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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Microwave-assisted solvent-free synthesis of biologically active novel heterocycles from 3-formylchromones

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Abstract A series of novel chromone derivatives have been synthesized employing 3-formylchromones and 5-acetyl-1,3-dimethylbarbituric acid as starting materials both under conventional heating method and microwave irradiation technique in good yields. The synthesized compounds were screened in vitro antibacterial activity against the representative panel of two Gram-positive bacteria and two Gram-negative bacteria. The synthesized compounds were also tested for their inhibitory action against three strains of fungus. The various compounds show potent inhibitory action against test organisms.

Keywords 3-Formylchromone · 5-Acetyl-1, 3-dimethylbarbituric acid · Microwave irradiation · Antibacterial activity · Antifungal activity

Introduction

Development of new antibacterial agents with novel structure and mode of action remains the primary goal of scientists for the solution of increasing bacterial resistance gained by microorganism to classical antibacterial agents (Moneer *et al.*, 2002). Chalcones and their derivatives are an attractive molecular scaffold for the search of new biologically active molecules. Also, chromones comprise a vast array of oxygen containing compounds ubiquitous in plants (Barton and Ollis, 1979). They form basic nucleus of flavones and have been recognized as the essential component of phamacophores of a large number of bioactive molecules (Dewick, 1994; Hansch *et al.*, 1990). Molecules containing chromone structure possess a myriad of biological activities ranging from antifungal (Prakash *et al.*, 2008), antioxidant (Kuroda *et al.*, 2009), neuroprotective (Larget *et al.*, 2000), HIV-inhibitory (Yu *et al.*, 2004), antibacterial (Deng *et al.*, 2000) to anticancer (Valenti *et al.*, 2000) activities. Chromone derivatives are also active at benzodiazepine receptors and on lipoxygenases and cyclooxygenases (Horton *et al.*, 2003). It was also sited in the literature that Hormothamnione, a naturally occurring styrylchromone isolated from the blue green algae, Hormothamnione enteromophoides has potent cytotoxicity to P-388 lymophocytic leukaemia and HL-60 human promyelocytic leukaemia cells (Alonso and Brossi, 1988; Grazul and Budzisz, 2009). Chalcones, the flavanoid and isoflavanoid precursors, display a wide spectrum of biological activities including antioxidant (Stevens *et al.*, 2003; Vogel *et al.,* 2008), antibacterial (Sugamoto *et al.*, 2008; Avila *et al.*, 2008), antimalarial, antileishmanial (Liu *et al.*, 2003; Quintin *et al.*, 2009; Suryawanshi *et al.*, 2008), anticancer (Lawrence *et al.*, 2006), antiangiogenic (Mojzis *et al.*, 2008), anti-infective, anti-inflammatory (Nowaskowska, 2007; Cheng *et al.*, 2008), antifungal (Lahtchev *et al.*, 2008), nitric oxide inhibition (Rojas *et al.*, 2002), tyrosinase inhibition (Nerya *et al.*, 2004), etc. It is also worth mentioning that the derivatives of chalcones particularly pyrazoline nucleus forms the core structure of many drugs and have been reported to possess widespread biological activities (Chimenti *et al.*, 2005; Khode *et al.*, 2009).

In view of variety of pharmacological properties exhibited by chromones, chalcones and its derivatives, we

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were prompted to undertake synthesis of chromonyl chalcones and conversion to other heterocycles like pyrazolines, pyrazoles, dibromoderivatives and dihydropyrimidines, which may show different or better physiological activities.

Results and discussion

Chemistry

Microwave-assisted organic reaction enhancement (MORE) is now a days a well-established technique for the synthesis of various heterocycles particularly from the viewpoint of green Chemistry. The e-Chemistry technique gives spectacular results viz-shorter reaction time, experimental simplicity, selectivity of products and easy work up, etc. (Kappe and Dallinger, 2009; Varma, 1999). It was found that 3-formyl chromone has emerged as a valuable synthon for incorporation of chromone moiety into a number of molecular frameworks, which forms important component of pharmacophores of a number of biologically active molecules of synthetic as well as natural origin (Raj *et al.*, 2008). To the best of our knowledge, no reports have so far made in the synthesis of chromonyl chalcones and its derivatives under microwave irradiation. This inspired us to go with these reactions. Due to exceptional reactivity of formyl group in 3-formylchromone, as well as the versatile biological activities of chromone and barbituric acid derivatives (Aboul and Wainer, 1997), two chromonyl chalcones **3a–b** were synthesized by employing 3-formylchromones **1a–b** and 5-acetyl-1,3-dimethylbarbituric acid **2** in the presence of mild base both under conventional heating and microwave irradiation method. The chalcones **3a–b** were synthesized in the presence of pyridine using ethanol as a solvent in conventional method, which took longer period for completion of reaction (7–7.5 h) with yields (78–79%) (Table 1). The reactions when carried out under microwave irradiation were completed within 6 min with substantial increase in yield of products (88%). Moreover, the reaction generated (E)-isomer only. The infrared (IR) spectrum of **3a** exhibited chromone and barbituric acid carbonyl groups at 1657 and 1719 cm^{-1}, respectively. The ^1H NMR spectrum showed *trans* olefinic protons H_a and H_b as ortho-coupled doublets at δ 9.26 ($J = 15.7$ Hz) and 7.84 (J = 15.7 Hz), respectively. The N–CH_3 protons of barbituric acid moiety were present as two sharp singlets at δ 3.37 and 3.39. The three aromatic protons of chromone moiety were discernible in the form of multiplet at δ 7.49–7.76 whereas C-2 and C-5 protons appeared as a singlet and doublet of doublet at δ 8.39 and 8.32, respectively. Further, the structure was confirmed by mass spectrum, which showed M^+ at *m/z* 354.

