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Antimicrobial and *in vitro* cytotoxicity of novel sulphanilamide condensed 1,2,3,4-tetrahydropyrimidines

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Abstract

The purpose of the study was to synthesize potent antimicrobial and cytotoxic agents by condensing sulphanilamide with 1,2,3,4-tetrahydropyrimidines. A new series of novel sulphanilamide condensed 1,2,3,4-tetrahydropyrimidines was prepared by reacting *N*-[(4-aminophenyl) sulphonyl]-3-oxobutanamide with urea/thiourea and aryl aldehyde in the presence of a catalytic amount of laboratory prepared chlorosulphonic acid as an efficient catalyst. The chemical structures of the synthesized compounds (7a-r) were confirmed by TLC, and the compounds were characterized by IR, ^{1}H NMR, mass spectra and elemental analysis. The synthesized compounds were evaluated for antimicrobial activity against the Gram-positive bacteria *Bacillus subtilis* and the Gram-negative bacteria *Escherichia coli* and for cytotoxicity against Vero cells. These compounds exhibited weak, moderate, or high antimicrobial activities and cytotoxicity. In particular, compound 7p exhibited the best antimicrobial activity and cytotoxicity of all the 1,2,3,4-tetrahydropyrimidine derivatives with antimicrobial activity MIC values of 11.4 μM and 12.1 μM against *B. subtilis* and *E. coli*, respectively, and a cytotoxicity CTC_{50} value of 19.0 μM against *Vero cells*. The sulphanilamide condensed 1,2,3,4-tetrahydropyrimidines generated here might prove interesting as potential antimicrobial cytotoxic agents.

Keywords: Sulphanilamide; Tetrahydropyrimidines; *B. subtilis*; *E. coli*; Antimicrobial; Cytotoxicity

1. Introduction

Throughout history, there has been a constant battle between humans and the multitude of microorganisms that cause infections and diseases. The treatment of bacterial infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Despite the many antibiotics and chemotherapeutics available, the emergence of old and new antibiotic-resistant bacterial strains in the last decades has generated a substantial need for new classes of

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anti-bacterial agents [1]. The increasing incidence of infection caused by the rapid development of bacterial resistance to most known antibiotics is a serious health problem. While many factors may be responsible for mutations in microbial genomes, the incorrect use of antibiotics has been thoroughly demonstrated to greatly increase the development of resistant genotypes. The need for effective therapies against multidrug-resistant bacterial strains has stimulated research into the design and synthesis of novel antimicrobial molecules [2].

In 1893, Pietro Biginelli reported the first synthesis of 3,4-dihydropyrimidin-2 (1*H*)-ones by one-pot cyclocondensation of an aromatic aldehyde, ethyl acetoacetate, and urea in ethanol solution using thermal heating [3]. In recent years, the interest in dihydropyrimidines (DHPMs) has increased rapidly because of the structural resemblance of DHPMs with clinically important Hantzsch pyridines [4,5]. The biologically active dihydropyridine molecules contain a 4-phenyl ring substituent positioned above and in the vertical plane of the 1,4-dihydropyridine ring, which has a flattened boat conformation [6]. Pyrimidine derivatives are a diverse and interesting group of drugs that have enormously important biological activities. Dihydropyrimidines and their derivatives have attracted increasing interest due to their therapeutic and pharmaceutical properties, such as antiviral, antitubercular [7,8], and antimicrobial [9–13] activities, antagonism of the human adenosine A_2A receptor [14], cyclooxygenase-2 inhibitory activity [15,16], tyrosine kinase inhibitory activity [17], antiamoebic activity [18], and cytotoxicity [19,20].

The chemical structure of sulphanilamide make its a valuable molecular template for the development of agents with a wide variety of biological activities [21]. Tetrahydropyrimidines are structurally similar to dihydropyrimidines. Thus, synthesizing new congeners by incorporating sulphanilamide with 1,2,3,4-tetrahydropyrimidinone moieties in a single molecular framework and evaluating their antimicrobial activities and cytotoxicities was considered to be worthwhile.

2. Experimental

2.1. Materials and methods

All chemicals were supplied by E. Merck (Germany) and S.D fine chemicals (India). Melting points were determined by the open tube capillary method and were uncorrected. The purity of the compounds was confirmed using thin layer chromatography (TLC) plates (silica gel G) in a solvent system, ethanol, chloroform, ethyl acetate (5:3:2), and spots were visualized under iodine vapours or UV light. IR spectrums were obtained using a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets). ^{1}H NMR spectra were recorded using a Bruker DRX-300 (300 MHz FT-NMR) spectrometer with DMSO-d_6 as the solvent and TMS as the internal standard. Mass spectra were obtained using Shimadzu LCMS 2010A software and the ESI ionization technique. Elemental analyses (C, H, and N) were performed using a Perkin Elmer model 240C analyser.

2.2. Preparation of N-[(4-aminophenyl) sulphonyl]-3-oxobutanamide (3)

Sulphanilamide 1 (0.01 M), ethyl acetoacetate 2 (0.01 M), and a catalytic amount of anhydrous potassium carbonate were mixed in the presence of 10 ml ethanol (95%) and refluxed for approximately 2.5 h. The colourless liquid that formed was then heated in a water bath to remove the alcohol that formed during the reaction. After allowing the reaction mixture to cool, crude crystals were obtained. Purification was performed by stirring crude crystals with cold diethyl ether for approximately 15 min using a mechanical stirrer. The solution was allowed to stand for 15 min and then filtered, resulting in the pure third compound of *N*-[(4-aminophenyl) sulphonyl]-3-oxobutanamide 3.

2.3. Preparation of 1,2,3,4-tetrahydropyrimidines by microwave irradiation method (7a-r)

The mixture of *N*-[(4-aminophenyl) sulphonyl]-3-oxobutanamide (0.005 M), urea/thiourea (0.0075 M), and aryl aldehyde (0.005 M) with a catalytic amount of chlorosulphonic acid in 10 ml of ethanol was subjected to microwave irradiation (300 W) for 14 min with 10 s intervals. The reactions were monitored with TLC using the ethanol, chloroform, ethyl acetate (4:3:3); solvent system. After the reaction was completed, the reaction mixture was cooled in a refrigerator and filtered. The precipitate obtained was washed thoroughly with water to remove unreacted urea/thiourea and dried. The crude solid product was crystallized with ethanol to generate the pure compounds (7a-r).

