Accepted Manuscript

Synthesis and biological evaluation of 2,5-disubstituted furan derivatives as P-glycoprotein inhibitors for Doxorubicin resistance in MCF-7/ADR cell

Ya-Sheng Li, Dong-Sheng Zhao, Xing-Yu Liu, Yi-Xian Liao, Hong-Wei Jin, Gao-Peng Song, Zi-Ning Cui

PII: S0223-5234(18)30346-5

DOI: 10.1016/j.ejmech.2018.04.012

Reference: EJMECH 10361

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 26 January 2018

Revised Date: 4 April 2018

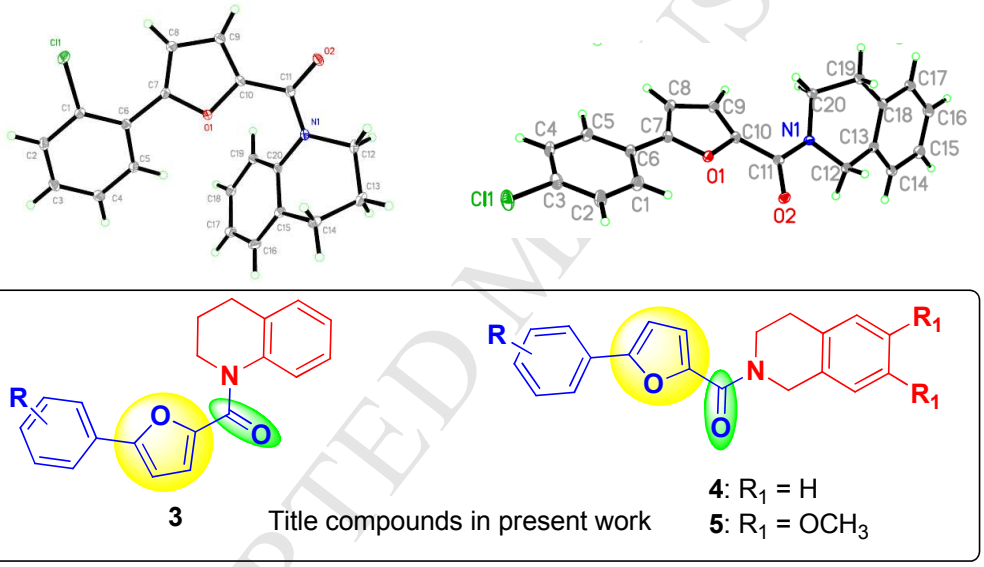
Accepted Date: 4 April 2018

Please cite this article as: Y.-S. Li, D.-S. Zhao, X.-Y. Liu, Y.-X. Liao, H.-W. Jin, G.-P. Song, Z.-N. Cui, Synthesis and biological evaluation of 2,5-disubstituted furan derivatives as P-glycoprotein inhibitors for Doxorubicin resistance in MCF-7/ADR cell, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.04.012.

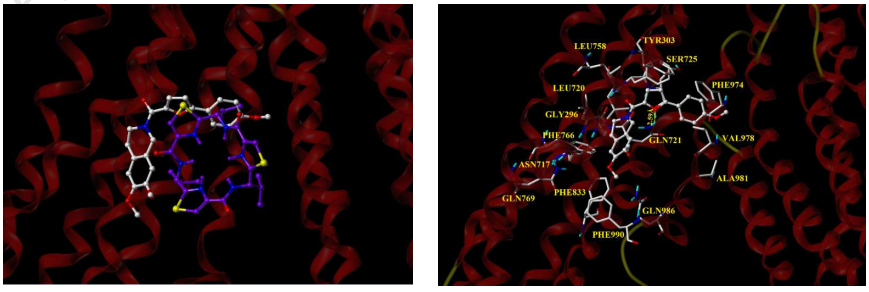
Graphical Abstract

Synthesis and biological evaluation of 2,5-disubstituted furan derivatives as P-glycoprotein inhibitors for Doxorubicin resistance in MCF-7/ADR cell

Ya-Sheng Li [a,#], Dong-Sheng Zhao [b,#], Xing-Yu Liu [a], Yi-Xian Liao [a], Hong-Wei Jin [c], Gao-Peng Song [d,*], Zi-Ning Cui [a,*]



5m: R = 4-OCH_3
P-gp inhibitory activity: EC_{50} = 0.89 ± 0.11 μ M
P_{app} = 5.3
Antiproliferative effect: 97.8 ± 1.9%
Folds of Doxorubicin: 9.2 ± 0.9



Synthesis and biological evaluation of 2,5-disubstituted furan derivatives as P-glycoprotein inhibitors for Doxorubicin resistance in MCF-7/ADR cell

Ya-Sheng Li [a, #], Dong-Sheng Zhao [b, #], Xing-Yu Liu [a], Yi-Xian Liao [a], Hong-Wei Jin [c], Gao-Peng Song [d,*], Zi-Ning Cui [a,*]

[a] State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Integrative Microbiology Research Centre, Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou 510642, China

[b] Department of Pharmacy, Quanzhou Medical College, Quanzhou 362100, China

[c] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

[d] College of Materials and Energy, South China Agricultural University, Guangzhou 510642, China

[#]Both authors contributed equally to this paper.

*Corresponding author. Tel.: +86-20-85288229; Fax: +86-20-85288229

E-mail address: ziningcui@scau.edu.cn (Z. C.), vinsin1021@126.com (G. S.)

Abstract

Multidrug resistance (MDR) is a tendency in which cells become resistant to structurally and mechanistically unrelated drugs, which is mediated by P-glycoprotein (P-gp). It is one of the noteworthy problems in cancer therapy. As one of the most important drugs in cancer therapy, doxorubicin has not good effectiveness if used independently. So targeting the P-gp protein is one of the key points to solve the MDR. Three series of furan derivatives containing tetrahydroquinoline or tetrahydroisoquinoline were designed and synthesized as P-gp inhibitors in this paper. Compound **5m** containing 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline possessed good potency against P-gp (EC_{50} = 0.89±0.11 μM). The preliminary structure–activity relationship and docking studies demonstrated that compound **5m** would be great promise as a lead compound for further study. Most worthy of mention is drug combination of doxorubicin and **5m** displayed antiproliferative effect of about 97.8%. This study provides highlighted P-gp inhibitor for withstanding malignant tumor cell with multidrug resistance especially doxorubicin resistance setting the basis for further studies.

Keywords: tetrahydroquinoline; inhibitors; P-glycoprotein; multidrug resistance; structure-activity relationship

1. Introduction

The overexpression of some transmembrane proteins, belonging to the ABC (ATP Binding Cassette) transporter family, led to Multidrug resistance (MDR) [1]. ABC transporters consumed the energy from the ATP hydrolysis to efflux xenobiotics out of cells. The overexpression of ABC transporter proteins in tumor cells caused MDR that is the main reason for the failure of antineoplastic treatment, because ABC transporters efflux chemotherapeutic agents out of tumor cells [2.3]. P-glycoprotein (P-gp), multidrug resistance-associated protein 1 (MRP1) and breast cancer resistance protein (BCRP) are the key ABC transporter proteins, which are mostly involved in MDR [4,5]. The development of P-gp modulators is one of the considerable strategies approached to overcome MDR in cancer therapy [6-13]. The P-gp inhibitors could be applied in co-administration with the antineoplastic drugs to restore drug sensitivity to tumor cells [14.15].

The most potent P-gp modulators, tariquidar and elacridar (**Figure 1**), subjected to clinical trials, showed high P-gp inhibition but poor selectivity due to their interaction with other ABC transporters such as MRP1 and BCRP [5]. Recently PET (positron emission tomography) studies using the corresponding ^{11}C-radiolabelled ligand showed that both inhibitors demonstrated high activity towards P-gp, as well as moderate activity towards BCRP [16-18].

Based on these lead compounds, some other P-gp potent modulators [19-26] such as MC18 [19,20], MC70 [21,22], MC113 [21,23], and MC266 [19,20] (**Figure 1**) bearing a tetrahydroisoquinoline fragment, were developed for improving the P-gp

selectivity. Among them, MC18 and MC70 were found to be P-gp inhibitors, whereas MC113 and MC266 were P-gp substrates.

Considering these findings, new P-gp ligands with better binding affinities and higher selectivity with respect to that of the lead compound are needed to be developed and discovered. In continuation of our research on the synthesis of biological heterocyclic compounds [27-33], a series of tetrahydroquinoline derivatives containing 5-phenyl-2-furan moiety were synthesized, and their P-gp potency and selectivity were evaluated. The SAR (structure-activity relationship) study and the docking research were discussed in this paper, and the choice of substituents such as fluorine atoms or methoxy groups was made for the additional purpose of developing useful tools with easy radiolabeling points for *in vivo* PET (Positron Emission Computed Tomography, ^{11}C or ^{18}F) analysis to check P-gp activity and expression.

2. Results and discussion

2.1. Chemical synthesis

The synthetic route of title compounds **3a**-**3o** (containing 1,2,3,4-tetrahydroquinoline), **4a**-**4o** (containing 1,2,3,4-tetrahydroisoquinoline) and **5a**-**5o** (containing 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) was described in **Scheme 1**. The key intermediates **2** (the different carboxylic acids **2**) were synthesized from substituted aniline by Meerwein arylation reaction according to the reported procedure [31.33]. Then a mixture of 5-substitutedphenyl-2-furancarboxylic acid **2** and thionyl chloride was refluxed in anhydrous toluene for 3.5 h to afford the various 5-phenyl-2-furancarbonyl chloride, which was added into tetrahydroquinoline in

anhydrous dichloromethane to react and obtain the title compounds **3** (**3a**-**3o**). With the different carboxylic acids **2** and 1,2,3,4-tetrahydroisoquinoline or 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, their coupling reaction was then performed in the presence of EDCI and HOBt to provide the different title compounds **4** (**4a**-**4o**) or **5** (**5a**-**5o**), respectively.

2.2. X-ray diffraction

X-ray single crystals of compounds **3b** and **4d** were obtained as described below in this section. In the case, well-shaped crystals were obtained by interfacial crystallization of *n*-hexane and dichloromethane. A summary of the experimental and crystallography data for compounds **3b** and **4d** were given in **Table S1** to **S3** (see Supplementary materials) and molecular structure of compounds **3b** and **4d** are depicted in **Figure 2**. Crystallographic data were collected at 113(2) K on a Rigaku XtaLAB P200 using a X-ray source Mo/Kα radiation (λ=0.71073 Å). The structure was solved by the direct method with SHELXS-97 [34,35] and refined by the full-matrix least-squares method on F2 data using SHELXL-97 [34,35]. All non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were finally included in their calculated positions. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited at the Cambridge Crystallographic Data Centre with CCDC numbers 1505007 (**3b**) and 1505009 (**4d**), respectively. Copies of the data can be obtained, free of charge, on application to CDC, 12 Union Road, Cambridge CB2 1EZ, UK.

2.3. Biological evaluation and SAR studies.

In this paper, three series of benzene substituted furyl derivatives were studied, and all the derivatives displayed good bioactive potency. *In vitro* data for the inhibition of P-gp and P_{app} (apparent permeability) was reported in **Table 1**. Cyclosporin A and verapamil were chosen as the positive controls. As depicted in **Table 1**, compounds with substituent at *para*-position of benzene ring exerted better bioactivity against P-gp (EC_{50}< 10 μM) than the other two substituents in benzene ring , suggesting the position of benzene ring played a key role in bioactivity. As shown in **Table 1**, compound **5m** containing 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline and methoxyl group at *para*-position of benzene ring showed the best bioactivity against P-gp (EC_{50} = 0.89 μM) among all the title compounds, which displayed better activity than both cyclosporin A and verapamil (EC_{50} = 83.68 μM and 20.54 μM against P-gp, respectively) and the similar to the MC113 [21,23]. Analysis of three series of potential inhibitors revealed that **5** (containing 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) displayed stronger bioactivity than both **3** (containing 1,2,3,4-tetrahydroquinoline) and **4** (containing 1,2,3,4-tetrahydroisoquinoline). For instance, compound **5m** showed better bioactivity (EC_{50} = 0.89 μM against P-gp) than **4m** (EC_{50} = 5.48 μM against P-gp) and **3m** (EC_{50} = 6.82 μM against P-gp). Similarly, compound **5j** displayed better bioactivity (EC_{50} = 9.23 μM against P-gp) than **4j** (EC_{50} = 12.45 μM against P-gp) and **3j** (EC_{50} = 15.46 μM against P-gp). These results indicated that the moiety of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline has a crucial influence on the inhibitory activity against P-gp. Like the best inhibitor **5m**, compounds **5l** and **5d** also exhibited strong inhibition against P-gp (EC_{50} = 4.34 μM

and 3.27 μM, respectively). What is more, all the compounds **5m**, **5l** and **5d** showed greater P_{app} values more than 2, which displayed better selectivity compared to verapamil, suggesting that they could be potential P-gp inhibitors. In addition, it was observed that electron-donating group (methoxyl group) and the halogen substituents (bromine atom and chlorine atom) at *para*-position of benzene ring are excellent options for future designs of P-gp inhibitor.

