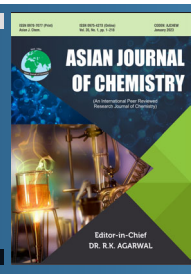
Asian Journal of Chemistry; Vol. 35, No. 1 (2023), 95-98

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.26877



Microwave Assisted Synthesis and Evaluation of Anticonvulsant Activity of Some 3-(3-(Substituted benzylidine)amino)phenyl-2-phenylquinazolin-4(3*H*)-ones

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Received: 25 October 2022; *Accepted*: 16 November 2022; *Published online*: 27 December 2022; AJC-21084

Herein, the synthesis of 3-(3-(substituted benzylidine)amino)phenyl-2-phenylquinazolin-4(3*H*)-ones from 3-aminophenyl-2-phenylquinazolin-4(3*H*)-ones is reported. The chemical structures of the synthesized compounds were confirmed by FT-IR, ^{1}H NMR and ^{13}C NMR studies. All compounds were evaluated for their anticonvulsant activity against maximal electroshock seizure method. The LD_{50} value was found to be 550 mg/Kg and the duration of tonic phase was reduced upto 1.1 s and that of stupor phase was reduced upto 70 s. The structure activity relationship of the compounds revealed that Schiff bases *viz.* 3-(3-benzylidineamino)phenyl-2-phenylquinazolin-4(3*H*)-one, 3-(3-(3-chlorobenzylidine)amino)phenyl-2-phenylquinazolin-4(3*H*)-one, 3-(3-(3-nitrobenzylidine)amino)phenyl-2-phenylquinazolin-4(3*H*)-one by significantly shorten the tonic and stupor phases of convulsions compared to controls, thus thereby these compounds demonstrated strong anticonvulsant potential.

Keywords: 4-(3*H*)-Quinazolinones, Microwave, Anticonvulsant, Maximal electroshock seizures.

INTRODUCTION

Epilepsy, a heterogeneous mix of disorders marked by neuronal hyper-excitability and hyper-synchronous neuronal firing [1]. According to the World Health Organization (WHO), about eighty out of every hundred persons living with epilepsy live in developing countries, with the mainstream of them lacking adequate medical care affects. Anticonvulsant substances are also referred to as antiepileptic drugs, which are currently widely offered on the market. About 70% of persons who have epilepsy are able to successfully control their seizures with the assistance of antiepileptic medications that are currently available on the market. However, these drugs have severe side effects such as hirsutism, ataxia, digestive problems and megaloblastic anaemia [2,3].

Quinazoline-4(3*H*)-one compounds are shown to have a variety of medicinal properties, including antioxidant, anticancer, antifungal, antibacterial, antimutagenic, anti-convulsant, anti-inflammatory, anti-HIV, CNS depressant and anti-inflammatory activities [4-6]. In recent years, chemists have paid a lot of attention to the therapeutic potential of quinazolinone derivatives in the pharmaceutical and medical fields.

A series of novel 3-{4-[2-amino-4-(substituted phenyl)-2*H*-[1,3]oxazin/thiazin-6-yl}-2- phenyl-3*H*-quinazolin-4-one derivatives were synthesized and evaluated for their anticonvulsant activity. For example, 3-{4-[2-amino-4-(4-nitrophenyl)-2*H*-[1,3]oxazin-6-yl}2-phenyl-3*H*-quinazolin- 4-one has shown significant activity against tonic seizure by the MES model and 3-{4-[2-amino-4-(4-nitrophenyl)-2*H*-[1,3]thiazin-6-yl}-2-phenyl-3*H*-quinazolin-4-one has shown significant activity against clonic seizure by scPTZ induced seizure model [7].

It was reported that *in vivo* studies were carried out the anticonvulsant effects of 2-((6,7-dimethoxy-4-oxo-2-phenylquinazolin-3(4*H*)-yl)amino)-*N*-(substituted phenyl)acetamides when utilized in chemically induced, electroshock and pharmacoresistant 6-Hz seizure models on mice, few compounds among those synthesized displayed outstanding anti-seizure effectiveness with no evidence of neurotoxicity or hepatotoxicity [8].

