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# 3D- QSAR STUDIES, BIOLOGICAL EVALUATION STUDIES ON SOME SUBSTITUTED 3-CHLORO-1-[5-(5-CHLORO-2-PHENYL-BENZIMIDAZOLE-1-YLMETHYL)-[1, 3, 4] THIADIAZOLE-2-YL]-AZETIDIN-2-ONE AS POTENTIAL ANTIMICROBIAL ACTIVITY

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A new series of 3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1, 3, 4] thiadiazole-2-yl]-azetidin-2-one derivatives [5a-o] has been synthesized and subjected to evaluate their antibacterial properties. All the synthesized compounds of the series displayed, remarkable activity in comparison to standard drug Norfloxacin and Clotrimazole. A number of descriptors were tested to adjudge a quantitative correlation between activity and structural features. However, significant correlations have emerged between activity and physicochemical parameters viz.quantum chemical, chemical descriptors, topological, chemical parameter. Moreover, results are interpreted on the basis of multiple regression and cross-validation methodology. The structures of the newly synthesized compounds were confirmed by analytical IR, $^{1}$NMR and mass spectral data. All the synthesized compounds have exhibited significant activity against the bacteria and fungi tested.

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*Keywords:* QSAR, HOMO, LUMO, MR azetidin-2-one, Antibacterial activity, Antifungal activities

## 1. Introduction

Interest in benzimidazole containing structures stems from their widespread occurrence in molecules that display a plethora of useful biological properties. Substituted benzimidazole derivatives have found commercial application in veterinarian medicine as anthelmintic agents and in such diverse human therapeutic areas as antiulcers [1], antihypertensive [2] antiviral [3] antifungal [4] anticancer [5] and antihistamines [6] to name just a few. In light of the affinity they display towards a variety of enzymes and protein receptors, medicinal chemists would certainly qualify them as 'privileged sub-structures' for drug design [7].However, little progress has been made in deducing antimicrobial behavior of benzimidazole derivatives [8], which need to be investigated thoroughly. It has been observed that resistance of several bacteria against commercially available drugs increases tremendously. As a result, there is an urgent need for new antibiotic agents, which would fight against bacterial infections. Azetidin-2-ones and their derivatives have been extensively investigated, considering the presence of β-lactam ring[7-15] moiety in their, structure just as in the case of highly popular β-lactam antibiotics. Quantitative structure-activity relationship (QSAR) studies have been investigated on the basis of the fact that the biological activity of a compound is a function of its physicochemical properties [17-22]. Physicochemical parameters, which represent structural features of the compounds-for example, hydrophobicity (lipophilicity) and steric properties govern the biological activity to a greater extent. For the sake of present purpose,

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QSAR analysis of synthesized benzimidazole compounds was performed based on the assumption of linear additive contributions of the different physicochemical properties. Multiple regression analysis was applied to generate QSAR models and to obtain statistical parameters, i.e. correlation coefficient *(r),* standard deviation (s), F-test, cross validated correlation coefficient ($r^2$cv), sum of square standard error (SPRESS), predicted residual sum of squares (PRESS) and sum of the squares of response value (SSY). These statistical data were utilized in order to have a judicious interpretation for effective QSAR models. The best-derived QSAR model was used to predict the activity of the tested compounds and to suggest structural features, which could be incorporated in order to manifest enhanced biological activity.

## 2. Expermintal

All the chemicals used were of analytical grade purity. Melting points were taken in open capillary tubes using an electric melting point apparatus. All the melting points reported are uncorrected. $^1$H NMR spectra were recorded at 300MHz with a Bruker advance DRX 300 instrument using TMS as an internal stranded. IR spectra were run on a Perkin Elmer model 377-spectrophotometer using KBr pellets. Analytical thin layer chromatography was performed using E. Merck Silica gel-G 0.50 mm plates (Merck No. 5700). A equimolar quantity of Polyphosphoric acid (0.03mol, 5gm) was taken in 250ml round bottom flask and (0.03mol, 6gm)of 4-chloro- o-Phenylenediamne and (0.03mol, 4gm) of benzoic acid were added to it and the mixture was heated at 170$^c$ for 6-9 hours with constant stirring. The mixture was cooled and poured in 200 ml of purified water with constant stirring. The pH of the mixture was adjusted to alkaline side by addition of sodium carbonate. The precipitated solid was filtered under pressure and washed with purified water and dried under vaccum.the crude product was recystallized from ethanol (1[15]). Compound 1 react (2-4 steps Prepared according to the literature procedure[16]) with ethylchloroacetate in the presence of anhyd. Potassium carbonate in dry acetone gave 5-chloro-2-phenyl-1h-benzimidazole acetic acid ethyl ester (2) which on treatment with thiasemicarbazide resulted in the formation of (2-(2-Phenyl-benzimidazole-1-yl)-acetyl))-thiourea (3). Dehydrated annulation of compound 3 with conc. sulphuric acid followed by 25% dilute ammonia solution treatment yielded 5-(2-Phenyl-benzimidazole-1-ylmethyl)-(1,3,4)thiadiazol-2-ylamine(4) which on condensation with various selected aromatic aldehydes,furnished,Schiff'sbases1-(5-Methyl-(1,3,4)thiadiazole-2-ylmethyl)-2-Phenyl 1Hbenzoimidazole(5) The Schiff bases (0.01mol) were condensed with chloroacetylchloride (0.025mol) and triethylamine (0.025 mol) in dry dioxane (50 ml). The mixture was stirred continuously for 21 hrs. The resulting contents were kept at room temperature for 24 hrs to complete the reaction. The contents were then poured on to the crushed ice. The solid material was filtered, washed with distilled water and recrystallised from warm ethanol.

