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ARTICLE TYPE

Multicomponent one-pot synthesis of highly functionalized Pyrrole-3-carbonitriles in aqueous medium and computational study

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One-pot green protocol in aqueous medium involving four-components is developed to synthesize highly-functionalized pyrroles in good yields. Two of the newly synthesized compounds were subjected to *in silico* analysis on the Pharm Mapper web-server and the human mitogen-activated protein kinase1 (MEK1) enzyme was identified as the potential target protein for these compounds. For target validation, MEK-1 inhibition was performed for two representative compounds (5g and 5h) using Docking simulations. These functionalized pyrroles were also measured at their respective IC_{50} values on human cancer cell lines for evaluating the biocompatibility. Several of these functionalized pyrrole molecules were found to possess higher growth inhibition activity relative to standard doxorubicin and cisplatin.

Owing to increasing concern and detrimental effects of volatile organic solvents, there is a serious endeavor to use water as solvent in many organic reactions.[1] The benefits of solvent-free multicomponent reactions (MCRs) numerous. Hence, MCRs are utilized in varied chemical processes. Due to cost-effectiveness, environmentally benign solvent-free methods are used to modernize synthetic procedures to create cleaner, safer, and easier performing conditions.[2] These features make MCRs well-matched for rapid assembly of complex molecules from easily available precursors in a one-pot procedure.[3]

Pyrroles are found in a many bioactive natural products with important properties, both in pharmacology and remarkable applications in materials science.[4] Poly-substituted pyrroles in particular have more significance due to their remarkable applications in bioactive molecules.[5] Literature survey details number of reports for the synthesis of pyrroles,[6] Favi *et al*. have reported one-pot reaction involving three-components for the synthesis of some multisubstituted pyrroles *via* catalyst and solvent-free conditions.[7a] Palmieri *et al*. have reported the synthesis of multisubstituted pyrroles under solvent-free conditions using selective intermediates.[7b] Jiang et al. have reported one-pot silver-catalyzed three-component reaction for the synthesis of multisubstituted pyrroles in dioxane at 100°C with 53-88% yield (3 h).[7c] Yan *et al.* have developed a method for the synthesis of multisubstituted pyrroles in DMF as a solvent at 80°C with 41-87 % yield (4 h) catalyzed by CuI in the presence of O_2.[7d] Wang *et al* have also reported highly efficient chemoselective synthesis of polysubstituted pyrroles via isocyanide-based multicomponent domino reaction, but with acetonitrile as a solvent and under reflux conditions.[7e] Thus, an efficient method for the synthesis of functionalized pyrroles under eco-efficient and economical conditions is still an attractive option. We earlier have reported few one-pot MCRs for different multisubstituted heterocycles using water as a solvent in absence and presence of reusable catalysts.[8] The concept of green chemistry and our continued pursuit for greener synthetic routes for heterocycles enthralled us to investigate the scope of coupling the reactions in water. For the first time, we report a convenient four component MCR protocol for synthesis of pyrroles using water as a solvent under catalyst-free conditions.

In preliminary experiments, the four-component reaction of benzaldehyde (**1a-j**), malononitrile (**2**), 3,4-dichlorophenyl isocyanide (**3**) and morpholine (**4**) in EtOH and H_2O as a solvent at room temperature, displayed no reaction (Table 1, entries 1, 5). At increased temperatures (80°C), using either EtOH or MeOH as the solvents, reaction occurred, but with low yields (Table 1, entries 2, 3). Then CH_3CN and H_2O were tried as solvents at 80ºC, which improved the reaction efficiency (Table 1, entries 4, 6). Reaction proceeded smoothly in both solvents at that temperature and yields were 5% higher in water (yield 75%). From the summarized results (Table 1) it could be inferred that increasing the polarity of the solvent facilitated the selective conversions in high yields. Keeping the green chemistry in view, further work to assess the scope of the protocol for different aromatic aldehydes was investigated under chosen optimum conditions with water as solvent.

