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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF A SERIES OF (SUBSTITUTED (4-CHLOROPHENYL)-N'-((2-CHLOROQUINOLINE-3-YL) METHELENE)-5-METHYL-1H-1,2,3-TRIAZOLE-4-CARBOHYDRAZIDE) DERIVATIVES

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ABSTRACT

A series of substituted1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4-carbohydrazide derivatives have been synthesized and assessed for their in vitro antimicrobial activity against three gram negative and two gram positive strains of bacteria and one fungal strain. Salt formation of 4-chloro aniline with sodium azide was taken place to form triazole moiety and was reacted to hydrazine hydrate to yield carbohydrazide. This carbohyrdazide was then condensed with various substituted quinolines to obtain various derivatives. In the present work we have synthesized some potent derivatives and characterizations of all novel compounds were conducted by IR, Mass, ^{1}HNMR and ^{13}C NMR spectral data. Broth dilution method was used to determine anti-bacterial and anti-fungal activities of synthesized organic compounds.

Keywords: Carbohydrazides, Quinolines, triazoles, Schiff base, Antimicrobial activity, MIC.

1. INTRODUCTION

One of the leading diseases are microbial infections spread by bacteria and fungi, which causes millions of deaths every year worldwide due to the lack of effective antimicrobial therapy and more resistance captured by micro organisms against conventional antibiotics [1]. Evolution of resistance to actual drugs is a constant growing phenomenon that has concerned researchers all over the world, and now has attained alarming levels for certain infections, so active research for the discovery and development of novel microbial agents is necessary. Quinoline scaffold possess unique physico-chemical properties and so that it is present in many classes of biologically-active compounds [2] as HIV inhibitor [3] antimicrobial [4-8], anti-tuberculosis [9-12], antimalarial [13], antifungal [14-15], anti-inflammatory [16], anti-oxidants [17-18]. On other hand, the recent anti fungal drugs are immensely toxic for example, amphotericin B, or are being ineffective due to presence of resistant stain for example- flucytosine and azoles. In treatment of fungal infection azoles remains the mainstay of therapy [19-20]. Triazoles are also described as an important family of heterocyclic compounds used in drug synthesis with various biological activities. Among them 1,2,4 triazole shows admirable safety profile immune kinetic characteristics for candida-albicans and Cryptococcus-neoformans due to its resistant activity [21]. 1,2,3-Triazole moieties are stable and potent for hydrogen bonding, which can be supportive binding for bio-molecular targets and ligands with high solubility [22]. Additionally, carbohydrazide derivatives also exhibit potent and broad spectrum of biological activities as anti-microbial and anti-inflammatory [23-24]. In present work we have synthesized novel series of carbohydrazide derivatives with excellent yield. All the prepared compounds were screened for their antimicrobial activity against various microbial strains.

