CHAPTER 5

Synthesis and biological studies of triazolopyridines and tetrazoles
SYNTHESIS AND BIOLOGICAL STUDIES OF TRIAZOLOPYRIDINES AND TETRAZONES

CHAPTER 5

Synthesis and biological studies of triazolopyridines and tetrazoles

5.1 Introduction

Recently, there has been renewed interest in multicomponent reactions, which have become foremost route of synthesizing many pharmacologically important heterocyclic intermediates [1-3]. Nitrogen rich triazolopyridines and tetrazoles are of substantial attention because of their application as analgesics, cancer therapeutics and also in many neurological related disorders [4-8]. With limited reports on synthetic procedure for spirotiazolo pyridine dicarbonitriles [9] and many procedures for tetrazoles [10] employ strong base and organic solvents for their synthesis. Most of these methods use conditions with hazardous solvents, expensive catalysts and tedious work up procedures. A synthetic method utilizing environmentally benign water as solvent and tetrabutyl ammonium bromide (TBAB) as mild water soluble catalyst serves as green approach. Considering these observations, an alternative green method was developed using water and TBAB to synthesize triazolo pyridine dicarbonitriles and tetrazoles.

5a: Synthesis and anti-cancer studies of triazolo[1,5-a]pyridine dicarbonitriles

5a.2 Results and Discussion

5a.2.1 Chemistry

Earlier report on synthesis of spirotiazolo pyridine dicarbonitriles Hussein, A. H. M. et al, [11] have used piperidine base in ethanol solvent to obtain the title compounds. In order to improve the methodology with the aim of greener approach we had
developed a simple and efficient method for the preparation of spirotriazolo pyridine dicarbonitriles 5 (a-l) (Table 1) by a four component reaction between aromatic/hetero aldehyde (1), malanitrile (2), cyanoacetohydrazie (3) and cyclohexanone (4) in presence of TBAB in water under reflux condition (Scheme 1).

Scheme 1: Synthesis of spirotriazolo pyridine dicarbonitriles

Table 1: A series of spirotriazolo pyridine dicarbonitriles and their anti-cancer activity

<table>
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<tr>
<th>Sl. No</th>
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<th>Time (Hr)</th>
<th>Yield (%)</th>
<th>IC₅₀ (µM)</th>
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SYNTHESIS AND BIOLOGICAL STUDIES OF TRIAZOLOPYRIDINES AND TETRAZOLES

5a.2.2 Biological studies

Given the anti-cancer activity of substituted triazolopyridines on lung cancer cells [12], we gained interest to study the activity of spirotriazolo pyridine dicarbononitriles on human lung carcinoma cell lines, the A549 cells. All the spirotriazolo pyridine dicarbononitriles were tested for their cytotoxic effect on A549 cells using MTT assay. Interestingly, most of these compounds induced considerable cytotoxicity on A549 carcinoma cell lines (Table 1).

5a.3 Experiments

5a.3.1 Chemistry

5a.3.1a General:

All reagents were commercially available reagent grade and were used without further purification. TLC was conducted on 0.25 mm silica gel plates (60F254, Merck). Column chromatography separations were obtained on silica gel (200-400 mesh). ♦H NMR spectra were recorded on Agilent/Bruker NMR 400 MHz instrument in DMSO-d₆ solvent. ♦C NMR spectra were obtained on Agilent NMR instrument at 100 MHz in] CDCl₃/DMSO-d₆ solvent. Chemical shifts are expressed in ppm downfield relative to TMS. LC-MS analysis was performed on Agilent LC-MS with electron ionization (ESI) +ve and –ve mode. Elemental analyses were recorded using Perkin Elmer CHNS analyzer.

