J. Chem. Sci. Vol. 124, No. 3, May 2012, pp. 625–631.

Synthesis of some new 2-oxo-1,4-disubstituted-1,2,5,6-tetrahydro-benzo[h]quinoline-3-carbonitriles and their biological evaluation as cytotoxic and antiviral agents

HASSAN M FAIDALLAH*, KHALID A KHAN and ABDULLAH M ASIRI
Department of Chemistry, Faculty of Science, King Abdulaziz University,
P.O. Box 80203 Jeddah 21589, Saudi Arabia
e-mail: hfaidallahm@hotmail.com

MS received 28 February 2011; revised 26 August 2011; accepted 22 September 2011

Abstract. A series of new 2-oxo-1,4-disubstituted-1,2,5,6-tetrahydro-benzo[h]quinoline-3-carbonitriles supported with various pharmacophoric functionalities at position-1 is described. N-formyl, N-nitroso, N-arylthiocarbamoyl, N-alkyl, N-sulfonyl, and N-acetyl derivatives, **5–10** were prepared. A novel fused triazole series was also prepared. The *in vitro* anticancer activity of twenty synthesized compounds, **4a, 4b, 7a2, 7b2** and **8b1** showed considerable cytotoxic activity against the three tested human tumor cell lines. Compounds **7a2** and **7b2** proved to be the most active with special effect against the human colon carcinoma HT29 and human breast cancer MCF 7 cell lines. Compounds **7b2** and **7f2** also exhibited significant antivirus activity against hepatitis-C virus.

Keywords. Synthesis; tetrahydrobenzo[h]quinolines; [1,2,4]triazolo[4,3-a]quinolines; cytotoxic agents; antiviral agents.

1. Introduction

During our ongoing studies aimed at the discovery of new structure leads endowed with diverse chemotherapeutic activities,[1–9] much concern has been given to the antimicrobial and antitumor potentials of some pyridines,[10–12] particularly 3-cyano-4,6-disubstituted-2(1*H*)-pyridinones. Some of these compounds were selected by the NCI to be evaluated for their antitumor potentials, where they exhibited promising broad-spectrum antitumor activity against several subpanel tumor cell lines. The results prompted further structure modification of the disubstituted-2(1*H*)-pyridinone scaffold by increasing compounds' lipophilicity via the synthesis of new 1,2,5,6-tetrahydrobenzo[h]quinoline analogs. The target compounds were substituted with formyl, acetyl, nitroso, benzenesulfonyl, thioureido and alkyl groups at position-1 that are believed to be responsible for the biological activity modulation in some relevant anticancer agents. The substitution profile of the main hexahydroquinoline ring was attempted to comprise some biologically active counterparts such as the thienyl and substituted phenyl rings, together with other pharmacophoric groups that would confer different electronic, lipophilic and steric environment. Moreover, it was considered worthwhile to utilize the *N*-acetyl derivatives as precursors for the synthesis of the fused-ring system triazolo[3,4-*a*]pyridine as an interesting structural variation, hoping to improve the anticipated chemotherapeutic activity.

Motivated by these facts, and in continuation of our interest in studying the synthesis and anticancer activities of 1,2,5,6-tetrahydrobenzo[h]quinoline analogs it was also considered of interest to confirm the ability of these synthesized compounds to inhibit the replication of the Hepatitis C virus (HCV).

2. Experimental

2.1 *General*

Melting points were determined on a Gallenkamp melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as an internal standard and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: *s*: singlet; *d*: doublet;

*For correspondence

m: multiplet; *q*: quartet. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254. The chalcones **3a–g** were prepared according to a reported procedure.[10] Physicochemical and analytical data of all compounds are listed in a table S1. ^{1}H NMR and ^{13}C NMR spectral data recorded are given in tables S2 and S3, respectively as supplementary data.

2.2 *3-Cyano-2-oxo-4-substituted-1,2,5,6-tetrahydrobenzo[h]quinolines **4a–f***

Method A: A mixture of the appropriate chalcone **3** (10 mmol), ethyl cyanoacetate (1.1 g, 10 mmol) and ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (30 m) was heated under reflux for 8 h. After being cooled to room temperature, the solid product formed was filtered, washed with water, dried and recrystallized from the DMF containing few drops of water.

