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TREATMENT AND PROPHYLAXIS - ORIGINAL PAPER

Antiamoebic activity of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-one library against *Acanthamoeba castellanii*

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Abstract

Acanthamoeba castellanii is a free-living amoeba which can cause a blinding keratitis and fatal granulomatous amoebic encephalitis. The treatment of *Acanthamoeba* infections is challenging due to formation of cyst. Quinazolinones are medicinally important scaffold against parasitic diseases. A library of nineteen new 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-one derivatives was synthesized to evaluate their antiamoebic activity against *Acanthamoeba castellanii*. One-pot synthesis of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones (**1**–**19**) was achieved by reaction of 2-amino-4,5-dimethoxybenzoic acid, trimethoxymethane, and different substituted anilines. These compounds were purified and characterized by standard chromatographic and spectroscopic techniques. Antiacanthamoebic activity of these compounds was determined by amoebicidal, encystation, excystation and host cell cytopathogenicity in vitro assays at concentrations of 50 and 100 μg/mL. The IC_{50} was found to be between 100 and 50 μg/mL for all the compounds except compound **5** which did not exhibit amoebicidal effects at these concentrations. Furthermore, lactate dehydrogenase assay was also performed to evaluate the in vitro cytotoxicity of these compounds against human keratinocyte (HaCaT) cells. The results revealed that eighteen out of nineteen derivatives of quinazolinones significantly decreased the viability of *A. castellanii*. Furthermore, eighteen out of nineteen tested compounds inhibited the encystation and excystation, as well as significantly reduced the *A. castellanii*–mediated cytopathogenicity against human cells. Interestingly, while tested against human normal cell line HaCaT keratinocytes, all compounds did not exhibit any overt cytotoxicity. Furthermore, a detailed structure-activity relationship is also studied to optimize the most potent hit from these synthetic compounds. This report presents several potential lead compounds belonging to 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-one derivatives for drug discovery against infections caused by *Acanthamoeba castellanii*.

Keywords Quinazolinone · Parasite · *Acanthamoeba* · Antiamoebic

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Introduction

Infectious diseases are one of the major contributors of mortality worldwide (Lozano et al. 2012). Parasites are responsible for over one million deaths per year (Ofir-Birin and Regev-Rudzki 2019), while around 200 million are at risk of parasitic infections (Saccoliti et al. 2019). Malaria, leishmania, trypanosomiasis, and amoebiasis are among the studied parasitic diseases; however, except malaria, most of the parasitic infections are considered as neglected diseases (Terrazas et al. 2010). Free-living amoebae are opportunistic pathogens which are abundantly present in soil and water. *Acanthamoeba* species can cause keratitis and mortal CNS infection called granulomatous amoebic encephalitis (Marciano-Cabral and Cabral 2003). The ability of *Acanthamoeba* to convert in dormant cysts make them challenging target for drug development. Due to the resistant, double walled protected cysts, most of the available drugs exhibit limited efficacy against *Acanthamoeba* infections (Khan 2006). Current recommendation of Centers for Disease Control and Prevention (CDC) against *Acanthamoeba* infections includes a mixture of drugs consist of chlorhexidine, pentamidine, fluconazole, and antibiotics (Lorenzo-Morales et al. 2015). However, despite the scientific progress and vast business of pharmaceutical and eye care industries, no single drug has been yet approved against *Acanthamoeba* infections (Khan et al. 2017). Besides, the poor prognosis, most of the drugs used to manage *Acanthamoeba* infections are known to cause high host cell cytotoxicity when used in high dose for long time. Hence, there is an urgent need to revisit the drug discovery process for the development of efficient, specific, and affordable antiprotozoal drugs against *Acanthamoeba* (Siddiqui et al. 2016).

