CHAPTER 4

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME 5-SUBSTITUTED-4-(SUBSTITUTED)AMINO-3-MERCAPTO-(4H)-1,2,4-TRIAZOLES
4.1 AIM OF PRESENT WORK

High-density lipoprotein (HDL) elevation has been of considerable interest to medicinal chemists due to its pivotal role in reverse cholesterol transport. CETP has gained most of the attention due to its unique role in HDL metabolism and inhibition of CETP is considered as an attractive target for atherosclerotic intervention. Sikorski J., et al. reported a series of 3-mercapto-1,2,4-triazoles as potential CETP inhibitors.\textsuperscript{295}

\[
\begin{align*}
  &\text{R}_1 = n-C_{13}H_{27}, \text{HCC(CH}_2)_n, \text{CH}_3(CH}_2)_6\text{S(CH}_2)_5, \text{CH}_3(CH}_2)_6\text{SCH}_2, \\
  &4-(n-C_6H_{13})\text{C}_6\text{H}_4\text{OCH}_2, 4-(n-C_7H_{15})\text{C}_6\text{H}_4\text{OCH}_2, 4-(n-C_8H_{17})\text{C}_6\text{H}_4(\text{CH}_2)_3 \\
  &\text{R}_2 = \text{cyclohexyl, substituted phenyl, 2-naphthyl, 3-pyridyl}
\end{align*}
\]

It was thought of interest to carry out systematic QSAR study of the above series using 2D and 3D descriptors to get insights into structural requirements for CETP inhibition. Separately, a series of novel 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles (230) was designed which exhibited a marked structural similarity with (229).

\[
\begin{align*}
  &\text{R}_1 = \text{H, CH}_3, \text{phenyl, substituted phenyl, benzyl, substituted benzyl, 4-pyridyl} \\
  &\text{R}_2 = \text{H, phenyl}
\end{align*}
\]

Thus, it was planned to synthesize a series of 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles (230) as potential HDL elevating and lipid lowering agents.
4.2 RESULTS AND DISCUSSION

4.2.1 Synthetic approach

Target 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles (234, 235) were synthesized using the widely utilized Hoggarth method (Scheme 2).³⁰⁴,³⁰⁵

\[
\text{AcOH} \xrightarrow{\text{H}^+, \text{CH}_3\text{OH}, \Delta} \text{AcO} \xrightarrow{\Delta} \text{AcO} \xrightarrow{\text{NH}_2\text{NH}_2\text{H}_2\text{O}, \Delta} \text{AcONHNH}_2
\]

(231)

(232)

\[
\text{CS}_2, \text{KOH}
\]

\[
\text{R}_2\text{NHNH}_2 \xrightarrow{\Delta} \text{R}_2\text{NHNH}_2 \xrightarrow{\Delta} \text{R}_2\text{NHNH}_2
\]

(233)

(234) \( R_2 = \text{H} \)

(235) \( R_2 = \text{Phenyl} \)

\( R_1 = \text{(substituted)phenyl, 4-pyridyl} \)

Scheme 2: Synthetic route of 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles

Esters of corresponding carboxylic acids (231) were synthesized by acid catalyzed esterification. These esters, on heating with hydrazine hydrate, underwent nucleophilic displacement of methoxy group of ester to corresponding acid hydrazides (232). These acid hydrazides (232) were condensed with carbon disulfide in methanolic potassium hydroxide to yield potassium 3-aroyldithiocarbazates (233). As reported by Reid and Heindel³⁰⁶, treatment of these dithiocarbazates (233) with hydrazines in aqueous medium affected ring closure to afford 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles (234 and 235). This modification avoids S-methylation of dithiocarbazates (233), results in a higher overall yields, and permits a shorter working time.
Compounds 234a and 234b were synthesized by a slightly modified method. Warming together carbon disulphide and hydrazine hydrate in an equimolar proportion yielded thiocarbohydrazide (236). The resulting thiocarbohydrazide was refluxed with formic acid and acetic acid for 4-5 h followed by removal of excess acid and cooling of the reaction mixture to afford compounds 234a and 234b, respectively.

4.2.2 Physical and spectral characteristics
5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles are pale yellow to colorless crystalline solids and are soluble in dichloromethane, chloroform, methanol and ethanol and insoluble in n-hexane. Compounds are soluble in dilute NaOH (10%w/w) and are insoluble in 1N HCl.

UV spectra
Characteristically, all compounds with amino group at 4th position (234a-234n), showed low absorption maxima as compared to the phenyl substituted compounds (235a-235r). The former absorbed in the range of 203.5 to 258.5 nm while the later absorbed in the range of 261 to 323 nm. Understandably, compound 234a showed lowest absorption (203.5 nm) owing to the presence of hydrogen at 5th position. Rest of the compounds exerted a similar pattern owing to the homology of phenyl ring at 5th position (λmax ~ 253). Most of the compounds from 235a-235r, showed absorption maximum near 300 nm.
Exceptions were 235b (with benzyl substitution) and 235p (with 2-NO₂ substitution), which showed abruptly high and low maxima of 265 and 323 nm, respectively.

**IR spectra**
Intense absorption band around 3295 cm⁻¹ indicates presence of aryl N-H group. Characteristically, compounds 234a-234n showed a doublet due to the presence of primary amino group while compounds 235a-235r showed a singlet absorption band due to the presence of a secondary aryl amino group. Compounds with O-H group on the aryl ring showed a characteristic broad band around 3400 cm⁻¹. Many absorption bands were found in the region of 3050-2900 cm⁻¹ owing to the presence of aryl C-H stretching vibrations. Compounds also showed strong absorption in the region of 1280-1060 cm⁻¹ due to the presence of C-S and C-N stretching vibrations.

**¹H NMR spectra**
The ¹H-NMR spectra of 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles were taken in CDCl₃, DMSO-d₆ or CDCl₃/DMSO-d₆ mixture. All the compounds showed similar pattern, indicating them to be of the homologous series. Aryl protons resonated at around δ 7.2-8.0. Also, compounds showed a characteristic broad singlet at δ 14.0 to 14.5 for N-H protons. The N-H protons were also found D₂O exchangeable. Protons of the S-H group did not give any signal, which is attributed to the well-known tautomerism seen with mercaptotriazoles between the thiol and thione groups.

**Mass Spectra**
All the 5-substituted-4-amino-3-mercapto-(4H)-1,2,4-triazoles (234a-234n) exhibited intense ion peak corresponding to the ion peak at (M+1). However, 5-substituted-4-phenylamino-3-mercapto-(4H)-1,2,4-triazoles (235a-235r) did not show corresponding molecular ions peak. Instead, all the compounds displayed strong M-91 peak corresponding to the removal of N-Ph radical. The compounds possessing chloro atom in the aromatic ring showed (M+2) peaks, about 30% as intense as the (M+1) peak due to the isotopic abundance. Compounds also displayed characteristic peak at (M-123) due to simultaneous of elimination of SH and N-Ph radical.
Table 9: Physical characteristics of 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles (234a-234n, 235a-235r)

$$
\text{N-N} \begin{array}{c}
\text{H} \\
\text{R}_1 \\
\text{NHR}_2
\end{array}
$$

(234) $R_2 = \text{H}$
(235) $R_2 = \text{Phenyl}$

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<tr>
<th>Sr. No.</th>
<th>Compd No.</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>m.p. (°C)</th>
<th>Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
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<th>Molecular weight</th>
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<td>H</td>
<td>H</td>
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<tr>
<td>2</td>
<td>234b</td>
<td>Me</td>
<td>H</td>
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<sup>a</sup> Yield values are approximate and based on experimental data.

