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1, 2- Dihydroquinoline sulphonamides synthesis, characterization, and biological evaluation

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Abstract

In medical chemistry, several quinoline compounds, whether synthetic or natural, are widely used. The present study's synthetic strategy is to synthesize 1,2-dihydroquinoline sulphonamide derivatives for antihelmintic activity and Cytotoxic Activity. The effective synthesis of sulfonamides with a 1,2-dihydroquinoline nucleus was the main goal of the research project. The reactions are conducted at a moderate temperature of 60-90 OC using the solvents methylene dichloride and tetrahydrofuran to produce final products with a high yield, short half-life, and high purity. This makes the processes environmentally benign. NMR, 13C NMR, and IR studies provided detailed characterizations of recently synthesized substances. The biological properties of the recently created compounds, such as their antibacterial and anti-inflammatory properties, were examined. All four of the compounds exhibited moderate cytotoxic activity when evaluated biologically.

Keywords: Quinoline, 1,2-Dihydroquinoline Sulphonamides, antihelmintic activity and Cytotoxic Activity

Introduction: Importance of Quinoline

Initially isolated quinoline, a heterocyclic base, from coal tar in 1834 and gave it the name Leukol Runge [1]. By heating the alkaloid cinchonine with alkali later in1842, Gerhardt [2] separated it and gave it the name quinoline. Petroleum products include a large number of quinoline compounds that have been isolated [3]. In medical chemistry, several quinoline compounds, whether synthetic or natural, are widely used. As bioactive compounds, quinoline motifs are often employed [4–7]. The synthesis of virucides, biocides, alkaloids, rubber compounds, and aromatic substances [8] all involves these heterocycles.

Furthermore, these compounds find use in the chemistry of transition element metal catalysts for polymerization and luminescent properties [9]. Refineries use quinoline derivatives as antifoaming agents [10]. Consequently, the synthesis of quinolines has piqued the curiosity of synthetic organic and medicinal chemists.

The major biological activities shown by the quinoline derivatives are discussed in the next section, which is based on the literature review.

Materials and Methods

- **Organic solvents and Reagents**

The organic solvents were purchased from SD Fine, Spectrochem, Sigma Aldrich and standard commercial sources are used without further purification.

- **Reaction conditions**

Room temperature reactions mentioned ranges between 20-30°C (throughout the year). For low temperatures reactions, ice-bath with sodium chloride (-5°C) was used. Magnetically stirred oil bath (or) a hotplate was used for high temperature reactions.

- **FT-IR**

The spectra were recorded using JASCO FTIR-4100 spectrophotometer as KBr disc in the range of 4000-400 cm-1. FT-IR data are reported as follows: Frequency (functional group).

- **^{1}H NMR and 13C NMR**

The 1H and 13CNMR spectra were recorded on JEOL-400 MHz spectrometers using deuteriated solvents (CDCl3& DMSO-d6) and Tetramethylsilane (TMS) as an internal standard. The chemical shift values were expressed in δ ppm in both 1H NMR (0-15 ppm) and 13C NMR (0-200 ppm) spectra.

^{1}H NMR data is reported in the following order: chemical shift (multiplicity, *J* value, number of protons and nature of proton). 13C NMR data are reported in the following order: chemical shift (numbered carbon atom).

- **Mass analyses**

Waters Alliance 2795 separations module and Waters Micro mass LCT mass detector was used to record LC-MS.

- **Elemental analysis**

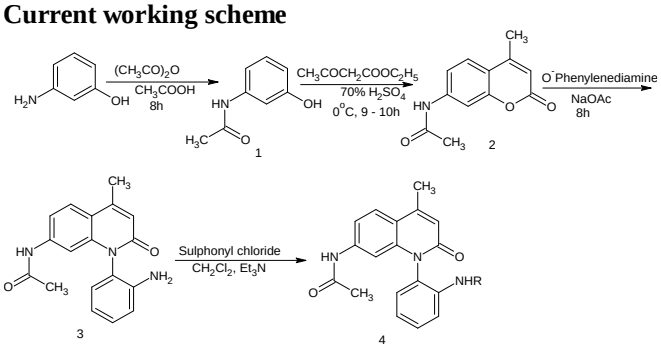
Elemental analysis (C, H and N) was performed on Elementarvario MICRO cube.