(5a)3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(2-hydroxy-4-methoxy-phenyl)-azetidin-2-one (KBr): 3168(N-H),3016(aromatic C-H),1633 (-C=N), 1699,1069(Ar-C-Cl) (-C=O), 698 (C-S-C),786 (-C-C1) cm'$^1$; $^!$H NMR (DMSO): 3.79 (s, 2H, -N-$CH_2$), 4.19 (s, 1H, -N-CH),5.62(s,1H,-C-OH), 5.05 (s, 1H, -CH-C1), 3.23 (s,3H, -$CH_3$), 7.05-8.10 (m, 12H, Ar-H); EI-MS: 550 ($M^+$ + 1).

(5b).3-chloro-4-(4-chlorophenyl)-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4] thiadiazole-2-yl]-azetidin-2-one IR (KBr): 3162(N-H),3030(aromatic C-H),1639 (-C=N), 1710 (-C=O), 708 (C-S-C), 1049(Ar-C-Cl) ,773 (-C-C1) cm'$^1$; $^!$H NMR (DMSO): 3.71 (s, 2H, -N-$CH_2$), 4.23 (s, 1H, -N-CH),5.05 (s, 1H, -CH-C1),7.01-8.14 (m, 12H, Ar-H); -MS m/z: 538 ($M^+$ + 1).

(5c).3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(2-nitro-phenyl)-azetidin-2-one IR (KBr): 3155(N-H),3014(aromatic C-H),1645 (-C=N), 1701 (-C=O), 1078(Ar-C-Cl) , 702 (C-S-C),771 (-C-C1) cm'$^1$; $^!$H NMR (DMSO): 3.72 (s, 2H, -N-$CH_2$), 4.20 (s, 1H, -N-CH),5.12 (s, 1H, -CH-C1),7.01-8.11 (m, 12H, Ar-H); -MS m/z: 551 ($M^+$ + 1).

(5d). 3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(4-nitro-phenyl)-azetidin-2-one-: IR (KBr): 3155(N-H),3014(aromatic C-H),1645 (-C=N), 1701 (-C=O), 1055(Ar-C-Cl), 702 (C-S-C),771 (-C-C1) cm'[1]; [!]H NMR (DMSO): 3.72 (s, 2H, -N-$CH_2$), 4.20 (s, 1H, -N-CH),5.12 (s, 1H, -CH-C1),7.01-8.11 (m, 12H, Ar-H); -MS m/z: 552 ($M^+$ + 1).

(5e).3-chloro-4-(4-chloro-2-nitrophenyl)-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-azetidin-2-oneIR(KBr):3155(N-H),3014(arC-H),1645(-C=N),1701( C=O), 1061(Ar-C-Cl),702 (C-S-C),771 (-C-C1) cm'[1]; [!]H NMR (DMSO): 3.78 (s, 2H, -N-$CH_2$),4.13(s, 1H, -N-CH),5.02 (s, 1H, -CH-C1),7.01-8.11 (m, 11H, Ar-H); MS m/z: 586 ($M^+$ + 1).

(5f)3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(2-ethoxy-phenyl)-azetidin-2-one : IR (KBr): 3143(N-H),3031(aromatic C-H),1621 (-C=N), 1705 (-C=O), 699 (C-S-C), 1051(Ar-C-Cl),762 (-C-C1) cm'[1]; [1]H NMR (DMSO): 2.42(s,3H-O-$CH_3$), 3.97 (s, 2H, -O-$CH_2$),3.70 (s, 2H, -N-$CH_2$), 4.11 (s, 1H, -N-CH),5.17 (s, 1H, -CH-C1),7.01-7.94 (m, 12H, Ar-H); -MS m/z: 550 ($M^+$ + 1).

(5g).3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(4-ethoxy-phenyl)-azetidin-2-one : IR (KBr): 3143(N-H),3031(aromatic C-H),1621 (-C=N), 1705 (-C=O), 699 (C-S-C), 1060 (Ar-C-Cl),762 (-C-C1) cm'[1]; [1]H NMR (DMSO): 2.42(s,3H-O-$CH_3$), 3.97 (s, 2H, -O-$CH_2$),3.70 (s, 2H, -N-$CH_2$), 4.11 (s, 1H, -N-CH),5.17 (s, 1H, -CH-C1),7.09-8.14 (m, 12H, Ar-H); -MS m/z: 549($M^+$ + 1).

