RSC Advances

This article can be cited before page numbers have been issued, to do this please use: O. abdelhafez, H. Ali, K. Amin, M. Abdalla and E. Ahmed, *RSC Adv.*, 2015, DOI: 10.1039/C4RA16228E.

View Article Online
DOI: 10.1039/C4RA16228E

Design, synthesis and anticancer activity of furochromone and benzofuran derivatives targeting VEGFR-2 tyrosine kinase

Omaima M. Abdelhafez,[a] Hamed I. Ali,[b, c] Kamelia M. Amin,[d]
Mohamed M. Abdalla, [e] Eman Y. Ahmed [a]

[a] *Chemistry of Natural Products Dept., National Research Center, Dokki, Egypt*

[b]*Pharmaceutical sciences Dept., Irma Lerma Rangel College of Pharmacy, Texas A&M Health Science Center, TX, USA*

[c]*Pharmaceutical Chemistry Dept., Faculty of Pharmacy, Helwan University, Egypt*

[d]*Pharmaceutical Chemistry Dept., Faculty of Pharmacy, Cairo University, Egypt*

[e] *Research unit, Mapco Pharmaceutical industries Balteim, Egypt*

Abstract:

In continuation of our work, concerning the relation between the anticancer and anti-vascular endothelial growth factor receptor (anti-VEGFR-2) activity of some synthesized compounds, we hereby, designed and prepared three new series of furochromone and benzofuran derivatives (carbonitriles, sulfonyl hydrazides and imides). The prepared compounds were evaluated for their *in vitro* VEGFR-2 inhibitory activity, their cytotoxicity on fifteen human cancer cell lines and their *in vivo* antiprostate cancer activity. The highest anti-VEGFR-2 activity was demonstrated by 6-acetyl-4-methoxy-7-methyl-5*H*-furo[3,2-g]chromen-5-one **(3)**, which exhibited the same IC_{50} value as the reference drug sorafenib (2.00×10^{-3} μM). On the other hand, most of the synthesized compounds showed potent cytotoxicity against most of the tested cell lines, in particular, the carbonitrile series **(4a,b** and **5a,d)** which exhibited the best activity with IC_{50} values ranging from (3.56×10^{-13} to 4.89×10^{-7} μM). Moreover, the imide series **(15, 16** and **17)** showed the most significant *in vivo* antiprostate cancer activity. In silico GOLD molecular docking study has been done to explore the binding mode of interaction of the

View Article Online
DOI: 10.1039/C4RA16228E

furochromone and benzofuran derivatives into VEGFR-2 kinase, and to reveal the correlation between IC_{50} (μM) of enzymatic inhibition of VEGFR-2 kinase and the Gold Score fitness for further therapeutic application.

Introduction

Angiogenesis of tumor mass is the process of forming new blood vessels from existing ones of host, and is strictly controlled by the balance of a number of angiogenic factors.[1] The imbalance is thought to affect angiogenesis, which is involved in the expansion and metastasis of solid tumors such as prostate, colon, breast, and gastric cancers.[2-6] The blockade of the tyrosine kinase VEGFR-2, (also known as Flk-1 for fetal liver kinase-1 or KDR for kinase insert domain–containing receptor),[7-9] signaling pathway may disrupt the angiogenesis process of solid tumor so that the developing tumor cells grow slowly or even cease to grow.[10,11] Antiangiogenic therapy is directed mainly at proliferating capillary endothelial cells and does not cause bone marrow suppression, gastrointestinal symptoms or hair loss.[12]

Some inhibitors of VEGFR-2 such as sunitinib [13] and sorafenib,[14] have been approved by the Food and Drug Administration for treating different kinds of tumors, also in literature chromone bioisosteres,[15,16] benzofuran,[17,18] carbonitrile,[19,20] hydrazide,[21,22] and imide derivatives,[23,24] were identified as anti-VEGFR-2 and anticancer agents.

The previously mentioned studies encouraged us to design some furochromone and benzofuran derivatives in order to evaluate their biological activities and molecular docking.

Chemistry

The carbonitriles **(4a-d** and **5a-d),** sulfonyl hydrazide**s (8a,b, 10a,b** and **12a-c)** and imides **(15, 16** and **17)** were efficiently synthesized according to the protocols outlined in **(schemes 1-3)**. All the starting materials of the synthesized compounds were prepared according to the reported methods, while the general reaction conditions, procedures and

View Article Online
DOI: 10.1039/C4RA16228E

compounds characterization of the newly synthesized derivatives were described in the experimental section.

Refluxing visnagin **(1)** in aqueous potassium hydroxide afforded the visnaginone derivative **(2)**,[25] which reacted with acetic anhydride in the presence of anhydrous sodium acetate under reflux to give the acetyl visnagin derivative **(3)**.[26] The reaction of compound **(3)** with the respective aromatic aldehyde namely, (benzaldehyde, 4-bromobenzaldehyde, 5-methyl furfural and 3,4,5-trimethoxybenzaldehyde),in the presence of malononitrile and/or ethylcyanoacetate and ammonium acetate afforded the iminocarbonitrile **(4a-d)** and the oxocarbonitrile **(5a-d)** derivatives respectively.

Moreover, khellinone **(7)** and acetyl khellin **(9)** were prepared using the same previously mentioned method of preparation of visnaginone **(2)** and acetyl visnagin **(3)** respectively. The Mannich bases **(11a-c)**[27] were prepared by the reaction of visnaginone **(2)** with some secondary amines namely, (piperidine, N-methyl piperazine and morpholine) in ethanol in the presence of formaline. The different aryl sulfonyl hydrazides **(8a,b, 10a,b** and **12a-c)** were synthesized by reacting the previously mentioned acetyl containing compounds **(2, 3, 7, 9** and **11a-c)** with *p*-toluene sulfonyl hydrazide in methanol and few drops of conc. HCL.

Furthermore, chlorosulphonation of visnagin **(1)** at 0°C afforded visnagin sulfonyl chloride **(13)**,[28] which upon stirring at room temperature with hydrazine hydrate 98% in 20 ml absolute ethanol and few drops of triethylamine gave the visnagin sulfonyl hydrazide **(14)**,[28] finally, the imide derivatives **(15, 16** and **17)** were obtained by refluxing **(14)** with the appropriate anhydrides namely, (phthalic, succinic and maleic anhydrides) in glacial acetic.

Results and Discussion

1) Biology

a) In vitro anti-VEGFR-2 screening

View Article Online
DOI: 10.1039/C4RA16228E

Studying the anti-VEGFR-2 activity of the tested compounds **(table 1)** showed that 6-acetyl-4-methoxy-7-methyl-5*H*-furo[3,2-g]chromen-5-one **(3)**, was the most active compound and exhibited the same IC_{50} value as the reference drug sorafenib (2.00×10^{-3} μM).

***b) In vitro* cytotoxicity screening**

The cytotoxicity of the tested compounds **(3, 4a-d, 5a-d, 8a,b, 10a,b, 11a-c, 12a-c, 14, 15, 16** and **17)** was determined on fifteen different human cancer cell lines **(tables 2a-d)**.

Screening the cytotoxicity of the tested compounds on cervical carcinoma **(KB),** leukemia **(K561)**, neuroblastoma **(NB-1)**, liver carcinoma **(HepG2)** cell lines, showed that compound **(4b)** has a very remarkable activity, with IC_{50} values ranging from (4.56×10^{-9} to 3.67×10^{-7} μM), compared to the reference drug used.

On ovarian carcinoma **(SKOV-3),** colonoadenocarcinoma **(RKOP27)**, melanoma **(SK-MEL-28),** neuroblastoma **(GOTO),** fibrosarcoma **(HT1080)** cell lines, compound **(5d)** was significantly potent with IC_{50} values ranging from (3.56×10^{-13} to 4.89×10^{-7} μM), exhibiting more potency than the reference drugs.

Regarding the CNS cancer **(SF-268)**, non-small lung cancer (**NCI H460**), leukemia (**HL60)** cell lines, it was found that compound **(5a)** (IC_{50} ranging from 2.33×10^{-11} μM to 3.67×10^{-9} μM) demonstrated a potent cytotoxicity among the other synthesized derivatives.

Estimation of the cytotoxicity of the prepared compounds on leukemia **(U937)**, prostate cancer **(PC-3)** cell lines revealed that compound **(4a)** showed a very pronounced potency ($IC_{50} = 4.39 \times 10^{-7}$ μM) and ($IC_{50} = 4.78 \times 10^{-8}$ μM) respectively compared to the reference drug and the other furochromone and benzofuran derivatives.

Evaluation of the cytotoxicity on melanoma **(G361)** cell lines showed that compound **(3)** exhibited a significant IC_{50} (3.67×10^{-8} μM), better than the reference drug aldesleukin ($IC_{50} = 6.66 \times 10^{-3}$ μM).

View Article Online
DOI: 10.1039/C4RA16228E

The previous screening of the bioactivity of the tested compounds on the presented panel of cell lines indicated that, the majority of the synthesized compounds were significantly potent than the comparative reference drugs used. The carbonitrile series **(4a,b** and **5a,d)** showed the best potency against all the cell lines compared to the reference drugs and to the rest of the newly synthesized furochromone and benzofuran derivatives.

***C) In vivo* antiprostate cancer activity:**

The results of the *in vivo* antiprostate cancer activity **(table 3)** proved that the most significant ED_{50} values were observed for the imide derivatives **(15, 16** and **17)** displaying (ED_{50} from 2.12 μM to 2.82 μM) which was better than the reference drug flutamide (ED_{50} = 11.60 μM).

2) Docking Studies

The computational docking methods are intensively applied to screen a variety of compounds, looking for new hits with specific binding affinities or by investigating a range of structural manipulation for an existing compound.[29] The protein-ligand docking program GOLD facilitates this approach for prediction of the binding mode of the compounds under investigation. GOLD is considered as one of the flexible docking programs that were tested on large test sets to give a 68% success rates on the CCDC/Astex validation set of 305 complexes taken from the Protein Data Bank (PDB).[30]

In this article, we have implemented the Goldscore as a search algorithmic function for "drug-like" which was reported to give superior results than the Chemscore function as a scoring function for GOLD.[31]This Goldscore fitness means the higher the values of fitness (**Table 4**) is the better binding interaction into the binding site.

$$\text{GOLD Fitness} = S_{hb\_ext} + S_{vdw\_ext} + S_{hb\_int} + S_{vdw\_int}$$

The fitness score is the negative sum of the component energy terms, such as protein-ligand external hydrogen bond energy (S_{hb_ext}), protein-ligand external van der Waals

View Article Online
DOI: 10.1039/C4RA16228E

energy (S_{vdw_ext}), protein-ligand intramolecular hydrogen bond energy (S_{hb_int}), and the ligand internal van der Waals energy (S_{vdw_int}), so that larger fitness scores are better.

The implemented Goldscore algorithmic function was initially evaluated for its accuracy into VEGFR-2 kinase. As a function of RMSD (root mean square deviation) of the runs done by flexible docking using GOLD 5.1, the docking poses were nearest to the experimental binding mode of the co-crystallized ligands of VEGFR-2[32]. Our docking results are matched with the reported requirements for successful scoring function,[33] being of RMSD ≤ 2.0Å from the experimental one.

