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Research Article

SYNTHESIS AND ANTI-MICROBIAL SCREENING OF SOME NOVEL QUINAZOLINONE DERIVATIVES

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ABSTRACT

A number of substituted quinazolinone are known for their biological importance like anti-microbial, anti-inflamatory, anticancer, antifungal, antimalarial, anti-viral, anti-psychotics activity. In the present investigation an attempt has been made for the synthesis of quinazolinone derivatives. Further these synthesized of quinazolinone derivatives has been condensed with various primary amine containing drug like sulfonalamide, sulfanilamide, acetamides, thiourea, urea, pyrimethamine and with aromatic amine like 2-amino benzoic acid, aniline. The synthesized compound have been confirmed by IR, and NMR spectral data. These compounds were also screened for various biological activities like anti-microbial activity by standard methods. The synthesized compound compound has shown moderate to good anti-microbial activity and some synthesized compound has shown significant as compared with standard.

Keywords: Synthesis, Thin layar chromatography, Anti-microbial activity, NMR, IR.

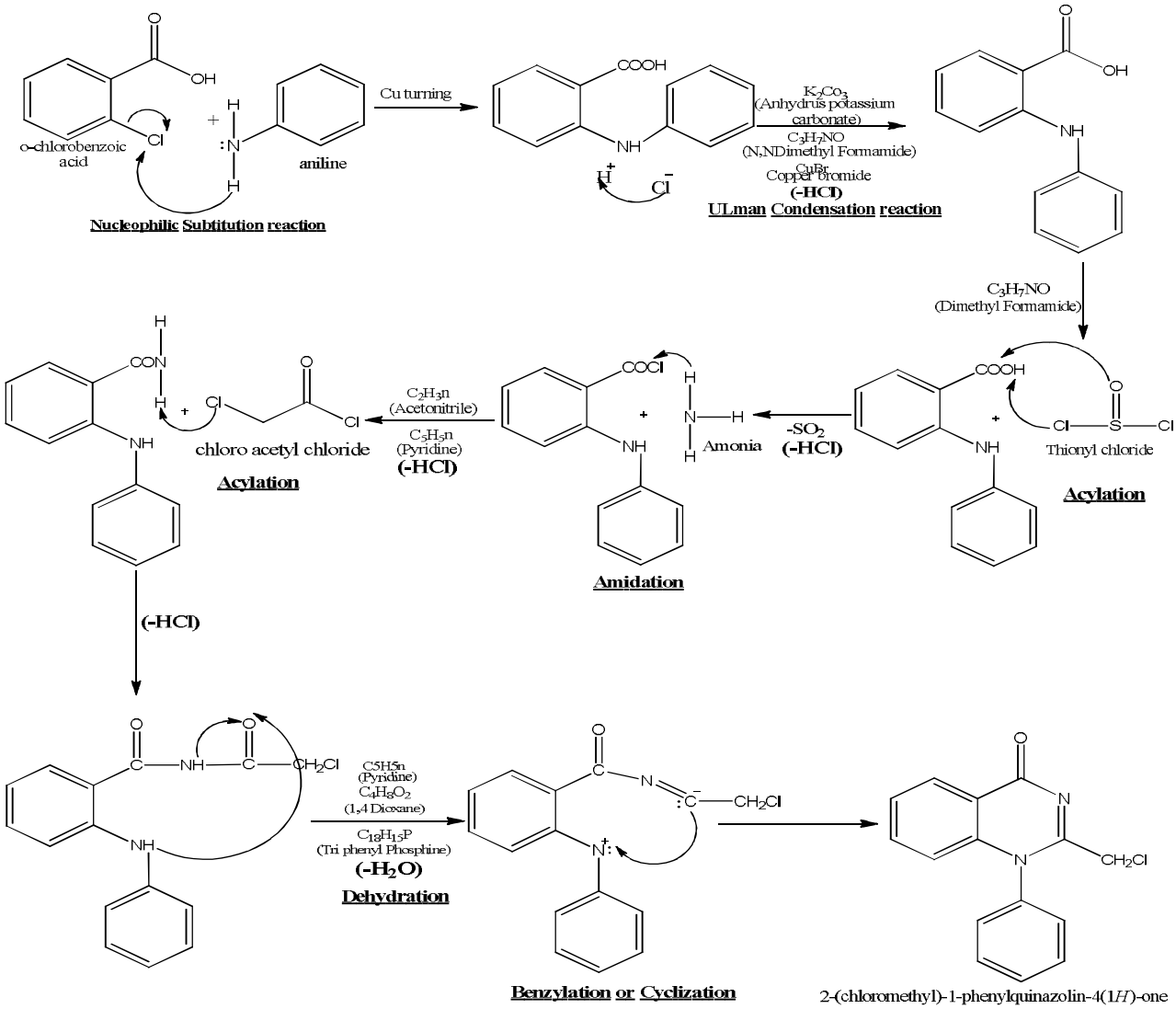
1. INTRODUCTION

Quinazolinone derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity. They are widely used in pharmaceuticals and agrochemicals. Several reports have been published on the biological activities of quinazolinone derivatives, including their anti-inflammatory (Kumar et al, 2002; Maggio et al, 2001; R.S.Giri et al 2009; E.Manivannan & S.C. Chaturvedi, 2001; A.Kumar et al, 2007; R.S Giri et al, 2010; E. Bansal et al, 2001), antimalarial (S.Zhu et al, 2010; S.Zhu et al 2009), antimicrobial, anti-fungal, antibacterial (G.P. Suresha et al 2011; M.S. Mahameda et al 2010; D.R. Patel et al 2011; D. Kohli et al 2009; N.B. Patel & J.C. Patel et al 2011; S.N. Pandeya et al 1999; A. Kumar et al 2011.), anticonvulsant (M. Zappala et al 2003; V. Jatav