CHAPTER 3
CHAPTER 3

DESIGN, SYNTHESIS AND INSECTICIDAL ACTIVITY OF NOVEL TRIAZENE AND TRIAZA-S-INDACENE PYRAZOLE DERIVATIVES HAVING AMINO ACID LINKERS

3.1 INTRODUCTION

Synthetic pyrazole-5-carboxamide derivatives such as tebufenpyrad and tolfenpyrad (figure 1) belong to an important kind mitochondrial respiration inhibitors, and they interrupt the mitochondrial electron transport by inhibition of NADH: ubiquinone oxidoreductase (complex I)\(^1\). Tebufenpyrad, discovered by Mitsubishi Kaaei Co., Ltd. in 1987, shows an excellent acracidal activity against phytophagous mites and Homoptera pests\(^2\). Tolfenpyrad, discovered by Mitsubishi Chemical Corp. (now Nihon Nohyaku Co.Ltd.) is one of the most important insecticides for control pests that are difficult to control, such as Hemiptera, Coleoptera, Diptera, Lepidoptera, and Acarina. It mainly provides contact activity against target pests on egg, larva, nymph and adult stages\(^3\). Because of their outstanding performance in controlling agricultural pests, pyrazole-5-carboxamide derivatives have attracted considerable attention for decades\(^4\).

[Figure 1]

Tebufenpyrad  

Tolfenpyrad

Figure -1

66
Synthetic 1-phenylpyrazoles such as fipronil (A) and ethiprole (B) (Figure 2) are important kind of insecticide, and the toxicity of 1-phenylpyrazoles to insects and mammals is attributable to their action at the GABA receptor as noncompetitive blockers of the GABA-gated chloride channel \textsuperscript{5-8}.

\[ \text{Fipronil A} \]

\[ \text{Ethiprole B} \]

**Figure 2**

Fipronil was the most important insecticides for control of soil insect on corn\textsuperscript{9} and fleas on cats and dogs\textsuperscript{10}. Ethiprole (B) is a new 1-phenylpyrazole insecticide effective against a broad spectrum of chewing and sucking insects with pronounced plant systemic activity\textsuperscript{11} as well as stored grain insect pests\textsuperscript{12}. Besides fipronil and ethiprole, vaniliprole (C)\textsuperscript{13}, acetoprole (D)\textsuperscript{14}, pyrafluprole (E)\textsuperscript{15} and pyriprole (F)\textsuperscript{16} (Figure 3) are among the 1-phenylpyrazole insecticides. In common, there are an electron-withdrawing group (cyano or acetyl), a sulfenyl (or sulfinyl) group, and an amino (or substituted amino) on the C\textsubscript{3}-position, C\textsubscript{4}-position and C\textsubscript{5}-position of the pyrazole ring, respectively.
Figure 3

It is noticed that vaniliprole, pyrafluprole and pyriprole could be prepared by reaction of the corresponding 5-amino pyrazole and proper aromatic aldehyde, and a subsequent reduction that results imine intermediate needed for pyrafluprole and pyriprole.\textsuperscript{15,16} There is significant progress in the study of the mechanism of pyrazole insecticides in recent years\textsuperscript{17}.

In the second chapter the synthesis of pyrazolo[3,4-\textit{b}]pyridine 3-carboxylate, pyrazolo[3,4-\textit{b}]quinoline-3-carboxylate by condensing various aromatic acetophenone cyclic, acyclic ketones and deeply discusses also studying Friedlander, Knowingly condensation name reactions.
3.2 PRESENT WORK

In this chapter we have discussed the synthesis of pyrrolo[2,3-d][1,2,3]triazine ester, pyrazolo[3,4-d][1,2,3]triazene-5-carbohydrazide, tetrahydro-1,2,8-triaza-s-indacene. Here we have used the compound 9 and 20a which we prepared in previous section (Chapter No.2, Page No.30,44). The pyrazolo[3,4-d]triazene and pyrazolo[3,4-b]pyridine were converted to succinimidoyl active ester which is then replaced by biological samples such as amino acids in slightly aqueous medium. A novel series of pyrazolo[3,4-d][1,2,3]triazene-5-carbohydrazide 30(a-d) have been synthesized from pyrazolo[3,4-d]triazine-5-carbohydrazide by cyclocondensation reaction with various aromatic aldehyde.

The synthesis of pyrazolo[3,4-b]pyridine derivatives, synthesis of succinimidoyl active ester and attachment of amino acids is depicted in the following retro synthesis.

i) Synthesis of succinimidoyl active ester.
ii) Attachment of amino acids to Pyrazolo[3,4-b] pyridines.

\[
\begin{align*}
\text{24} & \quad + \quad \text{Amino Acid} \quad \longrightarrow \quad \text{25} \\
\text{27} & \quad + \quad \text{Amino Acid} \quad \longrightarrow \quad \text{28}
\end{align*}
\]

iii) Reactions of pyrazolo[3,4-d][1,2,3]triazine-5-carboxyhydrazide.

\[
\begin{align*}
\text{33} & \quad \xrightarrow{+K'S} \quad \text{32} \quad \longrightarrow \quad \text{29} \\
\text{31a-d} & \quad \longrightarrow \quad \text{9}
\end{align*}
\]
3.3 RESULTS AND DISCUSSION

The starting ethyl 7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxylate obtained in previous chapter 2 is used for synthesis work. The C5-ester functional group in pyrazolo[3,4-d]triazine 9 is being important, used to attached amino acid linkers to obtain active pyrazole derivatives.

3.3.1 Synthesis of 7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxylic acid 23

![Scheme-I](image)

The pyrazolo triazines -5-carboxylate on basic hydrolysis with potassium hydroxide refluxed in ethanol furnish acid 23 in 72% yields. (Experiment No.1, Page No.112) Then PH of resulting mixtures adjusted with dil. HCL and precipitated solid was collected by filtration. The acid 23 was characterized by spectral and analytical data as follows. The IR of the compound shows broad singlet at 3400 for OH group and it is also confirmed by the ^1H NMR spectrum in DMSO-d_6 (Spectrum No.1, Page No.72) of this solid showed singlet at δ 15.80 for the acidic proton (OH) and another singlet at δ 9.20 was assigned for C_H. The aromatic protons are appeared at their respective chemical shift.
IR: 3400, 2287, 1735, 1620 cm$^{-1}$
Mass M/Z = 241M$^+$
Mol. Wt. = 241.21

Spectrum No. 1 $^1$H NMR (DMSO-d$_6$) -7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxylic acid, (23).
Spectrum No.2 $^{13}$C NMR (DMSO-$d_6$) - 7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxylic acid. (20).
Also elemental analysis is in agreement with the molecular formula C₁₁H₇N₅O₂. The $^{13}$C (Spectrum No.2, Page No.73) carbon also shows aromatic carbon are appeared from δ 120 to 132 and acid carbonyl carbon is appeared at δ 163 which confirms the acid groups. On the basis of this spectral and analytical methods structure 7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxylic acid was assigned to compound 23.