Synthesis and biological evaluation of substituted 1,2,4-triazoles
### Synthesis and biological evaluation of substituted 1,2,4-triazoles

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* Solvent of recrystallization: methanol
4.3 EXPERIMENTAL

General

All the melting points were determined in open capillaries in microprocessor based melting point apparatus model VMP-D (Veego make) and are uncorrected.

UV spectra were recorded on UV-VIS 160A Shimadzu spectrophotometer.

Infrared spectra were recorded in KBr on 8400S Shimadzu Fourier Transform spectrophotometer.

Proton Nuclear Magnetic Resonance spectra were taken on Bruker Avance 400 spectrophotometer at 400 MHz and the chemical shifts are given as parts per million (δ ppm) downfield from tetramethylsilane (TMS) as internal standard.

Mass spectra were obtained on Perkin-Elmer LC-MS PE Sciex API/65.

The elemental analyses were done on FLASH EA 1112 (Thermo Finnigan, Italy).

Thin layer chromatography was performed on ready-made Aluminum backed TLC GF\textsubscript{254} plates as well as on microscopic slides (2x7.5 cm) coated with silica gel G and spots were visualized by exposure to iodine vapors and UV radiation.
Synthesis and biological evaluation of substituted 1,2,4-triazoles

General procedure for the synthesis of substituted methyl carboxylates (231)
A mixture of aryl carboxylic acid (10 mmol) was refluxed in 20 ml of methanol in presence of concentrated sulfuric acid (98%) for 3-5 h. The reaction mixture was cooled or poured into ice-water and the crude ester (231) was separated and immediately used for the synthesis of acid hydrazides.

General procedure for the synthesis of substituted carboxylic acid hydrazides (196)
A mixture of crude methyl carboxylate (231) (10 mmol) and hydrazine hydrate (99%, 25 mmol) was refluxed in 20 ml of methanol for 3-5 h. The reaction mixture was cooled or poured into ice-water and the crude acid hydrazide (232) was separated and immediately used for the next step without purification.

Synthesis of thiocarbohydrazide (236)\textsuperscript{307}
To a vigorously stirred solution of hydrazine hydrate (25 g, 50 mmol), carbon disulfide (7.6 g, 10 mmol) was added dropwise. The temperature of the solution rose to 60°C. The reaction mixture was then heated at reflux for 30 minutes, cooled in an ice-bath for 30 min. and the precipitated thiocarbohydrazide was filtered off, washed with methanol, and air-dried to yield 6.7 g (63%) pale yellow thiocarbohydrazide (236) (63.3%) m.p. 170-171°C (171°C)\textsuperscript{307}. The crude product was used for the next step without purification.

Synthesis of 4-amino-3-mercapto-(4H)-1,2,4-triazole (234a)
A mixture of thiocarbohydrazide (236) (2.12 g, 20 mmol) and formic acid (15 ml) was refluxed for 6 h and the excess formic acid was distilled off under reduced pressure. The residual liquid was cooled to room temperature. The solid thus obtained was crystallized from water giving 1.69 g (73%) colorless crystalline product, which was characterized as 4-amino-3-mercapto-(4H)-1,2,4-triazole (234a), m.p. 165-166°C (166-167°C)\textsuperscript{307}.

ANALYSIS:

<table>
<thead>
<tr>
<th>Method</th>
<th>Value</th>
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<tr>
<td>Microanalysis</td>
<td>C\textsubscript{2}H\textsubscript{4}N\textsubscript{2}S (116.14)</td>
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<tr>
<td>TLC</td>
<td>Petroleum ether: Methanol (7:3); \textit{Rf} value = 0.45</td>
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<tr>
<td>IR (KBr, cm\textsuperscript{-1})</td>
<td>3240, 3101 (N-H)</td>
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<td>UV (\textit{\lambda}_{\text{max}}, nm, methanol)</td>
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Synthesis of 4-amino-3-mercapto-5-methyl-(4H)-1,2,4-triazole (234b)

A mixture of thiocarbohydrazide (236) (2.12 g, 20 mmol) and glacial acetic acid (15 ml) was refluxed for 4 h. Within an hour of refluxing, a solid started separating from the clear solution. The reaction mixture was cooled to room temperature and the excess acetic acid was distilled off under reduced pressure. The residual solid was crystallized from water giving 1.6 g (62%) colorless shining crystals, which was characterized as 4-amino-3-mercapto-5-methyl-(4H)-1,2,4-triazole (234b), m.p. 215-217°C (218°C) \(^{307}\).

**ANALYSIS:**

- **Microanalysis** : C\(_3\)H\(_6\)N\(_4\)S (130.17)
- **TLC** : Petroleum ether: Ethyl acetate (6:4); \(R_f\) value = 0.33
- **IR (KBr, cm\(^{-1}\))** : 3276, 3177 (N-H)
- **UV (A\(_{\text{max}}\), nm, methanol)** : 252.5

Synthesis of 4-amino-3-mercapto-5-phenyl-(4H)-1,2,4-triazole (234c)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, benzohydrazide (2.72 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 2.76 g (72%) of colorless crystalline flakes, which was characterized as 4-amino-3-mercapto-5-phenyl-(4H)-1,2,4-triazole (234c), m.p. 235-201°C (201-203°C) \(^{306}\).

**ANALYSIS:**

- **Microanalysis** : C\(_6\)H\(_8\)N\(_4\)S (192.24)
- **TLC** : Petroleum ether: Ethyl acetate (7:3); \(R_f\) value = 0.44
- **IR (KBr, cm\(^{-1}\))** : 3300, 3234 (N-H)
- **UV (A\(_{\text{max}}\), nm, methanol)** : 254.0

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*Synthesis and biological evaluation of substituted 1,2,4-triazoles*
Synthesis and biological evaluation of substituted 1,2,4-triazoles

Synthesis of 4-amino-5-benzyl-3-mercapto-(4H)-1,2,4-triazole (234d)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-phenylacetohydrazide (3.0 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.13 g (76%) of colorless crystalline product, which was characterized as 4-amino-5-benzyl-3-mercapto-(4H)-1,2,4-triazole (234d), m.p. 178-180°C (179-180°C) 308.

ANALYSIS:
Microanalysis : C_{9}H_{10}N_{4}S (206.27)
TLC : Petroleum ether: Ethyl acetate (7:3); R_{f} value = 0.33
IR (KBr, cm^{-1}) : 3286, 3240 (N-H)
UV (λ_{max}, nm, methanol): 253.0

Synthesis of 4-amino-3-mercapto-5-(2-methyl-phenyl)-(4H)-1,2,4-triazole (234e)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-methylbenzohydrazide (3.0 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 2.92 g (71%) of colorless crystalline product, which was characterized as 4-amino-3-mercapto-5-(2-methyl-phenyl)-(4H)-1,2,4-triazole (234e), m.p. 134-136°C (137°C) 309.

