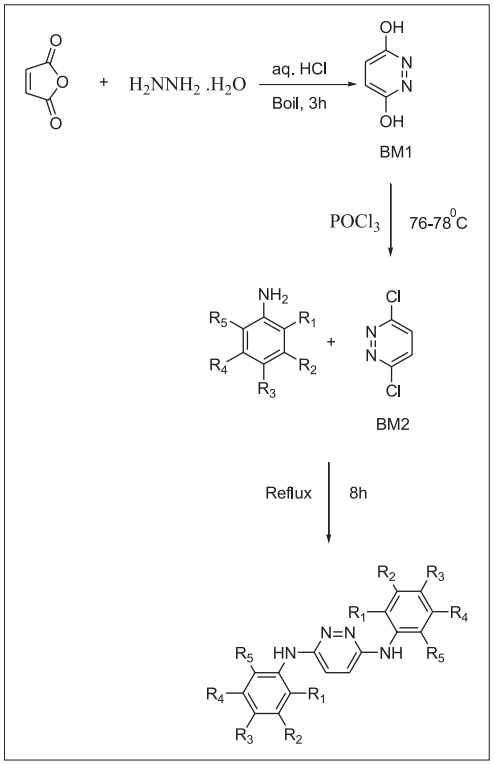

Figure 1: General synthetic scheme

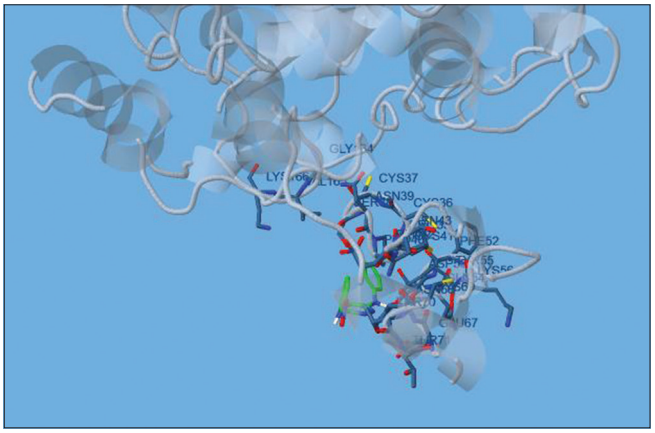

Figure 2: Stereo view of the complex formed by DHFR and the docked compound (AJ-23).The amino acids: GLY34, ASN39, CYS37, LYS 166, CYS36 AND CYS36 were involved in interaction with compounds

6.89 (d, *J* = 9.4 Hz, 1H), 7.15 (m, 2H, 4'-H), 7.28 (d, *J* = 9.4 Hz, 1H), 7.41 (d, *J* = 7.3 Hz, 2H, 2'-H), 8.0 (s, 1H, NH), 11.8 (s, 1H, NH). Analytical calculated value for $C_{16}H_{12}N_4I_2$: C, 37.38; H, 2.35; N, 10.9; I, 49.37. Analytical observed value for $C_{16}H_{12}N_4I_2$: C, 37.33; H, 2.38; N, 10.5; I, 49.34. m/z: 513.9.

AJ26 IR(KBr, cm^{-1}): 3410 (NH), 3042 (C-H Aromatic), 1659 (C = N). ^{1}H NMR (400MHz, DMSO-d_6):δ 6.62 (d, *J* = 8.0 Hz, 4H), 6.97 (d, *J* = 9.6 Hz, 1H), 7.28 (d, *J* = 9.6 Hz, 1H), 7.38 (d, *J* = 8.6 Hz, 4H), 8.3 (s, 1H, NH), 11.9 (s, 1H, NH). Analytical calculated value for $C_{16}H_{12}N_4I_2$: C, 37.38; H, 2.35; N, 10.9; I, 49.37. Analytical observed value for $C_{16}H_{12}N_4I_2$: C, 37.34; H, 2.31; N, 10.5; I, 49.33. m/z: 513.9.

