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# Synthesis and *In Vitro* Antimicrobial Evaluation of 6-Aryl-4-(1,1′-biphenyl-4-yl)-N-methyl-6H-1,3-thiazin-2-amines

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## ABSTRACT

*Spectral characterization and antimicrobial screening of new 6-aryl-4-(1,1'-biphenyl-4-yl)-N-Methyl-6H-1,3-thiazin-2-amines synthesized from 1-(1,1'-biphenyl-4-yl)-3-arylprop-2-en-1-ones using N-methyl thiourea have been described. The isolated crude products were subjected to column chromatography and used for further analysis. The in vitro antimicrobial study of all synthesized compounds exhibited bacterial activity equal or moderate to the commercial antibiotic moxifloxacin but they were moderate to less active against standard fungi amphotericin B.*

**Keywords:** Synthesis, N-Methyl-1,3-thiazin-2-amine, Bipenylethanone, Spectral characterization, Antimicrobial evaluation

## INTRODUCTION

Synthetic chemistry especially drug synthesis scenario mainly focused on the medicinally important scaffolds which are mainly contribute in the pharmaceutical field. Likewise the synthetic strategy is a promising attempt to discover new pharmaceuticals with very potent antimicrobials. The researchers are attempting to produce emerging Pharma targets by constructing various heterocycles with different side structural modifications. Since heterocycles are proved to be very active pharmaceuticals and among these, 2-amino-1,3-thiazines and their derivatives are very useful pharamacophore with N-C-S linkage exhibit variety of biological activities like antibacterial [1], beta amyloid cleaving enzyme-1 inhibitors [2], cannabinoid receptor agonist [3], cytoprotective toward heart and neurons [4] etc.

In earlier findings the compounds with biphenyl substitution exhibited variety of biological properties like anticancer [5], antibacterial [6] etc. In addition, the alkyl substituted amino units present in the aromatics shows variety of biological activities such as cytotoxic [7], antimicrobial [8] and enhancing antagonist activity of N-Methylated Endothelin-1 [9]. Earlier we have reported N-Methyl-1,3-thiazin-2-amines with naphthyl side chain in continuing our effort are reporting some N-Methyl-1,3-thiazin-2-amines with biphenyl core.

## MATERIALS AND METHODS

The chemicals used for the synthetic work are analytical grade and were purified by using literature procedure. Open capillary tubes were used in electro thermal apparatus for measuring the melting point and are uncorrected. The Thin Layer Chromatography (TLC) and column chromatography with silica gel-G were used to check the purity and purification of synthesized compounds. The IR spectra were recorded on Shimadzu Fourier Transform Infra-Red (FTIR) spectrometer using KBr pellets. The Proton Nuclear Magnetic Resonance ($^1$H-NMR) and Carbon-13 Nuclear Magnetic Resonance ($^{13}$C-NMR) spectra were recorded on Bruker (AMX-400 MHz) using $CDCl_3$ as solvent and Tetramethylsilane (TMS) as an internal standard (chemical shifts in δ ppm).

**Procedure for preparation of 1-(1,1′-biphenyl-4-yl)ethanone (2)**

The title compound was prepared by the acetylation of biphenyl using acetyl chloride in the presence of anhydrous aluminium chloride [10].

**Procedure for preparation of 1-(1, 1′-biphenyl-4-yl)-3-arylprop-2-en-1-ones (3a-g)**

A mixture of substituted benzaldehyde (0.01 M) and 1-(1,1′-biphenyl-4-yl)ethanone(0.01 M) in ethanol (95%, 50 ml), were heated over a water bath while a solution of sodium hydroxide (1.0 g in 5 ml of water) was added slowly during 15 min and heating was continued for another 15 min. The solution was cooled, the product thus obtained was filtered and recrystallized from ethanol.

