CHAPTER 6

RING TRANSFORMATION OF 1,3,4-OXADIAZOLES INTO [1,2,4]TRIAZOLO[3,4-b][1,3,4]THIADIAZINES

6.1 REVIEW ON [1,2,4]TRIAZOLO[3,4-b][1,3,4]THIADIAZINES
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6.1 REVIEWS ON THIADIAZINES

Heterocyclic chemistry and drug discovery are two fields which go hand in hand in the effective practice of medicinal chemistry. Heterocyclic chemistry furnishes indefinite number of potential pharmaceutical candidates many of which are successful drugs being effectively used against several deceases. Currently, heterocyclic small molecules or heterocyclic structural components form a majority of the drugs used in human medicine. Heterocyclic alkaloids were the active components in many natural remedies before the advancement of modern chemistry. Some are still in use today, for example morphine derivatives.

Gomtsyan in his article, “Heterocycles in drugs and drug discovery” [1], thought of the central role that heterocycles play in modern drug design. Heterocyclic entity is expected to improve pharmacological, pharmacokinetic, toxicological, and physicochemical properties of drug candidates to eventually afford drugs.

In pharmacology, almost all the drugs used in the treatment, cure, prevention, or diagnosis of diseases is a contribution of synthetic chemistry. Modern synthetic chemistry has made it possible to design a vast range of heterocycles. Combination of atoms C, N, O and S provides tens of thousands of systems, most of which can be substituted at various positions giving rise to a dumbfounding multifarious structures. [2]
One such significant finding of chemists is triazolothiadiazine ring. There are three classes of triazolothiadiazine heterocyclic rings found in the literature.

![Chemical structures](attachment://images.png)

R & R¹ are substituents

![Chemical structures](attachment://images.png)

R & R¹ are substituents

(275) 7H-[1,2,4]triazolo[3,4-b]-5H-[1,2,4]triazolo[5,1-b]-7H-[1,2,4]triazolo[3,4-b]-[1,3,4]thiadiazine [1,3,5]thiadiazine [1,3,5]thiadiazine

The commonly employed synthetic route for triazolothiadiazine system involves reaction between 4-amino-3-mercapto-1,2,4-triazoles (278) with α-haloketone derivatives. [3, 4] Till date this method has been used extensively for the synthesis of a wide range of 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine derivatives (279). [5, 6, 7]

![Reaction scheme](attachment://images.png)

(278) + (279)

Another simple and less reported method is by the direct ring transformation of S-substituted 1,3,4-oxdiazole (280) using hydrazine hydrate. [8, 9]
This 1,2,4-triazole fused heterocyclic ring has found extensive applications in the fields of medicine, agriculture and industry. We come across triazolothiadiazines especially 7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine ring (275) in several biologically active compounds making it a promising scaffold.

Albrecht and Sweet \cite{10} reported the synthesis of naphthotriazolothiadiazines (281) way back in 1976 and their usefulness as antidepressants.

\begin{center}
\includegraphics[width=0.5\textwidth]{image}
\end{center}

(280)

\begin{center}
R = R_1 = H, R_2 = H, OCH_3; R = CH_3, R_1 = R_2 = H; R = R_2 = H, R_1 = OCH_3
\end{center}

El-Dawy \cite{11} et al synthesized a series of 3,6-disubstituted-(7H)-triazolo[3,4-b][1,3,4]-thiadiazine derivatives (282) and screened them for antiparasitic activity using Ascaris vitulorum. They noticed that 6-substituted derivatives were generally more active than the 3-substituted ones.
Holla et al \[^{[12]}\] reported the synthesis and antibacterial activity of 7H-6-(5-nitro-2-furyl)-s-triazolo[3,4-b]-1,3,4-thiadiazines (283). The compounds exhibited good bactericidal activity.

Prasad et al \[^{[13]}\] synthesized a series of 3-aryloxyalkyl-6-aryl-7H-s-triazolo[3,4-b][1,3,4]thiadiazines (284) and screened them for analgesic and anti-inflammatory activities.
Thorwart et al \[^{14}\] synthesized and tested triazolothiadiazines (285) and some of their salts as 5-lipoxygenase inhibitors. Its salt (R\(_1\) = CH\(_3\)C, R\(_2\) = H, AB = CH\(_2\)CH\(_2\)).HCl. gave 62% inhibition of adjuvant-induced arthritis in rats at 50 mg/kg orally.

\[
R = \text{C}_6\text{H}_5, \text{4-BrC}_6\text{H}_4; \quad R_1 = \text{H}, \text{CH}_3; \quad R_2 = \text{H, Cl, CH}_3; \\
R_3 = \text{H}; \quad R_4 = \text{H, Cl}; \quad R_3R_4 = \text{OCH}_2\text{O}
\]

El-Emam et al \[^{15}\] reported 3-(1-adamantyl)-6-aryl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (286) as potential chemotherapeutic agents.

\[
R = \text{aryl, adamantyl}
\]

Turan-Zitouni et al \[^{16}\] synthesized triazolothiadiazines (287) starting from [(5,6,7,8-tetrahydro-2-naphthalenyl)oxy]acetic acid.
The compounds \((R_1 = H, R_2 = Cl, NO_2; R_1 = OH, R_2 = OCH_3)\) showed promising analgesic activity.

Laddi et al. \cite{17} reported various 7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin derivatives (287) and studied their antimicrobial and antituberculosis activities. Majority of the tested compounds showed antitubercular activity against \(H37 R_v\) at a concentration of 12.5 \(\mu g/mL\). They also exhibited good antimicrobial activity.

