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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

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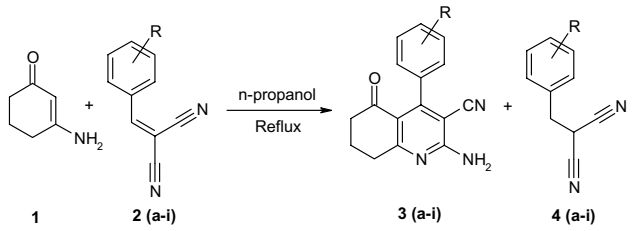
Abstract—A series of 2-amino-5-oxo-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile and various analogues have been synthesized in excellent isolated yields starting from various arylidenemalononitrile and 3-amino-2-cyclohexen-1-one in 1-propanol as solvent at reflux temperature in the absence of any added catalyst. All the synthesized compounds were evaluated for their antifungal activity. The relationship between functional group variation and biological activity of the evaluated compounds is discussed in the article.

1. Introduction

Many naturally occurring as well as synthetic compounds containing the pyridine scaffold exhibit interesting pharmacological properties.[1] As a consequence many efficient procedures have been reported in the literature for the synthesis of functionalized pyridines.[2] Pyridine is one of the most popular *N*-heteroaromatics incorporated into the structure of many pharmaceuticals. Among these, cyanopyridines with different alkyl and aryl groups were found to have antimicrobial,[3] antihypertensive,[4] cardiovascular,[5] anti-inflammatory, analgesic, antipyretic properties[6] as well as 1KK-β inhibitor properties.[7] The preparation of the 2-amino-3-cyanopyridine derivatives has been reported in the literature from chalcones on treatment with ammonium acetate via Michael-type condensation,[8] as well as via a one-pot coupling reaction of four components[9] acetophenone, benzaldehyde, malononitrile, and ammonium acetate in conventional heating mode or under microwave irradiation and by other methods.[10] The 2-amino-3-cyanopyridines are versatile intermediates for the synthesis of nitrogen heterocycles.[11]

As part of our ongoing development of efficient protocols for the preparation of biologically active heterocycles from common intermediates[12] and keeping in view the biological activity of the cyanopyridine derivatives along with the versatility of the organic synthon viz. cyclic enaminones and the cyano olefins, we herein report for the first time the synthesis of the novel 2-amino-5-oxo-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile



Scheme 1.

Keywords: Arylidenemalononitriles; Michael addition; Arylquinoline carbonitrile; Antifungal activity; Antiproliferative activity.
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and its analogues **3a**–**i** in excellent isolated yields. All the compounds **3a**–**i** were synthesized by Michael addition of various arylidenemalononitriles **2a**–**i** with 3-amino-2-cyclohexen-1-one **1** followed by aromatization in the absence of any added catalyst (Scheme 1). They were then evaluated for their antifungal activity.

2. Chemistry

The starting arylidenemalononitriles were synthesized by reacting the corresponding aromatic aldehyde and malononitrile in methanol in the presence of a catalytic amount of piperidine at room temperature, and the product arylidenemalononitriles **2a**–**i** formed were used as such for the reaction with the enaminone **1** in refluxing *n*-propanol for an appropriate time to afford the arylquinoline carbonitrile **3a**–**i** in excellent isolated yields. The results are recorded in Table 1. The products thus obtained **3a**–**i** were well characterized by their IR and ^{1}H NMR spectral analysis, additionally the elemental analyses were in conformity with the respective structures.

The IR spectra of **3a** showed sharp bands at 3422 and 3306 cm^{-1} (NH_2), 2215 cm^{-1} (CN), and 1685 cm^{-1} (C=O). The ^{1}H NMR spectrum showed triplet at δ 2.63, δ 2.13 and quintet at δ 1.72 for methylene protons of cyclohexanone ring. The by-product 2-(4-nitrobenzyl) malononitrile **4i** was isolated after the reaction of **1** with 2-(4-nitrobenzyledine) malononitrile **2i** and was completely characterized by spectral analysis. The formation of the reduced by-product **4i** indicates the dual role of arylidenemalononitrile as reactant as well as an oxidizing agent. The formation of hexahydroquinoline intermediate **5** was observed as a major product in the reaction mixture when equimolar amounts of **1** and **2i** were allowed to reflux in *n*-propanol. A reasonable mechanism for the formation of **3i** could be explained via initial Michael addition of **1** to **2i** followed by cyclization to hexahydroquinoline intermediate **5** which subsequently underwent oxidation in the presence of two molar excess of the cynoolefine **2i** to the fully aromatized product **3i**. The formation of nearly equimolar proportion of compound **4i** as compared to **3i** confirms the participation of **2i** in the hydrogen transfer process (Fig. 1). Additional evidence for the formation of fully aromatized product **3** was obtained by the X-ray crystallographic studies on **3f**. The ORTEP diagram of **3f**

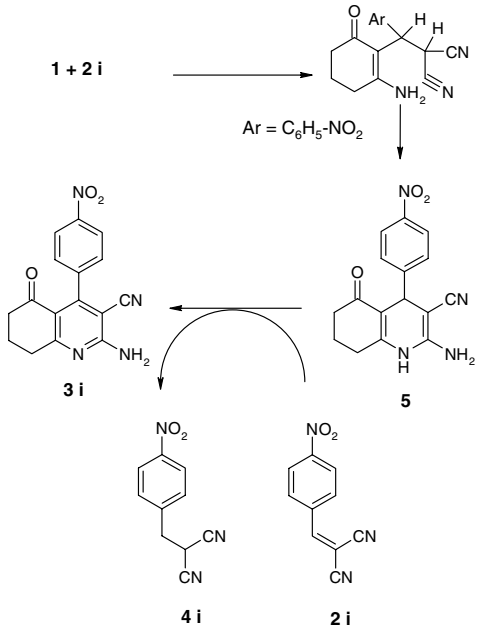


Figure 1. Mechanism.

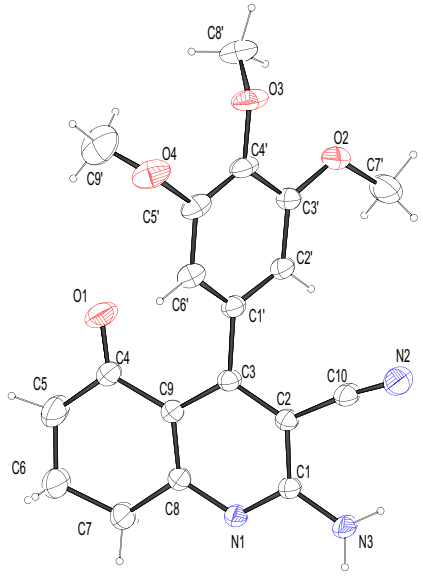


Figure 2. X-ray crystal structure of compound **3f**.

