Sequential Four-Component Synthesis And Antitumor Activity Screening of Spiro[chromene-indolo[2,1-*b*]quinazoline] And Spiro[indolo[2,1-*b*]Quinazoline-pyrano[3,2-*c*]chromene]

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Abstract

Chemotherapy is one of the most common types of treatment among cancer patients and by using potent chemicals and agents, tumor promotion was inhibited. Despite the usage of many chemical agents in cancer therapy, cancer is still incurable. It seems that the synthesis of new compounds with high efficiency on cancer cells and low side effects on normal cells will remain a critical challenge among researchers in this area. In the present work, a fast and straightforward process for the transformations involving tryptanthrins, malononitrile, some types of CH-acids such as 1,3-cyclohexanedione, dimedone, and 4-hydroxycoumarin resulting in preparing spiro[chromene-indolo[2,1-*b*]quinazoline] and spiro[indolo[2,1-*b*]quinazoline-pyrano[3,2-*d*]chromene] derivatives through sequential Knoevenagel/Michael/intramolecular cyclization sequences was reported. at room temperature. This protocol benefits some notable advantages including short reaction time, mild reaction condition, and simple purification, which make it interesting. Furthermore, it was carried out at room temperature, so it is according to green chemistry procedures. Also, antitumor screening of our new synthetic compounds (**4a-i**) was evaluated on pancreatic cancer cells (Panc1), breast cancer cells (MDA-MB-231), prostate cancer cells (PC3), and normal human adult dermal fibroblast cells (HDF) by using MTT assay using etoposide as a positive control. We found that 50% growth inhibitory concentration (IC₅₀) values of our synthetic compounds were not lower than etoposide against three cancer cell lines.

Introduction

Cancer is a major global problem with a high mortality rate. Chemotherapy is considered an effective platform in cancer therapy. However, the resistance to drugs and side effects of compounds on normal cells are the main unsolved problems. Therefore, the synthesis of new compounds with potent antitumor properties is required. In this respect, chromenes are an attractive group of compounds that are synthesized or occur in nature. Heterocyclic compounds bearing chromene moiety display a wide spectrum of biological properties including antileishmanial, antibacterial, antiproliferation, antioxidant, and antifungal. These compounds, due to their outstanding selective inhibitory activities of acetylcholinesterase and monoamine oxidase B enzymes exhibit excellent activity in the treatment of neurodegenerative diseases such as anti-Parkinson's anti-Alzheimer's and diseases [1-13].

As well, pyrano[3,2-*d*]chromen derivatives are a group of chromenes family which are prominent natural products with diverse biological properties including antimicrobial, antituberculosis, antioxidant, antifungal, anticoagulant [14-16].

On the other hand, alkaloids, characterized by an indolo[2,1-*b*]quinazoline core, present a broad range of potential biological activities too. In this regard, tryptanthrins, as natural alkaloids, are fascinating structures that have been considered as outstanding structures in drug discovery. These compounds are observed to be an aryl hydrocarbon receptor agonist, IDO-1 inhibitor, anti-cancer agent, antiparasitic, antineoplastic, and antileishmanial agent and have a vast range of immune-modulatory efficacies [17-21].

Owing to the importance and potential utility of the two above-mentioned structures, significant progress has been made in the synthesis of them which have been reported in the literature [22-23]. It is valuable to say that the connection of two structural motifs into one molecular structure might have importance and brings new enhancement properties, increasing their medicinal performance.

Numerous publications reported the synthesis of spiropyran derivatives through the reaction of isatin derivatives, malononitrile, and CH-acids such as dimedone and 4-hydroxycoumarin in presence of different catalysts [24-26].

To extend these works and intend to have two above-mentioned constructions in one molecule, we applied tryptanthrins instead of isatins. A few reports using tryptanthrin as a starting material are found in the literature. Some examples are presented in Scheme 1. Aldol reaction of tryptanthrins and acetophenones was reported by Itoh et al. in 2017 (Scheme 1a) [27]. Liang et al. synthesized a colorimetric probe for Cu^{2+} based on tryptanthrin in 2018 (Scheme 1b) [28]. Schepetkin et al. synthesized tryptanthrin-6-oxime (indolo(2,1-*b*)quinazoline-6,12-dion-6-oxime) and assessed their efficiencies on JNK activity (Scheme 1c) [29].

Previously, we reported the synthesis of spiro[indolo[2,1-*b*]quinazoline-pyrano[2,3-*c*]pyrazole] derivatives through the reaction of tryptanthrins, malononitrile and 3-methyl-pyrazolones (Scheme 1d) [30]. In this paper, we discover an efficient synthesis of a functionalized spiro[chromene-indolo[2,1-*b*]quinazoline] and spiro[indolo[2,1-*b*]quinazoline-pyrano[3,2-*d*]chromene] derivatives by reaction between tryptanthrins, malononitrile and some CH-acids such as 1,3-cyclohexanedione, dimedone and 4-hydroxycoumarin in CH_2Cl_2 , using Et_3N at room temperature (25°C), in 66% - 83% yields (Scheme 1e). Next, to find the antitumor activity of synthesized structures (**4a-i**), three cancer cell lines including pancreatic cancer cells (Panc1), breast cancer cells (MDA-MB-231), prostate cancer cells (PC3), and normal human adult dermal fibroblast cells (HDF) were cultured. By MTT colorimetric assay, we evaluated the antitumor activity of the compounds.

