e. (4-Oxo-2-phenyl-4H-thieno [3, 2-c] pyridin-5-yl)-acetic acid .
Fig 15: $^1$H NMR (DMSO-$d_6$) (4-Oxo-3-phenyl-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (24c)

Fig 16: $^{13}$C NMR (DMSO-$d_6$) (4-Oxo-3-phenyl-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (24c)
The $^1$H-NMR spectrum of 24a showed broad singlet at $\delta$ 12.90 ppm is due to carboxylic acid proton. The singlet at $\delta$ 7.89 ppm is for thiophene proton. The doublet at $\delta$ 7.78 ppm with $J = 7.5$ Hz and multiplate at $\delta$ 7.35-7.54 ppm due to the aromatic phenyl protons. The doublet in the 7.58 and 6.91 ppm with $J = 7.6$ Hz region due to the C$_6$-H and C$_7$-H proton respectively. The singlet in the $\delta$ 4.65 ppm region is due to the CH$_2$ group between nitrogen and acid. The $^{13}$C NMR spectrum of this solid showed the carboxylic acid carbonyl carbon at $\delta$ 173.1 ppm and pyridone carbonyl carbon at $\delta$ 156.40 ppm. All aromatic carbons appeared between $\delta$ 110.1-147.0 ppm. The mass spectrum showed peaks at 284.30 (M-H)$^-$. On the basis of above spectral and analytical data structure 24c was assigned to this compound i.e. (4-Oxo-3-phenyl-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid.

2.3.1 Antibacterial Activity

All compounds 21(a-e) and 24(a-e) were evaluated for in vitro antibacterial and antifungal activities against following stains

- *Escherichia coli* (MTCC 443)
- *Pseudomonas aeruginosa* (MTCC 1688)
- *Staphylococcus aureus* (MTCC 96)
- *Streptococcus pyogenes* (MTCC 442)

2.3.2 Antibacterial screening

The MICs of synthesized compounds were determined out by broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS) [58] (NCCLS, 2002). Antibacterial activity was screened against two Gram positive (*Staphylococcus aureus* MTCC 96 *Streptococcus pyogenes* MTCC 442) and two Gram
negative (Escherichia coli MTCC 443, Pseudomonas aeruginosa MTCC 1688) bacteria. Antifungal activity was screened against three fungal species (Candida albicans MTCC 227, Aspergillus niger MTCC 282 and Aspergillus clavatus MTCC 1323) bacteria. Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. Inoculum’s size for test strain was adjusted to 108 CFU (Colony Forming Unit) per milliliter by comparing the turbidity. DMSO was used as diluents to get desired concentration of compounds to test upon standard bacterial strains.

2.3.3 Antimicrobial testing

The MICs of synthesized compounds were carried out by broth microdilution method using DMSO as diluents to get desired concentration of compounds to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the compound concentrations. The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. All the tubes showing no visible growth (same as control tube) were subcultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each
synthesized compound was diluted obtaining 2000 µg/mL concentration as a stock solution. In primary screening 500, 250 and 200 µg/mL concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in second set of dilution against all microorganisms. The compounds found active in primary screening were similarly diluted to obtain 100, 62.5, 50 and 25 µg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

The results of antimicrobial screening of new compounds were expressed as the MIC values and are summarized.

