DESIGN, SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY STUDY OF SOME NOVEL SUBSTITUTED TETRAZOLO[1,5-α] QUINOLIN-4- YL DERIVATIVES

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ABSTRACT: The aim of the work was the synthesis of new substituted tetrazolo[1.5-α]quinoline-4-yl derivatives and evaluation of antimicrobial activity, which is important in terms of finding new drugs. The implementation of this approach can be made by Willgerodt-Kindler ReactionSo the reaction between substitutedtetrazolo[1,5-*a*] quinoline-4-carbaldehydes as a precursor, with sulfur and secondary amineswas investigated. It was established that the use of dimethylformamide as the reaction medium at 110-120 ºC. compound **5a**to **5c** shows moderate anti-bacterial activity against *Staphylococcus auras* (gram +ve) bacteria and *Proteusvulgaris* (gram –ve) bacteria. The compound **5c**showed more potency against fungi *Aspergillus niger*, while compounds **5a**and **5c**showed highly remarkable activity i.e. complete inhibition (full plate inhibition) against *Aspergillus niger*

Key words:tetrazole, Willgerodt-Kindler Reaction, antimicrobial activity,Streptomycin, Griseofulvin

I.INTRODUCTION

Synthesis of new tetrazolo[1.5-α]quinoline-4-yl derivatives is important in terms of searching new biologically active substances. In particular, compounds with tetrazoleand quinoline moiety in their structure, show a wide range of pharmacological activity anti-allergic,[1] anti-arrhythmic,[2]anti-inflammatory,[3] anti-fungal[4] and stimulants or sedatives on the central nervous system.[5]

Derivatives of quinonecontaining compounds have been described as potential anticancer agents in several publications[6,7]. In some articles it was shown the synthesis and study of antibacterial and antifungal activity series of tetrazolo[1.5-α]quinoline-4-yl[8,9]. Therefore, nowadays the search and expansion of combinatorial number of compounds are necessary for further synthesis of derivatives and their biological screening.

II.MATERIAL AND METHOD

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 open capillaries 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 1H-NMR and 13C-NMR spectra were recorded on 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 13C resonant frequency of 75 MHz. The Mass spectra (ESI-MS) were recorded on VG AUTOSPEC mass spectrometer and on Varian Inc. 410 prostar binary LC with 500 MS IT mass spectrometer. High-resolution mass spectra (HRMS) were obtained by using ESI-QTOF mass spectrometry.

Experimental

General procedure for synthesis

General procedure for the preparation of N-arylacetamides [2 a-c]:

Solution of aniline (5 mmol) and triethyl amine (2 mL) in DCM (10 mL) was cooled to 0-5 °C and added acetyl chloride (7 mmol) for 15 min with constant stirring.

After completion of reaction added ice-cold water and stirred for 10 min, extracted the reaction mixture using DCM (3 X 30 mL) and separate the organic layer fromaqueous layer. Wash the organic layer with dil. HCl (2 X 25 mL) and then with 10 % solution of NaHCO3 (2 X 25 mL). Then wash the organic layer with water (2 X 30 mL) and dried over anhydrous Na2SO4. The solvent was removed under reduced pressure to obtain the *N*-arylacetamides in sufficient purity.

General procedure for the preparation of 2-chloroquinoline-3-carbaldehydes [3 a-c]:

To a solution of *N*-arylacetamide **[51 a-c]** (5 mmol) in dry DMF (15 mmol) at 0-5 °C with stirring POCl3 (60 mmol) was added drop wise and the mixture stirred at 80-90 °C for time ranging between 4-16 h. The mixture was poured into crushed ice, stirred for 5 min and the resulting solid filtered, washed well with water and dried.

The compounds were purified by recrystallization from either ethyl acetate or acetonitrile.

General procedure for the preparation of tetrazolo[1,5-α]quinoline-4- carbaldehydes [4 a-c]:

2-chloroquinoline-3-carbaldehyde **[52 a-c]** (5 mmol), sodium azide (10 mmol), acetic acid (1 mL) and ethanol (10 mL) were taken in round bottom flask with mechanical stirrer and condenser. The reaction mixture was slowly heated and refluxed for 3-4 h. After the completion of reaction (checked by TLC), the product was filtered and washed with ethanol. The crude product was purified by triturated with volume ratio of chloroform and methanol (10:10 mL) to obtain the pure solid sample.

General procedure for the preparation of tetrazolo[1,5-a]quinolin-4- yl)(sec.amino)methanethiones [5 a-c]:

A mixture of tetrazolo[1,5-a] quinoline-4-carbaldehyde **[53 a-c]** (5 mmol), secondary amine (25 mmol), and fine powder of sulfur (12.5 mmol) in 25 mL of DMF was heated at 100-120 °C with stirring till the end of hydrogen sulfide liberation(from 15 to 45 min). The cooled reaction mixture was diluted with water (100 mL);the separated precipitate was filtered off and washed with sufficient water. Purification of the compounds was performed by recrystallization from ethanol or ethanol-DMF.

