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Evaluation of some new synthesis benzothiazole and benzimidazole derivatives as potential antimicrobial and anticancer agents

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ABSTRACT

There is an urgent global need to develop new antimicrobial and anti-cancer drugs. In the current study, the biological evaluation of some synthesized phenylsulfonyl of benzothiazole and benzimidazole moiety-containing pyrazolo [5, 1-c]-l, 2, 4-triazine derivative [6, 7, 11.12, 16 and 17], arylhydrazone derivatives and pyridazine derivative [20, 21, 25, 26, 29 and 29] was carried out for antimicrobial and anticancer activity. The synthesized compounds containing pyrazolo [5, 1-c]-l, 2, 4-triazine derivative [6, 7] exhibited higher activity against Staphylococcus aureus compared with control drug Chloramphenicol. While arylhydrazones 20 and 21 were found to be equal to the control drug. For antifungal activity, the compounds 6, 7, 20 and 21 were possessed the same potency as cycloheximide against Aspergillus fumigatus. The anticancer activity on Hepatocellular carcinoma (HEPG2) of the compounds 6, 7, 20 and 21 exhibited excellent activities, more potent than the reference drug. The findings of this study are worthwhile; however, further pharmaceutical and toxicological studies are recommended to be carried out.

1. Introduction

In recent decades, many outbreaks of antimicrobial resistance have been reported. This alarming phenomenon mainly results from the rapid development of microbial mutation and resistance to drugs, while innovations in the pharmaceutical industry are very slow and disproportionate to this growing risk, which requires new strategies to control these mutant pathogens and launching extensive investigations to develop new antimicrobial drugs (Cheng et al., 2016; Cole, 2013). Similarly, the increasing recurrence of carcinoid tumors worldwide with limited effective medications shows the constant need to develop new anticancer drugs (Ali et al., 2012). Benzothiazole and benzimidazole derivatives are notable pharmacological activities heterocyclic compounds (Anand and Wakode, 2017; Váradi et al., 2014). Their derivatives possess important biological activities (Dai et al., 2013; Telvekar et al., 2012), anti-tumour (Weekes and Westwell, 2009), anticancer (Solomon et al., 2009), antiproliferative agent (Racané et al., 2018), anthelmintic and antiprotozoal (Mavrova et al., 2010; Torres-Gómez et al., 2008), antibacterial (He et al., 2004), antifungal (Göker et al., 2002), antiphrastic (Kopańska et al., 2004). β -keto sulfones have attracted in synthetic chemists (Gopalan and Jacobs, 1990). The different pharmacological activities of heterocycles directed to synthesis benzothiazole and benzimidazole derivatives (Ali et al., 2016; Farag et al., 2008). The research program investigated synthesized β-ketosulphone benzimidazole and benzothiazole derivatives (1, 2) as an intermediate for the synthesis of new heterocyclic derivatives (Darweesh et al., 2016).

In continuation of a research program and an attempt to develop new antimicrobial and anticancer drugs, the current study was carried out, which aimed to evaluate some biological properties (antimicrobial and anticancer) of some synthesized heterocyclic derivatives containing pyrazoloazine and pyridazine incorporating benzothiazole and benzimidazole moieties.

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2. Materials and methods

2.1. Chemistry

All melting points were determined using an open glass capillary melting point apparatus. The infrared spectrophotometers using potassium bromide disks method. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury (VXR-300) NMR spectrometer at 300 and 75 MHz respectively using $CDCl_3$ and DMSO-d_6. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 e.v. β -keto sulfones 1 and 2 (Darweesh et al., 2014), 3-(4-chlorophenyl)-5-amino-1H-pyrazole (3) (Elnagdi et al., 1976), 2-cynomethylbenzothiazole (18) (Copeland and Day, 1943), diazonium salts of 5-amino-l,2,4-triazole (8) and 2-amino-1H-benzimidazole (13) (Elnagdi et al., 1979) were been ready by using methods from the literature.

2.2. The reaction of β -keto sulfones 1 and 2 with diazonium salt of heterocyclic amines 3, 8, 13 General procedure

Dazonium salt of (5-amino-3- phenylpyrazole (3), 3-amino-l, 2, 4-triazole (8), or 2-aminobenzimidazole (13) (2 mmol) was mixed to a stirred cold solution of β -keto sulfones 1 and 2 (2 mmol) in pyridine (30 ml) for 30 min at 0-5ºC. The mixture was stirred for 3 h after addition complete. The solid was obtained by filtration, washed thoroughly with H2O, dried. Finally, recrystallization from DMF/H2O to afford 6, 7, 11, 12, 16 and 17, respectively.