Method B: A one-pot mixture of the appropriate aldehyde **2** (10 mmol), 3,4-dihydro-2H-naphthalene-1-one **1** (1.46 g, 10 mmol), ethyl cyanoacetate (1.1 g, 10 mmol) and ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (50 ml) was refluxed for 6 h. The reaction mixture was allowed to cool, and the formed precipitate was filtered, washed with water, dried and recrystallized. IR (cm^{-1}): 3388–3263 (NH), 2226–2218 (CN), 1678–1672 (C=O).

2.3 *3-Cyano-1-formyl-2-oxo-4-substituted-1,2,5,6-tetrahydrobenzo[h]quinolines **5a–f***

A solution of the appropriate pyridinone **4** (3 mmol) in formic acid (5 ml) was heated under reflux for 3 h. The reaction mixture was poured on crushed ice (10 g) and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol as needles. IR (cm^{-1}): 2214–2208 (CN), 1676–1672 (C=O pyridone), 1665–1660 (C=O aldehyde).

2.4 *3-Cyano-1-nitroso-2-oxo-4-substituted-1,2,5,6-tetrahydrobenzo[h]quinolines **6a–f***

To an ice-cooled stirred solution of the appropriate starting material **4** (10 mmol) in acetic acid (15 ml), was added drop-wise, a solution of sodium nitrite (1.05 g, 15 mmol) in water (5 mL) over a period of 2 h. Stirring was maintained for further 2 h, and then the reaction mixture was left at room temperature overnight. The resulting solid product was filtered, washed with water, dried and recrystallized from ethanol as needles. IR (cm^{-1}): 2226–2215 (CN), 1673–1666 (C=O pyridone).

2.5 *3-Cyano-2-oxo-1-(substituted-thiocarbamoyl)-4-substituted-1,2,5,6-tertahydrobenzo[h] quinolines **7***

A mixture of appropriate starting compound **4** (10 mmol) and the appropriate isothiocyanate (11 mmol) in pyridine (10 ml) was heated under reflux for 6–8 h. After being cooled to room temperature, the reaction mixture was poured on ice cold water and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol as needles. IR (cm^{-1}): 2236–2222 (CN), 1675–1668 (C=O pyridone), 1237–1219 (C=S).

2.6 *3-Cyano-1,4-disubstituted-2-oxo-1,2,5,6-tetrahydrobenzo[h]quinolines **8a–f***

To a solution of the appropriate pyridone **4** (10 mmol) in pyridine (10 ml), was added the appropriate alkyl halide (10 mmol) and the mixture was heated under reflux for 3–4 h. The reaction mixture was allowed to attain room temperature, poured on ice cold water and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol. IR (cm^{-1}): 2230–2224 (CN), 1678–1672 (C=O pyridone).

2.7 *3-Cyano-2-oxo-1-(substituted-sulfonyl)-4-substituted-1,2,5,6-tetrahydrobenzo[h]quinolines **10 a–f***

A mixture of the appropriate start **4** (10 mmol) and appropriate sulfonyl chloride (10 mmol) in pyridine (10 ml) was heated under reflux for 4–6 h. After cooling to room temperature, the reaction mixture was poured on crushed ice and the separated solid product was filtered, washed with water, dried and recrystallized from ethanol. IR (cm^{-1}): 2236–2224 (CN), 1676–1668 (C=O pyridone), 1378–1372 and 1198–1172(SO_2).

2.8 *1-Acetyl-3-cyano-2-oxo-4-substituted-1,2,5,6-tetrahydrobenzo[h]quinolines **11a–f***

To a solution of the appropriate **4** (10 mmol) in acetic anhydride (10 ml), was added anhydrous sodium acetate (1.2 g, 15 mmol). The reaction mixture was refluxed for 4 h, cooled, and then poured on crushed

ice with vigorous stirring. The resulting solid product was filtered, thoroughly washed with water, dried and recrystallized from aqueous ethanol. IR (cm^{-1}): 2231–2218 (CN), 1727–1722 (C=O acetyl), 1674–1666 (C=O pyridone).