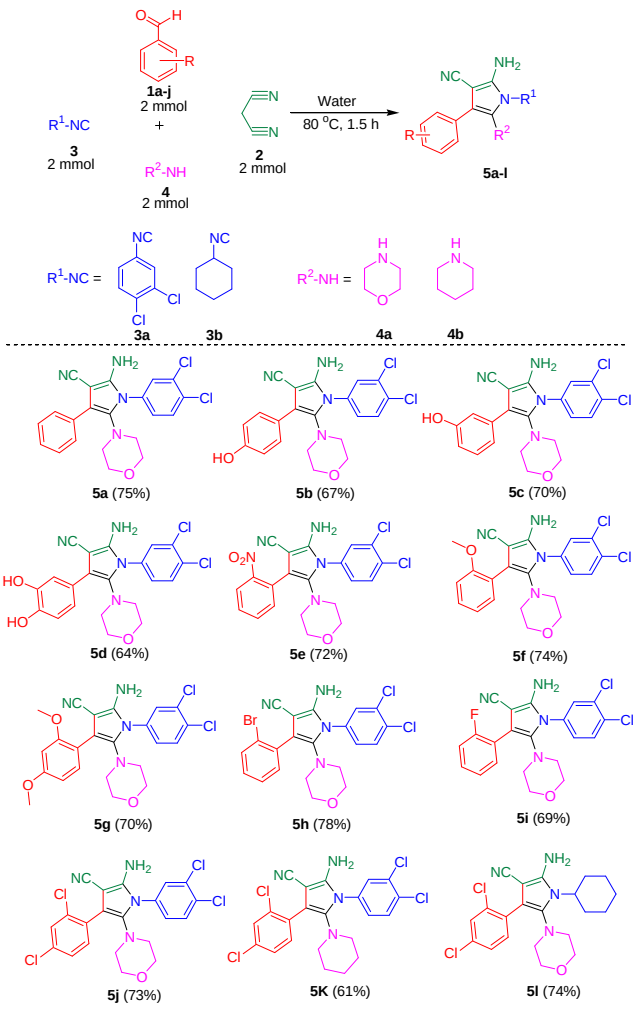
Table 1. Screening of reaction conditions for the MCR.

Entry	Product	Solvent	Temp (°C)	Time (h)	Yield [a](%)
1	**5a**	EtOH	rt	6	--[b]
2	**5a**	EtOH	80	4	45
3	**5a**	MeOH	80	4	40
4	**5a**	CH_3CN	80	2	70
5	**5a**	H_2O	rt	8	--[b]
6	**5a**	H_2O	80	1.5	75

[a] Isolated yields. [b] Product not found.

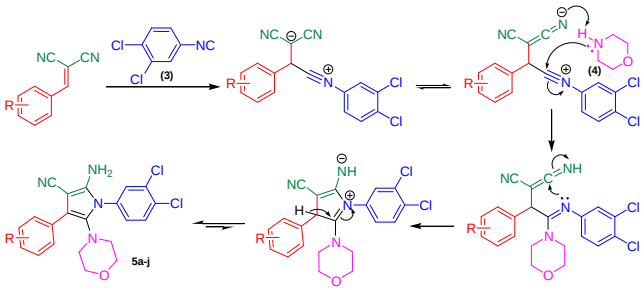
The scope of the MCR was further elaborated in this work.

Following the optimal conditions for the synthesis of **5a**, next we employed a variety of structurally different aldehydes (**1b-j**) in combination with malononitrile (**2**), 3,4-dichlorophenyl isocyanide (**3**) and morpholine (**4**) for the four component condensation reaction. The results are depicted in Scheme 1. The substrates of MCR bearing electron donating or electron-withdrawing groups on the aromatic ring proceeded smoothly and formed the corresponding multisubstituted pyrroles in good yields under the optimized conditions. All the reactions could be completed in less than 2 h.

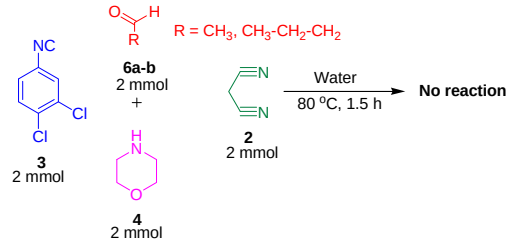


Scheme 1 Four-component reaction for the synthesis of Pyrroles (**5a-l**)

A plausible mechanism for the formation of pyrrole derivatives is proposed in Scheme 2. The intermediate arylidene-malononitrile from aromatic aldehyde and malononitrile could be generated *via* standard *Knoevenagael* condensation. This is followed by nucleophilic addition by 3,4-dichlorophenyl isocyanide. Morpholine undergoes nucleophilic attack of the resulting intermediate forming an intermediate/adduct, which undergoes cyclisation *via* intramolecular rearrangement yielding the pyrrole derivative as final product. The structures of all the pyrrole derivatives were identified and confirmed by their ^{1}H and ^{13}C NMR, FTIR, Mass spectra and elemental analysis. Furthermore, to validate the presence of the amino group in their structure, compounds were also characterized by ^{15}N NMR (GHSQC). However, when aliphatic aldehydes, such as acetaldehyde and n-butyraldehyde, were used, the corresponding polysubstituted pyrroles were not obtained (**Scheme 3**).