Holla et al. \cite{18} synthesized a series of 3,6-disubstituted-7H-s-triazolo(3,4-b)(1,3,4)thiadiazines (288) and studied their in vivo anthelmintic activity in albino mice. A number of compounds showed promising activity when given by the oral route.
Demchenko \[^{[19]}\] synthesized 6-Aryl-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine-7-carboxylates \((290)\) The antiradical and antioxidant activity of the products have been studied on the model of inhibition of NO formation in vitro.

Holla et al \[^{[20]}\] prepared a series of 7-arylidene-6-(2,4-dichlorophenyl)-3-aryloxymethyl/anilinomethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines \((291)\) and the compounds were tested for their antimicrobial activities against \textit{Escherichia coli}, \textit{Staphylococcus aureus} (Smith), \textit{Pseudomonas aeruginosa} (Gessard), \textit{Bacillus subtilis}, and \textit{Candida albicans}. A few among the selected compounds screened for in vitro anticancer activity showed good results.
Laskin et al [21] reported the synthesis and anticancer activity of fluorescent benzylidenetriazolothiadiazines (292). The compound (R = 4-pyridyl) showed IC\textsubscript{50} = 4 µM against human HeLa cervical carcinoma cells.

Ashok and Holla [22] synthesized a series of 7H-6-(substituted aryl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (293). Among all the compounds tested, the compounds (R = 4-Cl, 4-Br) exhibited maximum antibacterial as well as antifungal activities; Ciprofloxacin and Ciclopiroxolamine were used as standard drugs respectively.
Cai et al. [23] reported a series of 3,6-diaryl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (294) and analogs as activators of caspases and inducers of apoptosis. They claimed the compounds could be used to induce cell death in a variety of clinical conditions in which uncontrolled growth and spread of abnormal cells occurs.

**Ar**₁ = (un)substituted (hetero)aryl; **Q**₂ = (un)substituted alkyl, cycloalkyl, heterocyclyl, (hetero)aryl; **R**₁, **R**₂ = H, halo, amino, alkoxy, etc.; **X** = S, O or NR³ (wherein **R**³ = H or (un)substituted alkyl or aryl)

Al-Masoudi and Al-Soud [24] reported the anti-HIV and antitumor activities of new sulphonamide and carboxamide derivatives of acyclic C-Nucleosides of triazolothiadiazine analogs (295). Only the compound (296, **R** = C₆H₅) from the series inhibited HIV-2 replication in cell culture (EC₅₀ of 17.4 µg/mL), in comparison to the standard antiviral drugs efavirenz and
capravirine. None of the other compounds showed any kind of activity.

Skoumbourdis et al [25] reported the structure–activity studies on a series of substituted 7 H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine (297) and their efficacy as PDE4 inhibitors.

Tozkoparan et al [26] synthesized new 3,6-disubstituted 7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines (298) and were screened for their possible analgesic/anti-inflammatory, antioxidant activities and gastric toxicity. The compound (R = 3-CH₃O, X = F, Y = CH₂) was found to have both significant analgesic and consistent anti-
inflammatory activity without inducing any gastric lesions along with minimal lipid peroxidation.

![Chemical structure](image)

\[(298)\]

\[R = 2-\text{CH}_3\text{O}, 3-\text{CH}_3\text{O}; \quad X = \text{H, Cl, F}; \quad Y = (\text{CH}_2)_n, n = 1, 2\]

Bhat et al.\(^{27}\) reported the synthesis and in vitro antitumor activities of a series of 3-(2,4-dichloro-5-fluorophenyl)-6-(substituted phenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines \((299)\) against a panel of sixty cancer cell lines of leukaemia, non-small cell lung cancer, melanoma, ovarian cancer, prostate and breast cancer. The compound \((R = \text{Cl})\) showed promising antiproliferative activity with \(\text{GI}_{50}\) values in the range of 1.06-25.4 \(\mu\text{M}\).

![Chemical structure](image)

\[(299)\]

\[R = \text{H, 4-OCH}_3, 4-\text{CH}_3, 4-\text{Cl, 4-Br, 2,4-Cl}_2, 2,4-\text{Cl}_2-5-\text{F}\]
Ding et al.\cite{28} prepared a series of novel 4-(6-substituted-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)phenols (23). The results indicate that almost all the newly prepared compounds showed a moderate to good inhibiting effect on the growth of the stalk and the radical of the wheat and radish at a concentration of 10 μg/mL and 5 μg/mL. However, the compound (R= \( \text{CF}_3 \)) at a concentration 5 μg/mL, showed a little promoting effect on the growth of the stalk and the radical of the radish.

\[
\text{HO-} \begin{array}{c}
\text{N}\text{N}\text{N} \\
\text{N} \\
\text{S} \\
\text{R}_1
\end{array} \\
\text{R}_1= -\text{CF}_3, -\text{OC}_2\text{H}_5, -\text{C}_6\text{H}_5, 4-\text{Cl-}\text{C}_6\text{H}_4, 4-\text{Br-}\text{C}_6\text{H}_4, 4-\text{F-}\text{C}_6\text{H}_4, 4-\text{NO}_2-\text{C}_6\text{H}_4, 3-\text{NO}_2-\text{C}_6\text{H}_4, -\begin{array}{c}
\text{H} \\
\text{H} \\
\text{H}
\end{array} \\
4-\text{OCH}_3-\text{C}_6\text{H}_4, 3-\text{OCH}_3-\text{C}_6\text{H}_4, 2,4-\text{Cl}_2\text{C}_6\text{H}_3, 2,4-\text{F}_2\text{C}_6\text{H}_3
\]

Shehry et al.\cite{29} synthesized 3-((2,4-dichlorophenoxy)methyl)-1,2,4-triazolo thiadiazines and evaluated their anti-inflammatory and molluscicidal activity. The compound (301) showed significant anti-inflammatory activity in comparison with the standard Indomethacin.

\[
\text{R} \begin{array}{c}
\text{N} \\
\text{N} \\
\text{S} \\
\text{Ar}_1
\end{array} \\
\text{Ar} \\
\text{R}
\]

(301)
Gawad et al. [30] reported new 3-[5-(1H-Indol-3-yl)-1-phenyl-1H-pyrazol-3-yl]-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine incorporating indole nucleus and evaluated their antiviral activity against *herpes simplex type 1* (*HSV-1*). Compound (302) showed a moderate activity in reducing the number of plaques at 0.1 mg/mL concentration.

