Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(4):326-334

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Ring transformation of 1,3,4-oxadiazoles into novel [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines and their antimicrobial and antioxidant properties

Priscilla Patrao[a], A. M. A. Khader[a*], B. Kalluraya[a] and Vinayachandra[b]

[a]*Department of Studies in Chemistry, Mangalore University, Mangalagangothri, Karnataka, India*
[b]*Department of Studies in Applied Botany, Mangalore University, Mangalagangothri, Karnataka, India*

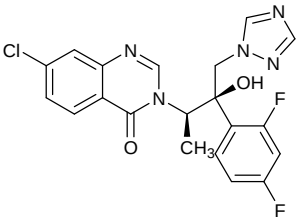
ABSTRACT

A series of new [1,2,4]triazolo[3,4-b][1,3,4]thiadiazines incorporating α and β-naphthoxy methyl moieties was synthesized starting from S-substituted 5-[(naphthalen-1/2-yloxy)methyl]-1,3,4-oxadiazole-2-thiols. The newly synthesized compounds were characterized by ^{1}H NMR, IR, mass and elemental analysis. The efficacy of the compounds was tried with regard to their antimicrobial and antioxidant properties.

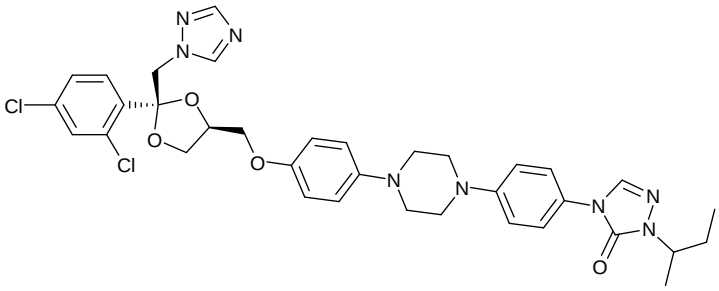
Keywords: Ring transformation; 1,3,4-oxadiazoles; triazolothiadiazines; antimicrobial; antioxidant.

INTRODUCTION

The discovery of penicillin and synthetic chemicals like sulphonamides in early years of 19th century began, a new era of antimicrobial chemotherapy. But over the years, the drugs have lost their significance because of the overexploitation and immune capability developed within disease organisms [1]. The overuse of antibiotics has become a cause of worry among many [2-4]. However, it has been almost two decades that the market has not seen an affirmative antibacterial compound. Considering these facts, with rationalizing the effects and synthetic methodologies a series of new triazolothiadiazines were designed, synthesized and tested for their antimicrobial property. In many drugs antioxidant action may also contribute to their pharmacological activity. Hence the reported compounds were also investigated for their antioxidant property.



Albaconazole (antifungal)



Itraconazole (antifungal)

In the past few decades, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention as a result of their synthetic and effective biological importance [5]. Albaconazole, Fluconazole, Isavuconazole, Itraconazole, Posaconazole, Ravuconazole, Terconazole, Voriconazole are few drugs of 1,2,4-triazole derivatives which are available in the market. N-bridged heterocyclic derivatives of 1,2,4-triazoles show varied biological activities [6]. 7H-[1,2,4]Triazolo[3,4-b][1,3,4]thiadiazines are a class of heterocycles with known antimicrobial [7] and analgesic activities [8, 9]. A large number of triazolothiadiazines have also been shown to exhibit antidepressant [10, 11], central nervous depressant [12], bactericidal, fungicidal [13], diuretic

activities [14] and also photographic coupling properties [15]. In addition, some triazolo[3,4-b]-[1,3,4]thiadiazines are reported to possess antiplatelet, antithrombic, [16], anticancer [17], antiviral [18], antitubercular [19], anti-inflammatory, antimolluscicidal [20], anti HIV [21] as well as antiemetic [22] properties.

On the other hand derivatives of α and β-naphthols have shown significant applications in the field of medicine. Many naphthalene containing drugs are available in the market, such as nafacillin, naftifine, tolnaftate, terbinafine etc with excellent antimicrobial activity. Several other synthetic derivatives possessing α and β-naphthols have also been reported which possess significant antimicrobial property [23-26]. Mkpenie et al., [27] have tested azo-2 naphthol and 2-napthol against human pathogenic microorganisms. Both were found equally effective against all the organisms tested. We have reported the synthesis of a new series of S-substituted 1,3,4-oxadiazoles containing α and β-naphthols and their antioxidant properties [28]. As a continuation of our work, in the present study we report s-triazolo-fused heterocycles, simultaneously containing naphthalene and triazolothiadiazine motifs synthesized by the intramolecular ring closure of S-substituted 1,3,4-oxadiazoles.

EXPERIMENTAL SECTION

Chemistry

Melting points were determined using open capillary method and are uncorrected. IR spectra were recorded on Shimadzu-FTIR infrared spectrometer in KBr (ν_{max} in cm^{-1}). The purity of the compounds was confirmed by thin layer chromatography using Merck silica gel 60 F254 coated aluminum plates. The ^1H NMR spectra were recorded on a Bruker AMX-400 (400 MHz) spectrometer using TMS as an internal standard. The chemical shifts are expressed in δ scale downfield from TMS and proton signals are indicated as s= singlet, d= doublet, t= triplet, q= quartet, m = multiplet. The mass spectra were recorded on a Jeol JMS-D 300 mass spectrometer operating at 70 eV. CHN analysis was carried out on a VARIO EL-III (Elementar Analysensysteme GmBH).

