SYNTHESIS OF CARBAMATE AND SULFONAMIDE ANALOGUES OF 2-(PHENYLTHIO) ANILINE AND THEIR ANTIMICROBIAL EVALUATION

K. Gowri¹, D. Srinivasulu¹*, M. Srinivasa Rao², VVPC. Narayana¹
¹Department of Chemistry, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.
²Biology Division, CSIR, IICT, Tarnaka, Hyderabad, Telangana, India.

ABSTRACT
A series of new 2-(phenylthio) phenyl substituted carbamate (3a-d) and N-(2-(phenylthio)) phenyl substituted sulfonamide derivatives (5a-d) were synthesized from 2-(phenylthio) aniline (1) using various pharmacologically active carbonochloridates (2a-d) and sulfonyl chlorides (4a-d) in the presence of a mild base. The structures of all the newly synthesized compounds were characterized by the IR, NMR (¹H & ¹³C) and mass analysis. Further, all the synthesized compounds were screened for the antimicrobial activity. Among all the tested compounds, 3c and 5b showed potent antibacterial and antifungal activities and these compounds might be used as antibiotic drugs.


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INTRODUCTION

Diphenyl sulfide is an important motif in drugs and drug intermediates. 2-[(2-Dimethylaminomethyl)phenylthio]-5-iodo-benzyl alcohol (IDAM) and 2-[(2-dimethylaminomethyl)phenylthio]-5-methoxyaniline (DAPP) have shown high in vitro and in vivo affinities and selectivities for serotonin transporter (SERT) relative to the dopamine, which plays a key-role in the regulation of serotoninergic function, numerous neurodegenerative, psychiatric disorders, and the norepinephrine transporters (DAT, NET) [1,2]. Sulfapyridine drugs are the first ever synthetic chemical compounds used for the cure and prevention of bacterial infections in human beings [3]. It has been estimated that 16-21% of annual antibiotic usage, making sulfonamide as the most important group of antibiotics consumed by human being.

Sulfonamides are among the most widely used antibacterial agents in the world, mainly because of their low cost, low toxicity and excellent activity against common bacterial diseases [4]. They constitute an important class of pharmaceutical compounds [5] with broad spectrum of biological activities including antimicrobial [6], analgesic [7], anti-inflammatory [8], anti-HIV [9], anticancer [10], anticonvulsant [11], antiviral [12], antitumor [13], antiplatelet aggregation [14] and antimalarial [15]. Also, in the supramolecular chemistry and supramolecular medicinal chemistry as it combined the features required for various biological activities and the metal co-ordination through phenylamino and sulfonamido groups [16]. Recently, sulfonamides are reported as highly efficient synthons for the preparation of various biologically active compounds [17]. Carbamate-bearing molecules play an important role in modern drug discovery and medicinal chemistry. Carbamates have been manipulated for use in the design of prodrugs as a means of achieving first-pass and systemic hydrolytic stability. Carbamates are playing a significant role in the human life and act as synthetic intermediates in medicinal, pharmaceutical industry and are ubiquitously found in a variety of medicinally bioactive compounds [19]. Generally, these compounds are prepared from phosgene [20, 21] or phosgene derivatives [22, 23]. Also, carbamates were found to exhibit various biological activities like antioxidant [24], antimicrobial [25], antidiabetic [26], anticancer [27], antitumour [28]. Recently, because of rapid development of combinatorial techniques in the field of drug discovery and owing to their medicinal and biological properties, carbamates have gained immense importance in the preparation of small molecule libraries [29]. Furthermore, organic carbamates serve a very important role as optimum protecting groups for amines and amino acids in organic synthesis and peptide chemistry.

The carbamate functionality imposes a degree of conformational restriction due to the delocalization of nonbonded electrons on nitrogen into the carboxyl moiety. In addition, the carbamate functionality participates in hydrogen bonding through the carboxyl group and the backbone NH. Therefore, substitution on the O- and N-termini of a carbamate offers opportunities for modulation of biological properties and improvement in stability and pharmacokinetic properties [30].

The impressive array of applications and ubiquitous medicinal and biological applications of sulfonamides and carbamates, led us to synthesize a new series, carbamate and sulfonamide analogues of 2-(phenylthio)aniline and evaluated their antimicrobial activity.

