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ORIGINAL ARTICLE

Synthesis and pharmacological evaluation of 9(10H)-acridone bearing 1,3,4-oxadiazole derivatives as antimicrobial agents

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Abstract In the present study a new acridone derivatives were synthesized. The newly synthesized compounds were characterized by IR, NMR and C, H, N, S analyses. All newly synthesized compounds were screened for their antibacterial (*Staphylococcus aureus*, *Streptococcus viridans* and *Escherichia coil*) and antifungal (*Gibberela*, *Cercospora arachidicola*, *Physolospora piricola* and *Fusarium oxysporum*) studies. The results revealed that all synthesized compounds have a significant biological activity against the tested microorganisms.

1. Introduction

Chemical modification of bioactive components is one of the most common approaches in drug discovery with improved therapeutic effect (Tan et al., 2006) and the wide occurrence of the heterocycles in bioactive natural products and pharmaceuticals has made them as important synthetic targets.

The acridone alkaloid, acronycine, was isolated from *Acronychia baueri* in 1948 and found to have potent anticancer activity. A series of naturally occurring acridone alkaloids were found to be potent molecules for inhibition of human Promyelocytic leukemia cells (Singh et al., 2009; Fadeyi et al., 2008). Glyfoline, another natural acridone alkaloid isolated from *Glycosmis citrifolia*, was found to be the most potent molecule for inhibition of human leukemic HL-60 cells (Shoji et al., 2007). Several 9-acridone derivatives with or without an alkyl side chain attached to the N-position were found to exhibit anticancer and antibacterial activities reported *in vitro* and *in vivo* anticancer activities of novel pyrimidoacridones, pyridophenoxadines, and pyrimidocarbazones (Sondhi et al., 2010) and suggested that anticancer topoisomerase II inhibitors, such as amonafide, amsacrine, doxorubicin, and ellipticine contain a planar chromophore, which can intercalate into the DNA helix. Pyrrolobenzodiazepine hybrid linked to acridone ring system designed to exhibit significant

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DNA-binding and shows promising *in vitro* anticancer activity (Hagiwara et al., 2008). The triazoloacridones (C1305 and C1533) showed potent antitumor activity towards a wide range of different experimental tumors *in vitro* and *in vivo*, including both murine and human colon carcinomas (Fig. 1). These studies showed that the anticancer activity is due to DNA-intercalation (Delmas et al., 2004). Later imidazoacridone derivatives showed very good cytotoxicity against number of human cancer cell lines. The most prominent analog, C1311, is currently in clinical trials (Fig. 1). It has been found that C1311 binds non-covalently to DNA to induce its cytotoxic effect (Boutefnouchet et al., 2010).

A series of 1-amino thioacridones were designed as DNA-intercalating agents or DNA-intercalating agents with covalent bond formation potential. Studies showed that one of the compounds exhibited the most promising anticancer activity and may be useful as a lead compound in the search for more potent anticancer agents (Hegde et al., 2004).

On the other hand, 1,3,4-oxadiazole is a versatile lead molecule for designing potential bioactive agents. The 1,3,4-oxadiazole derivatives have been found to exhibit diverse biological activities such as antimicrobial, (El-Emam et al., 2004; Küçükgüzel et al., 2002), antiviral (Küçükgüzel et al., 2006), anti-malarial (Akhter et al., 2009), analgesic (Unangast et al., 1992), anti-inflammatory (Khan et al., 2001), anti-convulsant (Kumar et al., 2008), hypoglycemic (Maslat et al., 2002) and other biological properties such as genotoxic studies (Farghaly et al., 2000) and lipid peroxidation inhibitor (Cao et al., 2003). Keeping in view of these and in continuation of our research on biologically active molecules, we hereby report the synthesis of some acridone derivatives using *Ullmann* condensation between 2-chlorobenzoic acid and 2-aminobenzoic acid followed by Friedel–Crafts acylation then screened all synthesized compounds for there *in vivo* antimicrobial activities.

