A “Green” Synthesis of N-(Quinoline-3-ylmethylene)benzohydrazide Derivatives and their Cytotoxicity Activities

Lingam Venkata Reddy, Suresh Babu Nallapati, Syed Sultan Beevi, Lakshmi Narasu Mangamoori, Khagga Mukkanti and Sarbani Pal*

a Center for Chemical Sciences and Technology and c Center for Biotechnology, Institute for Sciences and Technology, JNT University, Kukatpally, 500072 Hyderabad, India
b Department of Chemistry, MNR Degree and PG College, Kukatpally, 500072 Hyderabad, India

Relatamos algumas moléculas híbridas baseadas na N-(quinolina-3-ilmetileno)benzoidrazida, cuja síntese foi realizada usando-se 2-cloroquinolina-3-carbaldeído e uma variedade de hidrazidas substituídas, em PEG 400. O solvente PEG 400 foi recuperado e reutilizado diversas vezes na presente reação, mostrando-se eficiente em termos de rendimento dos produtos. Alguns dos compostos sintetizados mostraram atividade citotóxica significativa quando testados in vitro.

We report some hybrid molecules based on N-(quinoline-3-ylmethylene)benzohydrazide template the synthesis of which was carried out using 2-chloroquinoline-3-carbaldehyde and a variety of substituted hydrazides in PEG 400. The “green” solvent PEG 400 was recovered and reused for several times in the present reaction and was found to be effective in terms of product yield. Some of the compounds synthesized showed significant cytotoxic activity when tested in vitro.

Keywords: 2-chloroquinoline-3-carboxaldehyde, acylhydrazone, PEG 400, cytotoxicity

Introduction

A current rational approach of drug design characterized as “covalent bitherapy” involves linking of two molecules possessing individual inherent activity into a single agent, thus incorporating dual activity into a single hybrid molecule. Contemporary research in this area seems to endorse hybrid molecules as the next-generation drug candidates.1

The quinoline scaffold is prevalent in a variety of pharmacologically active compounds.2 They also occur widely in nature indicating nature’s preference for this fragment and identifying it as one of the so-called privileged structures. Compounds based on the quinoline moiety joined to other pharmacophore are of considerable interest because of their potential pharmacological properties. Hydrazones and substituted hydrazones on the other hand, because of their distinctive structural features and presence of azomethine group, continue to attract the attention of the medical researchers.3,4 This class of compounds show an extensive range of pharmacological properties especially antitumor activities.

In the light of these observations, we became interested in the synthesis, characterization and evaluation of pharmacological activities of hybrid molecules related to N-acylhydrazones and quinolines. It was thought worthwhile to combine these two potential pharmacophores to generate a new hybrid class of molecules and evaluate them for their synergistic cytotoxic activities. The hybrid molecule A (Figure 1) was expected to be cytotoxic due to the presence of hydrazone moiety as well as quinoline structure. Indeed, the fragment –CO−NH−N=CH−(2-hydroxyphenyl) was found to be responsible for potent antiproliferative activities of benzo[d]isothiazole-3-carboxylic acid (4-methoxy-benzylidene)-hydrazide (B, Figure 1).6,7 This compound showed the most marked effects on the ovarian cancer cell line (OVCAR log GI50 value −5.51) when tested in vivo. Thus, the common structural features between A and B prompted us to evaluate the cytotoxic potential of A in vitro. While the synthesis of compounds related to A have been reported earlier,8,9 their utility as potential cytotoxic agents has not been explored.

In performing the majority of organic transformations, solvents play an important role in mixing the ingredients to make the system homogeneous and allow molecular
interactions to be more efficient. Recently, control of environmental pollution caused by chemical reactions has become a challenging as well as inspiring issue and many strategies and techniques have been explored to overcome these harmful events, for example, conducting reaction in dry medium or the use of micro wave irradiation, solid support, ionic liquid, water etc. In case of catalysis it is customary to measure the efficiency of a catalyst by its environmental impact, how easily it can be exposed, how many times it can be recycled in addition to its low volatility, non flammability, solubility etc.