Table 1 Synthesis of compounds **3a–7** under thermal heating and Microwave irradiation

Entry	Product	Conventional heating method		Microwave irradiation[a]	
		Time	Yield (%)[b]	Time	Yield (%)[b]
1	**3a**	7 h	78	6 min	88
2	**3b**	7.5 h	79	6 min	88
3	**4a**	1 h	74	3 min	94
4	**4b**	1 h	74	3 min	92
5	**4c**	11 h	63	7 min	86
6	**4d**	10.5 h	65	6 min	86
7	**4e**	12 h	75	9 min	89
8	**5**	14 h	76	7 min	92
9	**6a**	20 min (RT)	82	–	–
10	**6b**	20 min (RT)	80	–	–
11	**7**	28 h (RT)	78	3 min	90

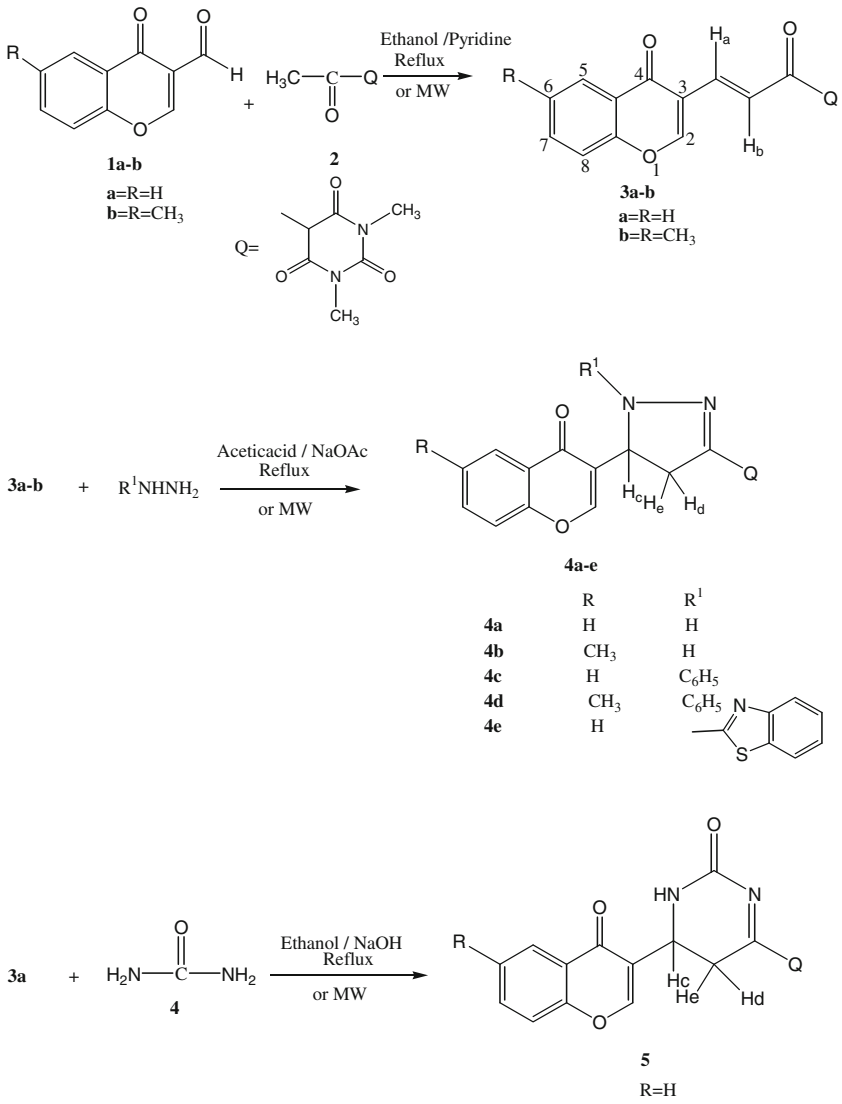
[a] Microwave equipment multimode with full power was used