Scheme 2 Credible reaction mechanism for the construction of Pyrroles (**5a-l**).



Scheme 3 Substrate Limitations of the MCR.

Computational analysis

The paradigm of targeting only single target in drug discovery has been shifted to more global and comparative analysis of multiple targets network by significant advancements in genomics. The identification of potential receptors for a given molecule/drug in cell or tissue using the proteomic approach was not very successful approach due to its laborious and time-consuming nature. In-silico methods, on the other hand, are proving to be very successful for probing protein targets for a given molecule within as short period of time with lesser investment. The Pharm Mapper server is one of the web-servers which are being used for this kind of investigations. Accordingly, two representative compounds (**5g** and **5h**) synthesized in the present study were subjected to *in silico* screening (see method section) for their target protein profiling on the PharmMapper web server.[9] Out of 100 different human proteins identified, the Mitogen-activated protein kinase enzyme (MEK-1) was selected as the potential target protein for **5g** and **5h** based on their best Fit Scores, 4.40 and 4.52, respectively. Total 9 pharmacophore features including hydrophobic center, positively-charged center, negatively-charged center, hydrogen bond acceptor vector, hydrogen bond donor vector, aromatic plane and metal interaction center were identified for both compounds, and are depicted as spheres in Figure 1a. Cleary, the presence of pyrrole ring, aromatic rings and amino group were found to be important structural features (Figure 1a) for their interactions with the receptor.

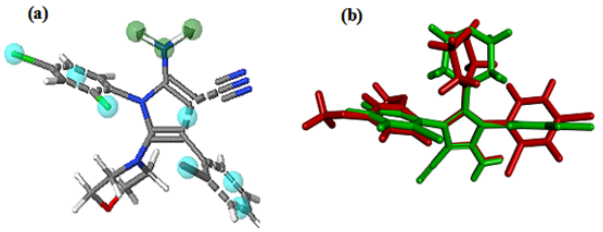


Figure 1: (a) Overlay of compound **5h** (in green) with the known inhibitor (in red) of protein, with RMSD (all atoms) <1.2 Å. Both compounds are depicted in sticks format. (b) Compound **5h** showing different pharmacophore features (in spheres) identified using the Pharm Mapper server.

Figure 1b shows the overlapping of **5h** with the known inhibitor of MEK-1, while the two-dimensional (2D) structure of the later is depicted in Figure 2. The root mean square deviation (RMSD) computed considering their all atoms was less than 1.2 Å, clearly suggesting a good three-dimensional (3D) structural correlation between both structures, along with the possibility of similar biological activity profiles. These enzymes (MEK-1 and MEK-2) play very important for the survival of normal cells, and are also expressed in various types of cancers, making them excellent targets for the drug discovery. Hence, *in silico* results obtained from the PharmMapper server indicate the potential of our compounds of being potential inhibitors of the MEK-1.

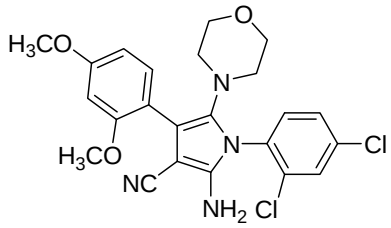


Figure 2: 2D structure of the known MEK-1 inhibitor identified using the Pharm Mapper.

In order to substantiate these observations and to validate the identified protein, both compounds (5g and 5h) were subsequently docked flexibly into the binding site of the protein using the Flexible algorithm module[10] of Discovery Studio program, installed on the centre for high performance computing (CHPC), South Africa. The computed CDocker interaction energy (CDE) of **5g** (-47.3) and **5h** (-41.2) suggested very good binding affinity of both compounds with the protein. The docked complexes of **5g** and **5h** with the protein were then visualized to get a deeper understanding of their modes of interactions responsible for their efficient host-guest relationship, and are diagrammatically represented in Figure 2a and 2b, respectively. A closer inspection of the Figure 2a revealed that compound 5g exhibits both hydrogen bonding and hydrophobic interactions with the protein. Specifically, two concurrent hydrogen bonds with Asn^{78} through morpholine oxygen, and single hydrogen bond with Arg^{234} *via* methoxy oxygen (-OCH_3) were observed in **5g**-protein complex. Moreover, both pyrroles and aryl ring showed hydrophobic interactions with the amino acid residues (Ala^{220} and Met^{219}) of the protein. Another donor-acceptor interaction between chloro substituent (Aryl) of **5g** and magnesium metal was also present.