Heterocyclic rings are one of the most important pharmacophores of several drugs and, therefore, potential source of drug development against various parasitic diseases (Hadda et al. 2013; Rice et al. 2015; Alho et al. 2014). Quinazolinones are widely used medicinally important scaffolds against a variety of infectious and noninfectious diseases (Tiwary et al. 2015). The quinazoline-4(3*H*)-one and its derivatives are also found naturally as an important part of numerous alkaloids (Mhaske and Argade 2006). Quinazolinone derivatives have shown promising antiinflammatory, anticonvulsant, antibacterial, antiviral, antifungal, and antiparasitic activities against malaria and leishmania (Giri et al. 2009; Jatav et al. 2008; Anwar et al. 2018a, b; Kumar et al. 2010; Gupta et al. 2008; Patel et al. 2015; Taha et al. 2017; Saad et al. 2016). To the best of our knowledge, there is no report for their effects against free-living amoebae. The antifungal and antiparasitic mode of action of quinazolinones is previously suggested to be the inhibition of ergosterol pathway (Masood et al. 2018), which is also known to be a rationale target for drug discovery against *Acanthamoeba*. Furthermore, several quinazolinone-containing compounds have paved their way to marketed drugs for different biological activities for example antifungal (Albaconazole), acetylcholinesterase inhibitor (Isaindigotone), and peroxisome proliferator–activated receptor (PPAR) gamma agonist (Balaglitazone). (Fig. 1) (Tiwary et al. 2015). Based on our interest in drug discovery against neglected diseases caused by free-living amoebae, a series of nineteen derivatives of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-one was synthesized to evaluate their in vitro antiamoebic effects and analyze the structure-activity relationship against *Acanthamoeba castellanii*.

In this report, we designed a simple one-pot synthetic route to afford pure, cost effective, and chemically diverse 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-one derivatives. These synthetic compounds were tested for in vitro antiamoebic activity against pathogenic *Acanthamoeba castellanii* for the first time. Most of the nineteen quinazolinone derivatives (Table S1) exhibited significant antiacanthamoebic effects, while structure-activity relationship (SAR) analysis identified that these compounds as novel antiparasitic lead compounds for drug development against *Acanthamoeba castellanii*. Furthermore, these compounds showed potent antiencystation and excystation effects whereas significantly reduced the cytopathogenicity against human cells. Due to unavailability of information about any synthetic scaffold against *Acanthamoeba* infections, current medicinal chemistry approach is anticipated to be of potential applications for drug development.

Experimental

Materials

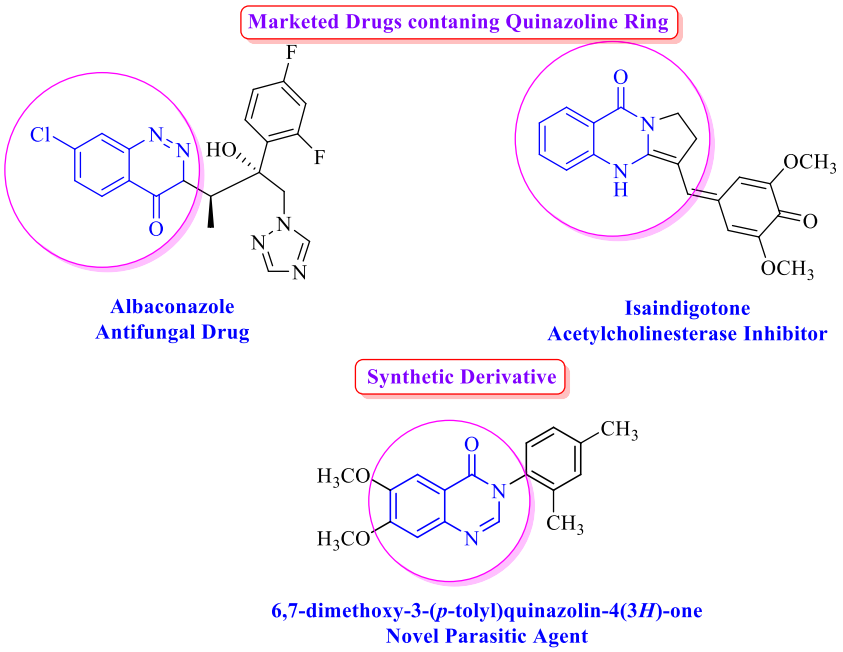
All reagents used in this study were purchased from Sigma until stated otherwise. Analytical grade solvents were used for synthesis and purification of compounds.

Methods

Synthesis of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones (1–19)

A library of nineteen analogs of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones was synthesized to study their antiacanthamoebic activities. Synthesis was accomplished by mixing 2-amino-4,5-dimethoxybenzoic acid (1 mmol) with trimethoxymethane (3 mmol) and several anilines derivatives (1 mmol) in AcOH under reflux until no starting material was found in the reaction mixture analyzed on thin layer chromatography plates (Scheme 1). The reaction mixture was precipitated upon the addition of water (100 mL), which were filtered, washed with ultrapure water, and dried under vacuum at elevated temperature (40 °C). The structures of these synthetic derivatives were

Fig. 1 Rationale of current study



elucidated by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) (Bruker 300 MHz and 400 MHz NMR spectrometers) and electron impact mass spectrometry (EI-MS) (JEOL MS Route 600 H spectrometer). Elemental analyses and high-resolution electron impact mass spectrometry (HREI-MS) data of all derivatives were found to be in agreeable range of theoretical predictions.

