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Diaryl Epoxypropanones as Synthons in Synthesis of Some Interesting Potential Anticancer and Antibacterial Heterocyclic Agents.

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ABSTRACT

3-(*p*-Chlorophenyl)-1-(2'-naphthyl)-2,3-epoxypropanone (2) is an important intermediate for the synthesis of a variety of different heterocyclic compounds with novel cyclic system. Compound 2 reacted with hydrazine hydrate, phenyl hydrazine, hydroxylamine hydrochloride as nitrogen nucleophiles to afford compounds 3-5, and with carbon disulfide and phenylisocyanate as a carbon electrophile to afford compounds 6 and 7. Furthermore, 6-(4-chlorophenyl) -2- hydrazino - 4- naphthaline- 2-yl- 1,6- dihydro - pyrimidin-5-one(8) reacted with *p*-chlorobenzaldehyde to give Schiff base9 which on cyclocondensation with thioglycolic acid affording compound 10. The new compounds 11-13 were synthesizedby the reaction of compound 8 with carbon disulfide, malononitrile and phenyl isothiocyanate. Also compound 8 reacted with aliphatic acid namely: acetic and formic acids and with sodium nitrite to give triazolopyrimidinones14, 15 and tetrazolopyrimidinone16. A novel series of acyclicnucleosides17a,b were synthesized by utilizing compound 8.Most of the tested compounds showed potent cytotoxic activity. However two compounds, 6 and 16, showed cytotoxic activity very close to that of the Doxorubicin as a reference drug against breast carcinoma cell line MCF-7. Also some synthesized compounds were screened for their antimicrobial activity. Among the assayed compounds derivatives 9 and 17a showed the highest antimicrobial activity.

Keywords: Oxiranylmethanone, pyrazoles, triazoles, tetrazoles,anticancer, antimicrobial.

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INTRODUCTION

Oxiranes (epoxides) are extremely reactive precursors that can be used to synthesize several types of organic compounds [1-6]. They have carcinogenic [6] and mutagenic effects and also are important in biosynthesis of marine organisms and toxin of some species of fungi[7].The reaction of aryl methylenecycloalkanones, pyrimidinones and thiazolones were studied [8-13] and some derivatives were found to possess anticancer activity [11,13]. In the present communication, the utilizing of 1,3-diaryl-2,3-epoxypropan-1-one (**2**) for the synthesis of some new heterocyclic compounds and investigation of their biological evaluation as anticancer and antimicrobial activity were reported.

EXPERIMENTAL

All melting points are uncorrected and were taken in open capillary tubes using Electrothermal apparatus 9100. Elemental microanalyses were carried out at Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, using VarioElementar and were found within ±0.5% of the theoretical values.Infrared spectra were recorded on a FT/IR-4100 Jasco-Japan, Fourier transform, Infrared spectrometer at cm^{-1} scale using KBr disc technique at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. ^1H NMR spectra were determined by using a Varian Gemini-300 MHZ NMR spectrometer at Central Services Laboratory, Cairo University, Cairo, Egypt, chemical shifts are expressed in δ (ppm) downfield from TMS as an internal standard. The mass spectra were measured with a GC MS-Qp1000EX Shimadzu, Cairo University,Cairo, Egypt.

General procedure for the synthesis of compounds 3,4.

A solution of compound **2** (0.01 mol) in acetic acid (20 mL) andhydrazine hydrate and/or phenyl hydrazine (0.01 mol) wasrefluxed for 8h, then left to cool, poured onto ice water. The obtained solid was filtered off, washed with water and crystallized fromdioxane.

5-(4-Chlorophenyl)-3-(naphthalene-2-yl)-1H-pyrazole (3).

Yield (50%); mp248-250˚C; IR (KBr) ν, cm^{-1}: 3234 (NH), 2928(C=N); ^1H NMR ($CDCl_3$,δppm): 6.92-7.89 (m, 12H, 11 Ar-H + pyrazoleH-4), 13.30 (s, 1H, NH, D_2O exchangeable). MS m/z:$M^{+\cdot}$304(100%), M^++2. 306(40%). Anal.Calcd. for $C_{19}H_{13}ClN_2$ (304.78): C, 75.00; H, 4.27; N, 9.20. found: C, 74.90; H, 4.30; N, 9.27**.**

5-(4-Chlorophenyl)-3-(naphthalene-2-yl)-1-phenyl-1H-pyrazole (4).

Yield (50%); mp 260-262˚C; IR (KBr)ν, cm^{-1}: 2800 (CH aromatic), 1620 (C=N); ^1H NMR ($CDCl_3$, δppm)6.96-8.87 (m, 17H,16 Ar-H +pyrazole H-4).MSm/z:M^+380(100%),M^++2, 382(30%), Anal.Calcd.for$C_{25}H_{17}ClN_2$ (380.88):C,78.94;H,4.47; N,7.36. found: C, 78.99; H, 4.50; N, 7.30**.**

5-(4-chlorophenyl)-3-(naphthalene-2-yl)-isoxazole(5).

A solution of compound **2** (0.01 mol) and hydroxylamine hydrochloride (0.01mol) in pyridine (20 mL) was refluxed for 10h., then left to cool. The cooled reaction mixture was acidified with cold dilute hydrochloric acid. The separated solid was filtered off**,** dried and recrystallized from benzene/pet.ether (40-60) to afford compound **5**.

Yield (50%); mp250-252 ˚C. IR (KBr) ν, cm^{-1}: 2800 (CH aromatic); ^1H NMR (DMSO, δ ppm) 6.91-7.93(m,12H, 11H-Ar + pyrazole H-4);MSm/z: M^+ 305 (10%),Anal.Calcd.for $C_{19}H_{12}ClNO$:(305.69): C,74.64;H, 3.93; N,4.58. found: C, 74.00; H, 4.12; N, 4.33**.**

5-(2'-Naphthyl)-4-[(4-chlorophenyl)-2-thioxo-[1,3]oxathiolan-5-yl]methanone (6).

