Applied Organic Chemistry Department[1], National Research Centre, Dokki; Pharmaceutical Chemistry Department[2], Faculty of Pharmacy, Ain-Shams University; Research Units[3], Hi-Care Pharmaceutical Co., Cairo, Egypt

Synthesis, docking studies and anti-inflammatory activity of some 2-amino-5,6,7,8-tetrahydroquinoline-3-carbonitriles and related compounds

O. I. Abd El-Salam[1], D. A. Abou El Ella[2], N. S. M. Ismail[2], M. Abdullah[3]

Received August 15, 2008, accepted October 3, 2008

Ass. Prof. Dr. Osama I. Abd El-Salam, Applied Organic Chemistry Department, National Research Centre, Dokki, Cairo – 12622, Egypt
oiel_salam@yahoo.com

Pharmazie 64: 147–155 (2009) *doi: 10.1691/ph.2009.8703*

A series of 2-amino-5,6,7,8-tetrahydroquinoline-3-carbonitriles **4a–c** has been synthesized and reacted with various reagents. Docking studies into the catalytic site of p38 mitogen activated protein kinase (MAPK) were used to identify potential anti-inflammatory lead compounds. The anticipated lead derivative 2-(pyridin-3-yl)-5-(4-methoxyphenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-amine **9b** was endowed with good binding energy. Also, the obtained products were evaluated for their *in vivo* anti-inflammatory activity as prostaglandin inhibitiors (% inhibition of edema and % inhibition of plasma PGE_2 at the two dose levels were determined). The tested compounds exhibited significant anti-inflammatory activity and minor ulcerogenic effects, when compared to the reference standard indomethacin, with product **9b** being of particular interest.

1. Introduction

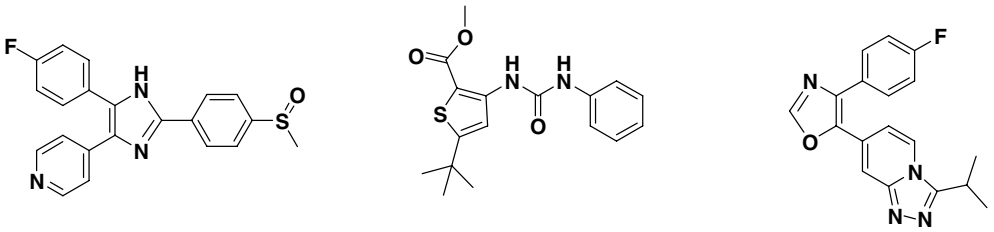
Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation in arthritis for decades. However, long-term clinical usage of NSAIDs is associated with significant side effects of gastrointestinal lesions, bleeding, and nephrotoxicity. Therefore the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area (Kontogiorgis and Hadjipavlou-Litina 2002; van Ryn et al. 2000; Beuck 1999).

In the late 1990s, the introduction of selective cyclooxygenase-2 (COX-2) inhibitors, such as celecoxib and rofecoxib, provided novel anti-inflammatory- analgesic agents with reduced gastrointestinal side effects (Praveen et al. 2005; Bombardier et al. 2000). However, the recent market withdrawal of rofecoxib and valdecoxib due to their adverse cardiovascular side effects clearly delineates the need to develop alternative anti-inflammatory agents with reduced toxicity (Dogne et al. 2005). Intracellular signaling pathways involving p38 mitogen activated protein kinase (MAPK) regulate the production of several pro-inflammatory cytokines and are considered as a focal point in the development of new therapeutic agents to treat inflammatory diseases such as rheumatoid arthritis (RA) (Kulkarni et al. 2006). p38 is a member of Ser/Thr kinases family of the mitogen-activated protein (MAP) kinase super family (Sandra et al. 2006). The four-p38 isoforms, p38-α, (also known as p38), p38-β, p38-γ, p38-δ varies based on their substrate specificity. Several reports indicate that p38a has a profound role in RA and is involved in the expression of cytokines such as tumor necrosis factor-a (TNF-a) and interleukin-1b (IL-1b) at both transcription and translation levels while the roles of p38-β, p38-γ, p38-δ have to be identified critically (Ravindra et al. 2008).

The interest in the development of p38 MAP kinase inhibitors is based on the expectations that p38 inhibiting drugs will treat the underlying cause of chronic inflammatory disease and cease their progression (Revesz et al. 2002). Although a number of structurally different inhibitors have been reported to inhibit p38 with varying degrees of selectivity, none has reached commercial status (Nayan et al. 2008).

The pyridinyl-imidazoles represented by SB203580 **1** were the first reported class of p38a inhibitors (Gallagher et al. 1995; Wilson et al. 1997). Following high throughout screening of the Bayer compound library, the commercially available thienyl urea **2** (Maybridge GK 00687) was identified as a reversible p38 inhibitor.

Many naturally occurring as well as synthetic compounds containing the quinoline scaffold exhibit interesting biological properties (Kouznetsov et al. 2005; Fadel et al. 2004; Crousse et al. 2000; Kobayashi et al. 2004). It has been reported that compounds containing quinoline, pyrimidine and tetrazole functionality exhibit anti-inflammatory activity (Marco-Contelles et al. 2006; Bekhit et al. 2004; Calhoun et al. 1995). Recently Gholap et al. (2007) synthesized a series of 5,6,7,8-tetrahydroquinoline analogues, based on the interesting pharmacological properties exhibited by compounds containing the pyridine scaffold. On the basis of these results and as a continuation of our ongoing effort in the field of pyridine derivatives (Abd-El-



1 2 3

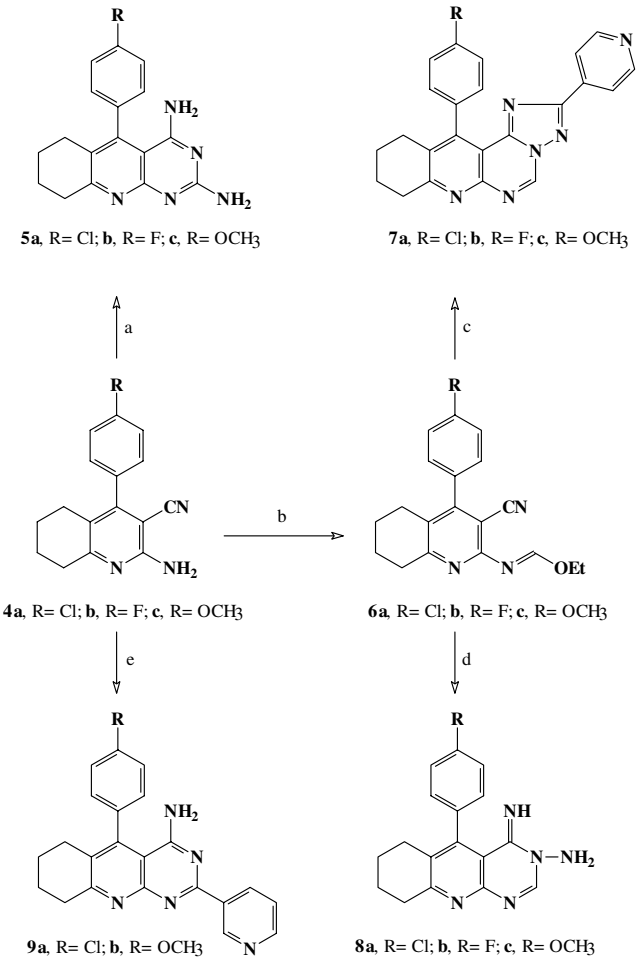
Salam and Mohamed 2005; Abd-El-Salam 2000; Attia et al. 1995), we are herein reporting the synthesis of a new series of 5,6,7,8-tetrahydroquinoline analogues and their anti-inflammatory evaluation, with the aim of developing novel anti-inflammatory lead compounds.