2.9 *4-Cyano-1-methyl-5-Substituted-6,7-diydro[1,2,4]triazolo[4,3-a]benzo[h]quinolines* ***12a–f***

A mixture of the appropriate 1-acetylpyridinone **11** (10 mmol) and hydrazine hydrate (0.9 g, 15 mmol) in ethanol (15 ml) was heated under reflux for 6–8 h. The reaction mixture was allowed to attain room temperature, poured on crushed ice and the precipitated solid product was filtered, washed with water, dried and recrystallized from DMF/water. IR (cm^{-1}): 2226–2218 (CN).

3. Biological evaluation

3.1 *In vitro MTT cytotoxicity assay*

All the procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of 10×10^3 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of the test compounds to give a final concentration of (100, 50, 25, 12.5, 3.125, 1.56, 0.78 μg/mL). DMSO was employed as a vehicle for dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HePG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mL penicillin potassium, 10,000 μg/mL streptomycin sulphate and 25 μg/mL amphotericin B), and 1% L-glutamine in 96-well flat bottom microplate at 37°C under 5% CO_2.

After 48 h of incubation, the medium was aspirated, 40 μL of MTT salt (2.5 μg/mL) were added to each well and incubated for further 4 h at 37°C under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 μL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm.[13,14] A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. The percentage change in viability was calculated according to the formula:

$$[\text{Reading of the sample}/(\text{Reading of the negative control} - 1)] \times 100$$

A probit analysis was carried for LC_{50} determinations using SPSS 11 program.

3.2 *In vitro effect on the replication of hepatitis-C virus in HCV-infected HepG2 hepatocellular carcinoma cell line*

Fifteen compounds were selected and tested by the Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Science and Technology Applications, Alexandria, Egypt.

3.2a *Cell Culture and RNA Extraction*: HepG2 cells were washed twice in EMEM media supplemented with 200 μM L-Glutamine, 100U Penicillin, 100 μg streptomycin and 25 μM HEPES buffer; *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (Bio Whittaker, USA). The cells were suspended in EMEM Culture media and then were left to adhere on polystyrene 6-well plates for 24 h in 37°C, 5% CO_2, 95% humidity incubator. The cells were washed twice from debris and dead cells using EMEM media and then infected with 2% HCV-infected serum in EMEM culture medium with 8% FBS. Each of the tested compounds was added at concentrations of 10, 25, 50, and 100 μg/mL. Positive and negative control cultures were included. After 96 h incubation another dose of the test compound was added and the cells were further incubated for another 96 h. The RNA was extracted following a method reported by El-Awady *et al.*[15] The positive strand and its replicating form (negative strand) of HCV were detected by RT-PCR using specific primers to the 5′-untranslated region of the virus.

4. Results and discussion

4.1 *Chemistry*

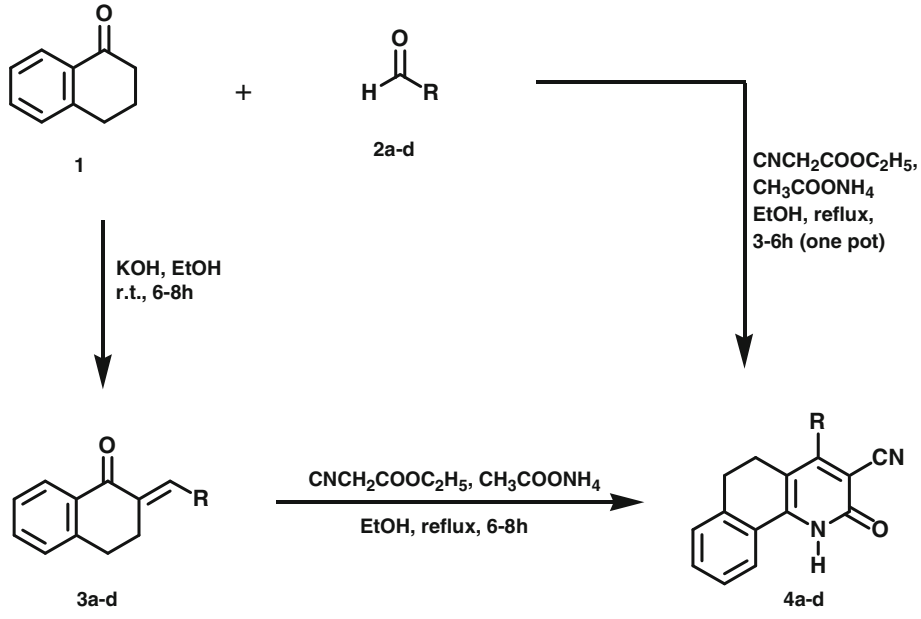
The synthetic strategies adopted for the preparation of the intermediate and target compounds are described in schemes 1 and 2. In scheme 1, TWO synthetic pathways were adopted to synthesize the target tetrahydrobenzo[h]quinolines **4** according to a reported

