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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antimalarial and antibacterial activities of 3-amino acid- and aryl amine-substituted 2-methyl-3*H*-quinalzolin-4-ones

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Abstract A new series of 2-methyl-3*H*-quinazolinones substituted at the third position with amino acids (**2**–**5**) and aryl amine (**6**, **7**) was designed, synthesized, and analyzed by infrared, NMR, and mass spectral analysis. Further, the compounds were screened for their in vivo antimalarial activity using the rodent malaria parasite *Plasmodium yoelii* (N-67) with the Swiss mice model. The compounds were also tested for their antibacterial activity.

Keywords Quinazolinone · Antimalarial · *Plasmodium yoelii* · Swiss mice model · Antibacterial

Introduction

The search for novel antimalarial drugs for treatment as well as for prophylaxis is of great concern in light of the current worldwide resurgence of malaria. Quinazolines and condensed quinazolines have received the attention of medicinal chemists due to their wide range of biological activities (Alagarsamy *et al.*, 2004), which include mainly anti-infective activities, such as antibacterial (Alagarsamy *et al.*, 2000) and antifungal activities, CNS activities such as analgesic (Nigam *et al.*, 1990), anti-inflammatory (Koizomi and Marakuni 1917), anticonvulsant (Manabu *et al.*, 1990) activities, and antimalarial activities (Michael and Leann 1998; Mei-Lin *et al.*, 1998; Kaylene, 1999; Joshi *et al.*, 2005). The quinazolin-4-one motif-based drugs that are on the market are known to possess side effects. In the present study it was envisaged that a drug molecule possessing the above-mentioned pharmacophore

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may be of advantage for antimalarial activity, because it is known to possess a broad spectrum of antimicrobial activity.

Chemistry

The quinazolin-4-ones incorporated with biologically friendly amino acids such as glycine (**2**), L-lysine (**3**), L-cysteine (**4**), and L-glutamic acid (**5**) were synthesized according to the route given in Scheme 1. The intention in designing a quinazolin-4-one motif incorporated with amino acids was to enhance the pharmacokinetic parameters such as PK_a and water solubility apart from nontoxic metabolites.

In general, anthranilic acid was acetylated using an acetylating reagent such as acetic anhydride to form *N*-acetyl anthranilic acid (**1**). The *N*-acetyl anthranilic acid was further treated with amino acid or aryl amine to cyclize in the presence of phosphorous pentoxide, to give the final compounds shown in Table 1. Compounds were analyzed based on infrared (IR), ^{1}H-NMR, and mass profiles. Elemental analysis was also performed for carbon, hydrogen, nitrogen, and oxygen. Calculated and found values were within $\pm$ 0.4% variance.

General

Melting points were determined in open capillaries on a LABINDIA digital melting-point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FTIR 283, spectrophotometer using KBr disks and are expressed as cm^{-1}. ^{1}H-NMR spectra were recorded on a Bruker DRX-300 (300 MHz, FTNMR) using $CDCl_3$ as solvent and TMS as internal standard and are expressed as δ ppm. Mass spectra (MS) were determined on Shimadzu LCMS 2010A, using ESI ionization technique in the positive mode (M + 1). Elemental analysis was performed using an Elemental