2. Investigations, results and discussion

2.1. Synthesis of the compounds

The synthesis of 2-amino-3-cyanopyridines was performed from arylidene-malononitriles and cyclohexanone in the presence of ammonium acetate using ethanol as a solvent (Attia et al. 1995; Elkholy and Morsy 2006). The amount of ammonium acetate was adjusted to get the maximum yield of the products **4**, it has been found that 8 mmol of the ammonium acetate furnished the maximum yield for 1 mmol of the reactants. Cyclization of **4** with chloroformamidine hydrochloride in diglyme (Harris et al. 1990), was proceeded to give 5-(4-substitutedphenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]-quinoline-2,4-diamine **5**, as white powder (Scheme 1). The methaneimidates **6**, as intermediates for further reactions, were obtained by the reaction of **4** with triethylorthoformate in the presence of a catalytic amount of acetic anhydride (Swelam et al. 1999). The obtained methanimidates **6** and 4-pyridinecarbohydrazide were refluxed in dioxane, to give 12-(4-substitutedphenyl)-2-(pyridin-4-yl)-8,9,10,11-tetrahydro-quinoline[2,3-*d*]-1,2,4-triazolo[5,1-*f*]pyrimidines **7**. Their ^{1}H NMR spectra showed the CH-pyrimidine signal at 9.3, 9.25 and 9.32 ppm for **7a**, **7b** and **7c**, respectively. The IR spectra of the products **7** showed the absence of bands corresponding to a CN group present in **6**. Further, when a well-stirred solution of **6** in ethanol hydrazine hydrate was added, the mixture was stirred at room temperature to afford 5-imino-6-substitutedphenyl-7,8,9,10-tetrahydropyrimido-[4,5-*b*]quinolin-4-amine **8**. When a mixture of **4** and pyridine-3-carbonitrile and sodium methoxide in isopropanol was refluxed for 48 and 60 h, furnished the formation of 2-(pyridin-4-yl)-5-substitutedphenyl-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-amine **9** (Taylor and McKillop 1970). Diazonium ion condensation with an adjacent nucleophilic function to form a five- or six-membered ring has proved valuable for synthesizing various nitrogen heterocycles. Among these are numerous 1,2,3-triazines that are formed *via* intramolecular attack of an electrophilic nitrogen function. Thus, 1,2,3-triazin-4-chloro or 4-hydroxy derivatives are obtained from the condensation of diazonium ion with an adjacent cyanide (Beck and Yahner 1976) or carboxamide (Ellis 1987), respectively. Thus, 1,2,3-triazines are obtained from the condensation of diazonium ion with an adjacent cyanide or carboxamide, respectively. When a suspension of **4a, c** in 20% alcoholic KOH solution was refluxed for 10 h, the products 2-amino-4-(4-substituted-phenyl)-5,6,7,8-tetrahydroquinoline-3-carboxamides **10**, were obtained as white crystalline material. Their IR spectra showed the absence of bands corresponding to CN group present in **4** and the amide CO bands are well represented by bands at 1669 and 1675 cm^{-1} for **10a** and **10b**, respectively. Diazotization of the product **10** with sodium nitrite in concentrated H_2SO_4–HOAc gave the triazino[4,5-*b*]quinolin-4-ol **11** (Scheme 2). Preparation of the required the 4-chloro-[1,2,3]triazino[4,5-*b*]quinolines **12** using standard reagents such as thionyl chloride or phosphorous oxychloride led to decomposition of **11**, which could be attributed to the instability of the triazine ring under these conditions. Nevertheless, the 4-chlorotriazines **12** were easily obtained from diazotization of an acetic acid solution of **4** with sodium nitrite in concentrated hydrochloric acid. Treatment of **12** with 2-aminopyridine led to normal halide displacement to give *N*-(pyridin-2-yl)-[1,2,3]-triazino[4,5-*b*]quinolin-4-amines **13**. Treatment of a mixture of **4** and ethylene diamine with carbon disulfide

Scheme 1 Reagents and conditions: (a) chloroformamidine hydrochloride, diglyme; (b) triethylorthoformate, acetic anhydride, reflux; (c) 4-pyridinecarbohydrazide, dioxane, reflux; (d) hydrazine hydrate, ethanol, rt; (e) 3-pyridinecarbonitrile, sodium methoxide, 2-propanol, reflux

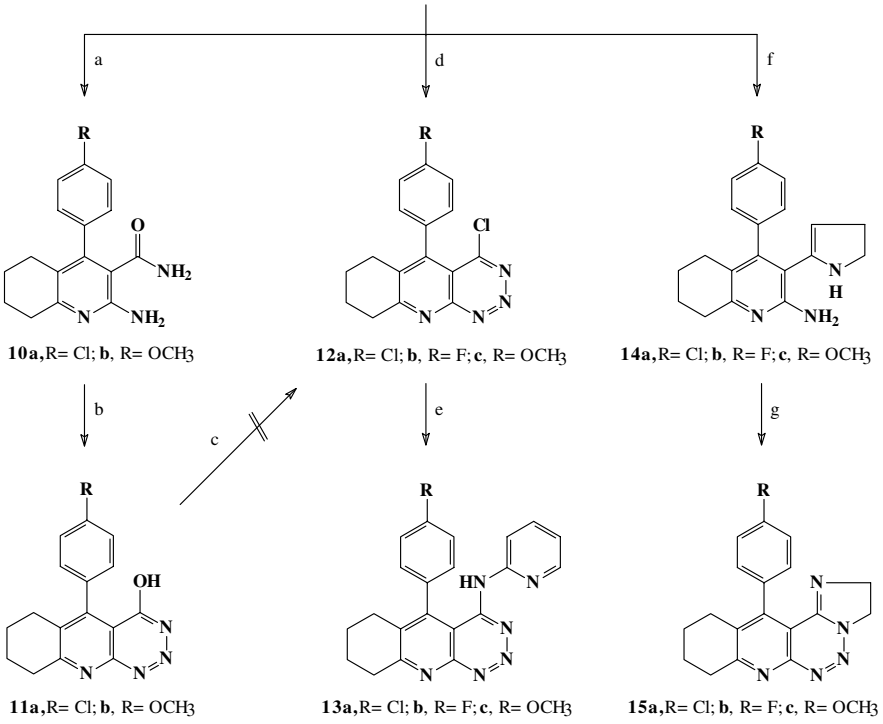


5a, R= Cl; **b**, R= F; **c**, R= OCH_3 **7a**, R= Cl; **b**, R= F; **c**, R= OCH_3

4a, R= Cl; **b**, R= F; **c**, R= OCH_3 **6a**, R= Cl; **b**, R= F; **c**, R= OCH_3

9a, R= Cl; **b**, R= OCH_3 **8a**, R= Cl; **b**, R= F; **c**, R= OCH_3

Scheme 2 Reagents and conditions: (a) 20% alcoholic KOH, reflux, 10 h; (b) sodium nitrite, H_2SO_4, acetic acid, −5 °C; (c) thionyl chloride or phosphorous oxychloride, reflux; (d) sodium nitrite, conc. HCl, acetic acid, −5 °C; (e) 2-aminopyridine, TEA, reflux; (f) ethylene diamine, carbon disulfide, reflux; (g) sodium nitrite, 5% HCl, −5 °C



and heating the reaction mixture on a steam bath for 8 h afforded compound **14**. The synthetic application of condensation of diazonium ion with an adjacent nitrogen function was extended to the synthesis of the tetracyclic **15**, which was prepared by the diazotization of the intermediate **14**. Thus, when a cold suspension of **14** in hydrochloric acid (5%) was treated with a solution of sodium nitrite in water and stirring the reaction at room temperature furnished products **15**.

The products obtained were well characterized by their MS, IR and ^{1}H NMR spectral analysis; additionally the elemental analyses were in conformity with the respective structures.

2.2. Molecular docking studies of p38 kinase inhibitors and binding conformation

This technique considered as direct molecular modeling where the 3D structure of the enzyme was known which used to detailed intermolecular interactions between the ligand and the target protein. An automated docking study was carried out using the crystal structure of inhibitor (3)/p38 kinase complex obtained from protein data bank website (pdb) entry 1ZZL; having resolution of 2.0 Å (Wilson et al. 1997). This regularized protein complex structure was used in determination of the active site that is mentioned in the literature (Wilson et al. 1997). The performance of the docking method on p38 inhibitors was evaluated by re-docking crystal ligand with 0.00621 RMSD value. Docking process was carried out for the test set of compounds **4**–**15**.

In the flexible-ligand-rigid enzyme docking, the enzyme was represented by six potential energy maps, namely, electrostatic, hydrogen bond, hydrophobic, and three van der Waals. Interactive docking using Mol table ligand was carried out for all the conformers of each compound of the test set (**4**–**15**) to the selected active site of p38. Each docked compound was assigned a score according to its fit in the ligand binding pocket (LBP)

The predicted binding energies of the compounds and the corresponding experimental values are also listed in the Table. The docking of ligand **3** and highly active molecule **9b** in to the active site of p38 was performed (Fig. 1).

The docking results were indicative that; firstly, the para-substitution of phenyl ring at position-5 by a methoxy group will increase the hydrophobic binding interaction with the deep hydrophobic pocket created by Thr106, Lys 53, Ala51 and Leu108. Secondly, the hydrogen bonding interactions have been found, between the crucial features of compounds with high docking scores and N−H group of Lys53, C=O of Tyr35 and Asp168. Moreover, replacement of the methoxy group on para-position of phenyl ring by halogen (electron withdrawing group) like Cl and F will decrease the docking value.

Alignment study of docked compound **9b** and ligand **3** with the binding pocket of p38 kinase protein (Fig. 1c) revealed that (i) tetrahydroquinolin ring system was perfectly aligned with triazolopyridine nucleus of ligand **3** (ii) *p*-methoxyphenyl side chain superimposed with *p*-flurophenyl side chain of ligand **3** (iii) additionally, both the ligand **3** and compound **9b** make same hydrogen bonding interaction with Asp 168.

Docking results provide useful information in understanding the structural features of the target and chemical features of the ligands. This was extended to the successful designing of highly active analogs of tetrahydroquinoline derivatives against p38 MAP kinase.