Results And Discussion

In present study, an efficient strategy to form spiro[chromene-indolo[2,1-*b*]quinazoline] and spiro[indolo[2,1-*b*]quinazoline-pyrano[3,2-*d*]chromene] derivatives **4a-i** was developed. Three-component domino reaction of tryptanthrins **1**, malononitrile **2**, and CH-acids **3a-c** in CH_2Cl_2 as a solvent, and Et_3N as a catalyst, at room temperature, led to the formation of target products (Scheme 2).

Tryptanthrins **1a-c** were formed *via* a reaction between isatoic anhydride and isatin derivatives in the presence of K_2CO_3 in dimethylformamide (Scheme 3) [30].

Initially, the reaction of tryptanthrin **1a**, malononitrile **2**, and 1,3-cyclohexadione **3a** were used as a model reaction in several solvents and temperatures to obtain an optimized condition. First, this reaction was examined in CH_2Cl_2 at room temperature (25°C) (Table 1, entry 1). Adding a base as a catalyst was

necessary for the formation of the tryptanthrin-malononitrile adduct in this solvent and the Knoevenagel product did not form without the basic catalyst. Et₃N as a catalyst was chosen and the reaction was carried out in a very short time, with a very good yield (Table 1, entry 2). The other solvents including water and ethanol were investigated and compared. In these solvents, the target compound did not form or the yield was very low.

Table 1
Optimizing of the reaction conditions for **4a**

Entry	Solvent	Catalyst	Temp. (°C)	Time (h)	Yield (%)
1	DCM	-	25	24	No reaction
2	DCM	Et ₃ N	25	7 min	82
4	H ₂ O	-	25	24	No reaction
5	H ₂ O	Et ₃ N	25	24	No target compound
6	H ₂ O	Et ₃ N	reflux	18	14
7	EtOH	-	25	24	No target compound
8	EtOH	Et ₃ N	25	24	35
9	EtOH	Et ₃ N	reflux	18	23

After that, the optimal reaction condition was applied to extend the domain of the synthetic procedure using tryptanthrin derivatives **1**, malononitrile **2**, and CH-acids **3a-c** leading to the construction of spiro[chromene-indolo[2,1-*b*]quinazoline] and spiro[indolo[2,1-*b*]quinazoline-pyrano[3,2-*c*]chromene] derivatives **4a-i** in very good yields (Table 2). These results showed that the time of tryptanthrins reaction with 1,3 cyclohexadione as a ketone was lower than dimedone and 4-hydroxycumarin and the yields of products were higher than the other used CH-acids. It's supposed that the steric effect has an effective role in this reaction. In these reactions, the substituent on tryptanthrin motif did not have remarkable efficacy on the reaction time.

Table 2. Preparation of spiro[chromene-indolo[2,1-*b*]quinazoline] and spiro[indolo[2,1-*b*]quinazoline-pyrano[3,2-*c*]chromene] derivatives **4a-i**

Entry	X	Product	Time (min)	Yield
1	H	4a	7	82
2	Cl	4b	6	85
3	Br	4c	5	87
4	H	4d	10	69
5	Cl	4e	7	66
6	Br	4f	8	67
7	H	4g	30	83
8	Cl	4h	20	72
9	Br	4i	20	81

A possible mechanism is shown in Scheme 4. First, tryptanthrin molecule **1a** and malononitrile **2**, in the basic medium, take part in the Knoevenagel condensation reaction to produce the adduct **5**. Next, dimedone **3a** attacks adduct **5** *via* its nucleophilic center to construct the Michael adduct **6**, followed by intermolecular *O*-cyclization to yield the final product **4a**.

Cytotoxic activity

First, to find antitumor properties of the synthesized compounds, cell lines were cultured, and next, the MTT colorimetric assay was performed. Fifty percent of growth inhibitory concentration (IC_{50}) values were determined after 48 h treatment (Table 3) [31]. DMSO (1%) as a negative control and Etoposide (a reference drug) as a positive control were used. Among ten compounds, no IC_{50} values lower than etoposide against three cancer cell lines were found. However, **4g** and **4h** showed some antitumor activities against PC3, Panc1, and MDA-MB231 cells respectively. Therefore, more studies will be required to find the potent agents in cancer therapy.

Table 3

The evaluation of the antitumor activity of the synthesized compounds **4a-i** against three different cancer cell lines (PC3, Panc1, MDA-MB-231) and normal human adult dermal fibroblast (HDF). Data is represented here as mean $\pm$ SD of three independent determinations.

Compounds ($IC_{50}/\mu M$)	PC3	Panc1	MDA-MB-231	HDF
4a	>100	>100	>100	>100
4b	80.01 $\pm$ 0.046	>100	92.15 $\pm$ 0.075	>100
4c	>100	>100	60.08 $\pm$ 0.041	>100
4d	>100	>100	>100	>100
4e	>100	>100	>100	>100
4f	72.17 $\pm$ 0.053	>100	>100	>100
4g	52.54 $\pm$ 0.031	47.03 $\pm$ 0.02	>100	>100
4h	56.35 $\pm$ 0.029	97.47 $\pm$ 0.076	>100	>100
4i	82.33 $\pm$ 0.06	76.22 $\pm$ 0.052	64.05 $\pm$ 0.046	>100
Etoposide	32.15 $\pm$ 0.021	24.35 $\pm$ 0.001	30.63 $\pm$ 0.014	>100

Conclusion

Spiro[chromene-indolo[2,1-*b*]quinazoline] and spiro[indolo[2,1-*b*]quinazoline-pyrano[3,2-*c*]chromene] derivatives were synthesized through the reaction of tryptanthrins, malononitrile, some CH-acids including dimedone and 4-hydroxycumarin through sequential Knoevenagel/Michael/Intramolecular cyclization sequences in CH_2Cl_2 at room temperature. Simple method, short reaction time, easy purification process without any chromatographic technique, and very good yield of the products are some advantages of the protocol. However, the evaluation of the antitumor activity of the synthetic compounds has no IC_{50} values lower than etoposide against three cancer cell lines. To find potent agents, more experiments will be essential.

