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ORIGINAL RESEARCH

The synthesis and evaluation of anti-acetylcholinesterase activity of some 4(3*H*)-quinazolinone derivatives bearing substituted 1,3,4- thiadiazole

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ABSTRACT

In the present study, a series of 4(3*H*)-quinazolinone derivatives **(5a-f)** were synthesized through the cylization reaction of substituted 1,3,4-thiadiazoles containing an aromatic primary amin and anthranilic acid in the presence of acetic anhydride and acetic acid. The structures of the synthesized compounds were confirmed by elemental analysis, IR, ^{1}H-NMR and mass spectroscopic (**5b** and **5f**) methods. Each derivative was evaluated for its ability to inhibit acetylcholinesterase (AChE) using a modification of Ellman's spectrophotometric method. Compounds 2-methyl-3-{4-[5-(ethylamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one (**5b)** and 2-methyl-3-{4-[5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one (**5d)** can be identified as promising anticholinesterase agents due to their inhibitory effect when compared with donepezil as a reference drug.

Keywords: 4(*3H*)-Quinazolinone, 1,3,4-thiadiazole, anti-acetylcholinesterase activity.

Introduction

According to the 2015 World Alzheimer Report, 46.8 million people worldwide are living with dementia in 2015. This number will almost double every 20 years. Much of the increase will take place in low and middle income countries (LMICs): in 2015, 58% of all people with dementia live in LMICs, rising to 63% in 2030 and 68% in 2050. The Report updates data on the prevalence, incidence, cost and trends of dementia worldwide. It also estimates how these numbers will increase in the future, leaving us with no doubt that dementia, including Alzheimer's disease and other causes, is one of the biggest global public health and social care challenges facing people today and in the future (1).

Cholinesterase (ChE) inhibitors constitute the only therapeutic class of agents approved for the symptomatic treatment of Alzheimer's disease (AD). These drugs enhance cholinergic function by inhibiting enzymes that degrade acetylcholine (ACh), thereby increasing the availability of ACh to stimulate nicotinic and muscarinic receptors within the brain. Four ChE inhibitors are currently licensed for the symptomatic treatment of AD: tacrine, donepezil, rivastigmine and galantamine. Although all of these agents increase levels of ACh in the brain, they differ substantially in pharmacological and pharmacokinetic profiles; for instance: enzymes inhibited, potency, brain selectivity, chemical class,

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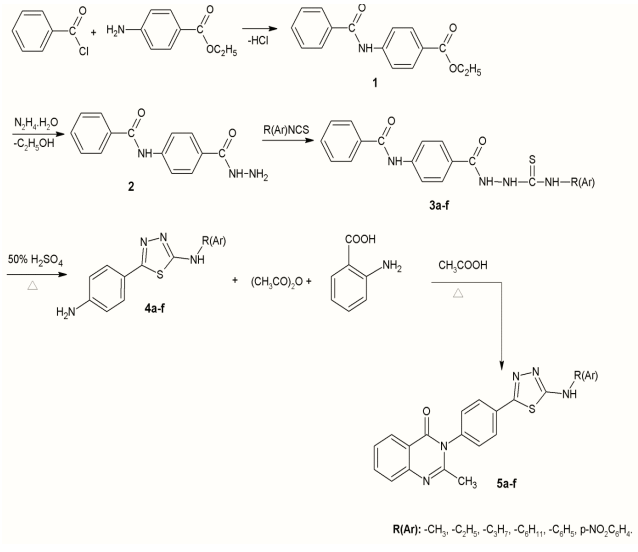
mechanism of action, metabolism and dose-dependent effects. Theoretically, such differences should differentiate the ChE inhibitors with respect to clinical efficacy and safety (2).

Scientists have widely studied on 1,3,4-thiadiazole derivatives as potential drugs to treat AD (3, 4). Additionally, 4(3*H*)-quinazoline compounds, which have diverse pharmacological activities (5-9) as well as important pharmacophore groups, exhibit cholinesterase inhibitory activity (10-12). Eventually, in this present study, the synthesis of compounds bearing both 4(3*H*)-quinazolinone and 1,3,4-thiadiazole moieties was achieved and the synthesized compounds wer evaluated for their ability to inhibit acetylcholinesterase (AChE) using a modification of Ellman's spectrophotometric method.

