# SYNTHESIS, CHARACTERISATION AND CYTOTOXICITY STUDY OF SOME NEW 1,2,4-TRIAZOLE DERIVATIVES

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# SYNTHESIS, CHARACTERISATION AND CYTOTOXICITY STUDY OF SOME NEW 1,2,4-TRIAZOLE DERIVATIVES

By

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# LIST OF SYMBOLS & ABBREVIATIONS

<sup>1</sup> H -NMR	Proton Nuclear Magnetic Resonance
<sup>13</sup> C-NMR	Carbon Nuclear Magnetic Resonance
IR	Infra-Red
Mol. Wt.	Molecular weight
HCl	Hydrochloric Acid
MeOD-d4	Deuterated Methanol
CDCl <sub>3</sub>	Deuterated Chloroform
DMSO- $d_6$	Deuterated Dimethyl sulfoxide
C <sub>5</sub> D <sub>5</sub> N	Deuterated Pyridine
D <sub>2</sub> O	Deuterium oxide
FT-IR	Fourier Transformer Infrared
1D-NMR	One Dimensional Nuclear Magnetic Resonance
2D-NMR	Two Dimensional Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
DEPT	Distortionless enhancement by Polarization Transfer
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HSQC	Heteronuclear Single Quantum Correlation
MIC	Minimum Inhibitory Concentration
TLC	Thin layer chromatography
$\mathbf{R}_{f}$	Retention factor
MHz	Mega Hertz
br.s	Broad Singlet
br.d	Broad Doublet

t	Triplet
q	Quartet
m	Multiplet
ppm	Parts per million
cm <sup>-1</sup>	Per centimeter
δ	Chemical shift
μg	Micrograms
mg	Milligram
g	Gram
mL	Milliliter
S	Singlet
d	Doublet
nm	Nanometer
°C	Degree Celsius
h	Hour
min	Minutes
conc.	Concentration
cm	Centimeter
µgmL <sup>-1</sup>	Microgram per milliliter
μL	Microliter
μΜ	Micromole
IC <sub>50</sub>	The half maximal inhibitory concentration
EC <sub>50</sub>	The half maximal effective concentration
$\lambda_{max}$	Lambda (Maximum Wavelength)

# SINTESIS, PENCIRIAN DAN KAJIAN SITOTOKSIK BAGI BEBERAPA TERBITAN BARU 1,2,4-TRIAZOL

## ABSTRAK

Dalam kajian ini, 36 sebatian baru 1,2,4-triazol dengan sistem gelang mono dan terlakur telah disintesis dan dicirikan. Sistem mono bagi terbitan 1,2,4-triazol mempunyai kumpulan yang berbeza terikat pada gelang triazol. Sistem mono yang pertama menunjukkan empat sebatian garam piridinium dan bes Schiff terikat pada gelang triazol manakala sistem mono yang kedua menunjukkan 24 sebatian garam piperidinium dan bes Schiff terikat pada gelang triazol. Sistem terlakur bagi terbitan 1,2,4-triazol termasuk tiga sebatian dengan gelang terlakur triazol-oksadiazol dan lima sebatian dengan gelang terlakur triazol-triazol. Kesemua sebatian ini telah dicirikan menggunakan spektroskopi Inframerah Transformasi Fourier (FT-IR) dan Resonan Nuklear Magnetik (NMR) dan analisa unsur karbon, hidrogen dan nitrogen (CHN). Beberapa sebatian telah dipilih untuk ujian sitotoksik dalam mencari agen anti kanser yang berpotensi, terutamanya untuk sel kanser payudara manusia (MCF-7) dan sel kanser kolon manusia (HCT 116). Sebanyak 35 sebatian yang terdiri daripada sembilan bahan perantara dan 26 hasil akhir telah dipilih untuk asai sitotoksik. Nilai IC<sub>50</sub> bagi beberapa sebatian yang tidak dapat ditentukan dalam asai MTT terhadap sel kanser MCF-7 atau HCT 116 dianggap tidak aktif. Asai MTT terhadap MCF-7 menunjukkan hanya sebatian 6A dan 7B sahaja yang mempunyai aktiviti sederhana dengan nilai IC50 masing-masing iaitu 38 and 53 µM. Lapan sebatian lain menunjukkan aktiviti anti-proliferasi yang sederhana lemah manakala enam sebatian lain menunjukkan aktiviti yang lemah. Asai MTT terhadap HCT 116 menunjukkan hanya sebatian **9A<sub>2</sub>-iii** dan **9B<sub>2</sub>-iii** yang melihatkan kesan sitotoksik yang sederhana dengan nilai IC<sub>50</sub> masing-masing iaitu 42 dan 56  $\mu$ M. Tiga sebatian lain menunjukkan kesan perencatan yang kurang ke atas proliferasi sel dan lapan sebatian yang lain menunjukkan aktiviti yang lemah dengan nilai IC<sub>50</sub> > 110. Keempat-empat terbitan baru 1,2,4-triazole yang berpotensi sebagai agen antiproliferatif yang baik ialah sebatian **6A** (IC<sub>50</sub>= 38  $\mu$ M) dan **7B** (IC<sub>50</sub>= 53  $\mu$ M) terhadap sel kanser payudara manusia (MCF-7) sementara sebatian **9A2-iii** (IC<sub>50</sub>= 42  $\mu$ M) dan **9B2-iii** (IC<sub>50</sub>= 56  $\mu$ M) terhadap sel kanser kolon manusia (HCT 116).

