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Synthesis and anticancer activity evaluation of naphthalene-substituted triazole spirodienones

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Abstract

Building on our previous work that discovered 1,2,4-triazole-spirodienone as a promising pharmacophore for anticancer activity, we have further diversified 1,2,4-triazole-spirodienone derivatives and synthesized a series of novel naphthalene-substituted triazole spirodienones to explore their antineoplastic activity. Of these, compound **6a** possesses remarkable *in vitro* cytotoxic activity by arresting cell cycle and inducing apoptosis in MDA-MB-231 cells. Subsequently, acute toxicity assay showed that **6a** at 20 mg/kg has no apparent toxicity to the major organ in mice. In addition, compound **6a** *in vivo* suppressed breast cancer 4T1 tumor growth. Taken together, these results indicate that compound **6a** may be a potential anticancer agent for further development.

Keywords

Naphthalene-substituted triazole; Spirodienones; Cytotoxicity; Anti-proliferation; MDA-MB-231; 4T1 breast tumor

1. Introduction

The naphthalene ring widely exists in biologically active natural products [1], partaking in anti-inflammatory [2], antibacterial [3], antioxidant [4], and antifungal [5] properties in molecules containing the naphthalene moiety (Fig. 1A). Moreover, naphthalene derivatives have been reported as potent topoisomerase inhibitors [6], cytoplasmic protein-tyrosine

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmech.2020.113039>.

phosphatase inhibitors [7], and microtubule inhibitors [8]. Naphthalene moiety has been found a good surrogate of the benzene ring to improve chemical and metabolic stability of the active molecule while retaining pharmacology [9,10] (Fig. 1B).

There has been a growing interest in the synthesis of [4,5] heterocyclic hybrids gathered via a spiro carbon [11,12] owing to their potential antitumor activity [13–15]. Moreover, 1,2,4-triazole-containing analogs have been reported to possess considerable antineoplastic activity [16,17], increase the solubility of the ligand, and significantly improve the pharmacological profile of the drug [18]. A series of 1,2,4-triazole–spirodienone conjugates were synthesized in our laboratory and evaluated for their antitumor activities, most of which were shown to inhibit cell proliferation against MDA-MB-231, HeLa, A549 and MCF-7 cell lines, as represented by **g-1p** (Fig. 2A) [19]. The proven antitumor activity of the triazole–spirodienone skeleton warrants further exploration of structural modifications that could improve potency as well as physicochemical properties.

We seek to further expand the study of structure–activity relationship of the lead triazole–spirodienone compounds, by introducing a naphthalene or a benzoheterocyclic derivative to potentially modify physicochemical properties such as lipid–water partition coefficient and to potentially increase metabolic sites of the compound structure such as the *a* position of naphthalene prone to oxidative metabolism. Such structural modifications could facilitate the absorption, distribution, and metabolism to reduce the toxicity of the compounds. With these in mind, we replace the A rings of the 1,2,4-triazole–spirodienones with naphthalene and other benzoheterocycles (Fig. 2B). Herein, we describe the synthesis and biological evaluation of naphthalene/benzoheterocyclics -substituted 1,2,4-triazole spirodienone conjugates.

2. Result and discussion

2.1. Chemistry

The designed derivatives were prepared following the procedures described in Fig. 3. The substituted *N*-cyanoimides (**2**) were prepared by an efficient one-pot procedure for the cyanoimidation of aldehyde (**1**). Then, the cyclization reaction of the corresponding *N*-cyanoimides led to the formation of 1,2,4-triazole derivatives (**3**) [20]. The acid (**4**) was first converted to acid chloride with oxalyl chloride in dichloromethane and the following acylation between amino (**3**) and acid chloride gave the *N*-(1,3-disubstituent-1*H*-1,2,4-triazol-5-yl)-2- phenoxyacetamides (**5**). Subsequently, the reduction of **5** gave 1,3-disubstituent-*N*-(2-phenoxyethyl)-1*H*-1,2,4-triazol-5-amines (**7**) [20]. Next, the target derivatives (**6** and **8**) were formed by an oxidative amination reaction of **5** and **7** using $\text{PhI}(\text{OAc})_2$ as oxidants and $\text{Cu}(\text{CF}_3\text{SO}_3)_2$ as a catalyst. All reactions were carried out as detailed in the experimental section, and the structures of all target conjugates were confirmed by analytical characterization using ^1H NMR, ^{13}C NMR, and HR-MS spectrometry.

2.2. Antiproliferative activity and structure-activity relationships

All synthesized compounds were tested for their antiproliferative activity against the triple-negative human breast carcinoma MDA-MB-231, the cervical epithelioid carcinoma HeLa, and a metastatic non-small cell lung carcinoma A549. The IC₅₀ values are summarized in Table 1. Bendamustine and Vorinostat were used as the reference compounds. As shown in Table 1, all designed compounds displayed potent antiproliferative activity against three cancer cell lines. Compounds exhibited the highest inhibitory activity against MDA-MB-231, with IC₅₀ values ranging from 0.03 to 0.26 μ M. Moreover, target compounds also show a strong inhibitory effect on HeLa (IC₅₀ ranging from 0.07 to 0.72 μ M) and A549 (IC₅₀ ranging from 0.08 to 2.00 μ M) cell lines.

Based on tumor cell inhibitory effects of reported conjugates, a preliminary structure-activity relationship was summarized. The introduction of different aromatic rings at R¹ positions of 1,2,4-tirazole conferred significant anticancer activity. We then introduced methyl, chloro, trifluoromethyl, and methoxy group into the phenyl ring of R² position, and the resulting compounds are less active. Compounds **6** were stronger cell proliferation inhibitors than the corresponding compounds **8**, indicating that the introduction of the carbonyl group on 3-position of the spirodienone ring increased the antitumor activity. Methyl group at R³ position caused an obvious reduction of antiproliferative activity against A549 cell line. Overall, compound **6a** is the most active conjugate, so we conducted subsequent assays to further assess compound **6a**.

3. ADME/T profile in silico

In silico prediction of ADME/T properties of 25 target compounds was computed by Discovery Studio. The results of these computations are summarized in Table 2. The aqua solubility parameter (solubility-level) is related to absorption and distribution. The prediction shows that most of our target compounds have low water solubility, prompting us to introduce a hydrophilic group into the existing molecule in further research. Moreover, BBB-level and absorption-level are all in the desirable range. Polar surface area (PSA) means van der Waals surface area of polar nitrogen and oxygen atoms, which should not be greater than 140 Å, and the values for the target molecules are in the range of 57.453–92.614 Å. It is evident from Table 2 that the great majority of target compounds follow Lipinski's rule. In general, these parameters of target molecules may help in the further development of new drug candidates.

3.1. Cell cycle analysis

As shown in Table 1, the MDA-MB-231 breast cancer cell line was more sensitive to our target compound than the other cell lines. Many antineoplastic agents exert their inhibition of cell proliferation by arresting the cell cycle. Therefore, we next observed cell cycle changes of MDA-MB-231 cells exposed to **6a** and **8c**. As shown in Fig. 4, treatment with 1 μ M of compound **6a** and **8c** caused the percentage of MDA-MB-231 cells in G1 phase to decrease significantly (from 74.39% to 39.14% and 55.31%), and cells in S phase to increase (from 19.84% to 36.99% and 21.86%). The results indicated that compound **6a** and **8c** were able to induce cell cycle arrest in the S phase.

3.2. Analysis of cell apoptosis

To evaluate the efficacy of compounds **6a** and **8c** in induction of cell death through apoptosis, we performed cell apoptosis assays in MDA-MB-231 cells treated with the compounds. After 48 h of treatment with **6a**, **8c**, or Combretastatin A4 (CA4) as a positive control, cell cycle was analyzed by flow cytometry with FITC-Annexin V/PI staining. As shown in Fig. 5, **6a** and **8c** could induce cell apoptosis in a dose-dependent manner. Compounds **6a** and **8c** induced both early and late apoptosis in MDA-MB-231 cells at 1 μ M.

3.3. Acute toxicity study

To evaluate the safety of compound **6a**, four groups of Kunming mice (n = 8, half male and half female) were administered either a single dose of compound **6a** at escalating doses (10 mg/kg, 20 mg/kg, 40 mg/kg) or a single dose of the vehicle control by intraperitoneal injection. No mortality occurred during the test, indicating the maximum tolerated dose of **6a** should be greater than 40 mg/kg. There were no remarkable clinical signs in the eyes, skin, and fur among mice administered 40 mg/kg body weight of tested compounds. But the gain of bodyweight of the highest dose group is lower than the other three groups (Fig. 6). Ascites were detected when we dissected female mice of the highest dose group, with decreased liver volume (Fig. S1, Supporting Information). These results indicate that 40 mg/kg of compound **6a** may induce partial damage on the liver of female mice.

3.4. Antitumor activity in vivo

The orthotopic 4T1 tumor-bearing mice were used to further evaluate the antitumor efficiency *in vivo*. The mice were randomly divided into four groups (n = 6) and treated with **6a** at 0 mg/kg, 1 mg/kg, 2 mg/kg dose by intraperitoneal injection daily. The positive control group was treated with doxorubicin at 1 mg/kg.

Mice treated with vehicle control showed significant tumor growth, reaching average volumes of 644 mm³ (Fig. 7A). In comparison, three different treatment groups showed varying degree of tumor growth inhibition (Fig. 7E). The antitumor effect of 2 mg/kg of compound **6a** (Fig. 7C) was comparable with doxorubicin at 1 mg/kg (Fig. 7D). Furthermore, the gross appearance of tumors (Fig. 7F) and the tumor weights (Fig. 7G) were consistent with the tumor volume. The inhibition ratio of tumor by **6a** at 1 mg/kg, **6a** at 2 mg/kg, doxorubicin at 1 mg/kg were 18.2%, 38.8%, and 45.1% when compared with the saline control at the end of the study, respectively.

The body weights of all four groups were barely changed during the experiment (Fig. 7H), indicating all treatments had no apparent toxicity. In comparison with the control group, the size of the spleen and liver of **6a** at 2 mg/kg group enlarged (Fig. S2, Supporting Information). The hematoxylin and eosin (H&E) stain slices of **6a** at 2 mg/kg groups showed extramedullary hematopoiesis in the liver and spleen, indicating that **6a** might have a slight inhibitory action on bone marrow. In addition, the levels of apoptosis occurred in tumor tissues were consistent with the results of antitumor effect (Fig. 8).

3.5. Potential target of promising compounds

To determine potential targets of these spirodienone compounds, all chemical structures were submitted to HitPickV2 [21], SuperPred [22] and SwissTargetPrediction [23], three different web servers for target prediction of bioactive molecules, but only the SwissTargetPrediction is feasible (when the SMLIES of small molecules were submitted, HitPickV2 give no corresponding target, and SuperPred say ‘No known targets were found/No targets could be predicted for your uploaded compound’). Table 3 lists the common target of all 25 compounds, ADORA2A and PDE10A are not the typical targets of antineoplastic agents. In addition, molecular docking was performed by Discovery Studio to view the potential interactions between the promising compound **6a** and the remaining seven targets (Fig. S3, Supporting Information). The docking results showed that Libdock Scores of AURKA and MAPK10 are higher than 100. In more detail, for these seven targets, the obtained docking poses of **6a** were compared with the co-crystallized compounds (Table S1, Supporting Information). In this sense, MAPK10 and JAK2 are the most promising target. **6a** has the same six interactions with 2B1P that co-crystallized compounds do: phenyl and naphthalene moiety of **6a** interact with ILE70, VAL196, ALA91, LEU206 and ILE124 residues of MAPK10 to build Pi-alkyl interaction, and the naphthalene interacts with MET146 residue to build a Pi-sulfur interaction. Additionally, **6a** has the same five interactions with 4C61 that co-crystallized compound do: carbon-hydrogen bond with the side chain of ASN981, GLY993, Pi-alkyl interaction with the side chain ALA880, LEU983, Pi-sigma interaction with the side chain LEU855.