EXPERIMENTAL
MATERIALS AND METHODS

All chemicals were purchased from Merck, Aldrich and S. D. Fine-chem. (India) for use without further purification. Solvents were distilled over the appropriate drying agents and degassed before use. Melting points were determined in open capillaries on Guna melting point apparatus and are uncorrected. IR spectra were recorded on JASCO FT-IR 5300 using KBr discs. 1H NMR and 13C NMR spectra were recorded on Bruker AV-400 spectrometer with operating frequency 400 MHz for 1H NMR and 100.6 MHz for 13C NMR. Mass spectra were recorded on an API 3000 mass spectrometer (positive mode and negative mode). The progress of the reaction was monitored by TLC on Merck silica plates. Results are presented as chemical shift δ in ppm, multiplicity, J values in Hertz (Hz). Multiplicities are shown as the abbreviations: s (singlet), d (doublet), m (multiplet).

General procedure for the synthesis of 2-(phenylthio)phenyl substituted carbamates (3a-d):

4-Nitrophenyl chloroformate (2a, 0.992 mmol, 200 mg) dissolved in 5 ml of THF was added dropwise to a solution of 2-(phenylthio)aniline (1, 0.993 mmol, 200 mg) in the presence of triethyl amine with stirring. After completion of addition, the reaction mixture was stirred at 50 °C for 3-5 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated under rota evaporator. The crude reaction mixture was purified by column chromatography using ethyl acetate and hexane as eluent (3:7) to obtain a pure compound, 4-nitrophenyl 2-(phenylthio)phenyl carbamate (3a). The remaining carbamate derivatives (3b-d) were synthesized by adopting the same procedure.

4-Nitrophenyl 2-(phenylthio)phenyl carbamate (3a):

Yellow solid, Yield 88%, mp: 221-223 °C; IR (KBr) v max cm⁻¹: 3295 (-NH, str), 1665 (-C=O, str), 1587 (-NO₂, str); 1H NMR (400 MHz, DMSO-d₆): δ (ppm) 6.90-7.38 (m, 9H, Ar-H), 7.91-7.93 (d, J = 8 Hz, 2H, Ar-H), 8.10-8.12 (d, 2H, J = 9.2 Hz, Ar-H), 9.03 (s, 1H, -NH); 13C NMR (100.6 MHz, DMSO-d₆): δ (ppm) 115.8, 122.8, 123.1, 126.1, 126.5, 128.3, 128.5, 129.3, 132.1, 134.6, 135.5, 135.7, 140.1, 152.7 (C=O), 164.3; MS (m/z): 367.0 (M+H)+.

4-Nitrobenzyl 2-(phenylthio)phenyl carbamate (3b):

Yellow solid, Yield: 86%, mp: 142-144 °C; IR (KBr) v max cm⁻¹: 3243 (-NH, str), 1727 (-C=O, str), 1520 (-NO₂, str); 1H NMR (400 MHz, DMSO-d₆): δ (ppm) 5.20 (s, 2H, OCH₃), 6.92-7.38 (m, 9H, Ar-H), 7.62-7.64 (d, 2H, J = 8 Hz, Ar-H), 8.20-8.22 (d, 2H, J = 8.8 Hz, Ar-H), 9.01 (s, 1H, -NH); 13C NMR (100.6 MHz, DMSO-d₆): δ (ppm) 65.3 (C aliphatic), 119.3, 123.7, 124.0, 127.0, 127.2, 128.0, 129.2, 131.0, 132.0, 135.2, 136.7, 143.3, 144.3, 147.6, 152.6 (C=O); MS (m/z): 379.0 (M+H)+.

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2,2,2-Trichloroethyl 2-(phenylthio)phenylcarbamate (3e): White solid, Yield: 90%, mp: 151-153 °C; IR (KBr) νmax, cm⁻¹: 3361 (-NH, str), 1753 (-C=O, str); ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 4.88 (s, 2H, -OCH₂), 7.23-7.35 (m, 9H, Ar-H), 9.52 (s, 1H, -NH); ¹³C NMR (100.6 MHz, DMSO-d₆): δ (ppm) 73.6 (C₅₇H₇₂Cl₃NO); MS (m/z): 774.4 (M+H)⁺.

4-Chlorophenyl 2-(phenylthio)phenylcarbamate (3d): White solid, Yield: 85%, mp: 162-164 °C; IR (KBr) νmax, cm⁻¹: 3363 (-NH, str), 1753 (-C=O, str); ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 6.99-7.38 (m, 9H, Ar-H), 7.41-7.43 (d, 2H, J = 8 Hz, Ar-H), 7.46-7.48 (d, 2H, J = 8 Hz, Ar-H), 9.30 (s, 1H, -NH); ¹³C NMR (100.6 MHz, DMSO-d₆): δ (ppm) 119.9, 125.2, 126.2, 127.4, 128.2, 129.4, 130.4, 131.2 (C-Cl), 131.4, 132.1, 135.2, 135.6, 144.2, 149.3, 152.2 (C=O); MS (m/z): 356.1 (M-H)⁻.