et al 2008; A.S. El-Azab et al 2012; S.K. Kashaw et al 2009.), and antitumor (S.L. Cao et al 2005; A.M. Al-Obaid et al 2009), activities. Moreover, large number of quinazolinone derivatives having substitution at 2 and 3 position by different heterocyclic moieties increases anti-inflammatory potential of quinazolinone derivatives. Similarly, various azetidinones (E. Bansal et al 2000; S.K. Srivastava et al 2000; A. Kumar et al 1990), and thiazolidinones (R. Yadav et al 2005; B. Goel et al 1999), have been reported to possess potent anti-inflammatory activity. Looking to the medicinal importance of 4(3*H*)- quinazolinone, 4-thiazolidinone, and azetidinones, we report here the synthesis of a new class of heterocyclic molecules in which all of these moieties are present and try to develop potential bioactive molecules. The structures of the compounds synthesized were assigned on the basis of elemental analysis, IR, 1H NMR, and anti-microbial activity. These compounds were evaluated for anti-microbial activity.

2. MATERIALS AND METHODS

All the melting points were determined. The purity is checked by TLC. IR Spectra's were recorded in KBr on shimatzu IR 8300 spectrophotometer. Analytical data were also confirmed from its H – NMR Spectra. The starting compound o-chloro benzoic acid and aniline has been prepared according to known method (Wasfy A A F et al 2003; Yassin F A et al 1999).

2.1 Mechanism of reaction scheme



2.2 Procedure

Step I.

A mixture o-chloro benzoic acid (0.10 Mole), different aniline derivative (0.17 Mole), anhydrous potassium carbonate (0.17 Mole), copper Powder (Dust) (50 mg) & n,n-dimethyl formamide (40 ml) was refluxed for 3.5 to 6 hrs. During reflux add 50 mg of copper bromide (in 3 times interval).

→ Cool → Pour in 1N HCl → Stir → Filter Wash with Water → Grey Solid Dry → Recrystallise →

Step II

A mixture of (I) (0.1 Mole), thionyl Chloride (0.2 Mole) and Ammonia (0.2 Mole) in 10 ml of Dimethyl Formamide was refluxed for 1-2 hrs to obtained Amide.

