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Original Article

Drug design and synthesis of 1, 3, 4-oxadiazolo cinnoline analogs as potent antitubercular agents

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ABSTRACT

A Series of 3-(5-substituted-1,3,4-oxadiazol-2-yl)-6-substitutedcinnolin-4-ol and 5-(4-hydroxy-6-substitutedcinnolin-3-yl)-1,3,4-oxadiazole-2(3)-thione were synthesized from 4-hydroxy-6-substitutedcinnoline-3-carbohydrazide. The structures of the newly synthesized compounds were confirmed by IR, NMR, Mass and elemental analysis. All the designed leads were optimised for ADMET parameters to predict drug likeliness using QIKPROP [GLIDE]. All the compounds were screened for their antibacterial and antifungal activity against various pathogenic microbes. Some of the compounds displayed very good antibacterial and antifungal activity remaining compounds showed moderate to good activity.

Keywords: Cinnoline,1,3,4-oxadiazole, QIKPROP, antitubercular, antifungal, MABA

INTRODUCTION

Oxadiazole is a five membered heterocyclic ring which is a versatile lead compound for designing potent bioactive agents. This interesting group of compound has diverse biological activities such as antimicrobial, anti-inflammatory, anti-tubercular, anti-hypertensive, anti-analgesic, anticonvulsant, anticancer, anti-HIV, hypogycemic and genotoxic etc [1]. Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is a tenacious and remarkably successful pathogen that has latently infected a one-third of the world population. It can be cured, the therapy takes at least 6-9 months and laborious and lengthy treatment brings with it dangers of non-compliance, significant toxicity and drug resistance and oppourtunistic fungal infections like Candidosis. Many of these diseases are fatal if untreated, and treatment has been complicated by the resistance of the microorganisms to the widely used drugs. The increasing emergence of drug resistance highlights the need to develop some novel active Anti-tubercular and Anti-fungal drugs. Cinnoline is a versatile lead molecule that has been investigated widely in medicinal chemistry due to its important pharmacological activities [2-4]. It have been reported to exhibit anti-microbial, anti-tubercular, anti-cancer, anti-malarial, anti-hypertensive, anti-pyretic, anti-thrombolytic, analgesic, anti-diabetic, anti-depressant, cardiotonic, anaesthetic, anxiolytic etc. Cinnoline ring system is an isosteric relative to either Quinoline or Isoquinoline, for urinary tract infection. This prompted us the synthesis of new congeners by fusing oxadiazole with Cinnoline hoping to get more potent anti-mycobacterial and anti-fungal activity.

MOLECULAR PROPERTIES PREDICTION

QikProp predicts physically significant descriptors and pharmaceutically relevant properties for organic structures. In addition to predicting molecular properties, QikProp [5] provides ranges for comparing a particular molecule's properties with those of 95% of known drugs. QikProp produces the following descriptors and properties like molecule name, Number of property or descriptor values that fall outside the 95% range of similar values for known drugs. Number of non-conjugated amine groups, Number of substituted groups, Number of carboxylic acid groups, Number of non-conjugated amide groups, Number of non-trivial, non-hindered ,rotatable bonds, Number of reactive functional groups, Predicted central nervous system activity on a -2 (inactive) to +2 (active) scale, Molecular weight of the molecule, Computed dipole moment of the molecule, Total

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solvent accessible surface area (SASA) , FOSA Hydrophobic component of SASA , FISA Hydrophilic component of the SASA, PISA carbon and attached hydrogen) component of the SASA,WPSA Weakly polar component of the SASA (halogens, P, and S),volume ,donor HB Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution, accptHB -Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution, QPpolrz- Predicted polarizability in cubic ångströms,QPlogPC16- Free energy of solvation in hexadecane, QPlogPoct- Free energy of solvation in octanol, QPlogPw- Free energy of solvation in water. QPlogPo/w- Predicted octanol/water partition coefficient. QPlogS Predicted aqueous solubility, log S. BIPCaco- Predicted apparent Caco-2 cell permeability in nm/sec using the Boehringer-Ingelheim scale, AffyPCaco- Predicted apparent Caco-2 cell permeability in nm/sec using the Affymax scale, QPlogBB-Predicted brain/blood partition coefficient, AffyPMDCK- Predicted apparent MDCK cell permeability in nm/sec using the Affymax scale, QPlogKp- Predicted skin permeability, QPLog Khsa- Prediction of binding to human serum albumin. All the title compounds were subjected to molecular properties prediction to check drug likeliness by QikProp v3.1 [GLIDE] software in order to filter the drugs for biological screening and the prediction scores are given in Table 1, 2&3.

