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4-Thiazolidinone derivatives: Synthesis, antimicrobial, anticancer evaluation and QSAR studies.

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Abstract: A series of 4-thiazolidinone derivatives (1–18) was synthesized and tested *in vitro* for its antimicrobial and anticancer potentials. In general, the synthesized compounds were found to be potent antimicrobial agents than anticancer agents. Anticancer screening results indicated that compound 13 (IC_{50} = 15.18 µM) was the most active anticancer agent and was more potent than standard drug, carboplatin (IC_{50} > 100 µM). Antimicrobial activity results indicated that 14 was the most active antimicrobial agent ($pMIC_{ec}$ = 2.14 µM) and may serve as important lead for the discovery of novel antimicrobial agents. The QSAR studies indicated that the antibacterial and antifungal activities of the synthesized derivatives against different microbial strains were governed by lipophillic parameter, log P, topological parameter, $\kappa\alpha_3$ and electronic parameters cos E and Nu. E.

Keywords: 4-Thiazolidinones; Antimicrobial; Anticancer

1. INTRODUCTION

Cancer encompasses many disease states generally characterized by abnormally proliferating cell and is a major and often fatal disease. However, the effect of anticancer drugs on solid tumors has been poor. Because the response of solid tumors to available anticancer chemotherapy has been reduced, new drugs with improved efficacy are desired.[1]

Infectious diseases are responsible for great number of deaths in the world population. The reduction of sensibility to antimicrobial agents in current use has been increasing for a great variety of pathogens and the resistance to multiple drugs is common for several microorganisms, especially for Gram positive bacteria. Infection by methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococci (VRE) presents a difficult problem for medicine. Given the evidence for the rapid global spread of resistant, there is need for discovery or optimization of antimicrobial agents active against these resistant strains is of paramount importance.[2]

Quantitative structure–activity relationships (QSARs) are among the most widely used techniques in rational drug design, which finds the mathematical relationship between

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physicochemical properties of compounds and their experimentally determined biological activities. Thus, the derived QSAR model can be subsequently used to predict the biological activities of new derivatives. A good QSAR model both enhances our understanding of the specifics of drug action and provides a theoretical foundation for lead optimization. Moreover, QSAR techniques increase the probability of success with reduced time and cost in drug discovery.[3]

Thiazolidin-4-one derivatives are known to exhibit diverse biological activities such as anticancer[4,5], antimicrobial[6,8], antiviral[9], antimycobacterial[10], analgesic and anti-inflammatory[11,12] activities.

In the light of abovementioned facts and in continuation of our research in the field of antimicrobial, anticancer agents and previously synthesized novel derivatives of 4-thiazolidinones [13-15], we hereby report synthesis, antimicrobial, anticancer evaluation of 4-thiazolidinone derivatives and their QSAR study.

2. EXPERIMENTAL

Melting points were determined in open capillary tubes on a sonar melting point apparatus and were uncorrected. Reaction progress was monitored by thin layer chromatography on silica gel sheets (Merck silica gel–G). ^{1}H nuclear magnetic resonance (^{1}H NMR) and C^{13} NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer using appropriate deuterated solvents and are expressed in parts per million (d, ppm) downfield from tetramethylsilane (internal standard). Infrared (IR) spectra were recorded on Perkin Elmer FTIR spectrometer using KBr pellets. Mass spectra were taken on Bruker Compass Data Analysis 4.0 Mass spectrometer.

2.1 General procedure for synthesis of 2-disubstituted-4-thiazolidinone derivatives

A mixture of (0.25 M) hipuric acid and excess of methanol (250 ml) with 1 ml of sulphuric acid was refluxed for 3–4 h in Round Bottom Flask (RBF). The mixture was cooled; the precipitated solid was separated by filtration and recrystallized from methanol to yield the methyl 2-benzamidoacetate. A mixture of methyl 2-benzamidoacetate (0.2 M) and excess of hydrazine hydrate (0.3 M), ethanol (250 ml) was refluxed for about 3 h and allowed to cool. The resultant solid was separated by filtration and recrystallized from ethanol to afford 2-benzamidoacetohydrazide. A mixture of 2-benzamido acetohydrazide (0.025 M) and required aromatic aldehydes (0.025 M) was refluxed in methanol (50 ml) in the presence of catalytic amount of glacial acetic acid for about 2 h. The reaction mixture was then cooled and the precipitated solid was separated by filtration and recrystallized from methanol to give the corresponding hydrazones of hippuric acid. A mixture of corresponding hydrazone of hippuric acid (0.015 M) and required amount of thioglycolic acid (0.015 M) in DMF (50 ml) containing a pinch of anhydrous zinc chloride was refluxed for about 6 h to yield 4-thiazolidinones (1-18). The reaction mixture was then cooled and poured onto the crushed ice. The solid thus obtained was filtered, washed with water, and the product was recrystallized from rectified spirit. [12]

2.1.1 N-(2-(2-(3-ethoxy-4-hydroxyphenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (1); IR (KBr pellets, cm^{-1}): 3610(OH),3317(NH), 3034 (C-H aromatic), 1638 (C=C aromatic), 1679 (C=O), 1279 (C-N), 748(C-S), 1178 (C-O-C str.,-OC_2H_5); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.25-7.91 (m, 8H, ArH), 8.10 (s, 1H, NH), 4.03(s, 1H, OH), 4.09(d, 2H, CH_2), 3.34(s, 2H, CH_2 of thiazolidinone), 1.36(t, 3H, CH_3 of -OC_2H_5), 3.94(m, 2H, CH_2 of -OC_2H_5).