2-(7-(4-Chlorophenyl)-3-(phenylsulfonyl) pyrazolo [5, 1-c] [1, 2, 4]-triazin-4-yl) benzothiazole (6)

Yield 78%; (mp. 170˚C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.42-8.13 (m, 13H, ArH's), 6.99 (s, 1H, pyrazolo-8-CH). MS (m/z): 503.03 (M+) (100); IR (KBr): υ: 1597 cm^{-1} (C=N). Anal. Found (calculated). For $C_{24}H_{14}N_5ClO_2S_2$ (%): C, 57.19 (57.20); H, 2.83 (2.80); S, 12.68 (12.72); N, 13.94 (13.90).

7-(4-Chlorophenyl)-4-(1-methyl-1H-benzimidazol-2-yl)-3-(phenylsulfonyl) pyrazolo [5, 1-c]-1, 2, 4-triazine (7)

Yield 72%; (mp. 188 ˚C); 1H NMR (300 MHz, DMSO-d6): δ 4.02 (s, 3H, NCH_3), δ 7.20-7.89 (m, 13H, ArH's), 6.89 (s, 1H, pyrazolo-8-CH). MS (m/z): 500 (M+) (100); IR (KBr): υ: 1597 cm^{-1} (C=N). Anal. Found (calculated) For $C_{25}H_{17}N_6ClO_2S$ (%): C, 59.96 (59.94); H, 3.39 (3.42); S, 6.41 (6.40); N, 16.80(16.78).

2-(3-(Phenysulfonyl) 1-[1, 2, 4] triazolo [5, 1-c][1, 2, 4]triazin-4-yl) benzothiazole (11)

Yield 72%; (mp. 205-206 ˚C); 1H NMR (300 MHz, DMSO-d6): δ 7.43-8.41 (m, 9H, ArH's), 8.57 (s, 1H, triazole-7-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 120.23, 121.53, 124.30, 125.32, 128.10, 129.57, 133.43, 133.02, 136.22, 152.54, 156.04, 156.05, 156.63; MS (m/z): 394(M+) (100); IR (KBr): υ: 1607 cm^{-1} (C=N). Anal. Found (calculated). For $C_{17}H_{10}N_6O_2S_2$ (%): C, 51.73 (51.77); H, 2.57(2.56); S, 16.23 (16.26); N, 21.34 (21.31).

4-(1-Methyl-1H-benzimidazol-2-yl)-3(phenylsulfonyl)-[1, 2, 4]-triazolo[5, 1-c] [1, 2, 4]-triazine (12)

Yield 72%; (mp. 110 ˚C); 1H NMR (300 MHz, DMSO-d6): δ 4.02 (s, 3H, NCH3), δ 7.20-8.04 (m, 9H, ArH's), 8.47 (s, 1H, triazole-7-CH). ^{13}C NMR (75 MHz, DMSO-d6): δ 31.97, 111.67, 121.43, 123.60, 128.10, 129.22, 133.36, 134.01, 136.02, 140.54, 141.11, 148.45, 148.57. MS (m/z): 391 (M+) (100); IR (KBr): υ: 1607 cm^{-1} (C=N). Anal. Found (calculated). For $C_{18}H_{13}N_7O_2S$ (%): 55.19 (C, 55.23); H, 3.25 (3.35); S, 8.21(8.19); N, 25.19 (25.05).

4-(Benzothiazol-2-yl)-3-(phenylsulfonyl)- [1, 2, 4] triazino [4, 3-a] benzimidazole (16)

Yield 70%;(mp. 166 ˚C); 1H NMR (300 MHz, DMSO-d6): δ 7.41-8.16 (m, 13H, ArH's). MS (m/z): 443 (M+) (100); IR (KBr): υ: 1607 cm^{-1} (C=N) Anal. Found (calculated). For $C_{22}H_{13}N_5O_2S_2$ (%): C, 59.56 (59.58); H, 3.01 (2.95); S, 14.42 (14.46); N, 15.76 (15.79).

4-(1-Methylbenzimidazol-2-yl)-3 (phenylsulfonyl)-1, 2, 4-triazin- o[4, 3-a] benzimidazole (17)

Yield 70%; (mp. 175 ˚C); 1H NMR (300 MHz, DMSO-d6): δ 4.02 (s, 3H, NCH_3), δ 7.38-7.94 (m, 13H, ArH's). MS (m/z): 440 (M+) (100); IR (KBr): υ: 1607 cm^{-1} (C=N). Anal. Found (calculated) For $C_{23}H_{16}N_6O_2S$ (%): C, 62.68 (62.72); H, 3.64 (3.66); S, 7.33 (7.28); N, 19.12 (19.08).

2.3. The reaction of β -keto sulfones 1 and 2 with benzene diazonium chloride

Sodium acetate trihydrate (4 g). was added to a stirred cold solution of keto-sulfone of benzothiazole 1 and benzimidazole 2 (10 mmol) in ethanol (25 ml), the mixture was cooled to 0°C and treated with aniline diazonium salt solution (10 mmol) with rapid stirring for 30 min and continued stirred for further 2h at 0°C then stored at 4°C for 6 h. The mixture was filtrated to collect the solid product, washed with water and dried; then recrystallized from ethanol to yield arylhydrazone 20 and 21 respectively.