To a warmethanolic sodium hydroxide solution [prepared by dissolving sodium hydroxide (0.01 mol) in ethanol (50 mL)], compound **2** (0.01 mol) and carbon disulphide (10 mL) were added. The mixture was heated on water bath at 80 ^{0}C under reflux for 10h, and then it was poured into water, neutralized by diluted hydrochloric acid. The formed solid was collected and recrystallized from benzene to give **6**.

Yield 65%; mp160-162 ˚C.IR (KBr)ν, cm^{-1}: 1425 (C=S), 1666 (C=O); ^{1}HNMR (DMSO, δppm): 3.31-3.34(d, J= 15 Hz, 1H, -CH), 3.68-3.71 (d, J=15 Hz, 1H, -CH), 6.97-7.86 (m, 11H, Ar-H). ^{13}C NMR(DMSO-d_6, δppm):52.11 (CH), 100.61 (CH), 124.20-139.80 (16C, Ar-C),197.00 (C=O), 209.60 (C=S); MSm/z: M$^+$384 (30%), Anal.Calcd. for $C_{20}H_{13}ClO_2S_2$(384.90): C, 62.40; H, 3.38; found C, 62.50 ; H,3.40.

4-(4-Cholorophenyl)-5-(2'-naphthone)-3-phenyloxazolidin-2-one (7).

A mixture of compound **2** (0.01 mol) and phenyl isocyanate, (0.01 mol) in dry benzene (20 mL) containing few drops of triethylaminewas refluxed for 12h. The solvent was evaporated and the obtained solid was filtered off, and recrystallized from isopropanol to give compound **7**.
Yield 69 %; mp 178-180 ^{0}C. IR (KBr) ν, cm^{-1}: 1700, 1677 (2C=O); ^{1}H NMR (DMSO, δ ppm): 4.15-4.17(d, J=10 Hz, 1H, -CH), 4.44-4.46(d, J= 10 Hz, 1H, -CH), 7.25-8.66(m, 16H, Ar-H); MSm/z: M$^+$427 (21%). Anal.Calcd. for $C_{26}H_{18}ClNO_3$(427.76):C,72.99; H,4.20; N,3.27; found: C,70.20 ; H,4.25; N,3.25.

2-[N'-(4'-Chlorobenzylidene)-hydrazino]-4-(4-chlorophenyl)-6-naphthalen-2-yl-1,6-dihydro-4H-pyrimidin-5-one(9).

A mixture of compound**8** (0.01mol) and equimolar amount of the aromatic aldehydes namely: *p*-chlorobenzaldehyde in ethanol (20 mL) and few drops of piperidine was refluxed for 8h. The solution was cooled, and the formed precipitate was filtered off and recrystallized from DMF to give compound **9.**

Yield (60%); mp280-282 ˚C; IR (KBr) ν, cm^{-1}: 3136, 3280 (2NH), 1740 (C=O); ^{1}HNMR ($CDCl_3$, δppm):3.68-3.70 (d, J=10 Hz, 1H, pyrimidine-H),3.84-3.88 (d, J=10 Hz, 1H, pyrimidine-H), 7.25-8.66 (m 17H, 15 Ar-H + 1H,benzylidene-H+ 1H, NH, D_2O exchangeable), 9.98 (s, 1H, NH, D_2O exchangeable).^{13}C NMR ($CDCl_3$, δppm): 63.40 (CH), 64.3 (CH), 127.0-136.60 (22 Ar-C), 142.81 (CN=N), 163.0 (C=N), 206.0 (C=O).MSm/z: M$^+$487 (25%). Anal.Calcd. for $C_{27}H_{20}Cl_2N_4O$(487.89):C,66.53; H,4.10; N,11.49; found: C,66.60 ; H,4.80; N,11.32.

4-(4-Chlorophenyl)-2-[2'-(4-chlorophenyl)-4'-oxothiazolidin-3'-ylamino]-6-naphthalen-2-yl-1,6-dihydro-4H-pyrimidin-5-one (10).

A mixture of compound **9** (0.01 mol) and equimolar amount of the thioglycolic acidin dry benzene (30 mL) was refluxed for 10h. After evaporation of the solvent under reduced pressure, the obtained product was filtered off and recrystallized from DMF to give compound **10**.

Yield 55%; mp230-232 ˚C; IR (KBr) ν, cm^{-1}: 3223, 3150 (2NH), 1725, 1677(2C=O); ^{1}HNMR (DMSO,δppm): 3.50-3.52 (d, J=10 Hz, pyrimidine–H), 3.66-3.68 (d, J=10 Hz, 1H, pyrimidine-H), 4.21 (s, 1H-thiazolidine), 5.56 (s, 2H, CH_2-thiazolidine), 7.10-7.83 (m, 16H, 15H, Ar-H+1NH D_2O exchangeable), 9.50 (s, 1H, NH, D_2O exchangeable); MS m/z :M$^+$560 (25%). Anal. calcd. for$C_{29}H_{22}Cl_2N_4O_2S$(561.49): C, 62.14, H, 3.92; N, 10.00; found: C, 62.20; H, 3.99; N, 10.30.

5-(4-Chlorophenyl)-7-naphthalen-2-yl-3-thioxo-2,3,7,8-tetrahydro-[1,2,4]-triazolo[4,3-a]- pyrimidin-6-one (11).

To a warmed ethanolic potassium hydroxide solution [prepared by dissolving potassium hydroxide (0.01mol) in ethanol (50 mL)], compound**8**(0.01mol) and carbon disulphide (10 mL) were added. The mixture was heated on water bath at 80 ^{0}C under reflux for 10h, and then it was poured into water, neutralized by diluted hydrochloric acid. The formed solid was collected and recrystallized from ethanol to give **11**.