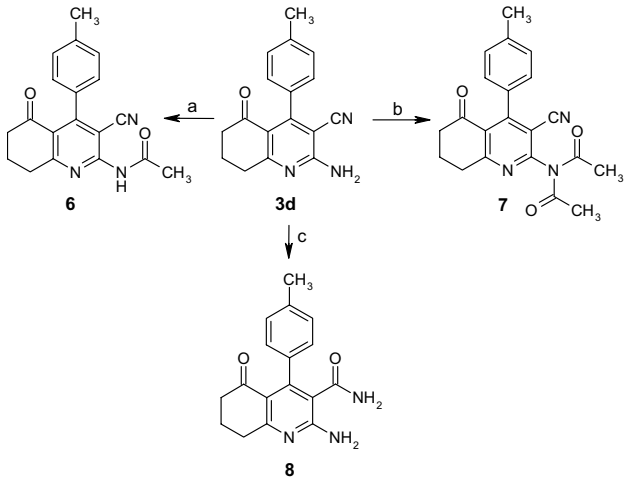
(Fig. 2) shows the formation of a fully aromatized product.

To study the significance of each group on core structure vis-à-vis the antifungal activity, the respective modifications of amino and cyano functional groups were carried out. Compound **3d** was selectively monoacetylated to **6** by using acetyl chloride in dry pyridine at reflux temperature for 3 h. The preparation of the diacylated derivative **7** was achieved by refluxing **3d** in acetic anhydride for 12 h. The conversion of the nitrile group in **3d** to the amide **8** was carried out by hydrolysis using 60% H_2SO_4 at 80 °C for 24 h (Scheme 2).

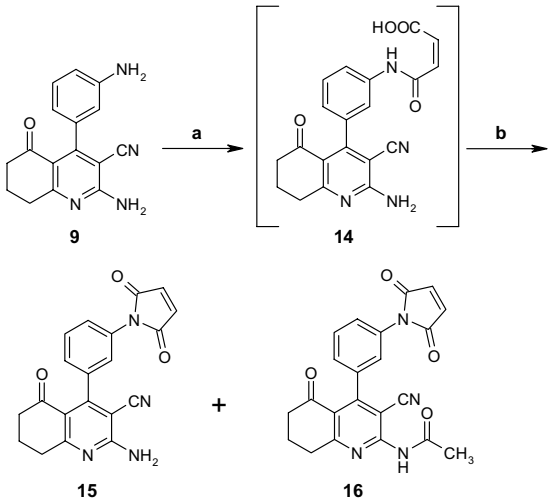
Table 1. Synthesis of compounds **3(a–i)**

Compound	R	Time	Yield[a]
3a	4-Cl	4	92
3b	4-F	4	94
3c	3-NO_2	4	95
3d	4-CH_3	5	87
3e	2-Cl	7	82
3f	3,4,5-OMe	5	89
3g	4-OMe	6	90
3h	H	7	81
3i	4-NO_2	4	90

[a] Isolated yields after column chromatography.



Scheme 2. Reagents and conditions: (a) pyridine, acetyl chloride, rt to reflux 3 h, 85%; (b) acetic anhydride, reflux 12 h, 90%; (c) 60% H_2SO_4, 80 °C 24 h, 88%.



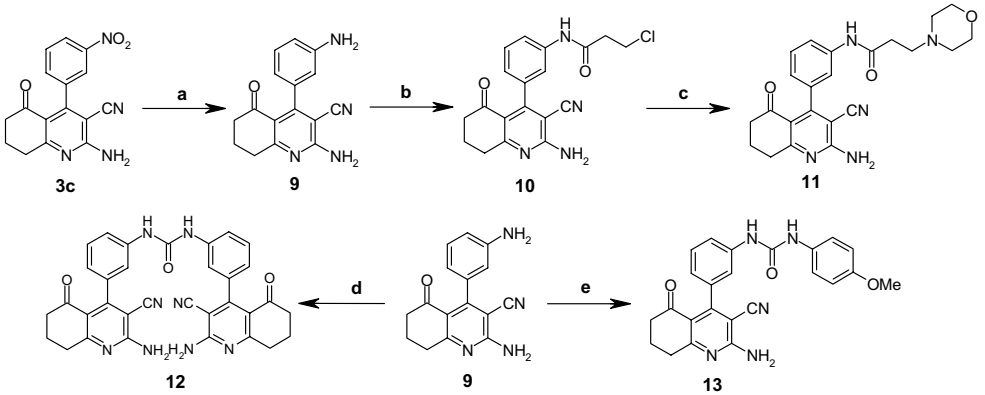
Scheme 4. Reagents and conditions: (a) maleic anhydride, THF, rt, 12 h, 95%; (b) acetic anhydride, NaOAc, 60 °C, 1 h, (**15**: 60%, **16**: 32%).

To modify the functionality in the aryl ring so as to incorporate groups which can enhance biological activity, the nitro derivative **3c** (Table 1) was reduced to 2-amino-5-oxo-4-(3-aminophenyl)-5,6,7,8-tetrahydroquinoline-3 carbonitrile **9** using $SnCl_2{\cdot}2H_2O$ in absolute ethanol. N-Acylation of **9** with 3-chloropropionyl chloride in THF and in the presence of $NaHCO_3$ gave chloropropionyl derivative **10**. Amination of **10** was carried out by refluxing it in morpholine for 4 h to afford **11**. Compound **12** was obtained by refluxing compound **9** and carbonyldiimidazole in dry THF for 16 h. The urea derivative **13** was obtained by the reaction of compound **9** with 4-methoxyphenylisocyanate in dry THF for 12 h at reflux temperature as shown in Scheme 3.

Likewise, reaction of **9** with maleic anhydride gave maleanilic acid derivative **14** which was used as such for the next step, without any further purification and characterization, to obtain a mixture of the desired maleimide derivative **15** and N-acylated product **16** (in 60% and 32% yield, respectively) by heating it with a mixture of fused NaOAc and acetic anhydride at 60 °C for 1 h as shown in Scheme 4. The products **15** and **16** were separated by column chromatography and completely characterized by spectral and elemental analyses.

3. Antifungal activity

All the synthesized compounds were screened for their in vitro antifungal activities. For preliminary screening the antifungal tests were carried out by disc-diffusion method as described earlier.[13] Amphotericin B (ergosterol synthesis inhibitor) and cycloheximide (protein synthesis inhibitor) were used as a positive control in the disc diffusion method. The yeast cell suspension for *Candida albicans* strains 1 and 2 (human pathogen), mycelial suspension for *Fusarium oxysporum* strains 1 and 2 (plant pathogen), and *Mucor* sp. (saprophyte) (100 μl) were spread on sterile YPG (yeast extract, 0.3%, peptone, 0.5%, and glucose, 1%) and PDA (potato, 20% dextrose, 2%) agar plates separately. Sterile Whatman filter paper No. 1 discs were placed on each



Scheme 3. Reagents and conditions: (a) $SnCl_2{\cdot}2H_2O$, ethanol, reflux 3 h, 97%; (b) 3-chloropropionyl chloride, THF, 0 °C–rt, 2 h, 92%; (c) morpholine reflux 4 h, 87%; (d) carbonyldiimidazole, THF, reflux 16 h, 80%; (e) 4-methoxyphenyl isocyanate, THF, reflux 12 h, 86%.