Despite the large variety of antiepileptic drugs presently available for therapy, approximately 25% of epileptic patients encounter major side effects and approximately 30% of patients

do not have adequate seizure control [9-11]. Herein, we have reported the synthesis of 3-(3-aminophenyl)-2-phenylquinazolin-4(3*H*)-one and related Schiff bases in a microwave oven since, it is necessary to continue to create antiepileptic medications that are efficient and have better safety profiles.

EXPERIMENTAL

All the chemicals and reagents were purchased from Sigma-Aldrich and Merck Ltd. USA. A scientific microwave oven (Catalyst system; Model: CATA 2R) with a 2450 MHz frequency and 700 W was used. Uncorrected melting points were measured using electrical melting point equipment in the open capillaries. The products were purified through recrystallization and the purity of the compounds was determined by a single spot-on TLC plate in appropriate solvent system and the spots were located by iodine. The progress of the reaction was monitored by TLC on silica gel coated glass plate. FT-IR Shimadzu Affinity-1 infrared spectrophotometer was used to record the FT-IR spectra. NMR spectra were recorded using a Bruker Avance II 400 spectrometer using TMS as internal standard.

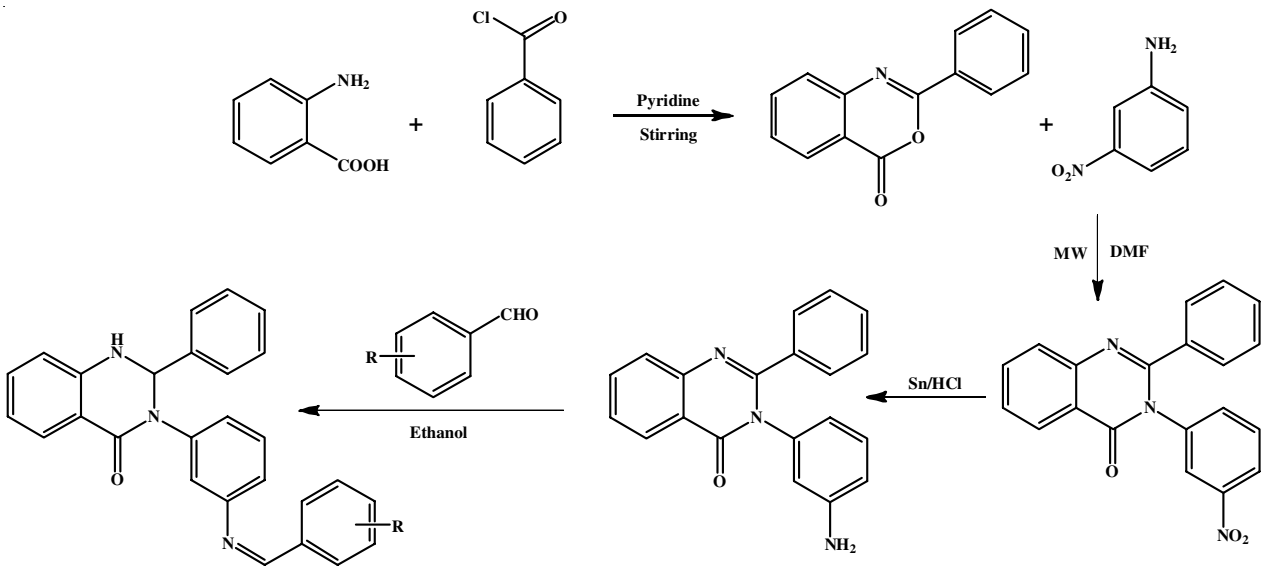
Synthesis of 2-phenyl-3,1-benzoxazin-4-one: To a solution of 2-aminobenzoic acid (0.01 mol) in pyridine (30 mL), benzoyl chloride (0.02 mol) was added and the mixture was shaken for 5 min and kept aside at room temperature for further 25 min with occasional shaking. The reaction mixture was treated with 5% $NaHCO_3$ solution (15 mL), filtered, washed with water, dried and the crude product was recrystallized from absolute ethanol (**Scheme-I**). Greyish solid in 86% yield, 3.78 g, m.p.: 180-184 ºC, R_f value: 0.8 (chloroform:ethyl acetate (50:50), IR (KBr, ν_{max}, cm^{-1}): 1078 (C-O), 1670 (C=N), 1581 (C=C), 1699 (C=O). ^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 7.43-7.75 (6H, m, aromatic), 8.16 (1H, ddd, *J* = 7.9, 1.4, 0.5 Hz), 8.48 (2H, dtd, *J* = 8.0, 1.6, 0.4 Hz).