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Yield 75%; mp 188-190^{0}C;IR (KBr) ν, cm^{-1}: 3200, 3129(2NH), 1685 (C=O), 1430(C=S); ^{1}HNMR($CDCl_3$,δppm): 3.27-3.30 (d, J=15Hz, 1H, pyrimidine-H), 3.56-3.59 (d,J=15,1H,pyramidine-H),7.11-7.95(m,11H,Ar-H), 10.20, 10.95 (2H, 2NH, D_2O exchangeable);^{13}C NMR ($CDCl_3$, δppm): 64.00 (CH), 65.90 (CH), 127.0-133.70 (16 Ar-C), 154.0 (C=N), 184.0 (C=S), 206.0 (C=O). MSm/z: M^+406 (20%). Anal.Calcd. for $C_{21}H_{15}ClN_4OS$(406.71):C,62.01; H, 3.69; N,13.77; found: C,62.40 ; H,3.31; N,13.80.

4-(4-Chlorophenyl)-2-[3',5'-dimethyl-pyrazolo-1-yl-6-(2'-naphthylpyrimidin)]-5-one (12).

A mixture of compound **8** (0.01mol) and acetyl acetone (0.01 mol) in absolute ethanol (20mL) was refluxed for 12h. The reaction mixture was concentrated, and the formed precipitate was filtered off and recrystallized from the DMF to give compound **12**.

Yield 55%; mp 230-232^o C. IR (KBr) ν, cm^{-1}: 3320 (NH), 1754 (C=O); ^{1}H NMR (DMSO-δppm): 2.26 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.30-3.32(d, J =10 Hz, 1H, pyrimidine-H), 3.35-3.37 (d, J =10 Hz, 1H, pyrimidine-H), 6.23(s, 1H, pyrazolo-H), 7.17-7.94 (m, 11H,Ar-H), 10.74 (s, 1H, NH,D_2O exchangeable); ^{13}C NMR (DMSO-δppm): 15.30 (CH_3), 17.70 (CH_3), 63.40 (CH), 65.10 (CH), 127.00-132.70 (16 Ar-C), 104.90, 143.10, 144.20 (3C-pyrazole), 163.00 (C=N), 206 (C=O).MSm/z:M^+,428 (15%).Anal.Calcd.for$C_{25}H_{21}ClN_4O$ (428.75): C, 70.03; H, 4.90; N, 13.06. found: C, 70.05; H, 4.79; N, 13.10.

4-Chlorophenyl-6-(2'-naphthyl)-2-(phenylaminothioxohydrazino)pyrimidin-5-one (13).

A mixture of compound **8** (0.01mol), phenyl isothiocyanate (0.01mol) and a catalytic amount of triethylamine in dry benzene (20 mL) was refluxed for 7h. The reaction mixture was concentrated, and the formed precipitate was filtered off and recrystallized from DMF to afford compound **13**.

Yield 55%; mp230-231˚C. IR (KBr) ν, cm^{-1}: 3289-3189 (broad, 4NH), 1680 (C=O), 1493(C=S); ^{1}H NMR (DMSO-δppm): 3.13-3.16(d, J = 15 Hz, 1H, pyrimidine-H), 3.32-3.35 (d, J= 15 Hz, 1H, pyrimidine-H), 7.85-8.09 (m, 18H, 16 Ar-H+2H, 2NH,D_2O exchangeable) , 6.91 (s, 1H, 1NH, D_2O exchangeable), 8.61 (s, 1H, NH,D_2O exchangeable). MSm/z: M^+499 (15%). Anal. Calcd. for$C_{27}H_{22}ClN_5OS$ (499.77): C, 64.88; H, 4.40; N, 14.00. found: C, 65.12; H, 4.31; N, 14.13.

General procedure for synthesis of compounds 14, 15.

A solution of compound **8**(0.01 mol) in acetic and/or formic acid (20 mL) was refluxed for 7h. The formed precipitate was filtered off and recrystallized from the DMF to afford compounds **14, 15.**

5-(4-Chlorophenyl)-3-methyl-7-(2'-naphthyl)triazolopyrimidin-6-one (14).

Yield65%; mp210-212 ˚C. IR (KBr) ν cm^{-1}:3251 (NH),1718 (C=O); ^{1}H NMR (DMSO-δppm): 2.40 (s, 3H, CH_3), 3.11-3.14 (d, J = 15 Hz, 1H, pyrimidine-H), 3.27-3.30 (d, J = 15 Hz, 1H, pyrimidine-H), 7.25-8.09 (m, 11H, Ar H), 9.98 (s, 1H, NH, D_2O exchangeable). MS m/z:M^+388 (5%). Anal. Calcd. for$C_{22}H_{17}ClN_4O$ (388.72): C, 67.97; H, 4.37; N, 14.40;found: C, 67.90; H, 4.42; N, 14.35.

5-(4-Chlorophenyl)-7-(2'-naphthyl)triazolopyrimidin-6-one (15).

Yield 55%; mp218-220 ˚C. IR (KBr) ν, cm^{-1}:3181 (NH), 1720 (C=O); ^{1}H NMR (DMSO-δppm): 3.15-3.18(d, J = 15 Hz, 1H, pyrimidine-H), 3.51-3.54(d, J = 15 Hz, 1H, pyrimidine-H), 7.20-8.12 (m, 12H, 11 Ar-H+triazole-H), 9.94(s, 1H, NH, D_2O exchangeable). MS m/z:M^+374 (7%). Anal. Calcd. for$C_{21}H_{15}ClN_4O$ (374.81): C, 67.29; H, 4.00; N, 14.94;found: C, 67.00; H, 4.31; N, 14.00.

7-(4-Chlorophenyl)-5-naphthalen-2-yl-tetrazolo-[1,5-a]-pyrimidin-6-one (16).

To an ice-cold solution of compound 8 (0.01mol) in glacial acetic acid (10 mL), a solution of sodium nitrite [prepared by dissolving sodium nitrite (0.01 mol) in water (3 mL)] was added dropwise in an ice-bath. The reaction mixture was allowed to stand overnight at room temperature and then was poured into water. The formed solid was filtered off, washed with water, dried and recrystallized from dioxin to afford**16**.

