BENI-SUEF UNIVERSITY JOURNAL OF BASIC AND APPLIED SCIENCES 3 (2014) 24–31

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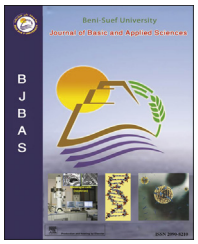

Microwave assisted synthesis of some novel acetazolamide cyclocondensed 1,2,3,4-tetrahydropyrimidines as a potent antimicrobial and cytotoxic agents

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ARTICLE INFO

Article history:
Available online 12 March 2014

Keywords:
Acetazolamide
Tetrahydropyrimidines
Cyclocondensation
Antimicrobial
Cytotoxicity

ABSTRACT

A new series of some novel acetazolamide cyclocondensed 1,2,3,4-tetrahydropyrimidines was prepared by reacting of *N*-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-3-oxobutanamide with urea/thiourea and appropriate aldehyde in the presence of catalytic amount of laboratory made p-toluenesulfonic acid as an efficient catalyst. Confirmation of the chemical structure of the synthesized compounds (**12a**–**n**) was substantiated by TLC, different spectral data IR, ^{1}H NMR, Mass spectra and elemental analysis were done. The synthesized compounds were evaluated for in-vitro antimicrobial and cytotoxicity against *Bacillus subtilis*, *Escherichia coli* and Vero cells. The titled compounds exhibited weak, moderate, or high in-vitro antimicrobial and cytotoxicity. Compounds **12c**, **12d**, **12g** and **12h**, exhibited potential antimicrobial and in-vitro cytotoxicity.

1. Introduction

The advent of microwave assisted technology in organic chemistry dates back to the mid 1980s and since the 1990s there has been a significant increase in the number of publications on Microwave Assisted Organic Reactions (MAOS) due to increased benefits associated with the process (Langa et al., 1997; Strauss and Trainor, 1995; Wathey et al., 2002). The promotion of microwave assisted reactions in organic

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Peer review under the responsibility of Beni-Suef University

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.bjbas.2014.02.003

chemistry has improved the speed, reduced cost, reduced energy spent making it a sustainable process and is widely heralded as "green chemistry" measures whose applications are promoted today to minimize the use of non renewable resources as well as polluting solvent, to reduce generation of secondary products which are often toxic and to reduce the emission of harmful gases (Prasad et al., 2012; Tucker, 2010; Wang et al., 2011). Microwave assisted reactions in organic chemistry achieve the facilitation of faster reactions under bulk conditions as well as promoting reduction of reaction time (Erdmenger et al., 2010).

Pyrimidine derivatives comprise a diverse and interesting group of drugs is extremely important for their biological activities. Dihydropyrimidine and their derivatives have attracted increasing interest owing to their therapeutic and pharmaceutical properties, such as antiviral, antitubercular (Desai et al., 2001; Singh et al., 2011; Prashantha Kumar et al., 2008), antimicrobial agent (Baldev et al., 2012; Bhuiyan et al., 2006; Shetty et al., 2009; Sharma et al., 2004; Vasudeva Rao et al., 2012; El-Sayed et al., 2012) antagonists of the human adenosine A2A receptor (Gillespie et al., 2008), cyclooxygenase-2 inhibitory activity (Orjales et al., 2008; Falcao et al., 2006), tyrosine kinase inhibitors, antiamoebic activity (Gangjee et al., 2010; Parveen et al., 2010) and cytotoxicity (Xie et al., 2009; Saritha Jyostna and Achaiah, 2010; Vasudeva Rao et al., 2012). The discovery during the 1930s that a dihydropyridine (dihydronicotinamide derivative, NADH), "hydrogen-transferring coenzyme" consequently became important in biological system, has generated numerous studies on the biochemical properties of dihydropyridines and their bioisosteres dihydropyrimidines. The search for more suitable preparation of dihydropyrimidinones continues today.

The chemical structure of acetazolamide provides a most valuable molecular template for the development of agents able to interact with a wide variety of biological activities (Karthikeyan et al., 2013; Kushwaha et al., 2012). Tetrahydropyrimidines are structurally similar to dihydropyrimidines. Hence, it was thought worthwhile to synthesize new congeners by incorporating acetazolamide with 1,2,3,4-tetrahydropyrimi dinones moieties in a single molecular frame work and to evaluate their antimicrobial and cytotoxicity.

