SYNTHESIS OF 4-HYDROXY-3-(1-HYDROXY-2-(SUBSTITUTED AMINO)ETHYL)-1-PHENYL/ METHYL QUINOLIN-2(1H)-ONE AS ANTICANCER AGENTS

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ABSTRACT

Objective: The current work is concerned with the synthesis of a series of 4-hydroxy-3-(1-hydroxy-2-(substituted amino)ethyl)-1-phenyl/methyl quinolin-2(1H)-one [III-a(1-5)/III-b(1-5)] and evaluation of its in vitro anticancer activity.

Methods: The starting material for linomide analogs was synthesized by following literature procedures. The carbonyl group was reduced to hydroxyl group using sodium borohydride, and the methyl group was brominated using bromine in acetic acid. Further bromine was nucleophilically substituted by primary amines. All the synthesized compounds were satisfactorily characterized by infrared, nuclear magnetic resonance, and mass spectral data. The synthesized compounds were tested for in vitro anticancer activity against MDA-MB cell line using the MTT assay method.

Results: Among all the synthesized compounds, compound [III-a1;R=C\textsubscript{6}H\textsubscript{5}], [III-b1;R=CH\textsubscript{3}], [III-a2;R=CH\textsubscript{3}], [III-b2;R=CH\textsubscript{3}], [III-a3;R=n-propylamine], [III-b3;R=n-propylamine], and [III-b4;R=methylamine] were found to be most cytotoxic with IC\textsubscript{50} value=25 µg against the MDA-MB cell line.

Conclusion: The results of screening studies concluded that compounds with (C\textsubscript{6}H\textsubscript{5} at C\textsubscript{3}) and (long chain aliphatic and cyclic amines at C\textsubscript{3}) position of quinolin-2-one ring showed moderate activity.

Keywords: Anticancer activity, Human mammary gland, Linomide.

INTRODUCTION

Cancer is characterized by uncontrolled growth of cells. It is a life-threatening disease affecting the people of all ages and is responsible for increase in the mortality rate globally. Inspite of availability of a large number of existing anticancer drugs, the development of new chemotherapeutics has always been one of the most noteworthy challenges due to non-selectivity and emergence of resistance by cancerous cells toward existing anticancer compounds [1-3]. Linomide is a nitrogen containing pharmacophore identified as a lead molecule for anticancer activity against MDA-MB cell lines [6-8]. It also inhibits angiogenesis and reduces the secretion of tumor necrosis factor alpha. In the present investigation, we thought of modifying linomide structure on ring nitrogen, carbonyl group of acetyl, and replacing phenyl ring by heterocyclic ring system.

METHODS

All the chemicals used were of analytical grade obtained from Sprague-Dawley, Fine and Spectrochem. Melting points of synthesized compounds were determined by thiel's melting point apparatus and are uncorrected. Fourier Transform infrared spectra were recorded on Shimadzu IRAffinity-1 spectrophotometer using KBr pellets. The 1H nuclear magnetic resonance (1H NMR) and 13C NMR were recorded on BrukerAvance II 400 NMR spectrometer using CDCl\textsubscript{3} or DMSO-d\textsubscript{6} as solvent and tetramethylsilane as internal standard; chemical shifts are expressed as δ values (ppm). The mass spectra were recorded on Waters, Q-TOP Micromass (liquid chromatography-mass spectrometry). The starting material for the synthesis of title compounds, as shown in Scheme 1, was synthesized following literature [9].

Synthesis of (±)-4-hydroxy-3-(1-hydroxyethyl)-1-phenyl/methyl quinolin-2(1H)-one [I-a/II-a]

A solution of 4-hydroxy-6-methyl/phenylquinolin-2(1H)-one (0.05 M) in 10 ml of absolute ethanol was placed in ice water bath, and then a solution of sodium borohydride (0.01 M) in 10 ml of cold water was added dropwise over a period of 1 hr. The mixture was then allowed to stand at room temperature for 10 minutes. 1 ml of dilute hydrochloric acid was added to destroy any residual borohydride and then transferred to separating funnel. 20 ml of ether was added, swirled, and the ether layer was separated, ether was removed under vacuum using rotaevaporator. The compound was dried, collected, and recrystallized using benzene.

