Research Paper

Two Dimensional Quantitative Structure Activity Relationship Prediction and Synthesis of Bioactive Newer 1,2,4-Triazole-Isoniazid Conjugates

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1,2,4-triazole and Schiff bases are active against antimicrobial and antitubercular activities. Isoniazid itself reacts as antitubercular drug. The two dimensional quantitative structure activity relationship study was used to predicts the antitubercular activity of the synthetic derivatives. Two dimensional quantitative structure activity relationship generated model using partial least squares regression method which predict the statistically significant r^2=0.8940, q^2=0.8197, pred_r^2=0.6675 and F test=50.6234. Two dimensional quantitative structure activity relationship generated equation of pMICs is denoted the antitubercular activity correlated with thermodynamic descriptor XAMostHydrophilic. Pharmacokinetic properties absorption, distribution, metabolism, excretion were also predicted which useful for design the derivatives. A designed derivatives of 1-(3-(substitutidene)amino)-5-(2,3,4,5-tetrafluoro-6-substitutedphenyl)-4H-1,2,4-triazol-4-yl)-3-(pyridin-4-yl)urea (B_1-B_{10}) were synthesized and spectrally evicted from fourier-transform infrared spectroscopy, ^{1}H Nuclear magnetic resonance, ^{13}C Nuclear magnetic resonance and Mass spectra as well as biologically evaluated against antitubercular and antimicrobial activities. From the biologically evaluated derivatives, compounds B_2, B_4, B_7, B8, B_9 and B_{10} were found to be active against the different antimicrobial species. Where as compound B_2 also active against antitubercular species.

Key words: Two dimensional quantitative structure activity relationship, partial least squares regression, 1,2,4-triazole, antimicrobial activity, antitubercular activity

From the last few decades, 1,2,4-triazole fused derivatives and itself it have gained high importance in the pharmaceutical industry as antimicrobial, antituberculer and antimalarial agents. Many antimicrobial drugs are available those having 1,2,4-triazole. Several significant biological activities, such as antimicrobial[1,2], antituberculosis[3], antimalarial[4], anticancer[5] and antioxidant[6] are described by 1,2,4-triazole. A number of routes are available for synthesis of 1,2,4-triazole[7-10]. From which we are synthesized 1,2,4-triazole directly from acid. Schif base is formation of carbon-nitrogen double bond which play wider role in synthetic organic chemistry. It is obtained from aldehydes and amines Schiff base creates attention for organic chemists because of its vital role in pharmaceutical chemistry. It gives significant activities like anticancer[11], antitumor[12], antituberculosis[13], antimicrobial[14], anticonvulsant[15]. Infectious deseases tuberculosis is an infectious disease which caused by *Mycobacterium tuberculosis (M. tuberculosis)*[16]. Today, tuberculosis is situted in the top five deases of global mortality. Approximately estimation is that arround 1 billion peoples are infected between years 2002 to 2018, around 150 million peoples are got sick and around 36 million people are died because of tuberculosis if new disease prevention and treatment measurements are not developed[17]. Isoniazid is one of the most effective antituberculosis drugs used for tuberculosis treatment.

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Some few decades, Two Dimensional Quantitative Structure Activity Relationship (2D-QSAR) are being applied in many areas of drug design. Accepted technique, 2D-QSAR is carried out to predicted the pharmacological activities which is a mathematical tool used to evaluate the toxicity of a compounds from its physiochemical properties of molecular structures. Our interest for synthesis of 1,2,4-triazole, isoniazid, Schiff base and flourine containing derivatives. We are referred literature review on 2D-QSAR study of biologically active 1,2,4-triazole, isoniazid, Schiff base and flourine derivatives[18-20]. 2D-QSAR were determined by VLifeMDS software and Partial Least Squares Regression (PLSR) method. 2D-QSAR predicted value of activity which are compared with actual biological activity. Swiss Absorption, Distribution, Metabolism, Excretion (ADME) tool is used for prediction of ADME properties.

My aim is to synthesized antimicrobial and antitubercular active agents. Our interest to developed 1,2,4-triazole scaffold as a novel bio-conjugates which are exhibited pharmacological properties as an antimicrobial[21] and an antituberculer[22] activities. For this purpose, we had decided to synthesized 1,2,4-triazole based conjugates which containing Schiff base and isoniazid in its final structure.

MATERIALS AND METHODS

General materials:

Analytical grade chemicals were used in synthesis. Fisher-Johns Melting Point apparatus was used to determined Melting points of synthesized compounds. Compounds purities were checked by Thin Layer Chromatography (TLC) on silica gel plates with visualization by Ultraviolet-light and iodine chamber. Fourier-Transform Infrared Spectroscopy (FTIR) spectra were analyzed by model FTIR 8400S and frequency obtained in cm^{-1} unit. 1H Nuclear Magnetic Resonance (NMR) and ^{13}C Nuclear Magnetic Resonance spectra were recorded at 400 MHz on Bruker Avance II spectrometer instruments in Dimethylsulfoxide (DMSO)-d_6. Chemical shifts were recorded in parts per million downfield from tetramethylsilane. Mass spectra were recorded on Liquid chromatography coupled to Mass Spectrometry (LC-MS). Structures and nomenclatures of the compounds are generated on Perkin Elmer ChemBioOffice Ultra 14.0.0.117 software. SwissADME online tool is used for prediction of absorption, distribution, metabolism, and excretion (ADME) properties.