# SYNTHESIS, CHARACTERISATION AND CYTOTOXICITY STUDY OF SOME NEW 1,2,4-TRIAZOLE DERIVATIVES

## ABSTRACT

In this study, 36 new 1,2,4-triazole compounds with mono and fused ring system have been synthesised and characterised. The mono system of 1,2,4-triazole derivatives have different moieties attached to the triazole ring. The first mono system showed four compounds of a pyridinium salt and a Schiff base moiety attached to the triazole ring, while the second mono system showed 24 compounds of a piperidinium salt and a Schiff base moiety attached to the triazole ring. The fused system of 1,2,4-triazole derivatives include three compounds with the triazoleoxadiazole fused ring and five compounds with the triazole-triazole fused ring. All these compounds were characterised using Fourier Transform Infrared (FT-IR) and Nuclear Magnetic Resonans (NMR) spectroscopy and carbon hydrogen nitrogen (CHN) elemental analysis. Some of the compounds were selected for cytotoxicity test in the search for potential anticancer agents, in particular for human breast tumor cells (MCF-7) and human colorectal tumour (HCT 116) cell. 35 compounds consisting of nine intermediates and 26 final compounds were tested for cytotoxicity assay. The IC<sub>50</sub> for some of the compounds which could not be determined in the MTT assay against MCF-7 or HCT 116 cells were considered inactive. The MTT assay findings against MCF-7 showed that only compounds 6A and 7B indicated moderate activity of IC<sub>50</sub> 38 and 53 µM, respectively. Eight other compounds showed moderate to weak anti-proliferation activity while six other compounds showed weak activity. The MTT assay against HCT 116 showed that only compounds 9A<sub>2</sub>-iii and 9B<sub>2</sub>-iii demonstrated moderate cytotoxic effect with IC<sub>50</sub> 42 and 56  $\mu$ M, respectively. Three other compounds demonstrated lesser inhibitory effect on cell proliferation while eight other compounds showed poor activity with the IC<sub>50</sub> of > 110. These four new 1,2,4-triazole derivatives which showed potentials to become promising anti-proliferative agents are compounds **6A** (IC<sub>50</sub>= 38  $\mu$ M) and **7B** (IC<sub>50</sub>= 53  $\mu$ M) against human breast tumor cells (MCF-7) while **9A2-iii** (IC<sub>50</sub>= 42  $\mu$ M) and **9B2-iii** (IC<sub>50</sub>= 56  $\mu$ M) against human colorectal tumour cells (HCT 116).

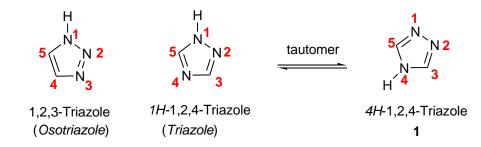
#### **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Background of the research work**

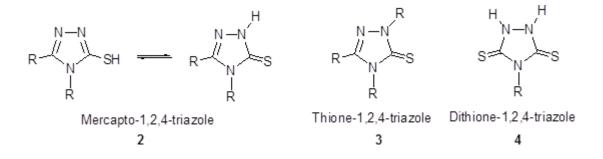
In the field of pharmaceutical organic chemistry, research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. Recently, the 5-membered ring heterocyclic compounds have proved their importance as being the centre of the biological activities (Kashyap et al., 2011). The nitrogen containing heterocycles are found in abundance in most of the medicinal compounds. Starting with imidazole as an important moiety of the medicinal agent, research work has led to the introduction of triazole which is an isostere of imidazole, in which the carbon atom of imidazole is isosterically being replaced by nitrogen (Bele and Singhvi, 2011).

Triazole is a 5-membered ring compound which contains two carbon and three nitrogen atoms. According to the position of the nitrogen atoms, the 5-membered triazole ring exists in two isomeric forms known as 1,2,3-triazole and 1,2,4-triazole, the former being known as *osotriazole*, and the latter as *triazole*. However, 1,2,4-triazole exists in two tautomeric forms of 1*H* and 4*H*-1,2,4-triazole (**1**), which is characterised by the position of the hydrogen (Finar, 1975, Singh and Chouhan, 2014, Shneine and Alaraji, 2016).



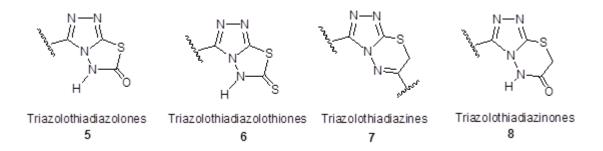
The discovery of 1,2,4-triazole ring represents an interesting class of compounds with promising biological activities (Valverde and Torroba, 2005) as antibacterial agent (Jayachandran et al., 2005; Matthew et al., 2007; Shaker, 2006; Swamy et al., 2010, ), antimicrobial agent (Udupi et al., 2000a; Udupi et al., 2000b; Foroumadi et al., 2003; Hussain et al., 2008; Sharma et al., 2010; Upmanyu et al., 2011; Patil et al., 2013), anti-mycobacterial (Klimesová, et al., 2004), anticonvulsant (Plech et al., 2014), antifungal ((Mali et al., 2009, Luo and Hu, 2006), antidepressant (Düğdü et al., 2014, Chelamalla et al., 2012, Wakale et al., 2013) and anti-tuberculosis (Kaplancikli et al., 2005, Nandha et al., 2013).

