One-Pot Synthesis of 4,6-Diaryl-2-oxo(imino)-1,2-dihydropyridine-3-carbonitrile; a New Scaffold for p38α MAP Kinase Inhibition

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Two series of new compounds with the general formula 4,6-diaryl-2-oxo-1,2-dihydropyridine-3-carbonitriles and their isosteric 2-imino derivatives were synthesized by the multicomponent reaction of the appropriate acetophenone, aromatic aldehyde, ammonium acetate, and malononitrile or ethyl cyanoacetate. The products were obtained with excellent yields. The prepared compounds were evaluated for their in vitro ability to inhibit p38 α-MAP kinase. Several compounds showed p38 MAP kinase inhibitory properties with IC_{50} as low as 0.07 μM. This is the first time to report compounds with such scaffold as p38 α-MAP kinase inhibitors. This asserts the potentiality of multicomponent reactions in drug discovery.

Introduction

The p38 MAP kinase is a serine/threonine kinase that is closely associated with the cytokine driven progression of rheumatoid arthritis, autoimmune diseases, and cancer. Almost all the pro-inflammatory, pro-oncogenic, and pro-angiogenic mediators are activators of the p38α MAP kinase cascade. Moreover, p38 MAP kinase is a key-player in the biosynthesis of pro-inflammatory cytokines, such as IL-1βΓand TNF-α both at the translational and the transcriptional level. Among the four identified p38 isoforms (p38α, p38βΓ, p38γ, and p38δ), the α-form is the fully studied.[1–3]

Thus, antagonists of this cascade may provide a multimodal strategy to counteract them. To date, p38α MAP kinase inhibitors may be classified into six main classes: diaryl heterocycles, particularly pyridinyl- and pyrimidinyl-imidazole derivatives, (e.g., SB203580, SB202190: Figure 1, left and middle), bicyclic 6,6-heterocycles (pyridone derivatives, Figure 1 right) and related structures, *N*,*N*′-diarylureas and related structures, substituted benzamides, diaryl ketones, and indole amides.[4–7]

Despite the efficient binding of p38α MAP kinase inhibitors from the diaryl heterocycle class to their target, the development of p38α MAP kinase inhibitors particularly from the pyridinyl-imidazole class into anti-inflammatory drugs was truncated by their severe liver toxicity, as the pyridinyl imidazoles were found to interact with hepatic cytochrome P450 enzymes through chelation of their iron by the lone pair of electrons of the pyridine and/or imidazole ring.[8] It remains unclear whether this side effect is caused by the pyridine or the imidazole ring.

It remains a medicinal chemistry challenge to design novel ligands to dissect the inhibition of p38α from interference with cytochrome P450.[8,9] Thus, herein we report the syntheses of novel diarylheterocycle derivatives that are devoid of the imidazole and the pyridine moiety; the core heterocycles are cyanopyridone and cyanoiminopyridines with the two aryls positioned meta to each other rather than vicinal. This is accomplished by one-pot multicomponent reaction of the appropriate acetophenone, appropriate aromatic aldehyde, ammonium acetate and malononitrile or ethyl cyanoacetate. The newly synthesized compounds were evaluated for their inhibitory activity toward p38α MAP kinase in vitro.

Results and Discussion

Chemistry. The general synthesis of the target 4,6-diaryl-2-imino-1,2-dihydropyridine-3-carbonitrile **1**−**7**, **15**−**17** and the isosteric 2-oxopyridine derivatives **8**−**14**, **18**−**20** is illustrated in Schemes 1 and 2. Briefly, a one-pot synthetic approach was utilized, whereby the respective acetophenone, the respective aromatic aldehyde, ammonium acetate and malononitrile or ethyl cyanoacetate were refluxed in ethanol for 10−14 h. Previously reported methods to this class of compounds include sequential reaction of the aldehyde with the acetophenone with the aldehyde to give the corresponding chalcone, followed by reaction with ammonium acetate and ethyl cyanoacetate or malononitrile.[10,11] In our methodology we have all the advantages of one-pot reaction over stepwise ones in addition to operational simplicity.[12]

In most of cases, the successful formation of the end product is indicated by the formation of a fine solid throughout the refluxing process, which was collected by filtration followed by crystallization from *N*,*N*-dimethylformamide (DMF)/ethanol in high yields. A variety of aromatic heteroaryl, aryl aldehydes both with electron withdrawing and electron donating substituents were employed, and they yielded the desired products under

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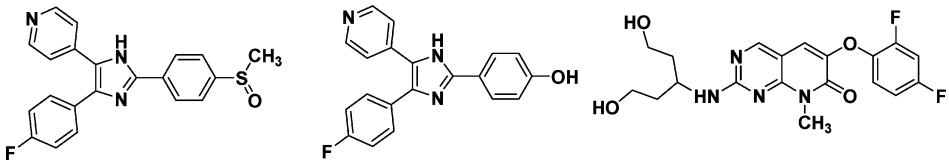
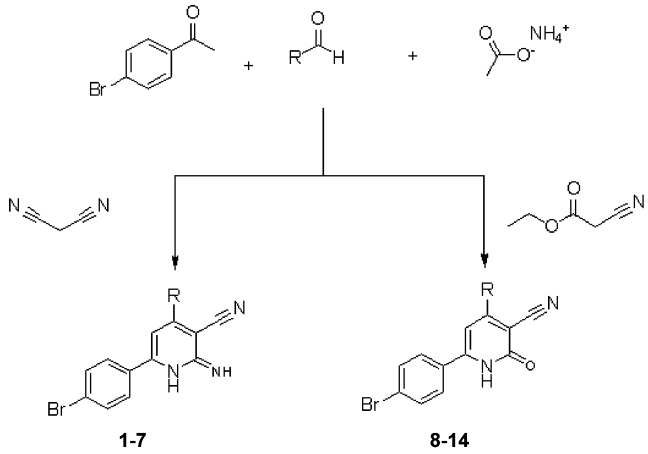