Experimental

Present Work

Based on the recent reports on the above pharmacological significance of quinolines and its derivatives, we undertook the comprehensive investigation on the general methods for the design, synthesis and characterization of quinoline derivatives such as sulphonamides and amides via simple and economically more convenient synthetic methods. Based on the survey of literature on quinolone containing sulphonamides and compounds containing 1,2-dihydroquinoline nucleus, it was found that compounds are pharmacologically very important therefore the objective of present work was focused to plan and synthesize 1,2-dihydroquinoline derivatives containing sulfonamide moiety.

Current working scheme



Synthesis procedure

Step-1: Synthesis of 3-hydroxy Acetanilide [1]

3-aminophenol (25g, 0.11 mol) taken in round bottom flask containing acetic anhydride (80 mL) and it was stirred for 8 h at 60oC under Nitrogen atmosphere. After reaction completion, excess acetic anhydride was removed off under reduced pressure; the residue was obtained which was dissolved in dichloromethane. The dichloromethane layer was washed with brine solution, dried over anhydrous Na2SO4 and concentrated to achieve compound **[1]**.

Step-2: Synthesis of intermediate acetamide [2]

3-hydroxy Acetanilide **[1]** (25g, 0.11 mol) was taken in ethyl acetoacetate (0.1mol) with 70% aq.H2SO4 (50 mL), and it was stirred for 9-10 h at 0 oC. After reaction completion, the reaction mixture was poured into ice cold water, solid separates out. The solid was filtered and washed with water. The crude product was recrystallized to obtain pure compound **[2]**.

Step-3: Synthesis of *N*-[1-(2-aminophenyl)-4-methyl-2-oxo-1,2-dihydroquinolin- 7-yl] acetamide [3]

Compound [2] (2.17g, 0.01 mole), o-phenylenediamine (0.01 mole, 1.08g) and sodium acetate (5 g) were taken in a round bottom flask containing glacial acetic acid (15 mL) and the resulting mixture was refluxed for 8 h. After completion reaction, the reaction mixture was bring down to room temperature. The separated solid was filtered and recrystallized from methanol: water (1:2) to give title compound [3].

Step-4: Synthesis of 1,2-dihydroquinoline Sulphonamides [4a-d]

Equimolar quantities of compound [3] (0.5 g, 0.001 mol), different substituted sulphonyl chlorides (0.001 mol) and triethylamine (0.57 g, 0.003 moles) were stirred in dry methylene chloride (10 mL) under N2 atmosphere at room temperature for 12 h. The reaction was monitored by TLC. After reaction completion, the mixture was washed with water and brine solution. Methylene chloride layer was dried over anhydrous Na2SO4 and evaporated under vacuum. The residue was purified by column chromatography using petroleum ether: ethyl acetate as eluent (7:3) to get Sulphonamide dihydroquinoline nucleus [4a-d] in good yield (scheme 2.1).

Biological Evaluation

Anthelmintic activity

Anthelmintic activity of all the series of compounds was carried out on Indian Earthworm (*Pheretima posthuma*), kept under standard vermicomposting medium with a tolerable supply of sustenance and fluids for about three weeks. Piperazine citrate (Standard drug) was procured from SD Fine Chemical Ltd., Mumbai. Normal saline (NS) 0.90% w/v of sodium chloride in distilled water was prepared for the analysis in the laboratory.

Cytotoxic Activity

Cell lines and Culture medium

MCF-7 cell lines were procured from NCCS, Pune and stock cells were cultured in DMEM with 10% neutralized FBS. Streptomycin (100 □g/ml), penicillin (100 IU/ml) and amphotericin B (5 µg/ml) in a cool atmosphere condition of 5% CO2 at 37□C until complex. In these cell lines, the cells were segregated with TPVG solution (0.02% EDTA, 0.2% trypsin 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). Cell culture and *in vitro* cytotoxicity assay. In this study, the cell line assay, *in vitro* MCF-7, H9C2 and A-549 cell lines were cultured [1, 3, 6, 1, 3, 7].

Results and Discussion

In this work, pharmacologically important novel dihydroquinoline derivatives were synthesized by the acetylation reaction of 3- Aminophenol, cyclisation of acylated aminophenol acetamide followed by substitution of o-phenylenediamine to get intermediate [3]. Compound [3]

was reacted with various substituted sulphonyl chlorides to get the dihydroquinoline derivatives with sulphonamide moiety [4a-d]. All the derivatives were obtained in good yield as tabulated in Table 1.