1-(benzothiazol-2-yl)-2-(2-phenylhyrazono)-2-(phenylsulfonyl) ethanone (20)

Yield 80%; (mp. 180 ˚C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.07-8.33 (m, 14H, ArH's) 11.11 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 113.05, 120.94, 122.74, 124.35, 125.29, 128.40, 129.20, 129.28, 133.52, 138.27, 139.40, 142.16, 160.25, 162.67, 182.35, 154.21. MS (m/z): 421 (M+) (100); IR (KBr):

υ: 1597 (C=N), 1630 (CO), 3215 (NH) cm^{-1} Anal. Found (calculated) For $C_{21}H_{15}N_3O_3S_2$ (%): C, 59.79(59.84); H, 3.62 (3.59); S 15.23(15.21); N 9.99 (9.97).

1-(1-Metylbenzimdazol-2-yl)-2-(2-phenylhyrazono)-2-(phenyl- sulfonyl) ethanone (21)

Yield 74%; (mp. 204 ˚C); ^1H NMR (300 MHz, DMSO-d6): δ 4.02 (s, 3H, NCH_3), 6.79-7.86 (m, 14H, ArH's) 11.01 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d6): δ 31.95 (NCH_3), 111.67, 114.62, 121.40, 121.43, 123.60, 128.10, 129.11, 129.22, 133.36, 134.01, 137.05, 139.45, 142.36, 141.11, 182.34, 145.21. MS (m/z): 418 (M+) (100); IR (KBr): υ: 1611 (C=N), 1648 (CO), 3543 (NH) cm^{-1} Anal. Found (calculated) for $C_{22}H_{18}N_4O_3S$ (%): C, 63.12 (63.14); H, 4.41 (4.34); S, 7.70 (7.66); N, 13.36 (13.39).

2.4. The reaction of arylhydrazone 20 and 21 with cyanomethyl benzothiazole (22) and with malonitrile

(5mmol), cyano-methyl-benzothiazole (22) or malonitrile, in ethanol (25 ml) was added to a solution of arylhydrazone 20 and 21 (5mmol). Drops of piperdine were added to the mixture, refluxed for 3-4 h then poured into ice-cold water. Dil. HCl was added to neutralize the mixture and formed a precipitate. The solid product was collected.by filtration. The crystals of the pyridazine derivative 25, 26, 29, and 30 were afforded by the recrystallized solid from DMF.

(4, 5)- (Bisbenzthiazol-2-yl)-1, 6-dihydro-6-imin-3-(phenylsulfonyl)-1-(phenyl) pyridazine (25)

Yield (50%); (mp. 258 ˚C); ^1H NMR (300 MHz, DMSO-d_6): δ 6.74-8.14 (m, 18H, ArH's), 11.88 (s, 1H, NH). MS (m/z): 577 (M+) (100); IR (KBr): υ: 1620 (C=N), 3332 (NH) cm^{-1} Anal. Found (calculated for $C_{30}H_{19}N_5O_2S_3$ (%): C, 62.35 (62.37); H, 3.34 (3.32); S, 16.68 (16.65); N, 12.08 (12.12).

5- (Benzthiazol-2-yl)-1, 6-dihydro-6-imino-3-(1-methylbenzimidazol-2-yl)-3-(phenylsulphonyl)-1-(phenyl) pyridazine (26)

Yield (55%); (mp. 258 ˚C); 1H NMR (300 MHz, DMSO-d_6): δ 4.02 (s, 3H, NCH_3), 6.67-8.14 (m, 18H, ArH's), 11.88 (s, 1H, NH). MS (m/z): 574 (M+) (100); IR (KBr): υ: 1620 (C=N), 3332 (NH) cm^{-1} Anal. Found (calculated). For $C_{31}H_{22}N_6O_2S_2$ (%): C, 64.75 (64.79); H, 3.89 (3.86); S, 11.14 (11.16); N, 14.65 (14.62).

4- (Benzthiazol-2-yl)-5- (cyano)-1, 6- dihydro-6-imin-3 (phenyl-sulfonyl)-1-(phenyl) pyridazine (29)

Yield (52%); (mp. 240 ˚C); 1H NMR (300 MHz, DMSO-d_6): δ 6.94-8.14 (m, 14H, ArH's), 11.88 (s, 1H, NH). MS (m/z): 469 (M+) (100); IR (KBr): υ: 1597 (C=N), 2233 (C≡N), 3332 (NH) cm^{-1} Anal. Found (calculated). For $C_{24}H_{15}N_5O_2S_2$ (%): C, 61.40 (61.39); H, 3.24 (3.22); S, 13.65 (13.66); N, 14.88 (14.92).