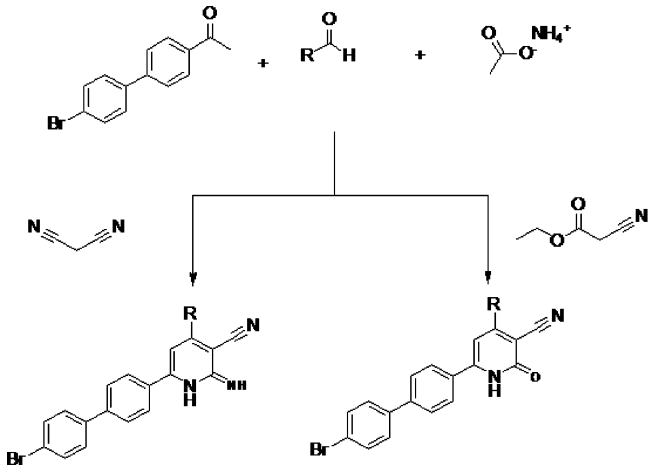
Figure 1. Structures of p38α MAP kinase inhibitors SB203580 (left), SB202190 (middle) and the 2-pyridone derivative Pamapimod (right) that are structurally relevant to our synthesized compounds.

Scheme 1



Compound	R
1,8	3,4,5-Trimethoxyphenyl
2,9	5-Methyl-2-furanyl
3,10	3-Pyridinyl
4,11	3,4-Dichlorophenyl
5,12	2,4-Dichlorophenyl
6,13	Indol-3-yl
7,14	4-Bromophenyl

Scheme 2



Compound	R
15,18	3,4,5-Trimethoxyphenyl
16,19	5-Methyl-2-furanyl
17,20	3,4-Dichlorophenyl

similar circumstances. This indicates the applicability of the procedure for the preparation of large libraries as well.

In ^{1}H NMR spectra the aromatic proton at position 5 of the dihydropyridine ring appeared as a singlet at a chemical shift ranging around 6.90 ppm. All compounds with 5-methyl-furan-2-yl substitution were indicated by a singlet at about 2.50 ppm indicating the deshielding effect of the neighboring oxygen atom. Meanwhile, compounds having trimethoxyphenyl substituent at position 4 of the 3-cyano-2-imino (oxo) pyridine ring showed two peaks at chemical shifts around 3.86 for 6 protons and at around 3.75 equivalent to 3 protons. Electron impact (EI) mass spectrometry of all products derived from 4-bromoacetophenone showed molecular ion peaks at M^+ and M^++2 as the element bromine is composed of two isotopes with different masses. In addition, the molecular ion peaks were also the base peaks signifying their stable characters. The infrared spectra (IR) of the 4,6-diaryl-2 imino-1,2-dihydropyridine-3-carbonitrile derivatives showed bands at a frequency around 3300 cm^{-1} corresponding to the NH and a band at around 2200 cm^{-1} corresponding to the CN function. On the other hand, the pyridone derivatives showed a band at around 1700 cm^{-1} for the lactam carbonyl. The correct elemental analysis of all compounds and the GC/MS of particular compounds, for example, compound 2 showed purity more than 95% (Figure 2).

Biology. All the final compounds were tested for their in vitro ability to inhibit p38α MAP Kinase inhibitory properties. First, the percentage inhibition at a screening dose of 10 μM was performed in triplicate, then the IC_{50} was determined for the compounds displaying a percentage of inhibition >60% by testing a range of 4 concentrations with 3 replicates per concentration. Results are summarized in Table 1. The nine active compounds as p38α MAP kinase inhibitors were of 4-bromophenyl substituent at position 6 and 2-oxo or 2-imino 3-cyano substituent on the pyridine ring. Derivatives with a 4-bromobiphenyl substituent at position 6 of the pyridine **15**−**20** were inactive; thus a bulky substituent at this position is deleterious for activity.

Compounds **1**, **2**, **3**, **6**, and **7** bear an imino group at position 2 of the pyridine ring, while compounds **8**, **11**, and **14** were of oxo substituent at the same position; compounds **8** and **11** with 2-oxo substituent were more active than their 2-imino congeners **1** and **4**, meanwhile, the 2-oxo analogue **14** was less active relative to its imino analogue **7**; this indicates that there is no preference for a carbonyl or an imino at position 2 of the pyridine ring for the MAP kinase inhibition. It should be borne in mind that the NH and O are isosteric functions.