5a.3.1b General procedure of synthesis of triazolo[1,5-a]pyridine dicarbononitriles:

In a 50 mL round bottom flask, 2-cyano acetohydrazide (3) (1.0 eq) and cycloexanone (4) (1.0 eq) were stirred at 90 °C in 8 mL of 10 mol% of TBAB solution for 20 mins. To this reaction mixture, an aromatic/hetero aldehyde (1) (1.0 eq) and malononitrile (2) (1.2 eq) were added and stirred further for 1-3 hrs at 100 °C. The reaction was
monitored by TLC using n-hexane and ethyl acetate eluent. After completion of the reaction, solid was filtered through Whatman filter paper 42, air dried and purified by column chromatography (scheme 1).

5a.3.1c Characterization data of novel triazolo[1,5-a]pyridine dicarbonitriles:

5a. (7-{(4-bromophenyl)-5-oxo-3,5-dihydro-1H-spiro[1,2,4]triazolo[1,5-a]pyridine-2,1'-cyclohexane]-6,8-dicarbonitrile): Brown colour solid. IR νmax (cm⁻¹): 3320, 2228, 1707; 

\[
\begin{align*}
\text{Br} & \\
\text{N} & \\
\text{O} & \\
\text{C} & \\
\text{N} & \\
\text{H} & \\
\text{C} & \\
\text{HN} & \\
\text{CN} & \\
\end{align*}
\]

\(^1^H\) NMR (DMSO-d\(_6\), 400 MHz δ in ppm): 7.75-7.71 (d, 2H, Ar-H), 7.52 (s, 1H, C-NH), 7.35-7.31 (d, 2H, Ar-H), 2.19 (s, 1H, N-NH), 1.82-1.71 (m, 4H, Ali-H), 1.42-1.31 (m, 6H, Ali-H); Anal. Calcd. for C\(_{19}\)H\(_{16}\)BrN\(_3\)O: C, 55.62; H, 3.93; N, 17.07; found C, 55.58; H, 3.90; N, 17.10 %; Mass m/z (MM: ES+APCI) (M+H)^+ 410.

5b.7-{(3-bromo-4-fluorophenyl)-5-oxo-3,5-dihydro-1H-spiro[1,2,4]triazolo[1,5-a]pyridine-2,1'-cyclohexane]-6,8-dicarbonitrile: Yellow solid. IR νmax (cm⁻¹): 3283, 2230, 1647; \(^1^H\) NMR (DMSO-d\(_6\), 400 MHz δ in ppm): 7.69 (s, 1H, Ar-H), 7.52 (s, 1H, C-NH), 7.41 (s, 1H, N-NH), 7.14-7.04 (m, 2H, Ar-H), 2.14 (s, 1H, N-NH), 1.72-1.60 (m, 4H, Ali-H), 1.39-1.23 (m, 6H, Ali-H); Anal. Calcd. for C\(_{19}\)H\(_{15}\)BrFN\(_3\)O: C, 53.29; H, 3.53; N, 16.35; found C, 53.32; H, 3.49; N, 16.31 %; Mass m/z (MM:ES+APCI) (M+H)^+ 428.

5c. 7-{(3-bromo-4-methoxyphenyl)-5-oxo-3,5-dihydro-1H-spiro[1,2,4]triazolo[1,5-a]pyridine-2,1'-cyclohexane]-6,8-dicarbonitrile: Yellow solid. IR νmax (cm⁻¹): 3338, 2230, 1685; \(^1^H\) NMR (DMSO-d\(_6\), 400 MHz δ in ppm): 7.35-7.25 (m, 3H, Ar-H), 7.04 (s, 1H, C-NH), 3.52 (s, 3H, -OCH\(_3\)), 2.57 (s, 1H, N-NH), 1.92-1.70 (m, 4H, Ali-H), 1.44-1.21 (m, 6H,
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Ali-H); $^{13}$C NMR (CDCl$_3$, 100 MHz δ in ppm) 167.28, 158.04, 157.13, 154.02, 131.19, 130.26, 128.07, 114.97, 113.32, 110.81, 87.90, 74.08, 56.19, 34.01, 26.21, 23.05; Anal. Calcd.

for C$_{20}$H$_{18}$BrN$_5$O$_2$: C, 54.56; H, 4.12; N, 15.91; found C, 54.50; H, 4.15; N, 15.88 %; Mass m/z (MM:ES+APCI) (M+H)$^+$ 440.