In this study, the newly synthesized compounds were docked into the target crystal structure of human Protein tyrosine kinase of VEGFR-2 kinase domain (pdb code: 3EWH) in complex with a pyridyl-pyrimidine benzimidazole inhibitor (K111). Many compounds revealed good Gold fitness score (Gold Score) values namely, compounds (**4a, 4b, 4d, 5a, 5b, 5c, 5d, 10b, 12a, 12b, 12c** and **15)** which revealed 72, 74, 79.85, 71.80, 70.86, 70.66, 81.17, 71.36, 74.71, 75.09, 78.28 and 72.88 respectively (**Table 4**). Almost all the docked compounds show the common hydrogen bond interactions (1-4) with D1046 (NH, C=O), K868 (NH), E885 (C=O), and R1027 (NH). Fitness of the best docked compounds takes place within RMSD of 2.39 – 6.27 Å as cited in **(Table 4)**. Such binding mode was identically similar to the native co crystallized ligand (K111) which binds with similar binding orientation involving similar amino acids used by docked compounds. This reveals that the interactions between the K868 (NH) and the small molecules will be crucial to inhibit the VEGFR-2 kinase activity.

Compound **(4a)** possess a high potential fitness (GoldScore: 72) into the binding site of the 3D macromolecule (PDB: 3EWH). Its high affinity is presumably attributed to its hydrogen bond formed between its 5-C=O group and D1046 (NH) amino acid and RMSD of 4.44 Å. In addition to the hydrophobic interaction of its 4`-phenyl pyridine moiety which occupies the hydrophobic pockets of the receptor site (I888, I889, I892, V898, V899, and I1044), as illustrated in **Figure 1**. Compound **(4b)** has a better potential fitness

View Article Online
DOI: 10.1039/C4RA16228E

(GoldScore: 74) into the binding site, forming one hydrogen bond between its Terminal 4``-Br and R1027 (NH) amino acid. In addition, both compounds (**4a** and **4b**) revealed external vdw of 52.43 and 53.55, respectively.

As shown in **(Figure 2)** and cited in **(Table 4)**, compound **(12a)** (GoldScore: 74.71) has a promising antiproliferative activity through its higher kinase binding affinity. Where it binds into VEGFR2 kinase through two hydrogen bonds between its 4-CH_3O and 6-O moieties and K868 (HN), and D1046 (HN) amino acids, respectively, where it revealed excellent RMSD of 2.39 Å. Furthermore its 7-piperidinyl moiety occupies the extended hydrophobic pocket similarly to the lipophilic trifluoromethyl-arene portion of the native cocrystallized (K111).

In addition to the role of the hydrogen bond and vdw interactions, the hydrophobic interaction plays a significant role to occupy the 3(4`-aryl pyridine) moiety of compounds **(4a-d** and **5a-d)**, and the 7-piperidinyl, N-Me piprazinyl, and morpholino moieties of compounds **(11a-c** and **12a-c)** into the extended hydrophobic pocket similarly to the lipophilic trifluoromethyl-arene portion of the native co-crystallized (K111).

In the analysis of GOLD docking results, a high correlation (R^2=0.712) between IC_{50} of (VEGFR-2) enzymatic inhibition and the GoldScore fitness for compounds **(4b, 4c, 5a, 5b, 5c, 8a**, **10a**, **10b, 11c, 12a, 16** and **17)** into (VEGFR-2) kinase is shown in **(Figure 3A)**.

Also well correlated result (R^2=0.687) is shown between IC_{50} (μM) against prostate cancer cell line (PC-3) and gold score fitness of compounds **(4a, 4d, 5d, 10a**, **10b, 11b, 11c, 12a, 12c** and **16**) **(Figure 3B)**.

On other hand the correlations between the biological results IC_{50} (μM) against different tumor cell lines namely, **(G361, HL60, K561, KB, RKOP27, SKOV-3, SK-MEL-28** and **U937)** were variably correlated and revealing correlation coefficients (R^2) of 0.67, 0.74, 0.73, 0.764, 0.66, 0.658, 0.79 and 0.686, respectively.

View Article Online
DOI: 10.1039/C4RA16228E

Conclusion

The previously mentioned discussions indicated that the more lipophilic agents may be antiangiogenic rather than cytotoxic agents, and suggested that VEGFR-2 kinase inhibition may not be critical for the cytotoxic responses of our compounds. For example, the phenyl **(5a)** and trimethoxy phenyl **(5d)** derivatives have a potent cytotoxic effects against different tumor cells such as HL60, U937, K561, G361, SK-MEL-28, PC-3, HT1080, HepG2, KB, SK OV-3, SF-268, NCI H460, and RKOP27 in IC_{50} of 10^{-6}-10^{-13} μM range. Whereas, their 4-bromophenyl derivative **(5b)** being more lipophilic inhibited these tumor cells in much lower IC_{50} of 10^{-3}-10^{-5} μM range, despite its higher VEGFR-2 inhibition (IC_{50} of 2.2 x 10^{-3} μM). The VEGFR-2 inhibition of compound **(5b)** is almost of the same potency of the referenc drug sorafenib being of (IC_{50} of 2.00 x 10^{-3} μM).

On the other hand, the in vivo results of these compounds are correlated with this outcome, where compound **(5b)** revealed better *in vivo* antiprostate cancer activity (ED_{50} = 13.11μM) than that of compounds **(5a** and **5d)** (ED50 = 26.0 and 28.6 μM, respectively). This suggests that their prostate cancer antiproliferative activities are mediated through VEGFR-2 pathway.

Likewise within the *in vivo* prostate cancer experiment, compounds **(15-17)** have (ED_{50} of 3.73, 2.56, 2.82, and 2.12 μM, respectively). Similarly, they have equipotent inhibitory activities against VEGFR-2 of (3.49 x 10^{-2}, 2.62 x 10^{-2}, 4.22 x 10^{-2}, and 2.17 x 10^{-2} μM, respectively).However, this correlation was deviated in the in vitro PC3 cell assay, where compound **(14** and **17)** have equipotent activities of IC_{50}: 1.25 x 10^{-5} and 5.0 x 10^{-5} μM, respectively, revealing highly more potent cytotoxic effect than compounds **(15** and **16)** of IC_{50}: 8.0 x 10^{-3} and 8.9 x 10^{-3} μM, respectively. This result may suggest the resistance of PC3 cell lines against compounds **(15** and **16)**, and indicates that *the in vitro* data may not necessarily reflect the *in vivo* effect.

View Article Online
DOI: 10.1039/C4RA16228E

Experimental

1) Chemistry

Melting points were determined on Electrothermal IA 9000 apparatus and were uncorrected. The infrared (IR) spectra were recorded using Nexus 670 FT-IR FT-Raman spectrometer as potassium bromide discs, at National Research Centre. The proton nuclear resonance (^{1}H NMR) spectra were determined on Varian mercury 500 MHz spectrometer, using tetramethylsilane (TMS) as the internal standard, at National Research Centre. The mass spectra were performed on JEOL JMS-AX500 mass spectrometer at the National Research Centre. The reactions were followed by thin layer chromatography (TLC) (Silica gel, aluminum sheets 60 F254, Merck) using benzene: ethyl acetate (8:2 v/v) as eluent and sprayed with iodine–potassium iodide reagent. The purity of the newly synthesized compounds was assessed by elemental analysis and was found to be higher than 95%.

Preparation of (6-hydroxy-4-methoxybenzofuran-5-yl)ethanone (2), 6-acetyl-4-methoxy-7-methyl-5*H*-furo[3,2-g]chromen-5-one (3).

Visnaginone **(2)**[25] and acetyl visnagin **(3)**[26] were prepared according to the reported methods.

General procedure for the preparation of different iminocarbonitriles (4a-d).

Acetyl visnagin (2.5 mmol) together with the respective aromatic aldehyde (2.5 mmol) namely, (benzaldehyde, 4-bromobenzaldehyde, 5-methyl furfural and 3,4,5-trimethoxybenzaldehyde), malononitrile (2.5mmol) and ammonium acetate (20 mmol) were refluxed for 10-14 hours in ethanol (30ml). The precipitate formed was filtered, washed with ethanol, dried and crystallized from DMF-ethanol (1:10).

2-Amino-6-(4-methoxy-7-methyl-5-oxo-5*H*-furo[3,2-g]chromen-6-yl)-4-phenylpyridine-3-carbonitrile (4a).

Yield: 90%; mp: 82-5 °C; Mol. wt.: 423.42. IR (cm^{-1}, KBr): 3343 (NH_2), 2206 (C≡N), 1617 (C=O, γ-pyrone), ^{1}H NMR (DMSO-d_6, δ, ppm): 2.40 (3H, s, CH_3), 3.78 (3H, s,

View Article Online
DOI: 10.1039/C4RA16228E

OCH_3), 6.64 (1H, s, CH), 6.66 (1H, d, H-3 benzofuran), 7.20-7.70 (5H, m, Ar-H), 7.30 (1H, s, CH-pyridine), 7.52 (1H, d, H-2 benzofuran), 11.70 (2H, s, NH_2, D_2O exchangeable). EIMS; m/z (R.A. %): 424 (M^++1) (65%), 393 (75%), 355 (100%), 213 (58%), 140 (57%), 91 (76%).

2-Amino-4-(4-bromophenyl)-6-(4-methoxy-7-methyl-5-oxo-5*H*-furo[3,2-g]chromen-6-yl)pyridine-3-carbonitrile (4b).

Yield: 71%; mp: 87-90°C; Mol. wt.: 502.32. IR (cm^{-1}, KBr): 3342 (NH_2), 2199 (C≡N), 1617 (C=O, γ-pyrone). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.90 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 6.64 (1H, s, CH), 6.66 (1H, d, H-3 benzofuran), 7.30-7.80 (4H, m, Ar-H), 7.38 (1H, s, CH-pyridine), 7.52 (1H, d, H-2 benzofuran), 10.38 (2H, s, NH_2, D_2O exchangeable). EIMS; m/z (R.A. %): 502/504 (M^+/M^++2) (80%), 469 (100%), 355 (70%), 213 (90%), 140 (60%), 91 (99%).

2-Amino-6-(4-methoxy-7-methyl-5-oxo-5*H*-furo[3,2-g]chromen-6-yl)-4-(5-methylfuran-2-yl)pyridine-3-carbonitrile (4c).

Yield: 70%; mp: 67-70°C; Mol. wt.: 427.41. IR (cm^{-1}, KBr): 3356 (NH_2), 2214 (C≡N), 1609 (C=O, γ-pyrone).^{1}H NMR (DMSO-d_6, δ, ppm): 2.41 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 5.1 (1H, d, H-4 furan), 5.9 (1H, d, H-3 furan), 6.60 (1H, s, CH), 6.62 (1H, d, H-3 benzofuran), 7.00 (1H, s, CH-pyridine), 7.52 (1H, d, H-2 benzofuran), 10.38 (2H, s, NH_2, D_2O exchangeable). EIMS; m/z (R.A. %): 427 (M^+) (70%), 330 (70%), 355 (100%), 214 (50%), 143 (65%), 83 (55%).

2-Amino-6-(4-methoxy-7-methyl-5-oxo-5*H*-furo[3,2-g]chromen-6-yl)-4-(3,4,5-trimethoxyphenyl)pyridine-3-carbonitrile (4d).

Yield: 90%; mp: 255-8°C; Mol. wt.: 513.50. IR (cm^{-1}, KBr): 3394 (NH_2), 2205 (C≡N), 1618 (C=O, γ-pyrone). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.75 (3H, s, CH_3), 3.71-3.74 (12H, m, OCH_3), 6.00 (2H, m, Ar-H), 6.54 (1H, s, CH), 6.60 (1H, d, H-3 benzofuran), 7.60 (1H, s, CH-pyridine), 7.78 (1H, d, H-2 benzofuran), 9.38 (2H, s, NH_2, D_2O exchangeable).