2. Experimental

2.1. Measurements

The percentage compositions of the elements (CHNS) for the compounds were determined using an elemental analyzer CHNS Model Fison EA 1108. The infrared spectra were recorded as potassium bromide disks using a Perkin–Elmer spectrophotometer GX. The ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded using the JEOL JNM-ECP 400 spectrometer. The purity of the synthesized compounds was checked by TLC silica gel coated plates obtained from Merck as stationary phase and solvent mixture of ethylacetate/chloroform as mobile phase at 25 °C.

2.2. Synthesis

2.2.1. Synthesis of diphenylamine-2,4′-dicarboxylic acid *1*

To a mixture of 2-chlorobenzoic acid (12.5 g, 0.08 mol), 4-aminobenzoic acid (10.97 g, 0.08 mol) and copper oxide powder (0.2 g) in (60 ml) of amyl alcohol, dry potassium carbonate (12 g) was slowly added and the mixture was allowed to reflux for 6 h at about (100 °C). The amyl alcohol was removed by rotary evaporator then mixture poured into (250 ml) of hot water, cooling and acidified with concentrated hydrochloric acid. The greenish black precipitate which formed was filtered, washed with cold water and collected. The crude acid was dissolved in aqueous sodium hydroxide solution, boiled in the presence of activated charcoal and filtered, on acidification

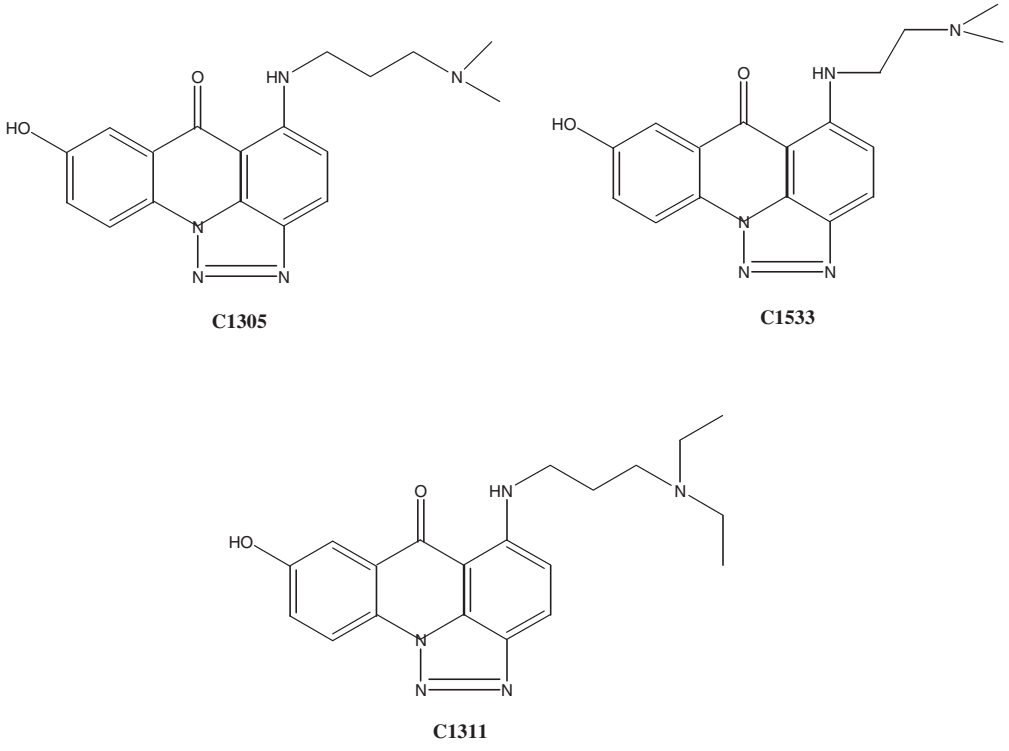


Figure 1 Examples of antitumor acridone derivatives.