(5h).3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(2,4-dichloro-phenyl)-azetidin-2-one-: IR (KBr): 3121(N-H),3047(aromatic C-H),1645 (-C=N), 1700 (-C=O), 1080(Ar-C-Cl), 709 (C-S-C),7.63 (-C-C1) cm'[1]; [!]H NMR (DMSO): 3.71 (s, 2H, -N-$CH_2$), 4.13 (s, 1H, -N-CH),5.02 (s, 1H, -CH-C1),7.03-8.10 (m, 11H, Ar-H); -MS m/z: 573 ($M^+$ + 1).

(5i).3-chloro-4-(2-chlorophenyl)-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl) [1,3,4]thiadiazole-2-yl]-azetidin-2-one:IR (KBr): 3162(N-H),3030(aromatic C-H),1639 (-C=N), 1710 (-C=O), 708 (C-S-C), 1068(Ar-C-Cl),773 (-C-C1) cm'[1]; [!]H NMR (DMSO): 3.71 (s, 2H, -N-$CH_2$), 4.23 (s, 1H, -N-CH),5.05 (s, 1H, -CH-C1),7.01-8.14 (m, 12H, Ar-H); -MS m/z: 538 ($M^+$ + 1).

(5j). 3-chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-phenyl-azetidin-2-one-: IR (KBr): 3140(N-H),3004(aromatic C-H),1661 (-C=N), 1701 (-C=O), 712 (C-S-C), 1074(Ar-C-Cl),771 (-C-C1) cm'[1]; [!]H NMR (DMSO): 3.78 (s, 2H, -N-$CH_2$), 4.13 (s, 1H, -N-CH),5.02 (s, 1H, -CH-C1),7.05-8.18 (m, 13H, Ar-H); -MS m/z506 ($M^+$ + 1).

(5k).3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(2-hydroxy-phenyl)-azetidin-2-one :IR(KBr):3133(N-H),3034(aromatic C-H),1671 (-C=N), 1698 (-C=O), 1065(Ar-C-Cl), 712 (C-S-C),767 (-C-C1) cm'[1]; [!]H NMR (DMSO): 3.62 (s, 2H, -N-$CH_2$), 4.04 (s, 1H, -N-CH),5.22 (s, 1H, -CH-C1), 5.41 (s, 1H, -C-OH),7.05-8.18 (m, 12H, Ar-H); -MS m/z: 521 ($M^+$ + 1).

(5l).3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(4-methoxy-phenyl)-azetidin-2-one :IR(KBr):3139(N-H),3014(aromatic C-H),1664 (-C=N), 1714 (-C=O), 719 (C-S-C), 1069(Ar-C-Cl),767 (-C-C1) cm'[1]; [1]H NMR (DMSO): 3.62 (s, 2H, -N-$CH_2$), 4.04 (s, 1H, -N-CH),5.22 (s, 1H, -CH-C1), 4.41 (s, 3H, -O-$CH_3$),7.05-8.18 (m, 12H, Ar-H); -MS m/z: 535 ($M^+$ + 1).