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

To a solution of 5-(α/β-Napthoxy-methyl)-1,3,4-oxadiazole-2-thiols **1** (10mmol, 1 eq) in acetic acid (10 ml) was added hydrazine hydrate (15 mmol, 1.5 eq) and the mixture was heated at 90^0C for 4 hrs. Completion of reaction was checked on TLC. The reaction mixture was concentrated and the residue was dissolved in 25 mL of $CHCl_3$ and successively washed with water (2 × 25mL), saturated brine solution (1 × 25 mL) and dried over anhydrous Na_2SO_4. Organic layer was removed under reduced pressure. Crude product was recrystallized using methylene dichloride to get the pure product.

6-methyl-3-[(naphthalen-1-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2a:

Yield 69%, m.p. 177-178 ^{0}C; IR (KBr) γ/cm^{-1}: 3047 (Ar C-H stretching), 2928 (Aliph C-H streching), 1631(C=N), 1577, 1508 (C=C), 1099 (C-O-C); ^1H NMR (400MHz, MeOD): δ 2.31 (s, 3H, CH_3), 3.75 (s, 2H, S-$\underline{CH_2}$), 5.50 (s, 2H, O-$\underline{CH_2}$), 7.15-7.17 (d, 1H, J=7.56 Hz, Naphthyl ring), 7.39-7.52 (m, 4H, Naphthyl ring), 7.80-7.82 (d, 1H, J=8.12 Hz,Naphthyl ring), 8.12-8.14 (d, 1H, J=8.32 Hz, Naphthyl ring); LC-MS (m/z): 311 (M+1), (M.F- $C_{16}H_{14}N_4OS$).

6-ethyl-3-[(naphthalen-1-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2b:

Yield 76.7%, m.p. 68-69^{0}C; IR (KBr) γ/cm^{-1}: 3055 (Ar C-H stretching), 2910 (Aliph C-H streching), 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, $\underline{CH_3}$ of ethyl group), 2.60-2.65 (q, 2H, J=7.32, $\underline{CH_2}$ of ethyl group), 3.73 (s, 2H, S-$\underline{CH_2}$), 5.52 (s, 2H, O-$\underline{CH_2}$), 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 ; LC-MS (m/z): 323 (M-1), (M.F- $C_{17}H_{16}N_4OS$).

3-[(naphthalen-1-yloxy)methyl]-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2c:

Yield 90%, m.p. 140-141^{0}C; IR (KBr) γ/cm^{-1}: 3055 (Ar C-H stretching), 2916 (Aliph C-H streching), 1630 (C=N), 1571, 1504 (C=C), 1093 (C-O-C); ^1H NMR (400MHz, MeOD): δ 4.27 (s, 2H, S-$\underline{CH_2}$), 5.64 (s, 2H, O-$\underline{CH_2}$), 7.22-7.24 (d, 1H, J=7.56 Hz, Naphthyl ring), 7.35-7.58 (m, 7H, 4H of Naphthyl ring & 3H phenyl ring), 7.80-7.82 (d, 1H, J=8.2 Hz, Naphthyl ring), 7.87-7.90 (d, 2H, J=7.28, phenyl ring), 8.14-8.16 (d, 1H, J=8.52, Naphthyl ring); LC-MS (m/z): 373 (M+1), (M.F- $C_{21}H_{16}N_4OS$).

6-(4-methoxyphenyl)-3-[(naphthalen-1-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2d:

Yield 77.3%, m.p. 168-169 ^{0}C; IR (KBr) γ/cm^{-1}: 3053 (Ar C-H stretching), 2916 (Aliph C-H streching), 1627 (C=N), 1600, 1512 (C=C), 1093 (C-O-C); ^1H NMR (400MHz, MeOD): δ 3.87 (s, 3H, -O$\underline{CH_3}$), 4.22 (s, 2H, S-$\underline{CH_2}$), 5.63 (s, 2H, O-$\underline{CH_2}$), 6.98-7.00 (d, 2H, J=7.0, 2H meta to –OCH_3 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, 2H ortho to $-OCH_3$ in phenyl ring), 8.14-8.16 (d, 1H, J=8.4, Naphthyl ring); LC-MS (m/z): 403 (M+1), (M.F- $C_{22}H_{18}N_4O_2S$).

6-(4-chlorophenyl)-3-[(naphthalen-1-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2e:
Yield 65.8%, m.p. 187-188 ^{0}C; IR (KBr) γ/cm^{-1}: 3057 (Ar C-H stretching), 2900 (Aliph C-H streching), 1628 (C=N), 1591, 1504 (C=C), 1093 (C-O-C); ^{1}H NMR (400MHz, $CDCl_3$): δ 3.88 (s, 2H, S-$\underline{CH_2}$), 5.58 (s, 2H, O-$\underline{CH_2}$), 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, 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, Naphthyl ring); LC-MS (m/z): 407 (M^++1), 409 (M+2), (M.F- $C_{21}H_{15}ClN_4OS$).