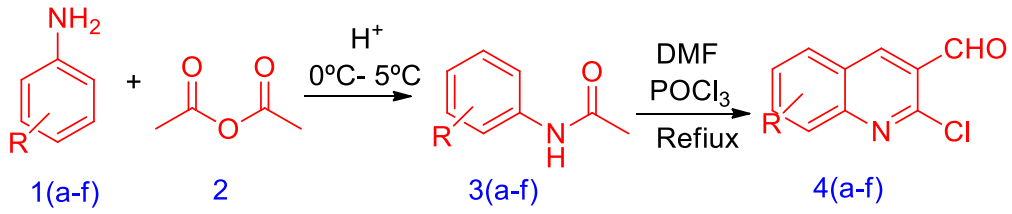
2. MATERIALS AND METHODS

2.1. General information

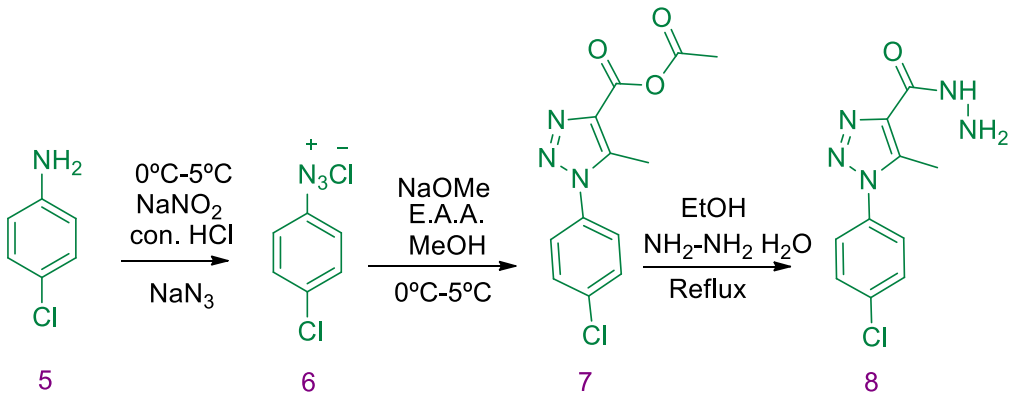
All starting materials and solvents used were pro analysis grade originated from Spectrochem, Sigma-Aldrich, Lobachemie. And Merck without further purification, *i.e.,* substituted aniline, E.A.A, DMF, ethanol, methanol, sodium hydroxide, dichloromethane, hexane, ethylacetate, hydrochloric acid, chloroform, and dimethylsulfoxide. Thin layer chromatography (TLC) was conducted by using aluminum plates 20x20 cm

coated by silica gel 60 F254 (Merck). All Final synthesized organic compounds were confirmed by mean of ^{1}HNMR, ^{13}C NMR and Mass and IR analysis. All melting points were recorded by melting point apparatus (uncorrected) using open capillary method. For all these conversions, progress of reaction was carried out by TLC plate. Visualization was made with ultra-violet (UV) light (254-365nm) or with iodine vapour. Solvents were evaporated with a BUCHI rotary evaporator.

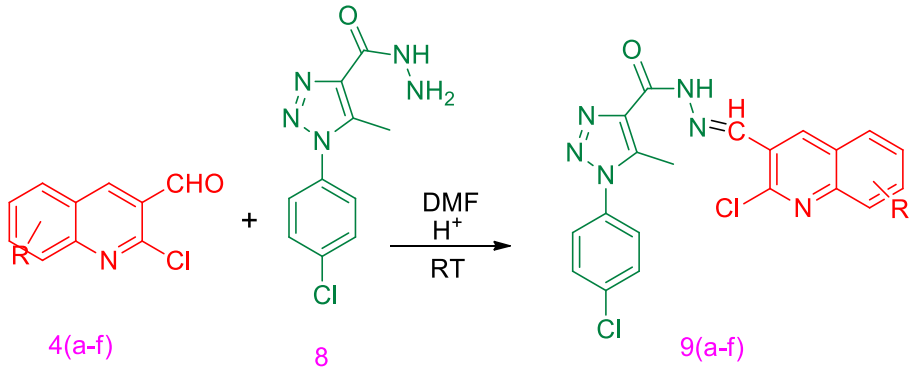
Mass spectra of the products were achieved from Mass spectrometer by Shimadzu GCMS-QP-2010 model using Direct Injection Probe technique. IR spectra were recorded on FTIR-8400 spectrometer using DRS prob. ^{1}HNMR and ^{13}C-NMR spectra were collected on a Bruker AVANCE II 400 MHz.400 MHz (^{1}H) and 100 MHz (^{13}C) using $CDCl_3$ and DMSO as solvents, chemical shift are expressed in δ ppm down filed from TMS as standard internal.



Scheme A: Route of synthesis of compounds 4(a-f)



Scheme B: Route of synthesis for compound 8



Scheme C: Route of synthesis for compounds 9(a-f).

2.2. Synthesis of various 2-chloroquinoline-3-carbaldehyde (4a-f)

Substituted aniline (1) (1mol) was taken in round bottom flask (RBF) at 0-5°C and acetic anhydride (1.5mol) was added drop wise in it with continuous stirring. After completion, catalytic amount of sulfuric acid (H_2SO_4) was added to generate acidic media. Reaction progress was observed by TLC. Reaction mass was precipitated using cold water to get product (white solid product). The product was filtered and dried (70-80%).