2.1.2. N-(2-(2-(4-chlorophenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide(2); IR (KBr pellets, cm^{-1}): 3322(NH), 2971 (C-H aromatic), 1633 (C=C aromatic), 1689 (C=O), 1289 (C-N), 729(C-S), 709(C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.56-8.69

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(m, 9H, ArH), 8.01(s, 1H, NH), 4.43 (d, 2H, CH_2), 3.50(s, 2H, CH_2 of thiazolidinone).

2.1.3.N-(2-(2-(4-(dimethylamino)phenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (3); IR (KBr pellets, cm^{-1}): 3214(NH), 3048 (C-H aromatic), 1601 (C=C aromatic), 1673 (C=O), 1227 (C-N), 738(C-S), 1367 (C-N str., aryl tertiary amine), 2928 (C-H str., CH_3).; ^{1}H NMR (DMSO-d_6, 400 MHz): 7.54-8.64 (m, 9H, ArH), 8.08(s, 1H, NH), 4.38(d, 2H, CH_2), 3.41(s, 2H, CH_2 of thiazolidinone), 2.87(s, 6H, $N(CH_3)_2$).

2.1.4. N-(2-(2-(2-chlorophenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (4); IR (KBr pellets, cm^{-1}): 3361(NH), 2891 (C-H aromatic), 1619 (C=C aromatic), 1688 (C=O), 1270 (C-N), 750(C-S), 700 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.54-8.01 (m, 9H, ArH), 8.03(s, 1H, NH), 4.01(d, 2H, CH_2), 3.42 (s, 2H, CH_2 of thiazolidinone)

2.1.5.N-(2-(2-(4-formylphenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (5); IR (KBr pellets, cm^{-1}): 2892(C-H str., CHO),3312(NH), 3060 (C-H aromatic), 1633 (C=C aromatic), 1672 (C=O), 1288 (C-N), 758(C-S);^{1}H NMR (DMSO-d_6, 400 MHz): 7.77-8.77 (m, 9H, ArH), 8.03(s, 1H, NH), 4.00(d, 2H, CH_2), 3.50(s, 2H, CH_2 of thiazolidinone), 8.88(s, 1H, CHO).

2.1.6. N-(2-(2-(2-methoxyphenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (6); Mp (°C); Yield –%; IR (KBr pellets, cm^{-1}): 3339(NH), 3037 (C-H aromatic), 1636 (C=C aromatic), 1665 (C=O), 1246 (C-N), 780(C-S), 1167 (C-O-C str.,-OCH_3); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.54- 7.96 (m, 9H, ArH), 8.18 (s, 1H, NH), 4.41(d, 2H, CH_2), 3.36(s, 2H, CH_2 of thiazolidinone), 3.83(s, 3H, OCH_3)

2.1.7. N-(2-oxo-2-(4-oxo-2-(3,4,5-trimethoxyphenyl)thiazolidin-3-ylamino)ethyl)benzamide (7); IR (KBr pellets, cm^{-1}): 3382(NH), 3006 (C-H aromatic), 1647 (C=C aromatic), 1697 (C=O), 1295 (C-N), 718(C-S), 1184 (C-O-C str.,- OCH_3); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.50-8.87 (m, 7H, ArH), 7.93(s, 1H, NH), 4.42(d, 2H, CH_2), 3.43(s, 2H, CH_2 of thiazolidinone), 3.74(s, 9H, $(OCH_3)_3$)

2.1.8. N-(2-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (8); IR (KBr pellets, cm^{-1}): 3383(NH), 2838 (C-H aromatic), 1645 (C=C aromatic), 1691 (C=O), 1299 (C-N), 756(C-S), 1162 (C-O-C str., -OCH_3); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.51-7.98 (m, 9H, ArH), 7.99(s, 1H, NH), 4.41(d, 2H, CH_2), 3.51(s, 2H, CH_2 of thiazolidinone), 3.85(s, 3H, OCH_3).

2.1.9. N-(2-(2-(4-bromophenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (9); IR (KBr pellets, cm^{-1}): 3316(NH), 3094 (C-H aromatic), 1631 (C=C aromatic), 1690 (C=O), 1286 (C-N), 709(C-S), 613 (C-Br); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.51-7.99 (m, 9H, ArH), 8.05(s, 1H, NH), 4.42(d, 2H, CH_2), 3.37(s, 2H, CH_2 of thiazolidinone)

2.*1.10. N-(2-(2-(3-nitrophenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (10)* Mp; IR (KBr pellets, cm^{-1}): 3341(NH), 3044 (C-H aromatic), 1629 (C=C aromatic), 1686 (C=O), 1246 (C-N), 712(C-S), 1347 (C-NO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.72-8.26 (m, 9H, ArH), 8.14(s, 1H, NH), 4.01(d, 2H, CH_2), 3.38(s, 2H, CH_2 of thiazolidinone)

2.1.11. N-(2-oxo-2-(4-oxo-2-phenylthiazolidin-3-ylamino)ethyl)benzamide (11); IR (KBr pellets, cm^{-1}): 3316(NH), 3071 (C-H aromatic), 1634 (C=C aromatic), 1688 (C=O), 1280 (C-N), 756(C-S);^{1}H NMR (DMSO-d_6, 400 MHz): 7.49-8.68 (m, 10H, ArH), 8.02(s, 1H, NH), 4.14(d, 2H, CH_2), 3.47(s, 2H, CH_2 of thiazolidinone)

2.1.12.N-(2-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (12); IR (KBr pellets, cm^{-1}):): 3603(OH), 3435(NH), 3027 (C-H aromatic), 1636 (C=C aromatic), 1676 (C=O), 1270 (C-N), 710(C-S), 1168 (C-O-C str.,-OCH_3); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.25-8.12 (m, 8H, ArH), 7.91(s, 1H, NH), 4.14(d, 2H, CH_2), 3.51(s,

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2H, CH_2 of thiazolidinone), 3.83 (s, 3H, (OCH_3), 4.41(s, 1H, OH).