Doxorubicin resistance is one of the thorniest problems in tumor therapy. As shown in **Table 2**, the simultaneous treatment with Doxorubicin and the title compounds increased Doxorubicin accumulation in overexpressing P-gp MCF-7/ADR cells. Compound **5m** displayed an increased Doxorubicin cell accumulation of 9.2- fold at 20 μM in the cells (**Table 2**). Compounds **5d** and **5l** increased Doxorubicin accumulation 6.8- and 5.0- fold, respectively (**Table 2**). At 20 μM the positive references Cyclosporin A and Verapamil showed an increase of Doxorubicin cell accumulation of 5.4- and 3.6- fold, respectively. The antiproliferative effect indicated that Doxorubicin cell accumulation induced by the title compounds improved that of the chemotherapeutic. At 5 μM, Doxorubicin itself demonstrated a low antiproliferative effect (5.3%) as listed in **Table 2**. In the presence of 20 μM compound **5m**, Doxorubicin inhibited cell growth of about 97.8%. Moreover, high antiproliferative effect was induced by Doxorubicin compounds **5d** and **5l** (89.6% and 79.8%). Mixed Cyclosporin A or Verapamil with Doxorubicin displayed good to moderate increase of antiproliferative effect (84.2% and 52.5%). The results of Doxorubicin antiproliferative effect as drugs combination with the title compounds

are well correlate with the increased Doxorubicin accumulation. These findings suggested that the drugs combination of Doxorubicin with the title compounds could improve Doxorubicin antiproliferative effect in MCF-7/ADR tumor cells.

Moreover, title compounds **5d**, **5l** and **5m** were evaluated in the absence of Doxorubicin for the cytotoxicity effects at 24 and 48 h, respectively. The cytotoxicity of tested compounds at 100 μM, checked in cell medium after treatment, was negligible (< 15% for each tested compound). Further study will be undergoing to evaluate *in vivo* bioactivity using some suitable animal models, such as the established patient-derived orthotopic xenograft (PDOX) nude mouse models where both efficacy and toxicity of the title compounds could be detected [36-39].

2.4. Docking study

Considering the inhibitory activity of title compounds, it was of interest to explore the binding to the active site of P-gp. Docking results revealed that compound **5m** was well located within P-gp. Binding models of compound **5m** and Cyclosporin A were depicted in **Figure 3**. Generally, a key hydrogen bond interaction could be observed between **O** of the furan ring and the **H** at amine of Gln721 (distance: 2.59Å). At the active site, the docking showed molecular skeleton shape from X-ray single crystal diffraction is folded. 2-Phenyl-furane moiety and 1,2,3,4-tetrahydroisoquinoline moiety have good site in this dock (**Figure 3**). The bending angle of amide bond revealed vital linking effect for those crucial pharmacophores. The hydrophobic effect of three *para*-methoxy groups (-OMe) at benzene ring and 1,2,3,4-tetrahydroisoquinoline to enhance the binding affinity with the enzyme. Such

orientations and interactions would form the foundation for inhibition of P-glycoprotein and increase Doxorubicin intracellular accumulation in MCF-7/ADR cells.

3. Conclusion

In this study, three series of 2,5-disubstituted furan derivatives were synthesized and evaluated as P-glycoprotein inhibitors in Doxorubicin resistance MCF-7/ADR cells. Their *in vitro* activities for the inhibition of P-gp and P_{app} (apparent permeability) were evaluated, and the results showed that compounds containing 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (series **5**) exhibited the best bioactivity among these three series of compounds. The primary structure-activity relationship (SAR) study showed that substituents at *para*-position of benzene ring in the molecule were favored to the bioactivity. Compound **5m** showed the highest inhibitory activity, which increased Doxorubicin accumulation 9.2- fold at 20 μM in overexpressing P-gp MCF-7/ADR cells. The docking results revealed interactions of compounds **5** within P-gp protein where a key hydrogen bond interaction could be observed between **O** of the furan ring and the **H** at amine of Gln721.

4. Experimental procedure

4.1. Chemistry

4.1.1. Materials and methods

All solvents and reagents were obtained from commercial sources without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance DRX spectrometer at 600 and 150 MHz or 400 and 100 MHz. Chemical shifts are reported

as δ values in parts per million (ppm), while tetramethylsilane (TMS) was used as an internal standard. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; td, triplet of doublets; ddd, doublet of doublet of doublets; brs, broad singlet; m, multiplet. Coupling constants (*J* values) are given in hertz (Hz). Compounds were dissolved in DMSO-d_6. Mass spectra were recorded on a Q-TOF Global mass spectrometer. Elemental analysis was carried out with a Flash EA 1112 elemantal analyzer. All the melting points were determined with a Cole-Parmer melting point apparatus while the thermometer was uncorrected. Thin-layer chromatography (TLC) was performed using Merck 60 F254 silica gel plates. Column chromatography was performed using silica gel (200-300 mesh, Qingdao, China) with a linear solvent gradient.

*4.1.2. General synthesis of compounds **3a-3o***

A mixture of 5-substituted phenyl-2-furancarboxylic acid **2** (100 mmol) and thionyl chloride (500 mmol) was refluxed in anhydrous benzene for 3.5 h. The excess of thionyl chloride and the solvent were distilled off, and the residue was dissolved in anhydrous dichloromethane. The solution of 5-substituted phenyl-2-furancarboxylic chloride in anhydrous dichloromethane was added into tetrahydroquinoline (110 mmol). The mixture was stirred and refluxed for 4 h. After cooling, the solvent was evaporated off under reduced pressure, and the solid was recrystallized from ethanol to obtain compounds **3a-3o.**

*4.1.2.1. (5-Phenylfuran-2-yl)(3,4-dihydroquinolin-1(2H)-yl)-Methanone (**3a**).* yellow liquid, yield 92.3%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.43 (d, *J* = 7.5 Hz, 2H), 7.37

(t, *J* = 7.6 Hz, 2H), 7.33-7.27 (m, 2H), 7.11 (td, *J* = 7.4, 1.1 Hz, 1H), 7.05-6.98 (m, 4H), 3.86 (t, *J* = 6.5 Hz, 2H), 2.80 (t, *J* = 6.5 Hz, 2H), 1.96 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.09, 154.61, 147.27, 139.23, 132.28, 129.66, 129.31, 129.00, 128.73, 126.09, 125.10, 124.50, 124.46, 119.17, 107.77, 44.65, 26.58, 24.16. ESIMS(*m/z*): 304.1330 $[M + H]^+$.

*4.1.2.2. [5-(2-Chlorophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone (**3b**).* light yellow solid, yield 88.7%, m.p. 146-148 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.54 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.35 (td, *J* = 7.7, 1.7 Hz, 1H), 7.31-7.28 (m, 2H), 7.18 (d, *J* = 3.6 Hz, 1H), 7.16 (d, *J* = 7.5 Hz, 1H), 7.12 (td, *J* = 7.4, 0.9 Hz, 1H), 7.05-7.02 (m, 2H), 6.97 (d, *J* = 8.0 Hz, 1H), 3.86 (t, *J* = 6.5 Hz, 2H), 2.80 (t, *J* = 6.5 Hz, 2H), 1.97 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.98, 150.66, 147.55, 139.16, 132.46, 131.22, 130.33, 130.08, 128.80, 128.60, 127.93, 127.83, 126.25, 125.22, 124.42, 118.60, 112.71, 44.63, 26.56, 24.13. Anal. Calcd. (%) for $C_{20}H_{16}ClNO_2$: C, 71.24; H, 4.61; N, 4.38. Found: C, 71.11； H, 4.77; N, 4.15.

*4.1.2.3. [5-(3-Chlorophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone (**3c**).* light yellow solid, yield 92.7%, m.p. 153-155 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.47-7.40 (m, 4H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.11 (m, 2H), 7.05-6.96 (m, 3H), 3.86 (t, *J* = 6.5 Hz, 2H), 2.80 (t, *J* = 6.5 Hz, 2H), 1.96 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.82, 152.80, 147.76, 139.18, 134.23, 132.60, 131.55, 131.23, 128.64, 128.62, 126.09, 125.27, 124.55, 124.01, 122.99, 119.18, 109.12, 44.55, 26.57, 24.16. Anal. Calcd. (%) for $C_{20}H_{16}ClNO_2$: C, 70.99; H, 4.89; N, 3.98. Found: C, 71.11； H, 4.77; N, 4.15.

*4.1.2.4. [5-(3-Chlorophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone (**3d**).* white solid, yield 90.1%, m.p. 159-160 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.42-7.34 (m, 3H), 7.33 – 7.28 (m, 2H), 7.17 (d, *J* = 3.6 Hz, 1H), 7.13 (t, *J* = 7.4 Hz, 1H), 7.05 (d, *J* = 3.6 Hz, 1H), 7.02 (t, *J* = 7.4 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 1H), 3.85 (t, *J* = 6.5 Hz, 2H), 2.80 (t, *J* = 6.5 Hz, 2H), 1.97 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.98, 153.40, 147.52, 139.13, 133.46, 132.35, 129.40, 128.74, 128.51, 126.12, 126.08, 125.17, 124.51, 119.15, 108.47, 44.67, 26.57, 24.15. Anal. Calcd. (%) for $C_{20}H_{16}ClNO_2$: C, 71.34; H, 4.47; N, 4.40. Found: C, 71.11； H, 4.77; N, 4.15.

*4.1.2.5. [5-(2-Fluorophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone (**3e**).* white solid, yield 82.6%, m.p. 150-151 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.42 (td, *J* = 7.7, 3.6 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.9 Hz, 1H), 7.16-7.10 (m, 4H), 7.05-7.01 (m, 2H), 6.96 (d, *J* = 7.9 Hz, 1H), 3.85 (t, *J* = 6.5 Hz, 2H), 2.80 (t, *J* = 6.5 Hz, 2H), 1.97 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.87 (d, $^{1}J_{C\text{-}F}$ = 243.5 Hz), 158.89, 153.14 (d, $^{4}J_{C\text{-}F}$ = 3.0 Hz), 147.71, 139.20, 132.51, 131.84 (d, $^{3}J_{C\text{-}F}$ = 8.7 Hz), 131.53 (d, $^{3}J_{C\text{-}F}$ = 8.6 Hz), 128.70, 126.12, 125.22, 124.52, 120.54 (d, $^{4}J_{C\text{-}F}$ = 2.6 Hz), 119.16, 115.70 (d, $^{2}J_{C\text{-}F}$ = 21.2 Hz), 111.09 (d, $^{2}J_{C\text{-}F}$ = 23.7 Hz), 109.07, 44.59, 26.56, 24.16. Anal. Calcd. (%) for $C_{20}H_{16}FNO_2$: C, 74.58; H, 4.94; N, 4.57. Found: C, 74.75; H, 5.02;N, 4.36.

*4.1.2.6. [5-(3-Fluorophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone (**3f**).* white solid, yield 88.9%, m.p. 156-158 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.41 (td, *J* = 7.7, 3.6 Hz, 1H), 7.30 (d, *J* = 7.4 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 1H),

7.17-7.10 (m, 4H), 7.05-7.00 (m, 2H), 6.95 (d, *J* = 8.0 Hz, 1H), 3.85 (t, *J* = 6.5 Hz, 2H), 2.80 (t, *J* = 6.5 Hz, 2H), 1.97 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.87 (d, $^{1}J_{C\text{-}F}$ = 243.6 Hz), 158.90, 153.13 (d, $^{4}J_{C\text{-}F}$ = 3.1 Hz), 147.71, 139.20, 132.51, 131.84 (d, $^{3}J_{C\text{-}F}$ = 8.8 Hz), 131.53 (d, $^{2}J_{C\text{-}F}$ = 8.6 Hz), 128.70, 126.12, 125.22, 124.52, 120.54 (d, $^{4}J_{C\text{-}F}$ = 2.6 Hz), 119.16, 115.70 (d, $^{2}J_{C\text{-}F}$ = 21.2 Hz), 111.09 (d, $^{2}J_{C\text{-}F}$ = 23.8 Hz), 109.07, 44.59, 26.56, 24.16. Anal. Calcd. (%) for $C_{20}H_{16}FNO_2$: C, 74.82; H, 5.21; N, 4.19. Found: C, 74.75; H, 5.02;N, 4.36.

4.1.2.7. [5-(4-Fluorophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone ***(3g)***. white solid, yield 90.5%, m.p. 149-150 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.46 (dd, *J* = 8.8, 5.4 Hz, 2H), 7.28 (d, *J* = 7.4 Hz, 1H), 7.23 (t, *J* = 8.8 Hz, 2H), 7.11 (td, *J* = 7.4, 1.1 Hz, 1H), 7.02 (dd, *J* = 7.9, 5.5 Hz, 2H), 6.98 (dd, *J* = 7.9, 5.8 Hz, 2H), 3.85 (t, *J* = 6.5 Hz, 2H), 2.80 (t, *J* = 6.5 Hz, 2H), 1.96 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.52 (d, *J* = 246.5 Hz), 159.06, 153.73, 147.29, 139.22, 132.33, 128.76, 126.70 (d, *J* = 8.4 Hz), 126.38 (d, *J* = 3.0 Hz), 126.09, 125.14, 124.52, 119.22, 116.42 (d, *J* = 22.0 Hz), 107.68, 44.65, 26.57, 24.16. Anal. Calcd. (%) for $C_{20}H_{16}FNO_2$: C, 74.86; H, 4.87; N, 4.52. Found: C, 74.75; H, 5.02;N, 4.36.