Scheme 1 Compound scheme

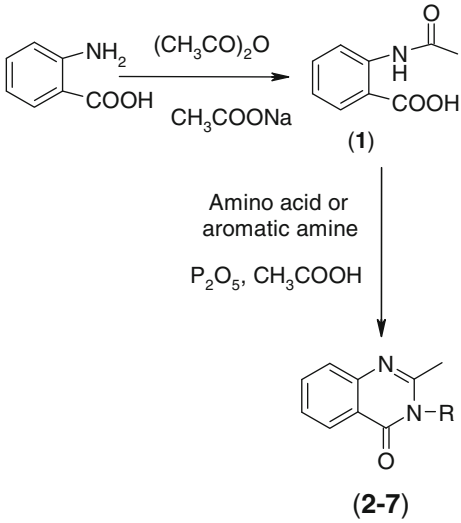
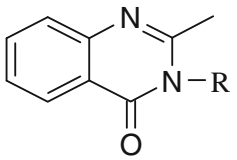
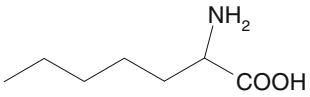
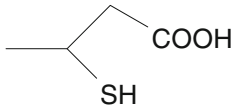
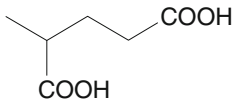
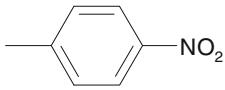
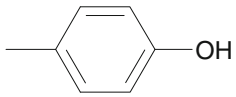


Table 1 Final compounds



Compounds	R	Yield (%)
2	—CH_2COOH	46.2
3		40.1
4		39.14
5		46.8
6		70.34
7		72.8

Vario EL III (Carloerba 1108). Reactions were monitored by TLC using silica gel type 60 F_{254} of E. Merck with 4% methanol in chloroform as the mobile phase. TLC plates were visualized in UV and with iodine vapors.

Procedure for the preparation of *N*-acetyl anthranilic acid (**1**)

Anthranilic acid (1 mol) was mixed with an equimolar quantity of sodium acetate and acetic anhydride (1.15 mol) and refluxed on a sand bath for 1 h. The reaction

mixture was then poured into a beaker containing water; the crude solid product separated was filtered and dried. The dried crude product was recrystallized from aqueous alcohol to get a brownish-yellow crystalline solid (4.7 g; 72.2%); m.p., 210°C. IR (KBr) cm^{-1}: 3367 (N–H), 2902 (CH_3), 1677 (CONH), 1718 (COOH), 1611 (C=C), 1218 (C–N), 1121 (C–O).

Procedure for the preparation of 3-amino acid-substituted 3*H*-quinazolin-4-ones (**2**–**5**)

To a solution of *N*-acetyl anthranilic acid (1 mol) in 20 ml of hot glacial acetic acid was added a phosphorus pentoxide (1.15 mol) and a corresponding amino acid (1.15 mol). The reaction mixture was refluxed for 3 h and the reaction monitored by TLC. Then the reaction mixture was poured into a beaker containing 100 ml of hydrochloric acid; crude products which separated out were filtered and dried. Crude products were recrystallized with aqueous ethanol. Further, the compounds were purified by column chromatography using silica gel (60-120) as the stationary phase and acetonitrile/ethyl acetate/methanol at a ratio of 1:1:1 as the mobile phase and are shown in Table 1.

*2-(2-Methyl-4-oxo-4H-quinazolin-3-yl)-ethanoic acid (**2**)*

Melting point, 201°C. IR (KBr) cm^{-1}: 3458 (OH), 2884 (CH_3), 1720 (COOH), 1677 (CONH), 1630 (C=C), 1588 (C=N), 1215 (C–N),1105 (C–O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.11 (3H, s, CH_3), 4.14 (2H, s, CH_2), 7.73–8.11(4H, m, ArH), 11.0 (1H, s, COOH). ESI-MS m/z: 219.2 (M + 1), 214, 167, 241, 82.

*2-Amino-6-(2-methyl-4-oxo-4H-quinazolin-3-yl)-hexanoic acid (**3**)*

Melting point, 197°C. IR (KBr) $cm^{-1;}$ 3470 (OH), 2897 (CH_3), 1723 (COOH), 1680 (CONH), 1631 (C=C), 1598 (C=N), 1210 (C–N), 1121 (C–O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.91 (3H, s, CH_3), 2.29–3.03 (8H, m, CH_2), 3.2 (1H, t, CH), 3.49 (1H, t, CH), 4.35 (2H, t, NH2), 7.65–7.91 (4H, m, ArH), 11.1 (1H, s, COOH). ESI-MS m/z: 290.2 (M + 1), 264, 177, 128.