2.1.13. N-(2-(2-(2-hydroxynaphthalen-1-yl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (13); IR (KBr pellets, cm^{-1}): 3624(OH), 3416(NH), 3060 (C-H aromatic), 1634 (C=C aromatic), 1697 (C=O), 1283 (C-N), 747(C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.50-8.05 (m,11H, ArH), 8.03(s, 1H, NH), 4.07(d, 2H, CH_2), 3.53(s, 2H, CH_2 of thiazolidinone), 4.46(s, 1H, OH)

2.1.14. N-(2-(2-(4-(diethylamino)phenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (14); IR (KBr pellets, cm^{-1}): 3399(NH), 3050 (C-H aromatic), 1639 (C=C aromatic), 1702 (C=O), 1266 (C-N), 712(C-S), 1353 (C-N str., aryl tertiary amine), 2895 (C-H str., CH_3); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.43-7.91 (m, 9H, ArH), 8.06(s, 1H, NH), 3.96(d, 2H, CH_2), 3.36(s, 2H, CH_2 of thiazolidinone), 2.89(m, 4H, $(CH_2)_2$), 1.14(t, 6H, $(CH_3)_2$).

2.1.15.N-(2-oxo-2-(4-oxo-2-p-tolylthiazolidin-3-ylamino)ethyl)benzamide (15); IR (KBr pellets, cm^{-1}): 3327(NH), 3069 (C-H aromatic), 1640 (C=C aromatic), 1688 (C=O), 1295 (C-N), 713(C-S), 2931 (C-CH_3); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.48-7.91 (m,9H, ArH), 7.98(s, 1H, NH), 3.99(d, 2H, CH_2), 3.47(s, 2H, CH_2 of thiazolidinone), 2.38(s, 3H, CH_3)

2.1.16. N-(2-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (16); IR (KBr pellets, cm^{-1}): 3613(OH),3367(NH), 3036 (C-H aromatic), 1639 (C=C aromatic), 1671 (C=O), 1238 (C-N), 708(C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.51-8.12 (m,9H, ArH), 7.95(s, 1H, NH), 3.97(d, 2H, CH_2), 3.48(s, 2H, CH_2 of thiazolidinone), 4.38(s, 1H, OH).

2.1.17. (E)-N-(2-oxo-2-(4-oxo-2-styrylthiazolidin-3-ylamino)ethyl)benzamide (17); IR (KBr pellets, cm^1): 3032 (C-H aromatic), 1567 (C=C aromatic), 1655 (C=O), 1223 (C-N), 2777(C-H str.,-CH=CH-), 777(C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.34-7.47 (m,10H, ArH), 7.96(s, 1H, NH), 3.95(d, 2H, CH_2), 3.34(s, 2H, CH_2 of thiazolidinone), 7.88(d, 1H, CH).

2.1.18. N-(2-(2-(3-methoxyphenyl)-4-oxothiazolidin-3-ylamino)-2-oxoethyl)benzamide (18); Mp (°C); Yield –%; IR (KBr pellets, cm^{-1}): 3316(NH), 3050 (C-H aromatic), 1635 (C=C aromatic), 1680 (C=O), 1260 (C-N), 711(C-S), 1193 (C-O-C str.,-OCH_3); ^{1}H NMR (DMSO-d_6, 400 MHz): 7.38-8.70 (m,9H, ArH), 7.98(s, 1H, NH), 3.97(d, 2H, CH_2), 3.38(s, 2H, CH_2 of thiazolidinone), 3.80 (s, 3H, OCH_3).

3. RESULTS AND DISCUSSION

3.1Antimicrobial assay

3.1.1Determination of MIC

The antimicrobial activity of synthesized compounds was performed against Gram-positive bacteria: *Staphylococcus aureus* MTCC 3160, *Bacillus subtilis* MTCC 441, Gram-negative bacterium: *Escherichia coli* MTCC 443 and fungal strains: *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 281 using tube dilution method.[17] Dilutions of test and standard compounds were prepared in double strength nutrient broth – I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi) [18]. The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 d (*A. niger*) and at 37 °C for 48 h (*C. albicans*) and the results were recorded in terms of MIC.

3.2.2 Determination of MBC/MFC

The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were determined by subculturing 100 µL of culture from each tube (which remained clear in the MIC determination) on fresh medium. MBC and MFC values represent the lowest concentration of compound that produces a 99.9% end point reduction.[19]

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3.3 Evaluation of anticancer activity

The anticancer activity of synthesized compounds (1-18) was determined against an oestrogen receptor positive human breast adenocarcinoma, MCF-7 (ATCC HTB-22) cancer cell line. The cell line was cultured in RPMI 1640 (Sigma) supplemented with 10% heat inactivated foetal bovine serum (FBS) (PAA Laboratories) and 1% penicillin/streptomycin (PAA Laboratories). Culture was maintained in a humidified incubator at 37 °C in an atmosphere of 5% CO_2. Anticancer activity of synthesized compounds at various concentrations was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) assay, as described by Mosmann, but with minor modification, following 72 h of incubation. Assay plates were read using a spectrophotometer at 520 nm. Data generated were used to plot a dose-response curve of which the concentration of test compounds required to kill 50% of cell population (IC_{50}) was determined. Anticancer activity was expressed as the mean IC_{50} of three independent experiments. [20]

3.4 QSAR Studies

The structures of synthesized compounds (1-18) were first pre-optimized with the Molecular Mechanics Force Field (MM^+) procedure included in Hyperchem 6.03[21] and the resulting geometries were further refined by means of the semiempirical method PM3 (Parametric Method-3). We chose a gradient norm limit of 0.04 kJ/A˚ for the geometry optimization. The lowest energy structure was used for each molecule to calculate physicochemical properties using TSAR 3.3 software for Windows. [22] Further, the regression analysis was performed using the SPSS software package. [23]

3.5 Results and Discussion

3.5.1 Chemistry

Thiazolidin-4-one derivatives (1-18) were synthesized using the synthetic procedure given in Scheme 1. The synthesized compounds were characterized by physicochemical as well as spectral means. Physicochemical properties and anticancer activity results of the synthesized compounds are presented in Table 1.