SYNTHESIS OF 1, 3, 4-OXADIAZOLO CINNOLINES

Synthesis of 4-substituted phenyl hydrazono (cyano) acetamide [6]: Sodium nitrite(0.02mol)dissolved in 20ml of water was added to a suspension of 4-substituted aniline(0.02mol)in 1N HCl (100ml). And the mixture was stirred for 1hr at 0-5 0C and filtered to obtain the clear **s**alt solution. This solution was added to the well stirred mixture of cyanoacetamide (0.02mol), ethanol (30ml) and water 400ml at 00C.sodium acetate (100g) was then added in small portion to keep the mixture alkaline and stirred the mixture for 3 hours at 00C.The precipitate was washed thoroughly with water, air dried and recrystallised from ethanol.

Synthesis of 4-amino-6-niitrocinnoline-3-carboxamide: A mixture of Cpd-1 (0.025mol), chlorobenzene (25mmol) and anhydrous aluminium chloride (50mmol) was stirred for 1hr and refluxed under anhydrous conditions, cooled and poured into ice and hydrochloride (25ml) was added while stirring. The residue thus obtained was washed with petroleum ether filtered and collected the filtrate and made alkaline with ammonia. The pure base was filtered and washed with DMF.

Synthesis of 4-hydroxy-6-substitutedcinnoline-3-carboxylic acid: 5ml of 10%Sodium hydroxide was added to the Cpd-2 and add 20 ml of ethanol and heated on a water bath for 30 min at 85C. And this mixture was cooled to room temperature and added to crush ice. The precipitate obtained was collected and washed thoroughly with water, Air dried and recrystalized from ethanol.

Synthesis of 4-Hydroxy-6-substitutedcinnoline-3-carbohydrazide.

***Preparation of ethyl 4-hydroxy-6-substituted vinnoline-3-carboxylate*:** This Compound was synthesized by refluxing Cpd-3 (0.0587 mol)with absolute ethanol (12 ml) in presence of conc. Sulphuric acid (0.5 ml) for 5 h at 40-50°C. Excess of alcohol was distilled off and allowed to cool in ice bath.

***Preparation of 4-hydroxy-6-substituted cinnoline-3-carbohydrazide*:** These Compounds were synthesized by refluxing a mixture of compound obtained from the above step (0.0602mol) with hydrazine hydrate (5 ml) in absolute ethanol (12 ml) for 8 hr at 30-40°C. The reaction mixture was cooled to room temperature and poured in ice with constant stirring.

Synthesis of 3-(5-substituted-1, 3,4-oxadiazol-2-yl)-6-substitutedcinnolin-4-ol: An equimolar mixture Cpd-4 (0.0054 mol) with various substituted benzoic acids (0.0054 mol) was refluxed with phosphorus oxychloride (5 ml) for 2-3 h on water bath at 100°C. Reaction mixture was cooled to room temperature and poured in ice. The precipitate obtained was filtered off, washed with water and further purified by Recrystallization.

***Synthesis of 5-(4-hydroxy-6-substitutedcinnolin-3-yl)-1,3,4-oxadiazole-2(3H)-thione*:** This compound was synthesized by the Cpd-4with carbon disulfide in an basic alcohol solution for 2-3 h on water bath at 85°C followed by acidification of the reaction mixture. It exhibits the thiol-thione tautomerism. The physicochemical properties of synthesised compounds given table:4