ANALYSIS:
Microanalysis : C_{9}H_{10}N_{4}S (206.27)

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TLC: Petroleum ether: Ethyl acetate (7:3); \( R_f \) value = 0.47
IR (KBr, cm\(^{-1}\)): 3285, 3130 (N-H)
UV (\( \lambda_{\text{max}} \), nm, methanol): 254.5, 335

Synthesis of 4-amino-3-mercapto-5-(3-methyl-phenyl)-(4H)-1,2,4-triazole (234f)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 3-methylbenzohydrazide (3.0 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25\(^\circ\)C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.33 g (81%) of colorless crystalline product, which was characterized as 4-amino-3-mercapto-5-(3-methyl-phenyl)-(4H)-1,2,4-triazole (234f), m.p. 134-136\(^\circ\)C (135\(^\circ\)C). \(^{309}\)

ANALYSIS:
Microanalysis: \( C_9 H_{10} N_4 S \) (206.27)
TLC: Petroleum ether: Ethyl acetate (7:3); \( R_f \) value = 0.51
IR (KBr, cm\(^{-1}\)): 3288, 3192 (N-H)
UV (\( \lambda_{\text{max}} \), nm, methanol): 254

Synthesis of 4-amino-3-mercapto-5-(4-methyl-phenyl)-(4H)-1,2,4-triazole (234g)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 4-methylbenzohydrazide (3.0 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25\(^\circ\)C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.1 g (75%) of light yellow crystalline product, which was characterized as 4-amino-3-
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mercaptop-5-(4-methyl-phenyl)-(4H)-1,2,4-triazole (234g), m.p. 210-212°C (210-211°C)\textsuperscript{309}.

ANALYSIS:
Microanalysis : C\textsubscript{9}H\textsubscript{10}N\textsubscript{4}S (206.27)
TLC : Petroleum ether: Ethyl acetate (7:3); R\textsubscript{f} value = 0.49
IR (KBr, cm\textsuperscript{-1}) : 3286, 3186 (N-H)
UV (\lambda_{max} nm, methanol): 255.0

Synthesis of 4-amino-5-(3,4-dichloro-phenyl)-3-mercapto-(4H)-1,2,4-triazole (234h)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 3,4-dichlorobenzohydrazide (4.1 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.07 g (78%) of colorless shining crystals, which was characterized as 4-amino-5-(3,4-dichloro-phenyl)-3-mercapto-(4H)-1,2,4-triazole (234h), m.p. 194-195°C.

ANALYSIS:
Microanalysis : C\textsubscript{9}H\textsubscript{8}Cl\textsubscript{2}N\textsubscript{4}S % Required: C (36.80), H (2.32), N (21.46) (261.13) % Found : C (36.66), H (2.36), N (21.58)
TLC : Petroleum ether: Ethyl acetate (7:3); R\textsubscript{f} value = 0.36
IR (KBr, cm\textsuperscript{-1}) : 3298, 3232 (N-H)
UV (\lambda_{max} nm, methanol): 253.5
\textsuperscript{1}H NMR (\delta) : \delta 7.40-7.74 (m, 9H, Ar-H), \delta 13.90 (s, 2H, NH-Ar)
(CDC\textsubscript{6}H\textsubscript{5}DMSO-d\textsubscript{6})
Mass (m/z) : 261(M), 262 (M+1), 228 (M-33)
Synthesis of 4-amino-3-mercapto-5-(4-methoxy-phenyl)-(4H)-1,2,4-triazole (234i)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 4-methoxybenzohydrazide (3.32 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 2.84 g (64%) of colorless crystalline product, which was characterized as 4-amino-3-mercapto-5-(4-methoxy-phenyl)-(4H)-1,2,4-triazole (234i), m.p. 226-227°C (226-227°C) 310.

ANALYSIS:

Microanalysis : C_{9}H_{10}N_{4}O_{9}S (222.27)

TLC : Petroleum ether: Ethyl acetate (7:3); Rf value = 0.35

IR (KBr, cm^{-1}) : 3310, 3144 (N-H)

UV (λ_{max}, nm, methanol): 256.5

Mass (m/z) : 222 (M), 223 (M+1), 206 (M-16)

Synthesis of 4-amino-5-(4-chloro-phenyl)-3-mercapto-(4H)-1,2,4-triazole (234j)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 4-chlorobenzohydrazide (3.4 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.50 g (79%) of colorless shining crystals, which was characterized as 4-amino-5-(4-chloro-phenyl)-3-mercapto-(4H)-1,2,4-triazole (234j), m.p. 207-209°C (209°C) 311.
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ANALYSIS:
Microanalysis: \( \text{C}_9\text{H}_7\text{CIN}_4\text{S} (226.69) \)
TLC: Petroleum ether: Ethyl acetate (7:3); \( R_f \) value = 0.43
IR (KBr, cm\(^{-1}\)): 3246, 3150 (N-H)
UV (\( \lambda_{\text{max}} \) nm, methanol): 253.5

Synthesis of 4-amino-3-mercapto-5-(2-methoxy-phenyl)-(4H)-1,2,4-triazole (234k)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-methoxybenzohydrazide (3.32 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 2.58 g (58%) of colorless crystalline product, which was characterized as 4-amino-3-mercapto-5-(2-methoxy-phenyl)-(4H)-1,2,4-triazole (234k), m.p. 135-137°C (137°C\(^3\)).

ANALYSIS:
Microanalysis: \( \text{C}_9\text{H}_{10}\text{N}_4\text{O}_3\) (222.27)
TLC: Petroleum ether: Ethyl acetate (7:3); \( R_f \) value = 0.43
IR (KBr, cm\(^{-1}\)): 3335, 3210 (N-H)
UV (\( \lambda_{\text{max}} \) nm, methanol): 254.0

Synthesis of 4-amino-5-(4-hydroxy-phenyl)-3-mercapto-(4H)-1,2,4-triazole (234l)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 4-hydroxybenzohydrazide (3.04 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature,
the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 2.58 g (62%) of light yellow crystalline product, which was characterized as 4-amino-5-(4-hydroxy-phenyl)-3-mercaptopo-(4H)-1,2,4-triazole (234l), m.p. 272-274°C.

**ANALYSIS:**

Microanalysis : C₈H₈N₄O₅S % Required: C (46.14), H (3.87), N (26.90) (208.24) % Found : C (45.89), H (3.94), N (27.04)

TLC : Petroleum ether: Ethyl acetate (6:4); Rₜ value = 0.40

IR (KBr, cm⁻¹) : 3308 (O-H), 3285, 3254 (N-H)

UV (λ_max, nm, methanol): 257.0

¹H NMR (δ) : δ 7.27-7.98 (m, 9H, Ar-H), δ 14.26 (s, 2H, NH-Ar) (CDCl₃/DMSO-d₆)

Mass (m/z) : 208 (M), 209 (M+1), 175 (M-33)

**Synthesis of 4-amino-3-mercaptopo-5-(2-nitro-phenyl)-(4H)-1,2,4-triazole (234m)**

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-nitrobenzohydrazide (3.62 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 2.80 g (59%) of light yellow crystalline product, which was characterized as 4-amino-3-mercaptopo-5-(2-nitro-phenyl)-(4H)-1,2,4-triazole (234m), m.p.234-235°C.

