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ORIGINAL RESEARCH

Synthesis and evaluation of fused pyrimidine derivatives as anti-inflammatory, antiproliferative and antimicrobial agents

Seekarajapuram Dinakaran Vachala · Keloth Kothari Srinivasan · Peralam Yegneswaran Prakash

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Abstract In this study, condensation of 2-amino-5-chloro-benzoic acid with benzoyl chloride afforded 2-benzamido-5-chloro benzoic acid which on cyclization with different primary amines yielded the corresponding 6-chloro-2-phenyl-3-substituted quinazolin-4(*3H*)-ones (V1–V8). The reaction was monitored by thin layer chromatographic analysis. The structure of the synthesized compounds was confirmed by spectral studies and elemental analysis. All these compounds were screened for their anti-inflammatory, antiproliferative and antimicrobial activities. Anti-inflammatory activity was done by rat paw oedema method. Compounds V6 and V5 showed a good percentage inhibition of inflammation at the 2nd and the 3rd h, respectively. Antiproliferative activity was carried out by MTT assay against Chang liver, Hep2 and HeLa cell lines. Compounds V1, V5 and V8 showed antiproliferative activity at low concentrations. The minimum inhibitory concentrations (MIC) of the synthesized compounds against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus flavus* was studied by microdilution assay. Compound V4 was found to be a potent antimicrobial agent against the organisms tested. Further, it was selected for time kill study. These data revealed that the compounds containing chloro- and/or fluoro-substituted phenyl ring or *N*,*N*-dimethyl ethyl substitutions in the quinazolin-4(*3H*)-ones led to increase of their biological activities.

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Keywords 4(3*H*)-Quinazolinone · Minimum inhibitory concentration · Time kill study · Anti-inflammatory · Antiproliferative · MTT assay

Introduction

The chemistry of heterocyclic compounds has been a thought-provoking field of study for a long time. The synthesis of novel 4(3*H*)-quinazolinone derivatives and investigation of their chemical and biological behaviours have gained more importance in recent decades in view of their biological and medicinal values.

Quinazolinone nucleus is a versatile lead molecule and has been the focus of great interest recently because its derivatives have been found to possess wide spectrum of pharmacological activities. Quinazolin-4(3*H*)-one derivatives are useful heterocycles, possessing potent pharmacological activities such as anticonvulsant (Ponnilavarasan *et al*., 2010), anti-inflammatory (Mosaad *et al*., 2010), antihelminthic (Rajiv *et al*., 2008), antitumor (Cao *et al*., 2005), antifungal (Chatrasal *et al*., 2010), antibacterial (Vachala *et al*., 2008), MAO inhibitory activities (Srivastava *et al*., 1980) and antiproliferative activities, as well as inhibitory effects for thymidylate synthase and poly-(*ADP*-ribose) polymerase (*PARP*) activities (Griffin *et al*., 1998). Furthermore, the 2-substituted quinazolinones in particular have been utilized as peptidomimetic scaffolds with specificity for cholecystokinin, angiotensin and certain cell adhesion receptors (Banihashemi *et al*., 1998). Several 2,3-disubstituted benzopyrimidine derivatives were synthesized and tested for different biological activities. The reports showed that the aryl substitution at 3rd position enhances the biological activity.

Several methods have been reported for the synthesis of 4(3*H*)-quinazolinone derivatives (McNamara *et al*., 1990). However, these methods suffer from drawbacks, such as longer reaction time, complicated workup, and use of expensive and hazardous chemicals with low yield (Varma *et al*., 1997). Usage of microwave irradiation (MWI) is well known for the synthesis of a variety of compounds wherein chemical reactions are accelerated because of selective absorption of microwave by polar molecules. The coupling of MWI with solid-supported reagents under solvent-free conditions provides unique chemical processes with special attribute, such as enhanced reaction rate, higher yield and greater selectivity. However, this technique requires an appreciable amount of solvent for adsorption of reactants and elution of products. In view of ongoing research on neat synthesis, when the solvent-free reactions are coupled with MWI, the same proved to be advantageous considering environmental reasons as well as due to their uniform heating effect and shorter reaction times.