6-(4-fluorophenyl)-3-[(naphthalen-1-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2f:
Yield 82%, m.p. 169-170^{0}C; IR (KBr) γ/cm^{-1}: 3061 (Ar C-H stretching), 2914 (Aliph C-H streching), 1629 (C=N), 1595, 1510 (C=C), 1097 (C-O-C), 1232 (C-F); ^{1}H NMR (400MHz, $CDCl_3$): δ 3.92 (s, 2H, S-$\underline{CH_2}$), 5.61 (s, 2H, O-$\underline{CH_2}$), 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); LC-MS (m/z): 391 (M+1), (M.F- $C_{21}H_{15}FN_4OS$).

3-[(naphthalen-1-yloxy)methyl]-6-[4-(trifluoromethyl)phenyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2g:
Yield 78.4%, m.p. 168-169^{0}C; IR (KBr) γ/cm^{-1}: 3062 (Ar C-H stretching), 2933 (Aliph C-H streching), 1625 (C=N), 1575, 1510 (C=C), 1327 (C-F of CF_3), 1120 (C-O-C); ^{1}H NMR (400MHz, MeOD): δ 4.31 (s, 2H, S-$\underline{CH_2}$), 5.64 (s, 2H, O-$\underline{CH_2}$), 7.21-7.23 (d, 1H, J=7.48 Hz, Naphthyl ring), 7.33-7.51 (m, 4H, Naphthyl ring), 7.73-7.75 (d, 2H, J=8.4 Hz, meta to $-NO_2$ in phenyl ring), 7.78-7.80 (d, 1H, J=8.2 Hz, Naphthyl ring), 8.01-8.03 (d, 2H, J=8.28 Hz, ortho to $-NO_2$ in phenyl ring), 8.11-8.13 (d, 1H, J=8.4 Hz, Naphthyl ring); LC-MS (m/z): 441 (M+1), (M.F- $C_{22}H_{15}F_3N_4OS$).

3-[(naphthalen-1-yloxy)methyl]-6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2h:
Yield 80.8%, m.p. 189-190^{0}C; IR (KBr) γ/cm^{-1}: 3057 (Ar C-H stretching), 2920 (Aliph C-H streching), 1628 (C=N), 1597 (C=C), 1519 (NO_2 asymmetric), 1344 (NO_2 symmetric), 1097 (C-O-C); ^{1}H NMR (400MHz, DMSO): δ 4.51 (s, 2H, S-$\underline{CH_2}$), 5.61 (s, 2H, O-$\underline{CH_2}$), 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); LC-MS (m/z): 418 (M+1), (M.F- $C_{21}H_{15}N_5O_3S$).

6-methyl-3-[(naphthalen-2-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2i:
Yield 67.2%, m.p. 119-120^{0}C; IR (KBr) γ/cm^{-1}: 3045 (Ar C-H stretching), 2922 (Aliph C-H streching), 1625(C=N), 1597. 1508 (C=C), 1116 (C-O-C); ^{1}H NMR (400MHz, MeOD): δ 2.36 (s, 3H, CH_3), 3.81 (s, 2H, S-$\underline{CH_2}$), 5.46 (s, 2H, O-$\underline{CH_2}$), 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); LC-MS (m/z): 311 (M+1), (M.F- $C_{16}H_{14}N_4OS$).

6-ethyl-3-[(naphthalen-2-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2j:
Yield 72%, m.p. 111-112^{0}C; IR (KBr) γ/cm^{-1}: 3057 (Ar C-H stretching), 2912 (Aliph C-H streching), 1624 (C=N), 1593. 1508 (C=C), 1118 (C-O-C); ^{1}H NMR (400MHz, MeOD): δ 1.23-1.26 (t, 3H, J=7.32 Hz, $\underline{CH_3}$ of ethyl group), 2.66-2.72 (q, 2H, J=7.36, $\underline{CH_2}$ of ethyl group), 3.80 (s, 2H, S-$\underline{CH_2}$), 5.45 (s, 2H, O-$\underline{CH_2}$), 7.17-7.20 (dd, 1H, J=9.0 Hz, Naphthyl ring), 7.34-7.48 (m, 3H, Naphthyl ring), 7.78-7.81 (m, 3H, Naphthyl ring); LC-MS (m/z): 325 (M+1), (M.F- $C_{17}H_{16}N_4OS$).

3-[(naphthalen-2-yloxy)methyl]-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2k:
Yield 84.1%, m.p. 133-134^{0}C; IR (KBr) γ/cm^{-1}: 3055 (Ar C-H stretching), 2918 (Aliph C-H streching), 1632 (C=N), 1577, 1506 (C=C), 1101 (C-O-C); ^{1}H NMR (400MHz, MeOD): δ 4.26 (s, 2H, S-$\underline{CH_2}$), 5.62 (s, 2H, O-$\underline{CH_2}$), 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, phenyl ring), 8.13-8.15 (d, 1H, J=8.4, Naphthyl ring); LC-MS (m/z): 373 (M+1), (M.F- $C_{21}H_{16}N_4OS$).

