CHAPTER-II

SYNTHESIS, CHARACTERIZATION OF 4(2-(2-OXOINDOLINE)AMINOCYCLOPENT-1-ENE)CARBOTHIOL)THIO DERIVATIVES AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES
PART: A

Synthesis, characterization of 4(2-(2-oxoindoline) aminocyclopent-1-ene carbothiol)thioderivatives and Evaluation of their Antimicrobial Activities

Introduction

Oxoindolines are aromatic heterocyclic compounds, which possess one benzene ring linked with pyrole in which keto group is present at 2\textsuperscript{nd} position and –C=N at 3\textsuperscript{rd} position.

![Oxoindoline structure](image)

(Z)-3-(methylimino) indolin-2-one

Oxoindolines possess wide variety of activities viz., antimicrobial, antiviral, antifungal, anti-inflammatory, analgesic activity etc. Some oxoindolines are used as new apoptosis inducers using a cell or program cell death, plays a crucial role in normal cell development and tissue homeostasis\textsuperscript{1,2}. Inappropriate apoptosis induction is known to result in excessive cell death, and cause degenerative diseases\textsuperscript{3}. A large number of oxoindoline derivatives have been incorporated into a wide variety of chemotherapeutical agents and is correlated to their apoptosis inducing ability\textsuperscript{4-6}.

Substituted N\textsuperscript{1}-(2-oxoindolin-3-ylidene)-benzohydride which is an isatin derivative, is employed an apoptosis inducer, using HTS (Harmonized Tariff
Schedule) assay and SAR study which led to the discovery of N\(^1\)-(4-bromo-5-methyl-2-oxoindolin-3-ylidene)-3,4,5 trimethoxybenzo hydrazide as potent apoptosis inducers.

Moreover, oxoindoline derivatives have been well studied and so far a variety of biological activities\(^7\)\(^-\)\(^9\) was investigated.

\[
\begin{align*}
R_1 &= H & R_2 &= \text{aryl or alkyl substituents.}
\end{align*}
\]

**Synthetic approach for the synthesis of oxoindolines:**

1) Condensation of aniline (I) with 2,2,2,-trichloroethane-1,1-diol and ammonium hydroxide gives an intermediate (II) which is isomerized in the presence of H\(_2\)SO\(_4\) resulting the formation of an isatin,(III) which is further treated with ammonium hydroxide leading to the formation of an oxoindoline derivatives (IV).
II) Substituted aromatic anhydride (V), treated with BuLi in THF and Diethyl acetate results the formation compound (VI), which on acid hydrolysis gives substituted isatins, (VII), which was further treated with ammonium hydroxide leads to the formation of substituted oxoindolines (VIII).

\[ \text{V} \xrightarrow{\text{BuLi, THF}} \text{VI} \xrightarrow{\text{Acid}} \text{VII} \xrightarrow{\text{NH}_2\text{OH}} \text{VIII} \]

Where X = Cl, Br, Where R = tBu.

III) Condensation of substituted aniline (IX) with 2,2,2-trichloroacetaldehyde and ammonium hydroxide results an oxime intermediate (X) which is isomerized to isatin(XI) in the presence of Con.H_2SO_4 and further reacts with hydroxylamine forming an oxoindoline(XII)^10.

\[ \text{IX} \xrightarrow{\text{2,2,2-trichloroacetaldehyde}} \xrightarrow{\text{NH}_2\text{OH, Na}_2\text{SO}_4} \text{XI} \xrightarrow{\text{NH}_2\text{OH}} \text{XII} \]

Where R = Cl, Br, H, OAC, etc.,
Synthesis of substituted oxoindoline derivatives:

IV) Chen et al\textsuperscript{11} reported that the 2-(oxoindoline-3-ylidene)-N-phenylhydrazine carboxamide derivatives (XIV) were prepared by the condensation of N-phenylhydrazine carboxamide with isatin & the synthetic scheme in presented in [Scheme-1]

\[
\begin{align*}
\text{R} & \quad \text{+} \quad X - R \\
\text{N} & \quad \text{H} \\
\text{O} & \quad \text{O}
\end{align*}
\]