*3-Mercapto-3-(2-methyl-4-oxo-4H-quinazolin-3-yl)-propionic acid (**4**)*

Melting point, 226°C. IR (KBr) cm^{-1}: 3469 (OH), 2890 (CH_3), 2590 (SH), 1721 (COOH), 1671 (CONH), 1630 (C=C), 1579 (C=N), 1219 (C–N), 1119 (C–O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.19 (3H, s, CH_3), 1.7 (1H, t, SH), 2.94 (2H, q, CH_2), 3.89 (1H, t, CH), 7.72–8.16 (4H, m, ArH), 11.1 (1H, s, COOH). ESI-MS m/z: 265.2 (M + 1), 250, 197, 119, 80.

*2-(2-Methyl-4-oxo-4H-quinazolin-3-yl)-pentandioic acid (**5**)*

Melting point, 213°C. IR (KBr) cm^{-1}: 3481 (OH), 2907 (CH_3), 1723 (COOH), 1680 (CONH), 1630 (C=C), 1589 (C=N), 1209 (C–N), 1112 (C–O). ^{1}H-NMR ($CDCl_3$,

300 MHz) δ: 2.32 (3H, s, CH_3), 2.95–3.11 (4H, m, $(CH_2)_2$), 4.52 (1H, t, CH), 7.74–8.19 (4H, m, ArH), 11.08 (1H, s, COOH), 11.28 (1H, s, COOH). ESI-MS m/z: 305.23 (M + 1), 289, 234, 186, 98.

Procedure for the preparation of compounds **6** and **7**

To a solution of *N*-acetyl anthranilic acid (1 mol) in 20 ml of hot glacial acetic acid was added a mixture of phosphorus pentoxide (1.15 mol) and aromatic amine (1 mol). The reaction mixture was refluxed for 3 h, then poured into a beaker containing 100 ml of 1 N sodium bicarbonate solution. The crude products which separated out were filtered and dried. Dried crude products were recrystallized with aqueous ethanol to get pure compounds and are shown in Table 1.

*1-(2-Methyl-4-oxo-4H-quinazolin-3-yl)-4-nitro benzene (**6**)*

Melting point, 124°C. IR (KBr) cm^{-1}: 2991 (CHAr), 2913 (CH_3), 1680 (CONH), 1629 (C=C), 1585 (C=N), 1550 (NO_2), 1202 (C–N), 1109 (C–O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.21 (3H, s, CH_3), 7.71–8.39 (4H, m, ArH), 7.90 (2H, d, ArH), 8.17 (2H, d, ArH), 10.9 (1H, s, COOH). ESI-MS m/z: 282.15 (M + 1), 267, 211, 184, 111, 74.

*1-(2-Methyl-4-oxo-4H-quinazolin-3-yl)-4-hydroxy benzene (**7**)*

Melting point, 190°C. IR (KBr) cm^{-1}: 3342 (OH), 2991 (CHAr), 2907 (CH_3),1680 (CONH), 1627 (C=C), 1585 (C=N), 1550 (NO_2), 1214 (C–N), 1109 (C–O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.07 (3H, s, CH_3), 7.84–8.31 (4H, m, ArH), 7.47 (2H, d, ArH), 7.51 (2H, d, ArH), 7.90 (2H, d, ArH), 8.9 (1H, s, OH). ESI-MS m/z: 253.2 (M + 1), 217, 176, 112, 81.

Pharmacology

Procedure for in vivo antimalarial screening

The in vivo efficacy of synthetic compounds was evaluated against a rodent malaria parasite *Plasmodium yoelii* (N-67) in the Swiss mice model (Neerja and Puri 2004; Singh *et al.*, 2002, 2004; Puri and Naresh 2000). Random-bred Swiss mice (23 ± 2 g) of either gender were obtained from breeding colonies at the laboratory animal facilities at CDRI, Lucknow, India. Each animal was inoculated intraperitoneally with 1×10^7 *P. yoelii* parasitized red blood cells (RBCs) and the day of inoculation was designated Day 0. Animals were maintained on a commercial pellet diet and water ad libitum under standard housing conditions.