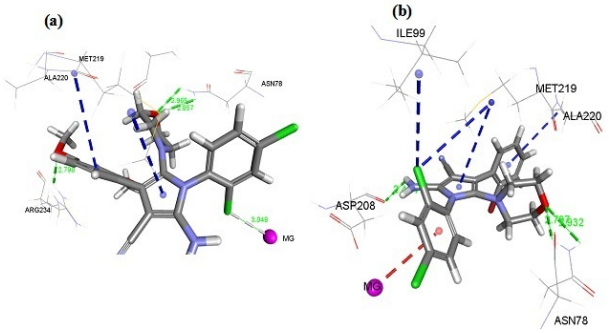


Figure 2: Complex of **5g** (a) and **5h** (b) with the MEK-1 protein (pdb code: 1S9J). Only interacting amino acid residues of protein are shown for clarity purpose. Compounds are depicted in sticks format, whereas the amino acids are shown in lines. Hydrogen bonds are shown as green dotted lines while the hydrophobic interactions are presented in blue dotted lines. Magnesium metal is shown in pink ball format.

Compound **5h** also interacted with the protein *via* three hydrogen bonds; two concurrent hydrogen bonds between its morpholine oxygen and Asn^{78}, and a single hydrogen bond between its hydrogen atom of amine (-NH_2) moiety and carbonyl oxygen of the Asp^{208}, along with hydrophobic interactions with Ile^{99}, Met^{219} and Ala^{220} amino acids of the protein. An electrostatic interaction (cation-π) between magnesium metal and dichlorophenyl ring of **5h** was also observed in the complex. Hence, the active participation of pyrrole ring, aromatic rings, chloro substituents of these compounds was found to be very important for locking their geometries in the active site of MEK-1 receptor, and were in agreement with their pharmacophore features identified using the Pharm Mapper web server.

***In vitro* cytotoxic activity**

Cell Culture: In this study we used MCF-7 (Human Breast cancer cell line), HT-29 (human Colon cancer cell line), and B16 (Murine melanoma cell line) which were purchased from National Centre for Cell Sciences (NCCS), Pune, India and were cultured aseptically using RPMI 1640&Dulbecco's modified eagles medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and penicillin (100 units mL^{-1})/streptomycin (100 mg mL^{-1}) at 37 °C, pH-7.2 and 5% CO_2. After attaining 80% confluence, the cells were trypsinized with 0.25 % Trypsin-EDTA and diluted with media to a fixed number of cells. Cisplatin (DDP) and Doxorubicin were used as reference drugs.

Cell viability assay for IC_{50}: The newly synthesized compounds (**5a-j**) were evaluated through *in vitro* cytotoxicity study for the estimation of IC_{50} value, i.e. cellular viability in the presence and absence of the test materials, which was determined by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide) assay as previously reported by Mosmann.[11] 100μl of medium containing cells at a density of $1x10^4$ cells/well were seeded into each well of 96 well plate and incubated overnight in a CO_2 incubator maintained 5% for 24 h at 37°C. Cell cultures were treated with varying concentrations (0.1-15 µM) of each compound made with 1:10 serial dilutions and left in contact for 24 h. After incubation, 50 µl of MTT reagent (5 mg of MTT/1 ml of PBS) was added and incubated for further 3 h. Eventually the media was pippeted out, violet colored formazen crystals were dissolved using 150 µl DMSO to each well. The reduction of MTT by mitochondrial dehydrogenase was measured at 560 nm using an ELISA reader. The percentage viable cells in each well were calculated from absorbance of purple color of formazan. All experiments were carried out in quadruples, in addition to maintaining a control (with solvent only) and reference standard drugs. The percentage of inhibition of each compound was calculated using the formula: % inhibition = (mean absorbance of treated cells/ Mean absorbance of control) X 100.

The cell viability values presented in Table 2 point out that the functionalized pyrrole-3-carbonitriles (**5a-j**) has revealed strong growth inhibition and fit for this activity against all the tested fanatic perilous cancer cell lines. Comparisons with the standard, some of the derivatives 5g and 5h have predominantly (in the same descending sequence) shown comparable or strong inhibition against all the three cell lines. In general most of these pyrroles acted efficiently on human cancer cell lines almost close to the concentration of standard and exhibited better inhibitory capability. As far as the structure-activity relationship is concerned, numerous correlations can be established from this data to support the nature of cytotoxic activity based on not only steric, but also electronic properties of aromatic ring substituents. This might be one of the promising reasons for the improvement in the activity of pyrroles, possibly due to drug-receptor interactions, i.e. ligational properties.