Based on the in silico target prediction and docking study, we first tested possible inhibition of AURKA, MAPK10, and JAK2, using **6a** and **6h** as test cases (Table 4). These compounds did not have any significant effect on AURKA and MAPK10 when tested at 100 μ M, but showed weak inhibition on the Janus kinases 2. The Janus kinases (JAK1, JAK2, JAK3, and TYK2) belong to the family of intracellular protein tyrosine kinases, which play an essential role in the signaling of a variety of cytokines [24]. Although the inhibitory potency of **6a** and **6h** toward JAK2 is not sufficient evidence of direct target determination, additional function study could validate the mechanism of action for the test compounds.

4. Conclusion

Building on our previous 1,2,4-triazole spirodienones, we synthesized a series of novel molecules with a naphthalene fragment as A ring and tested their antiproliferative activity. The naphthalene substituted analogs all demonstrated moderate to strong inhibitory effect against three cancer cell lines. Compound **6a** exhibited the strongest anticancer activity by inducing cell cycle arrest and cell apoptosis. Furthermore, compound **6a** significantly inhibited tumor growth in a metastatic 4T1 murine breast cancer model. Preliminary target modeling and enzyme assay suggest that compound **6a** is a potential JAK2 inhibitor for cancer therapy.

5. Experimental section

5.1. Chemistry

5.1.1. General chemistry methods—All solvents and reagents were obtained from commercial sources unless otherwise mentioned, and the solvents used were dried by conventional methods. All reactions were performed under a nitrogen atmosphere. The ^1H and ^{13}C NMR spectra were recorded on a Mercury 400 MHz NMR, Varian, Palo Alto, CA, USA and 600 MHz NMR. (CDCl_3 and $\text{DMSO}-d_6$ were the solvent and TMS was the internal standard). MS spectra were measured on Bruker Daltonics Data Analysis 3.4 Mass Spectrometer, Bruker, Karlsruhe, Germany and Thermo LTQ Orbitrap-XL Mass Spectrometer (Thermo Scientific, Waltham, MA, USA). A YRT-3 melting point instrument (Tianda Tianfa Company, Tianjin, China) was used for measuring melting points. HSGF 254 high-efficiency thin-layer chromatography silica gel plates were purchased from Huiyou Development Co., Yantai, Shangdong, China. Ltd. HSGF 254 thin-layer silica gel (300 mesh–400 mesh) was purchased from Ocean Chemical Plant (Qingdao, Shangdong, China).

5.1.2. Synthetic procedures—1.2.1 General procedure of the synthesis of methyl *N*-cyano-benzimidate (**2**): To a 50 ml flask were added benzaldehyde (1 mmol), cyanamide (3 mmol), *t*-BuONa (4 mmol), and MeOH (8 mL), the mixture was stirred for 0.5 h at room temperature, then NBS (4 mmol) was added. The resulting mixture was stirred for 12 h at 50 °C. After reaction, the reaction mixture was extracted with ethyl acetate; the organic layer was dried with anhydrous sodium sulfate and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel with petroleum ether/ethyl acetate as eluent to give **2**.

5.1.2.1. Methyl *N*-cyano-1-naphthimidate (2a**)**.: white solid, 180 mg, yield 86%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.05 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 8.2 Hz, 1H), 7.78 (d, J = 7.1 Hz, 1H), 7.64–7.55 (m, 3H), 4.20 (s, 3H).

5.1.2.2. Methyl *N*-cyano-2-naphthimidate (2b**)**.: white solid, 188 mg, yield 90%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.73 (s, 1H), 8.06 (d, J = 8.1 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.1 Hz, 1H), 7.65 (t, J = 7.4 Hz, 1H), 7.59 (t, J = 7.4 Hz, 1H), 4.12 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.7, 135.5, 132.2, 130.7, 129.7, 129.2, 128.8, 127.8, 127.3, 126.5, 123.8, 113.6, 56.9.

5.1.2.3. Methyl 1-bromo-*N*-cyano-2-naphthimidate (2c**)**.: yellow solid, 229 mg, yield 80%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.36 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.69 (t, J = 8.1 Hz, 1H), 7.64 (t, J = 8.1 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 4.17 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 179.3, 135.2, 131.8, 130.7, 128.8, 128.7 (2C), 128.5, 128.1, 123.6, 121.2, 112.6, 57.5.

5.1.2.4. Methyl *N*-cyano-2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbimidate (2d**)**.: yellow solid, 152 mg, yield 70%; ^1H NMR (400 MHz, Chloroform-*d*) δ 7.74 (dd, J = 8.6, 2.2 Hz, 1H), 7.64 (d, J = 2.2 Hz, 1H), 6.95 (d, J = 8.6 Hz, 1H), 4.34–4.27 (m, 4H), 4.01 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.5, 148.6, 143.4, 123.0, 122.3, 118.3, 117.6, 64.7, 64.1, 56.6.

5.1.2.5. Methyl N-cyanoquinoline-6-carbimide (2e): yellow solid, 155 mg, yield 74%; ^1H NMR (400 MHz, Chloroform-*d*) δ 9.05 (s, 1H), 8.78 (s, 1H), 8.34 (dd, J = 8.2, 2.2 Hz, 1H), 8.27–8.22 (m, 2H), 7.56–7.52 (m, 1H), 4.14 (s, 3H).

5.1.2.6. Methyl N-cyano-4-fluoro-1-naphthimide (2f): yellow solid, 180 mg, yield 86%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.20 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.78 (dd, d, J = 8.0, 5.1 Hz, 1H), 7.70–7.63 (m, 2H), 7.23 (d, J = 8.0 Hz, 1H), 4.20 (s, 3H).

5.1.2.7. Methyl N-cyano-9H-fluorene-2-carbimide (2g): yellow solid, 142 mg, yield 57%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.30 (s, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.85 (t, J = 8.0 Hz, 2H), 7.58 (d, J = 6.6 Hz, 1H), 7.44–7.37 (m, 2H), 4.06 (s, 3H), 3.95 (s, 2H).

5.1.3. General procedure of the synthesis of 1,3-disubstituted-1,2,4-triazole-5-amine (3)—A mixture of triethylamine (1.5 mmol) and phenylhydrazine hydrochloride (1.5 mmol) in methanol (3 mL) was stirred at room temperature for 30 min, then the N-cyanoimides (1 mmol) was added and the mixture was refluxed for 4 h. Then the solvent was removed in reduces pressure and the residue was purified by flash chromatography on silica gel to afford **3**.

5.1.3.1. 3-(Naphthalen-1-yl)-1-phenyl-1H-1,2,4-triazol-5-amine (3a): brown solid, 175 mg, yield 61%; ^1H NMR (400 MHz, Chloroform-*d*) δ 9.09 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 7.1 Hz, 1H), 7.90 (t, J = 7.6 Hz, 2H), 7.79 (d, J = 7.6 Hz, 2H), 7.59–7.48 (m, 5H), 7.43 (t, J = 7.4 Hz, 1H), 5.06 (s, 2H). ^{13}C NMR (151 MHz, CDCl_3) δ 159.6, 153.4, 136.9, 134.0, 130.9, 129.9, 129.8 (2C), 128.3, 128.1, 127.9, 127.7, 126.7, 126.6, 125.8, 125.2, 123.3 (2C).

5.1.3.2. 3-(Naphthalen-1-yl)-1-(p-tolyl)-1H-1,2,4-triazol-5-amine (3aa): yellow solid, 130 mg, yield 43%; ^1H NMR (400 MHz, Chloroform-*d*) δ 9.10 (d, J = 8.0 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.89 (t, J = 8.0 Hz, 2H), 7.58–7.48 (m, 5H), 7.35 (d, J = 8.0 Hz, 2H), 4.94 (s, 2H), 2.44 (s, 3H).

5.1.3.3. 3-(Naphthalen-1-yl)-1-(o-tolyl)-1H-1,2,4-triazol-5-amine (3ab): yellow solid, 117 mg, yield 39%; ^1H NMR (400 MHz, Chloroform-*d*) δ 9.08 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.90–7.86 (m, 2H), 7.56–7.36 (m, 7H), 4.70 (s, 2H), 2.33 (s, 3H).

5.1.3.4. 1,2,4 3-(Naphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-amine (3b): white solid, 232 mg, yield 81%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.53 (s, 1H), 8.16 (dd, J = 8.5, 1.3 Hz, 1H), 7.94–7.78 (m, 3H), 7.69–7.35 (m, 7H), δ 12 (s, 2H). ^{13}C NMR (101 Mhz, CDCl_3) δ 159.4, 154.3, 136.9, 133.8, 133.3, 129.9 (2C), 128.6, 128.3, 128.3, 128.2, 127.8, 126.4, 126.3, 125.6, 123.8, 123.5 (2C).

5.1.3.5. 3-(Naphthalen-2-yl)-1-(p-tolyl)-1H-1,2,4-triazol-5-amine (3ba): yellow solid, 271 mg, yield 90%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.55 (s, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.95–7.79 (m, 3H), 7.52–7.47 (m, 4H), 7.33 (m, 2H), 4.85 (d, J = 7.8 Hz, 2H), 2.43 (s, 3H).

5.1.3.6. 1-(4-Chlorophenyl)-3-(naphthalen-2-yl)-1H-1,2,4-triazol-5-amine

(3bc).: white solid, 192 mg, yield 60%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.54 (s, 1H), 8.16 (dd, J = 8.5, 1.7 Hz, 1H), 7.94–7.82 (m, 3H), 7.65–7.58 (m, 2H), 7.55–7.46 (m, 4H), 4.95 (s, 2H).

5.1.3.7. 3-(Naphthalen-2-yl)-1-(4-trifluoromethyl phenyl)-1H-1,2,4-triazol-5-amine

(3bd).: yellow solid, 191 mg, yield 54%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.54 (s, 1H), 8.16 (s, 1H), 7.90–7.82 (m, 7H), 7.50 (m, 2H), 5.03 (s, 2H).

5.1.3.8. 1-(4-Methoxyphenyl)-3-(naphthalen-2-yl)-1H-1,2,4-triazol-5-amine

(3bf).: yellow solid, 227 mg, yield 72%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.54 (s, 1H), 8.17 (dd, J = 8.5, 1.7 Hz, 1H), 7.93–7.82 (m, 3H), 7.54–7.46 (m, 4H), 7.05–7.00 (m, 2H), 4.94 (s, 2H), 3.85 (s, 3H).

