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SYNTHESIS OF 2-MERCAPTOPYRIMIDINYLQUINOLIN-2(1*H*)-ONE DERIVATIVES AS ANTIBACTERIAL AND ANTITUBERCULAR AGENTS

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A series of 2-mercaptopyrimidinylquinolin-2 (1*H*)-one substituted derivatives were synthesized and satisfactorily characterized by UV, IR, NMR and Mass spectral data. The synthesized compounds were evaluated for their antibacterial and antitubercular activity. Among all the compounds, compound IIIa2 and IIIa6 showed Minimum Inhibitory Concentration (MIC) of 0.2 μg/ml and were found to be highly potent than standard drug Ciprofloxacin (MIC=2μg/ml) against *Bacillus subtilis*. The synthesized compounds showed moderate antibacterial activity against *Escherichia coli* (MIC=12.5 μg/ml) and were found to be inactive against bacterial strains *Staphylococcus aureus* and *Pseudomonas aeruginosa*. However none of the compounds was active against *Mycobacterium tuberculosis* (H_{37}Rv strain) as compared to the standard drugs.

Since 1960's quinolones have been the center of interest and have also shown to possess ideal antibiotic properties like high potency, a broad spectrum of activity and good bioavailability[1,2]. Majority of the quinolones, clinically used belong to a class of Flouroquinolones. In general, Flouroquinolones were well tolerated but some of them have shown serious side effects like central nervours system toxicity, cardiac arrhythmias, convulsions, tendon rupture and hypoglycaemia[3]. Although the new generation antibacterials have shown improvement in potency, spectrum and pharmacokinetic properties but have faced a rapid increase of resistance especially with respect to Gram positive bacteria. Also many "old" bacterial pathogens, such as *Staphylococcus aureus* and *Mycobacterium tuberculosis*, have emerged with new forms of virulence and new patterns of resistance to antibacterial agents. *Mycobacterium tuberculosis* strains have become resistant to all the first line drugs leading to the multidrug-resistant TB (MDR-TB)[4].