***6-substituted-3-(5-phenyl-1,3,4-oxadiazol-2-yl)cinnolin-4-ol**:* UVʎmax 475.0, IR(KBr,cm^{-1}) 3181.97(OH group), 1398.14(NO2 stretching), 1559.66(C=N stretching), 1290.14(OH bending), 2921.63(azole moiety), 697.1(mono subsbenzene), 1027.87 (N-N stretching). ^{1}H NMR δppm: 7.0-8.12(Aromatic H) 2.72^{13}C NMR δppm: 130 - 134C[benzene], 146.89 –C-[oxadiazole] , 160.0 MS (m/z) 324

3-[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl]-6-substituted cinnolin-4-ol: UVʎmax 518.5, IR(KBr,cm^{-1}) 3199.33(NH2 stretching), 1609.31(C=N stretching), 1359.79(NO2 stretching), 1248.69(OH bending), 1021.12(N-N stretching), 758.85(di subs benzene), 2843.52(azole moiety). 1HNMR(δppm): 9.6(s,1H,OH group) ,3.8(s,2H,NH2),7.85(d,2H in ArH),6.8(d,2H,ArH) 7.4(m,3H in cinnoline). MS (m/z) 340

6-substituted-3-[5-(4-substitutedphenyl)-1,3,4-oxadiazol-2-yl]cinnolin-4-ol :UVʎmax 475.0 IR(KBr,cm1) 3389.28(OH group), 1600.63(C=N stretching), 2922.59(azole moiety), 1393.32 (NO2 stretching), 1011.19(N-N stretching), 1208.36(OH bending), 749.20(N-N stretching). 1HNMR(δppm) 7.25(s,1H in OHgroup) , 7.45 (m,2H, ArH7.4 (m,3H,cinnoline), 7.8(d,2H,ArH). MS (m/z) 414.

3-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-6-substituted cinnolin-4-ol : UVʎmax 473.0 , IR(KBr,cm1) 3422.06(OH group), 3114.47(Ar C-H stretching), 1608.34(C=N in Ar), 1525.42(C=C stretching), 1348.96(NO2 stretching), 1103.08(OH bending), 820.16(di subs benzene), 718.35(C-Cl stretching). 1HNMR(δppm) 5.57(s,1H in OH group) ,7.62(m,2H,ArH) 8.2-8.3(d,2H in ArH) ,7.4(m,3H in cinnoline). MS (m/z) 369

3-[5-(4-amino-2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]-6-substituted cinnolin-4-ol : UVʎmax473. IR(KBr,cm1) 3245.61(NH2 stretching), 3036.37(C-H in Ar ring), 1385.6(NO2 stretching), 1069.33(OH bending), 782.95(try subs benzene), 2948.35(azole moiety), 1698.02(C=N Stretching), 1534.1(C=C strctching). 1HNMR(δppm) 2.5(s,2H,NH2) ,6.9(s,1H,OH group) 7.8-7.9(m,3H,ArH)7.9-(m,3H,cinniline). MS (m/z) 308

6-substituted-3-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]cinnolin-4-ol :UVʎmax 478.0 IR(KBr,cm1) 3177.15(OH group),1338.36(NO2 stretching),1102.95(OH bending),1646.91(C=N in Ar), 1516.74(C=C stretching), 954.31(p-subs pyridine), 2923.56(Azole moiety) 1HNMR(δppm) 9.7(s,1H,OHgroup),7.6(d,2H in pyridine), 7.4 (m,3H,cinnoline), 7.85(d,2H in pyridine). MS (m/z) 324

6-substituted-3-[5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl]cinnolin-4-ol : UVʎmax 477.5,IR(KBr,cm1) 3412.42(OH group), 1395.25(NO2 stretching), 1655.59(C=N in Ar),1596.7(C=C stretching), 2922.59(azole moiety), 668.214(sub. Pyridine). 1HNMR(δppm) 7.25(s,1H in OHgroup) ,7.45 (m,2H, ArH7.4 (m,3H,cinnoline), 7.8(d,2H,ArH). MS (m/z) 324

3-[5-(3,5-disubstitutedphenyl)-1,3,4-oxadiazol-2-yl]-6-substituted cinnolin-4-ol: UVʎmax 377.0,IR(KBr,cm1) 3420.14(OH group), 1629.55 (C=C in Ar), 1541.81(C=N stretching),1399.1(NO2 stretching), 1107.9(OH bending), 850.41(tri subs benzene) 1HNMR(δppm) 7.25(s,1H in OHgroup) , 7.45 (m,2H,ArH7.4 (m,3H,cinnoline), 7.8 (d,2H,ArH). MS (m/z) 351