[b] All yields refer to isolated products

Condensation of α,β unsaturated ketone with nitrogen bases gives the corresponding pyrazolines (Wiley and Jarboe, 1967). Thus, the compounds **3a–b** when treated with variously substituted hydrazines under conventional heating procedure using glacial acetic acid and catalytic amount of sodium acetate afforded the products **4a–e** in 63–75% yield in a span of time period (1–12 h) (Scheme 1). However, microwave irradiation technique when applied increased yields of the products (86–94%) within shorter period of time (3–9 min) even in the absence of catalyst (Table 1). The IR spectrum of **4a** displayed a broad band at 3285 cm^{-1} due to the presence of NH group of pyrazoline moiety. The absorption bands for barbituric acid and chromone carbonyl groups were present in the region 1698 and 1649 cm^{-1}, respectively. The ^1H NMR spectrum of the compound showed the diagnostic singlet of C-2 olefinic proton of the chromone moiety at δ 8.20, which clearly indicated that the pyrone moiety in the compound didn't suffer ring cleavage by the attack of nitrogen base (Kostka, 1973). This fact was further supported by the presence of doublet of doublet of C-5 proton appeared at δ 8.14. The remaining three aromatic protons of chromone nucleus appeared as multiplet at δ 7.42–7.76. The presence of pyrazoline unit was established by three doublet of doublet at δ 3.66 (H_d), 4.01 (H_e) and 4.87 (H_c). A sharp singlet at δ 3.36 integrating for six protons was assigned to two N–CH_3 groups. The Mass spectrum of the compound showed M^+ at *m/z* 368.

Due to the remarkable pharmacological activities exhibited by dihydropyrimidine derivatives (Pani *et al.*, 2009), attempts were also made to synthesize dihydropyrimidine derivative of chalcone by reacting **3a** with urea in the

Scheme 1 Synthesis of compounds **3a–5**



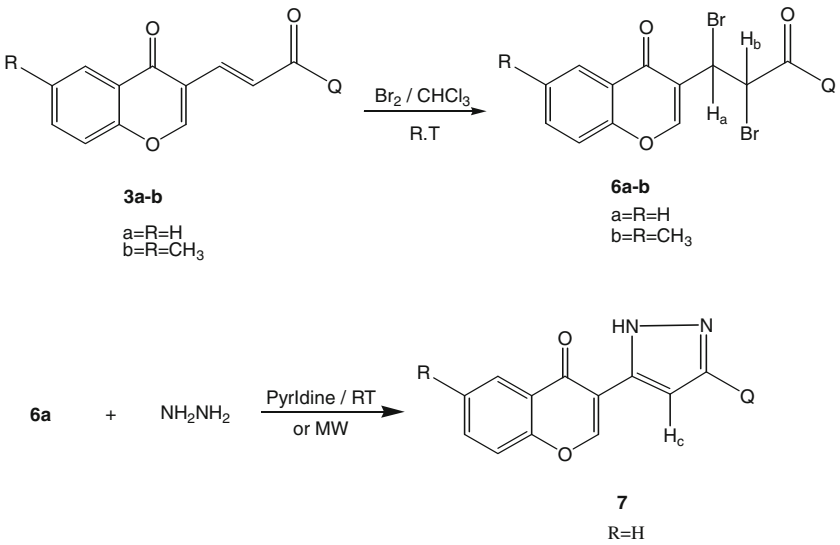
presence of NaOH in ethanol under thermal heating method and solvent free conditions (Scheme 1). The reaction mixture as visualized afforded **5** in quantitative yield (Table 1). The presence of dihydropyrimidine nucleus in **5** was established on the basis of ^{1}H NMR spectrum which showed two doublet of doublet at δ 3.20 (H_d), and 4.88 (H_c) whereas, the H_e proton at δ 3.42 is merged with two N–CH_3 protons of barbituric acid moiety. A broad singlet (D_2O exchangeable) at δ 14.12 was assigned to OH proton. The other prominent peaks as obtained are mentioned in the experimental part.

α,β-Dibromodihydrochalcones are interesting intermediates for the synthesis of various biologically active heterocycles such as pyrazoles, pyrazolines and pyrimidines. (Joshi and Wadodkar, 1981; Abdel-Rahman *et al.*, 2007). On the basis of these findings, bromination of heterochalcones was also attempted (Scheme 2). Thus, the reaction of heterochalcones **3a–b** with Br_2 in chloroform at room temperature afforded 2,3-dibromodihydrochalcones **6a–b** in quantitative yields. The ^{1}H NMR spectrum of the **6a** showed two doublets attributable to H_a and H_b protons at δ 5.56 and 7.76 with coupling constant of 11.3 Hz, which was in accordance to the erythro configuration. The other prominent peaks are given in the experimental part. Further, **6a** was converted to pyrazole derivative **7** in dry pyridine at room temperature and in solvent-free condition. The compound showed a sharp singlet at δ 7.94 due to H_c proton of pyrazole moiety in its ^{1}H NMR spectrum. A broad singlet at δ 10.31 was assigned to NH proton.