**Table 1:** Antibacterial screening of thieno[3,2-c]pyridine compounds 21(a-e)

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Ar</th>
<th>Gram-negative</th>
<th>Gram-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E.C. MTCC 443</td>
<td>P.A. MTCC 1688</td>
</tr>
<tr>
<td>21a</td>
<td>p-OMePh</td>
<td>250</td>
<td>100</td>
</tr>
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<td></td>
<td><img src="attachment.png" alt="p-OMePh" /></td>
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<td></td>
</tr>
<tr>
<td>21b</td>
<td>p'-FPh</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>21c</td>
<td>Ph</td>
<td>500</td>
<td>250</td>
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</tr>
<tr>
<td>21d</td>
<td>p'-CNPh</td>
<td>50</td>
<td>100</td>
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<tr>
<td></td>
<td><img src="attachment.png" alt="p'-CNPh" /></td>
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</tr>
</tbody>
</table>
The synthesized thiophene derivatives are found to exhibit good to excellent antibacterial activity. Some of the promising compounds and their antimicrobial activity compares with standard compounds as Ampicillin. From antibacterial activity data (Table 1), it is observed that compounds 21b, 21d and 21e, (Ar = p-FPh) (Ar = p-CNPh) and (Ar = p-
NO₂Ph) respectively are most active compounds. The antibacterial activity data showed that, compounds $21b$ (Ar = p -FPh, MIC = 100µg/mL) $21d$ (Ar = p –CNPh, MIC = 50µg/mL), $21e$ (Ar= p -NO₂Ph, MIC = 100µg/mL) are considered to be good active against *Escherichia coli*. Compounds $21a$, $21b$, $21d$ and $21e$ are considered as good active against *Pseudomonas aeruginosa* with MIC = 100µg/mL and MIC = 50µg/mL respectively. The compound $21e$ is also showed very good active against *Staphylococcus aureus*. with MIC = 100µg/mL. While Compound $21b$, $21d$, $21e$ are considered as good active against *Streptococcus pyogenes* with MIC = 100µg/mL. Overall compound $21b$, $21d$ and $21e$ show good activity against all bacterial stains.

Similarly compounds $21(a-e)$ (Table 2) it is observed that compounds $21a$, $21b$, and $21e$ (Ar= p-OMePh), (Ar = p-FPh) and (Ar= p -NO₂Ph) are most active compounds. Data of antifungal activity show that, compounds $21a$, $21b$ and $21e$ with MIC = 250µg/mL are considered to be good active against *Candida albicans*. Compounds $21a$, $21b$ and $21e$ are considered as good active against *Aspergillus niger* with MIC = 100µg/mL. $21a$, $21b$ and $21e$ are also considered as good active against *Aspergillus clavatus*. with MIC = 100µg/mL.

**Table 3:** Antibacterial screening of thieno[3,2-c]pyridine compounds $24(a-e)$

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Ar</th>
<th>Gram-negative</th>
<th></th>
<th>Gram-positive</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E.C. MTCC 443</td>
<td>P.A. MTCC 1688</td>
<td>S.A. MTCC 96</td>
<td>S.P. MTCC 442</td>
</tr>
<tr>
<td>$24a$</td>
<td>$p$-OMePh</td>
<td>250</td>
<td>100</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

**Table 4:** Antifungal screening of thieno[3,2-c]pyridine compounds 24(a-e)

<table>
<thead>
<tr>
<th>NO.</th>
<th>Ar</th>
<th>C.A. MTCC 227</th>
<th>A.N. MTCC 282</th>
<th>A.C. MTCC 1323</th>
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<td>100</td>
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<tr>
<td>24b</td>
<td>$p$-FPh</td>
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<td>100</td>
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<td>Ph</td>
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Ampicillin  

100 100 100 250

109
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From antibacterial activity data (Table 3), it is observed that compound 24e (Ar= p -NO<sub>2</sub>Ph) is most active compound. Data of antibacterial activity show that, compounds 24b (Ar = p -FPh, MIC = 100µg/mL) 24d (Ar = p –CNPh, MIC = 50µg/mL), 24e (Ar = p -NO<sub>2</sub>Ph, MIC = 100µg/mL) are considered to be good active against Escherichia coli. Compounds 24a, 24b, 24d and 24e are considered as good active against Pseudomonas aeruginosa with MIC = 100µg/mL and MIC = 50µg/mL respectively. Compounds 24e are also considered as good active against Staphylococcus aureus with MIC = 100µg/mL. While compounds 24a and 24b, 24d, 24e are considered as good active against Streptococcus pyogenes with MIC = 250 and 100µg/mL respectively. Overall compound 24e shows good activity against all bacterial stains. Data of antifungal activity (Table 4) show that, compounds 24a, 24b and 24e with MIC = 250µg/mL are considered to be good active against Candida albicans. Compounds 24a, 24b and 24e are considered as good active against Aspergillus niger with MIC = 100µg/mL. 24e is also good active against Aspergillus clavatus with MIC = 100µg/mL. We have compared antibacterial and antifungal activities based on standard drugs ampicillin and griseofulvin, respectively. The compounds showed moderate to good antibacterial and antifungal activities.