of the filtrate with concentrated of hydrochloric acid white precipitate of **1** was obtained which was washed with water and recrystallized from ethanol. Yield 78%, R_f 0.75, m.p. 208–210 °C. IR (KBr) t = 3453 (2O–H), 3367 (N–H), 3097 (C–H aromatic), 1702 (C═O), 1560 (C═C). ^{1}H NMR (DMSO-d_6) δ = 8.02–8.07 (d, 1H, Ar-H), 8.0–7.95 (d, 1H, Ar-H), 7.65–7.87 (d, 1H, Ar-H), 7.54–7.60 (d, 1H, Ar-H), 7.43–7.50 (d, 1H, Ar-H), 7.37–7.48 (d, 1H, Ar-H), 7.25–7.31 (d, 1H, Ar-H), 7.18–7.22 (t, 1H, Ar-H), 7.09–7.14 (t, 1H, Ar-H), 8.87 (s, 1H, N–H) (D_2O exchange, disappear), 9.05 (s, 1H, O–H) (D_2O exchange, disappear). ^{13}C NMR (DMSO-d_6) δ = 130.56–136.78 (12C, aromatic carbons), 173.47, 174.13 (2C, 2C═O). Elemental analysis ($C_{14}H_{11}NO_4$); calcd. C, 65.37; H, 4.31; N, 5.44; Found C, 65.43; H, 4.12; N, 5.89%.

*2.2.2. Synthesis of 9(10H)-acridone-2-carboxylic acid **2***

Compound **1** (5 g, 0.03 mol) was placed in a round bottom flask then it will add to concentrated sulfuric acid (25 ml), shaken well and heated on water bath at (100 °C) for 3 h. Appearance of yellow color indicated the completion of the reaction. Then, it was poured into hot water (150 ml). The yellow precipitate which formed was filtered, washed with water and collected. The sample of 9(10H)-acridone-2-carboxylic acid **2** was recrystallized from methanol (Goodell et al., 2006). Yield 90%, R_f 0.62, m.p. > 330 °C. IR (KBr) t = 3427 (O–H), 3363 (N–H), 3068 (C–H aromatic), 1705, 1685 (2C═O), 1573 (C═C). ^{1}H NMR (DMSO-d_6) δ = 8.12–8.18 (d, 1H, Ar-H), 7.95–8.02 (d, 1H, Ar-H), 7.54–7.65 (d, 1H, Ar-H), 7.52–7.64 (d, 1H, Ar-H), 7.25–7.32 (d, 1H, Ar-H), 7.24–7.27 (d, 1H, Ar-H), 7.16–7.21 (t, 1H, Ar-H), 7.05–7.12 (t, 1H, Ar-H), 8.67 (s, 1H, N–H) (D_2O exchange, disappear), 9.13 (s, 1H, O–H) (D_2O exchange, disappear). ^{13}C NMR (DMSO-d_6) δ = 131.23–135.97 (12C, aromatic carbons), 172.13, 174.65 (2C, 2C═O). Elemental analysis ($C_{14}H_9NO_3$); calcd. C, 70.29; H, 3.79; N, 5.86; Found C, 70.43; H, 3.02; N, 5.24%.

*2.2.3. Synthesis of 9(10H)-acridonyl chloride **3***

A mixture of (1 g, 0.004 mol) 9(10H)-acridone-2-carboxylic acid **2** and excess thionyl chloride (10 ml) was refluxed for 3 h, the excess of thionyl chloride was concentrate through high vacuum, the residue was quenched with ice and the solid separated was filtered and dried through pump to afford title compound **3** as white solid (Goodell et al., 2006). Yield 75%, R_f 0.53, m.p. 234–236 °C. IR (KBr) t = 3346 (N–H), 3027 (C–H aromatic), 1685, 1673 (2C═O), 1560 (C═C). ^{1}H NMR (DMSO-d_6) δ = 8.34–8.39 (d, 1H, Ar-H), 8.23–8.31 (d, 1H, Ar-H), 8.20–8.27 (d, 1H, Ar-H), 8.13–8.19 (d, 1H, Ar-H), 7.88–7.97 (d, 1H, Ar-H), 7.62–7.78 (d, 1H, Ar-H), 7.53–7.59 (t, 1H, Ar-H), 7.43–7.50 (t, 1H, Ar-H), 8.79 (s, 1H, N–H) (D_2O exchange, disappear). ^{13}C NMR (DMSO-d_6) δ = 132.76–137.11 (12C, aromatic carbons), 169.67, 172.45 (2C, 2C═O). Elemental analysis ($C_{14}H_8ClNO_2$); Calcd. C, 65.26; H, 3.13; N, 5.44; Found C, 65.14; H, 3.20; N, 5.37%.