5d.  7-(3,4-dimethoxyphenyl)-5-oxo-3,5-dihydro-1H-spiro[[1,2,4]triazolo[1,5-a]pyridine-2,1'-cyclohexane]-6,8-dicarbonitrile: White solid. IR $\nu$max (cm$^{-1}$): 3345, 2235, 1648; $^1$H NMR (DMSO-d$_6$, 400 MHz δ in ppm): 7.46 (m, 3H, C-NH), 7.15 (s, 1H, Ar-H), 6.92-6.90 (m, 2H, Ar-H), 3.71 (s, 6H, -CH$_3$), 2.12 (s, 1H, N-NH), 1.55-1.49 (m, 4H, Al-H), 1.36-1.29 (m, 6H, Al-H); Anal. Calcd. for C$_{21}$H$_{21}$N$_5$O$_3$: C, 64.44; H, 5.41; N, 17.89; found C, 64.40; H, 5.38; N, 17.92 %; Mass m/z (MM:ES+APCI) (M+H)$^+$ 392.

5e.  5-oxo-7-(4-propoxyphenyl)-3,5-dihydro-1H-spiro[[1,2,4]triazolo[1,5-a]pyridine-2,1'-cyclohexane]-6,8- dicarbonitrile: White solid. IR $\nu$max (cm$^{-1}$): 3340, 2221, 1652; $^1$H NMR (DMSO-d$_6$, 400 MHz δ in ppm): 7.84-7.79 (d, 2H, Ar-H), 7.52 (s, 1H, C-NH), 7.16-7.11 (d, 2H, Ar-H), 6.42-6.35 (q, 2H, -CH$_2$-), 2.17 (s, 1H, N-NH), 1.76-1.70 (m, 2H, -CH$_2$-), 1.60-1.52 (m, 4H, Al-H), 1.33-1.21 (m, 6H, Al-H); $^{13}$C NMR (CDCl$_3$, 100 MHz δ in ppm) 168.39, 158.18, 156.27, 153.69, 128.81, 123.42, 116.30, 115.27, 113.18, 84.01, 75.35, 70.42, 32.01, 24.00, 22.46, 21.84, 10.52; Anal. Calcd. for C$_{22}$H$_{23}$N$_5$O$_2$: C, 67.85; H, 5.95; N, 17.98; found C, 67.81; H, 5.98; N, 18.00 %; Mass m/z (MM:ES+APCI) (M+H)$^+$ 390.
5f. 7-(4-nitrophenyl)-5-oxo-3,5-dihydro-1H-spiro[1,2,4]triazolo[1,5-a]pyridine-2,1’-cyclohexane]-6,8-dicarbonitrile: Yellow solid. IR ν max (cm⁻¹): 3343, 2238, 1672; ¹H NMR (DMSO-d₆, 400 MHz δ in ppm): 8.39-8.28 (m, 2H, Ar-H), 7.64-7.56 (m, 2H, Ar-H), 7.41 (s, 1H, C-NH), 2.27 (s, 1H, N-NH), 1.70-1.66 (m, 4H, Ali-H), 1.28-1.18 (m, 6H, Ali-H); ¹³C NMR (CDCl₃, 100 MHz δ in ppm) 167.03, 159.26, 157.64, 148.47, 140.21, 130.35, 122.18, 115.8 114.13, 88.50, 74.25, 34.42, 27.18, 25.29; Anal. Calcd. for C₁₉H₁₆N₆O₃: C, 60.63; H, 4.28; N, 22.33; found C, 60.59; H, 4.30; N, 22.30 %; Mass m/z (MM:ES+APCI) (M+H)+ 377.

