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Mechanism of unusual formation of 3-(5-phenyl-3*H*-[1,2,4]dithiazol-3-yl)chromen-4-ones and 4-oxo-4*H*-chromene-3-carbothioic acid *N*-phenylamides and their antimicrobial evaluation

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ABSTRACT

6/6,7-Substituted 3-formylchromones (**9a–e**) react with 2 equivalents of 2-phenyl-4-dimethylamino-1-thia-3-azabuta-1,3-diene (**10**) or thiobenzamide (**11**) in refluxing toluene to furnish novel substituted 3-(5-phenyl-3*H*-[1,2,4]dithiazol-3-yl)chromen-4-ones (**12a–e**). However, reactions of substituted 2-anilino-3-formylchromones (**15a–d**) with thiobenzamide (**11**, 2 equivalents) in refluxing xylene furnish 4-oxo-4*H*-chromene-3-carbothioic acid *N*-phenylamide (**17a–d**) in high yields. A mechanistic rationalization of the conversion of 2-anilino-3-formylchromones (**15a–d**) to *N*-phenylamides (**17a–d**), and 3-formylchromones (**9a–e**) to the corresponding thioaldehydes, is proffered. All the compounds (**12a–e**, **17a–d**) display very high antifungal and antibacterial activities against a number of strains. Dithiazole **12d** exhibits a very high antifungal activity (MIC 5 μg/ml) against *Geotrichum candidum*, better than fluconazole (MIC 09 μg/ml) and also possesses good antibacterial activity (MIC 52 μg/ml) against *Shigella flexneri*.

1. Introduction

Heterocycles, both natural and synthetic, exhibit a wide range of interesting biological activities [1–6]. Among five-membered heterocycles dithiazoles, both 1,2,3- and 1,2,4-, are endowed with interesting biological activities, in particular, antimicrobial activity [7,8]. For instance 4-chloro-5-heteroimmino-1,2,3-dithiazoles (**1a–e**) have been evaluated against fungal species and a number of cancer cell lines; among these compounds (**1b–d**) exhibit the highest antifungal activity [9]. Similarly, *N*-arylimino-l,2,3-dithiazoles (**2**) possess antifungal activity against yeasts [10], whereas, 5-(4-chloro-[1,2,3]dithiazol-5-ylideneamino)-naphthalen-1-ol (**3**) possesses significant bactericidal as well as fungicidal activities; these are postulated to be potent inhibitors of enzymes such as serine proteases [11]. In fact, 1,2,4-dithiazole is the active moiety of the antifungal compound 5,6-dihydroimidazo[2,l-*c*]-l,2,4-dithiazole-3-thione (ethylenethiuram monosulfide **4**), which is obtained by auto-oxidation of the well-known fungicide disodium methylene-bisdithiocarbamate (Nabam) [12]. Compounds such as *N*-3-(1,2,4-dithiazole-5-thione)-β-resorcylcarbothioamide (**5**) display significant antifungal activity against various fungal strains (Fig. 1). These molecules are reported to possess diverse modes of action and their targets include enzymes such as leucine arylamidase, esterases, α-glucosidase, *N*-acetyl-β-glucosaminidase, lipases, and alkaline phosphatase [13].

At the same time, chromones of both synthetic and natural origin are also known to display valuable antifungal activity. Some recently discovered antifungal chromones includes 8-hydroxy-6-methyl-2,3-dihydro-cyclopenta[*b*]chromen-1,9-dione (**6**, Fig. 2), isolated from a carpophilus fungus [14] and synthetic 2-phenylchromone derivative 2-benzo[1,3]dioxol-5-yl-7-benzyloxy-chromen-4-one (**7**) [15]. Another important synthetic chromone based antifungal agent is *N*-isobutyl-2-(4-oxo-2-phenyl-4*H*-chromen-6-yl)-1*H*-benoimidazole-5-carboxamidine (**8**), which displays valuable antimycotic properties against *Candida albicans* and *Candida krusei*.

2. Chemistry

Recently, we had reported [16] a serendipitously discovered route to 3-(5-phenyl-3*H*-[1,2,4]dithiazol-3-yl)chromen-4-ones (**12a–e**).