View Article Online
DOI: 10.1039/C4RA16228E

EIMS; m/z (R.A. %): 512 (M^+-1) (65%), 496 (100%), 351 (20%), 204 (25%), 120 (15%), 100 (13%).

General procedure for the preparation of different oxocarbonitriles (5a-d).

Acetyl visnagin (2.5 mmol) together with the respective aromatic aldehyde (2.5 mmol) namely (benzaldehyde, 4-bromobenzaldehyde, 5-methyl furfural and 3,4,5-trimethoxybenzaldehyde), ethylcyanoacetate (2.5mmol) and ammonium acetate (20 mmol) were refluxed for 10-14 hours in ethanol (30ml). The precipitate formed was filtered, washed with ethanol, dried and crystallized from DMF-ethanol (1:10).

1,2-Dihydro-6-(4-methoxy-7-methyl-5-oxo-5*H*-furo[3,2-g]chromen-6-yl)-2-oxo-4-phenylpyridine-3-carbonitrile (5a).

Yield: 87%; mp: 100-1°C; Mol. wt.: 424.40. IR (cm^{-1}, KBr): 3123 (CH aromatic), 2210 (C≡N), 1624 (C=O, γ-pyrone). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.00 (3H, s, CH_3), 3.81 (3H, s, OCH_3), 6.00 (1H, s, CH-pyridine), 6.46 (1H, s, CH), 6.64 (1H, d, H-3 benzofuran), 7.10-7.30 (5H, m, Ar-H), 7.42 (1H, d, H-2 benzofuran), 8.70 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 424 (M^+) (60%), 364 (30%), 246 (38%), 169 (45%), 80 (100%).

4-(4-Bromophenyl)-1,2-dihydro-6-(4-methoxy-7-methyl-5-oxo-5*H*-furo[3,2-*g*]chromen-6-yl)-2-oxopyridine-3-carbonitrile (5b).

Yield: 75%; mp: 95-7°C; Mol. wt.: 503.30. IR (cm^{-1}, KBr): 3128 (CH aromatic), 2220 (C≡N), 1620 (C=O, γ-pyrone). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.70 (3H, s, CH_3), 3.63 (3H, s, OCH_3), 6.38 (1H, s, CH-pyridine), 6.44 (1H, s, CH), 6.66 (1H, d, H-3 benzofuran), 7.20-7.40 (4H, m, Ar-H), 7.40 (1H, d, H-2 benzofuran), 9.33 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 503/505 (M^+/M^++2) (67%), 359 (65%), 262 (85%), 114 (85%), 80 (100%).

View Article Online
DOI: 10.1039/C4RA16228E

1,2-Dihydro-6-(4-methoxy-7-methyl-5-oxo-5*H*-furo[3,2-g]chromen-6-yl)-4-(5-methylfuran-2-yl)-2-oxopyridine-3-carbonitrile (5c).

Yield: 70%; mp: 70-3°C; Mol. wt.: 428.39. IR (cm^{-1}, KBr): 3123 (CH aromatic), 2221 (C≡N), 1618 (C=O, γ-pyrone), ^{1}H NMR (DMSO-d_6, δ, ppm): 2.00 (3H, s, CH_3), 2.17 (3H, d, CH_3-furan), 3.73 (3H, s, OCH_3), 5.93 (1H, s, CH-pyridine), 6.10 (1H, d, H-4 furan), 6.42 (1H, s, CH), 6.62 (1H, d, H-3 benzofuran), 6.89 (1H, d, H-3 furan), 7.50 (1H, d, H-2 benzofuran), 9.00 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 428 (M^+) (60%), 330 (100%), 200 (50%), 143 (65%).

1,2-Dihydro-6-(4-methoxy-7-methyl-5-oxo-5*H*-furo[3,2-g]chromen-6-yl)-4-(3,4,5-trimethoxyphenyl)-2-oxopyridine-3-carbonitrile (5d).

Yield: 91%; mp: 104-7°C; Mol. wt.: 514.48. IR (cm^{-1}, KBr): 3122 (CH aromatic), 2216 (C≡N), 1627 (C=O, γ-pyrone). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.71 (3H, s, CH_3), 3.71-3.80 (12H, m, OCH_3), 5.60 (1H, s, CH-pyridine), 6.20 (2H, m, Ar-H), 6.54 (1H, s, CH), 6.61 (1H, d, H-3 benzofuran), 7.71 (1H, d, H-2 benzofuran), 8.88 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 513 (M^+-1) (60%), 410 (20%), 218 (55%), 105 (90%), 77 (100%).

Preparation of 1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)ethanone (7), 6-acetyl-4,9-dimethoxy-7-methyl-5*H*-furo[3,2-g]chromen-5-one (9), 1-(6-hydroxy-4-methoxy-7-(piperidin-1-ylmethyl)benzofuran-5-yl)-3-(5-methylfuran-2-yl)prop-2-en-1-one (11a), 1-(6-hydroxy-4-methoxy-7-(4-methylpiperazin-1-yl)methyl)benzofuran-5-yl)ethanone (11b), 1-(6-hydroxy-4-methoxy-7-(morpholinomethyl)benzofuran-5-yl)ethanone (11c).

Khellinone**(7)**[25], acetyl khellin**(9)**[26] and the Mannich bases **(11a-c)**[27]were prepared according to the reported methods.

View Article Online
DOI: 10.1039/C4RA16228E

General procedure for the preparation of different aryl sulfonylhydrazides (8a,b, 10a,b, 12a-c).

To the appropriate ketone **(2, 3, 7, 9** and **11a-c)** (0.02 mol),dissolved in methanol (15 ml), *p*-toluene sulfonyl hydrazide (0.025) dissolved in methanol (30 ml) was added, then 2-3 drops of conc. HCL were added to the reaction mixture. The reaction mixture was kept at room temperature overnight, where crystals of the product were separated out, filtered, dried and then crystallized from methanol.

5-(1-Tosylhydrazono-ethyl)-4-methoxy-benzofuran-6-ol (8a)

Yield: 87%; mp: 146-8°C; Mol. wt.: 374.41. IR (cm^{-1}, KBr): 3564 (OH aromatic), 3212 (NH), 1628 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.00 (3H, s, CH_3), 2.35 (3H, s, CH_3-toluene), 3.87 (3H, s, OCH_3), 5.00 (1H, s, OH, D_2O exchangeable), 6.22 (1H, d, H-3 benzofuran), 6.70 (1H, s, CH-benzofuran), 7.32-7.94 (4H, m, CH-toluene), 7.50 (1H, d, H-2 benzofuran), 9.00 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 374 (M^+) (65%), 250 (55%), 163 (90%), 79 (100%).

5-(1-Tosylhydrazono-ethyl)-4,7-dimethoxy-benzofuran-6-ol (8b)

Yield: 85%; mp: 205-7°C; Mol. wt.: 404.44. IR (cm^{-1}, KBr): 3490 (OH aromatic), 3225 (NH), 1617 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.88 (3H, s, CH_3), 2.35 (3H, s, CH_3-toluene), 3.87 (6H, s, OCH_3), 5.00 (1H, s, OH, D_2O exchangeable), 6.22 (1H, d, H-3 benzofuran), 7.32-7.94 (4H, m, CH-toluene), 7.50 (1H, d, H-2 benzofuran), 8.00 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 405 (M^++1) (70 %), 266 (50%), 183 (80%), 79 (100%).

6-(1-Tosylhydrazono-ethyl)-4-methoxy-7-methyl-furo[3,2-*g*]chromen-5-one (10a)

Yield: 82%; mp: 150-2°C; Mol. wt.: 440.47. IR (cm^{-1}, KBr): 3211 (NH), 3008 (CH aromatic), 2846 (CH aliphatic), 1628 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.88 (3H, s, CH_3), 2.00 (3H, s, CH_3-furochromone), 2.35 (3H, s, CH_3-toluene), 3.79 (6H, s, OCH_3), 6.66 (1H, d, H-3 benzofuran), 6.74(1H, s, CH-benzofuran), 7.23-7.66 (4H, m, CH-

View Article Online
DOI: 10.1039/C4RA16228E

toluene), 7.52 (1H, d, H-2 benzofuran), 10.47 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 439 (M^+-1) (60 %), 289 (70%), 186 (100%), 79 (80%).

6-(1-Tosylhydrazono-ethyl)-4,9-dimethoxy-7-methyl-furo[3,2-*g*]chromen-5-one (10b)

Yield: 80%; mp: 170-1°C; Mol. wt.: 470.49. IR (cm^{-1}, KBr): 3188 (NH), 3067 (CH aromatic), 2915 (CH aliphatic), 1631 (C=N). ^1H NMR (DMSO-d_6, δ, ppm): 1.22 (3H, s, CH_3), 1.90 (3H, s, CH_3-furochromone), 2.35 (3H, s, CH_3-toluene), 3.73 (3H, s, OCH_3), 6.69 (1H, d, H-3 benzofuran), 7.29-7.68 (4H, m, CH-toluene), 7.77 (1H, d, H-2 benzofuran), 11.47 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 439 (M^+-1) (60 %), 289 (70%), 183 (80%), 78 (100%).

5-(1-Tosylhydrazono-ethyl)-4-methoxy-7-piperidin-1-yl-methylbenzofuran-6-ol (12a).

Yield: 70%; mp: 258-9°C; Mol. wt.: 473.59. IR (cm^{-1}, KBr): 3450 (OH aromatic), 3062 (NH), 1611 (C=N). ^1H NMR (DMSO-d_6, δ, ppm): 0.99 (3H, s, CH_3), 2.39-2.49 (10H, m, CH_2-piperidine), 2.59 (3H, s, CH_3-toluene), 3.40 (2H, s, CH_2N), 3.48 (3H, s, OCH_3), 6.00 (1H, s, OH, D_2O exchangeable), 7.24 (1H, d, H-3 benzofuran), 7.27-7.46 (4H, m, CH-toluene), 7.70 (1H, d, H-2 benzofuran), 14.80 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 473 (M^+) (60%), 307 (65%), 226 (25%), 140 (40%), 66 (100%).

5-(1-Tosylhydrazono-ethyl)-4-methoxy-7-(4-methyl-piperazin-1-yl-methyl)-benzo-furan-6-ol (12b).

Yield: 71%; mp: 196-7°C; Mol. wt.: 488.60. IR (cm^{-1}, KBr): 3420 (OH aromatic), 3189 (NH), 1617 (C=N). ^1H NMR (DMSO-d_6, δ, ppm): 1.21 (3H, s, CH_3), 2.00 (3H, s, CH_3-piperidine), 2.30-2.50 (8H, m, CH_2-piperidine), 3.00 (3H, s, CH_3-toluene), 3.40 (2H, s, CH_2N), 3.78 (3H, s, OCH_3), 5.00 (1H, s, OH, D_2O exchangeable), 7.00 (1H, d, H-3 benzofuran), 7.37-7.70 (4H, m, CH-toluene), 7.90 (1H, d, H-2 benzofuran), 13.00 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 488 (M^+) (60%), 312 (25%), 229 (25%), 128 (32%), 65 (100%).

