Synthesis, characterization and biological activity of some sulfadriugs derivatives
تحضير وتشخيص بعض مشتقات أدوية السلفا ودراسة فعاليتها البايولوجية

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Abstract:
Sulfamethoxypyridiazine and sulfapyridine have been condensed with selected acyl chloride, namely benzoyl chloride, sebacoyl chloride and terephthaloyl chloride. The compounds were characterized by FTIR, $^1$H NMR and elemental analysis. The antibacterial activity of the studied compounds was determined against several clinical microbial isolates which are; Staphylococcus aureus and E.Coli by using different concentrations of each compound. The results shown the prepared compounds have varying degrees of inhibiting the test microorganisms.

Key words: Sulfa drugs, amide, sebacoyl chloride

Introduction:
The Sulfonamides are Synthetic antimicrobial agents with wide spectrum encompassing most gram-positive and many gram-negative organisms$^{1,2}$. The condensation product of sulfa drugs with aldehydes, ketones or their derivative are biologically very active$^3$. Beside having good complexing ability and the activity increase on complexation$^4$. Many chemotherapeutically important sulfa drugs like sulfapyridine, sulfadiazine etc. posses SO$_2$NH moiety which is an important toxophoric function$^5$ in addition the heterocyclic moiety which contain sulfur, oxygen or nitrogen atoms cause an enhanced the bioloical activites of sulfa drugs

Experimental Section
1- Materials and Measurements:-
All chemicals and solvents are obtained from Fluka and Aldrich chemical Co. and are used without further purification. Melting points were recorded on Gallenkamp melting points apparatus without correction. IR Spectra were measured on Shimadzu spectrophotometer as KBr pellets in the region 4000-400cm$^{-1}$, elemental analyses were performed on Euro vector EA 3000A(Italy). The $^1$HNMR spectra were recorded in DMSO-$d_6$ on Bruker 500MH$_z$ spectrometer using TMS an internal standard.

Synthesis of Sulfa drugs derivatives
Compound 1(SS$_3$):N-(4-(N-(6-methoxypyridazin-3-yl)sulfamoyl)phenyl)benzamide.
A 50 ml round bottomed flask was charged with 0.280g(0.001 mol) of sulphamethoxypyridazine, 0.141g(0.001 mol) of Benzoylechloride and 25ml CC$_4$. The mixture was refluxed for one hour. The yellow deposit which was formed was filtered off, washed with CC$_4$ and
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recrystalized from ethanol. Yield (64%) as yellow crystals m.p 193-195°C, elemental analysis, calculated: C, 56.24, H, 4.19, N, 14.57, S, 8.32, found: C, 56.64, H, 4.32, N, 14.71, S, 8.13

Compound 2 (SS2): N-(4-(N-pyridin-4-ylsulfamoyl)phenyl)benzamide A 50ml round bottomed flask was charged with 0.249g (0.001mol) of sulpha pyridine 0.141g (0.001 mol) of Benzoylchloride and 25ml CCl₄. The mixture was refluxed for 1.5 h. The resulting solid was collected, washed with CCl₄ and recrystalized from ethanol. Yield (65%) as yellow crystals m.p 210-211°C, elemental analysis, calculated: C, 61.18, H, 4.25, N, 11.89, S, 9.06, found: C, 61.54, H, 4.63, N, 11.61, S, 8.89

Compound 3 (SSP): N₁N₁₀-bis(4-(N-pyridin-2-ylsulfamoyl)phenyl)decanediamide:
The mixture of 0.498g (0.002 mol) of sulphapyridine and 0.238g (0.001mol) of Sebacoyl chloride was dissolved in 25ml of CCl₄. The mixture was refluxed for 1.5 h. The resulting solid was collected, washed with CCl₄ and then with acetone and recrystalized from ethanol. Yield (67%) as pale yellow crystals m.p 153 dec. elemental analysis, calculated: C, 57.81, H, 5.45, N, 12.64, S, 9.64, found: C, 58.03, H, 5.56, N, 12.21, S, 9.44

Compound 4 (SS₁): N₁N₄-bis(4-(N-pyridin-2-ylsulfamoyl)phenyl)terephthalamide
The mixture of 0.498g (0.002 mol) of sulphapyridine and 0.202g (0.001 mol) of Terephthalenchloride was dissolved in 25ml of CCl₄. The mixture was refluxed for 1h. The orange deposit which was formed was filtered off, washed with CCl₄ and then with acetone and recrystalized from ethanol. Yield (65%) as yellow crystals m.p > 300°C, elemental analysis, calculated: C, 57.31, H, 3.84, N, 13.36, S, 10.20, found: C, 57.76, H, 4.01, N, 13.71, S, 10.33

Determination of the biological activity of compounds:
A filter disk assay was used to determine the biological activity of the sulpha drugs against isolates of gram positive and gram negative bacteria included (Staphylococcus aureus and Escherichia coli) which were tested using plates of Muller- Hinton agar. The biological activity was defined as the clear zone of growth inhibition (11).