5.1.3.9. 1,3-Di(naphthalen-2-yl)-1H-1,2,4-triazol-5-amine (3bg).: yellow solid, 218 mg, yield 65%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.59 (s, 1H), 8.21 (d, J = 8.5 Hz, 1H), 8.08–7.99 (m, 2H), 7.97–7.82 (m, 6H), 7.80–7.76 (m, 1H), 7.58 (m, 2H), 7.49 (dd, J = 6.1, 3.2 Hz, 2H), 5.05 (s, 2H).

5.1.3.10. 3-(1-Bromonaphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-amine (3c).: yellow solid, 255 mg, yield 72%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.48 (d, J = 8.5 Hz, 1H), 7.88–7.83 (m, 3H), 7.72–7.60 (m, 3H), 7.57–7.53 (m, 3H), 7.43 (t, J = 7.4 Hz, 1H), 5.03 (s, 2H). ^{13}C NMR (151 MHz, DMSO) δ 155.1, 148.8, 132.1, 129.7, 128.0, 125.7, 125.1 (2C), 123.6, 123.4, 123.4, 123.2, 122.9, 122.8, 122.3, 118.6 (2C), 118.1.

5.1.3.11. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-1-phenyl-1H-1,2,4-triazol-5-amine (3d).: yellow solid, 232 mg, yield 79%; ^1H NMR (400 MHz, Chloroform-*d*) δ 7.61–7.51 (m, 7H), 7.40 (t, J = 8.0 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 5.12 (s, 2H), 4.28 (m, 4H).

5.1.3.12. 1-Phenyl-3-(quinolin-6-yl)-1H-1,2,4-triazol-5-amine (3e).: yellow solid, 218 mg, yield 76%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.92 (dd, J = 4.3, 1.7 Hz, 1H), 8.54 (d, J = 1.9 Hz, 1H), 8.45 (dd, J = 8.8, 1.7 Hz, 1H), 8.25 (dd, J = 8.4, 1.9 Hz, 1H), 8.17 (d, J = 8.8 Hz, 1H), 7.65 (m, 2H), 7.56 (m, 2H), 7.44 (m, 2H), 4.90 (s, 2H).

5.1.3.13. 3-(4-Fluoronaphthalen-1-yl)-1-phenyl-1H-1,2,4-triazol-5-amine (3f).: yellow solid, 213 mg, yield 70%; ^1H NMR (400 MHz, Chloroform-*d*) δ 9.12 (d, J = 8.0 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.09 (t, J = 7.3 Hz, 1H), 7.68–7.53 (m, 6H), 7.43 (t, J = 8.0 Hz, 1H), 7.21 (t, J = 8.0 Hz, 1H), 5.20 (s, 2H).

5.1.3.14. 3-(9H-Fluoren-1-yl)-1-phenyl-1H-1,2,4-triazol-5-amine (3g).: bright yellow solid, 156 mg, yield 48%; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.24 (s, 1H), 8.08 (d, J = 7.9 Hz, 1H), 7.84–7.79 (m, 2H), 7.63–7.61 (m, 2H), 7.56–7.54 (m, 3H), 7.45–7.28 (m, 3H), 5.04 (s, 2H), 3.94 (s, 2H).

5.1.3.15. 3-(9H-Fluoren-1-yl)-1-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-5-amine (3gd).: bright yellow solid, 164 mg, yield 43%; ^1H NMR (400 MHz, Chloroform-*d*) δ

8.25 (s, 1H), 8.10 (d, $J = 8.0$, 1H), 7.86–7.80 (m, 6H), 7.57 (d, $J = 7.4$ Hz, 1H), 7.40 (t, $J = 7.4$ Hz, 1H), 7.34 (t, $J = 7.4$, 1H), 4.91 (s, 2H), 3.97 (s, 2H).

5.1.4. General procedure of the synthesis of N-(1,3-disubstituted-1,2,4-triazol-5-yl)- 2-phenoxyacetamides (5)—Oxalyl chloride (6 mmol) was added dropwise into a solution of 4-methoxyphenoxyacetic acid (3 mmol) and DMF (1d) in dichloromethane (50 mL), the reaction mixture was stirred for 2 h at room temperature. The solution was concentrated to dryness under reduced pressure to give the acid chloride. Then dichloromethane (5 ml) was added into the acid chloride, next, the acid chloride solution was slowly added to the solution of **3** (1 mmol) and triethylamine (3 mmol) in dichloromethane (20 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was quenched with water, and the product was extracted with ethyl acetate, the organic layer was dried over anhydrous sodium sulfate and concentrated. The crude was purified by column chromatography on silica gel to afford **5**.

5.1.4.1. 2-(4-Methoxyphenoxy)-N-(3-(naphthalen-1-yl)-1-phenyl-1H-1,2,4-triazol-5-yl) acetamide (5a).: yellow solid, 160 mg, yield 36%; ^1H NMR (400 MHz, Chloroform- d) δ 9.08 (d, $J = 8.3$ Hz, 1H), 8.23 (d, $J = 7.1$ Hz, 1H), 8.00–7.89 (m, 2H), 7.68–7.39 (m, 8H), 6.94–6.77 (m, 4H), 4.59 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 155.0, 150.9, 145.0, 136.8, 134.0, 130.8, 130.4, 129.6 (2C), 129.0, 128.7, 128.5, 128.2, 127.0, 126.7, 126.3, 126.0, 125.2, 123.8 (2C), 122.6, 115.6 (2C), 115.0 (2C), 68.0, 55.7.

5.1.4.2. 2-(4-Methoxyphenoxy)-N-(3-(naphthalen-1-yl)-1-phenyl-1H-1,2,4-triazol-5-yl) propenamide (5a β).: brown solid, 155 mg, yield 33%; ^1H NMR (400 MHz, Chloroform- d) δ 9.03 (d, $J = 8.4$ Hz, 1H), 8.19 (dd, $J = 7.2, 1.2$ Hz, 1H), 7.97–7.86 (m, 2H), 7.59–7.50 (m, 5H), 7.48–7.45 (m, 2H), 6.84 (s, 4H), 4.69 (q, $J = 6.8$ Hz, 1H), 3.79 (s, 3H), 1.56 (d, $J = 6.8$ Hz, 3H).

5.1.4.3. 2-(4-Methoxyphenoxy)-N-(3-(naphthalen-1-yl)- 1-(p-tolyl)-1H-1,2,4-triazol-5-yl) acetamide (5aa).: yellow solid, 139 mg, yield 30%; ^1H NMR (400 MHz, Chloroform- d) δ 9.11 (s, 1H), 8.83 (s, 1H), 8.25 (s, 1H), 7.94 (s, 2H), 7.54 (m, 5H), 7.33 (s, 2H), 6.87 (s, 4H), 4.62 (s, 2H), 3.81 (s, 3H), 2.46 (s, 3H).

5.1.4.4. 2-(4-Methoxyphenoxy)-N-(3-(naphthalen-1-yl)- 1-(o-tolyl)-1H-1,2,4-triazol-5-yl) acetamide (5 ab).: brown solid, 162 mg, yield 35%; ^1H NMR (400 MHz, Chloroform- d) δ 9.10 (d, $J = 8.4$ Hz, 1H), 8.61 (s, 1H), 8.28 (d, $J = 7.1$ Hz, 1H), 7.94–7.89 (m, 2H), 7.61–7.47 (m, 3H), 7.47–7.38 (m, 2H), 7.36–7.33 (m, 2H), 6.82 (d, $J = 8.8$ Hz, 2H), 6.73 (d, $J = 8.8$ Hz, 2H), 4.53 (s, 2H), 3.78 (s, 3H), 2.30 (s, 3H).

5.1.4.5. 2-(4-Methoxyphenoxy)-N-(3-(naphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-yl) acetamide (5b).: yellow solid, 210 mg, yield 47%; ^1H NMR (400 MHz, Chloroform- d) δ 8.87 (s, 1H), 8.68 (s, 1H), 8.25 (d, $J = 8.6$ Hz, 1H), 7.97–7.85 (m, 3H), 7.61–7.45 (m, 7H), 6.88–6.81 (m, 4H), 4.60 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 166.8, 160.9, 155.1, 150.8, 145.5, 136.6, 134.0, 133.3, 129.7 (2C), 129.1, 128.7, 128.3 (2C), 127.8, 126.7, 126.4, 126.1, 123.9 (2C), 123.7, 115.6 (2C), 115.0 (2C), 68.0, 55.7.

5.1.4.6. 2-(4-Methoxyphenoxy)-N-(3-(naphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)propenamide (5bβ).: yellow solid, 186 mg, yield 40%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 8.24 (d, *J* = 8.5 Hz, 1H), 7.94–7.84 (m, 3H), 7.52–7.44 (m, 7H), 6.86–6.79 (m, 4H), 4.70 (q, *J* = 6.8 Hz, 1H), 3.79 (s, 3H), 1.58 (d, *J* = 6.8 Hz, 3H).

5.1.4.7. 2-(4-Methoxyphenoxy)-N-(3-(naphthalen-2-yl)-1-(p-tolyl)-1H-1,2,4-triazol-5-yl)acetamide (5ba).: yellow solid, 186 mg, yield 34%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.62 (s, 1H), 8.20 (d, *J* = 8.5 Hz, 1H), 7.93–7.89 (m, 2H), 7.88–7.84 (m, 1H), 7.53–7.49 (m, 2H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.30–7.25 (m, 2H), 6.84 (s, 4H), 4.56 (s, 2H), 3.78 (s, 3H), 2.43 (s, 3H).

5.1.4.8. 2-(4-Methoxyphenoxy)-N-(3-(naphthalen-2-yl)-1-(p-tolyl)-1H-1,2,4-triazol-5-yl)propenamide (5baβ).: yellow solid, 144 mg, yield 30%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.79 (s, 1H), 8.65 (s, 1H), 8.23 (d, *J* = 8.5 Hz, 1H), 7.93–7.83 (m, 3H), 7.51–7.48 (m, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.2 Hz, 2H), 6.85–6.79 (m, 4H), 4.69 (q, *J* = 6.8 Hz, 1H), 3.79 (s, 3H), 2.42 (s, 3H), 1.58 (d, *J* = 6.8 Hz, 3H).

5.1.4.9. N-(1-(4-Chlorophenyl)-3-(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)-2-(4-methoxyphenoxy)acetamide (5bc).: white solid, 150 mg, yield 31%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.63 (s, 1H), 8.27–8.17 (m, 1H), 7.95–7.90 (m, 2H), 7.88–7.84 (m, 1H), 7.54–7.48 (m, 4H), 7.44 (d, *J* = 8.4 Hz, 2H), 6.91–6.81 (m, 4H), 4.59 (s, 2H), 3.80 (s, 3H).

5.1.4.10. 2-(4-Methoxyphenoxy)-N-(3-(naphthalen-2-yl)-1-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-5-yl)acetamide (5bd).: yellow solid, 171 mg, yield 33%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.63 (s, 1H), 8.23–8.18 (m, 1H), 7.96–7.91 (m, 2H), 7.88 (dd, *J* = 9.0, 4.3 Hz, 1H), 7.75–7.71 (m, 4H), 7.55–7.50 (m, 2H), 6.90–6.86 (m, 4H), 4.60 (s, 2H), 3.80 (s, 3H).