View Article Online
DOI: 10.1039/C4RA16228E

5-(1-Tosylhydrazono-ethyl)-4-methoxy-7-morpholin-4-yl-methyl-benzofuran-6-ol (12c)

Yield: 75%; mp: 120-1°C; Mol. wt.: 475.56.IR (cm^{-1}, KBr): 3410 (OH aromatic), 3201 (NH), 1640 (C=N).^{1}H NMR (DMSO-d_6, δ, ppm): 1.00 (3H, s, CH_3), 2.22 (3H, s, CH_3-toluene), 2.47-3.49 (8H, m, CH_2-morpholine), 3.70 (2H, s, CH_2N), 3.98 (3H, s, OCH_3), 5.00 (1H, s, OH, D_2O exchangeable), 6.74 (1H, d, H-3 benzofuran), 7.37-7.66 (4H, m, CH-toluene), 7.90 (1H, d, H-2 benzofuran), 13.80 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 476 (M^++1) (60%), 345 (65%), 237 (45%), 159 (100%).

Preparation of 4-methoxy-7-methyl-5-oxo-5*H*-furo[3,2-*g*]chromene-9-sulfonylchloride (13) and 4-methoxy-7-methyl-5-oxo-5H-furo[3,2-*g*]chromene-9-sulfonylhydrazide (14).

Visnagin sulfonyl chloride **(13)**[28] and Visnagin sulfonyl hydrazide **(14)**[28] were prepared according to the reported methods.

General procedure for the preparation of different imino derivatives (15, 16 and **17).**

To a solution of visnagin-4-sulphonyl hydrazide (0.01 mol) in glacial acetic acid, the appropriate anhydrides namely, (phthalic anhydride, succinic anhydride and maleic anhydride) (0.005 mol) were added. The mixture was refluxed for 6-8 hours, poured onto ice cold water, the formed precipitate was filtered, washed with water and crystallized from glacial acetic acid to give the product.

4-Methoxy-7-methyl-5-oxo-5*H*-furo[3,2-*g*]chromene-9-sulfonic acid(1,3-dioxo-1,3-dihydro-isoindol-2-yl)amide (15).

Yield: 90%; mp: 160-1°C; Mol. wt.: 454.41. IR (cm^{-1}, KBr): 3160 (CH aromatic), 2925(CH aliphatic), 1740 (C=O, anhydride). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.20 (3H, s, CH_3), 4.00 (3H, s, OCH_3), 6.22 (1H, s, CH), 6.70 (1H, d, H-3 benzofuran), 7.78 (1H, d, H-2 benzofuran), 7.70-8.20 (4H, m, Ar-H), 13.00 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 454 (M^+) (80%), 382 (40%), 292 (76%), 185 (20%), 56 (100%).

View Article Online
DOI: 10.1039/C4RA16228E

4-Methoxy-7-methyl-5-oxo-5*H*-furo[3,2-*g*]chromene-9-sulfonic acid(pyrrolidine-2-5-dione-1-yl)amide (16).

Yield: 75%; mp: 190-2°C; Mol. wt.: 406.37. IR (cm^{-1}, KBr): 3166 (CH aromatic), 2927 (CH aliphatic), 1744 (C=O, anhydride). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.20 (3H, s, CH_3), 2.73 (4H, t , CH2-pyrrolidine-dione), 3.50 (3H, s, OCH_3), 6.11 (1H, s, CH), 6.60 (1H, d, H-3 benzofuran), 7.58 (1H, d, H-2 benzofuran), 9.00 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 406 (M^+) (60%), 342 (45%), 210 (66%), 191 (20%), 61 (100%).

4-Methoxy-7-methyl-5-oxo-5*H*-furo[3,2-*g*]chromene-9-sulfonic acid(pyrrol-2-5-dione-1-yl)amide (17)

Yield: 70%; mp: 270-2°C; Mol. wt.: 404.35. IR (cm^{-1}, KBr): 3170 (CH aromatic), 2930 (CH aliphatic), 1750 (C=O, anhydride). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.00 (3H, s, CH_3), 4.50 (3H, s, OCH_3), 6.31 (1H, s, CH), 6.60 (1H, d, H-3 benzofuran), 7.00 (2H, d, CH2-pyrrole-dione), 8.18 (1H, d, H-2 benzofuran), 10.00 (1H, s, NH, D_2O exchangeable). EIMS; m/z (R.A. %): 404 (M^+) (70%), 358 (49%), 217 (66%), 114 (40%), 66 (100%).

2) Biology

VEGFR-2 kinase activity assays by ELISA.[34]

The assay was performed in 96-well plates pre-coated with 20 μg mL^{-1} poly (Glu, Tyr) 4:1 using sorafenib and DMSO as positive and negative controls respectively, Experiments at varying concentrations (1nM-1μM) were performed in triplicate, where 85μL of an 8 μM ATP solution and 10 μL of the compound were added in each well.

The reaction was initiated by adding VEGFR-2 kinase (5 μL), followed by addition of 100 μL of anti-phosphotyrosine antibody (PY99; 1:500 dilution) then Goat anti-mouse IgG horseradish peroxidase (100 μL; 1 : 2000 dilution) diluted in T-PBS containing 5 mg mL^{-1} BSA was added. After each addition there was an incubation period of 1h at room temperature followed by washing.

Finally, 100 μL of (0.03% H_2O_2, 2 mg mL^{-1} *o*-phenylenediamine in citrate buffer 0.1 M, pH 5.5) was added and incubated at room temperature until color developed. The

View Article Online
DOI: 10.1039/C4RA16228E

reaction was terminated by the addition of 100 μL of 2 M H_2SO_4. The inhibition rate (%) was calculated using the following equation:

Inhibition rate (%) = [1-(A_{492}/ $A_{492Control}$)] x 100%, where, A_{492} was measured using a multiwell spectrophotometer (VERSAmax™).

***In vitro* cytoxicity screening.**[35]

Cells were plated in 96-multiwell plate (5000 to 40,000 cells /well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of plate, a solution of each tested compound was prepared by solubilizing each in DMSO 400-fold the desired final maximum test concentration.

After being incubated for 24 h, cells were treated with different concentrations of each compound under test, where an aliquot of frozen concentrate was diluted to twice the desired final maximum test concentration and four 10 fold serial dilutions were made to provide a total of five drug concentrations plus control. The cells were incubated with the compounds for 48 h at 37 ºC and in atmosphere of 5% CO_2. Control cells were treated with vehicle alone.

After 48 h, cells were fixed, washed and stained with Sulforhodamine B (SRB) (0.4 % w/v) dissolved in 1 % acetic acid for 10 min. Excess stain was washed with acetic acid and attached stain was solubilized with 10 mM trizma base. The absorbance was measured in an automated plate reader at wave length of 515 nm. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared with the reference drugs.

***In Vivo* evaluation of antiprostate cancer Activitiy.**[36]

Testosterone propionate (TP, 0.5 mg/kg) dissolved in cotton seed oil containing 5% ethanol was administered to castrated male Wistar rats treated with androgen, three days after castration, once daily for 5days alone or in combination with the tested compounds (10-30 mg/kg) suspended with 0.5% methylcellulose. The rats were sacrificed after final dosing, and both ventral prostates and seminal vesicles-coagulate glands were removed

View Article Online
DOI: 10.1039/C4RA16228E

and weighed. The antiprostate cancer activity was expressed as a percentage of inhibition of the TP effect.

3) Molecular Docking

GOLD software package, version 5.1 (Cambridge Crystallographic Data Centre, Cambridge, U.K.),[37] was used, the receptors were prepared using Hermes visualizer in the GOLD Suite, and the region of interest was selected as all the protein residues within the 10 Å of the reference ligands.

For pose selection and enrichment studies default values of speed settings and all other parameters were used. GOLD Score fitness algorithmic function was used with a default input and annealing parameters, which were managed within 4.0 and 2.5 Å for the van der Waals and H-bond interactions, respectively [38,39]

All the docked ligands were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10.

In this study, 10 genetic algorithm (GA) docking runs were used with internal energy offset. For pose reproduction analysis, the radius of the binding pocket was set as the maximal atomic distance from the geometrical center of the ligand plus 3Å. When the top three solutions attained RMSD values within 1.5 Å, docking was terminated.

The top ranked docking pose was retained for the 3D cumulative success rate analysis. Rescoring was conducted with the GOLD rescore option, in which poses would be optimized by the program. The top 10 ranked docking poses were selected based on their gold fitness scores and their reported mode of interactions, which were analyzed using Accelrys Discovery studio to reveal the hydrogen bond interaction and binding mode within the binding domain.

With respect to ligand flexibility, flipping of all planar RNR1R2 ring, NH-R ring, carboxylic acids –COOH were included, and the torsion angle distribution and post process rotatable bonds were set as default. All other Genetic Algorithm parameters were set as default settings.

View Article Online
DOI: 10.1039/C4RA16228E

The crystal structure of the (VEGFR-2) kinase domain in complex with *N*-[4-({3-[2-(methylamino)pyrimidin-4-yl]pyridin-2-yl}oxy)naphthen-1-yl]-6-(trifluorometh-yl)-1*H*-benzimidazol-2-amine (K111) co-crystalized ligand (pdb code: 3EWH) was used as target protein structure, which was retrieved from the Protein Data Bank (PDB). PDB sum was accessed for identification of the key amino acids and the flexible residues (E885, T916, and D1046) in the binding site. All residues within 10 Å radius from the origin: 13.237, -2.016 and 11.23 of the co-crystallized ligand coordinates.

Author Information

Corresponding Author

Phone: +20 202 37608284; fax: +20 202 33370931. E-mail address: dromaima45@gmail.com (O.M. Abdelhafez).

Abbreviations Used

IC_{50}, half maximal inhibitory concentration; ED_{50}, median effective dose; TLC, thin layer chromatography; DMSO, Dimethyl Sulfoxide; MOPAC, Molecular Orbital Package.

References:

(1) P. Carmeliet, R. K. Jain, *Nature*, 2000, 407, 249-57.

(2) J. Folkman, *Semin. Oncol.*, 2001, 28, 536-42.

(3) N. Ferrara, H. P. Gerber, J. Le Couter, *Nat. Med.*, 2003, 9, 669-76.

(4) P. Cohen, *Eur. J. Biochem.,* 2001, 268, 5001-10.

(5) E. Zwick, J. Bange, A. Ullrich, *Trends Mol. Med,* 2002, 8, 17-23.

(6) P. Traxler, G. Bold, E. Buchdunger, G. Caravatti, P. Furet, P. Manley, T. O'Reilly, J. Wood, J. Zimmermann, *J. Med. Res. Rev.,* 2001, 21, 499-512.

(7) M. Kowanetz, N. Ferrara, *Clin. Cancer ReS.,* 2006, 12, 5018–22.

(8) K. A. Houck, N. Ferrara, J. Winer, G. Cachianes, B. Li, D.W. Leung, *Mol. Endocrinol.,*1991, 5, 1806–14.

(9) P. N. Bernatchez, S. Soker, M. G. Sirois, *J. Biol. Chem.,* 1999, 274, 3147–54.

(10) B. Peruzzi, D. P. Bottaro, *Clin. Cancer Res.*, 2006, 12, 3657-60.

View Article Online
DOI: 10.1039/C4RA16228E

(11) J. Folkman, *Semin. Oncol.*, 2002, 29, 15-8.

(12) E. Wassberg, R. Christofferson, *Eur. J. Cancer*, 1997, 33, 2020-3.

(13) E. Cabebe, H. Wakelee, *Drugs Today (Barc),* 2006, 42, 387–98.

(14) R. C. Kane, A.T. Farrell, H. Saber, S. Tang, G. Williams, J.M. Jee, C. Liang,; B. Booth, N. Chidambaram, D. Morse, R. Sridhara, P. Garvey, R. Justice, R. Pazdur, *Clin. Cancer Res.,* 2006, 12, 7271–8.