Experimental

General

All of the chemical compounds in this study were purchased from Aldrich and Merck chemical companies. Melting points were measured on an Electro-thermal 9100 apparatus. FT-IR spectra were recorded using KBr disks on a Bruker tensor 27 spectrometer, and absorbencies were reported in cm^{-1} . ^1H and ^{13}C NMR spectra were measured with a Bruker DRX-300AVANCE spectrometer at 299.87 MHz. NMR spectra were obtained in solutions of $\text{DMSO}-d_6$ using TMS as an internal standard in ppm. Mass spectra were recorded on an Agilent Technologies 5975C VL MSD with a Tripe-Axis Detector mass spectrometer operating at an ionization potential of 70 eV.

General procedure for the synthesis of spiro[chromene-indolo[2,1- b]quinazoline] and spiro[indolo[2,1- b]quinazoline-pyrano[3,2- c]chromene] derivatives

General procedure for the synthesis of **4a**: A mixture of isatoic anhydride (1 mmol) and isatin (1 mmol), in the presence of K_2CO_3 (10 mol%) in dimethylformamide (5 mL), heating at reflux led to the formation of a yellow precipitate of tryptanthrin **1** during 30 minutes. It's filtered, washed with ethanol, and dried [30].

Then, a mixture of tryptanthrin **1** (1 mmol), malononitrile **2** (1 mmol) in the presence of Et_3N (10 mol%) was stirred in CH_2Cl_2 (5 mL) at room temperature. At once, an orange precipitate of tryptanthrin-malononitrile adduct was formed (followed by TLC). Afterward, 1,3-cyclohexanedione **3a** was added to the mixture. A solid product was formed after 7 minutes and completion of the reaction was confirmed by TLC. Then, the precipitated product was filtered and washed with diluted acidic water to obtain the pure product.

Biological activity

Cell culture and cell viability assay

Pancreatic cancer cells (Panc1), breast cancer cells (MDA-MB-231), prostate cancer cells (PC3), and, human adult dermal fibroblast cells (HDF) were received from Pasture Institute, Tehran, Iran. Cell culture condition was performed according to our previous report [30]. By using MTT assay, IC_{50} values of the synthesized compounds were investigated after 48 h treatment and the results are presented in Table 3.

2-Amino-5,12'-dioxo-5,6,7,8-tetrahydro-12' H -spiro[chromene-4,6'-indolo[2,1- b]quinazoline]-3-carbonitrile (4a): White solid, M.p.: 276-278°C, yield: 0.335 g (82%); IR (KBr) ($\bar{\nu}_{\text{max}}/\text{cm}^{-1}$): 3359, 3300, (NH_2), 3107 ($=\text{CH}$), 2900 (CH), 2196 (CN), 1675 ($\text{C}=\text{O}$), 1590, 1467 ($\text{C}=\text{C}$), 1343 ($\text{C}-\text{N}$), 762 (Ar). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.42 (1H, d , $^3J = 9.0$ Hz, ArH), 8.31 (1H, d , $^3J = 9.0$ Hz, ArH), 7.86 (1H, t , $^3J = 7.5$ Hz, ArH), 7.74 (1H, d , $^3J = 6.0$ Hz, ArH), 7.61 (1H, t , $^3J = 6.0$ Hz, ArH), 7.50-7.45 (4H, m , ArH and NH_2), 7.34 (1H, t , $^3J = 7.5$ Hz, ArH), 2.79-2.71 (2H, m , CH_2), 2.21-2.17 (2H, m , CH_2), 1.95-1.91 (2H, m , CH_2). ^{13}C NMR (75 MHz, $\text{DMSO}-$

d_6): δ 196.6 (C=O), 167.4 (C-NH₂), 163.3 (O-C=C), 159.5 (C=O), 158.7 (C=N), 147.2, 138.6, 135.6, 129.6, 129.6, 128, 127.8, 127.5, 126.8, 124.3, 120.7, 117.8, 116.2 (CN), 112.3 (O-C=C), 58.4 (C-CN), 48.3 (C_{spiro}), 36.6, 27.1 and 19.9 (3 CH₂). MS (EI): m/z (%) = 463 (0.4), 436 (2), 408 (M⁺, 66), 382 (13), 364 (19), 344 (33), 324 (11), 296 (100), 278 (1), 260 (42), 233 (21), 215 (9), 168 (1), 151 (6), 126 (5), 105 (23), 84 (11), 66 (20), 42 (20).