Furthermore, the fascinating piece of evidence at this point is, the aryl substituent on pyrrole afforded a chance to augment the cancer growth suppression. Dimethoxy substituted aryl derivative of pyrrole (**5g**) has shown excellent activity among all the derivatives tested against all the cell lines, in agreement with the docking predictions. It was found to be the stronger MEK-1 inhibitor based on the computed scoring function (CDE = -47.3). The electron rich nature of methoxy substituent and its engagement in hydrogen bonding with MEK-1 (Figure 2a) probably have stabilized their complexation, resulting in enhanced activity. Compound 5h showing comparative weaker MEK-1inhibition (CDE= -41.2), was the second most active anti-cancer agent especially against breast cancer cell line MCF-7 and B16. The compounds **5e,** with 2-NO_2, **5i**, with fluoro and **5j**, with dichloro substituent have shown relatively better activity in all cases relative to the standard drugs doxorubicin and cisplatin.

Table 2
*In vitro cytotoxic activity of pyrrole-3-carbonitrile derivatives (**5a-j**) on human cancer cell lines[a] (IC_{50} µM)[b].*

Entry	Compd	MCF-7 (Breast)	HT-29 (Colon)	B16 Cell line
1	**5a**	6.29±0.01	5.41±0.13	12.81±0.01
2	**5b**	5.76±0.01	4.49±0.07	10.48±0.09
3	**5c**	5.79±3.5	2.14±0.02	10.52±0.01
4	**5d**	4.55±0.02	2.47±0.013	10.72±0.03
5	**5e**	2.13±0.03	2.60±0.08	8.47±0.09
6	**5f**	3.86±0.03	3.35±0.06	7.54±0.15
7	**5g**	1.24±0.01	1.79±0.02	4.33±0.16
8	**5h**	1.35±0.01	1.47±0.04	4.61±0.01
9	**5i**	2.74±0.02	2.77±0.09	8.67±0.15
10	**5j**	2.74±0.02	1.85±0.05	9.04±0.04
11	Doxorubicin	1.58±0.03	--	--
12	Cisplatin	--	2.05±0.03	6.73±0.10

[a] Data represent as mean ± SEM values. Cytotoxicity as IC50 for each cell line is the concentration of compound which reduced by 50% the optical density of treated cell with respect to untreated cells using the MTT assay.
[b] Data represent as mean ± SEM values of these independent determinations.

Conclusions

In summary, we have described a practically simple and eco-friendly four-component coupling reaction in water, which is effective in synthesis of multi-functionalized pyrroles in catalyst-free conditions in shorter reaction times (< 2 h). This approach features products in good yields, with short reaction times and under relatively mild conditions. Easy accessibility of starting materials and catalyst-free reaction, as well as the use of environmentally benign water as solvent are the key advantages of the method. This methodology could prove to be a means for new synthetic fragments with unique properties. The synthesis of biologically relevant multi-functionalized pyrrole-3-carbonitriles directly relates to medicinal chemistry. The *in silico* screening and docking simulations revealed that reported compounds have competency to inhibit the MEK-1 enzyme, and thus have a great potential to act as anti-cancer agents under *in vivo* conditions.

The pyrrole-3-carbonitrile derivatives (**5a-j**) were synthesized in high yields and their application was promising as anti-cancer agents for a panel of human cancer cell lines. Specifically, some of these pyrrole-3-carbonitriles worked well for the growth inhibition in MCF-7 (Breast) and HT-29 (Colon) and found to be superior to standard cisplatin in terms of IC_{50} values.

The broad spectrum of anti-cancer activity displayed by these pyrrole-3-carbonitriles may be of interest for further derivatization and further *in vivo* and clinical studies in the hope of finding more active and selective anti-cancer agents.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedure and all the spectra of multisubstituted pyrrole derivatives **5a-j**. See DOI: 10.1039/b000000x/

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Apparatus and analysis

All chemicals used were reagent grade and were used as received without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded at 25°C at 400 MHz and 100 MHz (Bruker Avance) instrument respectively, using TMS as internal standard. Chemical shifts are given in parts per million (ppm). The FT-IR spectroscopy of samples was carried out on a Perkin Elmer Precisely 100 FT-IR spectrometer in the 400-4000 cm^{-1} region. ESI-MS spectra were determined on a LCQ ion trap mass spectrometer (Thermo Fisher, San Jose, CA, USA), equipped with an ESI source. Elemental analyses were carried out using a Perkin-Elmer CHNS Elemental Analyzer model 2400. Melting points were recorded on a hot stage melting point apparatus Ernst Leitz Wetzlar, Germany and were uncorrected. All the reactions and the purity of products were monitored using thin layer chromatography (TLC) on aluminum-backed plates coated with Merck Kieselgel 60 F254 silica gel, visualizing the spots under ultraviolet light and iodine chamber.