4- (1-Methylbenzimidazol-2-yl)-5- (cyano)-1, 6-dihydro-6-imin-3- (phenylsulfonyl)-1- (phenyl) pyridazine (30)

Yield (60%); (mp. 220 ˚C); ^1H NMR (300 MHz, DMSO-d6): δ 4.02 (s, 3H, NCH_3), 6.54-7.92 (m, 14H, ArH's), 11.88 (s, 1H, NH). MS (m/z): 466 (M+) (100); IR (KBr): υ: 1597 (C=N), 2233 (C≡N), 3332 (NH) cm^{-1} Anal. Found (calculated). For $C_{25}H_{18}N_6O_2S$ (%): C, 64.41 (64.36); H, 3.86 (3.89); S, 6.89 (6.87); N, 18.04 (18.01).

2.5. Antifungal activity

The synthesized compounds were screened (in vitro) for antifungal potential against selected fungi, namely *Aspergillus fumigatus* (RCMB 002003), *Geotrichum candidum* (RCMB 002006), *Candida albicans* (RCMB 005002) and *Syncephalastrum racemosum* (RCMB 005003) using Sabourad dextrose agar. The cultures of fungi were purified by a single spore isolation assay. The antifungal activity test was performed by the agar well diffusion method as mentioned in Choudhary and Thomsen (2001). Briefly, Sabouraud dextrose agar was prepared as the manufacturer's instructions, poured in sterile plates, left until solidified, and kept at room temperature upside-down for 15 minutes to remove the moisture. 0.1 ml of Fungal culture was swabbed over the sabouraud dextrose agar plates and left for about five minutes. Wells of size 6 mm were punched out on the agar plates using a good cutter. 100 µl of the tested samples (at a concentration of 10 mg/mL) was poured into the wells. All tested compounds were dissolved in dimethyl sulfoxide (DMSO), and the solvent was loaded separately as a negative control. Thereafter, the plates were incubated at 30˚C for 3-4 days. The plates were then inspected for the presence of a zone of inhibition. The inhibition zone was measured three times to get a mean value. A standard antifungal drug, cycloheximide was used as a positive control.

2.6. Antibacterial activity

Agar well diffusion method was used in the screening of the antibacterial potential of the synthesized compounds as mentioned in the literature (Choudhary and Thomsen, 2001). Briefly, bacterial strains were sub-cultured overnight prior of the experiment, these strains were *Staphylococcus aureus* (RCMB 000106) and *Bacillis subtillis* (RCMB 000107) as Gram positive bacteria, while a Gram-negatives were *Pseudomonas aeruginosa* (RCMB 000102) and *Escheirchia coli* (RCMB 000103) In a septic conditions, Petri-dishes containing Nutrient agar were prepared and bacterial strains were swabbed over the solidified agar. Wells (6mm) were made using sterile metallic bores and 100 µl of the tested compounds (concentration 10 mg/Ml) were loaded into the wells. All compounds were prepared

in dimethyl sulfoxide (DMSO) and DMSO was also loaded as a control. Chloramphenicol and Cephalothin were used as antibacterial standard drug. The plates were kept for incubation overnight for 24 hours and then the plates were inspected for the appearance of the zone of inhibition. Each inhibition zone was measured three times to get a mean value.

2.7. Anti-cancer activity

The synthesized compounds were sent to the Bioassay-Cell Culture Laboratory, for in-vitro primary antitumor screening on Hepatocellular carcinoma (HEPG2) (American Type Culture Collection). Cell viability was specified by the mitochondrial-dependent reduction of the yellow MTT (3-(4, 5- dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide) to purple formazan.

The following method was carried out in aseptic conditions using a Laminar flow cabinet (Baker, SG403INT, Sanford, ME, USA). Hepatocellular carcinoma (HEPG2) cell line was cultured in RPMI-1640 and MCF7 cell lines were cultured in DMEM. Cells were plated in 96-well plates (having about 10000 cells/well). The cultured plates were incubated incubation at 37°C for about 24 hours and 5% CO_2 atmosphere before treatment with the compounds to enable direct attachment of the cell to the plate. The investigated compounds were dissolved in DMSO. Different concentrations of the tested compounds (50, 25, 12.5, 6.25, 3.125 and 1.56 ug) were added to the cell monolayer. Thereafter, the plate was incubated for 48 hours at 37°C. After the incubation period, media were aspirated and a crystal violet solution (1%) was loaded to each well and left for around 30 minutes. The strain was removed, and the plates were rinsed using tap water until the removal of all excess stain. Then, glacial acetic acids (30%) were added to all wells and mixed thoroughly, subsequently, the absorbance of the plates was measured. The treated samples were compared with the non-treated (control). All tests were carried out in triplicate and the cell cytotoxic effect of each tested compound was measured (Mosmann, 1983).