Position 4 of the pyridine has been substituted by 3,4,5-trimethoxyphenyl,5-methyl-2-furanyl,3-pyridinyl,3,4-dichlorophenyl, 2,4-dichlorophenyl, 3-indolyl, and 4-bromophenyl moieties; almost all derivatives except those with 2,4-dichlorophenyl substitution were active. This finding indicates that a wide variety of aryls and with electron

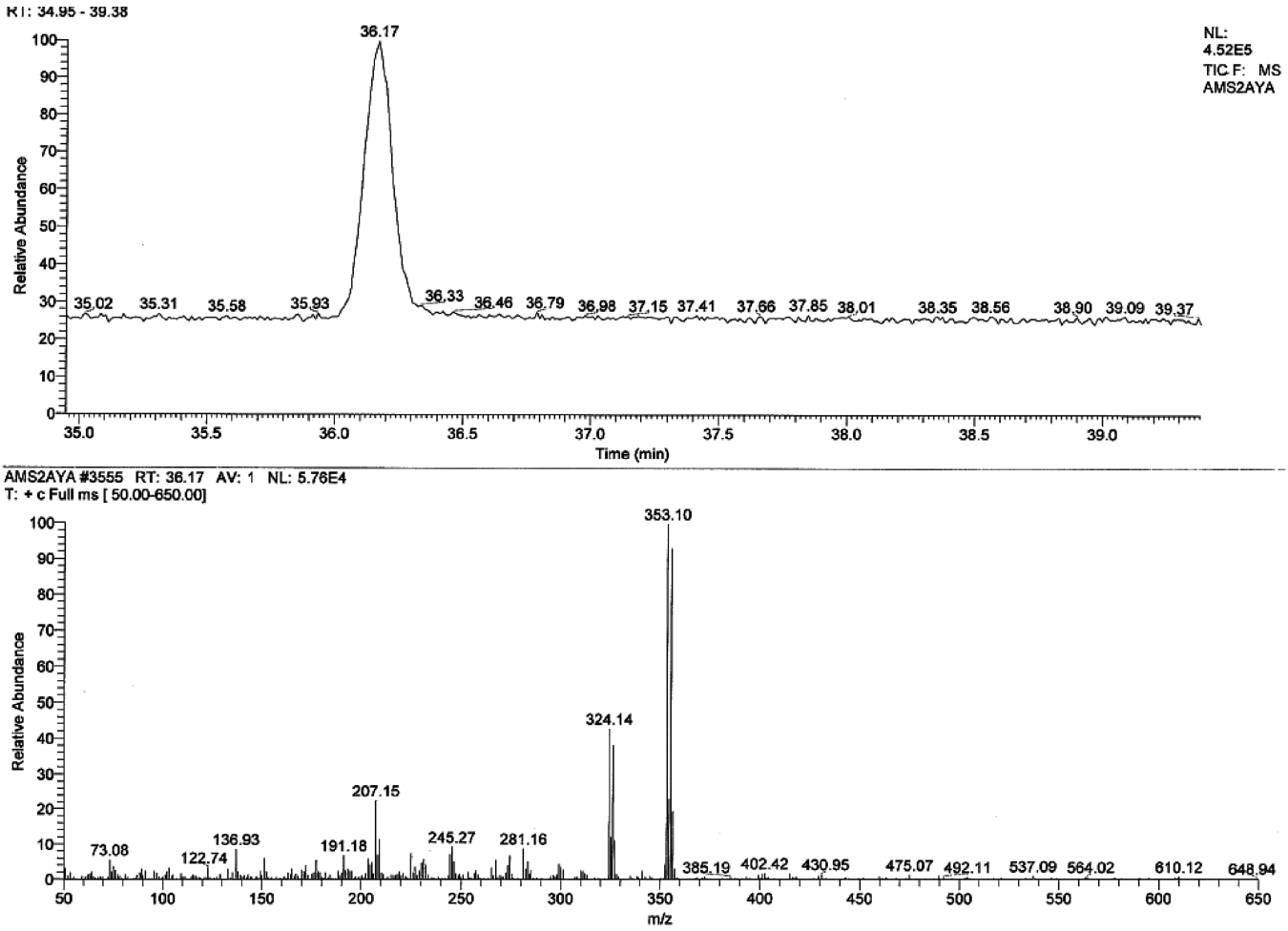


Figure 2. GC/MS of compound **2** showing purity of >95%, base peak as the molecular ion peak, and isotopic peak due to the bromine content of the molecule.

Table 1. Inhibitory Effects of the Synthesized Compounds on p38α MAP Kinase Enzyme

cpd. no.	% inhibition (10 μM)	IC_{50} (μM)	cpd. no.	% inhibition (10 μM)	IC_{50} (μM)
1	93	2	**11**	91	0.12
2	87	0.07	**12**	49	ND
3	86	3.7	**13**	17	ND
4	64	3.68	**14**	70	4.4
5	39	ND	**15**	18	ND
6	87	2.65	**16**	17	ND
7	83.5	0.76	**17**	35	ND
8	79	1.22	**18**	44	ND
9	58	ND	**19**	29	ND
10	8	ND	**20**	27	ND
SB203580	90	0.035			

withdrawing and electron donating substituent can be accommodated at position 4 and still retaining activity. The most active compound **2** is of the smallest substituent at position 4, and its energy minimized form showed planarity of its 2-methylfuranoyl substituent with the dihydropyridine ring; thus, steric and conformational aspects of the substituent at position 4 may be issues as well, Figure 3.

A docking experiment of compound **2** to p38α MAP kinase enzyme showed the nitrogen of the 2-imino substituent forms a crucial hydrogen bond with the NH of the backbone residue Met109; meanwhile its hydrogen forms hydrogen bonding with the carbonyl oxygen of the backbone residue His107. The cyano function nitrogen forms a hydrogen bond with the NH of the backbone Gly110. In addition, the 4-bromophenyl substituent at position 6 fills in a hydrophobic pocket circumferenced by hydrophobic amino acids, namely, Leu104, Ala51, Leu75, and Val38, Figures 4 and 5. Comparing our proposed interaction with the known inhibitor SB203580 (PDB 1A9U) it seems that the 2-imino-3-cyano part interacts with MAP kinase in a way similar to the pyridine of SB203580; the bromophenyl occupies the lipophilic pocket occupied by the corresponding fluorophenyl. The methylfuran is directed toward a pocket surrounded by Gly31, Ser32, and Ala34 with no specific interaction.

