Synthesis of Some New Kind of Antipyrine Carbo(thio)amide and 1,2,4-Triazole Derivatives and Comparing Their Anti-tubercular Activities

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Abstract
In this study, antipyrine (4-aminoantipyrine) was selected as the starting material and anti-tubercular activities were compared by carrying out the antipyrine nucleated carbothioamide and 1,2,4-triazole derivatives. The treatment of 4-aminoantipyrine with ethylbromoacetate, hydrazinhydrate and various isothiocyanates, respectively, followed by cyclic reaction by basic reaction resulted in the addition of a second ring of 1,2,4-triazole to the structure. The structures of the synthesized new antipyrine derivatives were determined by spectroscopic methods such as IR, NMR and mass spectrophotometry. For the new compounds synthesized, activity studies were performed against non-Gram bacteria.

Keywords: Antipyrine, 1,2,4-Triazole, Anti-tubercular Activity.

Bazı Yeni Antipirin Karbo (tiyo) amid ve 1,2,4-Triazol Türevlerinin Sentezi ve Anti-tuberküler Aktivitelerinin Karşılaştırılması

Öz
Bu çalışmada antipirin (4-Aminoantipirin) başlangıç maddesi olarak seçilerek karbotiyoamit ve 1,2,4-triazoltürevi bileşikler gerçekleştirilerek anti-tuberküler aktiviteleri karşılaştırılmıştır. 4-aminoantipirin sırasıyla etilbromoasetat, hidrazinhidrat ve çeşitli i佐(tiyo)sıyanatlar ile muameleri sonucu bazı ortamda reaksiyonu ile halkalama reaksiyonu gerçekleşmesi ile yapıya ikinci bir halka olan 1,2,4-triazol halkasının ilavesi gerçekleştirilmiştir. Sentezelenen yeni antipirin türevi bileşiklerin yapıları IR, NMR ve kütle spektrofotometresi gibi spektroskopik yöntemlerle aydınlatıldı. Sentezelenen yeni bileşikler, Gram olmayan bakteriye karşı aktivite çalışmaları gerçekleştirilmiştir.

Anahtar Kelimeler: Antipirin, 1,2,4-Triazol, Anti-tuberküler Aktivite.
1. Introduction

Antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) is the first pyrazole derivative to be used in pain and inflammation conditions and is active in many studies. Bioactive compounds obtained using this compound, also called 4-aminoantipyrine (1), are known in the literature (Jain et al. 2003, Gürsoy et al. 2000, Turan-Zitouni et al. 2001, Abu Elmaati 2002, Abdel-Latif 2006). Antipyrine-derived compounds; analgesics (Filho et al. 1998, Sondhi et al. 1999), anti-inflammatory (İsmail et al. 2007), antimicrobial (Mishra 1999, Raman et al. 2002, Raman et al. 2004) and anticancer (Sondhi et al. 2001) are compounds with bioactive properties (Bondock et al. 2008, Rostom et al. 2009).

\[
\begin{align*}
\text{(1)} & \quad \text{4-Aminoantipyrine} \\
\end{align*}
\]

The synthesis of heterocyclic compounds containing five-membered rings has become increasingly important in recent years due to their pharmacological properties. Examples of such compounds include Vorozol (2), Letrozole (3), Anastrozole (4) and Itraconazole (5), which are currently used in cancer treatment and contain an azole ring (Sun et al. 2004, Verrect et al. 2003).

\[
\begin{align*}
\text{(2)} & \quad \text{Vorozole} \\
\text{(3)} & \quad \text{Letrozole} \\
\text{(4)} & \quad \text{Anastrozole} \\
\end{align*}
\]
Pathogenic microorganisms have rapidly led to new drug discoveries due to their resistance to existing drugs. And this is of interest to medical chemists. Therefore, synthesis of simple or complex triazole compounds is important for the discovery of new drugs (Rostom et al. 2003, Turan-Zitouni et al. 1999, Kolavi et al. 2006, Holla et al. 2001, Demirbaş et al. 2004, Demirbaş et al. 2002). For this purpose, many working groups have begun to design and synthesize compounds containing triazole rings bearing different functional groups. The 1,2,4-triazole ring is included in the structure of many more therapeutically important drugs. For example; Fluconazole (9) (antifungal), Ribavirin (10) (antiviral), Rizatriptan (11) (antimigren), Alprazolam (12) (anxiolytic) are the best examples. It has been reported in the literature that these compounds have different biological and medicinal properties (Chandra et al. 2006, Dixit et al. 2006, İkizler et al, 1996, İkizler et al. 1999).

