SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY EVALUATION OF SUBSTITUTED-1,2,4-OXADIAZOL-3-YL) QUINOLIN-4(1*H*)-ONE DERIVATIVES

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ABSTRACT : Here we describe oxazole derivatives with potent antibacterial and antifungal activity. A new series of Substituted-1,2,4-Oxadiazol-3-Yl) Quinolin-4(1h)-One derivatives were synthesized using standard via amidoxime route. All the compounds presented here were obtained with high yields and under easy experimental conditions. Synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectra and mass spectral fragmentation. Synthesized compounds were screened against Bacillus substilis, Staphylococcus auras (gram +ve) and Escherichiacoli, Pseudomonas aeruginosa (gram –ve) bacteriafor antibacterial activity, as well as against Aspergillus niger and Candida albicans for antifungal activity. We were able to obtain compounds with higher or equal potency to the reference compounds (Ciprofloxacin and Amphotericine-B). Our data shows that a 9a to 9c were found to active against the bacteria as well as fungi tested. However, compounds 9a more active againstBacillus substilis and 9c more potent againstStaphylo. Compound 9b is more potent against Aspergillus niger andcompound 70c more active against Candida albicans as anti-fungal agent.

Key words:Oxazole, antimicrobial activity, Ciprofloxacin and Amphotericine-B.

I.INTRODUCTION

1,2,4-oxadiazole heterocyclic derivatives were reported as having important synthetic and biological activities potent H_3 receptor antagonist,[1] muscarinic agonist,[2] tyrosine kinase inhibitor,[3] anti-rhinoviral agents,[4]growth hormone secretagogues,[5] anti-inflammatory agents,[6] anti-tumor agents[7]and also used as an amide or ester bioisosteres.[8]. Therefore, we synthesized novel Substituted-1,2,4-Oxadiazol-3-Yl) Quinolin-4(1*h*)-One derivatives and screened their antimicrobial activity.

II.MATERIALS AND METHODS

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled and dried as necessary. Melting points of the synthesized compounds were determined in an open capillary and were uncorrected. The purity of the compounds was checked by TLC on silica gel G (Merck). IR spectra were recorded on Shimadzu FT-IR Affinity-1 spectrometer using KBr. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance 300 MHz spectrometer and Varian mercury plus 300 MHz spectrophotometer in CDCl3 and DMSO-*d6* as solvent and TMS as internal standard with 1H resonant frequency of 300 MHz and ^{13}C resonant frequency of 75 MHz. The Mass spectra (ESI-MS) were recorded on VGAUTOSPEC mass spectrometer. LC-MS spectra were recorded on Thermo Finnigan (Model-LCQ Advantage MAX) mass spectrometer. High resolution mass spectra (HRMS) were obtained by using ESI-QTOF mass spectrometry.

III.Experimental

General synthesis procedure

General procedure for the preparation of 1,4-dihydro-4-oxoquinoline-3- carbonitriles [4 a-c]:
Equimolar quantities of aniline **[1 a-c]** (1 mmol) and ethyl(ethoxymethylene)cyanoacetate **[2]** (1 mmol) were reflux in ethanol for a period of 3-4 h. The reaction mixture was cooled and the product was filtered and dried to give yellow needles of intermediate **[3 a-c]** as a mixture of *E* and *Z* isomers of sufficient purity in 85-90 % yield. This crud Intermediate **[3 a-c]** was suspended in Dowtherm [3:1 mixture of Diphenyl ether (Ph-O-Ph) & Biphenyl (Ph-Ph)] andnitrogen gas was bubbled through suspension within the completion of reaction for 8- 9 h at temperature range about 240-260 °C. After completion of reaction the mixture was allowed to cool at room temperature and the contents were poured into hexane. The resultant solid was collected and washed with hexane and twice with ethyl acetate gave brown tan solid which was recrystallized from methanol / DMF to obtain white compounds **[4 a-c]**.