procedure.[10] The first method involved the formation of the 2-arylidene-3,4-dihydro-2H-naphthalen-1-one **3** (chalcones) via Claisen–Schmidt condensation of the appropriate aryl or heteroaryl aldehydes **2** with 3,4-dihydro-2H-naphthalene-1-one **1**. These chalcones **3a–d**, in their turn, were allowed to react with ethyl cyanoacetate and ammonium acetate to yield the target 2-oxo-1,4-disubstituted-1,2,5,6-tetrahydrobenzo[h]quinoline-3-carbonitriles **4a–d**. On the other hand, the same compounds **4** could be directly prepared via one-pot multicomponent reaction (MCR) of the 3,4-dihydro-2H-naphthalene-1-one **1,** the aldehydes **2**, an excess of ammonium acetate and ethyl cyanoacetate in boiling ethanol. Such type of reactions has received considerable interest since it is easier to perform, gives higher yields and less time consuming. Therefore, a comparison of the data obtained from the above-mentioned synthetic methods revealed that the one-pot reaction was better in terms of yield percentage. The IR spectra of the tetrahydrobenzo[h]quinolines **4a–d** exhibited absorption bands at 2248–2218 cm^{-1} and 1678–1665 cm^{-1} for the CN and CO groups, respectively. Their structure was further confirmed from their ^{1}H NMR which showed beside the aromatic protons two multiplets at δ2.26–2.88 and 2.54–2.90 ppm corresponding to the H-5 and H-6 respectively.

The tetrahydrobenzo[h]quinolines **4a–d** obtained from scheme 1 were utilized as key intermediates for the synthesis of the target compounds in the second part (scheme 2). In this respect, heating **4** with formic acid resulted in the formation of the *N*-formyl derivatives **5**. Whereas, reacting **4** with sodium nitrite in the presence of cold acetic acid yielded the *N*-nitroso derivatives **6**.

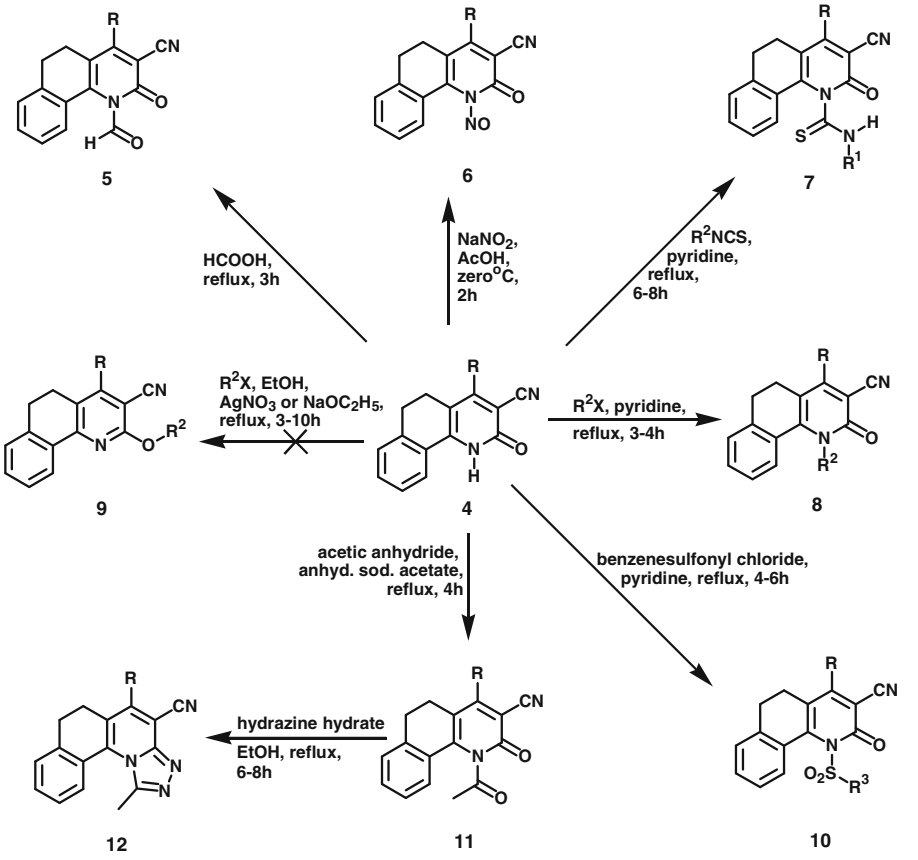
Condensation of **4** with the appropriate aryl isothiocyanate in alkaline medium afforded the corresponding *N*-arylthiocarbamoyl analogs **7**. The IR spectra of these compounds showed C=S absorption at 1240–1222 cm^{-1} as well as a C=O and CN absorptions in the regions of 1672–1667 and 2236–2218 cm^{-1}. The structures were further supported by ^{1}H NMR data. Furthermore, when compounds **4** were alkylated with the appropriate alkyl halide in the presence of sodium hydroxide, the targeted *N*-alkyl tetrahydrobenzo[h]quinolines **8** were formed, but in low yields. However, better yields were obtained when the reaction was carried out in pyridine as a basic solvent.