It is worth to mention that our compounds are devoid of the pyridine and the imidazole rings of known p38α MAP kinase inhibitors; thus, they are expected to be of lower iron chelating properties, interaction with CYP enzymes, and hence lower hepatotoxicity.[13]

3. Experimental Section

3.1. Chemistry. All reactions were performed with commercially available reagents, and they were used without further purification. Solvents were dried by standard methods and stored over molecular sieves. All reactions were monitored by thin-layer chromatography (TLC) carried out on precoated silica gel plates (ALUGRAM SILG/UV254), and detection of the components was made by short and long UV light. Melting points were determined in open capillaries

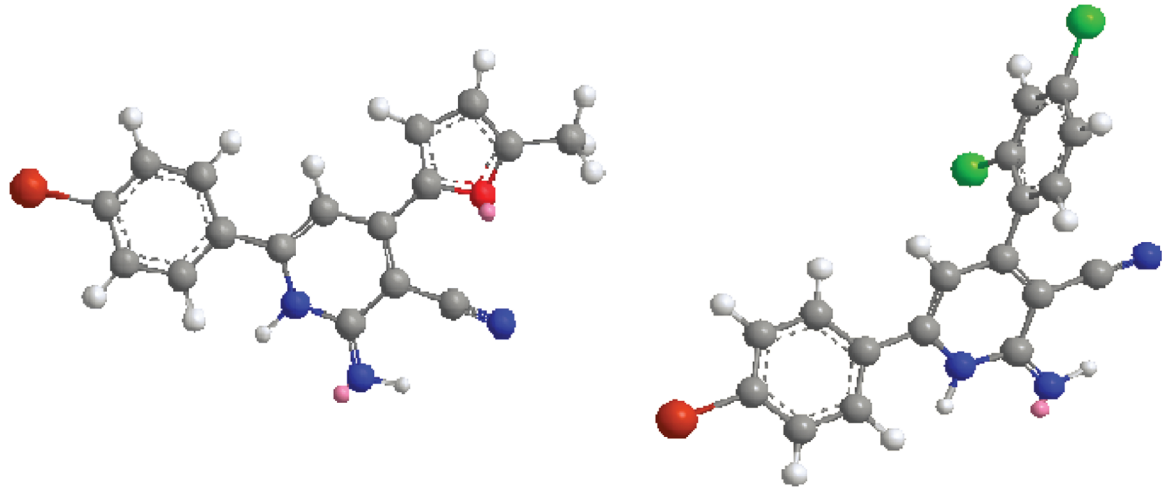


Figure 3. Energy minimized form of compound **2** (left) and compound **5** (right), showing coplanarity and non-coplanarity of the substituent at position 4 relative to the 2-iminopyridine ring, respectively.

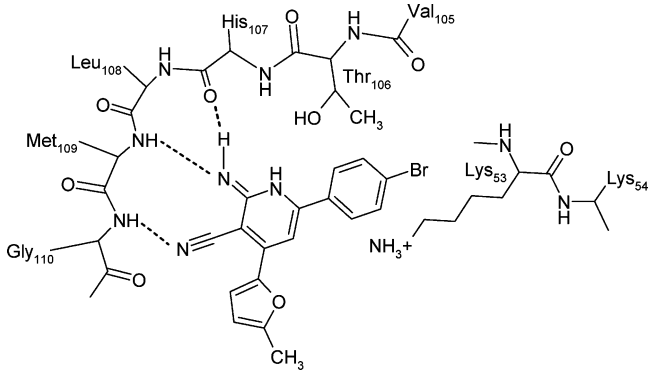


Figure 4. Schematic presentation of the interaction between the inhibitor 2 and human p38α MAP kinase enzyme. The bromophenyl is located in the hydrophobic pocket which is between Thr106 and Lys53; possible hydrogen bonding is shown in dashed lines.

using a Buchi Melting Point B-540 apparatus and are uncorrected. FTIR spectra were recorded on a Nicolet Avatar 380 spectrometer, ^{1}H NMR spectra were recorded on Varian Mercury VX-300 MHz spectrometer using DMSO-d_6 as a solvent; chemical shifts (δ) were reported in parts per million (ppm) downfield from TMS; multiplicities are abbreviated as follows: s, singlet; d, doublet; q, quartet; m, multiplet; dd, doublet of doublets; brs, broad. Mass spectra were made on Hewlett-Packard GC-MS, model 5890, series II at an ionization potential of 70 eV. Elemental analyses were performed by the Microanalytical Unit, Faculty of Science, Cairo University; the values found were within ±0.4% of the theoretical ones, unless otherwise indicated.

3.2. General Procedure for the Preparation of 4-Aryl-6-(4-bromophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile (1−7). 4-Bromoacetophenone (0.5 g, 2.5 mmol) together with the respective aromatic aldehyde (2.5 mmol), malononitrile (0.16 g, 2.5 mmol) and ammonium acetate (1.50 g, 20 mmol) were refluxed for 10−14 h in ethyl alcohol (30 mL). The precipitate formed was filtered, washed with ethyl alcohol and recrystallized from DMF-ethyl alcohol (1:10).