Biology

All the newly synthesized compounds were screened in vitro antibacterial activity against an assortment of two

Scheme 2 Synthesis of compounds **6a–7**



Gram-positive bacteria, *Staphylococcus aureus* SA 22, *Bacillus subtilis* MTCC 121, and two Gram-negative bacteria, *Escherichia coli* K12, *Salmonella typhimurium* MTCC 98, in vitro antifungal activity was tested against three fungal strains, *Candida albicans* IOA-109, *Aspergillus niger* (laboratory isolate) and *Aspergillus fumigatus* (laboratory isolate). The minimum inhibitory concentrations (MIC) of the tested compounds were 100 μg/ml. The newly generated compounds **3a–7** exerted significant inhibitory activity against the growth of the tested bacterial and fungal strains. The data (Table 2) reveal that compounds have significant influence on antibacterial profile of Gram-positive bacteria. The compounds also showed moderate to good inhibitory results against Gram-negative bacteria viz *S. typhimurium* and *E. coli*. Among the tested compounds, chalcones **3a** and **3b** showed more potent inhibitory activity against both types of bacteria. However, derivatives of chalcones exerted varying inhibitory action. Further antifungal screening revealed that the compounds **3a–7** exhibited moderate to good inhibition to *C. albicans* and lesser inhibition against *A. niger* and *A. fumigatus.*

Conclusion

In summary, we have described the synthesis of hitherto unknown 3-substituted chromone derivatives from readily synthesized starting materials, available reagents along with short reaction time, simple workup and isolation, etc., under green reaction conditions, make the current method feasible and attractive protocol for generation of novel heterocycles. The antimicrobial activity of compounds showed that various compounds are potent antimicrobial agents.

Experimental

Melting points were taken in Riechert Thermover instrument and are uncorrected. The IR spectra were recorded on Perkin Elmer RXI spectrometer in KBr, ^{1}H NMR on Bruker DRX-300 and Bruker Avance II 400 spectrometer using tetramethyl silane (TMS) as the internal standard and DMSO-d_6/$CDCl_3$ as solvent. Mass spectra were obtained on Jeol-SX-102 (FAB) spectrometer. The microanalytical data were collected on Elementar vario EL III elemental analyzer. 3-Formylchromone, substituted-3-formylchromone (Nohara *et al.*, 1974), 5-acetyl-1,3-dimethylbarbituric acid (Jursic and Neumann, 2001) and hydrazinobenzothiazole (Singh *et al.*, 1990) were synthesized by reported methods. All other chemicals used were purchased from Merck (Mumbai, India) and Fluka Chemicals (Switzerland). The purity of the compounds was checked by thin layer chromatography (TLC) on glass plates coated with silica gel G_{254} (Merck, Mumbai, India) using chloroform–methanol (3:1) mixture as mobile phase and visualized by iodine vapours. All the experiments under microwave irradiation were carried out in an unmodified domestic microwave oven (National, Model NN-S557WF, 1.3 KW, 2450 MHz).

Preparation of chalcones under thermal heating conditions

To a well stirred solution of 5-acetyl-1,3-dimethylbarbituric acid (5.05 mmol) in ethanol (25 ml) containing pyridine (0.5 ml) 3-formylchromone/substituted-3-formylchromone was added (5.05 mmol). The reaction mixture was then refluxed in a heating mantle for 7–7.5 h, cooled at room temperature. The bright yellow solid thus obtained was

Table 2 In vitro antibacterial and antifungal activity of compounds **3a–7**

Compounds	Gram positive		Gram negative		Fungal species		
	S. aureus	*B. subtilis*	*E. coli*	*S. typhimurium*	*C. albicans*	*A. niger*	*A. fumigatus*
Diameter of inhibition zone in mm at 100 μg/ml							
3a	15	20	20	16	16	8	10
3b	12	16	20	12	12	12	14
4a	16	13	15	12	14	8	14
4b	11	13	12	10	17	–	10
4c	–	–	8	12	15	–	12
4d	–	–	–	19	15	–	–
4e	13	10	18	9	14	14	–
5	8	–	15	11	–	–	–
6a	8	–	14	16	15	–	–
6b	12	–	15	10	17	–	–
7	14	12	12	12	–	–	–
Chloramphenicol	26	24	25	20	–	–	–
Nystatin	–	–	–	–	20	18	18
DMSO	–	–	–	–	–	–	–

–, No activity detected

filtered, washed with water, alcohol and dried to afford **3a–b**.