5.1.4.11. 2-(4-Methoxyphenoxy)-N-(1-(4-methoxyphenyl)-3-(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)acetamide (5bf).: white solid, 178 mg, yield 37%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.66 (s, 1H), 8.23 (d, *J* = 9.6 Hz, 1H), 7.91 (t, *J* = 8.9 Hz, 2H), 7.87–7.83 (m, 1H), 7.51–7.49 (m, 2H), 7.45 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 8.6 Hz, 2H), 6.86–6.80 (m, 4H), 4.59 (s, 2H), 3.87 (s, 3H), 3.78 (s, 3H).

5.1.4.12. 2-(4-Methoxyphenoxy)-N-(1-(4-methoxyphenyl)-3-(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)propenamide (5bfβ).: Yellow solid, 148 mg, yield 30%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.81 (s, 1H), 8.67–8.63 (m, 1H), 8.22 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.93–7.83 (m, 3H), 7.51–7.47 (m, 2H), 7.36–7.30 (m, 2H), 6.94–6.88 (m, 2H), 6.83–6.77 (m, 4H), 4.68 (d, *J* = 6.8 Hz, 1H), 3.83 (s, 3H), 3.77 (s, 3H), 1.58 (d, *J* = 6.8 Hz, 3H).

5.1.4.13. N-(1,3-Di(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)-2-(4-methoxyphenoxy)acetamide (5bg).: yellow solid, 160 mg, yield 32%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.94 (s, 1H), 8.70 (s, 1H), 8.30–8.25 (m, 1H), 8.03 (s, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 3H), 7.89–7.83 (m, 2H), 7.69 (d, *J* = 8.5 Hz, 1H), 7.62–7.56 (m, 2H), 7.53–7.50 (m, 2H), 6.80–6.74 (m, 4H), 4.58 (s, 2H), 3.76 (s, 3H).

5.1.4.14. N-(1,3-Di(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)-2-(4-methoxyphenoxy)propenamide (5bgβ): brown solid, 165 mg, yield 32%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.79 (s, 1H), 8.34 (s, 1H), 7.99–7.92 (m, 5H), 7.88 (t, *J* = 4.7 Hz, 1H), 7.83–7.79 (m, 1H), 7.65–7.58 (m, 3H), 7.54–7.51 (m, 2H), 6.77 (s, 4H), 4.69 (s, 1H), 3.78 (s, 3H), 1.59 (d, *J* = 6.8 Hz, 3H).

5.1.4.15. N-(3-(1-Bromonaphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-2-(4-methoxyphenoxy)acetamide (5c): brown solid, 200 mg, yield 39%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.97 (s, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 7.89 (s, 3H), 7.71–7.40 (m, 7H), 6.83 (m, 4H), 4.59 (s, 2H), 3.78 (s, 3H).

5.1.4.16. N-(3-(2,3-Dihydrobenzo[*b*] [1,4]dioxin-6-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-2-(4-methoxyphenoxy)acetamide (5d): brown solid, 192 mg, yield 42%; ¹H NMR (400 MHz, Chloroform-*d*) δ 7.67–7.64 (m, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.47 (m, 5H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.85–6.78 (m, 4H), 4.55 (s, 2H), 4.28 (m, 4H), 3.77 (s, 3H).

5.1.4.17. 2-(4-Methoxyphenoxy)-N-(1-phenyl-3-(quinolin-6-yl)-1H-1,2,4-triazol-5-yl)acetamide (5e): yellow solid, 147 mg, yield 36%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.96 (s, 1H), 8.92–8.89 (m, 1H), 8.66–8.60 (m, 1H), 8.47 (dd, *J* = 8.8, 1.9 Hz, 1H), 8.22 (d, *J* = 8.3 Hz, 1H), 8.16 (d, *J* = 8.8 Hz, 1H), 7.55–7.40 (m, 6H), 6.81 (m, 4H), 4.58 (s, 2H), 3.76 (s, 3H).

5.1.4.18. N-(3-(4-Fluoronaphthalen-1-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-2-(4-methoxyphenoxy)acetamide (5f): yellow solid, 230 mg, yield 50%; ¹H NMR (400 MHz, Chloroform-*d*) δ 9.10 (d, *J* = 8.3 Hz, 1H), 8.19 (t, *J* = 7.5 Hz, 2H), 7.66–7.48 (m, 7H), 7.25–7.19 (m, 1H), 6.84 (m, 4H), 4.60 (s, 2H), 3.79 (s, 3H).

5.1.4.19. N-(3-(9H-Fluoren-1-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-2-(4-methoxyphenoxy)acetamide (5g): bright yellow solid, 106 mg, yield 22%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.90 (s, 1H), 8.32 (s, 1H), 8.17 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.83 (dd, *J* = 10.8, 7.7 Hz, 2H), 7.58–7.53 (m, 3H), 7.52–7.44 (m, 3H), 7.39 (td, *J* = 7.5, 1.2 Hz, 1H), 7.32 (td, *J* = 7.4, 1.2 Hz, 1H), 6.86–6.80 (m, 4H), 4.58 (s, 2H), 3.95 (s, 2H), 3.78 (s, 3H).

5.1.4.20. N-(3-(9H-Fluoren-1-yl)-1-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-5-yl)-2-(4-methoxyphenoxy)acetamide (5gd): bright yellow solid, 111 mg, yield 20%; ¹H NMR (400 MHz, Chloroform-*d*) δ 8.30 (s, 1H), 8.15 (d, *J* = 7.7 Hz, 1H), 7.85 (dd, *J* = 15.1, 7.7 Hz, 2H), 7.74 (s, 4H), 7.57 (d, *J* = 7.4 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.34 (t, *J* = 7.3 Hz, 1H), 6.88 (s, 4H), 4.60 (s, 2H), 3.97 (s, 2H), 3.80 (s, 3H).

1.2.4 General procedure for the synthesis of N-(1,3-disubstituted-1,2,4-triazol-5-yl)-2-phenoxyethylamides (**7**). To a solution of **5** (0.1 mmol) in anhydrous THF (5 mL) was added LiAlH₄ (0.4 mmol) and AlCl₃ (0.2 mmol) at –20 °C under nitrogen. The reaction mixture was warmed to room temperature and continued to stir for 6 h. The reaction mixture was washed with 10% H₂SO₄ solution and then diluted with ethyl acetate. The organic layer was separated, dried over Na₂SO₄, and concentrated in reduced pressure. The residue was purified by flash chromatography on silica gel to afford **7**.

5.1.4.21. N-(2-(4-Methoxyphenoxy)ethyl)-3-(naphthalen-1-yl)-1-phenyl-1H-1,2,4-

triazol-5- amine (7a).: white solid, 34 mg, yield 80%; ^1H NMR (400 MHz, Chloroform-d) δ 9.11 (d, J = 8.5 Hz, 1H), 8.20 (d, J = 7.0 Hz, 1H), 7.90 (t, J = 7.3 Hz, 2H), 7.65 (d, J = 7.8 Hz, 2H), 7.58–7.51 (m, 5H), 7.42 (t, J = 7.4 Hz, 1H), 6.84 (m, 4H), 4.21 (t, J = 5.2 Hz, 2H), 3.95 (q, J = 5.2 Hz, 2H), 3.77 (s, 3H). ^{13}C NMR (151 MHz, cdCl_3) δ 160.1, 154.4, 154.2, 152.6, 137.0, 134.0, 131.1, 129.9 (2C), 129.7, 128.3 (2C), 128.0, 127.8, 126.7, 126.6, 125.7, 125.2, 123.5 (2C), 115.5 (2C), 114.8 (2C), 67.2, 55.7, 43.8.

5.1.4.22. N-(2-(4-Methoxyphenoxy)ethyl)-3-(naphthalen-1-yl)-1-(p-tolyl)-1H-1,2,4-

triazol-5- amine (7aa).: white solid, 30 mg, yield 68%; ^1H NMR (400 MHz, Chloroform-d) δ 9.13–9.07 (m, 1H), 8.19 (dd, J = 7.2, 1.4 Hz, 1H), 7.91–7.85 (m, 2H), 7.58–7.46 (m, 5H), 7.33 (d, J = 8.0 Hz, 2H), 6.87–6.79 (m, 4H), 4.20 (t, J = 5.0 Hz, 2H), 3.93 (q, J = 5.0 Hz, 2H), 3.76 (s, 3H), 2.43 (s, 3H).

5.1.4.23. N-(2-(4-Methoxyphenoxy)ethyl)-3-(naphthalen-2-yl)-1-phenyl-1H-1,2,4-

triazol-5- amine (7b).: white solid, 36 mg, yield 83%; ^1H NMR (400 MHz, Chloroform-d) δ 8.63 (s, 1H), 8.24 (d, J = 8.5 Hz, 1H), 7.95 (d, J = 9.3 Hz, 1H), 7.90 (d, J = 8.6 Hz, 1H), 7.88–7.84 (m, 1H), 7.60 (d, J = 7.9 Hz, 2H), 7.50 (dd, J = 9.1, 4.9 Hz, 4H), 7.42 (t, J = 7.3 Hz, 1H), 6.88–6.81 (m, 4H), 4.21 (t, J = 5.0 Hz, 2H), 3.95 (q, J = 5.0 Hz, 2H), 3.76 (s, 3H). ^{13}C NMR (151 MHz, cdCl_3) δ 159.6, 155.0, 154.2, 152.6, 136.9, 133.8, 133.4, 129.9 (2C), 128.6, 128.6, 128.10, 128.08, 127.7, 126.2, 126.1, 125.6, 124.0, 123.7 (2C), 115.6 (2C), 114.7 (2C), 67.3, 55.7, 43.8.

5.1.4.24. N-(2-(4-Methoxyphenoxy)ethyl)-3-(naphthalen-2-yl)-1-(p-tolyl)-1H-1,2,4-

triazol- 5-amine (7ba).: white solid, 27 mg, yield 60%; ^1H NMR (400 MHz, Chloroform-d) δ 8.62 (s, 1H), 8.22 (d, J = 8.5 Hz, 1H), 7.93 (d, J = 9.3 Hz, 1H), 7.91–7.81 (m, 2H), 7.51–7.42 (m, 4H), 7.32 (d, J = 7.7 Hz, 2H), 6.86–6.81 (m, 4H), 4.88 (s, 1H), 4.20 (s, 2H), 3.93 (s, 2H), 3.76 (s, 3H), 2.42 (s, 3H).

5.1.4.25. N-(2-(4-Methoxyphenoxy)propyl)-3-(naphthalen-2-yl)-1-phenyl-1H-1,2,4-

triazol- 5-amine (7b β).: white solid, 32 mg, yield 72%; ^1H NMR (400 MHz, Chloroform-d) δ 8.63 (s, 1H), 8.24 (d, J = 8.6 Hz, 1H), 7.96–7.93 (m, 1H), 7.88 (m, 2H), 7.51 (m, 6H), 7.39 (t, J = 7.1 Hz, 1H), 6.84 (q, J = 8.8 Hz, 4H), 4.95–4.88 (m, 1H), 4.64 (m, 1H), 3.95 (m, 1H), 3.76 (s, 3H), 3.60 (m, 1H), 1.37 (d, J = 6.1 Hz, 3H).