Acanthamoeba castellanii cultures

Acanthamoeba castellanii (ATCC 50492) belonging to T4 genotype, was routinely maintained in 75-cm^2 tissue culture flasks incubated at 30 °C with 10-mL PYG growth medium (Sissons et al. 2006). The composition of PYG growth medium is 0.75% w/v proteose peptone, 0.75% w/v yeast extract, and 1.5% w/v glucose. The flask normally reaches confluency within 48 h. Morphologically healthy trophozoites were obtained by replacing the old PYG medium with fresh phosphate buffer saline (PBS) and keeping the culture flasks on ice for 15 min followed by gentle tapping to detach the trophozoites. These trophozoites were transferred in a 50 mL conical tube and were centrifuged at 3000×*g* for 10 min. The supernatant was aspirated, and pellet was resuspended in 1 mL PBS, enumerated by using a hemocytometer.

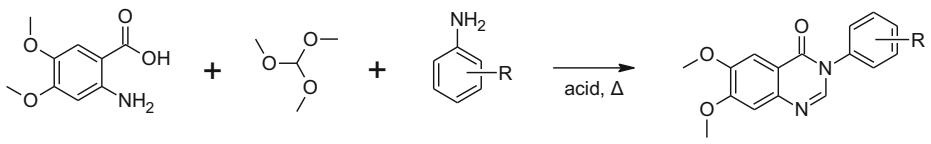
Amoebicidal assay

Acanthamoeba viability was determined by amoebicidal assay. The cidal effects of quinazolinones were determined by incubating 5 × 10^5 *Acanthamoeba castellanii* trophozoites/well at 50 and 100 μg/mL of quinazolinone derivatives and controls in 24-well plates in RPMI-1640 medium. The trophozoites were incubated at 30 °C for 24 h as described previously (Aqeel et al. 2012). Next, the viability was determined by Trypan blue exclusion assay (adding 0.1% Trypan blue aqueous solution for 5 min) which stains the dead cells. The unstained (live) cells were counted using a hemocytometer. *Acanthamoeba castellanii* treated with solvent control (1% MeOH) were considered as negative control, while standard amoebicidal drug chlorhexidine was used as a positive control.

Encystation assay

The encystation assay was performed to test the efficacy of quinazolinone derivatives for inhibiting the morphological differentiation of *Acanthamoeba castellanii* trophozoites into cysts. Briefly, 5 × 10^5 *Acanthamoeba castellanii* trophozoites were challenged with 100 μg/mL of quinazolinones in PBS in

Scheme 1 One pot synthetic route to 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-one derivatives.



3-aryl-6,7-dimethoxyquinazolin-4(3H)-ones
1-19

the presence of encystation medium (EM) consisting of 50 mM $MgCl_2$ (as triggering agent) and 10% glucose (for osmolality) in 24-well plates at 30 °C for 72 h (Abjani et al. 2016). Next, cysts formed were treated with (0.25% aqueous solution) sodium dodecyl sulfate (SDS) at room temperature for 10 min to dissolve the trophozoites, and the SDS-resistant mature cysts were numbered using a hemocytometer.

Excystation assay

Acanthamoeba castellanii cysts were prepared by inoculating 1×10^6 trophozoites on nonnutrient agar plates, then the plates were incubated at 30 °C for up to 14 days with routine observation until formation of mature double walled cysts. The nonnutrient agar plates were prepared by dissolving 1.5% bacteriological agar in ultrapure water followed by autoclaving the agar media and spreading on petri plates. Cyst formation was routinely observed under the microscope. Upon formation, double walled rounded cysts can easily be distinguished as compared to unorganized shape of trophozoites. The cysts were scraped and washed with PBS after 14 days, enumerated and stored at 4 °C. The suspension collected was centrifuged and resuspended in fresh media before usage in excystation assay. 1×10^5 preformed cysts were incubated with 100 μg/mL of quinazolinone derivatives and respective controls in growth medium PYG for 72 h to determine the excystation potential of these compounds (Dudley et al. 2009). Finally, trophozoites emerging from cysts are counted by using a hemocytometer.