Preparation of chalcones under microwave irradiation conditions

3-Formylchromone/substituted-3-formylchromone (5.05 mmol), 5-acetyl-1,3-dimethylbarbituric acid (5.05 mmol) and pyridine (0.3 ml) were mixed thoroughly in a mortar, and air dried. The reaction mixture was then transferred to an open Pyrex beaker and subjected to microwave irradiation (multimode, full power). The progress of reaction was monitored by TLC and on completion, the reaction mixture was slurred in water (40 ml). The solid thus obtained was filtered, washed with water, alcohol and dried to afford **3a–b**.

*(2E)-1-(1,3-Dimethyl-2,4,6-pyrimidinetrione-5-yl)-3-(4-oxo-4H-1-benzopyran-3-yl)-2-propene-1-one (**3a**)*

Purified by recrystallisation from chloroform. Bright yellow crystals; m.p., >300°C. Anal. Calcd. for $C_{18}H_{14}N_2O_6$ (%): C 61.02, H 3.98, N 7.91. Found (%): C 61.05, H 3.83, N 7.84. υ_{max}/cm^{-1} (KBr): 1657 (C=O, chromone), 1719 (C=O, barbituric acid). ^1H NMR ($CDCl_3$, 400 MHz): δ 3.37 (s, 3H, N–CH_3), 3.39 (s, 3H, N–CH_3), 7.49-7.76 (m, 3H, Ar–H), 7.84 (d, 1H, $J = 15.7$ Hz, H_b), 8.32 (dd, 1H, C-5), 8.39 (s, 1H, C-2), 9.26 (d, 1H, $J = 15.7$ Hz, H_a). MS (% rel int) *m/z* 354 (M^+, 80), 353 (60), 336 (30).

*(2E)-1-(1,3-Dimethyl-2,4,6-pyrimidinetrione-5-yl)-3-(6-methyl-4-oxo-4H-1-benzopyran-3-yl)-2-propene-1-one (**3b**)*

Purified by recrystallisation from chloroform. Bright yellow crystals; m.p., >300°C. Anal. Calcd. for $C_{19}H_{16}N_2O_6$ (%): C 61.95, H 4.38, N 7.61. Found (%): C 61.86, H 4.27, N 7.58. υ_{max}/cm^{-1} (KBr): 1666 (C=O, chromone), 1716 (C=O, barbituric acid). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.48 (s, 3H, CH_3), 3.38 (s, 3H, N–CH_3), 3.40 (s, 3H, N–CH_3), 7.31-7.46 (m, 3H, Ar–H), 7.87 (d, 1H, $J = 16.3$ Hz, H_b), 8.27 (s, 1H, C-2), 9.18 (d, 1H, $J = 15.8$ Hz, H_a). MS (% rel int) *m/z* 368 (M^+, 30), 367 (10).

Preparation of pyrazolines under thermal heating conditions

The mixture of **3a–b** (2.52 mmol), hydrazine hydrate (2.52 mmol)/phenylhydrazine (2.52 mmol)/hydrazinobenzothiazole (2.52 mmol) and sodium acetate (0.7 mmol) was refluxed in 10 ml of acetic acid for 1–12 h. After completion of the reaction (checked by TLC), the reaction mixture was cooled and the solid precipitated out was filtered, washed with water, acetone and dried to afford **4a–e**.

Preparation of pyrazolines under microwave irradiation conditions

The mixture of **3a–b** (2.52 mmol) and hydrazine hydrate (2.52 mmol)/phenylhydrazine (2.52 mmol)/hydrazinobenzothiazole (2.52 mmol) were mixed thoroughly in a mortar

and air dried. The reaction mixture was then transferred to an open Pyrex beaker and subjected to microwave irradiation (multimode, full power). The progress of reaction was monitored by TLC and on completion, the reaction mixture was slurred in water (25 ml). The solid obtained was filtered, washed with water, alcohol and dried to get **4a–e**.

*3-(1,3-Dimethyl-2,4,6-pyrimidinetrione-5-yl)-5-(4-oxo-4H-1-benzopyran-3-yl)pyrazoline (**4a**)*

Purified by recrystallisation from ethanol–DMF (3:2 v/v). White solid; m.p., >300°C. Anal. Calcd. for $C_{18}H_{16}N_4O_5$ (%): C 58.69, H 4.38, N 15.21. Found (%): C 58.54, H 4.43, N 15.12. υ_{max}/cm^{-1} (KBr): 1649 (C=O, chromone), 1698 (C=O, barbituric acid), 3285 (N–H, pyrazolinyl). ^1H NMR (DMSO-d_6, 400 MHz): δ 3.36 (s, 6H, 2N–CH_3), 3.66 (dd, 1H, $J = 19.0$ Hz, 6.0 Hz, H_d), 4.01 (dd, 1H, $J = 18.9$ Hz, 9.8 Hz, H_e), 4.87 (dd, 1H, $J = 9.7$ Hz, 6.0 Hz, H_c), 7.42–7.76 (m, 3H, Ar–H), 8.14 (dd, 1H, $J = 8.0$ Hz, 1.4 Hz, C-5), 8.20 (s, 1H, C-2). MS (% rel int) *m/z* 368 (M^+, 55), 367 (40).