4.1.2.8. [5-(2-Nitrophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone ***(3h)***. yellow solid, yield 83.8%, m.p. 161-163 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91 (d, *J* = 7.9 Hz, 1H), 7.71 (t, *J* = 7.3 Hz, 1H), 7.64-7.60 (m, 2H), 7.24 (d, *J* = 7.3 Hz, 1H), 7.10-7.03 (m, 3H), 6.96 (dd, *J* = 13.8, 3.6 Hz, 2H), 3.82 (t, *J* = 6.5 Hz, 2H), 2.79 (t, *J* = 6.5 Hz, 2H), 1.95 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.96, 149.49, 148.86, 147.56, 138.79, 133.06, 132.06, 130.58, 129.69, 129.03,

126.10, 125.21, 124.55, 124.36, 122.40, 118.53, 111.68, 44.85, 26.47, 24.05. Anal. Calcd. (%) for $C_{20}H_{16}N_2O_4$: C, 69.10; H, 4.51; N, 8.24. Found: C, 68.96; H, 4.63;N, 8.04.

*4.1.2.9. [5-(3-Nitrophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone (**3i**).* yellow liquid, yield 88.5%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (dd, *J* = 8.2, 1.6 Hz, 1H), 8.06 (s, 1H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.35-7.31 (m, 2H), 7.13 (td, *J* = 7.5, 1.0 Hz, 1H), 7.05 (d, *J* = 3.6 Hz, 1H), 7.03-6.97 (m, 2H), 3.87 (t, *J* = 6.5 Hz, 2H), 2.82 (t, *J* = 6.5 Hz, 2H), 1.98 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.78, 152.04, 148.77, 148.20, 139.05, 132.63, 131.10, 131.01, 130.50, 128.70, 126.04, 125.42, 124.51, 123.28, 119.13, 118.62, 110.07, 44.60, 26.57, 24.16. ESIMS(*m/z*): 349.1182 $[M + H]^+$.

*4.1.2.10. [5-(4-Nitrophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone (**3j**).* yellow solid, yield 93.0%, m.p. 161-162 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22 (d, *J* = 8.8 Hz, 2H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 3.7 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.14 (td, *J* = 7.4, 1.6 Hz, 1H), 7.03 (d, *J* = 3.7 Hz, 1H), 7.02-6.98 (m, 2H), 3.87 (t, *J* = 6.5 Hz, 2H), 2.81 (t, *J* = 6.5 Hz, 2H), 1.98 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.82, 152.18, 148.91, 147.06, 138.96, 135.34, 132.55, 128.80, 126.12, 125.40, 125.15, 124.77, 124.52, 119.15, 111.65, 44.73, 26.56, 24.12. Anal. Calcd. (%) for $C_{20}H_{16}N_2O_4$: C, 68.71; H, 4.86; N, 7.81. Found: C, 68.96; H, 4.63;N, 8.04.

*4.1.2.11. [5-(4-Methylphenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone (**3k**).* white solid, yield 89.2%, m.p. 152-155 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ

7.31 (d, *J* = 8.0 Hz, 2H, ArH), 7.28 (d, *J* = 7.4 Hz, 1H, QuH), 7.17 (d, *J* = 8.0 Hz, 2H, ArH), 7.10 (td, *J* = 7.4, 1.2 Hz, 1H, QuH), 7.03-6.99 (m, 1H), 6.98-6.95 (m, 3H), 3.85 (t, *J* = 6.5 Hz, 2H), 2.79 (t, *J* = 6.5 Hz, 2H), 2.29 (s, 3H), 1.96 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.10, 154.89, 146.88, 139.27, 138.64, 132.24, 129.86, 128.71, 127.02, 126.07, 125.05, 124.52, 124.45, 119.23, 107.03, 44.64, 26.58, 24.17, 21.29. Anal. Calcd. (%) for $C_{21}H_{19}NO_2$: C, 79.59; H, 6.21; N, 4.20. Found: C, 79.47; H, 6.03; N, 4.41.

4.1.2.12. [5-(4-Bromophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone ***(3l)***. yellow solid, yield 90.8%, m.p. 191-192 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.62-7.55 (m, 2H), 7.45-7.27 (m, 3H), 7.15-7.10 (m, 2H), 7.09-6.96 (m, 3H), 3.85 (t, *J* = 6.5 Hz, 2H), 2.79 (t, *J* = 6.5 Hz, 2H), 1.96 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.99, 153.45, 147.54, 139.13, 132.35, 132.30, 128.84, 128.75, 126.35, 126.08, 125.19, 124.52, 122.09, 119.15, 108.55, 44.68, 26.57, 24.15. Anal. Calcd. (%) for $C_{20}H_{16}BrNO_2$: C, 63.01; H, 4.08; N, 3.82. Found: C, 62.84; H, 4.22; N, 3.66.

4.1.2.13. [5-(4-Methoxyphenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone ***(3m)***. white liquid, yield 83.8%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.36 (d, *J* = 8.8 Hz, 2H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.11 (td, *J* = 7.4, 1.2 Hz, 1H), 7.04-7.01 (m, 1H), 6.97 (dd, *J* = 8.4, 5.8 Hz, 2H), 6.93 (d, *J* = 8.9 Hz, 2H), 6.88 (d, *J* = 3.6 Hz, 1H), 3.85 (t, *J* = 6.5 Hz, 2H), 3.77 (s, 3H), 2.79 (t, *J* = 6.5 Hz, 2H), 1.96 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.01, 159.14, 154.93, 146.55, 139.32, 132.18, 128.73, 126.11, 126.07, 125.02, 124.52, 122.47, 119.39, 114.79, 106.13, 55.70, 44.64, 26.58, 24.17. ESIMS(*m/z*): 334.1451 $[M + H]^+$.

4.1.2.14.

[5-(2',4'-di-Fluorophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone

(***3n*** *).* white solid, yield 92.6%, m.p. 133-136 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.40 (ddd, J = 11.6, 9.3, 2.5 Hz, 1H), 7.29 (d, J = 7.4 Hz, 1H), 7.20 (dd, J = 15.3, 8.5 Hz, 1H), 7.14-7.10 (m, 2H), 7.05-6.97 (m, 3H), 6.87 (t, J = 3.6 Hz, 1H), 3.86 (t, J = 6.5 Hz, 2H), 2.80 (t, J = 6.5 Hz, 2H), 1.97 (p, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.39 (dd, $^{1}J_{C\text{-}F}$ = 249.3 Hz, $^{3}J_{C\text{-}F}$ = 12.3 Hz), 158.92 (dd, $^{1}J_{C\text{-}F}$ = 253.2 Hz, $^{3}J_{C\text{-}F}$ = 12.4 Hz), 158.88, 147.87 (d, $^{4}J_{C\text{-}F}$ = 2.2 Hz), 147.38, 139.08, 132.41, 128.76, 127.82 (dd, $^{3}J_{C\text{-}F}$ = 9.94 Hz, $^{3}J_{C\text{-}F}$ = 9.99 Hz), 126.12, 125.23, 124.48, 118.96, 114.59 (dd, $^{2}J_{C\text{-}F}$ = 12.3 Hz, $^{3}J_{C\text{-}F}$ = 3.8 Hz), 112.78 (dd, $^{2}J_{C\text{-}F}$ = 21.9 Hz, $^{4}J_{C\text{-}F}$ = 3.2 Hz), 111.31 (d, $^{3}J_{C\text{-}F}$ = 10.1 Hz), 105.44 (dd, J = 25.9 Hz, J = 25.9 Hz), 44.66, 26.55, 24.13. Anal. Calcd. (%) for $C_{20}H_{15}F_2NO_2$: C, 70.60; H, 4.67; N, 3.95. Found: C, 70.79; H, 4.46; N, 4.13.

4.1.2.15.

[5-(2',6'-di-Fluorophenyl)-2-furanyl](3,4-dihydroquinolin-1(2H)-yl)-Methanone

(***4o*** *).* white liquid, yield 82.6%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.51-7.46 (m, 1H), 7.26-7.20 (m, 3H), 7.11-7.03 (m, 3H), 6.99-6.95 (m, 2H), 3.87 (t, J = 6.5 Hz, 2H), 2.79 (t, J = 6.5 Hz, 2H), 1.96 (p, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.18, 159.17 (dd, $^{1}J_{C\text{-}F}$ = 253.0, $^{3}J_{C\text{-}F}$ = 6.5 Hz), 148.27, 144.28, 138.80, 131.85, 131.44 (dd, $^{3}J_{C\text{-}F}$ = 10.6 Hz, $^{3}J_{C\text{-}F}$ = 10.5 Hz), 128.98, 126.13, 125.13, 124.29, 117.97, 114.37 (dd, $^{3}J_{C\text{-}F}$ = 5.4 Hz, $^{3}J_{C\text{-}F}$ = 5.5 Hz), 112.96 (dd, $^{2}J_{C\text{-}F}$ = 21.4, $^{4}J_{C\text{-}F}$ = 4.0 Hz),

107.71 (dd, $^{2}J_{C\text{-}F}$ = 16.3 Hz, $^{2}J_{C\text{-}F}$ = 16.3 Hz), 45.01, 26.50, 24.07. ESIMS(*m/z*): 340.1154 $[M + H]^{+}$.

*4.1.3. General synthesis of title compounds **4** and **5**.*

A mixture of 5-substituted phenyl-2-furancarboxylic acid **2** (100 mmol) and 1,2,3,4-tetrahydroisoquinoline (105 mmol) or 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (105 mmol) dissolved in anhydrous dichloromethane, their coupling reaction was then performed in the presence of EDCI (120 mmol) and HOBt (120 mmol) to provide the different title compounds **4** (**4a**-**4o**) or **5** (**5a**-**5o**), respectively.

*4.1.3.1. (5-Phenyl-2-furanyl)(3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4a**).* white solid, yield 95.1%, m.p. 105-106 ºC. ^{1}H NMR (600 MHz, DMSO-*d6*) δ 7.82 (d, *J* = 7.6 Hz, 2H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.27-7.13 (m, 6H), 4.81 (s, 2H), 3.97 (s, 2H), 2.97 (s, 2H); ^{13}C NMR (151 MHz, DMSO-*d6*) δ 158.48, 154.26, 146.48, 134.60, 133.19, 129.33, 128.98, 128.47, 128.39, 126.50, 126.36, 126.15, 124.03, 117.90, 107.16. Anal. Calcd. (%) for $C_{20}H_{17}NO_2$: C, 79.30; H, 5.78; N, 4.48. Found: C, 79.19; H, 5.65;N, 4.62.

*4.1.3.2. [5-(2-Chlorophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4b**).* yellow liquid, yield 97.1%, m.p. 118-119 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.89 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.61 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.50 (td, *J* = 7.6, 1.2 Hz, 1H), 7.45-7.41 (m, 1H), 7.28-7.20 (m, 6H), 4.77 (s, 2H), 4.01 (s, 2H), 2.96 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.86, 151.12, 147.33, 135.09, 133.62, 131.34, 1

30.43, 130.20, 129.11, 128.93, 128.26, 128.13, 127.06, 126.87, 126.71, 117.99, 112.56. ESIMS(*m/z*): 338.0953 $[M + H]^+$.

*4.1.3.3. [5-(3-Chlorophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4c**).* light yellow solid, yield 93.3%, m.p. 122-124 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88-7.75 (m, 2H), 7.57-7.42 (m, 2H), 7.31-7.16 (m, 6H), 4.78 (s, 2H), 3.96 (s, 2H), 2.97 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.99, 152.31, 148.95, 147.81, 135.90, 135.09, 133.58, 131.36, 131.30, 130.63, 128.92, 127.15, 126.82, 123.41, 118.81, 118.25, 110.35. Anal. Calcd. (%) for $C_{20}H_{16}ClNO_2$: C, 70.98; H, 4.85; N, 3.98. Found: C, 71.11； H, 4.77; N, 4.15.

*4.1.3.4. [5-(4-Chlorophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4d**).* yellow solid, yield 93.3%, m.p. 126-128 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.82 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 8.6 Hz, 2H), 7.30-7.16 (m, 6H), 4.81 (s, 2H), 3.95 (s, 2H), 2.97 (s, 2H); 13C NMR (151 MHz, DMSO-d_6) δ 158.95, 153.65, 147.23, 135.14, 133.69, 133.44, 129.59, 128.90, 128.72, 127.05, 126.92, 126.69, 126.30, 118.40, 108.40. Anal. Calcd. (%) for $C_{20}H_{16}ClNO_2$: C, 70.99; H, 4.86; N, 4.35. Found: C, 71.11； H, 4.77; N, 4.15.