Table 2. In vitro antifungal activity detection by using disc-diffusion method

Sr. No.	Compound (μg/disc)	Zone of inhibition (mm)														
		C. albicans 1			*C. albicans* 2			*F. oxysporum* 1			*F. oxysporum* 2			*Mucor*		
		40	60	100	40	60	100	40	60	100	40	60	100	40	60	100
1	**3a**	2	3	5	2	3	5	—[a]	4	7	—	4	7	—	7	10
2	**3b**	2	4	6	2	4	6	—	3	7	—	3	7	—	9	12
3	**3c**	3	4	7	3	4	7	3	4	7	3	4	7	—	9	15
4	**3d**	3	4	7	3	4	7	2	3	7	2	3	7	—	10	14
5	3e	—	2	4	—	2	4	2	3	6	2	3	6	—	7	10
6	**3f**	—	—	7	—	—	7	2	2	4	2	2	4	—	—	—
7	**3g**	—	3	4	—	3	4	—	—	6	3	—	6	—	—	—
8	**3h**	—	2	6	—	2	6	—	3	6	3	3	6	—	—	—
9	**3i**	—	3	5	—	3	5	—	2	3	—	2	3	—	—	—
10	**6**	—	3	7	—	3	7	—	3	5	—	3	5	—	—	—
11	**7**	—	4	6	—	4	6	—	2	4	—	2	4	3	7	10
12	**8**	—	2	4	—	2	4	—	—	4	—	2	4	—	—	—
13	**9**	—	3	6	—	3	6	2	3	5	2	3	5	5	7	10
14	**11**	2	2	7	2	2	7	2	3	5	2	3	5	—	—	—
15	**12**	3	3	7	3	3	7	—	2	6	—	2	6	—	—	—
16	**13**	2	3	6	2	3	6	—	—	2	—	—	2	—	—	—
17	**15**	3	5	7	3	5	7	3	3	7	3	3	7	—	—	—
18	**16**	2	2	7	2	2	7	2	2	6	2	2	6	8	9	11
19	Cycloheximide	2	4	7	2	4	7	3	5	7	3	5	7	—	—	—
20	Amphotericin B	4	7	12	4	7	12	2	6	9	3	6	9	5	7	10

Positive control, cycloheximide and Amphotericin B.
Negative control, DMSO (50% v/v).
[a] No inhibition.

plate. Different concentrations of the compounds were added on the filter paper discs. DMSO (50% v/v) without inhibitor was used as a control. The plates were then incubated at 28 °C for 24 h and antifungal activity was evaluated by measuring the diameter of zone of inhibition against that of the test organisms (Table 2).

Before discussing the structure–activity relationship it must be pointed out that the ratio between the highest and the lowest concentrations of compounds is only 2:3. The effective concentrations sometimes alter deceptively in the biological experiments because of conditions of living things. Moreover, since the antifungal assays are done using paper discs the structure–activity relationship is described purely on a qualitative basis with respect to substituents incorporated in the molecular skeleton.

It was observed that activity of the synthesized compounds was dependent on the substituent present at 3 or 4 position of the phenyl ring in 2-amino-3-cyano-4-arylpyridine moiety. In terms of structure–activity relationship, 2-amino-5-oxo-4-(3-nitro-phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile, **3c** with a nitro group substituent in the *meta* position showed, in general, a more potent antifungal activity than other compounds synthesized (Table 1). Thus the compound with *meta* substituted $-NO_2$ group **3c** had prominent activity against all the species studied than the compound with *para* substituted $-NO_2$ group **3i**. The compound **3i** did not have any activity against *Mucor* but compound **3c** showed good activity against *Mucor*. Compound **3h** without any substitution on phenyl ring showed poor activity against *C. albicans* 1 and 2, *F. oxysporum* 1 and no activity against *Mucor*, but showed comparable activity with those of the standards against *F. oxysporum* 2. The compound with halogen substituents showed good to moderate activity. Among the halogen substituted compounds **3e** showed the better activity than **3a** and **3b** against *F. oxysporum* species emphasizing the importance of the positioning of the functional group on the aryl ring. It was observed that compounds **3a**, **3b**, **3c**, and **3d** were effective against *C. albicans* 1 and 2 whereas compounds **3c**, **3d**, **3e**, and **3f** were more effective against *F. oxysporum* 1 and 2 and compounds **3a**–**e** were effective against *Mucor* sp. at higher concentration, the results are comparable with those of the standards. The studied compounds could be divided into two groups. One group includes compounds **3a** to **3i** which are generic and the second group includes compounds **6**–**16** with modification of functional groups present in parent compounds. N-Diacetylation of compound **3d** gave compound **7**, which showed poor activity as compared to **3d** in the case of *C. albicans* 1, 2 and *F. oxysporum* 1, 2. But in the case of *Mucor* compound **7** showed good activity, while compounds **6** and **7** which were acylated derivatives of compound **3d** had less activity than parent compound having free NH_2 group.

All the synthesized compounds **11**–**16** from Schemes 3 and 4 were effective against both the species of *C. albicans* and *F. oxysporum*, but compounds **11**–**15** did not show activity against *Mucor*. Compound **9** and its monoacylated maleimide derivative showed good activity against *Mucor*. Compounds **3c**, **3d**, and **12** showed activity comparable to that of cycloheximide. It was compound **16**,

the N-acetylated maleimide derivative, which showed the best activity against all the fungal species studied and greater activity than Amphotericin B against *Mucor*.

3.1. Yeast-hypha transition experiment

Most of the pathogenic fungi change their morphology viz. unicellular yeast or filamentous hypha for survival and proliferation in the host. The yeast-hypha transition experiment was carried out in a model non-pathogenic fungus *Benjaminiella poitrasii* to check the effect of compounds as described earlier.[14,15]

Yeast inoculum was grown in YPG medium for 24 h at 37 °C and the transition was studied in YP medium at 28 °C. The yeast cells were inoculated in YP broth (with and without compounds) at 28 °C for 4 h and the percentage of cells forming germ tubes was assessed as described earlier.[16]

Nikkomycin Z (chitin synthase inhibitor) was used as a positive control in the yeast-hypha transition experiment (Table 4). It was seen that compounds **3a**, **3b**, **3e**–**3g**, **3i**, **8**, **9**, **11**–**13**, **15**, and **16** exhibited 95–99% of inhibition while **3c**, **3d**, **3h**, **7**, and **6** showed 85–95% of inhibition at 20 μg/ml. Minimum inhibitory concentration (MIC) of the compounds was estimated by disc-diffusion method. Cycloheximide showed 2–7 mm inhibition in case of *C. albicans* and 3–7 mm inhibition for *F. oxysporum* at 40 μg/ml. While Amphotericin B showed 4–12 mm inhibition in *C. albicans*, 2–9 mm inhibition in *F. oxysporum*, and 5–10 mm inhibition in *Mucor* at 40 μg/ml. Therefore for other compounds the zone of inhibition 2 mm at 28 °C for 24 h was considered as minimum inhibitory concentration (MIC), Table 3.

Table 4. Effect of compounds on yeast-hypha transition

Group	Compounds (20 μg/ml)	Inhibition (%)
1	**3a**, **3b**, **3e**, **3f**, **3g**, **3i**, **8**, **9**, **11**, **12**, **13**, **15**, **16**	95–99
2	**3d**, **3h**, **7**, **6**	90–95
3	**3c**	85–90
4	Nikkomycin Z (4 μg/ml)	>90

Standard used was Nikkomycin Z (4 μg/ml).