2.3. Pharmacology

All the tested compounds showed potent anti-inflammatory activities with potent prostaglandin inhibition at the

Table: Results of docking score and *in vivo* ulcerogenic and anti-inflammatory activity

Compd.	Acute toxicity (LD_{50} mg kg^{-1} p.o.)	Ulcerogenic activity (UD_{50} mg kg^{-1} i.p.)	Docking score (kcal/mol)	Anti-inflammatory activity		
				Dose mg kg^{-1} p.o.	% Inhibition of oedema	% inhibition of plasma PGE_2
4a	1786.28	234.5	−70.10	2.5	76.12[c]	59.78[c]
				5	87.00[c]	72.00[a]
4b	1265.34	287.6	−78.88	2.5	86.77[b]	70.00[c]
				5	97.88[c]	82.00[c]
4c	1237.45	213.6	−84.25	2.5	87.00[a]	70.18[c]
				5	88.87[b]	82.16[c]
5a	1765.98	223.9	−68.93	2.5	78.99[c]	61.00[c]
				5	88.99[c]	73.00[c]
5c	1398.34	209.7	−74.10	2.5	84.11[a]	66.56[a]
				5	94.60[c]	79.87[a]
6a	1378.56	234.7	−61.00	2.5	70.45[c]	53.98[c]
				5	81.00[c]	71.00[c]
6b	1247.45	245.6	−65.61	2.5	71.12[c]	54.00[b]
				5	81.46[c]	73.56[c]
6c	1287.45	235.7	−66.76	2.5	71.89[c]	54.33[b]
				5	81.50[c]	74.32[a]
7b	1675.38	214.87	−72.7	2.5	83.22[a]	65.12[a]
				5	93.11[b]	78.54[c]
7c	1246.45	243.5	−76.72	2.5	86.00[a]	69.65[c]
				5	96.00[c]	81.50[c]
8a	1295.56	236.5	−64.43	2.5	71.00[c]	54.00[b]
				5	81.00[c]	72.00[c]
8b	1293.33	236.8	−69.17	2.5	75.11[b]	57.87[c]
				5	85.09[c]	70.63[b]
9a	1234.67	214.6	−71.46	2.5	82.16[b]	64.58[c]
				5	92.11[a]	77.76[c]
9b	1342.23	223.76	−91.87	2.5	88.19[a]	71.17[c]
				5	99.78[c]	83.40[c]
10a	1782.87	245.6	−67.8	2.5	75.66[b]	58.45[c]
				5	86.66[c]	71.09[b]
10b	1354.98	254.8	−69.21	2.5	73.78[a]	55.11[b]
				5	83.55[a]	68.65[b]
11a	1753.99	200.56	−75.12	2.5	85.00[a]	67.45[c]
				5	95.00[b]	80.65[c]
11b	1145.67	234.16	−85.30	2.5	87.99[b]	71.00[c]
				5	98.99[b]	83.00[c]
12a	1245.33	234.5	−67.41	2.5	71.16[c]	54.60[c]
				5	81.84[c]	75.00[c]
12b	1345.98	238.8	−67.92	2.5	72.98[b]	55.00[b]
				5	82.98[b]	76.09[c]
13a	1298.98	265.8	−70.10	2.5	74.00[b]	56.88[a]
				5	84.33[a]	69.55[a]
13c	1782.98	243.6	−75.40	2.5	85.77[b]	68.77[b]
				5	95.00[a]	81.00[b]
14a	1565.78	245.7	−65.12	2.5	70.00[c]	52.45[b]
				5	81.00[c]	70.12[b]
14b	2874.98	211.4	−71.32	2.5	81.22[c]	63.77[c]
				5	91.14[c]	76.98[c]
15a	1765.98	213.4	−70.25	2.5	79.58[c]	61.67[b]
				5	89.99[c]	74.30[a]
15b	1357.56	213.4	−70.40	2.5	80.56[c]	62.12[c]
				5	90.12[b]	75.39[a]
Indomethacin	2345.87	66.7	–	2.5	63.54[c]	49.16[a]
				5	73.43[c]	65.23[a]

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$

two dose levels tested. They are more potent than indomethacin and the degree of potency in descending order is **9b, 11b, 4b, 7c, 13c**, **11a**, **5c**, **7b**, **9a**, **14b**, **15b**, **15a**, **5a**, **4c**, **4a**, **10a**, **8b**, **10b**, **12b**, **12a**, **6c**, **6b**, **6a**, **8a** and **14a**.

The tested compounds of this series showed LD_{50} values above 1100 mg kg^{-1} p.o., with maximum in compound **14b** (2875 mg kg^{-1} p.o). All the results are depicted in the Table. Random screening of compounds and reference drug, indomethacin, was performed at two dose levels 2.5 and 5 mg kg^{-1} p.o.

The ulcerogenic activity (UD_{50} mg kg^{-1} i.p.) of the tested compounds and indomethacin are listed in the Table. All compounds are safer than indomethacin due to high ulcerogenic causing doses that were above 200 mg kg^{-1} i.p.

3. Experimental

3.1. Chemistry

Melting points were determined in open glass capillaries using an Electrothermal IA 9000 SERIES digital melting point apparatus (Electrothermal,

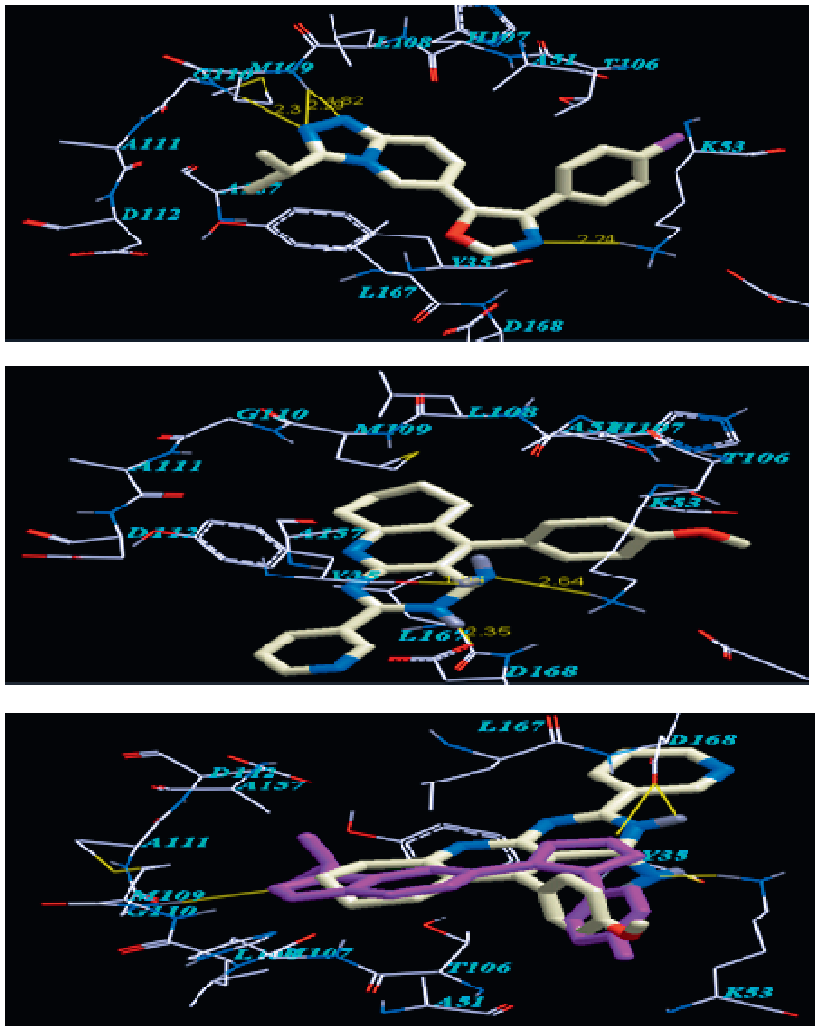


Fig. 1:
(a) and (b): the proposed binding mode of ligand **3** and compound **9b** inside the active site of p38 MAP kinase resulting from docking, respectively. The most important amino acids are shown together with their respective numbers. Compound **9b** form three hydrogen bonds with Lys53, Tyr35 and Asp168. (c): Alignment of docked compound **9b** and ligand **3** with the binding pocket

UK) and are uncorrected. Microanalyses were performed with all final compounds on an Elementar-Vario EL (Elementar-Vario EL, Germany) (Micro-analytical Unit, Central Services Laboratory, National Research Centre, Cairo; Egypt). Analyses of C, H, N were found to be within acceptable limits of theoretical values. The ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, USA). ^{1}H NMR spectra were run at 300 MHz in DMSO-d_6 as solvent. Chemical shifts δ are quoted in ppm and were related to that of the solvents. Mass spectra were recorded on a Shimadzu GCMS-QP 1000EX (EI, 70 eV) (Shimadzu, Japan) and Hewlett-Packard (EI, 70 eV) (Hewlett-Packard, USA). IR spectra were obtained with a Bruker-Vector 22 (Bruker, Rheinstetten, Germany). All the reactions were monitored using thin layer chromatography (TLC) using silica gel aluminum sheets $60F_{254}$ (Merck).

3.1.1. General procedure for the synthesis of compounds **4a–c**

A mixture of malononitrile (6.6 g, 100 mmol) and the proper aldehyde (100 mmol) in absolute ethanol (250 ml) was refluxed for 3 h. After addition of cylohexanone 10.3 ml (9.8 g, 100 mmol), and ammonium acetate (30.8 g, 400 mmol) the reaction mixture was refluxed for 5 h. The reaction mixture was concentrated to its volume and left to cool. The separated solid was filtered off, washed with aqueous ethanol (1 : 1), dried and crystallized from the proper solvent to yield compounds **4a–c**.