Among these heterocycles, the mercapto- (2), thione- (3) and dithione- (4) substituted 1,2,4-triazole ring systems have been well studied and so far a variety of biological activities have been reported for a large number of their derivatives. This 1,2,4-triazole system with sulphur substituted compounds represents promising biological activities due to the presence of the NCS (=N-C-S) moiety in the compounds (Shaker, 2006).

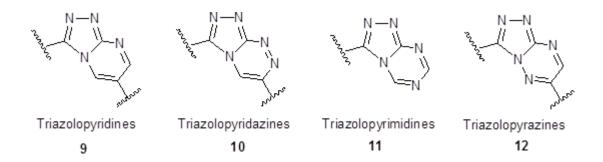


The mercapto-1,2,4-triazoles, **2** are of great utility in the organic chemistry synthesis because in the presence of various reagents, these compounds undergo different types of reactions to yield interesting fused heterocyclic compounds such as triazolothiadiazolones (**5**), triazolothiadiazolothiadiazolothiadiazines (**7**) and triazolothiadiazinones (**8**) (Holla et al., 2001; Abdel-Rahman et al., 2011; Siddiqui et

al., 2015). Compounds of triazoles fused to thiadiazines or thiadiazoles rings are also being incorporated into a wide variety of therapeutically important compounds, possessing a broad spectrum of biological activities (El Shehry et al., 2010, El-Sayed et al., 2010, Kamel and Abdo, 2014).



In the field of agrochemicals, the most common systems are generally 1,2,4triazoles fused to pyridines (9), pyridazines (10), pyrimidines (11) and pyrazines (12). Pyridine heterocyclic moiety plays a vital role in the development of medicinal agents which are present in many products such as drugs, vitamins, food, plant dyes, adhesives and herbicides (Katrizky et al., 1996; Mohamed, 2010).



Several studies have also been reported on the fused 1,2,4-triazoles derivatives with other heterocyclic moieties such as triazole ring, **13** (Othman et al., 2014), oxadiazole ring, **14** (Somani and Shirodkar, 2009, Deshmukh et al., 2011) and thiazole ring, **15** (Erian et al., 2003, Andrew et al., 2008). Various thiazole derivatives are proven to be efficient for various diseases. They showed good physiological activities and are considered as good therapeutic agents such as anti

tubercular agents (E1-Shaaer et al., 1998, Siddiquia et al., 2011) and antimicrobial agents (Guzeldemirci and Kucukbasmac, 2010, Liaras et al., 2011). Furthermore, diverse chemotherapeutic activities have also been ascribed to fused 1,2,4-triazolothiazole moieties as antimicrobial agents.



Schiff bases compounds have the imine or azomethine (-C=N-) functional group which is condensation products of the primary amines with carbonyl compounds. Schiff bases compounds have gained importance in medicinal and pharmaceutical fields due to their broad spectrum of biological activities. However, not many research work on the fused 1,2,4-trizole compounds with Schiff base moiety have been reported. This led to the formation of new ideas in the pharmacological and medicinal chemistry especially in the synthesis of therapeutically interesting molecules with better pharmacological functions for anticancer activity. Schiff bases of fused 1,2,4-triazoles have been found to possess extensive biological activities (Bekircan and Bektas, 2006; Bagihalli et al., 2008; Li et al., 2012, Farghaly et al., 2015; Gabr et al., 2015).

Cancer disease, a major worldwide problem is characterised by the proliferation and spreading of the abnormal cells. Therefore, the discovery and development of new potent and selective anticancer drugs are of high importance in modern cancer research. In spite of a large number of chemotherapeutic drugs available for medical usage, the increasing resistance made it necessary to continue the search for new potential anticancer drugs.

### **1.2** Problem statement

New findings have established the fact that fused 1,2,4-triazole ring contributed great significance in the field of medicinal chemistry due to their versatile biological properties (Asif, 2014). However, these types of derivatives targeting specifically on the anticancer activity have not been widely explored (Holla et al., 2002; Holla et al., 2003; Demirbas and Uğurluoğlu, 2004a; Demirbas and Uğurluoğlu, 2004b; Sztanke et al., 2008; Bhat et al., 2009; Al-Issa, 2013, Hassan et al., 2013; Kamel and Abdo, 2014). Since a large number of fused 1,2,4-triazoles derivatives have shown pharmacological properties as anticancer agents, this research work focused on the modification of 1,2,4-triazole scaffold into various bioactive structures and their subsequent evaluation for cytotoxicity activities focusing on the colon and breast cancer cell lines (HCT and MCF 7) which are two of the common cancers.