2. Experimental

2.1. Materials and methods

All chemicals were supplied by E.Merck (Germany) and SD fine chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system ethanol, chloroform, ethylacetate (6:3:1); the spots were located under iodine vapors or UV light. IR spectrums were obtained on a Perkin–Elmer 1720 FT-IR spectrometer (KBr Pellets). ^{1}H NMR spectra were recorded or a Bruker DRX-300 (300 MHz FT-NMR) spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Mass spectra were obtained using Shimadzu LCMS 2010A under ESI ionization technique.

2.1.1. *Preparation of N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-3-oxobutanamide (3)*

Acetazolamide **1** (0.01 M) and ethylacetoacetate **2** (0.01 M) were mixed in presence 10 ml of glacial acetic acid and refluxed for approximately 4.5 h. The colorless liquid formed was then heated on a water bath to remove the alcohol 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 20 min using a mechanical stirrer. Allowing it to stand for 15 min, followed by filtration, resulted in the third compound in a pure form of N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-3-oxobutanamide **3**.

2.2. General procedure

2.2.1. *Preparation of 1,2,3,4-tetrahydropyrimidines by microwave irradiation method(**12a–n**)*

The mixture of N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-3-oxobutanamide (0.005 M), urea/thiourea (0.0075 M), and appropriate aldehyde (0.005 M) with catalytic amount of p-toluenesulfonic acid in 10 ml of ethanol was subjected to microwave irradiation (300 W) for 10 min at the interval of 10 s. The reactions were monitored through TLC using 25 percent ethylacetate in pet ether as solvent system. After the reaction was complete, 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 recrystallized with ethanol to give the pure compounds (**12a–n**).

2.3. Analytical data

2.3.1. *N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-3-oxobutanamide (3)*

Light-bluish crystalline solid, M.P.: 188–190 °C; Yield: 74%; IR (KBr, cm^{-1}): 3332 (N–H), 2864 (AliC–H), 1734 (C=O, ketone), 1686 (C=O, amide), 1542 (C=C), 1326 (C–N); ^{1}H NMR (DMSO-d_6) δ: 2.09 (s, 3H, CH_3), 3.42 (s, 2H, CH_2), 9.44 (s, 1H, NH), 9.68 (s, 1H, NH); calculated for $C_8H_{10}N_4O_5S_2$: C, 31.37; H, 3.29; N, 18.29; found C, 31.42; H, 3.25; N, 18.35.

2.3.2. *N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-(3-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (12a)*

Dark-brownish solid, M.P.: 258–260 °C; Yield: 69%; IR (KBr, cm^{-1}): 3258 (N–H), 3146 (ArC–H), 2932 (AliC–H), 1663 (C=O, amide), 1571 (C=C), 1237 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.07 (s, 3H, CH_3), 3.63 (s, 3H, CH_3), 5.39 (s, 1H, CH), 7.33 (d, 2H, ArH), 7.84 (d, 2H, ArH), 8.88 (s, 1H, NH), 8.94 (s, 1H, NH), 9.62 (s, 1H, NH), 10.08 (s, 1H, NH); MS (*m*/*z*): (M + 1) calculated 472.00; Found 472.05; Calculated for $C_{16}H_{15}ClN_6O_5S_2$: C, 40.81; H, 3.21; N, 17.85; found C, 40.86; H, 3.19; N, 17.88.

2.3.3. *N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-(3-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (12b)*

Light-ash-colored solid, M.P.: 284–287 °C; Yield: 76%; IR (KBr, cm^{-1}): 3246 (N–H), 3146 (ArC–H), 2938 (AliC–H), 1628 (C=O, amide), 1557 (C=C), 1884 (C=S), 1176 (O–C); ^{1}H NMR (DMSO-

d_6) δ: 2.05 (s, 3H, CH_3), 3.58 (s, 3H, CH_3), 5.26 (s, 1H, CH), 6.48 (d, 2H, ArH), 7.18 (d, 2H, ArH), 9.16 (s, 1H, NH), 9.47 (s, 1H, NH), 9.78 (s, 1H, NH), 10.14 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 488.00; found 488.06. Calculated for $C_{16}H_{15}ClN_6O_4S_3$: C, 39.46; H, 3.10; N, 17.26; found C, 39.50; H, 3.07; N, 17.30.

*2.3.4. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12c**)*

Light-greenish-colored solid, M.P.: 292–295 °C; Yield: 81%; IR (KBr, cm^{-1}): 3316 (N–H), 3232 (ArC–H), 2944 (AliC–H), 1678 (C=O, amide), 1591 (C=C), 1342 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.04 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 5.47 (s, 1H, CH), 7.21 (d, 2H, ArH), 7.74 (d, 2H, ArH), 8.89 (s, 1H, NH), 9.31 (s, 1H, NH), 9.79 (s, 1H, NH), 9.94 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 472.00; found 471.96; Calculated for $C_{16}H_{15}ClN_6O_5S_2$: C, 40.82; H, 3.21; N, 17.85; found C, 40.79; H, 3.23; N, 17.82.