General procedure for the preparation of 1,4-dihydro-N-alkyl-4-oxoquinoline- 3-carbonitriles [5 a-c]:
A mixture of 1,4-dihydro-4-oxoquinoline-3-carbonitriles **[4 a-c]** (1 mmol), anhydrous K2CO3 (2 mmol), alkyl halide (5 mmol) and DMF (10 mL) was stirred at 120-140 °C for 5-8 h. The reaction mixture was poured into crushed ice; the solid obtained was filtered and washed with water to remove DMF and K2CO3 if any. After drying it gives solid **[5 a-c]** recrystallized by ethanol.

General procedure for the preparation of 1,4-dihydro-N'-hydroxy-1-alkyl-4- oxoquinoline-3-carboxamidines [6 a-c]:
A mixture of 1,4-dihydro-*N*-alkyl-4-oxoquinoline-3-carbonitriles **[5a-c]** (1mmol), hydroxylamine hydrochloride (4 mmol) and triethyl amine (2 mL) were reflux in methanol (20 mL) for 14-16 h, after formation of product (confirmed by TLC) the mixture was cooled to room temperature and 70 % of methanol was evaporated on rotary evaporator, The content was poured into ice cold water to give the yellowish compound which was washed with water and dried.

General procedure for the preparation of substituted-N-alkyl-3-(5-methyl- 1,2,4-oxadiazol-3-yl)quinolin-4(1H)-one [9 a-c]:
A mixture of 1,4-dihydro-*N'*-hydroxy-1-alkyl-4-oxoquinoline-3-carboxamidines **[6a-c]** (1 mmol) and acetic anhydride (1.2 mmol) in 20 mL of glacial acetic acid**[7]** was stirred at room temperature for 2 h gives the required product **[8a-c]**. TLC of a reaction shows only one new spot at this time. The mixture**[8a-c]** then refluxed for 1 h. The reaction mixture was concentrated to one-third of the original volume, water was added with vigorous stirring and the precipitate product was collected, washed with water and dried in air. The crud products were recrystallized by ethanol to give white compounds **[9 a-c]**