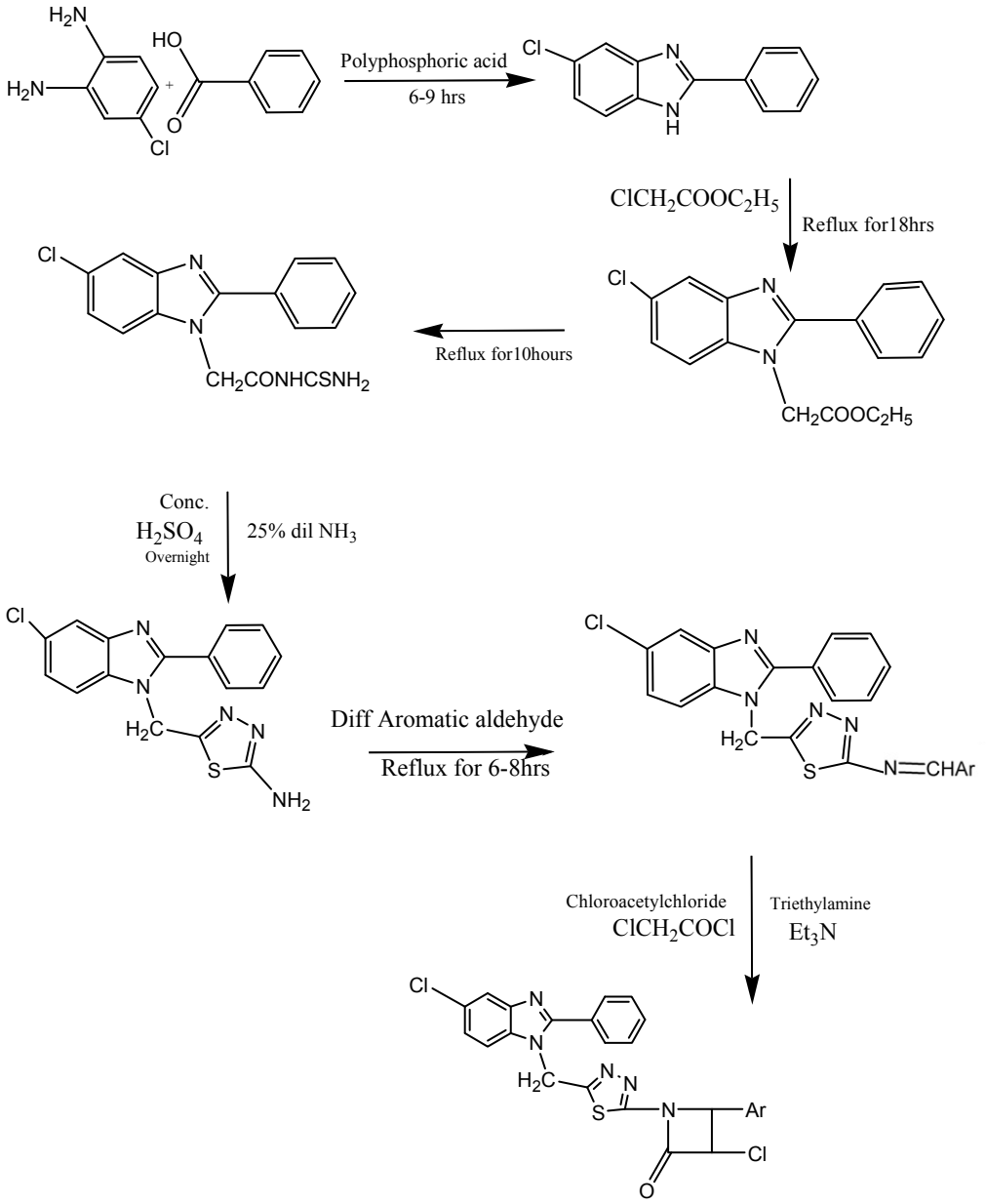
(5m).3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(4-hydroxy-phenyl)-azetidin-2-one ne:IR(KBr):3133(N-H),3034(aromatic C-H),1671 (-C=N), 1698 (-C=O), 1071(Ar-C-Cl), 712 (C-S-C),767 (-C-C1) cm'[1]; [!]H NMR (DMSO): 3.62 (s, 2H, -N-$CH_2$), 4.04 (s, 1H, -N-CH),5.22 (s, 1H, -CH-C1), 5.41 (s, 1H, -C-OH),7.05-8.18 (m, 12H, Ar-H); -MS m/z: 522 ($M^+$ + 1).

(5n).3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(3,4,5-trimethoxyphenyl)azetidin-2-one:IR(KBr):3124(N-H),3014(aromatic C-H),1682 (-C=N), 1714 (-C=O), 1049(Ar-C-Cl), 712 (C-S-C),760 (-C-C1) cm'[1]; [!]H NMR (DMSO):

3.62 (s, 2H, -N-$CH_2$), 4.04 (s, 1H, -N-CH), 4.28 (s, 9H, -O-$CH_3$),5.22 (s, 1H, -CH-C1), 7.05-8.18 (m, 10H, Ar-H); -MS m/z: 595 ($M^+$ + 1).

(5o).3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4]thiadiazole-2-yl]-4-(Dimethylamino-phenyl)azetidin-2-one:IR (KBr): 3124(N-H),3014(aromatic C-H),1682 (-C=N), 1714 (-C=O), 1058(Ar-C-Cl),712 (C-S-C),760 (-C-C1) cm'[1]; [!]H NMR (DMSO): 3.82 (s, 2H, -N-$CH_2$), 4.04 (s, 1H, -N-CH), 4.37 (s, 6H, -N-$CH_3$),5.22 (s, 1H, -CH-C1), 7.05-8.18 (m, 12H, Ar-H); -MS m/z: 548($M^+$ + 1).

SCHEME




3-Chloro-1-[5-(5-chloro-2-phenyl-benzoimidazol-1-ylmethyl)-[1,3,4]thiadiazol-2-yl]-azetidin-2-one