*2.3.5. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-(4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12d**)*

Ash-colored solid, M.P.: 304–307 °C; Yield: 84%; IR (KBr, cm^{-1}): 3272 (N–H), 3137 (ArC–H), 2968 (AliC–H), 1647 (C=O, amide), 1568 (C=C), 1872 (C=S), 1218 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.07 (s, 3H, CH_3), 3.50 (s, 3H, CH_3), 5.52 (s, 1H, CH), 7.31 (d, 2H, ArH), 7.84 (d, 2H, ArH), 8.88 (s, 1H, NH), 9.32 (s, 1H, NH), 9.54 (s, 1H, NH), 10.06 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 488.00; found 487.98; Calculated for $C_{16}H_{15}ClN_6O_4S_3$: C, 39.46; H, 3.10; N, 17.26; found C, 39.44; H, 3.12; N, 17.29.

*2.3.6. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12e**)*

Light-yellowish solid, M.P.: 321–323 °C; Yield: 74%; IR (KBr, cm^{-1}): 3378 (N–H), 3146 (ArC–H), 2958 (AliC–H), 1648 (C=O amide), 1545 (C=C), 1268 (C–O); ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, 3H, CH_3), 3.65 (s, 3H, CH_3), 5.71 (s, 1H, CH), 7.26–7.36 (t, 3H, ArH), 8.79 (s, 1H, NH), 9.24 (s, 1H, NH), 9.56 (s, 1H, NH), 10.08 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 482.00; found 482.06; Calculated for $C_{16}H_{15}N_7O_7S_2$: C, 39.91; H, 3.14; N, 20.36; found C, 39.87; H, 3.09; N, 20.40.

*2.3.7. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12f**)*

Ash-colored solid, M.P.: 308–3011 °C; Yield: 80%; IR (KBr, cm^{-1}): 3257 (N–H), 3156 (ArC–H), 2966 (AliC–H), 1648 (C=O, amide), 1588 (C=C), 1846 (C=S), 1177 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.07 (s, 3H, CH_3), 3.71 (s, 3H, CH_3), 5.58 (s, 1H, CH), 6.72 (d, 2H, ArH), 7.66 (d, 2H, ArH), 8.74 (s, 1H, NH), 9.24 (s, 1H, NH), 9.47 (s, 1H, NH), 10.01 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 498.00; found 498.04; Calculated for $C_{16}H_{15}N_7O_6S_3$: C, 38.63; H, 3.04; N, 19.71; found C, 38.59; H, 3.01; N, 19.7.

*2.3.8. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-(4-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12g**)*

Light-bluish-colored solid, M.P.: 342–345 °C; Yield: 85%; IR (KBr, cm^{-1}): 3247 (N–H), 3182 (ArC–H), 2938 (AliC–H), 1647 (C=O, amide), 1544 (C=C), 1182 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.02 (s, 3H, CH_3), 3.69 (s, 3H, CH_3), 5.51 (s, 1H, CH), 7.34 (d, 2H, ArH), 7.86 (d, 2H, ArH), 8.88 (s, 1H, NH), 9.47 (s, 1H, NH), 9.94 (s, 1H, NH), 10.06 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 455.00; found 455.04; Calculated for $C_{16}H_{15}FN_6O_5S_2$: C, 42.29; H, 3.33; N, 18.49; found C, 42.27; H, 3.37; N, 18.47.

*2.3.9. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-(4-fluorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12h**)*

Light-greenish solid, M.P.: 351–353 °C; Yield: 83%; IR (KBr, cm^{-1}): 3238 (N–H), 3154 (ArC–H), 2974 (AliC–H), 1666 (C=O, amide), 1573 (C=C), 1836 (C=S), 1153 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.09 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 5.42 (s, 1H, CH), 7.46 (d, 2H, ArH), 7.84 (d, 2H, ArH), 8.89 (s, 1H, NH), 9.34 (s, 1H, NH), 9.88 (s, 1H, NH), 10.06 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 471.00; found 470.96; Calculated for $C_{16}H_{15}FN_6O_4S_3$: C, 40.84; H, 3.21; N, 17.86; found C, 40.81; H, 3.25; N, 17.81.