5-(4-hydroxy-6-substituted cinnolin-3-yl)-1,3,4-oxadiazole-2(3H)-thione: UVʎmax 471.0,IR(KBr,cm1) 3213.79(NH in amine),3411.46(OH group),1650.77(C=C in Ar), 1515.7(C=N stretching), 1399.1(NO2 stretching), 1108.87(C=S stretching). 1HNMR(δppm) 7.25(s,1H in OHgroup) ,7.45 (m,2H, ArH7.4 (m,3H,cinnoline),7.8(d,2H,ArH). MS (m/z) 264.

BIOLOGICAL ACTIVITY

Anti -mycobacterial activity-microplate alamar blue assay method [7]: The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilutions of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml and inoculated with *Mycobacterium tuberculosis* (H37 RV strain): ATCC No- 27294.Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. Pyrazinamide, ciprofloxacin and Streptomycin were used as standard.

Anti-fungal activity [8]: Anti- Fungal Activity of all the synthesized compounds are evaluated against various fungal organisms *Candida albicans*, *Aspergillus niger*, *Trychopyton Rubrum* and *Monascus Purpuram* by Disc diffusion method using Standard clotrimazole discs as standard. The MIC values of the synthesized compounds against *Candida albicans*, *Aspergillus niger*, *Trychopyton Rubrum* and *Monascus Purpuram* was determined by serial dilution method**.**

RESULTS AND DISCUSSION

In the present study various novel 2,5 Di substituted 1,3,4-Oxadiazole derivatives fused with cinnoline were synthesized. The structure of the synthesized compounds was confirmed by IR, NMR, Mass spectral data. The IR spectrum of all the synthesized compound show bands in the region of showed the characteristic absorption band at 1609.29 cm-1, 3389.28 cm-1, 1582.31cm-1 arising from stretching vibration of bands (C=N,O-H,C=C respectively), 1393.21 cm-1, 749.20 cm-1 due to NO2 stretching, N-N bending, 782.91cm-1, 850.17 cm-1, 692.73cm-1 due to the substitution of benzene ring (O,P,M-substituted benzene), due to 3245.61cm-1 of RNH2 confirms the chemical structure of the compounds synthesized.

PMR spectra of the synthesized Oxadiazole derivatives shows 4 aromatic protons as a multiplet in 7.0-8.0 ppm, 1 Proton of hydroxyl group appeared as a singlet in 5.0-9.0 ppm, 4 protons of aromatic group appeared as a multiplet in 6.68-6.95 ppm. Thus the proton magnetic spectrum of the compound was in full agreement with its molecular formula, with regard to proton count and the chemical shift also. The Mass Spectral analysis of the synthesized compounds was performed and the mass spectrum of the compound was in agreement with its molecular weight. The synthesized compounds were tested for activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using Microplate alamar blue assay Method at the concentration of 0.19-100 µg/ml. The data of the anti-tubercular activity screening given in table: 5 reveal that the compound ox-1 to ox-9 inhibited the growth of *Mycobacterium tuberculosis* $H_{37}R_V$. It is interesting to notice that ox-4 and ox-5 Oxadiazole derivative inhibited mycobacterial growth with MIC of 12.5 µg/ml. All synthesized compounds shown good to moderate antifungal activity as given in table: 6&7

CONCLUSION

Provoked by the biological activity of the 1, 3,4-Oxadiazole and Cinnoline and in view of ongoing search for the most potent anti-tubercular and antifungal agents some novel 2,5 Di substituted derivatives of 1,3,4-Oxadiazole have been synthesized and their anti-tubercular activity studied**.** The structures of the synthesized compounds were confirmed by IR, NMR, and mass spectral analysis the proton magnetic spectrum of the compound was in full agreement with its molecular formula, with regard to proton count and the chemical shift. The Mass Spectral analysis of the synthesized compounds was performed, and the mass spectrum of the compound was in agreement with its molecular weight. *In vivo* absorption capabilities of the designed molecules were assessed by means of QIKPROP. All the lead compounds satisfied the rule indicating that they have good oral absorption and MABA method screening and disc diffusion studies showed good to moderate anti-tubercular and antifungal activity. Further studies on its possible mechanism and *in vivo* trials in experimental animals to broaden their Pharmacological assessment, may provide a new analogue that can overcome drug resistance, prolonged treatment, complex drug regimen and side effects involved in the treatment of infectious diseases.