Phosphorus oxychloride ($POCl_3$) (9mol) was taken in RBF and Dimethylformaide (3mol) was added drop wise in it very slowly. After completion of addition, N-phenylacetamide (3) (1mol) was added in RBF and was refluxed at 80ºC for 16-24 hours. In final step the reaction mass was poured in to crushed ice to get solid precipitates. (Yellow solid product) (60-70%).

2.3. Synthesis of 1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4-carbohydrazide (8)

To Form 1-azido-4-chlorobenzenechloride salt, mixture of HCl(6ml) and water (20ml) were taken in RBF and cooled. 4-chloroaniline (5.0g, 0.053mol) was added slowly in to the solution while temperature kept constant at 0-5°C. Solution of sodium nitrite (3.65g, 0.053mol) and sodium azide (3.44g, 0.053mol) were added drop wise. This reaction mass was stirred for 30min. After completion of the reaction extraction of the residue using chloroform was taken place to give 1-azido-4-chlorobenzene chloride salt (4.2g, 70.42%). To lead the formation of ethyl1-(4-chlorophenyl)-5-methyl-1-phenyl-1H-1,2,3-triazole-4-carboxylate derivatives azide (4.2g, 0.035mol) prepared substance was treated with E.A.A. (9.1g, 0.07mol) then reaction mixture was cooled at low temperature and then sodium methoxide (3.78g, 0.07mol) was added under inert atmosphere where methanol was taken as a solvent. The reaction mixture was stirred at RT. After the completion of reaction content was poured in to the crushed ice, the obtained residue was filtered, dried and re-crystallized from ethanol to give component (7) (6.5g, 80.39%). The carbohydrazide derivative can be prepared by dissolving ethyl1-(4-chlorophenyl)-5-methyl-1-phenyl-1H-1,2,3-triazole-4-carboxylate (6g,0.0259mol) in to ethanol (30ml) and then hydrazine hydrate (14 ml) was added drop wise and reaction mass was refluxed for 6 hr at 80ºC. After the completion of reaction, the reaction mass was cooled, residue was separated, filtered and washed with cold water to give product (5g, 88.80%).

2.4. General procedure for synthesis of substituted carbohydrazide (9a-f)

Carbohydrazide derivatives were prepared by dissolving substituted 2-chloroquinoline-3-carbaldehyde (1mol) in dimethylformamide and then catalytic amount of H_2SO_4 was added to generate acidic medium. 1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4 carbohydrazide (1mol) was added in the reaction mass. Reaction mass was Stirred for 10-15 min at RT. The precipitated reaction mass was filtered and washed by ice cold water and re-crystallized from ethanol.

2.5. Screening for Antimicrobial activity

In view of the biological importance of novel series of carbohydrazide derivatives, the synthesized compounds were screened for its antimicrobial activity. Activity was carried out at Department of bio-science, Saurashtra University, Rajkot. The compound was screened for antimicrobial activity against three gram-negative bacteria, namely *Escherichiacoli* (MTCC (Microbial Type Culture Collection) No. 443), *Pseudomonas aeruginosa* (MTCC No. 1688) and *Klebsiella pneumonia* (MTCC No. 109), two gram-positive bacteria namely *Bacillus cereus* [MTCC No. 430], *Staphylococcus aureus* (MTCC No. 96) and one anti fungal activity against *P. marneffei*. The strains were inoculated in nutrient broth, and kept for overnight culture at 37°C. All the activities were carried out using Furacin and itraconazole as standard drugs in minimum bacterial inhibitory concentration (MIC). The MIC is defined as the minimum inhibitory concentration able to inhibit any visible bacterial growth. Antibacterial activity was determined by broth micro dilution method performed in 30 well micro titer plate, using 2,3,5-triphenyl tetrazolium chloride (TTC) as an indicator for bacterial growth, by dissolving 5 mg of sample in 1 mL dimethylsulfoxide of solvent[25-27].

3. RESULT AND DISCUSSION

New series of carbohydrazide derivatives were prepared using triazole and quinoline as core moieties. All the compounds of presented work were characterized by analytical data i.e. ^{1}HNMR, ^{13}CNMR, IR, Mass Spectroscopy.