The use of a simple and widely available polymer, e.g., polyethylene glycol (PEG) as a nontoxic, inexpensive, non-ionic liquid solvent of low volatility has been explored in several reactions. In general, PEG being a biologically acceptable polymer has been used extensively in drug delivery and in bioconjugates as tool for diagnostics. It was already used successfully in different reactions like oxidation, reduction, Michael reaction, Baylis-Hillman reaction, Heck coupling, Suzuki-Miyaura cross coupling, synthesis of bezimidazole, etc. It was also used in enantioselective asymmetric synthesis like asymmetric dihydroxylation, asymmetric aldol reaction, etc. In addition, aqueous PEG solutions may often be used as substitute for expensive and often toxic phase transfer catalysts (PTCs). All these attractive “green” advantages encouraged us to study the PEG as alternative reaction medium for our purpose. While the synthesis of similar compounds represented by general formula “A” has been reported earlier, their preparation using PEG 400 is not known. Herein, we report the use of PEG-400 as a recyclable reaction medium for the synthesis of compound A (or 3, Scheme 1) at room temperature without using any acid or base catalysts.

Results and Discussion

Chemistry

The key step for the synthesis of N-(quinoline-3-ylmethylene)benzohydrazides (3) involved the reaction of 2-chloro-3-quinoline carboxaldehyde (1) with an appropriate hydrazide (2). Thus, the key intermediate 1 required for our synthesis was prepared from acetonilide according to a similar procedure reported in the literature (Scheme 1). Cyclization of acetonilide in the presence of POCl₃ and N,N-dimethyl formamide (DMF) leads to the formation of the desired intermediate 1. The other reactant, i.e., hydrazide (2), was prepared via esterification of the corresponding carboxylic acids with methanol in the presence of catalytic amount of concentrated H₂SO₄ followed by treating the resulting ester with hydrazine hydrate in methanol. The compound 3 was finally prepared successfully by condensation of 1 and 2 in PEG 400. The earlier method for the preparation of this type of compounds, e.g., N′-(2-chloroquinolin-3-yl)methylen)benzohydrazide, involved heating the appropriate reactants in ethanol for several hours. However, to establish a milder and environmentally friendly condition, we examined the reaction of 2-chloro-3-quinoline carboxaldehyde (1) with 2-methoxybenzohydrazide (2a) in various solvents at room temperature. The results of this study are summarized in Table 1. As indicated in Table 1 that no desired product (3a) was isolated when the reaction was performed in solvents such as benzene, chloroform and dichloromethane (entries 1, 2 and 3, Table 1), even after 48 h whereas only 37% of 3a was formed when iso-propanol was used as solvent (entry 4, Table 1). While the reaction time was reduced from 48 to 7 h when EtOH was used as a solvent (entry 5, Table 1), the best yield of 3a, however, was obtained in PEG 400 or 1,4-dioxane (entry 6 and 7, Table 1). Interestingly, the work-up procedure was found to be simple when PEG 400 was used. After completion of the reaction, the mixture was extracted with diethyl ether and the pure product 3a was isolated simply after purification via crystallization. The PEG 400 recovered was recycled without further purification. We have reused the recovered PEG 400 in the reaction of 1 with 2 at least for three times and 3a was isolated each time almost in similar yield. Thus, we decided...
to choose PEG 400 as a “green” and recyclable solvent for our further studies. Notably, no reaction was observed when water was used as a solvent (entry 8, Table 1) perhaps due to the poor solubility of reactants in water.

Table 1. Effect of solvent on the reaction of 2-chloro-3-quinoline carboxaldehyde (1) with benzohydrazide (2a)

| entry | solvent     | time / h | yield (%) |
|-------|-------------|----------|-----------|
| 1     | C2H5        | 48       | 0         |
| 2     | CHCl3       | 48       | 0         |
| 3     | DCM         | 48       | 0         |
| 4     | i-PrOH      | 48       | 37        |
| 5     | EtOH        | 7        | 64        |
| 6     | PEG 400     | 6        | 85 (83, 80, 79) |
| 7     | 1,4-Dioxane | 6.5      | 88        |
| 8     | Water       | 17       | 0         |

*a* All the reactions were performed using 1 (1.0 mmol) and 2a (1.0 mmol) in a solvent at room temperature under an open air condition. *b* Isolated yield. *c* Yields of 3a in recycled PEG 400 for 1st, 2nd and 3rd time.