Table:1 **QIKPROP MOLECULAR PROPERTIES PREDICTIONS**

Title	#stars	#rotor	#rtvFG	CNS	dipole	SASA	FOSA	FISA	PISA	WPSA	volume
OX-1	2	1	0	0	0.981	600.74	0	223.088	306.053	71.599	1022.944
OX-2	1	2	0	-2	2.348	562.458	0	125.561	365.3	71.597	950.03
OX-3	3	4	0	-2	8.347	574.986	0	180.492	322.895	71.599	973.11
OX-4	1	2	0	-2	10.409	547.406	0	125.571	374.868	46.966	922.023
OX-5	1	1	0	0	2.544	560.041	0	180.504	332.602	46.935	945.147
0X-6	1	2	0	-2	1.854	588.993	0	277.692	311.3	0	1001.354
OX-7	1	1	0	0	3.214	562.571	0	125.813	365.189	71.57	950.342
0X-8	1	3	0	-2	4.044	440.124	0	151.026	158.23	130.868	722.727
0X-9	1	1	0	0	10.024	648.476	0	363.339	283.206	1.931	1115.091

SCHEME OF REACTION

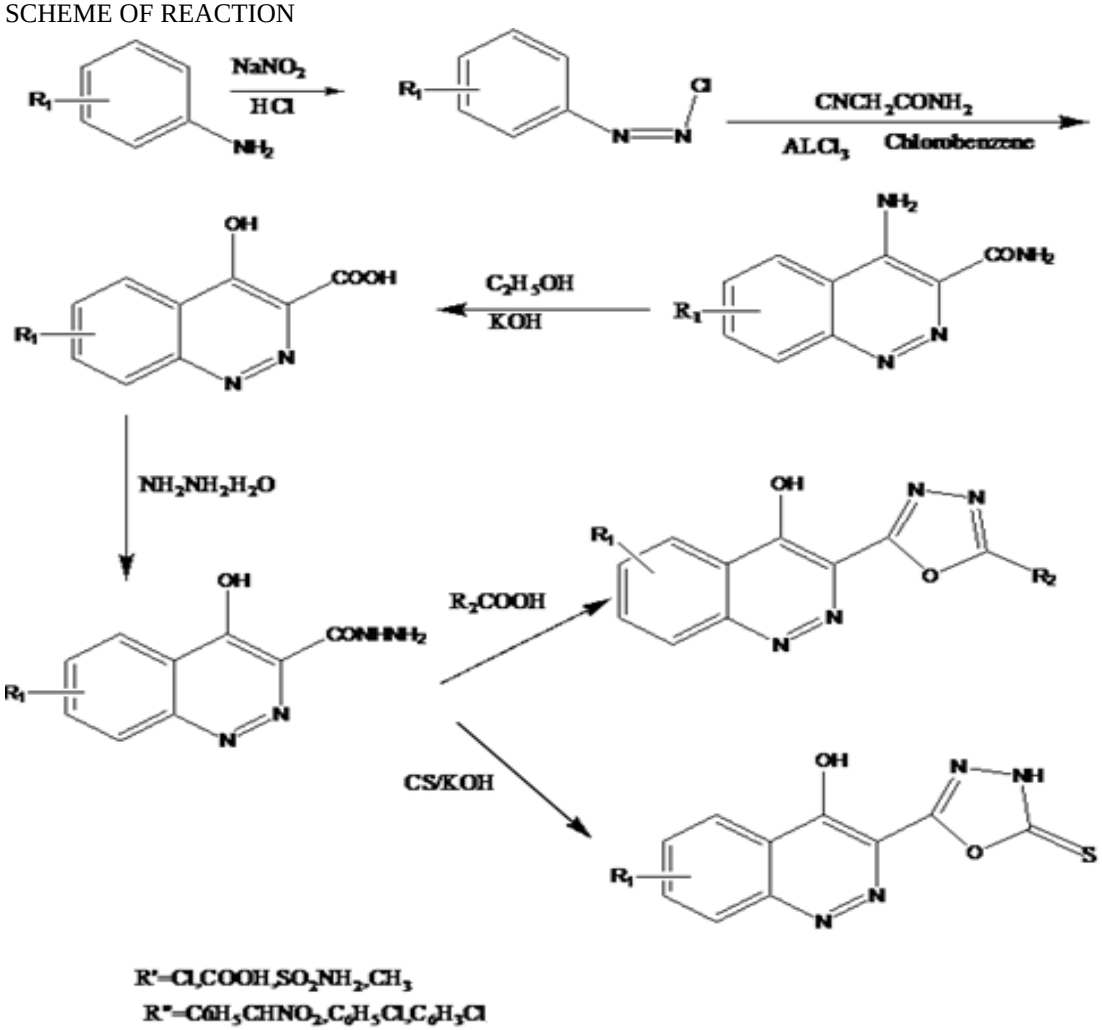

Table:2 **QIKPROP MOLECULAR PROPERTIES PREDICTIONS**

Title	QPpolrz	QPlogPC16	QPlogPoct	QPlogPw	QPlogPo/w	QPlogS	CIQPlogS	QPlogHERG	QPPCaco	QPlogBB	QPPMDCK
OX-1	34.713	11.149	16.23	10.85	2.882	-4.808	-4.983	-6.01	638.564	-0.533	751.639
OX-2	34.589	11.683	18.27	12.95	2.179	-4.63	-4.983	-5.888	192.439	-1.145	205.578
OX-3	38.608	13.813	24.85	19.77	-0.467	-3.9	-4.629	-6.087	3.551	-3.439	1.141