3.1.1.1. 2-Amino-4-(4-chlorophenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**4a**)

Yield: 17.6 g (62%), m.p. 258–259 °C (Dioxane/H_2O). IR (KBr, cm^{-1}): 3422, 3303 (NH_2), 2212 (CN). ^{1}H NMR (DMSO-d_6, ppm): 1.55–1.7 (m, 4 H, 2 CH_2), 2.29–2.38 (m, 2 H, CH_2), 2.56–2.74 (m, 2 H, CH_2), 6.6 (s, 2 H, NH_2, exchangeable with D_2O), 7.35 (d, 2 H, Ar–H), 7.55 (d, 2 H, Ar–H). MS, m/z (%): 283 [M^+] (100).
$C_{16}H_{14}ClN_3$ (283.76)

3.1.1.2. 2-Amino-4-(4-fluorophenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**4b**)

Yield: 14.7 g (55%), m.p. 262–263 °C (C_2H_5OH). IR (KBr, cm^{-1}): 3410, 3300 (NH_2), 2220 (CN). ^{1}H NMR (DMSO-d_6, ppm): 1.55–1.76 (m, 4 H, 2 CH_2), 2.27–2.35 (m, 2 H, CH_2), 2.61–2.7 (m, 2 H, CH_2), 6.6 (s, 2 H, NH_2, exchangeable with D_2O), 7.2–7.45 (m, 4 H, Ar–H). MS, m/z (%): 268 [$M^+ + 1$] (100).
$C_{16}H_{14}FN_3$ (267.30)

3.1.1.3. 2-Amino-4-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**4c**)

Yield: 12 g (43%); m.p. 232–233 °C (Dioxane/H_2O). IR (KBr, cm^{-1}): 3437, 3315 (NH_2), 2210 (CN). ^{1}H NMR (DMSO-d_6, ppm): 1.58–1.75 (m, 4 H, 2CH_2), 2.31–2.38 (m, 2 H, CH_2), 2.69–2.75 (m, 2 H, CH_2), 3.85 (s, 3 H, CH_3), 6.45 (s, 2 H, NH_2, exchangeable with D_2O), 7.0 (d, 2 H, Ar–H), 7.2 (d, 2 H, Ar–H). MS, m/z (%): 279 [M^+] (100).
$C_{17}H_{17}N_3O$ (279.34)

3.1.2. General procedure for the synthesis of compounds **5a–c**

A mixture of **4a–c** (5 mmol) and chloroformamidine hydrochloride (0.7 g, 6 mmol) in diglyme (5 ml) was stirred at 130–140 °C for 5 h. The reaction mixture was left to cool to room temperature. The obtained solid mass was diluted with dioxane and filtered. The crude hydrochloride was suspended in water, neutralized with TEA, filtered off, washed with water, dried, and crystallized from DMF/H_2O to give **5a–c**, as a white powder.

3.1.2.1. 5-(4-Chlorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinoline-2,4-diamine (**5a**)

Yield: 0.82 g (50%), m.p. 283–285 °C. IR (KBr, cm^{-1}): 3422, 3395, 3255, 3117 (NH_2). ^{1}H NMR (DMSO-d_6, ppm): 1.54–1.7 (m, 4 H, 2 CH_2), 2.34–2.39 (m, 2 H, CH_2), 2.69–2.74 (m, 2 H, CH_2), 6.2 (s, 2 H, NH_2, exchangeable with D_2O), 7.18 (s, 2 H, NH_2, exchangeable with D_2O), 7.25–7.32 (m, 4 H, Ar–H); MS, m/z (%): 326 [$M^+ + 1$] (100).
$C_{17}H_{16}ClN_5$ (325.80)

3.1.2.2. 5-(4-Fluorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinoline-2,4-diamine (**5b**)

Yield: 0.65 g (42%), m.p. 304 °C (decomp.). IR (KBr, cm^{-1}): 3410, 3300, 3125 (NH_2). ^1H NMR (DMSO-d_6, ppm): 1.55–1.76 (m, 4 H, 2 CH_2), 2.28–2.33 (m, 2 H, CH_2), 2.63–2.71 (m, 2 H, CH_2), 6.1 (s, 2 H, NH_2, exchangeable with D_2O), 6.9 (s, 2 H, NH_2, exchangeable with D_2O), 7.4 (m, 4 H, Ar—H). MS, m/z (%): 310 [$M^+ + 1$] (100).
$C_{17}H_{16}FN_5$ (309.34)

3.1.2.3. 5-(4-Methoxyphenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinoline-2,4-diamine (**5c**)

Yield: 0.63 g (39%), m.p. 296 °C (decomp.). IR (KBr, cm^{-1}): 3400, 3336, 3217, 3100 (NH_2). ^1H NMR (DMSO-d_6, ppm): 1.57–1.74 (m, 4 H, 2 CH_2), 2.32–2.37 (m, 2 H, CH_2), 2.67–2.73 (m, 2 H, CH_2), 3.68 (s, 3 H, CH_3), 5.92 (s, 2 H, NH_2, exchangeable with D_2O), 7.0 (s, 2 H, NH_2, exchangeable with D_2O), 7.05 (d, 2 H, Ar—H), 7.32 (d, 2 H, Ar—H). MS, m/z (%): 322 [$M^+ + 1$] (100).
$C_{18}H_{19}N_5O$ (321.38)

3.1.3. General procedure for the synthesis of compounds **6a–c**

A mixture of *o*-aminonitriles **4a–c** (10 mmol), triethylorthoformate (25 ml) and acetic anhydride (0.3 ml) was refluxed for 5 h. The solvent was removed under reduced pressure nearly to half volume and left to cool at room temperature. The resulting solid was filtered off, dried and crystallized to yield compounds **6a–c**, as white crystals.

3.1.3.1. 2-Ethoxymethyleneamino-4-(4-chlorophenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**6a**)

Yield: 2.65 g (78%), m.p. 145–147 °C (C_2H_5OH). IR (KBr, cm^{-1}): 2940, 2830 (CH_2), 2210 (CN), 1597 (N=CH). ^1H NMR (DMSO-d_6, ppm): 1.17 (t, 3 H, CH_3), 1.52–1.69 (m, 4 H, 2 CH_2), 2.38 (t, 2 H, CH_2), 2.73 (t, 2 H, CH_2), 4.25 (q, 2 H, CH_2), 7.15–7.3 (m, 4 H, Ar—H), 7.48 (s, 1 H, N=CH). MS, m/z (%): 340 [$M^+ + 1$] (37), 294 [$M^+ - OC_2H_5$] (78), 284 (17), 77 (100).
$C_{19}H_{18}ClN_3O$ (339.82)

3.1.3.2. 2-Ethoxymethyleneamino-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-quinoline-3-carbonitrile (**6b**)

Yield: 2.1 g (64%), m.p. 173–175 °C (C_2H_5OH). IR (KBr, cm^{-1}): 2925, 2852 (CH_2), 2220 (CN), 1610 (N=CH). ^1H NMR (DMSO-d_6, ppm): 1.22 (t, 3 H, CH_3), 1.53–1.72 (m, 4 H, 2CH_2), 2.36 (t, 2 H, CH_2), 2.75 (t, 2 H, CH_2), 4.25 (q, 2 H, CH_2), 6.95–7.2 (m, 4 H, Ar—H), 7.81 (s, 1 H, N=CH). MS, m/z (%): 324 [$M^+ + 1$] (100).
$C_{19}H_{18}FN_3O$ (323.36)

3.1.3.3. 2-Ethoxymethyleneamino-4-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**6c**)

Yield: 2.1 g (63%), m.p. 138–140 °C (decomp.; 2-propanol). IR (KBr, cm^{-1}): 2925, 2850 (CH_2), 2210 (CN), 1595 (N=CH). ^1H NMR (DMSO-d_6, ppm): 1.17 (t, 3 H, CH_3), 1.57–1.74 (m, 4 H, 2 CH_2), 2.3–2.37 (m, 2 H, CH_2), 2.68–2.73 (m, 2 H, CH_2), 3.85 (s, 3 H, CH_3), 4.25 (q, 2 H, CH_2), 7.14 (d, 2 H, Ar—H), 7.42 (d, 2 H, Ar—H), 7.87 (s, 1 H, N=CH). MS, m/z (%): 336 [$M^+ + 1$] (100).
$C_{20}H_{21}N_3O_2$ (335.4)

3.1.4. General procedure for the synthesis of compounds **7a–c**

A mixture of methanimidates **6a–c** (2 mmol) and 4-pyridinecarbohydrazide (2 mmol) in dioxane (25 ml) was refluxed for 10 h. After cooling, the obtained solid was filtered off, dried and crystallized from the proper solvent to afford compounds **7a–c**, as yellowish white powders.