Result and discussion
The 1:1 mol ratio reaction between sulphamethoxypyridine, Sulphapyridine and Benzoylchloride led to formation of compound (II), (I) in good yield, the resulting compound can be represented as followings in scheme 1.

Scheme 1
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The 2:1 molar ratio reaction between sulphapyridine with sebacoyl chloride, Terephthalen chloride had led to formation of compounds (III, IV), the resulting compounds can be represent as following in scheme 2.

\[
\text{SS}_1: \text{IR spectrum of compound SS}_1 \text{ (Fig. 1) show bands at } 3417 \text{ and } 3244 \text{ cm}^{-1} \text{ assignable to N-H stretching vibration in NHSO}_2 \text{ and NHCO residue respectively}^{(6,7)}. \text{ The spectrum show avery strong band at } 1699 \text{ cm}^{-1} \text{ attributed to C=O and at } 1637 \text{ cm}^{-1} \text{ to stretching vibration of C=N}. \text{ The two strong bands at } 1259 \text{ and } 1128 \text{ cm}^{-1} \text{ attributed to asym and sym stretching of O=S=O respectively}^{(7)}.
\]

\[
\text{SS}_2: \text{ The IR spectrum of SS}_2 \text{ compound show two strong band at } 3415 \text{ and } 3244 \text{ cm}^{-1} \text{ attributed to NH of (NHSO}_2) \text{ and NH (NHCO) respectively, a strong band at } 1689 \text{ cm}^{-1} \text{ attributed to C=O, the band at } 1637 \text{ cm}^{-1} \text{ attributed to C=N, the strong band at } 1265 \text{ cm}^{-1} \text{ attributed to C-N, and the two strong band at } 1379 \text{ and } 1126 \text{ cm}^{-1} \text{ attributed to asym. and sym. stretching of SO}_2 \text{ group}.
\]

\[
\text{SS}_3: \text{ The IR spectrum of SS}_3 \text{ show avery broad and strong band at } 3479 \text{ cm}^{-1} \text{ attributed to N-H stre. The band at } 1662 \text{ cm}^{-1} \text{ attributed to C=O and the strong band } 1527 \text{ cm}^{-1} \text{ attributed to N=N}^{(8)}. \text{ The asym and Symstr.of SO}_2 \text{ group appear as astrong bands at } 1311 \text{ and } 1155 \text{ cm}^{-1} \text{ respectively}.
\]

\[
\text{SSP: The IR spectrum of SSP}_1 \text{ show a broad band centred at } 3415 \text{ cm}^{-1} \text{ attributed to a combinds to N-H group. The strong band at } 2927 \text{ cm}^{-1} \text{ attributed to str. vibration of C-H of CH}_2 \text{Chain. The very strong band at } 1699 \text{ cm}^{-1} \text{ attributed to C=O. The medium band at } 1625 \text{ cm}^{-1} \text{ attributed to C=N of the pyridine ring, The asym and sym str. Of SO}_2 \text{ appear at } 1357 \text{ and } 1143 \text{ cm}^{-1} \text{ respectively}.
\]

**IR Spectra**

\[
\begin{align*}
\text{SS}_1 & : \text{The IR spectrum of compound SS}_1 \text{ (Fig. 1) show bands at } 3417 \text{ and } 3244 \text{ cm}^{-1} \text{ assignable to N-H stretching vibration in NHSO}_2 \text{ and NHCO residue respectively}^{(6,7)}. \text{ The spectrum show avery strong band at } 1699 \text{ cm}^{-1} \text{ attributed to C=O and at } 1637 \text{ cm}^{-1} \text{ to stretching vibration of C=N}. \text{ The two strong bands at } 1259 \text{ and } 1128 \text{ cm}^{-1} \text{ attributed to asym and sym stretching of O=S=O respectively}^{(7)}. \\
\text{SS}_2 & : \text{ The IR spectrum of SS}_2 \text{ compound show two strong band at3415 and } 3244 \text{ cm}^{-1} \text{ attributed to NH of (NHSO}_2) \text{ and NH (NHCO) respectively, a strong band at } 1689 \text{ cm}^{-1} \text{ attributed to C=O, the band at } 1637 \text{ cm}^{-1} \text{ attributed to C=N, the strong band at } 1265 \text{ cm}^{-1} \text{ attributed to C-N, and the two strong band at } 1379 \text{ and } 1126 \text{ cm}^{-1} \text{ attributed to asym. and sym. stretching of SO}_2 \text{ group}.
\end{align*}
\]