At this stage, it was thought of interest to study the effect of applying different alkylating conditions on such type of compounds. In this respect, compounds **4** were treated with the same alkyl halides in the presence of ethanolic silver nitrate[16] or sodium ethoxide[17,18] as basic catalysts in an attempt to obtain the *O*-alkyl derivatives **9**, nevertheless, such method failed to produce the targeted compounds. On the other hand, reacting compounds **4** with substituted sulfonyl



2, 3 and 4: R = **a**: C_6H_5; **b**: 4-BrC_6H_4; **c**: 4-$CH_3OC_6H_4$; **d**: 3,4-$(CH_3O)_2C_6H_3$; **e**: 3,4-$(OCH_2O)C_6H_3$; **f**: 2-thienyl

Scheme 1. Synthesis of 3-Cyano-2-oxo-4-substituted-1,2,5,6-tetrahydrobenzo[h]quinolines.



4-6,11 and 12: R = **a**: C_6H_5; **b**: 4-BrC_6H_4; **c**: 4-$CH_3OC_6H_4$; **d**: 3,4-$(CH_3O)_2C_6H_3$;**e**: 3,4-$(OCH_2O)C_6H_3$; **f**: 2-thienyl

7,8 and 10: R = **a1- a3**: C_6H_5; **b1- b3**: 4-BrC_6H_4; **c1- c3**: 4-$CH_3OC_6H_4$; **d1- d3**: 3,4-$(CH_3O)_2C_6H_3$; **e1- e3**: 3,4-$(OCH_2O)C_6H_3$; **f1- f3**: 2-thienyl

7a1-f1, $R^1 = C_6H_5$; **7a2-f2,** R^1 = 4-ClC_6H_4

8a1-f1, $R^2 = CH_3$; **8a2-f2,** $R^2 = C_2H_5$

10a1-f1, $R^3 = CH_3$; **10a2-f2,** $R^3 = C_6H_5$; **10a3-f3,** R^3 = 4-$CH_3C_6H_4$

Scheme 2. Synthesis of 3-Cyano-1,4-disubstituted-2-oxo-1,2,5,6-tetrahydrobenzo[h]quinolines.

chlorides in the presence of pyridine resulted in the introduction of a substituted sulfonyl moiety at position-1 to yield compounds **10**. Warming the starting compounds **4** with acetic anhydride in the presence of anhydrous sodium acetate afforded the *N*-acetyl derivatives **11**. In their turn, when compounds **10** were reacted with hydrazine hydrate, the targeted 5,6-dihydrobenzo[h][1,2,4]triazolo[3,4-a]quinolines **12** were successfully obtained.

4.2 *In vitro MTT cytotoxicity assay*

Twenty analogs namely; **4a–f, 5a–b, 6a–b, 7a1–a2, b2, 8a1–b1, f1, 10a1–b2, 11a–b,** and **12a** were selected to be evaluated for their *in vitro* cytotoxic effect via the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method[13,14] against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HePG2 and colon carcinoma HT29. The results are presented in table 1 as LC_{50} (μM) which is the lethal concentration of the compound which cause death of 50% of the cells in 24 h.

