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SHORT COMMUNICATION

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[3+2] cycloaddition synthesis of new (nicotinonitrile-chromene)-based bis(pyrazole) hybrids as potential acetylcholinesterase inhibitors

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Abstract

A [3+2] cycloaddition protocol was used to prepare two series of (nicotinonitrile-chromene)-based bis(pyrazoles) in 82%–91% yields. The protocol involved the reaction of nitrilimines, which were generated in situ by the action of triethylamine on hydrazonoyl chlorides, with the respective (nicotinonitrile-chromene)-based bis(enaminone). At the concentrations tested, bis(pyrazole) linked to 1-(4-methoxyphenyl) and 3-acetyl units had the best acetylcholinesterase and DPPH-free radical inhibitory activity.

1 | INTRODUCTION

Alzheimer's disease is one of the most well-known dementia-related diseases [1]. The first hypothesis proposed for the cause of Alzheimer's disease was that the acetylcholinesterase reduces acetylcholine levels, resulting in a decrease in nerve impulse transmission [2]. The use of acetylcholinesterase inhibitors as a symptomatic treatment for Alzheimer's disease is a viable strategy [3]. The acetylcholinesterase inhibitor donepezil, which has been approved by the FDA, is made up of two main components: indanone and piperidine [4]. Several studies looked into the inhibitory activity of donepezil analogues, which are made by substituting a chromene [5] or pyrazole [6] unit for the pharmacophore indanone. In addition, because of its potent neuroprotective activity [7], pyridine is used as an interesting bio-isosteric unit of piperidine [8].

[3+2] Cycloaddition reactions, in which a multiple bond system is added to a three-atom component, are a useful synthetic procedure for pyrazoles [9]. As a typical example, the reaction of the respective enaminones with nitrilimines [10]. The reaction yielded regioisomeric 4-aroyl-linked pyrazoles instead of the 5-aroyl derivative [11].

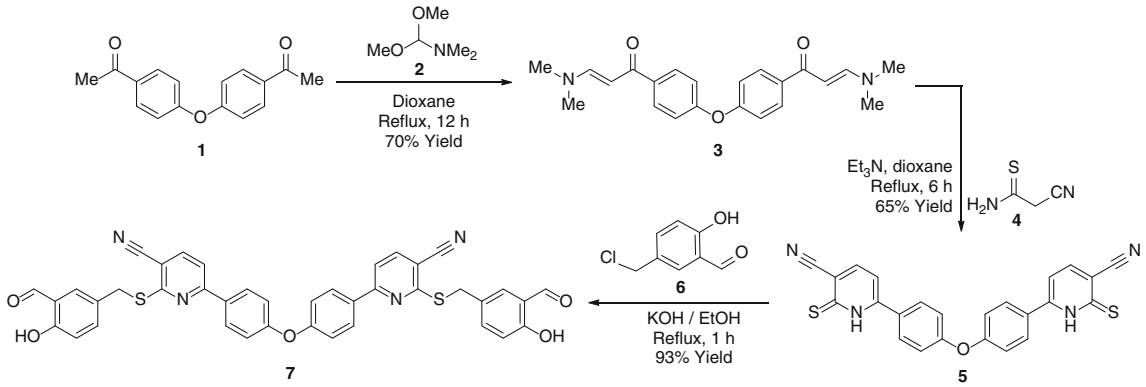
Pyrazole-based scaffolds have promising acetylcholinesterase inhibitory activity [12–14], in addition to a wide range of biological activities [15–17]. Our research group recently reported the promising inhibitory activity of nicotinonitrile-linked chromenes [18]. Inspired by the above, we pointed herein to investigate the inhibitory activity of bis(nicotinonitrile-chromene) hybrids with an additional pyrazole unit. The new pyrazole-linked hybrids are efficiently prepared utilizing the appropriate [3+2] cycloaddition protocol.