3. Results and discussion

3.1. Chemistry

The diazonium salts of 3-chloropheny-5-amino-1H-pyrazole (3), 5-amino-l,2,4-triazole (8) or 2-amino-1H-benzimidazole (13), was added to the cold solution of β-ketosulfones 1 and 2 in pyridine to afford intermediates 4, 5, 9, 10, 14 and 15 respectively which underwent cyclization to form pyrazolo [5, 1-c]-l, 2, 4- triazine 6 and 7, triazolo [5, 1-c](1, 2, 4) triazine and triazino [4, 3-a] benzimidazole derivatives (6, 7, 11, 12, 16 and 17) respectively (Fig. 1).

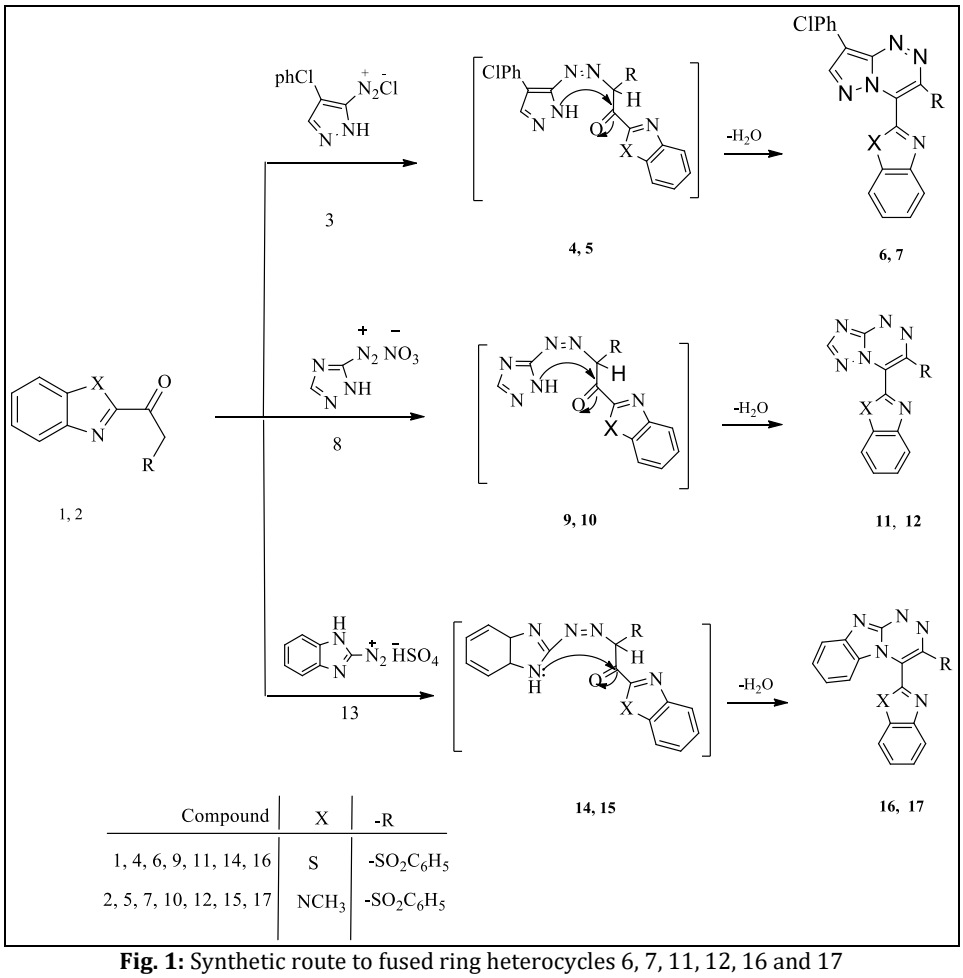
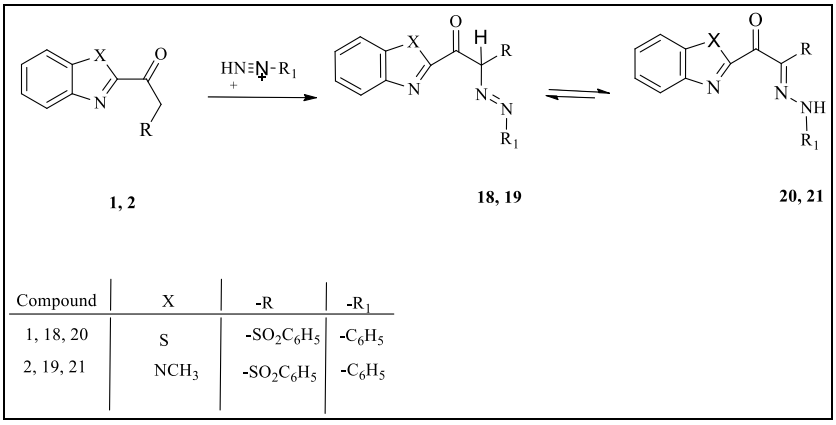