**2.4 Experimental section**

Experiment No: 1
2.4.1 Synthesis of (2E)-3-(4-Bromothien-2-yl) acryloyl chloride (16)

![Chemical structure](image)

To a 1L reaction flask containing suspension of (2E)-3-(4-Bromothien-2-yl) acryloyl acid (100 g, 0.43 mol) in chloroform (1000 ml) was added SOCl₂ (180.86 ml, 1.29 mol) and refluxed for 4 hours. Aliquot was removed from reaction mixture and absolute ethanol was added to convert acid chloride to stable ester for TLC. Reaction mixture was concentrated under vacuum. Yield: 107 g, (99%) (Crude is used for next step without purification)

**Experiment No: 2**

2.4.2 Synthesis of (2E)-3-(5-Bromothien-2-yl) acryloyl azide (17)

![Chemical structure](image)

To a 1L reaction flask containing NaN₃ (77.5 g, 1.19 mol) in water (300 ml) and 1,4-dioxane (300 ml) was added (2E)-3-(5-bromothien-2-yl) acryloyl chloride (100 g, 0.397 mol) in 1,4-dioxane (300 ml) over 30-35 minutes at 0 °C. The reaction mixture was stirred at room temperature for 3 hours and extracted twice with 500 ml of ethyl acetate. The combined organic layer was washed with sat. NaHCO₃ solution, and water followed by brine. Organic layer was dried with Na₂SO₄ and concentrated under vacuum to obtained 92.5 g (90%) (2E)-3-(5-bromothien-2-yl) acryloyl azide as light brown solid.
$^1$H NMR (300 MHz, CDCl₃): δ 6.40 (d, $J = 15$ Hz, 1H, olefin-H), 7.71 (s, 1H, thiophene-H), 7.83 (d, $J = 15$ Hz, 1H, olefin-H), 7.93 (s,1H, thiophene-H) ppm.

Experiment No: 3

2.4.3 Synthesis of 3-Bromothieno[3, 2-c]pyridine-4 (5H)-one (18)

To a 1L reaction flask containing diphenyl ether (450 ml) at 140 °C was added dropwise solution of (2E)-3-(4-bromothien-2-yl) acryloyl azide (90 g, 0.349 mol) in dichloromethane (400 ml). The pinch of iodine was added to reaction mixture. The temperature was increased up to 180 °C for 1 hr. The reaction mixture was cooled down to ambient temperature followed by addition of pet ether. The generated precipitate was collected by filtration. Washed with pet ether and dried under reduced pressure to obtained 55 g (68.5%) of 2-bromothieno [3, 2-c]pyridine-4 (5H)-one as brown solid.

$^1$H NMR (300 MHz, DMSO-$d_6$): δ 6.85 (d, $J = 7.2$ Hz, 1H, C₇H), 7.3 (d, $J = 7.2$ Hz, 1H, C₆H), 7.7(s, 1H, thiophene-H), 11.5 (bs, 1H) ppm.

Experiment No: 4

2.4.4 Synthesis of (2-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester (19)
In a 250ml round bottom flask containing solution of 2-bromo-5H-thieno[3,2-c]pyridin-4-one 12 (4.6 g, 20 mmol) in 100 ml acetone was added dropwise ethylbromoacetate (3.34 g, 20 mmol) at room temperature, followed by addition of K$_2$CO$_3$ (4.14 g, 30 mmol) and the resulting reaction mixture was reflux for 6 hour. The reaction was monitored by TLC (50% ethyl acetate in hexane). The reaction mixture was cooled to room temperature and filter. The solid is washed with acetone (100 ml) and filtrate was evaporated under vacuum. The crude product obtained was purified by flash column chromatography eluted with 30% ethyl acetate / hexane to obtain brown solid.

**Nature:** brown solid, **MP:** 140 °C; **Yield:** 92% (5.8 g)

**IR** (KBr): 3103, 2968, 1730, 1662, 1597 cm$^{-1}$;

$^1$H NMR (300MHz, DMSO-$d_6$) $\delta$ 1.20 (t, $J = 7.2$ Hz, 3H, CH$_3$), 4.15(q, $J = 7.2$ Hz, 2H, OCH$_2$), 4.77 (s, 2H, CH$_2$), 6.92 (d, $J = 7.2$ Hz, 1H, C$_7$H), 7.57 (s, 1H, C$_3$H), 7.58(d, $J = 7.2$ Hz, 1H, C$_6$H).