Synthesis of 3-(3-aminophenyl)-2-phenylquinazolin-4(3*H*)-one: An equimolar mixture of 2-phenyl-3,1-benzoxazin-4-one and 3-nitroaniline in sufficient DMF was irradiated under microwave at power 280 W for 20 min. The mixture was kept in ice-bath and solid thus obtained was recrystallized from absolute ethanol followed by the reduction of product using tin and conc. HCl (**Scheme-I**). Yellow-white solid in 72% yield, 3.8 g, m.p.: 290 ºC decom., R_f value: 0.7 (chloroform:ethyl acetate (50:50), IR (KBr, ν_{max}, cm^{-1}): 1238 (C-N), 1546 (C=N), 1583 (C=C), 1691 (C=O), 3495, 3381 (-NH_2); ^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 6.87 (1H, ddd, *J* = 8.2, 1.5, 1.4 Hz), 7.22 (1H, td, *J* = 8.2, 0.5 Hz), 7.30-7.72 (7H, m, aromatic), 7.78 (1H, ddd, *J* = 1.5, 1.4, 0.5 Hz), 8.14 (ddd, *J* = 7.9, 1.4, 0.5 Hz), 8.22 (dddd, *J* = 8.2, 1.8, 1.5, 0.4 Hz).

Synthesis of 3-(3-(substituted benzylidine)amino)-phenyl-2-phenylquinazolin-4(3*H*)-one: An equimolar mixture of 3-(3-aminophenyl)-2-phenylqinazolin-4(3*H*)-one and substituted aromatic aldehydes was irradiated under microwave in minimum quantity of ethanol at power 455 W at 70-75 ºC. The mixture was cooled to room temperature. Solid thus obtained was filtered, air dried and recrystallized from ethanol (**Scheme-I**).

3-(3-(4-Methoxybenzylidine)amino)phenyl)-2-phenylquinazolin-4(3*H*)-one (AQA-1): White crystalline solid in 75% yield, 1.17 g, m.p.: 60-64 ºC, R_f value: 0.65 (chloroform: ethyl acetate (50:50), Reaction time: 20 min, IR (KBr, ν_{max}, cm^{-1}): 1134 (C-N), 1190 (C-O), 1340 (C-H *str.*), 1645 (C=N), 1633 (N=C-H), 1710 (C=O), 1580 (C=C); ^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 3.81 (3H, s), 7.04-7.24 (3H, dt, *J* = 7.3, 1.5 Hz), 7.27-7.72 (11H, m, aromatic), 8.08-8.15 (3H, ddd, *J* = 7.9, 1.4, 0.5 Hz), 8.18 (1H, s); ^{13}C NMR (δ_c ppm, DMSO-d_6): 56.0, 114.3, 115.0, 120.4, 121.1, 124.5, 125.7, 127.1, 128.2, 130.1, 134.0, 144.0, 145.1, 146.9, 150.9, 159.8, 161.3.

3-(3-Benzylideneamino)phenyl-2-phenylquinazolin-4(3*H*)-one (AQB-2): Off white solid in 85% yield, 1.70 g, m.p.: 96-98 ºC, R_f value: 0.70 (chloroform:ethyl acetate (50:50), Reaction time: 20 min, IR (KBr, ν_{max}, cm^{-1}): 1128 (C-N), 1676 (C=N), 1637 (N=C-H), 1751 (C=O), 1544 (C=C); ^{1}H NMR



R = -H, *p*-OCH_3, *m*-Cl, *m*-NO_2, *p*-$N(CH_3)_2$, furan-2yl, benzimidazol-2yl

Scheme-I

(400 MHz, δ ppm, DMSO-d_6): 7.21 (1H, dt, *J* = 7.3, 1.5 Hz), 7.27-7.72 (12H, m, aromatic), 8.02-8.10 (5H, m), 8.19 (1H, s); ^{13}C NMR (δ_c ppm, DMSO-d_6): 115.0, 120.4, 121.1, 125.7, 127.8, 128.4, 129.6, 134.0, 144.0, 145.1, 146.9, 150.1, 161.3.