→ Residue → cool → filter & dry → Recrystallise

Step III

A mixture of (II) (20 Mili Mole), in dissolved in Pyridine (20 Mili Mole) and 50 ml of Acetonitrile. To this mixture add (2.0 ml) different Acyl derivative and reflux for 24-36 hrs and this mixture pour on Crushed ice and Solid Product was obtained.

→ Wash with Water → Filter & dry → Recrystallise

Step IV

A mixture of (III) (2.0 Mili Mole), in 10 ml of 1,4-Dioxane and stir with Triphenyl Phosphine (2.0 Mili Mole) & Pyridine (2.5 Mili Mole) continue stir for 4 hrs and add water (20 ml).

→ Filter & dry → Recystallise

3.0 SPECTRAL CHARACTERIZATION OF THE COMPOUNDS BY IR, ^{1}NMR

A4b

IR (KBr, cm^{-1}): 3063 (aromatic CH str.), 1692 (C=O)

^{1}H NMR (DMSO, δ(ppm)): 6.76-8.89(6H, aromatic ring) 3.39 (3H, CH_3)

R_f value: 0.75; **Boiling point:** 120-125^{o}C; **Percentage yield:** 56%.

A4c

IR (KBr, cm^{-1}): 3063 (aromatic CH str.), 1591 (C=O)

^{1}H NMR (DMSO, δ(ppm)): 6.82-7.26.59(6H, aromatic ring)

R_f value: 0.71; **Boiling point:** 85-90^{o}C; **Percentage yield:** 64%.

A4d

IR (KBr, cm^{-1}): 3058 (aromatic CH str.), 1737 (C=O), 1507(CH=CH)

^{1}H NMR (DMSO, δ(ppm)): 6.78-8.80(6H, aromatic ring) 2.44 (3H, CH_3)

R_f value: 0.65; **Boiling point:** 90-95^{o}C; **Percentage yield:** 69%.

A4e

IR (KBr, cm^{-1}): 3058 (aromatic CH str.), 1690 (C=O)

^{1}H NMR (DMSO, δ(ppm)): 6.72-8.24 (6H, aromatic ring) 3.58 (2H, CH_2)

R_f value: 0.63; **Boiling point:** 70-75^{o}C; **Percentage yield:** 67%.

B4b

IR (KBr, cm^{-1}): 3058 (aromatic CH str.), 1684 (C=O), 1118 (S)

^{1}H NMR (DMSO, δ(ppm)): 6.69-7.86 (6H, aromatic ring) 2.28 (3H, CH_3)

R_f value: 0.73; **Boiling point:** 55-60^{o}C; **Percentage yield:** 68%.

C_4a

IR (KBr, cm^{-1}): 3055 (aromatic CH str.), 1589 (C=O), 1519 (NO_2)

^{1}H NMR (DMSO, δ(ppm)): 7.01-8.73 (6H, aromatic ring) 2.86 (3H, CH_3)

R_f value: 0.55; **Boiling point:** 155-160^{o}C; **Percentage yield:** 55%.

C_4b

IR (KBr, cm^{-1}): 3061 (aromatic CH str.), 1626 (C=O), 1513 (NO_2),

^{1}H NMR (DMSO, δ(ppm)): 7.01-8.28 (6H, aromatic ring) 3.23 (3H, CH_3)

R_f value: 0.66; **Boiling point:** 80-85^{o}C; **Percentage yield:** 58%.

C_4c

IR (KBr, cm^{-1}): 3066 (aromatic CH str.), 1691 (C=O), 1475 (NO_2),

^{1}H NMR (DMSO, δ(ppm)): 7.25-8.26 (6H, aromatic ring)

R_f value: 0.60; **Boiling point:** 105-110^{o}C; **Percentage yield:** 66%.

D_4a

IR (KBr, cm^{-1}): 3047 (aromatic CH str.), 1691 (C=O), 692(C-Cl)

^{1}H NMR (DMSO, δ(ppm)): 7.02-7.89 (6H, aromatic ring) 2.40 (3H, CH_3)

R_f value: 0.74; **Boiling point:** 110-115^{o}C; **Percentage yield:** 62%.