2-amino-8'-chloro-5,12'-dioxo-5,6,7,8-tetrahydro-12' H -spiro[chromene-4,6'-indolo[2,1- b]quinazoline]-3-carbonitrile (4b): White solid, M.p.: 328-330°C, yield: 0.377 g (85%); IR (KBr) ($\bar{\nu}_{\max}/\text{cm}^{-1}$): 3341, 3300, (NH₂), 3075 (=CH), 2900 (CH), 2197 (CN), 1678 (C=O), 1600, 1466 (C=C), 1340 (C-N), 766 (Ar), 695 (C-Cl). ¹H NMR (300 MHz, DMSO- d_6): δ 8.40 (1H, d , ³ J = 9.0 Hz, ArH), 8.30 (1H, d , ³ J = 9.0 Hz, ArH), 7.87 (1H, t , ³ J = 7.5 Hz, ArH), 7.75 (1H, d , ³ J = 6.0 Hz, ArH), 7.65 (1H, d , ³ J = 6.0 Hz, ArH), 7.62-7.52 (4H, m , ArH and NH₂), 2.77-2.73 (2H, m , CH₂), 2.20-2.18 (2H, m , CH₂), 1.97-1.91 (2H, m , CH₂). ¹³C NMR (75 MHz, DMSO- d_6): δ 196.3 (C=O), 167.4 (C-NH₂), 162.8 (O-C=C), 159.3 (C=O), 158.7 (C=N), 147.4, 138.5, 137.6, 135.6, 131.7, 129.6, 128, 127.8, 126.9, 124.7, 120.8, 117.8, 117.5 (CN), 112.0 (O-C=C), 57.8 (C-CN), 48.4 (C_{spiro}), 36.6, 27.3 and 20 (3 CH₂). MS (EI): m/z (%) = 534 (0.07), 470 (0.1), 442 (M⁺, 75), 416 (14), 378 (43), 357 (8), 330 (100), 294 (44), 267 (30), 240 (8), 215 (11), 188 (10), 151 (7), 124 (6), 102 (9), 66 (37), 42 (14).

2-amino-8'-bromo-5,12'-dioxo-5,6,7,8-tetrahydro-12' H -spiro[chromene-4,6'-indolo[2,1- b]quinazoline]-3-carbonitrile (4c): Pink solid, M.p.: 341-344°C, yield: 0.425 g (87%); IR (KBr) ($\bar{\nu}_{\max}/\text{cm}^{-1}$): 3341, 3300, (NH₂), 3072 (=CH), 2900 (CH), 2198 (CN), 1669 (C=O), 1603, 1466 (C=C), 1343 (C-N), 771 (Ar), 600 (C-Br). ¹H NMR (300 MHz, DMSO- d_6): δ 8.34 (1H, d , ³ J = 9.0 Hz, ArH), 8.30 (1H, d , ³ J = 9.0 Hz, ArH), 7.87 (1H, t , ³ J = 7.5 Hz, ArH), 7.77 (1H, t , ³ J = 6.0 Hz, ArH), 7.74 (1H, s , ArH), 7.65 (1H, t , ³ J = 9.0 Hz, ArH), 7.59-7.53 (3H, m , ArH and NH₂), 2.75-2.71 (2H, m , CH₂), 2.22-2.18 (2H, m , CH₂), 2.00-1.89 (2H, m , CH₂). ¹³C NMR (75 MHz, DMSO- d_6): δ 196.3 (C=O), 167.5 (C-NH₂), 162.7 (O-C=C), 159.3 (C=O), 158.7 (C=N), 147.6, 138.7, 138.0, 135.6, 132.5, 128.0, 127.8, 127.5, 126.9, 120.8, 119.8, 117.9, 117.7 (CN), 112.0 (O-C=C), 57.9 (C-CN), 48.4 (C_{spiro}), 36.6, 27.3 and 20 (3 CH₂). MS (EI): m/z (%) = 488 (M⁺, 58), 462 (11), 442 (11), 422 (89), 402 (12), 376 (89), 338 (66), 319 (2), 297 (26), 271 (17), 241 (20), 215 (24), 188 (22), 164 (15), 142 (16), 105 (100), 86 (9), 66 (71), 42 (37).

2-Amino-7,7-dimethyl-5,12'-dioxo-5,6,7,8-tetrahydro-12' H -spiro[chromene-4,6'-indolo[2,1- b]quinazoline]-3-carbonitrile (4d): White solid, M.p.: 296-298°C, yield: 0.304 g (69%); IR (KBr) ($\bar{\nu}_{\max}/\text{cm}^{-1}$): 3375, 3283, (NH₂), 3165 (=CH), 2959 (CH), 2194 (CN), 1672 (C=O), 1597, 1467 (C=C), 1358 (C-N), 764 (Ar), ¹H NMR (300 MHz, DMSO- d_6): δ 8.42 (1H, d , ³ J = 9.0 Hz, ArH), 8.31 (1H, d , ³ J = 6.0 Hz, ArH), 7.86 (1H, t , ³ J = 9.0 Hz, ArH), 7.72 (1H, d , ³ J = 6.0 Hz, ArH), 7.61 (1H, t , ³ J = 9.0 Hz, ArH), 7.50-7.45 (3H, m , ArH and NH₂), 7.42-7.33 (2H, m , ArH), 2.66 (2H, AB quartet, $\Delta\delta$ = 0.09, J_{AB} = 18.0 Hz, CH₂), 2.09 (2H, AB quartet, $\Delta\delta$ = 0.08, J_{AB} = 16.5 Hz, CH₂), 1.04 (3H, s , CH₃), 0.99 (3H, s , CH₃), ¹³C NMR (75 MHz, DMSO- d_6): δ 196.0 (C=O), 165.1 (C-NH₂), 163.1 (O-C=C), 159.4 (C=O), 158.8 (C=N), 147.5, 138.9, 136.0, 135.4, 129.6, 127.8, 127.7, 127.6,

126.8, 124.2, 121.0, 117.7, 116.2 (CN), 111.6 (O-C=C), 58.5 (C-CN), 50.3 (C), 48.3 (C_{spiro}), 32.5 and 31.1 (2 CH₂), 27.8 and 27.5 (2 CH₃), MS (EI): m/z (%) = 474 (0.1), 436 (M⁺, 100), 410 (19), 392 (21), 372 (6), 351 (16), 324 (11), 296 (33), 278 (2), 259 (9), 233 (31), 215 (4), 190 (6), 164 (3), 140 (6), 120 (5), 102 (5), 83 (12), 55 (8).