*3-(1,3-Dimethyl-2,4,6-pyrimidinetrione-5-yl)-5-(6-methyl-4-oxo-4H-1-benzopyran-3-yl)pyrazoline (**4b**)*

Purified by recrystallisation from ethanol–DMF (3:2 v/v). White solid; m.p., >300°C. Anal. Calcd. for $C_{19}H_{18}N_4O_5$ (%): C 59.68, H 4.74, N 14.65. Found (%): C 59.53, H 4.83, N 14.49. υ_{max}/cm^{-1} (KBr): 1646 (C=O, chromone), 1699 (C=O, barbituric acid), 3241 (N–H, pyrazolinyl). ^1H NMR (DMSO-d_6, 400 MHz): δ 2.46 (s, 3H, CH_3), 3.34 (s, 6H, 2N–CH_3), 3.70 (dd, 1H, $J = 19.0$ Hz, 6.0 Hz, H_d), 4.03 (dd, 1H, $J = 19.0$ Hz, 9.7 Hz, H_e), 4.89 (dd, 1H, $J = 9.1$ Hz, 6.6 Hz, H_c), 7.31 (br s,1H, NH), 7.39–7.91 (m, 3H, Ar–H), 8.13 (s, 1H, C-2). MS (% rel int) *m/z* 382 (M^+, 100), 381 (70).

*1-Phenyl-3-(1,3-dimethyl-2,4,6-pyrimidinetrione-5-yl)-5-(4-oxo-4H-1-benzopyran-3-yl)pyrazoline (**4c**)*

Purified by recrystallisation from coloumn chromatography using benzene-ethylacetate (3:1 v/v) as the eluent. Pale yellow crystals; m.p., 236°C (dec). Anal. Calcd. for $C_{24}H_{20}N_4O_5$ (%): C 64.86, H 4.54, N 12.61. Found (%): C 64.81, H 4.61, N 12.53. υ_{max}/cm^{-1} (KBr): 1668 (C=O, chromone), 1715 (C=O, barbituric acid). ^1H NMR ($CDCl_3$, 400 MHz): δ 3.38 (s, 6H, 2N–CH_3), 3.71 (dd, 1H, $J = 18.5$ Hz, 7.9 Hz, H_d) 4.23 (dd, 1H, $J = 18.6$ Hz, 9.7 Hz, H_e), 4.49 (dd, 1H, $J = 10.0$ Hz, 7.9 Hz, H_c), 7.08–7.33 (m, 8H, Ar–H), 7.38 (dd, 1H, $J = 7.4$ Hz, 1.7 Hz, C-5), 8.27 (s, 1H, C-2). MS (% rel int) *m/z* 444 (M^+, 90), 443 (40), 391 (40).

*1-Phenyl-3-(1,3-dimethyl-2,4,6-pyrimidinetrione-5-yl)-5-(6-methyl-4-oxo-4H-1-benzopyran-3-yl)pyrazoline (**4d**)*

Purified by recrystallisation from coloumn chromatography using benzene-ethylacetate (3:1 v/v) as eluent. Pale yellow crystals; m.p., 247°C (dec). Anal. Calcd. for $C_{25}H_{22}N_4O_5$ (%): C 65.49, H 4.84, N 12.22. Found (%): C 65.42, H 4.93, N 12.16. υ_{max}/cm^{-1} (KBr): 1622 (C=O, chromone), 1712 (C=O, barbituric acid). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.25 (s, 3H, CH_3), 3.37 (s, 6H, 2N–CH_3), 3.70 (dd, 1H, $J = 18.6$ Hz, 7.9 Hz, H_d), 4.20 (dd, 1H, $J = 18.4$ Hz, 9.9 Hz, H_e), 4.45 (dd, 1H, $J = 10.0$ Hz, 7.9 Hz, H_c), 7.10–7.58 (m, 8H, Ar–H), 8.27 (s, 1H, C-2). MS (% rel int) *m/z* 458 (M^+, 75), 457 (50).