OX-4	36.423	12.118	19.13	11.96	2.174	-4.954	-5.493	-5.948	75.92	-1.608	75.226
OX-5	33.683	10.145	15.86	10.87	2.628	-4.441	-4.655	-5.959	638.419	-0.577	550.773
0X-6	33.563	10.677	17.79	12.97	1.931	-4.279	-4.665	-5.842	192.388	-1.182	150.571
OX-7	34.724	11.153	16.379	10.85	2.882	-4.809	-4.983	-6.009	635.063	-0.536	746.927
0X-8	34.967	12.055	19.161	14.30	1.007	-4.102	-4.824	-5.9	23.043	-2.358	8.403
0X-9	23.639	7.855	16.449	11.79	0.897	-2.897	-3.093	-4.149	366.204	-0.461	870.325

Table:3 **QIKPROP MOLECULAR PROPERTIES PREDICTIONS**

Title	QPlogKp	QPlogKhsa	Human Oral Absorption	Human Oral Absorption %	SA Fluorine	PSA	Rule Of Five	Rule Of Three	#non con
OX-1	-2.451	0.149	3	93.637	0	81.081	0	0	0
OX-2	-3.516	0.024	3	80.892	0	103.746	0	0	0
OX-3	-6.834	-0.499	2	21.103	0	191.402	1	1	0
OX-4	-4.361	0.097	3	72.57	0	126.122	0	0	0
OX-5	-2.417	0.079	3	92.315	46	81.082	0	0	0
0X-6	-3.483	-0.041	3	79.711	46	103.748	0	0	0
OX-7	-2.456	0.15	3	93.594	0	81.197	0	0	0
0X-8	-5.253	-0.115	2	57.23	0	148.592	0	0	0
0X-9	-3.649	-0.497	3	78.082	46	96.626	0	0	0