2-Amino-8'-chloro-7,7-dimethyl-5,12'-dioxo-5,6,7,8-tetrahydro-12' H -spiro[chromene-4,6'-indolo[2,1-b]quinazoline]-3-carbonitrile (4e): Pink solid, M.p.: 290-292°C, yield: 0.311 g (66%); IR (KBr) ($\bar{\nu}_{\max}/\text{cm}^{-1}$): 3381, 3300, (NH₂), 3097 (=CH), 2960 (CH), 2194 (CN), 1673 (C=O), 1599, 1468 (C=C), 1350 (C-N), 774 (Ar), 694 (C-Cl). ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.46 (1H, *d*, ³*J* = 9.0 Hz, ArH), 8.35 (1H, *d*, ³*J* = 6.0 Hz, ArH), 7.92 (1H, *t*, ³*J* = 7.5 Hz, ArH), 7.77 (1H, *d*, ³*J* = 9.0 Hz, ArH), 7.62-7.58 (5H, *m*, ArH and NH₂), 2.70 (2H, *s*, CH₂), 2.16 (2H, AB quartet, $\Delta\delta$ = 0.05, *J*_{AB} = 15 Hz, CH₂), 1.06 (6H, *s*, CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 196.0 (C=O), 165.4 (C-NH₂), 162.7 (O-C=C), 159.3 (C=O), 158.9 (C=N), 147.5, 139.7, 137.8, 135.5, 131.6, 129.6, 128.0, 127.8, 126.9, 124.5, 120.9, 117.6, (CN), 111.1 (O-C=C), 57.8 (C-CN), 50.3 (C), 48.4 (C_{spiro}), 32.5 and 28.0 (2 CH₂), 27.4 (2 CH₃). MS (EI): m/z (%) = 538 (83), 537 (27), 494 (18), 472 (M⁺+2, 8), 451 (0.9), 430 (8), 401 (2), 376 (100), 348 (12), 313 (20), 290 (2), 268 (11), 240 (15), 215 (11), 188 (11), 162 (39), 141 (1), 121 (74), 92 (40), 66 (43), 43 (15).

2-Amino-8'-bromo-7,7-dimethyl-5,12'-dioxo-5,6,7,8-tetrahydro-12' H -spiro[chromene-4,6'-indolo[2,1-b]quinazoline]-3-carbonitrile (4f): White solid, M.p.: 291-294°C, yield: 0.346 g (67%); IR (KBr) ($\bar{\nu}_{\max}/\text{cm}^{-1}$): 3374, 3300, (NH₂), 3082 (=CH), 2958 (CH), 2193 (CN), 1688 (C=O), 1599, 1465 (C=C), 1343 (C-N), 773 (Ar), 692 (C-Br). ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.34 (1H, *d*, ³*J* = 9.0 Hz, ArH), 8.30 (1H, *d*, ³*J* = 6.0 Hz, ArH), 7.87 (1H, *t*, ³*J* = 7.5 Hz, ArH), 7.72 (1H, *s*, Hz, ArH), 7.69 (1H, *m*, ArH), 7.67-7.56 (4H, *m*, ArH and NH₂), 2.65 (2H, AB quartet, $\Delta\delta$ = 0.06, *J*_{AB} = 18.0 Hz, CH₂), 2.11 (2H, AB quartet, $\Delta\delta$ = 0.05, *J*_{AB} = 16.5 Hz, CH₂), 1.02 (6H, *d*, ³*J* = 6.0 Hz, CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 196.2 (C=O), 165.5 (C-NH₂), 162.6 (O-C=C), 159.3 (C=O), 158.9 (C=N), 147.5, 138.5, 138.2, 135.6, 132.5, 128.1, 127.7, 126.9, 120.8, 119.8, 118.0 (CN), 117.6 (O-C=C), 57.8 (C-CN), 50.2 (C), 48.3 (C_{spiro}), 32.5 and 28.0 (2 CH₂), 27.4 (2 CH₃). MS (EI): m/z (%) = 516 (100), 515 (M⁺, 30), 488 (16), 452 (9), 431 (17), 403 (9), 376 (67), 351 (25), 311 (28), 293 (2), 266 (8), 240 (12), 215 (11), 189 (15), 165 (5), 140 (6), 140 (16), 112 (10), 83 (40), 55 (27).