2.4. Analytical data

2.4.1. N-(3-oxobutanoyl) pyrazine-2-carboxamide (3)

Colourless solid, M.P: 185–187 °C; yield: 63%; IR (KBr, cm^{-1}): 3386 (N–H), 2942 (Ali–C–H), 1726 (C=O, ketone), 1684e (C=O, amide), 1585 (C=C), 1358

(C–N), 1052 (S=O); ^{1}H NMR (DMSO-d6) δ: 2.07 (s, 3H, CH_3), 3.39 (s, 2H, CH_2), 4.08 (s, 2H, NH_2), 6.79 (d, 2H, Ar–H), 7.74 (d, 2H, Ar–H), 8.09 (s, 1H, NH); calculated for $C_{10}H_{12}N_2O_4S$: C, 46.87; H, 4.72; N, 10.93; found C, 46.92; H, 4.78; N, 10.98.

2.4.2. N-[(4-aminophenyl) sulphonyl] -6-methyl-2-Oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7a)

Colourless solid, M.P: 298–300 °C; yield: 73%; IR (KBr, cm^{-1}): 3282 (N–H), 3185(Ar–C–H), 2936 (Ali–C–H), 1677 (C=O, amide), 1588 (C=C), 1257 (O–C), 1068 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.78 (s, 3H, CH_3), 4.04 (s, 2H, NH_2), 5.41 (s, 1H, CH), 6.12 (s, 1H, NH), 6.15 (s, 1H, NH), 6.77 (d, 2H, Ar–H), 7.06–7.15 (m, 5H, Ar–H), 7.71 (d, 2H, Ar–H), 8.11 (s, 1H, NH); MS (*m/z*): (M+1) calculated 387.11; found 387.16; calculated for $C_{18}H_{18}N_4O_4S$: C, 55.95; H, 4.70; N, 14.50; found C, 55.89; H, 4.75; N, 14.56.

2.4.3. N-[(4-aminophenyl) sulphonyl]-6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7b)

Colourless solid, M.P: 326–328 °C; yield: 76%; IR (KBr, cm^{-1}): 3266 (N–H), 3178(Ar–C–H), 2934 (Ali–C–H), 1679 (C=O, amide), 1591 (C=C), 1867 (C=S), 1158 (O–C), 1044 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.76 (s,3H, CH_3), 2.12 (s, 1H, NH), 2.18 (s, 1H, NH), 4.11 (s, 2H, NH_2), 4.67 (s, 1H, CH), 6.77 (d, 2H, Ar–H), 7.06–7.15 (m, 5H, Ar–H), 7.71 (d, 2H, Ar–H), 8.06 (s, 1H, NH); MS (*m/z*): (M+1) calculated 403.08; found 403.03; calculated for $C_{18}H_{18}N_4O_3S2$: C, 53.71; H, 4.51; N, 13.92; found C, 53.76; H, 4.57; N, 13.87.

2.4.4. N-[(4-aminophenyl) sulphonyl]-6-methyl-4-(2-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7c)

Colourless solid, M.P: 337–339 °C; yield: 78%; IR (KBr, cm^{-1}): 3347 (N–H), 3263 (Ar–C–H), 2982 (Ali–C–H), 1676 (C=O, amide), 1574 (C=C), 1384 (O–C), 1053 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.74 (s, 3H, CH_3), 4.06 (s, 2H, NH_2), 5.49 (s, 1H, CH), 6.07 (s, 1H, NH), 6.14 (s, 1H, NH), 6.81 (d, 2H, Ar–H), 7.36 (d, 2H, Ar–H), 7.58 (s, 1H, Ar–H), 7.74 (d, 2H, Ar–H), 8.08 (s, 1H, Ar–H), 8.15 (s, 1H, NH); MS (*m/z*): (M+1) calculated 432.09; found 432.14; calculated for $C_{18}H_{17}N_5O_6S$: C, 50.11; H, 3.97; N, 16.23; found C, 50.16; H, 3.91; N, 16.28.

2.4.5. N-[(4-aminophenyl) sulphonyl]-6-methyl-4-(2-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7d)

Colourless solid, M.P: 351–353 °C; yield: 76%; IR (KBr, cm^{-1}): 3273 (N–H), 3147 (Ar–C–H), 2984 (Ali–C–H), 1678 (C=O, amide), 1558 (C=C), 1872 (C=S), 1264 (O–C), 1048 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.79 (s, 3H, CH_3), 2.07 (s, 1H, NH), 2.15 (s, 1H, NH), 4.03 (s, 2H, NH_2), 4.42 (s, 1H, CH), 6.79 (d, 2H, Ar–H), 7.37 (d, 2H, Ar–H), 7.55 (s, 1H, Ar–H), 7.73 (d, 2H, Ar–H), 8.04 (s, 1H, NH), 7.16 (s, 1H, Ar–H); MS (*m/z*): (M+1) calculated 448.07; found 448.13; calculated for $C_{18}H_{17}N_5O_5S_2$: C, 48.31; H, 3.83; N, 15.65; found C, 48.36; H, 3.77; N, 15.71.

2.4.6. N-[(4-aminophenyl) sulphonyl]-4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7e)

Colourless solid, M.P: 336–338 °C; yield: 73%; IR (KBr, cm^{-1}): 3297 (N–H), 3152 (Ar–C–H), 2967 (Ali–C–H), 1676 (C=O amide), 1583 (C=C), 1258 (C–O), 1063 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.76 (s, 3H, CH_3), 4.04 (s, 2H, NH_2), 5.51 (s, 1H, CH), 6.03 (s, 1H, NH), 6.11 (s, 1H, NH), 6.77 (d, 2H, Ar–H), 7.01–7.17 (m, 4H, Ar–H), 7.76 (d, 2H, Ar–H), 8.16 (s, 1H, NH); MS (*m/z*): MS (*m/z*): (M+1) calculated 421.07; found 421.02; calculated for $C_{18}H_{17}ClN_4O_4S$: C, 51.37; H, 4.07; N, 13.31; found C, 51.31; H, 4.13; N, 13.36.

