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ORIGINAL ARTICLE

Synthesis, and biological screening of chloropyrazine conjugated benzothiazepine derivatives as potential antimicrobial, antitubercular and cytotoxic agents

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Abstract A series of twenty new chloropyrazine conjugated benzothiazepines (**22**–**41**) have been synthesized with 58%–95% yields. The compounds were characterized by using different spectroscopic techniques including FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and mass spectrometry. The synthesized compounds (**22**–**41**) and their precursor chalcones (**2**–**21**) were evaluated for antitubercular and cytotoxic activities. Additionally, compounds **22**–**41** were also tested for antimicrobial activity. Among the chalcone series (**2**–**21**), compounds **7** and **14** showed significant

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antitubercular activities (MICs 25.51 and 23.89 μM, respectively), whereas among benzothiazepines (**22**–**41**), compounds **27** and **34** displayed significant antimicrobial (MICs 38.02 μM, 19.01 μM) and antitubercular (MIC 18.10 μM) activities. Compounds **7** and **41** displayed cytotoxic activities with IC_{50} of 46.03 ± 1 and 35.10 ± 2 μM respectively. All the compounds were evaluated for cytotoxic activity on normal human liver cell lines (L02) and found to be relatively less selective towards this cell line. The most active compounds identified through this study could be considered as potential leads for the development of drugs with possible antimicrobial, antitubercular, and cytotoxic activities.

1. Introduction

Heterocyclic compounds play a vital role in the design and development of drugs against different diseases. More than seventy percent of drugs used in therapy contain one or more heterocyclic rings. Out of the 24 small drug molecules approved in 2020 by Food and Drug Administration (FDA), USA, 22 drugs contain heterocyclic rings. Similarly, of the 10 top drugs prescribed in USA, six drugs contain heterocyclic rings. This scenario is due to the property of the heterocyclic rings to serve as useful templates to modulate physicochemical properties of the drug molecules including the hydrogen bond capacity, polarity, and lipophilicity. Presence of hetero atoms or heterocyclic nucleus within the molecules may further improve pharmacokinetic, pharmacodynamic, and toxicological profiles of the drug candidates and finally drug molecules (Gomtsyan, 2012). Some of the heterocyclic rings are considered as privileged because of their significant drug-like properties. Medicinal chemists usually club or conjugate two or more such privileged heterocycles to create biologically useful molecules as a part of drug discovery programs. In the recent past, the design and synthesis of heterocyclic hybrids have received greater attention due to their ease of synthesis and improved biological properties. The structures that evolve from such conjugation are usually rigid frameworks that can show the appended rings in a well-defined fashion that is necessary for molecular recognition of the biological target. Usually, the variable nature of these functionalities defines the selectivity on a privileged core for a particular target.

Pyrazine and 1,5-benzothiazepine rings are two privileged aromatic heterocycles of interest to organic and medicinal chemists because of their ease of synthesis and biological activities. Both these scaffolds are prominent in a variety of drugs used in the treatment of different complications. For example, pyrazine ring is found in drugs like pyrazinamide (PZA) and morinamide (antitubercular), favipiravir (anti-corona viral), glipizide (antidiabetic), amiloride and benzamil (diuretic), acipimox (antihyperlipidemic), and bortezomib (multiple myeloma). 1,5-benzothiazepine scaffold is present in calcium channel blockers for cardiovascular problems- Diltiazem and Clentiazem (Nagao et al., 1972, Chaffman and Brogden, 1985), atypical antipsychotic agents- Quetiapine and Clotiapine, and the antidepressant drug- Thiazesim (Kawakita et al., 1991, Geyer et al., 1970, Hopenwasser et al., 2004) (Fig. 1).

Pyrazine derivatives were reported to possess excellent antitubercular (Ambrożkiewicz et al., 2020, Bouz et al., 2020, Zitko et al., 2018, Miniyar et al., 2017, Zitko et al., 2016, Servusova-Vanaskova et al., 2015, Servusová et al., 2013, Zitko et al., 2013, Mangrolia and Osborne, 2020), anticancer (De Wang et al., 2020, Tantawy et al., 2020, Etaiw et al., 2020, Seo et al., 2020, Mamedova et al., 2019, Patil et al., 2019, Bhaskar et al., 2020), antimicrobial (Singh et al., 2020, Kucerova-Chlupacova et al., 2016, Kucerova-Chlupacova et al., 2015, Stepanić et al., 2019, Kucerova-Chlupacova et al., 2018), antioxidant (Cavalier et al., 2001, Zaki et al., 2018, Abu-Hashem and El-Shazly, 2018), analgesic and anti-inflammatory (Shankar et al., 2017, Aneesa et al., 2015, Bills et al., 1939, Bariwal et al., 2008), and anti-pellagra activities (El-Bayouki, 2011). Many authors have reviewed the synthesis, characterization, and applications of benzothiazepines (Deshmukh et al., 2016, Saha et al., 2015, El-Bayouki, 2013, Sekhar, 2014, Shaik et al., 2020a,b, Wu et al., 2017). This ring derivatives have shown promising anticancer (Gudisela et al., 2017, Ameta et al., 2012, Arya and Dandia, 2008, Ansari et al., 2008, Sharma et al., 1997, Upadhyay et al., 2012, Wang et al., 2020), antitubercular (Kendre et al., 2019), antibacterial and antifungal (Yan et al., 2019, Tongxiu et al., 2018, Pant et al., 2018, Mor et al., 2017, Patel et al., 2016, Li et al., 2017, El-Bayouki, 2013), antiviral (Di Santo and Costi, 2005, Kishor et al., 2017, Lokesh et al., 2017) activities. Representative structures of important pyrazine and benzothiazepine derivatives with potential antimycobacterial, antimicrobial, and anticancer activities are displayed below (Fig. 2).

In continuation for our interest in the synthesis and screening of heteroaryl chalcones (Shaik et al., 2020a,b, Lokesh et al., 2019a,b) and conjugated heterocycles (Palleapati et al., 2019, Lokesh et al., 2019a,b, Yazdan et al., 2015) as well as the potential bioactivities of conjugated heterocyclic derivatives, we prepared and screened novel chloropyrazine conjugated 1,5-benzothiazepine derivatives. Both pyrazine and 1,5-benzothiazepine rings exhibited promising antimicrobial, antitubercular and cytotoxic activities as discussed above. Hence, the molecules synthesized for our study were expected to possess collective effect on the proposed activities. We have previously described the antibacterial & antifungal activities of chloropyrazine chalcones (**2**–**21**) (Vegesna et al., 2017). Herein, we reported the synthesis of twenty new chloropyrazine conjugated benzothiazepines (**22**–**41**) as well as the antitubercular and cytotoxic activities of precursor chalcones (**2**–**21**), antimicrobial, antitubercular, and cytotoxic activities of chloropyrazine conjugated 1,5-benzothiazepine derivatives (**22**–**41**). The general structures of the chloropyrazine chalcones and chloropyrazine conjugated 1,5-benzothiazepines are portrayed in Fig. 3.