*2.3.10. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-6-methyl-4-(2-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12i**)*

Ash-colored solid, M.P.: 284–287 °C; Yield: 70%; IR (KBr, cm^{-1}): 3238 (N–H), 3148 (ArC–H), 2957 (AliC–H), 1648 (C=O, amide), 1554 (C=C), 1128 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.04 (s, 3H, CH_3), 3.71 (s, 3H, CH_3), 5.46 (s, 1H, CH), 7.38 (d, 2H, ArH), 7.92 (d, 2H, ArH), 8.78 (s, 1H, NH), 9.31 (s, 1H, NH), 9.72 (s, 1H, NH), 9.72 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 482.00; found 482.05; Calculated for $C_{16}H_{15}N_7O_7S_2$: C, 39.91; H, 3.14; N, 20.36; found C, 39.95; H, 3.10; N, 20.31.

*2.3.11. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-6-methyl-4-(2-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12j**)*

Light-yellowish solid, M.P.: 296–299 °C; Yield: 73%; IR (KBr, cm^{-1}): 3238 (N–H), 3173 (ArC–H), 2942 (AliC–H), 1674 (C=O, amide), 1584 (C=C), 1847 (C=S), 1166 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.04 (s, 3H, CH_3), 3.59 (s, 3H, CH_3), 5.57 (s, 1H, CH), 7.39 (d, 2H, ArH), 7.84 (d, 2H, ArH), 8.84 (s, 1H, NH), 9.35 (s, 1H, NH), 9.92 (s, 1H, NH), 10.13 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 498.00; found 497.94; Calculated for $C_{16}H_{15}N_7O_6S_3$: C, 38.63; H, 3.04; N, 19.71; found C, 38.59; H, 3.08; N, 19.66.

*2.3.12. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12k**)*

Light-yellowish solid, M.P.: 318–321 °C; Yield: 72%; IR (KBr, cm^{-1}): 3246 (N–H), 3174 (ArC–H), 2947 (AliC–H), 1674 (C=O, amide), 1572 (C=C), 1138 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.05 (s, 3H, CH_3), 3.68 (s, 3H, CH_3), 5.43 (s, 1H, CH), 7.46 (d, 2H, ArH), 7.87 (d, 2H, ArH), 8.74 (s, 1H, NH), 9.47 (s, 1H, NH), 9.89 (s, 1H, NH), 10.08 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 472.00; found 471.98; Calculated for $C_{16}H_{15}ClN_6O_5S_2$: C, 40.81; H, 3.21; N, 17.85; found C, 40.77; H, 3.24; N, 17.89.

*2.3.13. N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12l**)*

Light-bluish-colored solid, M.P.: 335–337 °C; Yield: 77%; IR (KBr, cm^{-1}): 3247 (N–H), 3133 (ArC–H), 2958 (AliC–H), 1683 (C=O, amide), 1528 (C=C), 1846 (C=S), 1158 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.04 (s, 3H, CH_3), 3.75 (s, 3H, CH_3), 5.53 (s, 1H, CH),

7.41 (d, 2H, ArH), 7.74 (d, 2H, ArH), 8.91 (s, 1H, NH), 9.45 (s, 1H, NH), 9.89 (s, 1H, NH), 10.06 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 488.0; found 488.06; Calculated for $C_{16}H_{15}ClN_6O_4S_3$: C, 39.46; H, 3.10; N, 17.26; found C, 39.41; H, 3.07; N, 17.30.

2.3.14. *N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-6-methyl-2-oxo-4-(pyridin-4-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12m**)*
Ash-colored solid, M.P.: 289–291 °C; Yield: 69%; IR (KBr, cm^{-1}): 3238 (N–H), 3166 (ArC–H), 2951 (AliC–H), 1664 (C=O, amide), 1592 (C=C), 1169 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.02 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 5.43 (s, 1H, CH), 7.29 (d, 2H, ArH), 7.82 (d, 2H, ArH), 8.88 (s, 1H, NH), 9.39 (s, 1H, NH), 9.92 (s, 1H, NH), 10.07 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 438.00; found 438.04; Calculated for $C_{15}H_{15}N_7O_5S_2$: C, 41.18; H, 3.46; N, 22.41; found C, 41.22; H, 3.39; N, 22.47.

2.3.15. *N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-6-methyl-4-(pyridin-4-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**12n**)*
Light-bluish-colored solid, M.P.: 356–358 °C; Yield 73%; IR (KBr, cm^{-1}): 3253 (N–H), 3148 (ArC–H), 2968 (AliC–H), 1654 (C=O, amide), 1538 (C=C), 1824 (C=S), 1136 (O–C); ^{1}H NMR (DMSO-d_6) δ: 2.06 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 5.43 (s, 1H, CH), 7.38 (d, 2H, ArH), 7.88 (d, 2H, ArH), 8.93 (s, 1H, NH), 9.39 (s, 1H, NH), 9.92 (s, 1H, NH), 10.08 (s, 1H, NH); MS (*m*/z): (M + 1) calculated 454.00; found 453.95; Calculated for $C_{15}H_{15}N_7O_4S_3$: C, 39.73; H, 3.33; N, 21.62; found C, 39.69; H, 3.38; N, 21.66.