2'-Amino-5',12-dioxo-5' H,12H-spiro[indolo[2,1-b]quinazoline-6,4'-pyrano[3,2-c]chromene]-3'-carbonitrile (4g): Purple solid, M.p.: 312-315°C, yield: 0.384 g (83%); IR (KBr) ($\bar{\nu}_{\max}/\text{cm}^{-1}$): 3354, 3300 (NH₂), 3069 (=CH), 2192 (CN), 1730, 1679 (C=O), 1601, 1465 (C=C), 1358 (C-N), 761 (Ar). ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.46 (1H, *d*, ³*J* = 9.0 Hz, ArH), 8.34 (1H, *d*, ³*J* = 9.0 Hz, ArH), 8.03 (1H, *d*, ³*J* = 9.0 Hz, ArH), 7.89 (2H, *d*, ³*J* = 6.0 Hz, ArH), 7.78 (2H, ³*J* = 9.0 Hz *dd*, ArH), 7.66-7.47 (5H, *m*, ArH, NH₂), 7.38 (1H, *t*, ³*J* = 6.0 Hz, ArH). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 162.2 (C=O), 159.3 (C-NH₂), 158.7 (C=O), 155.5 (O-C=C), 152.6, (C=N), 147.2, 139.2, 138.3, 137.0, 135.8, 134.5, 133.2, 130.4, 128.3 127.8, 127.0, 125.7, 125.2, 123.6, 121.0, 120.0, 117.2, 116.3, 112.8 (CN), 101.2 (O-C=C), 58.1 (C-CN), 49.0 (C_{spiro}). MS (EI): m/z (%) = 504

(0.4), 458 (M^+ , 64), 434 (0.9), 414 (21), 386 (7), 364 (3), 335 (11), 296 (100), 268 (19), 213 (3), 188 (4), 162 (21), 140 (3), 120 (28), 92 (19), 66 (22), 43 (15).

2'-Amino-8-chloro-5',12-dioxo-5' H,12H-spiro[indolo[2,1-b]quinazoline-6,4'-pyrano[3,2-c]chromene]-3'-carbonitrile (4h): white solid, M.p.: 320-322°C, yield: 0.358 g (72%); IR (KBr) ($\bar{\nu}_{\max}/\text{cm}^{-1}$): 3428, 3315 (NH_2), 3174 ($=\text{CH}$), 2205 (CN), 1721, 1676 ($\text{C}=\text{O}$), 1602, 1466 ($\text{C}=\text{C}$), 1353 ($\text{C}-\text{N}$), 766 (Ar), 660 ($\text{C}-\text{Cl}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.44 (1H, *d*, $^3J = 6.0$ Hz, ArH), 8.32 (1H, *d*, $^3J = 6.0$ Hz, ArH), 8.03-7.86 (5H, *m*, ArH and NH_2), 7.82-7.73 (2H, *m*, ArH), 7.67-7.56 (3H, *m*, ArH), 7.50 (1H, *d*, $^3J = 9.0$ Hz, ArH), ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 166.0 ($\text{C}=\text{O}$), 161.8 ($\text{C}-\text{NH}_2$), 159.3 ($\text{C}=\text{O}$), 159.2 ($\text{O}-\text{C}=\text{C}$), 158.7 ($\text{C}=\text{N}$), 155.9, 155.8, 152.6, 138.0, 136.8, 135.8, 134.5, 131.9, 130.3, 128.5, 127.9, 127.0, 125.7, 123.6, 120.9, 117.7, 117.3, 117.2, 113.0 (CN), 101.3 ($\text{O}-\text{C}=\text{C}$), 57.5 ($\text{C}-\text{CN}$), 49.1 (C_{spiro}). MS (EI): m/z (%) = 492 (M^+ , 74), 468 (5), 448 (16), 420 (6), 394 (4), 371 (1), 349 (1), 330 (100), 302 (13), 267 (21), 246 (8), 215 (4), 188 (4), 162 (22), 140 (1), 121 (41), 92 (22), 64 (12), 63 (13), 44 (4).

2'-Amino-8-bromo-5',12-dioxo-5' H,12H-spiro[indolo[2,1-b]quinazoline-6,4'-pyrano[3,2-c]chromene]-3'-carbonitrile (4i): White solid, M.p.: 331-334°C, yield: 0.435 g (81%); IR (KBr) ($\bar{\nu}_{\max}/\text{cm}^{-1}$): 3424, 3293 (NH_2), 3175 ($=\text{CH}$), 2206 (CN), 1724, 1680 ($\text{C}=\text{O}$), 1602, 1465 ($\text{C}=\text{C}$), 1355 ($\text{C}-\text{N}$), 766 (Ar), 693 ($\text{C}-\text{Br}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.39 (1H, *d*, $^3J = 9.0$ Hz, ArH), 8.33 (1H, *d*, $^3J = 6.0$ Hz, ArH), 8.03-7.97 (4H, *m*, ArH and NH_2), 7.91-7.80 (2H, *m*, ArH), 7.75 (2H, *d*, $^3J = 9.0$ Hz, ArH), 7.63-7.57 (2H, *m*, ArH), 7.50 (2H, *d*, $^3J = 9.0$ Hz, ArH). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 161.7 ($\text{C}=\text{O}$), 161.3 ($\text{C}-\text{NH}_2$), 159.3 ($\text{C}=\text{O}$), 159.2 ($\text{O}-\text{C}=\text{C}$), 158.6 ($\text{C}=\text{N}$), 155.8, 152.6, 147.2, 138.3, 137.0, 135.8, 134.5, 133.2, 128.5, 127.9, 127.0, 125.7, 123.6, 120.9, 120.0, 118.0, 117.3, 117.2, 113.0 (CN), 101.3 ($\text{O}-\text{C}=\text{C}$), 57.6 ($\text{C}-\text{CN}$), 48.9 (C_{spiro}). MS (EI): m/z (%) = 538 (83), 537 (M^+ , 27), 494 (18), 472 (8), 451 (0.9), 430 (8), 401 (2), 376 (100), 348 (12), 313 (20), 290 (2), 268 (11), 240 (15), 215 (11), 188 (11), 162 (39), 141 (1), 121 (74), 92 (40), 66 (43), 43 (15).

Declarations

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Conflicts of interest

The authors declare no competing financial interest.