2.4.7. N-[(4-aminophenyl) sulphonyl]-4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7f)

Colourless solid, M.P: 361–363 °C; yield: 77%; IR (KBr, cm^{-1}): 3286 (N–H), 3172 (Ar–C–H), 2978 (Ali–C–H), 1678 (C=O, amide), 1582 (C=C), 1868 (C=S), 1175 (O–C), 1069 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.74 (s,3H, CH_3), 2.03 (s, 1H, NH), 2.09 (s, 1H, NH), 4.07 (s, 2H, NH_2), 4.53 (s, 1H, CH), 6.73 (d, 2H, Ar–H), 7.01–7.18 (m, 4H, Ar–H), 7.73 (d, 2H, Ar–H), 8.07 (s, 1H, NH); MS (*m/z*): (M+1) calculated 437.05; found 437.01; calculated for $C_{18}H_{17}ClN_4O_3S_2$: C, 49.48; H, 3.92; N, 12.82; found C, 49.43; H, 3.96; N, 12.87.

2.4.8. N-[(4-aminophenyl) sulphonyl]-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7g)

Colourless solid, M.P: 321–323 °C; yield: 72%; IR (KBr, cm^{-1}): 3286 (N–H), 3159 (Ar–C–H), 2958

(Ali–C–H), 1676 (C=O, amide), 1573 (C=C), 1226 (O–C), 1044 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.72 (s, 3H, CH_3), 4.06 (s, 2H, NH_2), 5.48 (s, 1H, CH), 6.05 (s, 1H, NH), 6.10 (s, 1H, NH), 6.78 (d, 2H, Ar–H), 7.43 (s, 1H, Ar–H), 7.49 (s, 1H, Ar–H), 7.71 (d, 2H, Ar–H), 8.04 (d, 2H, Ar–H), 8.11 (s, 1H, NH); MS (*m*/*z*): (M+1) calculated 432.09; found 432.15; calculated for $C_{18}H_{17}N_5O_6S$: C, 50.11; H, 3.97; N, 16.23; found C, 50.16; H, 3.92; N, 16.29.

2.4.9. N-[(4-aminophenyl) sulphonyl]-6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7h)

Colourless solid, M.P: 364–366 °C; yield: 80%; IR (KBr, cm^{-1}): 3294 (N–H), 3176 (Ar–C–H), 2958 (Ali–C–H), 1677 (C=O, amide), 1584 (C=C), 1848 (C=S), 1186 (O–C), 1052 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.71 (s, 3H, CH_3), 2.04 (s, 1H, NH), 2.13 (s, 1H, NH), 4.05 (s, 2H, NH_2), 4.46 (s, 1H, CH), 6.71 (d, 2H, Ar–H), 7.41 (s, 1H, Ar–H), 7.48 (s, 1H, Ar–H),7.77 (d, 2H, Ar–H), 8.07 (d, 2H, Ar–H), 8.11 (s, 1H, NH); MS (*m*/*z*): (M+1) calculated 448.07; found 448.12; calculated for $C_{18}H_{17}N_5O_5S_2$: C, 48.31; H, 3.83; N, 15.65; found C, 48.36; H, 3.87; N, 15.71.

2.4.10. N-[(4-aminophenyl) sulphonyl]-4-(3-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7i)

Colourless solid, M.P: 323–325 °C; yield: 75%; IR (KBr, cm^{-1}): 3293 (N–H), 3174 (Ar–C–H), 2965 (Ali–C–H), 1676 (C=O, amide), 1548 (C=C), 1176 (O–C), 1061 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.76 (s,3H, CH_3), 4.08 (s, 2H, NH_2), 5.38 (s, 1H, CH), 6.02 (s,1H, NH), 6.11 (s, 1H, NH), 6.76 (d, 2H, Ar–H), 6.98–7.13 (m, 4H, Ar–H), 7.73 (d, 2H, Ar–H), 8.14 (s, 1H, NH); MS (*m*/*z*): (M+1) calculated 421.07; found 421.03; calculated for $C_{18}H_{17}ClN_4O_4S$: C, 51.37; H, 4.07; N, 13.31; found C, 51.43; H, 4.12; N, 13.36.

2.4.11. N-[(4-aminophenyl) sulphonyl]-4-(3-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7j)

Colourless solid, M.P: 374–376 °C; yield: 77%; IR (KBr, cm^{-1}): 3281 (N–H), 3178 (Ar–C–H), 2968 (Ali–C–H), 1683 (C=O, amide), 1593 (C=C), 1873 (C=S), 1188 (O–C), 1045 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.77 (s, 3H, CH_3), 2.02 (s, 1H, NH), 2.08 (s, 1H, NH), 4.06 (s, 2H, NH_2), 4.42 (s, 1H, CH), 6.79 (d, 2H, Ar–H), 6.96–7.10 (m, 4H, Ar–H), 7.72 (d, 2H, Ar–H), 8.03 (s, 1H, NH); MS (*m*/*z*): (M+1) calculated 437.05; found 437.11; calculated for $C_{18}H_{17}ClN_4O_3S_2$: C, 49.48; H, 3.92; N, 12.82; found C, 49.53; H, 3.97; N, 12.77.