### 3.3.2 Synthesis of active ester of pyrazolo[3,4-d]triazine 24

![Scheme-III](image)

The acid was successfully converted to the desired succinimidoyl active ester 24 for the linkage of biological moiety such as amino acids. Thus the reaction of 23 with $N$-hydroxysuccinimide in dry THF containing catalytic amount of dicyclohexylcarbodiimide (DCC) as protective additive is the non acid forming chemical water scavenger which do not form acids on reacting with water such scavenger includes acetals, ketals, ortho formates, dialkyl, diaryl and dicycloalkyl and diaryl carbodiimide particularly preferred is the water scavenger dicyclohexylcarbodiimide (DCCI). The carbodiimide react with by product water and to form urea or carbodiimide may convert carboxylic acid to its anhydride, thus urea being
formed by this route also. The formation of dicyclohexylurea in reactions has been confirmed by IR analysis. The reaction stirred for 7h and then TLC checks in toluene:acetone (8:2) afforded a colorless solid, which was recrystallized by ethanol (Experiment No.2, Page No.113). The solid 24 was characterized as follows, IR of the compound shows broad singlet at 1768 cm⁻¹ and 1739 cm⁻¹ carbonyl group and 1665 cm⁻¹ for amide carbonyl, it is also confirmed by the ¹H NMR spectrum in DMSO-d₆ of this solid showed singlet at 2.94 for four protons of two methyl groups of N-hydroxysuccinimide and another singlet at 8.47 for C₄H (Spectrum No.3, Page No.76) the elemental analysis is in agreement with the molecular formula C₁₅H₁₀N₆O₄. On the basis of this spectral and analytical methods structure was assigned to 7-Phenyl-7H-pyrrolo[3,4-d][1,2,3]triazene-5-carboxyacid 2,5-dioxopyrrolidine-1-yl-ester 24 this compound.
IR: 3326, 2929, 1668 cm\(^{-1}\)
Mass M/Z = 338 M\(^+\)
Mol. Wt. = 338.29

Spectrum No. 3 \(^1\)H NMR (CDCl\(_3\)) 7- Phenyl-7H-pyrrolo[3,4-d][1,2,3]triazene-5-carboxylic acid 2,5-dioxopyrrolidine-1-yl-ester (24).

76
Spectrum No. 4 $^{13}\text{C}$ NMR (CDCl$_3$) 7-Phenyl-7H-pyrrolo[3,4-\text{d}]\{1,2,3\}triazene-5-carboxylic acid 2,5-dioxopyrrolidine-1-\text{yl}-ester (24).
3.3.3 Synthesis of 2-(7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)-3-phenylpropanoic acid, 25a

![Scheme-IV](image)

After the successful synthesis of targeted active ester 24, the linking of different amino acids was carried out easily because of the polar N-O bond, during the reaction using aqueous DMSO as solvent we polarized the N-O bond by providing the polar protic solvents and hence it cleave in mild conditions and also increased the yield of the reaction. Thus the reaction of succinimidoyl active ester 24 with glycine in 10 % aq. DMSO containing catalytic amount of buffer solution of pH=7 stirred for 4h afforded compound 25 in 63% yield was recrystallized from ethanol. (Experiment No.3, Page. No.114) This the compound 25 was characterized as follows, IR spectrum of compound shows broad peak at 3326 cm⁻¹ for acid OH and 2932 for NH group 1627 cm⁻¹ for carbonyl. The ¹H NMR spectrum in CDCl₃ (Spectrum No.6, Page.No.79) of this solid showed the disappearance of singlet of four protons of 2 x CH₂ groups and the appearance of doublet at 5.05 for N-CH₂ and doublet at δ 7.64 for C₄H aromatic carbon, broad singlet at δ 8.27 for –NH and aromatic protons are their respective region, the elemental analysis agrees with molecular formula C₁₅H₁₀N₆O₅. On the basis of this spectral and analytical methods structure was assigned to 2-(7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)-3-phenylpropanoic acid 25a this compound.
IR: 3326, 2932, 1722, 1627 cm\(^{-1}\)
Mass M/Z : 298 M
Mol.Wt. 298.26

Spectrum No.6 \(^1\)H NMR (CDCl\(_3\)): 2-(7-phenyl-7H-pyrazolo [3, 4-d][1,2,3]triazine-5-carboxamido)-3-phenylpropanoic acid. (25a).
3.3.4 Synthesis of 2-(7-Phenyl-7\-H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)acetic acid 25b

After the successful linking of glycine, we planned for another amino acid i.e. D-L-phenylalanine. Thus the reaction of succinimidoxy active ester 24 with D-L-phenylalanine in 10 % aq. DMSO containing catalytic amount of buffer solution of pH-7 stirred for 6h and then TLC check in chloroform: methanol (8:2) afforded a colorless solid in 57% yield, (Experiment No.3, Page No.113) which was recrystallized by ethanol. Thus the compound 25b was characterized as follows, IR spectrum of compound shows broad peak at 3342 cm\(^{-1}\) for acid OH and 2929 for NH group. The \(^1\)H NMR spectrum in CDCl\(_3\) (Spectrum No.7, Page.No.81) of this solid showed triplet at \(\delta\) 4.35 for two protons of CH\(_2\)-Ph and doublet at \(\delta\) 5.37 for N-CH and also showed doublet for one proton of C\(_4\)H at \(\delta\) 8.34, broad singlet at \(\delta\) 9.42 for –NH. The aromatic protons are appeared in their respective region. The \(^{13}\)C NMR also shows peak at \(\delta\)174.6 acid carbonyl and \(\delta\) 159.4 for the amide carbonyl. The aromatic carbons are appeared in the respective region. The elemental analysis agrees with molecular formula C\(_{13}\)H\(_{10}\)N\(_6\)O\(_3\). On the basis of this spectral and analytical methods structure was assigned to 2(7-Phenyl-7\-H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)acetic acid 25b this compound.
IR: 3342, 2929, 1742, 1658 cm\(^{-1}\)

Mass M/Z = 388M\(^+\)

Mol. Wt. = 388.38

Spectrum No.7 \(^1\)H NMR (DMSO-\(d_6\)): 2-(7-Phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamidol)acetic acid (25b)
Spectrum No. 8 $^{13}$C NMR (DMSO-d$_6$): 2-(7-Phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)acetic acid (25b).
3.3.5 Synthesis of Benzyl 3-(7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)-4-oxopentanoate 25c

Similarly amino acid i.e. 5-((benzyloxy)carbonyl)-2-aminopentanoic acid was condensed with active ester 24. The reaction of succinimidoyl active ester 24 with 5-(benzyloxy)carbonyl)-2-aminopentanoic acid in 10% aq. DMSO containing catalytic amount of buffer solution of pH-7 stirred for 10h and then TLC check in chloroform: methanol (8:2) afforded a colorless solid in 57% yield which was purified by ethanol. (Experiment No.3, Page.No.114). Thus the 25c was characterized as follows, IR spectrum of this compound showed broad peak at 3226 cm\(^{-1}\) for acid OH and 2929 cm\(^{-1}\) for NH group. The \(^1\)H NMR spectrum in CDCl\(_3\) (spectrum No.9, page No.84) of this solid all six (3×CH\(_2\)) methylene protons are appeared from \(\delta\) 1.24 to 1.87 and the doublet at \(\delta\) 4.83 for N-CH and broad singlet at \(\delta\) 5.09 for –O-CH\(_2\). Also showed doublet for one proton of C\(_4\)H at \(\delta\) 7.87 and broad singlet at \(\delta\) 8.35 for –NH. The elemental analysis agrees with molecule formula C\(_{22}\)H\(_{18}\)N\(_6\)O\(_5\). On the basis of above spectral and analytical data structure benzyl 3-(7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)-4-oxopentanoate 25c was assigned to this compound.
IR: 3226, 1753, 1663, 1593 cm$^{-1}$

Mass M/Z = 447M$^+$

Mol. Wt. = 446.42

Spectrum No. 9 $^1$H NMR(CDC$_3$) : Benzyl 3-(7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)-4-oxopentanoate (25c).
Further the hydrolysis of the ester 20 was carried out for the preparation of acid 26. The compound 20 was refluxed in ethanol containing 10% aq. NaOH for 4h TLC check in chloroform: methanol (8:2). After the completion of the reaction the solution was poured into the water and acidifies with dil. HCL. The solid precipitated was collected by filtration and washed with water to remove the acid and recrystallized in DMF: ethanol (7:3) dried in oven yielded compound 26 in 72% yield (Experiment No. 4, page no.116) which was characterized by spectral and analytical method. The IR of the spectrum of this solid shows broad peak at 3347 cm$^{-1}$ that confirms the presence of the OH group in the compound and peak at 1733cm$^{-1}$ is for the carbonyl group. The $^1$H NMR spectrum in CDCl$_3$ of this solid showed the broad singlet at $\delta$ 11.92 for acidic proton of OH group and disappearance of triplet quartet signal of the ethoxy group, which confirms the hydrolysis of the ester group. The aromatic protons are appeared to their respective region (Spectrum No.10, Page. No.86). The elemental analysis is in agreement with the molecular formula C$_{16}$H$_{13}$N$_3$O$_2$. On the basis of above spectral and analytical data structure benzyl 1-Phenyl-1,5,6,7-tetrahydro-1,8-diaza-s-indacene-3-carboxylic acid 26 was assigned to this compound.
IR: 3465, 1742, 1610, 1593 cm\(^{-1}\)
Mass M/Z = 280 M\(^+\)
Mol.Wt. = 279.29