AJ27 IR(KBr, cm^{-1}): 3400-3000 (OH Broad Peak), 1659 (C = O), 1581 (C = N). ^{1}H NMR (400MHz, DMSO-d_6): δ 5.4 (s, 2H, OH), 6.59 (d, *J* = 7.2 Hz, 2H, 5'-H), 6.88 (d, *J* = 7.7 Hz, 2H, 4'-H), 6.98 (d, *J* = 9.6 Hz, 1H), 7.32 (d, *J* = 9.6 Hz, 1H), 7.35 (s, 2H, 2'-H), 9.2 (s, 1H, NH), 10.45 (s, 2H, COOH), 14.4 (s, 1H, NH). Analytical calculated value for $C_{18}H_{14}O_6N_4$: C, 56.55; H, 3.69; N, 14.65; O, 25.11. Analytical observed value for $C_{18}H_{14}O_6N_4$: C, 56.51; H, 3.66; N, 14.68; O, 25.15. m/z: 382.3.

AJ28 IR(KBr, cm^{-1}): 3458 (NH), 2954 (OH Aromatic), 1674 (C = O ester), 1626 (C = N), 1283 (C-O ester). ^{1} H NMR (400MHz, $CDCl_3$): δ 3.81 (s, 6H, OCH_3),3.82 (s, 6H, OCH_3), 3.83 (s, 6H, $COOCH_3$), 6.13 (s, 2H, 5'-H) 7.91 (d, *J* = 9.8 Hz, 1H), 7.28 (s, 2H, 2'-H), 7.36 (d, *J* = 9.8 Hz, 1H), 8.9 (s, 1H, NH), 13.8 (s, 1H, NH). Analytical calculated value for $C_{24}H_{26}O_8N_4$: C, 57.83; H, 5.26; N, 11.24; O, 25.68. Analytical observed value for $C_{24}H_{26}O_8N_4$: C, 55.74; H, 5.56; N, 11.79; O, 26.92. m/z: 498.2.

AJ29 IR(KBr, cm^{-1}): 3416 (NH), 2961 (C-H Aromatic), 1616 (C = N). ^{1}H NMR (400MHz, $CDCl_3$): δ 1.32 (m, 6H, *CH_3), 2.35 (s, 6H, CH_3), 2.61 (m, 4H, *CH_2), 6.47 (m, 2H, 3'-H), 6.68 (d, *J* = 2.6 Hz, 2H, 2'-H), 6.93 (d, *J* = 9.8 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 2H, 4'-H), 7.30 (d, *J* = 9.8 Hz, 1H), 8.3 (s, 1H, NH), 13.8 (s, 1H, NH). Analytical calculated value for $C_{22}H_{26}N_4$: C, 76.27; H, 7.56; N, 16.17. Analytical observed value for $C_{22}H_{26}N_4$: C, 76.24; H, 7.59; N, 16.19. m/z: 346.4.

AJ30 IR(KBr, cm^{-1}): 3325 (NH), 3033 (C-H Aromatic), 2799 (C-H). ^{1}H NMR (400MHz, $CDCl_3$): δ 2.33 (s, 3H, CH_3), 3.72 (s, 6H, OCH_3), 6.62 (d, *J* = 8.6 Hz, 2H, 5'-H), 6.67 (d, *J* = 2.6 Hz, 2H, 4'-H), 6.90 (d, *J* = 10.1 Hz, 1H), 7.27 (d, *J* = 10.1 Hz, 1H), 7.39 (s, 2H, 2'-H), 8.6 (s, 1H, NH), 13.3 (s, 1H, NH). Analytical calculated value for $C_{20}H_{22}O_2N_4$: C, 68.55; H, 6.33; N, 15.99; O, 9.13. Analytical observed value for $C_{20}H_{22}O_2N_4$: C, 68.53; H, 6.37; N, 15.96; O, 9.17. m/z: 350.1.

Docking study

All the synthesized compounds were docked into active site of DHFR and the results are presented as estimated free energy of binding in Table 2.

Antimicrobial activity

All the synthesized compounds were subjected to antimicrobial activity using five bacterial and two fungal

stains. The MIC of all the synthesized compounds were calculated and reported in tabular form [Table 3]. Ciprofloxacin was used as a standard against bacterial stains, and ketoconazole was used as a standard against fungal stains.

