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Syntheses and Antimicrobial Activities of Derivatives of 3-Amino-2-methyl-quinazolin-4-(3*H*)-one

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In the present study, 3-amino-2-methyl-quinazolin-4-(3*H*)-one (**1**) have been synthesized by the reaction of N-acetyl anthranilic acid with acetic anhydride followed by treating the mixture with hydrazine hydrate in pyridine. Compound **1** on treating with chloro acetyl chloride gives 3-chloroethanoylamino-2-methyl-quinazolin-4-(3*H*)-one (**2**). The final compounds **4a-g** were obtained by reacting the substituted derivative **2** with aliphatic and aromatic secondary amines. The structural assignment of this compound **4a-g** has been made on the basis of elemental analysis, IR and ^{1}H NMR data. The synthesized compounds were screened for *in vitro* growth inhibiting activity by determining the minimum inhibitory concentration against different strains of bacteria and fungi. Compounds **4a**, **4b**, **4e** and **4g** exhibit highest antibacterial activity and compounds **4a**, **4b**, **4d** and **4e** showed better antifungal activity.

Key Words: 3-Amino-2-methyl-quinazolin-4-(3*H*)-one, Antimicrobial activities.

INTRODUCTION

Quinazolin-4-(3*H*)-one is a versatile pharmacophore[1] and 2,3-disubstituted quinazolin-4-(3*H*)-one and 2,6,8-trisubstituted quinazolin-4-(3*H*)-one were reported to possess a wide variety of biological activities like hypnotic[2], antiallergic and antispasmodic[3], analgesic and antiinflammatory[4], cardiovascular[5] anticonvulsant[6], antiparkinsonian[7], CNS and antimicrobial[8], MAO inhibitors[9], CNS depressant[10], pyurvate oxidase inhibitor[11], psychotropic agent[12], anthelmintics[13], antifungal[14], *etc*. Therefore, in the present case it is decided to undertake the synthesis of various 3-(substituted)ethanoylamino-2-methyl quinazolin-4-(3*H*)-ones by treating 3-amino-2-methylquinazolin-4-(3*H*)-one with chloro acetyl chloride followed by reaction with different aliphatic and aromatic secondary amines in expectation of getting potent biodynamic agent and evaluate their antibacterial and antifungal activity.

EXPERIMENTAL

The melting point of the synthesized compounds were determined by open ended capillary tubes and are uncorrected. The purity of the compounds was checked by TLC.

The synthesized compounds were subjected to qualitative and quantitative elemental analysis. IR spectra were recorded on Jasco FT IR -470 spectrophotometer in KBr pellets. ^{1}H NMR spectra were recorded on Bruker-AC-300 MHz spectrometer using $CDCl_3$ and DMSO-d_6 as a solvent. Solubility of the synthesized compound was checked in different solvents at room temperature (28-30 °C). The sodium fusion extract of each compounds were prepared and was analyzed for nitrogen[15-17]. All the chemicals used were of high purity and synthetic grade.

General procedure for the synthesis of 3-amino-2-methyl quinazolin-4-(3*H*)-one: N-Acetylanthranilic acid[16-20] were prepared by refluxing anthranilic acid (2.7 g, 0.02 mol) for 1 h with glacial acetic acid (1.8 mL, 0.03 mol) and acetic anhydride (2.1 mL, 0.02 mol) and poured into cold distilled water and filtered it, washed with cold water and dry in air, recrystallize from hot distilled water (yield 74 %, m.p. 165-167 °C).

3-Amino-2-methylquinazolin-4-(3*H*)-ones was prepared by treating N-acetyl anthranilic acid (1.3 g, 0.01 mol) with acetic anhydride (1.1 mL, 0.01 mol) and refluxed under anhydrous condition for 4-5 h. Excess of acetic anhydride was then distilled off under reduced pressure. The reaction mixture was cooled down to room temperature. The mass obtained on solidification was used immediately for the next step because the formed intermediate benzoxazinone is unstable and highly reactive. The mixture of obtained solid (benzoxazinone) and hydrazine hydrate (0.1 M, 0.625 mL) in dried pyridine was refluxed for 4-5 h. After cooling it was poured with stirring into ice cold distilled water and kept overnight in the refrigerator. The solid so separated out was filtered, washed with cold water, dried and recrystallized from hot ethanol (wt. 750 mg, yield 60 %, m.p. 145 °C).