2.4. *Antimicrobial activity*

The in-vitro antibacterial activities were tested against Gram-positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* by standard serial dilution method using a stock solution of 100 μg/ml concentrations (Vasudeva Rao et al., 2012; Zhu et al., 2009). Double strength nutrient broth was used as culture media and dimethyl sulphoxide (DMSO) was used as solvent control. The stock solutions of the test compounds were serially diluted in test tubes containing 1 ml of sterile medium to get the different concentrations and then inoculated with 100 μl of suspension of respective microorganism in sterile saline. Norfloxacin (Nfn) was used as standard drug. The inoculated test tubes were incubated at 37 ± 1 °C for 24 h.

2.5. *In-vitro cytotoxicity*

Short-term in-vitro cytotoxicity assay was performed using Vero cells according to the standard procedure (Prashantha Kumar et al., 2010). SRB is a bright-pink aminoxanthene dye with two sulfonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues 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. After incubation, the solutions in the wells were flicked off and 100 μl of different concentrations (2–500 μg) of compounds were added to the cells and incubated at 37 °C for 3 days in 5% CO_2 atmosphere. The 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, each well 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 wells to solubilize the dye. The plates were vigorously shaken for 5 min, and the absorbance was measured using microtiter plate reader at 540 nm. The mean absorbance of triplicate was recorded. Mean absorbance taken from cells grown in the absence of the test compound was taken as 100% cell survival (control). Tamoxifen (Tfn) was used as standard drug. The percentage growth inhibition was calculated using the following formula:

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

3. Results and discussion

A series of 14 novel acetazolamide cyclocondensed 1,2,3,4-tetrahydropyrimidines of biological interest were synthesized and evaluated for antimicrobial and cytotoxicity, all the compounds were characterized by IR, ^{1}H NMR, MS and elemental analysis for their structures.

3.1. *Chemistry*

Synthesis of 1,4-dihydropyrimidines by adopting Biginelli synthetic protocol (Prashantha Kumar et al., 2009) involving one pot multicomponent reaction was performed by following steps as outlined in Fig. 1. In the first step, ethylacetoacetate **2** and acetazolamide **1** in presence 10 ml of glacial acetic acid reacted under neat conditions resulting in the formation of *N*-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-3-oxobutanamide **3** with the yield of 74 percent. The *N*-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-3-oxobutanamide was further taken for the Biginelli condensation reaction by reacting it with urea/thiourea and appropriate aldehyde in the presence of catalytic amount of p-toluenesulfonic acid. The advantages of the catalyst were better yields and do not require dry solvents.

The first step in the mechanism of the Biginelli reaction is the acid-catalyzed condensation of the urea with the aldehyde. This reaction begins with protonation of the aldehyde by the acid and is followed by attack of the amine from urea. Proton transfer steps then result in a protonated alcohol which leaves as water to

