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Preliminary communication

Synthesis and antifungal activity of some new 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones

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Abstract

Seven new 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones **4a–g** have been synthesized by the oxidation of 2-hydroxychalcone analogues of pyrazole **3a–g** with hydrogen peroxide (H_2O_2) in KOH–MeOH by Algar Flynn Oymanda (AFO) reaction. The structures of the compounds **4** were established by the combined use of ^{1}H NMR, IR and mass spectra. All the seven compounds were tested *in vitro* for their antifungal activity against three phytopathogenic fungi, namely *Helminthosporium* species, *Fusarium oxysporum* and *Alternaria alternata*. Five compounds **4a**, **4b**, **4c**, **4e** and **4f** were associated with substantially higher antifungal activity than commercial antifungal compound Actidione (cycloheximide) against all three phytopathogenic fungi.

Keywords: 3-Hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones; 2-Hydroxychalcone analogues of pyrazole; NMR; IR spectroscopy; Antifungal activity

1. Introduction

Flavonols are simply flavones in which a hydroxyl group substitutes the 3-position, and have considerable physiological, phylogenetic, chemosystematic, pharmacological, biosynthetic and analytical significance. They display many diverse types of biological properties such as antiproliferative [1,2], antiviral [3–5], antioxidant [6,7], antifungal [8–10] and anti-inflammatory [11]. They are also known to possess a good synthetic application; especially photooxygenation [12], superoxide anion [13] and reagents catalysed oxygenation reactions [14–19], which were non-enzymatic model reactions for the biological oxygenation of flavonols. On the other hand, substituted pyrazole ring also exhibits a broad spectrum of biological activities such as antidiabetic [20], antibacterial [21–23], antimicrobial [24–27] and herbicidal [28,29]. There has been a particular interest in the synthesis of flavonols with a wide array of groups at C-2. In view of these observations, it was envisaged in the present investigation to undertake the synthesis of a number of flavonols, having the pyrazole moiety at position C-2 with an aim to find new and more potent antifungal agents. We have synthesized some new 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones **4** as potential antifungal agents by the oxidation of 2-hydroxychalcone analogues of pyrazole **3** with hydrogen peroxide (H_2O_2) in KOH–MeOH by AFO reaction. The synthesized compounds **4a–g** were screened *in vitro* for their antifungal activity against three phytopathogenic fungi, namely *Helminthosporium* sp., *Fusarium oxysporum* and *Alternaria alternata* by Poisoned Food Technique.

2. Chemistry

Synthesis of 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones **4** is outlined in Scheme 1. Thus, oxidation of 2-hydroxychalcone analogues of pyrazole **3** with hydrogen peroxide (H_2O_2) in KOH–MeOH might afford the desired products

Abbrevations: AFO, Algar Flynn Oymanda reaction; MeOH, methanol; ppm, parts per million; PDA, potato dextrose agar.

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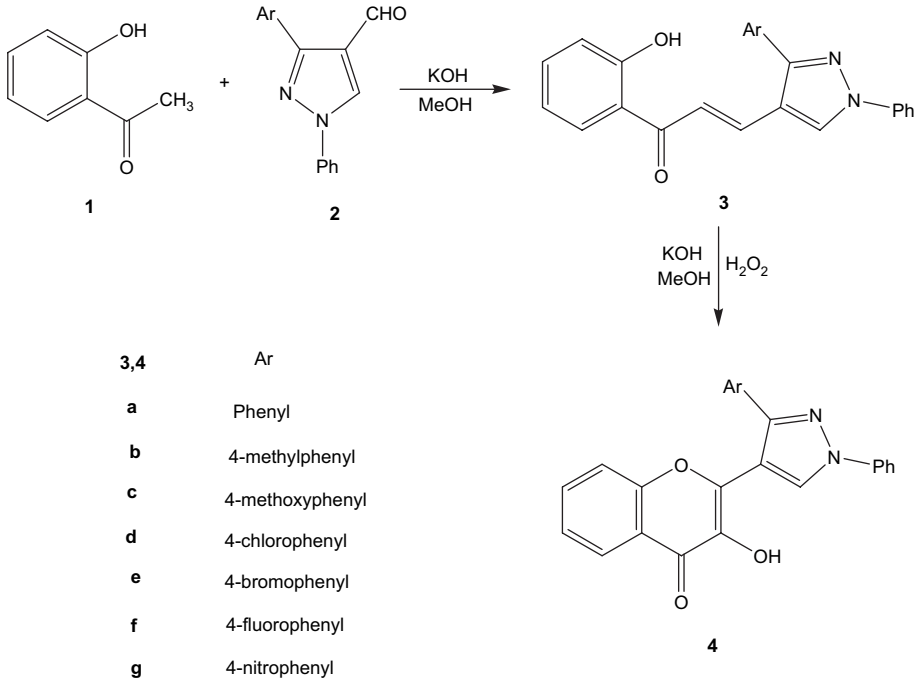