**ANALYSIS:**

Microanalysis : C₈H₈N₄O₅S % Required: C (40.50), H (2.97), N (29.52) (237.24) % Found : C (40.77), H (3.03), N (29.47)

TLC : Petroleum ether: Ethyl acetate (7:3); Rₜ value = 0.43

IR (KBr, cm⁻¹) : 3285, 3130 (N-H)

UV (λ_max, nm, methanol): 254.0
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\(^1\)H NMR (\(\delta\))

(CDCl\(_3\)/DMSO-d\(_6\))

Mass (m/z)

- \(\delta\) 7.20-7.59 (m, 9H, Ar-H), \(\delta\) 13.67 (s, 2H, NH-Ar)
- 238 (M), 239 (M+1), 205 (M-33)

**Synthesis of 4-amino-3-mercapto-5-(4-pyridyl)-(4H)-1,2,4-triazole (234n)**

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, isoniazide (2.74 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with hydrazine hydrate (1.6 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 2.39 g (62%) of bright yellow crystalline product, which was characterized as 4-amino-3-mercapto-5-(4-pyridyl)-(4H)-1,2,4-triazole (234n), m.p. 248-249°C (248°C)\(^3\)

**ANALYSIS:**

- Microanalysis: C\(_7\)H\(_7\)N\(_5\)S (193.23)
- TLC: Petroleum ether: Methanol (7:3); R\(_t\) value = 0.56
- IR (KBr, cm\(^{-1}\)): 3306, 3227 (N-H)
- UV (\(\lambda_{max}\), nm, methanol): 255.0

**Synthesis of 3-mercapto-5-phenyl-4-phenylamino-(4H)-1,2,4-triazole (235a)**

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, benzohydrazide (2.72 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.29 g (80%) of colorless crystalline product, which was characterized as 3-
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mercapto-5-phenyl-4-phenylamino-(4H)-1,2,4-triazole (235a), m.p. 204-206°C (204°C)\textsuperscript{313}.

**ANALYSIS:**

- **Microanalysis**: C\textsubscript{14}H\textsubscript{12}N\textsubscript{4}S (268.34)
- **TLC**: Petroleum ether: Ethyl acetate (7:3); R\textsubscript{T} value = 0.38
- **IR (KBr, cm\textsuperscript{-1})**: 3207 (N-H)
- **UV (λ\textsubscript{max}, nm, methanol)**: 301.0
- **Mass (m/z)**: 177 (M-91), 145 (M-123)

**Synthesis of 5-benzyl-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235b)**

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-phenyacetohydrazide (3.0 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.40 g (78%) of yellow crystalline flakes, which was characterized as 5-benzyl-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235b), m.p. 118-120°C.

**ANALYSIS:**

- **Microanalysis**: C\textsubscript{14}H\textsubscript{14}N\textsubscript{4}S (282.36) % Required: C (63.80), H (5.00), N (19.84) % Found: C (63.57), H (4.93), N (19.77)
- **TLC**: Petroleum ether: Ethyl acetate (5:5); R\textsubscript{T} value = 0.42
- **IR (KBr, cm\textsuperscript{-1})**: 3234 (N-H)
- **UV (λ\textsubscript{max}, nm, methanol)**: 265.0
- **\textsuperscript{1}H NMR (δ)**: δ 3.57 (s, 2H, CH\textsubscript{2}), δ 7.28-7.52 (m, 10H, Ar-H), δ 9.83 (s, 1H, NH-Ar) in DMSO-d\textsubscript{6}
- **Mass (m/z)**: 191 (M-91), 159 (M-123)
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Synthesis of 3-mercapto-5-(2-methyl-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235c)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-methybenzohydrazide (3.0 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitate, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.50 g (62%) of colorless crystalline product, which was characterized as 3-mercapto-5-(2-methyl-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235c), m.p. 177-179°C.

ANALYSIS:
Microanalysis: C_{15}H_{14}N_4S  
(282.36)  
% Required: C (63.80), H (5.00), N (19.84)
% Found: C (63.56), H (5.08), N (19.79)
TLC: Petroleum ether: Ethyl acetate (7:3); R_f value = 0.51
IR (KBr, cm^{-1}): 3109 (N-H)
UV (λ_max, nm, methanol): 296.0
^1H NMR (δ): δ 2.61 (s, 3H, CH_3), δ 7.31-7.89 (m, 9H, Ar-H), δ 14.33 (s, 1H, NH-Ar)
(Methanol-d_4/DMSO-d_6)
Mass (m/z): 191 (M+91), 159 (M-123)

Synthesis of 3-mercapto-5-(3-methyl-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235d)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 3-methybenzohydrazide (3.0 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the
solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.05 g (54%) of colorless amorphous product, which was characterized as 3-mercapto-5-(3-methyl-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235d), m.p. 155-156°C.

ANALYSIS:
Microanalysis : C_{15}H_{14}N_4S % Required: C (63.80), H (5.00), N (19.84) (282.36) % Found : C (63.98), H (5.04), N (19.94)
TLC : Petroleum ether: Ethyl acetate (7:3); Rf value = 0.36
IR (KBr, cm⁻¹) : 3092 (N-H)
UV (λ_{max}, nm, methanol): 301.5

¹H NMR (δ) : δ 2.42 (s, 3H, CH₃), δ 7.35-7.74 (m, 9H, Ar-H), δ 14.33 (s, 1H, (CDCl₃/DMSO-d₆) NH-Ar)
Mass (m/z) : 191 (M-91), 159 (M-123)

Synthesis of 3-mercapto-5-(4-methyl-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235e)
Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 4-methybenzohydrazide (3.0 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.38 g (60%) of colorless crystalline product, which was characterized as 3-mercapto-5-(4-methyl-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235e), m.p. 213-215°C.

ANALYSIS:
Microanalysis : C_{15}H_{14}N_4S % Required: C (63.80), H (5.00), N (19.84) (282.36) % Found : C (63.68), H (5.08), N (19.89)
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TLC

IR (KBr, cm\(^{-1}\))

UV (\(\lambda_{\text{max}}, \text{nm, methanol}\))

\(^1\)H NMR (\(\delta\))

Mass (m/z)

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**Synthesis of 5-(2-chloro-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235f)**

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-chlorobenzohydrazide (3.4 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.0 g (60%) of colorless crystalline product, which was characterized as 5-(2-chloro-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235f), m.p. 170-172°C.

**ANALYSIS:**

Microanalysis:

\(\text{C}_{14}\text{H}_{11}\text{CIN}_4\text{S} \quad \% \text{Required: C (55.53), H (3.66), N (18.50)}\)
\(\text{(302.78)} \quad \% \text{Found : C (55.23), H (3.62), N (18.47)}\)

TLC

IR (KBr, cm\(^{-1}\))

UV (\(\lambda_{\text{max}}, \text{nm, methanol}\))

\(^1\)H NMR (\(\delta\))

Mass (m/z)

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\(\delta \ 7.40-7.90 \text{ (m, 9H, Ar-H)}, \delta \ 14.46 \text{ (s, 1H, NH-Ar)}\)

\(\delta \ 7.40-7.90 \text{ (m, 9H, Ar-H)}\)

\(212 \text{ (M-91)}, 180 \text{ (M-123)}\)
Synthesis and biological evaluation of substituted 1,2,4-triazoles

Synthesis of 5-(3-chloro-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235g)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 3-chlorobenzenohydrazide (3.4 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.88 g (64%) of colorless crystalline product, which was characterized as 5-(3-chloro-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235g), m.p. 171-173°C.

ANALYSIS:

Microanalysis : C_{14}H_{11}ClN_{4}S
% Required: C (55.53), H (3.66), N (18.50)
(302.78) % Found : C (55.35), H (3.59), N (18.39)
TLC : Petroleum ether: Ethyl acetate (6:4); Rf value = 0.45
IR (KBr, cm\(^{-1}\)) : 3306 (N-H)
UV (\(\lambda_{\text{max}}\) nm, methanol): 301.0
\(^1\)H NMR (\(\delta\)) : \(\delta\) 7.48-7.89 (m, 9H, Ar-H), \(\delta\) 14.5 (s, 1H, NH-Ar)
(CDC\(_{3}\)/DMSO-d\(_{6}\))
Mass (m/z) : 212 (M-91), 180 (M-123)

Synthesis of 5-(4-chloro-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235h)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 4-chlorobenzenohydrazide (3.4 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid
obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.24 g (70%) of colorless amorphous product, which was characterized as 5-(4-chloro-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole \((235h)\), m.p. 162-164°C (162°C)\(^{313}\).