**Procedure for preparation of 6-Aryl-4-(1,1′-biphenyl-4-yl)-N-Methyl-6H-1,3-thiazin-2-amines (4a-g)**

A solution containing 1-(1,1′-biphenyl-4-yl)-3-arylprop-2-en-1-one (0.01 M ), N-Methylthiourea (0.01 M ) and KOH (0.02 M ) in ethanol (50 ml) was refluxed for 3-4 h and the reaction was monitored by TLC. After completion of the reaction, one third of the solvent was removed under reduced pressure, cooled to room temperature, poured on ice cold water and the solid product was filtered. The pure N-methyl-1,3-thiazin-2-amines were obtained by column chromatographic technique using benzene-ethyl acetate as eluting solvent.

**4-(1,1′-biphenyl-4-yl)-N-methyl-6-phenyl-6H-1,3-thiazin-2-amine (4a):** M.F: $C_{23}H_{20}N_2S$, Yield: 72%, M.P: 127°C; IR (KBr, $cm^{-1}$): 3441 (NH), 2917 ($CH_3$-Str. in N-$CH_3$), 1672 (C=N), 1489 (C=C), 1449 (C-NH), 1229 (C-S); $^1$H-NMR (δ, ppm): 5.41 (dd, 1H, $C_5$-H, $J_{1,2}$=2.1 and $J_{1,3}$=4.8 Hz), 5.71 (d, 1H, $C_6$-H, J=4.8 Hz), 3.33 (s, 3H, N-$CH_3$), 7.32-7.70 [m, 14H (Ar-H)+1H (NH)]; $^{13}$C-NMR (δ, ppm): 176.21 (C-2), 145.83 (C-4), 98.16 (C-5), 43.26 (C-6), 29.72 (N-$CH_3$) and 124.56-135.48 (Ar-C).

**4-(1,1′-biphenyl-4-yl)-6-(4-bromophenyl)-N-methyl-6H-1,3-thiazin-2-amine(4b):** M.F: $C_{23}H_{19}N_2BrS$, Yield: 74%, M.P: 72°C; IR (KBr, $cm^{-1}$): 3199 (NH), 2922 ($CH_3$-Str. in N-$CH_3$), 1678 (C=N), 1533 (C=C), 1481 (C-NH), 1266 (C-S); $^1$H-NMR (δ, ppm): 5.32 (d, 1H, $C_5$-H, J=4.8 Hz), 5.62 (d, 1H, $C_6$-H, J=3.6 Hz), 3.24 (s, 3H, N-$CH_3$), 7.26-7.61 [m, 13H (Ar-H)+1H (NH)]; $^{13}$C-NMR (δ, ppm): 175.76 (C-2), 141.35 (C-4), 97.25 (C-5), 39.56 (C-6), 28.67 (N-$CH_3$) and 124.85-130.66 (Ar-C). Mass Spectrum: m/z 435(molecular ion peak).

**4-(1,1′-biphenyl-4-yl)-6-(2-chlorophenyl)-N-methyl-6H-1,3-thiazin-2-amine (4c)**: M.F: $C_{23}H_{19}N_2ClS$, Yield: 65%, M.P: 96°C; IR (KBr, $cm^{-1}$): 3386 (NH), 2962 ($CH_3$-Str. in N-$CH_3$), 1632 (C=N), 1571 (C=C), 1423 (C-NH), 1298 (C-S); $^1$H-NMR (δ, ppm): 5.19 (d, 1H, $C_5$-H, J=6.4), 5.35 (dd, 1H, $C_6$-H, $J_{1,2}$=2.8 Hz, $J_{1,3}$=6.4 Hz), 3.32 (s, 3H, N-$CH_3$), 7.26-7.85 [m, 13H (Ar-H)+1H (NH)]; $^{13}$C-NMR (δ, ppm): 176.07 (C-2), 141.00 (C-4), 101.11 (C-5), 40.44 (C-6), 29.08 (N-$CH_3$) and 123.89-130.31 (Ar-C).