Yield 57%; mp 232-234˚C.IR (KBr) ν, cm^{-1}: 3336 (NH), 1720 (C=O); ^{1}H NMR ($CDCl_3$-δppm): 3.82-3.84(d, J = 10 Hz, 1H, pyrimidine-H), 3.71-3.73 (d,J =10Hz, 1H, pyrimidine-H), 6.90-7.98 (m, 11H, 11Ar-H), 9.44 (1H, NH,D_2O exchangeable). MSm/z: M^+375 (25%). Anal.Calcd.for$C_{20}H_{14}N_5OCl$ (375.70): C, 63.93; H, 3.73; N, 18.6; found: C, 63.32; H, 3.30; N, 18.60.

General procedure for synthesis of compound17a, b.

A mixture of compound **8** (0.01 mol), and monosaccharides, namely: D-glucose and/or D-arabinose (0.01 mol), in ethanol (50 mL) and a catalytic amount of acetic acid was heated at 80 ˚C for 1h. The formed precipitate was filtered on hot, washed with ethanol several times and dried to give compound **17a, b**.

4-(4-Chlorophenyl)-6-naphthalen-2-yl-2-[N'-(2,3,4,5,6-pentahydroxy-hexylidene)-hydrazino]-1,6-dihydro-4H-pyrimidin-5-one (17a).

Yield 65%; mp 210-211˚C. IR (KBr) ν, cm^{-1}: 3400-3280 (broad OH, NH), 1667(C=O), 1590 (HC=N); ^{1}H NMR (DMSO- δ ppm): 3.30-3.44 (m, 4H,the sugar protons congregated with the solvent signal), 3.43-3.46 (d, J=15 Hz, 1H, pyrimidine-H), 3.54-3.57 (d, J=15 Hz, 1H, pyrimidine-H), 3.60-4.22 (m, 2H, CH_2OH),4.70-5.14 (m, 5OH, D_2O exchangeable), 7.19-7.98 (m, 12H, 11Ar H + HC=N), 9.25, 10.14 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: M^+, 526 (37%). Anal. Calcd. for$C_{26}H_{27}ClN_4O_6$ (526.76): C, 59.28; H, 5.13; N, 10.63;found: C, 59.40; H, 5.00; N, 10.95.

4-(4-Chlorophenyl)-6-naphthalen-2-yl-2-[N'-(2,3,4,5-tetrahydroxy-pentylidene)-hydrazino]-1,6-dihydro-4H-pyrimidin-5-one 17b.

Yield 65%; mp220-222˚C. IR (KBr) ν, cm^{-1}: 3390-3210 (broad OH, NH) and 1676 (C=O), 1590 (HC=N); ^{1}H NMR (DMSO-δ ppm): 3.21-3.24 (d, J = 15 Hz, 1H, pyrimidine-H), 3.33-3.36 (d, J =15 Hz, 1H, pyrimidine-H),3.35-3.80 (m, 3H, the sugar protons congregated with the solvent signal),3.70-4.19 (m, 2H, CH_2OH), 4.40-4.73 (m, 4H, 4OH, D_2O exchangeable),7.27-8.09 (m, 12H, 11Ar H + HC=N),9.93, 10.25 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: M^+, 496(30%).Anal. Calcd.for$C_{25}H_{25}N_4O_5Cl$ (496.75): C, 60.44; H, 5.03; N, 11.27;found: C, 60.50; H, 5.00; N, 11.30.

Biological Evaluation

Anticancer screening

Chemicals

Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), tamoxifen, penicillin, and streptomycin were obtained from Sigma Chemical Company (Saint Louis, MO, USA). Human Tyrosine kinase (TRK) ELISA kit was purchase from Glory Science Co., Ltd (Del Rio, TX 78840, USA).

Cell lines and culturing

The cytotoxicity activity of the newly synthesized compounds was measured in vitro on breast cancer cell line MCF-7 obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf

serum (GIBCO), penicillin (100 U/mL) and streptomycin (100µg/ml) at 37^0Cin humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50 x 10^6 were grown in a 25 cm^2 flask in 5 ml of complete culture medium.

In-Vitro cytotoxicity assay

The antiproliferative activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previous reported standard procedure [14]. Cells were inoculated in 96-well microtiter plate (10^4 cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO at 1 mg/ml immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentration of tested compounds and doxorubicin (2, 5, 10, 20 or 40 µg/ml) were added to the cells. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h. at 37^0C and in atmosphere of 5% CO_2. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1.The results were compared to the antiproliferative effects of the reference control doxorubicin [15].

***In Vitro* Human Tyrosine Kinase (TRK)concentration assay**

The effect of tested compounds on the level of Human Tyrosine kinase (TRK) was determined in the MCF-7 cell line. The cells at a concentration of 0.50 x 10^6 were grown in a 25 cm^2 flask in 5 ml of DMEM culture medium and were treated with 20 µl of IC_{50} values of the compounds or the standard reference drug, Doxorubicin dissolved in DMSO, then incubated for 24 h at 37 ^{0}C, in a humidified 5% CO_2 atmosphere. The cells were harvested and homogenates were prepared in saline using a tight pestle homogenizer until complete cell disruption. To determine the level of TRK in samples, a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used. This assay is based on, add TRK to monoclonal antibody enzyme well which is pre-coated with human TRK monoclonal antibody, incubation; then, add TRK antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human TRK of sample were positively correlated and the optical density was determined at 450 nm. The level of TRK in samples was calculated (pmol/ml) as duplicate determinations from the standard curve.

Antimicrobial assay

Preparation of microbial suspension.