References

1. Foroumadi A, Emami S, Sorkhi M, Nakhjiri M, Nazarian Z, Heydar S, Ardestani S, Poorrajab F, Shafiee A. Chromene-based synthetic chalcones as potent antileishmanial agents: synthesis and biological activity. *Chem Biol Drug Des*. 2010;75: 590-596. doi:10.1111/j.1747-0285.2010.00959.x.

2. Alblewia FF, Okasha RM, Hritania ZM, Mohamed HM, El-Nassag MAA, Halawa AH, Mora A, Fouda AM, and Assiri MA. Antiproliferative effect, cell cycle arrest and apoptosis generation of novel synthesized antitumor heterocyclic derivatives based 4H-benzo[h]chromene, *Bioorg Chem*. 2019;87:560-571. doi:10.1016/j.bioorg.2019.03.059.
3. Mori J, Iwashima M, Takeuchi M, Saito H. A synthetic study on antiviral and antioxidative chromene derivative. *Chem Pharm Bull*. 2006;54:391-396. doi:10.1248/cpb.54.391.
4. Mekky AWM, Sanad SMH. Synthesis and in vitro study of new coumarin derivatives linked to nicotinonitrile moieties as potential acetylcholinesterase inhibitors. *J Heterocyclic Chem*. 2020;57(12):4278-4290. doi:10.1002/jhet.4134.
5. Srivastava P, Tripathi PN, Sharma P, Rai SN, Singh SP, Srivastava RK, Shankar S. Design and development of some phenyl benzoxazole derivatives as a potent acetylcholinesterase inhibitor with antioxidant property to enhance learning and memory. *Eur J Med Chem*. 2019;163:116-135. doi:10.1016/j.ejmech.2018.11.049.
6. Vina D, Matos MJ, Yanez M, Santana L, Uriarte E. 3-Substituted coumarins as dual inhibitors of AChE and MAO for the treatment of Alzheimer's disease. *Med Chem Comm*. 2012;3:213-218. doi:10.1039/C1MD00221J.
7. Matos MJ, Vina D, Janeiro P, Borges F, Santana L, Uriarte E. New halogenated 3- phenylcoumarins as potent and selective MAO-B inhibitors. *Bioorg Med Chem Lett*. 2010;20:5157-5160. doi:10.1016/j.bmcl.2010.07.013.
8. Matos MJ, Vina D, Quezada E, Picciau C, Delogu G, Orallo F, Santana L, Uriarte E. A new series of 3-phenylcoumarins as potent and selective MAO-B inhibitors. *Bioorg Med Chem Lett*. 2009;19:3268-3270. doi:10.1016/j.bmcl.2009.04.085.
9. Pisani L, Muncipinto G, Miscioscia TF, Nicolotti O, Leonetti F, Catto M, Caccia C, Salvati P, Soto-Otero R, Mendez-Alvarez E. Discovery of a novel class of potent coumarin monoamine oxidase b inhibitors: development and biopharmacological profiling of 7-[(3-chlorobenzyl)oxy]-4-[(methylamino)methyl]-2H-chromen-2-one methanesulfonate (NW-1772) as a highly potent, selective, reversible, and orally active monoamine oxidase B inhibitor. *J Med Chem*. 2009;52:6685-6706. doi:10.1021/jm9010127.
10. Wong KY, Duchowicz PR, Mercader AG, Castro EA. QSAR applications during last decade on inhibitors of acetylcholinesterase in Alzheimer's disease. *Mini Rev Med Chem*. 2012;12:936-946. doi:10.2174/138955712802762365.
11. Ma K, Thomason LA, McLaurin J. Preclinical, and clinical data for Alzheimer's disease. *Adv. Pharmacol*. 2012;64:177-212. doi:10.1016/B978-0-12-394816-8.00006-4.
12. Bingi C, Emmadi NR, Chennapuram M, Poornachandra Y, Kumar CG, Nanubolu JB, Atmakur K. One-pot catalyst free synthesis of novel kojic acid tagged 2- aryl/alkyl substituted-4H-chromenes and evaluation of their antimicrobial and anti-biofilm. *Bioorg Med Chem Lett*. 2015;25(9):1915-1919. doi:10.1016/j.bmcl.2015.03.034.