The structures of the newly synthesized compounds were established by spectroscopic methods for instance FT-IR, ^{1}H NMR, 13C NMR and LCMS or Mass spectra. Finally, all the synthesized compounds were evaluated for their *in-vitro* Antibacterial, and Anti-inflammatory and activity.

Physical characterization

Table 1: Physical characterization data of 1,2-dihydroquinoline sulfonamides

Compound	Molecular formula	Molecular weight	Yield (%)	Melting point (°C)
4a	C19H9N3O4S	385.44	82	189-190
4b	C25H23N3O4S	461.53	78	212-214
4c	C24H21N3O4S	447.51	72	200-201
4d	C23H19ClN4O4S	482.94	72	181-182

Spectral interpretation of the title compounds (4a-d)

Compound 4a

N-[1-(2-Methanesulfonylamino-phenyl)-4-methyl-2-oxo-1,2-dihydro-quinolin-7-yl] acetamide

IR: νmax/cm−1: 3445 (N-H), 2115 (CN), 1618 (CO), 1329–1136 (CF stretching);

^{1}H-NMR (CDCl3) δ: 7.34-7.28 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.26-7.12 (m, 7H, Ar- H & NH), 6.98-6.96 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.73-6.71 (d, *J* = 8.3 Hz, 1H, Ar-H), 2.98 (s, 3H, COCH3), 2.36 (s, 3H, CH3), 2.16 (s, 3H, CH3);

^{13}C-NMR (CDCl3) δ: 163.25, 135.75, 132.29, 129.97, 129.40, 122.92, 118.34, 114.30, 55.57, 28.27, 22.27;

MS: *m/z* = Cal. 385.44 (Found 386.1) (M+1);

Elemental analysis

Calculated: C, 59.21 %; H, 4.97%; N, 10.90%; Observed: C, 59.19%; H, 4.95%; N, 10.88%.

Compound 4b

N-{4-Methyl-2-oxo-1-[2-toluene-4-sulfonylamino)-phenyl]-4-methyl-2-oxo-1,2-dihydro-quinolin-7-yl}-acetamide

IR: νmax/cm−1: 3040 (N-H), 2346 (CN), 1616 (CO), 1383–1114 (CF stretching);

^{1}H-NMR (CDCl3) δ: 7.74-7.70 (m, 4H, Ar-H), 7.35-7.31 (m, 4H, Ar-H), 7.27- 7.26 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.99-6.97(t, 2H, Ar-H), 6.96–6.88 (m, 5H, Ar-H), 2.58 (s, 3H, COCH3), 2.36 (s, 3H, CH3), 2.16 (s, 3H, CH3) **(Fig 2.7)**;

^{13}C-NMR (CDCl3) δ: 173.28, 170.45, 163.80, 161.33, 154.55, 130.71, 130.63, 126.32, 126.24, 123.55, 123.52, 119.57, 119.35, 114.90, 114.66, 107.79, 80.08, 43.10, 28.27, 22.27 **(Fig 2.8)**; **MS:** *m/z* = Cal. 461.53; Found 462.2 (M+1) **(Fig 2.9)**;

Elemental analysis

Calculated: C, 65.06 %; H, 5.02%; N, 9.10%; Observed: C, 65.04%; H, 5.00%; N, 9.08% **(Fig 2.10)**.

Compound 4c

N-[1-(2-benzenesulfonylamino-phenyl)-4-methyl-2-oxo-1,2-dihydro-quinolin-7-yl} -acetamide

IR: νmax/cm−1: 3202 (N-H), 2228 (CN), 1586 (CO), 1311–1163 (CF stretching) **(Fig 2.11)**; **^{1}H-NMR (CDCl3) δ:** 7.43-7.29 (d, *J* = 8.7 Hz, 4H, Ar-H), 7.22-7.18 (m, 8H, Ar-H & NH), 6.96-6.94 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.72-6.69 (d, *J* = 8.3 Hz, 1H, Ar- H), 2.53 (s, 3H, CH3), 2.17 (s, 3H, CH3) **(Fig 2.12)**;

^{13}C-NMR (CDCl3) δ: 169.3, 141.2, 138.3, 134.8, 128.7 CF3, 125.9, 124.6, 115.9, 104.9, 28.7, 27.8; HPLC: 100% purity **(Fig 2.13)**;

MS: *m/z* = Cal. 447.51; Found 448.0 (M+1) **(Fig 2.14)**;

Elemental analysis

Calculated: C, 64.41 %; H, 4.73%; N, 9.39%;
Observed: C, 64.39%; H, 4.71%; N, 9.37% **(Fig 2.15)**.

6.2.4 Compound 4d

N-{1-[2-(6-Chloro-pyridine-2-sulfonylamino)-phenyl]-4-methyl-2-oxo-1,2-dihydro quinolin-7-yl}-acetamide [4d]

IR: νmax/cm−1: 3343 (N-H), 2332 (CN), 1669 (CO), 1314–1127 (CF stretching) **(Fig 2.16)**; **^{1}H-NMR (CDCl3) δ:** 7.43-7.29 (d, *J* = 8.7 Hz, 4H, Ar-H), 7.22-7.18 (m, 8H, Ar-H & NH), 6.96-6.94 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.72-6.69 (d, *J* = 8.3 Hz, 1H, Ar- H), 2.53 (s, 3H, CH3), 2.17 (s, 3H, CH3) **(Fig 2.17)**;

^{13}C-NMR (CDCl3) δ: 169.3, 141.2, 138.3, 134.8, 128.7 CF3, 125.9, 124.6, 115.9, 104.9, 28.7, 27.8; **HPLC:** 100% purity **(Fig 2.18)**;

MS: *m/z* = Cal. 482.94; Found 483.0 (M+1) **(Fig 2.19)**;

Elemental analysis:

Calculated: C, 57.20 %; H, 3.97%; N, 11.60%;
Observed: C, 57.18%; H, 3.95%; N, 11.58% **(Fig 2.20)**.

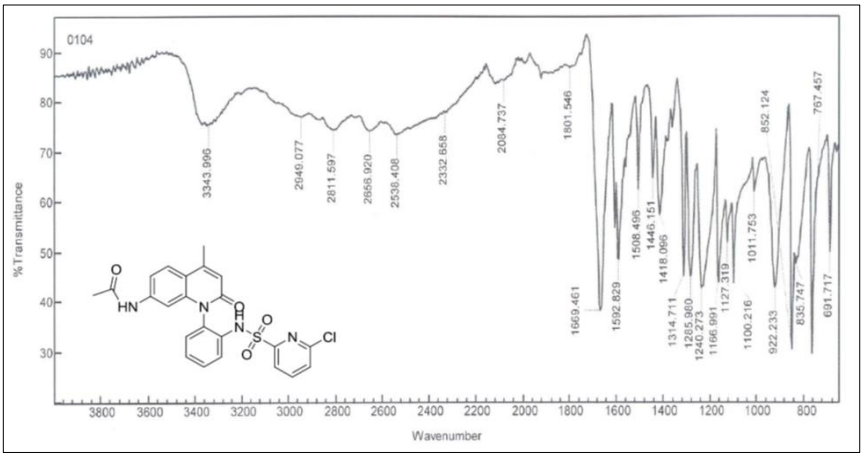


Fig 12: IR spectrum of compound **[4d]**

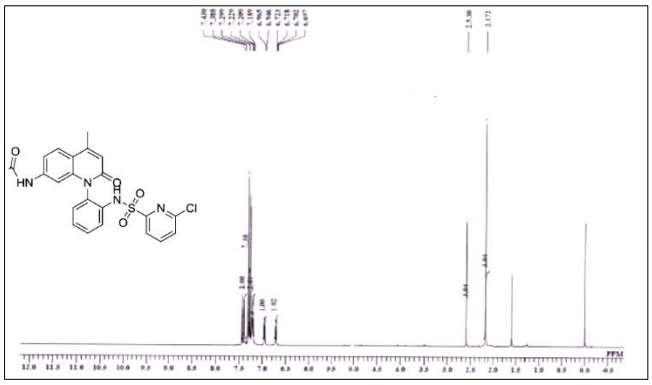


Fig 13: 1H NMR spectrum of compound **[4d]** in CDCl3

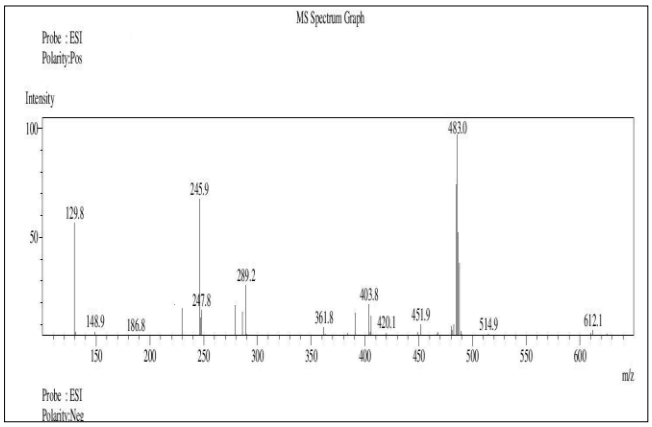


Fig 14: MS spectrum of compound [4d]

Biological evaluation

Anthelmintic activity

Table 2: Anthelmintic activity minimum concentration (MCs) values of (4a-d)

Test model	Conc. (mg/ mL)	Comps (4a-d)
P. postuma	15	IA
	25	IA
	35	IA
	45	IA
	50	AC

IA: Inactive; AC: Active

Table 3: Anthelmintic activity of 1,2 dihydroquinoline Sulfonamides (4a-d)

Sample	Concentration (mg/ml)	Time taken for paralysis(min)	Time taken for death (min)
Control	Normal Saline	142.33±0.49	167.17±0.87
DMF	10% in Saline	138.28±0.54	159.69±0.25
Piperazine citrate	50	39.17±0.48	57.00±0.58
4a	50	46.00±1.46	102.00±0.86
	100	35.00±1.59	56.50±0.76
4b	50	47.33±1.23	97.17±0.60
	100	70.00±0.82	83.17±0.60
4c	50	49.17±0.60	94.50±1.26
	100	37.33±1.23	48.67±0.71
4d	50	70.83±0.95	101.67±0.95
	100	38.50±0.76	49.00±0.58

Results are presented as mean ± SEM.

The compounds (4a-d) tested for anthelmintic activity against Pheretima posthuma (Indian earthworm) showed moderate outcomes with that of standard Piperazine citrate. Earthworms in control group showed paralysis time at 142.33±0.49 min and death time at 167.17±0.87 min. The concentration of 50 mg/mL and 100 mg/mL was chosen for the study, based on the initial studies carried out at concentrations of 15, 25, 35 and 45 mg/mL as given in Table 5.5. Standard drug piperazine citrate 50 mg/mL exhibited 49.17±0.48 and 57.00±0.58 min for paralysis and death time. Examination of anthelmintic activity revealed that 1,2-dihydroquinoline sulfonamide **4a and 4c** showed good activity against P. posthuma as reported in **Table 3.**

Cytotoxic activity

Table 4: Cytotoxic properties of test drugs (4a-d) against MCF-7 cell line

Compound	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
4a	1000	49.87±0.5	>1000±0.00
	500	34.89±0.2	
	250	25.89±0.2	
	125	11.89±0.2	
	62.5	9.11±0.3	
4b	1000	54.78±0.3	>1000±0.00
	500	29.68±0.2	
	250	19.78±0.1	
	125	11.87±1.8	
	62.5	5.55±0.2	
4c	1000	22.37±0.4	>1000±0.00
	500	21.02±0.3	
	250	16.32±0.3	
	125	11.53±0.2	
	62.5	7.28±0.4	
4d	1000	26.62±1.1	>1000±0.00
	500	23.70±0.3	
	250	19.55±0.4	
	125	10.77±0.6	
	62.5	6.72±0.4	

The cytotoxic effects of some newly synthesized compounds of 4a-4d against the MCF-7 (Breast carcinoma) cells were determined using MTT assay for cell viability. The cytotoxic potential of the free ligands was evaluated as well.

The retrieved MTT-formazan absorption values are summarized in Table 4. The 72 h exposure of both cell lines with the tested compounds resulted in a concentration dependent reduction of cell viability as assessed by the MTT assay, which enabled the construction of concentration–response curves. In addition the corresponding CTC50 values were derived in order to allow a quantitative merit for assessment of the relative potencies of the agents under investigation. The compounds showed moderate activity.

7. Conclusion

The effective synthesis of sulfonamides with a 1,2-dihydroquinoline nucleus was the main goal of the research project. The reactions are conducted at a moderate temperature of 60-90 OC using the solvents methylene dichloride and tetrahydrofuran to produce final products with

a high yield, short half-life, and high purity. This makes the processes environmentally benign. NMR, 13C NMR, and IR studies provided detailed characterizations of recently synthesised substances. The biological properties of the recently created compounds, such as their antibacterial and anti-inflammatory properties, were examined.
All four of the compounds exhibit moderate cytotoxic activity when evaluated biologically.

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