### 1.3 Objectives

The main objectives of this project are:

- 1. To synthesise and characterise the mono system of 1,2,4-triazole derivatives which are divided into compounds with pyridinium salt and Schiff base moieties and compounds with piperidinium salt and Schiff base moieties.
- 2. To synthesise and characterise the fused system of 1,2,4-triazole derivatives which are divided into compounds with triazole-oxadiazole ring and compounds with triazole-triazole ring.
- To evaluate the cyctotoxic activity of all these compounds on the colon and breast cancer cell lines.

## 1.4 Scope of the Study

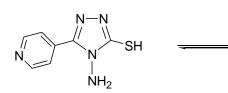
The research involved synthesizing some new 1,2,4-triazoles derivatives and characterisation work has been done using 1D and 2D NMR, FT-IR and CHNS elemental analysis. All these instruments are located at the School of Chemical Sciences. Samples for the anticancer work have been prepared and were sent to the Eman's Lab at Eureka.

#### **CHAPTER 2**

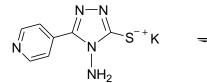
### LITERATURE REVIEW

#### 2.1 Compounds with 1,2,4-triazole core system

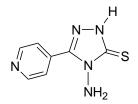
The discovery and development of new effective and selective anticancer drugs are of high importance in modern cancer researches (Ferlay et al., 2013). However, the fused 1,2,4-triazole compounds targeting specifically on the anticancer activity have not been widely explored. To date, only several studies on such compounds have been reported (Hou et al., 2011; Baviskar et al., 2012; Arul and Smith, 2014). Among the research work, the mercapto- and thione-substituted 1,2,4-triazole rings attached to a pyridine system have been of great interest due to the presence of the NCS (=N-C-S) moiety in the compounds (Shaker, 2006). 4-amino-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-thiol derivatives showed very promising results on anticancer activity. The two isomers of 4-amino-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-thiol molecule, known as thiol, **16** and thione, **17** exist in equilibrium (Creanga et al., 2010). The preparation of this 1,2,4-triazole derivatives produces two kinds of organic salts, **18** and **19**. (Ren and Jian, 2008).



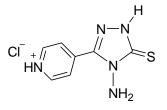
<sup>4-</sup>amino-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-thiol, **16** 



potassium 4-amino-5-(4-pyridyl)-4H-1,2,4-triazole-3-thiol, **18** 



4-amino-3-(4-pyridyl)-1*H*-1,2,4-triazole-5(4*H*)-thione, **17** 



4-(4-amino-5-thioxo-1*H*-1,2,4-triazol-3-yl)pyridinium chloride, **19** 

Preparation of 4-amino-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-thiol *via* cyclisation reaction of potassium 2-isonicotinoylhydrazinecarbodithioate gave a mixture of 1,2,4-triazole and its corresponding 1,3,4-oxadiazole derivatives, as shown in Figure 2.1. However, delicate work on the purification of this mixture afforded compound **18** in good purity but in low yield (Creanga et al., 2010).

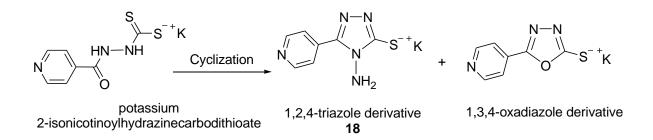
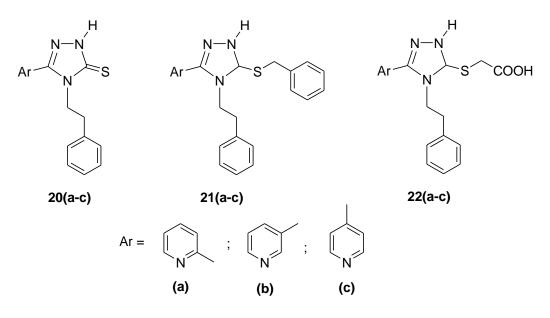
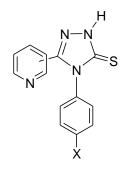


Figure 2.1: Cyclisation of 1,2,4-triazole and 1,3,4-oxadiazole derivatives

Iqbal et al. (1996) reported the synthesis of new 1,2,4-triazoles compounds, 3substituted thio-4-(2-phenylethyl)-5-(isomeric pyridyl)-1,2,4-triazoles, **21(a-c)** and **22(a-c)** derivatives from 4,5-disubstituted-1,2,4-triazoles **20(a-c)** using various isomers of pyridine carboxylic acid hydrazide with 2-phenylethyl isothiocyanate.

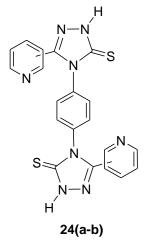


Iqbal et al. (1997) continued to synthesize a new series of 4-(4-halopheny)-3-(isomeric pyridyl)-1*H*-1,2,4-triazoles-3-thion derivatives, 23(a-c) through cyclisation reaction of 4-(isomeric pyridyl) thiosemicarbazide which was prepared from 4halophenyl isothiocyanates with isomers of pyridyl carboxylic acid hydrazide.