3.2.1. 6-(4-Bromophenyl)-2-imino-4-(3,4,5-trimethoxyphenyl)-1,2-dihydropyridine-3-carbonitrile (1). Yield 83%; mp 300−302 °C; IR (cm^{-1}): 3194, 2206; MS (EI): *m*/*z* 441 (M$^+$+2), 439 (M$^+$; 100%); ^{1}H NMR (DMSO-d_6): 7.73−7.20 (m, 4H, aromatic), 7.07 (s, 2H, C2 and C6 trimethoxyphenyl), 6.90 (s, 1H, C5 cyanopyridine), 3.83 (s, 6H, 2 × -OCH_3), 3.76 (s, 3H, -OCH_3); Anal. Calcd. for $C_{21}H_{18}BrN_3O_3$: C, 57.29; H, 4.12; N, 9.54. Found: C, 57.31; H, 4.13; N 9.54.

3.2.2. 6-(4-Bromophenyl)-2-imino-4-(5-methylfuran-2-yl)-1,2-dihydropyridine-3-carbonitrile (2). Yield 75%; mp 337−339 °C; IR (cm^{-1}): 3355, 2217; MS (EI): *m*/*z* 355 (M$^+$+2), 353 (M$^+$; 100%); ^{1}H NMR (DMSO-d_6): 8.20−7.71 (m, 4H, aromatic), 7.43−7.42 (d, 1H, C4 furan), 7.02 (s, 1H, C5 cyanopyridine), 6.36−6.35 (d, 1H, C3 furan), 2.42 (s, 3H, CH_3); Anal. Calcd. for $C_{17}H_{12}BrN_3O$: C, 57.65; H, 3.41; N, 11.86. Found: C, 57.68; H, 3.55; N, 11.53.

3.2.3. 6-(4-Bromophenyl)-2-imino-4-(pyridin-3-yl)-1,2-dihydropyridine-3-carbonitrile (3). Yield 85%; mp 309−310 °C; IR (cm^{-1}): 3359, 2210; MS (EI): *m*/*z* 352 (M$^+$+2), 350 (M$^+$; 100%); ^{1}H NMR (DMSO-d_6): 8.83 (s, 1H, C2 pyridine), 8.82−7.54 (m, 7H, aromatic), 6.90 (s, 1H, C5 cyanopyridine); Anal. Calcd. for $C_{17}H_{11}BrN_4$: C, 58.14; H, 3.16; N, 15.95. Found: C, 57.93; H, 3.61; N, 15.66.

3.2.4. 6-(4-Bromophenyl)-4-(3,4-dichlorophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile (4). Yield 75%; mp 369−370 °C; IR (cm^{-1}): 3365, 2216; MS (EI): *m*/*z* 419 (M$^+$+2), 417 (M$^+$; 100%); ^{1}H NMR (DMSO-d_6): 8.11−7.59 (m, 6H, aromatic), 7.36 (s, 1H, C2 dichlorophenyl), 6.87 (s, 1H, C5 cyanopyridine); Anal. Calcd. for $C_{18}H_{10}BrCl_2N_3$: C, 51.58; H, 2.40; N, 10.03. Found: C, 51.16; H, 2.43; N, 10.14.

3.2.5. 6-(4-Bromophenyl)-4-(2,4-dichlorophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile (5). Yield 70%; mp 235−237 °C; IR (cm^{-1}): 3281, 2160; MS (EI): *m*/*z* 419(M$^+$+2), 417 (M$^+$; 100%); ^{1}H NMR (DMSO-d_6): 8.22−7.62 (m, 8H, aromatic), 7.59 (s, 1H, C3 dichlorophenyl), 6.92 (s, 1H, C5 cyanopyridine); Anal. Calcd. for $C_{18}H_{10}BrCl_2N_3$: C, 51.58; H, 2.40; N, 10.03. Found: C, 51.70; H, 2.67; N, 10.41.

3.2.6. 6-(4-Bromophenyl)-2-imino-4-(1*H*-indol-3-yl)-1,2-dihydropyridine-3-carbonitrile (6). Yield 70%; mp 228−230 °C; IR (cm^{-1}): 3310, 2212; MS (EI): *m*/*z* 390 (M$^+$+2), 388 (M$^+$; 100%); ^{1}H NMR (DMSO-d_6): 8.56 (s, 1H, C2 indole), 8.55−7.20 (m, 9H, aromatic), 6.95 (s, 1H, C5 cyanopyridine); Anal. Calcd. for $C_{20}H_{13}BrN_4$: C, 61.71; H, 3.37; N, 14.39. Found: C, 62.02; H, 3.77; N, 14.09.