13. Saundane AR, Vijaykumar K, and Vaijinath AV. Synthesis of novel 2-amino- 4-(5'-substituted 2'-phenyl-1H-indol-3'-yl)-6-aryl-4H-pyran-3-carbonitrile derivatives as antimicrobial and antioxidant agents. *Bioorg Med Chem Lett*. 2013;23(7):1978–1984. doi:10.1016/j.bmcl.2013.02.036.
14. Mungra DC, Patel MP, Rajani DP, Patel RG. Synthesis and identification of b-aryloxyquinolines and their pyrano[3,2-c]chromene derivatives as a new class of antimicrobial and antituberculosis agents. *Eur J Med Chem*. 2011;46(9):4192-4200. doi:10.1016/j.ejmech.2011.06.022.
15. Zhang RR, Liu J, Zhang Y, Hou MQ, Zhang MZ, Zhou F, Zhang WH. Microwave-assisted synthesis and antifungal activity of novel coumarin derivatives: pyrano[3,2-c]chromene-2,5-diones. *Eur J Med Chem*. 2016;116:76–83. doi:10.1016/j.ejmech.2016.03.069
16. Krivogorsky B, Nelson AC, Douglas KA, Grundt P. Tryptanthrin derivatives as toxoplasma gondii inhibitor's structure activity relationship of the 6-position. *Bioorg Med Chem Lett*. 2013;23(4):1032-1035. doi:10.1016/j.bmcl.2012.12.024.
17. Kurandina DV, Eliseenkov EV, Khaibulova TS, Petrov AA, Boyarskiy VP. Copper-catalyzed C–N bond cross-coupling of aryl halides and amines in water in the presence of ligand derived from oxalyl dihydrazide: scope and limitation. *Tetrahedron*. 2015;71(14):7931-7937. doi:10.1016/j.tet.2015.07.071.
18. Sharma VM, Prasanna P, Seshu KVA, Renuka B, Rao CVL, Kumar GS, Narasimhulu CP, Babu PA, Puranik RC, Subramanyam D, Venkateswarlu A, Rajagopal S, Kumar KBS, Rao CS, Mamidi NVSR, Deevi DS, Ajaykumar R, Rajagopalan R. Novel indolo[2,1-b]quinazoline analogues as cytostatic agents: synthesis, biological evaluation and structure–activity relationship. *Bioorg Med Chem Lett*. 2002;12(17):2303-2307. doi:10.1016/S0960-894X(02)00431-6.
19. Yang SS, Li XS, Hu FF, Li YL, Yang YY, Yan JK, Kuang CX, Yang Q. Discovery of tryptanthrin derivatives as potent inhibitors of indoleamine 2,3-dioxygenase with therapeutic activity in lewis lung cancer (llc) tumor-bearing mice. *J Med Chem*. 2013;56(21): 8321-8331. doi:10.1021/jm401195n.
20. Kaur R, Manjal SK, Rawal RK, Kumar K. Recent synthetic and medicinal perspectives of tryptanthrin. *Bioorg Med Chem*. 2017;25(17):4533-4552. doi:10.1016/j.bmc.2017.07.003.
21. Pergola C, Jazzar B, Rossi A, Northoff H, Hamburger M. Sautebin L, Werz O. On the inhibition of 5-lipoxygenase product formation by tryptanthrin: mechanistic studies and efficacy in vivo. *Pharmacol*. 2012;165(3):765-776. doi:10.1111/j.1476-5381.2011.01605.x.
22. Zheng J, He M, Xie B, Yang L, Hu Z, Zhou HB, Dong C. Enantioselective synthesis of novel pyrano[3,2-c] chromene derivatives as AChE inhibitors via an organocatalytic domino reaction. *Org Biomol Chem*. 2018;16(3):472-479. doi:10.1039/C7OB02794J.
23. Roudbaraki SJ, Mansoor SS, Ghashang M. Aqueous media synthesis of pyrano[3,2-c]chromen derivatives using magnesium oxide nanoparticles as a recyclable catalyst. *Polycycl Aromat Compd*. 2019;41:211-222. doi:10.1080/10406638.2019.1576746.
24. Pattarawarapan M, Wiriya N, Hongsihsong S, Phakhodee W. Divergent synthesis of methylisatoid and tryptanthrin derivatives by $\text{Ph}_3\text{P}-\text{I}_2$ -mediated reaction of isatins with and without alcohols. *J Org Chem*. 2020;85(23):15743–15751. doi:10.1021/acs.joc.0c02403.

25. Amara R, Awad H, Chaker D, Bentabed-Ababsa G, Lassagne F, Erb W, Chevallier F, Roisnel T, Dorcet V, Fajloun Z, Vidal J, Mongin F. Conversion of isatins to tryptanthrins, heterocycles endowed with a myriad of bioactivities. *Eur J Org Chem.* 2019;31:5302-5312. doi:1002/ejoc.201900352.
26. Ahadi N, Bodaghifard MA, Mobinikhaledi A. Cu (II)- β -cyclodextrin complex stabilized on magnetic nanoparticles: A retrievable hybrid promoter for green synthesis of spiropyrans. *Appl Organometal Chem.* 2018;33(2):4738. doi:10.1002/aoc.4738.
27. Itoh T, Abe T, Choshi T, Nishiyama T, and Ishikuraa M. Synthesis of ($\pm$)-cephalanthrin a using baeyer-villiger oxidation. *Heterocycles.* 2017;95(1):507-516. doi:10.3987/COM-16-S(S)5 .
28. Liang Y, Wang X, Fang H, Han N, Wang C. A highly selective and sensitive colorimetric probe for Cu²⁺ determination in aqueous media based on derivative of tryptanthrin. *Anal Sci.* 2018;34(10):1111-1115. doi:10.2116/analsci.18P091.
29. Schepetkin IA, Khlebnikov AI, AS Potapov, Kovrizhina AR. Synthesis, biological evaluation, and molecular modeling of 11H-indeno[1,2-b]quinoxalin-11- one derivatives and tryptanthrin-6-oxime as C-JUN N-terminal kinase inhibitors. *Eur J Med Chem.* 2019;161:179-191. doi:10.1016/j.ejmech.2018.10.023.
30. Sadeghian Z, Bayat M, Safari F. Synthesis and in vitro antitumor activity evaluation of spiro[indolo[2,1-b]quinazoline-pyrano[2,3-c]pyrazole] via sequential four-component reaction. *J Mol Struct.* 2022;1250: 131759. doi:10.1016/j.molstruc.2021.131759.
31. Safari, H. Hosseini, M. Bayat, A. Ranjbar. Synthesis and evaluation of antimicrobial activity, cytotoxic and pro-apoptotic effects of novel spiro-4H-pyran derivatives. *RSC Adv.* 2019;9:24843–24851. doi:10.1039/C9RA03196K.

Scheme

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