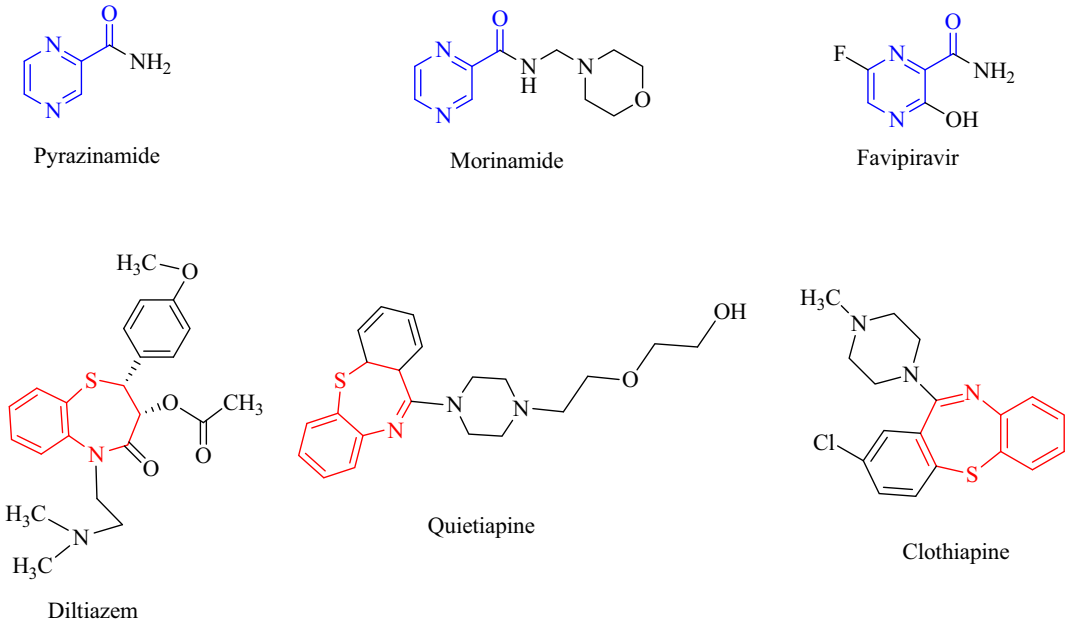


Fig. 1 Representative structures of clinically used pyrazine and benzothiazepine ring containing drugs.

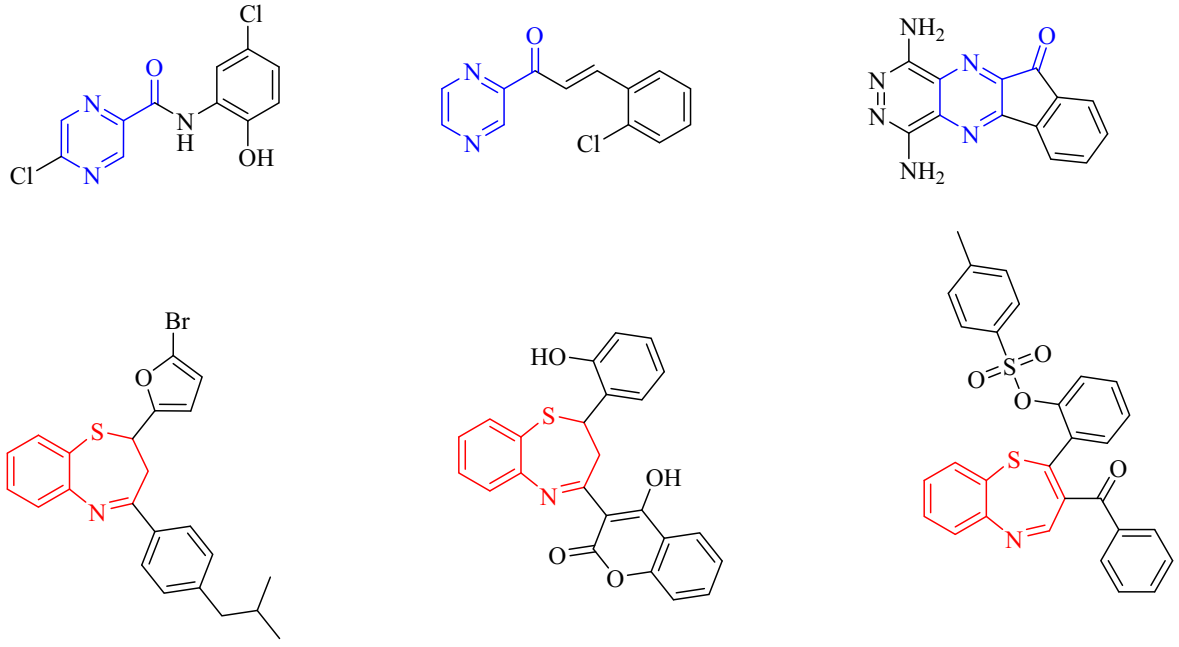
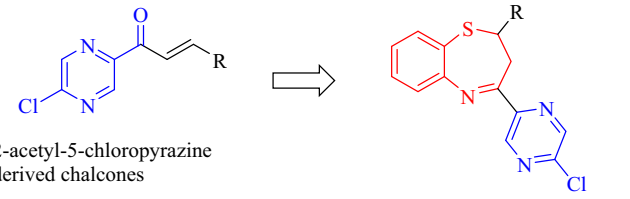


Fig. 2 Structures of selected pyrazine and 1,5-benzothiazepine derivatives with potent antimicrobial, antitubercular and anticancer activities.

2. Materials and methods

2.1. General

All solvents and reagents were obtained from S.D. Fine. Ltd. Mumbai, India and Sigma Aldrich Chemical Co (USA) and used without further purification. Pre-coated silica gel 60 F_{254} plates were used for thin-layer chromatography (TLC) and the spots on the TLC plates were visualized by UV lamp (254 nm). The benzothiazepines (**22**–**41**) were purified by recrystallization using ethanol. Melting points were determined on Boetius melting point apparatus in open capillary tubes and were uncorrected. FT-IR spectra were recorded on Bruker Vertex 80v spectrometer using potassium bromide discs and the absorption band values are given in cm^{-1}. Proton and carbon magnetic resonance (^{1}H NMR and ^{13}C NMR) were recorded on Bruker AMX 400 NMR spectrophotometer using Tetramethyl silane (TMS) as internal standard and the chemical shift values are given in parts per million (ppm) relative



2-acetyl-5-chloropyrazine derived chalcones

Chloropyrazine conjugated 1,5-benzothiazepine derivatives

Fig. 3 General structures of 2-acetyl-5-chloropyrazine chalcones and chloropyrazine conjugated 1,5-benzothiazepine derivatives.

to TMS. Mass spectra (MS) were recorded on Agilent 6100 QQQ ESI mass spectrophotometer by electron spray ionization technique.

2.2. Experimental

*2.2.1. General procedure for the synthesis of chalcones (**2**–**21**)*

To a mixture of 2-acetyl-5-chloropyrazine (0.001 M) and the appropriate aryl or heteroaryl aldehyde (0.001 M) was stirred in ethanol (7.5 mL) and to it aqueous solution of NaOH (40%, 7.5 mL) was added. The mixture was kept for 24 h and it was acidified with 1:1 mixture of hydrochloric acid and water, then it was filtered under vacuum and the product was washed with water (Vegesna et al., 2017).

*2.2.2. General procedure for the synthesis of chloropyrazine conjugated 1,5-benzothiazepines (**22**–**41**)*

To a mixture of 1 mmol of chalcones (**2**–**21**) and 1.5 mmol of 2-Aminothiophenol in dry methanol (50 mL), a catalytic amount of piperidine was added. The mixture was refluxed for 8 h. After cooling, the solid product separated was collected and washed with diethyl ether (2 × 50 mL) and cold methanol (2 × 50 mL). The crude solid was recrystallized from ethanol (Shaik et al., 2020a,b).

*2.2.2.1. 2,3-Dihydro-2-phenyl-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**22**).* FT-IR (KBr): 1584 (C═N), 1510 (C═C), 1395 (C—N), 663 (C—S), 879 (C—Cl), 3032 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.11 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.52 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.21 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.19–8.22 (11*H*, Ar—H); ^{13}H NMR (100 MHz, $CDCl_3$): 50.15 (C-2), 40.24 (C-3), 164.66 (C-4), 122.54, 126.44, 127.51, 127.81, 128.80, 133.31, 141.72, 144.46, 146.73, 150.32 and 151.45 (Ar-C's); MS (*m*/*z*): [M$^+$], 351.15, [M + 2], 353.15.