5.1.5. General procedure for synthesis of N-(1,3-disubstituted-1,2,4-triazol-5-yl) -1-oxa-4 -azaspiro [4,5]deca-6,9-dien-3,8-diones (6) and N-(1,3-disubstituted- 1,2,4-triazol-5-yl) -1-oxa-4-azaspiro [4,5] deca-6,9-dien-8-ones (8)

—A dried flask was charged, under a nitrogen atmosphere, with **5** or **7** (0.1 mmol), $\text{PhI}(\text{OAc})_2$ (0.25 mmol), and $\text{Cu}(\text{CF}_3\text{SO}_3)_2$ (0.015 mmol). The distilled dichloromethane (10 mL) was added. After stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuum and the residue was purified by flash chromatography on silica gel to give **6** or **8**.

5.1.5.1. 4-(3-(Naphthalen-1-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4,5]

deca-6,9-diene-3,8-dione (6a).: white solid, 19 mg, yield 90%, mp 182.2–185.1 °C; ^1H

NMR (400 MHz, Chloroform-*d*) δ 8.86 (d, J = 8.2 Hz, 1H), 8.13 (d, J = 7.2 Hz, 1H), 7.94 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 7.7 Hz, 1H), 7.62–7.46 (m, 8H), 6.65 (d, J = 10.0 Hz, 2H), 6.22 (d, J = 10.0 Hz, 2H), 4.59 (s, 2H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 183.8, 170.5, 162.6, 142.3, 142.1 (2C), 136.7, 134.3, 131.5 (2C), 131.0, 130.9, 130.3, 130.0 (2C), 128.8, 128.5, 127.4, 127.0, 126.4, 126.3, 125.5, 125.1 (2C), 88.6, 66.4. IR, ν/cm^{-1} : 2920, 1749, 1678, 1638, 1503, 1455, 1262, 1006. HRMS (ESI): Calculated for $\text{C}_{26}\text{H}_{19}\text{N}_4\text{O}_3$, $\{[\text{M}+\text{H}]^+\}$: 435.1452, found 435.1451.

5.1.5.2. 2-Methyl-4-(3-(naphthalen-1-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-

azaspiro-[4.5]deca-6,9-diene-3,8-dione (6a β): yellow solid, 19 mg, yield 82%, mp 184.4–187.0 °C; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.85 (d, J = 8.3 Hz, 1H), 8.13 (dd, J = 7.3, 1.3 Hz, 1H), 7.97–7.85 (m, 2H), 7.64–7.47 (m, 8H), 6.77 (dd, J = 10.0, 3.2 Hz, 1H), 6.45 (dd, J = 10.0, 3.2 Hz, 1H), 6.26–6.16 (m, 2H), 4.71 (q, J = 6.7 Hz, 1H), 1.57 (d, J = 6.7 Hz, 3H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 183.9, 172.8, 162.6, 143.3, 142.7, 142.5, 136.7, 134.3, 132.3, 131.0, 130.9, 130.5, 130.3, 129.9 (2C), 128.8, 128.5, 127.4, 127.1, 126.4, 126.3, 125.5, 125.0 (2C), 87.0, 73.4, 18.5. IR, ν/cm^{-1} : 2921, 1748, 1675, 1634, 1526, 1504, 1233, 1037. HRMS (ESI): Calculated for $\text{C}_{27}\text{H}_{20}\text{N}_4\text{NaO}_3$, $\{[\text{M}+\text{Na}]^+\}$: 471.1427, found 471.1422.

5.1.5.3. 4-(3-(Naphthalen-1-yl)-1-(p-tolyl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-

azaspiro[4.5]-deca-6,9-diene-3,8-dione (6aa): yellow solid, 16 mg, yield 87%, mp 198.1–198.9 °C; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.85 (d, J = 8.2 Hz, 1H), 8.12 (d, J = 7.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.89 (d, J = 9.1 Hz, 1H), 7.57–7.50 (m, 3H), 7.47 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.2 Hz, 2H), 6.66 (d, J = 10.1 Hz, 2H), 6.22 (d, J = 10.1 Hz, 2H), 4.57 (s, 2H), 2.48 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.5, 170.1, 162.2, 141.9 (2C), 140.2, 134.0 (2C), 133.9, 131.1 (2C), 130.7, 130.5, 130.2 (2C), 128.5, 128.2, 127.0, 126.9, 126.1, 126.0, 125.1, 124.7 (2C), 88.3, 66.0, 21.3. IR, ν/cm^{-1} : 2922, 1742, 1675, 1635, 1510, 1453, 1385, 1263, 1050. HRMS (ESI): Calculated for $\text{C}_{27}\text{H}_{20}\text{N}_4\text{NaO}_3$, $\{[\text{M}+\text{H}]^+\}$: 449.1608, found 449.1614.

5.1.5.4. 4-(3-(Naphthalen-1-yl)-1-(o-tolyl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-

azaspiro[4.5]-deca-6,9-diene-3,8-dione (6ab): yellow solid, 19 mg, yield 80%, mp 200.6–203.0 °C; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.88 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 7.2 Hz, 1H), 7.94–7.88 (m, 2H), 7.57–7.33 (m, 7H), 6.66 (d, J = 10.0 Hz, 2H), 6.25 (d, J = 10.0 Hz, 2H), 4.50 (s, 2H), 2.25 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.5, 169.9, 162.1, 143.0, 142.0 (2C), 136.0, 135.1, 134.0, 131.8, 131.2 (2C), 130.6 (2C), 130.5, 128.5, 128.2, 127.0, 126.8, 126.7, 126.4, 126.0 (2C), 125.1, 87.9, 65.9, 18.1. IR, ν/cm^{-1} : 3054, 1746, 1680, 1638, 1504, 1460, 1371, 1265, 1067. HRMS (ESI): Calculated for $\text{C}_{27}\text{H}_{20}\text{N}_4\text{NaO}_3$, $\{[\text{M}+\text{Na}]^+\}$: 471.1427, found 471.1424.

5.1.5.5. 4-(3-(Naphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]-

deca-6,9-diene-3,8-dione (6b): brown solid, 20 mg, yield 90%, mp 210.9–213.7 °C; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.58 (s, 1H), 8.16 (d, J = 9.8 Hz, 1H), 8.00–7.82 (m, 3H), 7.56–7.50 (m, 7H), 6.59 (d, J = 10.1 Hz, 2H), 6.15 (d, J = 10.0 Hz, 2H), 4.58 (s, 2H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 183.4, 170.3, 161.9, 142.5, 141.6 (2C), 136.2, 134.1,

133.2, 131.1 (2C), 130.0, 129.7 (2C), 128.6, 128.4, 127.8, 127.2, 126.8, 126.4, 126.1, 124.8 (2C), 123.6, 88.2, 66.0. IR, ν/cm^{-1} : 3053, 2919, 1740, 1678, 1637, 1594, 1528, 1503, 1450, 1236, 1003. HRMS (ESI): Calculated for $\text{C}_{26}\text{H}_{18}\text{N}_4\text{NaO}_3$, $\{[\text{M}+\text{Na}]^+\}$: 457.1271, found 457.1275.

5.1.5.6. 2-Methyl-4-(3-(naphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro-[4.5]deca-6,9-diene-3,8-dione (6b β): yellow solid, 17 mg, yield 71%, mp 186.0–189.7 °C; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.57 (d, J = 1.7 Hz, 1H), 8.16 (dd, J = 8.4, 1.7 Hz, 1H), 7.93–7.84 (m, 3H), 7.55–7.49 (m, 5H), 7.53–7.48 (m, 2H), 6.71 (dd, J = 9.9, 3.2 Hz, 1H), 6.38 (dd, J = 9.9, 3.2 Hz, 1H), 6.18–6.11 (m, 2H), 4.71 (q, J = 6.7 Hz, 1H), 1.57 (d, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.6, 172.6, 161.9, 142.9, 142.7, 142.1, 136.3, 134.1, 133.2, 131.9, 130.2, 129.6 (2C), 128.7, 128.4, 127.8, 127.3, 126.8, 126.5, 126.1, 124.9 (2C), 123.6, 86.6, 73.1, 18.2. IR, ν/cm^{-1} : 2926, 1744, 1680, 1638, 1595, 1502, 1453, 1371, 1265, 1018. HRMS (ESI): Calculated for $\text{C}_{27}\text{H}_{20}\text{N}_4\text{NaO}_3$, $[\text{M}+\text{Na}]^+$: 471.1428, found 471.1421.

5.1.5.7. 4-(3-(Naphthalen-2-yl)-1-(p-tolyl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6ba): yellow solid, 19 mg, yield 80%, mp 220.4–221.8 °C; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.59 (s, 1H), 8.17 (d, J = 8.6 Hz, 1H), 7.9–7.84 (m, 3H), 7.53 (m, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 6.62 (d, J = 9.8 Hz, 2H), 6.18 (d, J = 9.8 Hz, 2H), 4.60 (s, 2H), 2.50 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.5, 170.3, 161.8, 142.4, 141.8 (2C), 140.4, 134.1, 133.8, 133.2, 131.1 (2C), 130.2 (2C), 128.7, 128.4, 127.8, 127.4, 126.8, 126.4, 126.1, 124.8 (2C), 123.6, 88.2, 66.0, 21.4. IR, ν/cm^{-1} : 2924, 1748, 1678, 1638, 1515, 1385, 1268, 1067. HRMS (ESI): Calculated for $\text{C}_{27}\text{H}_{20}\text{N}_4\text{NaO}_2$, $\{[\text{M}+\text{Na}]^+\}$: 471.1428, found 471.1426.

5.1.5.8. 2-Methyl-4-(3-(naphthalen-2-yl)-1-(p-tolyl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6ba β): white solid, 17 mg, yield 70%, mp 193.1–195.0 °C; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.59–8.54 (m, 1H), 8.15 (dd, J = 8.5, 1.7 Hz, 1H), 7.94–7.83 (m, 3H), 7.53–7.47 (m, 2H), 7.44–7.39 (m, 2H), 7.34 (d, J = 8.2 Hz, 2H), 6.71 (dd, J = 10.0, 3.1 Hz, 1H), 6.39 (dd, J = 10.0, 3.1 Hz, 1H), 6.20–6.11 (m, 2H), 4.71 (q, J = 6.5 Hz, 1H), 2.47 (s, 3H), 1.58 (d, J = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.6, 172.6, 161.8, 143.0, 142.6, 142.2, 140.3, 134.1, 133.8, 133.2, 131.8, 130.2, 130.1 (2C), 128.7, 128.4, 127.8, 127.4, 126.8, 126.4, 126.1, 124.7 (2C), 123.6, 86.6, 73.1, 21.4, 18.2. IR, ν/cm^{-1} : 2926, 1744, 1679, 1638, 1515, 1456, 1372, 1265, 1037. HRMS (ESI): Calculated for $\text{C}_{28}\text{H}_{22}\text{N}_4\text{NaO}_3$, $\{[\text{M}+\text{Na}]^+\}$: 485.1584, found 485.1578.