![Chemical structure of compound 302](image)

Badr and Barwa [31] synthesized fused 1,2,4-triazole series, 6-(4-substituted phenyl)-3-(5-nitrofuran-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (303) and investigated their in vitro antibacterial activity. The tested compounds (R = Cl, Br) showed MIC value (25 µg/mL) equal to the standard Ampicillin against *Staphylococcus aureus*.

![Chemical structure of compound 303](image)

R = H, Br, OCH₃, Cl, NO₂
Koksal et al [32] reported the synthesis of a series of 3,6-diaryl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (304). All the compounds were evaluated for anti-inflammatory activity; in addition ulcerogenic activities of the compounds were also determined. The compounds exhibited anti-inflammatory activity at 100 mg/kg.

\[
\begin{align*}
\text{R} = & \text{C}_6\text{H}_5, 4\text{-ClC}_6\text{H}_4, 2\text{-naphthyl} \& \text{more} \\
\end{align*}
\]

Mohsen [33] synthesized 3-[(1H-indol-3-yl)methyl]-6-aryl-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines (305), and evaluated their ability to inhibit acetylcholinesterase (AChE).

\[
\begin{align*}
\text{R} = & \text{H, Cl, CH}_3, \text{NO}_2, -\text{N(CH}_3)_2 \\
\end{align*}
\]

Puthiyapurayil et al [34] reported the synthesis and antimicrobial properties of 6-(4-substituted phenyl) -3- (5-nitrofuran-2-yl) -7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (306). Compounds were found to be more sensitive to Gram-positive bacteria than Gram-negative bacteria.
Jakhar and Makrandhi [35] reported a series of 3-Aryl-6-(6-substituted-4-methylcinnolin-3-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines (307) and their antibacterial properties. All the tested compounds were found to show moderate to good activity against both Gram -ve and Gram +ve bacteria.

7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (275), incorporating a variety of scaffolds with different substituents are mentioned above. The above reports show the growing interest in thiadiazines because of their applications in various fields especially in medicine. A number of these molecules exhibiting several promising biological activities were the motivating factor for preparing a new series of the compounds based on it. Literature survey doesn’t mention many triazolothiadiazines with
α- and β-naphthoxy moieties. Hence 7H-[1,2,4] triazolo [3,4-b][1,3,4] thiadiazines incorporating α- and β-naphthols were synthesized by the less commonly reported method, i.e. by the direct ring transformation of S-substituted 1,3,4-oxadiazoles into 7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines. The compounds were screened for their antimicrobial and antioxidant properties.

6.2 MATERIALS AND METHODS
The triazolothiadiazine derivatives were prepared by the cyclization of S-substituted oxadiazoles. The preparation of S-substituted oxadiazoles (4E), starting from α- and β-naphthols (4A) through their esters (4B), hydrazides (4C), and oxadiazoles (4D) are discussed in detail in chapter 4 (Scheme 4).

The synthesis of triazolothiadiazine derivatives was performed as outlined in Scheme 6 in 65 to 90% yields. The S-substituted oxadiazoles (4E) were subjected to react with hydrazine hydrate in acetic acid to obtain the final compounds.
6.3 RESULTS AND DISCUSSION

Synthesis of S-substituted-5-[(naphthalen-1/2-yloxy)methyl]-1,3,4-oxadiazole-2-thiol ethanone compounds (4E) by reacting 5-[(naphthalen-1/2-yloxy)methyl]-1,3,4-oxadiazole-2-thiol (4D) with appropriate α-haloketones has been discussed in chapter 4. 6-substituted-3-[(naphthalen-1/2-yloxy)methyl]-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives (6A) were prepared in 65 to 90% yield by refluxing a mixture of S-substituted 1,3,4-oxadiazoles (4E) in acetic acid and hydrazine hydrate.

The structures of the newly synthesized compounds were established on the basis of IR, 1H-NMR, 13C-NMR, mass spectral and elemental analysis. Conversion of (4E) to (6A) was inferred by
the disappearance of a strong peak at ~1710 cm\(^{-1}\) which could have been attributed to carbonyl group in all the IR spectra of the products. The absence of C=O group strongly indicates the ring closure. A remarkable up field shift of S-CH\(_2\) resonance in \(^1\)H NMR from ~5.5 ppm in (4E) to ~4 ppm in (6Aa) was correlated to the absence of C=O group next to S-CH\(_2\) in thiadiazine ring.