3-(3-(3-Chlorobenzylidine)amino)phenyl)-2-phenylquinazolin-4(3*H*)-one (AQC-3): White crystalline solid in 70% yield, 1.52 g, m.p.: 90-92 ºC, R_f value: 0.68 (chloroform:ethyl acetate (50:50), Reaction time: 15 min. IR (KBr, ν_{max}, cm^{-1}): 755 (C-Cl), 1128 (C-N), 1654 (C=N), 1629 (N=C-H), 1701 (C=O), 1591 (C=C); ^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 7.14-7.82 (14H, m, aromatic), 8.03-8.10 (3H, ddd, *J* = 7.9, 1.4, 0.5 Hz), 8.17 (1H, s); ^{13}C NMR (δ_c ppm, DMSO-d_6): 115.0, 120.3, 121.4, 124.4, 125.7, 127.9, 128.7, 133.7, 134.0, 145.1, 146.9, 150.9, 161.2.

3(3-(Furan-2-ylmethylene)amino)phenyl-2-phenylquinazolin-4(3*H*)-one (AQD-4): White crystalline solid in 65% yield, 1.27g, m.p.: 102-104 ºC, R_f value: 0.65 (chloroform: ethyl acetate (50:50), Reaction time: 20 min IR (KBr, ν_{max}, cm^{-1}): 1105 (C-N), 1658 (C=N), 1627 (N=C-H), 1690 (C=O), 1580 (C=C) 3090 (furan C-H); ^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 6.38 (1H, dd, *J* = 3.5, 1.8 Hz), 7.01 (1H, dd, *J* = 3.5, 0.8 Hz), 7.14 (1H, dt, *J* = 8.1, 1.4 Hz), 7.28-7.81 (10H, m, aromatic), 8.00 (1H, s), 8.08-8.29 (3H, ddd, *J* = 7.9, 1.4, 0.5 Hz); ^{13}C NMR (δ_c ppm, DMSO-d_6): 112.0, 113.7, 115.0, 120.3, 121.4, 124.5, 125.7, 127.9, 129.6, 143.2, 144.0, 145.2, 146.8, 150.9, 161.1.

3-(3-(3-Nitrobenzylidine)amino)phenyl-2-phenylquinazolin-4(3*H*)-one (AQE-5): Yellow solid in 70% yield, 1.56 g, m.p.: 82-84 ºC, R_f value: 0.55 (chloroform:ethyl acetate (50:50). Reaction time: 15 min, IR (KBr, ν_{max}, cm^{-1}): 1346 (NO_2), 1155 (C-N), 1666 (C=N), 1604 (N=C-H), 1699 (C=O), 1595 (C=C); ^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 7.21 (1H, dt, *J* = 7.3, 1.5 Hz), 7.36-7.73 (10H, m, aromatic), 7.94 (1H, ddd, *J* = 8.0, 1.9, 1.7 Hz), 8.05-8.15 (4H, ddd, *J* = 7.9, 1.4, 0.5 Hz), 8.18 (1H, s), 8.71 (1H, td, *J* = 7.5, 1.4, 1.3 Hz); ^{13}C NMR (δ_c ppm, DMSO-d_6): 114.9, 120.2, 121.5, 123.3, 124.6, 125.8, 127.9, 128.5, 129.6, 134.0, 145.3, 146.8, 151.0, 161.2.