*2.2.4. Synthesis of 9(10H)-acridone-2-methylcarboxylate **4***

To 9(10H)-acridone-2-carboxylic acid **3** (0.5 g, 0.00194 mol), cold methanol (10 ml) was added readily and an instantaneous reaction occurred to give the title product. The crystals of ester were collected, filtered and washed with cold solution of 10% $NaHCO_3$, then with cold water (Goodell et al., 2006). Yield 83%, R_f 0.67, m.p. > 340 °C. IR (KBr) t = 3378 (N–H), 3024 (C–H aromatic), 1712, 1680 (C═O), 1567 (C═C). ^{1}H NMR (DMSO-d_6) δ = 3.88 (s, 3H, –OCH_3), 8.08–8.12 (d, 1H, Ar-H), 8.03–8.09 (d, 1H, Ar-H), 7.87–7.94 (d, 1H, Ar-H), 7.78–7.83 (d, 1H, Ar-H), 7.74–7.79 (d, 1H, Ar-H), 7.66–7.70 (d, 1H, Ar-H), 7.53–7.62 (t, 1H, Ar-H), 7.41–7.49 (t, 1H, Ar-H), 8.56 (s, 1H, N–H) (D_2O exchange, disappear). ^{13}C NMR (DMSO-d_6) δ = 22.14–23.32 (1C, –OCH_3), 133.53–139.02 (12C, aromatic carbons), 170.31, 174.86 (2C, 2C═O). Elemental analysis ($C_{15}H_{11}NO_3$); calcd. C, 71.14; H, 4.38; N, 5.53; Found C, 71.79; H, 4.09; N, 5.78%.

*2.2.5. Synthesis of 9(10H)-acridone-2-carboxylicacid hydrazide **5***

A mixture of 9(10H)-acridone-2-methylcarboxylate **4** (0.5 g, 0.00197 mol) and hydrazine hydrate (10 ml, 0.00591 mol) was refluxed for 3 h, then DMSO (1 ml) was added and the reflux continued for another 24 h. The crude product was obtained after distilling off the excess DMSO. Cooling filtering then washing with a little cold water, this product was employed in the next step without further purification. Yield 64%, R_f 0.82, m.p. 318–320 °C. IR (KBr) t = 3347–3256 ($NHNH_2$), 3024 (C–H aromatic), 1683, 1660 (2C═O), 1558 (C═C). ^{1}H NMR (DMSO-d_6) δ = 8.17–8.23 (d, 1H, Ar-H), 8.05–8.12 (d, 1H, Ar-H), 7.97–7.85 (d, 1H, Ar-H), 7.76–7.81 (d, 1H, Ar-H), 7.65–7.70 (d, 1H, Ar-H), 7.45–7.53 (d, 1H, Ar-H), 7.27–7.34 (t, 1H, Ar-H), 7.15–7.23 (t, 1H, Ar-H), 8.56 (s, 1H, N–H) (D_2O exchange, disappear), 8.76 (s, 2H, NH_2) (D_2O exchange, disappear). ^{13}C NMR (DMSO-d_6) δ = 131.80–136.73 (12C, aromatic carbons), 171.85, 174.60 (2C, 2C═O). Elemental analysis ($C_{14}H_{11}N_3O_2$); calcd. C, 66.40; H, 4.38; N, 16.59; Found C, 66.78; H, 4.67; N, 16.13%.