(15) J. Lee, S. Choi, Y. Lee, H. Lee, K. Kim, K. Ahn, H. Bae, H. Lee, E. Lee, K. Ahn, S. Ryu, J. Lü, S. Kim, *Mol. Cancer Ther.,* 2010, 9, 389-99.

(16) S. M. Sh. Atta, D. S. Farrag, A. M. K. Sweed, A. H. Abdel-Rahman, *Eur. J. Med .Chem.,*2010, 45, 4920-7.

(17) Y. Song, F. Dai, D. Zhai, Y. Dong, J. Zhang , B. Lu, J. Luo, M. Liu, Z. Yi, *Angiogenesis,* 2012, 15, 421–32.

(18) S.A. Galal, A. S. Abd El-All, M. M. Abdallah, H. I. El-Diwani, *Bioorg. Med. Chem. Lett.*, 2009, 19, 2420-8.

(19) J. T. Sisko, T. J. Tucker, M. T. Bilodeau, C.A. Buser, P.A. Ciecko, K. E. Coll, C. Fernandes, J. B. Gibbs, T. J. Koester, N. Kohl, J. J. Lynch, X. Mao, D. McLoughlin, C. M. Miller-Stein, L. D. Rodman, K. W. Rickert, L. Sepp-Lorenzino, J. M. Shipman, K. A. Thomas, B. K. Wong, G. D. Hartman., *Bioorg. Med. Chem. Lett.*, 2006, 16, 1146-50.

(20) A. H. Abadi, T. M. Ibrahim, K. M. Abouzid, J. Lehmann, H. N. Tinsley, B. D. Gary, G. A. Piazza, *Bioorg. Med. Chem.*, 2009, 17, 5974-82.

(21) T. Usui, H. S. Ban,J. Kawada, T. Hirokawa, H. Nakamura, *Bioorg. Med. Chem. Lett.*, 2008, 18, 285–8.

(22) P. De, M. Baltas, D. Lamoral-Theys, C. Bruyère, R. Kiss, F. Bedos-Belval, N. Saffon, *Bioorg. Med. Chem. Lett.*,2010, 18, 2537–48.

(23) M. Okaniwa, T. Imada, T. Ohashi, T. Miyazaki, T. Arita, M. Yabuki, A. Sumita, S. Tsutsumi, K. Higashikawa, T. Takagi, T. Kawamoto, Y. Inui, S. Yoshida, T. Ishikawa, *Bioorg. Med. Chem.*, 2012, 20, 4680-92.

View Article Online
DOI: 10.1039/C4RA16228E

(24) O. M. Abdel Hafez, K. M. Amin, N. A. Abdel-Latif, T. K. Mohamed, E. Y. Ahmed, T. Maher, Eur. J. Med. Chem., 2009, 44, 2967-74.

(25) E. Spath, W. Gruber, *Ber.,*1941, 74, 1492-500.

(26) W. S. Elhamouly, M. A. Abdel-Alim, O. M. Abdel-Hafez, H. A. Tawfeek, *Bull. Fac. Sci. Zagazig Univ.*, 1990, 12, 442-51.

(27) F. A. Ragab, S. L. El-Ansary, A. B. Hassan,Egypt. J. Pharm. Sci., 1992, 33, 931-42.

(28) M. H. A. Elgamal, N. M. M. Shalaby, H. Duddeck, D. Rosenbaum, *J. Heterocycl. Chem.*, 1987, 24, 721-4.

(29) M. L. Verdonk, J. C. Cole, M. J. Hartshorn, C. W. Murray, R. D. Taylor, *Proteins: Struct. Funct.Genet.*,2003, 52, 609–23.

(30) J. W. M. Nissink, C.W. Murray, M.J. Hartshorn, M. L. Verdonk, J. C. Cole, R. Taylor, *Proteins*, 2002, 49, 457–71.

(31) L. V. Marcel, C. C. Jason, J. H. Michael, W. M. Christopher, D. T. Richard, *Proteins: Struct. Funct. Genet.*, 2003, 52, 609–23.

(32) V. J. Cee, A. C. Cheng, K. Romero, S. Bellon, C. Mohr, D. A. Whittington, A. Bak, J. Bready, S. Caenepeel, A. Coxon, H. L. Deak, J. Fretland, Y. Gu, B. L. Hodous, X. Huang, J. L. Kim, J. Lin, A. M. Long, H. Nguyen, P. R. Olivieri, V. F. Patel, L. Wang, Y. Zhou, Hughes, P. Geuns, S. Meyer, Bioorg. Med. Chem. Lett*.,* 2009, 19, 424–7.

(33) R. Wang, Y. Wang, S. Lu, *J. Med. Chem.*, 2003, 46, 2287–303.

(34) L. Xi, J. Zhang, Z. Liu, J. Zhang, J. Yan, Y. Jin, J. Lin, *Org. Biomol. Chem.,* 2013, 11, 4367-78.

(35) M. R. Grever, S. A. Schepartz, B. A. Chabner, *Sem. Oncol*., 1992, 19, 622-38.

(36) I. Kinoyama, N. Taniguchi, E. Kawaminami, E. Nozawa, H. Koutoku, T. Furutani, M. Kudoh, M. Okada ,*Chem. Pharm. Bull.,* 2005*,* 53, 402-9.

(37) J. C. Cole, J. W. M. Nissink, R .Taylor, in Virtual Screening in Drug Discovery, ed. B. Shoichet and J. Alvarez, Taylor and Francis CRC Press: Boca Raton, FL, USA, 2005, pp. 379–416.

View Article Online
DOI: 10.1039/C4RA16228E

(38) G. Jones, P. Willett, *Curr. Opin.Biotechnol., 1995*, *6*, 652-6.

(39) G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol.,* 1997, 267, 727-48.

View Article Online
DOI: 10.1039/C4RA16228E

List of Tables

Table **1:** Anti-VEGFR-2 activity of the synthesized compounds.

Compound	Enzymatic inhibition $IC_{50}\mu M$
	VEGFR-2
3	2.00×10^{-3}
4a	-------
4b	2.70×10^{-3}
4c	2.90×10^{-3}
4d	8.40×10^{-3}
5a	1.01×10^{-2}
5b	2.20×10^{-3}
5c	6.30×10^{-3}
5d	1.22×10^{-2}
8a	2.62×10^{-2}
8b	5.70×10^{-3}
10a	3.20×10^{-3}
10b	3.20×10^{-3}
11a	1.20×10^{-2}
11b	5.20×10^{-3}
11c	1.63×10^{-2}
12a	4.65×10^{-2}
12b	1.48×10^{-2}
12c	1.79×10^{-2}
14	3.49×10^{-2}
15	2.62×10^{-2}
16	4.22×10^{-2}
17	2.17×10^{-2}
Sorafenib	2.00×10^{-3}

View Article Online
DOI: 10.1039/C4RA16228E

Table 2a: Cytotoxicity of the synthesized compounds.

Compound	$IC_{50}\mu M$				
	KB	**SK OV-3**	**SF-268**	**NCI H 460**	**RKOP27**
3	------	5.66×10^{-7}	2.56×10^{-9}	--------	--------
4a	------	4.35×10^{-3}	4.56×10^{-7}	2.67×10^{-3}	4.78×10^{-1}
4b	3.67×10^{-7}	-------	3.45×10^{-11}	2.39×10^{-10}	4.67×10^{-11}
4c	3.33×10^{-6}	8.36×10^{-3}	9.70×10^{-4}	8.80×10^{-3}	9.32×10^{-3}
4d	2.36×10^{-4}	4.40×10^{-4}	3.30×10^{-5}	6.40×10^{-3}	8.80×10^{-5}
5a	2.55×10^{-3}	-------	2.33×10^{-11}	4.62×10^{-11}	3.67×10^{-13}
5b	6.30×10^{-5}	3.42×10^{-3}	8.86×10^{-4}	8.56×10^{-6}	8.00×10^{-4}
5c	7.40×10^{-3}	6.34×10^{-3}	5.30×10^{-3}	8.70×10^{-4}	8.97×10^{-5}
5d	7.54×10^{-7}	2.67×10^{-7}	-------	1.45×10^{-10}	3.56×10^{-13}
8a	6.40×10^{-4}	4.60×10^{-5}	8.77×10^{-3}	2.27×10^{-3}	7.27×10^{-3}
8b	2.10×10^{-4}	7.23×10^{-5}	5.80×10^{-4}	8.56×10^{-4}	7.70×10^{-6}
10a	6.00×10^{-4}	3.00×10^{-4}	4.40×10^{-5}	6.60×10^{-5}	7.80×10^{-5}
10b	4.70×10^{-4}	4.00×10^{-3}	5.50×10^{-3}	4.40×10^{-3}	3.30×10^{-5}
11a	4.30×10^{-4}	5.50×10^{-5}	5.50×10^{-3}	3.30×10^{-3}	4.80×10^{-3}
11b	7.74×10^{-3}	5.50×10^{-5}	6.50×10^{-3}	5.50×10^{-3}	9.40×10^{-3}
11c	6.80×10^{-3}	7.70×10^{-3}	5.70×10^{-3}	9.47×10^{-3}	5.50×10^{-3}
12a	7.74×10^{-3}	4.40×10^{-4}	8.70×10^{-6}	6.60×10^{-5}	9.40×10^{-3}
12b	2.27×10^{-3}	6.60×10^{-4}	4.40×10^{-5}	4.79×10^{-3}	9.00×10^{-3}
12c	7.98×10^{-5}	3.33×10^{-2}	5.20×10^{-3}	9.00×10^{-4}	9.59×10^{-4}
14	6.98×10^{-3}	6.21×10^{-3}	4.60×10^{-3}	4.20×10^{-3}	1.25×10^{-5}
15	3.00×10^{-5}	7.00×10^{-3}	5.00×10^{-4}	6.00×10^{-4}	8.00×10^{-3}
16	4.50×10^{-4}	6.50×10^{-5}	4.30×10^{-5}	7.60×10^{-3}	8.90×10^{-3}
17	7.00×10^{-3}	5.00×10^{-3}	4.00×10^{-3}	3.00×10^{-3}	5.00×10^{-5}
Fluorouracil	**4.46×10^{-3}**	-------	-------	-------	-------
Doxorubicin	-------	**4.16×10^{-3}**	-------	-------	-------
Cytarabine	-------	-------	**7.68×10^{-3}**	-------	-------
Gemcitabine	-------	-------	-------	**2.13×10^{-3}**	-------
Capecitabine	-------	-------	-------	-------	**4.33×10^{-3}**

View Article Online
DOI: 10.1039/C4RA16228E

Table 2b: Cytotoxicity of the synthesized compounds.