*2.2.2.2. 2,3-Dihydro-2-(4-chlorophenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**23**).* FT-IR (KBr): 1590 (C═N), 1505 (C═C), 1395 (C—N), 660 (C—S), 841 (C—Cl), 3021 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.23 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.54 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.29 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.15–8.01 (10*H*, Ar—H).

*2.2.2.3. 2,3-Dihydro-2-(4-fluorophenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**24**).* FT-IR (KBr): 1593 (C═N), 1512 (C═C), 1397 (C—N), 665 (C—S), 922 (C—Cl), 921 (C-F), 3070 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.31 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.81 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.37 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.76–8.85 (10*H*, Ar—H).

*2.2.2.4. 2,3-Dihydro-2-(4-nitrophenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**25**).* FT-IR (KBr): 1591 (C═N), 1512 (C═C), 1369 (C—N), 681 (C—S), 854 (C—Cl), 3059 (Ar C—H), 1558 (N═O, asymmetric), 1341 (N═O, symmetric); ^{1}H NMR (400 MHz, $CDCl_3$): 5.33 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 4.01 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.73 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.70–8.56 (10*H*, Ar—H).

*2.2.2.5. 2,3-Dihydro-2-(2,4-fluorophenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**26**).* FT-IR (KBr): 1602 (C═N), 1518 (C═C), 1403 (C—N), 691 (C—S), 856 (C—Cl), 919 (C-F), 3060 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.45 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.76 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.51 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.66–8.61 (9H, Ar—H).

*2.2.2.6. 2,3-Dihydro-2-(2,4-dichlorophenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**27**).* FT-IR (KBr): 1592 (C═N), 1526 (C═C), 1382 (C—N), 688 (C—S), 945 (C—Cl), 3020 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.43 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.99 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.62 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.90–8.96 (9H, Ar—H).

*2.2.2.7. 2,3-Dihydro-2-(4-hydroxyphenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**28**).* FT-IR (KBr): 1596 (C═N), 1518 (C═C), 1388 (C—N), 667 (C—S), 915 (C—Cl), 3452 (Ar—OH), 3024 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 4.72 (s, 1H, Ar—OH), 5.23 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.51 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.28 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.20–8.19 (10*H*, Ar—H).

*2.2.2.8. 2,3-Dihydro-2-(4-methylphenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**29**).* FT-IR (KBr): 1588 (C═N), 1516 (C═C), 1390 (C—N), 678 (C—S), 888 (C—Cl), 2935 (Alkyl C—H), 3022 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 2.38 (s, 3H, Ar—CH3), 4.95 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.32 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.11 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 7.10–8.09 (10*H*, Ar—H).

*2.2.2.9. 2,3-Dihydro-2-(4-methoxyphenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**30**).* FT-IR (KBr): 1586 (C═N), 1519 (C═C), 1395 (C—N), 666 (C—S), 912 (C—Cl), 1219 (—O—CH3), 3045 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 3.88 (s, 3H, Ar—OCH3), 5.10 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.53 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.26 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 6.52–7.83 (10*H*, Ar—H).

*2.2.2.10. 2,3-Dihydro-2-(3-methoxy-4-hydroxyphenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**31**)*. FT-IR (KBr): 1600 (C═N), 1515 (C═C), 1398 (C—N), 665 (C—S), 896 (C—Cl), 1222 (—O—CH3), 3420 (Ar—OH), 3045 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 3.80 (s, 3H, Ar—OCH3), 4.91 (s, 1H, Ar—OH), 4.99 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.32 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.06 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 6.69–8.10 (9H, Ar—H).

*2.2.2.11. 2,3-Dihydro-2-(4-dimethylaminophenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**32**)*. FT-IR (KBr): 1651 (C═N), 1519 (C═C), 1391 (C—N), 671 (C—S), 910 (C—Cl), 1205 (—$N(CH_3)_2$), 3048 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 3.18 (s, 6H,-N(CH3)2, 5.28 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.60 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.41 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 6.65–8.07 (10*H*, Ar—H).

*2.2.2.12. 2,3-Dihydro-2-(3,4-dimethoxyphenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**33**)*. FT-IR (KBr): 1615 (C═N), 1512 (C═C), 1329 (C—N), 682 (C—S), 1229 (—O—CH_3), 3095 (Ar C—H).; ^{1}H NMR (400 MHz, $CDCl_3$): 3.83 (s, 3H, Ar—OCH_3), 3.88 (s, 3H, Ar—OCH3), 5.01 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.31 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.11 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 6.85–8.06 (9H, Ar—H).

*2.2.2.13. 2,3-Dihydro-2-(3,4,5-trimethoxyphenyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**34**)*. FT-IR (KBr): 1599 (C═N), 1508 (C═C), 1371 (C—N), 1226 (—O—CH3), 694 (C—S), 901 (C—Cl), 3014 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 3.70 (s, 6H, 2× Ar—OCH3), 3.95 (s, 3H, Ar—OCH_3), 5.05 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.29 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.10 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 6.78–8.12 (10*H*, Ar—H).

*2.2.2.14. 2,3-Dihydro-2-(pyridin-2-yl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**35**)*. FT-IR (KBr): 1595 (C═N), 1517 (C═C), 1396 (C—N), 672 (C—S), 886 (C—Cl), 1136 (—O—CH3), 3025 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.03 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.29 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.12 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 6.85–8.14 (10*H*, Ar—H).

*2.2.2.15. 2,3-Dihydro-2-(pyridin-3-yl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**36**)*. FT-IR (KBr): 1602 (C═N), 1504 (C═C), 1387 (C—N), 676 (C—S), 865 (C—Cl), 3031 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.51 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.77 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.56 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.45–8.49 (10*H*, Ar—H).

*2.2.2.16. 2,3-Dihydro-2-(pyridin-4-yl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**37**)*. FT-IR (KBr): 1610 (C═N), 1517 (C═C), 1391 (C—N), 692 (C—S), 859 (C—Cl), 3171 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.11 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.49 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.16 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.18–8.28 (10*H*, Ar—H).

*2.2.2.17. 2,3-Dihydro-2-(4-thienyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**38**)*. FT-IR (KBr): 1595 (C═N), 1525 (C═C), 1395 (C—N), 671 (C—S), 891 (C—Cl), 3012 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.29 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.72 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.49 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.10–8.12 (9H, Ar—H).

*2.2.2.18. 2,3-Dihydro-2-(2-furfuryl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**39**)*. FT-IR (KBr): 1583 (C═N), 1511 (C═C), 1385 (C—N), 699 (C—S), 875 (C—Cl), 3129 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 5.58 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.55 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.34 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 6.95–8.11 (9H, Ar—H).

*2.2.2.19. 2,3-Dihydro-2-(2-pyrrolyl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**40**)*. FT-IR (KBr): 1592 (C═N), 1527 (C═C), 1399 (C—N), 682 (C—S), 917 (C—Cl), 3321 (N—H), 3011 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 10.09 (s, 1H, —NH), 5.25 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.56 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.42 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 6.87–8.15 (9H, Ar—H).

*2.2.2.20. 2,3-Dihydro-2-(pyrazol-5-yl)-4-(5-chloropyrazin-2-yl)-1,5-benzothiazepine (**41**)*. FT-IR (KBr): 1588 (C═N), 1522 (C═C), 1387 (C—N), 669 (C—S), 911 (C—Cl), 3326 (N—H), 3051 (Ar C—H); ^{1}H NMR (400 MHz, $CDCl_3$): 10.25 (s, 1H, N—H), 5.51 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.54 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), 3.21 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b), 7.28–8.31 (8H, Ar—H).