3-Chloroethanoylamino-2-methyl-quinazolin-4-(3*H*)-ones was prepared by taking compound **2** (1.75 g, 0.01 mol) in round bottomed flask containing 40 mL of 1,4-dioxane and dissolved it completely by heating. Chloro acetyl chloride (0.011 mol) was added in dropwise fashion. When the addition was complete the contents were refluxed for 1 h in a water bath. The solvent was distilled off under reduced pressure and the product obtained was filtered and recrystallized from dioxane (wt. 1 g: yield 60 %; m.p. 200-202 °C).

Synthesis of 3-(substituted)ethanoylamino-2-methyl quinazolin-4-(3*H*)-ones were prepared by dissolving compound **3** (0.025 mol) in a round bottomed flask containing methanol (40 mL) and dissolve it completely by heating. The amine (0.03 mol) was added dropwise and the contents were refluxed for 5-6 h. After the evolution of HCl ceased, the excess of methanol was distilled off under reduced pressure and the resulting product was dried and recrystallized from hot ethanol and analyzed. Adopting the above procedure different compounds were synthesized and their characterization data are provided in Table-1. Yield and melting point of the products were determined and summarized below:

The reaction sequence leading to the formation of different title compounds is outlined in **Scheme-I**.

3-(N,N-Diethylamino)ethanoylamino-2-methyl-quinazolin-4-(3H)-one (4a): Yield: 60 %, m.p. 154-156 °C; IR (KBr, ν_{max}, cm^{-1}): 3313 (N-H stretch due to secondary amide), 3015 (aromatic C-H stretch), 2941, 2879 (alkyl C-H stretch), 1732, 1653 (aromatic overtones), 1698 (C=O stretch due to keto group), 1653 (C=N stretch due to quinazolin-4-one ring), 1558, 1540 (N-H bend due to secondary amide), 1595, 1474, 1456 (C=C stretch due to aromatic ring), 1434, 1379 (alkyl C-H bend), 1316, 1262, 1195 (C-N stretch due to 4-quinazolin one ring), 697, 767 (aromatic C=C-H bend out-of-plane), 697 (C-H bend CH_2 rocking vibration). ^{1}H NMR (DMSO-d_6, δ ppm): 7.75-7.40 (m, 4H, ArH), 7.27 (s, 1H, -NH-C=O-), 4.9 (s, 2H, -C=O-CH_2), 2.70 (q, 4H, N(CH_2CH_3)$_2$, 1.2 ((t, 6H, N(CH_2CH_3)$_2$, 1.0 (s, 3H, Ar-CH_3).

3-(N,N-Di-*n*-propylamino)ethanoylamino-2-methyl-quinazolin-4-(3*H*)-one (4b): Yield: 60 %, m.p. 98-100 °C; IR (KBr, ν_{max}, cm^{-1}): 3301 (N-H stretch due to secondary amide), 3011 (aromatic C-H stretch), 2961, 2818 (alkyl C-H stretch), 1867, 1843, 1792 (aromatic overtones), 1698 (C=O stretch due to keto group), 1652 (C=N stretch due to quinazolin-4-one ring), 1558, 1540 (N-H bend due to secondary amide), 1558, 1473, 1456 (C=C stretch due to aromatic ring), 1395 (alkyl C-H bend), 1316, 1262, 1198 (C-N stretch due to 4-quinazolin one ring), 772, 696 (aromatic C=C-H bend out-of-plane). ^{1}H NMR (DMSO-d_6, δ ppm): 7.75-7.4 (m, 4H, ArH), 7.27 (s, 1H, -NH-C=O-), 4.9 (s, 2H, -C=O-CH_2), 2.71 (t, 4H, -$CH_3CH_2CH_2$-N=), 1.6-1.7 (m, 4H, -$CH_3CH_2CH_2$-N=), 0.96-1.01 ((t, 6H, -$CH_3CH_2CH_2$-N=), 0.99 (s, 3H, Ar-CH_3).