V) Ghazaleh et al\textsuperscript{12} reported that 2-Oxoindolin-3-ylphosphonates (XV) were prepared by the reaction of substituted isatins with malanonitrile and diethylphosphites. [Scheme-2]
VI) The compounds [XVI-XIX] showed interesting anti-inflammatory and anti-analgesic activities.
II) E-4,5,6,7-tetrasubstituted-2-(4-(oxoindolineylideneamino)phenylsulphonyl)isoindoline1,3 diones (XXII) were synthesized by reflexing the 2-(oxoindoline-3ylidene amino) benzene sulphonamide with 4,5,6,7- tetra substituted benzofuran 1,3,dione. [Scheme-3]

A study of oxoindoline derivatives reveal a wide spectrum of biological activity, some of which are discussed below.

**Anti-inflammatory and Analgesic activity:**

2, 3 oxoindolin derivatives, such as 1-(1-((Ethyl (methyl) amino) methyl)-5-methyl-2-oxoindolin-3-ylidene)-4-(4-methyl pyridine-2-yl) thiosemicarbazide of Isatin (XXIII) possess analgesic and anti-inflammatory activity.
Muthu kumar et al\textsuperscript{13}, had synthesized 1-(1-((substituted) methyl)-5-methyl-2-oxoindolin-3-ylidene)-4-substituted pyridine -2-yl) thiosemicarbazide derivatives and studied the behavioral effects of Oxoindoline derivatives. The compounds possess anti-inflammatory activity and analgesic activity.

**Antiviral activity:**

It is evident from literature that oxoindoline derivatives are known to be associated with broad spectrum of biological activities like antibacterial, antifungal. Madiha farghaly et al\textsuperscript{14} had been synthesized 3-(2-(5-(aryl diazenyl)-4-methyl thiazol-2-yl)hydrazono Indolin-2-ones which were prepared by the reaction of Isatin $\beta$-thiosemicarbazone& anhydrides which showed good antibacterial and antifungal activity. 4,5,6,7-tetrachloro-2-((2-oxoindolin-3-ylidene)amino)isoindolin-1,3-dione showed antibacterial activity against *Bacillus subtilis* in about 25 mm inhibition zone.
Anti HIV-Activity:

Some novel Oxoindoline of isatins and its derivatives showed anti-HIV activity. V.V. Bolotov et al\textsuperscript{15} had synthesized Z-3-(2-oxoindolin-3-ylidene)-3,4-dihydroquinoxalin-2(1H)-one (XXV) by reacting with ethyl 2-oxoindoline-2-glyoxylate and o-amino phenol. The synthesized compounds were screened for anti HIV activity viz., (HIV-1(III B) in MT-4 cells).
Anti-Cancer Activity:

Nilantha Sirisoma et al\textsuperscript{16} had synthesized substituted N\textsuperscript{1}-(2-oxoindolin-3-ylidene)-3, 4, 5-trimethoxybenzohydrazides as inducers of apoptosis using proprietary cell and caspase-based ASAP HTS assay. They studied SAR studies of N\textsuperscript{1}-(4-bromo-5-methyl-2-oxoindolin-3-ylidene)-3,4,5-trimethoxybenzohydrazide (XXVI) as a potent apoptosis inducer with an IC\textsubscript{50} value of 0.24\mu m in human colorectal Carcinoma HCT116 cells.
PART: B

Present work:

Synthesis and characterization of Oxoindoline derivatives from 2-aminocyclopent-1-ene carbodithioic acid