5g. 5-oxo-7-(4-(trifluoromethyl)phenyl)-3,5-dihydro-1H-spiro[1,2,4]triazolo[1,5-a]pyridine-2,1’-cyclohexane]-6,8-dicarbonitrile: Yellow solid. IR ν max (cm⁻¹): 3329, 2240, 1637; ¹H NMR (DMSO-d₆, 400 MHz δ in ppm): 8.39-8.28 (m, 2H, Ar-H), 7.64-7.56 (m, 2H, Ar-H), 7.41 (s, 1H, C-NH), 2.27 (s, 1H, N-NH), 1.70-1.66 (m, 4H, Ali-H), 1.28-1.18 (m, 6H, Ali-H); ¹³C NMR (CDCl₃, 100 MHz δ in ppm) 167.03, 159.26, 157.64, 148.47, 140.21, 130.35, 122.18, 115.8 114.13, 88.50, 74.25, 34.42, 27.18, 25.29; Anal. Calcd. for C₂₀H₁₆F₃N₅O: C, 60.15; H, 4.04; N, 14.27; found C, 60.11; H, 4.07; N, 14.23 %; Mass m/z (MM:ES+APCI) (M+H)+ 400.

5h. 7-(4-(methylsulfonyl)phenyl)-5-oxo-3,5-dihydro-1H-spiro[1,2,4]triazolo[1,5-a]pyridine-2,1’-cyclohexane]-6,8-dicarbonitrile: White solid. IR ν max (cm⁻¹): 3341, 2232, 1657; ¹H NMR (DMSO-d₆, 400 MHz δ in ppm): 8.05-7.90 (m, 4H, Ar-H), 7.32 (s, 1H, C-NH), 3.84 (s, 3H, -CH₃), 2.30 (s, 1H, N-NH), 1.69-1.57 (m, 4H, Ali-H), 1.31-1.19 (m, 6H,
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$^{13}$C NMR (CDCl₃, 100 MHz $\delta$ in ppm) 168.32, 159.61, 158.09, 150.04, 140.19, 136.67, 130.79, 122.81, 116.02, 88.08, 74.21, 35.76, 26.85, 22.11; Anal. Calcd. for C$_{18}$H$_{15}$BrN$_6$O: C, 52.57; H, 3.68; N, 20.44; found C, 52.52; H, 3.70; N, 20.25%; Mass m/z (MM:ES+APCI) (M+H)$^+$ 411.

5i. 7-(6-bromopyridin-3-yl)-5-oxo-3,5-dihydro-1H-spiro[1,2,4]triazolo[1,5-a]pyridine-2,1'-cyclohexane]-6,8-dicarbonitrile: White solid. IR $\nu_{\text{max}}$ (cm$^{-1}$): 3353, 2239, 1647; $^1$H NMR (DMSO-d$_6$, 400 MHz $\delta$ in ppm): 8.35 (s, 1H, Py-CH), 7.83-7.80 (d, 1H, Ar-H), 7.56 7.53 (d, 1H, Ar-H), 7.40 (s, 1H, C-NH), 2.49 (s, 1H, N-NH), 1.75-1.65 (m, 4H, Ali-H), 1.30-1.18 (m, 6H, Ali-H); $^{13}$C NMR (CDCl$_3$, 100 MHz $\delta$ in ppm) 168.32, 159.61, 158.09, 150.04, 140.19, 134.67, 130.79, 122.81, 116.02, 88.08, 74.21, 35.76, 26.85, 22.11; Anal. Calcd. for C$_{20}$H$_{19}$N$_5$O$_3$S: C, 58.67; H, 4.68; N, 17.10; found C, 58.60; H, 4.70; N, 17.12%; Mass m/z (MM:ES+APCI) (M+H)$^+$ 410.