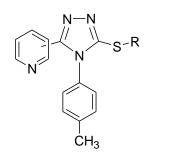
**23(a-c) a:** 2-pyridyl; **b:** 3-pyridyl; **c:** 4-pyridyl **X:** F, Cl, Br

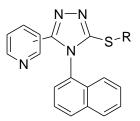
Banachiewicz et al. (2000) has reported two new systems of 4,4'-(1,4-phenylene)bis[5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol] and 4,4'-(1,4-phenylene)bis[5-(pyridin-2-yl)-4*H*-1,2,4-triazole-3-thiol], **24(a-b)** *via* the condensation of *N'*-substituted amidrazones and *p*-phenylenediisothiocyanate.



a: 2-pyridyl; b: 4-pyridyl

Zamani and his co-workers have conducted two independent studies in 2003 and 2004. The first study reported on the synthesis and characterisation of some new compounds, **25(a-c)** without any biological or pharmacological activity work (Zamani and Faghihi, 2003). Later, other synthesis work on pyridyl and naphthyl substituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives, **26(a-c)**, and their antimicrobial activity screening was reported (Zamani et al., 2004).

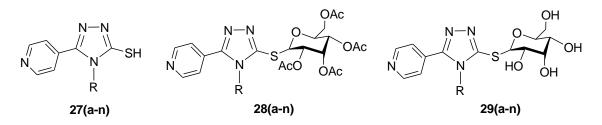




**25(a-c) a:** 2-pyridyl; **b:** 3-pyridyl; **c:** 4-pyridyl **R:** H(**a-c**); CH<sub>3</sub>(**a-c**); C<sub>2</sub>H<sub>5</sub>(**a-c**); CH<sub>2</sub>Ph(**a-c**); CH<sub>2</sub>COOH(**a-c**)

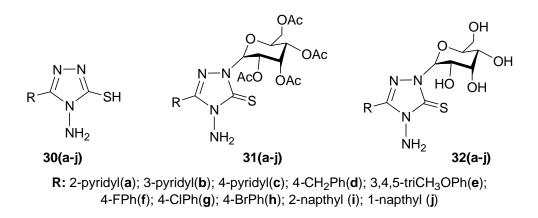
**26(a-c) a:** 2-pyridyl; **b:** 3-pyridyl; **c:** 4-pyridyl **R:** H(**a-c**); CH<sub>3</sub>(**a-c**); CH<sub>2</sub>Ph(**a-c**)

Zhang et al. (2008) reported two new series of *S*-nucleosides of 5-(4-pyridyl)-4-aryl-4*H*-1,2,4-triazole-3-thiols *via* direct glycosylation of 5-(4-pyridyl)-4-aryl-4*H*-1,2,4-triazole-3-thiols **27(a-n)** which gave the corresponding derivatives, **28(a-n)**. The removal of the protecting groups using ammonia gas in dry methanol afforded the final series, **29(a-n)**. All these compounds have been tested against three different type of anti-HIV-1, the reverse transcriptase (RT), protease (PR) and integrase (IN) activities but none of these compounds showed good anti-HIV activity.



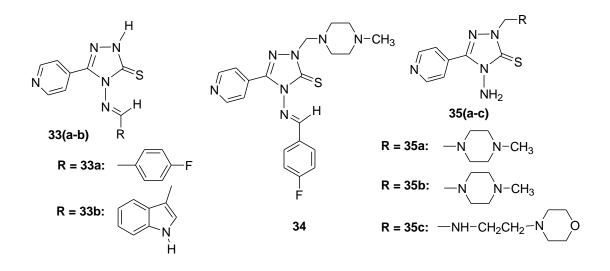
**R:** Ph(**a**); o-C<sub>2</sub>H<sub>5</sub>OPh(**b**); p-C<sub>2</sub>H<sub>5</sub>OPh(**c**); o-CH<sub>3</sub>OPh(**d**); p-CH<sub>3</sub>OPh(**e**); o-CH<sub>3</sub>Ph(**f**); m-CH<sub>3</sub>Ph(**g**); p-CH<sub>3</sub>Ph(**h**); o-BrPh(**i**); m-BrPh(**j**); p-BrPh(**k**);o-ClPh(**l**); m-ClPh(**m**); p-ClPh(**n**);

Nadeem and Ashraf (2012) modified Zhang's work using some novel Mannich bases to prepare the *N*-nucleosides of 4-amino-5-substituted-1,2,4-triazole-3-thiones whereby their *in-vitro* antimicrobial activity was also examined. The original 1,2,4-triazoles molecules **30(a-j)** portrayed lower antibacterial activity compared to the corresponding protected nucleosides **31(a-j)** and the unprotected nucleosides **32(a-j)** against some selected bacteria.

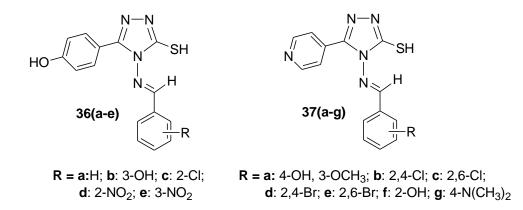


## 2.2 Schiff base 1,2,4-triazole derivatives

Recently, some well-known 1,3,4-oxadiazole and 1,2,4-triazole systems were developed by Bayrak et al. (2009). Some new Schiff base, **33(a-b)** and Mannich base with an aliphatic cyclic amine such as morpholine or methyl piperazine, **35(a-c)** compounds were prepared. The corresponding Mannich base of compound **34** was reported to possess some biological activities.