The antibacterial activity of the synthesized compounds was tested against*Bacillus subtilis*NRRL 543, *Staphylococcus aureus*NRRL B-313 (Gram-positive bacteria), *Escherichia coli NRRL B-210*, *Pseudomonas* NRRL B-23 (Gram-negative bacteria) using nutrient agar medium.The antifungal activity of the compounds was tested against *Candida albicans*NRRL Y-477 *and Aspergillusniger*NRRL 599using Sabouraud dextrose agar medium.

Determination of antimicrobial activity by Disk-Diffusion method.

All compounds were screened *in vitro* for their antimicrobial activity by agar diffusion method [16]. A suspension of the organisms were added to sterile nutrient agar media at 45 °C and the mixture was transferred to sterile Petri dishes and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer) An amount of 0.1 ml of the synthesized compounds was poured inside the holes. A hole filled with DMSO was also used as control. The plates were left for 1 h at room temperature as a period of pre-incubation diffusion to minimize the effects to variation in time between the applications of the different solutions. The plates were then incubated at 37 °C for 24 h and observed for antibacterial activity. The diameters of zone of inhibition were

measured and compared with that of the standard, the values were tabulated. Ciprofloxacin (50 µg/ml) and Fluconazole (50 µg/ml) were used as standard for antibacterial and antifungal activity respectively.

Determination of Minimum Inhibitory Concentration (MIC).

Minimum Inhibitory Concentration (MIC) of the test compounds were determined by agar streak dilution method [17]. 100 mg/ml stock solution of the synthesized compounds were made using DMSO as the solvent. From this stock solution, a range of concentration from (5 till 0.05 mg/ml) of the tested compounds solutions was mixed with the known quantities of molten sterile agar media aseptically. About 20 ml of nutrient agar medium for bacteria and Sabouraud dextrose agar medium for fungi containing the tested compound under study was dispensed into each sterile Petri dish. Then the media were allowed to get solidified. Microorganisms were then streaked one by one on the agar plates aseptically. After streaking, all the plates were incubated at 30 °C for 24 hours/48 hours for bacteria and fungi respectively. Then the plates were observed for the growth of microorganisms. The lowest concentration of the synthesized compounds inhibiting the growth of the given bacteria/fungus was considered as minimum inhibitory concentration (MIC) of the test compounds against that bacteria or fungi on the plate.

RESULTS AND DISCUSSION

Chemistry

3-(*p*-chlorophenyl)-1-(2'-naphthyl)prop-2-en-1-one (**1**)[18] reacted with hydrogen peroxide in alkaline medium to afford 3-(*p*-Chlorophenyl)-1-(2'-naphthyl)-2,3-epoxypropanone(**2**) [19], which is utilized as a key starting material in the synthesis of different novel heterocyclic compounds. Condensation of **2** with different nucleophiles namely: hydrazine hydrate, phenyl hydrazine in glacial acetic acid and hydroxylamine in pyridine gave compounds**3-5**. On the other hand, compound **2** reacted with carbon disulfide in ethanolic potassium hydroxide and phenyl isocyanate in dry benzene containing few drops of triethylamineto afford compounds **6** and **7** (Scheme 1). The structures of compounds **3-7** were in agreement with their spectral and analytical data. IR spectrum of **3** showed absorption band at 3234 (NH). ^{1}H NMR spectrum showed signals at δ 6.92-7.89 (m, 12H, 11 Ar-H + pyrazole-H) and at δ 13.30 (NH, D_2O exchangeable). It's mass spectrum showed a molecular ion peak at m/z 304 (100%) and at m/z 306 (M^++2, 40%). The IR spectrum of compound **4** showed absorption bands at 2800 and 1620 characteristic of CH-aromatic and C=N. The mass spectrum showeda molecular ion peak at m/z 380 (100%) and at m/z 382, M^++2(30%) supporting its molecular formula. The structure of isoxazole**5** was verified by elemental analysis, IR, ^{1}H NMR and mass spectroscopic data (cf. experimental). IR spectrum of compound **6** showed absorption bands at 1425, 1666 characteristic of C=S and C=O respectively. ^{13}C NMR spectrum of **6**showed signals at 52.1, 100.6 (2CH), C=O at 197.0, C=S at 209.6, the aromatic carbons, 16Ar-C, appeared at 124.20-139.80 and two signals in the sp^{3}carbon region at 52.11 and 100.61 ppm. It's^{1}H NMR spectrum showedtwo doublets at 3.31-3.34, 3.68-3.71 ppm for □-CH and □-CH. The mass spectrum showed a molecular ion peak at m/z 384 (30%). The phenyloxazolidinone**7** showed absorption bands in its IR spectrum at 1700 and 1677 (2C=O), while it's^{1}H NMR spectrum showed signals at 4.15-4.17 (d, □-CH) and at 4.44-4.46 (d, □-CH). Its mass spectrum showed molecular ion peak at m/z 427 (21%).

When compound **2** was refluxed with thiourea in ethanolic potassium hydroxide affordingthiopyrimidinone which on heating under reflux in ethanol with hydrazine hydrate giving 6-(4-chlorophenyl)-2-hydrazino-4-naphthaline-2-yl-1,6-dihydro-pyrimidin-5-one (**8**) [19] which is utilized as a key starting material for the synthesis of a novel heterocyclic compounds **9-16** and acyclic nucleoside **17a,b**.

Condensation of compound **8** with 4-chlorobenzaldehyde took place by heating under reflux, in ethanol, in the presence of piperidine where Schiff base**9** was produced which in turn underwent cyclocondensation with thioglycolic acid in dry benzene according to reported method [20, 21] to givethiazolidine derivative **10** (Scheme 2). The IR spectrum of **9** displayed absorption bands at 3136, 3280 (2NH) and 1740 (C=O). Compound **10** displayed absorption bands at 3223, 3150 (2NH) and 1725 and 1677 (2C=O).The ^{1}H NMR spectrum of compound **9** showed signals at 7.25-8.66 (m, 17H, 15 Ar-H + 1H benzlidine-H, 1H, NH, D_2O exchangeable), 9.98 (s, 1H, NH, D_2O

exchangeable); while compound **10** showed signals at 4.21 (s, 1H, thiazolidine-H) and at 5.56 (s, 2H, CH_2-thiazolidine), 7.10-7.83 (m, 16H, 15H, Ar-H+ 1H, NH, D_2O exchangeable), 9.50 (s, 1H, NH, D_2O exchangeable).The mass spectrum of **9**showed molecular ion peak($C_{27}H_{20}Cl_2N_4O$) at m/z 487 (25%), while that of **10** showed molecular ion peak ($C_{29}H_{22}Cl_2N_4O_2S$) at m/z 560 (25%).