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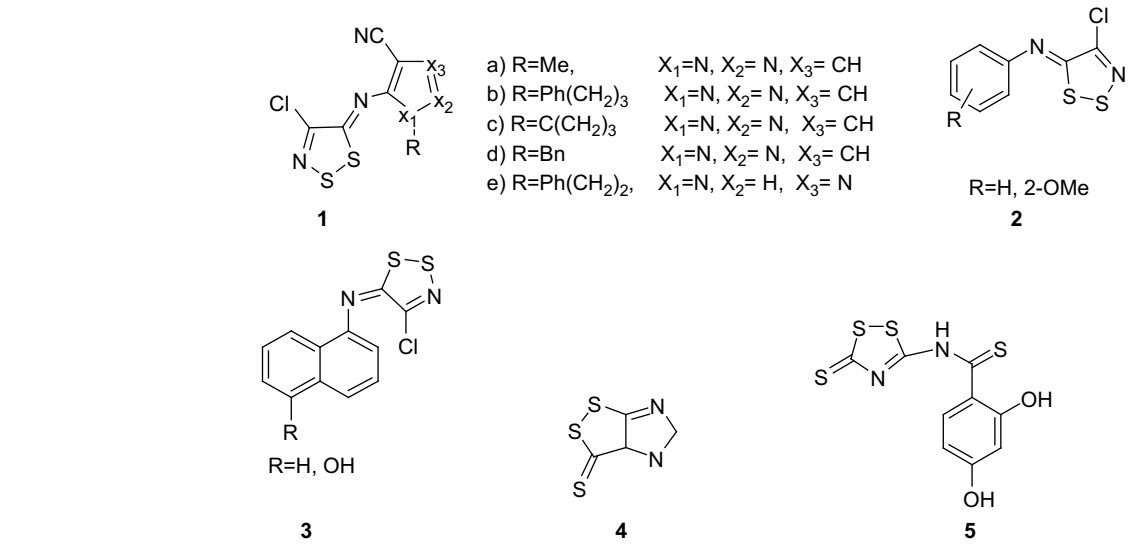


Fig. 1. Some antifungal and antibacterial dithiazole derivatives.

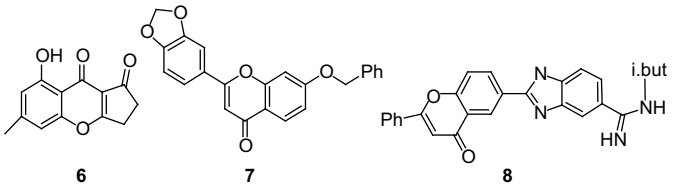


Fig. 2. Some chromone based antifungal and antibacterial agents

Thus, attempted cycloaddition of 2-phenyl-4-dimethylamino-1-thia-3-azabuta-1,3-diene (**10**) with substituted 3-formylchromones **9**, in sealed tube, led to the isolation of (**12a–e**) in good yields and the yields improved considerably using two equivalents of **10**. Subsequently, it was discovered that same dithiazoles (**12a–e**) were also obtained on reacting **9** with two equivalents of thiobenzamide (**11**) in refluxing toluene (Scheme 1).

Based on the above observations and detection of benzonitrile and DMF (the latter in the case of reaction with thioazadiene **10**), by HPLC, a plausible mechanism was postulated (Scheme 2); conversion of thioaldehydes to dithiazoles has precedent in literature [17–19].

Keeping in view the anticipated antifungal and antibacterial activities of these molecules, which encompass chromone and dithiazole moieties, it was decided to synthesize these molecules and evaluate their antifungal and antibacterial activity. It has been postulated that 3-formylchromone react with thiobenzamide/thiazadiene by initial attack on the aldehyde function [16] (Scheme 2), although more electron deficient C2 position is also present [20] therefore, it was also of interest to probe the mechanism of the formation of dithiazoles.

3. Results

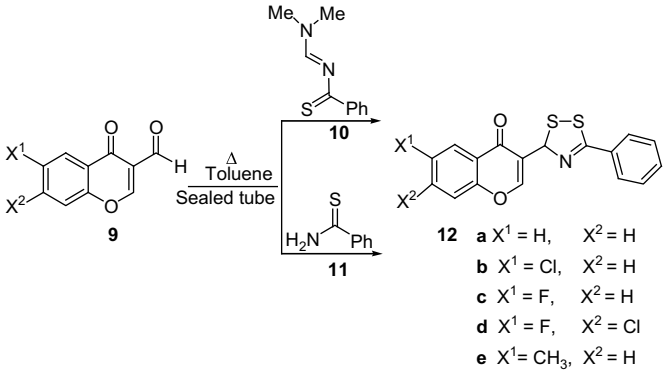
Because in the proposed mechanism (Scheme 2) the first step is thionation of the aldehyde moiety, reactions of benzaldehyde (**13**) with one and two equivalents of thioazadine (**10**)/thiobenzamide (**11**) in refluxing xylene were carried out. However, these reactions failed and no thionation was observed, indicating that the reaction doesn't start with aldehyde function. As the C2 of the chromone moiety has been reported to be highly electrophilic [20] and thinking that possibly the reaction may be starting with an attack on C2, the 2-*N*-methylanilino-3-formylchromone (**14**) [21] was reacted with two equivalents of thiobenzamide (**11**)/thioazadiene (**10**) under similar conditions; no reaction occurred as indicated by TLC and NMR analysis.