Having identified PEG 400 as a suitable and green solvent for the efficient preparation of 3a via the reaction of 1 and benzohydrazide (2a) at room temperature, we decided to examine the generality of this process in the preparation of other N- (quinoline-3-ylmethylene) benzohydrazide derivatives. Accordingly, a variety of functionalized benzohydrazides were reacted with 1 in PEG 400 at room temperature (Table 2). As evident from Table 2, the reactions proceeded well in all these cases, providing the desired products in good yields. The presence of electron donating, e.g., OMe (entry 1, Table 2), phenolic OH (entry 3, Table 2) or chloro (entry 8, Table 2), and electron withdrawing group, e.g., NO2 (entries 2 and 6, Table 2) present at the aryl ring of hydrazide 2, were well tolerated. A hydrazide 2d (entry 4, Table 2) prepared from a well known anti-inflammatory drug, mfenamic acid, also participated well in the present reaction and provided the expected product 3d in good yield. Apart from benzohydrides, we examined the reactivity of two 2-aryloxy acetoxyhydrazides in the present reaction both of which provided the corresponding products in good yields (entries 5 and 7, Table 2). All the compounds synthesized were well characterized by spectral (1H NMR, IR and MS) data. The presence of C=O and C=N groups were indicated by the appearance of stretching frequencies in the range of 1700-1650 and 1650-1590 cm⁻¹, respectively, in the IR spectra of compounds 3. Moreover, based on the earlier report that N-acylhydrazones derived from aromatic aldehydes in solution remained in the E form, because of the hindered rotation on the imine bond, we considered E-geometry in our cases. However, in few cases, e.g., for compounds 3e and 3g, the presence of a mixture of rotameric forms was detected (see the experimental section).

**Pharmacology**

To assess the cytotoxic activity potential of this class of compounds some of the compounds synthesized were tested against human lung adenocarcinoma cell line (A549) *in vitro*. Adenocarcinoma is the most common type of lung cancer that contains certain distinct malignant tissue architectural, cytological, or molecular features, including gland and/or duct formation and/or production of significant amounts of mucus. Adenocarcinomas account for approximately 40% of lung cancers. Adenocarcinoma is not as responsive to radiation therapy and is rather treated by surgically, for example by pneumonectomy or lobectomy. Thus small molecules that are active against human lung adenocarcinoma cell line may be useful for the development of potential agents to treat lung cancer. With this objective we conducted *in vitro* screen of compounds 3a-i and the results are summarized in Table 3. Since acylhydrazones may undergo hydrolysis to release toxic entities hence blank experiments were also carried out to confirm that the observed activity was due to the parent compounds but not their hydrolyzed products. All the compounds were tested at five different concentrations ranging from 1.0 to 25 µg mL⁻¹. At the concentration of 25 µg mL⁻¹ all the compounds showed significant inhibition and most of them showed reasonable dose responses across all the doses. The compounds 3b, 3c, 3d and 3h showed good inhibition at 25 µg whereas low to moderate inhibition was observed for compounds 3d, 3g, 3h and 3i at the lowest concentration, i.e., 1.0 µg tested. The other compounds were found to be either inactive or less effective at low concentrations. A known compound, i.e., etoposide (IC50 = 9.85 µmol L⁻¹) was used as a reference compound in this assay that showed 100% inhibition when tested at 25 µg mL⁻¹.

It is known that a number of acylhydrazone derivatives that showed activities in standard growth inhibition assays, were found to inhibit tubulin polymerization (which is the most probable primary mechanism of the action of these compounds). Tubulin-containing structures are important for diverse cellular functions, including chromosome segregation during cell division, intracellular transport, development and maintenance of cell shape, cell motility, and possibly distribution of molecules on cell membranes.
Table 2. Preparation of N-(quinoline-3-ylmethylene)benzohydrazide derivatives in PEG 400\textsuperscript{a}

| entry | benzohydrazide (2) | products (3) | time / h | yield\% |
|-------|--------------------|--------------|----------|----------|
| 1     | CONHNH₂OMe      | 2a          |          |          |
|       |                    | CONHNH₂     | 3a       | 6        | 85       |
| 2     | CONHNH₂NO₂      | 2b          |          |          |
|       |                    | CONHNH₂     | 3b       | 2        | 80       |
| 3     | CONHNH₂OH       | 2c          |          |          |
|       |                    | CONHNH₂     | 3c       | 4        | 82       |
| 4     | CONHNH₂        | 2d          |          |          |
|       |                    | CONHNH₂     | 3d       | 4        | 78       |
| 5     | CONHNH₂OCl₂     | 2e          |          |          |
|       |                    | CONHNH₂     | 3e       | 2        | 79       |
| 6     | CONHNH₂NO₂Cl₂  | 2f          |          |          |
|       |                    | CONHNH₂     | 3f       | 3        | 76       |
| 7     | OCH₂CONHNH₂     | 2g          |          |          |
|       |                    | CONHNH₂     | 3g       | 4.5      | 87       |
| 8     | CONHNH₂Cl       | 2h          |          |          |
|       |                    | CONHNH₂     | 3h       | 6        | 77       |
| 9     | CONHNH₂        | 2i          |          |          |
|       |                    | CONHNH₂     | 3i       | 2.5      | 75       |

\textsuperscript{a}All the reactions were performed using 1 (1.0 mmol) and 2 (1.0 mmol) in PEG 400 at room temperature under an open air condition. Isolated yield.
A “Green” Synthesis of N-(Quinoline-3-ylmethylene)benzohydrazide Derivatives

J. Braz. Chem. Soc.

The drugs that interact with tubulin cause its precipitation and sequestration to interrupt many important biologic functions that depend on the microtubular class of subcellular organelles. Thus, cytotoxicity shown by the present series of compounds (3) could be due to their covalent binding with β-tubulin thereby inhibiting the tubulin polymerization.