*4.1.3.5. [5-(2-Fluorophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4e**).* white solid, yield 90.9%, m.p. 137-138 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.87 (t, J = 7.8 Hz, 1H), 7.48-7.43 (m, 1H), 7.41-7.34 (m, 2H), 7.30-7.16 (m, 5H), 7.02 (t, J = 3.4 Hz, 1H), 4.78 (s, 2H), 4.00 (s, 2H), 2.98 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.86 (C=O), 158.75 (d, ^1J = 250.3 Hz, C-2, Ph), 148.97 (d, ^3J = 5.7 Hz, C-5, Fu), 147.17 (C-2, Fu), 135.11, 133.66, 130.77 (d, ^3J = 8.5 Hz, C-4, Ph), 128.91,

127.07, 126.91 (d, ^{4}J = 1.9 Hz, C-4, Fu), 126.69, 125.64 (d, ^{4}J = 3.2 Hz, C-5, Ph), 118.28 (2C), 117.82 (d, ^{2}J = 11.8 Hz, C-1, Ph), 116.86 (d, ^{2}J = 21.1 Hz, C-3, Ph), 111.67 (d, ^{3}J = 10.3 Hz, C-6, Ph). Anal. Calcd. (%) for $C_{20}H_{16}FNO_2$: C, 74.93; H, 4.87; N, 4.58. Found: C, 74.75; H, 5.02;N, 4.36.

*4.1.3.6. [5-(3-Fluorophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4f**).* white solid, yield 89.6%, m.p. 133-135 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.64 (t, *J* = 9.6 Hz, 1H), 7.54 (dd, *J* = 14.3, 7.8 Hz, 2H), 7.27-7.20 (m, 7H), 4.76 (s, 2H), 3.98 (s, 2H), 2.97 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.03 (d, ^{1}J = 243.5 Hz, C-3, Ph), 158.91 (C=O), 153.42 (d, ^{4}J = 3.2 Hz, C-5, Fu), 147.41(C-2, Fu), 135.14, 133.69, 132.05 (d, ^{3}J = 8.7 Hz, C-1, Ph), 131.72 (d, ^{3}J = 8.5 Hz, C-5, Ph), 128.91, 127.05, 126.89, 126.69, 120.61 (d, ^{4}J = 2.1 Hz, C-6, Ph), 118.34, 115.71 (d, ^{2}J = 21.2 Hz, C-2, Ph), 111.30 (d, ^{2}J = 23.6 Hz, C-4, Ph), 109.01. Anal. Calcd. (%) for $C_{20}H_{16}FNO_2$: C, 74.58; H, 4.86; N, 4.57. Found: C, 74.75; H, 5.02;N, 4.36.

*4.1.3.7. [5-(4-Fluorophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4g**).* white solid, yield 89.6%, m.p. 133-135 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88-7.84 (m, 2H, ArH), 7.36-7.32 (m, 2H, ArH), 7.26-7.19 (m, 5H), 7.13 (d, *J* = 3.6 Hz, 1H, FuH), 4.79 (s, 2H), 3.96 (s, 2H), 2.97 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.54 (d, ^{1}J = 246.2 Hz, C-4, Ph), 158.99, 158.98, 153.94, 146.99, 135.15, 133.72, 128.91, 127.05, 126.85 (d, ^{3}J = 8.4 Hz, 2C, C-2, C-6, Ph), 126.69, 126.57 (d, ^{4}J = 3.1 Hz, C-1, Ph), 118.46, 116.58 (d, ^{2}J = 22.1 Hz, C-2, C-5, Ph), 107.57. Anal. Calcd. (%) for $C_{20}H_{16}FNO_2$: C, 74.88; H, 5.21; N, 4.28. Found: C, 74.75; H, 5.02;N, 4.36.

*4.1.3.8. [5-(2-Nitrophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4h**).* yellow solid, yield 90.5%, m.p. 181-183 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.99 (d, *J* = 8.0 Hz, 1H), 7.94 (d, *J* = 7.6 Hz, 1H), 7.81 (td, *J* = 7.7, 1.1 Hz, 1H), 7.68 (t, *J* = 7.8 Hz, 1H), 7.28-7.14 (m, 6H), 4.81 (d, *J* = 102.6 Hz, 2H), 3.86 (s, 2H), 2.91 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.27, 150.01, 149.00, 147.59, 135.01, 133.50, 133.30, 130.68, 130.21, 128.93, 127.05, 126.71, 124.67, 122.61, 118.45, 111.78. Anal. Calcd. (%) for $C_{20}H_{16}N_2O_4$: C, 69.04; H, 4.78; N, 7.91. Found: C, 68.96; H, 4.63; N, 8.04.

*4.1.3.9. [5-(3-Nitrophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4i**).* yellow solid, yield 93.5%, m.p. 189-192 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.56 (t, *J* = 1.8 Hz, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 8.22-8.20 (m, 1H), 7.79 (t, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 3.6 Hz, 1H, FuH), 7.27-7.20 (m, 5H), 4.76 (s, 2H), 3.98 (s, 2H), 2.98 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.91, 152.37, 148.95, 147.78, 135.84, 135.13, 133.66, 131.34, 131.29, 130.65, 128.95, 127.11, 126.74, 123.31, 118.87, 118.23, 110.05. ESIMS(*m/z*): 349.1194 $[M + H]^+$.

*4.1.3.10. [5-(4-Nitrophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4j**).* yellow solid, yield 91.1%, m.p. 198-200 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.32 (d, *J* = 8.9 Hz, 2H, ArH), 8.04 (d, *J* = 8.9 Hz, 2H, ArH), 7.46 (d, *J* = 3.6 Hz, 1H, FuH), 7.27-7.20 (m, 5H), 4.75 (s, 2H), 3.96 (s, 2H), 2.97 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.84, 152.52, 148.58, 147.10, 135.58, 135.13, 133.60, 128.90, 127.10, 126.94, 126.73, 125.39 (2C), 124.96 (2C), 118.42, 111.63. Anal. Calcd. (%) for $C_{20}H_{16}N_2O_4$: C, 68.72; H, 4.86; N, 8.24. Found: C, 68.96; H, 4.63; N, 8.04.

4.1.3.11.

*[5-(4-Methylphenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4k**).*

white solid, yield 90.0%, m.p. 155-156 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.70 (d, *J* = 8.1 Hz, 2H, ArH), 7.30 (d, *J* = 8.1 Hz, 2H, ArH), 7.26-7.19 (m, 4H, QuH), 7.18 (d, *J* = 3.5 Hz, 1H, FuH), 7.07 (d, *J* = 3.6 Hz, 1H, FuH), 4.80 (s, 2H), 3.96 (s, 2H), 2.97 (s, 2H), 2.35 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.05, 155.06, 146.61, 138.63, 135.15, 133.76, 130.08 (2C), 128.93, 127.22, 127.03, 126.88, 126.68, 124.56 (2C), 118.46, 106.96, 21.36. Anal. Calcd. (%) for $C_{21}H_{19}NO_2$: C, 79.62; H, 5.89; N, 4.56. Found: C, 79.47; H, 6.03; N, 4.41.

4.1.3.12.

*[5-(4-Bromophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4l**).*

yellow solid, yield 88.1%, m.p. 188-189 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.76 (d, *J* = 8.4 Hz, 2H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.28-7.14 (m, 6H), 4.76 (s, 2H), 3.99 (s, 2H), 2.97 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.97, 153.72, 147.27 (s), 135.16, 133.71, 132.51 (2C), 129.07, 128.92, 127.08, 126.93, 126.72, 126.55 (2C), 122.09, 118.42, 108.49. Anal. Calcd. (%) for $C_{20}H_{16}BrNO_2$: C, 62.98; H, 4.05; N, 3.51. Found: C, 62.84; H, 4.22; N, 3.66.

4.1.3.13.

*[5-(4-Methoxyphenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone (**4m**).*

white solid, yield 93.2%, m.p. 136-138 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.74 (d, *J* = 8.7 Hz, 2H), 7.27-7.19 (m, 4H), 7.17 (d, *J* = 3.4 Hz, 1H), 7.06 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 3.5 Hz, 1H), 4.79 (s, 2H), 3.95 (s, 2H), 3.81 (s, 3H), 2.97 (s, 2H); ^{13}C

NMR (151 MHz, DMSO-d_6) δ 160.03, 159.07, 155.08, 146.29, 135.16, 133.79, 128.93, 127.03, 126.89, 126.69, 126.21 (2C), 122.68, 118.63, 115.00 (2C), 106.02, 55.74. Anal. Calcd. (%) for $C_{21}H_{19}NO_3$: C, 75.79; H, 5.94; N, 4.02. Found: C, 75.66; H, 5.74; N, 4.20.

4.1.3.14.

[5-(2',4'-di-Fluorophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone ***(4n)***. white solid, yield 90.1%, m.p. 129-131 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91 (dd, J = 15.3, 8.7 Hz, 1H), 7.46 (ddd, J = 11.6, 9.3, 2.5 Hz, 1H), 7.28-7.21 (m, 6H), 6.98 (t, J = 3.3 Hz, 1H), 4.78 (s, 2H), 3.98 (s, 2H), 2.97 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.43 (dd, ^1J = 249.1, ^3J = 12.2 Hz, C-4, Ph), 158.96 (dd, ^1J = 253.1, ^3J = 12.5 Hz, C-2, Ph), 158.82 (C=O), 148.31 (d, ^4J = 2.4 Hz, C-4, Ph), 147.16, 135.12, 133.66, 128.91, 128.37 (dd, ^3J = 10.0, ^3J = 4.2 Hz, C-6, Ph), 127.06, 126.91, 126.70, 118.24, 114.81 (dd, ^2J = 12.1, ^4J = 3.8 Hz, C-1, Ph), 113.02 (dd, ^2J = 21.9, ^4J = 3.5 Hz, C-5, Ph), 111.17 (d, ^3J = 9.8, C-5, Fu), 105.54 (dd, ^2J = 26.0 Hz, ^2J = 26.0 Hz, C-3, Ph). Anal. Calcd. (%) for $C_{20}H_{15}F_2NO_2$: C, 70.95; H, 4.59; N, 3.96. Found: C, 70.79; H, 4.46; N, 4.13.

4.1.3.15.

[5-(2',6'-di-Fluorophenyl)-2-furanyl](3,4-dihydro-2(1H)-isoquinolinyl)-Methanone ***(4o)***. white solid, yield 85.2%, m.p. 120-122 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.53 (dq, J = 8.3, 6.4 Hz, 1H), 7.34-7.12 (m, 7H), 7.04 (dd, J = 3.4, 1.6 Hz, 1H), 4.91 (d, J = 179.6 Hz, 2H), 4.01 (s, 2H), 2.96 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.22 (2C, dd, ^1J = 252.4, ^3J = 6.5 Hz, C-2, C-6, Ph), 158.59 (C=O), 148.11, 144.20,

135.00, 133.62, 131.38 (dd, ^{3}J = 10.7 Hz, ^{3}J = 10.7 Hz, C-4, Ph), 128.98, 127.03, 126.94, 126.70, 117.91, 114.32 (dd, ^{3}J = 5.5 Hz, ^{3}J = 5.5 Hz, C-5, Fu), 113.10 (2C, dd, ^{2}J = 21.5, ^{4}J = 3.9 Hz, C-3, C-5, Ph), 107.87 (dd, ^{2}J = 16.0 Hz, ^{2}J = 16.0 Hz, C-1, Ph). Anal. Calcd. (%) for $C_{20}H_{15}F_2NO_2$: C, 70.92; H, 4.31; N, 4.35. Found: C, 70.79; H, 4.46; N, 4.13.

4.1.3.16.

(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-phenyl-2-furyl)methanone (***5a*** *).*

white solid, yield 90.3%, m.p. 112-114 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (d, J = 7.9 Hz, 2H), 7.49 (t, J = 7.6 Hz, 2H), 7.39 (t, J = 7.3 Hz, 1H), 7.16 (dd, J = 14.4, 3.5 Hz, 2H), 6.86 (s, 1H), 6.79 (s, 1H), 4.74 (s, 2H), 3.92 (s, 2H), 3.73 (d, J = 4.2 Hz, 6H), 2.89 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.01, 154.72, 147.94, 147.86, 147.01, 129.88, 129.54, 129.02, 126.66, 125.34, 124.57, 118.28, 112.34, 110.41, 107.70, 56.01, 55.97. Anal. Calcd. (%) for $C_{22}H_{21}NO_4$: C, 72.89; H, 5.62; N, 3.64. Found: C, 72.71; H, 5.82; N, 3.85.

4.1.3.17.

(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(2-chlorophenyl)-2-furyl)methan one (***5b*** *).*

white solid, yield 88.1%, m.p. 133-134 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, J = 7.7 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.43 (t, J = 7.6 Hz, 1H), 7.24 (dd, J = 11.1, 3.5 Hz, 2H), 6.85 (s, 1H), 6.79 (s, 1H), 4.70 (s, 2H), 3.95 (s, 2H), 3.73 (d, J = 3.8 Hz, 6H), 2.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.83, 151.02, 147.95, 147.86, 147.33, 131.35, 130.43, 130.18, 129.09, 128.28, 128.14,

126.63, 125.22, 117.82, 112.56, 112.32, 110.38, 56.00, 55.96. Anal. Calcd. (%) for $C_{22}H_{20}ClNO_4$: C,66.69; H, 5.31; N, 3.38. Found: C, 66.42; H, 5.07; N, 3.52.

4.1.3.18.