General methods:

General procedure for synthesis of (E)-N-(5-(2,3,4,5-tetrafluoro-6-substitutedphenyl)-1,3,4-oxadiazol-2-yl)substitutedimine (A_1-A_{10}): An equimolar mixture of 2,3,4,5-tetrafluoro-6-substitutedbenzoic acid (0.02 mol), semicarbazide (0.02 mol) and substituted aldehyde (0.02 mol) were dissolved in methanol, which succeded by the addition of acetic acid (0.02 mol) and heat up to 90° around 30 min. Than, the addition of anhydrous zinc chloride (0.02 mol), reaction mass was heated around 5 h. The TLC was checked after each 1.5 h using water:methanol (3:7) mobile phase. When completed the reaction, cooled the reaction mass and pour over crushed ice. Obtained precipitates washed with water, followed by distilled water and crystallized from methanol to make a pure product (A). All the derivatives (A_1-A_{10}) are synthesized by this method (Table 1).

General procedure for the synthesis of (E)-N-(3-(substitutidene) amino)-5-(2,3,4,5-tetrafluoro-6-Substitutedphenyl)-4H-1,2,4-triazol-4-yl) isonicotinamide (B_1-B_{10}): An equimolar mixture of compound (A) (0.02 mol), isoniazid (0.02 mol) and substituted aldehyde (0.02 mol) were dissolved in methanol in a round bottom flask, then add KOH (0.02 mol) and heat up to 90° for 4-5 h. When completed the reaction cooled the reaction mass and pour over ice cold water. Obtained precipitates washed with water, followed by distilled water and crystallized from methanol to make pure product (B). All the derivatives (B_1-B_{10}) are synthesized by this method (Table 2).

Spectral data of compounds:

1-(4-Methylthio)phenyl)-N-(5-(2,3,4,5-tetrafluorophenyl)-1,3,4-oxadiazol-2-yl)-methanimine (A_1): Infrared (IR) (KBr) ν cm^{-1}: 3157, 3071 (C-H stretching, aromatic), 2988, 2921 (C-H stretching, aliphatic), 1593 (C=C stretching), 1524 (N-N stretching), 1485 (C=N stretching, oxadiazole), 1448 (C=N stretching, diazomethane), 1356 (C-H bending, SCH_3), 1247 (C-N stretching), 1142 (C-O-C stretching), 1093 (C-F stretching), 1003 (C-H out of plane), 812 (C-H bending, diazomethane), 754 (C-S stretching); 1H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.93 (s, ^1H, N=CH), 7.98 (s, 1H, tetrafluorophenyl), 7.60 (d, 2H, J=5.36 Hz, methyl(phenyl)sulfide), 7.30 (d, 2H, J=8.48 Hz, methyl(phenyl)sulfide), 2.49 (s, 3H, SCH_3).

TABLE 1: PHYSICAL DATA OF COMPOUNDS (A_1-A_{10})

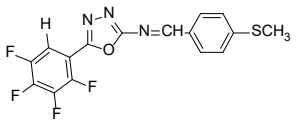
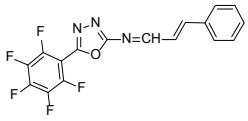
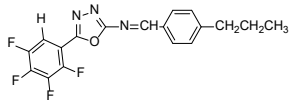
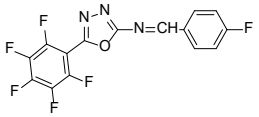
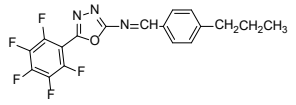
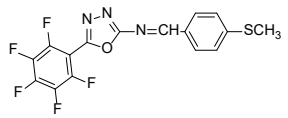
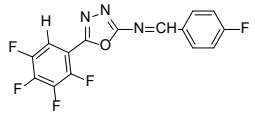
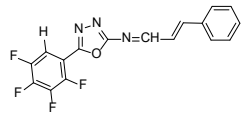
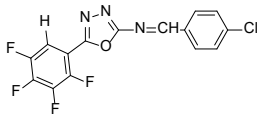
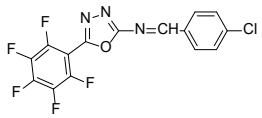
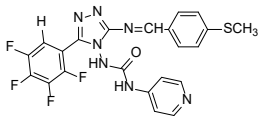
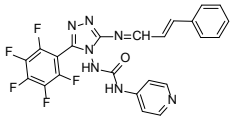
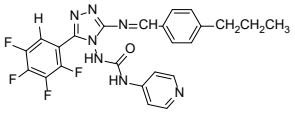
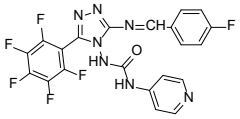
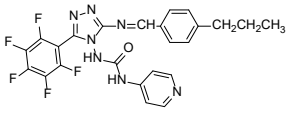
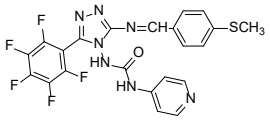
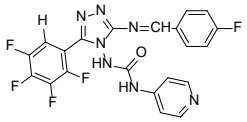
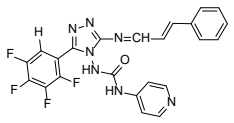
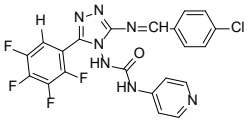
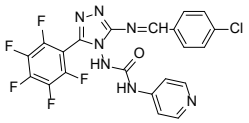
Code	IV	R_1	R_2	% yield	MP(°)	M W gm/mol	R_f	M.F.
A_1		H	4-Methylthiophenyl	73	179	367.32	0.5	$C_{16}H_9ON_3SF$
A_2		F	Styrene	77	185	365.26	0.5	$C_{17}H_8ON_3F_5$
A_3		H	4-Propylphenyl	74	194	363.31	0.6	$C_{18}H_{13}ON_3F_4$
A_4		F	4-Fluorophenyl	71	217	357.21	0.5	$C_{15}H_5ON_3F_6$
A_5		F	4-Propylphenyl	68	193	381.3	0.6	$C_{18}H_{12}ON_3F_5$
A_6		F	4-Methylthiophenyl	69	157	385.31	0.5	$C_{16}H_8ON_3SF_5$
A_7		H	4-Fluorophenyl	74	176	339.22	0.5	$C_{15}H_6ON_3F_5$
A_8		H	Styrene	75	181	347.27	0.5	$C_{17}H_9ON_3F_4$
A_9		H	4-Chlorophenyl	79	167	355.67	0.4	$C_{15}H_6ON_3F_4Cl$
A_{10}		H	4-Chlorophenyl	79	167	355.67	0.4	$C_{15}H_6ON_3F_4Cl$