Spectrum No. 10: \(^1\)H NMR (CDCl\(_3\)) : 1-Phenyl-1,5,6,7-tetrahydro-1,8-diaza-s-indacene-3-carboxyl
3.3.7 Synthesis of 1-Phenyl-1, 5, 6, 7-tetrahydro-1, 8-diaza-s-indacene-3-carboxylicacid2, 5-dioxo-pyrrolidin-1-yl-ester 27

The acid was successfully converted in to a desired succinimidoyl active ester 27 for the linkage of biological active moiety such as amino acids. Thus the reaction of 26 with N-hydroxysuccinimide in dry THF containing catalytic amount of dicyclohexylcarbodiimide (DCC) as water scavenger stirred at room temperature for 7h and then TLC check in chloroform: methanol 8:2 yielded 27 in 67%. (Experiment No.5, Page No.117) Thus the compound 27 was characterized as follows, IR of the compound shows broad singlet at 1768, 1762, cm⁻¹ and 1731 cm⁻¹ carbonyl group and it is also confirmed by the ¹H NMR spectrum in DMSO-d₆ which showed singlet at δ 2.98 for four protons of two methyl groups of N-hydroxysuccinimide and another singlet at δ 8.32 for C₄H (Spectrum No.11, Page. No.88). ¹³C NMR also clearly shows broad peak at δ 24.3 corresponds to two carbon of succinimidoyl and peak at δ 22.4, 32.3, and 34.6 for the three carbon of methylene carbon on pyridine and 169.5 for amide carbonyl carbon. The elemental analysis is in agreement with the molecular formula C₂₀H₁₆N₄O₄. On the basis of above spectral and analytical data structure benzyl 1-Phenyl-1,5,6,7-tetrahydro-1,8-diaza-s-indacene-3-carboxylicacid 2,5dioxo pyrrolidin-1-yl-ester 27 was assigned to this compound.
IR: 1768, 1762, 1731, 1610, 1592 cm\(^{-1}\)

Mass M/Z = 377M\(^+\)

Mol. Wt. = 376.37

Spectrum No. 11 \(^1\)H NMR (CDCl\(_3\)): 1-Phenyl-1, 5, 6, 7-tetrahydro-1, 8-diaza-s-indacene-3-carboxylic acid, 5-dioxo-pyrrolidin-1-yl-ester (27)
Spectrum No. 12 $^{13}$C NMR (CDCl$_3$): 1-Phenyl-1, 5, 6, 7-tetrahydro-1, 8-diaza-s-indacene-3-carboxylic acid, 2, 5-dioxo-pyrrolidin-1-yl-ester (27).
3.3.8 Synthesis of [(1-Phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino] acetic acid 28a

Scheme -IX

In the previous section we have studied the attachment of amino acids to triazole compounds. Now in this section the reaction on pyrazolo [3,4-b] pyridine ring was studied. After the successful synthesis of targeted active ester 27, the linking of the different amino acids was carried out. Thus the reaction of succinimidoyl active ester 27 with glycine in 10% aq. DMSO containing catalytic amount of buffer solution of pH-7 and stirring for 3h. The obtained solid was recrystallized by ethanol. (Experiment No.6, Page.No.118). The compound 28a was characterized as follows, the IR spectrum of compound shows broad peak at 3564 cm\(^{-1}\) for acid OH and 3352cm\(^{-1}\) for NH group 1650 cm\(^{-1}\) for carbonyl. The \(^1\)H NMR spectrum in CDCl\(_3\) (spectrum No.13, page no.91) of this solid showed multiplet and triplet at $\delta$ 1.72 to 2.19 for six protons of 3xCH\(_2\) groups and the appearance of doublet at $\delta$ 4.35 for CH\(_2\) and doublet for C\(_4\)H at $\delta$ 8.24, broad singlet at $\delta$ 8.42 for –NH and aromatic protons are appeared in their respective region. The elemental analysis agrees with molecular formula C\(_{18}\)H\(_{16}\)N\(_4\)O\(_3\). On the basis of above spectral and analytical data structure (1-Phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino] acetic acid 28a was assigned to this compound.
IR: 3564, 3352, 1650, 1610, 1593 cm$^{-1}$
Mass M/Z = 337M$^+$
Mol.Wt. = 336.34

Spectrum No.13 $^1$HNMR (CDCl$_3$): [(1-Phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino]-acetic acid (28a).
3.3.9 Synthesis of 1-Phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-S-indacene-3-carboxylic acid (1-benzyl2oxopropyl) amide 28b

After the successful attachment of glycine we had taken another amino acid i.e. D-L Phenylalanine. Thus the reaction of succinimidoyl active ester 27 with D-L Phenylalanine in 10 % aq. DMSO containing catalytic amount of buffer solution of pH-7 stirred for 8h. The obtained solid was recrystallized from ethanol. (Experiment No.6, Page.No.118). The compound 28b was characterized as follows, the IR spectrum of compound shows broad peak at 3496 cm⁻¹ for acid OH and 3326 for NH group 1649 cm⁻¹ for carbonyl. The 😝 NMR spectrum in (DMSO-dden) (spectrum No.13, page No.93) of this solid showed of multiplet and triplet at δ 2.01 to 2.95 for six protons of 3×CH₂ groups and doublet of two methylene protons at δ 4.84 for CH₂-Ph, the appearance of doublet at δ 5.42 for N-CH and also showed doublet for C4H pyridine at δ 8.26, broad singlet at δ 8.86 for –NH and aromatic protons are their respective region. The elemental analysis agrees with molecular formula C₂₅H₂₅N₄O₃. On the basis of above spectral and data structure28b1-Phenyl-1, 5, 6, 7-tetrahydro-1, 2,8-triaza-S-indacene-3-carboxylicacid (1benzyl 2-oxopropyl) 28b amide was assigned to this compound.
IR: 3564, 3352, 1649, 1609, 1590 cm⁻¹
Mass M/Z = 427M⁺
Mol.Wt. = 426.47

28 b

Spectrum No.13 ¹HNMR (DMSO-d₆): 1-phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-5-indacene-3-carboxylic acid (1benzyl2oxopropyl) amide (28b)
3.3.10 Synthesis of 3-Hydroxy-2-[(1-phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino]-propionic acid. 28c

Scheme -XI

The reaction of succinimidoyl active ester 27 with 2-amino-3-hydroxypropanoic acid in 10 % aq. DMSO containing catalytic amount of buffer solution of pH-7 stirred for 4h. The obtained solid was recrystallized from ethanol (Experiment No.6, Page No.118). The compound 28c was characterized as follows; the IR spectrum of compound shows broad peak at 3554 & 3328cm⁻¹ for OH groups and 2929 cm⁻¹ for NH group 1627 cm⁻¹ for carbonyl. The ¹H NMR spectrum in DMSO-d₆ (Spectrum No.14, Page No.95) of this solid showed of multiplet and triplet at δ 2.01 to δ 2.98 for six protons of 3x CH₂ groups and doublet of two methylene protons at δ 4.58 for CH₂-OH, the appearance of doublet at δ 5.58 for N-CH and doublet for aromatic C₄H at δ 8.30, broad singlet at δ 8.35 for –NH and broad singlet showed for two OH protons at δ 12.93 and the aromatic protons are their respective region. The elemental analysis agrees with molecular formula C₁₉H₁₈N₄O₄. On the basis of above spectral and data structure 3-Hydroxy-2-[(1-phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino]-propionic acid 28c was assigned to this compound.
IR: 3554, 3328, 2929, 1743, 1627 cm⁻¹