Compound	IC_{50} µM				
	Leukemia			Melanoma	
	HL60	U937	K561	G361	SK-MEL-28
3	3.67×10^{-8}	--------	--------	3.76×10^{-8}	--------
4a	4.67×10^{-8}	4.39×10^{-7}	--------	------	8.55×10^{-7}
4b	4.67×10^{-9}	4.56×10^{-7}	4.56×10^{-9}	5.33×10^{-8}	--------
4c	8.00×10^{-4}	9.86×10^{-3}	2.70×10^{-4}	6.20×10^{-4}	6.20×10^{-4}
4d	4.40×10^{-3}	6.60×10^{-3}	7.46×10^{-3}	634×10^{-3}	7.50×10^{-5}
5a	3.67×10^{-9}	5.87×10^{-7}	5.32×10^{-7}	7.43×10^{-8}	5.67×10^{-7}
5b	4.80×10^{-3}	8.00×10^{-3}	8.60×10^{-3}	7.00×10^{-3}	6.00×10^{-5}
5c	4.80×10^{-5}	4.80×10^{-3}	4.80×10^{-4}	4.80×10^{-5}	4.80×10^{-3}
5d	4.55×10^{-7}	5.44×10^{-7}	7.88×10^{-8}	3.34×10^{-7}	4.89×10^{-7}
8a	8.70×10^{-6}	5.40×10^{-3}	4.90×10^{-4}	6.60×10^{-5}	7.70×10^{-3}
8b	4.55×10^{-7}	5.44×10^{-7}	7.88×10^{-8}	3.34×10^{-7}	4.90×10^{-7}
10a	4.00×10^{-4}	6.00×10^{-6}	9.60×10^{-5}	7.70×10^{-3}	8.80×10^{-3}
10b	3.00×10^{-5}	9.00×10^{-3}	5.60×10^{-3}	8.89×10^{-3}	9.90×10^{-3}
11a	2.48×10^{-4}	9.60×10^{-5}	8.63×10^{-3}	7.50×10^{-3}	3.00×10^{-3}
11b	9.70×10^{-4}	4.00×10^{-4}	6.60×10^{-5}	9.90×10^{-5}	8.00×10^{-5}
11c	9.60×10^{-4}	9.70×10^{-4}	5.50×10^{-5}	7.70×10^{-3}	2.00×10^{-5}
12a	6.30×10^{-4}	5.00×10^{-5}	5.50×10^{-4}	8.80×10^{-4}	5.87×10^{-7}
12b	9.00×10^{-4}	6.00×10^{-3}	6.77×10^{-3}	8.90×10^{-5}	3.00×10^{-5}
12c	9.86×10^{-3}	4.40×10^{-3}	8.50×10^{-3}	5.50×10^{-3}	6.60×10^{-5}
14	8.00×10^{-3}	7.50×10^{-4}	4.30×10^{-3}	7.35×10^{-3}	3.40×10^{-5}
15	2.60×10^{-5}	5.00×10^{-3}	7.64×10^{-4}	8.00×10^{-5}	8.50×10^{-3}
16	7.53×10^{-4}	5.96×10^{-5}	6.00×10^{-6}	8.00×10^{-4}	8.00×10^{-4}
17	7.00×10^{-3}	7.70×10^{-3}	7.70×10^{-3}	8.60×10^{-3}	5.70×10^{-5}
Doxorubicin	1.13×10^{-3}	4.45×10^{-3}	6.66×10^{-3}	-------	-------
Aldesleukin	-------	-------	-------	6.66×10^{-3}	3.45×10^{-3}

View Article Online
DOI: 10.1039/C4RA16228E

Table 2c: Cytotoxicity of the synthesized compounds.

Compound	IC_{50} µM (Neuroblastoma)	
	GOTO	**NB-1**
3	4.89×10^{-7}	5.32×10^{-7}
4a	--------	5.78×10^{-7}
4b	6.55×10^{-9}	3.44×10^{-7}
4c	8.60×10^{-6}	7.90×10^{-5}
4d	3.00×10^{-4}	5.68×10^{-4}
5a	--------	--------
5b	8.60×10^{-6}	9.70×10^{-5}
5c	8.00×10^{-4}	3.64×10^{-4}
5d	4.66×10^{-9}	--------
8a	9.00×10^{-4}	3.00×10^{-5}
8b	8.60×10^{-4}	7.20×10^{-5}
10a	7.50×10^{-4}	7.20×10^{-4}
10b	6.41×10^{-4}	7.20×10^{-3}
11a	7.60×10^{-4}	5.00×10^{-5}
11b	8.60×10^{-3}	7.25×10^{-4}
11c	6.80×10^{-3}	7.00×10^{-3}
12a	8.60×10^{-3}	7.25×10^{-4}
12b	8.60×10^{-3}	7.20×10^{-3}
12c	8.00×10^{-5}	5.30×10^{-3}
14	8.50×10^{-3}	3.87×10^{-3}
15	4.63×10^{-5}	8.00×10^{-4}
16	7.40×10^{-4}	3.80×10^{-5}
17	9.60×10^{-3}	8.63×10^{-3}
Doxorubicin	4.73×10^{-3}	5.15×10^{-3}

View Article Online
DOI: 10.1039/C4RA16228E

Table 2d: Cytotoxicity of the synthesized compounds.

Compound	IC50 µM		
	PC-3	**HT1080**	**HepG2**
3	6.40×10^{-3}	5.43×10^{-7}	3.37×10^{-7}
4a	4.78×10^{-10}	8.90×10^{-7}	5.64×10^{-7}
4b	8.60×10^{-3}	5.65×10^{-7}	2.88×10^{-7}
4c	8.00×10^{-6}	4.64×10^{-3}	8.00×10^{-3}
4d	6.40×10^{-4}	8.08×10^{-5}	8.00×10^{-3}
5a	9.70×10^{-3}	7.68×10^{-7}	5.55×10^{-7}
5b	8.70×10^{-5}	7.00×10^{-3}	5.70×10^{-3}
5c	6.88×10^{-3}	8.70×10^{-3}	3.60×10^{-3}
5d	8.80×10^{-6}	2.34×10^{-7}	2.99×10^{-7}
8a	8.00×10^{-4}	9.70×10^{-3}	7.50×10^{-3}
8b	4.74×10^{-4}	9.70×10^{-4}	8.60×10^{-4}
10a	5.70×10^{-4}	9.76×10^{-5}	9.70×10^{-5}
10b	5.00×10^{-4}	9.70×10^{-3}	9.00×10^{-4}
11a	7.25×10^{-5}	8.00×10^{-3}	1.46×10^{-3}
11b	6.50×10^{-3}	3.46×10^{-4}	6.35×10^{-4}
11c	8.65×10^{-3}	3.57×10^{-3}	3.60×10^{-3}
12a	4.60×10^{-3}	4.97×10^{-3}	6.34×10^{-3}
12b	7.00×10^{-3}	8.60×10^{-3}	6.30×10^{-3}
12c	9.70×10^{-5}	7.90×10^{-3}	1.34×10^{-3}
14	1.25×10^{-5}	6.00×10^{-3}	7.60×10^{-3}
15	8.00×10^{-3}	7.80×10^{-4}	7.00×10^{-5}
16	8.90×10^{-3}	8.00×10^{-5}	7.00×10^{-3}
17	5.00×10^{-5}	4.60×10^{-3}	9.86×10^{-3}
Abiraterone acetate	6.70×10^{-6}	-------	-------
Imatinib	-------	13.24×10^{-5}	-------
Gemcitabine	-------	-------	3.44×10^{-3}

View Article Online
DOI: 10.1039/C4RA16228E

Table 3: *In vivo* antiprostate cancer activity of the synthesized compounds.

Compound	ED_{50} μM
3	13.11±0.004
4a	21.49±0.006
4b	23.64±0.005
4c	10.64±0.003
4d	8.00±0.004
5a	26±0.004
5b	13.11±0.004
5c	7.21±0.005
5d	28.6±0.004
8a	5.66±0.04
8b	6.22±0.04
10a	6.85±0.05
10b	7.53±0.06
11a	3.40±0.006
11b	16.14±0.006
11c	19.53±0.006
12a	14.68±0.005
12b	17.76±0.007
12c	5.14±0.005
14	3.73±0.004
15	2.56±0.005
16	2.82±0.006
17	2.12±0.007
Flutamide	11.60±0.09

Each ED_{50} value is the mean (three significant digits) ± SEM from five experiments. Level of statistical significance: $P < 0.01$ with respect to ED_{50}value of Flutamide as determined by ANOVA/Dunnett's.

View Article Online
DOI: 10.1039/C4RA16228E

Table 4: The flexible docking results (GOLD 5.1), regarding the GoldScore Fitness and external VDW of compounds docked intoVEGFR-2 kinase (3EWH).

Comp.	**Gold Score**	**External VDW**	Hydrogen bonds between atoms of compounds and amino acids of **VEGFR**				RMSD[a] (Å)
			Atoms of comp.	Amino acids	Distance (Å)	Angle (°)	
3	44.02	25.40	4-CH_3O 6-C=O	HN of K868 HN of R1051	1.97 1.95	166.6 164.8	6.96
4a	72.00	52.43	5-C=O	HN of D1046	2.32	164.3	4.44
4b	74.00	53.55	4``-Br	HN of R1027	2.45	152.0	4.16
4c	69.03	49.37	5-C=O	HN of D1046	2.07	164.8	4.73
4d	79.85	55.78	5-C=O 4``- CH_3O	HN of D1046 HN of R1027	2.01 2.11	159.7 152.9	5.65
5a	71.80	52.78	5-C=O	HN of D1046	2.05	165.7	4.68
5b	70.86	51.09	5-C=O	HN of D1046	2.05	165.4	4.63
5c	70.66	53.09	5-C=O	HN of D1046	2.23	171.3	4.77
5d	81.17	58.32	5-C=O	HN of D1046	2.48	162.0	5.48
8a	61.23	35.49	6-O	HN of K868	1.85	163.8	3.34
8b	63.93	37.98	4-O 6-O	HN of K868 HN of D1046	1.48 2.15	167.4 170.1	4.70
10a	68.08	45.62	4-O 6-O	^{1}HN of K868 ^{2}HN of K868	2.25 2.09	174.2 166.1	4.55
10b	71.36	41.51	4-O 6-O	^{1}HN of K868 ^{2}HN of K868	2.24 2.12	172.9 159.8	4.84
11a	57.89	39.98	Furan O 6-O	HN of K868 HN of D1046	2.37 1.85	171.5 171.3	3.86
11b	59.91	36.94	Furan O 6-O	HN of K868 HN of D1046	2.21 1.87	171.9 144.2	4.00
11c	58.27	38.14	Furan O 6-O	HN of K868 HN of D1046	2.27 2.05	174.0 160.1	3.42
12a	74.71	54.22	4-CH_3O 6-O	HN of K868 HN of D1046	2.22 2.16	165.7 151.4	2.39
12b	75.09	51.49	6-OH 5-CHNHNH	O=C of D1046 O=C of E885	1.94 2.25	141.6 115.5	6.27
12c	78.28	48.43	Furan O S=O	HN of D1046 HN of K868	2.20 1.75	161.9 162.0	5.85
14	57.17	27.14	Furan O 5-C=O S=O	HN of K868 HO of T916 HN of K868	1.64 1.92 1.85	154.2 139.7 164.0	2.40
15	72.88	51.88	S=ONH Furan O	O=C of D1046 HN of K868	1.48 1.87	127.6 164.4	2.40

View Article Online
DOI: 10.1039/C4RA16228E

Table 4: *(Continued)*

Comp.	Gold Score	External VDW	Hydrogen bonds between atoms of compounds and amino acids of **VEGFR**				RMSD[a] (Å)
			Atoms of comp.	Amino acids	Distance (Å)	Angle (°)	
16	60.02	37.25	Furan O	HN of K868	2.30	174.8	2.87
			S=O	HN of D1046	2.09	152.5	
17	60.65	38.98	4-O	^{1}HN of K868	1.85	151.4	4.28
			5-C=O	^{2}HN of K868	1.54	150.2	
			Pyran O	HN of D1046	2.15	154.9	
			S=O	HN of D1046	2.48	114.2	
K111[b]	80.74	60.92	Pyrimidine-N^{1}	^{1}HN of K868	2.46	139.4	1.83
			Naphthyloxy-O	^{2}HN of K868	2.34	114.7	
			Naphthyl-NH	OH of T916	1.90	154.9	