Conclusions

In conclusion, we have described the synthesis, and in vitro pharmacological properties of a number of novel N-(quinoline-3-ylmethylene)benzohydrazide derivatives. Syntheses of these compounds were carried out using 2-chloroquinoline-3-carboxaldehyde and a variety of substituted hydrazides under a mild reaction conditions. PEG 400 was identified as a green, effective and recyclable solvent for our synthesis. Some of the compounds synthesized showed significant cytotoxicity when tested against human lung adenocarcinoma cell line (A549) in vitro. Since quinoline and benzohydrazide derivatives have medicinal value, hence we believe that the present class of hydrazide derivatives represents an interesting profile for further experimental investigations especially in the area of anticancer research.

Experimental

General methods

Melting points were all determined by open glass capillary method on a Cintex melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer spectrometer in KBr pellets. 1H NMR spectra were recorded on a Bruker ACF-300 machine or a Varian 300 MHz spectrometer using DMSO-d6 as a solvent with tetramethylsilane as internal reference (TMS, δ = 0.00). 13C NMR spectra were recorded on a Bruker ACF-300 machine using either TFA (trifluoroacetic acid) or DMSO as a solvent. Chemical shift values of rotameric hydrogens whenever identified are presented within the parenthesis by assigning asterisk (*) mark along with that of other form. Elemental analyses were performed by Varian 3LV analyzer series CHN analyzer. Mass spectra were recorded on a Jeol JMCID-300 instrument. All solvents used were commercially available and distilled before use. All reactions were monitored by TLC on pre-coated silica gel plates (60 F 254; Merck). Column chromatography was performed on 100-200 mesh silica gel (SRL, India) using 10-20 fold excess (by weight) of the crude product. The organic extracts were dried over anhydrous Na2SO4.

The percentage of ap rotamer present was calculated by using the following formula

% of ap rotamer = (Iap / Iap + Isp) × 100

Iap = intensity of a singlet appeared in the 1H NMR of ap rotamer

Isp = intensity of the corresponding singlet appeared in the 1H NMR of sp rotamer

Preparation of 2-chloro-3-quinoline carboxaldehyde (1)

To an ice-cold solution of dimethylformamide (7.20 mL, 93.0 mmol) was added phosphorus oxychloride (24.0 mL, 260 mmol) dropwise and the mixture was stirred at 0 ºC for 45 min. To this mixture was added acetanilide (5.0 g, 37.0 mmol). The mixture was stirred initially at 0 ºC for 30 min and then heated at 75 ºC for 8 h. The mixture was cooled, poured into ice-water (300 mL) and stirred at 0-5 ºC for 45 min. The solid separated was filtered, washed with cold water (150 mL), dried and recrystallized from ethyl acetate to give the title compound as a light yellow solid (4.8 g, 68%); mp 146-147 ºC (lit27 148-149 ºC).

Table 3. In vitro MTT assay results of some of the N-(quinoline-3-ylmethylene)benzohydrazide derivatives

| entry | compounds | % of inhibition at various concentrationsa |
|-------|-----------|-------------------------------------------|
|       |           | 1 µg mL⁻¹ | 2 µg mL⁻¹ | 5 µg mL⁻¹ | 10 µg mL⁻¹ | 25 µg mL⁻¹ |
| 1     | 3a        | 8.00      | 12.61     | 27.38     | 31.69     | 43.38      |
| 2     | 3b        | 14.56     | 23.85     | 46.27     | 54.78     | 78.90      |
| 3     | 3c        | 17.86     | 23.52     | 30.12     | 67.89     | 89.32      |
| 4     | 3d        | 20.78     | 36.54     | 56.83     | 68.90     | 88.72      |
| 5     | 3e        | 10.30     | 16.30     | 18.46     | 19.07     | 20.92      |
| 6     | 3f        | 0         | 9.53      | 12.61     | 23.07     | 53.53      |
| 7     | 3g        | 21.53     | 22.61     | 27.38     | 31.69     | 43.38      |
| 8     | 3h        | 22.56     | 37.83     | 45.56     | 67.13     | 79.55      |
| 9     | 3i        | 26.15     | 29.83     | 32.61     | 38.15     | 66.76      |

aThe data presented are the average of three separate experiments. Etoposide (IC₅₀ = 9.85 µmol L⁻¹) was used as a reference compound.
General procedure for the synthesis of 3a-k