Novel 1,2,4-triazole analogs were synthesized and antitubercular activity results were evaluated with different pharmacophores (Figure 1 and Figure 2). Compound 5c which contain an allylic group at N-4 atom of 1,2,4-triazole ring showed excellent antitubercular activity compared with Streptomycin standard drug. Compound 5b also showed equivalent antitubercular activity to the
standard drug. Carbothioamide derivatives of compound 4b and 4c showed good-moderate activity against *Mycobacterium smegmatis* test microorganism. As a result, it has seen that the allylic and phenyl group of carbothioamide or 1,2,4-triazole derivatives containing antipyrine group showed very good antitubercular activity.

2. Materials and Methods

2.1. General Methods for Chemistry

All the chemicals used in this publication were obtained from Sigma-Aldrich and Merck without further purification. Melting points of the synthesized new compounds were obtained by using capillary tube in Stuart Brand SMP apparatus. Reaction times and purities were determined by thin layer chromatography. Infrared spectra were obtained by ATR apparatus on Perkin Elmer brand and 1600 serial IR devices. NMR spectra of the compounds were obtained from BRUKER AVENE II 400 MHz instrument in Karadeniz Technical University or Giresun University Central Research Laboratories. Mass spectra of the compounds were also obtained from Agilent Technologies branded 1260 Infinity 6230 TOF LC / MS model device at Giresun University Central Research Laboratory.

2.2. Ethyl N-(1,5-dimethyl-3-oxo-2-phenylpyrazolidin-4-yl)glycinate (2)

To a solution of the compound (1) (10 mmol) in THF (THF) was added an equivalent amount of triethylamine (20 mmol). The reaction was cooled to 0-5 °C and ethylbromoacetate (10 mmol) was added dropwise onto the mixture. The reaction mixture was stirred at room temperature for 24 hours. The triethylammonium salt formed after this time was removed by filtration. The solvent was evaporated under reduced pressure to precipitate the resulting oily substance from a mixture of n-butylacetate: ether (1: 2) to form a solid. The crude product obtained was purified by crystallization from ethyl acetate: petroleum ether (1: 2) mixture. Yield 70 %, m.p. 61-63 °C.

FT-IR (υmax, cm⁻¹): 3326 (NH), 3068 (aromatic CH), 1743 (C=O).

¹H NMR (DMSO-d₆, δ ppm): 1.14 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.14 (2H, s, CH₂), 2.71 (3H, s, CH₃), 3.81 (2H, s, CH₂), 7.21 (1H, d, J= 4.0 Hz, arH), 7.42 (4H, d, J= 8.0 Hz, arH), 8.98 (1H, s, NH).

¹³C NMR (DMSO-d₆, δ ppm): 9.06 (CH₃), 10.54 (CH₃), 14.58 (CH₃), 46.79 (CH₂), 60.48 (CH₂), 120.26 (antipirin C-5), arC: [120.53 (C), 122.65 (CH), 125.56 (CH), 125.81 (CH), 126.57 (CH), 129.36 (CH)], 136.04 (antipirin C-3), 161.86 (antipirin C-4), 172.43 (C=O).

EI MS m/z (%): 188.30 (100), 160.25 (32), 290.38 ([M+1]+, 30), 204.28 (18).
2.3. \(N\-[(N-(1,5\text{-dimethyl-3-oxo-2-phenylpyrazolidin-4-yl})\text{glycyl}]\text{amonium amide (3)}\)

Hydrazinhydrate (25 mmol) was added to a solution of compound 2 (10 mmol) in absolute ethanol and the reaction was refluxed for 10 hours. The mixture was purified by crystallization from ethanol by filtering the resulting white solid by standing in the cold overnight. Yield 65 %, m.p. 91-93°C.