Experimental section:

General experimental procedure for the synthesis of Pyrrole-3-carbonitriles derivatives:

A mixture of freshly distilled benzaldehyde (2.0 mmol) in water (5 mL) at room temperature, Malononitrile (2.0 mol) was magnetically stirred for 15 min at room temperature followed by addition of morpholine (2.0 mmol) with 3,4-dichlorophenyl isocyanide (2.0 mmol). Then, the reaction mixture was heated at 80 °C for 1h. The reaction progress was monitored by Thin Layer Chromatography (TLC) (EtOAc/hexane = 3:7). After completion of the reaction, the mixture was cooled to room temperature and the precipitated product was filtered, washed with water and EtOH then dried under vacuum. In most cases no further purification was necessary.

2-Amino-1-(2,4-dichlorophenyl)-5-morpholin-4-yl-4-phenyl-1*H*-pyrrole-3-carbonitrile (5a)

Off-white solid: mp 192-193°C; ^{1}H NMR (400 MHz, DMSO) δ = 3.58 (8H, t, *J* = 5.0 Hz), 7.41-7.47 (7H, m), 7.82 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 44.04, 65.86, 108.05, 113.98, 117.06, 119.23, 120.35, 122.99, 125.59, 129.47, 130.13, 130.54, 132.94, 135.07, 137.68, 140.68, 154.56; IR (KBr, cm^{-1}): 2203 (CN), 3275 (NH_2); MS (ESI), *m*/*z* = 436 (M+Na, 100%); Anal. Calcd ($C_{21}H_{18}Cl_2N_4O$): C 61.03, H 4.39, N 13.56%. Found: C 61.09, H 4.47, N 13.62%.

2-Amino-1-(2,4-dichlorophenyl)-4-(4-hydroxyphenyl)-5-morpholin-4-yl-1*H*-pyrrole-3-carbonitrile (5b)

Off-white solid: mp 215-216°C; ^{1}H NMR (400 MHz, DMSO) δ = 3.58 (8H, t, *J* = 5.0 Hz), 7.35-7.62 (7H, m), 7.82 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 44.04, 65.86, 115.37, 116.07, 116.28, 119.23, 120.35, 122.99, 126.61, 127.37, 129.40, 129.95 130.13, 130.53, 131.17, 140.68, 154.56; IR (KBr, cm^{-1}): 2226 (CN), 3274 (NH_2); MS (ESI), *m*/*z* = 430 (M+1, 100%); Anal. Calcd ($C_{21}H_{18}Cl_2N_4O_2$): C 58.75, H 4.23, N 13.05%. Found: C 58.82, H 4.28, N 13.07%.

2-Amino-1-(2,4-dichlorophenyl)-4-(3-hydroxyphenyl)-5-morpholin-4-yl-1*H*-pyrrole-3-carbonitrile (5c)

Off-white solid: mp 204-205°C; ^{1}H NMR (400 MHz, DMSO) δ = 3.58 (8H, t, *J* = 5.0 Hz), 7.40-7.45 (5H, m), 7.81 (1H, s), 8.41 (1H, s), 8.79 (2H, s, NH_2), 10.13 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO): δ 44.04, 65.86, 108.10, 113.15, 114.23, 116.04, 119.22, 120.35, 121.76, 122.01, 122.90, 130.12, 130.54, 130.57, 132.35, 140.67, 154.56, 157.81, 161.58; IR (KBr, cm^{-1}): 2242 (CN), 3276 (NH_2); MS (ESI), *m*/*z* = 430 (M+1, 100%); Anal. Calcd ($C_{21}H_{18}Cl_2N_4O_2$): C 58.75, H 4.23, N 13.05%. Found: C 58.84, H 4.32, N 13.09%.

2-Amino-1-(2,4-dichlorophenyl)-4-(3,4-dihydroxyphenyl)-5-morpholin-4-yl-1*H*-pyrrole-3-carbonitrile (5d)

Off-white solid: mp 226-227°C; ^{1}H NMR (400 MHz, DMSO) δ = 3.58 (8H, t, *J* = 5.0 Hz), 7.41-7.47 (4H, m), 7.81 (1H, s), 7.82 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 44.04, 65.86, 107.58, 110.19, 113.50, 116.11, 119.23, 120.35, 122.99, 127.01, 128.91, 130.13, 130.53, 132.70, 136.25, 138.15, 140.67, 141.70, 154.56; IR (KBr, cm^{-1}): 2289 (CN), 3274 (NH_2); MS (ESI), *m*/*z* = 446 (M+1, 100%); Anal. Calcd ($C_{21}H_{18}Cl_2N_4O_3$): C 56.64, H 4.07, N 12.58%. Found: C 56.71, H 4.16, N 12.65%.