3.5.2 Antimicrobial activity

The antimicrobial activity results (Table 2) indicated that the synthesized compounds were having excellent antimicrobial activity and compound 9 ($pMIC_{sa}$ = 1.84 µM/ml, Table 2) was the most potent antibacterial agent against *S. aureus*. Compound 14 was found to be most active against *E. coli* ($pMIC_{ec}$ = 2.14µM/ml). Compounds 2 and 4 (MIC_{bs} = 1.80µM/ml) were found to be active against *B. subtilis.* In the case of antifungal activity against *C. albicans*, and *A. niger,* compound 10 (MIC_{ca} = 2.11µM/ml, MIC_{an} = 1.81µM/ml) was found most effective among the synthesized series. All the above mentioned compounds having comparable antimicrobial potential to standard drugs norfloxacin (pMIC = 2.61 µM/ml) and fluconazole (pMIC = 2.64 µM/ml), may serve as important leads for the discovery of novel antimicrobial agents.

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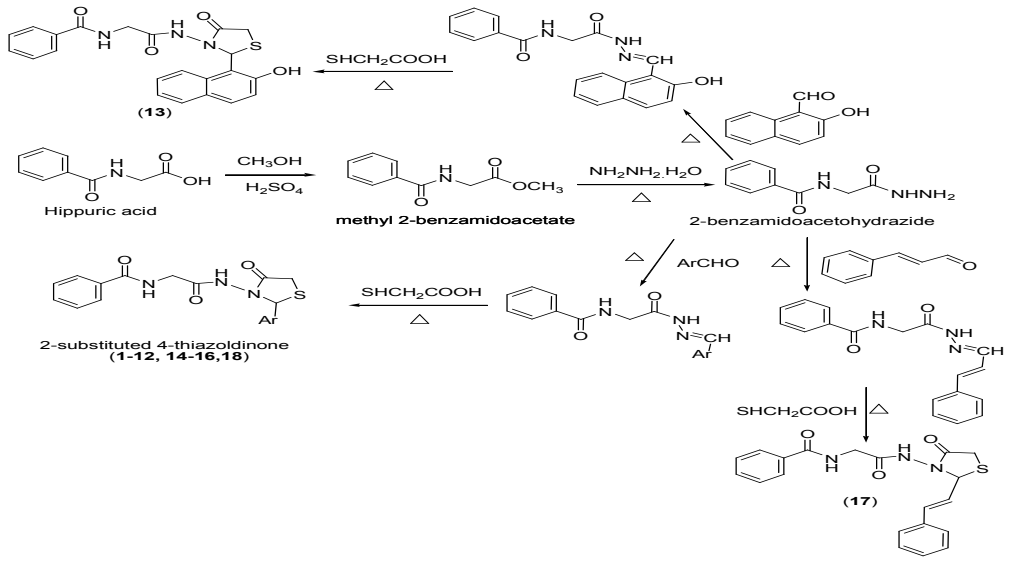
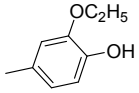
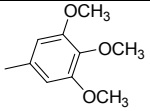
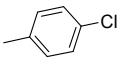
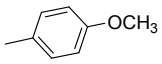
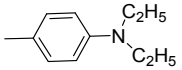
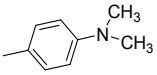
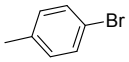
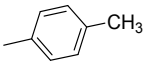
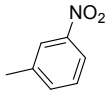
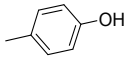
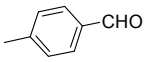
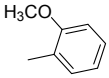
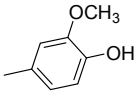
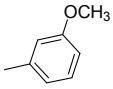


Scheme 1. Scheme for the synthesis of 4-thiazolidinone derivatives **(1-18)**

Comp.	Ar	Comp.	Ar	Comp.	Ar
1		7		13	--
2		8		14	
3		9		15	
4		10		16	
5		11		17	--
6		12		18	

In general, the results of MBC/MFC studies revealed that the synthesized compounds were bacteriostatic and fungistatic in action as their MFC and MBC values were (ranging from >0.11 to >0.14 μM/ml) 3-fold higher than their MIC values (a drug is considered to be bacteriosatic/fungistatic

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when its MFC and MBC values are 3- fold higher than its MIC value). [24]

Table 1. Physicochemical properties and anticancer activity of the synthesized 4-thiazolidinone derivatives **(1-18)**