3-(3-(4-Dimethylaminobenzylidine)amino)phenyl-2-phenylquinazolin-4(3*H*)-one (AQF-6): Yellowish white solid in 72% yield, 1.59 g, m.p.: 58-60 ºC, R_f value: 0.75 (chloroform: ethyl acetate (50:50). Reaction time: 18 min, IR (KBr, ν_{max}, cm^{-1}): 1124 (C-N), 1670 (C=N), 1649 (N=C-H), 1751 (C=O); ^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 2.94 (6H, s), 6.72-6.94 (3H, ddd, *J* = 8.5, 1.2, 0.4 Hz), 7.16 (1H, ddd, *J* = 1.6, 1.4, 0.5 Hz), 7.24-7.72 (10H, m, aromatic), 8.08-8.14 (3H, ddd, *J* = 7.9, 1.4, 0.5 Hz), 8.20 (1H, s); ^{13}C NMR (δ_c ppm, DMSO-d_6): 40.2, 112.2, 115.0, 120.2, 121, 124.6, 125.7, 127.1, 128.2, 134.0, 144.1, 146.9, 150.8, 161.1.

3-(3-(1*H*-Benzo[*d*]imidazol-2-y)methyleneamino)-phenyl-2-phenylquinazolin-4(3*H*)-one (AQG-7): Greyish solid in 85% yield, 1.59 g, m.p.: 116-120 ºC, R_f value: 0.68 (chloroform:ethyl acetate (50:50). Reaction time: 10 min, IR (KBr, ν_{max}, cm^{-1}): 1124 (C-N), 1670 (C=N), 1649 (N=C-H), 1751 (C=O); ^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 7.09-7.26 (2H, ddd, *J* = 7.8, 7.1, 1.4 Hz), 7.29-7.80 (11H, m, aromatic), 8.01 (1H, ddd, *J* = 7.8, 1.5, 0.4 Hz), 8.08-8.13 (4H, ddd, *J* = 7.9, 1.4, 0.5 Hz), 8.19 (1H, s); ^{13}C NMR (δ_c ppm, DMSO-d_6): 114.2, 115.0, 118.4, 120.3, 121.2, 124.5, 125.7, 127.8, 128.3, 129.6, 137.9, 144.1, 146.9, 151.0, 161.3.

Animals: Approved procedures and protocols used in the animal experiments under Institutional Animal Ethical Committee (IAEC) Protocol approval No. MET/IOP/M. PHARM/ 2013-14/IAEC/2. To determine the median lethal dose (LD_{50}) for acute toxicity tests in mice, OECD guidelines (no. 425) were adhered. Each animal was closely watched for symptoms of toxicity and mortality in the first 30 min following dosage, as well as periodically for a further 4 h and then every day after that for a period of 14 days. The number of mice that perished over a 48 h period was counted.

Anticonvulsant activity: The anticonvulsant activity of the synthesized compounds was determined by evaluation of the ability of the compounds to protect mice against electroshock induced convulsions, wherein electroshock (54 mA and 0.2 s) was applied through the corneal electrodes. If the compound reduces or abolishes the extensor phase of maximal electroshock convulsion, it is known to possess anticonvulsant potential. For each compound, a group of six male Swiss Albino mice (22-30 g) were used. Phenytoin 25 mg/Kg was considered as a reference for the anticonvulsant effect. The synthesized compounds, phenytoin were administered 30 min before application of electroshock. The hind limb tonic extensions were observed during next 30 min.

RESULTS AND DISCUSSION

Few substituted 3-(3-aminophenyl)-2-phenylquinazoline-4(3*H*)-one and their Schiff bases were synthesized using by microwave technique at 455 W (65% of total capacity of oven). The process for synthesizing novel molecule 2-phenyl-3,1-benzoxazin-4-one from 2-amino benzoic acid by condensing it with 3-nitroaniline followed by the reduction to obtain 3-(3-amino-phenyl)-2-phenylquinazolin-4(3*H*)-one. It was then converted into Schiff bases using various substituted aromatic aldehydes. The microwave technique is rapid and efficient resulting in the reduced reaction times up to 10-20 min. The synthesized compounds were obtained in moderate to good yields ranging from 64-82%. The synthesized compounds were confirmed on the basis of IR, wherein the characteristic peak for C=N (imine) was observed within 1693-1690 cm^{-1} in the ^{1}H NMR spectra, the characteristic δ for C-H benzylidine proton was observed within 8.00-8.20 ppm.