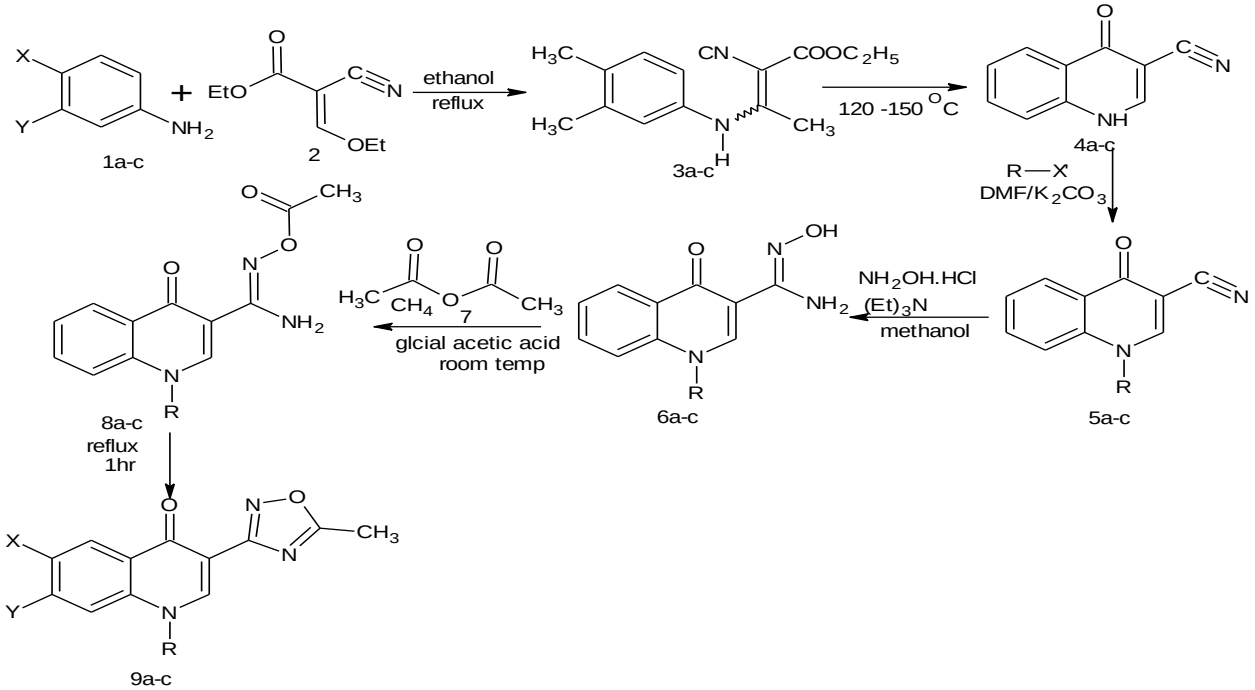

Figure: Reaction scheme for synthesis of substituted *1,2,4-oxadiazol-3-yl)quinolin-4(1H)-one*

Table 1.1: Synthesis of substituted-*N*-alkyl-3-(5-methyl-1,2,4-oxadiazol-3- yl) quinolin-4(1*H*)-one **[9a-**

C].

Compound	X	Y	R	Yield (%)
9a	Cl	H	$-CH_3$	75
9b	H	Cl	$-CH_2CH(CH_3)_2$	71
9c	H	Cl	$-CH_2-CH_2-CH_3$	82

Result and discussion

Characterization Data

6-chloro-1-methyl-3-(5-methyl-1,2,4-oxadiazol-3- yl) quinolin-4(1*H*)-one [9a]

Molecular Formula: $C_{13}H_{10}N_3O_2Cl$

Nature: White solid

M.P.: 244-246 °C

IR (KBr): 1639(C=O), 1598(nitrile), 1496 (C=C, stretching), 1114 (N – O), 1045, 819 (C - Cl) cm^{-1}.**^{1}H NMR(CDCl3, 300 MHz):** δ 2.62 (s, 3H, CH3), 3.90 (s, 3H, NCH3), 7.37 (d, 1H,C8H), 7.60 (d, 1H, C7H), 8.40 (s, 1H, C2H), 8.47 (s, 1H, C5H).**^{13}C NMR (CDCl3, 75 MHz):**δ 12.2, 41.3, 108.8, 117.3, 126.9, 128.4, 131.2, 132.7,138.0, 145.8, 164.6, 172.5, 175.0.**HRMS (ESI+) *m/z:***Cacld for C13H11N3O2Cl (M+1): 276.0529, found: 276.0534

7-chloro-1-isobutyl-3-(5-methyl-1,2,4-oxadiazol-3- yl) quinolin-4(1*H*)-one [9b]

Molecular Formula: C16H16N3O2Cl

Nature: Faint yellow solid

M.P.: 188-190 °C

IR (KBr): 1620 (C = O), 1469 (C = C, stitching aromatic (N – O), 1076, 862 (C - Cl) cm-1.**^{1}H NMR (CDCl3, 300 MHz):** δ 0.99 (d, 6H, 2CH3), 2.25 (m, 1H, CH), 2.58 (s, 3H, CH3),3.95 (d, 2H, CH2), 7.27 (s, 1H, C8H), 7.33 (d, 1H, C6H),8.33 (s, 1H, C2H), 8.46 (d, 1H, C5H).**^{13}C NMR (CDCl3, 75 MHz):** δ 12.1, 19.8, 27.6, 61.0, 108.8, 115.6, 125.1, 126.1. 129.5,138.7, 139.6, 145.9, 164.6, 172.9, 174.9.**HRMS (ESI+) *m/z:***Cacld for C16H16N3O2Cl(M+1): 318.0529, found: 318.10

7-chloro-3-(5-methyl-1,2,4-oxadiazol-3-yl)-1- propylquinolin-4(1*H*)-one [9c]