Table: 4 **Physicochemical properties of synthesised compounds.**

S.No	Comp code	M.WT	yield (%)	Colour	Solubility	M.Pt ^{0}C	R_f value*	#amidine	#amide
1	Ox-1	324.726	72%	red	DMSO	165	0.58	0	0
2	Ox-2	340.725	81%	red	DMSO	175	0.62	0	0
3	Ox-3	414.352	77.2%	Red	DMSO	163	0.63	0	0
4	Ox-4	369.723	78.1%	Red	DMSO	162	0.72	0	0
5	Ox-5	308.271	67.5%	Red	DMSO	173	0.65	0	0
6	Ox-6	324.27	72.5%	Red	DMSO	157	0.67	0	0
7	Ox-7	324.726	65%	Dark red	DMSO	187	0.58	0	0
8	Ox-8	351.278	80.3%	Red	DMSO	168	0.65	0	0
9	Ox-9	264.234	85%	Thick Red	DMSO	165	0.7	0	0

Table 5**:** ***In vitro*** **Anti - mycobacterial activity of the synthesized compounds-MABA Method**

Micro organism	COMP.CODE	MIC [µg/ml]
Mycobacterium tuberculosis $H_{37}R_V$	Ox-1	25
	Ox-2	25
	Ox-3	25
	Ox-4	12.5
	Ox-5	12.5
	Ox-6	100
	Ox-7	50
	Ox-8	50
	Ox-9	50

Table 6 : **Anti-fungal activity of the synthesized compounds**.

S.No	Micro organisms	Zone of inhibition (mm)									
		Compound (100 µg/ 20µl)									
		Ox1	Ox2	Ox3	Ox4	Ox5	Ox6	Ox7	Ox8	Ox9	Std*
1	*Candida albicans*	10	9	12	9	12	9	10	9	9	13
2	*Aspergillus niger*	9	9	8	9	10	10	12	7	10	12
3	*Trychopyton rubrum*	10	7	10	10	10	9	9	11	10	12
4	*Monascus purpuram*	10	9	8	8	9	10	6	9	10	11

TABLE: 7 **ANTIFUNGAL MINIMUM INHIBITORY CONCENTRATION BY SERIAL DILUTION METHOD**

Micro organism	MIC Values (µg/ml)								
	Ox1	Ox2	Ox3	Ox4	Ox5	Ox6	Ox7	Ox8	Ox9
Candida albicans	12.5	6.25	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Trychopyton rubrum	6.25	12.5	12.5	12.5	6.25	12.5	12.5	6.25	12.5
Aspergillus niger	12.2	12.2	6.25	12.2	12.2	12.2	12.2	12.2	12.2
Monascus purpuram	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

REFERENCES

1. A. Thirugnanasambanthan, S. Venkatraman and M. Senthil Palaniappan, Synthesis and antimicrobial screening of some substituted 1, 3, 4- Oxadiazole derivatives, J. Chem. Pharm. Res., 2012, 4(2):1217-1221
2. Stanczak A, Kawapiszewski W and Pakulska W. Synthesis and action on the central nervous system of some N2-substituted cinnoline derivatives. Pharamazie 1994,49:406–412.
3. Nargund, Badiger V and Yarnal SM. Synthesis and antibacterial activity of substituted 4-aryloxypyrimido 5, 4 cinnolines. Eur. J. Med. Chem. 1994, 29: 245-247.
4. Paola Barraja, Patrizia Diana and Antonino Lauria. Indolo[3,2-c]cinnolines with Antiproliferative, Antifungal, and Antibacterial Activity. Bioorg. Med .Chem. 1999, 7: 1591-1596.
5. QikProp: Descriptors and Properties, *Schrödinger Software Release* 2013.
6. Gayam krishna reddy, Hurmath unnissa S., Synthesis and biological evaluation of some 2,5-disubstituted 1,3,4-oxadiazole derivatives, Int.j.adv.pharm.res., 2013, 4(10) : 2427 – 2434
7. Maria C. S. Lourenco, Marcus V. N deSouza, Alessandra C Pinheiro, Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues. ARKIVOC 2007 (xv), 181-191.
8. Kishor S. Jain , Vijay M. Khedkar , Nikhilesh Arya , Prasad V. Rane ,Design, synthesis & evaluation of condensed 2H-4-arylaminopyrimidines as novel antifungal agents, Eur. J. Med. Chem.,2014(77) 166-175