3-(N,N-Di-isopropylamino)ethanoylamino-2-methyl-quinazolin-4-(3*H*)-one (4c): Yield: 60 %, m.p. 140-142 °C; IR (KBr, ν_{max}, cm^{-1}): 3300 (N-H stretch due to secondary amide), 3019 (aromatic C-H stretch), 2899, 2806 (alkyl C-H stretch), 1867, 1843, 1792 (aromatic overtones), 1698 (C=O stretch due to keto group), 1652 (C=N stretch due to quinazolin-4-one ring), 1558, 1540 (N-H bend due to secondary amide), 1456, 1474 (C=C stretch due to aromatic ring), 1388 (alkyl C-H bend), 1316, 1259, 1198 (C-N stretch due to quinazolin one ring), 697, 767 (aromatic C=C-H bend out-of-plane). ^{1}H NMR (DMSO-d_6, δ ppm): 7.75-7.40 (m, 4H, ArH), 7.27 (s, 1H, -NH-C=O-), 4.9 (s, 2H, -C=O-CH_2), 2.7 (m, 2H, -$CH(CH_3)_2$), 1.85 (m, 12H, -$CH(CH_3)_2$), 1.0 (s, 3H, Ar-CH_3).

3-(N,N-Di-butylamino)ethanoylamino-2-methyl-quinazolin-4-(3*H*)-one (4d): Yield: 60 %, m.p. 132-134 °C; IR (KBr, ν_{max}, cm^{-1}): 3337 (N-H stretch due to secondary amide), 3010 (aromatic C-H stretch), 2976, 2852 (alkyl C-H stretch), 1867, 1844, 1792 (aromatic overtones), 1698 (C=O stretch due to keto group), 1646 (C=N stretch due to quinazolin-4-one ring), 1558, 1540 (N-H bend due to secondary amide, 1474 (C=C stretch due to aromatic ring), 1456, 1395 (alkyl C-H bend), 1335, 1259 (C-N stretch due to 4-quinazolin one ring, 697, 767 (aromatic C=C-H bend out-of-plane). ^{1}H NMR (DMSO-d_6, δ ppm): 7.75-7.4 (m, 4H, ArH), 7.27 (s, 1H, -NH-C=O-), 4.9 (s, 2H, -C=O-CH_2), 2.31 (t, 4H, -$CH_3CH_2CH_2CH_2$-N=, 1.86 (m, 4H, -$CH_3CH_2CH_2CH_2$-N=), 1.68 (m, 4H, -$CH_3CH_2CH_2CH_2$-N=), 1.25 (t, 6H, -$CH_3CH_2CH_2$-N=), 1.2 (s, 3H, Ar-CH_3).

3-(Morpholin-1-yl)ethanoylamino-2-methyl-quinazolin-4-(3*H*)-one (4e): Yield: 60 %, m.p. 158-160°C; IR (KBr, ν_{max}, cm^{-1}): 3273 (N-H stretch due to secondary amide), 3029 (aromatic C-H stretch), 2943, 2850 (alkyl C-H stretch), 1867, 1844, 1792 (aromatic overtones), 1684 (C=O stretch due to keto group), 1650 (C=N stretch due to quinazolin-4-one ring), 1558, 1540 (N-H bend due to secondary amide), 1541, 1473 (C=C stretch due to aromatic ring), 1486, 1377 (alkyl C-H bend), 1335, 1267, 1144 (C-N stretch due to 4-quinazolin one ring), 763, 686 (aromatic C=C-H bend out-of-plane). ^{1}H NMR (DMSO-d_6, δ ppm): 7.75-7.40 (m, 4H, ArH), 7.27 (s, 1H, -NH-C=O-), 4.9 (s, 2H, -C=O-CH_2), 3.27 (t, 4H, -C_bH and C_eH of morpholine), 2.53 (t, 4H, -C_aHC_dH of morpholine), 1.2 (s, 3H, Ar-CH_3).