HaCaT keratinocyte (human normal cells) culture

HaCaT cells (CLS Cell Line Service, 300,493) were grown in supplemented RPMI-1640 with 10% of each FBS and Nu-serum, 2 mM glutamine, 1 mM pyruvate, 100 units/mL penicillin, 100 μg/mL streptomycin, vitamins, and nonessential amino acids in 75-cm^2 tissue culture flasks in a 5% CO_2 incubator with 95% humidity at 37 °C. The cells formed a confluent, uniform monolayer within 48 h. These cells were used for all cytotoxicity and cytopathogenicity assays. Cells were detached by using 2 mL trypsin, and cell suspension was transferred to 50-mL conical tube and centrifuged at 2500×*g* for 5 min. The obtained cell pellet was resuspended in 25-mL fresh cell growth media, and 200 μL of this suspension was used to seed each well of a 96-well plate until the formation of uniform monolayer after incubation in a 5% CO_2 incubator with 95% humidity (at 37 °C for 24 h).

Cytotoxicity assay

The cytotoxicity of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones was evaluated on human normal cells (HaCaT keratinocytes) by LDH cytotoxicity assay, as reported previously (Jeyamogan et al. 2018). Briefly, 100 μg/mL of these quinazolinone derivatives and respective controls were incubated with uniform monolayer of HaCaT cells in 96-well plates for 24 h at 37 °C in a humidified CO_2 incubator. Following this incubation, one set of untreated cells were taken as negative control, while the other set was further incubated with 0.1% Triton X-100 for 20 min for maximum cell death which was used as positive control. Next, the cell-free supernatants were collected from each well, and LDH was measured by plate reader at 492-nm wavelength using LDH cytotoxicity detection kit (Invitrogen) as described previously (Anwar et al. 2019a). The percent cell cytotoxicity was calculated by using following formula:

% cell cytotoxicity = (LDH released by cells with sample treatment − LDH measured in untreated cells)/(total LDH released by Triton X-100 treated cells − LDH measured in untreated cells) × 100.

Acanthamoeba castellanii–mediated host cells cytopathogenicity assay

The cytopathogenicity assays were carried out to test the effect of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones on the host cell cytotoxicity of *Acanthamoeba castellanii* as described previously (Sissons et al. 2005). Briefly, 5×10^5 *Acanthamoeba castellanii* were incubated with 100 μg/mL of these quinazolinone derivatives and respective controls for 2 h at 30 °C. Next, these cultures were transferred in 1.5-mL microcentrifuge tubes and were centrifuged at 3000×*g* for 5 min. The pellet was resuspended in fresh RPMI-1640, and these pretreated *Acanthamoeba castellanii* samples were incubated with HaCaT cells monolayer grown in each well of 96-well plates at 37 °C in a 5% CO_2 incubator with 95% humidity for 24 h. Following the incubation time, supernatants were mixed with LDH detection kit, and cytotoxicity was determined as mentioned above.

Statistical analysis

All presented results are representatives of several experiments ($n \geq 3$) performed in duplicate and are denoted as the mean ± standard error. Microsoft Excel worksheets were prepared for data obtained from biological assays, and for statistical significance, Student's *t* test was performed comparing test sample effects with solvent control which is 1% methanol solution. The threshold level of significance was $P < 0.05$, using two-sample *t* test and two-tailed distribution. * corresponds to $P < 0.05$.

Results

The structures of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-one (**1–19**) synthetic derivatives were elucidated by ^{1}H-NMR and EI-MS. Percent yields, R_f values, ^{1}H-NMR, EI-MS, and elemental analyses of all quinazolinones derivatives are presented in Table S1, while the original spectra are given in supplementary information. The spectroscopic properties of the most active compound are presented below as a representative example.

Spectral characterization of a representative 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-one (14)

^{1}H-NMR spectroscopy

The H^{1}-NMR spectrum of the most active compound **14** was recorded in DMSO-d_6 at 300 MHz. The characteristic peaks are discussed here. The compound comprises of the total 18 protons, the C-2 proton was the most downfield proton due to presence of two nitrogen atoms and resonated as δ 8.09 (s, 1H, H-1). The other two proton H-5 and H-8 of quinazolinone ring also appeared as δ 7.49 (s, 1H, H-5), 7.20 (s, 1H, H-8), respectively. H-3′ of aryl ring appeared at δ 7.22 (s, 1H, H-3′). While, H-5′ and H-6′ resonated as δ 7.17 (dd, $J_{5,6/6,5}$ = 8.0 Hz, 2H, H-5′, H-6′) with coupling constant value of 8.0 Hz respectively, the two methoxy proton appeared as δ 3.88 (s, 3H, OCH_3), and two methyl protons were resonated as δ 2.35 (s, 3H, CH_3) in their respective region as show in Fig. S1.