**4-(1,1′-biphenyl-4-yl)-6-(4-chlorophenyl)-N-methyl-6H-1,3-thiazin-2-amine (4d):** M.F: $C_{23}H_{19}N_2SCl$, Yield: 68 %, M.P: 103°C; IR (KBr, $cm^{-1}$): 3446 (NH), 2970 ($CH_3$-Str. in N-$CH_3$), 1673 (C=N), 1570 (C=C), 1424 (C-NH), 1266 (C-S); $^1$H-NMR (δ, ppm): 5.15 (dd, 2H, $C_5$-H and $C_6$-H, $J_{1,2}$=2.0 Hz, $J_{1,3}$=5.0 Hz), 3.28 (s, 3H, N-$CH_3$), 7.21-7.49 [m, 13H (Ar-H)+1H (NH)]; $^{13}$C-NMR (δ, ppm): 174.95 (C-2), 142.44 (C-4), 101.65 (C-5), 40.11 (C-6), 30.00 (N-$CH_3$) and 125.56-130.48 (Ar-C).

**4-(1,1′-biphenyl-4-yl)-6-(4-fluorophenyl)-N-methyl-6H-1,3-thiazin-2-amine (4e):** M.F: $C_{23}H_{19}N_2SF$, Yield: 65%, M.P: 88°C; IR (KBr, $cm^{-1}$): 3204 (NH), 2924 ($CH_3$-Str. in N-$CH_3$), 1677 (C=N), 1562 (C=C), 1488 (C-NH), 1266 (C-S); $^1$H-NMR (δ, ppm): 5.14 (d, 1H, $C_5$-H, J=3.6 Hz), 5.21 (b, s, 1H, $C_6$-H), 3.30 (s, 3H, N-$CH_3$), 7.30-7.70 [m, 13H (Ar-H)+1H (NH)]; $^{13}$C-NMR (δ, ppm): 176.02 (C-2), 139.81 (C-4), 99.95 (C-5), 40.33 (C-6), 29.71 (N-$CH_3$) and 125.51-135.86 (Ar-C).

**4-(1,1′-biphenyl-4-yl)-N-methyl-6-(4-methylphenyl)-6H-1,3-thiazin-2-amine (4f)**: M.F: $C_{24}H_{22}N_2S$, Yield: 58 %, M.P: 131°C; IR (KBr, $cm^{-1}$): 3335 (NH), 2921 ($CH_3$-Str. in N-$CH_3$), 1668 (C=N), 1517 (C=C), 1467 (C-NH), 1262 (C-S); $^1$H-NMR (δ, ppm): 5.01 (d, 1H, $C_5$-H, J=6.0 Hz), 5.12 (distorted dd, 1H, $C_6$-H, $J_{1,2}$=3.6 Hz, $J_{1,3}$=5.6 Hz), 3.17 (s, 3H, N-$CH_3$), 7.34-7.72 [m, 13H (Ar-H)+1H (NH)], 2.64 (s, 3H, -$CH_3$); $^{13}$C-NMR (δ, ppm): 174.17 (C-2), 146.0 (C-4), 102.82 (C-5), 41.51 (C-6), 29.73 (N-$CH_3$), 21.48 ($CH_3$)127.06-129.48 (Ar-C).

**4-(1,1′-biphenyl-4-yl)-6-(4-methoxyphenyl)-N-methyl-6H-1,3-thiazin-2-amine (4g):** M.F: $C_{24}H_{22}N_2OS$, Yield: 60 %, M.P: 122°C; IR (KBr, $cm^{-1}$): 3443 (NH), 2946 ($CH_3$-Str. in N-$CH_3$), 1673 (C=N), 1509 (C=C), 1459 (C-NH), 1247 (C-S); $^1$H-NMR (δ, ppm): 5.00 (d, 1H, $C_5$-H, J=5.6 Hz), 5.23 (dd, 1H, $C_6$-H, $J_{1,2}$=2.8 Hz, $J_{1,3}$=6.4 Hz), 3.29 (s, 3H, N-$CH_3$), 7.38-7.70 [m, 13H (Ar-H)+1H (NH)], 3.90 (s, 3H, -$OCH_3$); $^{13}$C NMR (δ, ppm): 169.00 (C-2), 145.81 (C-4), 101.00 (C-5), 41.00 (C-6), 26.69 (N-$CH_3$), 55.55,55.36 ($OCH_3$) and 127.04-139.88 (Ar-C).