TABLE 2: PHYSICAL DATA OF COMPOUNDS (B_1-B_{10})

Code	IV	R_1	R_2	% yield	MP(°)	M. W. gm/ mol	R_f	M.F.
B_1		H	4-Methylthiophenyl	70	209	501.46	0.68	$C_{22}H_{15}ON_7SF_4$
B_2		F	Styrene	76	226	499.4	0.63	$C_{23}H_{14}ON_7F_5$
B_3		H	4-Propylphenyl	72	218	497.45	0.65	$C_{24}H_{19}ON_7F_4$
B_4		F	4-Fluorophenyl	70	254	491.35	0.57	$C_{21}H_{11}ON_7F_6$
B_5		F	4-Propylphenyl	67	230	515.44	0.76	$C_{24}H_{18}ON_7F_5$
B_6		F	4-Methylthiophenyl	69	190	519.45	0.63	$C_{22}H_{14}ON_7SF_5$
B_7		H	4-Fluorophenyl	75	207	473.36	0.58	$C_{21}H_{12}ON_7F_5$
B_8		H	Styrene	73	201	481.4	0.69	$C_{23}H_{15}ON_7F_4$
B_9		H	4-Chlorophenyl	80	196	489.81	0.71	$C_{21}H_{12}ON_7F_4Cl$
B_{10}		F	4-Chlorophenyl	71	230	507.8	0.77	$C_{21}H_{11}ON_7F_5Cl$

N-(3-((4-(methylthio)benzylidene)amino)-5-(2,3,4,5-tetrafluorophenyl)-4H-1,2,4-triazol-4-yl)isonicotinamide (B_1): IR (KBr) ν cm^{-1}: 3393 (N-H stretching), 3159, 3067 (C-H stretching, aromatic), 2913, 2871 (C-H stretching, aliphatic), 1753 (C=O stretching), 1667 (N-H bending), 1597 (C=C stretching), 1573 (N-N stretching), 1491 (C=N stretching, oxadiazole), 1448 (C=N stretching, diazomethane), 1351 (C-H bending, SCH_3), 1253 (C-N stretching), 1097 (C-F stretching), 1009 (C-H out of plane), 824 (C-H bending, diazomethane), 750 (C-S stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.09 (s, 1H, N=CH), 7.78 (d, 2H, J=4.64 Hz, isoniazid), 7.56 (d, 2H, J=8.40 Hz, methyl(phenyl) sulfide), 7.37 (d, 2H, J=8.96 Hz, methyl(phenyl) sulfide), 7.17 (d, 2H, J=8.40 Hz, isoniazid), 6.66 (s, 1H, N-H), 2.45 (s, 3H, SCH_3); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 161.24 (N=CH), 156.79 (C_1 triazole), 153.06, 152.88 (C=O), 151.92, 151.42 (C_2 triazole), 146.97, 139.22, 137.80, 131.22, 129.53, 126.71, 125.47, 113.12, 108.82, 14.57 (SCH^3); Electrospray Ionisation Mass Spectrometry (ESI-MS) (m/z): 501.46 M^+, 503.46 $[M+2]^+$ peak calculated and 502 M^+, 504 $[M+2]^+$ peak found.

N-(3-pentafluorophenyl)-5-(((E)-3-phenylallylidene)amino)-4H-1,2,4-triazol-4-yl) isonico tinamide (B_2): IR (KBr) ν cm^{-1}: 3325 (N-H stretching), 3046 (C-H stretching, aromatic), 2964, 2913, 2889 (C-H stretching, aliphatic), 1716 (C=O stretching), 1684 (N-H bending), 1607 (C=C stretching), 1564 (N-N stretching), 1483 (C=N stretching, triazole), 1452 (C=N stretching, diazomethane), 1436 (C-H bending, CH_2), 1259 (C-N stretching), 1084 (C-F stretching), 998 (C-H out of plane), 923 (C-H bending, CH_2), 874 (C-H bending, diazomethane); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.80 (d, 2H, J=5.72 Hz, isoniazid), 8.72 (d, 1H, J=5.52 Hz, N=CH), 7.66 (d, 2H, J=7.12 Hz, phenyl ring), 7.60 (s, 1H, N-H), 7.53 (t, 2H, J=7.32 Hz, phenyl ring), 7.43 (t, 1H, J=7.36 Hz, phenyl ring), 7.29 (d, 1H, J=2.20 Hz, HC=CH-Ar), 7.02 (d, 2H, J=5.44 Hz, isoniazid), 6.77 (t, 1H, J=2.20 Hz, HC=CH-Ar); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 163.48 (N=CH), 156.55, 154.91 (C_1 triazole), 152.73 (C=O), 151.95 (C_2 triazole), 149.98, 143.51, 138.36, 134.86 (HC=CH), 130.67, 128.46, 126.63 (aromatic carbons), 121.38 (HC=CH), 111.73; ESI-MS (m/z): 499.40 M^+ peak calculated and 499 M+ peak found.