*(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(3-chlorophenyl)-2-furyl)methan one (**5c**).*

white solid, yield 93.3%, m.p. 138-140 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (s, 1H), 7.77 (d, J = 7.7 Hz, 1H), 7.52 (t, J = 7.8 Hz, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.28 (d, J = 3.5 Hz, 1H), 7.19 (d, J = 3.4 Hz, 1H), 6.85 (s, 1H), 6.79 (s, 1H), 4.71 (s, 2H), 3.91 (s, 2H), 3.73 (d, J = 4.7 Hz, 6H), 2.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.90, 153.07, 147.95, 147.86, 147.46, 134.37, 131.84, 131.51, 128.70, 126.64, 125.27, 124.18, 123.07, 118.15, 112.33, 110.38, 109.11, 56.01, 55.98. Anal. Calcd. (%) for $C_{22}H_{20}ClNO_4$: C,66.70; H, 4.82; N, 3.76. Found: C, 66.42; H, 5.07; N, 3.52.

4.1.3.19.

*(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(4-chlorophenyl)-2-furyl)methan one (**5d**).*

white solid, yield 94.9%, m.p. 139-142 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 8.3 Hz, 2H), 7.19 (dd, J = 3.4 Hz, 3.4 Hz, 2H), 6.85 (s, 1H), 6.79 (s, 1H), 4.72 (s, 2H), 3.90 (s, 2H), 3.73 (d, J = 4.6 Hz, 6H), 2.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.93, 153.59, 147.95, 147.86, 147.24, 133.43, 129.61, 128.74, 126.66, 126.30, 125.28, 118.26, 112.31, 110.44, 108.41, 56.01, 55.97. Anal. Calcd. (%) for $C_{22}H_{20}ClNO_4$: C,66.18; H, 5.36; N, 3.80. Found: C, 66.42; H, 5.07; N, 3.52.

4.1.3.20.

*(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(2-fluorophenyl)-2-furyl)methan one (**5e**).*

white solid, yield 83.3%, m.p. 115-118 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (t, J = 7.8 Hz, 1H), 7.46 (dd, J = 13.3, 7.3 Hz, 1H), 7.41 – 7.34 (m, 2H), 7.22 (d, J = 3.4 Hz, 1H), 7.01 (t, J = 3.2 Hz, 1H), 6.86 (s, 1H), 6.79 (s, 1H), 4.72 (s, 2H), 3.95 (s, 2H), 3.74 (d, J = 3.9 Hz, 6H), 2.89 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.84, 158.75 (d, ^{1}J = 250.2 Hz, C-2, Ph), 148.91 (d, ^{3}J = 2.7 Hz, C-5, Fu), 147.96, 147.86, 147.17, 130.77 (d, ^{3}J = 8.5 Hz, C-4, Ph), 126.90, 126.88, 126.64, 125.64 (d, J = 3.3 Hz, C-5, Ph), 125.25, 118.12, 117.83 (d, ^{2}J = 11.8 Hz, C-1, Ph), 116.87 (d, ^{2}J = 21.1 Hz, C-3, Ph), 112.31, 111.69 (d, ^{3}J = 10.4 Hz, C-6, Ph), 56.00, 55.96. Anal. Calcd. (%) for $C_{22}H_{20}FNO_4$: C,66.99; H, 5.01; N, 3.89. Found: C, 69.28; H, 5.29; N, 3.67.

4.1.3.21.

*(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(3-fluorophenyl)-2-furyl)methan one (**5f**).*

white solid, yield 88.4%, m.p. 125-126 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 (t, J = 8.5 Hz, 2H), 7.54 (dd, J = 14.7, 7.3 Hz, 1H), 7.29 – 7.18 (m, 3H), 6.86 (s, 1H), 6.80 (s, 1H), 4.71 (s, 2H), 3.92 (s, 2H), 3.73 (d, J = 4.2 Hz, 6H), 2.89 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.03 (d, ^{1}J = 243.5 Hz, C-3, Ph), 158.89, 153.36 (d, ^{4}J = 3.0 Hz, C-5, Fu), 147.95, 147.86, 147.42, 132.06 (d, ^{3}J = 8.7 Hz, C-1, Ph), 131.73 (d, ^{3}J = 8.6 Hz, C-5, Ph), 126.65, 125.28, 120.61 (d, ^{4}J = 2.3 Hz, C-6, Ph), 118.20, 115.71 (d, ^{2}J = 21.2 Hz, C-2, Ph), 112.32, 111.28 (d, ^{2}J = 23.7 Hz, C-4, Ph),

110.40, 109.00, 55.99, 55.96. Anal. Calcd. (%) for $C_{22}H_{20}FNO_4$: C, 69.56; H, 5.45; N, 3.33. Found: C, 69.28; H, 5.29; N, 3.67.

4.1.3.22.

(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(4-fluorophenyl)-2-furyl)methanone (***5g*** *).*

white solid, yield 91.8%, m.p. 130-132 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (dd, J = 7.8, 5.8 Hz, 2H), 7.34 (t, J = 8.6 Hz, 2H), 7.17 (d, J = 3.4 Hz, 1H), 7.13 (d, J = 3.4 Hz, 1H), 6.85 (s, 1H), 6.79 (s, 1H), 4.74 (s, 2H), 3.90 (s, 6H), 3.73 (d, J = 4.5 Hz, 2H), 2.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.54 (d, ^1J = 246.2 Hz, C-4, Ph), 158.98, 153.88, 147.94, 147.86, 147.00, 126.84 (d, ^3J = 8.3 Hz, 2C, C-2, C-6, Ph), 126.67, 126.60 (d, ^4J = 3.1 Hz, C-1, Ph), 125.32, 118.31, 116.60 (d, ^2J = 22.0 Hz, 2C, C-3, C-5, Ph), 112.32, 110.42, 107.58, 56.02, 55.97. Anal. Calcd. (%) for $C_{22}H_{20}FNO_4$: C, 68.99; H, 4.98; N, 3.92. Found: C, 69.28; H, 5.29; N, 3.67.

4.1.3.23.

(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(2-nitrophenyl)-2-furyl)methanone (***5h*** *).*

yellow solid, yield 92.8%, m.p. 158-161 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (d, J = 7.9 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.81 (t, J = 7.6 Hz, 1H), 7.68 (t, J = 7.8 Hz, 1H), 7.25 (d, J = 1.8 Hz, 1H), 7.14 (d, J = 3.4 Hz, 1H), 6.85 (s, 1H), 6.78 (s, 1H), 4.73 (d, J = 60.2 Hz, 2H), 3.83 (s, 2H), 3.74 (s, 6H), 2.82 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.40, 150.01, 149.02, 147.97, 147.87, 147.57, 133.32, 130.66, 130.23, 126.58, 125.05, 124.71, 122.65, 118.44, 112.28, 111.76, 110.46, 55.95 (2C,

CH_3O-). Anal. Calcd. (%) for $C_{22}H_{20}N_2O_6$: C, 64.97; H, 5.12; N, 6.53. Found: C, 64.70; H, 4.94; N, 6.86.

4.1.3.24.

(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(3-nitrophenyl)-2-furyl)methano ne (***5i*** *).*

yellow solid, yield 93.1%, m.p. 163-167 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.22 (dd, *J* = 13.2, 8.1 Hz, 2H), 7.78 (t, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 3.5 Hz, 1H), 7.24 (d, *J* = 3.3 Hz, 1H), 6.99 – 6.64 (m, 2H), 4.52 (s, 2H), 3.91 (s, 2H), 3.73 (d, *J* = 5.2 Hz, 6H), 2.82 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.82, 152.31, 148.90, 147.96, 147.88, 147.78, 131.32, 131.25, 130.62, 126.61, 125.19, 123.28, 118.83, 118.12, 112.30, 110.38, 110.02, 55.98, 55.97. Anal. Calcd. (%) for $C_{22}H_{20}N_2O_6$: C, 64.41; H, 4.65; N, 7.02. Found: C, 64.70; H, 4.94; N, 6.86.

4.1.3.25.

(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(4-nitrophenyl)-2-furyl)methano ne (***5j*** *).*

yellow solid, yield 91.7%, m.p. 179-181 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (d, *J* = 8.4 Hz, 2H), 8.06 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 3.4 Hz, 1H), 7.25 (d, *J* = 3.3 Hz, 1H), 6.86 (s, 1H), 6.79 (s, 1H), 4.71 (s, 2H), 3.89 (s, 2H), 3.73 (d, *J* = 5.4 Hz, 6H), 2.89 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.81, 152.45, 148.58, 147.97, 147.87, 147.08, 135.60, 126.64, 125.38 (2C), 125.16, 124.97 (2C), 118.25, 112.29, 111.63, 110.43, 56.02, 55.97. Anal. Calcd. (%) for $C_{22}H_{20}N_2O_6$: C, 64.92; H, 5.22; N, 6.60. Found: C, 64.70; H, 4.94; N, 6.86.

4.1.3.26.

*(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(p-tolyl)-2-furyl)methanone (**5k**):*

white solid, yield 96.8%, m.p. 143-144 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (d, J = 7.9 Hz, 2H), 7.29 (d, J = 7.9 Hz, 2H), 7.16 (d, J = 3.5 Hz, 1H), 7.06 (d, J = 3.5 Hz, 1H), 6.85 (s, 1H), 6.79 (s, 1H), 4.75 (s, 2H), 3.91 (s, 2H), 3.73 (d, J = 3.9 Hz, 6H), 2.87 (s, 2H), 2.34 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.03, 155.00, 147.94, 147.86, 146.62, 138.63, 130.08 (2C), 127.24, 126.66, 125.35, 124.55 (2C), 118.32, 112.33, 110.39, 106.94, 56.00, 55.95, 21.36. Anal. Calcd. (%) for $C_{23}H_{23}NO_4$: C, 72.91; H, 5.98; N, 3.58. Found: C, 73.19; H, 6.14; N, 3.71.

4.1.3.27.

*(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(4-bromophenyl)-2-furyl)methanone (**5l**).*

yellow solid, yield 82.3%, m.p. 168-171 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 (d, J = 8.1 Hz, 2H), 7.68 (d, J = 8.2 Hz, 2H), 7.21 (d, J = 3.4 Hz, 1H), 7.18 (d, J = 3.3 Hz, 1H), 6.85 (s, 1H), 6.78 (s, 1H), 4.73 (s, 2H), 3.91 (s, 2H), 3.73 (d, J = 4.7 Hz, 6H), 2.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.94, 153.63, 147.95, 147.86, 147.25, 132.50 (2C), 129.07, 126.66, 126.53 (2C), 125.27, 122.06, 118.25, 112.31, 110.41, 108.48, 56.01, 55.97. Anal. Calcd. (%) for $C_{22}H_{20}BrNO_4$: C, 59.55; H, 4.31; N, 3.38. Found: C, 59.74; H, 4.56; N, 3.17.

4.1.3.28.

(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(4-methoxyphenyl)-2-furyl)meth

*anone (**5m**).*

white solid, yield 92.8%, m.p. 179-180 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 (d, J = 8.2 Hz, 2H), 7.15 (d, J = 3.3 Hz, 1H), 7.05 (d, J = 8.2 Hz, 2H), 6.99 (d, J = 3.4 Hz, 1H), 6.85 (s, 1H), 6.79 (s, 1H), 4.76 (s, 2H), 3.91 (s, 2H), 3.81 (s, 3H), 3.73 (d, J = 3.9 Hz, 6H), 2.88 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.02, 159.07, 155.01, 147.93, 147.85, 146.29, 126.68, 126.20 (2C), 125.39, 122.70, 118.46, 115.00 (2C), 112.33, 110.41, 106.01, 56.01, 55.97, 55.74. Anal. Calcd. (%) for $C_{23}H_{23}NO_5$: C, 70.48; H, 5.92; N, 3.36. Found: C, 70.21; H, 5.89; N, 3.56.

4.1.3.29.

*(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(2,4-difluorophenyl)-2-furyl)met hanone (**5n**).*

white solid, yield 88.6%, m.p. 126-128 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (dd, J = 15.4, 8.4 Hz, 1H), 7.47 (t, J = 10.4 Hz, 1H), 7.27 (t, J = 8.5 Hz, 1H), 7.21 (d, J = 3.4 Hz, 1H), 6.98 (t, J = 3.2 Hz, 1H), 6.85 (s, 1H), 6.79 (s, 1H), 4.70 (s, 2H), 3.91 (s, 2H), 3.73 (d, J = 4.3 Hz, 6H), 2.88 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.42 (dd, ^{1}J = 249.0, ^{3}J = 12.3 Hz, C-4, Ph), 158.95 (dd, ^{1}J = 253.0, ^{3}J = 12.3 Hz, C-2, Ph), 158.81, 148.23 (d, ^{4}J = 2.2 Hz, C-4, Fu), 147.95, 147.86, 147.15, 128.35 (dd, ^{3}J = 9.9, ^{3}J = 4.0 Hz, C-6, Ph), 126.64, 125.23, 118.07, 114.83 (dd, ^{2}J = 12.1, ^{4}J = 3.8 Hz, C-1, Ph), 113.04 (dd, ^{2}J = 21.8, ^{4}J = 3.4 Hz, C-5, Ph), 112.30, 111.18 (d, ^{3}J = 9.5 Hz, C-5, Ph), 110.41, 105.57 (dd, ^{2}J = 26.0 Hz, ^{2}J = 26.0 Hz, C-3, Ph), 56.00, 55.96. Anal. Calcd. (%) for $C_{22}H_{19}F_2NO_4$: C, 65.94; H, 4.89; N, 3.71. Found: C, 66.16; H, 4.80; N, 3.51.