2-Amino-1-(2,4-dichlorophenyl)-5-morpholin-4-yl-4-(2-nitrophenyl)-1*H*-pyrrole-3-carbonitrile (5e)

Off-white solid: mp 180-181°C; ^{1}H NMR (400 MHz, DMSO) δ = 3.58 (8H, t, *J* = 5.0 Hz), 7.41-7.47 (6H, m), 7.81 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 44.04, 65.86, 111.75, 113.06, 116.32, 119.23, 120.35, 122.99, 125.39, 127.11, 130.13, 130.41, 130.53, 133.33, 135.02, 140.68, 146.75, 149.99, 154.56; IR (KBr, cm^{-1}): 2240 (CN), 3275 (NH_2); MS (ESI), *m*/*z* = 459 (M+1, 100%); Anal. Calcd ($C_{21}H_{17}Cl_2N_5O_3$): C 55.04, H 3.74, N 15.28%. Found: C 55.09, H 3.81, N 15.37%.

2-Amino-1-(2,4-dichlorophenyl)-4-(2-methoxyphenyl)-5-morpholin-4-yl-1*H*-pyrrole-3-carbonitrile (5f)

Off-white solid: mp 209-210°C; ^{1}H NMR (400 MHz, DMSO) δ = 3.42 (3H, s), 3.58 (8H, t, *J* = 5.0 Hz), 7.41-7.45 (6H, m), 7.82 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 44.03, 56.26, 65.86, 112.19, 113.29, 119.23, 120.37, 123.03, 124.37, 128.37, 128.39, 130.02, 130.08, 130.56, 131.37, 133.49, 133.93, 134.72, 135.93, 154.57; IR (KBr, cm^{-1}): 2227 (CN), 3275 (NH_2); MS (ESI), *m*/*z* = 444 (M+1, 100%); Anal. Calcd ($C_{22}H_{20}Cl_2N_4O_2$): C 59.60, H 4.55, N 12.64%. Found: C 59.67, H 4.59, N 12.72%.

2-Amino-1-(2,4-dichlorophenyl)-4-(2,4-dimethoxyphenyl)-5-morpholin-4-yl-1*H*-pyrrole-3-carbonitrile (5g)

Off-white solid: mp 231-232°C; ^{1}H NMR (400 MHz, DMSO) δ = 3.58 (8H, t, *J* = 5.0 Hz), 3.89 (3H, s), 3.90 (3H, s), 7.41-7.47 (4H, m), 7.81 (1H, s), 8.23 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 44.04, 56.08, 56.26, 65.86, 98.26, 107.67, 113.09, 114.16, 115.23,119.22, 120.35, 122.99, 130.12, 130.46, 130.53, 136.50, 140.67, 153.83, 154.56, 161.30, 166.77; IR (KBr, cm^{-1}): 2222 (CN), 3275 (NH_2); MS (ESI), *m*/*z* = 474 (M+1, 100%); Anal. Calcd ($C_{23}H_{22}Cl_2N_4O_3$): C 58.36, H 4.68, N 11.84%. Found: C 58.47, H 4.78, N 11.89%.

2-Amino-4-(2-bromophenyl)-1-(2,4-dichlorophenyl)-5-morpholin-4-yl-1*H*-pyrrole-3-carbonitrile (5h)

Off-white solid: mp 197-198 °C; ^{1}H NMR (400 MHz, DMSO) δ = 3.57 (8H, t, *J* = 5.0 Hz), 7.76-7.84 (6H, m), 8.55 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 44.03, 65.86, 112.19, 113.29, 119.23, 120.37, 123.03, 124.37, 128.37, 128.39, 130.02, 130.08, 130.56, 131.37, 133.49, 133.93, 134.72, 135.93, 154.57; IR (KBr, cm^{-1}): 2232 (CN), 3274 (NH_2); MS (ESI), *m*/*z* = 493 (M+1, 100%); Anal. Calcd ($C_{21}H_{17}BrCl_2N_4O$): C 51.24, H 3.48, N 11.38%. Found: C 51.35, H 3.58, N 11.44%.