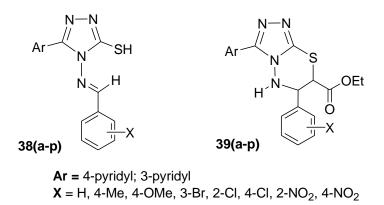


Valentina et al. (2009) have synthesised compounds **36(a-e)**, the Schiff base of 4-Amino-5-(4-hydroxyphenyl)-4*H*-1,2,4-triazole-3-thiol which showed good antioxidant activity. Moreover, Singh et al. (2010) have synthesised some new Schiff base of 4-amino-5-(4-pyridyl)-1,2,4-triazole-3-thiol compounds, **37(a-g)** and their antibacterial activities have been reported against four kinds of well-known bacteria, *S. aureus, E. coli, P. vulgaris* and *K. pneumonia*. However, all series showed mild antibacterial activity.

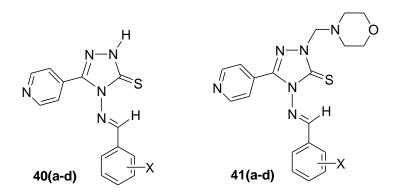


The composition of 4-(substituted benzylidene amino)-5-(isomeric pyridyl)-4H-1,2,4-triazole-3-thiols **38(a-p)** were accomplished in a one-step reaction by Foroughifar et al. (2010) *via* reacting the corresponding substrates of 4-amino-5-(isomeric pyridyl)-4H-1,2,4-triazole-3-thiols with various aromatic aldehydes in

refluxing acetic acid. All the derivatives obtained were used to synthesize of subsequent compounds of substituted triazolo[3,4-b][1,3,4]thiadiazines **39(a-p)**.



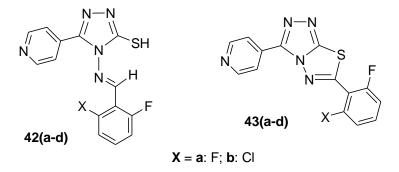
Koparir et al. (2010) prepared some new arylidene-amino compounds with Schiff base unit, 40(a-d), *via* the reaction of 4-amino-5-(4-pyridyl)-4*H*-1,2,4triazole-3-thione or 4-amino-5-(2-hydroxyphenyl)-4*H*-1,2,4-triazole-3-thione with a various aromatic aldehyde in anhydrous ethanol which was then converted into the corresponding Mannich base compounds, 41(a-d). It was reported that the thionethiol forms are two tautomers, whereby the first tautomer exists in pure solid form.



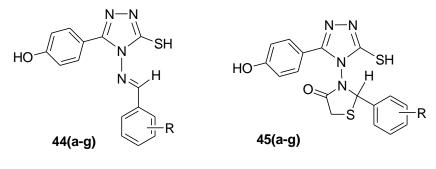
**X = a:** H; **b**: 4-OMe; **c**: 4-OH, 3-OMe; **d**: 2,4-dihydroxy

Abdel-Rahman et al. (2011) synthesised a new compounds of arylidene-amino compounds with Schiff base unit, **42(a-b)** which then undergo cyclisation reaction to

However, no biological activity studies were reported for these compounds.

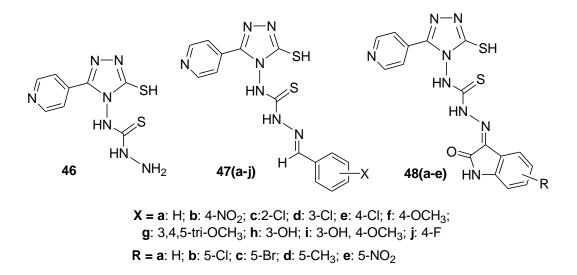


Some new phenolic Schiff base compounds, 44(a-g) have been synthesised from 4-amino-5-(4-hydroxyphenyl)-4*H*-1,2,4-triazole-3-thiol, followed by a condensation reaction with aromatic carbonyl compounds in refluxing methanol in the presence of traces of glacial ethanoic acid. Treatment of these compounds with thioglycolic acid gave compounds 45(a-g) (Rajput, 2012).

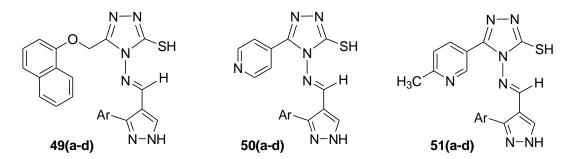


R = a: H; b: 2-Cl; c:4-Cl; d: 2,-CH<sub>3</sub>; e: 4-CH<sub>3</sub>; f: 2-OH; g: 2-NH<sub>2</sub>

Other significant Schiff base compounds were reported by Sachdeva et al. (2013) with good anti-inflammatory activities. 5-thiol-3-(4-pyridyl)-4*H*-1,2,4-triazol-4-yl-thiosemicarbazide, **46** was converted to 2-substituted-*N*-[3-(pyridin-4-yl)-5-sulfanyl-4*H*-1,2,4-triazol-4-yl]hydrazinecarbothioamide compounds, **47(a–j)** and **48(a–e)** via reaction of **46** with substituted aldehyde using the traditional technique and 1*H*-indole-2,3-di-ones using the microwave irradiation technique, respectively.