N-(3-((4-propylbenzylidene)amino)-5-(2,3,4,5-tetrafluorophenyl)-4H-1,2,4-triazol-4-yl) isonicotinamide (B_3): IR (KBr) ν cm^{-1}: 3417 (N-H stretching), 3068 (C-H stretching, aromatic), 2956, 2871 (C-H stretching, aliphatic), 1690 (C=O stretching), 1648 (N-H bending), 1603 (C=C stretching), 1506 (N-N stretching), 1443 (C=N stretching), 1357 (C-H bending, SCH_3), 1254, 1138 (C-N stretching), 1091 (C-F stretching), 957 (C-H out of plane), 936 (C-H bending, CH_2), 830 (C-H bending, diazomethane), 730 (C-S stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.76 (s, 1H, tetrafluorophenyl), 8.12 (s, 1H, N=CH), 7.89 (d, 2H, J=4.82 Hz, isoniazid), 7.68 (d, 2H, J=8.52 Hz, propylphenyl), 7.33 (d, 2H, J=8.63 Hz, propylphenyl), 7.11 (d, 2H, J=8.36 Hz, isoniazid), 6.35 (s, 1H, N-H), 2.61 (t, 2H, J=2.58 Hz, $CH_2CH_2CH_3$), 1.67 (m, 2H, J=2.86 Hz, $CH_2CH_2CH_3$), 0.92 (t, 3H, J=3.16 Hz, CH_3); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 163.71 (N=CH), 155.33, 153.46 (C_1 triazole), 152.06 (C=O), 150.96 (C_2 triazole), 150.28, 143.86, 140.15, 138.76, 136.02, 134.83, 129.36, 128.23, 127.84, 120.31, 111.32 (aromatic carbons), 38.13 (CH_2), 22.42 (CH_2), 13.11 (CH_3); ESI-MS (m/z): 497.45 M^+ peak calculated and 497 M^+ peak found

N-(3-((4-fluorobenzylidene)amino)-5-(perfluorophenyl)-4H-1,2,4-triazol-4-yl) isonicotinamide (B_4): IR (KBr) ν cm^{-1}: 3419 (N-H stretching), 3151, 3057 (C-H stretching, aromatic), 2926, 2882 (C-H stretching, aliphatic), 1702 (C=O stretching), 1684 (N-H bending), 1607 (C=C stretching), 1565 (N-N stretching), 1449 (C=N stretching), 1258 (C-N stretching), 1101 (C-F stretching), 998 (C-H out of plane), 870 (C-H bending, diazomethane); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.16 (s, 1H, N=CH), 7.72 (d, 2H, J=4.76 Hz, isoniazid), 7.61 (d, 2H, J=8.44 Hz, fluorophenyl), 7.43 (d, 2H, J=8.12 Hz, fluorophenyl), 7.21 (d, 2H, J=8.16 Hz, isoniazid), 6.39 (s, 1H, N-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 164.11 (N=CH), 156.21, 153.16 (C_1 triazole), 151.86 (C=O), 150.27 (C_2 triazole), 149.17, 143.42, 140.31, 138.64, 135.82, 133.83, 129.36, 128.54, 127.67, 119.54, 111.91; ESI-MS (m/z): Calculated 491.35 M+ peak calculated and 492 M^+ peak found.

N-(3-pentafluorophenyl)-5-((-4-propylbenzylidene)amino)-4H-1,2,4-triazol-4-yl) isonicotinamide (B_5): IR (KBr) ν cm^{-1}: 3413 (N-H stretching), 3056 (C-H stretching, aromatic), 2974, 2856 (C-H stretching, aliphatic), 1694 (C=O stretching), 1649 (N-H bending), 1605 (C=C stretching), 1506 (N-N stretching), 1443 (C=N stretching), 1357 (C-H bending, CH_3), 1256, 1139

(C-N stretching), 1093 (C-F stretching), 957 (C-H out of plane), 940 (C-H bending, CH_2), 838 (C-H bending, diazomethane); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.19 (s, 1H, N=CH), 7.93 (d, 2H, J=4.90 Hz, isoniazid), 7.66 (d, 2H, J=8.56 Hz, propylphenyl), 7.32 (d, 2H, J=8.63 Hz, propylphenyl), 7.12 (d, 2H, J=8.40 Hz, isoniazid), 6.36 (s, 1H, N-H), 2.61 (t, 2H, J=2.58 Hz, $CH_2CH_2CH_3$), 1.65 (m, 2H, J=2.90 Hz, $CH_2CH_2CH_3$), 0.93 (t, 3H, J=3.12 Hz, CH_3); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 162.94 (N=CH), 155.33, 153.49 (C_1 triazole), 152.16 (C=O), 150.91 (C_2 triazole), 150.38, 143.86, 140.15, 138.76, 135.83, 134.83, 129.36, 128.23, 127.84, 121.23, 111.53 (aromatic carbons), 36.43 (CH_2), 21.37 (CH_2), 13.18 (CH_3); ESI-MS (m/z): 515.44 M^+ peak calculated and 516 M^+ peak found.