6-(4-methoxyphenyl)-3-[(naphthalen-2-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2l:
Yield 71.8%, m.p. -139-140^{0}C; IR (KBr) γ/cm^{-1}: 3057 (Ar C-H stretching), 2914 (Aliph C-H streching), 1620 (C=N), 1600, 1512 (C=C), 1116 (C-O-C); ^{1}H NMR (400MHz, MeOD): δ 3.86 (s, 3H, -O$\underline{CH_3}$), 4.29 (s, 2H, S-$\underline{CH_2}$), 5.56 (s, 2H, O-$\underline{CH_2}$), 6.97-6.99 (d, 2H, J=7.0, 2H meta to $-OCH_3$ 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, 2H ortho to $-OCH_3$ in phenyl ring); LC-MS (m/z): 403 (M+1), (M.F- $C_{22}H_{18}N_4O_2S$).

6-(4-chlorophenyl)-3-[(naphthalen-2-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2m:
Yield 71.5%, m.p. 176-177^{0}C; IR (KBr) γ/cm^{-1}: 3057 (Ar C-H stretching), 2912 (Aliph C-H streching), 1629 (C=N), 1597, 1512 (C=C), 1118 (C-O-C); ^{1}H NMR (400MHz, $CDCl_3$): δ 3.95 (s, 2H, S-$\underline{CH_2}$), 5.50 (s, 2H, O-$\underline{CH_2}$), 7.17-7.19 (dd, 1H, J=9.0 Hz, Naphthyl ring), 7.36-7.45 (m, 5H, 3H of naphthyl ring & 2H meta to –Cl in phenyl ring), 7.72-7.77 (m, 5H, 3H of naphthyl ring & 2H ortho to –Cl in phenyl ring); LC-MS (m/z): 407 (M+1), 409 (M+2), (M.F- $C_{21}H_{15}ClN_4OS$).

6-(4-fluorophenyl)-3-[(naphthalen-1-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2n:
Yield 67.5%, m.p. 140-141 ^{0}C; IR (KBr) γ/cm^{-1}: 3057 (Ar C-H stretching), 2947 (Aliph C-H streching), 1627 (C=N), 1597, 1508 (C=C), 1118 (C-O-C), 1222 (C-F); ^{1}H NMR (400MHz, MeOD): δ 4.32 (s, 2H, S-$\underline{CH_2}$), 5.55 (s, 2H, O-$\underline{CH_2}$), 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); LC-MS (m/z): 391 (M+1), (M.F- $C_{21}H_{15}FN_4OS$).

3-[(naphthalen-2-yloxy)methyl]-6-[4-(trifluoromethyl)phenyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2o:
Yield 75%, m.p. 151.152 ^{0}C; IR (KBr) γ/cm^{-1}: 3059 (Ar C-H stretching), 2912 (Aliph C-H streching), 1625 (C=N), 1597, 1512 (C=C), 1319 (C-F of CF_3), 1118 (C-O-C); ^{1}H NMR (400MHz, MeOD): δ 4.38 (s, 2H, S-$\underline{CH_2}$), 5.58 (s, 2H, O-$\underline{CH_2}$), 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 – CF_3 in phenyl ring), 7.77-7.79 (m, 3H, Naphthyl ring), 8.08-8.10 (d, 2H, J=8.24 Hz, ortho to – CF_3 in phenyl ring); LC-MS (m/z): 441 (M+1), (M.F- $C_{22}H_{15}F_3N_4OS$).

3-[(naphthalen-2-yloxy)methyl]-6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine 2p:
Yield 75%, m.p. 161-162 ^{0}C; IR (KBr) γ/cm^{-1}: 3051 (Ar C-H stretching), 2924 (Aliph C-H streching), 1625 (C=N), 1597 (C=C), 1521 (NO_2 asymmetric), 1348 (NO_2 symmetric), 1118 (C-O-C); ^{1}H NMR (400MHz, DMSO): δ 4.52 (s, 2H, S-$\underline{CH_2}$), 5.53 (s, 2H, O-$\underline{CH_2}$), 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); LC-MS (m/z): 418 (M+1), (M.F- $C_{21}H_{15}N_5O_3S$).

Biological Evaluation

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^{o}C to standardize the culture to 10^{-5} CFU/ml.

Antibacterial and antifungal assay were carried out by disc diffusion method [29]. 0.1ml (10^{-5} 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 tested compound was placed on the surface of the medium and incubated at 37^{o}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 tested compound. Nystatin was used as positive reference standard against fungal strains. The results are recorded in Table **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 [30]. We determined the in vitro antioxidant activity 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 DPPH assay was based on the reported method [31]. Briefly, 1 mL of the solution of the compounds in DMSO (100 µg/mL) was diluted to 4 mL using methanol. To this 1mL of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) solution in methanol was added. The mixed solution was incubated at room temperature for 30 min. The absorbance of stable

DPPH was read at 517 nm using UV-Visible spectrophotometer and the remaining DPPH was calculated. Ascorbic acid was taken as standard. The free radical scavenging activity was expressed as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{[Ac - As]}{[Ac - Ab]} \text{ X } 100$$

Where *Ac* was the absorbance of the control, *As* for the sample and *Ab* for the blank (MeOH+DMSO). Each sample was assayed at 100 µg/mL and all the experiments were carried out in triplicate and the % RSC is shown in **Fig 1.**