3.2. Non-radioactive chitin synthase assay

Chitin synthase activity from *B. poitrasii* was estimated with and without compounds (4 μg/ml) using a non-radioactive chitin synthase assay according to Lucero et al.[17]

It was seen that compounds **3d**, **12**, **15**, and **16** inhibited 91–95% chitin synthase activity, **3a**–**3c**, **3e**–**3g** exhibited 80–90% of inhibition. The rest of the compounds exhibited substantial percentage of inhibition in the range of 60–80% (Table 5).

3.3. Antiproliferative activity: materials and methods

Human hepatocellular carcinoma Hep3B and human mammary adenocarcinoma (MCF-7) cell lines were obtained from American Type Culture Collection (Manassas, VA, USA), and maintained in our in-house National Cell Repository. Cells were maintained as a monolayer in culture medium consisting of nutrient media MEM supplemented with heat-inactivated fetal bovine serum (10%), penicillin (100 μg/ml), and streptomycin (100 μg/ml) (Invitrogen Life Technologies, MD, USA). The cells were grown at 37 °C in 5% CO_2 and humidified air atmosphere. Stock solutions of all compounds were

Table 3. Minimum inhibitory concentration values of the compounds against the fungal species using disc diffusion method

Sr. No.	Compound	Concentration of compounds (μg/disc)				
		Candida albicans 1	*Candida albicans* 2	*F. oxysporum* 1	*F. oxysporum* 2	*Mucor*
1	**3a**	40	40	60	60	60
2	**3b**	40	40	40	40	60
3	**3c**	40	40	20	20	60
4	**3d**	40	40	40	40	60
5	**3e**	60	60	40	40	60
6	**3f**	100	100	20	20	—[a]
7	**3g**	60	60	100	100	—
8	**3h**	60	60	60	60	—
9	**3i**	60	60	60	60	—
10	**6**	60	60	60	60	—
11	**7**	60	60	60	60	40
12	**8**	60	60	100	100	—
13	**9**	60	60	40	40	40
14	**11**	40	40	40	40	—
15	**12**	40	40	60	60	—
16	**13**	40	40	100	100	—
17	**15**	40	40	40	40	—
18	**16**	40	40	40	40	40
19	Cycloheximide	40	40	20	20	—
20	Amphotericin B	40	40	40	40	40

Standard used were cycloheximide and Amphotericin B.
[a] Not detected.

Table 5. In vitro antifungal activity of selected compounds for the inhibition of chitin synthase enzyme from *B. poitrasii*

Group	Compounds (4 μg/ml)	Inhibition (%)
1	**3d**, **12**, **15**, **16**	91–95
2	**3e**	86–90
3	**3a**, **3b**, **3c**, **3f**, **3g**	81–85
4	**9**	76–80
5	**13**	71–75
6	**3h**, **6**, **8**	66–70
7	**3i**, **7**, **11**	61–65
8	Nikkomycin Z	>90

prepared in DMSO (concentration of 4 mg/ml) and afterwards diluted to the required concentration in cell culture media. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was dissolved (1 mg/ml) in MEM (without phenol red) and filtered through a Millipore filter, 0.22 μm, before use. The generic compound **3c** and the functionalized derivative **16** prepared from **3c** which showed the highest antifungal activity were taken up for the determination of their antiproliferative activity.

3.4. Antiproliferative activity-MTT cell proliferation assay

Hep3B and MCF-7 cells were plated at a density of 10,000 cells per well in 96-well tissue culture plates. Cells were allowed to adhere for 24 h at 37 °C and then treated with various concentrations (0, 1, 10, 100, 1000, and 2000 μg/ml) of compounds diluted in culture medium, for additional 48 h. In the cells in control wells a culture medium consisting of corresponding concentration of DMSO only was added. After 48 h of drug treatment growth medium was removed from each well containing cells and fresh culture medium was added to each well. Cells were allowed to grow for another 24 h.

Thereafter, cell proliferation was assessed by replacing culture medium with 50 μl MEM containing 1 mg/ml MTT and subsequently incubated for additional 4 h at 37 °C. The medium was then aspirated off and formazan crystals were solubilized in 50 μl of 2-propanol. The optical density was read on a microplate reader at 570 nm using 630 nm as a reference filter against a blank prepared from cell-free wells. Absorbance given by cells treated with the carrier DMSO alone was taken as 100% cell growth. All assays were performed in triplicate.

3.5. Antiproliferative activity

The antiproliferative activity of all the three compounds, **3c** and **16** and Amphotericin B, was tested against human cancer cells MCF-7 and Hep3B, which were grown in vitro. Compounds **3c** and **16** as well as Amphotericin B at high doses were toxic to both MCF-7 (Fig. 3) and Hep3B (Fig. 4) cells. However, at lower doses MCF-7 cells were resistant to compound **3c** and the IC_{50} value was found to be 293.4 μg/ml. Both compound **16** and Amphotericin were similar in their toxicity to MCF-7 cells and their IC_{50} values were between 50 and 60 μg/ml. All the three compounds were identical in their effect on the survival of Hep3B cells. In fact none of the three

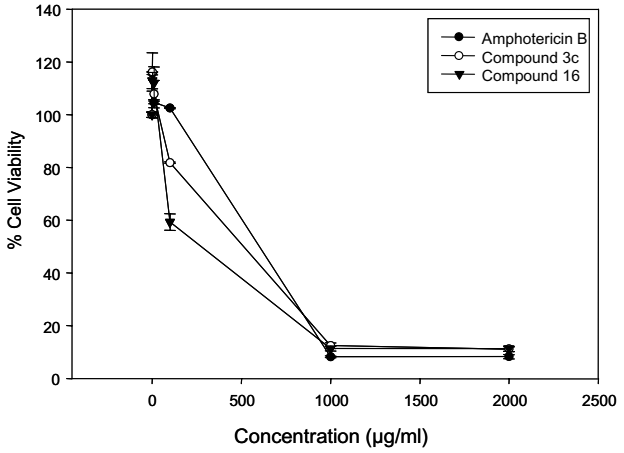


Figure 3. Antiproliferative activities of compounds in MCF-7 cells.

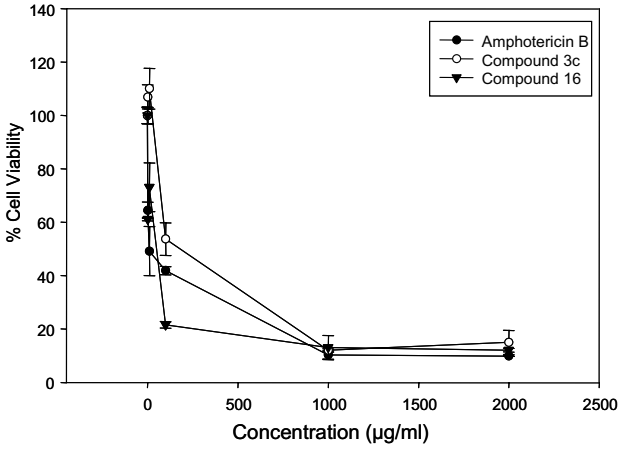


Figure 4. Antiproliferative activities of compounds in Hep3B cells.

compounds was toxic to the Hep3B cells up to a concentration of 400 μg/ml. At a concentration of 1 mg/ml or more all the three compounds were toxic to both the cells and inhibited growth.