General procedure for the synthesis of N-(2-(phenylthio)phenyl substituted sulfonamides (5a-d): 4-Nitrophenyl sulfonic chloride (4a, 0.902 mmol, 200 mg) dissolved in 5 ml of THF was added dropwise to a solution of 2-(phenylthio)aniline (1, 0.993 mmol, 200 mg) in the presence of triethylamine with stirring. After completion of addition, the reaction mixture was stirred at 50 °C for 3-5 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated under rotaevaporator. The crude reaction mixture was purified by column chromatography using ethyl acetate and hexane as eluent (3:7) to obtain a pure compound, 4-nitro-N-(2-(phenylthio)phenyl)benzenesulfonamide (5a). The remaining sulfonamide derivatives (5b-d) were synthesized by adopting the same procedure.

4-Nitro-N-(2-(phenylthio)phenyl)benzenesulfonamide (5a): Yellow solid, Yield: 88%, mp: 221-223 °C; IR (KBr) νmax, cm⁻¹: 3864 (-NH, str), 1383 (-SO₂, str); ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 6.43-7.49 (m, 9H, Ar-H), 8.12-8.14 (d, 2H, J = 8 Hz, Ar-H), 8.39-8.41 (d, 2H, J = 8.4 Hz, Ar-H), 10.01 (s, 1H, -NH); ¹³C NMR (100.6 MHz, DMSO-d₆): δ (ppm) 117.2, 120.7, 122.4, 124.0, 126.0, 127.2, 127.4, 128.2, 129.3, 131.2, 135.0, 137.9, 144.1 (C-SO₂), 150.1 (C-N₂O), MS (m/z): 385.0 (M-H)⁻.

4-Fluoro-N-(2-(phenylthio)phenyl)benzenesulfonamide (5b): Yellow solid, Yield: 89%, mp: 242-244 °C; IR (KBr) νmax, cm⁻¹: 3341 (-NH, str), 1387 (-SO₂, str); ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 6.48-7.41 (m, 9H, Ar-H), 7.42-7.44 (d, 2H, J = 8 Hz, Ar-H), 8.14-8.16 (d, 2H, J = 8 Hz, Ar-H), 10.12 (s, 1H, -NH); ¹³C NMR (100.6 MHz, DMSO-d₆): δ (ppm) 116.2, 120.6, 122.6, 126.6, 127.6, 128.0, 129.5, 130.2, 131.4, 135.9, 136.3 (C-SO₂), 137.1, 166.2 (C-F); MS (m/z): 378 (M+H₂O+H)⁺.

4-Bromo-N-(2-(phenylthio)phenyl)benzenesulfonamide (5c): Reddish brown solid, Yield: 88%, mp: 231-233 °C; IR (KBr) νmax, cm⁻¹: 3337 (-NH, str), 1388 (-SO₂, str); ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 6.41-7.41 (m, 9H, Ar-H), 7.88-8.00 (d, 2H, J = 8 Hz, Ar-H), 8.10-8.12 (d, 2H, J = 8.4 Hz, Ar-H), 10.23 (s, 1H, -NH); ¹³C NMR (100.6 MHz, DMSO-d₆): δ (ppm) 116.4, 117.2, 122.7, 126.6 (C-Br), 127.4, 127.8, 128.2, 129.6, 130.2, 131.4, 135.7, 136.0 (C-SO₂), 148.5; MS (m/z): 421.0 (M+H)⁺.

4-Chloro-3-nitro-N-(2-(phenylthio)phenyl)benzenesulfonamide (5d): White solid, Yield: 85%, mp: 219-221 °C; IR (KBr) νmax, cm⁻¹: 3337 (-NH, str), 1388 (-SO₂, str); ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 6.45-7.45 (m, 9H, Ar-H), 8.01-8.03 (d, 1H, J = 8.4 Hz, Ar-H), 8.19-8.21 (d, 1H, J = 8 Hz, Ar-H), 8.69 (s, 1H, Ar-H), 10.10 (s, 1H, -NH); ¹³C NMR (100.6 MHz, DMSO-d₆): δ (ppm) 116.2, 118.9, 121.8, 125.2, 126.4, 127.2, 128.9, 129.8, 131.0 (C-Cl), 131.2, 134.5, 135.1, 138.6 (C-SO₂), 136.2, 147.9 (C-NO₂), 150.2; MS (m/z): 421.0 (M⁺).