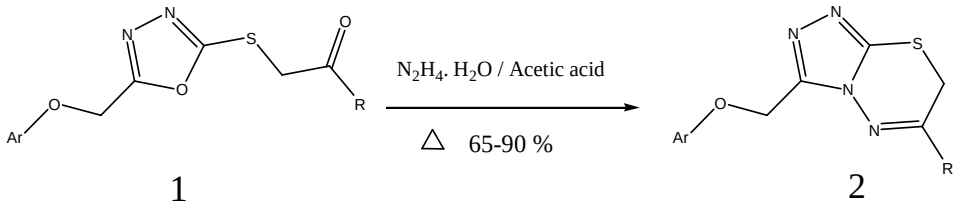
RESULTS AND DISCUSSION

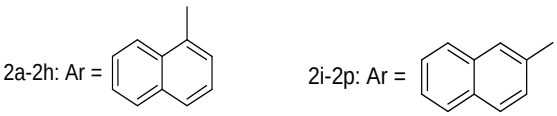
Chemistry

The wide application of triazolothiadiazines has led to the increased research interest in these molecules. Different methods have been employed to synthesize these triazolothiadiazines to get moderate to good yields. One of the most common methods widely reported is starting from amino mercaptotriazoles [32, 33], which are then condensed with various haloketones. Our research group has also reported the synthesis of triazolothiadiazines by the same method [6, 34-36]. But it is for the first time that we are reporting from our lab the intramolecular ring closure of substituted 1,3,4-oxadiazoles into triazolothiadiazines.

The methods of synthesis of heterocyclic esters, hydrazides, oxadiazoles and S-substituted oxadiazoles, the intermediates for the synthesis of triazolothiadiazine derivatives are already reported in our paper. 5-(α/β-Napthoxy-methyl)-1,3,4-oxadiazole-2-thiols were treated with a number of α-haloketones in the presence of triethylamine to get S-substituted oxadiazoles **1** [28].

The synthesis of triazolothiadiazine derivatives was performed as outlined in **Scheme 1** in 65 to 90% yields. The S-substituted oxadiazoles **1** were subjected to react with hydrazine hydrate in acetic acid to achieve the final compounds.





R= $-CH_3$, $-C_2H_5$, $-C_6H_5$, $-C_6H_4OCH_3$, $-C_6H_4Cl$, $-C_6H_4F$, $-C_6H_4CF_3$, $-C_6H_4NO_2$

Scheme 1

The structures of the newly synthesized compounds have been established on the basis of ^{1}H NMR, IR, mass spectral and elemental analysis. Conversion of **1** to **2** was free from any doubts with the disappearance of a strong peak at 1710 cm^{-1} in IR spectra of **2** which was prominent in **1** which could be attributed to carbonyl group. The missing C=O group strongly defends the ring closure. This change was accompanied by a remarkable upfield shift of methylene protons (S-CH_2) from ~5.5 ppm in **1** to ~4 ppm in **2**. This shift is correlated to the absence of C=O group next to S-CH_2 in thiadiazine ring. The ^{1}H NMR spectrum of compounds 2a-2p also showed two sharp singlets, one between δ 3.75 to 4.52 ppm for methylene protons of thiadiazine ring and another singlet between δ 5.45 to 5.6 ppm for –OCH_2 protons lying between naphthalene and oxadiazole rings. Apart from this, a pair of doublets corresponding to four protons (2 ortho and 2 meta) of phenyl ring and set of multiplets integrating for seven protons of naphthyl ring were seen in the region ranging from 7.1 to 8.3 ppm. The chemical shift was dependent on the nature of substituent group attached to phenyl ring. So in ^{1}H NMR spectra, all protons were seen according to the expected chemical shift and integral values. The physico-chemical and characterisation data of new 7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives is presented in **Table 1**.