Mass M/Z = 367M⁺

Mol. Wt. = 366.37

Spectrum No. 14 ¹H NMR (DMSO-d6): 3-Hydroxy-2-[(1-phenyl-1,5,6,7-tetrahydro-1,2,8-triazas-indacene-3-carbonyl)-amino]-propionic acid
3.3.11 Synthesis of 3-Methyl-2-[(1-phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino]-butyric acid 28d

As per above methods we had analogously prepared the next attachment of the amino acid i.e. L-Valine. Thus the reaction of succinimidoyl active ester 27 with L-Valine in 10 % aq. DMSO containing catalytic amount of buffer solution of pH-7 stirred for 6h. The obtained solid was recrystallized by ethanol (Experiment No.6, Page.No.118). The compound 28d was characterized as follows, IR spectrum of compound shows broad peak at 3467 cm\(^{-1}\) for acid OH and 3336 cm\(^{-1}\) for NH group 1632 cm\(^{-1}\) for carbonyl. The \(^1\)H NMR spectrum in DMSO-\(d_6\) (Spectrum No.15, Page. No.97) of this solid showed doublet at \(\delta\) 1.06 for six 2xCH\(_3\) methyl protons and multiplet and triplet at \(\delta\) 2.01 to \(\delta\) 2.98 for six protons of 3xCH\(_2\) groups, the triplet at \(\delta\) 4.42 for one proton of CH, appearance of doublet at \(\delta\) 5.47 for N-CH. The aromatic proton C\(_4\)H appeared doublet at \(\delta\) 8.27 and also doublet at \(\delta\) 8.35 for -NH. Also \(^13\)CMR showed peak at \(\delta\) 18.7, 18.9 for 2xCH\(_3\) carbon also shows \(\delta\) 57.2 ppm for N-CH carbon, the amide and acid carbonyl appeared at \(\delta\) 168.5 and 172.3 ppm respectively. The elemental analysis agrees with molecular formula C\(_{21}\)H\(_{22}\)N\(_4\)O\(_3\). On the basis of above spectral and data structure 3-Methyl-2-[(1-phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino]-butyric acid 28d was assigned to this compound.
IR: 3467, 3336, 1632 cm$^{-1}$
Mass M/Z = 378M$^+$
Mol.Wt. = 378.42

Spectrum No. 15 $^1$H NMR (DMSO-d6): 3-Methyl-2-[(1-phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl-amino]-butyric acid (28)
Spectrum No. 16 $^{13}$C NMR (DMSO-$d_6$): 3-Methyl-2-[(1-phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino]-butyric acid (28d)
Table 1: Reaction time and yield of amino acid derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time in hrs.</th>
<th>Amino Acid</th>
<th>Yield in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>25a</td>
<td>4</td>
<td>Glycine</td>
<td>57</td>
</tr>
<tr>
<td>25b</td>
<td>6</td>
<td>D-L Phenylamine</td>
<td>63</td>
</tr>
<tr>
<td>25c</td>
<td>10</td>
<td>Benzyl-L-Glutamate</td>
<td>58</td>
</tr>
<tr>
<td>28a</td>
<td>3</td>
<td>Glycine</td>
<td>67</td>
</tr>
<tr>
<td>28b</td>
<td>8</td>
<td>DL-Phenylamine</td>
<td>54</td>
</tr>
<tr>
<td>28c</td>
<td>4</td>
<td>2-Amino-3-hydroxypropanoic acid</td>
<td>58</td>
</tr>
<tr>
<td>28d</td>
<td>6</td>
<td>L-Valanine</td>
<td>53</td>
</tr>
</tbody>
</table>

From above table it was observed that the rate of reaction with linkers is depends upon steric interaction involved by amino acids. The benzyl-L-glutamate is bulkier than the other two amino acids i.e. DL-phenyl amine and glycine required higher reaction time (10 hrs) to condense with CO group although the O-N nitrogen bond is polar. Similarly, the DL-phenyl amine is bulkier than L-valanine and glycine required 8 hrs, while the reaction with amino acids was completes within 3 and 6 hrs respectively (Table 1).
3.3.12 Synthesis of 7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carbohydrazide

Further the compound 9 having ester functional group at the terminal of the pyrazole we planned to convert it into amide, refluxed in ethanol with excess hydrazine hydrate for 4 hrs. The obtained solid was recrystallized by ethanol.(Experiment No.7, Page No.121) The compound 29 was characterized as follows, the IR spectrum of compound shows 3468 cm⁻¹, 3448 cm⁻¹ doublet for NH₂ broad singlet at 3312 cm⁻¹ for -NH and 1668 cm⁻¹ for carbonyl amide. The ¹H NMR spectrum in DMSO-d₆ (spectrum No.17 page no.101) of this solid showed of broad singlet at δ 4.53 for -NH₂ another broad singlet at δ 10.01 for the -NH and sharp singlet at δ 9.42 was for C₄H. The aromatic protons are appeared at their respective region. The elemental analysis is in agreement with the molecular formula C₁₁H₈N₇O. On the basis of this data structure 7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carbohydrazide 29 was assigned to this compound.
IR: 3468, 3448, 3312, 1668, 1590 cm⁻¹
Mass M/Z = 255M⁺
Mol.Wt. = 255.24

Spectrum No. 17 $^1$HNMR (DMSO-d$_6$): 7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxydrazide 29.
3.3.13 Synthesis of 7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazine-5-carbohydrazide 31 (a-d)

Scheme - XIV

$\text{Ar; } 31, \text{a=Ph, } b=p\text{-cl-}C_{6}H_{4}; \text{c= m, } p\text{-OCH,C}_{6}H_{5}; \text{d= p-NO}_{2}\text{C}_{6}H_{4}$

Compound 29 was planned to convert into Schiff base by using a simple and convenient method to explore the chemistry. We have condensed different aromatic aldehydes (30) e.g. p-cl-aldehyde (31b) in the presence of catalytic amount of acetic acid, in ethanol refluxed for 4h. The obtained solid was recrystallized by methanol. (Experiment No.8, page No.121) The compound 31b was characterized as follows, the IR spectrum of compound shows a broad singlet at 3253 cm$^{-1}$ for $-\text{NH}$ and 1685 cm$^{-1}$ for carbonyl amide. The $^1$H NMR spectrum in DMSO-$d_6$ (Spectrum No.18, Page No.103) of this solid showed a broad singlet at $\delta$ 12.10 for the $-\text{NH}$ and another singlet at $\delta$ 9.49 for $-\text{NCH}$ and also shows a singlet at $\delta$ 8.59 for the proton on triazine ring. The aromatic protons are in their respective region shows clear parasyalloi substitution pattern. $^{13}$CMR clearly showed peak $\delta$ 157.8 for amide carbonyl, also peak at $\delta$148.6 for N-CH carbon. The elemental analysis is in agreement with the molecular formula $C_{18}H_{12}ClN_{7}O$. On the basis of above spectral and analytical data structure (E)-N'-(4-chlorobenzylidene)-7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazine-5-carbohydrazide. 31b was assigned to this compound. Analogously we had synthesized all the compound 31(a-d).
IR: 3253, 2241, 1632, 1590 cm⁻¹
Mass M/Z = 378M⁺
Mol.Wt. = 377.79

Spectrum No.18 ¹HNMR (DMSO-d₆): (E)-N’-(4-chlorobenzylidene)-7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxyrazide, (31b)
Spectrum No. 19 $^{13}$C NMR (CDCl$_3$): (E)-N’-(4-chlorobenzylidene)-7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazine-5-carbohydrazide (31b)
3.3.14 Synthesis of potassium salt of thiocarbazinate 32

![Scheme XV](image)

The triazene 5-carbohydrazide on reaction with carbon disulfide and potassium hydroxide in ethanol was reflux for 12h yielded the potassium salt of thiocarbazinate, the important intermediate for the synthesis new derivatives. The separated yellow color solid during the reaction was filtered washed with ethanol and dried. The compound 32 was used further for the synthesis of triazine derivatives.