Isatin (indole 2, 3-dione) is an endogenous compound, which were synthetically versatile substrates, useful for the synthesis of large variety of heterocyclic compounds \(^{17,18}\). The synthetic versatility of Isatin has stemmed from the interest in the biological and pharmacological properties of its derivatives. In nature, isatin is found in plants of the genus Isatis \(^{19}\), in Calanthe discolor on from the parotid gland of Bufo frogs \(^{20}\) and in humans as a metabolic adrenaline derivative. Isatin derivatives are reported to show other biological activities like antibacterial \(^{21-23}\), antifungal \(^{24-26}\), antiviral \(^{27-29}\), anti HIV \(^{30-32}\), antiprotozoal \(^{33,34}\), muscle relaxant \(^{35}\), antiallergic \(^{36}\), and anti-inflammatory activities \(^{37-41}\). Prompted by the biological properties of oxoindoline derivatives, it was decided to synthesize various oxoindoline derivatives of isatin with aryl substrates and to screen the synthesized compounds for their Antimicrobial activities. The oxoindoline derivatives were synthesized by condensing 2-aminocyclopent-1-ene carbodithioic acid (XXIX) (prepared from cyclopentanone (XXVII) and carbon disulphide (XXVIII) with Isatin (XXX). All the compounds gave satisfactory elemental analysis. IR, \(^1\)H, \(^{13}\)C-NMR
and Mass spectra were consistent with the assigned structures. All the synthesized compounds were screened for their Antibacterial and Antifungal Activities.

**Materials and Methods**

**General Experimental Procedure:**

All the chemicals used in the present study are of analytical grade and were obtained from local suppliers. Melting points were determined in open capillary tubes and are uncorrected. The physical constants of synthesized compounds are given in Table-1. The purity of the synthesized compounds was routinely checked by TLC on silica gel plates. $^1$H NMR and $^{13}$C-NMR spectra were recorded on 400 MHz Fourier-Transform-Nuclear Magnetic Resonance Spectrometer using TMS as an Internal standard (Chemical shifts in ppm); IR Spectra were recorded from KBr discs on Thermo Nicolet (Model: 6700) Spectrophotometer ($\nu_{\text{max}}$ cm$^{-1}$).

**General procedure for the synthesis of 2-amino cyclopent-1-ene carbodithioic acid (XXIX):**

An equimolar mixture of cyclopentanone (XXVII) and carbon disulphide (XXVIII) and liq.NH$_3$/ EtOH(100) were taken in 500ml R.B.Flask. The reaction mixture was stirred at 0°C for 1hr. The reaction was monitored by TLC, and after completion of the reaction, the solvent was evaporated under reduced pressure, to give yellow solid (XXIX). The crude was washed with ethyl acetate and dried. The
synthesized compound was recrystallized with chloroform and petroleum ether. The synthetic scheme is presented in [Scheme-4].

\[
\begin{align*}
\text{XXVII} + \text{XXVIII} + \text{liq.} \text{NH}_3 & \xrightarrow{0^\circ \text{C, 1hr}} \text{EtOH} \xrightarrow{\text{1hr}} \text{XXIX} \\
\text{O} & & \text{NH}_2 & & \text{S} & & \text{SH}
\end{align*}
\]

Scheme 4

2-amino cyclopent-1-ene carbodithioicacid (XXIX): The sample was recrystallized using chloroform and petroleum ether. Yield 82%

\text{m.p: 95}^\circ \text{C}

Elementary analysis for C\text{6}H\text{9}NS\text{2}:

\text{Required: C, 45.25, H, 5.70, N, 8.79, S, 40.26.}

\text{Found: C, 45.30, H, 5.73, N, 8.81, S, 40.23.}

\text{\textsuperscript{1}HNMR 90MHz (CDCl\textsubscript{3}) ppm:} \delta1.85 \text{ (s, 1H, SH)}, \delta2.09 \text{ (s, 2H, NH2)}, \delta2.58-2.76 \text{ (m, 6H, Cyclopentene methylene protons). (Fig: 1).}