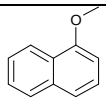
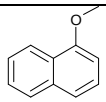
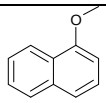
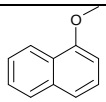
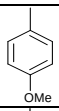
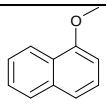
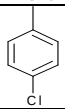
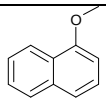
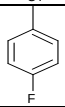
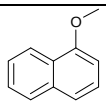
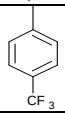
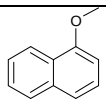
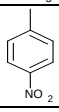
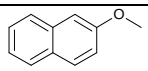
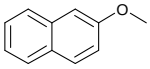
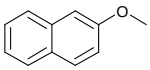
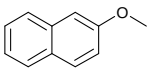
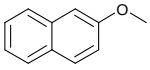
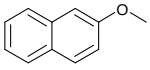
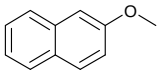
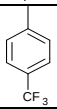
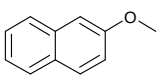
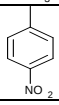
Sl. No.	Naphthyl group	R	Mol. Formula (mol. wt)	Melting Range (^{0}C)	Yield (%)	CHN Analysis *Found (calculated)*		
						C	H	N
2a		-CH_3	$C_{16}H_{14}N_4OS$ (310.37)	177-178	69.0	61.89 (61.92)	4.57 (4.55)	18.01 (18.05)
2b		-C_2H_5	$C_{17}H_{16}N_4OS$ (324.40)	68-69	76.7	62.91 (62.94)	5.01 (4.97)	17.23 (17.27)
2c			$C_{21}H_{16}N_4OS$ (372.44)	140-141	90.0	67.69 (67.72)	4.37 (4.33)	15.01 (15.04)
2d			$C_{22}H_{18}N_4O_2S$ (402.46)	168-169	77.3	65.60 (65.65)	4.55 (4.51)	13.87 (13.92)
2e			$C_{21}H_{15}ClN_4OS$ (406.88)	187-188	65.8	61.92 (61.99)	3.75 (3.72)	13.68 (13.77)
2f			$C_{21}H_{15}FN_4OS$ (390.43)	169-170	82.0	64.58 (64.60)	3.91 (3.87)	14.32 (14.35)
2g			$C_{22}H_{15}F_3N_4OS$ (440.44)	168-169	78.4	59.93 (59.99)	3.49 (3.43)	12.70 (12.72)
2h			$C_{21}H_{15}N_5O_3S$ (417.44)	189-190	80.8	60.34 (60.42)	3.65 (3.62)	16.75 (16.78)
2i		-CH_3	$C_{16}H_{14}N_4OS$ (310.37)	119-120	67.2	61.90 (61.92)	4.59 (4.55)	18.02 (18.05)
2j		-C_2H_5	$C_{17}H_{16}N_4OS$ (324.40)	111-112	72.0	62.87 (62.94)	5.02 (4.97)	17.23 (17.27)
2k			$C_{21}H_{16}N_4OS$ (372.44)	133-134	84.1	67.68 (67.72)	4.38 (4.33)	15.01 (15.04)
2l			$C_{22}H_{18}N_4O_2S$ (402.46)	139-140	71.8	65.57 (65.65)	4.59 (4.51)	13.89 (13.92)
2m			$C_{21}H_{15}ClN_4OS$ (406.88)	176-177	71.5	61.95 (61.99)	3.81 (3.72)	13.71 (13.77)
2n			$C_{21}H_{15}FN_4OS$ (390.43)	140-141	67.5	64.55 (64.60)	3.92 (3.87)	14.32 (14.35)
2o			$C_{22}H_{15}F_3N_4OS$ (440.44)	151-152	75.0	59.91 (59.99)	3.48 (3.43)	12.70 (12.72)
2p			$C_{21}H_{15}N_5O_3S$ (417.44)	161-162	75.0	60.38 (60.42)	3.67 (3.62)	16.74 (16.78)

Table 1: Physico-chemical and characterisation data of novel 6-(substituted)-3-[(naphthalen-1/2-yloxy)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives

Antimicrobial activity

The novel compounds were screened for antimicrobial as well as antioxidant properties. Antimicrobial assay was done through disc diffusion method using 10µg/ml of the test compounds. The investigation of antibacterial

screening data revealed that two of the bacterial strains viz *Staphylococcus aureus* and *Pseudomonas aeruginosa* are more susceptible towards the tested compounds compared to the other two strains *Escherichia coli* and *Bacillus subtilis*. Among the two fungal strains used, only *candida albicans* growth was inhibited by the compounds to certain extent, whereas *Aspergillus niger* showed complete resistance towards the test compounds.

Zone of inhibition in (mm)						
Compound (0.25mg/disc)	***Staphylococcus aureus***	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Aspergillus niger***	***Candida albicans***
	Antibacterial strains				**Antifungal Strains**	
2a	7	5	6	5	_	4
2b	6	5	7	8	_	5
2c	6	5	8	_	_	6
2d	8	7	6	5	_	4.5
2e	6	6	7	6	_	5
2f	8	6	8	_	_	5
2g	6	5	5	6	_	4.5
2h	7	5	8	5	_	4.5
2i	6	7	8	5	_	4
2j	6	5	8	5	_	4
2k	5	4	5	_	_	5
2l	8	4	6	_	_	4.5
2m	7	5	6	5	_	4.5
2n	6	5	6	_	_	5
2o	7	6	8	5	_	4.5
2p	6	4	6	4	_	5
Control	0	0	0	0	0	0
Streptomycin (10µg/ml)	23	21.9	16.1	19.5	_	_
Nystatin (10µg/ml)	–	–	–	–	19.9	18.2

Table 2: Antimicrobial activity of test compounds expressed as Avg ±SEM at a concentration of 10µg/ml