Aqueous suspensions of the synthetic compounds were prepared after making a paste with a few drops of Tween-80. The volume was adjusted so as to obtain the required 200 mg/kg dose in a volume of 0.5 ml. The treatment was administered intraperitoneally, once daily for 4 consecutive days, day 0 to day 3, to groups of five

P. yoelii-infected mice. One group of five mice was administered the aqueous vehicle used for preparing the suspension and served as untreated controls.

Thin blood smears were prepared from each animal on day 4, i.e., 24 h after the last treatment dose, and again on day 7. The degree of infection was microscopically recorded in terms of the number of *P. yoelii*-infected cells per 100 RBCs (i.e., percentage parasitemia). The mean value determined for each group of five mice was used to calculate the percentage suppression of parasitemia with respect to the untreated control group.

Antibacterial activity

In vitro antibacterial screening was performed for all six synthesized compounds against *S. aureus* and *E. coli* by the cup plate method. Sterile nutrient agar plates were prepared by pouring sterile agar into petri plates under aseptic conditions. One-tenth milliliter of the standardized organism was spread onto agar plates. Holes were prepared using a sterile borer 6 mm in diameter. Test compound solutions were placed in each hole separately. Then the plates were incubated at 4°C for 1 h to allow diffusion of the solution into the medium. Then all the bacterial plates were incubated at 37°C for 24 h. The zone of inhibition was measured (mm) against the standard kanamycin as a positive control and 10% DMSO in water as a solvent control. The concentration of test compounds as well as standard kanamycin was 100 μg.

Results and discussion

In the present studies, four 3-amino acid-substituted 3*H*-quinazolin-4-ones (**1**–**4**) were synthesized, and to compare and contrast the structure–activity relationships at the third position, we synthesized another two compounds with the aryl group incorporated at the third position of 3*H*-quinazolin-4-one. Their antimalarial activity

Table 2 Degree of parasitemia on days 4 and 7 in the six treated groups and relative suppression of parasitemia compared to the control group

Compound	Dose (mg/kg)	No. of mice	Mean % parasitemia on		Suppression of parasitemia on		Mean survival time ± SE (days)
			Day 4	Day 7	Day 4	Day 7	
2	200	5	10.50 ± 1.60	17.20 ± 0.00	15.05	11.79	6.60 ± 0.98
3	200	5	9.78 ± 1.30	17.50 ± 1.70	20.87	10.25	6.80 ± 1.39
4	200	5	11.90 ± 1.77	25.00 ± 3.00	3.72	NIL	7.40 ± 1.03
5	200	5	9.60 ± 1.73	18.45 ± 1.35	22.33	5.38	7.80 ± 0.97
6	200	5	10.43 ± 0.47	25.50 ± 2.50	15.61	NIL	7.00 ± 1.82
7	200	5	10.52 ± 1.33	16.80 ± 0.00	14.89	13.85	6.80 ± 0.58
Control		5	12.36 ± 1.14	19.50 ± 0.76	–	–	8.20 ± 0.86

(–) Mice remained negative during the entire observation period

was evaluated pharmacologically against the rodent malaria parasite *P. yoelii* (N-67) in the Swiss mice model. The degree of parasitemia on days 4 and 7 in the six treated groups and the relative suppression of parasitemia compared to the control group are summarized in Table 2. Although compounds **2**, **3**, **5**, and **7** showed a little antimalarial activity, surprisingly, the overall results revealed that none of the six compounds possess significant or potential antimalarial activity. None of the compounds showed antibacterial activity against either the gram-positive bacterium *S. aureus* or the gram-negative bacterium *E. coli* at a concentration of 100 μg.

In conclusion, the entitled compounds did not show any antimalarial or antibacterial activity, although better antimalarial and antibacterial activity was anticipated.

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