\text{IR KBr (cm}\textsuperscript{-1}): 3430\text{cm}^{-1} \text{(N-H str)}, 1628 \text{ cm}^{-1} \text{ (C=C), 1314cm}^{-1} \text{ (C-N), 2817 cm}^{-1} \text{(S-H), 1283 cm}^{-1} \text{(C=S). (Fig: 2).}
Fig: $^1$H NMR spectra of compound XXIX
Fig: 2 IR Spectra of Compound XXIX
Synthesis of (Z)-2-((2-Oxoindolin) amino cyclopentenecarbodithioic acid (XXXI)

An equimolar mixture of compound (XXIX) was condensed with Isatin (XXX) in 100ml of EtOH [Scheme-5]. The reaction mixture was stirred at room temperature for 2 hrs and heated in water bath for 2 hrs. The reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated under reducing pressure. The resulting crude was washed with ethyl acetate and synthesized compound (XXXI) was recrystallized with chloroform, petroleum ether (1:2) to give pale brown colour crystals. The title compound Z-2-((2-Oxoindoline)aminocyclopentene cabodithioic acid(XXXI), was directly used in further reaction.

![Scheme 5]

The structure of compound (XXXI) is established by IR & NMR spectra, as detailed below;

(Z)- 2- ((2-Oxoindolin) amino cyclopent-1-enecarbodithioic acid (XXXI): The sample was recrystallized using chloroform and petroleum ether. Yield 79%
m.p: 115°C.

Elemental analysis for C\textsubscript{14}H\textsubscript{12}N\textsubscript{2}OS\textsubscript{2}:

**Required:** C, 58.31; H, 4.19; N, 9.71; O, 5.55; S, 22.24.

**Found:** C, 58.28; H, 4.16; N, 9.73; O, 5.54; S, 22.24.

\textsuperscript{1}H NMR (CDCl\textsubscript{3}) ppm: δ1.85 (s, 1H, –SH), δ 2.64-2.76 (m, 6H, cyclopentene methylene protons), δ 3.76 (NH), δ 7.26-7.86 (m, 4H, Ar).

IR (KBr) cm\textsuperscript{-1}: 3397 cm\textsuperscript{-1} (NH), 1726 cm\textsuperscript{-1} (C=O), 1616 cm\textsuperscript{-1} (C=N), 1189 cm\textsuperscript{-1} (C=S).

Based on the above spectral data the structure of the compound was established as (XXXI)

**Synthesis of Oxoindoline derivatives (XXXII a-e):**

A slurry consisting of (Z)-2-((2-oxoindolin)aminocyclopentene)carbodiithioic acid (XXXI), in anhydrous EtOH (10ml) was treated with different 1,4 disubstituted aryl halo compounds in THF, drop wise, with cooling and shaking [scheme-6]. The reaction mixture was allowed to stand at room temperature for 1 hr with occasional shaking and later warmed on a steam bath for 30 min. The reaction was monitored by TLC. At the end of the reaction, the contents were cooled and the product obtained, was recrystallized from chloroform and petroleum ether. Six new compounds (XXXIIa-XXXIIe) were synthesized & characterized.
The physical constants of synthesized compounds XXXI and XXXII (a-e) were shown in the Table –1.
Table: 1  The physical constants of synthesized compounds XXXI and XXXII (a-e)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>m.p. °C</th>
<th>Yield (%)</th>
<th>Molecular formula</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>XXXI</td>
<td>H</td>
<td>115 °C</td>
<td>79</td>
<td>C&lt;sub&gt;14&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>288.39</td>
</tr>
<tr>
<td>XXXII(a)</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;ClO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>242 °C</td>
<td>72</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>408.49</td>
</tr>
<tr>
<td>XXXII(b)</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;Cl</td>
<td>128 °C</td>
<td>69</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>378.51</td>
</tr>
<tr>
<td>XXXII(c)</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;ClN</td>
<td>274-276 °C</td>
<td>73</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;19&lt;/sub&gt;N&lt;sub&gt;3&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>381</td>
</tr>
<tr>
<td>XXXII(d)</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>120 °C</td>
<td>63</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;15&lt;/sub&gt;ClN&lt;sub&gt;2&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>398.03</td>
</tr>
<tr>
<td>XXXII(e)</td>
<td>C&lt;sub&gt;5&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;Br</td>
<td>247-249 °C</td>
<td>67</td>
<td>C&lt;sub&gt;19&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;OS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>395.09</td>
</tr>
</tbody>
</table>
Characterization of synthesized compounds: (XXXII a-e)