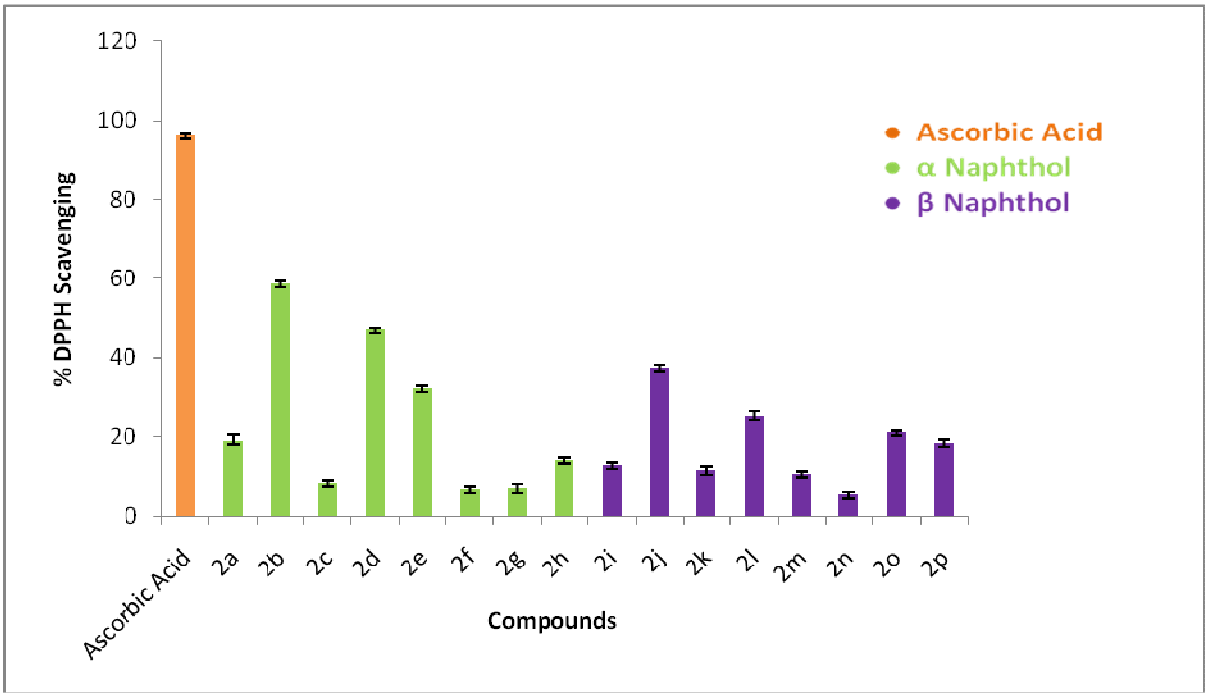


Fig I: % DPPH Radical scavenging assay of synthesized compounds (Avg ±SEM) at a concentration100µg/ml

Antioxidant activity

A freshly prepared DPPH solution exhibits a deep-purple color with an absorption maximum at 517 nm. This purple colour generally disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule, and convert them into colourless/bleached product. In this assay, we measured the DPPH initial absorbance and the absorbance once the potential antioxidant had been added. The reduction of absorbance is a

measure of free DPPH due to the action of the antioxidant. The antioxidant activity was expressed as the RSA% (Radical Scavenging Activity). We used ascorbic acid as reference standard. The results revealed that among the tested 16 compounds, 2b (R= $-C_2H_5$) showed highest activity (60%). Compounds 2d (R= ph-OMe), 2j (R= $-C_2H_5$), 2e (R= ph-Cl), and 2l (R= ph-OMe), were the other few compounds which showed moderate activity. 2a (R= $-CH_3$), 2o (R= ph-CF_3) and 2p (R= ph-NO_2) also showed slight activity in comparison with the standard ascorbic acid. The presence of groups like $-C_2H_5$ and –OMe appeared to be enhancing the capacity of the test compounds to quench DPPH radicals.

CONCLUSION

Continuing our interest to search for potent triazolothiadiazine motifs, we here in report the synthesis of a new class of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines prepared by the ring transformation of 1,3,4-oxadiazole derivatives incorporating α and β-naphthols through a simple yet efficient method. The series of compounds were examined for antibacterial and antioxidant properties. Few of the tested compounds showed moderate antimicrobial activity at a concentration of 10µg/ml against all the strains except for the antifungal strain *Trichoderma Viridae.* Few exhibited again moderate DPPH radical scavenging activity at a concentration 100 µg/ml, in comparison with the standard ascorbic acid.

Acknowledgements
One of the authors (P.P) is thankful to UGC for providing financial help for the research work through UGC-BSR scheme. Also thanks to the Head, IISc Bangalore and CDRI Lucknow, for spectral data, department of applied Botany, Mangalore University for the antimicrobial and antioxidant studies.