[a] Root mean square deviation,
[b] N-[4-({3-[2-(methylamino)pyrimidin-4-yl]pyridin-2- yl}oxy)naphthalen-1-yl]-6-(trifluorometh-yl)-1*H*- benzimidazol-2-amine

View Article Online
DOI: 10.1039/C4RA16228E

List of figures

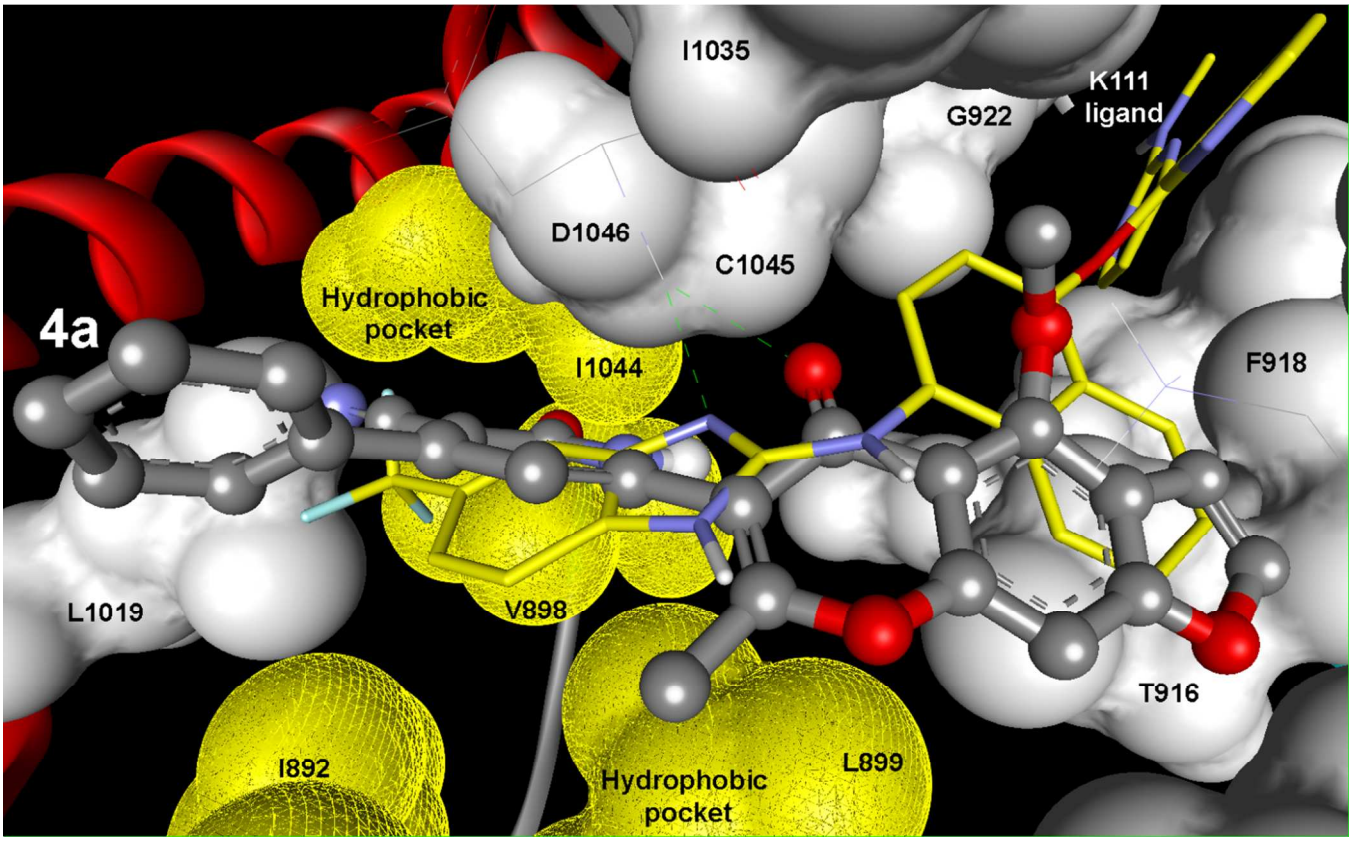


Figure 1: The docking fitness of compounds **4a** (cyan sticks) and **4b** (balls and sticks) involving flexible GOLD docking into VEGFR-2 kinase. They exhibited one hydrogen bond for each, shown as green dashed lines with D1046 and R1027, respectively. Protein is shown as solid backbone ribbon and the binding site is shown in solid surface view with labeled amino acids.

View Article Online
DOI: 10.1039/C4RA16228E

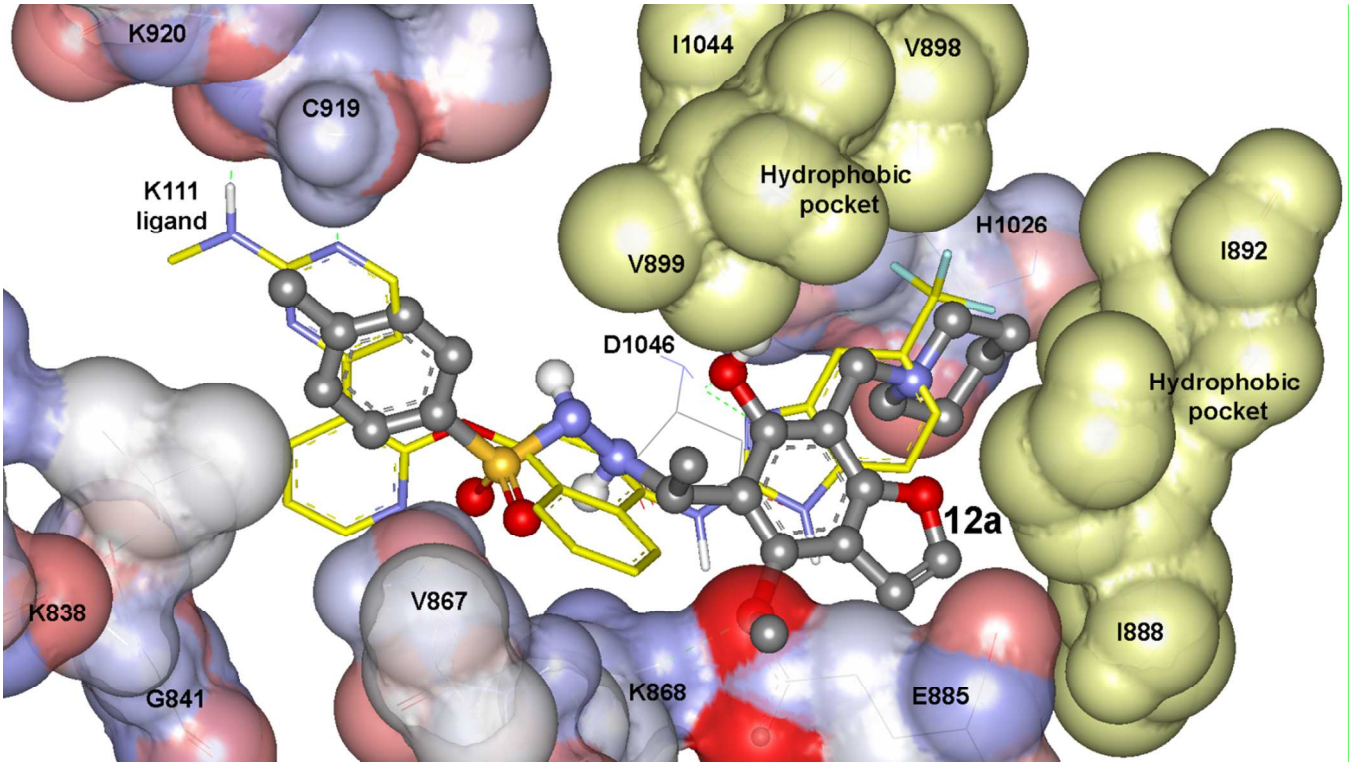


Figure 2: The binding affinity of compounds **12a** (balls and sticks) into the binding site of VEGFR-2 kinase within RMSD of 2.39 Å. It reveals GoldScore fitness of 74.71 and two hydrogen bonds (green dotted lines) with NH group of K868 and D1046.Its piperidine moiety is located between its two hydrophobic pockets similarly to the CF_3 group of the native ligand.

View Article Online
DOI: 10.1039/C4RA16228E

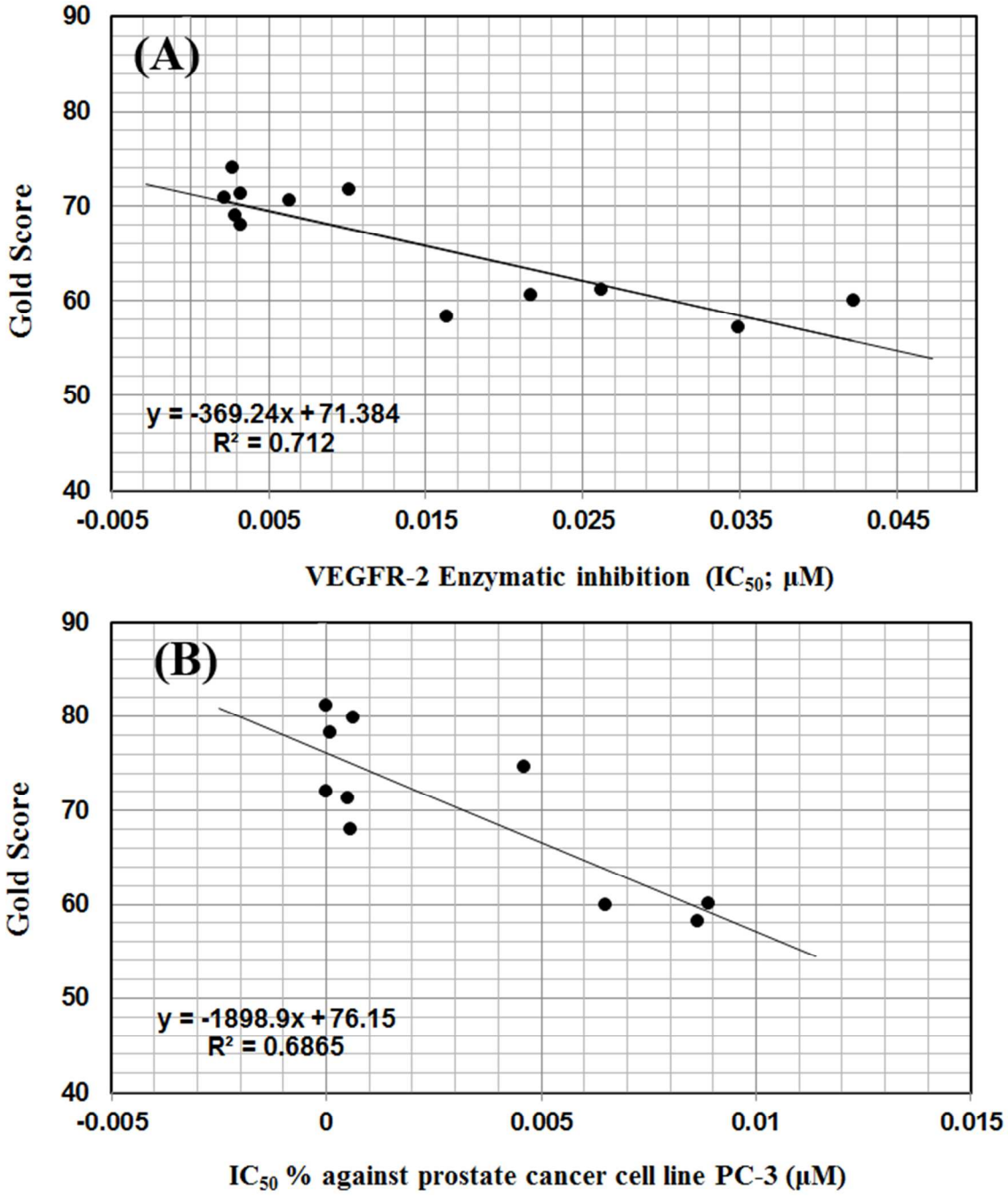
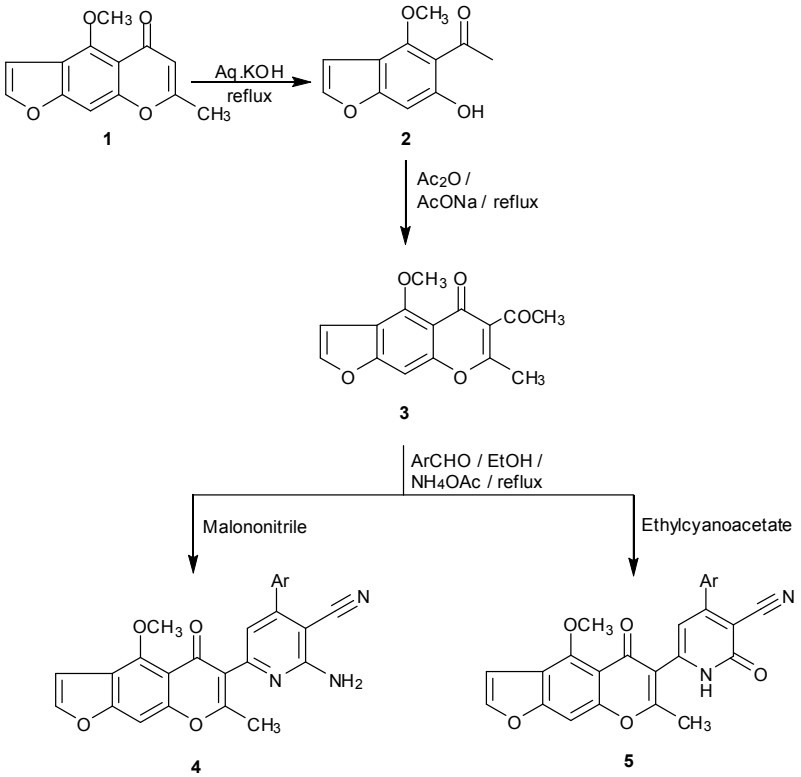