*2.2.6. Synthesis of 2-[5-thiol-1,3,4-oxadiazol-2-yl]-9(10H)-acridone **6***

To a stirred mixture of 9(10H)-acridone-2-carboxylicacid hydrazide **5** (0.5 g, 0.00169 mol) in ethanol (10 ml) and potassium hydroxide (0.0946 g, 0.00169 mol), carbon disulfide (0.202 ml, 0.00338 mol) was added slowly at (0 °C), and the mixture was refluxed for 24 h. The solvent was evaporated and the residue dissolved in water and acidified with dilute hydrochloric acid. The precipitate was filtered and washed with little cold water. The crude product was recrystallized from ethanol to give the desired product. Yield 87%, R_f 0.69, m.p. > 340 °C, IR (KBr) t = 3335 (N–H), 3062 (C–H aromatic), 2586 (S–H), 1678 (C═O), 1552 (C═C), 1332 (C═S), 1057 (C–O oxadiazole ring). ^{1}H NMR (DMSO-d_6) δ = 5.23–5.26 (s, 1H, S–H) (D_2O exchange), 8.10–8.16 (d, 1H, Ar-H), 7.78–7.81 (d, 1H, Ar-H), 7.57–7.64, 7.43–7.55, 7.36–7.40 (d, 1H, Ar-H), 7.27–7.33 (d, 1H, Ar-H), 7.20–7.24 (d, 1H, Ar-H), 7.15–7.18 (d, 1H, Ar-H), 7.09–7.12 (t, 1H, Ar-H), 7.00–7.06 (t, 1H, Ar-H), 8.64 (s, 1H, N–H) (D_2O exchange, disappear). ^{13}C NMR (DMSO-d_6) δ = 53.12–54.96 (2C, –C–O–C– oxadiazole), 130.79–135.68 (12C, aromatic carbons), 169.98 (1C, C═O). Elemental analysis ($C_{15}H_9N_3O_2S$); Calcd. C, 61.01; H, 3.07; N, 14.23; S, 10.86; Found C, 61.13; H, 3.51; N, 14.26; S, 10.92%.