4. Conclusion

A series of 2-amino-5,6,7,8-tetrahydro-5-oxo-4-phenylquinoline-3-carbonitrile and its various analogues have been synthesized in excellent isolated yields in the absence of any added catalyst by the reaction of 3-amino-cyclohexen-1one **1** with various arylidenemalononitriles **2** and their biological activities were evaluated. The dual role of **2** as a reactant in the Michael addition step and as an oxidizing agent in the aromatization contributing to the mechanism has been established with experimental evidence. The antifungal activity of compounds **3c**, **3d**, and **12** (40–100 μg/ml) showed activity comparable to that of cycloheximide (40–100 μg/ml) under similar experimental conditions. Compound **16** exhibited (2–11 mm) activity against all the test fungal strains. However a greater zone of inhibition with compound **16** was observed against *Mucor*. All the compounds have profound suppressive effect on yeast-hypha transition, exhibiting greater than 85% inhibition

at concentration 20 μg/ml. Compounds **3d**, **13**, **15**, **16**, and **17** exhibited more than 90% inhibition of chitin synthase activity comparable to that of Nikkomycin. All other compounds exhibited 61–90% inhibition of chitin synthase activity at concentration 4 μg/ml. The generic compound **3c** and the functionalized derivative **16** prepared from **3c** showed the most antifungal activity. The presence of halogen and nitrogen containing substituents on aryl ring seems to generate compounds with good antifungal activities. The antiproliferative activities of compounds **3c** and **16** were similar to that of Amphotericin B though only slight differences in the levels of toxicities to the two cell lines were observed. It was observed that at low doses the mammary adenocarcinoma cells MCF-7 were less sensitive to all the three compounds whereas hepatocellular carcinoma cells Hep-3B were more resistant to all the compounds. These results indicate that compounds **3c** and **16** do not have any profound effect on proliferation of human cancer cell lines at lower concentrations and their activity is comparable to that of Amphotericin B.

5. Experimental

5.1. General experimental procedures

All chemicals were of research grade and were used as obtained from Aldrich or Fluka. IR spectra were recorded on an ATIMATT-SON RS-1 Research Series FT-IR spectrometer. NMR spectra were recorded on Bruker AC-200, AC-400, and AC-500 spectrometers in $CDCl_3$/DMSO-d_6. The melting points were uncorrected and recorded on the BUCHI melting point instrument model B-540. Column chromatography was performed using silica gel (60–120 mesh size) purchased from Thomas Baker and TLC was carried out using aluminum sheets pre-coated with silica gel $60F_{254}$ purchased from Merck.

5.1.1. General procedures for the preparation of the compounds 3(a–i). A solution of 3-amino-2-cyclohexen-1-one **1** (2 g, 10 mmol) and arylidenemalononitrile **2** (30 mmol) was refluxed in 40 ml of *n*-propanol for the requisite time as shown in Table 1. After completion of reaction 1-propanol was removed under reduced pressure and the product was isolated by column chromatography using 60% ethyl acetate in pet ether as eluent.

5.1.1.1. 2-Amino-5-oxo-4-(4-chloro-phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3a). White solid; mp 289 °C; IR (cm^{-1}) 3422, 3306, 2215, 1685, 1648, 1537, 1461, 1243; ^1H NMR ($CDCl_3$+DMSO-d_6, [11:1] 200 MHz) δ 7.03 (d, $J = 8.5$ Hz, 2H), 6.77 (d, $J = 8.5$ Hz, 2H), 6.63 (br s, 2H), 2.63 (t, $J = 6.3$ Hz, 2H), 2.13 (t, $J = 6.3$ Hz, 2H), 1.72 (quin, $J = 6.3$ Hz, 2H); Anal. Calcd for $C_{16}H_{12}ClN_3O$: C, 64.54; H, 4.06; Cl, 11.91; N, 14.11. Found: C, 64.32; H, 4.15; Cl, 11.89; N, 14.24.

5.1.1.2. 2-Amino-5-oxo-4-(4-fluoro-phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3b). White solid; mp 286 °C; IR (cm^{-1}) 3397, 3309, 2215, 1684, 1649, 1511, 1460, 1217; ^1H NMR ($CDCl_3$+DMSO-d_6, [11:1] 200 MHz) δ 6.89–6.73 (m, 4H), 6.51 (br s, 2H), 2.66 (t, $J = 6.5$ Hz, 2H), 2.17 (t, $J = 6.5$ Hz, 2H), 1.75 (quin, $J = 6.5$ Hz, 2H); Anal. Calcd for $C_{16}H_{12}FN_3O$: C, 68.32; H, 4.30; F, 6.75; N, 14.94. Found: C, 68.45; H, 4.54; N, 14.82.

5.1.1.3. 2-Amino-5-oxo-4-(3-nitro-phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3c). White solid; mp 243 °C; IR (cm^{-1}) 3483, 3323, 2211, 1674, 1622, 1552, 1346, 755; ^1H NMR ($CDCl_3$+DMSO-d_6, [11:1] 200 MHz) δ 8.34–8.28 (m, 1H), 8.05 (t, $J = 1.9$ Hz, 1H), 7.64 (t, $J = 7.9$ Hz, 1H), 7.55–7.50 (m, 1H) 5.74 (br s, 2H), 3.04 (t, $J = 6.2$ Hz, 2H), 2.58–2.51 (m, 2H), 2.13 (quin, $J = 6.2$ Hz, 2H); ^{13}C NMR ($CDCl_3$+DMSO-d_6, [6:1] 125 MHz) δ: 192.8, 168.2, 158.6, 153.1, 146.1, 138.3, 132.2, 127.9, 121.3, 120.8, 115.2, 113.5, 90.2, 37.6, 32.2, 19.1; Anal. Calcd for $C_{16}H_{12}N_4O_3$: C, 62.34; H, 3.92; N, 18.17. Found: C, 62.40; H, 3.80; N, 18.02.

5.1.1.4. 2-Amino-5-oxo-4-(4-methyl-phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3d). White solid; mp 258 °C; IR (cm^{-1}) 3423, 3296, 2208, 1682, 1642, 1555, 1535, 1459; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.24 (d, $J = 8.0$ Hz, 2H), 7.08 (d, $J = 8.0$ Hz, 2H), 5.74 (br s, 2H), 2.99 (t, $J = 6.2$ Hz, 2H), 2.53 (t, $J = 6.2$ Hz, 2H), 2.40 (s, 3H), 2.09 (quin, $J = 6.2$ Hz, 2H); ^{13}C NMR ($CDCl_3$+DMSO-d_6, [6:1] 50 MHz) δ: 192.1, 167.3, 158.3, 155.6, 135.4, 133.3, 126.6, 125.4, 115.3, 113.7, 89.9, 37.5, 31.9, 19.3, 18.9; Anal. Calcd for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15. Found: C, 73.77; H, 5.62; N, 15.32.