5j. 7-(1-methyl-1H-imidazol-2-yl)-5-oxo-3,5-dihydro-1H-spiro[1,2,4]triazolo[1,5-a]pyridine-2,1'-cyclohexane]-6,8-dicarbonitrile: Off-white solid. IR $\nu_{\text{max}}$ (cm$^{-1}$): 3333, 2240, 1635; $^1$H NMR (DMSO-d$_6$, 400 MHz $\delta$ in ppm): 7.74 (s, 1H, C-NH), 7.25-7.22 (m, 2H, Imi-H), 3.87 (s, 3H, -CH$_3$), 2.05 (s, 1H, N-NH), 1.64-1.60 (m, 4H, Ali-H), 1.37-1.31 (m, 6H, Ali-H); $^{13}$C NMR (CDCl$_3$, 100 MHz $\delta$ in ppm) 170.75, 160.22, 159.93, 136.01, 127.99,
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120.48, 119.98, 112.04, 88.07, 74.01, 36.06, 33.12, 25.21, 22.52; Anal. Calcd. for 
C_{17}H_{17}N_{2}O: C, 60.88; H, 5.11 N, 29.24; found C, 60.85; H, 5.07; N, 29.35 %; Mass m/z 
(MM:ES+APCI) (M+H)^+ 336.

5k. 7-(1H-indol-3-yl)-5-oxo-3,5-dihydro-1H-spiro[[1,2,4]triazolo[1,5-a]pyridine-2,1'- 
cyclohexane]-6,8-dicarbonitrile: Brown solid. IR ν_{max} (cm^{-1}): 3342, 2230, 1657; ^{1}H NMR 
(DMSO-d_{6}, 400 MHz δ in ppm): 10.50 (s, 1H, Ind-NH), 7.88 (s, 1H, Ind-CH), 7.67 (s, 1H, C- 
NH), 7.44-7.40 (m, 4H, Ar-H), 2.16 (s, 1H, N-NH), 1.63-1.54 
(m, 4H, Ali-H), 1.48-1.39 (m, 6H, Ali-H); Anal. Calcd. For 
C_{21}H_{18}N_{6}O: C, 68.09; H, 4.90; N, 22.69; found C, 68.11; H, 4.85; N, 22.72 %; Mass m/z 
(MM:ES+APCI) (M+H)^+ 371.

5l. 7-(2-methyl-1H-indol-3-yl)-5-oxo-3,5-dihydro-1H-spiro[[1,2,4]triazolo[1,5-a]pyridine- 
2,1'-cyclohexane]-6,8-dicarbonitrile: Brown solid. IR ν_{max} (cm^{-1}): 3325, 2230, 1652; ^{1}H NMR 
(DMSO-d_{6}, 400 MHz δ in ppm): 8.35 (s, 1H, Ind-NH), 7.42-7.38 (m, 2H, Ar-H), 7.26 
(s, 1H, C-NH), 7.08-7.00 (m, 2H, Ar-H), 2.22 (s, 3H, -CH_{3}), 2.01 (s, 1H, N-NH), 1.43-1.39 
(m, 4H, Ali-H), 1.24-1.21 (m, 6H, Ali-H); Anal. Calcd. for 
C_{22}H_{20}N_{6}O: C, 68.73; H, 5.24; N, 21.86; found C, 68.77; H, 
5.22; N, 21.80 %; Mass m/z (MM:ES+APCI) (M+H)^+ 385.

5a.3.2 Biology

The anti-proliferative effect of spiro triazolo pyridine dicarbonitriles were tested 
against lung carcinoma cells was determined by the MTT dye uptake method as 
described previously (13). Briefly, the cells (5X10^{3} / ml) were incubated in triplicate in a 
96 well plate in the presence or absence of the indicated concentrations of compounds
in a final volume of 0.2 ml for different time intervals at 37 °C. Thereafter, 20 μl of MTT solution (5 mg/ml in PBS) was added to each well. After two hours of incubation at 37 °C, 0.1 ml of lysis buffer (20% SDS, 50% dimethylformamide) was added, incubation was done for 1 hour at 37 °C, and subsequently the optical density at 570 nm was measured by a Tecan plate reader.
5b: Synthesis and anti-cancer studies of tetrazoles