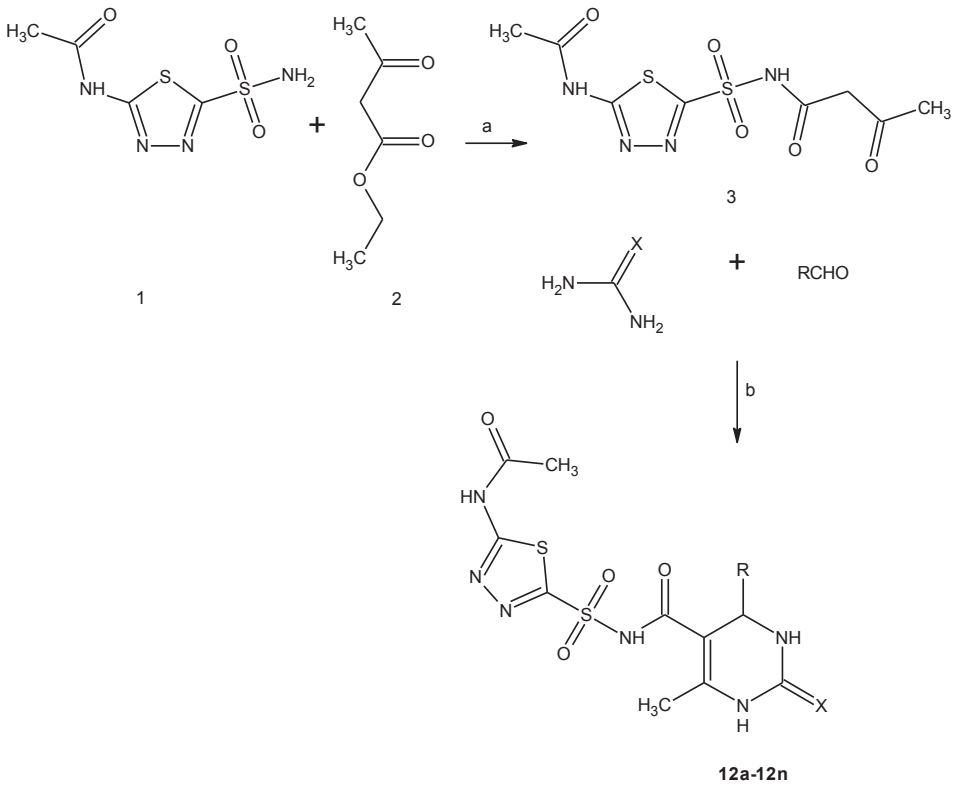


Fig. 1 – Synthesis of compounds (12a–12n). R for compounds: 12a: (3-chlorophenyl); 12b: (3-chlorophenyl); 12c: (4-chlorophenyl); 12d: (4-chlorophenyl); 12e:(3-nitrophenyl); 12f: (3-nitrophenyl); 12g: (4-flurophenyl); 12h: (4-flurophenyl); 12i: (2-nitorophenyl); 12j: (2-nitorophenyl); 12k: (2-chlorophenyl); 12l: (2-chlorophenyl); 12m: (4-pyridyl); 12n: (4-pyridyl). Reagents and conditions: (a) reflux 4.5 h, CH_3COOH; (b) C_2H_5OH, p-toluenesulfonic acid, and microwave irradiation (300 W) for 10 min.

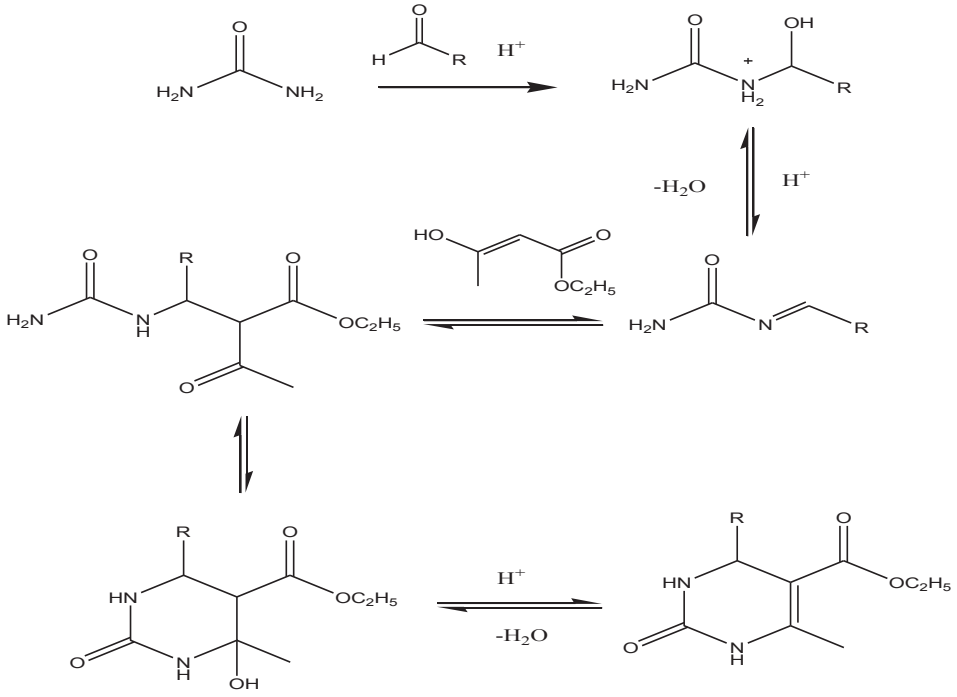


Fig. 2 – General mechanism of Biginelli tetrahydropyrimidines.