DISCUSSION

Starting material for the synthesis was obtained by the reaction between 3,6-pyridazine diol with phosphorous oxychloride [Table 1]. Final compounds were designed by the reaction between 3,6-dichlopyridazine and various anilines. An attempt was made to synthesize diphenylpyridazine analogs. All the reactions were monitored throughout by TLC. All the structures of final compounds were confirmed by IR, NMR, and mass spectrometry. The synthesized pyridazine analogs were screened for their antibacterial activity using broth microdilution method against *S. aureus, M. luteus*, *E. coli, P. aeruginosa* and *P. aurantiaca*. Ciprofloxacin was used as standard drug for comparison. The title compounds were also evaluated for their antifungal activity against *T. rubrum* and *C. laurentii* using Ketoconazole standard drug. The results revealed that synthesized compounds showed excellent broad-spectrum antimicrobial activity against all bacterial as well as fungal stains and compound AJ23 was found to be most active compound. However, since Nitroaromatic compounds are actually toxic and mutagenic and many are suspected or established carcinogens and which can be removed at this point in time. However, compound bearing hydroxyl group and carboxyl group was found to be most active next most active compound, i.e., Compound AJ27. From the literature, it has been observed that the incorporation of electron withdrawing substituent on phenyl ring causes increase in activity and are important structural requirement to be a good antimicrobial agent.

A careful analysis of all the data for the antimicrobial activity of all pyridazine analogs demonstrated an interesting finding that incorporation of one or more NO_2, Cl, Br groups in the skeleton causes increase in activity. Incorporation of fluorine in methyl group instead of the main skeleton does not yields good results. Compounds having–OCH_3 are less active than other compounds of the series. Addition of–COOH group on the main skeleton, increases the activity. Similarly, compounds bearing 2-iodo compounds are less active than 4-iodo compounds. Same finding is continued for bromine as well as chlorine-containing compounds in the same series. In drug receptor interaction study ligands were ranked according to docking score/estimated free energy of binding. The free energy of binding of ligands was in the range between −5.12 to −8.97 Kcal/mole. Top-ranked compound (AJ-23) and (AJ-12) with −8.97 and −7.31 Kcal/mole free energy of binding, respectively, were in correlation with wet laboratory experiments. The protein-ligand analysis also has shown its strong interactions with target protein and had six hydrogen bond interactions in (AJ-23) and five hydrogen bond interactions in (AJ-12). The excellent interactions of DHFR with all top ranked compounds indicated a high degree of coherent relationship between *in silico* approach and *in vitro* studies. Large numbers of hydrogen bond interactions exist between different amino acids of the DHFR and NO_2/hydroxyl/methoxyl group present in ring A, B, and heterocyclic ring C. High anti-microbial activities of the compounds demand further *in vivo* and clinical studies, and these compounds might find an important place in the new array of molecules targeting DHFR-dependent biological functions. Keeping in view, the biological and pharmacological importance of the pyridazine derivatives and biphenyl derivatives, it is our endeavor, to bring two important moieties into the single molecular frame by appropriate synthetic routes. This will stand not only as a source for new biologically active compounds but also as a model for molecular conjunction in the design of new drugs. The *in silico* results of the study were in good tune with the laboratory work experiments, and nearly 80% of docking results were same as that of *in vitro* experiments. Ligand-protein interactions were profoundly found in the present study and gave an insight for further evaluation of study up to molecular level.

CONCLUSIONS

All the test compounds (AJ11-AJ30) were screened for antimicrobial activity against five bacterial and two fungal stains, namely, *S. aureus, M. luteus, E. coli, P. aeruginosa, P. aurantiaca, T. rubrum, C. laurentii*. Most of the compounds showed remarkably good broad-spectrum activity ranging from 7.8 to 500 ug/ml. The remaining compounds have shown moderate activity. N^3, N^6-diphenylpyridazine-3,6-diamine is established as structural skeleton required for the antimicrobial activity. Compound AJ27 is found to be the most active compound. Docking studies revealed that carbonyl oxygen, NO_2, OH, and OCH_3 of the selected derivatives were involved in hydrogen bonding with various amino acids of the receptor. This confirms our hypothesis that conjugation of two pharmacophores might improve the pharmacological profile of synthesized compounds

Acknowledgment

The authors are thankful to Vice-chancellor Shoolini University for providing laboratory facilities.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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