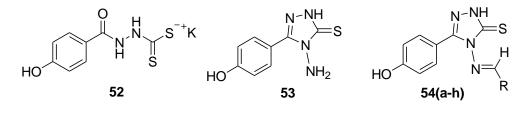


Some new compounds of 49(a-d), 50(a-d) and 51(a-d) can be obtained by refluxing the corresponding 4-amino-5-aryl-4*H*-1,2,4-triazole-3-thiol with 3-aryl-1*H*-pyrazole-4-carbaldehyde compounds in the presence of few drops of sulphuric acid as a catalyst in ethyl alcohol solvent. All these targeted compounds have been evaluated as potent antibacterial, antifungal and analgesic agents (Vijesh et al., 2013).



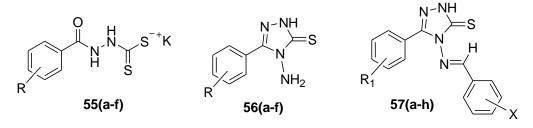
Ar = a: 2,4-dichlorophenyl; b: 4-thioanisyl; c:2,5-dichlorothiophene d:biphenyl

Ding et al. (2010) reported an intermediate of potassium dithiocarbamate salt, **52** which undergone a ring closure with a surplus of 85% hydrazine hydrate to produce a corresponding phenolic 1,2,4-triazole, **53**, followed by the formation of Schiff base compounds, **54(a-h)**.



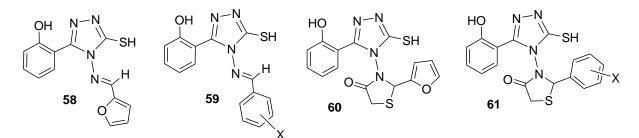
R = a: Ph; b: 4-F-Ph; c: 2-F-Ph; d: 4-Cl-Ph; e: 4-OCH<sub>3</sub>-Ph; f: 3-NO<sub>2</sub>-Ph; g: Ph-CH=CH-; h: 2-Furyl

Other potassium dithiocarbamate salts, **55(a-f)** were reported by Gajera et al. (2012). Treatment of the salts with an excess of hydrazine monohydrate initiated the ring closure into the corresponding 1,2,4-triazole compounds, **56(a-f)**, followed by the condensation reaction to form the Schiff base compounds, **57(a-h)**.



**R** = **a**: H; **b**: 2-NH<sub>2</sub>; **c**: 4-NH<sub>2</sub>; **d**: 4-NO<sub>2</sub>; **e**: 4-CH<sub>3</sub>; **f**: 4-Cl **R**<sub>1</sub>/X = **a**: H/4-NO<sub>2</sub>; **b**: 2-NH<sub>2</sub>/4-NO<sub>2</sub>; **c**: 4-NH<sub>2</sub>/4-NO<sub>2</sub>; **d**: 4-NO<sub>2</sub>/2-OH; **e**: 4-NO<sub>2</sub>/4-NO<sub>2</sub>; **f**: 4-CH<sub>3</sub>/4-NO<sub>2</sub>; **g**: 4-Cl/4-Cl; **h**: 4-Cl/4-OH

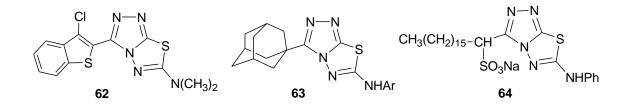
Wakale and Pattan (2015) reported a new Schiff base compounds of (E)-4-(substituted methylene amino)-5-(2-hydroxyphenyl)-4*H*-1,2,4-triazole-3-thiol, **58-59** *via* the synthesis pathway of 4-amino-1,2,4-triazole synthesis. Treatment of these compounds with thioglycolic acid in DMF produced two new series of 3-(3-(2hydroxyphenyl)-5-mercapto-4*H*-1,2,4-triazol-4-yl)-2-substituted-thiazolidine-4-one, **60-61**, respectively. All these compounds were tested for antimicrobial activity. The biological study showed that the new molecules **59**, **60** and **61** exhibited the strongest inhibition against the microbial strains.



**X** = **a**: F; **b**: 4-OH; **c**: 4-OCH<sub>3</sub>; **d**: 3-OCH<sub>3</sub>, 4-OH; **e**: 2-Cl

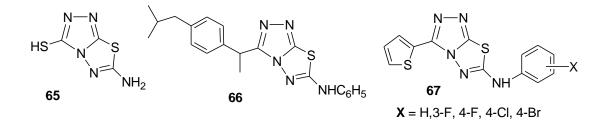
#### 2.3 1,2,4-Triazolo[4,3-b][1,2,4]thiadiazole derivatives

A variety of 1,2,4-triazolo[4,3-b][1,2,4]triazole compounds have been synthesised and their biological activity being reported. El-Ashry et al. (2006) synthesised 3-(3-chlorobenzo[b]-2-yl)-6-(*N*,*N*-dimethylamino)-1,2,4-triazolo[3,4-b][1,3,4]thiadiazole, **62** *via* microwave irradiation method. This technique was also used by Al-Abdullah et al. (2007) to synthesize 3-(1-adamantyl)-6-arylamino-1,2,4-triazolo[3,4-b][1,3,4]thiadiazole compound, **63** which showed good antimicrobial activities. Sodium 1-(6-phenylamino-1,2,4-triazolo[3,4b][1,3,4]thiadiazole-3-yl)heptadecane-1-sulfonate, **64** with good antibacterial activity was prepared by El-Sayed (2006).