5.1.1.5. 2-Amino-5-oxo-4-(2-chloro-phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3e). White solid; mp 286 °C; IR (cm^{-1}) 3392, 3308, 2218, 1680, 1647, 1543, 1462, 1377; ^1H NMR ($CDCl_3$+DMSO-d_6, [11:1] 200 MHz) δ 7.11–6.93 (m, 3H), 6.77–6.73 (m, 1H), 6.33 (br s, 2H), 2.64 (t, $J = 6.0$ Hz, 2H), 2.17–2.09 (m, 2H), 1.72 (quin, $J = 6.0$ Hz, 2H); Anal. Calcd for $C_{16}H_{12}ClN_3O$: C, 64.54; H, 4.06; Cl, 11.91; N, 14.11. Found: C, 64.41; H, 4.16; Cl, 11.72; N, 14.30.

5.1.1.6. 2-Amino-5-oxo-4-(3,4,5-trimethoxy-phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3f). Pale yellow solid; mp 219 °C; IR (cm^{-1}) 3417, 3308, 2213, 1681, 1651, 1555, 1459, 1093; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.38 (s, 2H), 5.73 (br s, 2H), 3.89 (s, 3H), 3.82 (s, 6H), 2.99 (t, $J = 6.2$ Hz, 2H), 2.55 (t, $J = 6.2$ Hz, 2H), 2.11 (quin, $J = 6.2$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 194.4, 169.1, 159.4, 157.6, 152.9, 138.0, 132.5, 118.3, 115.2, 104.4, 93.1, 60.8, 55.9, 39.4, 33.7, 20.7; Anal. Calcd for $C_{19}H_{19}N_3O_4$: C, 64.58; H, 5.42; N, 11.89. Found: C, 64.73; H, 5.60; N, 11.70.

5.1.1.7. 2-Amino-5-oxo-4-(4-methoxy-phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3g). White solid; mp 278 °C; IR (cm^{-1}) 3393, 3307, 2217, 1680, 1651, 1512, 1459, 1243; ^1H NMR ($CDCl_3$+DMSO-d_6, [11:1] 200 MHz) δ 6.72 (d, $J = 8.7$ Hz, 2H), 6.53 (d, $J = 8.7$ Hz, 2H), 6.44 (br s, 2H), 3.44 (s, 3H), 2.57 (t, $J = 6.2$ Hz, 2H), 2.09 (t, $J = 6.2$ Hz, 2H), 1.67 (quin,

J = 6.2 Hz, 2H); Anal. Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.76; H, 5.04; N, 14.45.

5.1.1.8. 2-Amino-5-oxo-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3h). White solid; mp 257 °C; IR (cm^{-1}) 3391, 3299, 2208, 1692, 1651, 1585, 1478, 1404, 1215, 1082, 930, 758, 669; ^1H NMR ($CDCl_3$+DMSO-d_6, [11:1] 200 MHz) δ 6.90–6.87 (m, 3H), 6.69–6.61 (m, 2H), 2.45 (t, J = 6.2 Hz, 2H), 1.91 (t, J = 6.2 Hz, 2H), 1.56 (quin, J = 6.2 Hz, 2H); Anal. Calcd for $C_{16}H_{13}N_3O$: C, 72.99; H, 4.98; N, 15.96. Found: C, 72.80; H, 5.05; N, 15.80.

5.1.1.9. 2-Amino-5-oxo-4-(4-nitro-phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (3i). White solid; mp 260 °C; IR (cm^{-1}) 3407, 3323, 2216, 1680, 1612, 1555, 1523, 1430, 1349, 1215, 929, 777, 669; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.31 (d, J = 8.5 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 5.76 (br s, 2H), 3.03 (t, J = 6.5 Hz, 2H), 2.54 (t, J = 6.5 Hz, 2H), 2.12 (quin, J = 6.5 Hz, 2H); Anal. Calcd for $C_{16}H_{12}N_4O_3$: C, 62.34; H, 3.92; N, 18.17. Found: C, 62.48; H, 3.79; N, 18.26.

5.1.1.10. Isolation of 2-(4-nitrobenzyl) malononitrile (4i). Compound **4i** was isolated after the reaction of **1** (1.8 mmol) with 2-(4-nitrobenzyledine) malononitrile **2i** (5.4 mmol) by column chromatography using 5% ethyl acetate in pet ether as eluent. (white solid 2.54 g, 80%); mp 156 °C; IR (cm^{-1}) 2256, 1526, 1444, 1421, 1350, 1215, 758, 669; ^1H NMR ($CDCl_3$, 200 MHz) δ: 8.29 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.5 Hz, 2H), 4.02 (t, J = 6.5 Hz, 1H), 3.40 (d, J = 6.5 Hz, 2H); ^{13}C NMR ($CDCl_3$+DMSO-d_6, [6:1] 50 MHz) δ 147.0, 140.2, 129.9, 123.2, 111.9, 34.7, 23.3; Anal. Calcd for $C_{10}H_7N_3O_2$: C, 59.70; H, 3.51; N, 20.89. Found: C, 59.74; H, 3.55; N, 20.95.

5.1.1.11. *N*-(3-Cyano-5,6,7,8-tetrahydro-5-oxo-4-*p*-tolylquinolin-2-yl) acetamide (6). To a solution of compound **3d** (0.5 g, 1.80 mmol) in dry pyridine (3 ml), acetyl chloride (0.16 ml, 2.16 mmol) was added dropwise at 0 °C in an inert atmosphere. The temperature was allowed to rise to reflux for 3 h. After completion of reaction pyridine was removed under reduced pressure to obtain a white solid. The solid obtained was washed with water, dried, and purified by column chromatography by using 30% ethyl acetate in pet ether as eluent to obtain **6** (0.489 g, 85% yield, white solid); mp 208–210 °C; IR (cm^{-1}) 3270, 2224, 1694, 1546, 1516, 1262, 1215, 766, 668; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.08 (br s, 1H), 7.26 (d, J = 8.0 Hz, 2H), 7.09 (d, J = 8.0 Hz, 2H), 3.16 (t, J = 6.2 Hz, 2H), 2.62 (t, J = 6.2 Hz, 2H), 2.52 (s, 3H), 2.41 (s, 3H), 2.17 (quin, J = 6.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 194.9, 169.8, 167.7, 158.0, 152.9, 139.0, 133.1, 129.0, 127.1, 122.8, 114.0, 100.5, 39.5, 33.7, 25.0, 21.3, 20.7; Anal. Calcd for $C_{19}H_{17}N_3O_2$: C, 71.46; H, 5.37; N, 13.16. Found: C, 71.62; H, 5.49; N, 13.10.

5.1.1.12. *N*-Acetyl-*N*-3-cyano-5,6,7,8-tetrahydro-4-(4-methylphenyl-5-oxo-4-*p*-tolylquinolin-2-yl) acetamide (7). A mixture of compound **3d** (0.277 g, 1 mmol) and acetic anhydride (5 ml) was refluxed for 12 h. After completion of reaction excess acetic anhydride was removed under reduced pressure. The residue obtained was added in water (15 ml) and extracted with ethyl acetate (3× 15 ml). The organic layer was separated, dried over anhydrous sodium sulfate, and evaporated to obtain the crude product. The pure product **7** was obtained by column chromatography using 15% ethyl acetate in pet ether as eluent. (0.325 g, 90%, yellow solid); mp 183–185 °C; IR (cm^{-1}) 2229, 1721, 1707, 1557, 1509, 1459, 1416, 1367, 1263, 1228, 1208, 1037, 979, 931, 823; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.28 (d, J = 8.2 Hz, 2H), 7.12 (d, J = 8.2 Hz, 2H), 3.26 (t, J = 6.3 Hz, 2H), 2.70 (t, J = 6.3 Hz, 2H), 2.42 (s, 3H), 2.37 (s, 6H) 2.24 (quin, J = 6.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 195.0, 171.2, 167.9, 157.8, 155.6, 139.3, 132.0, 129.0, 127.2, 126.6, 113.2, 110.9, 39.4, 33.3, 26.0, 21.2, 20.5; Anal. Calcd for $C_{21}H_{19}N_3O_3$: C, 69.79; H, 5.30; N, 11.63. Found: C, 69.68; H, 5.11; N, 11.44.