$^{13}$C NMR (300MHz, DMSO-$d_6$) $\delta$ 14.51, 50.28, 61.51, 101.32, 112.76, 127.34, 130.27, 135.19, 149.63, 157.09, 168.64 ppm.

**Analysis Calculated** for C$_{11}$H$_{10}$BrNO$_3$S (316.18) Calcd: C, 41.79; H, 3.19; N, 4.43%.

**Found:** C, 41.78; H, 3.20; N, 4.45%.

**MS; m/z :** 314.32, 316.33 (M-H)$^-$. 
2.4.5 Synthesis of (2-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (20)

In a 250ml round bottom flask containing the solution of 2-bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester 19 (5.7 g, 18 mmol) in 50 ml THF: water (1:1) mixture was added lithium hydroxide (1.15 g, 27 mmol) at room temperature. The reaction mixture was was stirred at room temperature for 16 hour. The reaction was monitored by TLC (50% ethyl acetate in hexane). The reaction mixture was concentrated under vacuum to remove THF and acidify with dil HCl. The reaction mixture was extracted with ethyl acetate. Organic layer dried with anhydrous Na₂SO₄ and evaporated under vacuum. The crude product obtained was purified by flash column chromatography eluted with 50% ethyl acetate / hexane to obtain brown solid.

**Nature:** brown solid, **MP:** 170 °C; **Yield:** 98% (5.1 g)

**1H NMR** (300MHz, DMSO-d₆) δ 4.69 (s, 2H, CH₂), 6.89 (d, J = 7.2 Hz, 1H, C₇H), 7.56 (s, 1H, C₃H), 7.57 (d, J = 7.2 Hz, 1H, C₆H), 13.0 (bs, 1H, COOH).

**13C NMR** (300MHz, DMSO-d₆) δ 50.21, 101.13, 112.58, 127.37, 130.32, 135.33, 149.54, 157.14, 170.05 ppm.

**Analysis Calculated** for C₉H₆BrNO₃S(288.12)  Calcd: C, 37.52; H, 2.10; N, 4.46%.

**Found:** C, 37.55; H, 2.11; N, 4.43%.
MS; m/z: 286, 288.14 (M-H)^−.

Experiment No: 6

2.4.6 Synthesis of (2-Aryl-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid 21(a-e)

\[
\begin{align*}
\text{Br} & \quad \text{ArB(OH)}_2, \text{Pd(PPh}_3)_4 \\
\text{K}_2\text{CO}_3, \text{water} & \quad \text{reflux, 4hr}
\end{align*}
\]

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<td>p-NO\textsubscript{2}Ph</td>
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In a 50 ml reaction flask containing compound (2-bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid 20 (1 mmol, 288 mg), boronic acid (1.5 mmol), K\textsubscript{2}CO\textsubscript{3} (3 mmol, 415 mg), tetrakistriphenylphosphine palladium (0) (60 mg, 5 mol %) was taken in H\textsubscript{2}O
The reaction mixture was heated at 90 °C for 4-6 hours. The reaction was monitored by TLC (40% ethyl acetate in hexane). The reaction mixture after cooling to room temperature was added in 20 ml water, stirred and extracted with ethyl acetate (3x15 ml). The aq. layer was acidified with dil. HCl. The product was filtered and dried under vacuum, The crude product obtained was purified by flash column chromatography eluting with 5% chloroform/ methanol with yields 75-85%.

**[2-(4-Methoxy-phenyl)-4-oxo-4H-thieno[3,2-c]pyridin-5-yl]-acetic acid (21a)**

**Nature:** Off white solid, **MP:** 168 °C; **Yield:** 80% (0.251 g)

**1H NMR** (300MHz, DMSO-\(d_6\)) \(\delta\) 3.78 (s, 3H, OCH\(_3\)), 4.71 (s, 2H, CH\(_2\)), 6.91 (d, \(J = 7.2\) Hz, 1H C\(_7\)H), 6.99 (d, \(J = 8.7\) Hz, 2H, ArH), 7.53 (d, \(J = 7.2\) Hz, 1H, C\(_6\)H), 7.65 (d, \(J = 8.7\) Hz, 2H, ArH), 7.72 (s, 1H, thiophene-H), 12.94 (bs, 1H, COOH).