Heating of compound **8** with carbon disulfide in the presence of ethanolic potassiumhydroxide afforded thioxotriazolopyrimidinone derivative **11**. The structure of **11** was confirmed on the basis of spectral and spectral data (cf. experimental). The IR spectrum showed absorption bands assignable to 2NH, C=O and C=S group. The ^{1}H-NMR spectrum revealed signals at 10.20 and 10.95 for 2NH groups and at 7.11-7.95 (m, 11H, Ar-H).^{13}C-NMR spectrum of **11**showed signals at 206.0 (C=O), 184.0 (C=S), 154.0 (C=N), 127.0-133.7 (16Ar-C), 64.0, 65.9 (2CH).

Condensation of derivative **8** with acetyl acetone in the presence of ethanol gave dimethylpyrazolopyrimidinone**12**. IR spectrum of **12** showed absorption bands at 3320 (NH) and at 1754 (C=O).It's^{1}H NMR spectrum showed signals at2.26 and 2.46 (2s, 6H, $2CH_3$), at 6.23 (s, 1H, pyrazole-H) and at 10.74 (s, 1H, NH, D_2O exchangeable). The mass spectrum showed molecular ion peak at m/z 428 (15%).

When compound **8** reacted withphenylisocyanate in dry benzene, aminothioxohydrazino-pyrimidinone**13** was obtained which showed, besides the correct values in elemental analysis, IR absorption bands at 3428-3189 (4 NH), 1680 (C=O) and at 1493 (C=S). The ^{1}H NMR spectrum showed signals at6.91 (s, 1H, NH, D_2O exchangeable), 7.25-8.09 (m, 18H, 16H, Ar-H + 2H, 2NH, D_2O exchangeable), 8.61 (s, 1H, NH, D_2O exchangeable). The mass spectrum of **13**showed molecular ion peak at m/z 499 (15%).

Heating compound **8** under reflux with aliphatic acids (acetic or formic acid) resulted in the formation of methyltriazolopyrimidinone**14** and triazolopyrimidinone**15** respectively(Scheme 2). IR spectrum of **14** showed absorption bands at 3251 (NH) and at 1718 (C=O). The ^{1}H-NMR spectrum of **14** showed signals at 2.40 ppm (s, 3H, CH_3) and at 9.98 ppm (s, 1H, NH, D_2O exchangeable), 7.25-8.09 (m, 11H, Ar-H), while that of **15** showed signals at 9.94 ppm (s, 1H, NH, D_2O exchangeable) and at 7.20-8.12 ppm (m, 12H, 11H, Ar-H + triazole-H).The mass spectrum of **14** showed molecular ion peak ($C_{22}H_{17}ClN_4O$) at m/z 388 (5%), while that of compound **15** ($C_{21}H_{15}ClN_4O$) at m/z 374 (7%).

Moreover, nitrosation of the hydrazino group of compound **8** afforded tetrazolopyrimidinone derivative **16**. Analytical and spectral data of **16** are in agreement with the proposed structure (cf. experimental).

Compound **8** was allowed to react with monosaccharides namely: D-glucose and D-arabinose in ethanol containing catalytic amount of glacial acetic acid to yield the corresponding compounds**17a,b**.The IR spectra of the obtained derivatives **17a,b**were characterized by the presence of broad absorptoion bands of OH and NH groups at the range of 3400-3210 cm^{-1}, while the HC=N group appeared at 1590 cm^{-1}. The ^{1}H NMR spectra revealed the alditol protons of the sugar part as multiplet signals at 3.30-3.80 ppm, OH protons as multiplet signals at 4.40-5.14, while the aromatic and the methane CH=N where represented as multiplet signals at 7.19-8.90 ppm. The 2NH protons were detected at 9.93 and 10.25 ppm.

Biological evaluation

Anticancer screening

Most of the clinical used antitumor agents possess significant cytotoxic activity in cell culture systems as cytotoxicity is one of chemotherapeutic targets of antitumor activity [22].The antiproliferative activity of the newly synthesized compounds was evaluated against human mammary carcinoma cell line (MCF-7) using Sulforhodamie B (SRB) colorimetric assay, in comparison with doxorubicin as reference drug [14]. The antiproliferative activities are expressed by median growth inhibitory concentration (IC_{50}) and provided in Table 1. From the results, it is evident that all the tested compounds displayed potent to moderate growth inhibitory activity, in particular compounds 1**6**and **6** (IC_{50} 4.20and 3.50 µg/ml, respectively) which were found to be potent and efficacious similar to doxorubicin (IC_{50} value 3.12 µg/ml).Protein kinases (PKs) are enzymes catalyze phosphorylation of different