**ANALYSIS:**

- Microanalysis : \(C_{14}H_{11}ClIN_4S\) (302.78)
- TLC : Petroleum ether: Ethyl acetate (5:5); \(R_f\) value = 0.37
- IR (KBr, cm\(^{-1}\)) : 3124 (N-H)
- UV (\(\lambda_{\text{max}},\) nm, methanol): 312.5

**Synthesis of 5-(3,4-dichloro-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235i)**

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 3,4-dichlorobenzohydrazide (4.1 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.92 g (73%) of pale yellow crystalline product, which was characterized as 5-(3,4-dichloro-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole \((235i)\), m.p. 175-177°C.

**ANALYSIS:**

- Microanalysis : \(C_{14}H_{10}Cl_2N_4S\) % Required: C (49.86), H (2.99), N (16.61)
  \(\text{(337.23)}\) % Found : C (50.12), H (3.05), N (16.70)
- TLC : Petroleum ether: Ethyl acetate (6:4); \(R_f\) value = 0.39
- IR (KBr, cm\(^{-1}\)) : 3092 (N-H)
- UV (\(\lambda_{\text{max}},\) nm, methanol): 307.0
- \(^1\text{H NMR (δ)}\) : \(δ 7.44-8.01\) (m, \(8H, \text{Ar-H}\)), \(δ 14.60\) (s, \(1H, \text{NH-Ar}\))
  (CDCl\(_3\)/DMSO-d\(_6\))
Synthesis of 5-(2-hydroxy-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235j)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-hydroxybenzohydrazide (3.04 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.70 g (65%) of colorless crystalline product, which was characterized as 5-(2-hydroxy-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235j), m.p. 195-234°C (195)\textsuperscript{313}.

ANALYSIS:

Microanalysis: $\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$ (284.34)

TLC: Petroleum ether: Ethyl acetate (6:4); $R_t$ value = 0.35

IR (KBr, cm$^{-1}$): 3415 (O-H), 3278 (N-H)

UV ($\lambda_{\text{max}}$, nm, methanol): 302.5

Synthesis of 5-(3-hydroxy-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235k)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 3-hydroxybenzohydrazide (3.04 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol
yielded 3.41 g (60%) of pale yellow crystalline product, which was characterized as 5-(3-hydroxy-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235k), m.p. 149-151°C.

**ANALYSIS:**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microanalysis</td>
<td>C_{14}H_{12}N_{4}OS (284.34) % Required: C (59.14), H (4.25), N (19.70)</td>
</tr>
<tr>
<td></td>
<td>% Found: C (58.88), H (4.32), N (19.65)</td>
</tr>
<tr>
<td>TLC</td>
<td>Petroleum ether: Ethyl acetate (7:3); R_{t} value = 0.35</td>
</tr>
<tr>
<td>IR (KBr, cm^{-1})</td>
<td>3566 (O-H), 3298 (N-H)</td>
</tr>
<tr>
<td>UV (λ_{max}, nm, methanol)</td>
<td>302.0</td>
</tr>
<tr>
<td>¹H NMR (δ)</td>
<td>δ 7.00 (s, 1H, OH), δ 7.27-7.50 (m, 9H, Ar-H), δ 14.26 (s, 1H, NH-Ar)</td>
</tr>
<tr>
<td>Mass (m/z)</td>
<td>193 (M-91), 161 (M-123)</td>
</tr>
</tbody>
</table>

**Synthesis of 5-(4-hydroxy-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235I)**

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 4-hydroxybenzohydrazide (3.04 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.09 g (72%) of colorless crystalline product, which was characterized as 5-(4-hydroxy-phenyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235I), m.p. 238-240°C (240°C)_{313}.

**ANALYSIS:**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microanalysis</td>
<td>C_{14}H_{12}N_{4}OS (284.34)</td>
</tr>
<tr>
<td>TLC</td>
<td>Petroleum ether: Ethyl acetate (6:4); R_{t} value = 0.37</td>
</tr>
<tr>
<td>IR (KBr, cm^{-1})</td>
<td>3448 (O-H), 3295 (N-H)</td>
</tr>
<tr>
<td>UV (λ_{max}, nm, methanol)</td>
<td>304.0</td>
</tr>
</tbody>
</table>

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Synthesis and biological evaluation of substituted 1,2,4-triazoles

Synthesis of 3-mercapto-4-phenylamino-5-(3,4,5-trimethoxy-phenyl)-(4H)-1,2,4-triazole (235m)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 3,4,5-trimethoxybenzohydrazide (4.52 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.59 g (64%) of colorless crystalline product, which was characterized as 3-mercapto-4-phenylamino-5-(3,4,5-trimethoxy-phenyl)-(4H)-1,2,4-triazole (235m), m.p. 187-188°C.

ANALYSIS:

Microanalysis: C_{17}H_{18}N_{4}O_{3}S % Required: C (56.97), H (5.06), N (15.63) % Found: C (57.23), H (5.12), N (15.58)

TLC: Petroleum ether: Ethyl acetate (6:4); R_f value = 0.34

IR (KBr, cm⁻¹): 3249 (N-H)

UV (λ_{max}, nm, methanol): 308.5

¹H NMR (δ): δ 3.90 (s, 9H, OCH₃), δ 7.16-7.36 (m, 7H, Ar-H), δ 14.19 (s, 1H, NH-Ar)

Mass (m/z): 267 (M-91), 235 (M-123)

Synthesis of 3-mercapto-5-(2-methoxy-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235n)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-methoxybenzohydrazide (3.32 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature,
the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.05 g (68%) of yellow crystalline product, which was characterized as 3-mercapto-5-(2-methoxy-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235n), m.p. 186-187°C.

**ANALYSIS:**

| Microanalysis | : C_{15}H_{14}N_{4}O_{2}S | % Required: C (60.38), H (4.73), N (18.78) (298.36) | % Found: C (60.66), H (4.80), N (18.83) |
| TLC | Petroleum ether: Ethyl acetate (7:3); R_f value = 0.50 |
| IR (KBr, cm^{-1}) | 3237 (N-H) |
| UV (λ_{max} nm, methanol): | 296.5 |
| ^1H NMR (δ) | δ 2.68 (s, 3H, OCH₃), δ 7.30-7.95 (m, 9H, Ar-H), δ 14.29 (s, 1H, NH-Ar) |
| Mass (m/z) | : 207 (M-91), 191 (M-107) |

**Synthesis of 5-(4-chloro-benzyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235o)**

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 4-chlorophenylacetohydrazide (3.68 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.06 g (64%) of light-brown amorphous product, which was characterized as 5-(4-chloro-benzyl)-3-mercapto-4-phenylamino-(4H)-1,2,4-triazole (235o), m.p. 119-121°C.