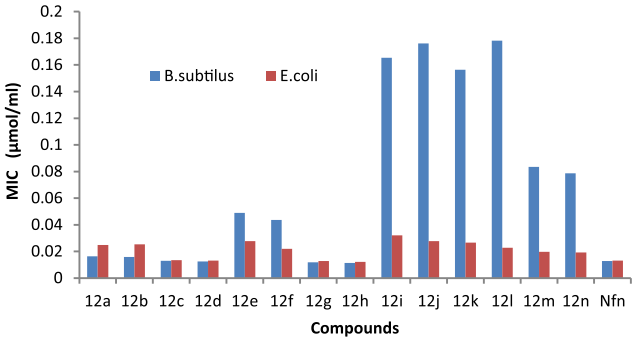


Fig. 3 – In-vitro antimicrobial activity of synthesized compounds and norfloxacin (Nfn).

form an N-acyliminium ion intermediate. (Oliver Kappe, 1997), subsequently enol form of the β-Keto ester attacks the N-acyliminum ion to generate an open chain Ureide which readily cyclizes to a tetrahydropyrimidines (Fig. 2). The reaction times were found to be 10 min. The IR spectra of compounds **12a-12n** showed strong absorption bands for amide group (1628–1683 cm^{-1}), aromatic C–H stretching (3137–3232 cm^{-1}) and aromatic C=C stretching (1528–1592 cm^{-1}). ^1H NMR spectrum of compounds **12a–12n** showed a methyl group protons singlet at (2.02–3.82 ppm), CH-R protons singlet at (5.26–5.71 ppm), aromatic protons doublet at (6.48–7.92 ppm) and amine protons singlet at (8.74–10.14 ppm). The elemental analysis results were within ±0.6% of the theoretical values. Totally, fourteen compounds **12a–n**, various substituted 1,2,3,4-tetrahydropyrimidines, were synthesized with the yield ranging from 69 to 85 percent. These conditions enable this method to be applicable for the synthesis of 1,2,3,4-tetrahydropyrimidines based heterocyclic compounds. The present protocol best describes the synthesis of 1,2,3,4-tetrahydropyrimidines. All the reported 1,2,3,4-tetrahydropyrimidines compounds were found to be novel and not reported elsewhere.

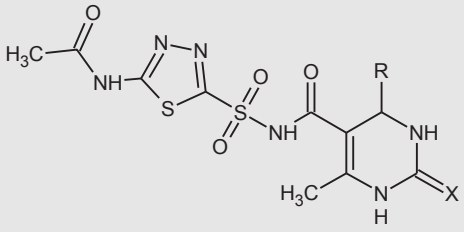
3.2. *Antimicrobial activity*

The synthesized compounds were subjected to in-vitro antimicrobial activity against Gram-positive bacteria *B. subtilis*, Gram-negative bacteria *E. coli*. The motive is to check the antimicrobial activity for the synthesized compounds. Antimicrobial activity of the synthesized compounds is shown in (Fig. 3 and Table 1). The data listed in (Fig. 3 and Table 1) clearly showed that most of the designed compounds exhibited good to moderate inhibitory activities toward the Gram-positive bacteria *B. subtilis*, Gram-negative bacteria *E. coli*. All the 1,2,3,4-tetrahydropyrimidines were potent antimicrobial agent, with an MIC value ranging from micromolar to submicromolar. Especially, compounds **12c**, **12d**, **12g** and **12h** showed the best antimicrobial activity (Fig. 3 and Table 1).

3.3. *In-vitro cytotoxicity*

The synthesized compounds were subjected to in-vitro cytotoxicity assay against Vero cells. The assay was performed by the sulforhodamine B (SRB) method (Prashantha Kumar et al., 2009). Almost all of the titled compounds exhibited weak, moderate, or high cytotoxicity. Compounds, **12c**, **12d**, **12g** and

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 (µmol/ml)	*E. coli* MIC (µmol/ml)	CTC_{50} (µg/ml) on Vero cells
1	**12a**	3-Chlorophenyl	O	0.0164	0.0248	27.75
2	**12b**	3-Chlorophenyl	S	0.0158	0.0254	22.50
3	**12c**	4-Chlorophenyl	O	0.0129	0.0134	12.5
4	**12d**	4-Chlorophenyl	S	0.0125	0.0131	10
5	**12e**	3-Nitrophenyl	O	0.0489	0.0277	38
6	**12f**	3-Nitrophenyl	S	0.0436	0.0219	31
7	**12g**	4-Flurophenyl	O	0.0118	0.0128	10
8	**12h**	4-Flurophenyl	S	0.0114	0.0121	08
9	**12i**	2-Nitorophenyl	O	0.1653	0.0321	68
10	**12j**	2-Nitorophenyl	S	0.1762	0.0278	74
11	**12k**	2-Chlorophenyl	O	0.1564	0.0267	120
12	**12l**	2-Chlorophenyl	S	0.1782	0.0228	116
13	**12m**	4-Pyridyl	O	0.0834	0.0198	20
14	**12n**	4-Pyridyl	S	0.0786	0.0192	16
15	Norfloxacin	Standard	–	0.0128	0.0132	–
16	Tamoxifen	Standard	–	–	–	12