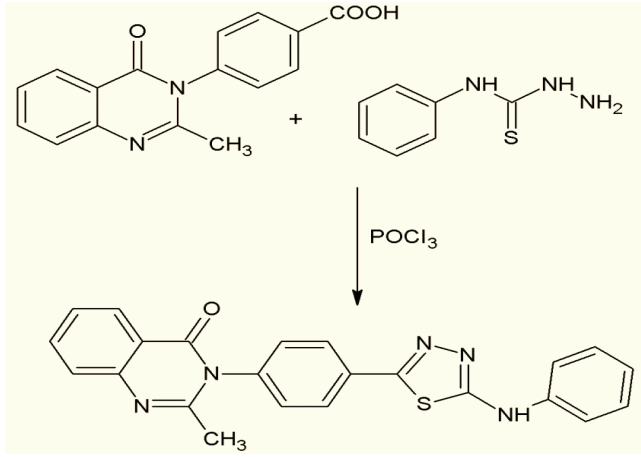
Chemistry

Various synthesis methods are reported about the cyclization of quinazolinone derivatives (13-16). ln this research, 2-methyl-3-substituted-4(3*H*)-quinazolinones were synthesized by cyclization of anthranilic acid and arylamines with acetic anhydride. The synthetic route of substituted 4(3*H*)-quinazolinones is represented in **Scheme 1**. The arylamines **4a-f** were prepared from ethyl 4-aminobenzoate by us (17). In a second step, the mixture of anthranilic acid and acetic anhydride were refluxed for 4 hours to form the intermediate 2-methylbenzoxazinone. The reaction medium was evaporated and then refluxed for 4 hours with **4a-f** in glacial acetic acid to obtain **5a-f** (18). We gained the 4(3*H*)-quinazolinones (**5a-f**) in a single-step and satisfied yield (66 -75 %).



Scheme 1. Synthesis of 2-methyl-3-{4-[5-(substitutedamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one (**5a-f**)

The compound **5e** was synthesized by Khalil and Habib with a different method (19). The synthetic route of 2-methyl-3-{4-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one is represented in **Scheme 2**.



Scheme 2. Synthesis of 2-methyl-3-{4-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one

Pharmacology

AChE Inhibition

All compounds were subjected to a slightly modified method of Ellman's test (23) in order to evaluate their potency to inhibit the AChE. The spectrophotometric method is based on the reaction of released thiocholine to give a coloured product with a chromogenic reagent 5,5-dithio-bis(2-nitrobenzoic)acid (DTNB). AChE, (E.C.3.1.1.7 from Electric Eel, 500 units), and donepezil hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatine, acetylthiocholine iodide (ATC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a 1700 Shimadzu UV-1700 UV–Vis spectrophotometer. Cholinesterase activity of the compounds was measured in 100 mM phosphate buffer (pH 8.0) at 25 °C, using ATC as substrates, respectively. DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control (**Table 1**) (24).

Enzymatic assay

Enzyme solutions were prepared in gelatin solution (1 %), at a concentration of 2.5 units/mL. AChE and compound solution

(50 µL) which is prepared in 2 % DMSO at a concentration range of 10^{-1}-10^{-6} mM were added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25 °C for 5 min. The reaction was started by adding DTNB (50 µL) and ATC (10 µL) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 mL buffer, 50 µL 2 % DMSO, 50 µL DTNB and 10 µL substrate. All processes were assayed in triplicate. The inhibition rate (%) was calculated by the following equation:

Inhibition % = $(A_C - A_I) / A_C \times 100$

Where A_I is the absorbance in the presence of the inhibitor, A_C is the absorbance of the control and AB is the absorbance of blank reading. Both of the values are corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Data were expressed as Mean ± SD.

Table 1. % AChE inhibition of the compounds and IC_{50} values

Comp.	AChE Inhibition (%)		
	1 mM	**0.1 mM**	**IC_{50} (mM)**
5a	11.62 ± 1.78	2.29 ± 2.32	> 1
5b	27.10 ± 2.51	14.21 ± 1.63	> 1
5c	8.22 ± 3.02	0.11 ± 2.01	> 1
5d	25.84 ± 0.59	11.43 ± 2.13	> 1
5e	7.7 ± 3.09	2.1 ± 2.33	> 1
5f	8.04 ± 2,97	2.01 ± 1.55	> 1
Donepezil	99.01 ± 4.89	95.52 ± 5.01	0.054 ± 0.002 (µM)