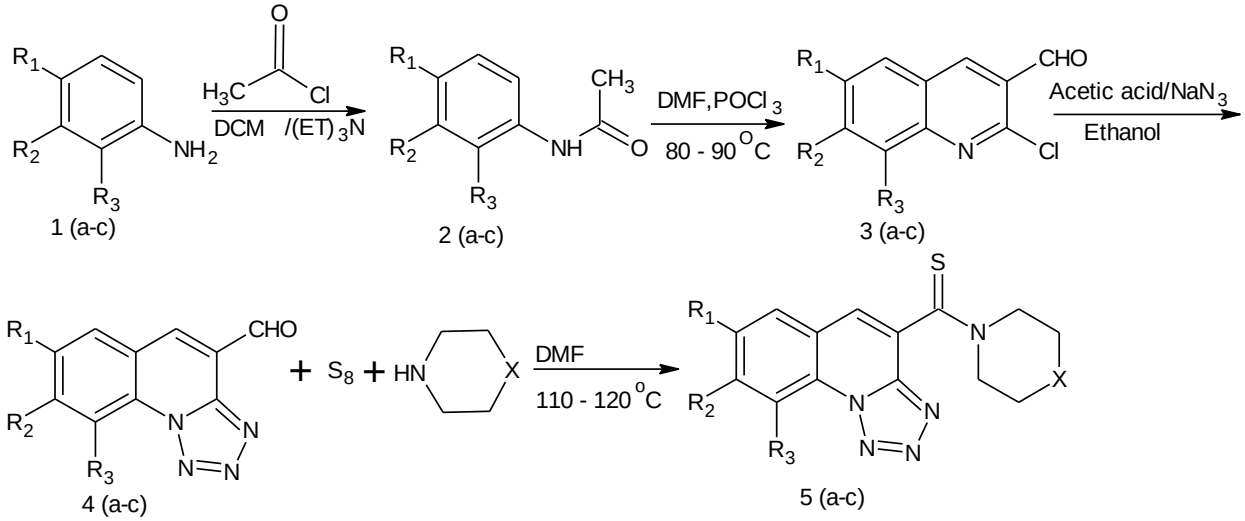

Fig:1 Reaction scheme for synthesis of *tetrazolo[1,5-α]quinolin-4- yl)*

Antimicrobial activity study

Anti-bacterial assay

For substituted-(tetrazolo[1,5-*a*] quinolin-4yl) derivatives **[54a-c]**the anti-bacterial activity was studied against the growth of *Bacillus substilis,Staphylococcus auras,* (gram +ve) and *Pseudomonas aeroginosa*, *Proteus vulgaris*(gram –ve) bacteria by the disc-fusion method[10] in nutrient agar medium at 100ppmconcentration in dimethyl sulfoxide (DMSO) **(Table 2)**. The results were comparedwith the activity of the standard anti-biotic Streptomycin (100mg/disc). Thesesolutions were added to each filter disc and DMSO was used as control for the bothseries 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 **[54 a-c]** are listed in **Table 2**.

Anti-fungal assay

The anti-fungal activity of substituted- (tetrazolo[1,5-*α*] quinolin-4yl) derivatives **[54 a-c]** was studied at 100ppm concentration assayed against the growth against *Aspergillus niger* and

Trichoderma ressi at 100 ppm concentration in dimethyl sulfoxide (DMSO) and Griseofulvin was used as the standard **(Table 2)** by tube dilution method[11].

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 **[54 a-c]** are described in **Table 2**.

Result and discussion

Characterization of synthesized compound

(4-methylpiperazin-1-yl) (tetrazolo[1,5-*a*] quinolin- 4-yl) methanethione (5a)

Molecular Formula: $C_{15}H_{16}N_6S$

Nature: Faint yellow solid

m.p.: 218-220 °C

IR (KBr): 1608, 1527, 1145, 1292 cm-1.

^{1}H NMR (CDCl3, 300 MHz):δ 2.36 (s, 4H, CH3 & CH), 2.69 (m, 3H, CH2 & CH), 3.54(m, 2H, CH2), 4.52 (m, 2H, CH2), 7.76 (t, 1H, *J* = 7.6 Hz,ArH), 7.86 (t, 1H, *J* = 7.6 Hz, ArH), 7.97 (d, 1H, *J* = 7.6Hz, ArH), 8.07 (s, 1H, ArH), 8.67 (d, 1H, ArH).

^{13}C NMR (CDCl3, 75 MHz):δ 45.4, 49.1, 52.4, 53.9, 54.5, 116.5, 123.4, 127.3, 128.2,129.2, 130.0, 131.3, 131.5, 144.3, 189.9.