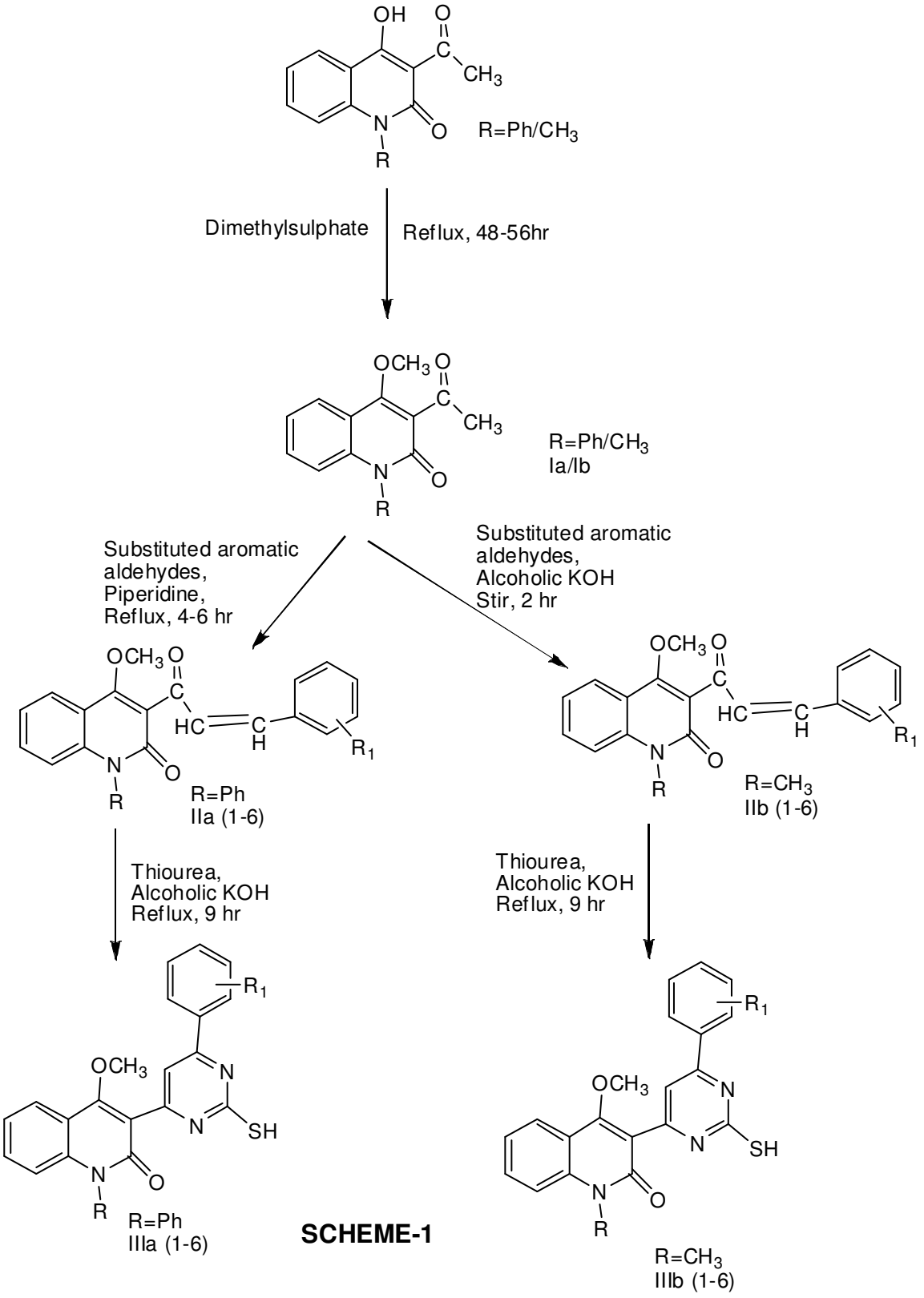
Quinolin-2(1*H*)-one ring system is present in many of the clinically used drugs such as Rebapimide as an anti-oxidant and anti-ulcer agent[5,6], Carteolol[7,8,9] to reduce normal and elevated intraocular pressure (IOP), Aripiprazoles an antipsychotic agent[10]. Quinolin-2(1*H*)-one derivatives have been reported to have anti-HIV, anticancer, antiviral, antiangiogenic, antibacteial activity etc[11,12]. In view of the above facts, we report the synthesis and *in-vitro* activities of 2-mercaptopyrimidinylquinolin-2(1*H*)-one derivatives.

The starting material, 3-acetyl-4-hydroxyquinolones were prepared as per the literature[13]. 3-Acetyl-4-hydroxyquinolone showed possibility of keto-enol tautomerism between 3rd and 4th position of the ring system. In order to prevent the tautomerism from occuring, hydroxy group of quinolones were subjected to methylation using distilled dimethyl sulphate which resulted in the formation of 3-acetyl-4-methoxyquinolones. Further Claisen-Schmidt condensation was carried out between 3-acetyl-4-methoxyquinolone derivatives and substituted aldehydes in the presence of ethanolic alkali to obtain 3-substitutedcinnamoyl-4-methoxyquinolones. In the final step, 3-substitutedcinnamoyl-4-methoxyquinolones were reacted with thiourea in equimolar quantities which resulted in formation of 2-mercaptopyrimidinylquinolones.

Biological activity

Antibacterial activity

The antibacterial activity was determined by minimum inhibitory concentration (MIC) method[14]. The twelve 2-mercaptopyrimidinylquinolin-2(1*H*)-one derivatives were tested against four bacterial strains, two Gram positive bacteria (*Bacillus subtilis* and


OH
O
C
CH3
N
O
R
R=Ph/CH3
Dimethylsulphate
Reflux, 48-56hr
OCH3
R=Ph/CH3
Ia/Ib
Substituted aromatic aldehydes, Piperidine, Reflux, 4-6 hr
Substituted aromatic aldehydes, Alcoholic KOH Stir, 2 hr
HC
H
R1
R=Ph
IIa (1-6)
R=CH3
IIb (1-6)
Thiourea, Alcoholic KOH Reflux, 9 hr
Thiourea, Alcoholic KOH Reflux, 9 hr
SH
R=Ph
IIIa (1-6)
R=CH3
IIIb (1-6)