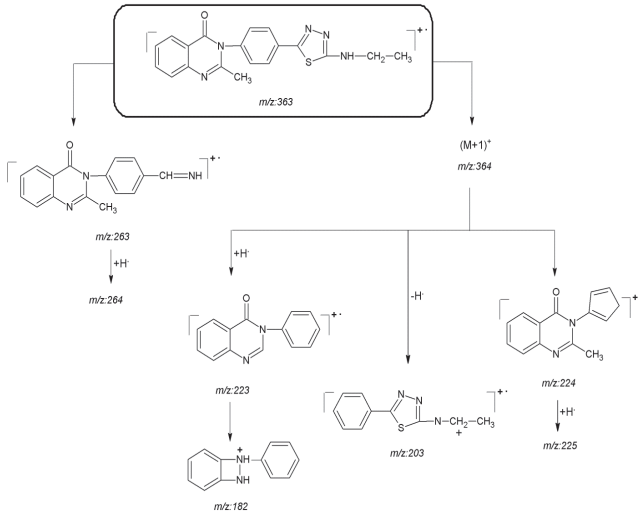
Results and Discussion

A series of 4(3*H*)-quinazolinone derivatives **(5a-f)** were synthesized through the cylization reaction of substituted 1,3,4-thiadiazoles containing an aromatic primary amin and anthranilic acid in the presence of acetic anhydride and acetic acid. The structures of the synthesized compounds were confirmed by elemental analysis IR, ^{1}H-NMR and mass spectroscopic (**5b** and **5f**) methods.

The IR spectra of substituted 4(3*H*)-quinazolinones were evaluated and the common bands of the main structure were observed in expected areas, correlating with the literature. The IR spectra of 4(3*H*)-quinazoline derivatives **5a-f** was shown strectching bands at 3343-3167 cm^{-1} and 3171-3003 cm^{-1} which have assigned to secondary amine N-H and aromatic =C-H, respectively. The asymmetric and symmetric strectching bands of C-H were observed at 2999-2849 cm^{-1}. In addition, the absorption bands at 1682-1668 cm^{-1} (quinazoline ring C=O), 1444-1609 cm^{-1} (aromatic C=C strectching band and N-H bending band), 816-835 cm^{-1} (characterized phenyl ring substitution bands) and 698-683 cm^{-1} (thiadiazole ring C-S-C stretching bands) were detected (20-22).

The ^{1}H-NMR spectra of the 2-methyl-3-[4-(5-substitutedamino-1,3,4-thiadiazole-2-yl)phenyl]-4(3*H*)-quinazolinones (**5a-f**) in DMSO-d_6 were evaluated and the chemical shifts of aliphatic and aromatic protons and N-H protone of seconder amine were observed correlating with the published data. In the ^{1}H-NMR spectra of these compounds, the signals representating the quinazoline ring methyl proton at second site appeared at 2.08-2.18 ppm as a singlet whereas methyl protons of **5c** compound appeared at 2.13-2.17 ppm as a double singlet. Also, secondary amine N-H resonances of these compounds and aromatic protons were observed in expected region (19).

EI-MS spectra of compounds **5b** and **5f** showed molecular ion (M^+) peaks which confirmed their molecular weights. The proposed mass fragmentation pathway of compound **5b** was given in **Scheme 3**.



Scheme 3. Proposed mass fragmentation pathway of compound **5b**

The anticholinesterase effects of synthesized compounds (**5a-f**) were determined by modified Ellman's spectrophotometric method (**Table 1**). Among these compounds **5b** with ethylamino substitution and **5d** with cyclohexylamino were found as the most active compounds. The inhibition percentages were calculated 27.10 and 14.21 % at 1 and 0.1 mM concentrations for compound **5b** and the inhibition percentages were calculated 25.84 and 11.43 % at 1 and 0.1 mM concentrations for compound **5d**. The IC_{50} values could not be defined none of all compounds. Compound **5a** showed moderate activity with the inhibition percentages 11.62 and 2.29 % at 1 and 0.1 mM concentrations. The other compounds **5c, 5e** and **5f** showed relatively weak activity and the inhibiton values were found less than 8.22 %. Standard drug Donepezil was studied at lower concentrations for the purpose of finding IC_{50} value and it was determined as 0.054 µM. None of the compounds showed comparable activity with donepezil and significant anticholinesterase activity contrary to expectations.

Experimental

All chemicals are commercially available and used as received. Melting points were determined on Schmelzpunktbestimmer SMP II and are uncorrected. Elemental analysis apparatus was Kleinfeld SMP-II. The IR spectra were recorded on Schimadzu FTIR 8400 S spectrometer. ^{1}H-NMR spectra were recorded on Bruker AVANCE-DPX 400 using TMS as an internal standard. The mass spectra were measured on Agilant 1100 LC-MS.