FT-IR (\(\nu_{\text{max}}, \text{cm}^{-1}\)): 3433 (NH\(_2\)), 3324 (NH), 3182 (NH), 3070 (aromatic CH), 1744 (C=O), 1677 (C=O).

\(^1\)H NMR (DMSO-\text{d6}, \(\delta\) ppm): 2.07 (3H, s, CH\(_3\)), 2.71(3H, s, CH\(_3\)), 2.78 (2H, s, CH\(_2\)), 3.78 (2H, brs, NH\(_2\)), 7.22 (1H, d, \(J=4.0\ \text{Hz}, \text{arH}\)), 7.40-7.43 (4H, m, arH), 8.25 (1H, s, NH), 8.78 (1H, s, NH).

\(^13\)C NMR (DMSO-\text{d6}, \(\delta\) ppm): 10.35 (CH\(_3\)), 38.71 (CH\(_3\)), 61.12 (CH\(_2\)), 120.50 (antipyrine C-5), arC: [122.31 (CH), 125.59 (2CH), 129.36 (2CH), 135.21 (C)], 136.01 (antipyrine C-3), 161.86 (antipyrine C-4), 172.80 (C=O).

EI MS \(m/z\) (%): 204.05 (100), 174.14 (62), 216.06 (41), 316.24 (38), 276.19 ([M+1]+, 28).

2.4. General Synthesis of Compounds 4a-4e:

To a solution of compound 3 (10 mmol) in absolute dichloromethane, phenyl isothiocyanate (for compound 4a), benzyl isothiocyanate (for compound 4b) or allyl isothiocyanate (for compound 4c) (20 mmol) was added dropwise and the mixture was stirred at room temperature for 24 hours. After this time, the precipitated solid was filtered off and purified by crystallization in the appropriate solvents.

2.4.1. \(N\-[(1,5\text{-dimethyl-3-oxo-2-phenylpyrazolidin-4-yl})\text{glycyl}]\text{amonium amide (4a)}\)

Crystallization solvent, ethanol. Yield 80 %, m.p. 175-177°C.

FT-IR (\(\nu_{\text{max}}, \text{cm}^{-1}\)): 3212 (NH), 3114 (NH), 3042 (aromatic CH), 1740 (C=O), 1631 (C=O).

\(^1\)H NMR (DMSO-\text{d6}, \(\delta\) ppm): 2.18 (3H, s, CH\(_3\)), 3.09 (3H, s, CH\(_3\)), 3.35 (2H, s, CH\(_2\)), 7.13 (2H, d, \(J=8.0\ \text{Hz}, \text{arH}\)), 7.31-7.37 (4H, m, arH), 7.44-7.55 (4H, m, arH), 8.92 (1H, s, NH), 9.67 (1H, s, NH), 9.74 (1H, s, NH), 10.14 (1H, s, NH).

\(^13\)C NMR (DMSO-\text{d6}, \(\delta\) ppm): 11.45 (CH\(_3\)), 36.04 (CH\(_3\)), 50.45 (CH\(_2\)), 120.65 (antipyrine C-5), arC: [124.29 (2CH), 125.12 (CH), 125.31 (CH), 126.88 (CH), 128.68 (2CH), 129.39 (2CH), 135.21 (C)].
2.4.2. \( N\)-\{(Benzylamino)carbonothioyl|amino\}-\(N'\)-(1,5-dimethyl-3-oxo-2-phenyl pyrazolidin-4-yl)glycyn amide (4b)

Crystallization solvent, ethanol. Yield 80 %, m.p. 168-170 °C.

FT-IR (\( \nu_{\text{max}} \), cm\(^{-1}\)): 3251 (NH), 3137 (NH), 3062 (aromatic CH), 1633 (C=O).

\(^1\)H NMR (DMSO-\(d_6\), \( \delta \) ppm): 2.15 (3H, s, CH\(_3\)), 2.80 (2H, s, CH\(_2\)), 3.08 (3H, s, CH\(_3\)), 4.60 (2H, s, CH\(_2\)), 7.21-7.39 (8H, m, arH), 7.47-7.51 (2H, m, arH), 8.19 (1H, s, NH), 8.69 (1H, s, NH), 9.35 (1H, s, NH), 9.96 (1H, s, NH).