A combination of two or more biologically active moiety could increase or decrease the activity. Keeping this aspect in mind, in view of the involvement of biological activities and the eco-friendly synthesis of 4(3*H*)-quinazolinone, the purpose of the present study is to construct some novel 6-chloro-2-phenyl-3-substitutued-4(3*H*)-Quinazolinone as potential anti-inflammatory, antiproliferative and antimicrobial agents.

Materials and methods

Experimental

Chemistry

The melting points of the synthesized compounds were determined in open capillary tubes and were uncorrected. The IR spectra of the compounds were recorded in the range of 500–4,000 cm^{-1} on Shimadzu FT-IR 8310 using KBr pellets. The ^{1}H-NMR spectra were recorded on Joel, model GSV-400 MHz spectrometer using $CDCl_3$/DMSO-d_6 as solvent. The chemical shifts were reported as parts per million downfield from tetramethylsilane (Me_4Si). Mass spectra were recorded on the Shimadzu GC-MS QP5050. The reactions were monitored by thin layer chromatographic analysis on precoated SiO_2 gel (HF_{254}, 200 mesh)-coated glass plates. The spots were visualized by UV light. Micro analyses for C, H and N were performed in Heraeus CHN Rapid Analyser. Satisfactory C, H and N analyses were obtained for all the compounds.

Synthesis of 2-benzamido-5-chloro benzoic acid

To a cooled solution of 5-chloroanthranilic acid (0.5 g) in 5 ml of 10% sodium hydroxide, 1 ml of benzoyl chloride was added dropwise with continuous shaking. After adding benzoyl chloride, the reaction mixture was stirred for 1 h. The crude product that separated out was filtered, washed with cold water and recrystallized using ethanol (Mahadik and Bhosale, 2004). The reaction was monitored by TLC analysis using $CHCl_3$:EtOH (0.5:2.5).

General procedure for the synthesis of quinazolin-4(3H)-one derivatives (V1–V8)

Equimolar amount of 2-benzamido-5-chloro benzoic acid and different amines were condensed in the presence of DCC under MWI for 8–14 min (Kidwai *et al*., 2004). Upon completion of reaction as monitored by TLC, the reaction mixture was cooled, ice cold water was added, and the solid separated was filtered off. Then, the product was washed with cold ethanol and recrystallized from ethanol.

Spectral data of the representative compounds

6-Chloro-3-(3-chloro-4-fluorophenyl)-2-phenylquinazolin-4(3H)-one (V1): IR (KBr) (cm^{-1}): 3039 (aromatic CH), 1683 (C=O), 1581 (C=N), 1099 (C–Cl), 1209 (C–F); ^{1}H-NMR (DMSO-d_6): 7.52–8.1 (m, 12H, aromatic-H); MS: 384(M^{+1}), 345, 322, 225, 176; *Anal.* Calc. for $C_{20}H_{11}Cl_2FN_2O$: C, 62.36; H, 2.88; N, 7.27. Found: C, 62.52; H, 2.76; N, 7.41.

6-Chloro-3-(4-fluorophenyl)-2-phenylquinazolin-4(3H)-one (V4): IR (KBr) (cm^{-1}): 2929 (aromatic CH), 1631 (C=O), 1575 (C=N), 1099 (C–Cl), 1188 (C–F); ^{1}H-NMR (DMSO-d_6): 7.37–7.51 (m, 12H, aromatic-H); MS: 350(M^{+1}), 317, 273, 176, 103. *Anal.* Calc. for $C_{20}H_{12}ClFN_2O$: C, 68.48; H, 3.45; N, 7.99. Found: C, 68.52; H, 3.63; N, 7.71.