2 | RESULTS AND DISCUSSION

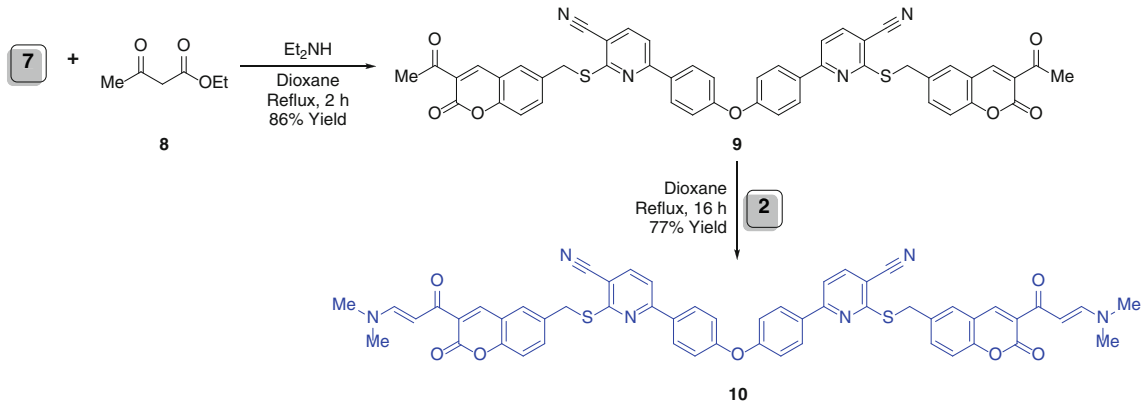
2.1 | Chemistry

At first, the bis(acetyl) derivative **1** [19] was reacted with dimethylformamide-dimethylacetal (DMF-DMA) **2** to give the corresponding bis(enaminone) **3** [20]. Then, bis(enaminone) **3** was cyclocondensed with cyanothioacetamide **4** in dioxane at reflux for 6 h in the presence of triethylamine to produce the bis(pyridinethione) **5** in 65% yield [21]. The bis(*S*-alkylation) of previous synthon with 5-chloromethyl-2-hybrdoxybenzaldehyde **6** in ethanolic potassium hydroxide solution yielded the nicotinonitrile-linked bis(salicylaldehyde) **7** (see Scheme 1 and Supporting information) [22].

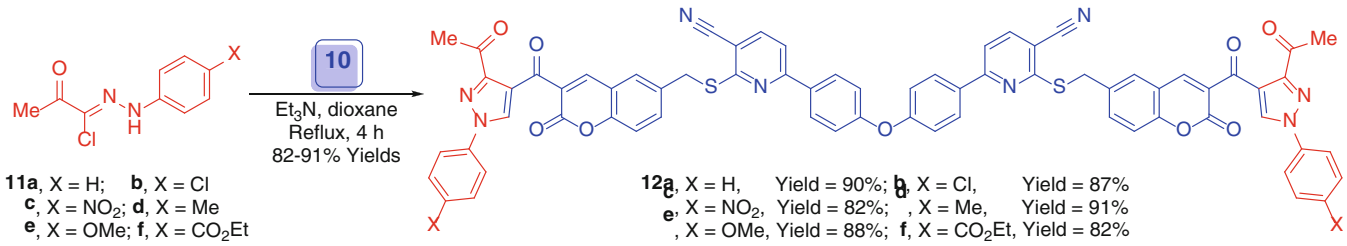
Cyclocondensation of bis(salicylaldehyde) **7** with ethyl acetoacetate **8** in dioxane in the presence of