(Z)-4-((2-(2-Oxindolin-3-ylidene)aminocyclopent-1-enecarbothioyl)thio) benzoic acid XXXII(a): The sample was recrystallized using chloroform and petroleum ether. Yield 72%

\[ \text{m.p: } 242^\circ C \]

Elemental analysis for \( C_{21}H_{16}N_{2}O_{3}S_{2} \):

\[ \text{Required: } C, 61.75; H, 3.95; N, 6.86; O, 11.75; S, 15.70. \]

\[ \text{Found: } C, 61.73; H, 3.92; N, 6.89; O, 11.77; S, 15.72 \]

HPLC: Mobile phase methanol

Purity: 99.52%

\( ^1\text{HNMR (DMSO-d}_6\text{)} \) ppm: \( \delta 2.3 \text{ (s,6H, cyclopentene methylene protons)} \), \( \delta 7.5-7.6 \text{ (s,4H Ar-H), } \delta 7.9-8.0\text{m,4H,Ar-H) } \delta 13.2 \text{ (-COOH). (Fig: 3)} \)

\[ \text{IR(KBr)} \text{cm}^{-1}: 3417\text{cm}^{-1} \text{ (N-H), } 3197\text{cm}^{-1}, 2923\text{cm}^{-1} \text{ (Aromatic C-H), } \]

\[ 1726\text{cm}^{-1} \text{ (C=O in -COOH), } 1616\text{cm}^{-1} \text{ (C=C aromatic), } \]

\[ 1189\text{cm}^{-1} \text{ (C=S). (Fig: 4).} \]

(Z)-benzyl-2-((2-Oxindolin-3-ylidene) amino) cyclo pent-1-ene carbo)dithioate XXXII(b) : The sample was recrystallized using chloroform and petroleum ether. Yield 69% .

HPLC: Mobile phase methanol

Purity: 99.31%
m.p: 128°C

Elemental analysis for C_{21}H_{20}N_{2}OS_{2}:

**Required:** C, 66.64; H, 4.79; N, 7.40; O, 4.23; S, 16.94.

**Found:** C, 66.66; H, 4.83; N, 7.37; O, 4.25; S, 16.93

$^1$H NMR (CDCl$_3$): δ2.0-2.79 (m, 6H, cyclopentene methylene protons), δ4.3-4.6 (s, 2H, S-CH$_2$-Ar), δ7.26-7.4 (m, 4H, Ar-H), δ7.5-7.81 (m, 5H, Ar-H), 12.9 (s, 1H, indole)-NH) (Fig: 5).

**IR (KBr) cm$^{-1}$:** 1718 (C=O), 1616 (C-N), 811 cm$^{-1}$ (C=S), 1183 cm$^{-1}$ (C=S-C-).

(Z)-4-aminophenyl-2-((2-oxoindolin-3-ylidene)aminocyclopent1-ene)carbodithioate XXXII(c): The sample was recrystallized using chloroform and petroleum ether. Yield 73%

m.p: 274-276°C

Elemental analysis for C_{20}H_{19}N_{3}OS

**Required:** C, 63.30; H, 4.52; N, 11.07; O, 4.22; S, 16.90.

**Found:** C, 63.29; H, 4.53, N, 11.05; O, 4.21; S, 16.06

$^1$H NMR (DMSO$_d_6$): δ2.1-2.7 (m, 6H, cyclopentene methylene protons), δ 5.9 (s, 2H, Ar-NH$_2$ protons), δ 6.6-7.0 (m, 4H, Ar-H), δ 7.26-7.81 (m, 4H, Ar-H), δ 11.33 (s, 1H, isatin NH).