2-Amino-1-(2,4-dichlorophenyl)-4-(2-fluorophenyl)-5-morpholin-4-yl-1*H*-pyrrole-3-carbonitrile (5i)

Off-white solid: mp 196-197°C; ^{1}H NMR (400 MHz, DMSO) δ = 3.58 (8H, t, *J* = 5.0 Hz), 7.41-7.47 (6H, m), 8.57 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 44.04, 65.86, 112.64, 113.69, 116.43, 116.64, 119.23, 119.47, 120.35, 122.99, 125.35, 125.38, 129.14, 130.13, 130.54, 136.49, 136.58, 140.67, 154.56; IR (KBr, cm^{-1}): 2229 (CN), 3273 (NH_2); MS (ESI), *m*/*z* = 432 (M+1, 100%); Anal. Calcd ($C_{21}H_{17}Cl_2FN_4O$): C 58.48, H 3.97, N 12.99%. Found: C 58.56, H 3.99, N 13.08%.

2-Amino-1,4-bis-(2,4-dichlorophenyl)-5-morpholin-4-yl-1*H*-pyrrole-3-carbonitrile (5j)

Off-white solid: mp 202-203°C; ^{1}H NMR (400 MHz, DMSO) δ = 3.58 (8H, t, *J* = 5.0 Hz), 7.42-7.47 (4H, m), 7.82 (1H, s), 7.82 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 44.04, 65.86, 108.05, 110.19, 112.08, 115.40, 119.22, 120.35, 122.99, 125.12, 126.77, 129.47, 130.12, 130.54, 132.22, 135.31, 137.20, 140.68, 154.56; IR (KBr, cm^{-1}): 2230 (CN), 3272 (NH_2); MS (ESI), *m*/*z* = 483 (M+1, 100%); Anal. Calcd ($C_{21}H_{16}Cl_4N_4O$): C 52.31, H 3.34, N 11.62%. Found: C 52.39, H 3.43, N 11.68%.

2-amino-1-(3,4-dichlorophenyl)-4-phenyl-5-(piperidin-1-yl)-1H-pyrrole-3-carbonitrile (5k)

Off-white solid: mp 189-190°C; ^{1}H NMR (400 MHz, DMSO) δ = 1.43-1.58 (6H, m), 2.60-2.83 (4H, m), 7.41-7.47 (7H, m), 7.82 (1H, s), 8.79 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 26.92, 28.34, 54.65, 108.05, 113.98, 117.06, 119.23, 120.35, 122.99, 125.59, 129.47, 130.13, 130.54, 132.94, 135.07, 137.68, 140.68, 154.56; IR (KBr, cm^{-1}): 2203 (CN), 3279 (NH_2); MS (ESI), *m*/*z* = 412 (M+1, 100%); Anal. Calcd ($C_{22}H_{20}Cl_2N_4$): C 64.24, H 4.90, N 13.62%. Found: C 64.32, H 4.97, N 13.65%.

2-amino-1-cyclohexyl-5-morpholino-4-phenyl-1H-pyrrole-3-carbonitrile (5l)

Off-white solid: mp 197-198°C; ^{1}H NMR (400 MHz, DMSO) δ = 1.47-1.52 (2H, m), 1.90-1.98 (4H, m), 2.46-2.95 (5H, m), 3.58 (8H, t, *J* = 5.0 Hz), 7.32-7.48 (5H, m), 8.78 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 23.72, 27.56, 34.79, 42.8, 44.04, 65.87, 108.76, 117.06, 119.23, 122.99, 125.59, 129.47, 130.54, 132.94, 137.68; IR (KBr, cm^{-1}): 2213 (CN), 3275 (NH_2); MS (ESI), *m*/*z* = 351 (M+1, 100%); Anal. Calcd ($C_{21}H_{26}N_4O$): C 71.97, H 7.48, N 15.99%. Found: C 71.99, H 7.57, N 16.04%.

Computational method: 3D structures of both representative compounds (5g and 5h) were submitted in the mol2 format on the PharmMapper web-server. The outputs were analyzed using the Discovery Studio visualizer. For docking, the crystal structure of MEK-1 (pdb code: 1S9J) was obtained from the protein data bank (http://www.rcsb.org). All native ligands and water molecules associated with the protein were removed. The protonation of protein was performed at physiological pH using the Prepare Protein algorithm in DS, followed by its minimization using the conjugate gradient algorithm with the CHARMm force field. Both representative compounds were geometrically optimized at DFT level using the combination of B3LYP functional and 6-31g [d, p] basis sets, in Gaussian 09.[12] Prior to docking, a binding sphere covering all the active site residues was generated using the Define and Edit Binding Site module embedded in DS. Docking of compounds was subsequently performed using the Flexible algorithm[10], and the best pose was selected based on the scoring function (-CDOCKER energy).