The compound 6Aa, in its IR spectrum (Figure 6.1) showed the characteristic peaks at 1631 cm\(^{-1}\) for C=N stretching and at 1577 and 1508 cm\(^{-1}\) for C=C stretching. The C-O-C stretching was observed at 1099 cm\(^{-1}\), whereas aromatic C-H and aliphatic C-H stretching were at 3047 cm\(^{-1}\) and 2928 cm\(^{-1}\) respectively. The \(^1\)H-NMR (400 MHz) spectrum of 6Aa (Figure 6.2) in MeOD showed a sharp signal at δ 2.31 ppm for -CH\(_3\) and another singlet at δ 3.75 ppm for -S-CH\(_2\)-. The two protons of -O-CH\(_2\)- resonated as a singlet at δ 5.50 ppm. A set of peaks in the aromatic region integrating to seven protons of naphthyl ring, viz at δ 7.15 ppm (d, 1H, J=7.56 Hz), at δ 7.39-7.52 ppm (m, 4H) and two doublets centred at δ 7.80 ppm and at δ 8.12 ppm with J=8.12 Hz and J=8.32 Hz respectively. In the \(^13\)C-NMR spectrum (Figure 6.3) there were sixteen signals accounting for sixteen distinct types of carbon atoms in the molecule. The signal at δ 23.31 was for -CH\(_3\) carbon and at δ 25.15 was for -S-CH\(_2\)-. The -O-CH\(_2\)- carbon atom resonated at δ 59.59. All the naphthyl and oxadiazole ring carbon atoms resonated at δ 106.75, 120.99, 121.46, 124.99, 125.46, 126.01, 126.52, 127.44, 134.03, 140.99, 148.80, 153.32 and 159.29. LC-Mass spectrum of 6Aa (Figure 6.4) had a fragmentation peak at m/z 168.1
because of the cleavage of the molecule at the ether linkage as follows.

\[
\begin{align*}
\text{Mass} &= 167 \\
\text{The observed molecular ion peak at } m/z 310.9 (M+H)^+ \text{ and a base peak at } m/z 312.2 (M+2H)^+, \text{ were consistent with its molecular formula } C_{16}H_{14}N_4OS.
\end{align*}
\]
Figure 6.1: IR spectrum of the compound 6Aa
Figure 6.2: $^1$H-NMR spectrum of the compound 6Aa
Figure 6.3: $^{13}$C-NMR spectrum of the compound 6Aa
Figure 6.4: LC-MS of the compound 6Aa