3.1.4.1. 12-(4-Chlorophenyl)-2-(pyridin-4-yl)-8,9,10,11-tetrahydroquinoline-[2,3-*d*]-1,2,4-triazolo[5,1-*f*]pyrimidine (**7a**)

Yield: 0.41 g (50%), m.p. 227–229 °C (dioxane). IR (KBr, cm^{-1}): 2940, 2830 (CH_2), 1620 (H=CN). ^1H NMR (DMSO-d_6, ppm): 1.52–1.69 (m, 4 H, 2 CH_2), 2.37–2.44 (m, 2 H, CH_2), 2.71–2.78 (m, 2 H, CH_2), 7.36–7.55 (m, 6 H, Ar—H + pyridine-H), 8.65 (d, 2 H, pyridine-H), 9.3 (s, 1 H, Pyrimidine-H). MS, m/z (%): 413 [$M^+ + 1$] (100).
$C_{23}H_{17}ClN_6$ (412.87)

3.1.4.2. 12-(4-Fluorophenyl)-2-(pyridin-4-yl)-8,9,10,11-tetrahydroquinoline-[2,3-*d*]-1,2,4-triazolo[5,1-*f*]pyrimidine (**7b**)

Yield: 0.33 g (42%), m.p. 241–243 °C (dioxane/H_2O). IR (KBr, cm^{-1}): 2925, 2852 (CH_2), 1627 (H=CN). ^1H NMR (DMSO-d_6, ppm): 1.53–1.67 (m, 4 H, 2 CH_2), 2.37–2.46 (m, 2 H, CH_2), 2.66–2.75 (m, 2 H, CH_2), 7.14–7.62 (m, 6 H, Ar—H + pyridine-H), 8.62 (d, 2 H, pyridine-H), 9.25 (s, 1 H, pyrimidine-H). MS, m/z (%): 397 [$M^+ + 1$] (100).
$C_{23}H_{17}FN_6$ (396.42)

3.1.4.3. 12-(4-Methoxyphenyl)-2-(pyridin-4-yl)-8,9,10,11-tetrahydro-quinoline[2,3-*d*]-1,2,4-triazolo[5,1-*f*]pyrimidine (**7c**)

Yield: 0.35 g (43%); mp: 263–265 °C (decomp., dioxane/H_2O). IR (KBr, cm^{-1}): 2925, 2850 (CH_2), 1612 (H=CN). ^1H NMR (DMSO-d_6, ppm): 1.57–1.75 (m, 4 H, 2 CH_2), 2.31–2.39 (m, 2 H, CH_2), 2.66–2.73 (m, 2 H, CH_2), 3.85 (s, 3 H, CH_3), 7.13 (d, 2 H, Ar—H), 7.38 (d, 2 H, Ar—H), 7.67 (d, 2 H, pyridine-H), 8.65 (d, 2 H, pyridine-H), 9.32 (s, 1 H, pyrimidine-H). MS, m/z (%) 409 [$M^+ + 1$] (100).
$C_{24}H_{20}N_6O$ (408.46)

3.1.5. General procedure for the synthesis of compounds **8a–c**

To a well-stirred solution of methaneimidates **6a–c** (3 mmol) in C_2H_5OH (25 ml), hydrazine hydrate (3 ml) was added, and then the mixture was stirred at room temperature until a solid started to be formed. Stirring was continued for additional 2 h; the solid formed was filtered off and crystallized from the proper solvent to afford **8a–c**, as a white powder.

3.1.5.1. 5-Imino-6-(4-chlorophenyl)-7,8,9,10-tetrahydropyrimido[4,5-b]-quinolin-4-amine (**8a**)

Yield: 0.9 g (92%), m.p. 248–250 °C (dioxane/H_2O). IR (KBr, cm^{-1}): 3420, 3144, 3100 (NH_2, NH). ^1H NMR (DMSO-d_6, ppm): 1.52–1.69 (m, 4 H, 2 CH_2), 2.37–2.44 (m, 2 H, CH_2), 2.71–2.78 (m, 2 H, CH_2), 6.66 (s, 2 H, NH_2, exchangeable with D_2O), 7.35–7.42 (m, 4 H, Ar—H), 8.48 (s, 1 H, pyrimidine-H), 8.85 (s, 1 H, NH, exchangeable with D_2O). MS, m/z (%): 326 [$M^+ + 1$] (100).
$C_{17}H_{16}ClN_5$ (325.8)

3.1.5.2. 5-Imino-6-(4-fluorophenyl)-7,8,9,10-tetrahydropyrimido[4,5-*b*]-quinolin-4-amine (**8b**)

Yield: 0.8 g (86%), m.p. 285–287 °C (dioxane/H_2O). IR (KBr, cm^{-1}): 3400, 3132, 3112 (NH_2, NH). ^1H NMR (DMSO-d_6, ppm): 1.53–1.69 (m, 4 H, 2 CH_2), 2.39–2.47 (m, 2 H, CH_2), 2.66–2.75 (m, 2 H, CH_2), 6.75 (s, 2 H, NH_2, exchangeable with D_2O), 7.17–7.32 (m, 4 H, Ar—H), 8.27 (s, 1 H, pyrimidine-H), 8.9 (s, 1 H, NH, exchangeable with D_2O). MS, m/z (%): 310 [$M^+ + 1$] (100).
$C_{17}H_{16}FN_5$ (309.34)

3.1.5.3. 5-Imino-6-(4-methoxyphenyl)-7,8,9,10-tetrahydropyrimido[4,5-*b*] quinolin-4-amine (**8c**)

Yield: 0.72 g (75%), m.p. 255–257 °C (decomp.; C_2H_5OH). IR (KBr, cm^{-1}): 3452, 3128, 3098 (NH_2, NH). ^1H NMR (DMSO-d_6, ppm): 1.57–1.75 (m, 4 H, 2CH_2), 2.31–2.39 (m, 2 H, CH_2), 2.66–2.73 (m, 2 H, CH_2), 3.87 (s, 3 H, CH_3), 6.35 (s, 2 H, NH_2, exchangeable with D_2O), 7.13 (d, 2 H, Ar—H), 7.45 (d, 2 H, Ar—H), 8.27 (s, 1 H, pyrimidine-H), 8.65 (s, 1 H, NH, exchangeable with D_2O). MS, m/z (%): 322 [$M^+ + 1$] (100).
$C_{18}H_{19}N_5O$ (321.38)

3.1.6. General procedure for the synthesis of compounds **9a, b**

A mixture of **4a, c** (10 mmol) and pyridine-3-carbonitrile (1.05 g, 10 mmol) and sodium methoxide (1.08 g, 20 mmol) in 2-propanol (50 ml) was refluxed for 48 h or 60 h, respectively. The solvent was removed under reduced pressure and the residue was stirred with ice water. The crude product was filtered off, washed thoroughly with water, C_2H_5OH (50%), dried and crystallized from the proper solvent to yield compounds **9a, b**, as white powders.

3.1.6.1. 2-(Pyridin-3-yl)-5-(4-chlorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]-quinolin-4-amine (**9a**)

Yield: 2.9 g (75%), m.p. 284–286 °C (dioxane/H_2O). IR (KBr, cm^{-1}): 3477, 3137 (NH_2), 2945 (CH_2), 1626 (HC=N). ^1H NMR (DMSO-d_6, ppm): 1.53–1.71 (m, 4 H, 2CH_2), 2.37–2.44 (m, 2 H, CH_2), 2.71–2.78 (m, 2 H, CH_2), 6.2 (s, 2 H, NH_2, exchangeable with D_2O), 7.35–7.42 (m, 5 H, Ar—H + pyridine-H), 7.97 (m, 1 H, pyridine-H), 8.47 (d, 1 H, pyridine-H), 8.92 (s, 1 H, pyridine-H). MS, m/z (%): 388 [$M^+ + 1$] (7), 171 (100).
$C_{22}H_{18}ClN_5$ (387.86)

3.1.6.2. 2-(Pyridin-3-yl)-5-(4-methoxyphenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-amine (**9b**)

Yield: 0.72 g (75%), m.p. 255–257 °C (dec.; C_2H_5OH). IR (KBr, cm^{-1}): 3409, 3112 (NH_2), 2929 (CH_2), 1619 (H=CN). ^1H NMR (DMSO-d_6, ppm): 1.57–1.75 (m, 4 H, 2 CH_2), 2.31–2.39 (m, 2 H, CH_2), 2.66–2.73 (m, 2 H, CH_2), 3.67 (s, 3 H, CH_3), 5.6 (s, 2 H, NH_2, exchangeable with D_2O), 7.15 (d, 2 H, Ar—H), 7.41 (m, 3H, Ar—H + pyridine-H), 7.95 (m, 1 H, pyridine-H), 8.45 (d, 1 H, pyridine-H), 8.9 (s, 1 H, Pyridine-H). MS, m/z (%): 384 [$M^+ + 1$] (100).
$C_{23}H_{21}N_5O$ (383.45)

3.1.7. General procedure for the synthesis of compounds **10a, b**

A suspension of **4a, c** (10 mmol) in 20% alcoholic KOH solution (100 ml) was refluxed for 10 h. After cooling, H_2O (30 ml) was added to the reaction mixture and the whole was left to stand overnight. The separated white crystalline product was filtered off, dried and crystallized from DMF/H_2O to afford **10a, b**.