4.1.3.30.

*(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-(5-(2,6-difluorophenyl)-2-furyl)methanone (**5o**).*

white solid, yield 86.4%, m.p. 120-122 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 – 7.48 (m, 1H), 7.36 – 7.22 (m, 3H), 7.03 (d, J = 1.7 Hz, 1H), 6.79 (s, 2H), 4.69 (s, 2H), 3.97 (s, 2H), 3.74 (s, 6H), 2.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.20 (2C, dd, ^{1}J = 252.4 Hz, ^{3}J = 6.6 Hz, C-2, C-6, Ph), 158.56, 148.10, 147.96, 147.85, 144.14, 131.36 (dd, ^{3}J = 10.7 Hz, ^{3}J = 10.7 Hz, C-4, Ph), 126.55, 125.19, 117.71, 114.31 (dd, ^{3}J = 5.6 Hz, ^{3}J = 5.6 Hz, C-5, Fu), 113.10 (2C, dd, ^{2}J = 19.8 Hz, ^{4}J = 5.4 Hz, C-3, C-5, Ph), 112.33, 110.43, 107.87 (dd, ^{2}J = 16.0 Hz, ^{2}J = 16.0 Hz, C-1, Ph), 55.97, 55.94. Anal. Calcd. (%) for $C_{22}H_{19}F_2NO_4$: C, 66.41; H, 4.66; N, 3.40. Found: C, 66.16; H, 4.80; N, 3.51.

4.2. Biological investigations.

4.2.1. Cell lines

MCF-7/ADR (resistant to Doxorubicin), as the breast cancer cell line of human origin, was routinely cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 100 μg/mL streptomycin, 2 mM glutamine, and 100,000 U/mL penicillin in a humidified incubator with a 5 % CO_2 atmosphere at 37 ^{o}C. Caco-2 cells were grown in DMEM with 10% heat-inactivated fetal calf serum, 100 μg/mL streptomycin, 2 mM L-glutamine and 100 U/mL penicillin. The cells were trypsinized twice a week with trypsin/ethylenediaminetetraacetic acid (EDTA) (0.02%/0.02%) and the medium

was changed twice a week.

4.2.2. Determination of the cytotoxicity of tested compounds to HeLa cells

HeLa cells were seeded at 5000 cells per well in 96-well plates in DMEM supplemented with 10% FBS (Invitrogen), which were washed once with PBS after culture overnight and incubated with FBS-free medium supplied with 100 μM tested compounds as indicated. The survival cells were examined with the Cell Counting Kit-8 (Dojindo) after 4-hour incubation, and the survival percentage of cells without compounds treatment was set as 100% as a control.

4.2.3. Cell antiproliferative effect

The antiproliferative effect was examined through the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) assay [10,11,40,41] with minor modifications. In the absence or presence of test compounds, the cells were seeded to 96-well plates for 48 h and 10 μL of freshly prepared MTT solution (5 mg/mL) was added in each well. Then the plate was incubated at 37 ^{o}C for 3-4 h in a humidified atmosphere of 5% CO_2. After removal of MTT solution, 200 μL of EtOH/DMSO (v:v/1:1) was added into each well to dissolve the blue formazan solid crystals. The optical density was checked at 570 and 650 nm wavelenghts by Victor3 (Perkin-Elmer Life Sciences).

4.2.4. Intracellular Doxorubicin accumulation

The effects of intracellular Doxorubicin accumulation by compounds **5m**, **5d**, **5l**, Cyclosporin A, and Verapamil was examined by flow cytometry. In MCF-7/ADR cells, all the compounds were prepared at 20 μM for 2 days exposure, and during the

second day Doxorubicin was supplied at 50 μM. After incubation and removal of the cell media, trypsin-EDTA was applied to detach the cells from the plates. Cells were harvested and washed twice in ice-cold PBS (pH 7.4), then which were placed on ice (less than 1 h) until analysis. Fluorescence measurements of individual cells were evaluated using a Becton-Dickinson FACScan. The mean fluorescence intensity in the Doxorubicin-treated cells, established 100% as the positive control, and the auto-fluorescence of untreated cells established 0% as the negative control.

4.2.5. Effect of antiproliferative drug combination

In MCF-7/ADR, the compounds were performed at 20 μM; Doxorubicin at 5 μM; Verapamil and Cyclosporin A were used as positive references. The incubation of the compounds together with Doxorubicin for 2 days, after two wash steps with complete medium, by Doxorubicin for 1 day. The analysis was performed by the MTT assay. On the first day, 10,000 cells/well were plated in 96-well plates in 200 μL, and on the second day, the compounds alone or in combination were added. Eight blank control wells (untreated cells) and eight wells for each treatment were applied in each experiment. 0.5 mg/mL MTT was added to each well and the supernatant was removed after 1-h incubation at 37 ^{o}C. Then 100 μL DMSO was added and the absorbance values were recorded at 570 and 630 nm on the microplate reader SpectraCount (Packard-USA). The cell growth inhibitory activity was shown as percentage of control (untreated cells).

4.3 Molecular docking

Molecular docking was performed on Surflex-Dock module of Sybyl 8.0 [42].

The protein crystal structure of P-gp (PDB ID: 4Q9I) obtained from Protein Data Bank was used as the receptor for molecular docking study. The 3D structure of compound **5m** was drawn and optimized with SYBYL package. The docking procedure was started with the protomol generation, which was created using a ligand-based approach (native ligand for P-gp structure). Proto threshold was set to 0.5 and proto bloat was kept at 0 as a default parameter. For docking, max conformation and max rotation values were 20 and 100, respectively. Pre-dock and Post-dock energy minimization methods were also applied. Docking results were compared by the total score values. The pose with the higher total-score value was considered as the best one. After the end of molecular docking, the interactions of the docked domain with ligand were analyzed.

Acknowledgments

We acknowledge the financial supports from the National Key Research and Development Program of China (2017YFD0200504), the National Key Project for Basic Research (973 Program, 2015CB150600), the Special Fund for the Research and Construction of Public Service Ability (2016A020217014), the Pearl River S&T Nova Program of Guangzhou (201506010029).

References

1. M.M. Gottesman, T. Fojo, S.E. Bates, Multidrug resistance in cancer: role of ATP-dependent transporters, Nat. Rev. Cancer 2 (2002) 48-58.
2. R. Krishna, L.D. Mayer, Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in

influencing the pharmacokinetics of anticancer drugs, Eur. J. Pharm. Sci. 11 (2000) 265-283.

3. B. Verhalen, R. Dastvan, S. Thangapandian, Y. Peskova, H.A. Koteiche, R.K. Nakamoto, E. Tajkhorshid, H.S. Mchaourab, Energy transduction and alternating access of the mammalian ABC transporter P-glycoprotein, Nature 543 (2017) 738-741.
4. G. Szakacs, J.K. Paterson, J.A. Ludwig, C. Boothgenthe, M.M. Gottesman, Targeting multidrug resistance in cancer, Nat. Rev. Drug Discov. 5 (2006) 219-234.
5. N.A. Colabufo, F. Berardi, M. Contino, M. Niso, R. Perrone, ABC pumps and their role in active drug transport, Curr. Top. Med. Chem. 9 (2009) 119-129.
6. D. Waghray, Q.H. Zhang, Inhibit or evade multidrug resistance P-glycoprotein in cancer treatment, J. Med. Chem. DOI: 10.1021/acs.jmedchem.7b01457.
7. T. Liu, Y.B. Peng, X. Li, L. Liu, F. Liu, L. He, A novel delocalized lipophilic cation-chlorambucil conjugate inhibits P-glycoprotein in HepG2/ADM cells, Bioorg. Med. Chem. 25 (2017) 5461-5467.
8. A. Palmeira, E. Sousa, M.H. Vasconcelos, M.M. Pinto, Three decades of P-gp inhibitors: skimming through several generations and scaffolds, Curr. Med. Chem. 19 (2012) 1946-2025.
9. A. Palmeira, E. Sousa, M.H. Vasconcelos, M. Pinto, M.X. Fernandes, Structure and ligand-based design of P-glycoprotein inhibitors: a historical perspective, Curr. Pharm. Des. 18 (2012) 4197-4214.

10. Q.Q. Qiu, B.M. Liu, J. Cui, Z. Li, X. Deng, H. Qiang, J.M. Li, C. Liao, B. Zhang, W. Shi, M.B. Pan, W.L. Huang, H. Qian, Design, synthesis, and pharmacological characterization of *N*-(4-(2(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)yl)ethyl) phenyl)quinazolin-4-amine derivatives: novel inhibitors reversing P-glycoprotein-mediated multidrug resistance, J. Med. Chem. 60 (2017) 3289-3302.
11. Q.Q. Qiu, W. Shi, Z. Li, B. Zhang, M.B. Pan, J. Cui, Y.X. Dai, W.L. Huang, H. Qian, Exploration of 2-((pyridin-4-ylmethyl)amino)nicotinamide derivatives as potent reversal agents against P-glycoprotein-mediated multidrug resistance, J. Med. Chem. 60 (2017) 2930-2943.
12. C.Y. Chen, C.M. Lin, H.C. Lin, C.F. Huang, C.Y. Lee, T.C. Tou, C.C. Hung, C.S. Chang, Structure activity relationship study of novel 2-aminobenzofuran derivatives as P-glycoprotein inhibitors, Eur. J. Med. Chem. 125 (2017) 1023-1035.
13. P. Joshi, R.A. Vishwakarma, S.B. Bharate, Natural alkaloids as P-gp inhibitors for multidrug resistance reversal in cancer, Eur. J. Med. Chem. 138 (2017) 273-292.
14. S. Nobili, I. Landini, B. Giglioni, E. Mini, Pharmacological strategies for overcoming multidrug resistance, Curr. Drug Targets 7 (2006) 861-879.
15. S. Modok, H.R. Mellor, R. Callaghan, Modulation of multidrug resistance efflux pump activity to overcome chemoresistance in cancer, Curr. Opin. Pharmacol. 6 (2006) 350-354.
16. M. Bauer, R. Karch, M. Zeitlinger, J. Stanek, C. Philippe, W. Wadsak, M.

Mitterhauser, W. Jaeger, H. Haslacher, M. Mueller, O. Langer, Interaction of ^{11}C-tariquidar and ^{11}C-elacridar with P-glycoprotein and breast cancer resistance protein at the human blood-brain barrier, J. Nucl. Med. 54 (2013) 1181-1187.

17. J. Mullauer, R. Karch, J.P. Bankstahl, M. Bankstahl, J. Stanek, T. Wanek, S. Mairinger, M. Muller, W. Loscher, O. Langer, C. Kuntner, Assessment of cerebral P-glycoprotein expression and function with PET by combined [^{11}C]inhibitor and [^{11}C]substrate scans in rats, Nucl. Med. Biol. 40 (2013) 755-763.
18. B. Dorner, C. Kuntner, J.P. Bankstahl, M. Bankstahl, J. Stanek, T. Wanek, G. Stundner, S. Mairinger, W. Loscher, M. Muller, O. Langer, T. Erker, Synthesis and small-animal positron emission tomography evaluation of [^{11}C]-elacridar as a radiotracer to assess the distribution of P-glycoprotein at the blood-brain barrier, J. Med. Chem. 52 (2009) 6073-6082.
19. N.A. Colabufo, F. Berardi, M. Cantore, M.G. Perrone, M. Contino, C. Inglese, M. Niso, R. Perrone, A. Azzariti, G.M. Simone, L. Porcelli, A. Paradiso, Small P-gp modulating molecules: SAR studies on tetrahydroisoquinoline derivatives, Bioorg. Med. Chem. 16 (2008) 362-373.
20. A. van Waarde, N.K. Ramakrishnan, A.A. Rybczynska, P.H. Elsinga, F. Berardi, J.R. de Jong, C. Kwizera, R. Perrone, M. Cantore, J.W.A. Sijbesma, R.A. Dierckx, N.A. Colabufo, Synthesis and preclinical evaluation of novel PET probes for P-glycoprotein function and expression, J. Med. Chem. 52 (2009) 4524-4532.
21. N.A. Colabufo, F. Berardi, M. Cantore, M.G. Perrone, M. Contino, C. Inglese, M. Niso, R. Perrone, A. Azzariti, G.M. Simone, A. Paradiso, 4-Biphenyl and

2-naphthyl substituted 6,7-dimethoxytetrahydroisoquinoline derivatives as potent P-gp modulators, Bioorg. Med. Chem. 16 (2008) 3732-3743.