3-(Piperidine-1-yl)ethanoylamino-2-methyl-quinazolin-4-(3*H*)-one (4f): Yield: 60 %, m.p. 160-164 °C; IR (KBr, ν_{max}, cm^{-1}): 3305 (N-H stretch due to secondary amide), 3038 (aromatic C-H stretch), 2943, 2850 (alkyl C-H stretch), 1867, 1844, 1792 (aromatic overtones), 1684 (C=O stretch due to keto group), 1647 (C=N stretch due to quinazolin-4-one ring), 1546, 1473 (C=C stretch due to aromatic ring), 1521, 1540 (N-H bend due to secondary amide), 1456, 1377 (alkyl C-H bend), 1333, 1268, 1159 (C-N stretch due to 4-quinazolin one ring), 766, 689 (aromatic C=C-H bend out-of-plane). ^{1}H NMR (DMSO-d_6, δ ppm): 7.75-7.40 (m, 4H, ArH), 7.27 (s, 1H, -NH-C=O-), 4.9 (s, 2H, -C=O-CH_2), 2.51 (t, 4H, -C_aH,C_eH of piperidine), 1.86 (m, 6H, C_b,C_c,C_d of piperidine), 1.2 (s, 3H, Ar-CH_3).

3-(Pyrrolidine-1-yl)ethanoylamino-2-methyl-quinazolin-4-(3*H*)-one (4g): Yield: 60 %, m.p. 84-86 °C; IR (KBr, ν_{max}, cm^{-1}): 3311 (N-H stretch due to secondary amide), 3030 (aromatic C-H stretch), 2960, 2800 (alkyl C-H stretch), 1732, 1683 (aromatic overtones), 1716, 1698 (C=O stretch due to keto group), 1647 (C=N stretch due to quinazolin-4-one ring), 1558, 1540 (N-H bend due to secondary amide), 1507, 1473, 1456 (C=C aromatic stretch), 1473, 1400 (alkyl C-H bend), 1365, 1250, 1150 (C-N stretch due to 4-quinazolin one ring), 764 (aromatic C=C-H bend out-of-plane). ^{1}H NMR (DMSO-d_6, δ ppm): 7.75-7.40 (m, 4H, ArH), 7.27 (s, 1H, -NH-C=O-), 4.9 (s, 2H, -C=OCH_2), 2.51 (t, 4H, -C_aH, C_dH of pyrrolidine), 1.47-1.35 (m, 4H, C_b,C_c of pyrrolidine).

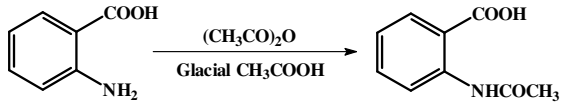
Screening for antimicrobial activity: The synthesized compound were evaluated for antibacterial and antifungal activities. The minimum inhibitory concentrations[21] were determined by liquid dilution method. The bacterial strain used were *Bacillus subtilis* (MTCC-69), *Pseudomonas aeruginos*a (MTCC-424), *Staphylococcus aureus* (MTCC-96) and *Proteus mirablis* (MTCC-425). The fungal strain used are *Candida albicans* (MTCC-277), *Aspergillus niger* (MTCC-282) and *Trichoderma viridae*.

For the antibacterial activity, the standard drug used was norfloxacin[22] and the stock solution (1000 µg/mL) was prepared in DMF. The nutrient broth containing beef extract, yeast extract, peptone, sodium chloride and water in proper composition was used as culture media. The stock culture was incubated for 48 h at 37 °C and the MIC of synthesized compound in nutrient broth was determined by varying the concentration (10-160 µg/mL). The concentration of norfloxacin in nutrient broth was taken between (2-9 µg/mL).

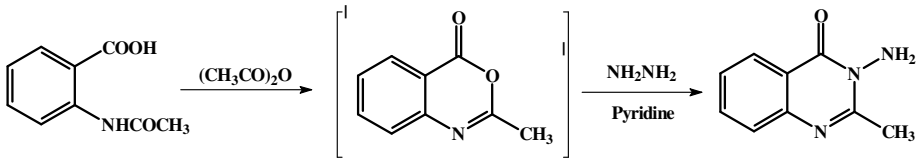
For the antifungal activity the stock solution of clotrimazole[23] (1000 µg/mL) and the saborauds dextrose medium was used as a culture medium in the present study and the incubation was done at 30 °C for 3 days. The concentration of synthesized compound in nutrient media was taken between (10-160 µg/mL) and the standard drug concentration was taken between (2-9 µg/mL) and the MIC of the title compound was determined and given in Tables 2 and 3.