SCHEME-1

Table-1
Antibacterial activity data of the title compounds

Compd	R	R_1	Minimum inhibitry concentration in μg/ml			
			Gram positive		Gram negative	
			B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
IIIa1	C_6H_5	H	1.6	50	50	50
IIIa2	C_6H_5	4-Cl	0.2	100	100	100
IIIa3	C_6H_5	3-OCH_3	0.4	50	100	50
IIIa4	C_6H_5	4-OH	3.125	50	100	50
IIIa5	C_6H_5	4-F	0.8	25	100	50
IIIa6	C_6H_5	4-Br	0.2	25	50	50
IIIb1	CH_3	H	0.4	50	25	50
IIIb2	CH_3	4-Cl	0.8	50	12.5	50
IIIb3	CH_3	3-OCH_3	0.8	50	25	25
IIIb4	CH_3	4-OH	6.25	50	12.5	50
IIIb5	CH_3	4-F	0.8	50	50	100
IIIb6	CH_3	4-Br	25	50	50	100
Ciprofloxacin			2	2	2	<4

Staphylococcus aureus) and two Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Ciprofloxacin was used as a standard drug with MIC=2 μg/ml against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and <4 μg/ml against *Pseudomonas aeruginosa*. The MIC was determined in the range of concentrations from 100-0.2 μg/ml.

Antibacterial evaluation showed that few derivatives were potent against *Bacillus subtilis* than the standard Ciprofloxacin. Compounds with R=C_6H_5, R_1-4-Cl (**III**a2) and R=C_6H_5; R_1-4-Br (**III**a6) were found to be the most potent against *Bacillus subtilis* with MIC value of 0.2 μg/ml. This may be probably due to the electronic effect of halogen substituents at para position of aryl ring. The compounds (**III**a3) and (**III**b1) showed good activity at MIC of 0.4 μg/ml. With respect to *Escherichia coli* strain, compounds (**III**b2) and (**III**b4) were found to show moderate activity. The compounds were found to be inactive against the bacterial strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). The results of antibacterial activity are summarized in Table-1.

Antitubercular activity

The antitubercular activity of derivatives was assessed against H_{37}Rv of *Mycobacterium tuberculosis* (Vaccine strain) using microplate Alamar Blue Assay (MABA)[15]. The activity of the derivatives was determined by MIC method. MIC was determined in the range of concentrations from 100-0.8 μg/ml. Pyrazinamide, Streptomycin and Ciprofloxacin were used as the reference standards with MIC of 3.125 μg/ml, 6.25 μg/ml and 3.125 μg/ml respectively. The results of antitubercular activity are summarized in Table-2.

The biological studies showed that all the twelve 3-(2-mercapto-6-phenyl/substitutedphenylpyrimidin-4-yl)-4-methoxy-1-phenyl/methylquinolin-2(1*H*)-ones {**III**a (1-6)/**III**b (1-6)} showed significant antibacterial and moderate antitubercular activity. *Bacillus subtilis* was found to be the most sensitive strain. Two compounds, compound (**III**-a2) and (**III**a6) were found to show better antibacterial activity then the standard drug ciprofloxacin.

Experimental

Melting points of the synthesized compounds were determined by Thiels melting point apparatus and are uncorrected. FT-IR spectra were recorded on Shimadzu IRAFFINITY-1 spectrophotometer by using KBr pellets. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 NMR spectormeter by using $CDCl_3$ or DMSO as solvent and TMS as internal standard, chemical shifts are expressed as δ values (ppm). Mass spectra were recorded on Waters,

Table-2
Antitubercular activity data of the title compounds

Compd	R	R_1	MIC (μg/ml)
IIIa1	C_6H_5	H	50
IIIa2	C_6H_5	4-Cl	50
IIIa3	C_6H_5	3-OCH_3	50
IIIa4	C_6H_5	4-OH	50
IIIa5	C_6H_5	4-F	50
IIIa6	C_6H_5	4-Br	50
IIIb1	CH_3	H	>100
IIIb2	CH_3	4-Cl	>100
IIIb3	CH_3	3-OCH_3	>100
IIIb4	CH_3	4-OH	50
IIIb5	CH_3	4-F	>100
IIIb6	CH_3	4-Br	50
Pyrazinamide			3.125
Streptomycin			6.25
Ciprofloxacin			3.125

Q-TOF Micromass (LC-MS) and λ_{max} was recorded on UV-1800 Shimadzu UV spectrophotometer using dimethyl formamide as a solvent. The reactions were monitored by thin layer chromatography (TLC) using precoated Silica gel-G plates as stationary phase and iodine vapours as visualizing agent.