6-Chloro-3-(3-chlorophenyl)-2-phenylquinazolin-4(3H)-one (V5): IR (KBr) (cm^{-1}): 2924 (aromatic CH), 1631 (C=O), 1570 (C=N), 1097 (C–Cl); ^{1}H-NMR (DMSO-d_6): 7.53–7.8 (m, 12H, aromatic-H); MS: 366(M^{+1}), 350, 336, 255, 201, 127, 104. *Anal.* Calc. for $C_{20}H_{12}Cl_2N_2O$: C, 65.41; H, 3.29; N, 7.63. Found: C, 65.58; H, 3.51; N, 7.82.

6-Chloro-3-(2-(dimethyl amino)ethyl)-2-phenylquinazolin-4(3H)-one (V6): IR (KBr) (cm^{-1}): 2945 (aromatic CH), 1630 (C=O), 1556 (C=N); ^{1}H-NMR (DMSO-d_6): 7.47–7.59 (m, 8H, aromatic-H), 2.48 (t, 2H, –CH_2–), 3.21 (t, 2H, –CH_2–); MS: 327(M^{+1}), 317, 152, 111, 98. *Anal.* Calc. for $C_{18}H_{18}ClN_3O$: C, 65.95; H, 5.53; N, 12.82. Found: C, 65.72; H, 5.28; N, 12.76.

6-Chloro-3-(4-chlorophenyl)-2-phenylquinazolin-4(3H)-one (V8): IR (KBr) (cm^{-1}): 2958 (aromatic CH), 1645 (C=O), 1595 (C=N), 1085 (C–Cl); ^{1}H-NMR (DMSO-d_6): 7.83–8.1 (m, 12H, aromatic-H); MS: 366(M^{+1}), 317, 211, 121, 104, 98. *Anal.* Calc. for $C_{20}H_{12}Cl_2N_2O$: C, 65.41; H, 3.29; N, 7.63. Found: C, 65.28; H, 3.53; N, 7.46.

Biological activities

Anti-inflammatory activity

Animals

Albino rats of *Wistar* strain of either sex, weighing 150–180 g, were selected by random sampling technique and used in the study of anti-inflammatory activity. The animals had free access to standard commercial diet and water ad libitum, and rats were kept in rooms maintained at 22 ± 1°C with a 12-h light/dark cycle.

Carrageenan-induced paw oedema

The anti-inflammatory activity of the test compounds was carried out by carrageenan-induced paw oedema method (Winter *et al*., 1962). Carboxy methyl cellulose (0.5% w/v CMC) was selected as vehicle to suspend the standard drug and the test compounds. The animals were weighed, marked for identification and divided into 10 groups each group containing six animals. Oedema was induced in the left hind paw of all rats by subcutaneous injection of 0.1 ml of 1% (w/v) carrageenan in normal saline (0.9%) into their footpads. The 1st group was kept as control and was given the respective volume of the solvent. The 2nd to 9th groups were given an aqueous suspension of the synthesized compounds in a dose of 100 mg/kg body mass. The 10th group was administered ibuprofen in a dose of 100 mg/kg (standard). All the test compounds and the standard drug were administered orally, 1 h before the carrageenean injection. The paw volume of each rat was measured using a digital plethysmometer (UGO Basil, Italy), just before the carrageenean injection (0 h) and then hourly for 5 h post administration of the carrageenan injection. The percentage inhibition of paw volume for each test group was calculated using the following equation:

$$\text{Percentage of inhibition } (\%) = 100(1 - (a - x/b - y))$$

where a = mean paw volume of treated animals after carrageenan injection, x = mean paw volume of treated animals before carrageenan injection, b = mean paw volume of control animals after carrageenan injection, and y = mean paw volume of control animals before carrageenan injection