2.4.12. N-[(4-aminophenyl) sulphonyl]-4-(3-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7k)

Colourless solid, M.P: 331–333 °C; yield: 75%; IR (KBr, cm^{-1}): 3288 (N–H), 3174(Ar–C–H), 2972 (Ali–C–H), 1677 (C=O, amide), 1552 (C=C), 1148 (O–C), 1052 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.73 (s, 3H, CH_3), 4.05 (s, 2H, NH_2), 5.43 (s, 1H, CH), 6.05 (s, 1H, NH), 6.13 (s, 1H, NH), 6.71 (d, 2H, Ar–H), 6.79 (d, 2H, Ar–H), 6.86 (s, 1H, Ar–H), 7.17 (s, 1H, Ar–H), 7.75 (d, 2H, Ar–H), 8.07 (s, 1H, NH); MS (*m*/*z*): (M+1) calculated 405.10; found 405.16; calculated for $C_{18}H_{17}FN_4O_4S$: C, 53.46; H, 4.24; N, 13.85; found C, 53.51; H, 4.29; N, 13.91.

2.4.13. N-[(4-aminophenyl) sulphonyl]-4-(3-fluorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7l)

Colourless solid, M.P: 381–383 °C; yield: 79%; IR (KBr, cm^{-1}): 3282 (N–H), 3194 (Ar–C–H), 2969 (Ali–C–H), 1679 (C=O, amide), 1566 (C=C), 1883 (C=S), 1182 (O–C), 1047 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.74 (s, 3H, CH_3), 2.05 (s, 1H, NH), 2.11 (s, 1H, NH), 4.02 (s, 2H, NH_2), 4.53 (s, 1H, CH), 6.76 (d, 2H, Ar–H), 6.80 (d, 2H, Ar–H), 6.89 (s, 1H, Ar–H), 7.17 (s, 1H, Ar–H), 7.77 (d, 2H, Ar–H), 8.06 (s, 1H, NH); MS (*m*/*z*): (M+1) calculated 421.07; found 421.13; calculated for $C_{18}H_{17}FN_4O_3S_2$: C, 51.42; H, 4.08; N, 13.32; found C, 51.47; H, 4.13; N, 13.39.

2.4.14. N-[(4-aminophenyl) sulphonyl]-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7m)

Colourless solid, M.P: 339–341 °C; yield: 77%; IR (KBr, cm^{-1}): 3312 (N–H), 3157 (Ar–C–H), 2972 (Ali–C–H), 1675 (C=O, amide), 1558 (C=C), 1184 (O–C), 1055 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.75 (s, 3H, CH_3), 4.01 (s, 2H, NH_2), 5.49 (s, 1H, CH), 6.08 (s, 1H, NH), 6.17 (s, 1H, NH), 6.76 (d, 2H, Ar–H), 7.05 (d, 2H, Ar–H), 7.18 (d, 2H, Ar–H), 7.73 (d, 2H, Ar–H), 8.17 (s, 1H, NH); MS (*m*/*z*): (M+1) calculated 421.07; found 421.02; calculated for $C_{18}H_{17}ClN_4O_4S$:

C, 51.37; H, 4.07; N, 13.31; found C, 51.31; H, 4.12; N, 13.31.

2.4.15. N-[(4-aminophenyl) sulphonyl]-4-(4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7n)

Colourless solid, M.P:371–373 °C; yield 80%; IR (KBr, cm^{-1}): 3298 (N–H), 3178(Ar–C–H), 2972 (Ali–C–H), 1681 (C=O, amide), 1558 (C=C), 1846 (C=S), 1182 (O–C), 1065 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.76 (s, 3H, CH_3), 2.03 (s, 1H, NH), 2.14 (s, 1H, NH), 4.06 (s, 2H, NH_2), 4.51 (s, 1H, CH), 6.77 (d, 2H, Ar–H), 7.05 (d, 2H, Ar–H), 7.18 (d, 2H, Ar–H), 7.73 (d, 2H, Ar–H), 8.04 (s, 1H, NH); MS (*m/z*): (M+1) calculated 437.05; found 437.11; calculated for $C_{18}H_{17}ClN_4O_3S_2$: C, 49.48; H, 3.92; N, 12.82; found C, 49.53; H, 3.96; N, 12.88.

2.4.16. N-[(4-aminophenyl) sulphonyl]-4-(4-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7o)

Colourless solid, M.P: 324–326 °C; yield 73%; IR (KBr, cm^{-1}): 3294 (N–H), 3188 (Ar–C–H), 2964 (Ali–C–H), 1679 (C=O, amide), 1573 (C=C), 1184 (O–C), 1069 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.75 (s, 3H, CH_3), 4.01 (s, 2H, NH_2), 5.49 (s, 1H, CH), 6.08 (s, 1H, NH), 6.17 (s, 1H, NH), 6.76 (d, 2H, Ar–H), 7.05 (d, 2H, Ar–H), 7.18 (d, 2H, Ar–H), 7.73 (d, 2H, Ar–H), 8.17 (s, 1H, NH); MS (*m/z*): (M+1) calculated 405.10; found 405.14; calculated for $C_{18}H_{17}FN_4O_4S$: C, 53.46; H, 4.24; N, 13.85; found C53.51; H, 4.18; N, 13.89.

2.4.17. N-[(4-aminophenyl) sulphonyl]-4-(4-fluorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7p)

Colourless solid, M.P: 353–355 °C; yield 79%; IR (KBr, cm^{-1}): 3296 (N–H), 3186 (Ar–C–H), 2982 (Ali–C–H), 1682 (C=O, amide), 1585 (C=C), 1861 (C=S), 1186 (O–C), 1053 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.72 (s, 3H, CH_3), 2.06 (s, 1H, NH), 2.11 (s, 1H, NH), 4.09 (s, 2H, NH_2), 4.47 (s, 1H, CH), 6.76 (d, 2H, Ar–H), 6.90 (d, 2H, Ar–H), 7.08 (d, 2H, Ar–H), 7.71 (d, 2H, Ar–H), 8.11 (s, 1H, NH); MS (*m/z*): (M+1) calculated 421.07; found 421.13; calculated for $C_{18}H_{17}FN_4O_3S_2$: C, 51.42; H, 4.08; N, 13.32; found C, 51.47; H, 4.02; N, 13.36.