5.1.5.9. 4-(1-(4-Chlorophenyl)-3-(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro-[4.5]deca-6,9-diene-3,8-dione (6bc): brown solid, 17 mg, yield 82%, mp 219.7–223.1 °C; ^1H NMR (400 MHz, Chloroform-*d*) δ 8.55 (s, 1H), 8.12 (d, J = 10.2 Hz, 1H), 7.95–7.82 (m, 3H), 7.59–7.46 (m, 6H), 6.65 (d, J = 10.1 Hz, 2H), 6.20 (d, J = 10.1 Hz, 2H), 4.59 (s, 2H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.4, 170.3, 162.1, 142.6, 141.6 (2C), 136.0, 134.8, 134.2, 133.2, 131.3 (2C), 129.9 (2C), 128.7, 128.5, 127.8, 127.0, 126.9, 126.5, 126.2, 126.1 (2C), 123.5, 88.3, 66.0. IR, ν/cm^{-1} : 2923, 1748, 1679, 1638, 1499,

1458, 1263, 1012. HRMS (ESI): Calculated for $C_{26}H_{17}ClN_4NaO_3$, $\{[M+Na]^+\}$: 491.0881, found 491.0876.

5.1.5.10. 4-(3-(Naphthalen-2-yl)-1-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6bd): yellow solid, 19

mg, yield 76%, mp 178.1–181.5 °C; 1H NMR (400 MHz, Chloroform-*d*) δ 8.55 (s, 1H), 8.12 (d, J = 10.0 Hz, 1H), 7.94–7.84 (m, 5H), 7.73 (d, J = 8.3 Hz, 2H), 7.52 (dd, J = 7.0, 2.4 Hz, 2H), 6.68 (d, J = 10.1 Hz, 2H), 6.23 (d, J = 10.1 Hz, 2H), 4.61 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.3, 170.2, 162.3, 142.8, 141.4 (2C), 139.4, 134.3, 133.2, 131.9 (q, J = 33.5 Hz), 131.4 (2C), 131.2, 128.7, 128.6, 127.9, 126.99, 126.96, 126.93, 126.6, 126.3, 124.8 (2C), 123.5, 120.7 (q, J = 271.4 Hz), 88.4, 66.0. IR, $\nu_{cm^{-1}}$: 2920, 1691, 1457, 1261, 1018. HRMS (ESI): Calculated for $C_{27}H_{18}F_3N_4O_3$, $\{[M+H]^+\}$: 503.1326, found 503.1315.

5.1.5.11. 4-(1-(4-Methoxyphenyl)-3-(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6bf): white solid, 19 mg, yield 80%, mp 188.8–

191.0 °C; 1H NMR (400 MHz, Chloroform-*d*) δ 8.59–8.55 (m, 1H), 8.14 (dd, J = 8.5, 1.7 Hz, 1H), 7.93–7.88 (m, 2H), 7.87–7.83 (m, 1H), 7.52–7.48 (m, 2H), 7.47–7.43 (m, 2H), 7.06–7.01 (m, 2H), 6.64–6.57 (m, 2H), 6.20–6.14 (m, 2H), 4.57 (s, 2H), 3.90 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.5, 170.4, 161.7, 160.7, 142.5, 141.8 (2C), 134.1, 133.2, 131.1 (2C), 129.0, 128.7, 128.4, 127.8, 127.4, 126.8, 126.5 (2C), 126.4, 126.1, 123.6, 114.7 (2C), 88.1, 66.0, 55.7. IR, $\nu_{cm^{-1}}$: 2932, 1747, 1679, 1637, 1513, 1460, 1385, 1253, 1026. HRMS (ESI): Calculated for $C_{27}H_{20}N_4NaO_4$, $\{[M+Na]^+\}$: 487.1377, found 487.1375.

5.1.5.12. 4-(1-(4-Methoxyphenyl)-3-(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)-2-methyl-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6bf β): white

solid, 17 mg, yield 72%, mp 184.7–187.2 °C; 1H NMR (400 MHz, Chloroform-*d*) δ 8.57 (dd, J = 1.7, 0.8 Hz, 1H), 8.15 (dd, J = 8.5, 1.7 Hz, 1H), 7.94–7.82 (m, 3H), 7.55–7.49 (m, 2H), 7.49–7.41 (m, 2H), 7.08–6.99 (m, 2H), 6.75–6.67 (m, 1H), 6.44–6.35 (m, 1H), 6.23–6.11 (m, 2H), 4.70 (q, J = 6.7 Hz, 1H), 3.90 (s, 3H), 1.57 (d, J = 6.7 Hz, 3H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 183.9, 172.9, 162.1, 161.0, 143.4, 143.1, 142.6, 134.4, 133.6, 132.1, 130.5, 129.4, 128.9, 128.7, 128.1, 127.7, 127.1, 126.8 (2C), 126.7, 126.4, 123.9, 114.9 (2C), 86.8, 73.4, 56.0, 18.6. IR, $\nu_{cm^{-1}}$: 2921, 1744, 1680, 1638, 1514, 1460, 1373, 1253, 1026. HRMS (ESI): Calculated for $C_{28}H_{22}N_4NaO_4$, $\{[M+Na]^+\}$: 501.1533, found 501.1529.

5.1.5.13. 4-(1,3-Di(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6bg): white

solid, 22 mg, yield 89%, mp 184.3–186.8 °C; 1H NMR (400 MHz, Chloroform-*d*) δ 8.60 (s, 1H), 8.18 (dd, J = 8.5, 1.7 Hz, 1H), 8.07–8.01 (m, 2H), 7.94 (m, 4H), 7.89–7.85 (m, 1H), 7.66–7.60 (m, 3H), 7.54–7.50 (m, 2H), 6.56 (d, J = 10.0 Hz, 2H), 6.13 (d, J = 10.0 Hz, 2H), 4.57 (s, 2H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 183.8, 170.8, 162.4, 143.0, 142.0 (2C), 134.5, 133.9, 133.7, 133.6, 133.3, 131.4 (2C), 130.2, 129.0, 128.8 (2C), 128.4, 128.2, 128.1, 128.0, 127.6, 127.2, 126.8, 126.5, 124.1, 124.0, 122.7, 88.6, 66.4. IR, $\nu_{cm^{-1}}$: 2921, 1746, 1676, 1638, 1503, 1453, 1281, 1045. HRMS (ESI): Calculated for $C_{30}H_{20}N_4NaO_3$, $\{[M+Na]^+\}$: 507.1428, found 507.1427.

5.1.5.14. 4-(1,3-Di(naphthalen-2-yl)-1H-1,2,4-triazol-5-yl)-2-methyl-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6bgβ): brown solid, 19 mg, yield 72%, mp 174.7–176.3 °C; ¹H NMR (400 MHz, Chloroform-d) δ 8.61 (d, *J* = 1.7 Hz, 1H), 8.19 (dd, *J* = 8.5, 1.7 Hz, 1H), 8.07–8.00 (m, 2H), 8.00–7.83 (m, 5H), 7.69–7.59 (m, 3H), 7.56–7.47 (m, 2H), 6.70 (dd, *J* = 10.1, 3.2 Hz, 1H), 6.31 (dd, *J* = 10.1, 3.1 Hz, 1H), 6.12 (m, 2H), 4.72 (q, *J* = 6.7 Hz, 1H), 1.57 (d, *J* = 6.7 Hz, 3H); ¹³C NMR (151 MHz, Chloroform-d) δ 183.9, 173.1, 162.4, 143.2 (2C), 142.5, 134.5, 133.9, 133.7, 133.6, 133.24, 132.17, 130.5, 130.2, 129.0, 128.8, 128.7, 128.4, 128.2, 128.1, 128.0, 127.7, 127.2, 126.8, 126.5, 124.0, 123.7, 122.6, 87.0, 73.4, 18.6. IR, ν_{cm⁻¹}: 3054, 1742, 1679, 1637, 1600, 1528, 1507, 1451, 1372, 1264, 1037. HRMS (ESI): Calculated for C₃₁H₂₂N₄NaO₃, {[M+Na]⁺}: 521.1584, found 521.1584.

5.1.5.15. 4-(3-(1-Bromonaphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6c): brown solid, 18 mg, yield 70%, mp 210.9–214.9 °C; ¹H NMR (400 MHz, Chloroform-d) δ 8.46 (d, *J* = 8.5 Hz, 1H), 7.87 (dd, *J* = 8.0, 3.9 Hz, 2H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.679–7.60 (m, 7H), 6.63 (d, *J* = 10.0 Hz, 2H), 6.22 (d, *J* = 10.0 Hz, 2H), 4.59 (s, 2H); ¹³C NMR (101 MHz, Chloroform-d) δ 183.6, 170.2, 162.2, 141.7, 141.6 (2C), 136.2, 134.7, 132.6, 131.3 (2C), 130.0, 129.7 (2C), 129.2, 128.4, 128.2, 127.9, 127.8, 127.6, 127.5, 124.7 (2C), 123.5, 88.4, 66.1. IR, ν_{cm⁻¹}: 2924, 1750, 1678, 1638, 1596, 1504, 1452, 1262, 1004. HRMS (ESI): Calculated for C₂₆H₁₈BrN₄O₃, {[M+H]⁺}: 513.0557, found 513.0555.

5.1.5.16. 4-(3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6d): white solid, 19 mg, yield 82%, mp 200.7–202.4 °C; ¹H NMR (400 MHz, Chloroform-d) δ 7.58–7.48 (m, 7H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 9.8 Hz, 2H), 6.12 (d, *J* = 9.8 Hz, 2H), 4.55 (s, 2H), 4.29 (s, 4H); ¹³C NMR (101 MHz, Chloroform-d) δ 183.5, 170.3, 161.5, 145.2, 143.6, 142.1, 141.7 (2C), 136.3, 131.0 (2C), 129.8, 129.6 (2C), 124.8 (2C), 123.5, 119.8, 117.5, 115.6, 88.1, 66.0, 64.5, 64.3. IR, ν_{cm⁻¹}: 2919, 1749, 1679, 1639, 1590, 1500, 1278, 1065. HRMS (ESI): Calculated for 442.1277, C₂₄H₁₈N₄NaO₅, {[M+Na]⁺}: 465.1169, found 465.1170.

5.1.5.17. 4-(1-Phenyl-3-(quinolin-6-yl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6e): brown solid, 17 mg, yield 78%, mp 196.6–198.0 °C; ¹H NMR (400 MHz, Chloroform-d) δ 8.93 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.54 (d, *J* = 1.9 Hz, 1H), 8.40 (dd, *J* = 8.8, 1.9 Hz, 1H), 8.23 (dd, *J* = 8.4, 1.7 Hz, 1H), 8.17 (d, *J* = 8.8 Hz, 1H), 7.57–7.52 (m, 5H), 7.44 (dd, *J* = 8.4, 4.3 Hz, 1H), 6.61–6.51 (m, 2H), 6.19–6.08 (m, 2H), 4.57 (s, 2H); ¹³C NMR (101 MHz, Chloroform-d) δ 183.4, 170.4, 161.2, 151.0, 148.7, 142.7, 141.6 (2C), 136.8, 136.2, 131.2 (2C), 130.1, 129.9, 129.7 (2C), 128.2, 128.1, 127.4, 125.9, 124.8 (2C), 121.7, 88.2, 66.0. IR, ν_{cm⁻¹}: 3056, 1745, 1678, 1638, 1595, 1499, 1477, 1266, 1002. HRMS (ESI): Calculated for C₂₅H₁₈N₅O₃, {[M+Na]⁺}: 458.1224, found 458.1228.