SCHEME 1 Synthesis of nicotinonitrile-linked bis(salicylaldehyde) **7**



SCHEME 2 Synthesis of (nicotinonitrile-chromene)-based bis(enaminone) **10**

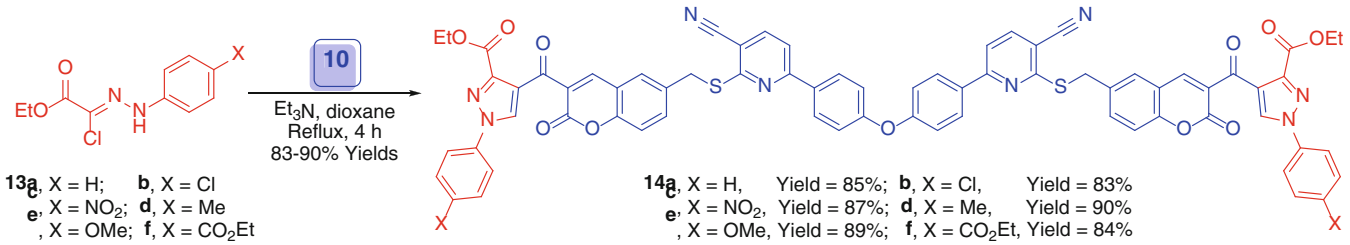


SCHEME 3 Synthesis of (nicotinonitrile-chromene)-based bis(pyrazoles) **12**

diethylamine afforded bis(chromene) **9** [23]. Next, the previous intermediate was reacted with DMF-DMA **2** in dioxane to prepare the (nicotinonitrile-chromene)-based bis(enaminone) **10** in 77% yield (see Scheme 2 and Supporting information) [24].

Next, a [3+2] cycloaddition protocol was used to prepare the target acyl-linked bis(pyrazoles) [9]. Therefore, the (nicotinonitrile-chromene)-based bis(enaminone) **10** was reacted with the respective hydrazonoyl chlorides **11** in dioxane in the presence of triethylamine at reflux for 4 h (see Scheme 3). As detected by TLC analyses, a sole product was isolated in each case. The reaction yielded the regioisomeric acetyl-linked bis(pyrazoles) **12** in 82%–91% yields (for the detailed mechanism, see Supporting information) [10, 25].

Using a similar protocol, another series of ethoxycarbonyl-linked bis(pyrazoles) **14** was prepared, in 83%–90% yields, utilizing the hydrazonoyl chlorides **13** (see Scheme 4). The ^{1}H-NMR spectra of the obtained products revealed a singlet signal at δ 9.14–9.38, in series **12**, and at δ 9.23–9.43, in series **14**, which corresponded to pyrazole-H protons (see Supporting information).



SCHEME 4 Synthesis of ethoxycarbonyl-linked bis(pyrazoles) **14**

TABLE 1 The AChE inhibitory activity of new bis(pyrazoles) **12(14)**

				Inhibition percentage	
Entry	Product	X	σ_p	At 25 μM	At 50 μM
1	**12a (14a)**	H	0.00	49.4 (35.6)	64.3 (50.2)
2	**12b (14b)**	Cl	0.23	26.7 (15.9)	41.4 (22.5)
3	**12c (14c)**	NO_2	0.78	11.8 (5.3)	18.1 (9.9)
4	**12d (14d)**	Me	−0.17	62.4 (34.6)	75.7 (59.3)
5	**12 e (14 e)**	OMe	−0.27	71.3 (45.1)	85.6 (67.8)
6	**12f (14f)**	CO_2Et	0.45	15.2 (9.0)	23.5 (14.3)
7	**Donepezil**	—	—	91.6	94.5

TABLE 2 The DPPH-free radical inhibitory activity of new bis(pyrazoles) **12(14)**

	12a	**12b**	**12c**	**12d**	**12 e**	**12f**	**14a**	**14b**	**14c**	**14d**	**14 e**	**14f**	**Ascorbic acid**
Inhibition percentage	45.7	31.3	16.4	73.5	84.7	24.0	24.6	17.2	8.5	37.9	44.6	12.4	88.7