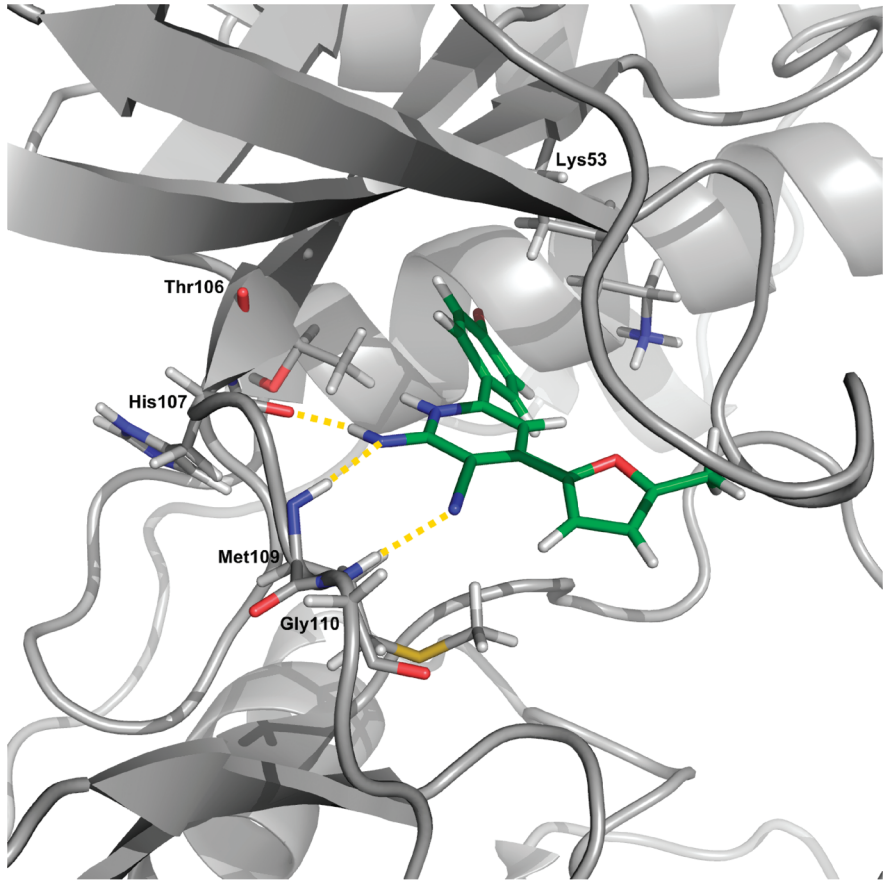


Figure 5. 3D interaction of compound **2** with human p38α MAP kinase; the NH interacts by hydrogen bonding with the backbone residues His107 and Met109, meanwhile CN interacts by hydrogen bonding with Gly110 residue.

3.2.7. 4,6-Bis-(4-bromophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile (7). Yield 80%; mp 284−285 °C; IR (cm^{-1}): 3355, 2217; MS (EI): *m/z* 429 (M^++2), 427 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 7.77−7.56 (m, 8H, aromatic), 6.79 (s, 1H, C5 cyanopyridine); Anal. Calcd. for $C_{18}H_{11}Br_2N$: C, 50.38; H, 2.58; N, 9.79. Found: C, 50.29; H, 2.19; N, 10.13.

3.3. General Procedure for the Preparation of 4-Aryl-6-(4-bromophenyl)-2-oxo-1,2-dihydropyridine-3 Carbonitrile Derivatives (8−14). 4-Bomoacetophenone (0.5 g, 2.5 mmol) together with the aromatic aldehyde (2.5 mmol), ethyl cyanoacetate (0.28 g, 2.5 mmol) and ammonium acetate (1.50 g, 20 mmol) were refluxed for 10−14 h in ethyl alcohol (30 mL). The precipitate formed was filtered, washed with ethyl alcohol, dried and recrystallized fom DMF-ethyl alcohol (1:10).

3.3.1. 6-(4-Bromophenyl)-2-oxo-4-(3,4,5-trimethoxyphenyl)-1,2-dihydropyridine-3-carbonitrile (8). Yield 80%; mp 320−322 °C; IR (cm^{-1}): 3265, 2248, 1641; MS (EI): *m/z* 442 (M^++2), 440 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 12.78 (s, 1H, NH), 7.87−7.73 (m, 4H, aromatic),7.06 (s, 2H, C2 and C6 trimethoxyphenyl), 6.94 (s, 1H, C5 pyridone), 3.86 (s, 6H, 2 × -OCH_3), 3.75 (s, 3H, -OCH_3); Anal. Calcd. for $C_{21}H_{17}BrN_2O_4$: C, 57.16; H, 3.88; N, 6.35. Found: C, 57.44; H, 3.55; N, 6.00.

3.3.2. 6-(4-Bromophenyl)-4-(5-methylfuran-2-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (9). Yield 80%; mp 335−337 °C; IR (cm^{-1}): 3378, 2221, 1657; MS (EI): *m/z* 356 (M^++2), 354 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 7.82−7.76 (m, 4H, aromatic), 7.73−7.61 (d, 1H, C4 furan), 7.00 (s, 1H, C5 pyridone), 6.50−6.49 (d, 1H, C3 furan), 3.34 (s, 3H, CH_3); Anal. Calcd. for $C_{17}H_{11}BrN_2O_2$: C, 57.49; H, 3.12; N, 7.89. Found: C, 57.77; H, 3.32; N, 7.56.

3.3.3. 6-(4-Bromophenyl)-2-oxo-4-(pyridin-3-yl)-1,2-dihydropyridine-3-carbonitrile (10). Yield 70%; mp 330−332 °C; IR (cm^{-1}): 3200, 2222, 1666; MS (EI): *m/z* 353 (M^++2), 351 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 12.94 (s, 1H, NH), 8.92 (s, 1H, C2 pyridine), 8.90−7.59 (m, 7H, aromatic), 7.03 (s, 1H, C5 pyridone); Anal. Calcd. for $C_{17}H_{10}BrN_3O$: C, 57.98; H, 2.86; N, 11.93. Found: C, 57.66; H, 3.12; N, 11.64.