Synthesis of (±)-3-(2-bromo-1-hydroxyethyl)-4-hydroxy-1-phenyl/methyl quinolin-2(1H)-one [I-b/II-b]

A solution of 1-a/b (0.01 M) in acetic acid (8 ml) was cooled to below 20°C by immersion in ice bath, then bromine (1.6 g, 0.01 M) in acetic acid (3 ml) was added dropwise with vigorous stirring over a period of 1 hr. After completion of addition of bromine, the mixture was stirred for 1 hr at room temperature and then diluted with 2.5 ml of water. The mixture was neutralized by the addition of 3 ml of 40% sodium hydroxide solution. The precipitate obtained was filtered, washed with water and dried.

SYNTHESIS OF 4-HYDROXY-3-(1-HYDROXY-2-(SUBSTITUTED AMINO)ETHYL)-1-PHENYL/ METHYL QUINOLIN-2(1H)-ONE AS ANTICANCER AGENTS
Synthesis of (±)-4-hydroxy-3-(1-hydroxy-2-(substituted amino)ethyl)-1-phenyl/methyl-quinolin-2(1H)-one (III-a (1-5)/III-b (1-5))

A mixture of II-a/II-b (0.05 M) and substituted primary amines (0.05 M) in 20 ml of absolute ethanol was heated under reflux for 12-16 hrs. After completion of the reaction, the excess solvent was distilled off under reduced pressure using rotoevaporator. The product was poured into crushed ice, filtered, washed thoroughly with water, and recrystallized using suitable solvent. The purity of all the newly synthesized compounds was ascertained by thin-layer chromatography using (n-hexane:ethyl acetate) in the ratio 7:3 as the mobile phase, silica gel G as stationary phase and iodine vapors as visualizing agent. The structural data for the synthesized compounds are given in Table 1.

### Anticancer activity

**MTT assay method [10]**

The human mammary gland (MDA-MB) cell line was obtained from the National Centre for Cell Sciences, Pune, India. The cell lines were maintained in 96 wells of microtiter plate containing minimum essential medium (MEM) media supplemented with 10% heat-inactivated fetal calf serum, containing 5% of mixture of gentamicin (10 µg), penicillin (100 units/ml), and streptomycin (100 µg/ml) in presence of 5% CO₂ at 37°C for 48-72 hrs. The supernatant from the plate was removed, and fresh MEM solution was added and treated with different concentration of test compound appropriately diluted with dimethyl sulfoxide (DMSO). Control group contained only DMSO. After 48 hrs of incubation at 37°C in a humidified atmosphere with 5% CO₂ the medium was replaced with MTT solution (20 µl, 5 mg per ml in sterile phosphate buffered saline) for further 4 hrs incubation. The supernatant was carefully aspirated, and the precipitated crystals of “Formazan blue” were solubilized by adding DMSO (100 µl).

In vitro growth inhibition of test compound was assessed by determination of conversion of MTT into “Formazan blue” by living cells. Optical density (OD) of the sample was measured at 492 nm. The results represent the mean of five readings (Table 2). The percent cell lysis was calculated using the following formula:

\[
\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD at control}} \times 100
\]

### RESULTS

#### Characterization of the synthesized compounds

**(±)-4-Hydroxy-3-(1-hydroxyethyl)-1-phenylquinolin-2(1H)-one (I-a)**

Yield: 70.76%, m.p: 167°C, IR (KBr, cm⁻¹): 3213.41 (-OH); 3064.89, 3032.10 (aromatic -C-H); 2922.16 (aliphatic-C-H str.); 1646.42 (-C=O amide), 1H NMR (DMSO-d_6, δ ppm): 17.15 (s, 1H, -OH); 8.2-6.4 (m, 9H, Ar-H); 3.93 (t, 1H, -CHOH); 3.36 (s, 1H, -OH of CHOH); 2.6 (s, 3H, -CH_3).

**III-a (1-5)**

| Compound | R  | R_1          |
|----------|----|--------------|
| III-a1   | C_6H_5 | CH_3-CH_2-NH- |
| III-a2   | C_6H_5 | CH_2-NH-       |
| III-a3   | C_6H_5 | CH_2-CH_2-NH-  |
| III-a4   | C_6H_5 | ⋯-NH-         |
| III-a5   | C_6H_5 | CH_2-NH-       |
| III-b1   | CH    | CH_3-NH-       |
| III-b2   | CH    | CH_2-CH_2-NH-  |
| III-b3   | CH    | CH_2-NH-       |
| III-b4   | CH    | ⋯-NH-         |
| III-b5   | CH    | CH_2-NH-       |