Molecular Formula: C15H14N3O2Cl

Nature: White solid

M.P.: 188-190 °C

IR (KBr): 1649 (C – H, stretching aromatic), 1597(CH = CH, stretching) 1477 (C = C, Streching), 1085 (C = O), 848 (C – Cl, streching) cm-1.**^{1}H NMR (CDCl3, 300 MHz):** δ 1.04 (t, 3H, CH3), 1.94 (m, 2H, CH2), 2.61 (s, 3H, CH3),4.14 (t, 2H, CH2), 7.36 (d, 1H, *J* = 8.6 Hz, C6H), 7.40 (s,1H, C8H), 8.40 (s, 1H, C2H), 8.49 (d, 1H, *J* = 8.6 Hz,C5H).**^{13}C NMR (CDCl3, 75 MHz):** δ 11.0, 12.1, 22.0, 55.4, 109.0, 115.3, 125.1, 126.1, 129.5,138.8, 139.4, 145.4, 164.6, 172.9, 174.9.**HRMS (ESI+) *m/z:***Cacld for C15H15N3O2Cl (M+1): 304.0849, found: 304.0847.

Anti-bacterial, Anti-fungal Screening

The novel heterocyclic compounds i.e. substituted-*N*-alkyl-3-(5-methyl-1,2,4- oxadiazol-3-yl) quinolin-4(1*H*)-ones **[9a-c]** have been tested for their anti-microbial response such as anti-bacterial and anti-fungal activities against different bacteria and fungi by the disc-fusion method in nutrient agar medium at 100 ppm concentration in dimethyl sulfoxide (DMSO). Stock solutions of the synthesized compounds were prepared by dissolving in DMSO. These solutions were added to each filter disc and DMSO was used as control.

Anti-bacterial assay

The anti-bacterial activity of substituted-*N*-alkyl-3-(5-methyl-1,2,4-oxadiazol-3-yl)quinolin-4(1*H*)-ones **[9a-c]** was studied at 100 ppm concentration assayed against the growth of *Bacillus substilis, Staphylococcus auras* (gram +ve) and *Escherichiacoli, Pseudomonas aeruginosa* (gram –ve) bacteria by the disc-fusion method[9] in nutrient agar medium at 100 ppm concentration in dimethyl sulfoxide (DMSO) **(Table 2)**. The results were compared with the activity of the standard anti-biotic Ciprofloxacin (100mg/disc).

These solutions were added to each filter disc and DMSO was used as control for the both series of

experiments. The cultures were prepared in Mueller-Hinton broth for all the bacteria and incubated for 24 h at 37 ± 1°C. Testing was carried out in Mueller-Hinton broth at pH 7.4 and the serial dilution technique was applied. The microorganisms were grown overnight in Mueller-Hinton broth at 37 ± 1°C and the final inoculums size was 105 CFU ml-1 for the anti-bacterial assay. A set of tubes containing only inoculated broth was kept as controls. After incubation for 24 h at 37 ± 1°C, each experiment was duplicated to define the correct values. The results of anti-microbial potency for compounds **[9a-c]**, are listed in **Table 1**.

Anti-fungal assay

The anti-fungal activity[10] of substituted-*N*-alkyl-3-(5-methyl-1,2,4-oxadiazol-3- yl)quinolin-4(1*H*)-ones **[9a-c]** was studied at 100 ppm concentration assayed against the growth against *Aspergillus niger* and *Candida albicans* at 100ppm concentration in dimethyl sulfoxide (DMSO) and Amphotericin-B was used as the reference compound **(Table 1)**,

The fungi were incubated for 24 h at 37 ± 1°C. The two-fold serial dilution technique was applied. The microorganisms were grown overnight in Triptic soy broth at 37 ± 1°C and the final inoculum size was 1 X 106 spore per mL for the antifungal assay. A set of tubes containing only inoculated broth was kept as a control.

After incubation for 48 h at 37 ± 1 °C, each experiment was duplicated in order to define correct values. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliter of agar media was poured into each Petri-dish. Excess of suspension was decanted and the plates were dried in an incubator for 48 h at 37 °C. Zones of Inhibition were measured in mm and compared with the Standard at 100 ppm concentration. The results for anti-fungal assay of compounds **[9a-c]**are described in **Table 1**.