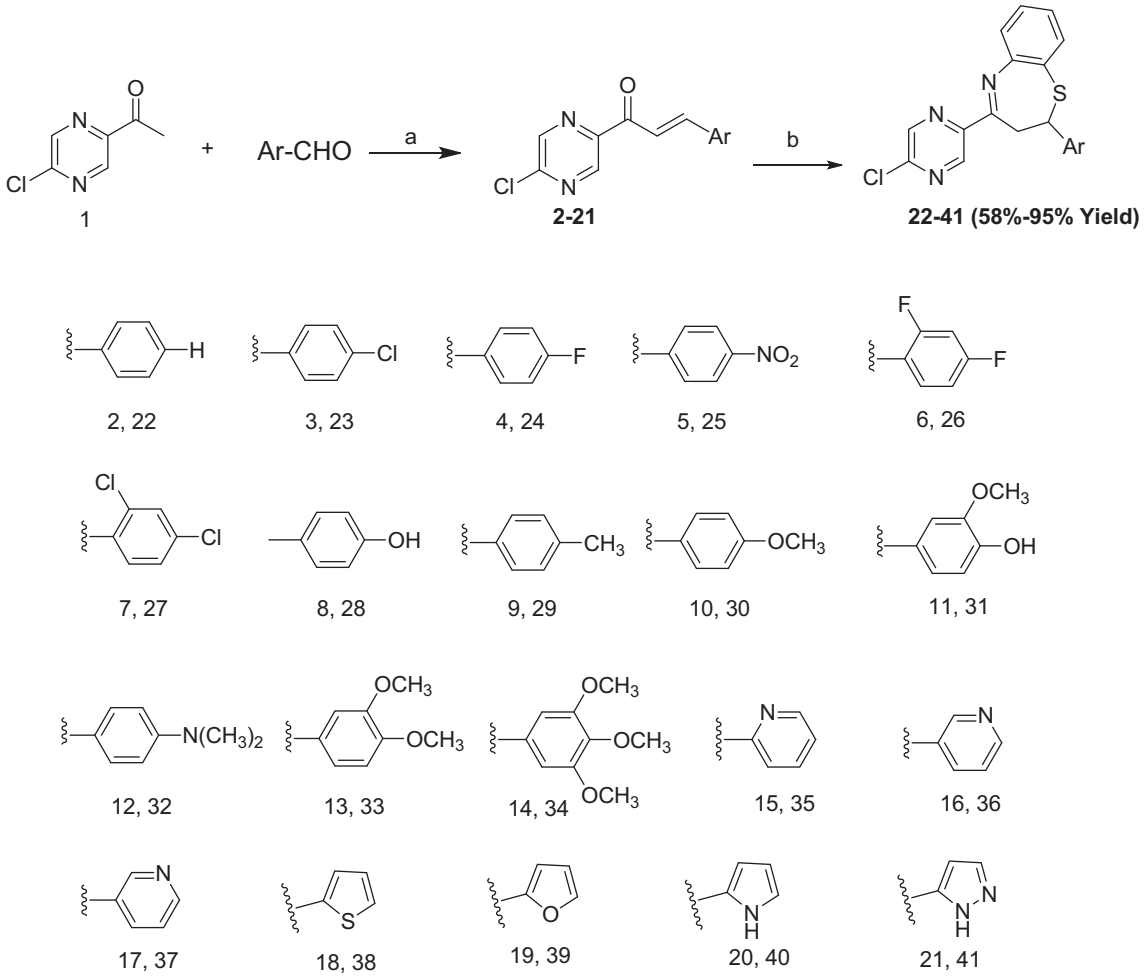
2.3. Antimicrobial activity

The antimicrobial (antibacterial and antifungal) activities of the novel chloropyrazine clubbed benzothiazepines (**22**–**41**) was evaluated against selected bacterial and fungal strains using standard experimental procedures as described in the literature (Vegesna et al., 2017). The standard strains were procured from the American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The bacterial strains selected for the study were *Bacillus subtilis* (ATCC-60511), *Staphylococcus aureus* (ATCC-11632), *Escherichia coli* (ATCC-10536), *Pseudomonas aeruginosa* (ATCC-10145) whereas the fungal strains include *Aspergillus niger* (ATCC-6275) and *Candida tropicalis* (ATCC-1369) respectively. Ciprofloxacin was used as positive control for antibacterial studies and fluconazole for antifungal activity. Antibacterial activity was performed using nutrient agar medium whereas Potato Dextrose-Agar medium was used for antifungal testing. 2.048 mg of each test compound was taken in vials separately. Then 2 mL of methanol was added.

Thus, a solution with a concentration of 1.024 mg/mL was obtained. All the experiments were carried out in triplicate and the results are presented as the mean of three independent experiments. The microbial strains were grown at 37 °C in their respective nutrient medium and diluted in sterile nutrient broth medium to get a suspension containing 10^7 cells/mL and this suspension was used as the inoculum. All the test tubes were incubated for 18 h at 37 °C. A similar experiment with inoculum, medium and methanol without compound was furthermore performed to confirm that there is no inhibitory effect of methanol used for the dilutions. The test tube number in which the first sign of the growth of the organism observed was noted using a spectrophotometer. The MIC was determined for all the compounds by taking that concentration used in the test tube number just before the test tube number where the first sign of growth observed (Kasetti et al., 2020).

2.4. Antitubercular activity

Chalcone precursors (1–21) and conjugated 1,5-benzothiazepine derivatives (**22–41**) were evaluated for antitubercular activity using the same procedure which we described in our previous paper (Kishor et al., 2017, Lokesh et al., 2019a, b, Palleapati et al., 2019) on *Mycobacterium tuberculosis* $H_{37}Rv$ strain (procured from National Institute of Tuberculosis, Chennai, India) using pyrazinamide (Sigma-Aldrich, USA) as reference drug. Broth dilution assay was employed to determine the minimum inhibitory concentration (MIC) of each compound. All the test compounds were dissolved separately in dimethyl sulfoxide (DMSO, (Merck Life Sciences Private Limited, Mumbai, India) and then diluted twice at the required concentration. A frozen culture in Middle brook 7H9 broth added with 10% albumin-dextrose-catalase and 0.2% glycerol (Himedia, Mumbai, India) was melted and diluted in broth to 10^5 cfu mL^{-1} (colony forming unit/mL) dilutions. The final concentration of DMSO in the assay medium was 1.3%. Then, each U-tube was inoculated with 0.05 mL of standardized culture and incubated at 37 °C for 21 days. The growth in the U-tubes was compared with visibility in opposition to a negative control (without drug and inoculum), positive control (without drug), and with standard pyrazinamide. The MIC values obtained in μg/mL were converted into μM for the standard drug pyrazinamide and the target compounds (**2–41**) in order allow comparison in molecular level.



Scheme 1 Synthesis of target compounds (**2–21**) and (**22–41**): (a) Ethanol, NaOH, room temperature (b) 2-Aminothiophenol, Piperidine/Ethanol, reflux.