*Table 1. Physical constants of the synthesized compounds 4a-o & 5a-o*

| Comp. | R | M.F. | Yield (%) | M.P.(°) | $R_f$ Value | LogP | UV max |
|---|---|---|---|---|---|---|---|
| 4a | 2-hydroxy-4-methoxy | $C_{24}H_{19}N_5ClO_2S$ | 72 | 201-204 | 0.251 | 2.345 | 245nm |
| 4b | 4-chlorophenyl | $C_{23}H_{16}N_5Cl_2S$ | 78 | 194-197 | 0.316 | 2.479 | 253nm |
| 4c | 2-nitrophenyl | $C_{23}H_{16}N_6O_2ClS$ | 80 | 210-215 | 0.430 | 2.567 | 235nm |
| 4d | 4-nitrophenyl | $C_{23}H_{16}N_6O_2ClS$ | 65 | 220-224 | 0.170 | 3.673 | 270nm |
| 4e | 4-chloro-2-nitrophenyl | $C_{23}H_{15}N_6O_2Cl_2S$ | 67 | 175-178 | 0.226 | 4.654 | 265nm |
| 4f | 2-ethoxyphenyl | $C_{25}H_{21}N_5OClS$ | 61 | 190-192 | 0.385 | 3.568 | 256nm |
| 4g | 4-ethoxyphenyl | $C_{25}H_{21}N_5OClS$ | 59 | 223-226 | 0.237 | 2.451 | 265nm |
| 4h | 2,4-Dichlorophenyl | $C_{23}H_{15}N_5Cl_3S$ | 65 | 241-244 | 0.267 | 3.451 | 278nm |
| 4i | 2-chlorophenyl | $C_{23}H_{16}N_5Cl_2S$ | 68 | 193-195 | 0.196 | 3.902 | 280nm |
| 4j | Phenyl | $C_{23}H_{17}N_5ClS$ | 62 | 175-177 | 0.503 | 4.531 | 227nm |
| 4k | 2-hydroxyphenyl | $C_{23}H_{17}N_5OClS$ | 76 | 217-219 | 0.459 | 3.213 | 224nm |
| 4l | 4-methoxyphenyl | $C_{24}H_{19}N_5OClS$ | 80 | 234-236 | 0.481 | 3.121 | 289nm |
| 4m | 4-hydroxyphenyl | $C_{23}H_{17}N_5OClS$ | 56 | 233-235 | 0.362 | 4.021 | 272nm |
| 4n | 3,4,5-trimethoxyphenyl | $C_{26}H_{23}N_5O_3ClS$ | 61 | 260-263 | 0.612 | 3.789 | 240nm |
| 4o | Dimethylamino phenyl | $C_{25}H_{22}N_6ClS$ | 64 | 280-283 | 0.266 | 4.612 | 248nm |
| 5a | 2-hydroxy-4-methoxy | $C_{26}H_{19}Cl_2N_5O_3S$ | 66 | 246-248 | 0.586 | 4.543 | 271nm |
| 5b | 4-chlorophenyl | $C_{25}H_{16}Cl_3N_5OS$ | 70 | 262-264 | 0.498 | 5.432 | 286nm |
| 5c | 2-nitrophenyl | $C_{25}H_{16}Cl_2N_6O_3S$ | 74 | 250-254 | 0.545 | 5.786 | 335nm |
| 5d | 4-nitrophenyl | $C_{25}H_{16}Cl_2N_6O_3S$ | 70 | 281-284 | 0.625 | 6.543 | 303nm |
| 5e | 4-chloro-2-nitrophenyl | $C_{25}H_{15}Cl_3N_6O_3S$ | 72 | 234-236 | 0.742 | 6.547 | 326nm |
| 5f | 2-ethoxyphenyl | $C_{27}H_{21}Cl_2N_5O_2S$ | 67 | 291-293 | 0.562 | 6.812 | 3456nm |
| 5g | 4-ethoxyphenyl | $C_{27}H_{21}Cl_2N_5O_2S$ | 61 | 299-301 | 0.632 | 5.654 | 312nm |
| 5h | 2,4-Dichlorophenyl | $C_{25}H_{15}Cl_4N_5OS$ | 70 | 226-228 | 0.551 | 5.980 | 318nm |
| 5i | 2-chlorophenyl | $C_{25}H_{16}Cl_3N_5OS$ | 64 | 201-202 | 0.623 | 7.654 | 300nm |
| 5j | Phenyl | $C_{25}H_{17}Cl_2N_5OS$ | 65 | 230-232 | 0.674 | 5.786 | 321nm |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5k | 2-hydroxyphenyl | $C_{25}H_{17}Cl_2N_5O_2S$ | 73 | 271-272 | 0.590 | 6.231 | 365nm |
| 5l | 4-methoxyphenyl | $C_{26}H_{19}Cl_2N_5O_2S$ | 70 | 241-243 | 0.661 | 6.328 | 359nm |
| 5m | 4-hydroxyphenyl | $C_{25}H_{17}Cl_2N_5O_2S$ | 60 | 235-237 | 0.659 | 6.723 | 290nm |
| 5n | 3,4,5-trimethoxyphenyl | $C_{28}H_{23}Cl_2N_5O_4S$ | 66 | 301-303 | 0.720 | 5.875 | 357nm |
| 5o | Dimethylamino phenyl | $C_{27}H_{22}Cl_2N_6OS$ | 75 | 290-295 | 0.512 | 7.651 | 360nm |

