Synthesis, characterization and antibacterial evaluation of new pyridyl-thiazole hybrids of sulfonamides

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ABSTRACT
Background and Aims: Sulfonamide drugs are a very old and noted group of small molecules, and are still one of the most important antimicrobial compounds. In this study, starting from sulfonamide drugs, new original compounds containing frequent and functional rings such as thiazole and pyridine were synthesized and their antimicrobial effects were evaluated.
Methods: Eighteen new compounds were synthesized by converting the 4-amino group of the sulfonamides to thiourea, and continued by thiazole ring closure. Characterization of the compounds was carried out by FT-IR, 1H-NMR and 13C-NMR and HRMS. MIC values were obtained in antimicrobial activity studies, which were carried out by Broth Microdilution method.
Results: Compounds 3p-r had an effect of 32 µg/ml against B. spizizenii. In addition, compounds 3d-f and 3p-r each showed effect against different gram-positive bacteria. Compound 3r had an MIC of 128 µg/mL against gram-negative organisms. The rest of the series did not affect gram-negative bacteria. In the study, chloramphenicol and sulfamethoxazole were used as standards.
Conclusion: Sulfanilamide and sulfadiazine derivatives showed higher inhibitory effects compared to the rest of the series. 3d-f and 3p-r showed inhibitor activity against gram-positive bacteria, conversely to the standard drug sulfamethoxazole, which possibly means that the mechanism of action is not same.
Keywords: Sulfonamide, thiazole, antibacterial

INTRODUCTION
Infectious diseases have been an incessant problem for human over the years. Gram positive and gram negative bacteria cause diverse infectious reactions. Among the microbial strains, E. coli produce lethal toxins, which can be contaminated from unwashed foods (Donnenberg & Whittam, 2001). P. aeruginosa and K. pneumoniae cause respiratory diseases (Gellatly & Hancock, 2013; Bengoechea & Pessoa 2018; Russo & Marr 2019). B. spizizenii is a subspecies of the Bacillus family and may produce typical Bacillus infections (Drobniewski, 1993). S. aureus, E. faecalis, S. epidemidis and vancomycin-resistant enterococcus (VRE) are other pathogenic microorganisms involved in this study. Many drug classes and targets are identified for treatment of these diseases. One of the most important classes among them is the sulfonamides, with many drugs identified; sulfamethoxazole, sulfapyridine, sulfadiazine etc. (Supuran, 2017). Advances in the development of new drugs are dependent on synthesis of original molecules and evaluation of their activity on different microbial strains. Pyridine and thiazole are frequent rings used in approved and investigational drugs because they provide ionizable groups and/or good pharmacokinetical properties. Besides, pyridyle-
thiazole analogs are an investigational pharmacophore against different targets. The feature for pyridyle-thiazole analogs is their potential for complex formation of cofactors as they are bearing close nitrogen atoms on related rings. (Kashyap et al., 2011; Hamada, 2018; Ertas et al., 2018) Similar sulfonamide derivatives have been reported in a variety of pharmacological activities such as sodium channel inhibitors, anticancer or anti-inflammatory properties in the literature (Sun et al. 2014; El-Sayed et al., 2010). In this study, we synthesized 18 novel pyridyle-thiazole sulfonamide hybrids to evaluate their antimicrobial properties.

MATERIALS AND METHODS

Chemistry

The reactants necessary for the synthesis process were purchased from Sigma Aldrich Chemical Corp. Melting point of title molecules were accomplished by a Stuart melting point apparatus and experiments performed in duplicate. Infra-red spectrums were recorded by Perkin Elmer Spectrom Two using attenuated total reflection (ATR) method. 1H-NMR and 13C-NMR spectrums were recorded in Bruker 300 MHz UltraShield NMR and Bruker 75 MHz UltraShield NMR, respectively. DMSO-d6 was used as solvent and TMS was used as standard. High-resolution mass spectrums were recorded in Shimadzu 8,040 LC/MS/MS ITTOF system by the electron spray method (ESI).

Synthesis of sulfonamide-thioureas (1a-f)

Related sulfonamide drug (100 mmol), equal mole (100 mmol) ammonium thiocyanate was dissolved in distilled water by HCl addition (Figure 1). The mixture was refluxed for 8 hours and then left to reach room temperature. The precipitation was collected and recrystallized from ethanol.

Synthesis of bromoacetylpyridines (2a-c)

Acetyl pyridine derivatives were dissolved in hydrobromic acid solution (48%) and bromine added drop wise. The reaction mixture was stirred for 1 hour and the precipitation collected. It was crystallized from ethanol.