3.1. Spectral data

3.1.1. (E)-1-(4-chlorophenyl)-N'-((2-chloroquinolin-3-yl)methylene)-5-methyl-1H-1,2,3-triazole-4-carbohydrazide (9a)

White colored yield: 88%; ***M.P.***:152ºC; ***Chemical Formula:*** $C_{20}H_{14}C_{l2}N_6O$; ***^{1}HNMR (400MHz,CDCl$_3$)(δ ppm):*** 10.76(s,1H,NH), 9.032 (s,1H,quinoline ring), 8.745 (S,1H,N-NH), 8.012-7.275 (M,8H,Ar ring), 2.714(s,3H,CH_3); ***^{13}CNMR (100 MHz,CDCl$_3$) (δ ppm):*** 156 (s, C=O), 153 (s, quinoline fused ring), 150 (s,C=N in triazole ring), 146 (s,C-Cl in aromatic ring), 140 (s,CH=N), 134 (s, in chloro benzene ring), 133 (s, C-Cl in quinoline ring), 131 (s, chloro benzene ring), 130(s), 129.67 (s), 129.24-129.03 (m), 128.12 (s),

127.90 (d, J = 3.2 Hz), 126 (s,C-CH_3), 126 (s, chloro benzene ring), 126 – 126 (m, Aromatic ring), 11(s,CH_3); ***IR (KBr, cm^{-1})***: 3500, 3300 (starching CH_3 Sp^3 hybridization), 3200 (=C-H), 1600 (-C=O), 1560 (aromatic), 1500 (aromatic), 1400 (aromatic), 1250 (bending C=N); ***Mass m/z:*** 424.06.

3.1.2. (E)-N'-((2-chloro-6,8-dimethylquinolin-3-yl) methylene)-1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4-carbohydrazide (9b)

White colored yield: 84%; ***M.P.:*** 183°C; ***Chemical Formula:*** $C_{22}H_{18}C_{l2}N_6O$; ***^{1}HNMR (400MHz,CDCl$_3$-d)(δ ppm):*** 10.591 (s,1H,N-NH), 8.895 (s,1H,fused quinoline ring), 8.720 (s,1H,CH=N), 7.600-7.257 (m,6H, aromatic ring), 2.721(s,6H,2CH_3 in Quinoline), 2.490 (s.3H,CH_3 in triazole); ***^{13}CNMR (100MHz,CDCl$_3$)(δ ppm):*** 152 (s,C=O), 141 (s,C=Cl quinoline ring), 140 (s, C=N), 138 (s,), 133(s, C-Cl aromatic ring), 132.13-128.51 (m, C-CH_3 ring), 124.78 (s),121.97-119.70 (m, C- CH_3 quinoline ring), 16.35 (s,2CH_3 quinoline ring),12.40 (s,CH_3 in triazole); ***IR (KBr, cm^{-1}):*** 3406 (starching CH_3 SP^3 hybridization in triazole ring), 3100 (starching SP^3 hybridization in quinoline ring), 3000 (=C-H), 1610 (-C=O), 1400(aromatic), 1570 (aromatic), 1450 (aromatic), 1250 (bending C=N), 750 (disubstituted ring); ***Mass m/z:*** 452.06.

3.1.3. (E)-N'-((6-bromo-2-chloroquinolin-3-yl) methylene)-1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4-carbohydrazide (9c)

Yellow colored yield: 80%; ***M.P.:***198°C; ***Chemical Formula:*** $C_{20}H_{13}BrCl_2N_6O$; ***^{1}HNMR (400MHz,CDCl$_3$-d)(δ ppm):*** 9.542 (s,1H,N-NH), 8.927 (s,1H,CH=N), 8.729 (s,1H, quinoline ring), 8.043-7.266 (m,7H, Aromatic), 2.722 (s,3H,CH_3 in triazole); ***^{13}C NMR (100 MHz, CDCl$_3$)(δ ppm):*** 156.32 (s, C=O), 153.82 (s, fused quinoline ring), 150.47 (s, N=C in triazole ring), 146.78 (s, C-Cl in quinoline), 140.81 (s, C=N), 134.52 (s), 133.90 (s, C-Cl in chloro benzene), 132.90 (s), 130.84 (s, aromatic), 130.33 (s, aromatic), 129.15 (t, J = 2.8 Hz, aromatic), 127.17 (s, aromatic), 126.92 (s, aromatic), 126.45-126.07 (m,C-CH_3), 126.04 (s, chloro benzene), 117.75 (s, C-Br), 11.06 (s,CH_3***); IR (KBr, cm^{-1}):*** 3420 (starching CH_3 sp^3 hybridiaztion), 3030 (=C-H), 1610 (-C=O), 1550 (aromatic), 1430 (aromatic), 1230 (bending C=N), 730, 650 (disubstitued); ***Mass m/z***: 501.9.