Figure 3: (A) The correlation between IC_{50}(µM) of enzymatic inhibition of VEGFR-2 kinase and the GoldScore fitness for compounds **4b, 4c, 5a, 5b, 5c, 8a**, **10a**, **10b, 11c, 12a, 16** and**17**. (**B**) The correlation between IC_{50}(µM) against prostate cancer cell line (PC-3) and the GoldScore fitness for compounds **4a, 4d, 5d, 10a**,**10b, 11b, 11c, 12a, 12c** and **16**into VEGFR2 kinase.

View Article Online
DOI: 10.1039/C4RA16228E

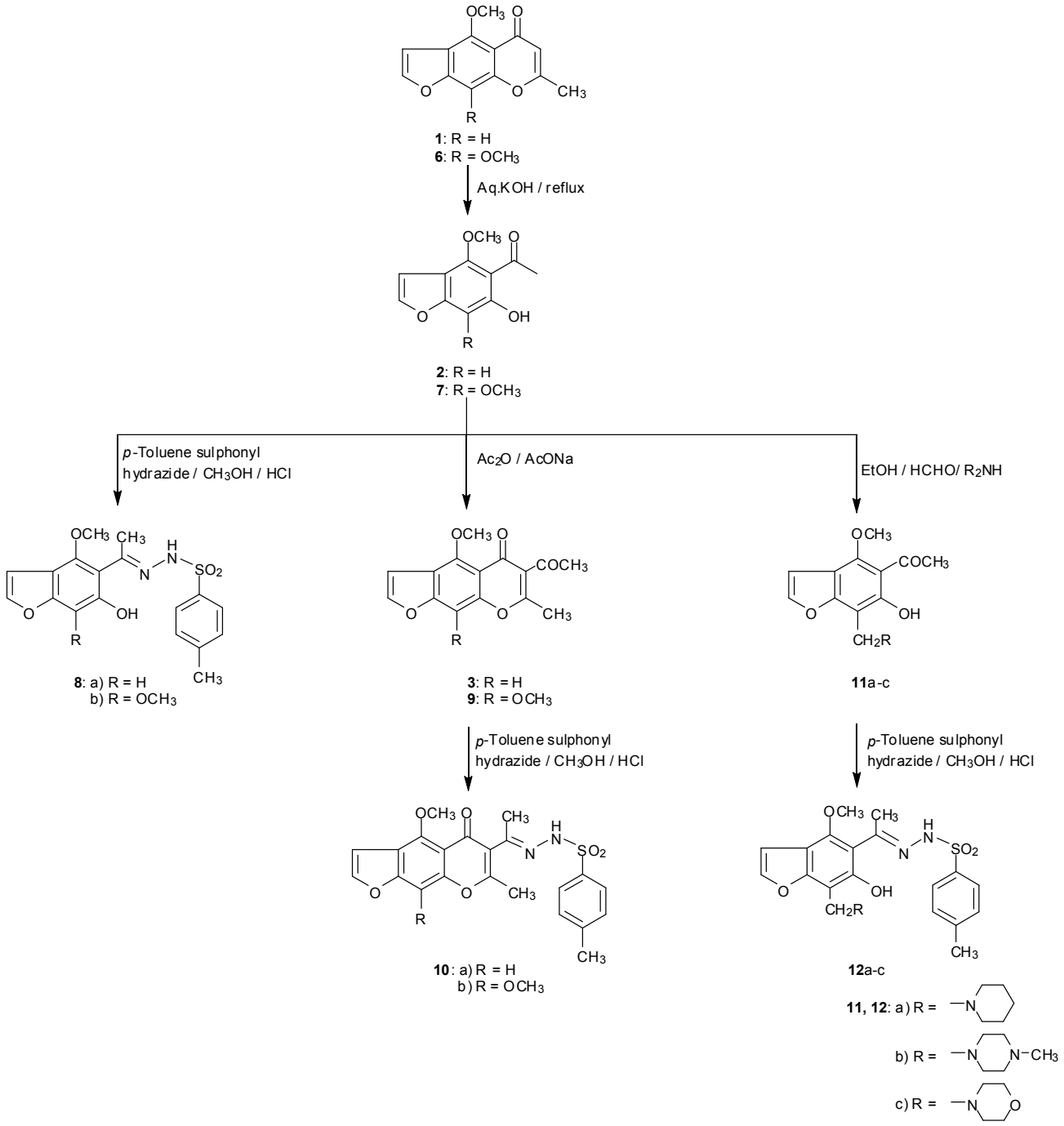
Scheme 1



4,5: a) Ar = Phenyl
b) Ar = 4-Bromopheny
c) Ar = 5-Methyl furan
d) Ar = 3,4,5-Trimethoxyphenyl

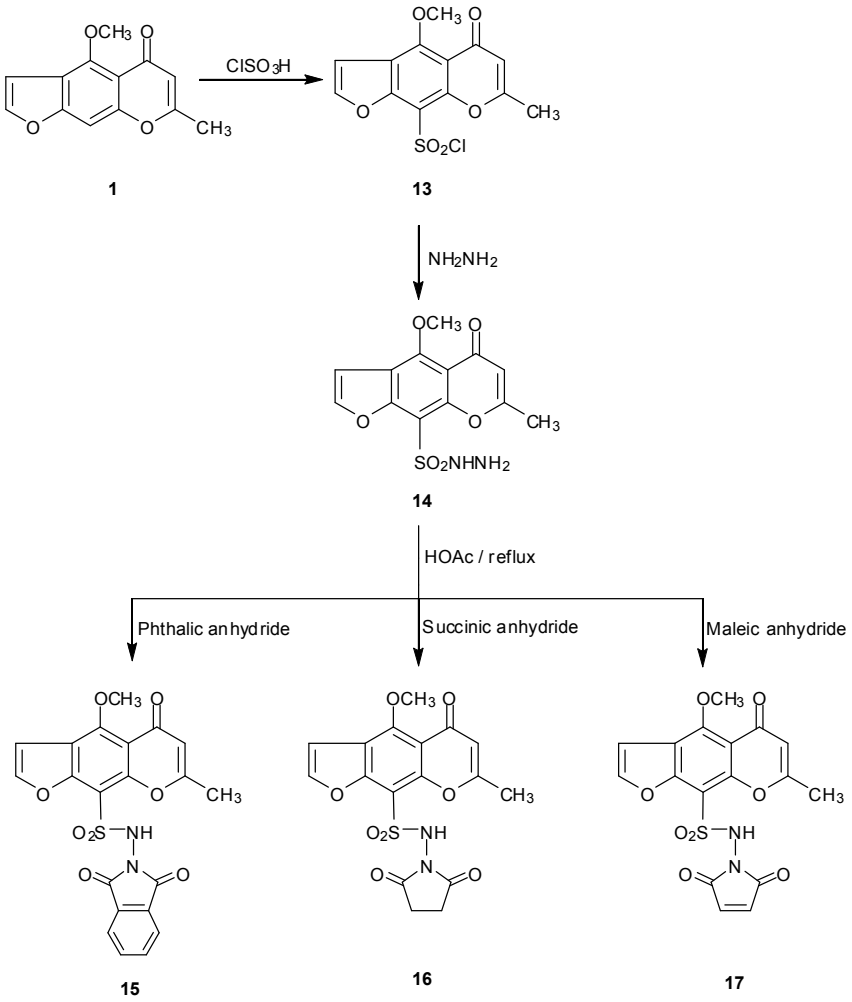
View Article Online
DOI: 10.1039/C4RA16228E

Scheme 2



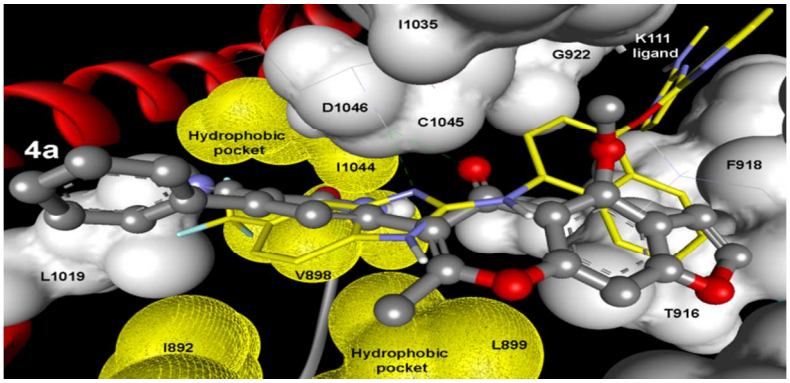
View Article Online
DOI: 10.1039/C4RA16228E

Scheme 3



View Article Online
DOI: 10.1039/C4RA16228E

Table of contents entry



Furochromone and benzofuran derivatives were synthesized, docked and evaluated for their anti-VEGFR-2 activity, cytotoxicity, and *in vivo* antiprostate cancer activity.