3.3.4. 6-(4-Bromophenyl)-4-(3,4-dichlorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (11). Yield 70%; mp 289−290 °C; IR (cm^{-1}): 3310, 2211, 1692; MS (EI): *m/z* 420 (M^++2), 418 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 8.04−7.55 (m, 6H, aromatic), 7.41 (s, 1H, C2 dichlorophenyl), 7.02 (s, 1H, C5 pyridone); Anal. Calcd. for $C_{18}H_9BrCl_2N_2O$: C, 51.46; H, 2.16; N, 6.67. Found: C, 51.75; H, 2.46; N, 6.50.

3.3.5. 6-(4-Bromophenyl)-4-(2,4-dichlorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (12). Yield 75%; mp 368−370 °C; IR (cm^{-1}): 3276, 2223, 1640; MS (EI): *m/z* 420 (M^++2), 418 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 12.90 (s, 1H, NH), 7.87−7.58 (m, 6H, aromatic), 7.55 (s, 1H, C3 dichlorophenyl), 6.90 (s, 1H, C5 pyridone); Anal. Calcd. for $C_{18}H_9BrCl_2N_2O$: C, 51.46; H, 2.16; N, 6.67. Found: C, 51.67; H, 2.22; N, 6.36.

3.3.6. 6-(4-Bromophenyl)-4-(1*H*-indol-3-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (13). Yield 85%; mp 260−

261 °C; IR (cm^{-1}): 3268, 2218, 1670; MS (EI): *m/z* 391(M^++2), 389 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 12.71 (s, 1H, NH), 8.68 (s, 1H, C2 indole), 8.51−7.22 (m, 8H, aromatic), 7.02 (s, 1H, C5 pyridone); Anal. Calcd. for $C_{20}H_{12}BrN_3O$: C, 61.56; H, 3.10; N, 10.77. Found: C, 61.13; H, 3.12; N, 10.67.

3.3.7. 4,6-Bis(4-Bromophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (14). Yield 90%; mp 320−322 °C; IR (cm^{-1}): 3277, 2219, 1639; MS (EI): *m/z* 430 (M^++2), 428 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 12.90 (s, 1H, NH), 7.87−7.65 (m, 8H, aromatic), 6.90 (s, 1H, pyridone); Anal. Calcd. for $C_{18}H_{10}Br_2N_2O$: C, 50.27; H, 2.34; N, 6.51. Found: C, 50.51; H, 2.60; N, 6.30.

3.4. General Procedure for the Preparation of 4-Aryl-6-(4′-bromo-biphenyl-4-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile Derivatives (15−17). 4′-(4-Bromophenyl)acetophenone (0.7 g, 2.5 mmol) together with the respective aromatic aldehyde (2.5 mmol), malononitrile (0.160 g, 2.5 mmol), and ammonium acetate (1.5 g, 20 mmol) were refluxed for 10−14 h in ethyl alcohol (30 mL). The precipitate formed was filtered, washed with ethyl alcohol, and recrystallized fom DMF-ethyl alcohol (1:10).

3.4.1. 6-(4′-Bromobiphenyl-4-yl)-2-imino-4-(3,4,5-trimethoxyphenyl)-1,2-dihydropyridine-3-carbonitrile (15). Yield 90%; mp 307−309 °C; IR (cm^{-1}): 3200, 2207; MS (EI): *m/z* 517 (M^++2), 515 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 7.84−7.23 (m, 8H, aromatic), 7.10 (s, 2H, C2 and C6 trimethoxyphenyl), 7.06 (s, 1H, C5 cyanopyridine), 3.84 (s, 6H, 2 × -OCH_3), 3.75 (s, 3H, -OCH_3); Anal. Calcd. for $C_{27}H_{22}BrN_3O_3$: C, 62.80; H, 4.29; N, 8.14. Found: C, 63.09; H, 4.42; N, 8.55.

3.4.2. 6-(4′-Bromobiphenyl-4-yl)-2-imino-4-(5-methylfuran-2-yl)-1,2-dihydropyridine-3-carbonitrile (16). Yield 80%; mp 300−301 °C; IR (cm^{-1}): 3311, 2208; MS (EI): *m/z* 431 (M^++2), 429 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 8.22−7.24 (m, 8H, aromatic), 7.17−7.04 (d, 1H, C4 furan), 6.78 (s, 1H, C5 cyanopyridine), 6.39−6.38 (d, 1H, C3 furan), 2.50 (s, 3H, CH_3); Anal. Calcd. for $C_{23}H_{16}BrN_3O$: C, 64.20; H, 3.75; N, 9.77. Found: C, 64.31; H, 3.49; N, 10.14.

3.4.3. 6-(4′-Bromobiphenyl-4-yl)-4-(3,4-dichlorophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile (17). Yield 90%; mp 298−300 °C; IR (cm^{-1}): 3359, 2210; MS (EI): *m/z* 495 (M^++2), 493 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 8.04−7.64 (m, 10H, aromatic), 7.04 (s, 1H, C2 dichlorophenyl), 6.90 (s, 1H, C5 cyanopyridine); Anal. Calcd. for $C_{24}H_{14}BrCl_2N_3$: C, 58.21; H, 2.85; N, 8.49. Found: C, 58.49; H, 3.09; N, 8.19.