**III-b (1-5)**

Yield: 73.69%, m.p: 70°C, IR (KBr, cm⁻¹): 3392.79 (-OH); 3099.61 (aromatic -C-H); 2976.16, 2931.80 (aliphatic-C-H str.); 1652.01 (-C=O amide), 1H NMR (CDCl_3, δ ppm): 16.79 (s, 1H, -OH); 8.1-7.0 (m, 4H, Ar-H); 3.75 (t, 1H, -CHOH); 3.72 (s, 3H, -N-CH_3); 3.5 (s, 1H, -OH of CHOH); 2.7 (s, 3H, -CH_3).

**(±)-4-Hydroxy-3-(1-hydroxyethyl)-1-methylquinolin-2(1H)-one (I-b)**

Yield: 73.69%, m.p: 70°C, IR (KBr, cm⁻¹): 3392.79 (-OH); 3099.61 (aromatic -C-H); 2976.16, 2931.80 (aliphatic-C-H str.); 1652.01 (-C=O amide), 1H NMR (CDCl_3, δ ppm): 16.79 (s, 1H, -OH); 8.1-7.0 (m, 4H, Ar-H); 3.75 (t, 1H, -CHOH); 3.72 (s, 3H, -N-CH_3); 3.5 (s, 1H, -OH of CHOH); 2.7 (s, 3H, -CH_3).
Table 2: In vitro anticancer activity of 4-hydroxy-3-(1-hydroxy-2-(substituted amine)ethyl)-1-phenyl/methylnolin-2 (1H)-one derivatives

| S. No. | Compound                        | Concentration (µG) | OD at 492 nm | % of cell lysis as observed | IC₅₀  |
|--------|--------------------------------|-------------------|-------------|-----------------------------|-------|
| 1      | III-a1                         | 10                | 0.495       | No lysis                    | 25 µg |
|        |                                | 20                | 0.469       | No lysis                    |       |
|        |                                | 25                | 0.300       | 50 lysis                    |       |
|        |                                | 30                | 0.295       | 100 lysis                   |       |
|        |                                | 50                | 0.289       | 100 lysis                   |       |
| 2      | III-a2                         | 10                | 0.488       | No lysis                    |       |
|        |                                | 20                | 0.424       | No lysis                    |       |
|        |                                | 25                | 0.420       | No lysis                    |       |
|        |                                | 30                | 0.413       | No lysis                    |       |
|        |                                | 50                | 0.384       | No lysis                    |       |
| 3      | III-a3                         | 10                | 0.549       | No lysis                    |       |
|        |                                | 20                | 0.511       | No lysis                    |       |
|        |                                | 25                | 0.500       | No lysis                    |       |
|        |                                | 30                | 0.461       | No lysis                    |       |
|        |                                | 50                | 0.386       | No lysis                    |       |
| 4      | III-a4                         | 10                | 0.614       | No lysis                    | 30-50 µg|
|        |                                | 20                | 0.531       | No lysis                    |       |
|        |                                | 25                | 0.520       | No lysis                    |       |
|        |                                | 30                | 0.514       | No lysis                    |       |
|        |                                | 50                | 0.396       | 100 lysis                   |       |
| 5      | III-a5                         | 10                | 0.687       | No lysis                    | 30-50 µg|
|        |                                | 20                | 0.672       | No lysis                    |       |
|        |                                | 25                | 0.595       | No lysis                    |       |
|        |                                | 30                | 0.587       | No lysis                    |       |
|        |                                | 50                | 0.396       | 100 lysis                   |       |
| 6      | III-b1                         | 10                | 0.687       | No lysis                    | 25 µg  |
|        |                                | 20                | 0.645       | No lysis                    |       |
|        |                                | 25                | 0.295       | 50 lysis                    |       |
|        |                                | 30                | 0.290       | 100 lysis                   |       |
|        |                                | 50                | 0.285       | 100 lysis                   |       |
| 7      | III-b2                         | 10                | 0.783       | No lysis                    | 25 µg  |
|        |                                | 20                | 0.719       | No lysis                    |       |
|        |                                | 25                | 0.310       | 50 lysis                    |       |
|        |                                | 30                | 0.300       | 50 lysis                    |       |
|        |                                | 50                | 0.295       | 100 lysis                   |       |
| 8      | III-b3                         | 10                | 0.628       | No lysis                    |       |
|        |                                | 20                | 0.615       | No lysis                    |       |
|        |                                | 25                | 0.578       | No lysis                    |       |
|        |                                | 30                | 0.572       | No lysis                    |       |
|        |                                | 50                | 0.557       | No lysis                    |       |
| 9      | III-b4                         | 10                | 0.535       | No lysis                    |       |
|        |                                | 20                | 0.520       | No lysis                    |       |
|        |                                | 25                | 0.490       | No lysis                    |       |
|        |                                | 30                | 0.490       | No lysis                    |       |
|        |                                | 50                | 0.458       | No lysis                    |       |
| 10     | III-b5                         | 10                | 0.675       | No lysis                    |       |
|        |                                | 20                | 0.567       | No lysis                    |       |
|        |                                | 25                | 0.511       | No lysis                    |       |
|        |                                | 30                | 0.507       | No lysis                    |       |
|        |                                | 50                | 0.456       | No lysis                    |       |
| 11     | Vincristine (standard)          | 30                | 1.153       | 90                           |       |