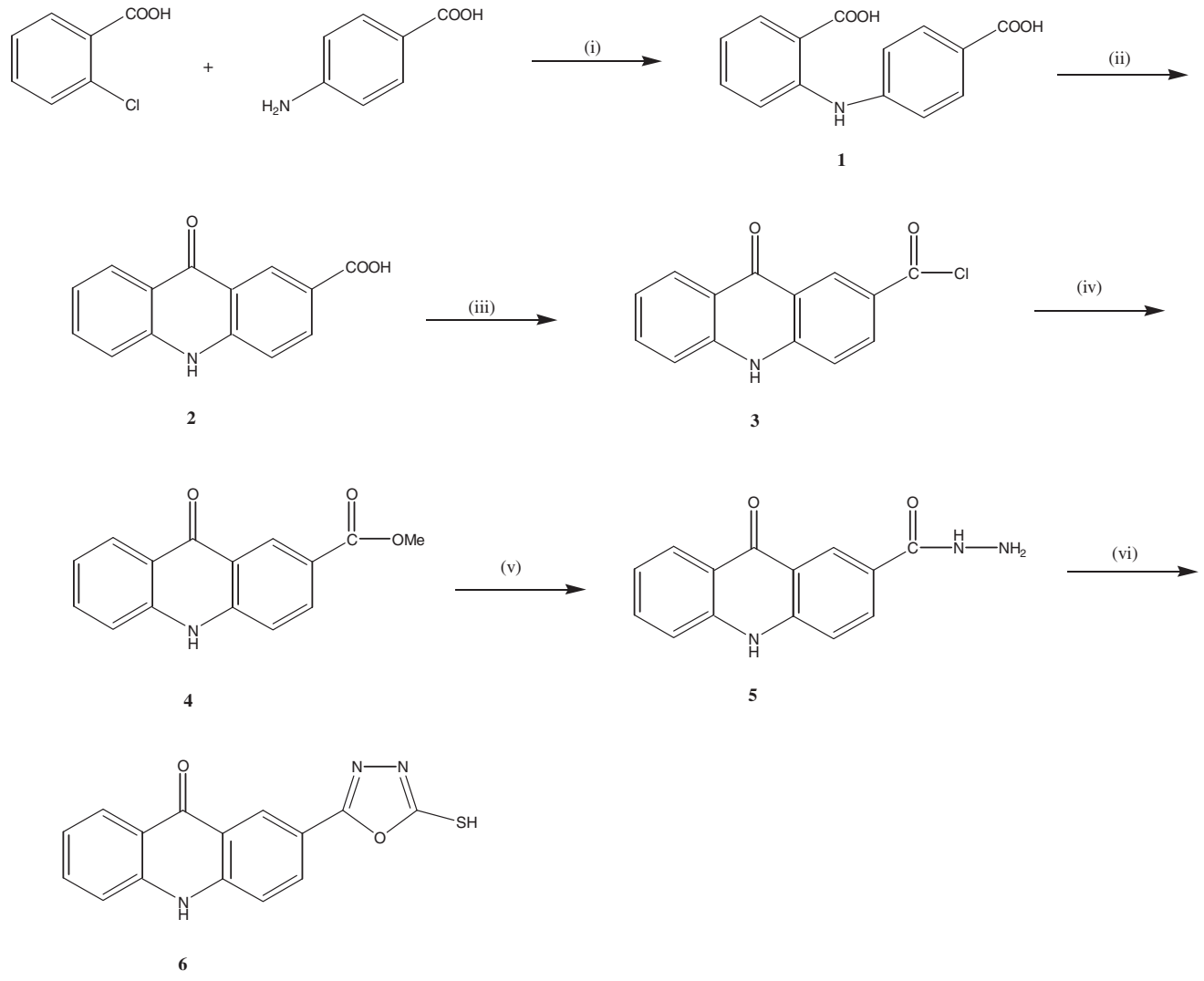
2.3. Antimicrobial tests

All the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus*, *Streptcoccus viridans* and *Escherichia coli*. Disk diffusion method (Cruickshank et al., 1975; Collins 1976) was used for

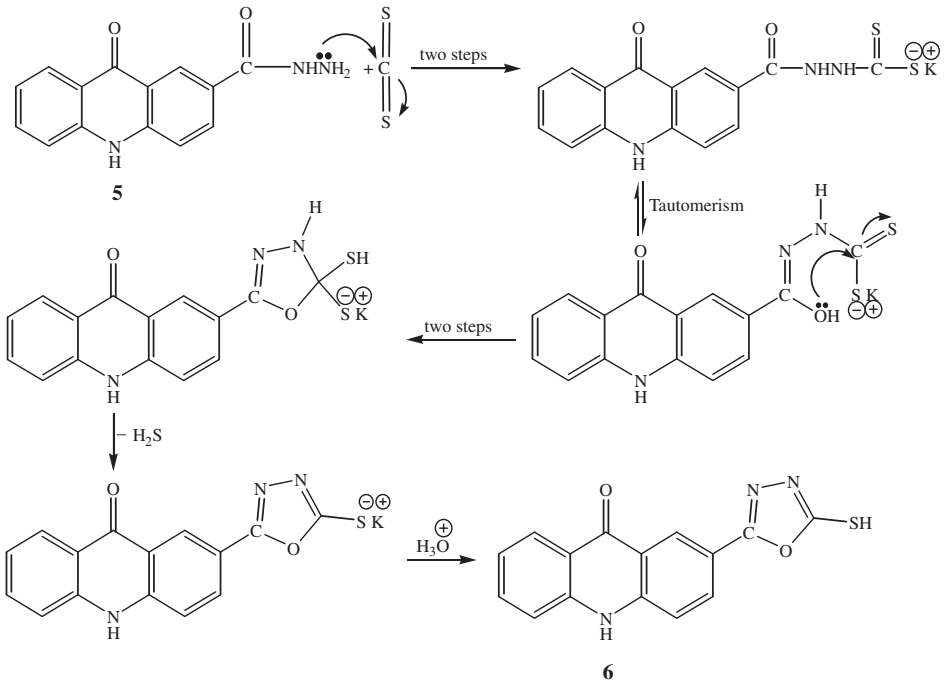
Table 1 Antibacterial and antifungal data for the synthesized compounds.

Comp. no.	Antibacterial activity data in MIC (μg/mL)			Antifungal activity data in MIC (μg/mL)			
	S. aureus	*S. viridans*	*E. coli*	*Gibberela*	*C. arachidicola*	*P. piricola*	*F. oxysporum*
1	13	14	13	34	52	14	37
2	15	17	26	45	23	67	53
3	26	18	13	56	34	47	62
4	34	29	17	15	22	38	56
5	20	22	20	66	56	17	34
6	21	36	27	19	29	50	64
Ampicillin trihydrate (std.)	16	5	21	23	12	10	34
Fluconazole (std.)	38	32	27	15	29	26	31
DMF (control)	–	–	–	–	–	–	–

determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using DMF. One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C



Scheme 1 Reagents and conditions: (i) CuO/K_2CO_3, (ii) H_2SO_4/Δ, (iii) $SOCl_2$, reflux 3 h, (iv) MetOH, (v) N_2H_4, DMSO, reflux 24 h, and (vi) CS_2, KOH, EtOH, reflux 24 h.