The obtained data revealed that, the three tested human tumor cell lines exhibited variable degree of sensitivity profiles towards twelve of the tested compounds namely; **4a–b, d, 5a–b, 7a2–b2**, **8a1–b1, f1, 10b2** and **11a**. Among these, the human colon carcinoma HT29 cell line showed pronounced sensitivity against compounds **7a2** and **7b2** with LC_{50} values of 9.2 and 8.3 μM, respectively. Moreover, a remarkable cytotoxic potential was displayed by compound **8b1** against the same cell line (13.1 μM). Meanwhile,

Table 1. Cytotoxic effects (LC_{50}; μM)[a] of the active compounds on some human tumor cell lines using the MTT assay.

Compd no.	Human colon carcinoma HT29	Human hepatocellular carcinoma HePG2	Human breast cancer MCF 7
4a	20.7	37.8	47.6
4b	18.4	25.4	33.8
4d	76.8	–[b]	–
5a	40.9	40.3	–
5b	34.7	46.8	52.8
7a2	9.2	22.9	3.1
7b2	8.3	21.5	2.2
8a1	55.5	48.5	63.4
8b1	13.1	31.6	10.1
8f1	83.2	–	–
10b2	64.4	46.8	–
11a	76.2	–	–
Doxorubicin[c]	12.1	1.69	2.14

[a]LC50: Lethal concentration of the compound which causes death of 50% of cells in 24 h (μM).
[b]Totally inactive against this cell line.
[c]Positive control cytotoxic agent

compounds **4a** and **4b** revealed an obvious cytotoxicity profile against colon carcinoma HT29 with LC_{50} values 20.7and 18.4 μM, respectively.

Moreover, compounds **5a, 5b, 8a1** and **10b2** were able to exhibit moderate activity against the same cell line with LC_{50} values range of 34.7–64.4 μM. Whereas, mild activity was displayed by compounds **4d, 8f1** and **11a** at LC_{50} range of 76.2–83.2 μM. Furthermore, the growth of the human hepatocellular carcinoma HePG2 cell line was found to be moderately inhibited by nine of the active compounds namely; **4a–b, 5a–b, 7a2, b2, 8a1, b1** and**10b2**, with LC_{50} values range of 21.5–48.5 μM. Among these, the highest cytotoxic activity was displayed by compounds **4b, 7a2** and **7b2** which were almost equipotent (LC_{50} values 25.4, 22.9 and 21.5 μM, respectively). On the other hand, human breast cancer MCF 7 was proved to be the least sensitive among the cell lines tested as it was affected by only seven of the test compounds. However, an outstanding growth inhibition potential was shown by compounds **7a2, 7b2** and **8b1** as evidenced from their LC_{50} values (3.1, 2.2 and 10.1 μM, respectively). The rest four active compounds namely **4a, 4b, 5b** and **8a1** showed moderate to mild activity against the same cell line with LC_{50} values of 47.6, 33.8, 52.8 and 63.4 μM, respectively (table 1). Further interpretation of the results revealed that, compounds **4a, 4b, 7a2, 7b2** and **8b1** showed considerable broad spectrum of cytotoxic activity against the three tested human tumor cell lines. In particular, compound **7b2** proved to be the most active member in this study with a broad spectrum of activity against the tested cell lines, with special effectiveness against the human colon carcinoma HT29 and human breast cancer MCF 7 cell lines (LC_{50} values 8.3 and 2.2 μM, respectively) (table 1). A close examination of the structure of the active compounds showed that the 4-bromophenyl counterpart at position-4 of the tetrahydrobenzo[h]quinoline skeleton is the most favourable substituent when compared with other analogs. Moreover, the thiocarbamoyl substituent at position-1 (as in compounds **7a2** and **7b2**) was responsible for the high activity displayed by these analogs. Substitution at position-1 with an aldehyde, alkyl, alkylsulfonyl or acetyl functionalities resulted in moderate to weakly active compounds (**5, 8, 10** and **11**), whereas cyclization of the acetyl compounds **11** to the bicyclic 6,7-dihydro[1,2,4]triazolo[3,4-a]benzo[h]quinolines **12** led to complete abolishment of activity.