*Table 2. Antimocrobial activity of the compounds 5a-5o.*

| Compounds | Zone of Inhibition in (mm) | | | | |
|---|---|---|---|---|---|
| | S. Aureus | B. Subtillis | E. Coli | A. niger | C. albicana |
| **5a** | 3.984 | 3.654 | 3.956 | 4.943 | 5.345 |
| **5b** | 4.154 | 4.556 | 4.140 | 4.189 | 4.160 |
| **5c** | 4.458 | 4.546 | 4.161 | 4.160 | 4.133 |
| **5d** | 4.542 | 4.056 | 4. 510 | 4.041 | 4.030 |
| **5e** | 4.450 | 4.542 | 3.591 | 4.020 | 4.235 |
| **5f** | 4.324 | 4.543 | 4.852 | 4.820 | 4.711 |
| **5g** | 4.590 | 4.589 | 4.678 | 4.665 | 4.624 |
| **5h** | 4.678 | 7.680 | 4.563 | 4.402 | 4.456 |
| **5i** | 4.651 | 4.356 | 4.056 | 4.180 | 4.759 |
| **5j** | 4.906 | 3.271 | 4.154 | 4.045 | 4.495 |
| **5k** | 3.238 | 4.002 | 4.161 | 4.160 | 4.233 |
| **5l** | 4.430 | 4.136 | 4. 210 | 4.041 | 4.231 |
| **5m** | 4.449 | 4.571 | 4.291 | 4.020 | 4.275 |
| **5n** | 4.419 | 4.143 | 3.052 | 4.220 | 4.011 |
| **5o** | 4.481 | 4.089 | 4.178 | 4.565 | 3.624 |
| **Norfloxacin** | 4.907 | 4.606 | 4.975 | ------ | -------- |
| **Clotrimazole** | -------- | ---------- | --------- | 3.51 | 3.80 |

### 2.1 Biological and QSAR Studies

The newly obtained derivatives were evaluated for in vitro antibacterial activity against Escherichia coli ATCC 13607, Staphylococcus aureus ATCC 2943, Bacillus subtilis ATCC 6633 and antifungal activity against Aspergillus niger ATCC 16404 and Candida albicans ATCC 10231. Nutrient agar and Saboured dextrose agar were employed for bacterial and fungal growth, respectively. Minimal Inhibitory Concentrations (MIC) were determined by means of standard twofold serial dilution method using agar media[25] and reported in Table 2. Stock solutions of tested compounds were prepared in DMSO at a concentration of 1 mg/mL. Suspension containing approximately $10^7$CFUs/mLof of bacteria and $10^6$ CFUs/mL of fungi were prepared from broth

cultures. Bacterial and fungal plates were made in triplicate and incubated at 37 $^0$C within 16–24 h for bacteria and 48–72 h for fungi. Norfloxacin and Clotrimazole were also screened under similar conditions as reference antibacterial and antifungal drug, respectively. MIC is defined as the lowest concentration of compound that inhibited visible growth. The correlation of observed activity, in terms of MIC (μg/mL) of reported compounds with different structural parameters, systematic QSAR investigations have been carried out using the model proposed by Hansch and coworkers[17].To The activity data (MIC) represents the concentration of compounds that inhibited visible growth. The same are further expressed as –log MIC on molar basis and used as dependent variables to get linear relationship in QSAR model. The calculated parameters used in present studies include Vander Waals volume (VDW), Connolly accessible area (CAA), Connolly molecular area (CMA), Connolly solvent excluded area (CSEV), dipole-dipole energy (DDENE), partition coefficient (log P) Table 3. The above-mentioned parameters were calculated by using Chem 3D 6.0 software[21]. Further, HOMO and LUMO energies were calculated by semi empirical PM3 studies using MOPAC 6.0 package[22].Multiple linear regression (MLR) analysis was used to investigate the correlation between biological activity and physicochemical properties. The MLR was performed by using the VALSTAT[24] by the stepwise method. The highest correlation of independent variables with dependent variable was chosen for deriving the QSAR model. The statistical values, multiple correlation coefficient (r), standard errors (s), cross validation $r^2$ ($q^2$) and standard error of prediction (SPRESS) were used to evaluate the obtained QSAR models. Several combinations of independent variables were firstly attempted using three variables (one representative from each property) for individual models, and then, more variables were added in order to optimize the statistical values but not more than five independent variables were used. The best model derived from the MLR analysis was used to predict the inhibitory activity of the synthesized compounds. Calculated parameters and correlation matrix needed are shown in Tables 3, 4 and 5.The resulting mono parametric models are depicted in Eqs. 1-6, along with statistical parameters of the regression. No outliers have been determined the equations were derived using the entire data set (n=15).