$$C_{10}H_{12}N_{2}O_{5}S$$

Mol. Wt.: 310.37
In the IR spectrum of the compound 6Am (Figure 6.5), the C=N stretching was seen at 1629 cm\(^{-1}\) and C=C stretching at 1597 and 1512 cm\(^{-1}\). The C-O-C stretching was observed at 1118 cm\(^{-1}\) and the characteristic peak for C-Cl was seen at 831 cm\(^{-1}\). The aromatic and aliphatic C-H stretches were seen at 3057 and 2912 cm\(^{-1}\) respectively. The \(^1\)H-NMR spectrum of the compound in CDCl\(_3\) (Figure 6.6) showed two sharp singlets, one at \(\delta\) 3.95 ppm for two protons of -S-CH\(_2\)- and another at \(\delta\) 5.50 ppm for -O-CH\(_2\)- protons. Out of seven naphthyl ring protons, one resonated at \(\delta\) 7.19 ppm (d, 1H, J=9.0 Hz) while three more naphthyl ring protons and two protons of phenyl ring meta to -Cl group appeared as a multiplet at \(\delta\) 7.36-7.45 ppm. Another multiplet was observed at \(\delta\) 7.72-7.77 ppm for three naphthyl ring protons and for two protons ortho to -Cl in the phenyl ring. The \(^{13}\)C-NMR spectrum (Figure 6.7) of 6Am was consistent with the structure. While -S-CH\(_2\)- and -O-CH\(_2\)- carbon atoms appeared at \(\delta\) 22.79 and \(\delta\) 59.00, the rest of the aromatic carbon atoms and oxadiazole carbon atoms resonated at \(\delta\) 107.97, 118.55, 123.97, 126.53, 126.78, 127.53, 128.85, 129.05, 129.27, 129.48, 132.06, 134.01, 136.88, 141.56, 149.57, 154.38 and 155.76. The mass and the stability of the compound was confirmed with the LC-mass spectrum (Figure 6.8) showing the molecular ion -cum- base peak at \(m/z\) 407.3 (M)\(^+\) and its chlorine isotopic peak at \(m/z\) 409.1 (M+2H)\(^+\), agreeing with the molecular formula C\(_{21}\)H\(_{15}\)ClN\(_4\)OS.
Figure 6.5: IR spectrum of the compound 6Am
Figure 6.6(a): ¹H-NMR spectrum of the compound 6Am
Figure 6.6(b): $^1$H-NMR spectrum of the compound 6Am
Figure 6.7: $^{13}$C-NMR spectrum of the compound 6Am
Figure 6.8: LC-MS of the compound 6Am
The formation of the compound 6Ac was evident by its IR, $^1$H-, $^{13}$C-NMR and mass spectra. In the IR spectrum (Figure 6.9) the peaks at 3055 cm$^{-1}$ and 2916 cm$^{-1}$ corresponded with the aromatic and aliphatic stretching. The $\nu_{C=N}$ at 1571 cm$^{-1}$ and $\nu_{C=O}$ at 1093 cm$^{-1}$ were observed. The $^1$H-NMR spectrum in MeOD (Figure 6.10) showed two singlets integrating for two protons each at $\delta$ 4.27 ppm for -S-CH$_2$- and at $\delta$ 5.64 ppm for -O-CH$_2$-. One of the naphthyl ring protons was identified with a doublet centred at $\delta$ 7.22 ppm with coupling constant J=7.56 Hz. Four more naphthyl ring protons and three phenyl ring protons appeared as a multiplet at $\delta$ 7.35-7.58 ppm. A set of three doublets, centred at $\delta$ 7.80 ppm (J=8.2 Hz) for a naphthyl proton, $\delta$ 7.87 ppm (J=7.28 Hz) for phenyl ring proton and at $\delta$ 8.14 ppm (J=8.52 Hz) accounted for all the protons in the molecule. The $^{13}$C-NMR spectrum of 6Ac (Figure 6.11) showed 18 distinct types of carbon atoms. The -SCH$_2$- and -OCH$_2$- resonances were at $\delta$ 22.92 and $\delta$ 59.86 respectively. The resonances for naphthyl ring, oxadiazole ring and phenyl ring carbon atoms appeared at $\delta$ 106.9, 121.03, 121.45, 125.04, 125.45, 126.02, 126.51, 127.47, 128.92, 131.97, 133.22, 134.05, 141.76, 149.58, 153.40 and 155.45. The LC-Mass spectrum of the compound (Figure 6.12) was in complete agreement with its structure and the molecular formula C$_{21}$H$_{16}$N$_4$OS, showing a molecular ion peak was found at $m/z$ 372.8 (M+H)$^+$ and base peak was at $m/z$ 374.2 (M+2H)$^+$. 
Figure 6.9: IR spectrum of the compound 6Ac
Figure 6.10(a): $^1$H-NMR spectrum of the compound 6Ac
Figure 6.10(b): $^1$H-NMR spectrum of the compound 6Ac
Figure 6.11: $^{13}$C-NMR spectrum of the compound 6Ac
Figure 6.12: LC-MS of the compound 6Ac
For the compound 6Aj, the IR spectrum (Figure 6.13) showed characteristic peaks at 3057 cm\(^{-1}\) and 2912 cm\(^{-1}\) for aromatic and aliphatic C-H stretching respectively. The peaks at 1624 cm\(^{-1}\) and at 1118 cm\(^{-1}\) corresponded with C=N and C-O-C bond stretching. C=C stretching was seen at 1593 and 1508 cm\(^{-1}\). In the \(^1\)H-NMR spectrum of the compound in MeOD (Figure 6.14), a triplet centred at \(\delta\) 1.25 ppm (3H) with coupling constant \(J=7.32\) Hz and a quartet centred at \(\delta\) 2.68 ppm (3H) with coupling constant \(J=7.36\) Hz clearly proved the presence of ethyl substituent. Two sharp singlets were seen at \(\delta\) 3.80 ppm and at \(\delta\) 5.45 ppm each integrating for two protons, correlated to -S-CH\(_2\)- and -O-CH\(_2\)-protons. A doublet of doublets centred at \(\delta\) 7.18 ppm with coupling constant \(J=9.0\) Hz integrated for one proton of naphthyl ring. Six more naphthyl ring protons appeared as a set of three multiplets at \(\delta\) 7.34-7.38, 7.44-7.48 and 7.78-7.81 ppm integrating for one, two and three protons respectively. The Figure 6.15 presents the \(^{13}\)C-NMR spectrum of the compound 6Aj. The signals at \(\delta\) 9.66 and \(\delta\) 24.34 represent the carbon atoms of -CH\(_3\)- and -CH\(_2\)- respectively. The -SCH\(_2\)- and -OCH\(_2\)- carbon atoms resonated at \(\delta\) 29.95 and \(\delta\) 58.85. The rest of the naphthyl ring and thiadiazine ring carbon atoms resonated at \(\delta\) 107.80, 118.50, 123.96, 126.552, 126.76, 127.52, 128.82, 129.44, 134.01, 141.31, 148.91, 155.71 and 162.75. The LC-Mass spectrum (Figure 6.16) showed a fragmentation peak at \(m/z\) 181.1 corresponding to the cleavage of the ether linkage.
The molecular ion peak was observed at $m/z$ 324.9 ($\text{M+H}^+$) and the base peak at $m/z$ 326.5 ($\text{M+2H}^+$) in the positive mode. The peaks found in the spectrum matched with the structure and the molecular formula $\text{C}_{17}\text{H}_{16}\text{N}_4\text{OS}$ of the compound $6\text{Aj}$. 
Figure 6.13: IR spectrum of the compound 6Aj
Figure 6.14(a): $^1$H-NMR spectrum of the compound 6Aj
Figure 6.14(b): $^1$H-NMR spectrum of the compound 6Aj
Figure 6.15: $^{13}$C-NMR spectrum of the compound 6Aj
The elemental analysis, mass, IR, $^1$H- and $^{13}$C-NMR data obtained correlated well with the structures and molecular formulae of the synthesized compounds. The IR and $^1$H-NMR data of the rest of the compounds are given at the bottom of the Table 6.1.
Table 6.1: Physico-chemical and characterization data of novel 6-(substituted)-3-[(naphthalen-1/2-yloxy)methyl]-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Naphthyl group</th>
<th>R</th>
<th>Mol. Formula (mol.wt)</th>
<th>Melting Range</th>
<th>Yield (%)</th>
<th>Chemical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>6Aa</td>
<td>-CH₃</td>
<td></td>
<td>C₁₆H₁₄N₄OS (310.37)</td>
<td>177-178</td>
<td>69.0</td>
<td>61.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-C₂H₅</td>
<td>C₁₆H₁₄N₄OS (324.40)</td>
<td>68-69</td>
<td>76.7</td>
<td>62.93</td>
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<tr>
<td>6Ac</td>
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<td></td>
<td>C₁₆H₁₄N₄OS (372.44)</td>
<td>140-141</td>
<td>90.0</td>
<td>67.69</td>
</tr>
<tr>
<td>6Ad</td>
<td></td>
<td></td>
<td>C₂₂H₁₈N₄O₄S (402.46)</td>
<td>168-169</td>
<td>77.3</td>
<td>65.69</td>
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<td>6Ae</td>
<td></td>
<td></td>
<td>C₂₁H₁₆ClN₄OS (406.88)</td>
<td>187-188</td>
<td>65.8</td>
<td>61.90</td>
</tr>
<tr>
<td>6Af</td>
<td></td>
<td></td>
<td>C₂₁H₁₆F₃N₄OS (390.43)</td>
<td>169-170</td>
<td>82.0</td>
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<td></td>
<td>C₂₂H₁₈F₃N₄OS (440.44)</td>
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<td>78.4</td>
<td>60.00</td>
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<td></td>
<td>C₂₁H₁₆N₄O₄S (417.44)</td>
<td>189-190</td>
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<td></td>
<td>C₁₆H₁₄N₄OS (310.37)</td>
<td>119-120</td>
<td>67.2</td>
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<tr>
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<td>-C₂H₅</td>
<td></td>
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<td></td>
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<td>176-177</td>
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<tr>
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<td>67.5</td>
<td>64.65</td>
</tr>
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<td>6Ao</td>
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<td></td>
<td>C₂₂H₁₈F₃N₄OS (440.44)</td>
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<td>75.0</td>
<td>60.05</td>
</tr>
<tr>
<td>6Ap</td>
<td></td>
<td></td>
<td>C₂₁H₁₆N₄O₄S (417.44)</td>
<td>161-162</td>
<td>75.0</td>
<td>60.38</td>
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</table>