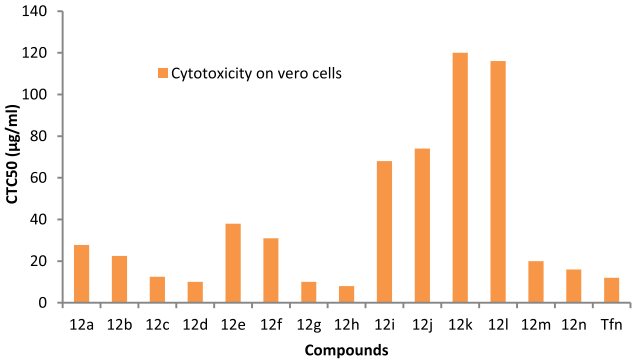


Fig. 4 – In-vitro cytotoxicity of synthesized compounds and tamoxifen (Tfn).

12h exhibited significant cytotoxic activity with lesser CTC_{50} value (Fig. 4 and Table 1). The goal of the cytotoxicity screening in the discovery of new antibacterial is not necessarily to identify the best compound but rather to identify active hits that have attributes that are good enough to become successful drugs after chemo type optimization (Peternel et al., 2009). In other words, the main goal of the initial cytotoxicity screen is compound classification and not investigation of the mechanism of cytotoxicity. In general, cytotoxicity screens are applied only for compounds that pass the desired antibacterial activity criteria in the first-line antibacterial efficacy screening campaign. By using such an approach, seemingly little concern is given to missing important leads that may be less potent but safe. Therefore, it is paramount to perform antibacterial efficacy and cytotoxicity assays in parallel, resulting in instant determination of the CTC_{50}/MIC ratio and thereby of safety margins.

Analyzing the activities of the synthesized compounds, the following structure activity relationships (SARs) were obtained. The fifth position of 1,2,3,4-tetrahydropyrimidines contain N-{[5-(acetylamino)-1,3,4-thiadiazol-2-yl]sulfonyl}-3-oxobutanamide group contributed toward antimicrobial and cytotoxicity activity and fourth positions of 1,2,3,4-tetrahydropyrimidines contain substituted phenyl and hetero aromatic ring responsible antimicrobial and cytotoxicity potency (Prashantha Kumar et al., 2009). Substituted atom or group of atom must be strong electron withdrawing nature for potent activity because it decreases electron density in the ring due to inductive effect. Fluoride and chloride substitution at fourth position of phenyl ring showed potent antimicrobial and cytotoxicity because of strong electron withdrawing nature due to inductive effect. Substitution of chloro group at third position of phenyl ring showed potent action when compare with nitro atom. Introduction of heterocyclic ring at forth position it showed moderate antimicrobial and cytotoxicity. The second position sulfur substituted derivatives most potent when compare with oxygen atom. Among the compounds reported here in, compounds (**12c**, **12d**, **12g** and **12h**) is arguably the most potent when compare with current therapeutic agent norfloxacin and tamoxifen because fluoride and chloride substituted phenyl ring present at 4th position of 1,2,3,4-tetrahydropyrimidines it enhances the antimicrobial and cytotoxicity (Figs. 3 and 4 and Table 1).

4. Conclusion

A series of novel 1,2,3,4-tetrahydropyrimidines of biological interest were synthesized and analyzed for their structures. The libraries of compounds were prepared by using laboratory made p-toluenesulfonic acid as an efficient catalyst when compare with Lewis acid. The importance of substitutions at the fourth positions of 1,2,3,4-tetrahydropyrimidines was studied toward the antimicrobial and cytotoxicity. The antimicrobial and cytotoxicity data revealed that the all synthesized compounds proved to be active against the test organism *B. subtilis*, *E. coli*, and Vero cells. Almost all of the titled compounds exhibited weak, moderate, or high antimicrobial and cytotoxicity. Some of new derivatives showed an in-vitro antimicrobial activity against *B. subtilis*, *E. coli* better than that of norfloxacin and cytotoxicity against *Vero cells* better than that of tamoxifen. Among the compounds reported here in, compounds (**12c**, **12d**, **12g** and **12h**) is arguably the most potent, our present study makes it an interesting compound when compared to the current therapeutic agents and are considered the candidates to investigate further for the same.

Acknowledgments

The authors wish to thank Sunrise University for research support. Also thank the Molecules Research Laboratory for in-vitro biological evaluation, Chennai, India.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bjbas.2014.02.003.

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