RESULTS AND DISCUSSION

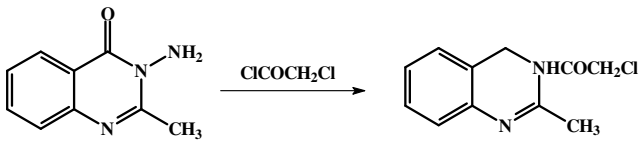
3-(Substituted)ethanoylamino-2-methylquinazolin-4-(3*H*)-one (**4a-g**) was synthesized by refluxing N-acetylanthranilic acid with acetic anhydride followed by adding hydrazine hydrate. The 3-amino-2-methylquinazolin-4(3*H*)-ones (**1**) obtained was reacted with chloro acetyl chloride and then by secondary amines to get the substituted derivatives (**4a-g**) as shown in **Scheme-I**. The physical and analytical data of the compounds were collected and presented in Table-1. Yield of the compounds (**4a-g**) falls in the range of 60-80 %.The spectral (IR, NMR) and analytical data are in good agreement with there structures.



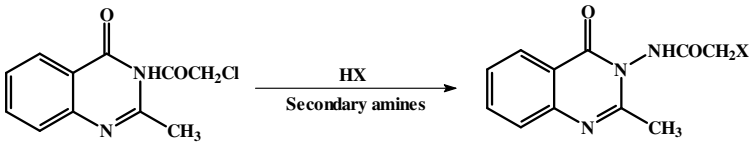
Step-I: Synthesis of N-acetyl anthranilic acid



Step-II: Synthesis of 3-Amino-2-methylquinazolin-4-(3*H*)-ones (**1**)



Step-III: Synthesis of 3-Chloroethanoyl amino-2-methyl-quinazolin-4-(3*H*)-ones (**2**)



Step-IV: Synthesis of 3-(dialkyl amino)ethanoylamino-2-methylquinazolin-4-(3*H*)-one (**4a-g**)

Scheme-I

TABLE-1
PHYSICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

Compd.	X	Yield (%)	m.p. (ºC)	m.f.	m.w.	% N	
						Calcd.	Found
4a	$N(CH_2CH_3)_2$	60	154-56	$C_{15}H_{20}N_4O_2$	288	19.4	20.170
4b	$N(CH_2CH_2CH_3)_2$	65	98-100	$C_{17}H_{24}N_4O_2$	316	17.7	17.944
4c	$NCH(CH_3)_2CH(CH_3)_2$	68	140-42	$C_{17}H_{24}N_4O_2$	316	17.7	17.940
4d	$N(CH_2CH_2CH_2CH_3)_2$	62	132-34	$C_{19}H_{28}N_4O_2$	344	16.2	16.890
4e	-Morpholino	70	158-60	$C_{15}H_{18}N_4O_3$	302	18.5	17.070
4f	-Piperidino	66	160-64	$C_{16}H_{20}N_4O_2$	300	18.6	17.250
4g	-Pyrrolidino	62	84-86	$C_{15}H_{18}N_4O_2$	286	19.5	20.210

TABLE-2
MIC OF THE TITLE COMPOUNDS (FOR ANTIBACTERIAL ACTIVITY IN µg/mL)

Compd.	*Pseudonas aeruginosa*	*Bacillus subtilis*	*Stapylococcus aureus*	*Proteus mirabilis*
4a	92	96	136	112
4b	138	114	152	56
4c	138	158	136	118
4d	154	112	132	156
4e	98	132	114	156
4f	116	136	92	158
4g	74	152	88	134
Norfloxacin	8	5	6	4

TABLE-3
MICs OF THE TITLE COMPOUND (FOR ANTIFUNGAL ACTIVITY IN µg/mL)

Compd.	*Aspergillus niger*	*Candida albicans*	*Trichoderma viridae*
4a	88	84	72
4b	112	158	154
4c	134	138	156
4d	154	112	92
4e	94	96	116
4f	118	98	134
4g	72	92	76
Clotrimazole	6	8	9

Screening results of the antibacterial and antifungal activity (Tables 2 and 3) reveals that the MIC of the standard drug norfloxacin was found to be 4-8 µg/mL. The antibacterial activity was shown by all of the synthesized compound and the most active compound (56-98 µg/mL) include **4a**, **4b**, **4e** and **4g**. For the antifungal activity, the MIC of the clotrimazole was found to be 6-9 µg/mL fungitoxicity was shown by all of the synthesized compounds and most active compounds (72-98 µg/mL) include **4a**, **4b**, **4d** and **4e**.

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