3-Acetyl-4-methoxy-1-phenyl/methyl quinolin-2(1*H*)-one (Ia/Ib)

A solution of 3-acetyl-4-hydroxy-1-phenyl/methylquinolin-2(1*H*)-one (10 mmol) and dimethylsulphate (1.051 ml, 11.1 mmol) in acetone (100 ml), potassium carbonate (1.531g, 11.1 mmol) was added and the mixture was refluxed for 48-56 hr. The reaction completion was monitored with the help of TLC by using ethyl acetate : chloroform in the ratio of 1:1 as mobile phase. Removal of solvent by distillation left a reddish-orange solid which was suspended in water (100 ml), collected by filtration and dried. Thus, obtained compound was purified by suspending the compound in 20% solution of sodium carbonate. It was then filtered and dried.

3-Acetyl-4-methoxy-1-phenylquinolin-2(1*H*)-one (Ia)

Yield : 74%, m.p. : 70-72^0, R_f value : 0.62, λ_{max} : 278.80 nm. IR (KBr cm^{-1}): 3076, 3016 (aromatic –C-H), 2997, 2949 (aliphatic –C-H str.), 1705 (-C=O acetyl), 1639 (-C=O amide), 1080 (-C-O-C). ^{1}H NMR (DMSO-d_6 δ ppm): 8.0-6.0 (m, 9H, ArH), 4.0 (s, 3H, OCH_3), 2.6 (s, 3H, $COCH_3$).

3-Acetyl-4-methoxy-1-methylquinolin-2(1*H*)-one (Ib)

Yield : 78%, m.p. 82-84^0, R_f value : 0.67, λ_{max} : 225.20 nm. IR (KBr): 3008 (aromatic –C-H), 2974, 2956, 2918 (aliphatic –C-H str.), 1683 (-C=O acetyl), 1614 (-C=O amide), 1124 (-C-O-C). ^{1}H NMR (DMSO-d_6): 8.0-7.2 (m, 4H, ArH); 3.8 (s, 3H, O-CH_3), 3.5 (s, 3H, N-CH_3), 2.5 (s, 3H, $COCH_3$).

3-Substitutedcinnamoyl-4-methoxy-1-phenylquinolin-2(1*H*)-one derivatives {IIa (1-6)}

A mixture of **I**a (0.01M) and substituted aromatic aldehyde (0.012M) was dissolved in 30 ml of n-butanol under heating. Then 0.3 ml of glacial acetic acid and 0.3 ml of piperidine were added. The reaction mixture was refluxed for 4-6 hr and the completion of the reaction was monitored by TLC by using ethyl acetate: chloroform in the ratio of 1:1 as mobile phase. The solvent was then removed by vacuum distillation using rota evaporator. The residue was triturated with 20-30 ml of ethanol until a precipitate was formed, filtrated and recrystallized using suitable solvent.

4-Methoxy-3-[3-(3-methoxyphenyl) acryloyl]-1-phenylquinolin-2(1*H*)-one (IIa3)

Yield : 63%, m.p. 263-65^0, R_f value : 0.85, λ_{max} : 211.70 nm. IR (KBr): 3103, 3061, 3008 (aromatic –C-H), 2935, 2835 (aliphatic –C-H, str), 1653 (-C=O), 1608 (-C=O amide), 1074 (-C-O-C). ^{1}H NMR (DMSO-d_6): 7.1-8.1 (m, 13H, ArH), 7.6 (d, 1H, CHβ); 7.2 (d, 1H, CHα); 3.6 (s, 3H, O-CH_3), 3.4 (s, 3H, N-CH_3).