N-(3-((4-(methylthio)benzylidene)amino)-5-(pentafluorophenyl)-4H-1,2,4-triazol-4-yl) isonicotinamide (B_6): IR (KBr) ν cm^{-1}: 3417 (N-H stretching), 3157, 3068 (C-H stretching, aromatic), 2979, 2913 (C-H stretching, aliphatic), 1750 (C=O stretching), 1685 (N-H bending), 1597 (C=C stretching), 1573 (N-N stretching), 1489 (C=N stretching, oxadiazole), 1448 (C=N stretching, diazomethane), 1356 (C-H bending, SCH_3), 1241 (C-N stretching), 1090 (C-F stretching), 1013 (C-H out of plane), 813 (C-H bending, diazomethane), 750 (C-S stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.24 (s, ^{1}H, N=CH), 7.88 (d, 2H, J=4.94 Hz, isoniazid), 7.54 (d, 2H, J=8.48 Hz, methyl(phenyl) sulfide), 7.39 (d, 2H, J=8.92 Hz, methyl(phenyl) sulfide), 7.19 (d, 2H, J=8.40 Hz, isoniazid), 6.64 (s, 1H, N-H), 2.46 (s, 3H, SCH_3); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 162.54 (N=CH), 156.46 (C_1 triazole), 153.26, 152.78 (C=O), 151.21 (C_2 triazole), 147.04, 139.32, 137.68, 130.29, 129.53, 126.67, 125.42, 113.12, 108.72 (aromatic carbons), 14.18 (SCH_3); ESI-MS (m/z): 519.45 M^+, 521.45 $[M+2]^+$ peak calculated and 520 M^+, 522 $[M+2]^+$ peak found.

N-(3-((4-Fluorobenzylidene)amino)-5-(2,3,4,5-tetrafluorophenyl)-4H-1,2,4-triazol-4-yl) isonicotinamide (B^7): IR (KBr) ν cm^{-1}: 3358 (N-H stretching), 3162, 3051 (C-H stretching, aromatic), 2937, 2867 (C-H stretching, aliphatic), 1712 (C=O stretching), 1652 (N-H bending), 1612 (C=C stretching), 1564 (N-N stretching), 1454 (C=N stretching), 1257 (C-N stretching), 1103 (C-F stretching), 996 (C-H out of plane), 874 (C-H bending, diazomethane); 1H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.84 (s, 1H, tetraflurophenyl), 8.18 (s, 1H, N=CH), 7.76 (d, 2H, J=4.76 Hz, isoniazid), 7.58 (d, 2H, J=8.36 Hz, fluorophenyl), 7.45 (d, 2H, J=8.16 Hz, fluorophenyl), 7.19 (d, 2H, J = 8.20 Hz, isoniazid), 6.42 (s, 1H, N-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 163.27 (N=CH), 156.23 (C_1 triazole), 154.12, 152.39 (C=O), 151.46 (C_2 triazole), 146.83, 139.68, 137.54, 130.37, 129.26, 126.34, 125.21, 112.86, 109.17; ESI-MS (m/z): 473.36 M^+ peak calculated and 473 M^+ peak found.

N-(3-(((E)-3-phenylallylidene)amino)-5-(2,3,4,5-tetrafluorophenyl)-4H-1,2,4-triazol-4-yl)isonicotinamide (B_8): IR (KBr) ν cm^{-1}: 3339 (N-H stretching), 3045 (C-H stretching, aromatic), 2968, 2918, 2894 (C-H stretching, aliphatic), 1720 (C=O stretching), 1680 (N-H bending), 1607 (C=C stretching), 1564 (N-N stretching), 1482 (C=N stretching, triazole), 1446 (C=N stretching, diazomethane), 1436 (C-H bending, CH_2), 1260 (C-N stretching), 1084 (C-F stretching), 998 (C-H out of plane), 922 (C-H bending, CH_2), 872 (C-H bending, diazomethane); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.88 (s, 1H, tetraflurophenyl), 8.76 (d, 2H, J=5.68 Hz, isoniazid), 8.71 (d, 1H, J=5.56 Hz, N=CH), 7.64 (d, 2H, J=7.16 Hz, phenyl ring), 7.54 (t, 2H, J=7.28 Hz, phenyl ring), 7.45 (t, 1H, J=7.40 Hz, phenyl ring), 7.31 (d, 1H, J=2.16 Hz, HC=CH-Ar), 7.06 (d, 2H, J=5.40 Hz, isoniazid), 6.77 (t, 1H, J=2.24 Hz, HC=CH-Ar), 6.52 (s, 1H, N-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 163.37 (N=CH), 156.33, 153.77 (C_1 triazole), 152.67 (C=O), 151.62 (C_2 triazole), 150.39, 143.25, 138.36, 134.83 (HC=CH), 130.56, 128.46, 126.53, 121.34 (HC=CH), 111.87; ESI-MS (m/z): 481.40 M^+ peak calculated and 481 M^+ peak found.

N-(3-((4-chlorobenzylidene)amino)-5-(2,3,4,5-tetrafluorophenyl)-4H-1,2,4-triazol-4-yl) isonicotinamide (B_9): IR (KBr) ν cm^{-1}: 3375 (N-H stretching), 3036 (C-H stretching, aromatic), 2964, 2873 (C-H stretching, aliphatic), 1712 (C=O stretching), 1678 (N-H bending), 1598 (C=C stretching), 1563 (N-N stretching), 1443 (C=N stretching), 1269 (C-N stretching), 1110 (C-F stretching), 993 (C-H out of plane), 838 (C-H bending, diazomethane), 815 (C-Cl stretching); 1H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.74 (s, 1H, tetrafluorophenyl), 8.18 (s, 1H, N=CH), 7.75 (d, 2H, J=4.72 Hz, isoniazid), 7.64 (d, 2H, J=8.52 Hz, chlorophenyl), 7.47 (d, 2H, J=7.96 Hz, chlorophenyl), 7.17 (d, 2H, J=8.12 Hz, isoniazid), 6.42 (s, 1H, N-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 161.97 (N=CH), 155.82, 153.42 (C_1 triazole), 152.27 (C=O), 151.19 (C_2 triazole), 150.26, 143.15, 138.24, 130.31,

128.46, 126.13, 111.32; ESI-MS (m/z): 489.81 M^+ peak calculated and 490 M^+ peak found.