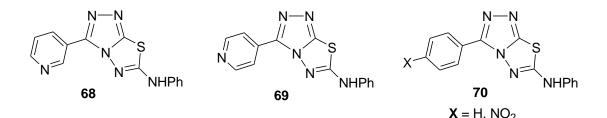


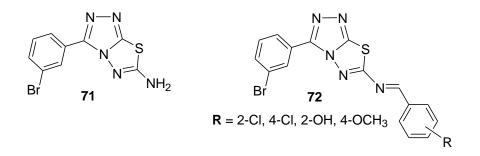
Compounds with a similar scaffold of novel 1,2,4-triazolo[3,4b][1,3,4]thiadiazole derivatives were reported by several groups. Salih (2008) reported the preparation and characterisation of 3-mercapto-6-amino-1,2,4triazolo[3,4-b][1,3,4]thiadiazole, **65** while Shrivastava et al. (2010) reported 3-(2-(4isobutylphenyl)propyl)-6-phenyl amine[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, **66** with their good screening results as antimicrobial, analgesic and anthelmintic

activities. Al-Omar (2010) reported the synthesis of 3-(2-thienyl)-6-(4-sub-phenylamine)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole, **67** *via* microwave irradiation technique and their antimicrobial activity.



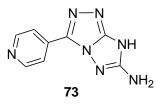
Other novel compounds encompassing of 1,2,4-triazole and 1,3,4-thiadiazole were also synthesised and characterised. Omprakash et al. (2011) reported a new compound of *N*-phenyl-3-(pyridin-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-phenyl amino, **68** having good antimicrobial activity. Abdel-Rahman et al. (2011) have reported new derivative of 3-(pyrid-4-yl)-6-phenylamino-1,2,4-triazolo[4,3-b][1,3,4]thiadiazole, **69**, showing anti-inflammatory and antimicrobial activities. Pandeya et al. (2012) synthesised two derivatives, **70** of 3(4-nitrophenyl)-*N*-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6-phenyl amino and 3(phenyl)-*N*-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6-phenyl amino with their antifungal and antibacterial activities. Harcharan et al. (2012) have reported a new compound of 3-(3-bromophenyl) [1,2,4]-triazolo[3,4-b][1,3,4]thiadiazol-6-amine, **71**, which was then converted to the corresponding new Schiff base compounds, **72**. All these compounds showed good antibacterial and antifungal activities.





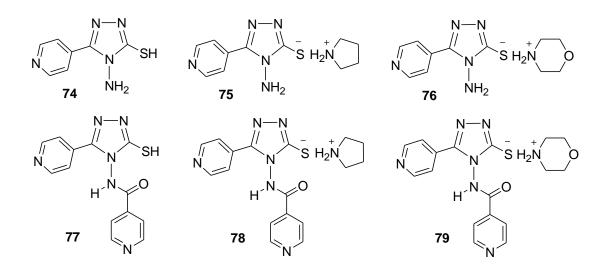
#### 2.4 1,2,4-Triazolo[4,3-b][1,2,4]triazole derivatives

A new system of fused 1,2,4-triazolo-triazole derivative such as 3-(pyridin-4-yl)6-amino-7*H*-1,2,4-triazolo[4,3-b][1,2,4]triazole was reported by Abdel-Rahman et al. (2011). The compound showed promising results for anti-inflammatory and antimicrobial activities.



#### 2.5 1,2,4-Triazolo-3-thiolate derivatives

In this interesting study, Chemica et al. (2011) reported the interaction of 4amino-5-(4-pyridyl)-4*H*-1,2,4-triazole-3-thiol, **74** with a cyclic secondary amines such as pyrrolidine and morpholine in anhydrous dimethyl sulfoxide formed the corresponding quaternary salts of pyrrolidinium 4-amino-5-(pyridin-4-yl)-4*H*-1,2,4triazole-3-thiolate, **75** and morpholine-4-ium 4-amino-5-(pyridin-4-yl)-4*H*-1,2,4triazole-3-thiolate, **76**, respectively. Similar reactions of *N*-(3-mercapto-5-(4pyridyl)-4*H*-1,2,4-triazol-4-yl) isonicotinamide, **77** with morpholine and pyrrolidine yielded the quaternary salts of pyrrolidinium 4- isonicotinamido-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiolate, **78** and morpholine-4-ium 4- isonicotinamido-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiolate, **79**, respectively. These new salts were screened for their antibacterial and antifungal activity.



## 2.6 1,3,4-Oxadiazole-2-thiolate derivatives

Rutavicius et al. (2003) have also reported the interaction of 5-(4-pyridyl)-1,3,4-oxadiazole-2-thiol with piperidine or morpholine in dimethylformamide under reflux. The equivalent ratio of piperidine or morpholine in the solvent undergo cleavage of oxazole ring but in the presence of excess piperidine or morpholine in dimethyl sulfoxide, piperidinium 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiolate, **80** and morpholin-4-ium 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiolate, **81**, were formed, respectively.