REFERENCES

[1] Richard Wise. *J. Antimicrob. Chemother.*, **2011**, 66, 1939.
[2] MM Swann; KL Baxter; HI Field. Report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine. London: Her Majesty's Stationery Office, **1969**.
[3] House of Lords Select Committee on Science and Technology. Session 1997–1998. Seventh Report. Resistance to Antibiotics and other Antimicrobial Agents. London: Her Majesty's Stationery Office, **1998**.
[4] World Health Organization. Antimicrobial resistance: no action today, no cure tomorrow. http://www.who.int/dg/speeches/2011/WHD_20110407/en/
[5] Jyoti Sharma; Shamim Ahmad; M Shamsher Alam. *J. Chem. Pharm. Res.*, **2012,** 4(12), 5157-64.
[6] BS Holla; B Kalluraya; KR Sridhar; E Drake; LM Thomas; KK Bhandary; MJ Levine. *Eur. J. Med. Chem.*, **1994**, 29(4), 301-308.
[7] NF Eweiss; AA Bahajaj. *J. Heterocycl. Chem.*, **1987**, 24(4), 1173-82.
[8] G Turan Zitouni; ZA Kaplacikli; K Erol; FS Kilias. *Farmaco.*, **1999**, 54(4), 218-223.
[9] K Shashikala Devi; M Ramaiah; GK Vanita; K Veena; VP Vaidya. *J. Chem. Pharm. Res.*, **2011**, 3(1), 445-451.
[10] WL Albrecht; FW Sweet. U.S. Pat. 3,954,983 (1976), *Chem. Abstr*., **1976**, 85, 78172g.
[11] A Brucato; A Coppola; S Gianguzza; PM Provenzano. *Boll. Soc. Ital. Biol. Sper.*, **1978**, 54(11), 1051-57.
[12] WL Albrecht; WD Jones. U.S. Pat. 3,954,981 (1976), *Chem. Abstr*., **1976**, 85, 177501v.
[13] P Molina; M Alajarin; MJ Perez de Vega; MC Foces-Foces; F Hernandez Cano; RM Claramunt; J Elguero. *J. Chem. Soc. Perkin. Trans. I, Organic and Bio-Organic Chemistry (1972-1999).*, **1987**, 8, 1853-60.
[14] WL Albrecht. U.S. Pat. 3,954,984 (1976), *Chem. Abstr*., 1976, 85, 78173h.
[15] Y Kawashima; YH Ishikawa; S Kida; T Tanaka; K Masuda. Jpn. (Kokai) 61,260,085 (1986), *Chem. Abstr*., **1987**, 106, 138475x.
[16] K Rehse; U Brummer; E Unsold. *Die Pharmazie.*, **1998**, 53(12), 820-824.
[17] HB Shivarama; RB Sooryanarayana; BK Sarojini; PM Akberali; KN Suchetha. *Eur. J. Med. Chem*., **2006**, 41(5), 657-663.
[18] HB Shivarama; PM Akberali; MK Shivananda. *Farmaco.*, **2001**, 56(12), 919-927.
[19] GVS Kumar; YR Prasad; BP Mallikarjuna; SM Chandrashekar. *Eur. J. Med. Chem*., **2010**, 45(11), 5120-29.
[20] MF El Shehry; AA Abu-Hashem; EM El-Telbani. *Eur. J. Med. Chem*., **2010**, 45(5), 1906-11.
[21] NA Al-Masoudi; YA Al-Soud. *Nucleosides, Nucleotides and Nucleic Acids.*, **2008**, 27(9), 1034-44.
[22] J Thomas. *Ger.Offen*., 2403357, (1974); *Chem. Abstr*., **1974**, 81, 136153.
[23] Mustafa Yildiz; Askin Kiraz; Basaran Dulger. *J. Serb. Chem. Soc*., **2007**, 72(3), 215-224.
[24] Zeynep Ates-Alagoz; Mehmet Alpl; Canan Kus; Sulhiye Yildiz; Erdem Buyukbingol; Hakan Goker; *Arch. Pharm.*, **2006**, 339(2), 74-80.
[25] GK Nagaraja; GK Prakash; MN Kumaraswamy; VP Vaidya; KM Mahadevan. *Arkivoc (xv)*, **2006**, 160-168.
[26] GK Nagaraja; GK Prakash; MN Kumaraswamy; VP Vaidya; KM Mahadevan; *Arkivoc (xv)*, **2006**, 142-152.
[27] V Mkpenie; G Ebong; IB Obot; B Abasiekong. *E-Journal of chemistry*, **2008**, 5(3), 431-434.

[28] Priscilla Patrao; AMA. Khader; Balakrishna Kalluraya; Vinayachandra. *Der Pharma Chemica,* **2013**, 5 (2), 24-32.
[29] PA Wayne. National Committee for Clinical Laboratory Standards (NCCLS). Performance standard for antimicrobial disk susceptibility test. 7th ed. Approved standard NCCLS document M2-A7. **2000**.
[30] T Kulisic; A Radonic; V Katalinic; M Milos. *Food. Chem*., **2004**, 85(4), 633-640.
[31] AK Tiwari. *Curr. Sci*., **2004,** 86(8), 1092-1102.
[32] Zafer Asım Kaplancıklı; Gu¨lhan Turan-Zitouni; Ahmet O¨ zdemir; Gilbert Revial. *Eur. J. Med. Chem*., **2008**, 43(1), 155-159.
[33] Bantval Shivarama Holla, Balladka Kunhanna Sarojini, Balikekodi Sooryanarayana Rao, Padiyath Mohammed Akberali, Nalilu Suchetha Kumari, Veena Shetty, *Il. Farmaco.*, **2001**, 56(8), 565-570.
[34] Kalluraya Balakrishna, P. Vishwanatha, M. Isloor Arun, B. Ganesh Rai, *Journal of Indian Council of Chemists*, **1999,** 16(2), 26-28.
[35] Kalluraya Balakrishna, Chimbalkar Ramesh, *Oriental Journal of Chemistry* **1995**, 11(3), 242-245.
[36] Kalluraya Balakrishna, D'Souza Alphonsus, Holla B. Shivarama, *Indian J. Chem., Sect B.,* **1995,** 34B(11), 939-943.