2.4.18. N-[(4-aminophenyl) sulphonyl]-6-methyl-2-oxo-4-(pyridin-4-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7q)

Colourless solid, M.P: 363–365 °C; yield 78%; IR (KBr, cm^{-1}): 3287 (N–H), 3184 (Ar–C–H), 2984 (Ali–C–H), 1679 (C=O, amide), 1574 (C=C), 1179 (O–C), 1058 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.72 (s, 3H, CH_3), 4.07 (s, 2H, NH_2), 5.45 (s, 1H, CH), 6.02 (s, 1H, NH), 6.09 (s, 1H, NH), 6.79 (d, 2H, Ar–H), 7.46 (d, 2H, Ar–H), 7.78 (d, 2H, Ar–H), 8.10 (s, 1H, NH), 8.64 (d, 2H, Ar–H); MS (*m/z*): (M+1) calculated 388.10; found 388.14; calculated for $C_{17}H_{17}N_5O_4S$: C, 52.70; H, 4.42; N, 18.08; found C, 52.76; H, 4.46; N, 18.14.

2.4.19. N-[(4-aminophenyl) sulphonyl] -6-methyl-4-(pyridin-4-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (7r)

Colourless solid, M.P: 317–319 °C; yield 73%; IR (KBr, cm^{-1}): 3258 (N–H), 3192(Ar–C–H), 2936 (Ali–C–H), 1677 (C=O, amide), 1583 (C=C), 1891 (C=S), 1138 (O–C), 1064 (S=O); ^{1}H NMR (DMSO-d6) δ: 1.74 (s, 3H, CH_3), 2.03 (s, 1H, NH), 2.15 (s, 1H, NH), 4.04 (s, 2H, NH_2), 4.52 (s, 1H, CH), 6.79 (d, 2H, Ar–H), 7.45 (d, 2H, Ar–H), 7.77 (d, 2H, Ar–H), 8.06 (s, 1H, NH), 8.65 (d, 2H, Ar–H); MS (*m/z*): (M+1) calculated 355.09; found 355.14; calculated for $C_{16}H_{14}N_6O_2S$: C, 54.23; H, 3.98; N, 23.71; found C, 54.29; H, 3.95; N, 23.77.

2.5. Antimicrobial activity

The *in vitro* antibacterial activities of the novel compounds against the Gram-positive bacteria *Bacillus subtilis* and the Gram-negative bacteria *Escherichia coli* were tested by a standard serial dilution method using a stock solutions of 100 μg/ml [22,23]. Double strength nutrient broth was used as the culture medium and dimethyl sulphoxide (DMSO) was used as the solvent control. The stock solutions of the test compounds were serially diluted in test tubes containing 1 ml of sterile medium to obtain different concentrations and then inoculated with a 100 μL of suspension of the indicated microorganism in sterile saline. Norfloxacin was used as the standard drug. The inoculated test tubes were incubated at 37 ± 1 °C for 24 h.

2.6. In vitro cytotoxicity

Short-term *in vitro* cytotoxicity assays were performed using Vero cells (African Monkey kidney cells)

following the standard procedure [24]. Sulphorhodamine B (SRB) is a bright-pink amino-xanthine dye with two sulphonic groups. Under mild acidic conditions, SRB binds to basic amino acid residues of proteins in trichloroacetic acid fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. Vero cells were incubated with SRB, and then, the SRB solution was removed. Then, the cells and incubated at 37 °C for 3 days in a 5% CO_2 atmosphere with 100 μl of the different compounds at different concentrations (2–500 μg). Microscopic examinations were performed, and observations were recorded every 24 h. After, 72 h, 50% trichloroacetic acid (25 μl) was added to each well, and the plates were incubated for 1 h at 4 °C. The supernatant was then removed, and the cells were washed with water, air-dried, and stained with SRB for 30 min. The unbound dye was removed by washing with 1% acetic acid, and the plates were air dried. Tris base (10 mM, 100 μl) was added to the wells to solubilize the dye. The plates were vigorously shaken for 5 min, and absorbance was measured using a microtitre plate reader at 540 nm. The mean absorbance of triplicate wells was recorded. The mean absorbance value of cells grown in the absence of the test compounds was taken as 100% cell survival (control). The percentage growth inhibition was calculated using the following formula:

$$\text{Growth inhibition}\% = 100 - \left[\frac{\text{sample absorbance}}{\text{control absorbance}}\right] \times 100$$

3. Results and discussion

A series of 18 novel sulphanilamide cyclocondensed 1,2,3,4-tetrahydropyrimidines of biological interest were synthesized and evaluated for their antimicrobial activities and cytotoxicities, and all compounds were characterized by IR, ^{1}H NMR, MS and elemental analysis of their structures.