3.3.15 Synthesis of 4-Amino-5-(7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazin-5-yl)-4H-[1,2,4] triazole-3-thiol 33

![Scheme XVI](image)
The intermediate potassium salt 32 was used to cyclise with hydrazine hydrate in water refluxed for 18-20 h. TLC check in chloroform: methanol (8:2). The reaction was cooled to room temperature and neutralized with dilute HCL. The separated solid was collected by suction and washed with water; afforded colorless solid was recrystallized from DMF-Water (80:20) yielded compound 33 in 62% yield (Experiment No.9, page no.124). The compound 33 was characterized as follows, the IR spectrum of compound shows broad singlet at 3341 cm⁻¹ for –NH₂ and 2560 cm⁻¹ for mercapto (SH) group. The ¹H NMR spectrum in DMSO-_-d₆ of this solid showed of broad singlet at δ 11.28 for the –SH (D₂O exchangeable) and another singlet at δ 8.94 for –C₄H and also shows singlet at δ 6.01 for two protons of NH₂ (Spectrum No.20, Page. No.107). ¹³CMR showed δ 162.6 for the carbon adjacent to mercapto group, i.e. (C-SH) δ 156.7 for the other carbon in triazine ring. The aromatic protons are appeared in their respective region. The elemental analysis is in agreement with the molecular formula C₁₂H₉N₉S. On the basis of above spectral and analytical data structure 33 was assigned to this compound.
IR: 3341, 2560, 1709, 1610, 1590 cm$^{-1}$

Mass $M/Z = 312^*$

Mol. Wt. = 311.23

Spectrum No. 20 $^1$H NMR (DMSO-$d_6$): 4-Amino-5-(7-phenyl-7H-pyrrolo[3,4-$d$][1,2,3]triazin-5-yl)-4H-[1,2,4]triazole-3-thiol (33)
Spectrum No.21 $^{13}$C NMR (DMSO-$d_6$): (E)-N'-(4-chlorobenzylidene)-7-phenyl-7$H$-pyrrolo[3,4-][1,2,3]triazine-5-carboxyhydrazide.33
3.4 EXPERIMENTAL BIOASSAY

The stored grain insect *Callosobruchus Chinensis Linn* was collected from Manmad of Food Corporation of India warehouse. The insect were brought to laboratory and cultured in propylene plastic containers on disinfected dry gram seeds at temperature 26±2 °C; RH; 65-70%, photoperiod (L: D, 12: 12) and the culture was maintained under laboratory conditions. The fresh adult insects were used for toxicity testing against compounds. The stock solutions were prepared for all testing chemical compounds by using DMF as solvent and further concentration of each target compound were made as 100 ppm to 500 ppm concentration in distilled water. The adult were selected for toxicity testing. Petri dishes were labeled as a 100 ppm, 200ppm, 300ppm, 400ppm, and 500ppm. The filter papers were put in each Petri dish according to their inner diameter size; 5-6 drops of each concentration were smeared with disinfected dried gram seeds and kept in respective petriplates and 10 fresh adult were released in each Petri dishes. The control set was arranged with DMF and mortality was observed for 24 hrs exposure.

The LC_{50} value Heterogeneity, variance and fiducial limits were calculated following finney’s method (1971)

Table II: Insecticidal activity of new triazine and triaza-s-indacene pyrazole derivatives against *Callosobruchus Chinensis L* after 24h exposure at ambient temperature 26±2.

<table>
<thead>
<tr>
<th>Compd No.</th>
<th>Structure</th>
<th>Dose in PPM</th>
<th>% Mortality</th>
<th>LC_{50} ± SE</th>
<th>Heterogeneity</th>
<th>Variance</th>
<th>Fiducial Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td><img src="image_url" alt="Structure" /></td>
<td>100</td>
<td>30</td>
<td>1.7379 ± 0.9957</td>
<td>1.9831</td>
<td>0.9915</td>
<td>M_1 = 2.1915, M_2 = -1.7114</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>25a</td>
<td></td>
<td>100</td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>50</td>
<td>40</td>
<td>40</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3175 ± 0.8483</td>
<td>0.1427</td>
<td>0.7197</td>
<td>M₁ = 1.7824</td>
<td>M₂ = -1.543</td>
<td></td>
</tr>
<tr>
<td>25b</td>
<td></td>
<td>100</td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>40</td>
<td>40</td>
<td>10</td>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4678 ± 0.8946</td>
<td>9.9591</td>
<td>0.8004</td>
<td>M₁ = 1.867</td>
<td>M₂ = -1.64</td>
<td></td>
</tr>
<tr>
<td>25c</td>
<td></td>
<td>100</td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>50</td>
<td>20</td>
<td>60</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3448 ± 0.8841</td>
<td>28.0485</td>
<td>0.7817</td>
<td>M₁ = 1.8615</td>
<td>M₂ = -1.6043</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>100</td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>30</td>
<td>30</td>
<td>70</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4092 ± 0.9453</td>
<td>1.8793</td>
<td>0.8936</td>
<td>M₁ = 2.0016</td>
<td>M₂ = -1.7081</td>
<td></td>
</tr>
<tr>
<td>28b</td>
<td></td>
<td>100</td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>40</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3083 ± 0.8446</td>
<td>292.100</td>
<td>0.7135</td>
<td>M₁ = 1.772</td>
<td>M₂ = -1.538</td>
<td></td>
</tr>
<tr>
<td>28d</td>
<td></td>
<td>100</td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>80</td>
<td>40</td>
<td>70</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9478 ± 0.8283</td>
<td>7.1006</td>
<td>0.6861</td>
<td>M₁ = 1.6002</td>
<td>M₂ = -1.6466</td>
<td></td>
</tr>
<tr>
<td>31b</td>
<td></td>
<td>100</td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>60</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.8654 ± 0.6786</td>
<td>4.5432</td>
<td>0.7654</td>
<td>M₁ = 1.454</td>
<td>M₂ = -1.24</td>
<td></td>
</tr>
<tr>
<td>31d</td>
<td></td>
<td>100</td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>60</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6509 ± 0.9208</td>
<td>2.1132</td>
<td>0.8479</td>
<td>M₁ = 2.0660</td>
<td>M₂ = -1.64661</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td></td>
<td>100</td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>50</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2052 ± 0.8333</td>
<td>-1.1857</td>
<td>0.6945</td>
<td>M₁ = 1.7143</td>
<td>M₂ = -1.552</td>
<td></td>
</tr>
</tbody>
</table>

8,23,24,28a, 28c 30b, 30c

Do not show the activity

The data are the average of three replicates and significant at P< 0.05%
The insecticidal activities of the new triazene and triaza-s-indacene pyrazole derivatives against stored grain pest *Callosobruchus chinensis* L were investigated and depicted in Table-II. The compound 28d shows excellent insecticidal activity against *C. Chinensis* L (LC₅₀ = 0.9478 ± 0.8283) due to amide as well as acid, isopropyl (aliphatic chain) group which increase the substitution pattern at the chiral carbon while other title compounds show good to moderate insecticidal activity for 24h exposure.