Scheme 1.

3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones **4** in high yields [30,31].

Accordingly, a solution of 2-hydroxychalcone **3a** in KOH–MeOH was treated with hydrogen peroxide by stirring at 0–5 °C for about 4–5 h. Usual work-up of the reaction afforded the pure crystalline product **4a** in 61% yield. Encouraged by the feasibility of our strategy for **4a**, we studied oxidative cyclization of a wide range of substituted 2-hydroxychalcones **3b–g** with hydrogen peroxide under similar conditions. The 2-hydroxychalcone analogues of pyrazole **3** were obtained by the condensation of 2-hydroxyacetophenone **1** with 1-phenyl-3-arylpyrazole-4-carboxaldehydes **2** in KOH–MeOH [32]. The structures of all the new 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones **4** and 2-hydroxychalcone analogues of pyrazole **3** were confirmed by their spectral data (*IR*, ^1H *NMR and MS*) and elemental analysis.

3. Biological results and discussion

All the seven compounds were tested *in vitro* for their antifungal activity against three phytopathogenic fungi, namely *Helminthosporium* sp., *F. oxysporum* and *A. alternata* (Table 1 and Fig. 1). Five of these compounds **4a**, **4b**, **4c**, **4e** and **4f** exhibited good antifungal activity than commercial antifungal compound Actidione (cycloheximide) against all three phytopathogenic fungi. Compound **4c** showed maximum inhibition against *Helminthosporium* sp. (77.0), *F. oxysporum* (64.2) and *A. alternata* (77.3) at 1000 ppm. Compound **4b** was found more effective against *Helminthosporium* sp. (76.1). It also inhibited the growth of *F. oxysporum* (55.7) and *A. alternata* (61.8) at 1000 ppm. Compound **4a** also inhibited the growth of *Helminthosporium* sp. (71.2), *F. oxysporum* (51.7) and *A. alternata* (52.8). Compound **4f** inhibited the growth of *Helminthosporium* sp. (58.6) and *A. alternata* (50.9) while the growth of *F. oxysporum* (16) was inhibited in less extent. Compound **4e** displayed almost same inhibition against all three phytopathogenic fungi at all concentrations. Compound **4d** did not show any percentage inhibition on the growth of *F. oxysporum*.

A careful analysis of percentage inhibition data revealed some interesting trends (Table 1 and Fig. 1). (i) The compounds **4a–g** inhibited the growth of phytopathogenic fungi significantly at different concentrations. The inhibitory effects of the compounds **4a–g** were directly correlated with concentrations. Hence, as concentration of the compounds **4a–g** increased (100–1000 ppm), the inhibition against all three phytopathogenic fungi also increased. The fungi are completely inhibited at higher level of concentrations (<1000 ppm). (ii) Comparison of the inhibition data of compounds **4a–g** suggested that by replacement of proton of aryl ring of pyrazole moiety (at 3-position) in 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones **4a–g** with electron releasing groups the antifungal activity increased while as the aryl proton is replaced with electron withdrawing group the antifungal activity decreased. The most striking examples in compounds **4a–g** are **4c** and **4g** compared with the parent compound **4a**. In compound **4c** the aryl proton is replaced with methoxy group which exhibited significant level of antifungal activity against all three fungi while in compound **4g** the aryl proton replaced with nitro group which is almost inactive towards all three fungi.