3.1.7.1. 2-Amino-4-(4-chlorophenyl)-5,6,7,8-tetrahydroquinoline-3-carboxamide (**10a**)

Yield: 1.97 g (64%), m.p. 292–294 °C. IR (KBr, cm^{-1}): 3594, 3335 (NH_2), 1669 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 1.53–1.71 (m, 4 H, 2 CH_2), 2.37–2.44 (m, 2 H, CH_2), 2.71–2.78 (m, 2 H, CH_2), 5.4 (s, 2 H, NH_2, exchangeable with D_2O), 6.34 (s, 2 H, NH_2, exchangeable with D_2O), 7.28–7.34 (m, 4 H, Ar–H). MS, m/z (%): 302 [$M^+ + 1$] (100).
$C_{16}H_{16}ClN_3O$ (301.77)

3.1.7.2. 2-Amino-4-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinoline-3-carboxamide (**10b**)

Yield: 1.6 g (54%), m.p. 272–274 °C. IR (KBr, cm^{-1}): 3535, 3290 (NH_2), 1675 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 1.57–1.75 (m, 4 H, 2 CH_2), 2.31–2.39 (m, 2 H, CH_2), 2.66–2.73 (m, 2 H, CH_2), 3.67 (s, 3 H, CH_3), 5.6 (s, 2 H, NH_2, exchangeable with D_2O), 6.37 (s, 2 H, NH_2, exchangeable with D_2O), 7.15 (d, 2 H, Ar–H), 7.41 (m, 2 H, Ar–H). MS, m/z (%): 298 [$M^+ + 1$] (100).
$C_{17}H_{19}N_3O_2$ (297.35)

3.1.8. General procedure for the synthesis of compounds **11a, b**

To a cold solution of $NaNO_2$ (0.21 g, 3.1 mmol) in conc. H_2SO_4 (5 ml) a suspension of the 2-amino-3-carbamoyl derivatives **10a, b** (3 mmol) in AcOH (20 ml) was slowly added. Stirring was continued for 1 h. It was then filtered and the filtrate was poured onto ice. The crude product was filtered, dissolved in NaOH (5%), treated with charcoal, filtered, and acidified with AcOH, giving compound **11a, b**.

3.1.8.1. 5-(4-Chlorophenyl)-6,7,8,9-tetrahydro-[1,2,3]-triazino[4,5-b] quinolin-4-ol (**11a**)

Yield: 0.32 g (34%), m.p. 183–185 °C (dioxane/H_2O). IR (KBr, cm^{-1}): 3227 (br, OH). ^{1}H NMR (DMSO-d_6, ppm): 1.53–1.71 (m, 4 H, 2 CH_2), 2.37–2.44 (m, 2 H, CH_2), 2.71–2.78 (m, 2 H, CH_2), 7.26 (d, 2 H, Ar–H), 7.35 (d, 2 H, Ar–H), 9.1 (br s, 1 H, OH, exchangeable with D_2O). MS, m/z (%): 313 [$M^+ + 1$] (100).
$C_{16}H_{13}ClN_4O$ (312.75)

3.1.8.2. 5-(4-Methoxyphenyl)-6,7,8,9-tetrahydro-[1,2,3]-triazino[4,5-*b*]-quinolin-4-ol (**11b**)

Yield: 0.5 g (54%), m.p. 146–148 °C (2-propanol/H_2O). IR (KBr, cm^{-1}): 3214 (br, OH). ^{1}H NMR (DMSO-d_6, ppm): 1.57–1.75 (m, 4 H, 2 CH_2), 2.31–2.39 (m, 2 H, CH_2), 2.66–2.73 (m, 2 H, CH_2), 3.67 (s, 3 H, CH_3), 7.15 (d, 2 H, Ar–H), 7.41 (m, 2 H, Ar–H), 9.24 (s, 1 H, OH, exchangeable with D_2O). MS, m/z (%): 309 [$M^+ + 1$] (100).
$C_{17}H_{16}N_4O_2$ (308.33)

3.1.9. General procedure for the synthesis of compounds **12a–c**

To a well-stirred cold suspension (−5 °C) of **4a–c** (10 mmol) in conc. HCl (20 ml) and AcOH (20 ml) a solution of $NaNO_2$ [1 g, 12 mmol/water (10 ml)] was added drop-wise. After addition, the ice bath was removed and stirring was continued at room temperature for 2 additional hours. The crude products were crystallized from dioxane/H_2O to give **12a–c**.

3.1.9.1. 4-Chloro-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-[1,2,3]-triazino[4,5-*b*]-quinoline (**12a**)

Yield: 1.85 g (56%), m.p. 297–299 °C. IR (KBr, cm^{-1}): 3088 (CH-aromatic), 2987 (CH-aliphatic), 1638 (C=N). ^{1}H NMR (DMSO-d_6, ppm): 1.53–1.67 (m, 4 H, 2 CH_2), 2.25–2.34 (m, 2 H, CH_2), 2.55–2.73 (m, 2 H, CH_2), 7.35 (d, 2 H, Ar–H), 7.55 (d, 2 H, Ar–H). MS, m/z (%): 332 [$M^+ + 1$] (100).
$C_{16}H_{12}Cl_2N_4$ (331.2)

3.1.9.2. 4-Chloro-5-(4-fluorophenyl)-6,7,8,9-tetrahydro-[1,2,3]-triazino[4,5-*b*]-quinoline (**12b**)

Yield: 1.7 g (55%), m.p. 275–277 °C. IR (KBr, cm^{-1}): 3090 (CH-aromatic), 2991 (CH-aliphatic), 1641 (C=N). ^{1}H NMR (DMSO-d_6, ppm): 1.54–1.73 (m, 4 H, 2 CH_2), 2.26–2.33 (m, 2 H, CH_2), 2.6–2.68 (m, 2 H, CH_2), 7.43 (d, 2 H, Ar–H), 7.52 (d, 2 H, Ar–H). MS, m/z (%): 315 [$M^+ + 1$] (100).
$C_{16}H_{12}ClFN_4$ (314.74)

3.1.9.3. 4-Chloro-5-(4-methoxyphenyl)-6,7,8,9-tetrahydro-[1,2,3]-triazino[4,5-*b*]quinoline (**12c**)

Yield: 1.8 g (56%), m.p. 188–190 °C. IR (KBr, cm^{-1}): 3090 (CH-aromatic), 2986 (CH-aliphatic), 1637 (C=N). ^{1}H NMR (DMSO-d_6, ppm): 1.57–1.73 (m, 4 H, 2 CH_2), 2.29–2.35 (m, 2 H, CH_2), 2.69–2.75 (m, 2 H, CH_2), 3.87 (s, 3 H, CH_3), 7.23 (d, 2 H, Ar–H), 7.57 (d, 2 H, Ar–H). MS, m/z (%): 327 [$M^+ + 1$] (100).
$C_{17}H_{15}ClN_4O$ (326.78)

3.1.10. General procedure for the synthesis of compounds **13a–c**

A mixture of **12a–c** (3 mmol), 2-aminopyridine (0.28 g, 3 mmol) and TEA (3 ml) in dioxane (50 ml) was refluxed for 4 h. The reaction mixture was left to cool, poured onto ice-water and then treated with few drops of HCl. The crude precipitate was filtered off, washed thoroughly with H_2O, dried and crystallized from the proper solvent to afford **13a–c**, as a yellowish white powder.

3.1.10.1. 5-(4-Chlorophenyl)-6,7,8,9-tetrahydro-*N*-(pyridin-2-yl)[1,2,3]-triazino[4,5-*b*]quinolin-4-amine (**13a**)

Yield: 0.65 g (56%), m.p. 319–321 °C (DMF/H_2O). IR (KBr, cm^{-1}): 3345 (NH). ^{1}H NMR (DMSO-d_6, ppm): 1.54–1.68 (m, 4 H, 2 CH_2), 2.27–2.37 (m, 2 H, CH_2), 2.56–2.73 (m, 2 H, CH_2), 6.7 (d, 1 H, pyridine-H), 6.81 (d, 1 H, pyridine-H), 7.3–7.48 (m, 5 H, Ar–H + pyridine-H), 8.21 (d, 1 H, pyridine-H), 8.39 (s, 1 H, NH, exchangeable with D_2O). MS, m/z (%): 389 [$M^+ + 1$] (100).
$C_{21}H_{17}ClN_6$ (388.85)

3.1.10.2. 5-(4-Fluorophenyl)-6,7,8,9-tetrahydro-*N*-(pyridin-2-yl)[1,2,3]-triazino[4,5-*b*]quinolin-4-amine (**13b**)

Yield: 0.36 g (32%), m.p. 224–226 °C (dioxane/H_2O). IR (KBr, cm^{-1}): 3390 (NH). ^{1}H NMR (DMSO-d_6, ppm): 1.54–1.73 (m, 4 H, 2 CH_2), 2.26–2.33 (m, 2 H, CH_2), 2.6–2.68 (m, 2 H, CH_2), 6.65 (d, 1 H, pyridine-H), 6.79 (t, 1 H, pyridine-H), 7.3–7.43 (m, 3 H, Ar–H + pyridine-H), 8.21 (d, 1 H, pyridine-H), 7.51 (d, 2 H, Ar–H), 8.54 (s, 1 H, NH, exchangeable with D_2O). MS, m/z (%): 373 [$M^+ + 1$] (100).
$C_{21}H_{17}FN_6$ (372.4)

3.1.10.3. 5-(4-Methoxyphenyl)-6,7,8,9-tetrahydro-*N*-(pyridine-2-yl)[1,2,3]-triazino-[4,5-*b*]quinolin-4-amine (**13c**)

Yield: 0.35 g (59%), m.p. 218–220 °C (dioxane/H_2O). IR (KBr, cm^{-1}): 3298 (NH). ^{1}H NMR (DMSO-d_6, ppm): 1.57–1.73 (m, 4 H, 2 CH_2), 2.29–2.35 (m, 2 H, CH_2), 2.69–2.75 (m, 2 H, CH_2), 3.87 (s, 3 H, CH_3), 6.65 (d, 1 H, Pyridine-H), 6.79 (t, 1 H, Pyridine-H), 7.23 (d, 2 H, Ar–H), 7.3–7.43 (m, 3 H, Ar–H + pyridine-H), 8.19 (d, 1 H, Pyridine-H), 8.41 (s, 1 H, NH, exchangeable with D_2O). MS, m/z (%): 385 [$M^+ + 1$] (100).
$C_{22}H_{20}N_6O$ (384.43)

3.1.11. General procedure for the synthesis of compounds **14a–c**

To a mixture of **4a–c** (10 mmol) and ethylene diamine (1.19 g, 1.3 ml; 20 mmol) was added drop-wisely carbon disulfide (2.28 g, 1.8 ml, 30 mmol). The reaction mixture was heated on a steam bath for 8 h. After cooling, it was triturated with water and the separated solid was filtered off, dried and crystallized from the proper solvent to yield compounds **14a–c**.