5.1.1.13. 2-Amino-5,6,7,8-tetrahydro-5-oxo-4-*p*-tolylquinoline-3-carboxamide (8). A solution of 60% aqueous H_2SO_4 (10 ml) and compound **3d** (1.2 g, 4.33 mmol) was heated at 80 °C for 24 h with stirring. The reaction mixture was cooled to room temperature, diluted with water, and neutralized by 10% aqueous sodium hydroxide solution. The resulting precipitate was filtered, dried, and purified by column chromatography by using 70% ethyl acetate in pet ether to obtain **8** (1.124 g, 88% yield, white solid); mp 250 °C; IR (cm^{-1}) 3400, 3300, 3250, 3150, 1678, 1655, 1605, 1551, 1215; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.22 (d, J = 8.2, 2H), 7.06 (d, J = 8.2 Hz, 2H), 6.43 (br s, 2H), 5.39 (s, 1H), 4.95 (s, 1H), 2.95 (t, J = 6.2 Hz, 2H), 2.49 (t, J = 6.2 Hz, 2H), 2.39 (s, 3H), 2.07 (quin, J = 6.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 193.0, 166.5, 163.4, 155.2, 146.8, 134.1, 134.0, 126.0, 115.2, 115.1, 37.8, 31.5, 19.5, 19.2; Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.25; H, 5.69; N, 14.15.

5.1.1.14. 2-Amino-4-(3-aminophenyl)-5,6,7,8-tetrahydro-5-oxoquinoline-3-carbonitrile (9). A mixture of nitro derivative **3i** (0.695 g, 1 mmol) and $SnCl_2{\cdot}2H_2O$ (2.54 g, 5 mmol) was heated up to reflux in absolute ethanol (40 ml) for 3 h. After completion of reaction, ethanol was removed under reduced pressure. Water (60 ml) was added to the oily residue and the mixture was neutralized to pH 7 –8 by adding aqueous saturated $NaHCO_3$ solution followed by extraction with ethyl acetate (3× 30 ml). The combined ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain the pure product **9** (0.608 g, 97% yield, yellow solid). mp 276–278 °C; IR (cm^{-1}) 3397, 3363, 3313, 2219, 1655, 1457, 1285; ^1H NMR ($CDCl_3$+DMSO-d_6, [11:1] 200 MHz) δ 6.95 (t, J = 8.0 Hz, 1H), 6.57–6.52 (m, 1H), 6.33–6.30 (m, 2H), 3.06 (br s, 2H), 2.73 (t, J = 6.2 Hz, 2H), 2.25 (t, J = 6.2 Hz, 2H), 1.83 (quin, J = 6.2 Hz, 2H); ^{13}C NMR ($CDCl_3$+DMSO-d_6, [6:1] 100 MHz) δ: 192.5, 167.6, 158.5, 156.4, 145.3, 137.4, 127.1, 115.9, 114.6, 114.1, 113.1, 112.0, 90.5, 37.9, 32.3, 19.3: Anal. Calcd for $C_{16}H_{14}N_4O$: C, 69.05; H, 5.07; N, 20.13; Found: C, 69.27; H, 5.24; N, 20.11.

5.1.1.15. *N*-(3-(2-Amino-3-cyano-5,6,7,8-tetrahidro-5-oxoquinolin-4-yl)phenyl)-3-chloropropanamide (10). To a solution of compound **9** (0.504 g, 1.8 mmol) and $NaHCO_3$ (0.152 g, 1.8 mmol) in THF, chloropropionyl chloride (0.18 ml, 1.8 mmol) was added at 0 °C over a period of 10 min and then stirred for 2 h at room temperature. After completion of reaction, THF was removed under reduced pressure to obtain a yellow solid. The solid was washed with excess water, dried and finally washed with pet ether to obtain a pure product. (0.614 g, 92% yield, yellow solid); mp 284 °C (dec); IR (cm^{-1}) 3440, 3315, 3219, 2216, 1666, 1626, 1586, 1553, 1447, 1421, 1320, 1284, 886, 774; ^1H NMR ($CDCl_3$, 200 MHz) δ 10.31 (s, 1H), 7.86 (br s, 2H), 7.76 (d, $J = 7.5$ Hz, 1H), 7.59 (s, 1H), 7.49 (t, $J = 7.5$ Hz, 1H), 6.99 (d, $J = 7.5$ Hz, 1H), 4.03 (t, $J = 6.2$ Hz, 1H), 3.07 (t, $J = 6.0$ Hz, 2H), 2.99 (t, $J = 6.2$ Hz, 2H), 2.57 (t, $J = 6.0$ Hz, 2H), 2.14 (t, $J = 6.0$ Hz, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 193.8, 169.4, 167.9, 159.9, 156.9, 138.6, 128.3, 122.0, 118.3, 117.3, 116.7, 115.2, 91.0, 40.7, 40.0, 39.0, 33.4, 20.4; Anal. Calcd for $C_{19}H_{17}ClN_4O_2$: C, 61.88; H, 4.65; Cl, 9.61; N, 15.19. Found: C, 61.98; H, 4.60; Cl, 9.51; N, 15.08.

5.1.1.16. *N*-(3-(2-Amino-3-cyano-5,6,7,8-tetrahidro-5-oxoquinolin-4-yl)phenyl)-3-morpholinopropanamide (11). A solution of compound **10** (0.150 g, 0.4 mmol) and excess morpholine (15 ml) was heated to reflux for 4 h. After completion of reaction, morpholine was removed under reduced pressure to obtain a residue. Water (30 ml) was added to the residue and neutralized by diluted HCl to obtain a yellow solid which was further filtered and washed with 1:1 ethyl acetate and pet ether mixture to get the pure product. (0.148 g, 87% yield, yellow solid); mp 162–164 °C; IR (cm^{-1}) 3396, 3322, 3211, 2215, 1731, 1651, 1629, 1584, 1557, 1462, 1376; ^1H NMR ($CDCl_3$, 200 MHz) δ 10.94 (s, 1H), 7.68 (s, 1H), 7.40–7.37 (m, 2H), 6.95–6.89 (m, 1H), 5.64 (s, 2H), 3.83 (t, $J = 4.8$ Hz, 4H), 3.01 (t, $J = 6.2$ Hz, 2H), 2.74 (t, $J = 6.2$ Hz, 2H), 2.63 (t, $J = 4.8$ Hz, 4H), 2.57–2.51(m, 4H), 2.11 (quin, $J = 6.2$ Hz, 2H); ^{13}C CMR ($CDCl_3$, 100 MHz) δ 193.1, 169.2, 168.2, 159.0, 156.2, 137.8, 137.5, 127.3, 121.0, 117.8, 116.9, 116.4, 114.4, 91.2, 65.7, 53.1, 51.9, 38.3, 32.8, 32.3, 19.8; Anal. Calcd for $C_{23}H_{25}N_5O_3$: C, 65.86; H, 6.01; N, 16.70. Found: C, 65.78; H, 6.10; N, 16.58.