2.5. Cytotoxic activities

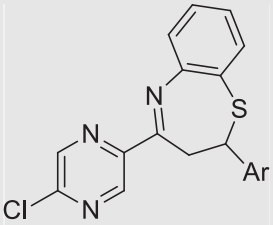
DU-145 (prostate cancer) and normal liver (L02) cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagels Medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Trypsin, EDTA were purchased from Sigma chemicals (St.Louis,MO). Fetal bovine serum (FBS) was purchased from Arrow Labs, 96 well flat bottom tissue culture plates were purchased from Tarson. The *in vitro* cytotoxic activity was performed for both chalcones **(2–21)** and benzothiazepines (**22–41**) on prostate cancer cell lines (DU-145) by Mosmann's MTT assay (Mosman, 1983) and their IC_{50} values were determined. The cytotoxic activity of the target compounds was compared with the standard drug methotrexate (Mtx). In principle, the assay is based on the reduction of MTT (3-(4,5-dime thylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to formazan (blue-purple colored) product, due to the action of mitochondrial reductase enzyme inside the living cells. DU-145 cells were cultured in Dulbecco's Modified Eagle Medium **(**DMEM) at 37 °C and humidified at 5% CO_2. The test compounds (**2–41**) were dissolved in 0.1% DMSO to make their stock solutions. From these stock solutions, different dilutions of the compounds were made with sterile water to attain the required final concentrations. Briefly, the cells were placed on 96-well plates at 100 µL total volume with a density of 1×10^4 cells per well and were allowed to adhere for 24 h. Later, the medium was replaced with fresh media containing different dilutions of the test compounds and allowed to incubate for another 48 h at 37 °C in DMEM with 10% fetal bovine serum (FBS) medium. Afterwards, the medium was replaced with 90 µL of fresh DMEM without FBS. The wells were treated with 10 µL of MTT reagent (5 mg/mL of stock solution in DMEM without FBS) and incubated at 37 °C for 3-–4 h. The formed blue crystals of formazan were dissolved in 200 µL of DMSO, and the optical density was determined at 570 nm using micro plate reader. Assay was carried out in triplicate for three independent experiments. The results had good reproducibility between replicate wells with standard errors below 10%. The cytotoxic activity results measured as IC_{50} values in µg/mL were converted into µM. All the compounds were also evaluated for their cytotoxicity on normal human liver cell lines (L02) to determine their toxicity by using the identical protocol as described above.

3. Results and discussion

3.1. Chemistry

Choloropyrazine based chalcones (**2–21**) and conjugated 1,5-benzothiazepines (**22–41**) described here were synthesized following the synthetic routes outlined in Scheme 1. To prepare chalcones **2–21**, 2-acetyl-5-chloropyrazine was treated with substituted aromatic and heteroaromatic aldehydes in ethanol under basic conditions to obtain yields between 55% and 97% (Vegesna et al., 2017). Intermediates **2–21** were refluxed with 2-aminothiophenol to obtain target benzothiazepines **22–41** with yields between 58% and 95% (Table 1). The compound **22** was analyzed for molecular formula $C_{19}H_{14}ClN_3S$, m.p. 166–168 °C, well supported by a M^+ peak at *m/z* 351.85 and also a satellite peak at *m/z* 353.85 with 3:1 intensity in its electron spray ionization mass spectrum. The FT-IR spectrum (cm^{-1}) of the compound **22** showed the characteristic bands at 1584 (C═N), 1510 (C═C), 1395 (C─N), 663 (C─S), 844 (C─Cl), and 3032 (Ar C─H). The ^{1}H NMR spectrum of compound **22** showed three characteristic peaks of C2-H and C3-CH_2 protons of 1,5-benzothiazepine ring at 5.11 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C2-H), 3.52 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C3-H-3a), and 3.21 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C3-H-3b). The spectrum also accounted for the other twelve aromatic protons in between δ 7.19–8.22. The ^{13}C NMR spectrum of compound **22** accounted for all the carbons whose resonance appeared at the following δ values: 50.15 (C-2), 40.24 (C-3), 164.66 (C-4), 122.54, 126.44, 127.51, 127.81, 128.80, 133.31, 141.72, 144.46, 146.73, 150.32 and 151.45 (Ar-C's). The values are consistent with the proposed structure for the compound. Based on the above spectral data and elemental analysis, the structure of compound **22** was confirmed as 2,3-dihydro-2-phenyl-4-(5-chl oropyrazin-2-yl)-1,5-benzothiazepine (Fig. 4). Similarly, other **19** compounds exhibited their characteristic spectral features in their FT-IR and ^{1}H NMR spectra. The general structures and physicochemical properties of compounds **22–41** are shown in Table 1.

Table 1 Chemical structures and physicochemical properties of compounds **22–41**.



22-41

Entry	Ar	% Yield	m.p. °C
22	phenyl	90	166–168
23	4-chlorophenyl	95	155–157
24	4-fluorophenyl	92	171–173
25	4-nitrophenyl	83	185–187
26	2,4-difluorophenyl	79	121–123
27	2,4-dichlorophenyl	88	133–135
28	4-hydroxyphenyl	63	196–198
29	4-methylphenyl	79	148–150
30	4-methoxyphenyl	66	205–207
31	3-methoxy-4-hydroxyphenyl	73	252–254
32	4-dimethylaminophenyl	91	175–177
33	3,4-dimethoxyphenyl	81	166–168
34	3,4,5-trimethoxyphenyl	75	210–212
35	2-pyridinyl	65	211–213
36	3-pyridinyl	58	118–120
37	4-pyridinyl	66	131–133
38	2-thienyl	69	150–152
39	2-furfuryl	80	142–144
40	2-pyrrolyl	75	175–177
41	5-pyrazolyl	82	232–236

3.2. Antimicrobial activity

All the compounds synthesized through this study were evaluated for their antimicrobial, antitubercular, and cytotoxic activities employing standard protocols. The compounds were classified into three different categories based on their structural features. Physicochemical properties modulated were based on the "R" group. Electron withdrawing or donating groups on phenyl ring (Tables 2–5) were either substituted at para position (**3**–**5**, **8**–**10** & **12; 23**–**25**, **28**–**30,** and **32**), ortho and para positions (**6**, **7**, **26** & **27**), or meta & para positions (**11**, **13**, **14**, **31**, **33** and **34**). Bioisosteric substitution of phenyl ring with pyridyl, thienyl, furfuryl, pyrrolyl, and pyrazolyl systems resulted in compounds **15**–**21** and **35**–**41** as chalcone and benzothiazepine derivatives.

3.2.1. Antibacterial activity

Compounds **22**–**41** were evaluated for their antibacterial activity (Table 2) against *Bacillus subtilis*, *Staphylococcus aureus*,