### 3.5 Conclusion

A new class of amino acid linked pyrrolo[3,4-d][1,2,3]triazine, tetrahydro-1,2,8-triaza-s-indacene were obtained in good yield from pyrazolo triazene-5-carboxylate 9 with simple workup and clean products. Also, novel series of [1,2,3]triazine-5-carboxyhydrazide 31(a-d) and triazene[1,2,4]triazol-3-thiol 33 were synthesized in good yields. The insecticidal activities of the new triazene and triaza-s-indacene pyrazole derivatives against stored grain pest *Callosobruchus chinensis* L were investigated showed that compounds 28a, 28c, 9, 33, 28c, 9, 28b, 28a, 28d showed higher (LC₅₀ = 1.3448 ± 0.8483 to 1.2052 ± 0.8333) LC₅₀, compounds 9, 28b, 28a, 28d (LC₅₀ = 1.42092 ± 0.9453 to 1.7379 ± 0.9957 1.4092) showed moderate and compound 28d showed excellent insecticidal activity against *C. Chinensis* L (LC₅₀ = 0.9478 ± 0.8283) for 24h exposure. The compounds containing amide acid and higher branching groups found to have higher activity while the compounds 23, 24, 31b, 31c, 8, 26, 27, 28a do not showed any activity against these species.
3.6 EXPERIMENTAL PART

Experiment No.1

Synthesis of 7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxylic acid (23)

\[
\begin{align*}
&\text{EtO} \\
&\text{EtOH, KOH} \\
&\text{HCl} \\
&\text{reflux} \\
&\text{7-8h} \\
\end{align*}
\]

\[\text{N} \]
\[\text{Ph} \]

\[\text{23} \]

72%

Scheme-I

Procedure:

A solution of 9 (0.27 g, 10 mmol) in ethanol (25 ml) and 10% aq. NaOH (2mL) was heated at reflux temperature for 7-8 h (TLC check toluene: acetone 8:2). The ethanol was removed under reduced pressure and the residue dissolved in water (50 ml). The mixture was acidified with dil. HCl and the resulting precipitate was filtered by suction and washed with water, dried and recrystallized from ethanol to afford 23 as, colorless solid.

7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxylic acid (23)

Recrystallized from ethanol, m.p. 205-207 °C, Yield 72 %, colorless solid.

IR (KBr): 3400 bs (OH), 1610 (C=N), 1533(C=C) cm\(^{-1}\)

\(^1\)H NMR (DMSO-\(d_6\)) \(\delta = 7.43-8.77\) (m, 5H, Ar H), 9.26 (s, 1H, Ar H), 15.80 (bs, 1H, OH).
$^{13}$C NMR (DMSO-$d_6$) $\delta$ 117.7, 118.8, 119.4, 128.3, 129.8, 130.6, 132.5, 138.3, 143.3, 161.2, 165.3; MS (70 eV) m/z (%): 242 (M$^+$).

Anal. Calcd for C$_{11}$H$_7$N$_5$O$_2$ Mol. Wt. (241.21); Calcd: C 54.77, H 2.93, N 29.03; Found: C 54.67, H 2.87, N 29.18.

Experiment No.2

Synthesis of 7-Phenyl-7H-pyrrolo[3,4-d][1,2,3]triazene-5-carboxyacid 2,5-dioxopyrrolidine-1-ylester (24)

Scheme-II

Procedure: A solution of 23, (0.24g, 10mmol), N-hydroxysuccinimide (0.115g, 10mmol) and dicyclohexylcarbodiimide (DCC) (0.20g, 10 mmol) as water scavenger in dry tetrahydrofuran (25 ml) was stirred at room temperature for 7h (TLC check toluene: acetone 8:2). Then the solvent was removed under reduced pressure. The gummy mass obtained was stirred in ethanol, the solid precipitated out was filtered by suction, washed with ethanol, dried and recrystallized from ethanol to afford 24 as colorless solid.

7-Phenyl-7H-pyrrolo[3,4-d][1,2,3]triazene-5-carboxyacid 2,5-dioxopyrrolidine-1-ylester(24)

Recrystallized from ethanol, m.p. 226-227 °C, Yield 67 %, colorless solid.
IR (KBr): 2948 (C-H arom), 1768 (C=O), 1739, 1665 (C=O amide), 1596 (C=C), cm⁻¹

¹H NMR (CDCl₃) δ 2.97 (s, 4H, 2×CH₂), 7.27-7.74 (m, 5H, Ar H), 8.26 (s, 1H, Ar H).

¹³C NMR (CDCl₃). δ 23.8, 25.5, 30.0, 34.3, 116.2, 122.5, 125.0, 127.6, 129.0, 129.8, 135.3, 138.4, 150.8, 157.3, 169.0. MS (70 eV) m/z (%): 338 (M).


**Experiment No.3**

**Synthesis of 2-(7-phenyl-7H-pyrazolo [3,4-d][1,2,3]triazine-5-carboxamido)-3-phenylpropanoic acid. 25 (a-c)**

![Scheme-III](image)

**General Procedure:**

A solution of succinimidoyl active ester 21 (1.69g, 5 mmol) was dissolved in dimethylsulfoxide (5 ml) and drops wise added in to the solution of glycine, (D, L)-phenylalanine (5 mmol) in 10 % aq. dimethylsulfoxide (5 ml) at 20 °C with constant stirring. After this the buffer solution of pH-7 (0.5 ml) was added drop wise in to above
solution. Then the reaction mixture was stirred at 45-55 °C for 7 h (TLC check in toluene: acetone 8:2) and the solution was poured in to cold water (20 ml) and neutralized by dil. HCl, the solid separated out was filtered by suction, washed with water, dried and recrystallized from ethanol to afford 25a in 50-55 % yield.

**2-(7-phenyl-7//-pyrazolo[3,4-r][1,2,3]triazine-5-carboxamido)-3-phenylpropanoic acid(25a)**

Recrystallized from ethanol, m.p. 285-286°C, Yield 53%, colorless solid.

IR (KBr): 3326 (OH), 2932 (NH), 1668 (C=O, amide), 1540(C=C) cm⁻¹.

1H NMR (CDCl₃) δ 5.05 (d, 2H, J = 5.7 Hz, N CH₂), 7.38-7.52 (m, 5H, Ar H), 7.64 (d, 1H, J = 7.8 Hz, ArH), 8.27 (s, 1H, N-H); MS (70 eV) m/z (%): 298 (M).

Anal. Calcd. for C₁₃H₁₀N₆O₃ Mol. Wt. (298.26); Calcd C, 52.35; H, 3.38; N, 28.18.

Found: C, 52.42; H, 3.35; N, 28.23.

**2-(7-Phenyl-7//-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)acetic acid (25b)**

Recrystallized from ethanol, m.p. 305-306°C, Yield 57%, colorless solid.

IR (KBr) 3326 (bs, OH), 2929 (bs, NH), 1627 (C=O, amide), cm⁻¹

1H NMR (CDCl₃): δ 4.35 (m, 2H, CH₂), 5.37 (d, 1H, N CH), 7.46 -7.61 (m, 5H, Ar H), 7.95 (d, J = 8.2Hz, 2H, Ar H), 7.99 (m, 3H, Ar H ); 8.34 (d, J = 8.7 Hz 1H, ArH), 9.42 (s, 1H NH )

13C NMR (CDCl₃). δ 18.3, 19.1, 24.3, 25.2, 29.6, 33.2, 47.4, 57.5, 93.5, 112.4, 119.7, 128.5, 129.6, 136.6, 138.0, 147.4, 158.9, 172.3. MS (70 eV) m/z (%): 389 (M⁺).

Anal. Calcd. for C₂₀H₁₆N₆O₃ Mol. Wt. (388.88); Calcd C, 61.85; H, 4.15; N, 21.64.

Found: C, 61.72; H, 4.35; N, 21.43.
Benzyl3-(7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carboxamido)-4-oxopentanoate (25c)

Recrystallized from ethanol, m.p. 318-319°C, Yield 58%, colorless solid.

IR (KBr) 3326 (bs, OH), 2929 (bs, NH), 1733, 1627 (C=O, amide), cm⁻¹

¹H NMR (CDCl₃) δ 1.31 (t, 2H, CH₂), 1.57 (m, 2H, CH₂), 1.64 (t, 2H, CH₂), 4.83 (m, 1H, N CH), 5.09 (s, 2H, OCH₂), 7.43-7.71 (m, 10H, ArH), 7.71 (d, J = 7.8 Hz, 1H, NH), 8.35 (s, 1H, Ar H);

Anal. Calcd. for C₂₄H₂₂N₆O₅ Mol.Wt.(474.17); Calcd. C, 60.75; H, 4.67; N, 17.71.