22. A. Azzariti, A.E. Quatrale, L. Porcelli, N.A. Colabufo, M. Cantore, G. Cassano, G. Gasparre, G. Iannelli, S. Tommasi, M.A. Panaro, A. Paradiso, MC70 potentiates doxorubicin efficacy in colon and breast cancer in vitro treatment, Eur. J. Pharmacol. 670 (2011) 74-84.
23. S. Mairinger, T. Wanek, C. Kuntner, Y. Doenmez, S. Strommer, J. Stanek, E. Capparelli, P. Chiba, M. Mueller, N.A. Colabufo, O. Langer, Synthesis and preclinical evaluation of the radiolabeled P-glycoprotein inhibitor [^{11}C] MC113, Nucl. Med. Biol. 39 (2012) 1219-1225.
24. N.A. Colabufo, F. Berardi, M.G. Perrone, M. Cantore, M. Contino, C. Inglese, M. Niso, R. Perrone, Multidrug resistance-reverting agents: 2-aryloxazole and 2-arylthiazole derivatives as potent BCRP or MRP1 inhibitors, ChemMedChem 4 (2009) 188-195.
25. N.A. Colabufo, M. Contino, M. Cantore, E. Capparelli, M.G. Perrone, G. Cassano, G. Gasparre, M. Leopoldo, F. Berardi, R. Perrone, Naphthalenyl derivatives for hitting P-gp/MRP1/BCRP transporters, Bioorg. Med. Chem. 21 (2013) 1324-1332.
26. E. Capparelli, L. Zinzi, M. Cantore, M. Contino, M.G. Perrone, G. Luurtsema, F. Berardi, R. Perrone, N.A. Colabufo, SAR studies on tetrahydroisoquinoline derivatives: The role of flexibility and bioisosterism to raise potency and selectivity toward P-glycoprotein, J. Med. Chem. 57 (2014) 9983-9994.

27. G.P. Song, S.M. Li, Z.W. Lei, Y.B. Li, J.H. Li, Y.X. Liao, Z.N. Cui, Synthesis and biological evaluation of acylated oligorhamnoside derivatives structurally related to mezzettiaside-6 with cytotoxic activity, Org. Biomol. Chem. 14 (2016) 6691-6702.

28. G.P. Song, J.Z. Li, H. Tian, Y.S. Li, D.K. Hu, Y. Li, Z.N. Cui, Synthesis and antitumor activity of novel *N*-benzoyl-*N*'-substituted pyrimidinyl (thio)semicarbazide derivatives, Lett. Drug Des. Discov. 13 (2016) 329-334.

29. G.P. Song, D.S. Zhao, D.K. Hu, Y.S. Li, H.W. Jin, Z.N. Cui, Design, synthesis and biological evaluation of novel tetrahydroisoquinoline derivatives as potential PDE4 inhibitors, Bioorg. Med. Chem. Lett. 25 (2015) 4610-4614.

30. G.P. Song, S.M. Li, H.Z. Si, Y.B. Li, Y.S. Li, J.H. Fan, Q.Q. Liang, H.B. He, H.M. Ye, Z.N. Cui, Synthesis and bioactivity of novel xanthone and thioxanthone L-rhamnopyranosides, RSC Adv. 5 (2015) 36092-36103.

31. Z.N. Cui, Y. Li, Y. Ling, J. Huang, J.R. Cui, R.Q. Wang, X.L. Yang, New class of potent antitumor acylhydrazone derivatives containing furan, Eur. J. Med. Chem. 45 (2010) 5576-5584.

32. Y.S. Li, D.K. Hu, D.S. Zhao, X.Y. Liu, H.W. Jin, G.P. Song, Z.N. Cui, L.H. Zhang, Design, synthesis and biological evaluation of 2,4- disubstituted oxazole derivatives as potential PDE4 inhibitors, Bioorg. Med. Chem. 25 (2017) 1852-1859.

33. Y.S. Li, H. Tian, D.S. Zhao, D.K. Hu, X.Y. Liu, H.W. Jin, G.P. Song, Z.N. Cui, Synthesis and bioactivity of pyrazole and triazole derivatives as potential PDE4

inhibitors, Bioorg. Med. Chem. Lett. 26 (2016) 3632-3635.

34. G.M. Sheldrick, SHELXS97 and SHELXL97, University of Göttingen: Germany 1997.

35. Bruker Saint and SHELXTL. In Bruker AXS Inc. Madison, Wisconsin, USA 1999.

36. T. Murakami, A.S. Singh, T. Kiyuna, S.M. Dry, Y.F. Li, A.W. James, K. Igarashi, K. Kawaguchi, J.C. Delong, Y. Zhang, Y. Hiroshima, T. Russell, M.A. Eckardt, J. Yanagawa, N. Federman, R. Matsuyama, T. Chishima, K. Tanaka, M. Bouvet, I. Endo, F.C. Eilber, R.M. Hoffman, Effective molecular targeting of CDK4/6 and IGF-1R in a rare *FUS-ERG* fusion *CDKN2A*-deletion doxorubicin-resistant Ewing's sarcoma patient-derived orthotopic xenograft (PDOX) nude-mouse model, Oncotarget 7 (2016) 47556-47564.

37. T. Kiyuna, T. Murakami, Y. Tome, K. Kawaguchi, K. Igarashi, Y. Zhang, M. Zhao, Y.F. Li, M. Bouvet, F. Kanaya, A. Singh, S. Dry, F.C. Eilber, R.M. Hoffman, High efficacy of tumor-targeting *Salmonella typhimurium* A1-R on a doxorubicin- and dactolisib-resistant follicular dendritic-cell sarcoma in a patient-derived orthotopic xenograft PDOX nude mouse model, Oncotarget 7 (2016) 33046-33054.

38. K. Miyake, T. Murakami, T. Kiyuna, K. Igarashi, K. Kawaguchi, M. Miyake, Y.F. Li, S.D. Nelson, S.M. Dry, M. Bouvet, I.A. Elliott, T.A. Russell, A.S. Singh, M.A. Eckardt, Y. Hiroshima, M. Momiyama, R. Matsuyama, T. Chishima, I. Endo, F.C. Eilber, R.M. Hoffman, The combination of temozolomide-irinotecan regresses a

doxorubicin-resistant patient-derived orthotopic xenograft (PDOX) nude-mouse model of recurrent Ewing's sarcoma with a FUS-ERG fusion and *CDKN2A* deletion: Direction for third-line patient therapy, Oncotarget 8 (2017) 103129-103136.

39. K. Miyake, T. Murakami, T. Kiyuna, K. Igarashi, K. Kawaguchi, Y.F. Li, A.S. Singh, S.M. Dry, M.A. Eckardt, Y. Hiroshima, M. Momiyama, R. Matsuyama, T. Chishima, I. Endo, F.C. Eilber, R.M. Hoffman, Eribulin regresses a doxorubicin-resistant Ewing's sarcoma with a *FUS-ERG* fusion and *CDKN2A*-deletion in a patient-derived orthotopic xenograft (PDOX) nude mouse model, J. Cell. Biochem. 119 (2018) 967-972.
40. J. Sargent, C. Taylor, Appraisal of the MTT assay as a rapid test of chemosensitivity in acute myeloid leukaemia, Brit. J. Cancer 60 (1989) 206-210.
41. S. Jabbar, P. Twentyman, J. Watson, The MTT assay underestimates the growth inhibitory effects of interferons, Brit. J. Cancer 60 (1989) 523-528.
42. G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J. Comput. Chem. 19 (1998) 1639-1662.

Figure captions

Figure 1. Development of P-gp modulators

Figure 2. (A, B) Molecular structure of **3b**. Displacement ellipsoids are drawn at the 30% probability level. (C, D) Molecular structure of **4d**. Displacement ellipsoids are drawn at the 30% probability level.

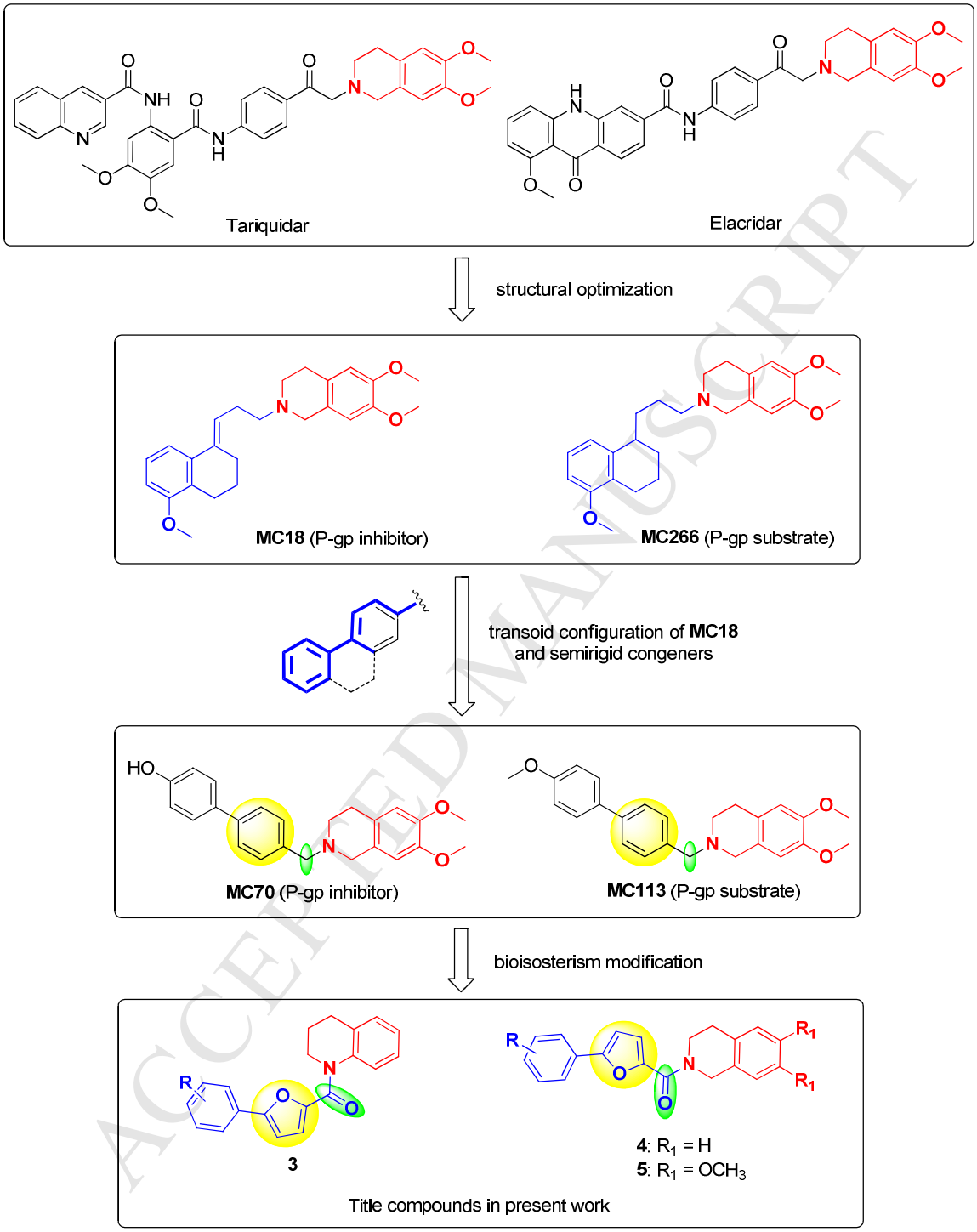
Figure 3. Models of P-glycoprotein and docking of compound **5m**. (A, B) The entire P-glycoprotein structure binding to **5m**. (C, D) The catalytic domain binding to **5m** overlaid with Cyclosporin A (purple). (E) The electronic surface distribution of **5m** at the catalytic domain. (F) The overview of binding model.

Scheme 1. The synthetic route of the title compounds **3a**-**3o**; **4a**-**4o** and **5a**-**5o**. Reagents and conditions: (a) $NaNO_2$, hydrochloricacid, 0 - 5 ºC, 3h; (b) furoicacid, $CuCl_2$ (cat.), acetone - H_2O, rt, 5h (two steps); (c) HOBt, EDCI, anhydrous dichloromethane, 0 ºC – r.t., 3h; (d) $SOCl_2$, anhydrous toluene, reflux; (e) tetrahydroquinoline , anhydrous dichloromethane, reflux, 4 h. R = **a**: H; **b**: 2-Cl; **c**: 3-Cl; **d**: 4-Cl; **e**: 2-F; **f**: 3-F; **g**: 4-F; **h**: 2-NO_2; **i**: 3-NO_2; **j**: 4-NO_2; **k**: 4-CH_3; **l**: 4-Br; **m**: 4-OCH_3; **n**: 2,4-di-F; **o**: 2,6-di-F.