**IR (KBr) cm$^{-1}$:** 3196 (NH$_2$), 1699 (C=O), 1266 (C-N), 1186 (C=S).
Fig: $^1$H NMR spectra of compound XXXIIa
Fig: 4 IR spectra of XXXIIa
Fig: 5 $^1$H NMR spectra of XXXIIb
(Z) 4-chlorophenyl 2- ((2-Oxoindolin 3-ylidene) aminocyclopent-1-ene carbodithioate XXXII(d): The sample was recrystallized using chloroform and petroleum ether. Yield 63% m.p: 120\(^0\)C, Elemental analysis for C\(_{20}\)H\(_{15}\)ClN\(_2\)OS\(_2\): 
Required: C, 60.21; H, 3.79; Cl, 8.89; N, 7.02; O, 4.01; S, 16.08. 
Found: C, 20.24; H, 3.80; Cl, 8.88; N, 7.0; O, 4.03; S, 16.06. 
\(^1\)H NMR (DMSOd\(_6\)) ppm: \(\delta\) 2.1- 2.6 (m, 6H, cyclopentene methylene protons) \(\delta\) 7.30-7.8 (br, m, 8H, Ar-H), \(\delta\) 13.7 (br, 1H, isatin-NH). [Fig.6]. 
IR(KBr) cm\(^{-1}\): 3215 cm\(^{-1}\) (NH), 1705 cm\(^{-1}\) (C=O), 1620 cm\(^{-1}\) (C=N), 1286 cm\(^{-1}\) (C-N), 1180 cm\(^{-1}\) (C=S). 

(Z) 5,6 diaminopyridine-3-yl- 2- ((2-Oxoindolin 3-ylidene) aminocyclopent-1-ene carbodithioate XXXII(e): The sample was recrystallized using chloroform and petroleum ether. Yield 67% m.p: 247-249\(^0\)C. 
Elemental analysis for C\(_{19}\)H\(_{17}\)N\(_5\)OS\(_2\). Required: C, 57.70; H, 4.33; N, 17.71; O, 4.05; S, 16.21.
Found: C, 57.72; H, 4.35; N, 17.69; O, 4.02; S, 16.19.

1H NMR (DMSO-d$_6$): δ 1.87-2.273 (m, 6H, Cyclopentene methylene protons), δ6.3, δ7.8 (s, 2H, -NH$_2$), δ 6.87-7.67(d, 2H, Ar-H), δ13.4(s,1H, isatin –NH)

IR(KBr)cm$^{-1}$: 3430 cm$^{-1}$ (N-H, isatin), 1724 cm$^{-1}$ (C=O), 1572, 1591, 1684 cm$^{-1}$ (C=C aromatic str), 1305, 1281 cm$^{-1}$ (C-N), 1174 cm$^{-1}$ (C=S), 761.3 cm$^{-1}$ (-C-Br). [Fig.7].
Fig: $^1$H NMR spectra of compound XXXIIId.
Fig: 7 IR spectra of compound XXXIIe
Results and Discussions:

All the synthesized compounds from Scheme-4, Scheme-5 and Scheme-6 were characterized by the IR Spectra, 1H-NMR and elemental analysis. This new procedure provides the first example of three component and catalyst free synthesis of oxoindoline derivatives. The reactions under catalyst free conditions are considerably safe, nontoxic, environmentally friendly and inexpensive. The absence of catalyst for the reaction allows, avoiding the use of moisture sensitive and heavy metal lewis acids. This method, based on catalyst free reaction with solvent is the most simple and convenient and would be applicable for the synthesis of different types of oxoindoline derivatives. These reactions proceeded smoothly under mild conditions, and no undesirable side reactions were observed. The oxoindoline derivatives XXXII (a-e) were synthesized for the first time.
PART: C

Bioevaluation of oxoindoline derivatives with 2-aminocyclopent-1-ene carbodithioic acid