5.1.5.18. 4-(3-(4-Fluoronaphthalen-1-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6f): brown solid, 19 mg, yield 82%, mp 192.1–195.5 °C; ¹H NMR (400 MHz, Chloroform-d) δ 8.98–8.84 (m, 1H), 8.19–8.10 (m, 2H), 7.62–7.53 (m, 7H), 7.22 (s, 1H), 6.65 (d, *J* = 8.6 Hz, 2H), 6.22 (d, *J* = 8.6 Hz, 2H),

4.59 (s, 2H); ^{13}C NMR (151 MHz, Chloroform- d) δ 183.5, 170.1, 161.7, 160.8, 159.1, 142.0, 141.8 (2C), 136.3, 132.1 (132.10, 132.07, J = 18 Hz), 131.1 (2C), 130.0, 129.7 (2C), 128.3 (128.40, 128.34, J = 36 Hz), 128.0, 126.4, 126.1, 124.7 (2C), 123.9 (124.03, 123.93, J = 62 Hz), 122.9 (122.96, 122.93, J = 16 Hz), 120.8 (120.85, 120.81, J = 23 Hz), 109.0 (109.09, 108.95, J = 80 Hz)p, 88.2, 66.0. IR, ν/cm^{-1} : 2920, 1747, 1678, 1638, 1595, 1500, 1477, 1454, 1268, 1005. HRMS (ESI): calculated for $\text{C}_{26}\text{H}_{17}\text{FN}_4\text{NaO}_3$, $\{[\text{M}+\text{Na}]^+\}$: 475.1177, found 475.1180.

5.1.5.19. 4-(3-(9H-Fluoren-2-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]-deca-6,9-diene-3,8-dione (6g): yellow solid, 20 mg, yield 82%, mp 236.1–239.6 °C; ^1H NMR (400 MHz, Chloroform- d) δ 8.24 (s, 1H), 8.10 (dd, J = 8.0, 1.6 Hz, 1H), 7.83 (dd, J = 8.0, 6.2 Hz, 2H), 7.59–7.51 (m, 6H), 7.40 (t, J = 7.4 Hz, 1H), 7.36–7.31 (m, 1H), 6.60–6.54 (m, 2H), 6.17–6.12 (m, 2H), 4.58–4.57 (m, 2H), 3.96 (s, 2H); ^{13}C NMR (101 MHz, Chloroform- d) δ 183.5, 170.3, 162.2, 143.8, 143.6, 143.4, 142.3, 141.7 (2C), 141.2, 136.3, 131.1 (2C), 129.9, 129.7 (2C), 128.3, 127.2, 126.9, 125.3, 125.1, 124.8 (2C), 123.0, 120.3, 120.0, 88.2, 66.0, 36.9. IR, ν/cm^{-1} : 3061, 1750, 1677, 1638, 1596, 1513, 1457, 1235, 1006. HRMS (ESI): Calculated for $\text{C}_{29}\text{H}_{20}\text{N}_4\text{NaO}_3$, $\{[\text{M}+\text{Na}]^+\}$: 495.1428, found 495.1433.

5.1.5.20. 4-(3-(9H-Fluoren-2-yl)-1-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-3,8-dione (6gd): yellow solid, 22 mg, yield 78%, mp 248.6–251.0 °C; ^1H NMR (400 MHz, Chloroform- d) δ 8.22 (s, 1H), 8.11–8.04 (m, 1H), 7.85 (dd, J = 8.2, 4.4 Hz, 4H), 7.72 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 7.4 Hz, 1H), 7.43–7.32 (m, 2H), 6.73–6.61 (m, 2H), 6.28–6.17 (m, 2H), 4.60 (s, 2H), 3.97 (s, 2H); ^{13}C NMR (101 MHz, Chloroform- d) δ 183.4, 170.2, 162.5, 143.8, 143.7, 143.6, 142.5, 141.4 (2C), 141.1, 139.3, 131.9, 131.6, 131.4 (2C), 127.9, 127.5, 127.3, 126.93, 126.92 (126.98, 126.95, 126.91, 126.87, J = 150 Hz), 125.3, 125.2, 124.7 (2C), 123.0, 120.3, 120.1, 88.3, 65.9, 36.9. IR, ν/cm^{-1} : 2923, 1744, 1678, 1638, 1616, 1525, 1449, 1267, 1001. HRMS (ESI): Calculated for $\text{C}_{30}\text{H}_{19}\text{F}_3\text{N}_4\text{NaO}_3$, $\{[\text{M}+\text{Na}]^+\}$: 563.1301, found 563.1296.

5.1.5.21. 4-(3-(Naphthalen-1-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]-deca-6,9-dien-8-one (8a): yellow solid, 10 mg, yield 48%, mp 179.4–180.6 °C; ^1H NMR (400 MHz, Chloroform- d) δ 9.08 (d, J = 8.4 Hz, 1H), 8.19 (d, J = 7.2 Hz, 1H), 7.96–7.81 (m, 2H), 7.69–7.37 (m, 8H), 6.57 (d, J = 10.1 Hz, 2H), 5.97 (d, J = 10.1 Hz, 2H), 4.29 (t, J = 6.2 Hz, 2H), 3.91 (t, J = 6.2 Hz, 2H); ^{13}C NMR (101 MHz, Chloroform- d) δ 184.8, 160.5, 152.9, 143.8 (2C), 137.6, 134.0, 130.8, 130.1, 129.5 (2C), 129.1 (2C), 128.8, 128.4, 127.8, 127.4, 126.8, 126.4, 125.9, 125.5 (2C), 125.2, 87.9, 66.1, 50.0. IR, ν/cm^{-1} : 2926, 1673, 1631, 1593, 1561, 1503, 1067. HRMS (ESI): Calculated for $\text{C}_{26}\text{H}_{21}\text{N}_4\text{O}_2$, $\{[\text{M}+\text{H}]^+\}$: 421.1659, found 421.1665.

5.1.5.22. 4-(3-(Naphthalen-1-yl)-1-(p-tolyl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-dien-8-one (8aa): brown solid, 9mg, yield 40%, mp 170.6–172.1 °C; ^1H NMR (400 MHz, Chloroform- d) δ 9.07 (d, J = 8.2 Hz, 1H), 8.20–8.15 (m, 1H), 7.88 (t, J = 9.0 Hz, 2H), 7.60–7.46 (m, 3H), 7.43–7.41 (m, 2H), 7.25 (s, 2H), 6.58 (d, J = 10.1 Hz, 2H), 6.00 (d, J = 10.1 Hz, 2H), 4.28 (t, J = 6.2 Hz, 2H), 3.87 (t, J = 6.2 Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (151 MHz, Chloroform- d) δ 184.8, 160.3, 152.7, 143.8 (2C), 139.0,

135.1, 134.0, 130.8, 130.0 (3C), 129.1 (2C), 128.3, 127.7, 127.4, 126.7, 126.4, 125.8, 125.3 (2C), 125.1, 87.7, 66.1, 49.8, 21.2. IR, ν/cm^{-1} : 2923, 1691, 1459, 1377, 1042. HRMS (ESI): Calculated for $\text{C}_{27}\text{H}_{23}\text{N}_4\text{O}_2$, $\{[\text{M}+\text{H}]^+\}$: 435.1815, found 435.1815.

5.1.5.23. 4-(3-(Naphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-dien-8-one (8b): yellow solid, 13 mg, yield 60%, mp 186–9–191.0 °C; ^1H NMR (400 MHz, Chloroform-d) δ 8.57–8.52 (m, 1H), 8.15 (dd, J = 8.6, 1.7 Hz, 1H), 7.94–7.83 (m, 3H), 7.52–7.38 (m, 7H), 6.55–6.47 (m, 2H), 5.96–5.87 (m, 2H), 4.29 (t, J = 6.2 Hz, 2H), 3.93 (t, J = 6.2 Hz, 2H); ^{13}C NMR (101 MHz, Chloroform-d) δ 184.8, 159.9, 153.8, 143.5 (2C), 137.6, 133.9, 133.3, 129.6 (2C), 129.0 (2C), 128.9, 128.6, 128.3, 128.1, 127.8, 126.5, 126.3, 125.7, 125.5 (2C), 123.8, 87.9, 66.1, 50.1. IR, ν/cm^{-1} : 2923, 1674, 1634, 1594, 1560, 1503, 1466, 1071. HRMS (ESI): Calculated for $\text{C}_{26}\text{H}_{20}\text{N}_4\text{NaO}_2$, $\{[\text{M}+\text{Na}]^+\}$: 443.1478, found 443.1482.

5.1.5.24. 4-(3-(Naphthalen-2-yl)-1-(p-tolyl)-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-dien-8-one (8ba): brown solid, 10 mg, yield 47%, mp 163.6–165.2 °C; ^1H NMR (400 MHz, Chloroform-d) δ 8.53 (s, 1H), 8.14 (d, J = 10.1 Hz, 1H), 7.94–7.83 (m, 3H), 7.49 (dt, J = 6.2, 3.4 Hz, 2H), 7.36 (d, J = 8.2 Hz, 2H), 7.24 (d, J = 8.2 Hz, 2H), 6.52 (d, J = 10.1 Hz, 2H), 5.95 (d, J = 10.1 Hz, 2H), 4.28 (t, J = 6.2 Hz, 2H), 3.89 (t, J = 6.2 Hz, 2H), 2.41 (s, 3H); ^{13}C NMR (151 MHz, Chloroform-d) δ 185.0, 159.8, 153.6, 143.6 (2C), 139.1, 135.1, 133.9, 133.3, 130.0 (2C), 129.1 (2C), 128.6, 128.2, 128.1, 127.8, 126.4, 126.2, 125.7, 125.5 (2C), 123.8, 87.8, 66.1, 50.0, 21.2. IR, ν/cm^{-1} : 2925, 1676, 1635, 1595, 1562, 1506, 1455, 1378, 1071. HRMS (ESI): Calculated for $\text{C}_{27}\text{H}_{22}\text{N}_4\text{NaO}_2$, $\{[\text{M}+\text{Na}]^+\}$: 457.164035, found 457.1629.

5.1.5.25. 2-Methyl-4-(3-(naphthalen-2-yl)-1-phenyl-1H-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]deca-6,9-dien-8-one (8bb): brown solid, 12 mg, yield 53%, mp 186.6–189.2 °C; ^1H NMR (400 MHz, Chloroform-d) δ 8.54 (s, 1H), 8.15 (dd, J = 8.5, 1.7 Hz, 1H), 7.96–7.81 (m, 3H), 7.54–7.38 (m, 7H), 6.54 (dd, J = 10.1, 3.1 Hz, 1H), 6.49 (dd, J = 10.0, 3.1 Hz, 1H), 5.96 (dd, J = 10.0, 2.1 Hz, 1H), 5.82 (dd, J = 10.1, 2.1 Hz, 1H), 4.62–4.55 (m, 1H), 3.95 (m, 1H), 3.53 (t, J = 9.3 Hz, 1H), 1.45 (d, J = 6.0 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-d) δ 184.9, 160.0, 153.8, 144.6, 143.6, 137.7, 133.9, 133.3, 129.6 (2C), 129.5, 128.8, 128.6, 128.3, 128.2, 128.1, 127.8, 126.5, 126.3, 125.7, 125.6 (2C), 123.8, 88.2, 73.9, 56.4, 18.5. IR, ν/cm^{-1} : 2919, 1675, 1634, 1562, 1505, 1467, 1378, 1070. HRMS (ESI): Calculated for $\text{C}_{27}\text{H}_{22}\text{N}_4\text{NaO}_2$, $\{[\text{M}+\text{Na}]^+\}$: 457.1635, found 457.1641.