4. Conclusion

Using easily obtainable compounds **1** and **2** we have prepared a new series of 2-hydroxychalcone analogues of pyrazole **3** and 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones

Table 1
In vitro antifungal activity of **4a–g** using Poisoned Food Technique

Compounds	Concentrations (in ppm)	% Inhibition of phytopathogenic fungi[a]		
		Hs	Fo	Aa
4a	Control	0 ± 0.12	0 ± 0.05	0 ± 0.11
	100	31.0 ± 0.04	33.9 ± 0.12	15.0 ± 0.55
	500	50.5 ± 0.32	39.2 ± 0.18	41.5 ± 0.36
	1000	71.2 ± 0.39	51.7 ± 0.10	52.8 ± 0.44
4b	Control	0 ± 0.05	0 ± 0.11	0 ± 0.04
	100	73.8 ± 0.07	50.0 ± 0.07	60.0 ± 0.05
	500	73.8 ± 0.07	51.4 ± 0.07	61.8 ± 0.05
	1000	76.1 ± 0.04	55.7 ± 0.05	61.8 ± 0.05
4c	Control	0 ± 0.12	0 ± 0.05	0 ± 0.11
	100	59.7 ± 0.03	55.5 ± 0.04	73.5 ± 0.10
	500	59.7 ± 0.04	55.3 ± 0.07	75.4 ± 0.05
	1000	77.6 ± 0.05	64.2 ± 0.05	77.3 ± 0.09
4d	Control	0 ± 0.05	0 ± 0.11	0 ± 0.04
	100	0 ± 0.05	0 ± 0.04	0 ± 0.04
	500	1.19 ± 0.05	0 ± 0.04	38.2 ± 0.13
	1000	21.4 ± 0.43	0 ± 0.04	38.2 ± 0.13
4e	Control	0 ± 0.05	0 ± 0.11	0 ± 0.04
	100	39.0 ± 0.11	34.2 ± 0.11	25.4 ± 0.05
	500	39.2 ± 0.23	35.7 ± 0.04	29.0 ± 0.05
	1000	39.2 ± 0.23	41.4 ± 0.05	34.5 ± 0.05
4f	Control	0 ± 0.12	0 ± 0.05	0 ± 0.11
	100	12.3 ± 0.10	8.9 ± 0.20	30.1 ± 0.27
	500	28.7 ± 0.27	8.9 ± 0.05	43.3 ± 0.17
	1000	58.6 ± 0.32	16.0 ± 0.12	50.9 ± 0.10
4g	Control	0 ± 0.25	0 ± 0.35	0 ± 0.0
	100	1.31 ± 0.25	8.57 ± 0.10	0 ± 0.0
	500	1.31 ± 0.25	11.4 ± 0.35	16.1 ± 0.35
	1000	14.4 ± 0.25	14.2 ± 1.11	16.1 ± 0.35
Ac (Cy)	Control	0 ± 0.005	0 ± 0.11	0 ± 0.04
	100	38.5 ± 0.07	39.2 ± 0.13	40.2 ± 0.05
	500	41.5 ± 0.02	43.4 ± 0.05	45.5 ± 0.12
	1000	50.0 ± 0.05	50.2 ± 0.06	50.6 ± 0.10

Ac, Actidione; **Cy**, cycloheximide.
Mean of three replicates, ±standard deviation.
[a] Hs, *Helminthosporium* species; Fo, *Fusarium oxysporum*; Aa, *Alternaria alternata*.

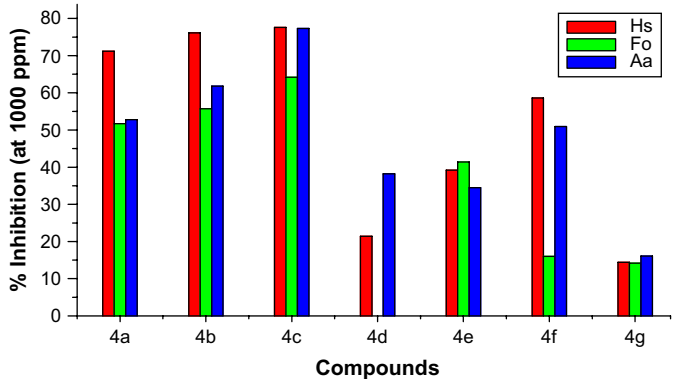

Fig. 1. Comparison of antifungal activity of compounds **4a–g** against three fungi at concentration 1000 ppm.