**13C NMR** (300MHz, DMSO-\(d_6\)) \(\delta\) 50.14, 55.73, 101.53, 115.09, 119.05, 121, 125.96, 127.66, 131.30, 134.61, 142.58, 146.67, 158.22, 159.91, 170.22 ppm.

**Analysis Calculated** for C\(_{16}\)H\(_{13}\)NO\(_4\)S (315.35) Calcd: C, 60.94; H, 4.16; N, 4.44%.

**Found:** C, 61.00; H, 4.18; N, 4.42%.

**MS; m/z :** 314.30 (M-H)-.

**[2-(4-Fluoro-phenyl)-4-oxo-4H-thieno[3,2-c]pyridin-5-yl]-acetic acid (21b)**

**Nature:** Off white solid, **MP:** 184 °C; **Yield:** 85% (0.257g)

**1H NMR** (300MHz, DMSO-\(d_6\)) \(\delta\) 4.72 (s, 2H, CH\(_2\)), 6.91 (d, \(J = 7.2\) Hz, 1H, C\(_7\)H), 7.27 (t, \(J = 8.7\) Hz, 2H, ArH), 7.58 (d, \(J = 7.2\) Hz, 1H, C\(_6\)H), 7.75-7.83 (m, 2H, ArH), 7.84 (s, 1H, C\(_3\)H), 12.98 (bs, 1H, COOH).

**13C NMR** (300MHz, DMSO-\(d_6\)) \(\delta\) 50.15, 101.48, 116.47, 116.76, 120.68, 128.32, 128.43, 129.99, 131.23, 135.05, 141.28, 147.39, 158.25, 160.87, 164.13, 170.20 ppm.
(4-Oxo-2-phenyl-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (21c)

**Nature:** Off white solid, **MP:** 155°C; **Yield:** 84% (0.238 g)

**IR** (KBr): 3464, 3090, 3000, 1747, 1650, 1572 cm⁻¹;

**¹H NMR** (300MHz, DMSO-d₆) δ 4.67 (s, 2H, CH₂), 6.89 (d, J = 7.2 Hz, 1H, C₇H), 7.30-7.50 (m, 3H, ArH), 7.54 (d, J = 7.2 Hz, 1H, C₆H), 7.80 (d, J = 7.5 Hz, 2H, ArH), 7.87 (s, 1H, thiophene-H), 12.90 (bs, 1H, COOH).

**¹³C NMR** (300MHz, DMSO-d₆) δ 50.62, 101.28, 120.59, 126.20, 128.81, 129.72, 131.28, 133.38, 135.31, 142.22, 147.23, 158.30, 170.5 ppm.

**Analysis Calculated** for C₁₅H₁₀FNO₃S (303.31)  
Calcd: C, 59.40; H, 3.32; N, 4.62%.

**Found:** C, 59.40; H, 3.34; N, 4.63%.

**MS; m/z :** 302.16 (M-H)⁻.

[2-(4-Cyano-phenyl)-4-oxo-4H-thieno[3,2-c]pyridin-5-yl]-acetic acid (21d)

**Nature:** Off white solid, **MP:** 175°C; **Yield:** 75% (0.232 g)

**¹H NMR** (300MHz, DMSO-d₆) δ 4.72 (s, 2H, CH₂), 6.61 (d, J = 6.9 Hz, 1H, C₇H), 6.95 (d, J = 6.9 Hz, 1H, C₆H), 7.90-8.30 (m, 4H, ArH), 8.08 (s, 1H, C₃H), 12.96 (bs, 1H, COOH).

**¹³C NMR** (300MHz, DMSO-d₆) δ 50.37, 101.50, 110.72, 119.17, 123.38, 126.79, 131.20, 133.57, 135.90, 137.68, 140.13, 148.63, 158.27, 170.12 ppm.