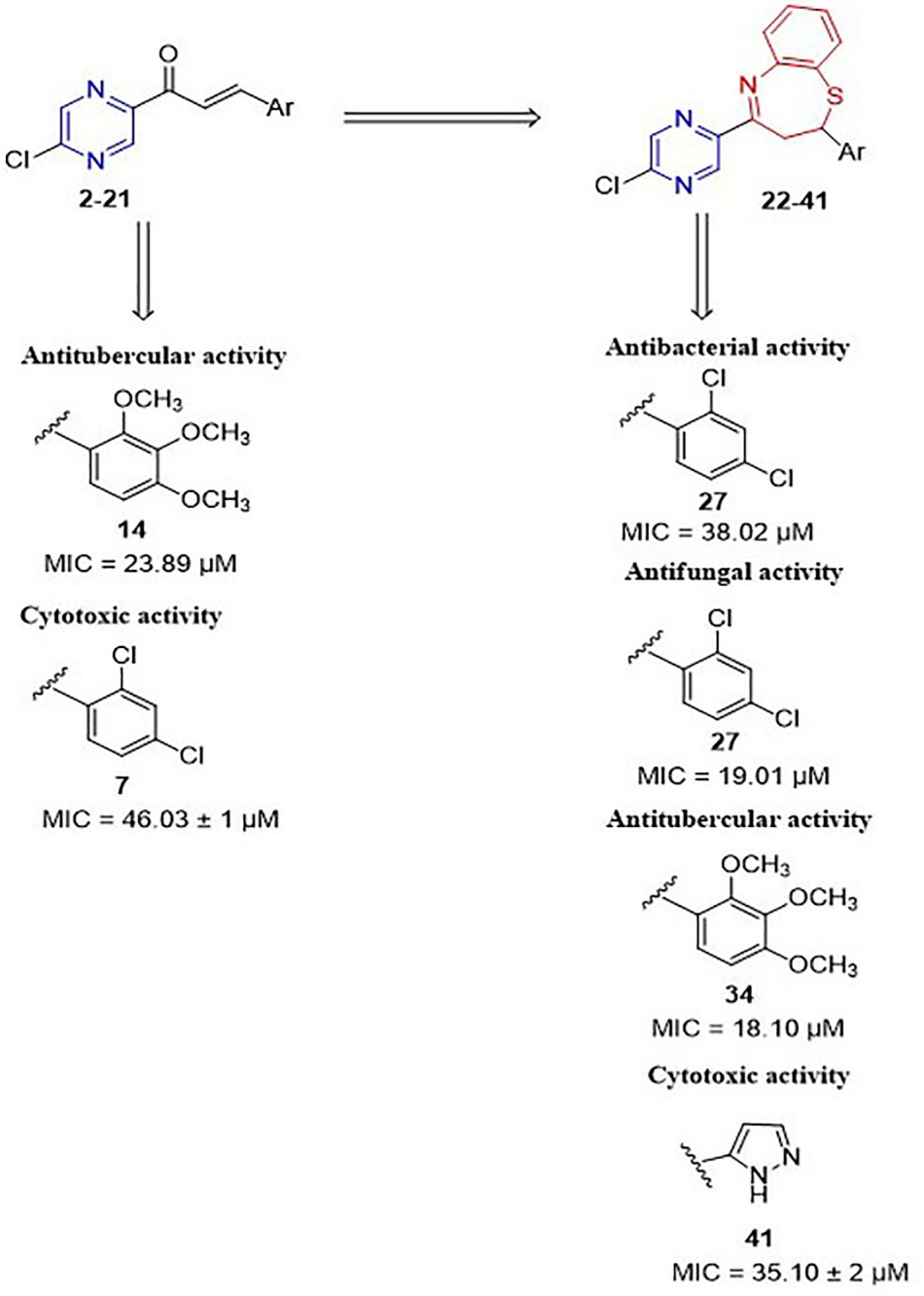
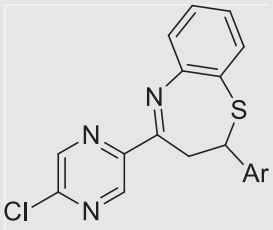


Fig. 4 Summary of the antibacterial, antifungal, antitubercular, and cytotoxic activities of chalcones (**2**–**21**) and benzothiazepines (**22**–**41**).

Table 2 Antibacterial activity of benzothiazepines **22–41** (expressed as MIC in μM).



22-41

Entry	Ar	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*
22	phenyl	> 200	> 200	> 200	> 200
23	4-chlorophenyl	82.83	82.83	82.83	82.83
24	4-fluorophenyl	86.52	86.52	86.52	86.52
25	4-nitrophenyl	80.63	80.63	80.63	80.63
26	2,4-difluorophenyl	41.25	41.25	41.25	41.25
27	2,4-dichlorophenyl	**38.02**	**38.02**	**38.02**	**38.02**
28	4-hydroxyphenyl	> 200	> 200	> 200	> 200
29	4-methylphenyl	> 200	174.92	> 200	> 200
30	4-methoxyphenyl	> 200	> 200	> 200	> 200
31	3-methoxy-4-hydroxyphenyl	> 200	> 200	> 200	> 200
32	4-dimethylaminophenyl	162.05	> 200	> 200	> 200
33	3,4-dimethoxyphenyl	> 200	> 200	> 200	> 200
34	3,4,5-trimethoxyphenyl	144.81	144.81	72.40	144.81
35	2-pyridinyl	> 200	> 200	> 200	> 200
36	3-pyridinyl	181.38	> 200	181.38	> 200
37	4-pyridinyl	181.38	181.38	181.38	181.38
38	2-thienyl	178.83	> 200	> 200	178.83
39	2-furfuryl	> 200	> 200	> 200	> 200
40	2-pyrrolyl	> 200	> 200	> 200	> 200
41	5-pyrazolyl	> 200	> 200	> 200	> 200
Ciprofloxacin		145.71	145.71	72.85	72.85

The bold numbers indicate the activity of most potent compounds.

Escherichia coli, and *Pseudomonas aeruginosa* using ciprofloxacin as standard.

The benzothiazepine series (**22–41**) (Table 2) overall antibacterial activity ranged from 38 to > 200 μM. For compounds **22–25, 28–30,** and **32**, the para position was substituted with either H (**22**), Cl (**23**), F (**24**), NO_2 (**25**), OH (**28**), CH_3 (**29**), OCH_3 (**30**), and $N(CH_3)_2$ (**32**). Compound **22** with H substituent showed inactivity with MICs > 200 μM against four bacterial species. Replacing H substituent (**22**) with electron withdrawing substituents chlorine (Cl) (**23**) fluorine (F) (**24**), or nitro (NO_2) (**25**) resulted in MICs between 80 and 86 μM suggesting an improvement in the activity against four bacterial species over compound 22. Changing the para position of electron withdrawing to electron donating groups OH (**28**), CH_3 (**29**), OCH_3 (**30**), and $N(CH_3)_2$ (**32**), a trend similar to chalcones **8–10** and **12** (Vegnesa et al., 2017) was observed causing a significant loss in potency with MICs between 162 and > 200 μM indicating that electron withdrawing groups are favored over donating groups at the para position of the phenyl ring.

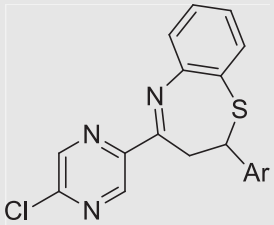
Based on our data obtained from compounds **23–25**, **28–30,** and **32**, we decided to synthesize few compounds having electron withdrawing and electron donating groups at ortho/para and meta/positions. As seen in compounds **26** and **27**, they showed 2-fold improvement in the antibacterial activity (38.02–41.25 μM) over the compounds **23–25** with electron withdrawing groups (**23–25**; 80.63–86.52 μM), the most active being compound **27** (MIC 38.02 μM). However, in case of disubstituted chalcones with electron donating groups **31** and **33**, it resulted in MICs between > 200 μM, suggesting loss of activity similar to compounds **28–30**.

Among the bioisosteres (**35–41**), there was no improvement in the activity in comparison to the standard ciprofloxacin. The MICs varied between 178- > 200 μM. The most potent among all benzothiazepines was compound **27** (MIC 38.02 μM) having two- to four-fold improvement in activity over ciprofloxacin against four bacterial species. Overall, chalcones (Vegesna et al., 2017) fared better over benzothiazepine derivatives. Comparing between the two series, it can be inferred that in chalcone and benzothiazepine series, physicochemical property was modulated by electron withdrawing groups in ortho/para positions thereby improving the antibacterial activity.

3.2.2. Antifungal activity

The antifungal activity (Table 3) of benzothiazepine series (**22–41**) ranged from 19 to > 200 μM. Compound **22** with H sub-

Table 3 Antifungal activities of benzothiazepines (**22–41**) (expressed as MIC in μM).