Synthesis of N-(pyridyle)thiazolyl sulfonamides (3a-r)

Equal moles (10 mmol) of bromoacetyl pyridine (2a-c) and sulfonamide thiourea (1a-f) derivatives were dissolved in ethanol and refluxed for 4 hours. The precipitated product was filtered, poured into water at room temperature and neutralized by sodium acetate. If precipitation did not occur, water was added to provide precipitation. The products were recrystallized from ethanol.

N-(4,6-dimethylpyrimidin-2-yl)-4-((4-(pyridine-2-yl)thiazol-2-yl)amino)benzene-sulfonamide (3a)

Yield: 77%, m.p. 253°C, IR νmax (cm⁻¹): 3351.25 (N-H), 3055.31 (N-H), 3005.95, 2927.74 (Ar C-H). 1H-NMR (300 MHz, DMSO-d6, δ, ppm): 2.26 (6H, s, CH3), 6.75 (1H, s, Ar), 7.31-7.35 (1H, m, Ar), 7.65 (1H, s, Ar), 7.84-7.92 (3H, m, Ar), 7.97 (2H, d, J: 8.89 Hz, Ar), 8.06 (2H, d, J: 7.93 Hz, Ar), 8.57-8.61 (1H, m, Ar), 10.75 (1H, s, NH), 11.46 (1H, s, NH). 13C-NMR (75 MHz, DMSO-d6, δ, ppm): 23.45, 108.63, 116.15, 121.05, 123.27, 130.22, 132.45, 137.83, 144.92, 149.89, 150.87, 152.36, 156.83, 162.97 HR-MS (M+H) pred: 439.1005, found: 439.1002.
N-(4,6-dimethylpyrimidin-2-yl)-4-((4-(pyridine-3-yl)thiazol-2-yl)amino)benzenesulfonamide (3b)
Yield: 66%, m.p. 265°C, IR νmax (cm−1): 3052.88 (N-H), 2925.56 (Ar–CH). 1H-NMR (300 MHz, DMSO-d6, δ, ppm): 2.25 (6H, s, CH3), 6.75 (1H, s, Ar), 7.47-7.55 (1H, m, Ar), 7.65 (1H, s, Ar), 7.85 (2H, d, J = 8.62 Hz, Ar), 7.98 (2H, d, J = 8.93 Hz, Ar), 8.29 (1H, d, J = 8.07 Hz, Ar), 8.53 (1H, br, s, Ar), 9.17 (1H, br, s, Ar), 10.78 (1H, s, NH), 11.51 (1H, s, NH). 13C-NMR (75 MHz, DMSO-d6, δ, ppm): 23.44, 106.43, 114.12, 116.19, 124.27, 130.25, 132.51, 134.31, 144.84, 147.50, 147.81, 149.03, 156.31, 163.20, 167.82. HR-MS (M+H+): pred: 439.1005, found: 439.1002.

N-(4,6-dimethylpyrimidin-2-yl)-4-((4-(pyridine-4-yl)thiazol-2-yl)amino)benzenesulfonamide (3c)
Yield: 60%, m.p. 266°C, IR νmax (cm−1): 3264.27 (NH), 3185.09 (N-H), 3103.63 (Ar–CH). 1H-NMR (300 MHz, DMSO-d6, δ, ppm): 2.26 (6H, s, CH3), 6.76 (1H, s, Ar), 7.87 (2H, d, J = 8.97 Hz, Ar), 7.98 (2H, d, J = 8.94 Hz, Ar), 8.17-8.29 (3H, m, Ar), 8.79-?? (2H, m, Ar), 10.93 (1H, s, NH), 11.55 (1H, s, NH). 13C-NMR (75 MHz, DMSO-d6, δ, ppm): 23.44, 114.12, 116.44, 121.96, 120.31, 132.91, 144.52, 145.93, 156.79, 163.46. HR-MS (M+H+): pred: 439.1005, found: 439.1001.

N-(pyridine-2-yl)-4-((4-(pyridine-3-yl)thiazol-2-yl)amino)benzenesulfonamide (3f)
Yield: 64%, m.p. 247.4°C, IR νmax (cm−1): 3241.06 (N-H), 3100-2785 (Ar–C). 1H-NMR (300 MHz, DMSO-d6, δ, ppm): 6.80 (1H, s, Ar), 7.24 (1H, br, s, Ar), 7.46 (1H, br, s, Ar), 7.61 (1H, br, s, Ar), 7.82-7.88 (6H, m, Ar), 8.31 (1H, m, Ar), 8.53 (1H, br, s, Ar), 9.18 (1H, d, J = 2.01 Hz, Ar), 10.77 (1H, s, NH), 12.63 (1H, s, NH). 13C-NMR (75 MHz, DMSO-d6, δ, ppm): 106.28, 108.43, 115.96, 117.64, 123.92, 124.29, 124.84, 125.21, 127.11, 127.90, 128.85, 130.46, 133.58, 134.61, 144.40, 144.64, 143.79, 147.75, 148.88, 163.27, 169.06.