**Analysis Calculated** for C₁₆H₁₀N₂O₃S (310.33)  
Calcd: C, 61.93; H, 3.25; N, 9.03%.

**Found:** C, 61.99; H, 3.26; N, 9.03%.

**MS; m/z :** 284.30 (M-H)⁻.
MS; m/z : 309.35 (M-H)^-.

[2-(4-Nitro-phenyl)-4-oxo-4H-thieno[3,2-c]pyridin-5-yl]-acetic acid (21e)

**Nature:** Yellow solid, **MP:** 159°C; **Yield:** 82% (0.270 g)

**1H NMR** (300MHz, DMSO-d_6) δ 4.63 (s, 2H, CH₂), 6.94 (d, J = 7.2 Hz, 1H, C₇H), 8.05 (d, J = 8.7 Hz, 2H, ArH), 8.12 (s, 1H, thiophene-H), 8.23 (d, J = 7.2 Hz, 1H, C₆H), 8.33 (d, J = 8.7 Hz, 2H, ArH), 12.98 (bs, 1H, COOH).

**13C NMR** (300MHz, DMSO-d_6) δ 50.40, 101.17, 124.67, 124.90, 126.95, 129.13, 139.70, 144.53, 148.84, 148.02, 146.87, 158.28, 170.20 ppm.

**Analysis Calculated** for C₁₅H₁₀N₂O₅S (330.32)  Calcd: C, 54.54; H, 3.05; N, 8.48%.

**Found:** C, 54.58; H, 3.05; N, 8.51%.

MS; m/z : 329.23 (M-H)^-.

**Experiment No:** 7

2.4.7 Synthesis of (3-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester (22)

In a 250ml round bottom flask containing solution of 3-bromo-5H-thieno[3,2-c]pyridin-4-one 18 (4.6 g, 20 mmol) in 100 ml acetone was added ethylbromoacetate (3.34 g, 20 mmol) dropwise at room temperature, followed by addition of K₂CO₃ (4.14 g, 30 mmol) and resulting reaction mixture was reflux for 6 hour. The reaction was monitored by TLC
(50% ethyl acetate in hexane). The reaction mixture cooled to room temperature and filter. The solid is washed with acetone (100 ml) and filtrate was evaporated under vacuum. The crude product obtained was purified by flash column chromatography eluted with 30% ethyl acetate / hexane to obtained brown solid.

**Nature:** brown solid, **MP:** 132 °C; **Yield:** 90% (5.75 g)

**IR** (KBr): 3058, 2958, 1738, 1650, 1589 cm⁻¹;

**¹H NMR** (300MHz, DMSO-\(d_6\)) 1.22 (t, \(J = 7.2\) Hz, 3H, CH₃), 4.13 (q, \(J = 7.2\) Hz, 2H, OCH₂), 4.75 (s, 2H, CH₂), 6.98 (d, \(J = 7.2\) Hz, 1H, C₇H), 7.61 (d, \(J = 7.2\) Hz, 1H, C₆H), 7.74 (s, 1H, C₂H).

**¹³C NMR** (300MHz, DMSO-\(d_6\)) δ 14.53, 50.21, 61.50, 101.71, 107.6, 124.41, 124.72, 135.71, 149.19, 157.47, 168.74 ppm.

**Analysis Calculated** for C₁₁H₁₀BrNO₃S(316.18) Calcd: C, 41.79; H, 3.19; N, 4.43%.

**Found:** C, 41.82; H, 3.21; N, 4.41%.

**MS; m/z :** 314.22, 316.08 (M-H⁻).

**Experiment No: 8**

**2.4.8 Synthesis of (3-Bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (23)**

![Chemical structure](image)

In a 250ml round bottom flask containing solution of 2-bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid ethyl ester **22** (5.7 g, 18 mmol) in 50 ml THF: Water (1:1) was
added lithium hydroxide (1.15 g, 27 mmol) and stirred at room temperature for 16 hours. The reaction was monitored by TLC (50% ethyl acetate in hexane). The reaction mixture was concentrated in vacuum to remove THF, and then acidify with dil HCl and extracted with ethyl acetate. The organic layer was dried with anhydrous Na₂SO₄ and evaporated under vacuum. The crude product was purified by flash column chromatography eluted with 50% ethyl acetate / hexane as brown solid.