Antimicrobial Activities

The synthesized compounds were tested for antibacterial activity against bacteria: Viz, *E.Coli, P.aeruginosa, P.Vulgaris, S.aureus, B.Cereus, B.subtilis*, and against fungi Viz *C.albicans* and *S. Cerevisia*, at concentrations of 25, 50, 100, 200µg/ml. 36g of Nutrient agar Media (Hi-Media) and Potato dextrose agar were mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterile nutrient agar medium was used for bacterial inoculation and the sterile Potato dextrose agar was used for fungal inoculation with test organisms. The inoculation was done under aseptic conditions and when the medium was in molten state, then poured into petri dishes. The solidified plates were bored with 6mm diameter cork borer. The plates with wells were used for the antimicrobial studies. The antimicrobial activities of the compounds were carried out by well diffusion method.

Well diffusion method

The synthesized compounds were dissolved in DMSO and the following concentration viz as 25µg, 50µg, 100µg and 200µg were prepared. Each concentration of these compounds was tested against different bacterial pathogens. Antibacterial activity was tested by using agar well diffusion method\textsuperscript{42}. The inoculated sterilized nutrient agar
media was poured into petri dishes and allowed to solidify. 6mm Wells were made on the agar surface, into each of these wells, (50μg) of the test compound/reference standard/control was added by using a micropipette. Streptomycin was used as standard reference and DMSO was used as a control (solvent) which did not possess any inhibition zone. The plates were incubated at 37°C for 24 hours for bacterial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

**Antibacterial activity**

The antibacterial activity of the synthesized compounds of 25μg, 50μg, 100μg and 200μg concentrations were tested against gram positive bacteria *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441) and *Bacillus cereus* (MTCC 430), gram negative bacteria *Pseudomonas aeruginosa* (MTCC 424) *Escherichia coli* (MTCC 443) and *Proteus vulgaris*. And the inhibition zones were calculated and recorded in Table-II

**Antifungal activity**

All the synthesized compounds were evaluated *in vitro* for antifungal activity by using agar well diffusion method. The test organisms are *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170) were used. They were grown on potato
dextrose agar medium. The plates were incubated at 28°C for 24 hrs and the zone of inhibition was measured in mm. Fluconazole was used as a standard reference and DMSO was used as a control (solvent), which did not possess any inhibition zone. The results of the antimicrobial activities are summarized in Table II.
Table II. Antimicrobial activities of the synthesized compounds XXIX, XXXI and XXXII (a-e).

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>Conc. of the Compound µg/ml</th>
<th>Zone of inhibition in mm</th>
<th>Gram negative bacteria</th>
<th>Gram positive bacteria</th>
<th>Fungi</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>E.coli</td>
<td>P.aeruginosa</td>
<td>P.Vulgaris</td>
</tr>
<tr>
<td>XXXIX</td>
<td>200</td>
<td>16</td>
<td>13</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>13</td>
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*NA* Not active
Fig-11: Inhibition zones of the compound XXIX against test pathogens.
**Fig-12:** Inhibition zones of the compound XXXI against test pathogens.
Fig-13: Inhibition zones of the compound XXXII(a) against test pathogens.
Fig-14: Inhibition zones of the compound XXXII(b) against test pathogens.
Fig-15: Inhibition zones of the compound XXXII(c) against test pathogens.
Fig-16: Inhibition zones of the compound XXXII(d) against test pathogens.
Fig-17: Inhibition zones of the compound XXXII(e) against test pathogens.
Results and Discussions:

The synthesized compounds XXIX, XXXI & XXXII (a-e) were tested in vitro antimicrobial activity. The activity was reported by measuring the diameter of inhibition zone in mm, against gram-positive, gram-negative bacteria & fungi and was presented in Table-2. The inhibition zones were photographed and presented(Fig 11-Fig 17)

In fig-11 the synthesized compound XXIX exhibits the antimicrobial activities against tested pathogens used in this study. In gram negative bacteria the minimum inhibition concentration (MIC) value was exhibited with 50µg/ml for E.coli, P.aeruginosa and 25µg/ml for P.Vulgaris. In gram positive bacteria, the MIC value was identified with 25µg/ml for S.aureus and 100µg/ml for B.cereus and B.subtilis. In fungi the MIC value was 100µg/ml for C.albicans and 50µg/ml for S.cerevisiae.