Fig. 1: Synthetic route to fused ring heterocycles 6, 7, 11, 12, 16 and 17

The structures of compounds (6, 7, 11, 12, 16 and 17) were characterized based on their spectral data and elemental analyses. The formation of pyrazoles and triazoles derivatives (6, 7, 11, 12) was confirmed by the disappearance of NH band in IR spectra. The ^{1}H NMR spectra of compounds (6, 7, 11, 12, 16 and 17) displayed a multiplet signal between δ (7.20-8.41) ppm which were attributed to aromatic protons. While a singlet signal of (CH) in pyrazoles and triazoles derivatives (6, 7, 11, 12) appeared around δ (6.89-8.57). A singlet signal δ 4.02 (N-CH3) appeared in the spectra of benzimidazole derivatives (7, 12 and 17). The C13 NMR spectra of compounds 11 and 12 displayed the most characteristic carbon signals, CH3 signals appeared around δ 31.97, C=N signal appeared around δ 141.0-157.0 and aromatic carbon peaks at range δ 111.0-136.0. In addition, the mass spectra of synthesized compounds were agreement with expected structures.

Coupled of the β-ketosulfones 1 and 2 with diazotized aniline, to afford arylhydrazone derivatives 20 and 21 (Fig. 2) as starting materials for synthesized biologically interesting pyridazine derivatives (Kaji et al., 1984). The β-ketosulfones 1 and 2 coupled with diazotized aromatic amines such as aniline, to give the corresponding arylhydrazone derivatives 20 and 21 (Fig. 2). The obtained arylhydrazones 20 and 21 have been utilized as starting materials for the synthesis of a pyridazine ring systems, which are considered as interesting biologically molecules.



Compound	X	-R	-R_1
1, 18, 20	S	$-SO_2C_6H_5$	$-C_6H_5$
2, 19, 21	NCH_3	$-SO_2C_6H_5$	$-C_6H_5$

Fig. 2: Synthetic route to arylhydrazone derivatives 20 and 21

Thus, the arylhydrazones 20 and 21 condensed with 2-cynomethylbenzothiazole (22) and malonnitril under reflux in ethanol with catalytic piperidine to formed pyridazine derivatives 25, 26, 29 and 30 respectively (Fig. 3). The obtained pyridazines structures were constructed based on their spectral data and elemental analyses. As in IR spectra, the appearance of the absorption band in obtained compounds near 3332 cm^{-1} indicated the presence of NH and band around (1597-1620) due to C=N, while (C≡N) band at 2233 appeared in 29 and 30. From^{1}H NMR spectra a singlet signals near 11.88 ppm due NH protons in all synthesized compounds. The structure of the obtained compounds was also confirmed by their mass spectra.

3.2. Antimicrobial evaluation

The synthesized compounds were screened for them in vitro antibacterial activity against *Streptococcus aureus* and *Bacillis subtilis* as Gram-positive bacteria and *Escherichia coli* and *Salmonella typhimurium* as Gram-negative bacteria. Synthesized compounds were also evaluated for them in vitro antifungal potential against some fungal strains, namely *Aspergillus fumigatus*, *Candida albicans, Geotricum candidum, and Syncephalastrum racemosum*. Microorganisms were tested against the activity of solutions of concentrations (5 µg/mL) and using inhibition zone diameter (IZD) in mm as an indicator for the antimicrobial activity (agar well diffusion assay). The fungicide drugs; Cycloheximide and the bactericide drugs; Chloramphenicol and Cephalothin were used as references to determine the efficacy of the tested compounds under the same conditions. The results are summarized in Table 1 and Table 2. Generally, the most susceptible bacteria to the tested compounds were the gram positives (*Staphylococcus aureus* and *Bacillus subtilis*). Whereas the most susceptible fungi to the examined derivatives were *Aspergillus fumigatus* Also, all other microorganisms also showed varied sensitivity to the tested compounds.

The results in Table 1 revealed that 2- (7- (4-Chlorophenyl)-3- (phenylsulfonyl) pyrazolo [5, 1-c][1, 2, 4]- triazin-4-yl) benzothiazole (6) and 7-(4-Chlorophenyl)-4- (1-methyl- 1H- benzimidazol-2-yl)-3- (phenylsulfonyl) pyrazolo [5, 1-c]-1,2 ,4- triazine (7) were found to be more active compared to the standard drug Chloramphencol against Staphylococcus aureus, while the compounds: arylhydrazones 20 and 21, were found to be equipotent to Staphylococcus aureus. As well, the results of the antifungal activity of the synthesized compounds showed that the compounds 6, 7, 20 and 21 were equipotent to the standard drug cycloheximide against *Aspergillus Fumigatus*. Moreover, some compounds, like derivatives numbers 11, 12, 16, 17, 25 and 26 showed moderate activity against *Staphylococcus aureus*.