Biological Activity
Antimicrobial activity
Antibacterial activity

   The antibacterial activity of various new synthetic compounds 3(a-d) and 5(a-d) were screened against two gram positive bacteria, *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96) and two gram negative bacteria, *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 741) using agar well diffusion method [31-33]. Streptomycin was used as standard. 200 μg of the test compounds were dissolved in 1 ml of DMSO solvent. Centrifuged pellets of bacteria from 24 h old culture containing approximately 10⁶ colony forming unit (CFU) per mL was spread on the surface of Muller Hinton Agar (MHA) plates. Nutrient agar medium was prepared by suspending nutrient agar 20 g in one liter of distilled water (PH 7.0), autoclaved and cooled to 45 °C. Then it was seeded with 10 mL of prepared inocula to have 10⁶ CFU/mL. Petri dishes were prepared by pouring 75 mL of seeded nutrient agar. Wells were created in medium with the help of a sterile metallic borer and the test solution was added. Experimental plates were incubated for 24 h. After incubation, diameter zone of inhibition formed around the well was measured in millimeters in comparison to the standard. All test were carried out in triplicate and the results are presented in Table 2.

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Antifungal activity
All the newly synthesized compounds were screened for the antifungal activity against fungal strains, *C. albicans*, *S. cerevisiae*, *A. niger* and *A. flavus* using poison plate method [34] with respect to standard drug, Amphotericin-B. Five kinds of fungal strains were incubated in PDA at 25 ± 1 °C for 5 days to get new mycelium for antifungal assay, and then a mycelia disc of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. The inoculated plates were incubated at 25 ± 1 °C for 5 days. Dimethyl sulfoxide (DMSO) solvent was added as negative control to determine possible inhibitory activity of the solvent, Amphotericin-B was used as standard. For each treatment, three replicates were carried out and mean of diameter zone of inhibition was calculated. The results are shown in Table 3.

RESULTS AND DISCUSSIONS
Chemistry
2-(Phenylthio) phenyl substituted carbamate (3a-d) and N-(2-(phenylthio)phenyl substituted sulfonamide derivatives 5(a-d) were synthesized by using 2-(phenylthio)aniline (1) as represented in Scheme 1. Various carbonochloridates (2a-d) and sulfonyl chlorides (4a-d) dissolved in THF was added dropwise to a solution of 2-(phenylthio)aniline (1) in the presence of triethylamine at 20 °C with stirring. After completion of addition, the reaction mixture was raised to temperature 45–50 °C and stirred for 3–5 h. Progress of the reaction was checked by the TLC. After completion of reaction, the reaction mixture was filtered to remove triethylamine hydrochloride and concentrated by rotaevaporator to obtain crude compounds, (3a-d) and (5a-d). Further, the crude compounds were purified by short column chromatography using silica gel and 30% ethyl acetate and hexane as eluent to obtain pure final compounds, 2-(phenylthio)phenyl substituted carbamate (3a-d) and N-(2-(phenylthio)phenyl substituted sulfonamide derivatives (5a-d) in good yields as given Table 1.

SCHEME 1: Synthesis of 2-(phenylthio)phenyl substituted carbamate (3a-d) and N-(2-(phenylthio)phenyl sulfonamide (5a-d) derivatives.

Table 1. Physical data of the synthesized compounds (3a-d) and (5a-d).

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Time (h)</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>3.5</td>
<td>88</td>
<td>221-223</td>
</tr>
<tr>
<td>3b</td>
<td>3.5</td>
<td>86</td>
<td>142-144</td>
</tr>
<tr>
<td>3c</td>
<td>3.0</td>
<td>90</td>
<td>151-153</td>
</tr>
<tr>
<td>3d</td>
<td>4.0</td>
<td>85</td>
<td>162-164</td>
</tr>
<tr>
<td>5a</td>
<td>3.0</td>
<td>88</td>
<td>221-223</td>
</tr>
<tr>
<td>5b</td>
<td>4.5</td>
<td>89</td>
<td>242-244</td>
</tr>
<tr>
<td>5c</td>
<td>4.5</td>
<td>88</td>
<td>231-233</td>
</tr>
<tr>
<td>5d</td>
<td>5.0</td>
<td>85</td>
<td>219-221</td>
</tr>
</tbody>
</table>
Structures of all the newly synthesized compounds (3a-d) and (5a-d) were characterized by IR, $^1$H NMR, $^{13}$C NMR and mass spectral analysis. The IR spectra of all the compounds contained absorption bands at 3295-3361, 1383-1388, 1665-1753 cm$^{-1}$ corresponding to –NH, –SO$_2$ and –C=O respectively. $^1$H NMR spectra for –CONH and –SO$_2$NH protons resonated as singlets in the region $\delta$ 9.01-9.52 and 10.01-10.23 ppm respectively. The aromatic protons resonated as multiplets and doublets in the region $\delta$ 6.41-7.49 and 7.41-8.41 ppm. In $^{13}$C NMR spectra, some of the functional groups are assigned in support of the characterization of the title compounds. Chemical shift values in the region $\delta$ 152.2-152.7 and 136.0-144.1 ppm were assigned to C=O and C-SO$_2$ functional groups respectively. Further, the structures of the titled compounds (3a-d) and (5a-d) were confirmed by the molecular ion peaks in the mass spectra.