Found: C, 60.84; H, 4.54; N, 17.83.

Experiment No. 4

Synthesis of 1-Phenyl-1, 5, 6, 7-tetrahydro-1, 8-diaza-s-indacene-3-carboxylic acid (26)

![Scheme-IV](image)

**Procedure:**
A solution of 20, 0.30 g (10 mmol) in ethanol (25 ml) and NaOH (10% aq. 2 mL) was heated at reflux temperature for 4h. (TLC check, toluene: acetone 8:2). Then the ethanol was removed under reduced pressure and the residue dissolved in water (50 ml). The
mixture was acidified with con. HCl and the resulting precipitate was filtered by suction and washed with water, dried in oven.

1-Phenyl-1,5,6,7-tetrahydro-1,8-diaza-s-indacene-3-carboxylic acid (26)
Recrystallized from DMF: ethanol (8:2), m.p. 205-207 °C, Yield 72%, colorless solid.
IR (KBr): 3347 (OH), 1733 (C=O), 1533 (C=C), cm⁻¹
¹H NMR (DMSO-ᴅ) δ = 1.90 (m, 2H, CH₂), 3.54 (m, 4H, 2×CH₂), 7.24-8.23 (m, 5H, Ar H), 8.74 (s, 1H, Ar H), 13.26 (bs, 1H, OH). MS (70 eV) m/z (%): 280 (M⁺).
Anal. Calcd. for C₁₆H₁₃N₃O₂: Mol.Wt.(279.29); Calcd. C 68.81; H, 4.69; N, 15.05; Found. C, 68.71; H, 4.67; N, 15.18;

Experiment No. 5
Synthesis of 1-Phenyl-1, 5, 6, 7-tetrahydro-1, 8-diaza-s-indacene-3-carboxylic acid2, 5-dioxo-pyrrolidin-1-yl-ester (27)

**Scheme-V**

Procedure:
A solution of 26 0.27 g (10 mmol), N-hydroxsuccinimide (10 mmol) and dicyclohexyl carbodiimide (DCC) (10 mmol) as water scavenger in dry tetrahydrofuran (25 ml) was stirred at room temperature for 7 h. (TLC check, toluene: acetone 8:2). Then the solvent was removed under reduced pressure. The gummy mass obtained was stirred in ethanol, the solid precipitated out was filtered by suction, washed with ethanol, dried in oven.
Recrystallized from ethanol, m.p. 226-227 °C, Yield 67%, colorless solid.

IR (KBr): 3546, 2948, 1768, 1739, 1596, 1498, 1301 cm⁻¹.

¹H NMR (CDCl₃) δ = 2.29 (t, 2H, CH₂), 2.97 (s, 4H, 2CH₂), 3.14 (m, 4H, 2CH₂), 7.42-7.57 (m, 5H, Ar H), 8.28 (s, 1H, Ar H).

¹³C NMR (CDCl₃) δ 23.8, 25.5, 30.0, 34.3, 116.1, 122.4, 125.0, 127.6, 129.4, 129.8, 135.3, 138.4, 150.8, 157.2, 169.3.

Anal. Calcd. for C₂₀H₁₆N₄O₄ : Mol.Wt.376.37; Calcd. C, 63.82; H, 4.28; N, 14.89; Found. C, 63.73; H, 4.37; N, 14.82.

Experiment No. 6

Synthesis of Pyrazolo[3,4-b]pyridines with Amino Acid linkers 28 (a-d)

![Chemical structure](image)

General Procedure:

A solution of succinimidoyl active ester 23 1.88 g (5 mmol) was dissolved in dimethylsulfoxide (5 ml) and drops wise added in to the solution of glycine, L-valine or (D, L)-phenylalanine (5 mmol) in 10 % aq. dimethylsulfoxide (5 ml) at 20 °C with
constant stirring. After this the buffer solution of pH7 (0.5 ml) was added drop wise in to above solution. Then the reaction mixture was stirred at 45-55 °C for 7 h (TLC check, toluene: acetone 8:2) and the solution was poured in to cold water (20 ml) and neutralized by dil. HCl, the solid separated out was filtered by suction, washed with water, dried and recrystallized from ethanol to afford 28(a-d) as colourless solid in 50-55 % yield.

[(1-Phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino]-acetic acid (28a)

Recrystallized from ethanol, m.p. 205-207 °C, Yield 67%, colorless solid.

IR: (KBr): 3564,(OH) 3352 (NH), 1653(amide), 1533(C=C), 1415 and 1320. cm⁻¹;

¹H NMR (CDCl₃) δ = 1.72 (m, 2H, CH₂), 1.94-2.19 (m, 4H, 2×CH₂), 4.35 (d, J = 6.1Hz, 2H, N-CH₂), 7.22-8.22 (m, 5H, Ar H), 8.25 (s, 1H, Ar H), 8.42 (s, 1H, NH). MS (70 eV) m/z (%):337( M⁺1)


1-Phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-S-indacene-3-carboxylie acid (1-benzyl2oxopropyl) amide (28b)

Recrystallized from ethanol, m.p. 205-207 °C, Yield 54%, colorless solid.

IR (KBr): 3496 (OH), 3326 (NH), 2929, 1739 (C=O), 1625 (amide), 1533(C=C) and 1415 cm⁻¹;
\(^1\)H NMR (DMSO-\(d_6\)) \(\delta = 2.01\) (m, 2H, CH\(_2\)), 2.28-2.73 (m, 4H, 2\(\times\)CH\(_2\)), 4.62 (d, 2H, CH\(_2\)), 5.58 (d, \(J = 7.2\) Hz 1H, N CH), 7.20-7.84 (m, 10H, ArH), 8.24 (d, 1H, ArH), 8.34 (s, 1H, NH). MS m/z : 427 M+1


3-Hydroxy-2-[(1-phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino]-propionic acid (28c)

Recrystallized from ethanol, m.p. 227-228 °C, Yield 58%, colorless solid.

IR (KBr): 3554(OH), 3328 (NH), 1743 (C=O), 1627 (amide), 1569 (C=C), 1496, and 1320 cm\(^{-1}\);

\(^1\)H NMR (DMSO-\(d_6\)) \(\delta = 1.96\) (m, 2H, CH\(_2\)), 2.48-2.73 (m, 4H, 2\(\times\)CH\(_2\)), 4.58 (d, \(J = 6.1\) Hz, 2H, CH\(_2\)), 5.58 (d, \(J = 7.2\) Hz 1H N-CH), 7.42-8.31 (m, 5H, Ar H), 8.30 (s, 1H, Ar H), 8.35 (s, 1H, NH), 12.91 (bs, 2H, CH\(_2\)-OH & CO-OH ) MS (70 eV) m/z (%): 366 (M).

Anal. Calcd. for C\(_{19}\)H\(_{18}\)N\(_4\)O\(_4\): Mol. Wt.366.76; Calcd. C, 62.29, H 4.95, N 15.29;


3-Methyl-2-[(1-phenyl-1,5,6,7-tetrahydro-1,2,8-triaza-s-indacene-3-carbonyl)-amino]-butyric acid (28d)

Recrystallized from ethanol, m.p. 215-217 °C, Yield 53%, colorless solid.

IR (KBr): 3467 (OH), 3336 (NH), 1738 (C=O), 1632 (amide). cm\(^{-1}\)

\(^1\)H NMR (DMSO-\(d_6\)) \(\delta = 1.06\) (d, \(J = 6.6\)Hz, 6H, 2\(\times\)CH\(_3\)), 2.01 (m, 2H, CH\(_2\)), 2.11-2.98 (m, 4H, 2\(\times\)CH\(_2\)), 4.42 (t, 1H, CH), 5.47 (t, \(J = 6.6\)Hz, 1H, N CH), 7.36-8.31 (m, 5H, Ar H), 8.31 (s, 1H, Ar H), 8.43 (s, 1H NH).
Experiment No. 7

Synthesis of 7-phenyl-7H-pyrazolo[3,4-d][1,2,3]triazine-5-carbohydrazide 29.