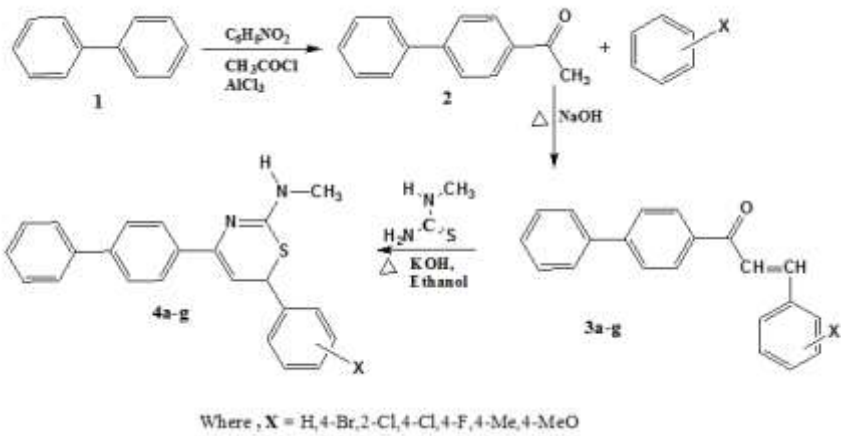
**Microbiology**

The disc diffusion method was used to determine the antimicrobial assay and the disc of 6 mm diameter was prepared, sterilized, dried and saturated with the compounds in Dimethyl Sulfoxide (DMSO). All the clinically isolated bacterial and fungal strains used for microbial assay were obtained from Microbial Type Culture Collection (MTCC) Centre, Chandigarh, India. The test compounds with10 µg/ml in each disc using DMSO was placed in petri dish and the zone of inhibition was measured to evaluate the antimicrobial assay.

## RESULTS AND DISCUSSION

The ongoing drug discovery mainly concentrates very potent medicinally important scaffold to be obtained from viable and ready synthetic strategy. Compounds bearing 1,3-thiazine and 2-amino-1,3-thiazine moieties are potent antimicrobials and their core structure is present in number of medicinally important pharmaceuticals and naturally occurring antibiotics e.g., Cephalosporin. Consequently, alkyl amino group particularly N-Methylamino moiety imparts the enhanced biological activity of pharmacophores which include increased proteolytic stability and membrane permeability of peptides [11], increased insecticidal activity of acyclic nitroethene [12], antimicrobial [13] etc.

Based on the synthetic utility of 2-amino-1,3-thiazines, we have synthesized some pharmaceutically important N-Metyl-1,3-thiazin-2-amines in simple following methodology. The 1-(1,1′-biphenyl-4-yl)ethanone (2) is obtained by acetylation of (1) in the presence of anhydrous aluminum chloride which on further treatment with substituted benzaldehydes in the presence of base sodium hydroxide afford substituted 1-(1,1′-biphenyl-4-yl)-3-aryl prop-2-en-1-ones (3a-g) on further treatment with N-methylthiourea in the presence of ethanolic KOH gives 6-Aryl-4-(1,1′-biphenyl-4-yl)-N-methyl-6H-1,3-thiazin-2-amines (4a-g). The formation of N-Methyl-1,3-thiazin-2-amine is given in Scheme 1.




**Scheme 1: Formation of N-Methyl-1,3-thiazin-2-amine**

The mechanism involves the formation of Michael adduct and its subsequent heterocyclization with a tautomeric change [14]. The structure proposed for the synthesized compounds were based on the spectral data obtained from IR, NMR and Mass and also evidenced from the previous work [15]. The obtained IR absorption bands of compounds 4a-4g shows their characteristics stretching frequencies ($cm^{-1}$) of NH (3200-3450), $CH_3$ in N-$CH_3$ (2920-2970), C=N (1630-1680), C=C (1480-1580), C-NH (1420-1480), and C-S (1220-1300). The presence of N-Methyl group is conformed from the IR stretching frequency band around 2920 $cm^{-1}$ which are not detected in simple 2-amino-1,3-thiazines.