Mass spectrometry

The EI-MS spectra of the active compound 14 showed molecular ion peak at *m/z* 310, in agreement with the molecular formula $C_{18}H_{18}N_2O_3$. The fragment ion at *m/z* 295 was due to the loss of one of the methyl groups from molecular ion. The key fragments are presented in Fig. S2.

Quinazolinones inhibited the viability of *Acanthamoeba castellanii*

Amoebicidal assay was performed at 50 and 100 μg/mL concentrations of each quinazolinone derivative. Result revealed that all compounds inhibited the number of viable *A. castellanii* trophozoites at 100 μg/mL except compound **5**. Whereas, at 50 μg/mL, ten out of nineteen tested compounds including **1**, **2**, **7**, **8**, **10**, **11**, **12**, **13**, **14**, and **17** showed significant amoebicidal activity (Fig. 2). The level of significance was calculated with respect to solvent control which is 1% methanol. Compounds **11**, **12**, **13**, **14,** and **17** showed most pronounced amoebicidal effects consistently at both concentrations. These results demonstrate the potential applications of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones as novel antiacanthamoebic agents.

Quinazolinones altered the phenotypic conversion of *Acanthamoeba castellanii*

The process of conversion of pathogenic and reproductive trophozoite form into dormant cyst is called encystation. Encystation assays were performed to test whether these compounds can cause inhibition of this morphological transformation. The results revealed the consistency in encystment behavior of *Acanthamoeba castellanii* when treated with quinazolinones. All compounds inhibited the encystation at 100 μg/mL except compounds **5**, while compounds **7**, **15**, **16**, **17**, **18,** and **19** were found to be most effective (Fig. 3a). Conversely, the cysts are converted back to trophozoites when the conditions are suitable, and nutrients are available. Since cysts are more resistant against treatment, the excystation is the main reason for recurrence of infection. Most of the drugs used currently against *A. castellanii* infections have shown limited efficacy against cysts. The excystation assay results revealed that quinazolinones also inhibited the reemergence of trophozoites at 100 μg/mL when treated with preformed mature cysts of *Acanthamoeba castellanii*. Notably, compound **15** showed comparable results as positive control chlorhexidine (Fig. 3b). Representative images of both assays after treatment compound 15 are presented in Figs. S3 and S4. These results show that 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones act as promising candidate for drug development against *Acanthamoeba castellanii*.

Quinazolinones showed minimal cytotoxicity and reduced the *Acanthamoeba*-mediated host cell cytopathogenicity

The cytotoxicity evaluation of quinazolinones against human normal cells (HaCaT keratinocytes) by lactate dehydrogenase (LDH) assays showed that all compounds produced less than 20% cytotoxic effects at 100 μg/mL (Fig. 4a). Compound **3** and **8** exhibited 17 and 19% cytotoxicity, respectively, while compounds **7**, **10**, **12,** and **14** showed no toxicity at 100 μg/mL. On the other hand, *Acanthamoeba castellanii* is known to induce programmed cell death in host cells via a phosphatidylinositol 3-kinase-dependent mechanism which results in its cytopathogenicity (Sissons et al. 2005). Host cell cytotoxicity was determined by LDH assay as a secondary screen to study the antiacanthamoebic effects of quinazolinones. *Acanthamoeba*-mediated cytopathogenicity assay revealed that these compounds reduced the host cells cytotoxicity of amoeba. The pretreatment of *Acanthamoeba castellanii* with quinazolinones at 100 μg/mL significantly reduced the cytopathogenicity, notably compound **7** protected the cells as much as positive control standard drug chlorhexidine (Fig.