HRMS (ESI+) *m/z:*Cacld for C15H17N6S (M+1): 313.1226, found: 313.1229.

8-methyltetrazolo[1,5-*a*] quinolin-4- yl) (morpholino)methanethione (5b)

Molecular Formula: C15H15N5OS

Nature: Faint yellow solid

m.p.: 244-246 °C

IR (KBr): 1624, 1602, 1527, 1114, 1029 cm-1.

^{1}H NMR(CDCl3, 300 MHz):δ 2.64 (s, 3H, CH3), 3.54 (m, 3H, CH2 & CH), 3.94 (m, 3H, CH2 & CH), 4.52 (m, 2H, CH2), 7.45 (d, 1H, ArH), 7.84 (d, 1H, ArH), 8.11 (s, 1H, ArH), 8.37 (s, 1H, ArH).

^{13}C NMR (CDCl3, 75 MHz):δ 22.0, 49.6, 52.9, 66.1, 66.3, 116.2, 121.1, 125.7, 129.0,129.7, 130.0, 132.2, 143.3, 144.2, 190.6.

HRMS (ESI+) *m/z:*Cacld for C15H16N5OS (M+1): 314.1061, found: 314.1070.

7-ethyltetrazolo[1,5-*a*]quinolin-4-yl)(4- methylpiperazin-1-yl)methanethione (5c)

Molecular Formula: C17H20N6S

Nature: Yellow solid

m.p.: 234-236 °C

IR (KBr): 1600, 1575, 1527, 1490, 1143 $cm^{-1.}$

^{1}H NMR (CDCl3, 300 MHz):1.33 (t, 3H, *J* = 7.6 Hz, CH3), 2.36 (s, 4H, CH3 & CH), 2.68 (m, 3H, CH2 & CH), 2.84 (q, 2H, *J* = 7.5 Hz, CH2), 3.54 (m, 2H, CH2), 4.53 (m, 2H, CH2), 7.72 (d, 1H, *J* = 8.6 Hz, ArH), 7.77 (s, 1H, ArH), 8.04 (s, 1H, ArH), 8.51 (d, 1H, *J* = 8.5 Hz, ArH).

^{13}C NMR (CDCl3, 75 MHz):δ 15.2, 28.4, 45.3, 49.0, 52.2, 53.8, 54.5, 116.3, 123.5, 127.0, 125.5, 128.2, 131.2, 131.9, 144.0, 144.7, 190.4.

HRMS (ESI+) *m/z:*Cacld for C17H21N6S (M+1): 341.1540, found: 341.1542.

Table 1: Physical characterization of synthesized compound substituted-(tetrazolo[1,5-*a*]quinolin-4-yl)(sec.amino)methanethiones **[5 a-c]**

Compound	R1	R2	R3	X	Yield(%)
5a	H	H	H	NCH_3	83
5b	H	CH_3	H	O	88
5c	C_2H_5	H	H	NCH_3	86

Table 2. Anti-microbial activity of substituted-(tetrazolo[1,5-*a*] quinoline-4yl) **[5 a-c]**

Compound	Zone of inhibition in mm					
	Anti-bacterial activity				Anti-fungal activity	
	Bacillus substilis	*Staphylo. aureus*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Aspergillus niger*	*Tricho. Ressi*
5a	-	**10**	-	**10**	-	-
5b	-	**10**	-	**10**	-	-
5c	-	**9**	-	**10**	**11**	-
Streptomycin	20	15	10	16	-	-
Griseofulvin	-	-	-	-	**10**	**10**

VI.CONCLUSION

The compounds substituted- (tetrazolo[1,5-*a*] quinolin-4yl) derivatives **[5 a-c]**were synthesized and spectral study like IR, ^{1}HNMR,^{13}CNMR and MS were done to conform their structural details. In biological activity study antibacterial and antifungal properties were carried out.

The investigation of anti-bacterial screening of compounds **[5 a-c]** against*B. substilis*, *S. aureus*, *P. aeruginosa*, *P.vulgaris* and anti-fungal activity against *A. niger* and *T. ressi* revealed that compound **5a**to **5c** shows moderate anti-bacterial activity against *Staphylococcus auras* (gram +ve) bacteria and *Proteusvulgaris* (gram –ve) bacteria. The compound **5c**showed more potency against fungi *Aspergillus niger*, while compounds **5a**and **5c**showed highly remarkable activity i.e. complete inhibition (full plate inhibition) against *Aspergillus niger*than standard Griseofulvin. However, all the compound **5a**to **5c**were found to be inactive, against *Bacillus substilis*, *Pseudomonas aeruginosa* and *Trichoderma ressi* organisms i.e no one compounds showed zone of inhibition.

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