D_4b

IR (KBr, cm^{-1}): 3333 (aromatic CH str.), 1668 (C=O), 743 (C-Cl),

^{1}H NMR (DMSO, δ(ppm)): 7.06-8.66 (6H, aromatic ring), 3.32 (2H, CH_2Cl)

R_f value: 0.69; **Boiling point:** 70-75^{o}C; **Percentage yield:** 63%.

D_4c

IR (KBr, cm^{-1}): 3052 (aromatic CH.), 1668 (C=O), 743(C-Cl)

^{1}H NMR (DMSO, δ(ppm)): 7.05-8.55 (6H, aromatic ring)

R_f value: 0.77; **Boiling point:** 120-125^{o}C; **Percentage yield:** 70%.

E_4a

IR (KBr, cm^{-1}): 3047(aromatic CH str.), 1871(C-O str. of Acid), 1500(NO_2)

^{1}H NMR (DMSO, δ(ppm)): 7.39-8.22 (7H, aromatic ring), 2.57 (3H, CH_3)

R_f value: 0.59; **Boiling point:** 80-85^{o}C; **Percentage yield:** 57%.

E_4b

IR (KBr, cm^{-1}): 3047 (aromatic CH str.), 1650 (C-O str.), 668-734(C-Cl)

^{1}H NMR (DMSO, δ(ppm)): 7.39-8.11 (6H, aromatic ring), 3.55 (2H, CH_2-Cl)
R_f value: 0.73; **Boiling point:** 85-90^{o}C; **Percentage yield:** 59%.

E4c
IR (KBr, cm^{-1}): 3069 (aromatic CH str.), 1744 (C=O), 1500(NO_2)
^{1}H NMR (DMSO, δ(ppm)): 6.58-8.15(6H, aromatic ring)
R_f value: 0.66; **Boiling point:** 80-85^{o}C; **Percentage yield:** 61%.

E4e
IR (KBr, cm^{-1}): 3060 (aromatic CH str.), 1697 (C=O), 1505 (NO_2), 2924 (C-H)
^{1}H NMR (DMSO, δ(ppm)): 6.60-7.99(6H, aromatic ring) 3.58 (2H, CH_2)
R_f value: 0.77; **Boiling point:** 85-90^{o}C; **Percentage yield:** 66%.

4.0 Antibacterial Activity
The synthesized compounds were tested *staphylococcus aureus* (S.A), *E. coli* (E.C) and *pseudomonas aeruginosa* (P.A) bacteria.
The stock solutions of compounds were prepared at a concentration of 5mg/ml & from stock solution the disc were prepared at a concentration of 100µg/ml. The testing was done on muller hinton agar plates by swabbing the agar plates with respective cultures, and placing the disc on it and incubating at 37°C for 24 hrs. the above results were obtained.

4.1 Procedure
1. Label each sterile Petri plate with the name of different bacterium to included (E.coli, P. aeruginosa, S. aureus).
2. Pour the nutrient agar media in the Petri plate when temperatures of media reach about 50^{o}C.
3. Allow the poured Petri plates until it solidify
4. Spread the 100µl of test micro-organism was inoculated by the spread technique by the spreader.
5. NO: 1 Whattmann filter paper was placed in the pre-labeled agar Petri plate.
6. Each disc was pressed down to insure complete contact the agar surface.
7. Add the 10µl of test sample and 10µl standard sample solvent against the different micro-organism by micro-pipette.

Table 4.1: Antimicrobial activities of newly synthesised compounds zone of inhibition (100%)