HR-MS (M+H+): pred: 410.0740, found: 410.0736.

N-(pyridine-2-yl)-4-((4-(pyridine-3-yl)thiazol-2-yl)amino)benzenesulfonamide (3h)
Yield: 65%, m.p. 290.5°C, IR νmax (cm−1): 3320.74 (N-H), 3185.88 (N-H), 3114.95-2923.28 (Ar–C). 1H-NMR (300 MHz, DMSO-d6, δ, ppm): 2.29 (3H, s, Ar), 6.14 (1H, s, Ar), 7.85-7.91 (7H, m, Ar), 8.63 (2H, d, J = 6.09 Hz, Ar), 10.90 (1H, s, Ar), 11.29 (1H, s, NH). 13C-NMR 69
(75 MHz, DMSO-d$_6$ δ, ppm): 12.54, 95.82, 109.60, 116.95, 120.52, 129.00, 131.29, 141.25, 145.30, 150.68, 158.12, 162.99. HR-MS (M+H): pred: 414.0689, found: 414.0695.

4-((4-(pyridine-2-yl)thiazol-2-yl)-amino)-N-(thiazol-2-yl)benzenesulfonamide (3m)

Yield: 72%, m.p. 266.8°C, IR νmax (cm$^{-1}$): 3279.64 (N-H), 3149.35-2811.30 (Ar C-H). ¹H-NMR (300 MHz, DMSO-d$_6$ δ, ppm): 6.81 (1H, d, J: 4.62 Hz, thiazole), 7.24 (1H, d, J: 4.62 Hz, thiazole), 7.3304-7.38 (1H, m, Ar), 7.66 (1H, s, thiazole), 7.68-7.80 (2H, m, Ar), 8.5-7.95 (3H, m, Ar), 8.09 (1H, d, J: 7.89 Hz, Ar), 8.59-8.61 (1H, m, Ar) 10.74 (1H, s, NH), 12.64 (1H, s, NH). ¹³C-NMR (75 MHz, DMSO-d$_6$ δ, ppm): 108.42, 108.73, 116.68, 121.20, 123.34, 124.82, 127.84, 134.56, 138.12, 144.42, 149.61, 150.57, 152.13, 163.05. HR-MS (M+H): pred: 416.0304, found: 416.0297.

Antimicrobial activity

Minimum inhibitory concentration assay

Antimicrobial activity test was applied to various gram positive and gram negative bacteria strains including, E. coli (ATCC8739), S. aureus (ATCC6538), B. spizizenii (ATCC6633), K. pneumoniae (Clinical isolate), P. aeruginosa (ATCC9027), E. faecalis (ATCC29212), S. epidermidis (ATCC12228), VRE (Clinical isolate). These organisms were inoculated to mid-log phase in Muller Hinton Broth (MHB) at 37°C. Broth microdilution procedure is a more user-friendly method that enables testing of multiple antimicrobial agents. The broth microdilution method was carried out in accordance with the relevant 2018 CLSI standard. Compounds were dissolved in DMSO below 1% concentration and were added to 96-well plates. These compounds were 2-fold serially diluted to make different concentrations, from 0.5 to 256 mM. Bacterial inoculum suspensions were prepared at a final concentration of 1x10$^5$ cfu/ml and plates were incubated at 37°C for 24 hours. Positive or negative controls were set to wells with and without bacteria, respectively. Sulfamethoxazole and chloramphenicol were used as standards. The MIC was determined by visual inspection after the change in turbidity. Experiments were performed in triplicate.