N-(3-((4-chlorobenzylidene)amino)-5-(pentafluorophenyl)-4H-1,2,4-triazol-4-yl) isonicotinamide (B_{10}): IR (KBr) ν cm^{-1}: 3396 (N-H stretching), 3034 (C-H stretching, aromatic), 2951, 2872 (C-H stretching, aliphatic), 1716 (C=O stretching), 1658 (N-H bending), 1593 (C=C stretching), 1556 (N-N stretching), 1446 (C=N stretching), 1273 (C-N stretching), 1105 (C-F stretching), 994 (C-H out of plane), 848 (C-H bending, diazomethane), 818 (C-Cl stretching); 1H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.16 (s, 1H, N=CH), 7.71 (d, 2H, J=4.80 Hz, isoniazid), 7.63 (d, 2H, J=8.56 Hz, chlorophenyl), 7.49 (d, 2H, J=7.96 Hz, chlorophenyl), 7.15 (d, 2H, J=8.08 Hz, isoniazid), 6.41 (s, 1H, N-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 162.67 (N=CH), 155.21, 153.26 (C_1 triazole), 152.17 (C=O), 151.42 (C_2 triazole), 150.67, 143.64, 138.46, 130.23, 128.67, 126.34, 111.33; ESI-MS (m/z): 507.80 M^+ peak calculated and 508 M^+ peak found.

RESULTS AND DISCUSSION

Compounds were synthesized in two steps which first intermediate (E)-N-(5-(2,3,4,5-tetrafluoro-6-substitutedphenyl)-1,3,4-oxadiazol-2-yl) substitutedimine (A_1-A_{10}) were synthesized from 2,3,4,5-tetrafluoro-6-substitutedbenzoic acid using Lewis acid as catalyst, which held on Perkin-Elmer apparatus and obtained intermediates were spectrally evaluated from IR and ^{1}H NMR spectra. IR is given band at 1485 cm^{-1} (Oxdiazole C=N), 1448 cm^{-1} (diazomethane C=N) and 1093 cm^{-1} (C-O-C) and ^{1}H NMR is given a singlet signal at 8.93 δ ppm (N=CH) which are sufficient for identification of intermediates. Final compounds 1-(3-(substitutidene)amino)-5-(2,3,4,5-tetrafluoro-6-substitutedphenyl)-4H-1,2,4-triazol-4-yl)-3-(pyridin-4-yl) urea were synthesized from intermediates using isoniazid and KOH as a catalyst. Obtained compounds characterized from IR, ^{1}H NMR, ^{13}C NMR and Mass spectrum. In IR spectrum, bend obtained at 1271-1245 cm^{-1} for (C-N) instead of 1093 cm^{-1} (C-O-C) to indicate the formation of the final compound. In ^{1}H NMR, signals obtained singlet at 10.10-10.30 δ ppm and 6.20-6.45 δ ppm for (N-H). ^{13}C NMR gives the signal at 161.10-161.40 δ ppm for (N=CH), 156.79 δ ppm for (C_1 triazole) and 151.62 δ ppm for (C_2 triazole). Both step products were purified by methanol and solvents used in reaction were distilled out and dried it using dry sieves as the usual manner.

Compounds were evicted for antimicrobial susceptibility. The broth dilution method was used to quantitative measure of the *in vitro* antimicrobial activity[23-25]. Compounds were tested against two gram positive bacterial strains; *Staphylococcus aureus* (MTCC-96) and *Streptococcus pyogenes* (*S. pyogenus*) (MTCC-443), two gram-negative bacterial strains; *Escherichia coli* (*E. coli*) (MTCC-442) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (MTCC-441), and for fungi, three species, *Candida albicans* (*C. albicans*) (MTCC-227), *Aspergillus niger* (MTCC-282), and *Aspergillus clavatus* (MTCC-1323) are used. Institute of Microbial Technology, Chandigarh is procured the strains and species. To evaluating the activity of compounds against microorganisms, Minimum Inhibitory Concentrations (MICs) were measured which is the lowest concentration of the assayed antimicrobial agent to inhibits the visible growth of the microorganism tested and its expressed in µg/ml and DMSO is used as diluents/vehicles. Chloramphenicol, ciprofloxacin and norfloxacin are used as standard drug. The results depicted in Table 3 revealed that compounds B_8 active against *E. coli*, B_9 against P. aeruginosa, B_2 and B_7 against S. pyogenus. From the above active compounds B_2 contains extended conjugation and B_7, B_8, B_9 having electron withdrawing groups which indicate electron withdrawing group containing substituents and extending conjugation very effective against the antibacterial species. The antifungal screening data displayed in Table 3 which gives the variable inhibitory effects against fungal species. Greseofulvin and nystatin are used as a standard antifungal drug. From the screened compounds B_4, B_{10} activity against *C. albicans*, which are having electron withdrawing groups. Drug susceptibility and MICs determination of the compounds against *M. tuberculosis* H37Rv were performed by the by L-J medium agar micro dilution method[26-28]. Rifampicin and isoniazid are used as standard drug. The MIC levels of screened compounds (B_1-B_{10}) against *M. tuberculosis* are given in Table 4 which explained that the compounds, showen variable inhibitory effects on the growth of the tested *M. tuberculosis* H37Rv strains. From the tested compounds, B2 having extended conjugation and B4 having electron withdrawing groups, which both are increase the electrone density of compounds so that they shown active against *M. tuberculosis* H37Rv.