Synthesis of 2-methyl-3-{4-[5-(substitutedamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-ones [5a-f]

Anthranilic acid (0.002 mol, 0.274 g) and 0.6 mL acetic anhydride were refluxed under anhydrous condition for 4 h. Excess of acetic anhydride was distilled off under reduced pressure. To the mixture obtained, 4a-f (0.01 mol) in glacial acetic acid was added and refluxed for 4 h, and the obtained reaction mixture was poured into crushed ice and kept overnight. The solid was filtered, washed with water, dried and recrystallized from ethanol (18).

2-Methyl-3-{4-[5-(substitutedamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one [5a-f]

2-Methyl-3-{4-[5-(methylamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one [5a]

M.p. 274-275 °C; yield 67 %; brown crystals; IR (υ_{max}, cm^{-1}): 3343 (N-H); 2920 (C-H); 1674 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.17 (s, 3H, -CH_3), 2.95 (d, 3H, *J*: 4.80 Hz, -NH-C<u>H</u>$_3$), 7.53 (t, 1H, quinazoline C_6-H), 7.57 (d, 2H, *J*: 8.00 Hz protons in meta position of thiadiazole), 7.68 (d, 1H, *J*: 8.40 Hz, quinazoline C_8-H), 7.86 (t, 1H, quinazoline C_7-H), 7.94 (d, 2H, *J*: 8.52 Hz, protons in ortho position of thiadiazole), 7.98 (d, 1H, *J*: 4.80 Hz, -NH), 8.11 (dd, 1H, *J*: 7.80, *J*: 1.00 Hz, quinazoline C_5-H); Anal. calc. for $C_{18}H_{15}N_5OS$ (349.41): C, 61.87; H, 4.33; N, 20.04; S, 9.18 %. Found: C, 61.49; H, 4.05; N, 19.94; S, 8.79 %.

2-Methyl-3-{4-[5-(ethylamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one [5b]

M.p. 255-256 °C; yield 69 %; white powder; IR (υ_{max}, cm^{-1}): 3308 (N-H.), 2982 (C-H), 1672 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.22 (t, 3H, –CH_2-C<u>H</u>$_3$), 2.18 (s, 3H, -CH_3), 3.32-3.38 (-NH–C<u>H</u>$_2$ was over shadow by DMSO peak), 7.51-7.58 (m, 3H, quinazoline C_6-H and protons in meta position of thiadiazole), 7.68 (d, 1H, *J*: 7.79 Hz, quinazoline C_8-H), 7.85 (t, 1H, quinazoline C_7-H), 7.94 (d, 2H, *J*: 8.52 Hz, protons in ortho position of thiadiazole), 8.03 (1H, t, -NH), 8.11 (dd, 1H, *J*: 7.98 Hz, *J*: 1.25 Hz, quinazoline C_5-H). Anal. calc. for $C_{19}H_{17}N_5OS$ (363.44): C, 62.79; H, 4.71; N, 19.27; S, 8.82 %. Found: C, 62.36; H, 4.65; N, 19.11; S, 8.64 %. (+) ES-MS (70 eV): *m/z (%)*: 364 (81) $(M+1)^+$.

2-Methyl-3-{4-[5-(propylamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one [5c]

M.p. 240-242 °C; yield 72 %; cream crystals; IR (υ_{max}, cm^{-1}): 3167 (N-H), 2965, 2934 (C-H), 1682 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 0.95 (t, 3H, -CH_2-CH_2-C<u>H</u>$_3$), 1.60-1.68 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_3), 2.13, 2.17 (2s, 3H, -CH_3), 3.21-3.42 (-C<u>H</u>$_2$-CH_2-CH_3 was over shadow by DMSO peak), 7.14-11.09 (m, 9H, Ar-H ve -NH). Anal. calc. for $C_{20}H_{19}N_5OS$ (377.46): C, 63.64; H, 5.07; N, 18.55; S, 8.49 %. Found: C, 62.21; H, 5.97; N, 18.71; S, 8.15 %.