4 in good yields. Many of these compounds **4a**, **4b**, **4c**, **4e** and **4f** showed excellent activity as displayed in Table 1 and Fig. 1. The effect of presence of substituents such as methoxy and nitro in the aryl ring of pyrazole moiety of compounds **4** on antifungal activity has been shown.

5. Experimental

Melting points were determined in open capillaries with electrical melting point apparatus and are uncorrected. The IR spectra were obtained with a Buck Scientific IR M-500 spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker (300 MHz) spectrometer using tetramethylsilane as an internal standard. All the new compounds gave satisfactory analytical results (within ±0.4 of the theoretical values).

1-Phenyl-3-arylpyrazole-4-carboxaldehydes **2a–g** needed in this study were prepared by Vilsmeier–Haack reaction of acetophenone phenylhydrazones [33].

*5.1. 2-Hydroxychalcone analogues of pyrazole (**3a–g**)*

To a solution of KOH (1.12 g, 0.02 mol) in methanol (50 ml) was added 2-hydroxyacetophenone **1** (1.36 g, 0.01 mol) and 1-phenyl-3-(4-methylphenyl) pyrazole-4-carboxaldehydes **2** (2.62 g, 0.01 mol) at 0–5 °C. The reaction mixture was stirred overnight at room temperature. Then, this reaction mixture was poured over crushed ice and acidified with dil. HCl. The yellow solid thus obtained was filtered, washed with water and dried. The crude product was crystallized with chloroform–ethanol to afford pure 2-hydroxychalcones **3a–g**.

The physical, analytical and spectral data of new 2-hydroxychalcone analogues of pyrazole **3** are given below.

*5.1.1. 1-(2-Hydroxyphenyl)-3-(1,3-diphenyl-4-pyrazolyl) prop-2-en-1-one (**3a**)*

Yield 64%; mp 162–163 °C; IR (KBr): 1638 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.92 (s, 1H, OH), 8.41 (s, 1H), 7.40 (d, 1H, $J = 8.4$ Hz), 7.04 (d, 1H, $J = 8.4$ Hz), 8.03 (d, 1H, $J = 15.3$ Hz), 6.93 (m, 1H), 7.49–7.56 (m, 7H), 7.73–7.85 (m, 5H). Anal. Cald. for $C_{24}H_{18}N_2O_2$: C, 78.69, H, 4.92, N, 7.65. Found: C, 78.52, H, 4.93, N, 7.85.

*5.1.2. 1-(2-Hydroxyphenyl)-3-[(1-phenyl-3-(4-methylphenyl)-4-pyrazolyl)] prop-2-en-1-one (**3b**)*

Yield 58%; mp 143–145 °C; IR (KBr): 1638.6 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.47 (s, 3H, CH_3), 12.94 (s, 1H, OH), 8.4 (s, 1H), 8.02 (d, 1H, $J = 15.3$ Hz), 7.63 (d, 2H, $J = 7.8$ Hz), 7.35 (d, 2H, $J = 7.8$ Hz), 7.09 (d, 1H, $J = 8.4$ Hz), 7.40 (d, 1H, $J = 8.4$ Hz), 6.93 (m, 1H), 7.48–7.55 (m, 4H), 7.79–7.84 (m, 3H). Anal. Cald. for $C_{25}H_{20}N_2O_2$: C, 78.95, H, 5.26, N, 7.37. Found: C, 78.60, H, 5.41, N, 7.51.

*5.1.3. 1-(2-Hydroxyphenyl)-3-[(1-phenyl-3-(4-methoxyphenyl)-4-pyrazolyl)] prop-2-en-1-one (**3c**)*

Yield 54%; mp 150–152 °C; IR (KBr): 1638 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.91 (s, 3H, OCH_3), 12.94 (s, 1H, OH), 8.4 (s, 1H), 7.04 (d, 1H, $J = 8.4$ Hz), 7.39 (d, 1H, $J = 8.4$ Hz), 7.67 (d, 2H, $J = 8.7$ Hz), 7.08 (d, 2H, $J = 8.7$ Hz), 6.94 (m, 1H), 8,02 (d, 1H, $J = 15.3$ Hz), 7.82–7.84 (m, 3H), 7.48–7.55 (m, 4H). Anal. Cald. for $C_{25}H_{20}N_2O_3$: C, 75.76, H, 5.05, N, 7.07. Found: C, 75.28, H, 4.92, N, 6.96.