TABLE 3: ANTIMICROBIAL ACTIVITY DATA OF COMPOUNDS (B_1-B_{10})

Code	Minimal Bactericidal Concentration (µg/ml)				Minimal Fungicidal Concentration (µg/ml)		
	E. coli	*P. aeruginosa*	*S. aureus*	*S. pyogenus*	*C. albicans*	*A. niger*	*A. clavatus*
	MTCC 443	MTCC 1688	MTCC 96	MTCC 442	MTCC 227	MTCC 228	MTCC 1323
B1	250	62.5	125	250	>1000	>1000	1000
B2	250	100	150	25	500	500	500
B3	500	150	100	500	500	500	400
B4	500	500	500	200	200	200	250
B5	125	250	150	100	500	500	250
B6	250	100	125	250	500	500	250
B7	125	100	150	12.5	1000	1000	1000
B8	50	125	250	125	>1000	>1000	500
B9	250	12.5	100	62.5	1000	1000	500
B10	100	62.5	250	200	250	250	250
Drug	Micromolar (µg/ml)						
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	25	25	50	50	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Greseofulvin	-	-	-	-	500	100	100

Note: All the values are presented as mean of six experiments. Antimicrobial activity is zero for 2P DMSO which used as control and diluent

TABLE 4: ANTITUBERCULAR ACTIVITY AND 2D-QSAR DATA OF THE COMPOUNDS (B1-B10)

Code	H37Rv MIC in µg/ml				Discriptors Used
	Actual	Actual	Predicted	Residual	XA Most Hydrophilic
	MIC	pMIC	pMIC	pMIC	
B1	125	-2.09	-2.04	0.05	-7.5752
B2	25	-1.39	-1.45	-0.06	-7.5750
B3	100	-2.00	-2.04	-0.04	-7.5752
B4	12.5	-1.09	-1.15	-0.06	-7.5749
B5	100	-2.00	-1.74	-0.26	-7.5751
B6	100	-2.00	-1.74	-0.26	-7.5754
B7	50	-1.69	-1.45	-0.24	-7.5750
B8	62.5	-1.79	-1.74	0.05	-7.5749
B9	125	-2.09	-2.33	-0.14	-7.5753
B10	62.5	-1.79	-1.74	-0.05	-7.5749
Drug	Micromolar (µg/ml)				
Isoniazid	0.20				
Rifampicin	40				

Note: pMIC=(1/log MIC) value used for 2D-QSAR determination. All the values of MIC presented as mean of six experiments. Antitubercular activity is zero for 2P DMSO which used as control and diluent

SAR study suggested that 3, 4, and 5 substituted 1,2,4-triazole is required for more potent antimicrobial and antitubercular activity[29,30]. At Position-3 HC=N Schiff base and aromatic ring are more favorable to increase the antimicrobial and antitubercular activity. At position-4 isoniazid increased the antitubercular activity and at position-5 aromatic ring increased the antimicrobial activity due to increased resonance. 1,2,4-Triazole is itself increased the activity due to double bonds between carbon and nitrogen atom. The arrangement of the substitution position and groups are also effective to increase the biological effects. Resonance with 1,2,4-triazole derivatives are given optimum activity against antimicrobial species. Position-3 contains C=N Schiff base and electron withdrawing group having aromatic substitution which favorable to increase the biological activity. Toxophoric C=N linkage of Schiff base is increased the antimicrobial activity[31]. Isoniazid is itself an antitubercular drug and its derivatives are potent antitubercular activity against *M. tuberculosis* H37Rv (MTB) and multi-drug resistant *M. tuberculosis* (MDR-TB)[32]. Fluorinated compounds are given the potent antimicrobial activity. A fluorine atoms are increased the pharmacological properties due to more electronegativity, low polarizability, small atom size and strong carbon-fluorine bond. Cinnamal phenyl have exhausted more effective activity due to extending conjugation. The arrangement of groups and rings also affects better to good biological activities[33]. We are further process the *in vivo* activity for more potent compounds.

2D-QSAR analysis is used to understand observed biological properties and determines the structural parameter control biological activity. A large number of molecular descriptors (physicochemical, spatial, electronic and topological parameters) are usually used in the QSAR analysis using Vlife MDS software. The various physicochemical descriptors calculated the free energy change due to drug receptor complex and topological structure descriptors are used for alignment independent descriptor, which both independent descriptors are considered as independent variables. Spatial parameters are given the steric features of the drug molecules which required to fit with the receptor. Non-covalent bonding between the drug molecules and the receptors, which is described the electronic descriptors. QSAR analysis regression was performed using pMIC values as dependent variables and calculated parameters as independent variables. Manually selecting and placing molecules in the training and test sets comprising of 8 and 2 molecules, respectively.