*1-Benzothiazolyl-3-(1,3-dimethyl-2,4,6-pyrimidinetrione-5-yl)-5-(4-oxo-4H-1-benzopyran-3-yl)pyrazoline (**4e**)*

Purified by recrystallisation from chlorofom-methanol (3:2 v/v). Brown crystals; m.p., 291°C (dec). Anal. Calcd. for $C_{25}H_{19}N_5O_5S$ (%): C 59.87, H 3.82, N 13.96. Found (%): C 59.81, H 3.88, N 13.84. υ_{max}/cm^{-1} (KBr): 1647 (C=O, chromone), 1709 (C=O, barbituric acid). ^1H NMR ($CDCl_3$, 400 MHz): δ 3.37 (s, 6H, 2N–CH_3), 4.02 (dd, 1H, $J = 19.3$ Hz, 4.8 Hz, H_d) 4.24 (dd, 1H, $J = 19.2$ Hz 10.6 Hz, H_e), 5.57 (dd, 1H, $J = 10.6$ Hz, 4.7 Hz, H_c), 7.18–7.94 (m, 7H, Ar–H), 8.15 (dd,1H, $J = 8.0$ Hz, 1.6 Hz, C-5), 8.41 (s, 1H, C-2). MS (% rel int) *m/z* 502 (M^+, 40), 501 (20), 354 (30).

Preparation of dihydropyrimidine under thermal heating condition

A mixture of **3a** (5 mmol), urea (7 mmol) and NaOH (14 mmol) was refluxed in ethanol (20 ml) for 14 h. The reaction mixture was then cooled, and poured into ice-cold water (50 ml), acidified with conc.HCl. The crude product thus obtained was filtered washed with acetone and dried to afford **5**.

Preparation of Dihydropyrimidine Under Microwave Irradiation Condition

The mixture of **3a** (5 mmol), urea (7 mmol) and NaOH (10 mmol) was grounded well using a mortar and pestle. The reaction mixture was then transferred to an open Pyrex beaker and exposed to microwave irradiation (multimode, full power). The progress of reaction was monitored by TLC and on completion the reaction mixture was slurred in water (25 ml). The solid obtained was filtered, washed with water, acetone and dried to afford **5**.

*4-(1,3-Dimethyl-2,4,6-pyrimidinetrione-5-yl)-6-(4-oxo-4H-1-benzopyran-3-yl)-5H,6H-dihydropyrimidine-2-one (**5**)*

Purified by recrystallisation from ethanol. White powder, m.p., 279–281°C. Anal. Calcd. for $C_{19}H_{16}N_4O_6$ (%): C 57.58, H 4.07, N 14.14. Found (%): C 57.42, H 3.91, N 14.12. υ_{max}/cm^{-1} (KBr): 1651 (C=O, chromone), 1716 (C=O, barbituric acid), 3511 (OH). ^1H NMR ($CDCl_3$, 300 MHz): δ 3.20 (dd, 1H, $J = 16.5$ Hz, 3.9 Hz, H_d), 3.37 (s, 6H, 2N–CH_3), 3.42 (dd, 1H, H_e), 4.88 (dd, 1H, $J = 9.9$ Hz 3.9 Hz, H_c), 7.44–7.76 (m, 3H, Ar–H), 8.02 (s, 1H, C-2), 8.24 (dd, 1H, $J = 7.8$ Hz, 0.9 Hz, C-5), 14.12 (br s, 1H, OH). MS (% rel int) *m/z* 396 (M^+, 80), 395(50).

General procedure for the preparation of 2,3-dibromoderivatives of chalcones

Bromine (5 mmol) was added dropwise with vigorous stirring to a solution of **3a–b** (5 mmol) in 12 ml of chloroform over a period of 20 min. After complete addition of Br_2 the reaction mixture was allowed to stand for 2 h. The dibromoderivatives **6a–b** were precipitated, filtered off, and washed with 20 ml of ether to remove the excess of bromine.

*1-(1,3-Dimethyl-2,4,6-pyrimidinetrione-5-yl)-2,3-dibromo-3-(4-oxo-4H-1-benzopyran-3-yl) propan-1-one (**6a**)*

Purified by recrystallisation from ethanol. Yellow solid; m.p., 242°C (dec). Anal. Calcd. for $C_{18}H_{14}N_2O_6Br_2$ (%): C 42.05, H 2.74, N 5.45. Found (%): C 42.14, H 2.75, N 5.42. υ_{max}/cm^{-1} (KBr): 1657 (C=O, chromone), 1729 (C=O, barbituric acid). ^1H NMR ($CDCl_3$, 400 MHz): δ 3.42 (s, 3H, N–CH_3), 3.44 (s, 3H, N–CH_3), 5.56 (d, 1H, $J =$ 11.3 Hz, H_a), 7.44–7.72 (m, 3H, Ar–H), 7.76 (d, 1H, $J =$ 11.3 Hz, H_b), 8.15 (s, 1H, C-2), 8.34 (dd, 1H, $J = 7.9$ Hz, 1.5 Hz, C-5). MS (% rel int) *m/z* 514 (M^+, 55), 513 (30), 434 (30), 354 (30).