*5.1.4. 1-(2-Hydroxyphenyl)-3-[(1-phenyl-3-(4-chlorophenyl)-4-pyrazolyl)] prop-2-en-1-one (**3d**)*

Yield 62%; mp 165–166 °C; IR (KBr): 1639.2 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 12.88 (s, 1H, OH), 8.40 (s, 1H), 7.97 (d, 1H, $J = 15.3$ Hz), 7.41 (d, 1H, $J = 8.4$ Hz), 7.04 (d, 1H, $J = 8.4$ Hz), 7.68 (d, 2H, $J = 8.4$ Hz), 7.48 (d, 2H, $J = 8.4$ Hz), 6.95 (m, 1H), 7.50–7.56 (m, 4H), 7.80–7.83 (m, 3H). Anal. Cald. for $C_{24}H_{17}N_2O_2Cl$: C, 71.82, H, 4.24, N, 6.98. Found: C, 71.29, H, 4.36, N, 7.15.

*5.1.5. 1-(2-Hydroxyphenyl)-3-[(1-phenyl-3-(4-bromophenyl)-4-pyrazolyl)] prop-2-en-1-one (**3e**)*

Yield 51%; mp 171–173 °C; IR (KBr): 1638.2 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 12.87 (s, 1H, OH), 8.41 (s, 1H), 7.42 (d, 1H, $J = 8.4$ Hz), 7.05 (d, 1H, $J = 8.4$ Hz), 7.69 (d, 2H, $J = 8.1$ Hz), 7.63 (d, 2H, $J = 8.1$ Hz), 6.95 (m, 1H), 7.48–7.56 (m, 4H), 7.80–7.84 (m, 3H), 7.93 (d, 1H, $J = 15.3$ Hz). Anal. Cald. for $C_{24}H_{17}N_2O_2Br$: C, 64.72, H, 3.82, N, 6.29. Found: C, 64.19, H, 3.80, N, 6.42.

*5.1.6. 1-(2-Hydroxyphenyl)-3-[(1-phenyl-3-(4-fluorophenyl)-4-pyrazolyl)] prop-2-en-1-one (**3f**)*

Yield 61%; mp 184–186 °C; IR (KBr): 1638 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 12.87 (s, 1H, OH), 8.40 (s, 1H), 7.69 (d, 2H, $J = 8.4$ Hz), 7.61 (d, 2H, $J = 8.4$ Hz), 7.55 (d, 1H, $J = 8.4$ Hz), 7.05 (d, 1H, $J = 8.4$ Hz), 6.92–6.97 (m, 1H), 7.47–7.52 (m, 3H), 7.79–7.83 (m, 3H), 7.97 (d, 1H, $J = 15.3$ Hz), 7.37–7.41 (m, 1H). Anal. Cald. for $C_{24}H_{17}N_2O_2F$: C, 75.00, H, 4.43, N, 7.29. Found: C, 74.89, H, 4.35, N, 6.99.

*5.1.7. 1-(2-Hydroxyphenyl)-3-[(1-phenyl-3-(4-nitrophenyl)-4-pyrazolyl)] prop-2-en-1-one (**3g**)*

Yield 64%; mp 195–197 °C; IR (KBr): 1640 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 12.86 (s, 1H, OH), 8.31 (d, 2H, $J = 9.0$ Hz), 8.05 (d, 2H, $J = 9.0$ Hz), 8.41 (s, 1H), 7.96 (d, 1H, $J = 15.3$ Hz), 6.95 (m, 1H), 7.47–7.57 (m, 5H), 7.73–7.85 (m, 4H). Anal. Cald. for $C_{24}H_{17}N_3O_4$: C, 70.07, H, 4.14, N, 10.22. Found: C, 69.78, H, 4.04, N, 9.92.