**ANALYSIS:**

| Microanalysis | : C_{15}H_{13}ClN_{4}S | % Required: C (56.87), H (4.41), N (17.68) (316.81) | % Found: C (56.67), H (4.39), N (17.77) |
Synthesis and biological evaluation of substituted 1,2,4-triazoles

TLC: Petroleum ether: Ethyl acetate (7:3); Rf value = 0.42
IR (KBr, cm\(^{-1}\)): 3223 (N-H)
UV (\(\lambda_{\text{max}}\), nm, methanol): 262.5

\(^1\text{H NMR}\) (\(\delta\), ppm): \(\delta\) 3.98 (s, 2H, \(\text{CH}_2\)), \(\delta\) 7.23-7.44 (m, 9H, Ar-H), \(\delta\) 14.06 (s, 1H, NH-Ar)
Mass (m/z): 226 (M-91), 194 (M-123)

Synthesis of 3-mercapto-5-(2-nitro-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235p)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 2-nitrobenzohydrazide (3.62 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.63 g (58%) of light-brown amorphous product, which was characterized as 3-mercapto-5-(2-nitro-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235p), m.p. 189-191°C.

ANALYSIS:
Microanalysis: \(\text{C}_{14}\text{H}_{11}\text{N}_2\text{O}_2\text{S}\) % Required: C (53.66), H (3.54), N (22.35)
\(\text{M}(313.33)\) % Found: C (53.47), H (3.50), N (22.46)
TLC: Ethyl acetate; Rf value = 0.33
IR (KBr, cm\(^{-1}\)): 3314 (N-H)
UV (\(\lambda_{\text{max}}\), nm, methanol): 261.0, 323.0
\(^1\text{H NMR}\) (\(\delta\), ppm): \(\delta\) 7.12-8.01 (m, 9H, Ar-H), \(\delta\) 9.31 (s, 1H, NH-Ar)
Mass (m/z): 222 (M-91), 190 (M-123)
Synthesis and biological evaluation of substituted 1,2,4-triazoles

Synthesis of 3-mercapto-5-(4-nitro-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235q)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, 4-nitrobenzohydrazide (3.62 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 3.57 g (57%) of light yellow crystalline product, which was characterized as 3-mercapto-5-(4-nitro-phenyl)-4-phenylamino-(4H)-1,2,4-triazole (235q), m.p. 128-130°C.

ANALYSIS:

Microanalysis : C_{14}H_{11}N_{5}O_{2}S % Required: C (53.66), H (3.54), N (22.35) (313.33) % Found : C (53.42), H (3.59), N (22.48)

TLC : Petroleum ether: Ethyl acetate (6:4); R_t value = 0.37

IR (KBr, cm^{-1}) : 3271 (N-H)

UV (λ_{max}, nm, methanol): 315.0

^1H NMR (δ) (CDCl_3/DMSO-d_6) : δ 7.29-7.94 (m, 9H, Ar-H), δ 10.47 (s, 1H, NH-Ar)

Mass (m/z) : 222 (M-91), 190 (M-123)

Synthesis of 3-mercapto-4-phenylamino-5-(4-pyridyl)-(4H)-1,2,4-triazole (235r)

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in 20 ml of methanol and stirred for 10 minutes. To it, isoniazide (2.74 g, 20 mmol) was added at once. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added and the reaction mixture was stirred at 20-25°C. After 3 h, 100 ml of diethyl ether was added to get precipitates, which were filtered and washed with diethyl ether. The precipitates were mixed with phenyl hydrazine (3.46 g, 32 mmol) and 2 ml of water and the solution was refluxed for 1 h until the color of the solution became clear green. After cooling to room temperature, the solution was poured in ice-water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water and dried. Recrystallization from methanol yielded 4.25 g (79%) of
Synthesis and biological evaluation of substituted 1,2,4-triazoles

pale yellow crystalline product, which was characterized as 3-mercapto-4-phenylamino-5-(4-pyridyl)-(4H)-1,2,4-triazole (235r), m.p. 155-158°C.

ANALYSIS:
Microanalysis $\text{C}_{13}\text{H}_7\text{N}_5\text{S}$ % Required: C (57.97), H (4.12), N (26.00)
(269.32) % Found : C (58.21), H (4.05), N (25.89)
TLC : Petroleum ether: Methanol (8:2); $R_f$ value = 0.52
IR (KBr, cm$^{-1}$) : 3308 (N-H)
UV ($\lambda_{max}$, nm, methanol): 225.5, 315.0
$^1$H NMR (δ) (CDCl$_3$/DMSO-d$_6$) $\delta$ 7.67-8.01 (m, 9H, Ar-H), $\delta$ 8.96 (s, 1H, NH-Ar)
Mass (m/z) : 178 (M-91), 146 (M-123)
Sample Name : LMPB-224

Acquired By : DHARMESH BHATT

<table>
<thead>
<tr>
<th>Date Acquired</th>
<th>Vial</th>
<th>Injection Volume</th>
<th>Data file</th>
<th>Method File</th>
<th>Data processed</th>
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</table>

**MS Spectrum Graph**

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**Polarity : Neg Peak No 2 Ret. Time 0.417 Averaged D\-06-MMP\-LMPB-224.lcd**
Current Data Parameters
- NAME: Jan02-2009
- EXPNO: 360
- PROCNO: 1

F2 - Acquisition Parameters
- Date: 20090106
- Time: 9:59
- INSTRUM: spect
- PROBHD: 5 mm PABBO BB-
- PULPROG: zg30
- TD: 65536
- SOLVENT: DMSO
- NS: 16
- DS: 2
- SWH: 12019.230 Hz
- FIDRFS: 0.183399 Hz
- AQ: 2.7263477 sec
- RG: 406
- DW: 41.600 usec
- TE: 293.1 K
- DI: 1.00000000 sec
- TDO: 1

- CHANNEL f1
  - NUC1: 1H
  - P1: 10.90 usec
  - PL1: -3.00 dB
  - SFO1: 400.1324008 MHz

F2 - Processing parameters
- SI: 32768
- SF: 400.1299646 MHz
- WOW: EM
- SSB: 0
- LB: 0.30 Hz
- GB: 0
- PC: 1.00
4.4 PHARMACOLOGICAL SCREENING

All the synthesized compounds were evaluated for their antihyperlipidemic potential using 'Poloxamer 407 induced hyperlipidemia in rat' model. Percentage reduction of total cholesterol and triglycerides; and percentage increase in HDL levels were measured and compared against control and standard (atorvastatin) groups.

4.4.1 General conditions for experimental animals

The pharmacological experiments were carried out using Sprague Dawley rats of either sex. Inhouse breeding of these animals was carried out at the Department of Pharmacology, Zydus Research Centre, Ahmedabad (India). The animals were housed at 24 ± 1°C and air humidity of 50-70% with 14 h light and 10 h dark cycle. The animals were given standard chow diet. Tap water was given ad libitum, unless specified in any particular method. For all the studies, animals were selected at random. The experiment was performed as per the guidelines of Institutional Animal Care Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under Ministry of Animal Welfare Division, Government of India, New Delhi, India. A copy of the certificate has been attached as Annexure.