3.5. General Procedure for the Preparation of 4-Aryl-6-(4′-bromobiphenyl-4-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile Derivatives (18−20). 4′-(4-Bromophenyl)acetophenone (0.7 g, 2.5 mmol) together with the aromatic aldehyde (2.5 mmol), ethyl cyanoacetate (0.28 g, 2.5 mmol), and ammonium acetate (1.50 g, 20 mmol) were refluxed for 10−14 h in ethyl alcohol (30 mL). The precipitate formed was filtered, washed with ethyl alcohol, and recrystallized fom DMF-ethyl alcohol (1:10).

3.5.1. 6-(4′-Bromobiphenyl-4-yl)-2-oxo-4-(3,4,5-trimethoxyphenyl)-1,2-dihydropyridine-3-carbonitrile (18). Yield 85%; mp 320−322 °C; IR (cm^{-1}): 3350, 2217, 1640; MS (EI): *m/z* 518 (M^++2), 516 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 8.04−7.40 (m, 8H, aromatic), 7.15 (s, 2H, C2 and C6 trimethoxyphenyl), 6.90 (s, 1H, C5 pyridone), 3.87 (s, 6H, 2 × -OCH_3), 3.75 (s, 3H, -OCH_3); Anal. Calcd. for $C_{27}H_{21}BrN_2O_4$: C, 62.68; H, 4.09; N, 5.41. Found: C, 62.98; H, 4.08; N, 5.21.

3.5.2. 6-(4′-Bromobiphenyl-4-yl)-4-(5-methylfuran-2-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (19). Yield 80%; mp 320−322 °C; IR (cm^{-1}): 3332, 2208, 1750; MS (EI): *m/z* 432 (M^++2), 430 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 8.05−7.56 (m, 8H, aromatic), 7.47−7.36 (d, 1H, C4 furan), 7.03 (s, 1H, C5 pyridone), 6.43−6.41 (d, 1H C3 furan), 2.61 (s, 3H, CH_3); Anal. Calcd. for $C_{23}H_{15}BrN_2O_2$: C, 64.05; H, 3.51; N, 6.50. Found: C, 63.80; H, 3.69; N, 6.0.

3.5.3. 6-(4′-Bromobiphenyl-4-yl)-4-(3,4-dichlorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (20). Yield 85%; mp 300−304 °C; IR (cm^{-1}): 3220, 2202, 1722; MS (EI): *m/z* 496 (M^++2), 494 (M^+; 100%); ^{1}H NMR (DMSO-d_6): 8.12−7.42 (m, 11H, aromatic), 6.87 (s, 1H, C5 pyridone); Anal. Calcd. for $C_{24}H_{13}BrCl_2N_2O$: C, 58.09; H, 2.64; N, 5.65. Found: C, 58.01; H, 2.68; N, 5.39.

3.6. Biology. 3.6.1. p38 MAP Kinase Assay.[14] Microtiter plates were coated with 50 μL/well of the p38α MAPK substrate ATF-2 (10 μg/mL in TBS) for 1.5 h at 37 °C. After washing three times with bidistilled water, the remaining open binding sites were blocked with blocking buffer (BB; 0.05% Tween 20 (Bio-Rad), 0.25% BSA, 0.02% NaN_3 in TBS) for 30 min at room temperature. Plates were washed again, 50 μL of the respective test solution was filled into the wells, and the plates were incubated for 1 h at 37 °C. Test solutions containing 12 ng/well p38α MAPK were diluted in kinase buffer (50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 10 mM β-Glycerophosphate, 100 μg/mL BSA, 1 mM Dithiothreitol, 0.1 mM Na_3VO_4, 100 μM rATP) with or without test substance (10^{-4}−10^{-8} M).

Test substances were dissolved in DMSO to form stock solutions of 10^{-2} M; all further dilution steps were carried out in kinase buffer. After subsequent washing, plates were blocked again with BB for 15 min followed by a fourth washing step. Wells were filled with 50 μL of the primary AB; Phospho-ATF-2 (Thr69/71)-Antibody (1:500 in BB) and incubated for 1 h at 37°L of the secondary AB; Antirabbit IgG-AP-Antibody (alkaline phosphatase conjugated) (1:4000 in BB). Then 100 μL of 4-NPP (Nitrophenylphosphate) was pipetted in each well after a final washing step, and the color development was measured 1.5−2 h later with an enzyme-linked immunosorbent assay reader linked equipped with SOFTmax PRO software at 405 nm.[14]

3.6.2. Molecular Modeling. 3.6.2.1. Energy Minimization. The compounds were drawn on ChemSketch 11 software and saved as mol file; the latter were subjected to energy minimization using the AM1 procedure. The drawn compounds were first subjected to MM2 followed by the semiempirical AM1 in the MOPAC module of Chem3D Ultra 9.0; the methods were repeated consecutively until the rms gradient <0.01 and <0.1, respectively.

3.6.2.2. Docking Procedure. The X-ray structures of p38α MAP kinase enzyme (PDB ID code: 1WBW) for docking

were downloaded from the official pdb database and prepared with the Protein Preparation Wizard using default settings. This tool is available in Maestro, the GUI of the Schrödinger Software package. The co-crystallized ligand and the binding site with its residues were identified. The old ligand was removed and redocked to the protein to reveal the different types of interaction as a validation for the coming docking procedure. Compound **2** was prepared with the tool LigPrep from the Schrödinger software package, using the OPLS_2005 force field. The docking was done by using the Induced Fit Docking protocol with the XP scoring function. The functional form of the OPLS force field is very similar to the AMBER force field. The lowest energy conformation was selected as the best.[15]

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