The observed chemical shifts and multiplicity pattern of synthesized compounds 4a-4g revealed three types of protons present in the aliphatic region corresponding to H-5, H-6 and N-Methyl protons. Between H-5 and H-6, the H-5 proton resonates at a slightly shielded region as doublet except compounds 4a and 4d (gives doublet of doublets). The benzylic proton (H-6) gives three different multiplicity pattern such as for compounds 4c, 4d, 4f and 4g observed doublet of doublets and 4a and 4b show doublets. The compound 4e gives as broad singlet for H-6 proton. The appearance of doublet of doublets with $J_{1,2}$=2.0-3.0 and $J_{1,3}$= > 3.0 Hz revealed the long range coupling of H-6 proton probably with N-H proton owing to their reduced multiplicity in $D_2O$ experiment.

The $^{13}$C-NMR spectral data of synthesized compounds assist to prove the formation of methylaminothiazines. The observed $^{13}$C-NMR signals of respective thiazines (4a-4g) are consistent with the corresponding carbon resonance position such as the chemical shift values (δ, ppm)=28-30 (N-$CH_3$), 40-43 (C-6), 97-101 (C-5), 139-146 (C-4), 169-176 (C-NH) and 124-139 (ArC) respectively. The Mass spectrum of compound 4b shows the molecular ion peak at m/z: 435.

**Antimicrobial screening**

The compounds 4a-4g were screened for their *in vitro* antimicrobial activities against two Gram-positive and two Gram-negative bacteria's and fungi by using moxifloxacin (Bacteria) and amphotericin-B (Fungi) as standards. The results are reproduced in Table 1 and show equal to moderate bacterial activities as reference to standard. The fungal activities are less as compared to tested bacterial activities. The substituent's as 4-methyl, 4-methoxy and 4-bromo are more active against both type of bacterial strains. Synthesized thiazines 4a-4g exhibits moderate to less active than standard amphotericin B and among all compounds, the 4-methyl and 4-bromo (4b and 4f) are more active against tested fungal strains.

**Table 1: Assay of antimicrobial activity**

| S. No. | Microbes | Zone of inhibition (mm in diameter) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Bacteria** | **Control** | **Standard*** | **4a** | **4b** | **4c** | **4d** | **4e** | **4f** | **4g** |
| 1 | *Escherichia coli* | - | 25 | 16 | 21 | 15 | 18 | 18 | 18 | 24 |
| 2 | *Klebsiella pneumoniae* | - | 18 | 11 | 16 | 12 | 10 | 10 | 18 | 18 |
| 3 | *Bacillus subtilis* | - | 22 | 15 | 21 | 15 | 20 | 19 | 25 | 23 |
| 4 | *Staphylococcus aureus* | - | 24 | 16 | 20 | 17 | 22 | 18 | 23 | 21 |
| | **Fungi** | | | | | | | | | |
| 5 | *Aspergillus flavus* | - | 10 | 06 | 08 | 05 | 06 | 08 | 08 | 06 |
| 6 | *Aspergillus niger* | - | 12 | 05 | 10 | 06 | 06 | 07 | 10 | 08 |

*Moxifloxacin (Bacteria); *Amphotericin B (Fungi)

## CONCLUSION

We have described the synthesis, spectral studies and microbial evaluation of 6-aryl-4-(1,1′-biphenyl-4-yl)-N-methyl-6H-1,3-thazine-2-amines using N-Methylthiourea and 1-(1,1′-biphenyl-4-yl)-3-aryl prop-2-en-1-ones in the presence of KOH. The synthesized compounds are characterized by spectral techniques and screened for their antimicrobial activity using moxifloxacin and amphotericin-B as the standards.

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