In vitro antiproliferation assay

In vitro antiproliferative activity was performed by MTT assay method (Mosmann, 1983). Exponentially growing cancerous cells were harvested from 75 cm^2 tissue culture flask and a stock cell suspension (1×10^5cell/ml) was prepared. A 96-well flat bottom tissue culture plate was seeded with 2×10^3 cells in 0.1 ml of MEM (Eagle's minimum essential medium) medium supplemented with 10% FBS (foetal bovine serum) and allowed to attach for 24 h. After 24 h of incubation, the cells were treated with test compounds. Dimethylsulfoxide (0.2% DMSO) was used as a solvent to prepare the test solutions. The cells in the control wells were treated only with the medium containing 0.2% DMSO. Each treatment was performed in triplicate. After 24 h of incubation, drug containing media was removed and washed with 100 µl of phosphate buffered saline. To each well of the plate, 100 µl of MTT reagent was added and incubated for 4 h at 37°C. After the 4th h of incubation, the plate was inverted on tissue paper to remove the MTT reagent. To solubilize the formazan crystals, 100 µl of DMSO was added to each well. The absorbance was measured at 540 nm. The IC_{50} values were calculated.

Antimicrobial activity

Microbial strains

Transfer culture of bacterial strains: *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922); *and fungal strains Candida albicans* (ATCC 24433) and *Aspergillus flavus* (ATCC 15517) from the stock collection were carried out on to Muller Hinton Agar (MHA) and Sabourauds Dextrose Agar (SDA) plates, respectively. The cultures were passaged aseptically thrice to ensure purity.

Microdilution assay

The broth micro dilution test was performed using sterile, disposable, multiwell microdilution plates (96 U-shaped wells) in 12 × 8 format (Venugopal *et al*., 2007). Column 1 was the media control, containing 200 µl of the medium to determine the sterility of the medium. Column 11 was the test compound growth control with 100 µl of sterile medium and 100 µl of the test compound. It determines the purity of the compound tested. Column 12 was the growth control which contains 100 µl of medium and 100 µl of diluted inoculum suspension.

Initially, 100 µl of medium (MHB for bacteria and RPMI for fungi) were added to all the wells of the plate except the media control, where 200 µl was added. Next, 100 µl of each of the test compounds were added to their respective wells in column 2. Column 2 contains 100 µl of the highest concentration of the drugs and 100 µl of the medium. By performing a serial dilution from column 2 to column 10, varying concentrations of the test compounds ranging from 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and

0.39 μg/ml were achieved. Column 10 had 100 μl of the lowest concentration of the drugs and 100 μl of the medium. Finally, 100 μl of the diluted inoculum suspension was added to wells from column 10 to column 2. The micro dilution plates were incubated at 37°C and observed for interpretation of bacterial and fungal MIC values after 24 and 48 h respectively. The last well in the dilution series that does not demonstrate growth indicated by turbidity and/or a pellet of microorganisms in the bottom of the vessel corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial agent.

Time kill study

The compounds which showed good antimicrobial activity at low concentration were considered for time kill study. Test tubes with 5 ml Mueller–Hinton broth (Difco) for *S. aureus, P. aeruginosa* and *E. coli* (Difco) and RPMI broth (Difco) *C. albicans, A. flavus* were used. Test drug (V4) concentrations were chosen to comprise dilutions at the MIC range 1 mg/ml. A sensitivity threshold of 5×10^6 CFU/ml was used to determine the 99.9% killing. The inoculum for *S. aureus, P. aeruginosa* and *E. coli* and *C. albicans* was prepared by diluting a 16 h broth culture to obtain the correct inoculum 5×10^6 CFU/ml. In case of *A. flavus*, a spore suspension was adjusted to 5×10^6 CFU/ml by counting using a cell counter. After vortexing briefly, 1 ml of each diluted inoculum was delivered by pipette and plated for viability counts (0 h). Only tubes with an initial inoculum within the range 5×10^6 CFU/ml were acceptable for the study.

The viability counts of test drugs (V4) suspensions were performed at 2, 4, 6, 12, and 24 h by plating 0.1 ml aliquots of inoculum of each test microorganisms onto Trypticase soy agar (TSA) for bacteria and Sabourauds dextrose agar (SDA) for fungi. The plates were incubated for up to 48 h, and colony counts were noted for the recovery, yielding 30–300 colonies. The lower limit of sensitivity of colony counts was 250 CFU/ml.