OD: Optical density

(±)-4-Hydroxy-3-(1-hydroxy-2-(propylamino)ethyl)-1-phenylquinolin-2(1H)-one (III-a1)

Yield: 77.45%, m.p: 150°C, IR (KBr, cm⁻¹): 3408.22 (-OH); 3202.43 (-NH); 3076.61, 3064.89 (aromatic -C-H); 2962.24 (aliphatic -C-H str.); 1631.78 (-C=O amide).

(±)-4-Hydroxy-3-(1-hydroxy-2-(methylamino)ethyl)-1-phenylquinolin-2(1H)-one (III-a2)

Yield: 80.12%, m.p: 133°C, IR (KBr, cm⁻¹): 3446.79 (-OH); 3202.43 (-NH); 3076.61, 3064.89 (aromatic -C-H); 2962.24 (aliphatic -C-H str.); 1631.78 (-C=O amide).
6.5 (m, 9H, Ar-H); 4.04 (t, 1H, -CHOH); 3.25 (s, 1H, -OH of CHOH); 3.22 (s, 3H, -CH3); 2.17 (d, 2H, -CH2); 2.05 (s, 1H, -NH). (±)3-(2-(Cyclopropylamino)-1-hydroxyethyl)-1-hydroxy-1-phenyl-quinolin-2(1H)-one (III-a1)

Yield: 72.56%, m.p. 135°C, IR (KBr, cm⁻¹): 3474.81 (-OH); 3244.27 (-CONH); 2976.16, 2931.72 (aliphatic -C-H str.); 1643.35 (-C=O amide), (-NH); 3067.27 (aromatic -C-H); 2999.16, 2929.87 (aliphatic -C-H str.); 1635.64 (-C=O amide), (-NH); 3044.27 (aromatic -C-H); 2924.09 (aliphatic -C-H str.); 1518.47, 1456.34 (aromatic -C=C); 1419.2, 1234.99 (aliphatic -C=C); 1033.27, 835.33 (aliphatic -C-OCH3); 691.8, 547.64, 481.08 (aromatic). 1H NMR (CDCl3, δ ppm): 8.18 (s, 1H, -OH); 8.1-7.1 (m, 10H, Ar-H); 7.38 (t, 1H, -CHOH); 7.37 (s, 3H, -N-CH3); 7.36 (s, 1H, -OH of CHOH); 7.33 (s, 3H, -CH3); 2.17 (d, 2H, -CH2); 2.05 (s, 1H, -NH).

CONCLUSION

A series of (±)-3-hydroxy-1-hydroxy-2-(substituted amino)ethyl)-1-phenyl/methylquinolin-2(1H)-one derivatives were synthesized and evaluated for their anticancer activity by MTT assay method. From the obtained result, it was concluded that, among all, compound (III-a1), (III-b1), and (III-b2) were found to be most potent with IC50 value=25 µg whereas compound (III-a4) and (III-a5) showed moderate activity with IC50 value=30-50 µg and were therefore found to be active against the in vitro anticancer activity. The results of screening studies suggested that compounds with C6H5 at C4 and long chain aliphatic and cyclic amines at C3 position of quinolin-2-one ring showed moderate activity.

Thus, research work was undertaken for substitution at 3rd position of quinolin-2-one ring. The encouraging results shown may lead to the development of novel anticancer drugs, if explored further.

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