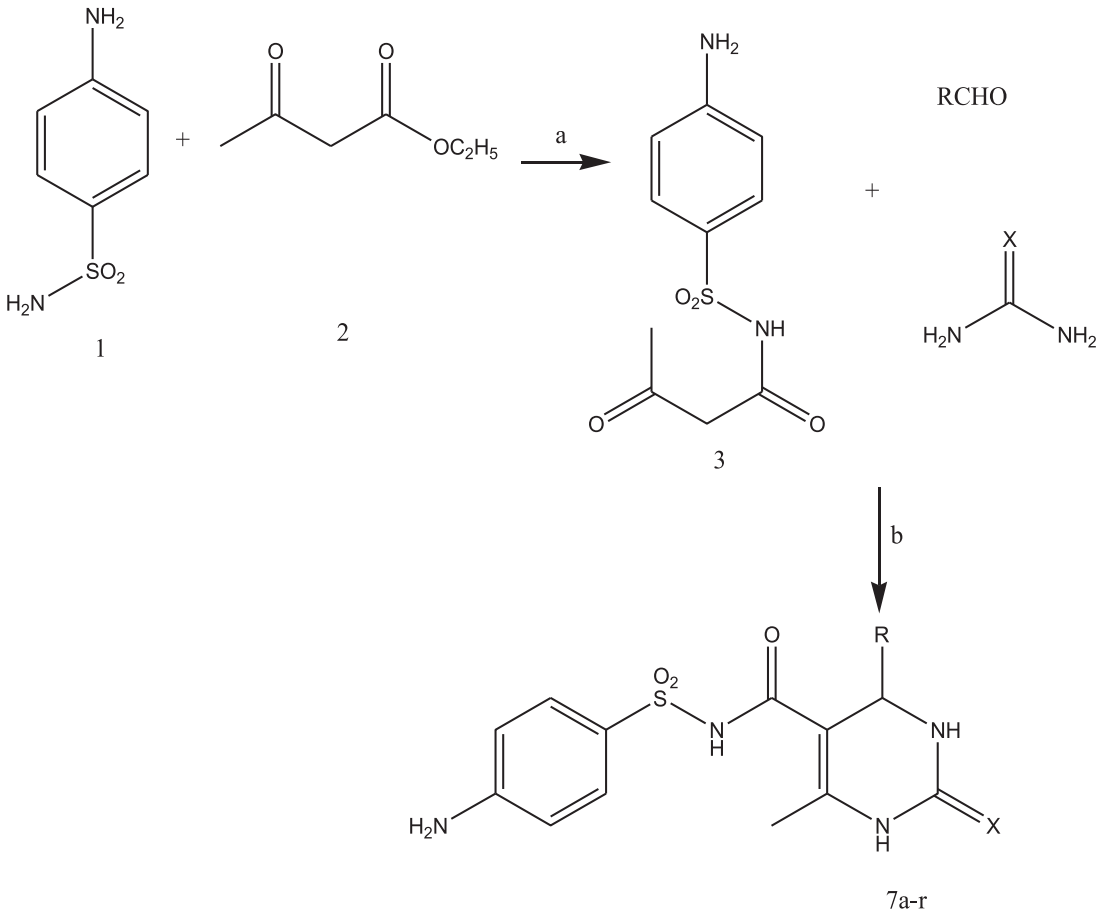


Fig. 1. Synthesis of compounds (7a-r). Reagents and conditions: (a) reflux 2.5 h, catalytic amount of anhydrous K_2CO_3 and 10 ml of ethanol (95%); (b) C_2H_5OH, chloro sulphonic acid, and microwave irradiation (300 W) for 14 min.

3.1. Chemistry

Synthesis of 1,2,3,4-tetrahydropyrimidines using the Biginelli synthetic protocol [24], which involves a one pot multicomponent reaction, was performed following the steps as outlined in Fig. 1. In the first step, ethyl acetoacetate 2 and sulphanilamide 1 in the presence of a catalytic amount of anhydrous potassium carbonate and 10 ml of ethanol (95%) were reacted under net conditions resulting in the formation of *N*-[(4-aminophenyl) sulphonyl]-3-oxobutanamide 3, with a 63% yield. The β-keto ester reacted more readily with sulphonamide groups than with aromatic primary amines. The *N*-[(4-aminophenyl) sulphonyl]-3-oxobutanamide was used for the Biginelli condensation reaction; it was reacted with urea/thiourea and aryl aldehyde in the presence of a catalytic amount of chlorosulphonic acid. The advantages of using the catalyst were better yields and allowing the reaction to occur without dry solvents.

The one-pot synthetic method was developed for 1,2,3,4-tetrahydropyrimidines using microwave irradiation. Microwave technology has been proven to be better than conventional heating in the synthesis of these compounds, as time and side products are decreased and yield is improved with microwave irradiation. We succeeded in widening the scope of the first step of the reaction by using unconventional active methylene compounds. The first step in the Biginelli reaction is the acid-catalysed condensation of urea with the aldehyde [25]. This reaction begins with protonation of the aldehyde by the acid and is followed by an attack of the amine by urea. Then, proton transfer steps result in the generation of a protonated alcohol, which is converted to water to form an N-acyliminium ion intermediate [26]. The subsequent enol form of the β-Keto ester attacks the N-acyliminium ion to generate an open chain ureide, which readily cyclizes into a tetrahydropyrimidine (Fig. 2). The reaction time was 14 min. The IR spectra of compounds 7a-r are showed strong absorption bands for the amine group (3258–3347 cm^{-1}), amide group (1675–1683 cm^{-1}), aliphatic C–H stretching (2934–2984 cm^{-1}), aromatic C–H stretching (3152–3263 cm^{-1}), aromatic C=C stretching (1548–1593 cm^{-1}), C–O (1138–1258 cm^{-1}), S=O (1044–10690 cm^{-1}), and C=S (1846–1883 cm^{-1}). The ^{1}H NMR spectra of compounds 7a-r showed a methyl group protons singlet at (2.02–2.08 ppm), CH–R protons singlet at (5.39–5.52 ppm), aromatic protons multiplet at (6.96–7.72 ppm) and amine protons singlet at (8.92–10.26 ppm). The mass spectra and elemental analysis results were within ±0.6% of the theoretical values. Eighteen substituted 1,2,3,4-tetrahydropyrimidines, named compounds 7a-r, were synthesized with yields ranging from 72% to 80%. These conditions allow this method to be applicable for the synthesis of 1,2,3,4-tetrahydropyrimidine-based heterocyclic compounds. The present protocol is the best described for the synthesis of 1,2,3,4-tetrahydropyrimidines. All reported 1,2,3,4-tetrahydropyrimidines compounds were found to be novel and were not reported elsewhere.

3.2. Antimicrobial activity

The synthesized compounds were analyzed for their *in vitro* antimicrobial activities against the Gram-positive bacteria *B. subtilis* and the Gram-negative bacteria *E. coli*. Sulphanilamides are competitive inhibitors of para-aminobenzoic acid (PABA), which is essential for the biosynthesis of foliate coenzymes in the synthesis of purine and pyrimidine bases in bacteria [21]. The antimicrobial activities of the synthesized compounds are shown in Fig. 3 and Table 1, and most of the designed compounds exhibited good to moderate or high antimicrobial activities against the Gram-positive bacteria *B. subtilis* and the Gram-negative bacteria *E. coli.* All 1,2,3,4-tetrahydropyrimidines were potent antimicrobial agents, with MIC values ranging from micromolar to submicromolar levels. In particular, compound 7p showed the best antimicrobial activity of all of the 1,2,3,4-tetrahydropyrimidine derivatives, with MIC values of 11.4 μM and 12.1 μM for *B. subtilis* and *E. coli*, respectively.