Time-kill assay results were analysed by determining the numbers of strains which yielded $\log_{10}^{-1}$ CFU/ml in comparison with counts at 0 h, for test drugs at different time intervals (Pankuch *et al*., 1994). Test drugs were considered microbicidal at the MIC concentration that reduced the original inoculum by ≥ 3 $\log_{10}^{-1}$ CFU/ml (99.9%) and microbistatic, if inoculum was reduced by <3 $\log_{10}^{-1}$ CFU/ml.

Results and discussion

Chemistry

The synthesis of hitherto unreported title compounds were prepared as outlined in Fig. 1. Condensation of 2-amino-5-chlorobenzoic acid with benzoyl chloride gave 2-benzamido-5-chloro benzoic acid which on cyclization with different primary amines by MWI yielded the corresponding 6-chloro-2-phenyl-3-substituted quinazolin-4(*3H*)-ones (V1–V8). The physicochemical properties of the synthesized compounds are given Table 1.

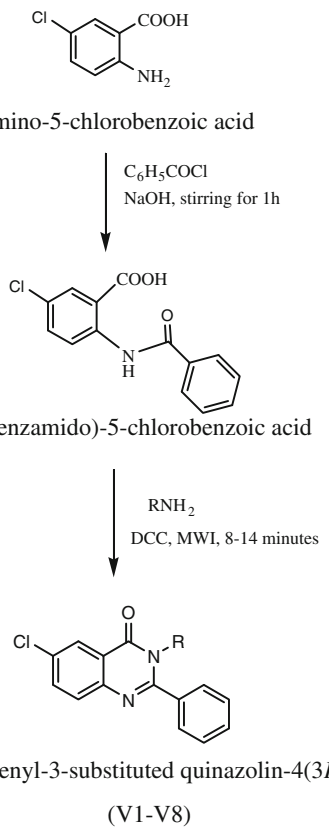

Fig. 1 Synthetic route to 6-chloro-2-phenyl-3-substituted quinazolin-4(3H)-ones

The postulated structures of the synthesized compounds were in accordance with IR, ^{1}H-NMR, mass, and elemental analyses. In the IR spectra of compounds V1–V8, the carbonyl absorption bands were observed in the region of 1,630–1,690 cm^{-1}. The C–H stretching band was seen around 2,929–3,052 cm^{-1}, whereas the –C=N stretching band was observed around 1,556–1,580 cm^{-1}. In the ^{1}H-NMR spectra of these compounds, the methylene protons appeared as singlet at region of 1.3–1.8, while the aromatic protons were seen as multiplet in the range of 7.35–8.10. Mass spectra showed the corresponding molecular ion peaks for all the compounds. Satisfactory C, H and N analyses were obtained for all the compounds.

All the synthesized compounds (V1–V8) were screened for the anti-inflammatory activity by carrageenan-induced rat paw oedema method. Carrageenan is a pro-inflammatory agent, induces the inflammation by releasing inflammatory mediators such as histamine, serotonin and cytokines leads

Table 1 Physicochemical properties of the synthesized compounds

Compound code	R	Mol. formula	Reaction time (min)	Yield (%)	Melting point (°C)	Retention factor (R_f)
V1	3-Chloro-4-fluorophenyl	$C_{20}H_{11}Cl_2FN_2O$	13	77	224	0.60
V2	4-Fluorobenzyl	$C_{21}H_{14}ClFN_2O$	10	82	198	0.81
V3	4-Chlorobenzyl	$C_{21}H_{14}Cl_2N_2O$	11	83	208	0.81
V4	4-Fluorophenyl	$C_{20}H_{12}ClFN_2O$	12	85	184	0.56
V5	3-Chlorophenyl	$C_{20}H_{12}Cl_2N_2O$	14	77	148	0.70
V6	*N*,*N*-Dimethyl ethyl	$C_{18}H_{18}ClN_3O$	11	89	202	0.80
V7	4-Methoxyphenyl	$C_{21}H_{15}ClN_2O_2$	9	84	190	0.67
V8	4-Chlorophenyl	$C_{20}H_{12}Cl_2N_2O$	8	81	220	0.82