5b.2 Results and Discussion

5b.2.1 Chemistry

Tetrazoles are well established as analgesics and in anti-cancer drug therapy. Valsatan, Irbesartan, Omisartan are few well known tetrazoles moieties to exhibit anti-hypertensive effect. There are many synthetic methodologies reported for tetrazole formation. But, we were interested to employ greener method for tetrazole synthesis. Thus, we came up with a novel method where a substituted aromatic/heterocyclic carbonitrile (6) and sodium azide (7) in presence of TBAB in water reflux condition gave us desired products with considerably high yield (Scheme 2). This method was validated for its efficacy by considering few reported molecules 8(a-f) and also synthesized few novel tetrazoles 8(g-l) (Table 2).

\[
\text{TBAB, Water} \quad \begin{array}{c}
\text{R-CN} \\
6
\end{array} \quad \begin{array}{c}
\text{NaN}_3 \\
7
\end{array} \quad \begin{array}{c}
\text{Reflux, 3-5 Hrs} \\
\end{array} \quad \begin{array}{c}
\text{R} \\
\end{array} \quad \begin{array}{c}
\text{N-N} \\
\end{array} \quad \begin{array}{c}
\text{N} \\
\end{array} \quad \begin{array}{c}
\text{H} \\
\end{array} \\
8(a-l)
\]

R- substituted aromatic/heterocyclic carbonitrile

Scheme 2: Synthesis of tetrazoles

Table 2: A series of tetrazoles and their anti-cancer activity

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<tr>
<th>Sl. No</th>
<th>8(a-l)</th>
<th>Time (Hr)</th>
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5b.2.2 Biological studies

All the tetrazoles 8(a-l) were tested against lung carcinoma cells, which was determined by the MTT dye uptake method as described previously [13] (Table 2). The compound 8c was found to be most effective with its inhibition of A549 cancer cells.

5b.3 Experiments

5b.3.1 Chemistry

5b.3.1a General:

All reagents were commercially available reagent grade and were used without further purification. TLC was conducted on 0.25 mm silica gel plates (60F254, Merck). Column chromatography separations were obtained on silica gel (200-400 mesh). 1H NMR spectra were recorded on Agilent NMR 400 MHz instrument in CDCl3 solvent. 13C NMR spectra were obtained on Agilent NMR instrument at 100 MHz in CDCl3 solvent. Chemical shifts are expressed in ppm downfield relative to TMS. LC-MS analysis was performed on Agilent LC-MS with electron ionization (ESI) +ve and -ve mode.

5b.3.1b General procedure of synthesis of tetrazoles:

To a round bottom flask, substituted aromatic/heterocyclic carbonitriles (6) (1 eq), sodium azide (7) (1.2 eq) and 10 mol % of TBAB in water were stirred at 100 °C for
stipulated time (Table 2). The reaction was monitored by TLC using n-hexane and ethyl acetate eluent. After completion of the reaction, solid was filtered through Whatman filter paper 42, air dried to get crude tetrazoles 8(a-l) and further purified by column chromatography (Scheme 2).

5b.3.1c Characterization data of novel tetrazoles:

8g. 5-(5-fluoro-2-methoxyphenyl)-1H-tetrazole: IR $v_{\text{max}}$ (cm$^{-1}$): 3313, 1696, 1021; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.61 (s, 1H, Ar-H), 7.19-7.00 (m, 2H, Ar-H), 6.09 (s, 1H, NH), 3.05 (s, 3H, CH$_3$); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 161.99, 155.61, 152.14, 118.48, 117.56, 114.64, 113.25, 55.53; Mass m/z (MM:ES+APCI) (M+H)$^+$ 195.

8h 2-(1-chloro-2-(1H-tetrazol-5-yl)vinyl)aniline: IR $v_{\text{max}}$ (cm$^{-1}$): 3351, 1687, 1033; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.61-7.57 (m, 2H, Ar-H), 7.55-7.51 (m, 2H, Ar-H), 6.10 (s, 1H, NH), 5.40 (s, 1H, Ethylene H), 4.20 (s, 2H, NH$_2$); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 159.45, 136.45, 132.81, 130.15, 129.03, 127.87, 126.74, 124.06, 96.61; Mass m/z (MM:ES+APCI) (M+H)$^+$ 222.