3.3. In vitro cytotoxicity

The synthesized compounds were subjected to an *in vitro* cytotoxicity assay against Vero cells. The assay was performed using the sulphorhodamine B (SRB) method. These 1,2,3,4-tetrahydropyrimidines act primarily by impairing cell reproductive integrity. Thus, affected cells may remain alive and may continue to perform many of their functions but are unable to reproduce successfully [20]. The synthesis of DNA, RNA and other cell constituents may continue at normal rates. However, the cell either fails to divide, forming a giant cell, commences a division that cannot be completed, or divides unsuccessfully, resulting in chromosomes being unequally shared between the daughter cells or being damaged in some way; these chromosomal lesions may eventually cause cell death [22]. The investigated compounds had a wide range of cytotoxic activities categorized as weak, moderate, or high. Compound 7p

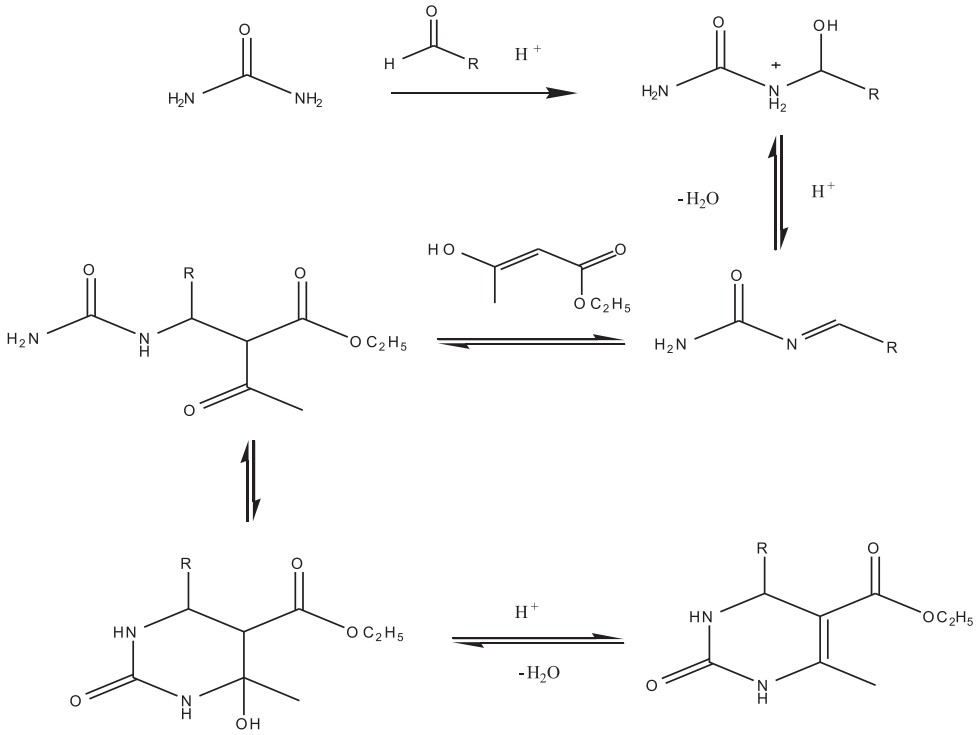


Fig. 2. General mechanism of Biginelli 1,2,3,4-tetrahydropyrimidines.

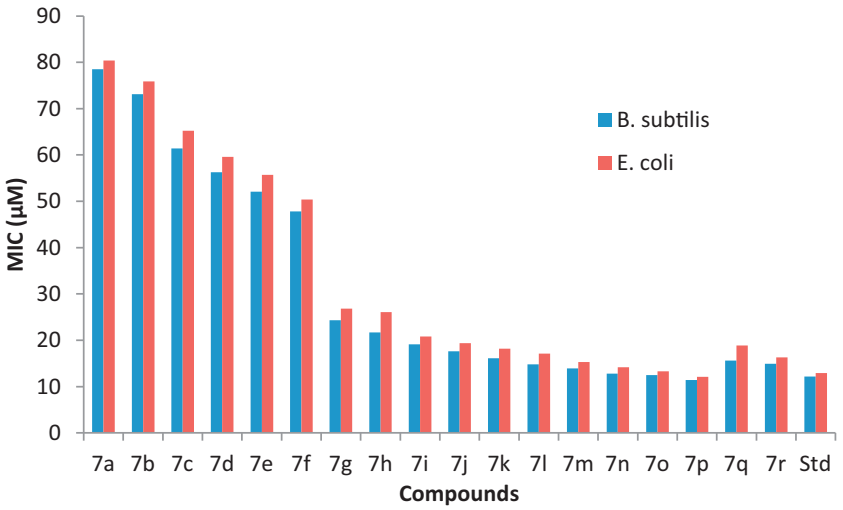


Fig. 3. *In Vitro* antimicrobial activity of compounds (7a-r) and Norfloxacin (Std.).

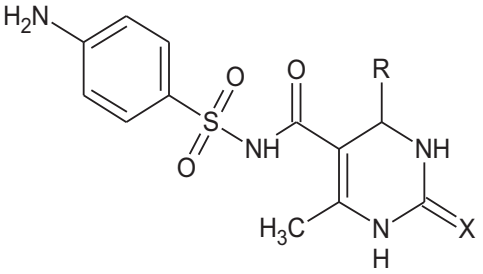
exhibited significant cytotoxicity against Vero cells, with a CTC_{50} value of 19.0 μM (Fig. 4 and Table 1).

3.4. Structural activity relationship

By analysing the activities of the synthesized compounds, the following structure activity relationships (SAR) were obtained. The fifth position of 1,2,3,4-tetrahydropyrimidines contains a sulphanilamide group that contributes to their antimicrobial activities and cytotoxicities [15]. Sulphanilamide is a competitive inhibitor of PABA. Incorporation of sulphanilamide in 1,2,3,4-tetrahydropyrimine enhances its antimicrobial and cytotoxic potencies because it enhances the affinity of the compound for the PABA receptor. The 4th position of 1,2,3,

Table 1
Synthesized 1,2,3,4-tetrahydropyrimidines: *In Vitro* antimicrobial and cytotoxicity.