cascade inflammation reactions in short period time and later (up to 6 h) further augmentation of inflammation reaction is mediated by prostaglandins (PGs) derived from arachidonic acid by the action of prostaglandin H synthase [also referred as cyclooxygenase (COX)]. The present study illustrated that all the compounds are having anti-inflammatory activity. The inhibition of inflammation was given in Table 2. The percentage inhibition of paw oedema and the percentage potency of the compounds were represented in Figs. 2 and 3. At 1st h, compound V3 showed very good anti-inflammatory activity. The percentage inhibition of inflammation was found to be 72.9. Other compounds did not show any inhibitory activity at 1st h. At the 2nd h, all the compounds were showing good anti-inflammatory activity. Compounds V6 and V5 showed 88.42 and 86.8% inhibitions of paw oedema, respectively. The standard drug showed 65.3% anti-inflammatory activity. At the 3rd h and the 4th h, compound V5 showed maximum inhibition of inflammation, and it was found to be 81.3 and 79.1%, respectively. At the 5th h, compound V3 showed good inhibition of inflammation when compared with all the other compounds. Compound V2 was found to be active only at the 2nd h. After the 2nd h, it was not showing any anti-inflammatory activity. These data revealed that the presence of 4-chlorophenyl, 4-fluorobenzyl and *N*,*N*-dimethyl ethyl substitution in the structure played a major role in their anti-inflammatory activity.

In vitro antiproliferative activity was done for all the compounds by MTT assay using different cell lines. The IC_{50} values were summarized in Table 3. Compounds V1, V5 and V8 showed antiproliferative activity at lower concentrations (<6.25 μg/ml against Chang liver cells and Hep2 cells and <25 μg/ml against HeLa cells). Compounds V2, V4 and V7 showed the activity at moderate concentrations (IC_{50}:10.87–50 μg/ml). The IC_{50} of the standard drug was found to be 1.72, 18.27 and 1.25 μg/ml against Chang liver cells, Hep2 cells and HeLa cells, respectively. Compound V3 had IC_{50} value ≫50 μg/ml against Chang liver cells and Hep2 cells and >200 for HeLa cells. These results showed that the compounds containing fluoro- or chloro-substituted phenyl ring at the 3rd position might be responsible for their antiproliferative potency.

Minimum inhibitory concentration of the test compounds were determined by micro dilution assay using 96 well plates and the results were given in Table 4. Compound V4 showed very good antimicrobial activity, and it

Table 2 Anti-inflammatory activity of the synthesized compounds V1–V8

Compound code	Mean paw volume ± SEM					
	0 h	1 h	2 h	3 h	4 h	5 h
Ctrl	1.1150 ± 0.0437	1.2075 ± 0.0405	1.4175 ± 0.1931	1.2250 ± 0.0484	1.2825 ± 0.0862	1.2400 ± 0.0754
V1	1.1175 ± 0.0454	1.1875 ± 0.0301	1.3525 ± 0.0366	1.1700 ± 0.0587	1.3075 ± 0.0614	1.2500 ± 0.0374
V2	0.7875 ± 0.2477	1.1525 ± 0.1026	1.2850 ± 0.0595	1.1350 ± 0.0134	1.2400 ± 0.0422	1.2875 ± 0.0539
V3	1.1125 ± 0.0823	1.1375 ± 0.0798	1.2650 ± 0.0977	1.1950 ± 0.0803	1.2325 ± 0.0807	1.1825 ± 0.0859
V4	1.1050 ± 0.0378	1.2200 ± 0.0460	1.2675 ± 0.0800	1.2100 ± 0.0816	1.2750 ± 0.0750	1.2100 ± 0.0861
V5	1.0225 ± 0.0263	1.1325 ± 0.0269	1.0625 ± 0.0269	1.0600 ± 0.0248	1.0575 ± 0.0144	1.1250 ± 0.0284
V6	1.0850 ± 0.0719	1.1825 ± 0.0698	1.1200 ± 0.0631	1.1875 ± 0.0409	1.1650 ± 0.0539	1.2150 ± 0.0441
V7	1.0225 ± 0.0287	1.1200 ± 0.0363	1.1875 ± 0.0466	1.2100 ± 0.0510	1.1400 ± 0.0363	1.1750 ± 0.0328
V8	1.0450 ± 0.0393	1.1350 ± 0.0357	1.1100 ± 0.0274	1.2300 ± 0.0722	1.1425 ± 0.0832	1.1825 ± 0.0527
Ibuprofen	1.0925 ± 0.0437	1.0350 ± 0.0456	0.9875 ± 0.0411	0.9600 ± 0.0474	0.9500 ± 0.0397	0.9600 ± 0.0543