In fig-12 the synthesized compound XXXI exhibits the potent antimicrobial activities against gram negative bacteria and gram positive bacteria. In gram negative bacteria MIC value was noticeable with 50µg/ml for E.coli and P.aeruginosa. In case of P.Vulgaris the MIC value was noticeable with 25µg/ml. In gram positive bacteria, the MIC value was noticeable with 25µg/ml for S.aureus ,B.cereus and B.subtilis. In fungi the MIC value was observed with 25µg/ml for C.albicans and S.cerevisiae. The results
indicate that the compound XXXI exhibits potent antimicrobial activity when compared with standard reference streptomycin and fluconazole.

In **fig-13** the synthesized compound XXXIIa exhibits moderate antimicrobial activities against test pathogens used in this study. In gram negative bacteria the minimum inhibition concentration (MIC) value was observed with 50µg/ml for *E.coli*, *P.aeruginosa* and 25µg/ml for *P.Vulgaris*. In gram positive bacteria, the MIC value was noticeable with 25µg/ml for *S.aureus* and in case of *B.cereus* and *B.subtilis* observed with 50µg/ml. In fungi the MIC value was noticeable with 100µg/ml for *C.albicans* and 25µg/ml for *S.cerevisiae*.

The synthesized compound XXXIIb (**fig-14**) exhibits slightest antimicrobial activities against gram negative bacteria and gram positive bacteria. In gram negative bacteria MIC value was observed with 200µg/ml for *E.coli* and 50µg/ml for *P.Vulgaris*. In gram positive bacteria the MIC value was noticeable with 50µg/ml for *S.aureus*. In fungi the MIC value was 100µg/ml for *S.cerevisiae*.

The synthesized compound XXXIIc (**fig-15**) exhibits the potent antimicrobial activities against gram negative bacteria and gram positive bacteria. In gram negative bacteria MIC value was observed with 25µg/ml for *E.coli* and *P.Vulgaris*. The MIC value was observed with 50/ml for *P.aeruginosa*. In gram positive bacteria the MIC value was noticeable with 50µg/ml for *S.aureus*, *B.cereus* and 100µg/ml for *B.subtilis*. In fungi
the MIC value was observed with 50µg/ml for *C.albicans* and 25µg/ml for *S.cerevisiae*.

In fig-16 the synthesized compound XXXIIId exhibits less antimicrobial activities, when compared with reference standards used in this study. In gram negative bacteria the MIC value was observed with 200µg/ml for *E.coli, P.aeruginosa* and in case of *P.Vulgaris* the MIC value was noticeable with 50µg/ml. In gram positive bacteria the MIC value was observed with 50µg/ml for *S.aureus* and 100µg/ml for *B.cereus*. In fungi the MIC value was noticeable with 100µg/ml for *C.albicans* and 25µg/ml for *S.cerevisiae*.

The synthesized compound XXXIIle (fig-17) exhibits potent antibacterial activity against gram negative and gram positive bacteria when compared with reference standard streptomycin. The minimum inhibition concentration (MIC) value was observed with 25µg/ml for both gram positive and gram negative bacteria. The compound XXXIIle did not possess inhibition zones for fungi used in this study.

In general the antibacterial activity of oxoindoline derivatives XXXII (a), XXXII(c), XXXII (e), showed more activity than XXXII (b), and XXXII (d). For the antifungal activity, the compounds XXXII (a), XXXII (b), XXXII(c) showed potent activity against *S.cerevisiae*. Compounds with carboxylic and amine substituents showed better activity than other substituents, so the synthesized compounds are a good source of biological properties.
References:


