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RESEARCH ARTICLE

Synthesis and Anti Proliferative Activity of Isatin Incorporated Quinoxaline Derivatives

Gautham Naidu, Sravanthi Siliveri, Radhika Tumma, V. Harinadha Babu*, B. Madhava Reddy

G. Pulla Reddy College of Pharmacy, Hyderabad, Andhra Pradesh, India.

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ABSTRACT

A novel isatin incorporated quinoxaline derivatives have been synthesized by employing hybridization approach and structures were confirmed on the basis of physical and spectral data. In anti-proliferative screening, compounds 5b, 5c and 5d showed promising activity against MDAMB cell line at IC50 values of 4.10, 4.79 and 5.15 µM.

KEYWORDS

Isatin, Quinoxaline Hydrazide, Mannich Reaction, Anti-Proliferative Activity

INTRODUCTION

Isatin (1*H*-indole-2,3-dione) is the core moiety in the recently introduced tyrosine kinase inhibitors such as Sunitinib, Toceranib, Semaxanib[1] etc. According to literature, isatin and its derivatives reported to possess an extensive range of biological activities such as antibacterial[2], anti-inflammatory[3], analgesic[4], anti fungal[5], anti viral[6], anti tubercular[7], anti depressant[8] and anti-cancer activities[9]. On the other hand Quinoxaline is the basic nucleus in antitumor antibiotic Echinomycin[10] and the drug Chloroquinoxaline sulphonamide[11] which is an antineoplastic quinoxaline derivative act by poisoning topoisomerase. 2- Quinoxaline and its derivatives demonstrate excellent activities like anti viral[12], anti cancer[13], anti bacterial[14-18], anti plasmodial[19,20], anti fungal[21] and anti-inflammatory[22]. Hybridization is one of the new approaches to design new drugs with improved biological activity with respect to the corresponding lead compounds.

***Address for Correspondence:**
Dr. V. Harinadha Babu
Department of Pharmaceutical Chemistry,
G. Pulla Reddy College of Pharmacy, India.
E-Mail Id: dr.harinathbabu@rediffmail.com

Inspired by the cytotoxic property of isatin and quinoxaline heterocycles, in the present work an attempt was made to synthesize some novel isatin-quinoxaline hybrids hoping the resulting compounds will have good cytotoxic activity. Quinoxaline hydrazide was incorporated with isatin to give 2-(3-methyl quinoxalin-2-yloxy) acetohydrazide (4). Five new derivatives were synthesized by acetylation (5a), benzylation (5b) and Mannich reaction of compound (4) which led to compounds 5(c-e).

EXPERIMENTAL SECTION

Melting points (^{o}C) were determined on Analab melting point apparatus by open capillary method and were uncorrected. The IR spectra were recorded on Schimadzu FTIR spectrophotometer by using 1**%** potassium bromide discs. ^{1}H NMR spectra was recorded on Varian 400 MHz spectrometer using DMSO-d_6 as solvent and tetra methyl silane as an internal standard and mass spectra on Agilent 6430 triple quadruple LC-MS system. Thin layer chromatography (TLC) using E-Merck 0.25 mm silica gel plates and visualization was accomplished with UV light (256nm).

General Procedure for the Synthesis of 3-methyl quinoxalin-2-ol (1)

ortho phenylenediamine (0.1 mol, 10.8 g) was dissolved in suitable amount of toluene by the application of heat (if necessary) and to which was added ethyl pyruvate (0.1 mol, 11.6 g) drop wise with stirring. This mixture was heated on a water bath for 30 min. The completion of the reaction was monitored by TLC. Compound thus formed was filtered, dried and recrystallized from methanol and further confirmation was done by IR Spectrum.

Synthesis of Ethyl [(3-methylquinoxalin-2-yl) oxy] acetate (2)

3-methyl quinoxalin-2-ol (0.1 mol, 16 g) was dissolved in acetone (15 ml) and to which was added ethyl chloroactetate (0.1 mol,14.05 ml), dry potassium carbonate (3g) and refluxed at 50-60^{o}C for 24 h. Excess acetone was distilled off and allowed to cool. The residue obtained was poured into water contained in a separating funnel, 10-15 ml of chloroform was added and shaken. Chloroform thus separated was dried by using anhydrous magnesium sulphate and concentrated to give the residue and was recrystallized from methanol.

Synthesis of 2-[(3-methylquinoxalin-2-yl) oxy] acetohydrazide (3)

To a solution of ethyl [(3-methylquinoxalin-2-yl)oxy] acetate **(2)** (0.1mol) in methanol, was added hydrazine hydrate (99%, 20ml) and heated to reflux for 5 h on a water bath at 60-70^{o}c. Then it was cooled and the reaction mixture was poured into crushed ice with stirring kept aside for 1-2 h for the precipitate to settle. The resultant precipitate was filtered, washed with water, dried and recrystallized from methanol.

Synthesis of 2-[(3-methylquinoxaline-2-yl) oxy]-*N*'-(2-oxo-1,2-dihydro-3-*H*-indole-3-ylidene) acetohydrazide (4)

A mixture of 2-[(3-methylquinoxalin-2-yl)oxy]acetohydrazide **(3)** (0.01mol, 2.32g) and isatin (0.01 mol, 2.36g)was taken in a RB flask containing 20-25 ml of DMF to the catalytic amounts of glacial acetic acid and heated to reflux for 4 h at 120 ˚C. The completion of the reaction was monitored by TLC. Reaction mixture was poured into crushed ice, solid product thus obtained was filtered, washed with water and recrystallized from aq. methanol.

Preparation of N'-(1-acetyl-2-oxoindoline-3-ylidene)-2-(3-methyl quinoxalin-2-yloxy) acetohydrazide (5a)

A mixture of 2-[(3-methylquinoxaline-2-yl) oxy]-*N*'-(2-oxo-1,2-dihydro-3-*H*-indole-3-ylidene)acetohydrazide (0.01 mol, 3.63 g) (4) and acetic anhydride (10 ml) was taken in RBF and heated under reflux for 2-3 h at 130-140˚C by monitoring the reaction with TLC. Reaction mixture was poured into ice cold water to get the precipitate .The precipitate was filtered on a suction pump, washed thoroughly with water and the crude product was recrystallized from methanol.

Preparation of N'-(1-benzyl-2-oxoindoline-3-ylidene)-2-(3-methyl quinoxalin-2-yloxy) acetohydrazide (5b)

A mixture of 2-[(3-methylquinoxaline-2-yl)oxy]-*N*'-(2-oxo-1,2-dihydro-3-*H*-indole-3-ylidene)acetohydrazide (0.01 mol, 3.63 g) (5) and benzyl chloride 0.01 mol (2 ml) was taken into a conical flask containing DMF and the mixture was stirred at 120-130˚C for 7-8 h on a magnetic stirrer. The completion of the reaction was monitored by TLC and the reaction mixture was poured into ice cold water to get the precipitate. The precipitate was filtered on a suction pump, washed thoroughly with water and the crude product was recrystallized from methanol.

Preparation of N'-(1-((dimethyl amino) methyl)-2-oxoindoline-3-ylidene)-2-(3-methyl quinoxalin-2-yloxy) acetohydrazide (5c)

A mixture of 2-[(3-methylquinoxaline-2-yl)oxy]-*N*'-(2-oxo-1,2-dihydro-3-*H*-indole-3-ylidene)acetohydrazide (0.01 mol , 3.63 g) and 0.01 mol of dimethyl amine HCl and 2 ml of formaldehyde was taken in conical flask and was stirred on a magnetic stirrer for 8 h at 50-60˚c. The precipitate obtained was filtered,

washed thoroughly with distilled water and dried.

Preparation of 2-(3-methyl quinoxalin-2-yloxy)-N'-(2-oxo-1-(piperazine-1-yl—methyl) indolin -3-ylidine) acetohydrazide (5d)

A mixture of 2-[(3-methylquinoxaline-2-yl)oxy]-*N*'-(2-oxo-1,2-dihydro-3-*H*-indole-3-ylidene)acetohydrazide (0.01 mol, 3.63 g) (4) was taken into a conical flask containing DMF (20 mls), piperazine (0.01 mol, 1.5 ml) and 2ml of formaldehyde was added and the mixture was stirred under a magnetic stirrer at 110˚C for 8 h .The reaction mixture was poured into ice cold water and the precipitate obtained was filtered, washed thoroughly with distilled water and dried.

Preparation of 2-(3-methyl quinoxalin-2-yloxy)-N' (1-(morpholinomethyl)-2-oxoindolin-3-ylidine) acetohydrazide (5e)

A mixture of 2-[(3-methylquinoxaline-2-yl)oxy]-*N*'-(2-oxo-1,2-dihydro-3-*H*-indole-3-ylidene)acetohydrazide (0.01 mol, 3.63 g) (4) was taken into a conical flask containing DMF, (20 ml), morpholine (0.01 mol, 1.3 ml) and 2 ml of formaldehyde was added and the mixture was stirred under a magnetic stirrer at 130-140^{o}c for 4 h. The reaction mixture was poured into ice cold water and the precipitate was filtered, washed thoroughly with distilled water on a Buchner funnel and dried.

Spectral Data

N'-(1-acetyl-2-oxoindoline-3-ylidene)-2-(3-methyl quinoxalin-2-yloxy) acetohydrazide (5a)

Yield: 56%, melting point: 274-276^{o}c, TLC system: ethyl acetate and n-hexane-30:70, ^{1}H NMR (300 MHz, $CDCl_3$+DMSO d_6), δ ppm δ11.04(s, 1H, NH), δ (7.0-8.0)(8 H Ar- H) δ5.32(s, 2H, OCH_2), δ2.86(s, 3H, CH_3) δ3.6(s, 3H, OCH_3). Mass (m/z): 403, (M+1): 404.

N'-(1-benzyl-2-oxoindoline-3-ylidene)-2-(3-methyl quinoxalin-2-yloxy acetohydrazide (5b)

Yield: 58%, melting point: 183-185^{o}c, TLC system: ethyl acetate and n-hexane-50:50, ^{1}H NMR (300 MHz, $CDCl_3$+DMSO d_6), δ ppm δ11.04(s, 1H, NH), δ(7.0-8.0)(13 H Ar- H) δ5.32(s, 2H, OCH_2), δ2.86(s, 3H, CH_3), δ5.12(s, 2H, -CH_2). Mass (m/z): 451, (M+1): 452.

N'-(1-((dimethylamino) methyl)-2-oxoindoline-3-ylidene)-2-(3-methylquinoxalin-2-yloxy) acetohydrazide (5c)

Yield: 52%, melting point: 155-156^{o}c, TLC system: ethyl acetate and n-hexane-50:50, ^{1}H NMR (300 MHz, $CDCl_3$+DMSO d_6), δ ppm δ11.40(s, 1H, NH), δ7.0-8.0(8H Ar- H),δ5.32(s, 2H, OCH_2), δ 5.71 (s, 2H, N-CH_2) δ2.69 (s, 3H, CH_3), δ2.38(s, 6H, 2-CH_3), . Mass (m/z): 418, (M+1): 419.

2-(3-methylquinoxalin-2-yloxy)-N'-(2-oxo-1-(piperazine-1-yl—methyl)indolin-3-ylidine) acetohydrazide (5d)

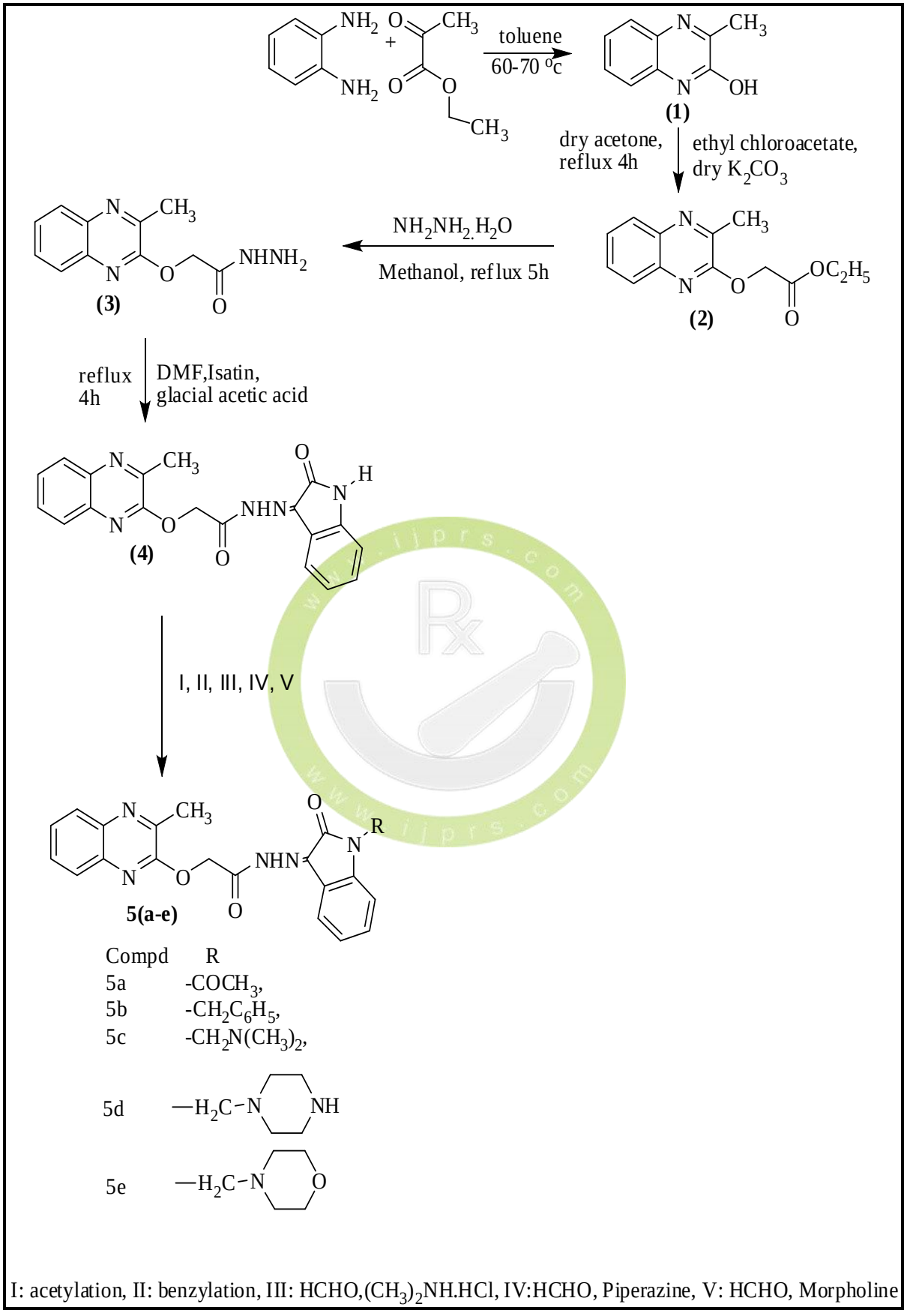
Yield: 55%, melting point: 191-192^{o}c, TLC system: ethyl acetate and n-hexane-30:70, ^{1}H NMR (300 MHz, $CDCl_3$+DMSO d_6), δ ppm δ11.20 (s, 1H, NH), δ 10.86 (s, 1H, NH of piperazine), δ7.0-8.0 (8H Ar- H),δ5.12 (s, 2H, OCH_2), δ 4.62 (s, 2H, N-CH_2) δ2.69 (s, 3H, CH_3), δ2-2.5(t, 8H, methylene protons of piperazine), . Mass (m/z): 459, (M+1): 460.

2-(3-methyl quinoxalin-2-yloxy)-N' (1-(morpholinomethyl)-2-oxoindolin-3-ylidine) acetohydrazide (5e)

Yield: 55%, melting point: 173-174^{o}c, TLC system: ethyl acetate and n-hexane-30:70, ^{1}H NMR (300 MHz, $CDCl_3$+DMSO d_6), δ ppm δ12.63(s, 1H, NH), δ7.0-8.0(8H Ar- H),δ5.03(s, 2H, OCH_2), δ 4.62 (s, 2H, N-CH_2) δ2.69 (s, 3H, CH_3), δ2-2.5(t, 8H, methylene protons of morpholine), . Mass (m/z): 459, (M+1): 460.