22-41

Entry	Ar	*A. niger*	*C. tropicalis*
22	phenyl	181.91	181.91
23	4-chlorophenyl	41.41	41.41
24	4-fluorophenyl	43.26	43.26
25	4-nitrophenyl	40.31	40.31
26	2,4-difluorophenyl	20.62	20.62
27	2,4-dichlorophenyl	**19.01**	**19.01**
28	4-hydroxyphenyl	173.98	173.98
29	4-methylphenyl	174.92	174.92
30	4-methoxyphenyl	167.59	> 200
31	3-methoxy-4-hydroxyphenyl	> 200	> 200
32	4-dimethylaminophenyl	162.05	> 200
33	3,4-dimethoxyphenyl	38.84	155.37
34	3,4,5-trimethoxyphenyl	36.20	36.20
35	2-pyridinyl	90.69	45.34
36	3-pyridinyl	45.34	45.34
37	4-pyridinyl	> 200	90.69
38	2-thienyl	**44.70**	**44.70**
39	2-furfuryl	93.61	187.23
40	2-pyrrolyl	> 200	187.77
41	5-pyrazolyl	187.23	187.23
Fluconazole		84.14	63.10

The bold numbers indicate the activity of most potent compounds.

stituent showed the lowest antibacterial activity with MIC of 181 μM against two fungal species in comparison to compounds 23–34. Replacing H substituent (**22**) with electron withdrawing substituents chlorine (Cl) (**23**), fluorine (F) (**24**), or nitro (NO_2) (**25**) resulted in MICs between 40 and 43 μM suggesting 4.2–4.5-fold improvement in the activity against two fungal species, respectively, over compound **22**. Changing the para position from electron withdrawing to electron donating groups OH (**28**), CH_3 (**29**), OCH_3 (**30**), and $N(CH_3)_2$ (**32**) resulted in a trend similar to chalcones **8**–**10** and **12** causing a significant drop in the activity with MICs between 162 and 174 μM against A. niger and 173.98–> 200 μM against C. tropicalis.

Electron withdrawing groups at ortho/para positions as seen in compounds **26** and 27 showed two-fold improvement in the antibacterial activity (19.01–20.62 μM) over compounds **23**–**25** with electron withdrawing groups (23–**24**; 40.31–43.26 μM). Compounds **26** & **27** (MICs 20.62 & 19.01 μM) had similar antifungal activity. However, in case of electron donating groups **31** (meta/para) and **33** (meta/para), it resulted in MICs between 38.84- > 200 μM with compound **33** (MIC 38.84 μM) having better activity against *A. niger* over compound **30** (MIC 167.59 μM vs *A. niger*).

The MICs of bioisosteres **35**–**41** varied between 44 and 751 μM. Among the six-membered pyridine ring containing bioisosteres (**35**–**37**), compound **36** showed the best activity of 45.34 μM. Although among five-membered heterocycles, the 2-thienyl containing benzothiazepine **38** showed the best activity of 44.70 μM; it was comparatively less than standard fluconazole. The most potent among all benzothiazepines identified was compound 27 (MIC 19.01 μM) having three- to four-fold improvement in activity over fluconazole. Overall, benzothiazepines were found to have comparable activity with chalcones (Vegesna et al., 2017).

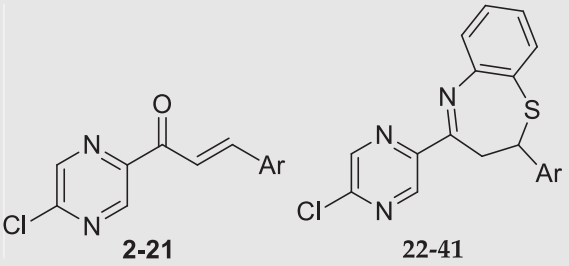
3.3. Antitubercular activity

Chalcone (**2**) with H substituent showed the modest antifungal activity with MIC 130.78 μM against *M. tuberculosis* (Table 4). Replacing H substituent (**2**) with electron withdrawing substituents chlorine (Cl) (**3**) and fluorine (F) (**4**) resulted in MICs between 57 and 60 μM suggesting nine-fold enhancement in the activity against two fungal species. Substitution of halogens with a nitro group **5** resulted in four-fold decrease in activity over **3** and **4**. It was observed that compounds **8**, **9**, **10,** and **12** having electron donating groups caused a significant drop in the activity (MICs 116–491 μM).

Having electron withdrawing groups at ortho and para position of phenyl ring resulted in further improvement in antitubercular activity by two-fold (**7** vs **3**, MICs 25.51 vs 57.32 μM; **6** vs **4**, MICs 28.50 vs 60.91 μM). Substituting meta and para position with electron donating groups (compounds **11** and **13**) showed modest activity (MICs 52–110 μM), with compound **13** having better activity over compound **11**. Interestingly, substituting the para and the two meta positions with methoxy substituent **14** improved the activity to 23.89 μM. Compound **14** was identified as the most potent of all the synthesized chalcones having 17-fold improved activity over pyrazinamide (MIC 412.76 μM). The next in potency among the chalcone series was compound **7** having MIC of 25.51 μM. Bioisoster-based chalcones **15**–**21** did not show any improvement in activity with MICs of 521–1095 μM.

The benzothiazepine series (**22**–**41**) fared slightly better than the chalcones (Table 4). The overall antitubercular activity ranged from 18 to 1497 μM. Compound **22** with H substituent showed the modest antibacterial activity with MIC of 181.89 μM. Substituting H substituent (**22**) with electron withdrawing substituents chlorine (Cl) (**23**) and fluorine (F) (**24**) resulted in MICs between 43 and 82 μM, suggesting two- to + improvement in the activity against *M. tuberculosis* over compound **22**. However, inserting a 4-nitro (**25**) substituent surprisingly proved to be deleterious for the activity in comparison to compounds **23** and **24**. This trend was found to be similar to chalcones **3**, **4** in comparison to **5**. Changing the para position from electron withdrawing to electron donating groups OH (**28**), CH_3 (**29**), OCH_3 (**30**), and $N(CH_3)_2$ (**32**) resulted in attenuation in activity with MICs between 167 and > 500 μM, which highlights the importance of electron withdrawing group on the potency.

Table 4 Antitubercular activities of chalcones (**2**–**21**) and benzothiazepines (**22**–**41**) (expressed as MIC in μM).



#	Ar	MIC values (μM) of *M. tuberculosis* H37Rv	#	MIC values (μM) of *M. tuberculosis* H37Rv
2	phenyl	130.78	22	181.89
3	4-chlorophenyl	57.32	23	82.83
4	4-fluorophenyl	60.91	24	43.26
5	4-nitrophenyl	220.93	25	322.54
6	2,4-difluorophenyl	28.50	26	41.25
7	2,4-dichlorophenyl	**25.51**	27	38.02
8	4-hydroxyphenyl	491.02	28	> 500
9	4-methylphenyl	123.69	29	174.92
10	4-methoxyphenyl	116.49	30	167.59
11	3-methoxy-4-hydroxyphenyl	110.07	31	160.85
12	4-dimethylaminophenyl	444.83	32	324.11
13	3,4-dimethoxyphenyl	52.50	33	38.84
14	3,4,5-trimethoxyphenyl	**23.89**	34	**18.10**
15	2-pyridinyl	> 500	35	> 500
16	3-pyridinyl	> 500	36	> 500
17	4-pyridinyl	> 500	37	> 500
18	2-thienyl	> 500	38	> 500
19	2-furfuryl	> 500	39	> 500
20	2-pyrrolyl	> 500	40	> 500
21	5-pyrazolyl	> 500	41	> 500
Pyrazinamide		412.76		

The bold numbers indicate the activity of most potent compounds.