3.1.11.1. 4-(4-Chlorophenyl)-5,6,7,8-tetrahyro-3-(4,5-dihydro-*1H*-imidazolin-2-yl)quinolin-2-amine (**14a**)

Yield: 2.1 g (64%), m.p. 234–236 °C (2-propanol). IR (KBr, cm^{-1}): 3435, 3292, 3200 (NH_2, NH). ^{1}H NMR (DMSO-d_6, ppm): 1.55–1.7 (m, 4 H, 2 CH_2), 2.29–2.38 (m, 2 H, CH_2), 2.56–2.74 (m, 2 H, CH_2), 3.54 (m, 4 H, 2 CH_2), 6.63 (s, 2 H, NH_2, exchangeable with D_2O), 7.34 (d, 2 H, Ar–H), 7.54 (d, 2 H, Ar–H), 8.39 (s, 1 H, NH, exchangeable with D_2O). MS, m/z (%): 327 [$M^+ + 1$] (100).
$C_{18}H_{19}ClN_4$ (326.82)

3.1.11.2. 4-(4-Fluorophenyl)-5,6,7,8-tetrahyro-3-(4,5-dihydro-*1H*-imidazolin-2-yl)quinolin-2-amine (**14b**)

Yield: 2 g (67%), m.p. 245–247 °C (C_2H_5OH). IR (KBr, cm^{-1}): 3439, 3288, 3230 (NH_2, NH). ^{1}H NMR (DMSO-d_6, ppm): 1.55–1.76 (m, 4 H, 2 CH_2), 2.27–2.35 (m, 2 H, CH_2), 2.61–2.7 (m, 2 H, CH_2), 3.55 (m, 4 H, 2 CH_2), 6.45 (s, 2 H, NH_2, exchangeable with D_2O), 7.36 (m, 4 H, Ar–H), 8.35 (s, 1 H, NH, exchangeable with D_2O). MS, m/z (%): 310 [M^+] (100).
$C_{18}H_{19}FN_4$ (310.37)

3.1.11.3. 4-(4-Methoxyphenyl)-5,6,7,8-tetrahyro-3-(4,5-dihydro-*1H*-imidazolin-2-yl)quinolin-2-amine (**14c**)

Yield: 2.2 g (70%), m.p. 267–269 °C (C_2H_5OH). IR (KBr, cm^{-1}): 3420, 3278, 3112 (NH_2, NH). ^{1}H NMR (DMSO-d_6, ppm): 1.58–1.75 (m, 4 H,

2 CH_2), 2.31–2.38 (m, 2 H, CH_2), 2.69–2.75 (m, 2 H, CH_2), 3.52 (m, 4 H, 2 CH_2), 3.85 (s, 3 H, CH_3), 6.45 (s, 2 H, NH_2, exchangeable with D_2O), 7.11 (d, 2 H, Ar–H), 7.36 (d, 2 H, Ar–H), 8.47 (s, 1 H, NH, exchangeable with D_2O). MS, m/z (%): 322 [M^+] (100).
$C_{19}H_{22}N_4O$ (322.4)

3.1.12. General procedure for the synthesis of compounds **15a–c**

To an ice cold stirred suspension of **14a–c** (5 mmol) in 5% HCl (20 ml) a solution of $NaNO_2$ (0.37 g, 5.5 mmol) in H_2O (5 ml) was slowly added. After addition was completed, the reaction mixture was stirred at room temperature for 1 h. It was then neutralized with 1N $NaHCO_3$ and kept in a freezer. The solid formed was collected by filtration, washed with water and dried. It was dissolved in $CHCl_3$, treated with charcoal, filtered and the solvent was removed under reduced pressure. The obtained residue was solidified by trituration with ether, and the obtained solid was crystallized from the proper solvent to afford compounds **15a–c**.

3.1.12.1. 8-Chlorophenyl-5,6,9,10,11,12-hexahydroimidazo[2′,1′ : 6,5] [1,2,3]-triazino[4,5-*b*]quinoline (**15a**)

Yield: 0.7 g (42%), m.p. 183–185 °C (C_2H_5OH). IR (KBr, cm^{-1}): 3112 (CH-aromatic), 2933 (CH-aliphatic). ^{1}H NMR (DMSO-d_6, ppm): 1.55–1.7 (m, 4 H, 2 CH_2), 2.29–2.38 (m, 2 H, CH_2), 2.56–2.74 (m, 2 H, CH_2), 3.54 (m, 4 H, 2 CH_2), 7.33 (d, 2 H, Ar–H), 7.52 (d, 2 H, Ar–H). MS, m/z (%): 338 [$M^+ + 1$] (100).
$C_{18}H_{16}ClN_5$ (337.81)

3.1.12.2. 8-Fluorophenyl-5,6,9,10,11,12-hexahydroimidazo[2′,1′ : 6,5] [1,2,3]-triazino[4,5-*b*]quinoline (**15b**)

Yield: 0.56 g (36%), m.p. 155–157 °C (2-propanol). IR (KBr, cm^{-1}): 3075 (CH-aromatic), 2932 (CH-aliphatic). ^{1}H NMR (DMSO-d_6, ppm): 1.55–1.76 (m, 4 H, 2 CH_2), 2.27–2.35 (m, 2 H, CH_2), 2.61–2.7 (m, 2 H, CH_2), 3.55 (m, 4 H, 2 CH_2), 7.24 (d, 2 H, Ar–H), 7.44 (d, 2 H, Ar–H). MS, m/z (%): 321 [M^+] (100).
$C_{18}H_{16}FN_5$ (321.35)

3.1.12.3. 8-Methoxydphenyl-5,6,9,10,11,12-hexahydroimidazo[2′,1′ : 6,5]-[1,2,3]-triazino[4,5-*b*]quinoline (**15c**)

Yield: 0.62 g (37%), m.p. 168–170 °C (C_2H_5OH). IR (KBr, cm^{-1}): 3100 (CH-aromatic), 2944, 2835 (CH-aliphatic). ^{1}H NMR (DMSO-d_6, ppm): 1.58–1.75 (m, 4 H, 2 CH_2), 2.31–2.38 (m, 2 H, CH_2), 2.69–2.75 (m, 2 H, CH_2), 3.55 (m, 4 H, 2 CH_2), 3.84 (s, 3 H, CH_3), 7.12 (d, 2 H, Ar–H), 7.34 (d, 2 H, Ar–H). MS, m/z (%): 333 [M^+] (100).
$C_{19}H_{19}N_5O$ (333.39)

3.2. Molecular modeling

Docking Study was performed Using Molsoft ICM software. Downloading the crystal structure of p38 MAP kinase enzyme complexed with inhibitor **3** was carried out from protein data bank website (pdb) entry 1ZZL. Regularization and optimization for protein and ligand was performed. Determination of the essential amino acids in binding site and compared with that present in literature. The performance of the docking method was evaluated by re-docking crystal ligand into the assigned active site of p38 MAP kinase. Interactive docking using Mol table ligand was carried out for all the conformers of each compound of the test set (**4–15**) to the selected active site. Each docked compound was assigned a score according to its fit in the ligand binding pocket (LBP) (Table).

3.3. Pharmacology

3.3.1. Animals

Animals were obtained from the animal house colony of the National Research Center, Cairo, Egypt. All animals were allowed free access to water and were kept on a constant standard diet. All procedures involving animals were carried out in accordance with the guide for the care and use of laboratory animals (National Academy of Science of Egypt) and were approved by the Animals Studies Committee at Washington University. Adult male albino rats, weighing 150–180 g, fasted for 12 h and used for determining the anti-inflammatory activity and LD_{50}.

3.3.2. Evaluation of anti-inflammatory activity

The inhibitory activity of the studied compounds on carrageenan-induced rat's paw edema was carried out according to the method of Winter et al. (1962, 1963). Groups of rats, each of 8 animals were orally dosed with the test compounds at a dose level of 2.5 and 5 mg/kg an hour before carrageenan challenge. Foot paw edema was induced by sub planter injection of 0.05 ml of 1% suspension of carrageenin in saline into the planter tissue of one hind paw. An equal volume of saline was injected to the other hind paw and served as control. Four hours after drug administration the animal was decapitated, blood was collected and the paws were rapidly excised.
The average weight of edema was estimated for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated; then percentage protection against edema was estimated (Table). Indomethacin (2.5 and 5 mg/kg) was employed as standard reference against which the test compounds were compared.