2.2 | Acetylcholinesterase inhibitory activity

Ellman method was used to test the anti-acetylcholinesterase activity of new hybrids **12(14)** at concentrations of 25 and 50 μM, using the reference donepezil (inhibition percentages of 91.6 and 94.5, respectively) [26]. All findings are recorded in Table 1. At a concentration of 25 μM, the acetyl-linked bis(pyrazoles) **12d** and **12 e** demonstrated the best anti-acetylcholinesterase activity with inhibition percentages of 62.4 and 71.3, respectively. Other tested hybrids from acetyl-linked series **12** in addition to all hybrids from ethoxycarbonyl-linked series **14** showed lower activity with inhibition percentages below 50. Their inhibition percentages ranged from 9.0 to 49.4. When tested at a concentration of 50 μM, all hybrids had significantly higher AChE inhibitory activity. Six hybrids with inhibition percentages greater than 50 have been tested. Hybrids **12a**, **12d**, and **12 e** showed the best inhibitory activity with inhibition percentages of 64.3, 75.7, and 85.6, respectively, whereas their ethoxycarbonyl-analogues **14a**, **14d**, and **14 e** showed decreased activity with inhibition percentages of 50.2, 59.3, and 67.8, respectively. Other hybrids **12b**, **12c**, and **12f**, as well as their analogues in series **14** showed lower inhibitory activity, with inhibition percentages ranging from 9.9 to 41.4.

2.3 | Antioxidant activity

Several studies have looked into the efficacy of antioxidant therapeutic strategies in the treatment of AD [27]. The new hybrids were also assessed for their anti-DPPH-free radicals at a concentration of 25 μg/mL using the standard ascorbic acid (inhibition percentage of 88.7) (see Table 2) [28]. The acetyl-linked bis(pyrazoles) **12d** and **12 e** showed the best antioxidant activity with inhibition percentages of 73.5 and 84.7, respectively. Other tested hybrids from demonstrated lower activity with inhibition percentages below 50. Their inhibition percentages ranged from 8.5 to 45.7.

2.4 | Structure–activity relationship

Series **12(14)** are connected to an aryl unit at pyrazole-C1, where various substituents (X) of different electronic properties are attached at para-positions. In the case of series **12**, we discovered that compound **12 e**, which is linked to the *p*-OMe unit (σ_p of −0.27) [29], had the best anti-acetylcholinesterase activity, with an inhibition percentage of 85.6 at 50 μM. Compound **12c**, on the other hand, was linked to the *p*-NO_2 unit (σ_p of 0.78) and showed the least activity, with an inhibition percentage of 18.1 at the previously tested concentration. Therefore, we can deduce that the inhibitory potency increases as the electron releasing of the substituent X increases. We also discovered that the acyl unit attached to pyrazole-C3 has a significant impact on the inhibitory activity of **12(14)**. The tested acetyl-linked series **12** inhibited acetylcholinesterase more effectively than the other ethoxycarbonyl-linked series **14**. Hybrid **14 e**, with 1-(4-methoxyphenyl) and 3-ethoxycarbonyl units, demonstrated an inhibition percentage of 67.8 at 50 μM. Hybrid **12 e**, with the same 1-aryl unit but a 3-acetyl unit, showed significant potency at the same concentration tested, with an inhibition percentage of 85.6.

As shown in Tables 1 and 2, we can observe same impact of the electronic properties of substituent (X) and the acyl units on the results of antioxidant activity as in the anti-acetylcholinesterase activity. Therefore, hybrid **12 e**, with acetyl and *p*-OMe units, showed the best antioxidant activity with inhibition percentage of 84.7, while hybrid **14c**, with ethoxycarbonyl and *p*-NO_2 units, displayed the least activity with inhibition percentage of 8.5.

3 | CONCLUSION

Two series of (nicotinonitrile-chromene)-linked bis(pyrazoles) were produced in good yields using a [3+2] cycloaddition protocol. Generally, the electronic properties of the para-substituent attached to the arene unit at pyrazole-C1 are related to the anti-acetylcholinesterase and antioxidant activity. Additionally, the acyl unit attached to pyrazole-C3 has a significant impact on the inhibitory activity of the new hybrids. The 3-acetylpyrazole hybrid attached to a *p*-OMe unit had the best inhibitory activity.

4 | EXPERIMENTAL

4.1 | General procedure for the synthesis of 3-acyl-linked bis(pyrazoles) 12(14)

A mixture of bis(enaminone) **10** (5 mmol) and the appropriate hydrazonyl chlorides **11a–11f** or **13a–13f** (10 mmol) in dioxane (20 ml) containing triethylamine (1 ml) was heated at reflux for 4 h. The reaction mixture was evaporated and the obtained solid was washed with cold ethanol, filtered, and then recrystallized from the appropriate solvent.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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