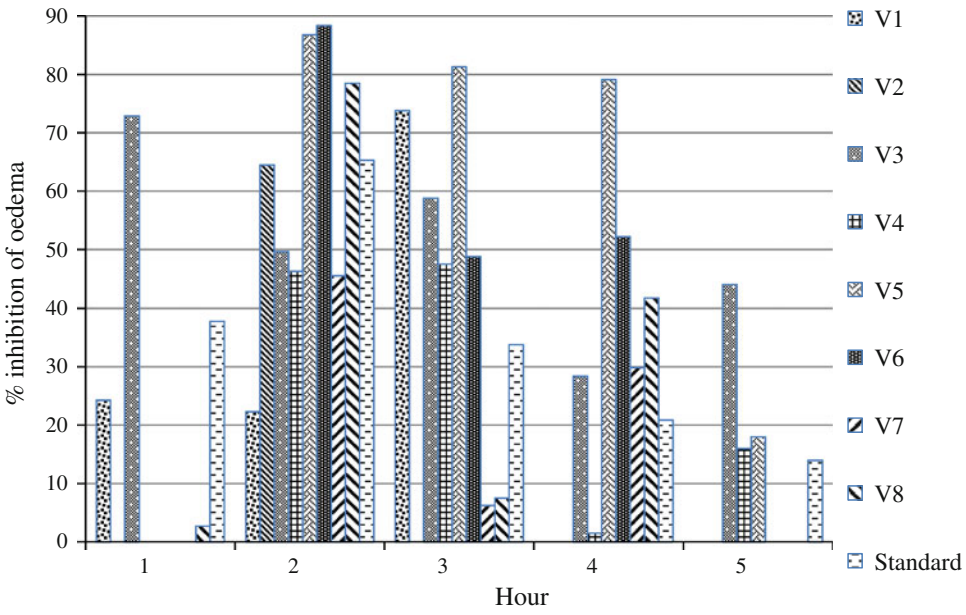

Fig. 2 Percentage inhibition of paw oedema at 100 mg/kg body weight

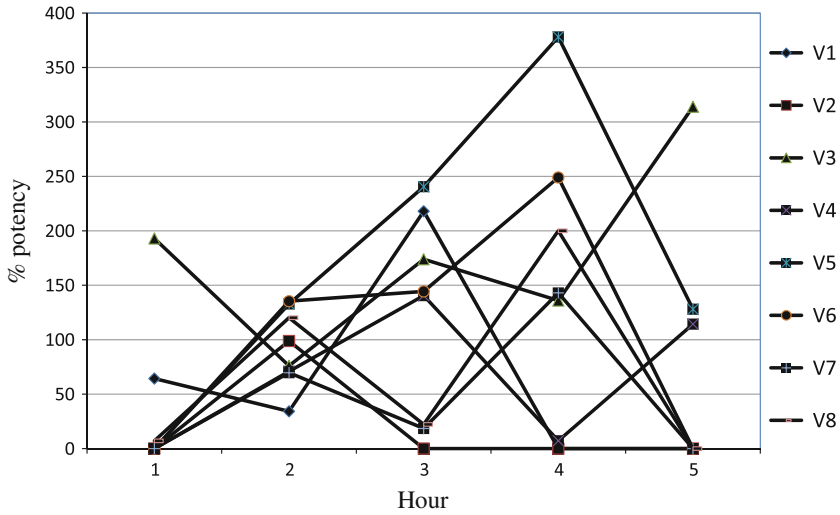

Fig. 3 Percentage potency of the test compounds

Table 3 In vitro anti-cancer activity of the test compounds by MTT assay

Compound code	50% inhibitory concentration (μg/ml)		
	Chang liver cells	HeLa cells	Hep2 cells
V1	<6.25	<25	<6.25
V2	17.95	41.48	10.87
V3	>>50	>200	>>50
V4	16.58	<25	10.77
V5	<6.25	<25	<6.25
V6	33.39	>200	20.49
V7	>25	<50	12.5
V8	6.25	<25	<25
Cisplatin	1.72	18.27	<1.25

Table 4 Minimum inhibitory concentration of the test compounds V1–V8

Compound code	*P. aeroginosa*	*S. aureus*	*E. coli*	*C. albicans*	*A. flavus*
V1	>100	>100	>100	>100	>100
V2	3.12	0.78	1.56	12.5	25
V3	3.12	0.78	1.56	12.5	25
V4	0.78	0.39	0.39	1.56	6.25
V5	25	3.12	6.25	>100	>100
V6	6.25	1.56	3.12	50	100
V7	3.12	3.12	1.56	12.5	>100
V8	12.5	3.12	12.5	>100	>100

Table 5 Time kill study of the test compound V4

Test strains	No (%) of strains with the indicated $\log_{10}^{-1}$ CFU/ml at time intervals				
	2 h	4 h	6 h	12 h	24 h
P. aeroginosa (ATCC 27853)	6 (66.7)	6 (66.7)	9 (100)	9 (100)	9 (100)
S. aureus (ATCC 25023)	3 (33.3)	6 (66.7)	9 (100)	9 (100)	9 (100)
E. coli (ATCC 25922)	3 (33.3)	6 (66.7)	9 (100)	9 (100)	9 (100)
C. albicans (ATCC 24433)	4 (44.4)	6 (66.7)	9 (100)	9 (100)	9 (100)
A. Flavus (ATCC 15517)	1 (11.1)	2 (22.2)	8 (88.9)	6 (66.7)	9 (100)