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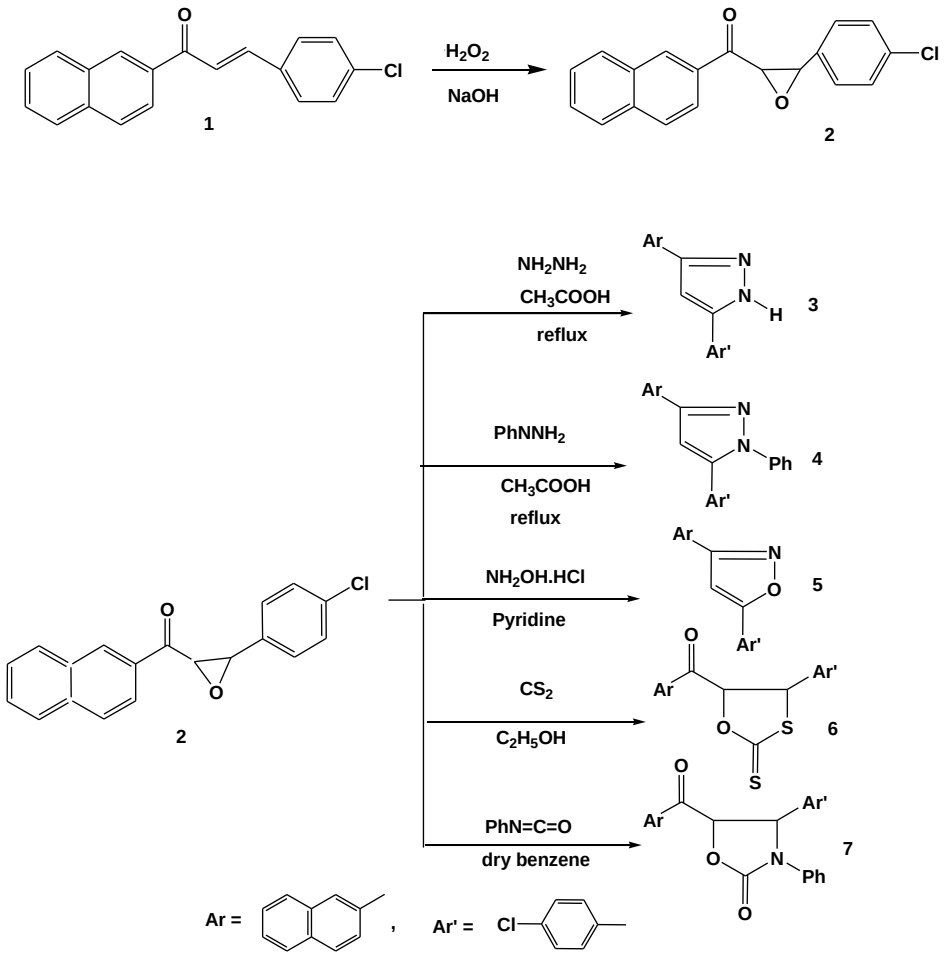
cellular substrates. Phosphorylation in turn regulates various cellular functions. Normally, their activity is stringently regulated. However, under pathological conditions PKs can be deregulated, leading to alterations in the phosphorylation and resulting in uncontrolled cell division, inhibition of apoptosis, and other abnormalities and consequently to diseases [23]. Various cancers and other diseases are known to be caused or accompanied by deregulation of the phosphorylation. Inhibition of PKs has been shown to be a promising therapeutic strategy. Many PK inhibitors (PKIs) have been produced and tested in clinic by now. These molecules have a low molecular weight and most of them bind to protein kinases competing with ATP for the ATP-binding site [24].The discovery of newer inhibitors has provided an opportunity of many researchers in the future.

We synthesized a novel series of new heterocyclic compounds in an attempt to use them as inhibitor for human TRK. The results showed that most of the tested compounds showed potent inhibition against human TRK in human breast cancer cell line MCF-7 as compared to the inhibition for the untreated cells as listed in Table 1. Nearly two compounds, **16**and **6** which had a good cytotoxicity activity were found to be potent and selective similar to the positive drug, doxorubicin (85%) against human TRK with percentage of inhibition values were 73, 82% respectively as compared with control untreated cells. From the foregoing result it is clear that compound **6** has a cytotoxicity activity and inhibition activity to human TRK nearly was very close to that of doxorubicin in human breast cancer cell line MCF-7 (Table 1).



Scheme 1: Synthesis of compounds 3-7

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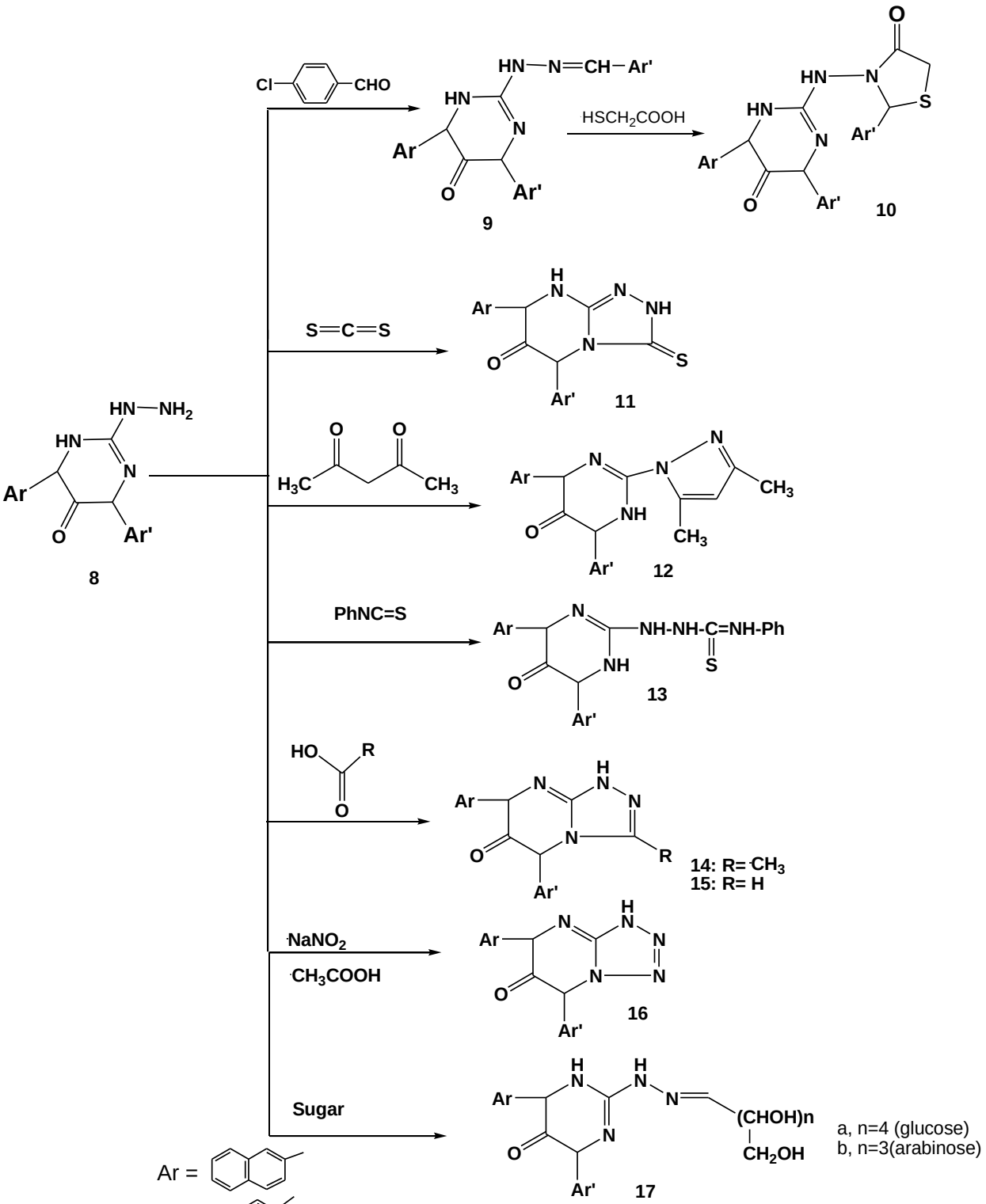