\(^{13}\)C NMR (DMSO-\(d_6\), \( \delta \) ppm): 10.48 (CH\(_3\)), 36.03 (CH\(_3\)), 37.80 (CH\(_2\)), 47.92 (CH\(_2\)), 120.54 (antipyrine C-5), arC: [123.02 (CH), 124.13 (CH), 126.18 (CH), 126.72 (CH), 126.95 (CH), 127.07 (CH), 127.44 (CH), 127.51 (CH), 128.38 (CH), 128.57 (CH)], 139.61 (antipyrine C-3), 140.12 (antipyrine C-4), 162.21 (C=O), 183.21 (C=S).

EI MS \( m/z \) (%): 380.24 (100), 375.24 (81), 443.44 ([M+H\(_2\)O+1]\(^+\), 55), 391.32 (46), 407.21 (49), 425.42 ([M+1]\(^+\), 31).

2.4.3. \( N\)-\{(Allylamino)carbonothioyl|amino\}-\(N'\)-(1,5-dimethyl-3-oxo-2-phenyl pyrazolidin-4-yl)glycyn amide (4c)

Crystallization solvent, ethanol. Yield 80 %, m.p. 178-180 °C.

FT-IR (\( \nu_{\text{max}} \), cm\(^{-1}\)): 3277 (NH), 3080 (aromatic CH), 1636 (C=O).

\(^1\)H NMR (DMSO-\(d_6\), \( \delta \) ppm): 2.10 (3H, s, CH\(_3\)), 3.08 (3H, s, CH\(_3\)), 3.32 (2H, s, CH\(_2\)), 4.10 (2H, s, CH\(_2\)), 5.02-5.17 (2H, m, CH\(_2\)), 5.79-5.84 (1H, m, CH), 7.29-7.35 (3H, m, arH), 7.47-7.51 (2H, m, arH), 7.85 (2H, s, 2NH), 8.06 (1H, s, NH), 8.60 (1H, s, NH), 9.33 (1H, s, NH).

\(^{13}\)C NMR (DMSO-\(d_6\), \( \delta \) ppm): 11.13 (CH\(_3\)), 36.04 (CH\(_3\)), 40.59 (CH\(_2\)), 46.41 (CH\(_2\)), 46.96 (CH\(_2\)), 115.69 (antipyrine C-5), arC: [115.82 (C), 124.13 (2CH), 126.72 (CH), 129.50 (CH), 135.16 (CH), 135.47 (CH)], 135.53 (antipyrine C-3), 153.95 (antipyrine C-4), 162.27 (C=O), 182.74 (C=S).

EI MS \( m/z \) (%): 330.13 (100), 325.18 (51), 429.17 (31), 375.30 ([M+1]\(^+\), 15).
2.4.4. \(N^2\)-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1\(H\)-pyrazol-4-yl)-(\{[2-phenyl ethyl]amino|carbonothioyl|amino\)glycynamide (4d)

Crystallization solvent, acetone. Yield 76 \%, m.p. 189-191 °C.

FT-IR (\(\nu_{\text{max}}\), cm\(^{-1}\)): 3316 (NH), 3241 (NH), 3064 (aromatic CH), 1635 (C=O).

\(^1\)H NMR (DMSO-\(d_6\), \(\delta\) ppm): 2.05 (3H, s, CH\(_3\)), 2.82 (2H, d, \(J= 8.0\) Hz, CH\(_2\)), 3.07 (3H, s, CH\(_3\)), 3.32 (2H, s, CH\(_2\)), 3.64 (2H, s, CH\(_2\)), 7.18-7.35 (8H, m, arH), 7.47-7.51 (2H, m, arH), 7.72 (2H, s, 2NH), 8.58 (2H, s, 2NH).