3-Substitutedcinnamoyl-4-methoxy-1-methylquinolin-2(1*H*)-one derivatives {IIb (1-6)}

A mixture of **I**b (0.05M) and substituted aromatic aldehydes (0.05M) was dissolved in 10% ethanolic KOH (2.2ml). Then the mixture was stirred for 2 hr and the completion of the reaction was monitored by TLC by using ethyl acetate: chloroform in the ratio of 1:1 as mobile phase. The compound obtained was separated by filtration and washed properly with water and further recrystallized using suitable solvent.

4-Methoxy-1-methyl-3-[3-(4-nitrophenyl) acryloyl] quinolin-2-(1*H*)-one (IIb3)

Yield : 41%, m.p. >300^0, R_f value : 0.87, λ_{max} 297.50 nm. IR (KBr): 3107, 3078 (aromatic –C-H), 2939, 2881, 2848 (aliphatic –C-H str); 1708 (-C=O), 1645 (-C=O amide), 1529 and 1346 (-NO_2), 1109 (-C-O-C). ^{1}H NMR (DMSO-d_6): 8.4-6.9 (m, 8H, ArH), 7.9 (d, 1H, CHβ); 7.1 (d, 1H, CHα), 3.6 (s, 3H, O-CH_3), 3.4 (s, 3H, N-CH_3).

3-(2-Mercapto-6-phenyl/substitutedphenylpyrimidin-4-yl)-4-methoxy-1-phenyl/methylquinolin-2(1*H*)-one {IIIa(1-6)/IIIb(1-6)}

A mixture of **II**a/**II**b (0.01M) and thiourea (0.01M) in 20 ml of ethanolic KOH (0.01178M) was heated under reflux for 9 hr and then cooled and poured into crushed ice. To this, a solution of 5% of glacial acetic acid was added. The precipitate obtained was filtered, washed with water and recrystallized from chloroform. The purity of all the newly synthesized compounds were ascertained by TLC using ethyl acetate: petroleum ether in the raio 2:1 as a mobile phase.

3-[2-Mercapto-6-(3-methoxyphenyl) pyrimidin-4-yl]-4-methoxy-1-phenylquinolin-2(1*H*)-one (IIIa3)

Yield : 58%, m.p. 168-70^0; R_f value : 0.52, λ_{max} 239.20 nm. IR (KBr): 3062, 3008 (aromatic –C-H); 2937, 2833 (aliphatic –C-H str), 1639 (-C=N), 1600 (-C=O amide), 1157, 1045 (-C-O-C ether). ^{1}H NMR (DMSO-d_6): 15.61 (s, 1H, S-H), 7.92-6.98 (m, 14H, ArH); 3.80 (s, 3H, O-CH_3), 3.62 (s, 3H, N-CH_3), ^{13}C NMR (DMSO-d_6), 198.27, 187.47, 171.25, 168.78, 154.24, 146.25, 139.51, 137.33, 135.85, 131.97, 130.78, 130.25, 129.14, 128.97, 127.49, 126.21, 125.30, 125.05, 122.57, 118.89, 116.80, 31.15, 30.89; LC-MS: 468 [M$^+$1].

3-[2-Mercapto-6-(4-nitrophenyl) pyrimidin-4-yl]-4-methoxy-1-methylquinolin-2(1*H*)-one (IIIb3)

Yield : 68%, m.p. 144-46^0; R_f value : 0.37: λ_{max} 282.20 nm. IR (KBr): 3080, 3032 (aromatic –C-H), 2941, 2889 (aliphatic –C-H str), 1647 (-C=N), 1622 (-C=O amide), 1562 and 1367 (-NO_2), 1076 (-C-O-C ether). ^{1}H NMR (DMSO-d_6): 16.78 (s, 1H, S-H), 8.08-6.65 (m, 9H, ArH), 3.50 (s, 3H, O-CH_3), 2.72 (s, 3H, N-CH_3). ^{13}C NMR (DMSO-d_6): 205.90, 189.17, 173.40, 154.40, 141.09, 134.64, 131.75, 131.20, 125.31, 125.04, 122.63, 121.56, 114.10, 113.82, 112.97, 105.43, 31.00, 28.56 LC-MS: 421 [M$^+$1].

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