386
6b: IR (KBr) ν/cm⁻¹: 3055 (Ar-H stretching), 2910 (aliph C-H stretching), 1625 (C=N), 1596, 1508 (C=C), 1093 (C-O-C); ¹H-NMR (400MHz, MeOD): δ 1.15-1.19 (t, 3H, J=7.32 Hz, -CH₃ of ethyl group), 2.60-2.65 (q, 2H, J=7.32 Hz, -CH₂- of ethyl group), 3.73 (s, 2H, -S-CH₂-), 5.52 (s, 2H, -O-CH₂-), 7.17-7.19 (d, 1H, J=7.4 Hz, naphthyl ring), 7.40-7.52 (m, 4H, naphthyl ring), 7.81-7.83 (d, 1H, J=8.0 Hz, naphthyl ring), 8.12-8.14 (d, 1H, J=8.32 Hz, naphthyl ring).

6d: IR (KBr) ν/cm⁻¹: 3053 (Ar-H stretching), 2916 (aliph C-H stretching), 1627 (C=N), 1600, 1512 (C=C), 1093 (C-O-C); ¹H-NMR (400MHz, MeOD): δ 3.87 (s, 3H, -OCH₃), 4.22 (s, 2H, -S-CH₂-), 5.63 (s, 2H, -O-CH₂-), 6.98-7.00 (d, 2H, J=7.0 Hz, 2H meta to -OCH₃ in phenyl ring), 7.22-7.24 (d, 1H, J=7.64 Hz, naphthyl ring), 7.35-7.51 (m, 4H, naphthyl ring), 7.80-7.82 (d, 1H, J=7.76 Hz, naphthyl ring), 7.86-7.88 (d, 2H, J=7.0 Hz, 2H ortho to -OCH₃ in phenyl ring), 8.14-8.16 (d, 1H, J=8.4, naphthyl ring).

6e: IR (KBr) ν/cm⁻¹: 3057 (Ar-H stretching), 2900 (aliph C-H stretching), 1628 (C=N), 1591, 1504 (C=C), 1093 (C-O-C); ¹H-NMR (400MHz, CDCl₃): δ 3.88 (s, 2H, -S-CH₂-), 5.58 (s, 2H, -O-CH₂-), 7.15-7.16 (d, 1H, J=7.6 Hz, naphthyl ring), 7.33-7.47 (m, 6H, 4H of naphthyl ring & 2H meta to –Cl in phenyl ring), 7.67-7.71 (d, 2H, J=8.8 Hz, 2H ortho to –Cl in phenyl ring), 7.76-7.78 (d, 1H, J=8.4 Hz, naphthyl ring), 8.14-8.16 (d, 1H, J=8.4 Hz, naphthyl ring).

6f: IR (KBr) ν/cm⁻¹: 3061 (Ar-H stretching), 2914 (aliph C-H stretching), 1629 (C=N), 1595, 1510 (C=C), 1097 (C-O-C), 1232 (C-
F; $^1$H-NMR (400MHz, CDCl$_3$): $\delta$ 3.92 (s, 2H, -S-CH$_2$-), 5.61 (s, 2H, -OCH$_2$-), 7.12-7.19 (m, 3H, naphthyl ring), 7.35-7.49 (m, 4H, 2H of naphthyl ring & 2H meta to -F in phenyl ring), 7.78-7.81 (m, 3H, 1H of naphthyl ring & 2H ortho to -F in phenyl ring), 8.16-8.18 (d, 1H, J=8.12, naphthyl ring).

6h: IR (KBr) v/cm$^{-1}$: 3057 (Ar-H stretching), 2920 (aliph C-H stretching), 1628 (C=N), 1597 (C=C), 1519 (NO$_2$ asymmetric), 1344 (NO$_2$ symmetric), 1097 (C-O-C); $^1$H-NMR (400MHz, DMSO): $\delta$ 4.51 (s, 2H, -S-CH$_2$-), 5.61 (s, 2H, -O-CH$_2$-), 7.28-7.30 (d, 1H, J=7.6 Hz, naphthyl ring), 7.38-7.54 (m, 4H, naphthyl ring), 7.85-7.87 (d, 1H, J=8.0 Hz, naphthyl ring), 8.07-8.09 (d, 1H, J=8.4 Hz, naphthyl ring), 8.12-8.14 (d, 2H, J=9.0 Hz, 2H meta to -NO$_2$ in phenyl ring), 8.28-8.30 (d, 2H, J=9.0 Hz, ortho to -NO$_2$ in phenyl ring).