To a cold solution of 2-chloroquinoline-3-carboxaldehyde (2.07 g, 0.011 mol) in PEG 400 (5.0 mL) was added an appropriate hydrazide (0.01 mol) and the solution was stirred vigorously at room temp for the time indicated in Table 2. The progress of the reaction was monitored by TLC. After completion of the reaction the mixture was diluted with diethyl ether (25 mL), stirred for 10 min and then allowed to settle. The ether layer separated was collected, washed with cold water (2 × 15 mL), dried over anhydrous Na₂SO₄, filtered and concentrated. The crude compound isolated by re-crystallization (EtOH) to furnish the desired product.

The PEG 400 layer was collected and recycled in the next reaction.

N’-((2-Chloroquinolin-3-yl)methylene)-2-methoxybenzo-hydrazide (3a)

Light orange solid; yield: 2.88 g (85%); mp 142-144 °C (EtOH); Rf = 0.45 (ethyl acetate:hexane, 2:3); MS m/z 339 (M+100%), 341 (M+2) 3:1 ratio; IR (KBr) ν max/cm⁻¹: 3432, 3266, 2956, 1676, 1649, 1607, 1578; ¹H NMR (DMSO-d₆, 300 MHz) δ 12.10 (1H, NH, s, D,O exchangeable), 9.00 (s, 1H, –CH=N–), 8.90 (s, 1H, 4-H), 8.25 (d, 1H, J 8.1 Hz, ArH), 8.00 (m, 3H, ArH), 7.90 (t, 1H, J 8.1 Hz, ArH), 7.70 (t, 2H, J 7.8 Hz, ArH). Elemental analysis found: C 63.63, H 4.15, N 12.37%.

N’-((2-Chloroquinolin-3-yl)methylene)-4-nitrobenzo-hydrazide (3b)

Yellow solid; yield: 2.83 g (80%); mp 258-260 °C (EtOH); Rf = 0.65 (ethyl acetate:hexane, 2:3); MS m/z 354 (M+100%), 356 (M+2) 3:1 ratio; IR (KBr) ν max/cm⁻¹: 3437, 3181, 2853, 1670, 1617, 1597, 1523; ¹H NMR (DMSO-d₆, 300 MHz) δ 12.50 (s, 1H, NH, D,O exchangeable), 9.05 (s,1H, –CH=N–), 8.95 (s,1H, 4-H), 8.40 (d, 2H, J 8.7 Hz, ArH), 8.30 (m, 3H, ArH), 8.00 (d, 1H, J 8.4 Hz, ArH), 7.90 (t,1H, J 6.9 Hz, ArH), 7.70 (t,1H, J 7.5 Hz, ArH). Elemental analysis found: C 57.33, H 3.19, N 15.58; C₁₆H₁₄ClN₂O₃ requires C 57.56, H 3.13, N 15.79%.

N’-((2-Chloroquinolin-3-yl)methylene)-2-hydroxybenzo-hydrazide (3c)

Off white solid; yield: 2.67 g (82%); mp 158-160 °C (EtOH); Rf = 0.36 (ethyl acetate:hexane, 2:3); MS m/z 325 (M+100%), 327 (M+2) 3:1 ratio; IR (KBr) ν max/cm⁻¹: 3196, 3051, 1669, 1590, 1556; ¹H NMR (DMSO-d₆, 300 MHz) δ 12.20 (s, 1H, NH, D,O exchangeable), 11.75 (s,1H, OH, D,O exchangeable), 9.00 (s,1H, –CH=N–), 8.95 (1H, s, 4-H), 8.45 (d,1H, J 7.5 Hz, ArH), 8.00 (d,1H, J 8.4 Hz, ArH), 7.90 (t, 2H, J 7.2 Hz, ArH), 7.70 (t,1H, J 7.5 Hz, ArH), 7.50 (t, 1H, J 7.2 Hz, ArH), 7.00 (t, 2H, J 7.8 Hz, ArH). Elemental analysis found: C 62.45, H 3.67, N 12.66; C₁₆H₁₄ClN₂O₂ requires C 62.68, H 3.71, N 12.90%.