*5.2. 3-Hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones (**4a–g**)*

General procedure. To a well-stirred solution of 2-hydroxychalcones **3** (2.0 g, 0.007 mol) in MeOH (20 ml) and aq. KOH (10 ml, 20%), cooled at 5–10 °C, was added 30% H_2O_2 (10 ml) dropwise over 1 h. The reaction mixture was further stirred for 4–5 h. The resulting light yellow reaction mixture was poured on crushed ice and neutralized with dil. HCl. The light yellow solid thus obtained was filtered, washed with water and dried. The crude product was crystallized with chloroform–ethanol to afford pure 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones **4**.

The physical, analytical and spectral data of 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones **4a–g** are given below.

*5.2.1. 3-Hydroxy-2-(1,3-diphenyl-4-pyrazolyl) chromone (**4a**)*

Yield 61%; mp 209–210 °C; IR (KBr): 3306 cm^{-1} (–OH str.), 1609 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.86 (s, 1H), 8.24 (dd, 1H, $J = 1.5$, 8.1 Hz), 7.88 (d, 2H, $J = 7.5$ Hz), 7.71–7.74 (m, 2H), 7.49–7.59 (m, 6H), 7.36–7.41 (m, 2H), 6.90 (d, 1H, $J = 8.4$ Hz). Anal. Cald. for $C_{24}H_{16}N_2O_3$: C, 75.79, H, 4.21, N, 7.36. Found: C, 74.95, H, 4.08, N, 7.51; MS: *m/z*, M^+ 380.

*5.2.2. 3-Hydroxy-2-[(1-phenyl-3-(4-methylphenyl)-4-pyrazolyl)] chromone (**4b**)*

Yield 58%; mp 221–224 °C; IR (KBr): 3284 cm^{-1} (–OH str.), 1611 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.49 (s, 3H, CH_3), 8.86 (s, 1H), 8.25 (dd, 1H, $J = 1.5$, 7.8 Hz), 7.87 (d, 2H, $J = 7.8$ Hz), 7.30 (d, 2H, $J = 7.8$ Hz), 6.97 (d, 1H, $J = 8.4$ Hz), 7.53–7.63 (m, 5H), 7.36–7.40 (m, 2H). Anal. Cald. for $C_{25}H_{18}N_2O_{3:}$, C, 76.14, H, 4.57, N, 7.11. Found: C, 75.88, H, 4.73, N, 7.24; MS: *m/z*, M^+ 394.

*5.2.3. 3-Hydroxy-2-[(1-phenyl-3-(4-methoxyphenyl)-4-pyrazolyl)] chromone (**4c**)*

Yield 52%; mp 202–204 °C; IR (KBr): 3298 cm^{-1} (–OH str.), 1611 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.93 (s, 3H, CH_3), 8.85 (s, 1H), 8.25 (dd, 1H, $J = 1.5$, 8.1 Hz), 7.87 (d, 2H, $J = 7.8$ Hz), 7.67 (d, 2H, $J = 8.7$ Hz), 7.03 (d, 2H, $J = 8.7$ Hz), 7.50–7.57 (m, 4H), 7.36–7.40 (m, 2H). Anal. Cald. for $C_{25}H_{18}N_2O_{4:}$ C, 73.17, H, 4.39, N, 6.83. Found: C, 73.35, H, 4.38, N, 6.88; MS: *m/z*, M^+ 410.

*5.2.4. 3-Hydroxy-2-[(1-phenyl-3-(4-chlorophenyl)-4-pyrazolyl)] chromone (**4d**)*

Yield 61%; mp 244–246 °C; IR (KBr): 3278 cm^{-1} (–OH str.), 1613 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.86 (s, 1H), 8.25 (dd, 1H, $J = 1.5$, 8.1 Hz), 7.85 (d, 2H, $J = 8.4$ Hz), 7.67 (d, 2H, $J = 8.4$ Hz), 7.40–7.51 (m, 7H), 6.98 (d, 1H, $J = 8.4$ Hz). Anal. Cald. for $C_{24}H_{15}N_2O_3Cl$: C, 69.40, H, 3.61, N, 6.75. Found: C, 69.62, H, 3.68, N, 6.62; MS: *m/z*, M^+ 414.