S.No	Sample	Dissolve solvent	Pseudomonas aeruginosa (P.A.)	Escherichia Coli (E.C.)	Staphylococcus aureus (S.A)	Sreptococcus (S.C)
1	A4a	Chloroform(C)	++	++	++	+++
2	A4b	Chloroform(C)	+++	+++	+++	++
3	A4c	Chloroform(C)	++	++	+++	++
4	A4d	Chloroform(C)	++	++	+++	+++
5	A4e	Chloroform(C)	++	++	++	++
6	B4a	Chloroform(C)	++	++	++	++
7	B4b	Chloroform(C)	+++	+++	++	++
8	B4c	Chloroform(C)	++	+++	+++	+++
9	B4d	Chloroform(C)	+++	+++	+++	++
10	B4e	Chloroform(C)	++	+++	++	++
11	C4a	Chloroform(C)	++	++	++	++
12	C4b	Chloroform(C)	++	++	++	++
13	C4c	Chloroform(C)	++	+++	+++	+++
14	C4d	Chloroform(C)	++	+++	++	+++
15	C4e	Chloroform(C)	++	++	+++	+++
16	D4a	Chloroform(C)	+++	+++	++	++
17	D4b	Chloroform(C)	+++	+++	++	++
18	D4c	Chloroform(C)	++	++	++	++
19	D4d	Chloroform(C)	+++	+++	++	++
20	D4e	Chloroform(C)	+++	+++	+++	+++
21	E4a	Chloroform(C)	+++	+++	++	++
22	E4b	Chloroform(C)	+++	+++	+++	++
23	E4c	Chloroform(C)	++	++	+++	+++

Concentration = 100 µg/ml
Greatest inhibition zone = +++
Average inhibition zone = ++
Good inhibition zone = +

Table 4.2: MINIUM INHIHITION CONCENTRATION (M.I.C.) 50%

S. No	Sample	Dissolve solvent	Pseudomonas aeruginosa (P.A.)	Escherichia Coli (E.C.)	Staphylococcus aureus (S.A)	Sreptococcu (S.C)
1	A4b	Chloroform(C)	++	+++	++	++
2	B4b	Chloroform(C)	++	++	++	++
3	D4a	Chloroform(C)	++	++	+++	+
4	D4d	Chloroform(C)	+++	++	++	++
5	E4a	Chloroform(C)	++	++	+++	+++
6	F4c	Chloroform(C)	++	+++	++	++

Concentration = 50 µg/ml
Greatest inhibition zone = +++
Average inhibition zone = ++
Good inhibition zone = +

Table 4.3: MINIUM INHIHITION CONCENTRATION (M.I.C.) 25%

S. No	Sample	Dissolve solvent	Pseudomonas aeruginosa (P.A.)	Escherichia Coli (E.C.)	Staphylococcus aureus (S.A)	Sreptococcu (S.C)
1	A4b	Chloroform(C)	+++	+++	++	++
2	B4b	Chloroform(C)	++	++	++	++
3	D4a	Chloroform(C)	+++	++	+++	+++
4	D4d	Chloroform(C)	+++	++	++	++
5	E4a	Chloroform(C)	++	++	+++	+++
6	F4c	Chloroform(C)	++	+++	++	++

Concentration = 25 µg/ml
Greatest inhibition zone = +++
Average inhibition zone = ++
Good inhibition zone = +

5.0 RESULT AND DISCUSSION

The Zone of inhibition & Minimum Inhibitory Concentration was determined by the disk plate method. Ceftriaxone was employed during the procedures as reference. The Minimum Inhibitory Concentration the synthesized compounds range between 50-100µg/ml. A4b, B4b, D4a, D4d, E4a were found moderately active, A4b, B4b, D4a and D4d were found to have more avtivity compared with ceftriaxone. Test compound were found to be more sensitive towords *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

6.0 CONCLUSION

From the date of the Table number 2.1 of anti-microbial activity, it is clearly concluded that the synthesized compounds are promisingly significant, good anti-microbial agents. As per the results of screening it is clearly indicated that the compounds of the scheme have shown good anti-microbial activity equipotent with the standard drugs. While A4b, B4b, D4a and D4d were found to have good activity compared with Ceftriaxone as a standard drug.

This is because of the presence of groups like – CH_3, -NH_2, -F, -S-, C_6H_5 at the different positions of phenyl nucleus and hetrocyclic system attached to quinazolinone nucleus which is attached to molecule.

From the above results one can establish that the systhesized substituted quinazolinone can be rich source for the exploitation. Therefore in search of new generation of the active compounds, it may be worthwhile to explore the possibility in this area or by making or introducing different functional groups or 2nd amines or by cyclization as substitution. Which may results into better pharmacological agents?

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