**Scheme 2**

\[
\begin{align*}
\text{SS}_3 & : \text{The IR spectrum of SS}_3 \text{ show avery broad and strong band at } 3479 \text{ cm}^{-1} \text{ attributed to N-H stre. The band at } 1662 \text{ cm}^{-1} \text{ attributed to C=O and the strong band } 1527 \text{ cm}^{-1} \text{ attributed to N=N}^{(8)}. \text{ The asym and Symstr.of SO}_2 \text{ group appear as astrong bands at } 1311 \text{ and } 1155 \text{ cm}^{-1} \text{ respectively}.
\end{align*}
\]

**HNMR Spectra**

\[
\begin{align*}
\text{SS}_1 & : \text{The } ^1\text{HNMR spectrum of SS}_1 \text{ in DMSO-d}_6 \text{ (Fig. ) show a singlet broad at } 5.9 \text{ppm attributed to NH proton of NHSO}_2 \text{ moiety}^{(9)}. \text{ While the very broad signal at } 10.7 \text{ ppm attributed to NH proton of NHCOH}_2 \text{moiety}^{(10)}. \text{ The assignment of other aromatic protons are presented in Fig.}
\end{align*}
\]

\[
\begin{align*}
\text{SS}_2 & : \text{ The } ^1\text{HNMR spectrum of SS}_2 \text{ (Fig. ) show a singlet signal at } 6.5 \text{ppm attributed to proton of NHSO}_2 \text{ While the protons of NHCO appear at } 10.72 \text{ as a singlet signal. The aromatic proton appear in the region } 6.8 \text{ – 8 ppm}.
\end{align*}
\]

\[
\begin{align*}
\text{SS}_3 & : \text{ The } ^1\text{HNMR spectrum of SS}_3 \text{ show the signal of methoxy protons at } 3.83 \text{ ppm and the aromatic protons in the region } 6.8 \text{ – 8 ppm, the signal of proton of NHSO}_2 \text{ moiety appear at } 6.5 \text{ ppm while proton of NHCO appear at } 10.8 \text{ ppm}.
\end{align*}
\]
SSP: The $^1$HNMR spectrum of SSP$_1$ (Fig. ) show three distinguish signal at aliphatic region attributed to CH$_2$ chain the first signal attributed to 8H centered at δ1.23 ppm the second signal attributed to 4H of the two methylene groups (b) appear at δ1.47ppm and the third signal centered at δ2.17ppm attributed to 4H of the two methylene groups(-COCH$_2$). The aromatic protons appear in the region δ6.6-7.7 ppm. The broad signal at δ8ppm attributed to NH proton of NHSO$_2$moiety. While the NH proton of NHCO moiety appear at δ10.5 ppm.

Fig. 1: IR spectrum of SS2
Fig. 2: IR spectrum of ssp

Fig. 3: $^1$H NMR spectrum of SSP
The biological activity of the Sulfa drugs:

The results of antibacterial activity of the sulphadrugs were shown in Table(1) and figures (5 and 6). Sulfa drugs were the first synthetic drugs with widespread antibiotic activity to be put into clinical use\(^\text{(12)}\), once sulfanilamide was recognized as an active antimicrobial agent, scientists synthesized thousands of sulfonamides to test for bactericidal activity. It was later realized that sulfonamides do not actually kill bacteria; they interfere with bacterial growth and replication. Sulfa drugs are bacteriostatic. They inhibit an enzyme necessary for the biosynthesis of folic acid in bacteria. Folic acid is necessary for the biosynthesis of thymine and the purine bases, the building blocks of DNA\(^\text{(12-14)}\).

The prepared compounds in this study were shown very effective against gram negative strain\((Escherichia coli)\) but less active against gram positive strain\((Staphylococcus aureus)\). It has been postulated that cell membrane of \((Escherichia coli)\) contains many condensed fat layers compared with \((Staphylococcus aureus)\)\(^\text{(15)}\). The chemicals and antibiotics or antiseptics face difficulty in penetrating these membranes and, therefore, their effectiveness is diminished, this may be justified due to the ionic combination between each complex and the phospholipids of the bacterial cell wall, which led to destroy the cell membrane and then led to inhibit the microbial growth and may change the cell protein nature (Denaturation) and increase the permeability of the cell membranes\(^\text{(16)}\), as many types of antibacterial compounds\(^\text{(17)}\).
Fig 5: The antibacterial activity of sulpha drugs against *E. coli*

Fig 6: The antibacterial activity of sulpha drugs against *S. aureus*

Table 1: The antibacterial activity of sulfa drugs.

<table>
<thead>
<tr>
<th>Bacterial Isolated comp. No.</th>
<th>Inhibition zone (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>30</td>
</tr>
</tbody>
</table>

References:

(11) J. Collee, A. Fraser, B. Marmion, and A. Bimon, Practical Medical microbiology, 14th, p. 978, 1996.