N’-((2-Chloroquinolin-3-yl)methylene)-2-(2,3-dimethylphenylamino)benzohydrazide (3d)

Yellow solid; yield: 3.34 g (78%); mp 218-220 °C (EtOH); Rf = 0.40 (ethyl acetate:hexane, 2:3); MS m/z 428 (M+100%), 430 (M+2) 3:1 ratio; IR (KBr) ν max/cm⁻¹: 3309, 3213, 3039, 2920, 1674, 1629, 1616, 1577; ¹H NMR (DMSO-d₆, 300 MHz) δ 12.30 (3s, 1H, NH, D,O exchangeable), 9.40 (s,1H, NH, D,O exchangeable), 8.95 (s,1H, –CH=N–), 8.90 (1H, s, 4-H), 8.25 (d,1H, J 8.4 Hz, ArH), 8.08-7.70 (4H, ArH, m), 7.35 (t,1H, J 7.2 Hz), 7.10-6.85 (m, 5H, ArH), 2.30 (s, 3H, CH₃), 2.10 (s, 3H, CH₃); ¹C NMR (TFA, 75 MHz) δ 168.3, 148.8, 148.1, 145.5, 143.1, 141.1, 140.3, 137.8, 137.6, 133.3, 133.2, 132.8, 131.9, 131.6, 130.6, 129.2, 128.9, 128.8, 127.9, 126.5, 126.4, 123.4, 120.7, 19.9, 13.5. Elemental analysis found: C 70.25, H 4.74, N 13.14; C₁₆H₁₄ClN₂O requires C 70.01, H 4.93, N 13.06%.

N’-((2-Chloroquinolin-3-yl)methylene)-2-(3,5-dichlorophenoxy)acetohydrazide (3e)

White solid; yield: 3.22 g (79%); mp 198-200 °C (EtOH); Rf = 0.62 (ethyl acetate:hexane, 2:3); MS m/z 408 (M+100%); IR (KBr) ν max/cm⁻¹: 3192, 3113, 2978, 1685, 1635, 1616, 1583; ¹H NMR (DMSO-d₆, 300 MHz) δ 12.10 (12.00*, s, 1H, NH, D,O exchangeable), 9.03 (8.98*, s, 1H, –CH=N–), 8.70 (8.46*, s,1H, ArH), 8.22 (8.13*, d,1H, J 12 Hz, ArH), 7.98 (d,1H, J 8 Hz, ArH), 7.88 (t,1H, J 8 Hz, ArH), 7.70-7.34 (m, 3H, ArH), 7.20 (m,1H, ArH), 5.20 (s, 2H, OCH₂) (ap and sp rotamer ratio 75:25, h ap = 0.67, Iap/Isp = 0.22, % of ap = (0.67/0.67 + 0.22) × 100 = 75); ¹¹C NMR (DMSO-d₆, 75 MHz) δ 165.8, 162.0, 161.4, 158.1, 145.3, 133.2, 132.0, 130.7, 129.7, 128.7 (2C), 125.4, 121.1, 118.5, 113.7 (2C), 113.6, 55.3. Elemental analysis found: C 52.67, H 2.71, N, 10.41; C₁₆H₁₄Cl₂N₂O₂ requires C 52.90, H 2.96, N 10.28%.

N’-((2-Chloroquinolin-3-yl)methylene)-2,4-dinitrobenzo-hydrazide (3f)

Yellow solid; yield: 3.03g (76%); mp 142-144 °C (EtOH); Rf = 0.6 (ethyl acetate:hexane, 2:3); MS m/z 399 (M+100%), 401 (M+2) 3:1 ratio; IR (KBr) ν max/cm⁻¹: 3414, 3250, 2916, 1665, 1630, 1619, 1582, 1563; ¹H NMR (DMSO-d₆, 300 MHz) δ 12.40 (1H, NH, s, D,O exchangeable), 8.69 (1H, s, –CH=N–), 8.68 (s,1H, 4-H), 8.26 (d,1H, J 8 Hz, ArH), 8.00-7.86 (m, 2H, ArH), 7.72
N’-(2-Chloroquinolin-3-yl)methylene)-2-phenoxyacetohydrazide (3g)