### Biological Activity

#### Antimicrobial activity

**Antibacterial activity**

The antibacterial activity of newly synthesized compounds (3a-d) and (5a-d) were screened against two gram positive bacteria, *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96) and two gram negative bacteria, *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 741) using agar well diffusion method [31-33]. Streptomycin was used as a standard. The diameter zone of inhibition of newly synthesized compounds (3a-d) and (5a-d) were measured in millimeters (mm) and the results are presented in Table 2.

**Table 2. Antibacterial activity of newly synthesized compounds (3a-d) and (5a-d).**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Diameter of inhibition zone, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
</tr>
<tr>
<td>3a</td>
<td>19</td>
</tr>
<tr>
<td>3b</td>
<td>17</td>
</tr>
<tr>
<td>3c</td>
<td>18.5</td>
</tr>
<tr>
<td>3d</td>
<td>17.5</td>
</tr>
<tr>
<td>5a</td>
<td>18</td>
</tr>
<tr>
<td>5b</td>
<td>19.5</td>
</tr>
<tr>
<td>5c</td>
<td>17</td>
</tr>
<tr>
<td>5d</td>
<td>19</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>20</td>
</tr>
</tbody>
</table>

The antibacterial results revealed that the activity of these compounds is influenced by substituents present on the phenyl ring. Among the compounds 3c and 5b showed potent antibacterial activity towards all the tested strains due to the presence of trichloro and 4-fluoro substituents respectively. Compound 3a showed potent activity against *B. subtilis* and *P. aeruginosa* due to presence of 4-NO$_2$ group and compound 3d showed potent activity against *P. aeruginosa* due to presence of 4-Cl in the moiety. Compound 5a showed potent activity against *B. subtilis* and *S. aureus*. Compound 5c showed potent activity against *S. aureus*. The remaining titled compounds showed moderate to good activities towards all the tested strains.

#### Antifungal activity

All the newly synthesized compounds were screened for antifungal activity against the fungi, *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger* and *Aspergillus flavus* using poison plate method [34] with respect to standard drug, Amphotericin-B. The diameter zone of inhibition for the newly synthesized compounds (3a-d) and (5a-d) were measured in millimeters and the results are presented in Table 3.

**Table 3. Antifungal activity of newly synthesized compounds (3a-d) and (5a-d).**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Diameter of inhibition zone, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td>3a</td>
<td>19</td>
</tr>
<tr>
<td>3b</td>
<td>15</td>
</tr>
<tr>
<td>3c</td>
<td>21.5</td>
</tr>
<tr>
<td>3d</td>
<td>17</td>
</tr>
<tr>
<td>5a</td>
<td>19</td>
</tr>
<tr>
<td>5b</td>
<td>22</td>
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<tr>
<td>5c</td>
<td>14</td>
</tr>
<tr>
<td>5d</td>
<td>16</td>
</tr>
<tr>
<td>Amphotericin-B</td>
<td>23.5</td>
</tr>
</tbody>
</table>
Results revealed that among the tested compounds, 3c and 5b showed potent antifungal activity against all the fungal strains due to presence of trichloro and 4-fluoro substituents. Compound 3a showed potent activity against C. albicans and 5a showed against C. albicans and S. cerevisiae. The remaining titled compounds showed moderate to good activities against all the fungal strains.

CONCLUSION
We have synthesized a series of new 2-(phenylthio)phenyl substituted carbamates (3a-d) and N-(2-(phenylthio)phenyl substituted sulfonamide derivatives (5a-d) in good yields and evaluated their antimicrobial activity. Compounds 3c and 5b showed potent antibacterial and antifungal activities among all the tested compounds. Finally, it is concluded that these compounds might be used as antibiotic drugs and to develop chemotherapeutic agents in future. The accomplished results could be advantageous for further improvements, preparation of carbamate and sulfonamide derivatives having substituent heterocyclic functionality to offer library of compounds to explore their potential biological activities.

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AUTHORS’ STATEMENT
The authors declare that the article content has no conflicts of interest.

REFERENCES