Table 1. Biological evaluation of the title compounds

Table 2. Capability of the synthesized P-gp modulating agents to increase Doxorubicin intracellular accumulation in MCF-7/ADR cell line

1



2

3 **Figure 1.** Development of P-gp modulators

4

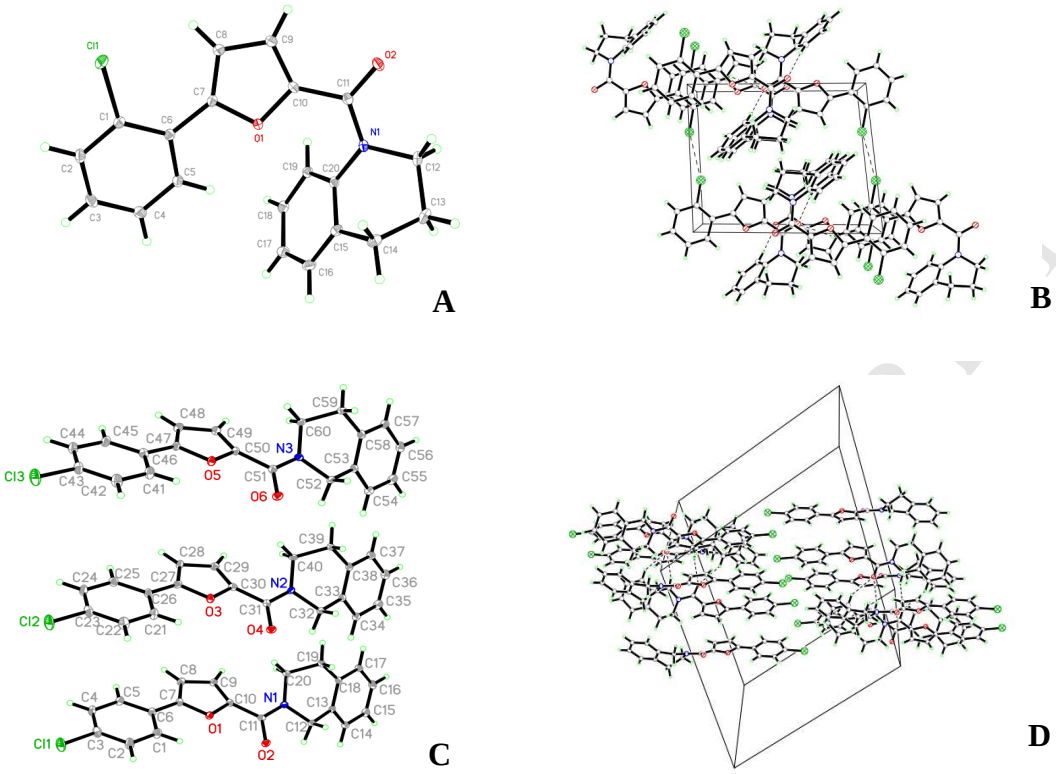


Figure 2. (A, B) Molecular structure of **3b**. Displacement ellipsoids are drawn at the 30% probability level. (C, D) Molecular structure of **4d**. Displacement ellipsoids are drawn at the 30% probability level.

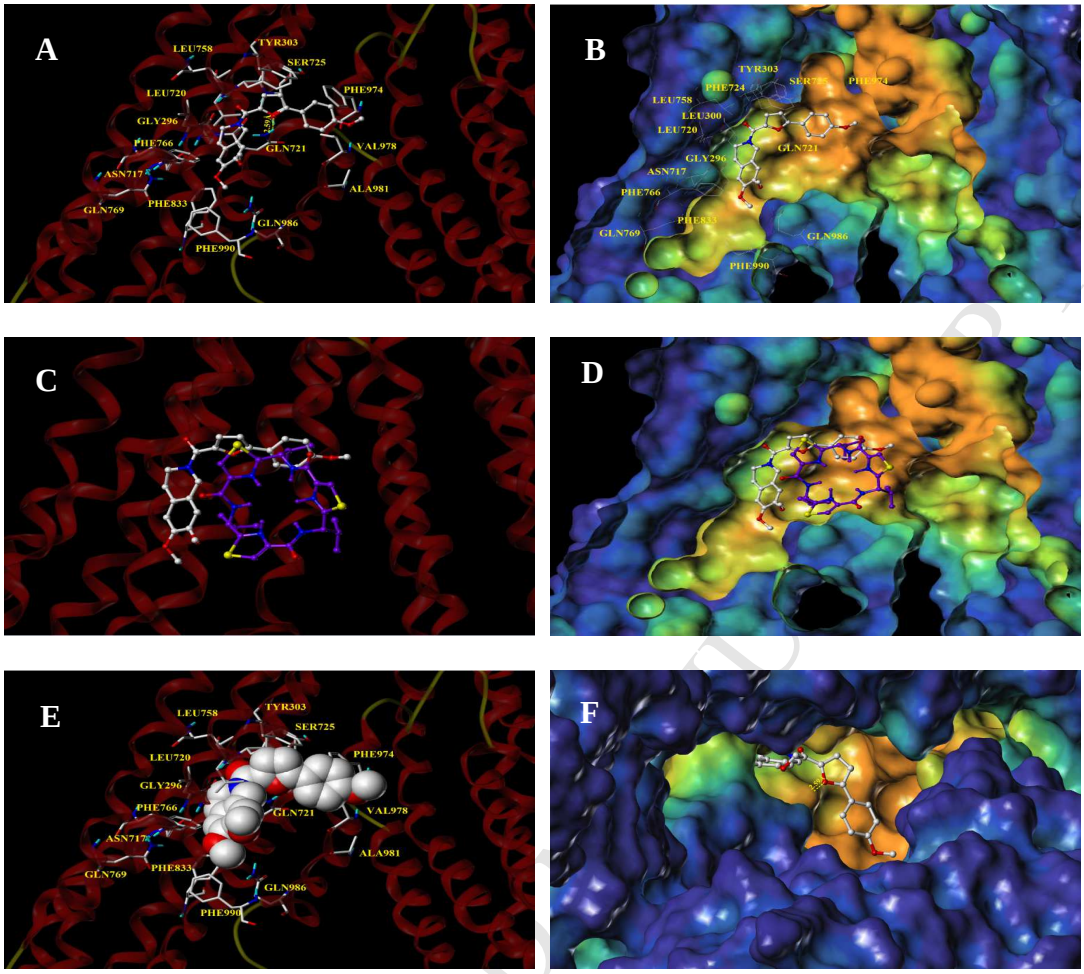
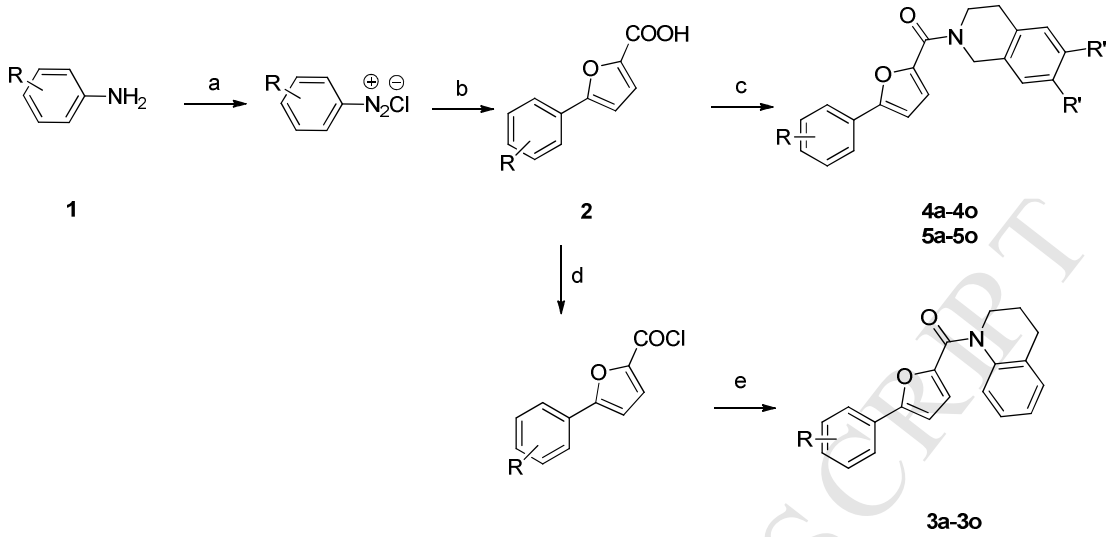
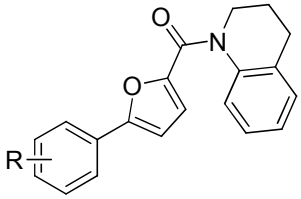
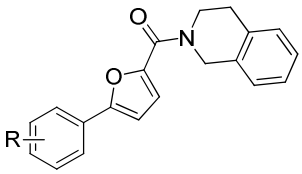
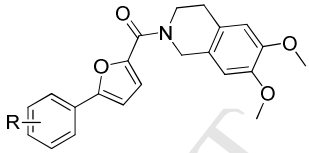


Figure 3. Models of P-glycoprotein and docking of compound **5m**. (A, B) The entire P-glycoprotein structure binding to **5m**. (C, D) The catalytic domain binding to **5m** overlaid with Cyclosporin A (purple). (E) The electronic surface distribution of **5m** at the catalytic domain. (F) The overview of binding model.



Scheme 1. The synthetic route of the title compounds **3a-3o**; **4a-4o** and **5a-5o**. Reagents and conditions: (a) $NaNO_2$, hydrochloricacid, 0 - 5 ºC, 3h; (b) furoicacid, $CuCl_2$ (cat.), acetone - H_2O, rt, 5h (two steps); (c) HOBt, EDCI, anhydrous dichloromethane, 0 ºC – r.t., 3h; (d) $SOCl_2$, anhydrous toluene, reflux; (e) tetrahydroquinoline , anhydrous dichloromethane, reflux, 4 h. R = **a**: H; **b**: 2-Cl; **c**: 3-Cl; **d**: 4-Cl; **e**: 2-F; **f**: 3-F; **g**: 4-F; **h**: 2-NO_2; **i**: 3-NO_2; **j**: 4-NO_2; **k**: 4-CH_3; **l**: 4-Br; **m**: 4-OCH_3; **n**: 2,4-di-F; **o**: 2,6-di-F.

Table 1. Biological evaluation of the title compounds [a]

Compd.	 (**3a-3o**)		 (**4a-4o**)		 (**5a-5o**)	
R-	P-gp [b]	P_{app}	P-gp [b]	P_{app}	P-gp [b]	P_{app}
H	10.93±0.99^{i}	13.5	8.29±0.61^{ghi}	12.2	6.72±0.58^{ij}	9.6
2-Cl	13.73±1.03^{f}	4.6	10.25±0.91^{gh}	10.3	8.18±0.82^{hi}	8.2
3-Cl	17.41±1.26^{de}	7.3	18.22±1.31^{c}	7.9	14.72±1.28^{de}	7.9
4-Cl	8.28±0.91^{hi}	6.2	6.73±0.76^{ij}	4.8	3.27±0.63^{k}	10.1
2-F	17.92±1.24^{de}	7.4	14.77±1.07^{de}	3.7	10.89±0.94^{fg}	6.2
3-F	27.39±1.55^{b}	5.9	20.48±1.64^{b}	4.0	17.75±1.73^{c}	4.6
4-F	13.20±1.19^{fg}	5.8	10.37±0.87^{g}	3.8	8.48±0.53^{hi}	3.6
2-NO_2	19.34±1.31^{cd}	6.7	15.20±0.98^{d}	4.9	13.87±1.15^{e}	4.9
3-NO_2	29.11±1.46^{b}	4.9	20.37±1.22^{b}	8.6	16.34±1.38^{cd}	5.4
4-NO_2	15.46±1.28^{ef}	8.7	12.45±1.06^{f}	7.8	9.23±1.21^{gh}	3.5
4-CH_3	9.14±1.01^{hi}	4.8	8.12±1.13^{hi}	7.5	5.23±1.10^{jk}	6.0
4-Br	9.21±0.98^{hi}	9.7	7.82±1.02^{i}	6.3	4.34±1.21^{k}	5.8
4-OCH_3	6.82±0.58^{i}	5.6	5.48±0.42^{j}	6.0	0.89±0.11^{l}	5.3
2,4-di-F	17.33±1.21^{de}	4.7	12.66±0.92^{ef}	4.9	10.43±1.16^{gh}	4.5
2,6-di-F	19.58±1.42^{cd}	7.9	15.34±1.01^{d}	5.3	12.98±1.08^{ef}	3.9
				cyclosporin A	83.68±3.12^{a}	9.8
				verapamil	20.54±0.64^{c}	1.2

[a] Data are the mean of three-independent determinations of triplicate samples.

[b] EC_{50} μM±SEM.

The letters a-l denoted the results of difference significance analysis. Means followed by the same letter within the same column are not significantly different ($p \geq 0.05$, Fisher's LSD multiple comparison test).

Table 2. Capability of the synthesized P-gp modulating agents to increase Doxorubicin intracellular accumulation in MCF-7/ADR cell line [a]

Compd.	Folds of Doxorubicin (50 μM) intracellular accumulation	Antiproliferative effect (%) (5 μM Doxorubicin)
Doxorubicin	1.0	5.3±1.1[f]
20 μM **5d** + Doxorubicin	6.8±0.6[b]	89.6±1.8[b]
20 μM **5l** + Doxorubicin	5.0±0.7[c]	79.8±1.7[d]
20 μM **5m** + Doxorubicin	9.2±0.9[a]	97.8±1.9[a]
20 μM Cyclosporin A + Doxorubicin	5.4±0.8[c]	84.2±2.1[c]
20 μM Verapamil + Doxorubicin	3.6±0.7[d]	52.5±1.5[e]

[a] Data are the mean of three-independent determinations of triplicate samples.
The letters a-e denoted the results of difference significance analysis. Means followed by the same letter within the same column are not significantly different ($p \geq 0.05$, Fisher's LSD multiple comparison test).

Highlights

- 5-Phenyl-2-furan derivatives containing tetrahydro(iso)quinoline were synthesized.
- Most compounds showed good inhibitory activity against P-gp.
- SAR and molecular simulation studies were conducted.
- Compound **5m** could increase Doxorubicin accumulation in overexpressing P-gp MCF-7/ADR cells.
- Drug combination of Doxorubicin and **5m** displayed antiproliferative effect about 97.8%.