Scheme 2

for 24 h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. The MIC (μg/mL) values of the tested compounds against the tested bacteria strains are recorded in Table 1.

On the other hand, the newly prepared compounds were screened for their *in vitro* antifungal activity against *Cibberela*, *Cercospora arachidicola, Physolospora piricola* and *Fusarium oxysporum* in DMSO by the serial plate dilution method (Khan, 1997; Varma, 1998). All the fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of the corresponding species. Agar media (20 ml) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch wells were made into each well labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The MIC (μg/mL) values of the tested compounds against the tested fungal strains are recorded in Table 1.

3. Results and discussions

3.1. Chemistry

Ullmann condensation of 2-chlorobenzoic acid with 4-aminobenzoic acid was carried out by refluxing the reactants in the presence of copper oxide and potassium carbonate in amyl alcohol medium afforded diphenylamine-2,4′-dicarboxylic acid **1**. In this reaction as copper catalyzed nucleophilic aromatic substitution on aryl halide bearing electron withdrawing group. Further this dicarboxylic acid was converted into 9(10H)-acridone-2-carboxylic acid **2**, by reacting with conc. sulfuric acid (Goodell et al., 2006). Title compound 2-[5-thiol-1,3,4-oxadiazol-yl]-9(10H)-acridone **6** was synthesized by refluxing equimolar mixture of 9(10H)-acridone-2-carboxylicacid hydrazide **5** with potassium hydroxide and excess of carbon disulfide in ethanol (10 ml) for 6 h (Scheme 1).

The suggested mechanism of 2-[5-thiol-1,3,4-oxadiazol-2-yl]-9(10H)-acridone **6** is depicted in Scheme 2.

3.2. Characterization of the synthesized compounds

The structures of the resulting compounds were established by elemental analysis, IR and NMR. The proposed structure given to 9(10H)-acridone-2-carboxylicacid hydrazide **5** was support by IR analysis which showed band at 3347–3256 cm^{-1} due to stretching vibration of $NHNH_2$ moiety (Silverstien et al., 2005). Its ^1H NMR spectrum showed two D_2O exchangeable signal at 8.56 and 8.76 ppm assignable for the $NHNH_2$ moiety. Furthermore, the ^{13}C NMR spectrum showed two C═O signals at 171.85 and 174.60 ppm.

Formation of 2-[5-thiol-1,3,4-oxadiazol-yl]-9(10H)-acridone **6** was confirmed by recording its IR, NMR and elemental analysis. IR spectrum of compound **6** showed absorption at 3097 cm^{-1} due to the aromatic stretching. An absorption band at 1594 cm^{-1} assignable for C═N group, band at 1057 cm^{-1} is due to stretching of oxadiazole ring and the absorption band appeared at 664 cm^{-1} is for C–S group (Fuloria et al., 2009). The ^1H NMR spectrum of **6** showed multiplet in the region of δ 8.07–7.00 assignable to aromatic protons. Similarly a singlet appeared at δ, 5.03 due to the one proton of the S–H group (Jayashankar et al., 2009).

3.3. Antimicrobial studies

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were *S. aureus*, *Streptococcus viridans* and *Escherichia coil*. For antifungal, *Gibberela*, *C. arachidicola*, *P. piricola* and *F. oxysporum* were used as microorganisms. Both antimicrobial studies were assessed by minimum inhibitory concentration (MIC). The data are summarized in Table 1, and show that all compounds display certain activity against the tested microorganisms.

From SAR we can see that the antibacterial and antifungal activity of the synthesized compounds may be due the presence of the versatile pharmacophore which might increase the lipophilic character of the molecules, which facilitate the crossing through the biological membrane of the microorganism and thereby inhibit their growth.

4. Conclusion

The preparation procedure follow in this work for synthesis of the title compounds offers reduction in the reaction time, operation simplicity, cleaner reaction, easy work-up and improved yields. All spectroscopic analysis confirmed the proposed structures of these compounds. Biological activity data have shown that the synthesized compounds have a significant biological activity against the tested microorganisms.

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