*1-(1,3-Dimethyl-2,4,6-pyrimidinrtrione-5-yl) -2,3-dibromo- 3- (6-methyl-4-oxo-4H-1-benzopyran-3-yl) propan-1-one (**6b**)*

Purified by recrystallisation from ethanol. Yellow solid; m.p., 202–204°C. Anal. Calcd. for $C_{19}H_{16}N_2O_6Br_2$ (%); C 43.21, H 3.05, N 5.30. Found (%): C 43.13, H 3.13, N 5.22. υ_{max}/cm^{-1} (KBr): 1664 (C=O, chromone), 1730 (C=O, barbituric acid). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.47 (s, 3H, CH_3), 3.42 (s, 3H, N–CH_3), 3.43 (s, 3H, N–CH_3), 5.57 (d, 1H, $J =$ 11.2 Hz, H_a), 7.38–7.75 (m, 3H, Ar–H), 7.77(d, 1H, $J =$ 11.3 Hz, H_b), 8.14 (s, 1H, C-2). MS (% rel int) *m/z* 528 (M^+, 40) 527 (10), 526 (20), 448 (10) 368 (30).

Preparation of pyrazole under thermal heating condition

Hydrazine hydrate (2.5 mmol) was added with vigorous stirring to a solution of **6a** (2.5 mmol) in 10 ml of dry pyridine at room temperature. The reaction mixture was allowed to stand for 28 h. The pyrazole derivative **7** precipitated was filtered, washed with water, ethanol and dried.

Preparation of pyrazole under microwave irradiation

A mixture of **6a** (2.5 mmol) and hydrazine hydrate (2.5 mmol) was mixed well and air dried. The reaction mixture was subjected to microwave irradiation (multimode, full power) in an open Pyrex beaker. On completion (as checked by TLC), the reaction mixture was slurred in water (20 ml). The crude product obtained was filtered, washed with water, ethanol and dried.

*3-(1,3-Dimethyl-2,4,6-pyrimidinetrione-5-yl)-5-(4-oxo-4H-1-benzopyran-3-yl)pyrazole (**7**)*

Purified by recrystallisation from ethanol–DMF (3:2 v/v), White powder; m.p., >300°C. Anal. Calcd. for $C_{18}H_{14}N_4O_5$ (%): C 59.02, H 3.85, N 15.29. Found (%): C 59.14, H 3.79, N 15.22. υ_{max}/cm^{-1} (KBr): 1654 (C=O, chromone), 1710 (C=O, barbituric acid), 3247 (NH, pyrazole). ^1H NMR (DMSO-d_6, 300 MHz): δ 3.20 (s, 6H, 2N–CH_3), 6.97–7.39 (m, 3H, Ar–H), 7.77 (d, 1H, $J =$ 7.5 Hz, C-5), 7.94 (s, 1H, pyrazole), 9.56 (s, 1H, C-2), 10.31 (br s, 1H, NH). MS (% rel int) *m/z* 366 (M^+, 90), 365 (50).

Biological activity

Antibacterial studies

All the synthesized compounds were dissolved to prepare a stock solution of 1 mg/ml using DMSO. The antibacterial activity of test compounds and standard chloramphenicol was done by filter paper disc method (Bauer *et al.*, 1966). Media with DMSO was set up as control. All cultures were routinely maintained on NA (nutrient agar) and incubated at 37°C for overnight. The culture was centrifuged at 1000 rpm and pellets were resuspended and diluted in sterile NSS to obtain viable count 10^5 cfu/ml. Approximately, 0.1 ml of diluted bacterial culture suspension was spread with the help of spreader on NA plates uniformly. Sterile 8-mm discs (Hi-media Pvt Ltd) were impregnated with the test compounds. Antibiotic disc, chloramphenicol (30 μg/disc Hi-Media) was used as control. The disc was placed on the plate. Each plate had one control disc impregnated with the solvent. The plates were then

incubated for 24 h at 37°C, and the resulting zones of inhibition (in mm) were measured.

Antifungal studies

The synthesized compounds were dissolved in DMSO. Media with DMSO was set up as control. All cultures were routinely maintained on SDA and incubated at 28°C. Spore formation of filamentous fungi was prepared from 7 day old culture in sterile normal solution (8% NaCl) and approximately diluted to obtain 10^5 cfu/ml. The inoculums of non-sporing fungi, *C. albicians* was performed by growing the culture in SD broth at 37°C for overnight. The culture was centrifuged at 1,000 rpm and pellets were resuspended and diluted in sterile NSS to obtain viable count 10^5 cfu/ml. Approximately, 0.1 ml of diluted fungal culture suspension was spread with the help of spreader on SDA plates uniformly. Sterile 8 mm discs (Hi-media Pvt Ltd) were impregnated with the test compounds. Antibiotic disc, nystatin (30 μg/disc Hi-Media) was used as control. The disc was placed onto the plate. Each plate had one control disc impregnated with the solvent. The plates were incubated at 28°C for filamentous fungi for 72 h or more, while for *C. albicans* plates were incubated at 37°C for 18–48 h. Antifungal activity was determined by measuring the diameters of the inhibition zone.

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