![Scheme-VII]

**Procedure**: A mixture of compound 9 0.27 g. (10 mmol) and hydrazine hydrate (0.05 mL, 1 mmol) in ethanol (10mL) was refluxed 4h TLC check, chloroform: methanol, (8:2). The reaction mixture after cooling was poured over cold water. Recrystallized from ethanol, m.p. 325-326 °C, Yield 75%, colorless solid.

IR (KBr): 3468(d, NH$_2$), 3312 (bs, NH), 1668 (C=O, amide), cm$^{-1}$.

$^1$H NMR (DMSO-$d_6$) δ 4.60 (s, 2H, NH$_2$), 7.43-7.95 (m, 5H, Ar H), 9.42 (s, 1H, ArH), 9.96 (s, 1H, NH), MS (70 eV) m/z (%): 256 (M$^+$).

Anal. Calcd. For C$_{11}$H$_7$N$_7$O, Mol. Wt. 255.24; Calcd. C, 51.76; H, 3.55; N, 38.41.

Found: C, 51.67; H, 3.65; N, 38.31.
Experiment No.8

Synthesis of 7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazine-5-carbohydrazide 31(a-d)

Scheme-VIII

30; Ar, a= Ph, b=P-Cl-C₆H₄, c=m, p-OCH₃C₆H₄, d= p-NO₂C₆H₄

General Procedure (31a-d): Equimolar quantities of pyrrolo[2,3-d][1,2,3]triazine-5-carbohydrazide 5 0.25 g (10 mmol) and different aromatic aldehyde (2 mmol) was refluxed in alcohol for 4 h in the presence of few drops of glacial acetic acid. TLC check, chloroform: methanol, (8:2) the reaction mixture after cooling was poured in cold water. Precipitated solid was filtered and dried in oven. The crude solid was recrystallized in DMF–water (9:2).

(E)-N'-benzylidene-7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazine-5-carbohydrazide (31a)

Recrystallized from DMF–water (9:2). m.p. 352-353 °C, Yield 75%, colorless solid

IR (KBr) : 3203 (N-H), 2235 (C=N), 1653 (amide) cm⁻¹

¹H NMR (DMSO-d₆) δ 7.47-7.99 (m, 1 OH, Ar H), 8.59 (s, 1H, Ar H ), 9.50 (s, 1H, CH), 12.08 (s, 1H, NH), MS m/z : 343.


Found: C, 62.72; H, 3.75; N, 28.42.
(E)-N’-(4-chlorobenzylidene)-7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazine-5-carbohydrazide (31b)

Recrystallized from DMF-water (9:2), m.p. 348-349°C, Yield 78%, colorless solid.

IR (KBr): 3253 (NH), 2241 (C=N), 1685 (amide) cm⁻¹.

¹H NMR (DMSO-d₆) δ 7.48-7.56 (m, 5H, Ar H), 7.60 (m, 1H, Ar H), 7.63 (d, J = 7.8 Hz, 2H, Ar H), 7.75 (d, J = 8.1 Hz, 2H, Ar H), 8.59 (s, 1H, Ar H), 9.49 (s, 1H, C-H), 12.10 (s, 1H, NH).

¹³C NMR (DMSO-d₆) 116.3, 119.8, 123.6, 128.6, 129.8, 130.2, 132.3, 134.3, 135.8, 138.3, 139.4, 148.6, 149.3, 157.8. MS (70 eV) m/z (%): 377(M⁺).


Found: C, 57.42; H, 3.35; N, 25.82.

(E)-N’-(3,4-dimethoxybenzylidene)-7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazine-5-carbohydrazide (31c)

Recrystallized from DMF-water (9:2), m.p. 338-340°C, Yield 82%, colorless solid.

IR (KBr): 3238 (N-H), 2241 (C=N), 1681 (amide), 1602. cm⁻¹

¹H NMR (DMSO-d₆) δ 3.79 (s, 6H, 2xCH₃), 7.03-7.33 (m, 5H, Ar H), 7.47 (d, J = 7.8 Hz, 2H, Ar H), 7.57 (s, 1H, Ar H), 8.48 (s, 1H, Ar H), 9.46 (s, 1H, C-H), 11.94 (s, 1H, NH). MS (70 eV) m/z (%): 403(M⁺).


Found: C, 59.62; H, 4.35; N, 24.42.

(E)-N’-(4-nitrobenzylidene)-7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazine-5-carbohydrazide (31d)

Recrystallized from DMF-water (9:2), m.p. 327-329°C, Yield 85%, colorless solid.
IR (KBr): 3241 (N-H), 2218 (C=N), 1659 (amide), 1596 cm⁻¹

¹H NMR (DMSO-d₆) δ 7.48-7.99 (m, 5H, Ar H), 8.14 (d, J = 7.8 Hz, 2H, Ar H), 8.27 (d, J = 7.8 Hz, 2H, Ar H), 8.70 (s, 1H, Ar H), 9.51 (s, 1H, CH), 12.34 (s, 1H, NH), MS (70 eV) m/z (%): 388(M⁺).


Found: C, 55.62; H, 3.25; N, 28.72.

Experiment No. 9

Synthesis of 4-Amino-5-(7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazin-5-yl)-4H-[1,2,4]triazole-3-thiol (33)

Procedure: A suspension of potassium thiocarbazinate 32 0.38 g (10 mmol) in water (5 mL) and hydrazine hydrate 0.17 ml (99%, 3 mmol) was heated for 18–20 h at 100 °C with occasional shaking. The color of the reaction mixture changed to green with the evolution of hydrogen sulfide gas. A homogeneous reaction mixture was obtained during the reaction process. (TLC check, chloroform: methanol 8:2). The reaction mixture was cooled at room temperature. The reaction is poured in water and on acidification with dil. HCl the required triazole was precipitated out. The precipitated solid was collected by filtration, washed with water and dried in oven.
4-Amino-5-(7-phenyl-7H-pyrrolo[3,4-d][1,2,3]triazin-5-yl)-4H-[1,2,4]triazole-3-thiol (33)

Recrystallized from DMF-water (9:2), m.p. 325-326 °C, Yield 75%, colorless solid.

IR (KBr): 3341 (d, NH), 2560 (SH), 1596 cm⁻¹

¹H NMR (DMSO-δ6) δ 5.97 (d, 2H, NH₂), 7.23-7.97 (m, 5H, Ar H), 9.21 (s, 1H, Ar H), 11.20 (s, 1H, SH);

¹³C NMR (75 MHz, DMSO-δ6), 110.3, 115.2, 121.2, 123.6, 128.4, 130.3, 137.6, 139.5, 143.6, 144.7, 145.7, 151.7, 156.7, 162.6. MS (70 eV) m/z (%): 312(M⁺).


Found: C, 46.42; H, 2.85; N, 40.32.

3.7 REFERENCES


[4] (a) I. Okada; S. Suzuki; S. Okui; Y. Takahashi; T. Fukuchi; T. Nakajima, Preparation of [(5-pyrazolylcarboxamido) alkyl] pyridine derivatives and


[15] S. Okui; N. Kyomura; T. Fukuchi; K. Tanaka; M. Katsurada; K. Okano; N. Sumitani; A. Miyauchi; A. Yabe, Preparation of 4-amino-1-phenyl-3-cyanopyrazole derivatives and process for producing the same, and pesticides containing the same as the active ingredient. WO 2001000614,(2001).
[16] S. Okui; N. Kyomura; T. Fukuchi; K. Okano; L. He; A. Miyauchi, Preparation process of pyrazole derivatives in pest controllers containing the same as the active ingredient. WO 2002010153,(2002).