8i (4-(1H-tetrazol-5-yl)phenyl)(2,6-dichloropyridin-3-yl)methanone: IR $v_{\text{max}}$ (cm$^{-1}$): 3297, 1692, 1038; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.76-7.74 (d, 1H, pyr-H), 7.50-7.44 (m, 5H, Pyr-H &Ar-H), 6.40 (s, 1H, NH); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 189.90,
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154.65, 151.43, 146.03, 139.81, 137.22, 136.18, 129.34, 125.96, 122.71, 121.05; Mass m/z (MM:ES+APCI) (M+H)^+ 320.

8j 5-chloro-4-([4-chlorophenyl](1H-tetrazol-5-yl)methyl)-2-methylaniline: IR \( \nu_{\text{max}} \) (cm\(^{-1}\)):

\[ \begin{align*}
3325, 1700, 1027; & \\
{^1}H \text{ NMR (CDCl}_3, 400 \text{ MHz) } \delta & \quad 7.32-7.26 \text{ (m, 4H, Ar-H), 7.07 (d, 1H, Ar-H), 6.68 (s, 1H, Ar-H), 6.45 (s, 2H, NH), 5.47 (s, 1H, CH), 3.75 (s, 2H, NH)}_2, 2.10 \text{ (s, 3H, CH}_3), {^13}C \text{ NMR (CDCl}_3, 100MHz) } \delta & \quad 148.21, 146.98, 139.00, 136.12, 132.43, 129.18, 128.25, 126.97, 122.16, 114.38, 57.81; \text{ Mass m/z (MM:ES+APCI) (M-H)^- 332.}
\end{align*} \]

8k 1-(5-{1H-tetrazol-5-yl}thiophen-2-yl)ethan-1-one: IR \( \nu_{\text{max}} \) (cm\(^{-1}\)):

\[ \begin{align*}
3295, 1686, 1030; & \\
{^1}H \text{ NMR (CDCl}_3, 400 \text{ MHz) } \delta & \quad 7.62 \text{ 7.59 (m, 2H, thiophene-H), 6.25 (s, 1H, NH), 2.59 (s, 3H, CH}_3), {^13}C \text{ NMR (CDCl}_3, 100 MHz) } \delta & \quad 190.31, 143.74, 142.16, 137.09, 113.11, 131.25, 24.48; \text{ Mass m/z (MM:ES+APCI) (M-H)^- 193.}
\end{align*} \]

8l 5-(1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-1H-pyrazol-5-yl)-1H-tetrazole: IR \( \nu_{\text{max}} \)

\[ \begin{align*}
\text{(cm}\(^{-1}\)) & \quad 3321, 1689, 1026; {^1}H \text{ NMR (CDCl}_3, 400 \text{ MHz) } \delta & \quad 8.51 \text{ (s, 1H, Diaz-H), 8.08 (s, 1H, pyrazol-H), 7.57 (s, 2H, Ar-H), 6.35 (s, 1H, NH), } {^13}C \text{ NMR (CDCl}_3, 100MHz) } \delta & \quad 1469.19, 131.18, 125.77, 123.59, 121.82, 120.00, 115.04; \text{ Mass m/z (MM:ES+APCI) (M+H)^+ 349.}
\end{align*} \]
5.4 References


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Appendices
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$^1$H NMR spectrum of compound 5c

Mass spectrum of 5c
$^{13}\text{C}$ NMR spectrum of compound 5c
1H NMR spectrum of compound 5i

Mass spectrum of compound 5i
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$^{13}$C NMR spectrum of compound 5i
$^1$H NMR spectrum of compound 5j

Mass spectrum of 5j
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13C NMR spectrum of compound 5j
1H NMR spectrum of compound 8i

Mass spectrum of 8i
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13C NMR spectrum of compound 8i
\( ^1H \) NMR spectrum of compound 8k
Mass spectrum of 8k
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$^{13}$C NMR spectrum of compound 8k

$^{1}$H NMR spectrum of compound 8l
Mass spectrum of 8l

$^{13}$C NMR spectrum of compound 8l