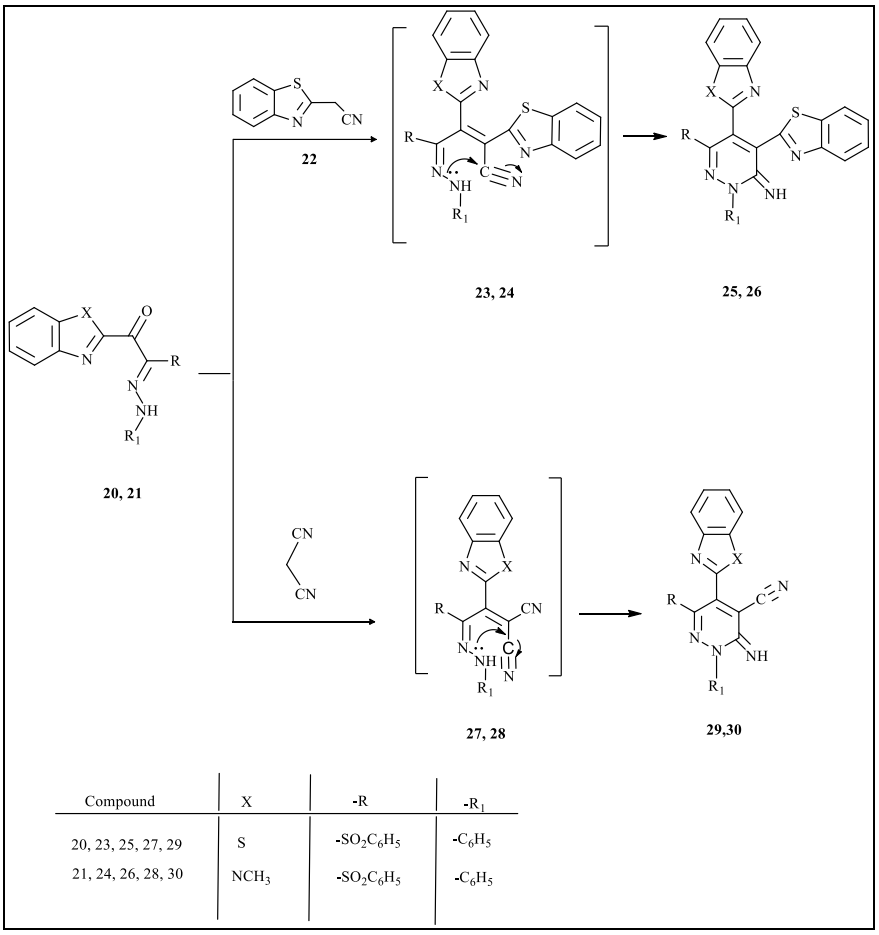


Fig. 3: Synthetic route to pyridazine derivative 25, 26, 29 and 30

Table 1: Antibacterial activities of the synthesized compounds

Tested compounds	Gram (+)		Gram (-)	
	Staphylococcus aureus (SA)	*Bacillus subtilis* (BS)	*Escherichia coli anaerobic* (EC)	*Salmonella typhimurium* (ST).
6	25.4±0.03	23.3±0.07	16.5±0.04	13.4±0.03
7	25.3±0.04	25.3±0.02	23.3±0.09	20.2±0.07
11	21.5±0.04	19.2±0.05	16.6±0.04	13.6±0.01
12	23.3±0.01	21.4±0.08	17.3±0.06	15.9±0.05
16	20.7±0.03	18..7±0.01	19.1±0.02	13.0±0.08
17	21.2±0.03	19.5±0.01	18.2±0.01	15.5±0.02
20	24.5±0.02	30.3±0.03	22.4±0.02	22.4±0.05
21	24.4±0.01	25.7±0.05	22.6±0.70	20.1±0.03
25	19.4±0.08	14.4±0.20	17.1±0.05	15.5±0.02
26	20.5±0.03	17.6±0.02	14.0±0.03	12.3±0.01
29	10.4±0.01	8.2±0.04	11.1±0.06	10.0±0.06
30	11.1±0.03	9.4±0.01	10.0±0.02	11.2±0.05
Chloramphenicl	24. 5±0.05	32.4±0.03	-	-
Cephalothin	-	-	24.3±0.02	28.5±0.08

Data are presented as mean ±SD. Mean zone of inhibition in mm ± Standard Deviation. The good diameter is 6 mm.

Table 2: Antifungal activities of the synthesized compounds

Tested compounds	Aspergillus Fumigatus (AF)	Candida albicans (CA)	Geotricum candidum	Syncephalastrum racemosum
6	25.3±0.02	11.8±0.04	22.4±0.02	10.9±0.04
7	25.7±0.06	10.4±0.08	21.6±0.05	12.0±0.05
11	12.9±0.08	10.5±0.03	10.7±0.03	13.5±0.06
12	14.2±0.50	12.9±0.05	13.7±0.06	11.3±0.02
16	14.8±0.01	12.7±0.06	11.3±0.2	13.6±0.03
17	16.5±0.06	13.5±0.01	12.4±0.3	10.7±0.04
20	25.2±0.01	9.4±0.07	21.9±0.6	12.6±0.04
21	25.0±0.8	10.7±0.03	21.5±0.4	12.1±0.02
25	23.6±0.07	10.3±0.04	19.5±0.06	11.3±0.04
26	10.5±0.0	9.5±0.03	13.4±0.03	NA
29	7.3±0.05	6.9±0.02	9.2±0.06	NA
30	6.8±0.04	5.7±0.08	8.7±0.05	NA
cycloheximide	25.2±0.04	27.0±0.01	22.4±0.5	23.1±0.3

NA: not activity. Data are presented as mean ± SD.