Anticonvulsant activity: The LD_{50} was calculated by using the software AOT425StatPgm as 550 mg/kg. The exact doses used to assess the activity of the synthesized compounds were dose I, which was 55 mg/kg (about 1/10th of the LD_{50}) and dose II, which was 80 mg/kg (~1.5 time of dose I). The maximal electroshock induced seizures is feasible animal model to evaluate the compound for potential as anticonvulsant activity. A 54 mA current applied to the ear pinna electrodes for 0.2 s was enough to cause the classic tonic clonic convulsions with the recognizable stupor phase and straub tail phases. The absence of straub tail stages, the shortening of the duration of these distinct phases and the recovery of the animals were investigated and compared to the control. It was found that the duration of severe tonic phase was reduced up to 1.1 to 2.4 s at

TABLE-1
ANTICONVULSANT EFFECT OF SOME 3-((SUBSTITUTED BENZYLIDINE)-AMINO)-PHENYL-2-PHENYLQUINAZOLIN-4(3*H*)-ONES IN MICE USING MES METHOD

Compound code	Dose (mg/kg)	Duration in seconds (mean) In sec			Recovery/death
		Tonic	Straub tail	Stupor	
Electroshock (control)	54 mA for 0.3 s	5.0 ± 0.000	Present	184 ± 0.836	Recovery
Phenytoin	25	2.6 ± 0.547	Absent	42 ± 2.074	Recovery
AQA-1	55	3.4 ± 0.137	Absent	135 ± 1.150	Recovery
	80	2.1 ± 0.123*	Absent	91.2 ± 0.836*	Death
AQB-2	55	1.3 ± 0.114*	Absent	105 ± 0.235*	Recovery
	80	1.1 ± 0.135**	Absent	81 ± 0.141*	Recovery
AQC-3	55	1.8 ± 0.745*	Absent	102 ± 2.074*	Recovery
	80	1.2 ± 0.4472**	Absent	70 ± 1.304**	Death
AQD-4	55	2.7 ± 0.1321	Absent	110 ± 1.210*	Recovery
	80	1.4 ± 0.2344	Absent	99.3 ± 0.516	Death
AQE-5	55	1.4 ± 0.238**	Absent	115 ± 1.2345*	Recovery
	80	1.2 ± 0.447**	Absent	80 ± 0.5624**	Recovery
AQF-6	55	1.7 ± 0.745*	Absent	113 ± 1.643*	Recovery
	80	1.3 ± 0.126**	Absent	81.2 ± 1.304**	Recovery
AQG-7	55	3.1 ± 0.247	Absent	173 ± 1.00	Recovery
	80	2.4 ± 0.355	Absent	135 ± 3.00	Recovery

N = 6, in each group; *$p < 0.05$; **$p < 0.01$; NS: Non-significant; one-way ANOVA followed by Dunnett's test. Value expressed as Mean ± SEM

dose 80 mg/ kg and up to 1.3 to 3.4 s at dose 55 mg/kg and that of stupor phase was reduced up to 70 to 135 s at dose 80 mg/kg and 102 to 173 s at dose 55 mg/kg compared to control (Table-1).

Conclusion

An effort to improve the anticonvulsant effects, few novel quinazolinones were synthesized using microwave technology, which substantially reduced the reaction times up to few minutes. The quinazolinone derivatives were synthesized with good yields and their structure were confirmed through the spectral analysis. The synthesized compounds were also evaluated for its anticonvulsant potential and it was found that quinazolin-4(3*H*)-one Schiff bases *viz*. 3-(3-benzylideneamino)phenyl-2-phenylquinazolin-4(3*H*)-one (**AQB-2**), 3-(3-(3-chlorobenzylidine)amino)phenyl-2-phenylquinazolin-4(3*H*)-one (**AQC-3**) and 3-(3-(3-nitrobenzylidine)amino)phenyl-2-phenylquinazolin-4(3*H*)-one (**AQE-5**) showed the substantial anticonvulsant potential by reducing the duration of the tonic and stupor phases of convulsions compared to control.

ACKNOWLEDGEMENTS

The authors are thankful to The Principal, Management Mumbai Education Trust's Institute of Pharmacy, Bhujbal Knowledge City, Nashik (India) for providing the necessary research facilities. Thanks are also due to The Director, SAIF, Punjab University, Chandigarh, India for the structural analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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