RESULTS AND DISCUSSION

The synthesis of title compounds is outlined in Scheme-1. 3-methylquinoxalin-2-ol (**1**) was synthesized by cyclization of o-phenylenediamine with ethyl pyruvate in toluene. This on reaction with ethyl chloroactetate followed by condensation with 99% hydrazine hydrate afforded 2-[(3-methylquinoxaline-2-yl)oxy]acetohydrazide (**3**). Condensation of compound **(3)** with isatin in DMF gave 2-[(3-methylquinoxalin-2- yl)oxy]-



Scheme: 1

N'-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)acetohydrazide **(4).** From compound (**4**), different derivatives were prepared acetylation (5a) benzylation (5b) and Mannich addition (5c, 5d and 5e) on NH of indole.

Purity of all the compounds in the present work was checked by TLC and melting points. The products obtained are confirmed by ^{1}H NMR, IR and MASS spectral data.

Biological Activity

Cytotoxic potential of all the synthesized compounds was evaluated *in vitro* against a panel of three cell lines - human lung cancer cell line (A549), human breast cancer cell line (MDAMB) and human prostate cancer cell line (DU145) using MTT-micro cultured tetrazolium assay method[23]. Doxorubicin was used as the reference drug and the results are summarized in Table-1 and Figure-1.

The results were represented as percentage of cytotoxity/viability. From the percentage of cytotoxicity, the IC_{50} values are calculated and presented in the Table.1. Among all the compounds, compounds 5b, 5c and 5d showed significant activity against MDAMB cell line.

Cytotoxicity Against Three Different Cell Lines

Cytotoxicity of synthesized compounds was tested against three cell lines, by MTT assay method, and all the experiments were carried out in triplicates.

All the three types of cell lines are seeded to flat bottom 96 well plates (10,000 cells/100 µl) and cultured in the medium(Dulbecco's modified eagle's) containing 10% serum. Incubated for 24h in a 5% CO_2 humid chamber so that the cells adhere to the surface. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was dissolved in PBS at 5 mg/mL and sterile filtered. Different concentrations of the compounds were added to the adhered cells. After 48 hours MTT solution (10ul per well) was added to the culture plate. Cells were further incubated in the CO_2 chamber for 2 h. Following this, media was removed and 100 µl of DMSO was added. Absorbance was measured at 562 nm in a multimode micro plate reader (Tecan GENios).

Table 1: Anti-Cancer data of synthesized compounds on different cell lines

S.No	Compound	IC_{50} (µM)		
		A549	SKNSH	MDAMB
1	5a	>100	>100	20.35±5.02
2	5b	>100	61.79±0.30	4.10±0.11
3	5c	>100	>100	4.79±0.34
4	5d	>100	>100	5.15±0.09
5	5e	>100	78.14±0.97	40.35±5.02
6	Doxorubicin(10µM)	8.05±0.37	8.5±0.17	5.96±0.13

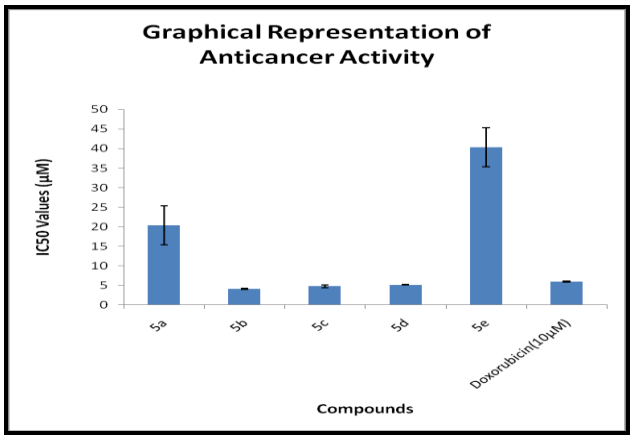


Figure 1: Graphical representation of anticancer activity

CONCLUSION

In this investigation, five new isatin-quinoxaline hybrids (**5a-e**) were synthesized by condensing 2-[(3-methylquinoxalin-2-yl)oxy] acetohydrazide with isatin, and the structures of the resulting compounds were characterized on the basis of spectral data. All the compounds were screened *in vitro* against anti-cancer activity, and the results of the present study indicate that the synthesized compounds have more activity against MDAMB cell line when compared to other two cell lines.

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