Scheme 2. Synthesis of compounds 9-17

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Table 1: *In vitro*cytotoxic activity and the percent of TRK inhibition

Compound No.	IC_{50} (µg/mL)	% of TRK inhibition
2	11.20	13
6	3.50	83
7	9.00	19
8	18.20	11
11	12.10	16
12	9.30	8.5
16	4.20	73
17a	16.00	10
E	9.80	17
DMSO	----	---
Doxorubicin	3.12	85

Table 2: Inhibition zone in mm as a criterion of antimicrobial and antifungal activities of the newly synthesized compounds.

Compound No.	Microorganism inhibition zone diameter (mm)					
	Gram $^{+}$ve Bacteria		Gram $^{-}$ve Bacteria		Fungi	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeuroginosa*	*Candida albicans*	*Aspergillusniger*
3	19	17	18	18	20	17
4	14	15	13	12	15	13
6	20	17	17	17	17	20
7	15	12	13	13	12	-ve
9	22	18	20	21	21	18
10	-ve	13	13	14	12	-ve
14	20	17	18	18	19	16
16	13	-ve	11	11	-ve	-ve
17a	22	22	20	21	22	18
Ciprofloxacin	22	24	24	23	-ve	-ve
Fluconazole	-ve	-ve	-ve	-ve	22	24

Highly active = (inhibition zone > 20 mm)
Moderately active =(inhibition zone 15 - 20 mm)
Slightly active = (inhibition zone 11 - 14 mm)
Inactive = (inhibition zone < 11 mm)

Table 3: MIC in mg/mL of the newly synthesized compounds against microorganisms.

Compound No.	Gram $^{+}$ve Bacteria		Gram $^{-}$ve Bacteria		Fungi	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeuroginosa*	*Candida albicans*	*Aspergillus niger*
3	0.49	0.53	0.50	0.53	0.45	0.50
6	0.45	0.53	0.53	0.51	0.53	0.60
9	0.10	0.50	0.45	0.40	0.10	0.20
14	0.45	0.53	0.50	0.50	0.49	0.60
17a	0.08	0.08	0.45	0.49	0.08	0.15

Antimicrobial activity

All the newly synthesized compounds werescreened for their *in vitro* antibacterial activityagainst two strains of Gram positive bacteria*(Staphylococcus aureus, Bacillussubtilis*), and twostrains of Gram negative bacteria (*Escherichia coli,Pseudomonas aeruginosa*) using ciprofloxacin as astandard drug (100 µg/mL). They were also evaluatedfor their *in vitro* antifungal activity against themycotic strains (*Candida albicans*and *Aspergillusniger*)

using fluconazole as a standard antifungaldrug (100 µg/mL). Agar-diffusion method wasused in this investigation for determination of thepreliminary antibacterial and antifungal activity andthe results were recorded for each tested compoundas the average diameter of inhibition zones (IZ) ofbacterial or fungal growth around the discs in mm(Table 2). The minimal inhibitory concentrations (MIC)were determined for compounds showing promisinggrowth inhibition, using the twofold serial dilutionmethod [17]. The MIC (µg/mL) values against thetested bacterial and fungal isolates are presented inTable 3.

According to Table 2 and 3, it is clear that compounds**4, 7, 10** and **16** displayed less activity against all the tested microorganisms. Compounds **3**,**6** and **14** showed moderate activity. While compounds **9** and **17a**showed goodactivity towards the tested microorganisms.

CONCLUSION

In the present study, the synthesis of new series of heterocyclic compounds was carried out for anticancer and antimicrobial evaluation utilizing 3-(*p*-Chlorophenyl)-1-(2'-naphthyl)-2,3-epoxypropanone (**2**) as the key starting compound. The results showed that two compounds **6** and **16**, which had goodcytotoxic activity and inhibition activity to human TRK nearly very close to that of Doxorubicin in human breast cancer cell line MCF-7.The antimicrobial evaluation revealed that the new synthesized compounds **3**, **6**, **9**, **14** and **17a** showed the best antimicrobial activity against the tested microorganisms. Compounds **9** and **17a** showed good activity against Gram +ve and Gram –vebacteria,fungai, while the other compounds showed moderate activity.

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