\(^13\)C NMR (DMSO-\(d_6\), \(\delta\) ppm): 15.41 (CH\(_3\)), 35.21 (CH\(_3\)), 36.07 (CH\(_2\)), 46.18 (2CH\(_2\)), 119.65 (antipyrine C-5), arC: [124.11 (CH), 126.56 (2CH), 126.73 (2CH), 128.81 (2CH), 129.10 (2CH), 129.51 (CH), 135.46 (C), 139.74 (C)], 140.41 (antipyrine C-3), 142.21 (antipyrine C-4), 165.36 (C=O), 181.79 (C=S).

2.4.5. \(N^1\)-(Anilinocarbonyl)amino]-\(N^2\)-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1\(H\)-pyrazol-4-yl)glycynamide (4e)

Crystallization solvent, ethanol. Yield 82 \%, m.p. 205-207 °C.

FT-IR (\(\nu_{\text{max}}\), cm\(^{-1}\)): 3292 (NH), 3232 (NH), 3062 (aromatic CH), 1708 (C=O), 1668 (C=O).

\(^1\)H NMR (DMSO-\(d_6\), \(\delta\) ppm): 2.19 (3H, s, CH\(_3\)), 3.02 (3H, s, CH\(_3\)), 4.35 (2H, s, CH\(_2\)), 6.91-7.48 (10H, m, arH), 7.97 (1H, s, NH), 8.56 (1H, s, NH), 8.78 (2H, d, \(J= 8.0\) Hz, 2NH).

\(^13\)C NMR (DMSO-\(d_6\), \(\delta\) ppm): 35.55 (CH\(_3\)), 36.59 (CH\(_3\)), 60.21 (CH\(_2\)), 108.73 (antipyrine C-5), arC: [118.49 (CH), 119.01 (CH), 122.10 (CH), 123.83 (CH), 124.79 (CH), 126.61 (CH), 129.06 (CH), 129.17 (CH), 129.23 (CH), 129.52 (CH), 135.48 (C), 140.38 (C)], 152.99 (antipyrine C-3), 154.06 (antipyrine C-4), 156.50 (C=O), 162.61 (C=O).

2.5. General Synthesis of Compounds 5a-5e

Compound 4 and NaOH solution (2 \%) were refluxed for 4 hours. Then, the crude mixture was acidified to pH 4 with 37% HCl by cooling to room temperature. The resulting solid was filtered and purified by crystallization from the appropriate solvents.

2.5.1. \(4\{-[5\text{-Mercapto-4-phenyl-1,2,4-triazol-3-yl}methyl]amino\}-1,5\text{-dimethyl-2-phenyl pyrazolidin-3-one (5a)}\)

Crystallization solvent, ethyl acetate. Yield 71 \%, m.p. 201-203°C.
FT-IR (ν_max, cm⁻¹): 3280 (NH), 3094 (aromatic CH), 1698 (C=O).

1H NMR (DMSO-d₆, δ ppm): 2.18 (3H, s, CH₃), 2.48 (3H, s, CH₃), 3.03 (2H, s, CH₂), 6.89-7.55 (3H, m, arH), 9.03 (1H, s, NH), 13.32 (1H, s, SH).

13C NMR (DMSO-d₆, δ ppm): 11.65 (CH₃), 36.63 (CH₃), 42.62 (CH₂), 109.55 (antipyrine C-5), arC: [118.22 (CH), 118.35 (CH), 121.97 (CH), 123.78 (CH), 126.56 (CH), 129.07 (CH), 129.14 (CH), 129.40 (CH), 129.52 (CH), 129.84 (CH), 135.53 (2C)], 142.39 (antipyrine C-3), 152.33 (antipyrine C-4), 160.12 (triazole C-3), 161.57 (triazole C-5).

EI MS m/z (%): 345.27 (100), 361.22 (41), 252.17 (31), 415.28 ([M+1]+, 11).

2.5.2. 4-{[(4-Allyl-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]amino}-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (5b)

Crystallization solvent, acetone. Yield 76 %, m.p. 210-212 °C.

FT-IR (ν_max, cm⁻¹): 3277 (NH), 3081 (aromatic CH), 1698 (C=O), 1591 (C=N).

1H NMR (DMSO-d₆, δ ppm): 2.10 (3H, s, CH₃), 2.99 (3H, s, CH₃), 4.10 (2H, s, CH₂), 5.03-5.18 (2H, m, CH₂), 5.79-5.83 (1H, s, CH), 7.33-7.35 (3H, m, arH), 7.46-7.48 (2H, m, arH), 7.94 (1H, s, NH), 8.65 (1H, s, NH).