6i: IR (KBr) v/cm$^{-1}$: 3045 (Ar-H stretching), 2922 (aliph C-H stretching), 1625(C=N), 1597. 1508 (C=C), 1116 (C-O-C); $^1$H-NMR (400MHz, MeOD): $\delta$ 2.36 (s, 3H, -CH$_3$), 3.81 (s, 2H, -S-CH$_2$-), 5.46 (s, 2H, -O-CH$_2$-), 7.14-7.16 (d, 1H, J=7.56 Hz, naphthyl ring), 7.36-7.48 (m, 4H, naphthyl ring), 7.78-7.80 (d, 1H, J=8.14 Hz, naphthyl ring), 8.11-8.13 (d, 1H, J=8.26 Hz, naphthyl ring).

6k: IR (KBr) v/cm$^{-1}$: 3055 (Ar-H stretching), 2918 (aliph C-H stretching), 1632 (C=N), 1577, 1506 (C=C), 1101 (C-O-C); $^1$H-NMR (400MHz, MeOD): $\delta$ 4.26 (s, 2H, -S-CH$_2$-), 5.62 (s, 2H, -O-CH$_2$-), 7.21-7.23 (d, 1H, J=7.56 Hz, naphthyl ring), 7.33-7.57 (m, 7H, 4H of naphthyl ring & 3H phenyl ring), 7.78-7.80 (d, 1H, J=8.16 Hz,
naphthyl ring), 7.86-7.88 (d, 2H, J=7.4 Hz, phenyl ring), 8.13-8.15 (d, 1H, J=8.4, naphthyl ring).

6l: IR (KBr) ν/cm⁻¹: 3057 (Ar-H stretching), 2914 (aliph C-H stretching), 1620 (C=N), 1600, 1512 (C=C), 1116 (C-O-C); ¹H-NMR (400MHz, MeOD): δ 3.86 (s, 3H, -OCH₃), 4.29 (s, 2H, -SCH₂-), 5.56 (s, 2H, -O-CH₂-), 6.97-6.99 (d, 2H, J=7.0 Hz, 2H meta to -OCH₃ in phenyl ring), 7.20-7.23 (dd, 1H, J=7.64 Hz, naphthyl ring), 7.35-7.54 (m, 3H, naphthyl ring), 7.78-7.81 (m, 3H, naphthyl ring), 7.91-7.93 (d, 2H, J=7.0 Hz, 2H ortho to -OCH₃ in phenyl ring).

6n: IR (KBr) ν/cm⁻¹: 3057 (Ar-H stretching), 2947 (aliph C-H stretching), 1627 (C=N), 1597, 1508 (C=C), 1118 (C-O-C), 1222 (C-F); ¹H-NMR (400MHz, MeOD): δ 4.32 (s, 2H, -S-CH₂-), 5.55 (s, 2H, -O-CH₂-), 7.16-7.21 (m, 3H, naphthyl ring), 7.34-7.52 (m, 3H, naphthyl ring) 7.77-7.79 (m, 3H, 1H of naphthyl ring & 2H meta to -F in phenyl ring), 7.97-8.01 (m, 2H, ortho to -F in phenyl ring).

6o: IR (KBr) ν/cm⁻¹: 3059 (Ar C-H stretching), 2912 (aliph C-H stretching), 1625 (C=N), 1597, 1512 (C=C), 1319 (C-F of -CF₃), 1118 (C-O-C); ¹H-NMR (400MHz, MeOD): δ 4.38 (s, 2H, -S-CH₂-), 5.58 (s, 2H, -O-CH₂-), 7.18-7.21 (dd, 1H, J=9.0 Hz, naphthyl ring), 7.34-7.53 (m, 3H, naphthyl ring), 7.72-7.74 (d, 2H, J=8.4 Hz, meta to -CF₃ in phenyl ring), 7.77-7.79 (m, 3H, naphthyl ring), 8.08-8.10 (d, 2H, J=8.24 Hz, ortho to -CF₃ in phenyl ring).

6p: IR (KBr) ν/cm⁻¹: 3051 (Ar C-H stretching), 2924 (aliph C-H stretching), 1625 (C=N), 1597 (C=C), 1521 (NO₂ asymmetric), 1348 (NO₂ symmetric), 1118 (C-O-C); ¹H-NMR (400MHz, DMSO): δ 4.52
(s, 2H, S-CH$_2$), 5.53 (s, 2H, -O-CH$_2$), 7.21-7.24 (dd, 1H, J=9.0 Hz, naphthyl ring), 7.35-7.60 (m, 3H, naphthyl ring), 7.79-7.85 (m, 3H, naphthyl ring), 8.15-8.17 (d, 2H, J=7.2 Hz, 2H meta to –NO$_2$ in phenyl ring), 8.28-8.30 (d, 2H, J=9.0 Hz, ortho to –NO$_2$ in phenyl ring).