RESULTS AND DISCUSSION

Chemistry

Tested compounds were synthesized successfully by the proposed methods in 60- 85% yield. NH stretchings were observed above 3.000 cm$^{-1}$ and C-H stretchings were observed between 2.800-3.100 cm$^{-1}$ in the IR spectra. All protons matched with the presented methods in 60 - 85% yield. NH stretchings were observed at ~10.8 and ~11.55 ppm. Two protons matched with the expectations in -H-NMR. NH protons for 3a-o were observed as two separated singlet peaks around d ~10.8 and ~11.55 ppm. Supporting the literature information, SO$_2$NH$_2$ containing (3p-r) molecules NH stretchings were observed at d 7.23 ppm and the other NH group stretchings observed at d ~10.8 ppm like the rest in the series (Gowda, Jyothi, & D’Souza, 2002; Başar, Tunca, Bülbül, & Kaya, 2016). The hydrogen on the neighborhood of the pyridine nitrogen was observed approximately d 8-9 ppm for pyridine/pyrimidine containing molecules as singlet or mixed by aromatic hydrogens as multiplet. Most of the single aromatic hydrogens at thiazole and/or isoxazole rings were observed around d 8.2 and 6.2 ppm, respectively. Some of them were interfered by other aromatic peaks and observed as multiplet. Methyl groups on 1-3 and 10-12 were observed.
around δ ~2.25 ppm. In the 13C NMR, methyl-containing compounds gave a δ ~20 ppm peak. All other carbons were aromatics, thus were observed between δ 120-160 ppm. Finally, HRMS results proved the structures of the molecules.

**Antimicrobial activity**

Antimicrobial activity of the tested compounds are given in Table 1. Among the series, 6 of the 18 compounds exhibited lower MIC values than 256 μg/mL. The MIC value of 3d, sulfadiazine-thiazole 2-pyridyl derivative was 64 μg/mL against S. aureus. The other sulfadiazine derivatives (3e, 3f) were also found more active compared to the rest of the series. 3-pyridyle derivative (3e) exhibited 128 μg/mL against S. aureus and E. faecalis. Compound 3e also showed 32 μg/mL against S. epidermidis. 4-pyridyle derivative of sulfadiazine (3f) exhibited 64 μg/mL against S. epidermidis. Sulfanilamide derivatives (3p-r) exhibited 32 and 128 μg/mL against B. spizizenii and S. aureus, respectively. Besides, 3q exhibited 64 μg/mL against VRE, as the only effective compound against this microorganism. 3r exhibited 128 μg/mL against E. coli and P. aeruginosa as the only compound that showed inhibitory activity against gram-negative species along with 3q. The standard drug sulfamethoxazole showed 4, 32 and 64 μg/mL against E. coli, P. aeruginosa and S. aureus, respectively. The other standard drug chloramphenicol showed 8-16 μg/mL against all microorganisms as a wide spectrum antibiotic. None of the compounds showed inhibitory effect against K. pneumoniae, and there was only one compound (3r), which showed inhibition against gram-negative bacteria growth. The compounds were derived from two main parts, those which are sulfonamide derivatives and the position of pyridyl substitution. To point this out, 6 compounds seemed to be more effective compared to the rest of the series, and these 6 compounds were sulfanilamide and sulfadiazine derivatives. There was no significant data difference between 2, 3 or 4-pyridyl derivatives of each sulfonamide derivative. Considering these results, it was interpreted that activity was mainly related to the sulfonamide part. Besides, the pyridyl position did not make an important change in inhibition. High inhibitor potential (32 μg/mL) was provided by compound 3p-r, which supports the SAR feature that an unsubstituted amine group is important for activity in sulfanilamide derivatives.

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### Table 1. Screening for MIC of the compounds 3a-r using microdilution method.

| Cpd. | Gram-Negative Bacteria | Gram-Positive Bacteria |
|------|------------------------|------------------------|
|      | E. coli | P. aeruginosa | K. pneumoniae | B. spizizenii | S. aureus | E. faecalis | S. epidermidis | VRE |
| 3a   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3b   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3c   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3d   | >256   | >256   | >256   | >256   | >64   | >256   | >128   | >256   |
| 3e   | >256   | >256   | >256   | >256   | >128   | >128   | >32    | >256   |
| 3f   | >256   | >256   | >256   | >256   | >256   | >256   | >64    | >256   |
| 3g   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3h   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3i   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3j   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3k   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3l   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3m   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3n   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3o   | >256   | >256   | >256   | >256   | >256   | >256   | >256   | >256   |
| 3p   | >256   | >256   | >256   | >32    | >128   | >256   | >256   | >256   |
| 3q   | >128   | >128   | >256   | >32    | >128   | >256   | >256   | >64    |
| 3r   | >128   | >128   | >256   | >32    | >128   | >256   | >256   | >128   |
| C    | >8     | >16    | >8     | >16    | >8     | >8     | >8     | >8     |
| S    | >4     | >32    | >256   | >256   | >64    | >256   | >256   | >256   |

C* Chloramphenicol S* Sulfamethoxazole
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