*5.2.5. 3-Hydroxy-2-[(1-phenyl-3-(4-bromophenyl)-4-pyrazolyl)] chromone (**4e**)*

Yield 52%; mp 232–234 °C; IR (KBr): 3280 cm^{-1} (–OH str.), 1612 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.86 (s, 1H), 8.26 (dd, 1H, $J = 1.5$, 8.1 Hz), 7.86 (d, 2H, $J = 7.8$ Hz), 7.55 (d, 2H, $J = 7.8$ Hz), 7.39–7.47 (m, 3H),

7.62–7.72 (m, 4H), 6.99 (d, 1H, $J = 8.4$ Hz). Anal. Cald. for $C_{24}H_{15}N_2O_3Br$: C, 62.75, H, 3.27, N, 6.10. Found: C, 62.93, H, 3.22, N, 6.04; MS: *m/z*, M^+ 459.

*5.2.6. 3-Hydroxy-2-[(1-phenyl-3-(4-fluorophenyl)-4-pyrazolyl)] chromone (**4f**)*

Yield 58%; mp 226–228 °C; IR (KBr): 3300 cm^{-1} (–OH str.), 1610 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.86 (s, 1H), 8.24 (dd, 1H, $J = 1.5$, 8.1 Hz), 7.51–7.91 (m, 9H), 7.06–7.22 (m, 3H). Anal. Cald. for $C_{24}H_{15}N_2O_3F$: C, 72.36, H, 3.77, N, 7.04. Found: C, 72.30, H, 3.70, N, 6.9; MS: *m/z*, M^+ 398.

*5.2.7. 3-Hydroxy-2-[(1-phenyl-3-(4-nitrophenyl)-4-pyrazolyl)] chromone (**4g**)*

Yield 57%; mp 237–239 °C; IR (KBr): 3297 cm^{-1} (–OH str.), 1609 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.82 (s, 1H), 8.20 (dd, 1H, $J = 1.5$, 8.1 Hz), 8.37 (d, 2H, $J = 8.4$ Hz), 8.13 (d, 2H, $J = 8.4$ Hz), 7.45–7.59 (m, 6H), 7.06–7.18 (m, 2H). Anal. Cald. for $C_{24}H_{15}N_3O_5$: C, 67.76, H, 3.53, N, 9.88. Found: C, 67.62, H, 3.32, N, 9.51; MS: *m/z*, M^+ 425.

6. *In vitro* biological assay

6.1. Medium

Potato dextrose agar (PDA) medium was used for biological assays. PDA medium was prepared by boiling 200 gm potato chips in 1 l of distilled water, filtering the extracts and making the final volume 1 l. To this 1% dextrose was added (pH adjusted at 5.5), after this 2% agar–agar was added and autoclaved at 121 °C for 20 min.

6.2. Test phytopathogenic fungi

Three phytopathogenic fungi, namely *Helminthosporium* sp., *F. oxysporum* and *A. alternata* were used for biological assays. The synthesized compounds **4a–g** and commercial antifungal compound Actidione (cycloheximide) were screened *in vitro* for their antifungal activity against these fungi by Poisoned Food Technique [34,35].

6.3. Biological procedure

Potato dextrose agar medium was prepared in the flasks and sterilized. To this medium requisite quantity of the samples of compounds **4a–g** were added so as to get desirable final concentrations, i.e. 100 ppm, 500 ppm, and 1000 ppm. The samples were thoroughly mixed by stirring. The medium was then poured into sterilized Petri dishes. The mycelial discs were taken from the cultures of the test fungi previously grown on PDA medium for seven days, were used for the purpose of inoculation in the centre of Petri dishes aseptically. Suitable controls were kept where the cultured discs were grown under same conditions on PDA medium without any sample compounds. The plates were inoculated at 28 ± 1 °C. The efficacy in each was determined by measuring radial mycelial growth. The radial growth of the colony was measured in two directions at right angles to each other, and the average of three replicates was recorded in each case. Data were expressed as percent inhibition over control from the size of the colonies, and subjected to two-way analysis of variance (ANOVA).

The percentage inhibition given in Table 1 was calculated using the formula [36]:

$$\text{Percentage inhibition} = (C - T) \times 100/C$$

where C = diameter of the fungus colony in the control plate after 96 h;
T = diameter of the fungus colony in tested plates after the same period.

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