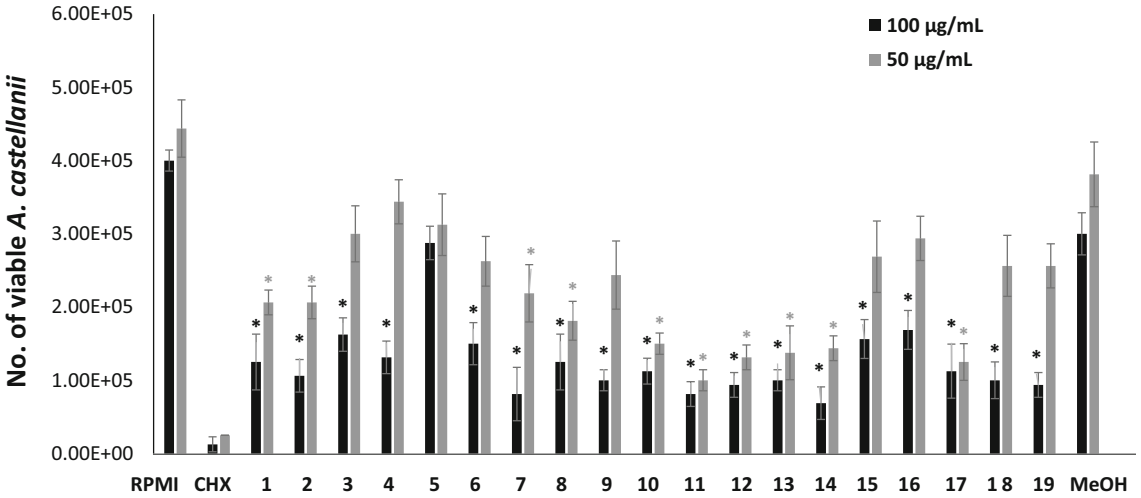


Fig. 2 Amoebicidal activity against *Acanthamoeba castellanii* was assessed by exposing amoebae with quinazolinone derivatives. The viability of amoeba was determined by Trypan blue staining. Untreated amoebae, and amoebae treated with solvent only were the negative controls while chlorhexidine was used as positive controls

4b). While the untreated *Acanthamoeba castellanii* caused 80% toxicity against HaCaT normal cell line.

Discussion

A. castellanii is considered as a challenging facultative protist which is the causative agent of blinding eye infection *Acanthamoeba* keratitis infection which is fairly common (1 in 10,000) in contact lens wearers (Moore et al. 1987). Furthermore, it causes a rare but fatal CNS infection known as granulomatous amoebic encephalitis with an associated rate of over 90% mortality (Visvesvara et al. 2007). Unfortunately, despite the high mortality rate and severity of *Acanthamoeba* infections, currently, there has been no positive effort in the drug development by pharmaceutical companies. A number of drugs, natural compounds, and nanomaterials have shown potential (Debnath et al. 2012; García-Davis et al. 2018; Anwar et al. 2018a, b), but yet, these diseases have not been included in the mainstream for drug discovery (Seal 2003), since there are only a few identified molecular targets to inhibit the key physiological functions of *A. castellanii* which include, ergosterol biosynthesis (Thomson et al. 2017), cysteine synthase (Wu et al. 2018a), and apoptosis (Wu et al. 2018b) which corresponds to the fundamental limitations in development of effective therapeutics.

In this study, we synthesized a series of functionally diverse nineteen variants of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones and determined their antiamoebic activity against *A. castellanii* for the first time. Quinazolinones are biologically active class of heterocyclic compounds and are known to exhibit antimicrobial properties against a variety of pathogens including bacteria, viruses, fungi, and parasites (Wang et al. 2013). Quinazolinone derivatives have been shown to exhibit antiamoebic effects against *Entamoeba histolytica* and *Dictyostelium discoideum* (Tariq et al. 2018; Rifkin 2002). Recently, our group has shown the antiamoebic effects of 3-aryl-8-methylquinazolin-4(3H)-ones against *Acanthamoeba*