General structure of 1,2,3,4-tetrahydropyrimidines



S. no	Compound	R	X	*B. subtilis* MIC (μM)	*E. coli* MIC (μM)	CTC_{50} (μM)on *Vero Cells*
1	7a	Phenyl	O	78.5	80.4	484.4
2	7b	Phenyl	S	73.1	75.9	388.0
3	7c	2-Nitrophenyl	O	61.4	65.2	299.3
4	7d	2-Nitrophenyl	S	56.3	59.6	275.1
5	7e	2-Chlorophenyl	O	52.1	55.7	304.7
6	7f	2-Chlorophenyl	S	47.8	50.4	222.4
7	7g	3-Nitorophenyl	O	24.3	26.8	180.9
8	7h	3-Nitorophenyl	S	21.7	26.1	143.1
9	7i	3-Chlorophenyl	O	19.1	20.8	123.8
10	7j	3-Chlorophenyl	S	17.6	19.4	110.0
11	7k	3-Flurophenyl	O	16.1	18.2	96.5
12	7l	3-Flurophenyl	S	14.8	17.1	73.8
13	7m	4-Chlorophenyl	O	13.9	15.3	42.8
14	7n	4-Chlorophenyl	S	12.8	14.2	36.6
15	7o	4-Flurophenyl	O	12.5	13.3	34.6
16	7p	4-Flurophenyl	S	11.4	12.1	19.0
17	7q	4-Pyridyl	O	15.6	18.9	74.9
18	7r	4-Pyridyl	S	14.9	16.3	57.0
19	Norfloxacin	Standard	–	12.2	12.9	–
20	Tamoxifen	Standard	–	–	–	35.0

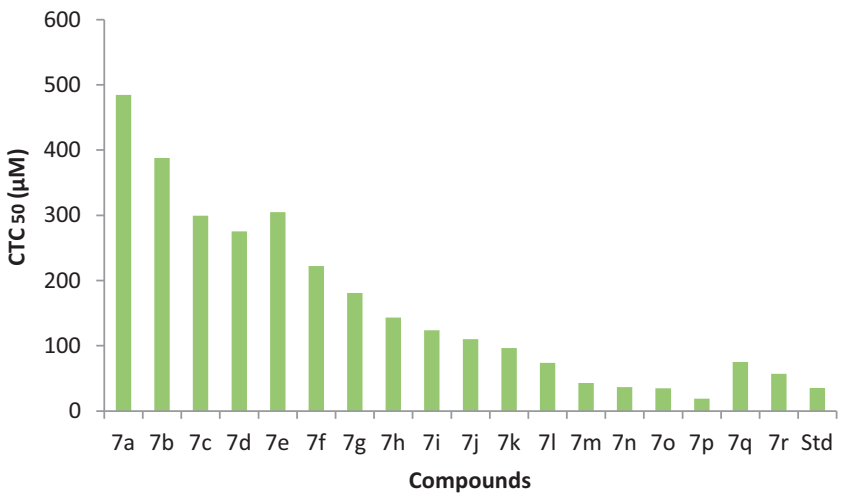


Fig. 4. *In Vitro* cytotoxicity of compounds (7a-r) and Tamoxifen (Std.).

4-tetrahydropyrimine contains an aryl ring, specifically a phenyl ring, and one hydrogen in that ring must be substituted with a strong electron withdrawing group to improve the compound's antimicrobial and cytotoxic potencies; this substitution decreases the electron density in the aryl ring due to the inductive effect. Fluoride and chloride substitution at the 4th position of the phenyl ring generated compounds with potent antimicrobial and cytotoxic actions because of the strong electron withdrawing nature of fluoride and chloride. Substitution of fluro and chloro groups at the third position of the phenyl ring generated compounds with more potent antimicrobial and cytotoxic actions than substitution with a nitro group at that position. Compounds with a heteroaryl group substitution at the 4th position of the phenyl ring showed moderate antimicrobial and cytotoxic actions. The following order was found for the potency of different substituents at different positions within the phenyl ring at the 4th position of 1,2,3,4-tetrahydropyrimidines: 4-fluoro > 4-chloro > 3-fluoro > 4-pyridyl > 3-chloro > 3-nitro > 2-chloro > 2-nitro > H. The second position sulphur-substituted derivatives of 1,2,3,4-tetrahydropyrimidines were showed most potent antimicrobial and cytotoxic effect when compared with oxygen atom substituted molecules. Among the compounds reported here, compound 7p is arguably the most potent when compared with the current therapeutic agents norfloxacin and tamoxifen because the substitution of fluoride in the phenyl ring present at the 4th position of 1,2,3,4-tetrahydropyrimidine enhances the antimicrobial and cytotoxic actions of compound 7p (Figs. 3 and 4 and Table 1).

4. Conclusions

A series of novel 1,2,3,4-tetrahydropyrimidines of biological interest were synthesized, and their structures were analyzed. A compound library was prepared using laboratory prepared chlorosulphonic acid as an efficient catalyst as compared to Lewis acid. The incorporation of sulphanilamide into 1,2,3,4-tetrahydropyrimidine enhanced its antimicrobial and cytotoxic potencies. The antimicrobial and cytotoxicity assays showed that all synthesized compounds were active against the Gram-positive bacteria *B. subtilis*, the Gram-negative bacteria *E. coli* and Vero Cells. The investigated compounds showed a range of antimicrobial and cytotoxic activities that could be categorized as weak, moderate, or high. Compared to the antimicrobial and cytotoxic actions of norfloxacin and tamoxifen, compound 7p was the most potent compound of those developed in this study. Thus, we generated interesting compounds when compared to the current therapeutic agents, and these compounds should be considered candidates for further investigation for therapeutic use.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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