Compounds	**Zone of inhibition in mm**					
	Gram-positive bacteria		**Gram-negative bacteria**		**Fungi**	
	Bacillus substilis	*Staphylo. aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Candida albicans*
9a	11.23	10.12	8.13	9.12	9.67	9.33
9b	10.65	10.34	9.11	10.23	11.12	9.45
9c	10.34	11.34	7.65	8.11	9.96	10.12
Ciprofloxacin	21.34	33.33	21.11	22.23	NA	NA
Amphotericine-B	NA	NA	NA	NA	15.34	14.23

The concentration of test compounds was 100 µg/ml using solvent DMSO, '-'means no zone of inhibition, NA- Not applicable. Standard reference as anti-bacterial drug. standard reference as anti-fungal drug.

IV.CONCLUSION

Then compounds substituted-*N*-alkyl-3-(5-methyl-1,2,4-oxadiazol-3-yl)quinolin-4(1*H*)-ones (70a-70c) were synthesized and were characterized by IR, NMR and Mass spectroscopy.

The synthesized compounds were evaluated for their anti-bacterial activity against *B. substilis, S. aureus, E. coli, P. aeruginosa* and anti-fungal activity against *A. niger* and *C. albicans*. Most of the compounds showed moderate to good antibacterial and anti-fungal activity as compare to Ciprofloxacin and Amphotericine-B standard used. The results show that compounds **9a** to **9c** were found to active against the bacteria as well as fungi tested. However, compounds **74 a** more active against *Bacillus substilis* and **9c** more potent against *Staphylo.* Compound 9b is more potent against *Aspergillus niger* and compound

9c more active against *Candida albicans* as anti-fungal agent.

REFERENCES

1. Clitherow, J. W.; Beswick, P.; Irving, W. J.; Scopes, D. I. C.; Barnes, J. C.;Clapham, J.; Brown, J. D.; Evans, D. J.; Hayes, A. G. *Bioorg. Med. Chem. Lett.***1996**, *6*, 833.
2. Orlek, B. S.; Blaney, F. E.; Brown, F.; Clark, M. S.; Hadley, M. S.; Hatcher, J.;Riley, G. J.; Rosenberg, H. E.; Wadsworth, H. J.; Wyman, P. *J. Med. Chem.***1991**, *34*, 2726.
3. Vu, C. B.; Corpuz, E. G.; Merry, T. J.; Pradeepan, S. G.; Bartlett, C.; Bohacek, R.S.; Botfield, M. C.; Eyermann, C. J.; lynch, B. A.; MacNeil, I. A.; Ram, M. K.;Van Schravendijk, M. R.; Violette, S.; Sawyer, T. K. *J. Med. Chem.* **1999**, *42*,4088.
4. Diana, G. D.; Volkots, D. L.; Nitz, T. J.; Bailey, T. R.; Long, M. A.; Vescio, N.;Aldous, S.; Pevear, D. C.; Dutko, F. J. *J. Med. Chem*. **1994**, *37*, 2421.
5. Ankersen, M.; Peschke, B.; Hansen, B. S.; Hansen, T. K. *Bioorg. Med. Chem.Lett.* **1997**, *7*, 1293.
6. Nicolaides, D. N.; Fylaktakidou, K. C.; Litinas K. E.; Hadjipavlou-Litina, D. *Eur.J. Med. Chem*. **1998**, *33*, 715.
7. Chimirri, A.; Grasso, S.; Montforte, A. M.; Rao, A.; Zappala, M. *Farmaco***1996**,*51*, 125.
8. (a) Diana, G. D.; Volkots, D. L.; Nitz, T. J.; Bailey, T. R.; Long, M. A.; Vescio,N.; Aldous, S.; Pevear, D. C.; Dutko, F. J. *J. Med. Chem.* **1994**, *37*, 2421. (b)Borg, S.; Vollinga, R. C.; Labarre, M.; Payza, K.; Terenius, L.; Luthman, K. *J.Med. Chem*. **1999**, *42*, 4331.
9. Bauer, A. W.; Kirby, W. M.; Sherris, J. C. Am. J. Clin.Pathol. 1966, 39, 493-496.
10. Varma, R. S. Editor, Antifungal Agents: Past, Present and Future prospects, National Academy of Chemistry and Biology, Lucknow, India, 1998.