Off white solid; yield 2.96 g (87%); mp 168-170 °C (EtOH); Rf = 0.86 (ethyl acetate:hexane, 2:3); MS m/z 340 (M+100%); IR (KBr) νmax/cm⁻¹: 3279, 3194, 2939, 1681, 1614, 1597, 1585; ¹H NMR (DMSO-d₆, 300 MHz) δ 12.10 (12.00*, s, 1H, NH, DClN), 9.02 (8.99*, s, 1H, =CHN), 8.85 (8.46*, s, 1H, ArH), 8.22 (8.18*, m, 1H, ArH), 7.98 (d, 1H, J 8 Hz, ArH), 7.84 (m, 1H, ArH), 7.70 (m, 1H, ArH), 7.52 (m, 2H, ArH), 7.00 (m, 3H, ArH), 5.23 (4.70*, s, 2H, CH=N) (ap and sp rotameric ratio 54:46, Iₙ = 0.60, Iₚ = 0.50, % of ap = (0.60/0.60 + 0.50) × 100 = 54). Elemental analysis found: C 51.26, H 2.29, N 17.39; C₁₂H₁₀ClIN₂O₃ requires C 51.08, H 2.52, N 17.52%.

4-Chloro-N’-(2-chloroquinolin-3-yl)methylene)benzohydrazide (3h)

Off white solid; yield 2.65 g (77%); mp 268-270 °C (EtOH); Rf = 0.52 (ethyl acetate:hexane, 2:3); MS m/z 344 (M+100%) 346 (M+2) 3:1 ratio; IR (KBr) νmax/cm⁻¹: 3174, 3053, 2902, 1651, 1618, 1593, 1552; ¹H NMR (DMSO-d₆, 300 MHz) δ 12.40 (s, 1H, NH, DClN), 8.95 (s,1H, –CH=N–), 8.94 (s,1H, 4-H), 8.25 (d,1H, J 8.1 Hz, ArH), 8.00 (m, 3H, ArH), 7.85 (t, 1H, J 6.9 Hz, ArH), 7.76-7.65 (m, 3H). Elemental analysis found: C 59.51, H 3.90; C₁₂H₁₀ClIN₂O₃ requires C 59.32, H 3.22, N 12.21%.

N’-(2-Chloroquinolin-3-yl)methylene)benzohydrazide (3i)

White solid; yield 2.32 g (75%); mp 208-210 °C (EtOH) (lit² 202-205 °C); Rf = 0.71 (ethyl acetate:hexane, 2:3); MS m/z 309 (M+100%) 311 (M+2) 3:1 ratio; IR (KBr) νmax/cm⁻¹: 3748, 3182, 2923, 1698, 1618, 1600, 1563; ¹H NMR (DMSO-d₆, 300 MHz) δ 12.30 (s, 1H, NH, D₂O exchangeable), 8.98 (s, 1H, –CH=N–), 8.97 (s,1H, 4-H), 8.25 (d,1H, J 8.1 Hz, ArH), 7.98 (m, 3H, ArH), 7.86 (m, 1H, ArH), 7.74-7.57 (m, 4H, ArH). Elemental analysis found: C 63.34, H 4.08, N 12.30; C₁₂H₁₀ClIN₂O requires C 63.63, H 4.15, N 12.37%.

Biological assay

Chemical and reagents: Dulbecco’s modified eagle medium (DMEM), L-glutamine, streptomycin and penicillin were obtained from Sigma-Aldrich, USA. Foetal bovine serum was procured from PAA Biotech, Germany. All other fine chemicals/reeagents used in this study were of cell culture grade and obtained from Sigma-Aldrich and/or Merck.

Cell line and culture conditions: A549 (human lung adenocarcinoma cell line) was obtained from National Centre for Cell Science, Pune, India. The cells were grown in DMEM culture medium supplemented with 2 mmol L⁻¹ L-glutamine, 10% FBS, penicillin (50 IU per mL) and streptomycin (50 µg mL⁻¹) at a temperature of 37 °C in a humidified incubator with a 5% CO₂ atmosphere.

MTT assay for cytotoxicity: The viability of the cells was assessed by MTT (3, 4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Fifteen cells (1 × 10⁴) were plated in a 96-well plate. After 24 h, they were treated with different concentration (0-10 µg mL⁻¹) of different test compounds diluted appropriately with culture media for 48 h. Cells grown in media containing equivalent amount of DMSO served as positive control and cells in medium without any supplementation were used as negative control. After the treatment, media containing compound were carefully removed. 100 µL of 0.4 mg mL⁻¹ MTT in PBS was added to each well and incubated in the dark for 4 h. 100 µL of DMSO was added to each well and kept in an incubator for 4 h for dissolution of the formed formazan crystals. Amount of formazan was determined by measuring the absorbance at 540 nm using an ELISA plate reader. The data were presented as percent post treatment recovery (% of live cells), whereas the absorbance from non-treated control cells was defined as 100% live cells.

Supplementary Information

Supplementary information (copies of ¹H NMR spectra of all the compounds synthesized) is available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgments

The author (S. Pal) thanks Mr. M. N. Raju, the chairman of M. N. R. Educational Trust for his constant encouragement.