5.1.1.17. 1,3-Bis(3-(2-amino-3-cyano-5,6,7,8-tetrahydro-5-oxoquinolin-4-yl)phenyl) urea (12). A solution of compound **10** (0.200 g, 0.71 mmol) and carbonyldiimidazole (0.058 g, 0.35 mmol) in dry THF (60 ml) was heated to reflux and maintained at reflux for 18 h. After completion of reaction, THF was removed under reduced pressure to obtain a pale yellow solid. The solid was washed with water, dried, and then washed with cold ethyl acetate to obtain the pure product **12** (0.335 g, 80% yield, pale yellow solid); mp 285–287 °C; IR (cm^{-1}) 3377, 2219, 1646, 1589, 1542, 1460, 1377; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.80 (s, 2H), 7.70 (s, 4H), 7.38–7.25 (m, 6H), 6.78 (d, $J = 7.5$ Hz, 2H), 2.97–2.91 (m, 4H), 2.57–2.41 (m, 4H), 2.03–1.97 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 193.7, 169.2, 159.8, 157.1, 152.3, 139.1, 138.6, 128.3, 120.7, 117.5, 116.7, 116.5, 115.2, 91.0, 39.0, 33.3, 20.4; Anal. Calcd for $C_{33}H_{26}N_8O_3$: C, 68.03; H, 4.50; N, 19.23. Found: C, 68.25; H, 4.32; N, 19.42.

5.1.1.18. 1-(3-(2-Amino-3-cyano-5,6,7,8-tetrahydro-5-oxoquinolin-4-yl)phenyl)-3-(4-methoxyphenyl) urea (13). A solution of compound **10** (0.250 g, 0.89 mmol) and 4-methoxyphenyl isocyanate (0.11 ml, 0.89 mmol) was refluxed in dry THF (60 ml) for 6 h. After completion of reaction THF was removed under reduced pressure and the solid obtained was washed with water, dried, and recrystallized from acetone to obtain a pure solid **13** (0.330 g, 86% yield, pale yellow); mp 225–227 °C; IR (cm^{-1}) 3405, 3320, 3168, 2215, 1712, 1675, 1650, 1598, 1560, 1541, 1461, 1377, 1294, 1244; ^1H NMR ($CDCl_3$+DMSO-d_6, [11:1] 400 MHz); δ 8.08 (s, 1H), 7.85 (s, 1H), 7.40 (s, 1H), 7.21–7.19 (m, 4H), 6.93 (br s, 2H), 6.69–6.67 (m, 3H), 3.64 (s, 3H), 2.94 (t, $J = 6.2$ Hz, 2H), 2.38 (t, $J = 6.2$ Hz, 2H), 1.98 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 193.8, 169.3, 159.9, 157.2, 154.5, 152.6, 139.4, 138.6, 132.6, 128.3, 120.4, 120.0, 117.3, 116.7, 116.4, 115.3, 113.9, 91.1, 55.0, 39.0, 33.4, 20.4; Anal. Calcd for $C_{24}H_{21}N_5O_3$: C, 67.44; H, 4.95; N, 16.38. Found: C, 67.19; H, 4.89; N, 16.45.

5.1.2. Synthesis of compounds 15 and 16. To a stirred solution of maleic anhydride (0.177 g, 1.8 mmol) in dry THF (15 ml) was added a solution of amine compound **9** (0.500 g, 1.8 mmol) in dry THF (60 ml) dropwise at room temperature over a period of 20 min and the reaction mixture was further stirred for 12 h. After completion of reaction, THF was removed under reduced pressure to obtain maleanilic acid derivative **14** in quantitive yield, which was used for the next step without any further purification and characterization. A mixture of maleanilic acid derivative **14**, acetic anhydride (10 ml), and fused sodium acetate (75 mg) was heated in an oil bath at 60 °C for 1h. When complete consumption of starting material was observed along with the formation of two new spots on TLC, the reaction mixture was cooled to room temperature and acetic anhydride was removed under reduced pressure to obtain mixture of products, which were separated by flash column chromatography using 15% ethyl acetate in pet ether as eluent to get **15** (0.386 g, 60% yield) and **16** (0.230 g, 32% yield).

5.1.2.1. 2-Amino-5,6,7,8-tetrahydro-5-oxo-4-(3-(2,5-dioxo-2*H*-pyrrol-1-(5*H*)-yl)phenyl)quinoline-3-carbonitrile (15). Gray solid; mp 262–264 °C (dec); IR (cm^{-1}) 3465, 3357, 2222, 1702, 1683, 1461, 1377, 833; ^1H NMR ($CDCl_3$+DMSO-d_6, [11:1] 200 MHz) δ 7.20–7.11 (m, 2H), 6.90–6.87 (m,1H), 6.85–6.80 (m, 1H), 6.55 (s, 2H), 6.47 (br s, 2H), 2.64–2.56 (m, 2H), 2.14 (t, $J = 6.2$ Hz, 2H), 1.80–1.67 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 194.1, 169.8, 169.6, 160.0, 156.1, 138.8, 134.7, 131.3, 128.5, 126.5, 125.8, 124.9, 116.8, 115.2, 91.3, 39.0, 33.5, 20.5; Anal. Calcd for $C_{20}H_{14}N_4O_3$: C, 67.03; H, 3.94; N, 15.63. Found: C, 67.15; H, 3.80; N, 15.52.

5.1.2.2. *N*-3-Cyano-5,6,7,8-tetrahydro-5-oxo-4-(3-(2,5-dioxo-2,4-pyrrol-(5*H*)phenyl)quinolin-2-yl) acetamide (16). White solid; mp 242–245 °C; IR (cm^{-1}) 3359, 2229, 1717, 1693, 1557, 1537, 1492, 1462, 1378, 1245, 992, 827, 701; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.11 (br s, 1H), 7.59–7.51 (m, 2H), 7.33–7.31 (m, 1H), 7.22–7.17 (m, 1H), 6.83 (s, 2H), 3.17 (t, $J = 6.4$ Hz, 2H), 2.63 (t, $J = 6.4$ Hz, 2H), 2.52 (s, 3H), 2.17 (quin, $J = 6.4$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.9, 167.5, 167.1, 165.8, 153.5, 152.0, 135.7, 132.7, 129.7, 126.7, 124.9, 124.0, 123.1, 121.5, 112.2, 37.4, 31.4, 21.5, 18.7. Anal. Calcd for $C_{22}H_{16}N_4O_4$: C, 66.00; H, 4.03; N, 13.99. Found: C, 66.20; H, 4.19; N, 13.81.

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Supplementary data

Single-crystal data and CIF file of compound **3f** (CCDC # 282502) have been deposited at the Cambridge Crystallographic Data Centre and can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html(or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1123-336-033; or email: deposit@ccdc.ac.uk).^1H and ^{13}C NMR spectra of the synthesized compounds are available as supporting information. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.08.009.

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