Interestingly, all compounds exhibited almost less activity against *Candida albicans* and almost all compounds exhibited low antibacterial activities against the gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*). It is believed that the differences between the sensitivity of the Gram-positive and the Gram-negative bacteria are related to the thickness of the bacterial cell wall, the gram-negative have impenetrable cell wall, because of the presence of an outer membrane covering the peptidoglycan layer which makes it more resistant to antibiotics (Nazzaro et al., 2013). The antimicrobial activity relationship of the synthesized compounds **6** and 7 revealed that the maximum activity was observed with compounds 6 and 7, having a pyrazolo-triazine with chloro substituent in the phenyl group incorporating benzothiazole and benzimidazole nucleus. However, the current study provides some interesting compounds as potent antimicrobial drugs. A similar study on a series of synthesized benzothiazole derivatives reported potent antimicrobial activity against some bacterial strains, namely *Bacillus subtilis*, *Escherichia coli*, *Streptomyces griseus* and also found effective against some fungal strains, namely *Candida albicans* and *Aspergillus niger* (Soni et al., 2010). Also, various previous studies showed that some benzimidazole and benzoxazole exhibited excellent results against some bacterial strains and benzothiazole against some fungal strains (Padalkar et al., 2016; Tahlan et al., 2019).

3.3. Anticancer evaluation

The synthesized compounds were preliminarily screened for their cytotoxic activity (in vitro) against human cancer cell line including Hepatocellular carcinoma (HEPG2). Table 3, shows the in-vitro cytotoxic activity of the newly synthesized compounds at a concentration of 50 µM, where seven compounds revealed anticancer activity percentage against the tested human cancer cell line.

Some of the synthesized compounds gave cytotoxic activity inhibition of cell viability at concentration 50µg, Using the MTT method, to calculate their IC_{50} (µM) value which corresponds to the concentration required for 50% inhibition of cell viability (Table 3).

Vinblastine is a widely used anticancer agent, it was used as a reference anticancer drug in the current study. The tested derivatives showed significant activity against the HEPG2 cancer cell line, where some compounds showed moderate or no activity.

The compounds 6, 7, 20 and 21 presented excellent activities with IC_{50} values, which recorded 2.45, 2.55,1.22 and 1.12 µg, respectively (Table 3), more potent than the reference drug (Vinblastine, IC_{50} value 2.60µM), while compounds 11, 12, 16 and 17 were found to be slightly less effective than the reference drug. The compounds 29 and 30 exhibited no activity (Table 3). Interestingly, the current results are in agreement with previous studies, which showed that some synthesized derivatives of benzothiazole and benzimidazole possessed noticeable anticancer activity (Youssef et al., 2012; Xiang et al., 2012).

Table 3: Anticancer activity (IC_{50}, µM) of of the synthesized compounds against human cancer cell line (HEPG2)

Tested compounds	Cytotoxicity[a,b](IC_{50}) (µg)
Vinblastine	2.60
6	2.45
7	2.55
11	3.83
12	3.67
16	4.21
17	3.95
20	1.22
21	1.12
29	NA
30	NA

[a] IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.; [b] Values are means of three experiments.; *NA*: No activity.

4. Conclusion

There is an intrinsic need for new antimicrobial and anticancer drugs, benzothiazole still considered as one of the most versatile classes of compounds with various biological activities. In the current study, a series of pyrazolo [5, 1-c]-l, 2, 4- triazindine, pyridazine derivative with phenylsulfonyl of benzothiazole and benzimidazole moiety were synthesized and evaluated for their antimicrobial and anticancer activities. Some compounds exhibited potent antibacterial, antifungal and anticancer activity. Although, this in vitro screening requires further pharmaceutical investigations, such as in vivo studies using experimental animals, understanding the mode of action of these compounds, possible toxicity or side effects, drug interactions and many more.

List of symbols

mp	*Melting point*
Anal	*Elemental analysis*
DMF	*Dimethylformamide*
$CDCl_3$	*Deuterated chloroform*
DMSO	*Dimethyl sulfoxide*
m/z	*mass to charge ratio*
MS	*Mass spectrometry*
C^{13} NMR	*Carbon 13 nuclear magnetic resonance*
^1H NMR	*Proton nuclear magnetic resonance*

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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