4.4.2 Antihyperlipidemic activity of 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles

All the synthesized compounds (234a-234n, 235a-235r) were subjected to screening for antihyperlipidemic activity in hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Method used</th>
<th>Poloxamer 407 induced hyperlipidemia in rat $^{300}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals used</td>
<td>Sprague Dawley (S.D.) rats</td>
</tr>
<tr>
<td>Dose of poloxamer</td>
<td>1.0 ml of 30% w/v aqueous solution</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>Dose of test compounds</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>Dose of standard drug</td>
<td>50 mg/kg (Atorvastatin)</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Oral (in sodium CMC suspension form)</td>
</tr>
<tr>
<td>Treatment period</td>
<td>24 hours</td>
</tr>
</tbody>
</table>
Method
Two hundred and four Albino S.D. rats of either sex, weighing 180-250 g were divided in thirty-four groups of six rats each as shown in the following table.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Title</th>
<th>Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Poloxamer 407 (1.0 ml, 30% w/v solution, i.p.)</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>Poloxamer 407 (1.0 ml, 30% w/v solution, i.p.), atorvastatin (50mg/kg, orally)</td>
</tr>
<tr>
<td>3-34</td>
<td>Test</td>
<td>Poloxamer 407 (1.0 ml, 30% w/v solution, i.p.), test compounds (50mg/kg, orally)</td>
</tr>
</tbody>
</table>

First group was maintained as a control group, second group as a standard group and other thirty-two groups were employed as test groups. The control group received 1.0 ml of 30% w/v solution of poloxamer 407, whereas standard group and test groups received 50 mg/kg of atorvastatin and test compounds in form of sodium CMC suspension, respectively, along with poloxamer 407 solution. Blood samples were collected before and after 24 h of the treatment from the retino-orbital plexures of rat eye. Blood samples were analysed for serum cholesterol, triglycerides and HDL levels by using their respective enzyme immuno assay kits (Transasia Ltd., Mumbai, India). Percentage decrease in the levels of cholesterols and triglycerides were calculated using the following formula (Table 10).

\[
\% \text{ Decrease in lipid levels} = \frac{C_c - C_t}{C_c - C_n} \times 100
\]

\((C_c = \text{lipid level of the control group}, C_t = \text{lipid level of the test group}, C_n = \text{mean normal lipid levels})\)

Percentage increase in the levels of HDL was calculated using the following formula (Table 10).

\[
\% \text{ Increase in HDL-C levels} = \frac{C_t - C_c}{C_t - C_n} \times 100
\]

\((C_c = \text{HDL level of the control group}, C_t = \text{HDL level of the test group}, C_n = \text{mean normal HDL levels})\)
Statistical Analysis

The mean values were calculated for each parameter and compared in different groups. All results were expressed as % Mean ± SEM. The statistical differences between various groups were evaluated by One Way Analysis Of Variance (ANOVA) followed by Tukey's test. Data were considered significant at P<0.05. Statistical analysis was performed using GraphPad statistical software.
Table 10: Antihyperlipidemic activity of 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles (234a-234n, 235a-235r) on Poloxamer 407 induced hyperlipidemia in rat

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Compound</th>
<th>% Decrease in lipid levels ± SEM</th>
<th>% Increase in HDL ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TC</td>
<td>TG</td>
</tr>
<tr>
<td>1</td>
<td>Atorvastatin</td>
<td>78.88 ± 1.25</td>
<td>39.51 ± 0.85</td>
</tr>
<tr>
<td>2</td>
<td>234a</td>
<td>73.03 ± 2.15</td>
<td>69.61 ± 1.58</td>
</tr>
<tr>
<td>3</td>
<td>234b</td>
<td>23.0 ± 0.86</td>
<td>8.43 ± 1.25</td>
</tr>
<tr>
<td>4</td>
<td>234c</td>
<td>-17.05 ± 0.25</td>
<td>19.84 ± 1.44</td>
</tr>
<tr>
<td>5</td>
<td>234d</td>
<td>1.72 ± 0.39</td>
<td>35.70 ± 2.27</td>
</tr>
<tr>
<td>6</td>
<td>234e</td>
<td>-20.22 ± 1.52</td>
<td>24.70 ± 0.97</td>
</tr>
<tr>
<td>7</td>
<td>234f</td>
<td>-22.00 ± 1.29</td>
<td>-1.72 ± 0.25</td>
</tr>
<tr>
<td>8</td>
<td>234g</td>
<td>-27.09 ± 1.48</td>
<td>25.59 ± 0.98</td>
</tr>
<tr>
<td>9</td>
<td>234h</td>
<td>16.75 ± 0.46</td>
<td>3.92 ± 0.05</td>
</tr>
<tr>
<td>10</td>
<td>234i</td>
<td>-10.57 ± 0.75</td>
<td>7.56 ± 0.28</td>
</tr>
<tr>
<td>11</td>
<td>234j</td>
<td>3.44 ± 0.21</td>
<td>24.32 ± 1.88</td>
</tr>
<tr>
<td>12</td>
<td>234k</td>
<td>18.70 ± 0.29</td>
<td>58.60 ± 2.87</td>
</tr>
<tr>
<td>13</td>
<td>234l</td>
<td>-17.12 ± 1.87</td>
<td>-19.25 ± 1.28</td>
</tr>
<tr>
<td>14</td>
<td>234m</td>
<td>12.6 ± 1.22</td>
<td>-20.56 ± 0.86</td>
</tr>
<tr>
<td>15</td>
<td>234n</td>
<td>57.99 ± 0.44</td>
<td>69.17 ± 2.96</td>
</tr>
<tr>
<td>16</td>
<td>235a</td>
<td>-16.96 ± 0.85</td>
<td>44.60 ± 2.01</td>
</tr>
<tr>
<td>17</td>
<td>235b</td>
<td>18.20 ± 0.78</td>
<td>15.28 ± 1.22</td>
</tr>
<tr>
<td>18</td>
<td>235c</td>
<td>22.27 ± 0.78</td>
<td>48.0 ± 1.55</td>
</tr>
<tr>
<td>19</td>
<td>235d</td>
<td>6.08 ± 0.15</td>
<td>28.52 ± 0.89</td>
</tr>
<tr>
<td>20</td>
<td>235e</td>
<td>-27.82 ± 2.58</td>
<td>6.92 ± 0.34</td>
</tr>
<tr>
<td>21</td>
<td>235f</td>
<td>-20.61 ± 1.46</td>
<td>15.29 ± 0.33</td>
</tr>
<tr>
<td>22</td>
<td>235g</td>
<td>59.57 ± 1.58</td>
<td>53.62 ± 1.99</td>
</tr>
<tr>
<td>23</td>
<td>235h</td>
<td>15.56 ± 1.98</td>
<td>69.83 ± 1.50</td>
</tr>
<tr>
<td>24</td>
<td>235i</td>
<td>40.58 ± 1.54</td>
<td>69.14 ± 2.00</td>
</tr>
<tr>
<td>25</td>
<td>235j</td>
<td>17.30 ± 0.98</td>
<td>22.50 ± 1.45</td>
</tr>
<tr>
<td>26</td>
<td>235k</td>
<td>5.80 ± 0.08</td>
<td>6.01 ± 0.15</td>
</tr>
</tbody>
</table>
Synthesis and biological evaluation of substituted 1,2,4-triazoles

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>235l</td>
<td>-30.44 ± 2.66</td>
<td>-25.49 ± 1.23</td>
<td>-0.03 ± 0.20</td>
</tr>
<tr>
<td>28</td>
<td>235m</td>
<td>-19.17 ± 1.25</td>
<td>35.22 ± 1.85</td>
<td>-7.13 ± 1.40</td>
</tr>
<tr>
<td>29</td>
<td>235n</td>
<td>12.78 ± 0.87</td>
<td>65.11 ± 1.59</td>
<td>-6.18 ± 0.46</td>
</tr>
<tr>
<td>30</td>
<td>235o</td>
<td>-6.81 ± 0.23</td>
<td>55.01 ± 1.85</td>
<td>8.44 ± 0.88</td>
</tr>
<tr>
<td>31</td>
<td>235p</td>
<td>-2.31 ± 0.56</td>
<td>-5.41 ± 0.55</td>
<td>-25.58 ± 1.68</td>
</tr>
<tr>
<td>32</td>
<td>235q</td>
<td>30.07 ± 1.25</td>
<td>72.41 ± 2.25</td>
<td>-26.91 ± 1.20</td>
</tr>
<tr>
<td>33</td>
<td>235r</td>
<td>53.66 ± 0.88</td>
<td>45.68 ± 1.44</td>
<td>34.01 ± 1.27</td>
</tr>
</tbody>
</table>

* Significantly different than control (P<0.0001).