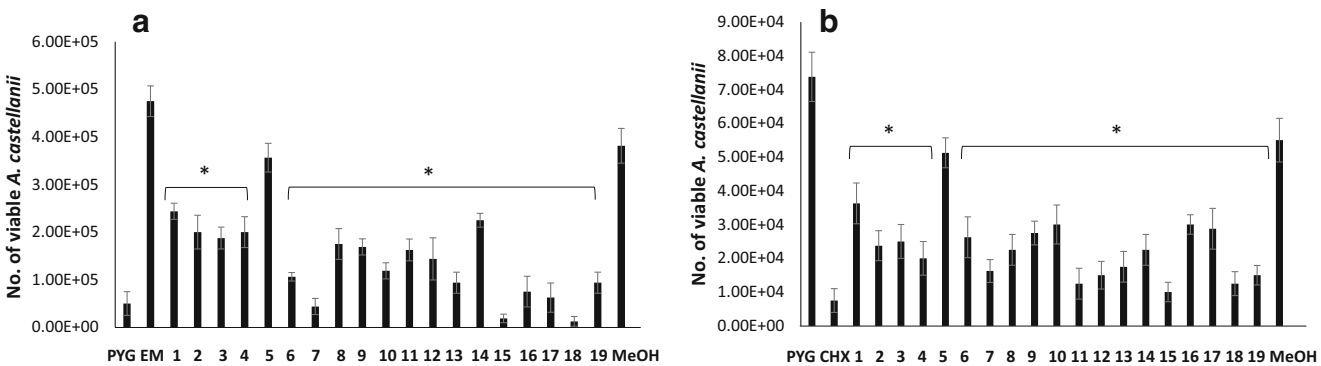


Fig. 3 **a** Encystation assay. 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones inhibited the *Acanthamoeba castellanii* encystation at 100 µg/mL. After 72 h incubation, 0.25% SDS resistant cysts were enumerated using a hemocytometer. **b** Excystation assays. After 72 h incubation, the amoebae trophozites re-emerged from cysts were counted using a hemocytometer

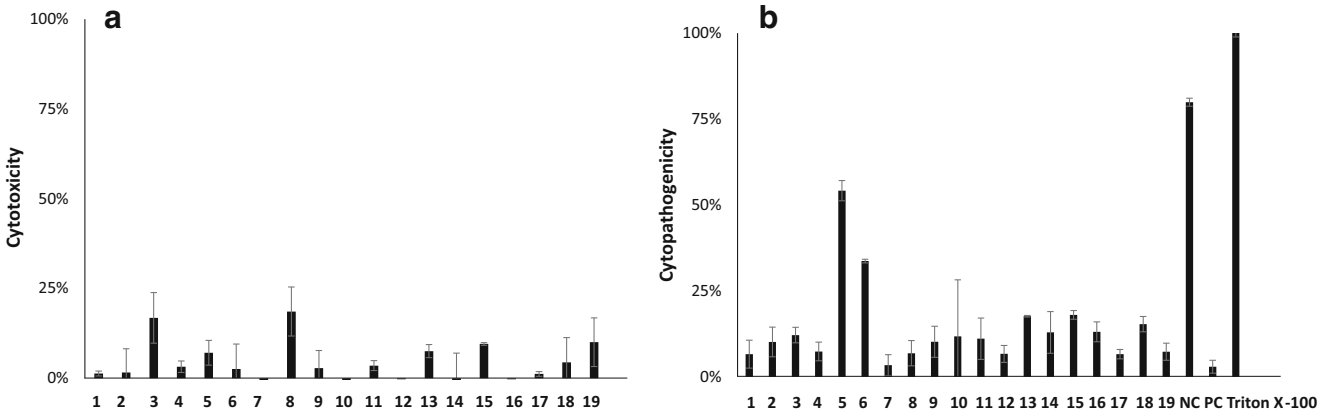


Fig. 4 **a** In vitro cytotoxicity against human normal cell line (HaCaT keratinocytes). 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones did not exhibit much cytotoxicity against HaCaT cells at 100 μg/mL. The negative control values for cytotoxicity assays were obtained by incubating cells with RPMI-1640 alone, and positive control values were obtained by 100% cell death using 0.1% Triton X-100. **b** Cytopathogenicity assay for determination of *Acanthamoeba castellanii*–mediated host cell cytotoxicity against HaCaT cells. *Acanthamoeba castellanii* caused around 80% cytotoxicity against human cells. On the other hand, the pretreatment of 100 μg/mL of these quinazolinones significantly inhibited the amoeba-mediated host cells cytotoxicity

castellanii (Anwar et al. 2019b). However, their mode of action has not been clearly understood yet. A few quinazolinones have previously shown inhibition of enzyme cholinesterase (Yan et al. 2012) which is also known to affect the invasion of Acanthamoeba on human cells. Moreover, the new triazole derivative of quinazolinone albaconazole has shown high in vitro anti-*Trypanosoma cruzi* activity by inhibiting a specific inhibitor of sterol C14α-demethylase (Urbina et al. 2000). We suggest that the mode of action may be similar against Acanthamoeba. Albeit, these compounds are new and require exploration of mechanism for each and individual derivatives which is a subject of our future studies; the above discussion provide a valid rationale for the selection of quinazolinone class of compounds. The optimized procedure for cyclization of anthranilic acid and aniline derivatives is straightforward, cost effective, and can feasibly scaled up for bulk synthesis. Therefore, it is anticipated that these compounds hold promise in antimicrobial chemotherapy against *Acanthamoeba castellanii*. The therapeutic efficacy (antiacanthamoebic vs cytotoxic activity) of 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones suggested that these compounds hold potential for in vivo studies.