13C NMR (DMSO-d₆, δ ppm): 36.07 (CH₃), 36.67 (CH₃), 42.19 (CH₂), 46.96 (CH₂), 114.89 (antipyrine C-5), arC: [115.69 (C), 123.64 (CH), 124.11 (CH), 126.43 (CH), 126.70 (CH), 129.50 (CH), 135.49 (CH)], 135.52 (antipyrine C-3), 136.90 (antipyrine C-4), 162.21 (triazole C-3), 163.57 (triazole C-5).

2.5.3. 4-{[(4-Benzyl-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]amino}-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (5c)

Crystallization solvent, acetone. Yield 73 %, m.p. 198-200 °C.

FT-IR (ν_max, cm⁻¹): 3272 (NH), 3061 (aromatic CH), 1633 (C=O), 1537 (C=N).

1H NMR (DMSO-d₆, δ ppm): 2.01 (3H, s, CH₃), 3.08 (3H, s, CH₃), 4.11 (2H, s, CH₂), 4.73 (2H, s, CH₂), 7.21-7.35 (8H, m, arH), 7.47-7.51 (2H, m, arH), 8.69 (1H, s, NH), 13.71 (1H, s, SH).

13C NMR (DMSO-d₆, δ ppm): 10.36 (CH₃), 11.15 (CH₃), 36.03 (CH₂), 47.92 (CH₂), 118.70 (antipyrine C-5), arC: [123.04 (CH), 124.13 (CH), 126.09 (CH), 126.72 (CH), 127.07 (CH), 127.44 (CH), 127.69 (CH), 128.10 (CH), 128.57 (CH), 129.03 (CH), 135.49 (C), 135.70 (C)], 136.37 (antipyrine C-3), 139.86 (antipyrine C-4), 154.11 (triazole C-3), 162.31 (triazole C-5).
2.5.4. 4-{{[5-Mercapto-4-(2-phenylethyl)-4\(^H\)-1,2,4-triazol-3-yl]methyl}amino}-1,5-dimethyl-2-phenyl-1,2-dihydro-3\(^H\)-pyrazol-3-one (5d)

Crystallization solvent, ethanol. Yield 73 %, m.p. 212-213 °C.

FT-IR (\(\nu_{\text{max}}, \text{cm}^{-1}\)): 3311 (NH), 3065 (aromatic CH), 1634 (C=O), 1574 (C=N).

\(^1\)H NMR (DMSO-\(d_6\), \(\delta\) ppm): 2.05 (3H, s, CH\(_3\)), 2.81 (2H, s, CH\(_2\)), 3.07 (3H, s, CH\(_3\)), 3.63 (2H, s, CH\(_2\)), 4.45 (2H, s, CH\(_2\)), 7.11-7.48 (10H, m, arH), 8.66 (1H, s, NH), 14.59 (1H, s, SH).

\(^{13}\)C NMR (DMSO-\(d_6\), \(\delta\) ppm): 11.30 (CH\(_3\)), 15.78 (CH\(_3\)), 48.10 (CH\(_2\)), 49.28 (CH\(_2\)), 50.12 (CH\(_2\)), 107.10 (antipyrine C-5), arC: [110.12 (CH), 112.52 (CH), 118.37 (CH), 120.10 (CH), 120.83 (CH), 121.02 (CH), 122.20 (CH), 123.85 (CH), 124.10 (CH), 125.33 (CH), 134.10 (C), 135.21 (C)], 140.18 (antipyrine C-3), 143.86 (antipyrine C-4), 156.62 (triazole C-3), 160.37 (triazole C-5).

2.5.5. 4-{{[5-Hydroxy-4-phenyl-4\(^H\)-1,2,4-triazol-3-yl]methyl}amino}-1,5-dimethyl-2-phenyl-1,2-dihydro-3\(^H\)-pyrazol-3-one (5e)

Crystallization solvent, ethanol. Yield 75 %, m.p. 223-225 °C.