Comp.	M. Formula	M. Pt. (°C)	M. Wt.	R_f value*	% yield	IC_{50} (µM) (MCF-7)
1	$C_{20}H_{21}N_3O_5S$	195-197	415.12	0.61	68	180.67
2	$C_{18}H_{16}ClN_3O_3S$	201-203	389.86	0.59	75	48.75
3	$C_{20}H_{22}N_4O_3S$	178-180	398.48	0.68	82	>250.95
4	$C_{18}H_{16}ClN_3O_3S$	166-168	389.86	0.72	74	225.72
5	$C_{19}H_{17}N_3O_4S$	186-188	383.42	0.60	77	>260.81
6	$C_{19}H_{19}N_3O_4S$	182-184	385.44	0.75	83	207.56
7	$C_{21}H_{23}N_3O_6S$	155-157	445.49	0.67	78	>224.47
8	$C_{19}H_{19}N_3O_4S$	161-163	385.44	0.68	72	67.456
9	$C_{18}H_{16}BrN_3O_3S$	150-152	434.31	0.59	69	66.77
10	$C_{18}H_{16}N_4O_5S$	191-193	400.41	0.71	80	62.44
11	$C_{18}H_{17}N_3O_3S$	222-224	355.41	0.58	71	>281.37
12	$C_{19}H_{19}N_3O_5S$	174-176	401.44	0.59	76	234.16
13	$C_{22}H_{19}N_3O_4S$	209-211	421.47	0.72	83	15.18
14	$C_{22}H_{26}N_4O_3S$	181-183	426.53	0.66	65	112.54
15	$C_{19}H_{19}N_3O_3S$	157-159	369.44	0.55	63	35.19
16	$C_{18}H_{17}N_3O_4S$	170-172	371.41	0.50	76	>269.24
17	$C_{20}H_{19}N_3O_3S$	144-146	381.45	0.54	79	83.89
18	$C_{19}H_{19}N_3O_4S$	234-236	385.44	0.62	81	179.02
5-FU						0.0052
Carboplatin						> 100

*TLC mobile phase : Benzene

3.5.3 Anticancer activity

Anticancer activity results (Table 1) indicated that besides having excellent antimicrobial activity, the synthesized compounds were also having good anticancer activity against MCF 7 cancer cell line and compounds **2**, **8**, **9**, **10**, **13**, **15** and **17** showed more potent anticancer activity than carboplatin (IC_{50} > 100 µM). Compounds **13** (IC_{50} = 15.18 µM) was found to be most potent anticancer agent.

3.6 SAR (Structure Activity Relationship) studies

The antimicrobial and anticancer results revealed that the nature of the substituents has a considerable impact on the biological activities of thiazolidin-4-one derivatives and the following structure activity relationship (SAR) can be deduced:

1. Presence of electron withdrawing groups (-Cl, -NO_2, -Br, Compounds **4, 9** and **10**) on moiety improved the antimicrobial activity of the synthesized compounds against *C. albicans, A. niger, S. aureus* and *B. subtilis*.
2. Presence of electron releasing groups (Compounds **14** and **15**) on moiety improved the antibacterial and anticancer activity of the synthesized compound against *E. coli* and MCF-7 (ATCC HTB-22) cancer cell line.
3. The presence of fused aromatic ring substitution (2-OH naphthaldehyde, Compound **13**) in moiety improves anticancer activity of synthesized compounds against an oestrogen receptor positive human breast adenocarcinoma, MCF-7 (ATCC HTB-22) cancer cell line.
4. From these result we may conclude that different structural requirements are required for a compound to be effective against different targets.[25]

The above mentioned findings are summarized in Fig. 1.

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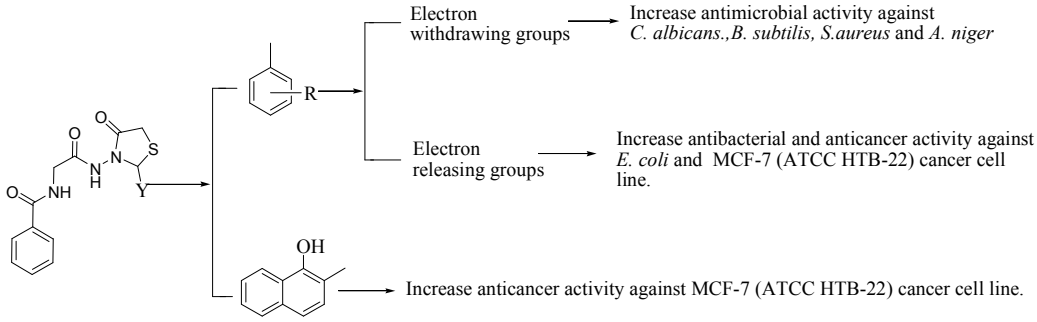

Fig. 1. Structural requirements for the antimicrobial and anticancer activities of 4-thiazolidinone derivatives (**1-18**).

3.7 QSAR study

In order to identify the substituent effect on the antimicrobial activity, quantitative structure activity relationship (QSAR) studies were undertaken, using the linear free energy relationship (LFER) model described by Hansch and Fujita (1964).[26] Biological activity data determined as MIC values were first transformed into pMIC values (*i.e.* –log MIC) and used as dependent variables in QSAR study (Table 2). The different molecular descriptors selected for the present study and the values of selected descriptors are presented in Table 3.

In the present study, a dataset of 18 thiazolidinone derivatives (**1–18**) was used for linear regression model generation. The standard drugs norfloxacin and fluconazole were not included in model generation because of dissimilarity in structure with synthesized compounds. Different outliers are identified against different microorganisms, and the models have been developed after removal of the outliers (compound numbers in brackets) *B. subtilis* (**2, 4, 6, 7, 9** and **17**), *E. coli* (**4, 7, 8, 10, 14** and **16**), *C. albicans* (**1**, **6**, **7**, **9**, **10**, **15** and **18**) and *A. niger* (**4, 6, 9, 10, 11, 16** and **18**). In multivariate statistics, it is common to define three types of outliers.[27]