**Nature:** brown solid, **MP:** 175 °C; **Yield:** 97% (5.05 g)

**¹H NMR (300MHz, DMSO-**d₆**) δ:** 4.67 (s, 2H, CH₂), 6.95 (d, J = 7.2 Hz, 1H, C₇H), 7.60 (d, J = 7.2 Hz, 1H, C₆H), 7.73 (s, 1H, C₂H), 13.05 (bs, 1H, COOH).

**¹³C NMR (300MHz, DMSO-**d₆**) δ:** 50.15, 101.50, 107.63, 124.23, 124.73, 135.85, 149.08, 157.50, 170.15 ppm.

**Analysis Calculated** for C₉H₆BrNO₃S (288.12)  
Calcd: C, 37.52; H, 2.10; N, 4.46%.  
**Found:** C, 37.52; H, 2.09; N, 4.45%.

**MS; m/z:** 286.34, 288.14 (M-H⁻).

**Experiment No:** 9

2.4.9 Synthesis of (3-Aryl-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (24)

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In a 50 ml reaction flask containing compound (3-bromo-4-oxo-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid 23 (1 mmol, 288 mg), boronic acid (1.5 mmol), K₂CO₃ (3 mmol, 415 mg), tetrakistriphenylphosphine palladium (0) (60 mg, 5 mol %) was taken in H₂O (10 ml). The reaction mixture was heated at 90 ℃ for 4-6 hours. The reaction was monitored by TLC (30% ethyl acetate in hexane). The reaction mixture after cooling to room temperature was added 20ml water, stirred and extracted with ethyl acetate (3x15 ml). The aq. layer was acidified with dil. HCl. The product was filter and dried under vacuum, The crude product obtained was purified by flash column chromatography eluted with 50% ethyl acetate / hexane with yields 70-78%.

[3-(4-Methoxy-phenyl)-4-oxo-4H-thieno[3,2-c]pyridin-5-yl]-acetic acid (24a)

**Nature:** Off white solid, **MP:** 166 ℃; **Yield:** 75% (0.236 g)
$^1$H NMR (300MHz, DMSO-$d_6$) δ 3.75 (s, 3H, OCH$_3$), 4.68 (s, 2H, CH$_2$), 6.90 (d, $J$ = 7.6 Hz, 1H C$_7$H), 6.94 (d, $J$ = 8.9 Hz, 2H, ArH), 7.58 (d, $J$ = 7.6 Hz, 1H, C$_6$H), 7.62 (d, $J$ = 8.9 Hz, 2H, ArH), 7.74 (s, 1H, thiophene-H), 12.94 (bs, 1H, COOH).

$^{13}$C NMR (300MHz, DMSO-$d_6$) δ 49.3, 55.8, 110.1, 114.9, 128.5, 128.7, 129.6, 132.0, 139.6, 141.6, 151.3, 156.40, 160.9, 172.9 ppm.

Analysis Calculated for C$_{16}$H$_{13}$NO$_4$S (315.35) Calcd: C, 60.94; H, 4.16; N, 4.44%.

Found: C, 60.98; H, 4.15; N, 4.45%.

MS; m/z : 314.35 (M-H)$^-$.

[3-(4-Fluoro-phenyl)-4-oxo-4H-thieno[3,2-c]pyridin-5-yl]-acetic acid (24b)

Nature: Off white solid, MP: 188$^\circ$C; Yield: 76% (0.230 g)

$^1$H NMR (300MHz, DMSO-$d_6$) δ 4.65 (s, 2H, CH$_2$), 6.90 (d, $J$ = 7.6 Hz, 1H, C$_7$H), 7.25 (t, $J$ = 8.9 Hz, 2H, ArH), 7.55 (d, $J$ = 7.6 Hz, 1H, C$_6$H), 7.75-7.80 (m, 2H, ArH), 7.88 (s, 1H, C$_3$H), 12.92 (bs, 1H, COOH).

$^{13}$C NMR (300MHz, DMSO-$d_6$) δ 49.6, 110.0, 116.0, 129.1, 129.7, 132.0, 132.1, 139.6, 141.6, 151.0, 156.40, 162.9, 173.2 ppm.

Analysis Calculated for C$_{15}$H$_{10}$FNO$_3$S (303.31) Calcd: C, 59.40; H, 3.32; N, 4.62%.