FT-IR (\(\nu_{\text{max}}, \text{cm}^{-1}\)): 3291 (NH), 3206 (NH), 3092 (aromatic CH), 1697 (C=O), 1595 (C=N).

\(^1\)H NMR (DMSO-\(d_6\), \(\delta\) ppm): 2.19 (6H, s, 2CH\(_3\)), 3.39 (2H, s, CH\(_2\)), 6.92 (2H, m, arH), 7.23-7.52 (8H, m, arH), 8.87 (1H, s, NH), 8.94 (1H, d, \(J= 12.0\) Hz, NH).

\(^{13}\)C NMR (DMSO-\(d_6\), \(\delta\) ppm): 11.65 (CH\(_3\)), 36.56 (CH\(_3\)), 48.21 (CH\(_2\)), 108.71 (antipyrine C-5), arC: [118.42 (CH), 118.51 (CH), 118.85 (CH), 122.05 (CH), 122.15 (CH), 122.24 (CH), 123.84 (CH), 126.62 (CH), 129.05 (CH), 129.16 (CH), 135.48 (C), 140.43 (C)], 153.10 (antipyrine C-3), 154.13 (antipyrine C-4), 156.53 (triazole C-3), 162.64 (triazole C-5).
Figure 1. Synthetic route for compounds 2-5.

2.6. Procedure of Minimal Inhibition Concentration (MIC)

To determine the minimum amount of substance that exhibits antimicrobial activity, the microdilution fluid method is applied in liquid medium and the minimum inhibition concentration (MIC) is determined as micrograms / milliliters (µg / mL) (Woods et al. 2003). For the determination of antimicrobial activity, liquid medium was used to determine the antifungal activity of Mueller-Hinton liquid (MHB, pH.7.3) (Difco, Detroit, MI) and serial dilutions were made with 0.1 ml for microdilution tests. McFarland 0.5 turbidity (1 x 108 cfu / mL) from overnight inoculated microorganism cultures of dissolved chemicals was prepared for reconstitution and diluted 1:10 and 0.005 ml microorganism (final assay concentration 5 x 104 cfu / well) to each well. The plates were incubated at 35 °C for 16-24 hours under aerobic conditions. The MIC value was completely inhibited by the growth of the microorganism in the micro-dilution wells and was determined as the lowest
antimicrobial concentration detectable with the naked eye. Streptomycin (4 μg) and standard solvent control were used as standard control drugs.

Table 1. Determination of Anti-tubercular Activity of the New Compounds by Microorganisms and Minimum Inhibition Concentrations (MIC) method

| Comp. No | Ms (µg / mL) |
|----------|--------------|
| 2        | 125          |
| 3        | 31.25        |
| 4a       | 15.6         |
| 4b       | 250          |
| 4c       | 7.81         |
| 4d       | 125          |
| 4e       | -            |
| 5a       | 15.6         |
| 5b       | 3.9          |
| 5c       | 1.95         |
| 5d       | 250          |
| 5e       | 250          |
| Str.     | 4            |

*Ms*: *Mycobacterium smegmatis* ATCC607,  
Str.: Streptomycin, (—): no activity.

3. Results and Discussion

In this study, antipyrine (1) was chosen as the starting material and the corresponding carbo(thio)amide (4a-4e) derivatives were obtained using different iso(thio)cyanates. Subsequently, the corresponding 1,2,4-triazole derivatives (5a-5e) were synthesized by cyclic reaction in basic medium. Anti-tubercular activities of all compounds obtained were examined. Of these, compound 5c with allyl group in structure has shown better anti-tubercular activity (1.95 µg / mL) even than the standard drug streptomycin (4 µg / mL). In addition, compound 5b with the benzyl group in structure, showed equivalent activity against *Mycobacterium smegmatis* (Ms) to standard drug streptomycin. The carbothioamide compound 4c having allyl group in its structure showed also moderate anti-tubercular activity (Table 1).

4. Conclusions and Recommendations

This study covers the synthesis of 1,2,4-triazole derivatives with different pharmacophores and investigation of their anti-tubercular activity. Compound 5b and 5c showed excellent activity against the test microorganism of Ms compared with standard drug Streptomycin.
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