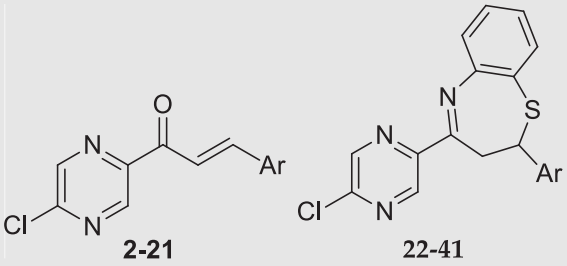
Electron withdrawing groups at ortho/para positions as seen in compounds **26** and **27** showed either similar or two-fold improvement in the antitubercular activity (38.02–41.25 μM) over the compounds **23**–**25** with electron withdrawing groups (**23**–**24**; 43.26–82.83 μM), the most active being compound **27** (MIC 38.02 μM). Additionally, in case of electron donating groups **31** (meta/para), **33** (meta/para), it resulted in MICs between 38.84 and 160.85 μM, with compound **33** having 4.3-fold better activity over compound **30** (MIC 167.59 μM). Interestingly, compound **34** having three methoxy groups at positions 3, 4, and 5 on the phenyl ring resulted in two-fold improvement in activity over compound **33**, a trend found similar in chalcones (**14** vs **13**).

Introducing bioisosteric replacement for phenyl ring did not show any improvement in MICs as compounds **35**–**41** displayed inactivity with MICs > 500 μM. The activity trend of benzothiazepines were similar to chalcones (**27** vs **7** and **34** vs **14**). The most potent compound identified was **34** (MIC 18.01 μM) having 23-fold improvement in activity over pyrazinamide. Overall, benzothiazepines were found to have antitubercular activity comparable with chalcones (**34** vs **14;** MICs 18.01 vs 23.89 μM).

3.4. Cytotoxic activities

All the 40 compounds were evaluated for their cytotoxic activity against prostate cancer cell line, **DU-145**, employing MTT assay (Table 5). Among the chalcone series, compound **7** was found to be the most potent with IC_{50} 46.03 ± 1 μM, whereas in benzothiazepine series, compound **41** had 1.3-fold better activity over **7** with the IC_{50} of 35.10 ± 2 μM. The activity of these compounds was found to be 3–4–fold less than the standard, methotrexate (IC_{50} = 11 ± 1 μM). The compounds **4**, **6**, **26,** and **35** were next in activity with IC_{50} values 124.27 ± 1, 121.18 ± 2, 72.19 ± 2 and 51.01 ± 1 μM, respectively. All the other compounds exhibited modest to inactivity with MICs ranging from 99.82 ± 2 to > 200 μM. The structure activity relationship (SAR) features of chalcones and benzothiazepines indicated that electron withdrawing group (Cl) at ortho and para positions (**7**) and 5-pyrazolyl heterocyclic ring (**41**) played a key role in cytotoxic activity. The compounds were also tested against the human normal liver cells (**L02**) and were found to be less selective towards this cell type as the IC_{50} is beyond the highest concentration tested. (Table 6). A summary of the antibacterial,

Table 5 Cytotoxic activity results of chalcones (**2**–**21**) and benzothiazepines (**22**–**41**) (IC_{50} values in μM).



#	Ar	DU-145	#	DU-145
2	phenyl	> 200	22	122.21 ± 1
3	4-chlorophenyl	> 200	23	119.08 ± 2
4	4-fluorophenyl	124.27 ± 1	24	156.82 ± 2
5	4-nitrophenyl	> 200	25	> 200
6	2,4-difluorophenyl	121.18 ± 2	26	72.19 ± 2
7	2,4-dichlorophenyl	**46.03 ± 1**	27	99.82 ± 2
8	4-hydroxyphenyl	> 200	28	> 200
9	4-methylphenyl	> 200	29	> 200
10	4-methoxyphenyl	> 200	30	> 200
11	3-methoxy-4-hydroxyphenyl	> 200	31	> 200
12	4-dimethylaminophenyl	> 200	32	121.54 ± 2
13	3,4-dimethoxyphenyl	> 200	33	> 200
14	3,4,5-trimethoxyphenyl	> 200	34	158.39 ± 2
15	2-pyridinyl	> 200	35	51.01 ± 1
16	3-pyridinyl	> 200	36	> 200
17	4-pyridinyl	> 200	37	181.38 ± 2
18	2-thienyl	> 200	38	> 200
19	2-furfuryl	> 200	39	> 200
20	2-pyrrolyl	> 200	40	> 200
21	5-pyrazolyl	> 200	41	**35.10 ± 2**
Methotrexate		11 ± 1		

Data presented as mean ± SD (n = 3). All the compounds and the standard drug were dissolved in DMSO and then diluted with culture medium containing 0.1% DMSO. The control cells were treated with culture medium containing 0.1% DMSO.
The bold numbers indicate the activity of most potent compounds.

antifungal, antitubercular, and cytotoxic activities of chalcones (**2**–**21**) and benzothiazepines (**22**–**41**) is depicted in Fig. 4.

4. Conclusions

In our ongoing projects on the synthesis of compounds with potential antimicrobial, antitubercular, and cytotoxic activities, we have here reported 20 new compounds bearing benzothiazepine nucleus and evaluated our 20 previously reported chalcones for antitubercular and cytotoxic activities. Biological data indicated that benzodiazepines demonstrated excellent antifungal and antitubercular activities. It was observed that the electronic property (electron withdrawing and electron releasing) of the substituents on the phenyl ring was instrumental for the difference in the potency of the compounds. For instance, among benzothiazepines, electron withdrawing groups resulted in excellent antimicrobial and antifungal activity. Structure activity relationship studies of chalcones indicated that electronic properties did not play a key role for antitubercular activities as evident by compounds **7** and **14** whereas a similar phenomenon was observed for benzothiazepine **34**. None of the compounds showed any improvement in cytotoxic activity over the standard drug methotrexate. All the compounds were found to be relatively less selective towards the human normal liver cell line LO2. Further studies are under progress to assess the computational binding characteristics of chalcones and benzodiazepines, which can give a fair understanding of newer analogs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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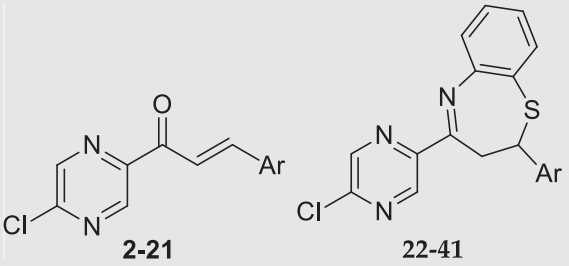
Table 6 Cytotoxic activity (IC_{50} in μg/mL) of chalcones (2–21) and benzothiazepines (22–41).



Entry	Ar	L02	#	L02
2	phenyl	> 50	22	> 40
3	4-chlorophenyl	> 50	23	> 40
4	4-fluorophenyl	> 50	24	> 40
5	4-nitrophenyl	> 50	25	> 40
6	2,4-difluorophenyl	> 50	26	> 40
7	2,4-dichlorophenyl	> 50	27	> 40
8	4-hydroxyphenyl	> 50	28	> 40
9	4-methylphenyl	> 50	29	> 40
10	4-methoxyphenyl	> 50	30	> 40
11	3-methoxy-4-hydroxyphenyl	> 50	31	> 40
12	4-dimethylaminophenyl	> 50	32	> 40
13	3,4-dimethoxyphenyl	> 50	33	> 40
14	3,4,5-trimethoxyphenyl	> 50	34	> 40
15	2-pyridinyl	> 50	35	> 40
16	3-pyridinyl	> 50	36	> 40
17	4-pyridinyl	> 50	37	> 40
18	2-thienyl	> 50	38	> 40
19	2-furfuryl	> 50	39	> 40
20	2-pyrrolyl	> 50	40	> 40
21	5-pyrazolyl	> 50	41	> 40
Methotrexate		–		

Data presented as mean ± SD (n = 3). All the compounds and the standard drug were dissolved in DMSO and then diluted with culture medium containing 0.1% DMSO. The control cells were treated with culture medium containing 0.1% DMSO.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2020.102915.

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