Table 2. Antimicrobial activity (pMIC in µM/ml) of synthesized compounds

Comp.	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{sa}$	$pMIC_{an}$	$pMIC_{ca}$
1	1.22	1.82	0.92	1.52	0.92
2	1.80	1.49	1.19	1.19	1.19
3	1.20	1.50	1.50	1.20	1.50
4	1.80	1.80	1.49	1.80	1.19
5	1.19	1.49	0.88	1.19	1.49
6	1.49	1.19	1.49	1.79	1.79
7	0.95	1.25	1.55	1.55	1.55
8	1.19	1.19	1.49	1.19	1.19
9	0.94	1.54	1.84	1.54	1.84
10	1.51	1.20	1.20	1.81	2.11
11	1.45	1.45	1.15	0.85	1.15
12	1.21	1.51	1.81	1.21	1.51
13	1.53	1.23	1.53	1.53	1.23
14	1.53	2.14	1.53	1.53	1.53
15	1.47	1.47	1.47	1.17	0.87
16	1.17	1.17	1.77	1.77	1.17
17	1.56	1.86	0.96	1.26	1.56
18	1.19	1.49	1.49	1.79	2.09
SD	0.25	0.27	0.29	0.29	0.36
Std.	2.61*	2.61*	2.61*	2.64*	2.64**

*Norfloxacin **Fluconazole

As there was no difference in the activity (Table 2) as well as the molecular descriptor range (Table 3) of these outliers when compared to other synthesized compounds, these outliers belong to the category of Y outliers (substances for which the reference value of response is invalid.

Preliminary analysis was carried out in terms of correlation analysis. A correlation matrix constructed for antibacterial activity against *B. subtilis* is presented in Table 4. The correlation of molecular descriptors with their antimicrobial activity against different strains is given in Table 5. In general, high colinearity ($r > 0.5$) was observed between different parameters. The high interrelationship was observed between Ne and W ($r = 0.995$) and low interrelationship was observed between Cos E and HOMO ($r = -0.087$, Table 4).

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Table 3. Values of selected descriptors calculated for QSAR studies

Comp.	Cos E	log P	MR	$^0\chi$	$\kappa\alpha_3$	W	Ne	LUMO	HOMO	μ
1	-2.12	1.58	108.12	20.80	5.36	2514.00	34431.60	-0.29	-8.75	3.78
2	-1.39	2.29	100.02	18.52	4.79	1877.00	27578.20	-0.48	-9.09	2.49
3	1.94	1.56	108.92	20.10	5.13	2333.00	32503.40	-0.27	-8.44	1.70
4	-0.50	2.29	100.02	18.52	4.59	1839.00	28029.90	-0.42	-9.00	1.88
5	7.17	1.45	101.81	19.23	4.75	2104.00	30277.40	-0.88	-9.21	2.59
6	0.69	1.52	101.68	19.23	4.68	2028.00	31216.10	-0.30	-8.80	3.38
7	12.95	1.01	114.60	22.38	5.43	2936.00	39949.90	-0.39	-8.95	3.69
8	-0.18	1.52	101.68	19.23	4.88	2104.00	29594.20	-0.24	-8.91	1.76
9	-0.51	2.56	102.84	18.52	4.88	1877.00	28154.20	-0.56	-9.04	2.76
10	8.69	1.72	102.54	20.10	4.95	2276.00	31868.50	-1.12	-9.34	4.42
11	0.94	1.77	95.21	17.65	4.40	1676.00	25929.80	-0.26	-8.99	2.71
12	2.31	1.23	103.37	20.10	4.92	2274.00	32848.10	-0.36	-8.84	4.09
13	-4.28	2.49	113.36	21.09	4.35	2609.00	35959.30	-0.78	-8.85	3.61
14	9.39	2.25	118.42	21.51	5.63	2851.00	38341.30	-0.24	-8.44	5.38
15	-5.30	2.24	100.26	18.52	4.66	1877.00	28296.20	-0.30	-8.90	2.94
16	-5.83	1.49	96.91	18.52	4.64	1877.00	27759.40	-0.26	-8.95	1.22
17	5.20	3.99	130.67	23.05	6.12	3898.00	37017.60	-0.69	-8.65	2.74
18	-0.55	1.52	101.68	19.23	4.88	2066.00	30022.10	-0.24	-8.93	1.21

From the correlation Table 4, it was observed that lipophilic parameter, log P was found to be the dominating descriptor for antibacterial activity of the synthesized compounds against *B. subtilis* (Eq. 1).

QSAR model for antibacterial activity against *B. subtilis*

$pMIC_{bs}$ = 0.348 log P + 0.719 Eq. 1

n = 12 r = 0.849 q^2 = 0.632 s = 0.087 F = 25.85

Here and thereafter, n - number of data points, r - correlation coefficient, q^2 -cross validated r^2 obtained by leave one out method, s - standard error of the estimate and F - Fischer statistics.

The developed QSAR model for antibacterial activity (Eq. 1) indicated that there is a positive correlation between log P and antibacterial activity of the synthesized compounds against *B. subtilis*. This is indicated by low antibacterial activity value of compound **7** ($pMIC_{bs}$ = 0.95 μM/ml) having low log P value (1.01).