3.1.4. (E)-N'-((2-chloro-6-methylquinolin-3-yl) methylene)-1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4-carbohydrazide (9d)

White colored yield: 82%; ***M.P.:***176°C; ***Chemical Formula:***$C_{21}H_{16}Cl_2N_6O$***; ^{1}HNMR (400MHz,CDCl$_3$-d)(δ ppm)***: 10.681 (s,1H,N-NH), 8.836 (s,1H,CH=N), 8.741 (s,1H,quinoline ring), 7.905-7.273 (m,7H, aromatic), 2.717 (s,3H, CH_3 in quinoline ring), 2.540 (s,3H, CH_3 in triazole ring); ***^{13}C NMR (100 MHz,CDCl$_3$) (δ ppm)***: 155.52 (d, J = 200.3 Hz, C=O), 150.47 (s, fused quinoline ring), 142.17 (d, J = 285.2 Hz, triazole ring), 136.12-124.66 (m, aromatic), 21.69 (s,CH_3 in quinoline ring), 11.06 (s,CH_3 in triazole ring); ***IR (KBr, cm^{-1}):*** 3450 (starching CH_3 sp^3 hybridization in CH_3), 3120 (starching CH_3 sp^3 in quinoline), 3080 (=C-H), 2890 (N-H), 1600 (-C=O), 1530 (aromatic), 1430 (aromatic), 1270(bending C=N), 750 (para disubstituted); ***Mass m/z:*** 438.08.

3.1.5. (E)-N'-((2-chloro-5,8-dimethylquinolin-3-yl) methylene)-1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4-carbohydrazide (9e)

Yellow colored yiled: 82%; ***M.P.:*** 201°C; ***Chemical Formula:*** $C_{22}H_{18}Cl_2N_6O$; ***^{1}HNMR (400MHz,CDCl$_3$-d)(δ ppm):*** 10.599 (s,1H,N-NH), 8.895 (s,1H,quinoline ring),8.720 (s,1H,CH=N), 7.600-7.267 (m, 6H,aromatic), 2.721 (s,6H,2CH_3 in quinoline), 2.490 (s,3H,CH_3 in triazole); ***^{13}C NMR (100 MHz,CDCl$_3$) (δ ppm):*** 155.19 (d, J = 283.5 Hz,C=O), 150.47 (s, fused quinoline), 141.95 (d, J = 285.3 Hz, triazole ring), 137.43-123.54 (m, aromatic), 20.14(d, J = 172.2 Hz, CH_3 in quinoline), 11.06 (s, CH_3 in triazole); ***IR (KBr, cm^{-1}):*** 3400 (starching CH_3 sp^3 hybridization in CH_3), 3120 (sp^3 in quinoline), 3060 (=C-H), 1600 (-C=O), 1570 (aromatic), 1450 (aromatic), 1290 (bending C=N), 740 (disubstituted); ***Mass m/z:*** 452.06.

3.1.6. (E)-5-chloro-N'-((2-chloro-6,7-dimethylquinolin-3-yl)methylene)-1-(4chlorophenyl)-1H-1,2,3-triazole-4-carbohydrazide (9f)

Yellow colored solid yield: 80%; ***M.P.***:187°C; ***Chemical Formula:*** $C_{22}H_{18}Cl_2N_6O$; ***^{1}HNMR (400MHz, CDCl$_3$-d)(δ ppm):*** 10.664 (s, 1H,N-NH), 8.899 -7.275 (m, 8H,aromatic), 2.962-2.441 (m, 9H,3-CH_3); ***^{13}CNMR (100 MHz,CDCl$_3$) (δ ppm):*** 157.92-148.87 (m, C=O and Ar ring), 145.07 -136.64 (m, Ar ring and C=N), 136.12-124.45 (m, Ar ring), 21.63-18.43 (m,CH_3), 11.06 (s,CH_3); ***IR (KBr, cm^{-1}):*** 3410

(starching CH_3 sp^3 hybridization in CH_3), 3120 (sp^3,CH_3), 3050 (=C-H), 1610 (-C=O), 1550 (aromatic), 1420 (aromatic), 1250 (bending C=N), 770 (disubstituted); ***Mass m/z:*** 452.04.