QSAR model for S. Aureus
BA =[4.7231(±0.183385)]+StrBE[-0.00249098(±0.000959259)]
n=15, r=0.821034, r^2=0.674097, variance=0.105331, std=0.324548, F=31.026.....1
QSAR model for B. Subtillis
BA= [4.67526( ± 0.223377)] +StrBE [-0.00243428( ± 0.00113716)] +DipL [-0.0768013( ± 0.0777552)]
n=15, r=0.832077, r^2=0. 892352, variance=0.139234, std=0.373141, F=15.7533.....2
QSAR model for E. coli
BA=[4.56686(± 0.216945)]+StrBE [-0.00366331( ± 0.00151133)] +TorE [-0.0221713( 0.0227936)]
n=15, r=0.830696, r^2=0.920056, variance=0.140274, std=0.374531, F=15.5847.....3
BA= [2.52931( ± 3.07013)] +DipE [-2505.96( ± 1848.11)] +DipL [2505.91( ± 1848.13)] +HOMO[-0.299262(±0.339542)]
n=15, r=0.940606, r^2=0.88474, variance=0.0291845, std=0.170835, F=35.8217.....4
QSAR model for A. niger
BA = [4.98717( ± 0.486757)] +LUMO [0.422503( ± 0.153794)] +BE[-0.186712( ± 0.117363)]+DipL[-0.135476(±0.0794105)]
n=15,r=0.91609,r^2=0.850058,variance=0.0534298,std=0.231149,F=13.004.....5
QSAR model for C. albicana
BA=[4.66037(±0.180173)]+StrBE[-0.00229287(±0.000972849)]+DipL[-0.107023(±0.0653869)]
n=15, r=0.858649, r^2=0.737278, variance=0.113739, std=0.337252, F=22.4504 ...6

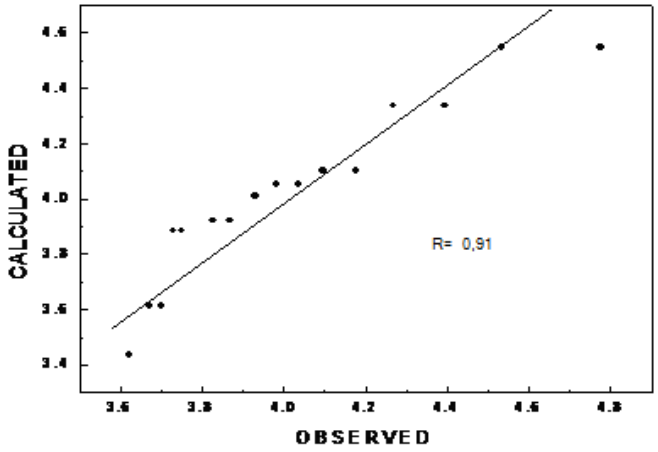



*Fig. 3. Plots of observed vs. calculated and observed vs. predicted activity of against E.Coli*

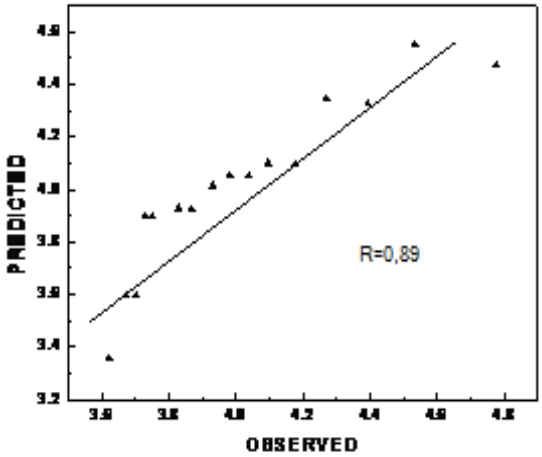



*Fig. 4. Plots of observed vs. calculated and observed vs. predicted activity against A. niger*

*Table 3. Calculated physico-chemical parameters of the compounds.*

| S.No. | HOMO | LUMO | Dipole Energy | Strech Energy | Stech bend energy | Torsion energy | Dipole length | Bend Energy |
|---|---|---|---|---|---|---|---|---|
| 5a | -7.72108 | -1.73927 | 3.85121 | 129.966 | -8.22454 | 18.1594 | 7.07674 | 32.0539 |
| 5b | -8.62655 | -0.686022 | 3.76773 | 6.25512 | -1.65284 | -0.58201 | 5.17066 | 29.6984 |
| 5c | -8.65548 | -0.687851 | 3.93823 | 6.3893 | -1.69169 | -0.338994 | 5.04646 | 29.8875 |
| 5d | -8.71065 | -0.888798 | 4.10803 | 6.08257 | -1.529 | -4.67359 | 4.41909 | 30.0214 |
| 5e | -8.66524 | -0.700145 | 3.82608 | 6.42995 | -1.68565 | 1.4222 | 5.22871 | 29.5951 |
| 5f | -7.99055 | -0.663117 | 4.40578 | 9.15987 | -1.52731 | 4.25048 | 4.81611 | 30.2639 |
| 5g | -8.13336 | -0.715728 | 4.33364 | 8.7707 | -1.50199 | 0.545449 | 7.01467 | 29.7314 |
| 5h | -8.07344 | -0.687741 | 4.17745 | 9.51539 | -1.64337 | 2.64631 | 5.29105 | 30.6205 |
| 5i | -8.26894 | -0.91695 | 4.44794 | 8.1308 | -1.31449 | 3.80641 | 7.91143 | 30.562 |
| 5j | -8.96708 | -0.944754 | 0.74873 | 9.67002 | -1.33446 | 25.8829 | 7.21486 | 31.8442 |
| 5k | -8.69043 | -0.833993 | 9.35988e | 10.3209 | -1.45319 | 14.2989 | 10.2871 | 31.7791 |
| 5l | -8.29894 | -0.592622 | -0.72892 | 10.0876 | -1.45319 | -1.75164 | 5.76081 | 29.7897 |
| 5m | -8.4527 | -1.00524 | 0.112016 | 9.59837 | -1.2462 | 6.45914 | 8.19172 | 30.5129 |
| 5n | -8.77663 | -0.981942 | 1.03041 | 21.9933 | -1.16709 | 16.3105 | 8.3754 | 30.9537 |
| 5o | -8.8087 | -0.846746 | 0.893568 | 23.3292 | -1.40079 | 17.7638 | 12.1102 | 30.8414 |