6.4 BIOLOGICAL EVALUATION

6.4.1 Antimicrobial Activity
The in vitro antimicrobial activity was carried out against 24 hrs culture of four bacterial strains, Gram positive (Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633) and Gram negative (Escherichia coli ATCC 8739 and Pseudomonas aeruginosa ATCC 27853). Bacterial strains used in this study were obtained from National Chemical Laboratory, Pune, India. The bacterial strains were maintained on nutrient agar slants. Two hundred micro litre of overnight grown culture of each organism was dispensed into 20 mL of sterile nutrient broth and incubated for 4-5 hrs at 37 °C to standardize the culture to $10^{-5}$ CFU/mL.
Table 6.2: Antimicrobial activity of test compounds expressed as Avg±SEM at a concentration of 10 μg/mL

<table>
<thead>
<tr>
<th>Compound (10μg/mL)</th>
<th>Staphylococcus aureus</th>
<th>Bacillus subtilis</th>
<th>Pseudomonas aeruginosa</th>
<th>Escherichia coli</th>
<th>Aspergillus niger</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>6Aa</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>6Ab</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>8</td>
<td>–</td>
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<td>–</td>
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<td>5</td>
<td>–</td>
<td>4.5</td>
</tr>
<tr>
<td>6Ae</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>6Af</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>–</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>6Ag</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>–</td>
<td>4.5</td>
</tr>
<tr>
<td>6Ah</td>
<td>7</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>–</td>
<td>4.5</td>
</tr>
<tr>
<td>6Ai</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>5</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>6Aj</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>6Ak</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>6Al</td>
<td>8</td>
<td>4</td>
<td>6</td>
<td>–</td>
<td>–</td>
<td>4.5</td>
</tr>
<tr>
<td>6Am</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>–</td>
<td>4.5</td>
</tr>
<tr>
<td>6An</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>–</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>6Ao</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>5</td>
<td>–</td>
<td>4.5</td>
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<tr>
<td>6Ap</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Streptomycin</strong></td>
<td><strong>23</strong></td>
<td><strong>21.9</strong></td>
<td><strong>16.1</strong></td>
<td><strong>19.5</strong></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Nystatin</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>19.9</strong></td>
<td><strong>18.2</strong></td>
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</table>
Antibacterial and antifungal assay were carried out by disc diffusion method \cite{26}. 0.1 mL \((10^{-5} \text{ CFU/mL})\) of 24 hrs old bacterial culture was placed on Mueller Hinton agar medium and spread throughout the plate by spread plate technique. The compounds were tested at 10 mg/mL concentration against both bacterial and fungal strains. DMSO was used as a vehicle. Sterile paper discs (6 mm in diameter) impregnated with the test compound was placed on the surface of the medium and incubated at 37 °C for 24 hrs. Antibacterial activity was recorded by measuring the diameter of zone of inhibition. Streptomycin was used as positive reference standard. The entire test was performed in triplicate. The antifungal activity was assayed by inoculating the fungal spores on the potato dextrose agar (PDA) medium pre-impregnated with discs containing test compounds. Nystatin was used as positive reference standard against fungal strains. The results are recorded in Table 6.2.

6.4.2 DPPH radical scavenging assay

Antioxidants are defined as substances that, even at low concentration, significantly delay or prevent oxidation of easily oxidizable substrates. Normally, defence against the highly reactive free radicals causing oxidation can be accomplished by the organism using antioxidants. The most popular screening assays for the estimation of the antioxidative potential of chemical components use commonly available instrumentation. They have been developed to be fast and easy. Most of them require a
spectrophotometric measurement and a certain reaction time in order to obtain reproducible results \cite{27}. The in vitro antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) test.

The stable free radical DPPH is a useful reagent to investigate the scavenger properties of compounds. The details of DPPH assay has been explained in Chapter 3. Each sample was assayed at a concentration of 100 μg/mL and all the experiments were carried out in triplicate and the % RSC is shown in Figure 6.17.

![Figure 6.17: % DPPH Radical scavenging assay of synthesized compounds (Avg ±SEM) at a concentration 100 μg/mL](image)

The investigation of antibacterial screening data revealed that Staphylococcus aureus and Pseudomonas aeruginosa are more susceptible towards the tested compounds compared to Escherichia coli and Bacillus subtilis. Among the two fungal strains used, only Candida albicans growth was inhibited by the compounds to a
certain extent, whereas *Aspergillus niger* showed complete resistance towards the test compounds. The antioxidant activity carried out through DPPH assay revealed that among the tested compounds, 6Ab (R= -C₂H₅) showed the highest activity (60%), but much less than the standard (93%). The compounds 6Ad (R = C₆H₄-OCH₃), 6Aj (R = C₂H₅), 6Ae (R = C₆H₄-Cl), and 6Al (R = C₆H₄-OCH₃), were the other few compounds which showed moderate activity. 6Aa (R = CH₃), 6Ao (R = C₆H₄-CF₃) and 6Ap (R= C₆H₄-NO₂) also showed slight activity in comparison with the standard ascorbic acid. The presence of groups like -C₂H₅ and -OMe appeared to be enhancing the capacity of the test compounds to quench DPPH radicals.

### 6.5 EXPERIMENTAL

**General Procedure for the preparation of 6-substituted-3-[(naphthalen-1/2-yloxy)methyl]-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (6a-6p)**

To a solution of S-substituted 1,3,4-oxadiazole 5 (10 mmol, 1 eq) in acetic acid (10 mL) was added hydrazine hydrate (15 mmol, 1.5 eq) and the mixture was heated at 90 °C for 4 hrs. Completion of reaction was checked on TLC. The reaction mixture was concentrated and the residue was dissolved in 25 mL of CHCl₃ and successively washed with water (2 × 25 mL), saturated brine solution (1 × 25 mL) and dried over anhydrous Na₂SO₄. The organic layer was removed under reduced pressure. The crude products were recrystallized from methanol.
6.6 REFERENCES