4.3 *Antiviral activity*

Compounds **4a**,**b**,**d**,**f**, **5d**, **6b**,**d**, **7b2**,**d2**,**f2**, **8b1**,**d1**,**f1**, **11a** and **12d** were investigated for their *in vitro* effect on the replication of hepatitis-C virus in HepG2 hepatocellular carcinoma cell line infected with the virus. Out of these compounds only two derivatives **7b2** and **7f2** were able to inhibit the hepatitis-C virus RNA (+) and (−) strands at 10–100 μg/mL concentration range.

The rest of the series were either inactive or exhibited insignificant activity.

5. Conclusion

The present paper describes the synthesis of new 2-oxo-1,4-disubstituted-1,2,5,6-tetrahydro-benzo[h]quinoline-3-carbonitriles as possible anticancer and antiviral agents. In this series, position 1 and 4 were critical in modulating the biological activity. In one instance tetrahydrobenzo[h]quinoline derivatives having *p*-bromophenyl substituent on position-4 were found to increase the activity and on the other hand, *N*-arylthiocarbamoyl group on position-1 depicted a significant enhancement in the anticancer activity. Compounds **7a2** and **7b2** were found to be the most active in this series. To our surprise, cyclization of the acetyl compounds **11** to the bicyclic 6,7-dihydro[1,2,4]triazolo[3,4-a]benzo[h]quinolines **12** led to complete abolishment of activity. Out of 15 compounds only two derivatives **7b2** and **7f2** were able to inhibit the hepatitis-C virus.

Supporting information

The electronic supporting information can be seen in www.ias.ac.in/chemsci for tables S1, S2 and S3.

Acknowledgements

The authors are thankful to the authorities of the Bioassay-Cell Culture laboratory, *in vitro* bioassays on human tumor cell lines for drug discovery unit, National Research Center (NRC), Cairo, Egypt, for their efforts in performing the MTT cytotoxicity assay.

References

1. Rostom S A F, Shalaby M A and El-Demellawy M A 2003 *Eur. J. Med. Chem.* **38** 959
2. Al-Saadi M S M, Rostom S A F and Faid Allah H M 2005 *Saudi Pharmaceutical Journal (SPJ)* **13** 89
3. Rostom S A F 2006 *Bioorg. Med. Chem.* **14** 6475
4. Al-Saadi M S M, Rostom S A F and Faid Allah H M 2008 *Arch. Pharm. Chem. Life Sci.* 341 181
5. Al-Saadi M S M, Faid Allah H M and Rostom S A F 2008 *Arch. Pharm. Chem. Life Sci.* **341** 424
6. Rostom S A F, Ashour H M A and Abd El Razik H A 2009 *Arch. Pharm. Chem. Life Sci.* **342** 299
7. Rostom S A F, Ashour H M A, Abd El Razik H A, Abd El Fattah A H and El-Din N N 2009 *Bioorg. Med. Chem.* **17** 2410
8. Rostom S A F 2010 *Bioorg. Med. Chem.* **18** 2767
9. Faidallah H M, Rostom S A F and Al-Saadi M S 2010 *J. King Abdulaziz University; Science (JKAU: Sci.)* **22** 177
10. Al-Saadi M S M, Rostom S A F and Faid Allah H M 2005 *Alex. J. Pharm. Sci.* **19** 15
11. Faid Allah H M, Al-Saadi M S and Rostom S A F 2008 *Saudi Pharmaceurical Journal (SPJ)* **16** 33
12. Rostom S A F, Hassan G S and El-Subbagh H I 2009 *Arch. Pharm. Chem. Life Sci.* **342** 584
13. Mosmann T 1983 *J. Immunol. Methods* **65** 55
14. Denizot F, Lang R 1986 *J. Immunol. Methods* **22** 271
15. El-Awady M K, Ismail S, El-Sagheer M, Sabour Y A, Amr K S and Zaki E A 1999 *Clinica Chimica Acta* **283** 1
16. Hopkins G C, Jonak J P, Tieckelmann H and Minnemeyer H J 1966 *J. Org. Chem.* **31** 3969
17. Kornblum H, Berrigan P J and Le Noble W J 1963 *J. Am. Chem. Soc.* **85** 1141
18. Kornblum H, Seltzer R and Haberfield P 1963 *J. Am. Chem. Soc.* **85** 1148