Log P is the logarithm of the ratio of the concentrations of the un-ionized solute in two solvents, which is calculated according to following equation, where o is octanol and w is un-ionized water

$\log P_{o/w} = \log([solute_o] / [solute_w])$

The hydrophobic effect is the major driving force for the binding of drugs to their receptor targets in pharmacodynamics, and is based on the log P contribution of each atom. Each atom in a molecule contributes to the log P by the amount of its atomic parameter multiplied by the degree of exposure to the surrounding solvent.[28]

The developed QSAR model (Eq. 1) was cross validated by q^2 value (q^2 = 0.632) obtained by

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leave one out (LOO) method. The value of q^2 more than 0.5 indicated that the model developed is a valid one. As the observed and predicted values are close to each other (Table 6), the QSAR model for antibacterial activity against *B. subtilis* (Eq. 1) is a valid one.[29] The plot of predicted $pMIC_{bs}$ against observed $pMIC_{bs}$ (Fig. 2) also favors the developed model expressed by Eq. 2. Further, the plot of observed $pMIC_{bs}$ *vs* residual $pMIC_{bs}$ (Fig. 3) indicated that there was no systemic error in model development as the propagation of error was observed on both sides of zero. [30]

Table 4. Correlation matrix for the antibacterial activity of the synthesized compounds against *B. subtilis*

	$pMIC_{bs}$	Cos E	log P	$^{0}\chi$	$\kappa\alpha_3$	W	Ne	LUMO	HOMO	μ
$pMIC_{bs}$	1.000									
Cos E	0.199	1.000								
log P	0.849	-0.124	1.000							
$^{0}\chi$	0.233	0.340	0.307	1.000						
$\kappa\alpha_3$	-0.098	0.508	-0.115	0.621	1.000					
W	0.268	0.372	0.358	0.991	0.647	1.000				
Ne	0.296	0.358	0.380	0.987	0.618	0.995	1.000			
LUMO	-0.337	-0.422	-0.125	-0.216	0.234	-0.164	-0.160	1.000		
HOMO	-0.030	-0.087	0.226	0.456	0.542	0.510	0.515	0.667	1.000	
μ	0.657	0.520	0.422	0.643	0.423	0.652	0.683	-0.337	0.103	1.000

Table 5. Correlation of antibacterial, antifungal and antimicrobial activities of synthesized compounds with their molecular descriptors

Descriptors	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{an}$	$pMIC_{ca}$
Cos E	0.199	0.289	0.291	0.789
log P	0.849	0.386	-0.056	0.195
MR	0.346	0.495	0.480	0.666
$^{0}\chi_v$	0.233	0.466	0.650	0.718
$^{0}\chi$	0.233	0.522	0.633	0.696
$^{1}\chi_v$	0.287	0.442	0.588	0.656
$^{1}\chi$	0.359	0.515	0.581	0.633
$^{2}\chi_v$	0.309	0.425	0.539	0.612
$^{2}\chi$	0.366	0.430	0.436	0.600
$^{3}\chi_v$	0.138	0.291	0.504	0.524
$^{3}\chi$	0.156	0.199	0.070	0.464
κ_1	0.179	0.528	0.642	0.768
κ_2	0.116	0.624	0.512	0.763
κ_3	-0.065	0.791	0.197	0.748
$\kappa\alpha_1$	0.135	0.563	0.684	0.775
$\kappa\alpha_2$	0.064	0.682	0.564	0.767
$\kappa\alpha_3$	-0.098	0.838	0.257	0.745
R	0.287	0.442	0.588	0.656
J	-0.413	-0.246	-0.001	-0.195
W	0.268	0.550	0.443	0.676
Te	-0.097	-0.440	-0.730	-0.687
Ee	-0.283	-0.306	-0.805	-0.722
Ne	0.296	0.292	0.807	0.721
SA	0.189	0.592	0.591	0.755
IP	0.030	-0.227	-0.281	-0.566
LUMO	-0.337	-0.013	0.045	-0.241
HOMO	-0.030	0.227	0.281	0.566
μ	0.657	-0.043	0.701	0.482

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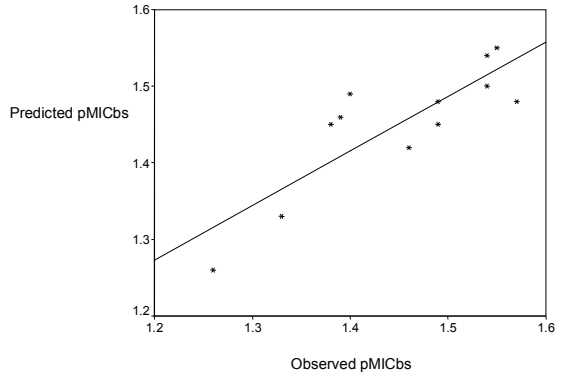

Fig. 2. Plot of observed $pMIC_{bs}$ against predicted $pMIC_{bs}$ by Eq. 1.

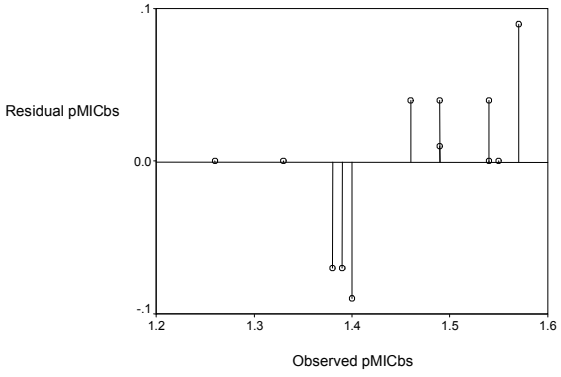

Fig. 3. Plot of observed $pMIC_{bs}$ against residual $pMIC_{bs}$ by Eq. 1.

In case of antibacterial activity against *E. coli*, topological parameter, Kier's alpha third order shape index ($\kappa\alpha_3$, Table 5) was found most dominant in expressing antibacterial activity of the synthesized compounds against *E. coli.* So, QSAR model for antibacterial activity against *E. coli* (E 2) was developed using $\kappa\alpha_3$.

QSAR model for antibacterial activity against *E. coli*

$pMIC_{ec} = 0.342\ \kappa\alpha_3 - 0.177$

Eq. 2

n = 12 r = 0.838 q^2 = 0.531 s = 0.109 F = 23.62

As in case of antibacterial activity against *B. Subtilis,* antibacterial activity of the synthesized compounds against *E. coli* is also positively correlated with their kα3 values which means that antibacterial activity of the synthesized compounds against *E. coli* will increase with increase in their $\kappa\alpha_3$ values (Tables 2 and 3).