Structure-activity relationship

Among the library of nineteen 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones (**1–19**), the most active compound was **14** having two methyl groups at *para* to each other, respectively. Changing the positions of methoxy on aryl ring in compound **19** resulted in slight decreased activity; however, this compound has shown significant antiamoebic effect. Compound **11** with *para* methyl substitution also showed good activity; however, less active as compared with disubstituted analogue, it showed that methyl group at *ortho* position is also contributing in the activity (Fig. 5). Compounds **7** and **12** bearing thiomethyl and chloro group also showed good antiamoebic activity. Compound **7** with thiomethyl group at *para* position was more active as compared to *ortho* substituted chloro compound **12**. All these compounds

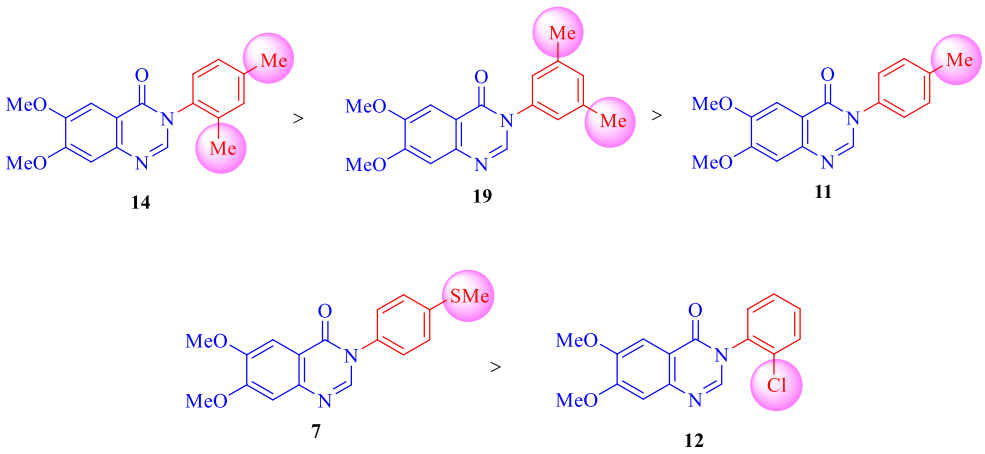


Fig. 5 Structure-activity relationship of compounds

were also found to inhibit excystation and encystation of *Acanthamoeba castellanii* (Fig. 5). While comparing these 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones with previously reported 3-aryl-8-methylquinazolin-4 (3*H*)-ones (Anwar et al. 2019b), the structure-activity relationship of orientation of different functional groups presented consistent antiamoebic effects. For example, presence of methyl groups at both *ortho* and *para* positions (this study) gave most potent effects similarly as compared to floro groups present at *ortho* and *para* positions (previous study). Moreover, electron-donating thiomethyl group at *para* position gave more antiamoebic effects (this study) similarly as compared with the electron donating methoxy group at *para* position (previous study). However, the comparative results of both studies established that electron-donating methoxy groups in ring A of the quinazolinones presented in this study produced more antiamoebic effects as compared with single methyl group on the same ring.

Conclusions

3-Aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones were synthesized by facile one-pot reaction in high yields and purity by varying the aniline part. All compounds were thoroughly characterized by ^{1}H-NMR, EI-MS, elemental analysis, and HREI-MS. These compounds were screened for antiamoebic effects against *Acanthamoeba castellanii*. All compounds showed significant amoebicidal effects especially compounds **7**, **11**, **12**, **14**, and **13**, while compounds **7** and **15** showed pronounced inhibition of encystation and excystation processes of *Acanthamoeba castellanii*. These compounds also abolished the *Acanthamoeba*-mediated host cells cytopathogenicity determined by LDH assay. Moreover, all of these compounds exhibited minimal cytotoxicity when tested against human normal cell line HaCaT keratinocytes. A brief SAR was also developed to understand the functional requirements to tune the antiacanthamoebic activity of these compounds. Hence, these new 3-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones derivatives hold potential for the development of effective antiacanthamoebic agents.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethical approval Not required.

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