References

1. Muregi, F. W.; Ishih, A.; Drug Dev. Res. 2010, 71, 20.
2. Kaur, K.; Jain, M.; Reddy, R. P.; Jain, R.; Eur. J. Med. Chem. 2010, 45, 3245.
3. Tributino, J. L. M.; Duarte, C. D.; Corrêa, R. S.; Dorigueto, A. C.; Ellena, J.; Romeiro, N. C.; Castro, N. G.; Miranda, A. L. P.; Barreiro, E. J.; Fraga, C. A. M.; Bioorg. Med. Chem. 2009, 17, 1125.

4. Zheng, L.-W.; Wu, L.-L.; Zhao, B.-X.; Dong, W.-L.; Miao, J.-Y.; Bioorg. Med. Chem. 2009, 17, 1125.

5. Vera-DiVaio, M. A. F.; Freitas, A. C. C.; Castro, H. C.; de Albuquerque, S.; Cabral, L. M.; Rodrigues, C. R.; Albuquerque, M. G.; Martins, R. C. A.; Henriques, M. G. M. O.; Dias, L. R. S.; Bioorg. Med. Chem. 2009, 17, 295.

6. Vicini, P.; Incerti, M.; Doytchinova, I. A.; Colla, P. L.; Busonera, B.; Loddo, R.; Eur. J. Med. Chem. 2006, 41, 624.

7. Terzioğlu, N.; Gürsoy, A.; Eur. J. Med. Chem. 2003, 38, 781.

8. Dubey, P. K.; Rao, S. S.; Reddy, P. V. P.; Heterocycl. Commun. 2003, 9, 411.

9. Khalil, M. A.; El-Sayed, O. A.; El-Shamy, H. A.; Archiv der Pharmacie 1993, 326, 489.

10. Haimov, A.; Neumann, R.; Chem. Commun. 2002, 876.

11. Santaniello, E.; Fiecchi, A.; Manzocchi, A.; Ferraboschi, P.; J. Org. Chem. 1983, 48, 3074.

12. Kumar, R.; Chaudhary, P.; Nimesh, S.; Chandra, R.; Green Chem. 2006, 8, 356.

13. Chandrasekhar, S.; Narshimu, ch.; Saritha, B.; Sultana, S. S.; Tetrahedron Lett. 2004, 45, 5865.

14. Chandrasekhar, S.; Narshimu, C.; Sultana, S. S.; Reddy, N. R.; Org. Lett. 2002, 4, 4399.

15. Liu, L.; Zhang, Y.; Wang, Y.; J. Org. Chem. 2005, 70, 6122.

16. Kidwai, M.; Jahan, A.; Bhatnagar, D.; J. Chem. Sci. 2010, 122, 607.

17. Chandrasekhar, S.; Narshimu, Ch.; Sultana, S. S.; Reddy, N. R.; Chem. Commun. 2003, 1716.

18. Chandrasekhar, S.; Narshimu, C.; Reddy, N. R.; Sultana, S. S.; Tetrahedron Lett. 2004, 45, 4581.

19. Sondhi, S. M.; Dinodia, M.; Jain, S.; Kumar, A.; Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem. 2009, 48, 1128.

20. Palla, G.; Predieri, G.; Domiano, P.; Tetrahedron 1986, 42, 3649.

21. Palmer, R. B.; Andersen, N. H.; Bioorg. Med. Chem. Lett. 1996, 6, 2173.

22. Subramanian, J.; Govindan, R.; J. Clin. Oncol. 2007, 25, 561.

23. Zhang, H.; Drewe, J.; Tseng, B.; Kasibhatla, S.; Cai, S. X.; Bioorg. Med. Chem. 2004, 12, 3649.

24. Datta, N. J.; Khunt, R. C.; Parikh, A. R.; J. Inst. Chem. 2000, 72, 133.

25. Borchhardt, D. M.; Mascarello, A.; Chiaradia, L. D.; Nunes, R. J.; Oliva, G.; Yunes, R. A.; Andricopulo, A. D.; J. Braz. Chem. Soc. 2010, 21, 142.

26. Reddy, L. V.; Suman, A.; Beevi, S. S.; Mangamoori, L. N.; Mukkanti, K.; Pal, S.; J. Braz. Chem. Soc. 2010, 21, 98.

27. Meth-Cohn, O.; Narhe, B.; Tarnowski, B.; J. Chem. Soc., Perkin Trans. 1 1981, 1520.

Submitted: January 14, 2011
Published online: June 21, 2011