inhibited the growth of all the test organisms at very low concentration. The MICs of compound V4 against *P. aerugenosa, S. aureus, E. coli, C. albicans* and *A. flavus* was found to be 0.78, 0.39, 0.39, 1.56, and 6.5 μg/ml, respectively. Compound, V2, V3 and V7 were having almost the same MIC values. Compound V1 was not showing microbial growth inhibition at 100 μg/ml concentration. Its MIC value was found to be >100 μg/ml in all the test strains used. The most potent compound V4 was further studied for the time kill study. The result was produced in Table 5. The growth of *P. aeroginosa*, *S. aureus*, *E. coli* and *C. albicans* was completely inhibited at the 6th h. At the 24th h, it prevented the growth of *A. flavus*. Compound V4 was found to be bactericidal against the strains tested. It also worked as a fungicidal against *C. albicans*. Initially compound V4 was not effectively reducing the growth of *A. flavus*. However, after 24 h of incubation, it showed complete inhibition of the fungal growth. These reports revealed that the presence of 4-fluorophenyl substitution at the 3rd position in the quinazolin-4(3H)-one (benzopyrimidinone) nucleus showed microbicidal activity at low concentrations.

Conclusion

Some novel 2,3,6-trisubstituted Quinazolin-4(*3H*)-one derivatives have been synthesized by adapting a modified strategy which has the advantage of employing non-drastic reaction conditions to give high yields. All the compounds were screened for their biological activities. The results revealed that the groups like, 4-fluorophenyl, 4-chlorophenyl, 4-fluorobenzyl and *N*,*N*-dimethyl ethyl substitutions at the 3rd position in Quinazolin-4(*3H*)-one nucleus led to increase their biological activities.

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