2-Methyl-3-{4-[5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl]phenyl}quinazolin-4(3*H*)-one [5d]

M.p. 244-245 °C; yield 66 %; cream powder; IR (υ_{max}, cm^{-1}): 3306 (N-H), 2915, 2849 (C-H), 1668 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.00-2.20 (m, 10H, cyclohexyl -CH_2-), 2.18 (s, 3H, -CH_3), 3.52-3.70 (bs, 1H, cyclohexyl -CH-), 7.51-7.57 (m, 3H, quinazoline C_6-H and protons in meta position of thiadiazole), 7.68 (d, 1H, *J*: 8.33 Hz, quinazoline C_8-H), 7.86 (t, 1H, quinazoline C_7-H), 7.93 (d, 2H, *J*: 7.92 Hz, protons in ortho position of thiadiazole), 8.00 (d, 1H, *J*: 7.34 Hz, -NH), 8.12 (dd, 1H, *J*: 7.98 Hz, *J*: 1.23 Hz, quinazoline C_5-H). Anal. calc. for $C_{23}H_{23}N_5OS$ (417.53); C, 66.16; H, 5.55; N, 16.77; S, 7.68 %. Found: C, 65.83; H, 5.39; N: 16.25; S, 7.38 % .

2-Methyl-3-{4-[5-(phenylamino)-1,3,4-thiadiazol-2-yl] phenyl}quinazolin-4(3*H*)-one [5e] (19)

M.p. 272-275 °C; yield 70 %; white powder; IR (υ_{max}, cm^{-1}): 3279 (N-H), 2999, 2969 (C-H), 1672 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.08 (s, 3H, -CH_3), 7.34-7.88 (m, 13H, Ar-H), 10.21 (s, 1H, -NH). Anal. calc. for $C_{23}H_{17}N_5OS$ (411.48): C, 67.13; H, 4.16; N, 17.02; S, 7.79 %. Found: C, 66.80; H, 4.06; N, 17.43; S, 8.15 %.

2-Methyl-3-(4-{5-[(4-nitrophenyl)amino)-1,3,4-thiadiazol-2-yl}phenyl)quinazolin-4(3*H*)- one [5f]

M.p.〉 350 °C; yield 75 %, green powder; IR (υ_{max}, cm^{-1}): 3273 (N-H), 2932 (C-H), 1670 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.16 (s, 3H, -CH_3), 7.51-8.28 (m, 12H, Ar-H), 11.35 (s, 1H, -NH). Anal. calc. for $C_{23}H_{16}N_6O_3S$ (456.47): C, 60.52; H, 3.53; N, 18.41; S, 7.02 %. Found: C, 59.18; H, 3.76; N, 17.89; S, 7.13 %. (+) ES-MS (70 eV): *m/z (%)* = 457 (100) $(M+1)^+$.

Conclusion

We have successfully developed a single-step and efficient synthesis of new 4(*3H*)-quinazolinone derivatives using anthranilic acid and substituted 1,3,4-thiadiazoles bearing an aromatic primary amin under acidic conditions. The structures of the synthesized compounds were confirmed by elemental analysis, IR, ^{1}H-NMR and mass spectroscopic (**5b** and **5f**) methods. Compounds **5b** and **5d** can be identified as promising anticholinesterase agents due to their inhibitory effect when compared with donepezil as a reference drug.

1,3,4-Tiyadiazol içeren bazı 4(3*H*)-kinazolinon türevlerinin sentezi ve anti-asetilkolinesteraz aktiviteleri

ÖZ

Bu çalışmada, aromatik pirimer amin içeren sübstitüe 1,3,4-tiyadiazoller ile antranilik asitin asetik asit ve asetik anhidritli ortamdaki siklizasyonundan bir seri 4(3*H*)-kinazolinon türevi **(5a-f)** sentez edildi. Bileşiklerin yapıları IR, ^{1}H-NMR, kütle spektroskopisi (**5b** ve **5f**) ve elementel analiz yöntemleri kullanılarak aydınlatıldı. Bileşiklerin aktivite tayini Ellman'ın modifiye edilmiş spektrofotometrik yöntemi kullanılarak yapıldı. Donepezil standardı ile karşılaştırıldığında bileşik **5b** (2-metil-3-{4-[5-(etilamino)-1,3,4-tiyadiazol-2-il] fenil}kinazolin-4(3*H*)-on) ve bileşik **5d** (2-metil-3-{4-[5-(siklohegzilamino)-1,3,4-tiyadiazol-2-il]fenil}kinazolin-4(3*H*)-on) inhibitör etkilerine göre antikolinesteraz ajanları olarak tanımlanabilirler.

Anahtar kelimeler: 4(*3H*)-Kinazolinon, 1,3,4-tiyadiazol, anti-asetilkolinesteraz aktivite

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