5.2. Cell proliferation assay

Breast cancer cell lines, MDA-MB-231, the cervical cancer cell line, HeLa, and the non-small cell lung carcinoma cell line, cell lines were purchased from ATCC. A549 were routinely cultured in DMEM medium supplemented with 10% FBS, 4 mM glutamine, 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin and 0.25 $\mu\text{g}/\text{mL}$ amphotericin. Cultures were maintained in 5% CO_2 at a temperature of 37 °C. The cells were plated in 24-well plates at a density of 20,000 per well in 10% FBS DMED medium. The cells were then treated with synthesized triazole-spirodienone conjugates separately at 5 different doses ranging from 0.01 mM to 1 nM for 5 days, while equal treatment volumes

of DMSO were used as vehicle control. Cell numbers were counted with a cell viability analyzer (BeckmanCoulter). The ratio of drug-treated viable cell numbers to vehicle-treated viable cell numbers were defined as percentage viability. IC₅₀ values were obtained from dose-response curves for each tested compound.

5.3. Cell cycle assay

Cells (5×10^5 cells/mL) were seeded in six-well plates and treated with compounds at different concentrations for 24 h. The cells were then harvested by trypsinization and washed twice with cold PBS. After centrifugation and removal of the supernatants, cells were resuspended in 400 μ L of $1 \times$ PBS buffer. After adding 10 μ L of PI the cells were incubated at room temperature for 15 min in the dark. The stained cells were analyzed by a flow cytometer (BD Accuri C6).

5.4. Apoptosis detection assay

Cells (5×10^5 cells/mL) were seeded in six-well plates and treated with compounds at different concentrations for 24 h. The cells were then harvested by trypsinization and washed twice with cold PBS. After centrifugation and removal of the supernatants, cells were resuspended in 400 μ L of $1 \times$ binding buffer which was then added to 5 μ L of annexin V-FITC and incubated at room temperature for 15 min. After adding 10 μ L of PI the cells were incubated at room temperature for another 15 min in the dark. The stained cells were analyzed by a flow cytometer (BD Accuri C6).

5.5. In vivo antitumor effect

The orthotopic 4T1 tumor-bearing mice were divided into four groups ($n = 6$) randomly. When the tumor volume reached around 100 mm², mice were respectively treated with vehicle control, **6a** at 1 mg/kg, **6a** at 2 mg/kg, and doxorubicin at 1 mg/kg by intraperitoneal injection every day. While body weight and tumor volumes were measured and recorded every day. At the end of treatment, the major organs and tumors were harvested and photographed. Sections prepared from tumor and organs were histopathologically examined and H&E stained, and then imaged by microscope.

5.6. Statistical analysis

All data were presented as mean $\pm$ standard deviation (SD). Statistical significance was performed by two-tailed Student's t-tests for two groups and one-way ANOVA for multiple groups, and $p < .05$, $p < .01$, and $p < .001$ were considered as a significant difference (remarked with *, **, and ***, respectively).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 2. (A) A representative 1,2,4-triazole-spirodienone g-1p from a previous study; (B) introduction of a naphthalene or a benzoheterocycle moiety to triazole-spirodienones.

R^1 =1-naphthalene, 2-naphthalene, 6-quinoline, 1-bromo-2-naphthalene, 1-fluoro-4-naphthalene, 2-9H-fluorene, 6-[2,3-dihydrobenzo[b][1,4]dioxine] R^2 =phenyl, p-toluene, 2-naphthalene, p-chlorobenzene, o-toluene, p-anisole, p-(trifluoromethyl)benzene R^3 =H, CH_3

Fig. 3.
Synthetic route of target derivatives.

Fig. 4.
Cell cycle analysis of MDA-MB-231 cells treated with (a) DMSO; (b) CA4; (c) 6a; (d) 8c.

Fig. 5.
Effects of **6a**, **8c** and CA4 on the induction of cell apoptosis in MDA-MB-231 cells.

Fig. 6.
Body weights of all the groups measured daily post treatments.

Fig. 7.

Tumor volume of each mouse in the groups treated with A) saline; B) 6a at 1 mg/kg; C) 6a at 2 mg/kg; D) dox at 1 mg/kg; E) average tumor volumes and F) Image of isolated tumors; G) average weights of tumors; H) body weights change of all four groups. The significant differences have been indicated, and *, ** and *** represent $p < .05$, $p < .01$ and $p < .001$, respectively.

Fig. 8.
H&E images of major organ and tumor tissues after different treatment, scale bar = 50 μ m.

Table 1

Cytotoxic activity of synthesized compounds on selected tumor cell lines.

Compound	R ¹	R ²	R ³	IC ₅₀ (μM)	
				MDA-MB-231	HeLa A549
6a	1- <u>naphthyl</u>	Phenyl	H	0.05	0.07 0.08
6aβ	1- <u>naphthyl</u>	Phenyl	methyl	0.10	0.11 0.18
6aa	1- <u>naphthyl</u>	<i>p</i> -methylphenyl	H	0.04	0.14 0.14
6 ab	1- <u>naphthyl</u>	<i>o</i> -methylphenyl	H	0.12	0.10 0.13
6b	2- <u>naphthyl</u>	Phenyl	H	0.07	0.12 0.14
6bβ	2- <u>naphthyl</u>	Phenyl	methyl	0.07	0.12 0.31
6ba	2- <u>naphthyl</u>	<i>p</i> -methylphenyl	H	0.03	0.15 0.11
6baβ	2- <u>naphthyl</u>	<i>p</i> -methylphenyl	methyl	0.10	0.53 1.21
6bc	2- <u>naphthyl</u>	<i>p</i> -chlorophenyl	H	0.07	0.72 0.40
6bd	2- <u>naphthyl</u>	<i>p</i> -(trifluoromethyl)phenyl	H	0.07	0.39 0.46
6bf	2- <u>naphthyl</u>	4-methoxy-phenyl	H	0.06	0.20 0.26
6bfβ	2- <u>naphthyl</u>	4-methoxy-phenyl	methyl	0.05	0.14 0.86
6bg	2- <u>naphthyl</u>	2- <u>naphthyl</u>	H	0.05	0.21 0.25
6bgβ	2- <u>naphthyl</u>	2- <u>naphthyl</u>	methyl	0.06	0.30 0.30
6c	1-bromo-2- <u>naphthyl</u>	Phenyl	H	0.05	0.29 0.10
6d	6-[1,4-benzodioxane]	Phenyl	H	0.10	0.07 0.17

Compound	R ¹	R ²	R ³	IC ₅₀ (μM)		
				MDA-MB-231	Hela	A549
6e	6-quinoline	Phenyl	H	0.05	0.11	0.17
6f	1-fluoro-4- <u>naphthyl</u>	Phenyl	H	0.08	0.20	0.32
6g	2-9 <i>H</i> -fluorenyl	Phenyl	H	0.07	0.11	0.15
6gd	2-9 <i>H</i> -fluorenyl	<i>p</i> -(trifluoromethyl)phenyl	H	0.08	0.13	0.18
8a	1- <u>naphthyl</u>	Phenyl	H	0.11	0.15	0.43
8aa	1- <u>naphthyl</u>	<i>p</i> -methylphenyl	H	0.26	0.57	1.52
8b	2- <u>naphthyl</u>	Phenyl	H	0.23	0.37	0.36
8bβ	2- <u>naphthyl</u>	Phenyl	methyl	0.13	0.26	0.32
8ba	2- <u>naphthyl</u>	<i>p</i> -methylphenyl	H	0.16	0.52	2.00
Bendamustine				13.28	>20	–
Vorinostat				3.52	4.52	–

Table 2

In silico ADME parameters of target compounds.

	Standard range	Solubility-level	BBB-level	Absorption-level	logP	PSA-2D	MW	HBD	HBA
	0-5	1-4	0-3	-2-7	<140	<500	<10		
6a	1	2	0	4.132	74.754	434.44	0	5	
6aβ	1	1	0	4.616	74.754	448.47	0	5	
6aa	2	2	0	3.41	86.015	435.43	0	6	
6 ab	1	1	0	4.618	74.754	448.47	0	5	
6b	1	2	0	4.132	74.754	434.44	0	5	
6bβ	1	1	0	4.616	74.754	448.47	0	5	
6ba	1	1	0	4.618	74.754	448.47	0	5	
6aβ	1	1	0	5.102	74.754	462.49	0	5	
6bc	1	1	0	4.797	74.754	468.89	0	5	
6bd	1	1	0	5.074	74.754	502.44	0	5	
6f	1	2	0	4.116	83.684	464.47	0	6	
6fβ	1	2	0	4.6	83.684	478.49	0	6	
6bg	1	1	0	5.041	74.754	484.50	0	5	
6gβ	1	4	1	5.525	74.754	498.53	0	5	
6c	1	1	0	4.881	74.754	513.34	0	5	
6d	2	3	0	3.01	92.614	442.42	0	7	
6e	1	1	0	4.618	74.754	448.47	0	5	
6f	1	1	0	4.338	74.754	452.43	0	5	
6g	1	1	0	4.887	74.754	472.49	0	5	
6gd	1	4	1	5.829	74.754	540.49	0	5	
8a	1	1	0	5.101	57.453	420.46	0	5	
8aa	1	1	0	5.587	57.453	434.48	0	5	
8b	1	1	0	5.101	57.453	420.46	0	5	
8bβ	1	1	0	5.478	57.453	434.48	0	5	
8ba	1	1	0	5.587	57.453	434.48	0	5	

Table 3

Predicting results of SwissTargetPrediction.

Target Name	Typical Target of Anticancer Agent?	PDB Code	Libdock Score
AURKB	Y	4AF3	93.448
ADORA2A	Target for immunosuppressive disorders, inflammatory tissue damage, and neurodegenerative diseases.	–	–
MAPK8	Y	4QTD	91.398
AURKA	Y	4BY1	100.574
MAPK10	Y	2B1P	135.906
IGF1R	Y	3I81	94.451
PDE10A	Possible target for new anti-psychotic or -diabetic drugs.	–	–
KDR	Y	5EW3	–
JAK2	Y	4C61	96.227

Table 4Inhibitory effect (IC_{50}) on AURKA, MAPK10 and JAK2.

Target		6a	6h
AURKA	Repeat 1	>100 mM	>100 mM
	Repeat 2	>100 mM	>100 mM
	Average	>100 mM	>100 mM
	SD	–	–
MAPK10	Repeat 1	>100 mM	>100 mM
	Repeat 2	>100 mM	>100 mM
	Average	>100 mM	>100 mM
	SD	–	–
JAK2	Repeat 1	41.3 mM	20.9 mM
	Repeat 2	76.6 mM	21.4 mM
	Average	59.0 mM	21.2 mM
	SD	25	0.4