3.3.3. Estimation of plasma prostaglandin E_2 (PGE_2)

Heparinized blood samples were collected from rats (n = 8), plasma was separated by centrifugation at 12 000 × g for 2 min at 4 °C and immediately stored frozen 20 °C until use.
The designs correlate-EIA prostaglandin in E_2 (PGE_2) kit is a competitive immune assay for the quantitative determination of PGE_2 in biological fluids. The kit uses a monoclonal antibody to PGE_2 to bind, in a competitive manner, the PGE_2 in the sample. After a simultaneous incubation at room temperature the excess reagents were washed away and the substrate was added. After a short incubation time the enzyme reaction was stopped and the yellow color generated was read on a micro plate reader (DYNATCH, MR 5000) at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE_2 in either standard or samples. The percentage inhibition of plasma PGE_2 for each compound was estimated (Table) (Herrmann et al. 1990; Weithmann et al. 1994).

3.3.4. Evaluation of acute toxicity study

The test compounds were administered orally at different dose levels in separate groups of animals. After 24 h of drug administration percent mortality in each group was observed. From the data obtained, the lethal dose (LD_{50}) was calculated by the method of Smith (1960).

3.3.5. Evaluation of ulcerogenic activity

Ulcerogenic activity was determined according to the method of Verma et al. (1981). In this method, adult albino rats, fasted 24 h prior to the administration of drugs, were divided into groups of ten animals each. Water was allowed *ad libitum* to the animals. The test compounds and standard drugs were given intraperitoneally and the animals sacrificed 8 h after drugs treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of (a) shedding of epithelium (b) petechial and frank haemorrhage and (c) erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

References

Abd El-Salam OI (2000) Synthesis of some new pyrimido[5,4-*a*]-Indolizines. Monatsh Chem 131: 959–965.

Abd El-Salam OI, Mohamed SF (2005) Synthesis and preliminary antibacterial activity of some 4-substituted-N^1-2-pyridylsulfanilamide derivatives. J Chin Chem Soc 52: 1157–1164.

Attia A, Abo-Ghalia MH, Abd El-Salam OI (1995) Synthesis of 3,5-disubstituted pyridines as antimicrobial agents. Pharmazie 50: 455–459.

Beck JR, Yahner JA (1976) Synthesis of [l]benzo-thieno[3,2-d]-v-triazine derivatives. A unique diazonium ion cyclization. J Org Chem 41: 1733–1734.

Bekhit AA, El-Sayed OA, Aboulmagd E, Park J (2004) Tetrazolo[1,5-*a*]quinoline as a potential promising new scaffold for the synthesis of novel anti-inflammatory and antibacterial agents. Eur J Med Chem 39: 249–255.

Beuck M (1999) Nonsteroidal anti-inflammatory drugs: A new generation of cyclooxygenase inhibitors. Angew Chem Int Ed 38: 631–633.

Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJN (2000) Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N Engl J Med 343: 1520–1528.

Calhoun W, Carlson RP, Crossley R, Datko LJ, Dietrich S, Healtherington K, Marshall LA, Meade PJ, Opalko A, Shepherd RG (1995) Synthesis and anti-inflammatory activity of certain 5,6,7,8-tetrahydroquinolines and related compounds. J Med Chem 38: 1473–1481.

Crousse B, Bégué J, Bonnet-Delpon D (2000) Synthesis of 2-CF_3-tetrahydroquinoline and quinoline derivatives from CF_3-N-aryl-aldimine. J Org Chem 65: 5009–5013.

Dogné J, Supuran CT, Pratico D (2005) Adverse cardiovascular effects of the coxibs. J Med Chem 48: 2251–2257.

Elkholy YM, Morsy MA (2006) Facile synthesis of 5,6,7,8-tetrahydro-pyrimido[4,5-b]quinoline derivatives. Molecules 11: 890–903.

Ellis GP (1987) The chemistry of heterocyclic compounds (Synthesis of fused heterocycles) John Wiley & Sons, New York, p. 217.

Fadel F, Titouani SL, Soufiaoui M, Ajamay H, Mazzah A. (2004) Synthése de nouveaux dérivés tétrahydroquinoléines et quinoléines via la réaction d'aza-Diels-Alder suivie d'aromatisation. Tetrahedron Lett 45: 5905–5908.

Gallagher TF, Fier-Thompson SM, Garigipati RS, Sorenson ME, Smietana JM, Lee D, Bender PE, Lee JC, Laydon JT, Chabot-Fletcher MC, Breton JJ, Adams JL (1995) 2,4,5-triarylimidazole inhibitors of IL-1 biosynthesis. Bioorg Med Chem Lett 5: 1171–1176.

Gholap AR, Toti KS, Shirazi F, Kumari R, Bhat MK, Deshpande MV, Srinivasan KV (2007) Synthesis and evaluation of antifungal properties of a series of the novel 2-amino-5-oxo-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile and its analogues. Bioorg Med Chem 15: 6705–6715.

Harris NV, Smith C, Bowden K (1990) Antifolate and antibacterial activities of 5-substituted 2,4-diaminoquinazolines. J Med Chem 33: 434–444.

Herrmann F, Lindemann A, Gauss J, Mertelsmann R (1990) Cytokine-stimulation of prostaglandin synthesis from endogenous and exogenous arachidonic acids in polymorphonuclear leukocytes involving activation and new synthesis of cyclooxygenase. Eur J Immunol 20: 2513–2516.

Kobayashi K, Yoneda K, Miyamoto K, Morikawa O, Konishi H (2004) A convenient synthesis of quinolines by reactions of o-isocyano-β-methoxystyrenes with nucleophiles. Tetrahedron 60: 11639–11645.

Kontogiorgis CA, Hadjipavlou-Litina DJ (2002) Non steroidal anti-inflammatory and anti-allergy agents. Curr Med Chem 9: 89–98.

Kouznetsov VV, Mendez LYV, Gomez CMM (2005) Recent progress in the synthesis of quinolines. Curr Org Chem 9: 141–161.

Kulkarni RG, Sastry GN, Achaiah G (2006) Targets for anti-inflammatory and anti-arthritic agents. Curr Pharm Des 12: 2437–2454.

Marco-Contelles J, León R, López MG, Garcìa AG, Villarroya M (2006) Synthesis and biological evaluation of new 4 H-pyrano[2,3-*b*]quinoline derivatives that block acetylcholinestearase and cell calcium signals, and cause neuroprotection against calcium overload and free radicals. Eur J Med Chem 41: 1464–1469.

Nayana MR, Sekhar YN, Kumari NS, Mahmood SK, Ravikumar M (2008) CoMFA and docking studies on triazolopyridine oxazole derivatives as p38 MAP kinase inhibitors. Eur J Med Chem 43: 1261–1269.

Praveen PN, Chen Q, Knaus E (2005) Synthesis and biological evaluation of 1,3-diphenylprop-2-yn-1-ones as dual inhibitors of cyclooxygenases and lipooxygenases. Bioorg Med Chem Lett 15: 4842–4845.

Ravindra GK, Achaiah G, Sastry GN (2008) Molecular modeling studies of phenoxypyrimidinyl imidazoles as p38 kinase inhibitors using QSAR and docking. Eur J Med Chem 43: 830–838.

Revesz L, Di Padova FE, Buhl T, Feifel R, Gram H, Hiestand P, Manning U, Wolf R, Zimmerlin AG (2002) SAR of 2,6-diamino-3,5-difluoropyridinyl substituted heterocycles as novel p38 MAP kinase inhibitors. Bioorg Med Chem Lett 12: 2109–2112.

Sandra P, Maria JP, Pedro MC, Victoria SM, Ana RG, Piero C, Federico Jr. M, Cristina M (2006) Phosphorylation of p38 by GRK2 at the docking groove unveils a novel mechanism for inactivating p38 MAPK. Curr Biol 16: 2042–2047.

Smith QE (1960) Pharmacological screening tests progress in medicinal. chemistry, vol. 1, Butterworth, London.

Swelam SA, Abd El-Salam OI, Zaki MEA (1999) Synthesis of some pyrazolo[3,4-d]pyrimidines and their triazole and tetrazole derivatives. J Serb Chem Soc 64: 655–662.

Taylor EC, McKillop A (1970) Advances in organic chemistry: Methods and results (The chemistry of cyclic enaminonitriles and o-aminonitriles). John Wiley & Sons, Inc (Interscience Publishers), New York, vol. 7, p. 237.

van Ryn J, Trummlitz G, Pairet M (2000) COX-2 selectivity and inflammatory processes. Curr Med Chem 7: 1145–1161.

Verma M, Sinha JN, Gujrati VR, Bhalla TN, Bhargava KP, Shanker K (1981) A new potent anti-inflammatory quinazolone. Pharmacol Res Commun 13: 967–969.

Weithmann KU, Jeske S, Schlotte V (1994) Effect of leflunomide on constitutive and inducible pathways of cellular eicosanoid generation. Agents Actions 41: 164–170.

Wilson K, McCaffrey P, Hsiao K, Pazhanisamy S, Galullo V, Bemis G, Fitzgibbon J, Caron P, Murcko M, Su M (1997) The structural basis for the specificity of pyridinylimidazole inhibitors of p38 MAP kinase. Chem Biol 4: 423–431.

Winter CA, Risely EA, Nuss GW (1962) Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med 111: 544–547.

Winter CA, Risely EA, Nuss GW (1963) Anti-inflammatory and antipyretic activities of indomethacin, 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid. J Pharmacol Exp Ther 141: 369–376.