Found: C, 59.44; H, 3.30; N, 4.61%.

MS; m/z : 302.31 (M-H)$^-$.  

(4-Oxo-3-phenyl-4H-thieno[3,2-c]pyridin-5-yl)-acetic acid (24c)

Nature: Off white solid, MP: 152$^\circ$C; Yield: 78% (0.221 g)

$^1$H NMR (300MHz, DMSO-$d_6$) δ 4.65 (s, 2H, CH$_2$), 6.91 (d, $J$ = 7.6 Hz, 1H, C$_7$H), 7.35-7.54 (m, 3H, ArH), 7.58 (d, $J$ = 7.6 Hz, 1H, C$_6$H), 7.78 (d, $J$ = 7.5 Hz, 2H, ArH), 7.89 (s, 1H, Thiophene-H), 12.90 (bs, 1H, COOH).
$^{13}$C NMR (300MHz, DMSO-$d_6$) δ 49.5, 110.1, 116.0, 127.5, 128.8, 129.3, 132.1, 136.4, 139.6, 141.6, 147.00, 156.40, 173.1 ppm.

**Analysis Calculated** for C$_{15}$H$_{11}$NO$_3$S (285.32) Calcd: C, 63.14; H, 3.89; N, 4.91%.

**Found:** C, 63.14; H, 3.90; N, 4.90%.

**MS; m/z :** 284.32 (M-H)$^{-}$.

**[3-(4-Cyano-phenyl)-4-oxo-4H-thieno[3,2-c]pyridin-5-yl]-acetic acid (24d)**

**Nature:** Off white solid, **MP:** 161 °C; **Yield:** 70% (0.216 g)

$^1$H NMR (300MHz, DMSO-$d_6$) δ 4.70 (s, 2H, CH$_2$), 6.65 (d, $J$ = 7.2 Hz, 1H, C$_7$H), 6.94 (d, $J$ = 7.2 Hz, 1H, C$_6$H), 7.95-8.35 (m, 4H, ArH), 8.12 (s, 1H, C$_3$H), 12.98 (bs, 1H, COOH).

$^{13}$C NMR (300MHz, DMSO-$d_6$) δ 49.5, 110.1, 112.4, 115.8, 128.4, 129.6, 132.1, 132.53, 140.5, 140.0, 142.0, 151.1, 156.50, 172.15 ppm.

**Analysis Calculated** for C$_{16}$H$_{10}$N$_2$O$_3$S (310.33) Calcd: C, 61.93; H, 3.25; N, 9.03%.

**Found:** C, 61.98; H, 3.24; N, 9.05%.

**MS; m/z :** 309.35 (M-H)$^{-}$.

**[3-(4-Nitro-phenyl)-4-oxo-4H-thieno[3,2-c]pyridin-5-yl]-acetic acid (24e)**

**Nature:** Yellow solid, **MP:** 171 °C; **Yield:** 76% (0.250 g)

$^1$H NMR (300MHz, DMSO-$d_6$) δ 4.72 (s, 2H, CH$_2$), 6.90 (d, $J$ = 7.6 Hz, 1H, C$_7$H), 8.12 (d, $J$ = 8.3 Hz, 2H, ArH), 8.15 (s, 1H, thiophene-H), 8.25 (d, $J$ = 7.6 Hz, 1H, C$_6$H), 8.30 (d, $J$ = 8.3 Hz, 2H, ArH), 12.96 (bs, 1H, COOH).

$^{13}$C NMR (300MHz, DMSO-$d_6$) δ 49.5, 110.20, 121.6, 128.4, 129.6, 132.1, 139.6, 141.6, 142.3, 148.48, 151.05, 156.50, 172.15 ppm.

**Analysis Calculated** for C$_{15}$H$_{10}$N$_2$O$_5$S (330.32) Calcd:C, 54.54; H, 3.05; N, 8.48%.
Found: C, 54.60; H, 3.07; N, 8.50%.

**MS; m/z :** 329.20 (M-H)^-.

**2.5 Conclusion**

1) New thienopyridines substituted with electron donating and electron withdrawing phenyl groups are synthesized by simple method in good yields.

2) The compounds showed moderate to good antibacterial and antifungal activities.

**2.6 References**