PLSR method is generated significant QSAR model[34], which is considered on the basis of statistical parameters, correlation coefficient (r), squared correlation coefficient (r^2), predictive r^2 for external test set (pred r^2) for external validation, and Fischer's value (F). External validation for the activity of the test set was predicted using the model developed by the training set[35]. The cross-validated coefficient, q2 was calculated[36]. The significance of the models, hence obtained is derived based on a calculated Z score[37].

pMIC (pMIC=log(1/MIC)) values are applied for the 2D-QSAR prediction of *M. tuberculosis* H37Rv. PLSR methodology was used up for the 2D-QSAR prediction of the compounds from VLife MDS software, which counting the term selection criterion as r^2, q^2, pred_r^2 and F test. The training and test sets of compounds were selected by the sphere exclusion method and the models were validated by both internal and external validation procedures. The model gives the following equation for pMIC prediction.

pMIC=+2957.0432
XAMostHydrophilic+22398.1532

$N_{training}$=5, N_{test}=5, Degree of freedom=6, r^2=0.8940, q^2=0.8197, F test=50.6234, r^2_se=109.5612, q^2_se=142.9109, pred_r^2=0.6675, pred_r^2se=367.2893

The equation explains 89 % (r^2=0.8940) of the total variance in the training set as well as it has internal (q^2) and external (pred_r^2) predictive ability of ~81 % and ~66 % respectively. The F test=50.6234 shows the statistical significance of the model. The model is incorporated with XA Most Hydrophilic parameters with their corresponding values for each molecule in the selected model given in Table 3. The descriptor XA Most Hydrophilic in the model is indices the highest hydrophilicity of the compounds correlate with the antitubercular activity. The positive correlation suggests that antitubercular activity directly proportional to highest hydrophilicity of 1,2,4-triazole derivatives. The model is validated by Z_{Score} R^2=5.53757, Z_{Score} Q^2=3.57107, Best Rand R^2=0.40359, Best Rand Q^2=-0.00449, Alpha Rand R^2=0.0000, Alpha Rand Q^2=0.0050, ZScore Pred R^2=1.57248, best Rand Pred R^2=0.33061, Alpha Rand Pred R^2=0.1000. The randomization test suggests that the developed model has a probability of less than 1 % that the model is generated by chance.

The observed MIC values are near to predicted MIC values. Predicted values, Residual values and used descriptors shown in Table 3. Fitness graph Predicted vs Actual data given in graph.

It is mandatory to study the pharmacokinetics properties, i.e., absorption in the body, distribution into the different compartments, metabolism by organs and elimination through the body. Computational studies of the ADME parameters are mandatory to designed the molecules which prioritize for synthesis[38]. Hence, the ADME study is an essential step for checking the drug-likeness. ADME studies of the synthesised compounds (C_1-C_{10}) were carried out using the Swiss ADME tool[39]. QSAR and drug-likeness are also predicted an octanol/water partition coefficient (log Po/w), Topological Polar Surface Area (TPSA), Hydrogen Bond Acceptor (HBA), Hydrogen Bond Donor (HBD), Lipinski Rule and synthetic accessibility which are tabulated in Table 5.

Lipinski rule of five is given by Lipinski *et al*.[40] in 1997, the rule of five is based on exact norms to predict the drug-likeness of a molecule which having a pharmacological activities. These norms are log P<5, number of HBD<5, HBA<10 and M.W≤500 Da. This rule is used in preselect molecules which presenting good ADME properties that must have a medicament in the organism. Here, we are used the SwissADME property calculator (http://www.SwissADME.com) to measured the four parameters of Lipinski's rule. The number of rotatable bonds that are to be lesser than 10 have a good oral bioavailability[41].

In conclusion, biologically active isoniazid and Schiff bases having 1,2,4-triazole derivatives 1-(3-(Substitutidene)amino)-5-(2,3,4,5-tetrafluoro-6-substitutedphenyl)-4H-1,2,4-triazol-4-yl)-3-(pyridin-4-yl)urea was synthesized. These derivatives are contained antimicrobial and antitubercular active toxophores isoniazid, 1,2,4-triazole and Schiff base. From this toxophoric unit isoniazid is increased antitubercular activity while 1,2,4-triazole and Schiff base increased the antimicrobial activity. The derivatives also having the fluorinated molecules which are increased the biological activity of compounds because of its electron withdrawing nature. From the biological results, we concluded an electron withdrawing group containing substituents and extending conjugation increased the biological activity. We are also performed the 2D-QSAR for *M. tuberculosis* H37Rv using VLife MDS software which suggested that antitubercular activity of the compounds are directly proportional to most hydrophilicity of the compounds.

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TABLE 5: *IN SILICO* ADMET PROPERTIES DATA OF THE COMPOUNDS (B_1-B_{10})

Code	RB	HBA	HBD	MR	Log S	GI absorption and BBB Permeant	Log Kp (cm/s)	SA	TPSA	Lipinski Rule
B_1	6	9	1	111.70	-5.10	High and no	-6.45	3.72	85.06	1
B_2	7	10	1	116.62	-5.29	Low and no	-6.52	3.81	85.06	1
B_3	8	9	1	121.31	-9.46	Low and no	-5.93	3.88	85.06	1
B_4	6	7	1	106.65	-5.12	Low and no	-6.71	3.61	85.06	1
B_5	8	10	1	121.27	-5.89	Low and no	-5.97	3.88	85.06	2
B_6	6	9	1	111.70	-5.10	High and no	-6.45	3.72	85.06	1
B_7	6	10	1	106.69	-4.96	High and no	-6.67	3.61	85.06	1
B_8	7	9	1	116.67	-5.13	High and no	-6.48	3.81	85.06	1
B_9	6	9	1	111.74	-5.39	High and no	-6.39	3.6	85.06	1
B_{10}	6	10	1	111.70	-5.55	Low and no	-6.43	3.6	85.06	1

Note: pMIC=(1/log MIC) value used for 2D-QSAR determination. All the values of MIC presented as mean of six experiments. Antitubercular activity is zero for 2P DMSO which used as control and diluent

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