Table 1: List of substituted Hydrazones with Time, % yield and M.P.

Compound ID	Substitutions R	Time (min)	Yield %	MP (^oC)
9a	H	10	88	152
9b	6,7- dimethyl	15	84	183
9c	6-bromo	12	80	198
9d	6-methyl	15	82	176
9e	6,8-dimethyl	17	82	201
9f	5,8-dimethyl	15	80	187

Table 2: Optimization of yield by various solvents

Solvents	Time(min)	% yield
Hexane	60	10
Diethyl ether	40	10
Dichloromethane	35	60
Methanol	30	70
Ethanol	30	75
Dimethylformamide	≤15	≤80

All compounds showed strong absorption around at 1600 cm^{-1} due to the carbonyl group, the compounds showed strong absorption around 1250 cm^{-1} due to C=N group, also the compounds showed absorption around 3300 cm^{-1} due to the presence of sp^3 hybridized CH_3. All the compounds showed singlet around at 10.6 δ ppm due to one proton of =N-N-H. The compounds showed significant multiplates at 158-147 δ ppm due to the presence of carbonyl group in ^{13}CNMR.

All the synthesized compounds were screened for their anti-microbial activities against three gram negative and two gram positive strains of bacteria and one fungal strain by broth dilution method. MIC values of the appraised compounds are recorded in (Table 3). According to the results we found that Derivatives **9a, 9b** and **9f** were exhibited moderate activity against *E. coli*. **9b** and **9c** possess moderate activity against *P. aeruginosa*. **9b, 9d** and **9f** compounds showed moderate activity against *Kl. Pneumoniae*. **9a** and **9f** showed moderate activity against *Bacillus Cereus*. Compound **9a, 9c, 9d** and **9e** exhibited moderate activity against *S. Aureus*.

For anti fungal activity derivative **9b, 9d, 9e** and **9f** showed moderate activity against *P. marneffei*.

Table 3: Anti bacterial/anti-fungal activity [microgram/ml] in terms of Minimum Inhibition Concentration of synthesized compounds

Compound Id	Minimim inhibition concentration(MIC)(microgram /ml)					
	Anti-bacterial activity					Anti- fungal activity
	Gram Negative Bacteria			Gram Positive Bacteria		Fungus
	E. coli MTCC 443	*P. aeruginosa* MTCC 1688	*K.pneumoniae* MTCC 109	*B.Cereus* MTCC 430	*S. aureus* MTCC 96	*P.marneffei* Wild stain
9a	25	50	100	25	50	500
9b	12.5	25	25	50	100	100
9c	50	50	100	50	25	250
9d	100	25	50	100	50	100
9e	25	50	100	100	25	50
9f	12.5	50	25	25	100	50
Furacin	25	25	50	50	50	-
Intraconozole	-			-		100

E. coli: Escherichia coli, P.aeruginosa: Pseudomonas aeruginosa, K. pnemoniae: Klebsiella pneumonia, S. aureus: Staphpyococcus aureus, P. marneffei: penicillium marneffei.

4. CONCLUSION

All the compounds were successfully synthesized by the conventional heating method with high amount of yield at RT and purified by Column chromatography. All synthesized compounds were characterized by different spectroscopic techniques like ^{1}HNMR, ^{13}CNMR, IR and Mass analysis. The novel compounds were shown their antimicrobial activity against three gram negative strains and two gram positive of bacteria as well as one fungal strain. From present studies we came to know that all the

compounds emerged out as potent moderate biological active agents. They are helpful as active pharmaceutical ingredient. Further work on these compounds may help for discovery of lead molecule in future.

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