*Table 4. Correlation matrix of used molecular descriptors*

| | **$HOMO_{ene}$** | **$LUMO_{ene}$** | DE | **SE** | **SBE** | **TE** | **DipL** | **BE** |
|---|---|---|---|---|---|---|---|---|
| $HOMO_{ene}$ | 1.000 | | | | | | | |
| $LUMO_{ene}$ | 0.720 | 1.000 | | | | | | |
| DE | 0.503 | 0.574 | 1.000 | | | | | |
| SE | 0.242 | 0.255 | 0.480 | 1.000 | | | | |
| SBE | 0.106 | 0.275 | 0.336 | 0.548 | 1.000 | | | |
| TE | 0.650 | 0.643 | 0.423 | 0.521 | 0.407 | 1.000 | | |
| DipL | 0.182 | 0.0149 | 0.086 | 0.766 | 0.412 | 0.595 | 1.000 | |
| BE | 0.539 | 0.219 | 0.320 | 0.515 | 0.258 | 0.509 | 0.579 | 1.000 |

*Table 5. Cross-validation parameters.*

| **Eq.** | **PRESS** | **SSY** | **PRESS/SSY** | **$S_{PRESS}$** | **SDEP** | **$r^2_{CV}$** | **$r^2_{bsp}$** |
|---|---|---|---|---|---|---|---|
| 1 | 0.156 | 1.009 | 0.155 | 0.105 | 0.098 | 0.913 | 0.892 |
| 2 | 0.414 | 0.926 | 0.447 | 0.172 | 0.160 | 0.749 | 0.718 |
| 3 | 0.294 | 0.807 | 0.364 | 0.144 | 0.135 | 0.787 | 0.757 |
| 4 | 0.273 | 0.719 | 0.380 | 0.140 | 0.131 | 0.779 | 0.759 |
| 5 | 0.214 | 0.873 | 0.553 | 0.321 | 0.127 | 0.749 | 0.734 |
| 6 | 0.134 | 0.769 | 0.471 | 0.132 | 0.139 | 0.721 | 0.772 |

## 3. Results and discussion

Good antibacterial activity was observed in 5b, 5c, 5e, 5g, 5h, against *B. Subtilis* compounds 5h, 5i, 5j, showed good activity against *S. aureus* compounds 5a,5b, 5d, 5f,5g showed significant activity against A. niger and whereas compounds 5a,5d,5f,5i, showed noticeable activity against *E. coli.* Compound 5a, 5b, 5d, 5e, 5f, 5h showed marked activity against *A-niger* and *C. albicans.* Conclusively, a series of 3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1, 3, 4] thiadiazole-2-yl]-azetidin-2-one derivatives have been synthesized as potent antimicrobial agents. Furthermore, QSAR studies performed on these compounds have revealed that the positive coefficient of the HOMO, LUMO, BendEnergy descriptor, which relates to the hydrophobicity of the molecule, suggested that an increase in the lipophilicity might increase the activity. This corresponds to the presence of hydrophobic binding site in the 3-Chloro-1-[5-(5-chloro-2-phenyl-benzimidazole-1-ylmethyl)-[1,3,4] thiadiazole-2-yl]-azetidin-2-one. Previously we have done 2D QSAR studies K.F. Ansari, et.al[16] . On the basis of 2D QSAR studies we prepare new design molecules with syntheses and here done again 15 new molecules with QSAR Studies. All the compounds exhibited significant antibacterial and antifungal activities.

## 4. Conclusion

Conclusively, a variety of azetidine derivatives have been successfully synthesized in appreciable yields and screened in vitro for their antimicrobial activities against both strains of Gram-positive and Gram-negative bacteria. Moreover, moreover, quantitative structure–activity relationship studies revealed that the antimicrobial activities of these synthesized derivatives against the test microorganisms are mainly governed by the molar refractivity, a polarizability parameter. Thus a proper substitution of the group with high polarizability at N-6 position probably improves the potency of these derivatives as antibacterial and antifungal agents. The effect of modification at this site will be the subject of further optimization and investigation. Such a QSAR evaluation would open future perspectives to use these compounds as new lead compounds in clinical trials.

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