Table 6. Observed, predicted and residual antimicrobial activities of the synthesized compounds obtained by developed QSAR models.

Comp.	$pMIC_{bs}$			$pMIC_{ec}$			$pMIC_{an}$			$pMIC_{ca}$		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.22	1.27	-0.05	1.82	1.65	0.17	1.52	1.37	0.15	0.92	1.23	-0.31
2	1.80	1.52	0.28	1.49	1.46	0.03	1.19	1.14	0.05	1.19	1.26	-0.06
3	1.20	1.26	-0.06	1.50	1.58	-0.08	1.20	1.30	-0.10	1.50	1.36	0.15
4	1.80	1.52	0.28	1.80	1.39	0.40	1.80	1.16	0.64	1.19	1.28	-0.09
5	1.19	1.22	-0.04	1.49	1.45	0.04	1.19	1.23	-0.05	1.49	1.51	-0.03
6	1.49	1.25	0.24	1.19	1.42	-0.24	1.79	1.26	0.53	1.79	1.32	0.47
7	0.95	1.07	-0.12	1.25	1.68	-0.43	1.55	1.55	0.00	1.55	1.69	-0.13
8	1.19	1.25	-0.06	1.19	1.49	-0.30	1.19	1.21	-0.02	1.19	1.29	-0.10
9	0.94	1.61	-0.67	1.54	1.49	0.05	1.54	1.16	0.38	1.84	1.28	0.56
10	1.51	1.32	0.19	1.20	1.52	-0.31	1.81	1.28	0.52	2.11	1.56	0.55
11	1.45	1.34	0.12	1.45	1.33	0.13	0.85	1.09	-0.24	1.15	1.33	-0.17
12	1.21	1.15	0.06	1.51	1.50	0.00	1.21	1.32	-0.11	1.51	1.37	0.14
13	1.53	1.59	-0.06	1.23	1.31	-0.09	1.53	1.42	0.11	1.23	1.17	0.06
14	1.53	1.50	0.03	2.14	1.75	0.39	1.53	1.50	0.04	1.53	1.58	-0.05
15	1.47	1.50	-0.03	1.47	1.42	0.05	1.17	1.17	0.00	0.87	1.14	-0.27
16	1.17	1.24	-0.06	1.17	1.41	-0.24	1.77	1.15	0.63	1.17	1.12	0.05
17	1.56	2.11	-0.54	1.86	1.92	-0.05	1.26	1.45	-0.19	1.56	1.45	0.11
18	1.19	1.25	-0.06	1.49	1.49	0.00	1.79	1.22	0.57	2.09	1.28	0.81

Electronic parameter, cosmic total energy (cos E) was found to be effective in describing the antifungal activity of the synthesized compounds against *C. albicans* (Eq. 3, Table 5).

QSAR model for antifungal activity against *C. albicans*

$pMIC_{ca} = 0.0300 \cos E + 1.297$

Eq. 3

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n = 11 r = 0.789 q^2 = 0.503 s = 0.113 F = 14.82

In case of antifungal activity of the synthesized compounds against *A. niger,* electronic parameter, nuclear energy (Nu. E) was found to be the most dominating descriptor for describing the antifungal activity of the synthesized compounds against *A. niger* (Eq. 4, Table 5).

QSAR model for antifungal activity against *A. niger*

$pMIC_{an}$ = 0.000033 Nu. E + 0.232

Eq. 4

n = 11 r = 0.807 q^2 = 0.529 s = 0.105 F = 16.78

The validity and predictability of the QSAR models for antimicrobial activity of the synthesized compounds against *E. coli, C. albicans and A. niger* (Eqs. 2-4) was cross validated by their high q^2 values (q^2 = 0.531, 0.503 and 0.529 respectibely) obtained by leave one out (LOO) method. Further, as the observed and predicted values are close to each other (Table 4), the QSAR model for antimicrobial activity of the synthesized compounds against *E. coli, C. albicans and A. niger* (Eqs. 2-4) are valid ones.[29] The high residual values observed in case of outliers justify their removal while developing QSAR models. It is important to mention a fact here that no significant correlation was observed between antibacterial activity of synthesized compounds against *S. aureus* and their calculated molecular descriptors.

It was observed from developed QSAR models [Eq. 1-4] that the antibacterial and antifungal activities of the synthesized 4-thiazolidinone derivatives against different microbial strains were governed by lipophillic parameter, log P, topological parameter, $\kappa\alpha_3$ and electronic parameters cos E and Nu. E.

When biological activity data lies in the narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies.[31] The minimum standard deviation (Table 2) observed in the antimicrobial activity data justifies its use in QSAR studies.

7.8 Conclusion

A series of 4-thiazolidinone derivatives (**1–18**) was synthesized and tested *in vitro* for its antimicrobial and anticancer potentials. In general, the synthesized compounds were found to be potent antimicrobial agents than anticancer agents. Anticancer screening results indicated that compound **13 (**IC_{50} = 15.18 μM) was the most active anticancer agent and was more potent than standard drug, carboplatin (IC_{50} > 100 μM). Antimicrobial activity results indicated that **14** was the most active antimicrobial agent ($pMIC_{ec}$ = 2.14 μM) and may serve as important lead for the discovery of novel antimicrobial agents. The QSAR studies indicated that the antibacterial and antifungal activities of the synthesized derivatives against different microbial strains were governed by lipophillic parameter, log P, topological parameter, $\kappa\alpha_3$ and electronic parameters cos E and Nu. E.

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