TC - Total cholesterol
TG - Triglycerides
HDL - High density lipoprotein
SEM - Standard error of mean
Fig. 13: Antihyperlipidemic activity of 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles (234a-234n) on total cholesterol and triglycerides in Poloxamer 407 induced hyperlipidemia in rats

![Graph showing % Decrease of total cholesterol and triglycerides for different compounds](image)

**Compound No.**

Fig. 14: Antihyperlipidemic activity of 5-substituted-4-(substituted)amino-3-mercapto-(4H)-1,2,4-triazoles (235a-235r) on total cholesterol and triglycerides levels in Poloxamer 407 induced hyperlipidemia in rats

![Graph showing % Decrease of total cholesterol and triglycerides for different compounds](image)

**Compound No.**
Synthesis and biological evaluation of substituted 1,2,4-triazoles

Fig. 15: Antihyperlipidemic activity of 5-substituted-4-(substituted)amino-3-mercaptop-(4H)-1,2,4-triazoles (234a-234n) on HDL levels in Poloxamer 407 induced hyperlipidemia in rats

![Graph showing antihyperlipidemic activity of compounds 234a-234n on HDL levels](image)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>234a</td>
<td></td>
</tr>
<tr>
<td>234b</td>
<td></td>
</tr>
<tr>
<td>234c</td>
<td></td>
</tr>
<tr>
<td>234d</td>
<td></td>
</tr>
<tr>
<td>234e</td>
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<td>234f</td>
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<tr>
<td>234i</td>
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<tr>
<td>234k</td>
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<tr>
<td>234l</td>
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</tr>
<tr>
<td>234m</td>
<td></td>
</tr>
<tr>
<td>234n</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 16: Antihyperlipidemic activity of 5-substituted-4-(substituted)amino-3-mercaptop-(4H)-1,2,4-triazoles (235a-235r) on HDL levels in Poloxamer 407 induced hyperlipidemia in rats

![Graph showing antihyperlipidemic activity of compounds 235a-235r on HDL levels](image)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>235a</td>
<td></td>
</tr>
<tr>
<td>235b</td>
<td></td>
</tr>
<tr>
<td>235c</td>
<td></td>
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<td>235d</td>
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<tr>
<td>235e</td>
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<td>235g</td>
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<td>235h</td>
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<td>235i</td>
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<td>235q</td>
<td></td>
</tr>
<tr>
<td>235r</td>
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</tbody>
</table>
4.4.3 Results and discussion

Treatment of rats with 1.0 ml of 30% w/v solution of poloxamer 407 produced significant elevation of total blood cholesterol (from 66.15 ± 2.30 to 244.30 ± 1.46 mg/dL) and total triglycerides (from 54.56 ± 1.85 to 4980 ± 6.68 mg/dL). However, HDL levels were not affected significantly. Atorvastatin, the antihyperlipidemic drug used as a standard drug at a dose of 50 mg/kg, decreased cholesterol levels by 78.9% and triglycerides by 39.5% and increased HDL levels by 23.1%.

Many of the test compounds showed significant reduction in serum cholesterol levels. Compound 234a was found to be the most potent with 73.0% reduction in serum cholesterol levels, which was found to be comparable to that of atorvastatin. It also produced significant decrease in serum triglyceride levels (69.6%). However, it suffered from the fact that it produced remarkable reduction in serum HDL levels indicating unsuitability of 234a as a possible lead molecule.

In general, compounds 235a-235r were found to be better at reducing serum cholesterol and triglycerides levels than compounds 234a-234n. Among compounds 234a-234n, besides compound 234a, compound 234n, with 4-pyridyl substitution at the 5th position, produced significant decrease in the serum cholesterol levels (58.0%) and serum triglycerides (69.2%). However, as seen with 234a, compound 234n lowered serum HDL levels (79.7%). Compound 234k showed a significant reduction in serum triglycerides (58.6%), although other lipid parameters were poorly affected. Also, none of the compounds showed significant elevation of serum HDL levels except 234l (25.3%). However, compound 234l showed modest elevation of serum cholesterol and triglycerides levels.

Among compounds 235a-235r, almost all the compounds produced significant reduction in serum triglyceride levels. Compounds 235c-235e, with 2-Me, 3-Me and 4-Me substitution on the aryl ring at C-5, respectively, showed very good increase in serum HDL levels (43%, 43% and 52%, respectively). Compounds 235c and 235d showed good activity on other lipid parameters. Compound 235c showed 23% and 48% decrease in serum cholesterol and triglycerides levels, respectively, while compound 235d showed 6% and 28.5% decrease in serum cholesterol and triglycerides levels, respectively. Compound 235e elevated serum cholesterol levels by 28%.

Interesting observations were drawn from the results of compounds 235g-235i. Compounds 235g and 235h, with 3-Cl and 4-Cl substitutions at aryl ring, showed good reduction in serum cholesterol levels (59.6% and 15.6%, respectively) and serum
Synthesis and biological evaluation of substituted 1,2,4-triazoles

triglycerides levels (53.6% and 69.8%, respectively). However, compounds produced negative effect on serum HDL levels (77.7% and 2.3% decrease in serum HDL levels). Excellent results were obtained for 3,4-dichloro substituted derivative (235i). Compound 235i stood out as the best among all compounds with 40.6% decrease in serum cholesterol levels, 69.1% decrease in serum triglycerides and 40.8% increase in serum HDL levels. Compounds 235m-235o showed promising results on serum triglyceride levels (35-65% decrease). However, they failed to affect other lipid parameters. Compound 235q with 4-NO₂ substitution on the aryl ring showed highest reduction of serum triglycerides levels (72.4%) along with good decrease (30.1%) in serum cholesterol levels. However, HDL levels were reduced by 27% making it a poor lead candidate. Compound 235r, with 4-pyridyl substitution at C-5, stood out as second best candidate as it affected all the lipid parameters favorably. Serum cholesterol levels were dropped by 53.7%, serum triglycerides were reduced by 43.7% and a rise of 34% was observed in serum HDL levels.

With respect to substitutions at C-4, following order of activity was seen among the test compounds on serum cholesterol levels:

\[
H > 3\text{-Cl-phenyl} > 4\text{-pyridyl} > 3,4\text{-di-Cl-phenyl}
\]

As seen from the results, electron releasing substitutions are deleterious to the activity. Following is the order of activity for reduction in triglyceride levels:

\[
4\text{-NO}_2\text{-Ph} > H > 4\text{-pyridyl} > 3,4\text{-di-Cl-phenyl} > 2\text{-OCH}_3\text{-Ph} > 4\text{-Cl-benzy}l > 3\text{-Cl-phenyl}
\]

Few compounds also showed significant elevation of HDL levels. Electron releasing group at 2\text{nd} and 3\text{rd} position and electron withdrawing groups at 3\text{rd} and 4\text{th} position on the aryl ring at C-5 tend to increase the activity. In addition, substitution of the aryl moiety with heteroaryl moiety increased the activity significantly. In fact, 234n and 235r with 4-pyridyl substitution at C-5 significantly affected all the lipid parameters.