However, when the substituted 2-anilino-3-formylchromones (**15a–d**) were reacted with 2 equivalents of thiobenzamide (**11**), 4-oxo-4*H*-chromene-3-carbothioic-*N*-phenylamides (**17a–d**) were obtained in high yields (Scheme 3, Table 1), but no formation of (2-phenylamino-3-(5-phenyl-3*H*-[1,2,4]dithiazol-3-yl)-chromen-4-one (**16**) was observed.

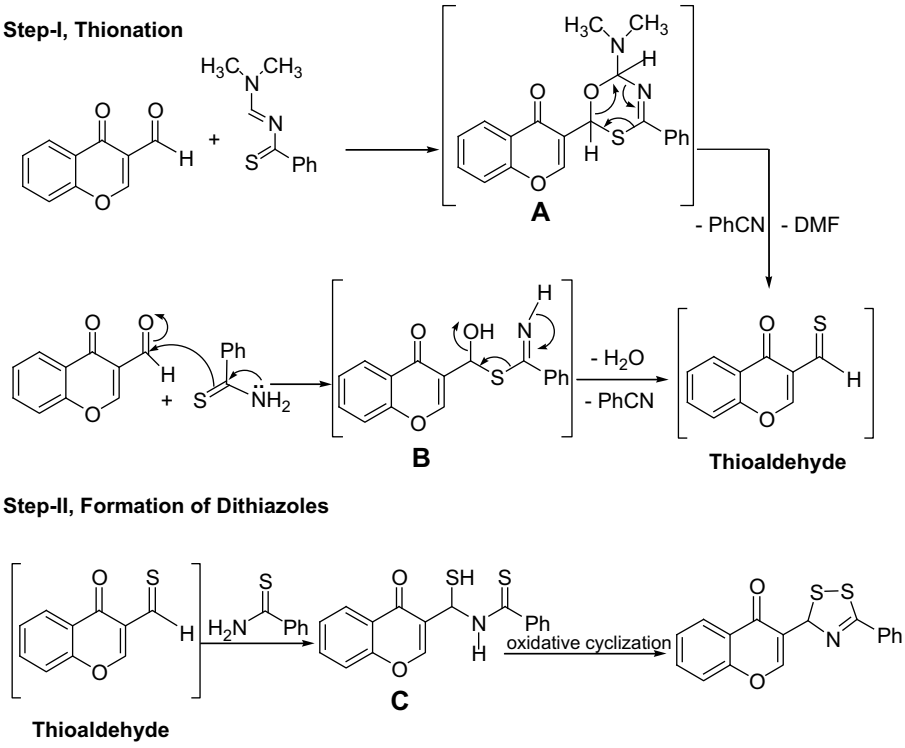
All the products (**17a–d**) were characterized by detailed spectroscopic analysis (^{1}H and ^{13}C NMR, IR and mass) and microanalytical data. The structure of **17c** was further confirmed X-ray crystallographically (Fig. 3) [22].

4. Discussion

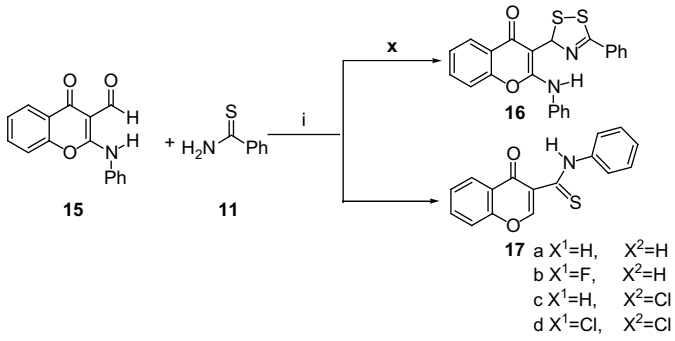
Formation of **17a–d** clearly indicates that the reaction indeed starts with an initial attack on C2, instead of earlier proposed involvement of aldehyde function [16]. Mechanistically, the formation of **17** can be rationalized as outlined in Scheme 4; such opening and recyclization of chromone ring is precedented [21]. Apparently, reactions of 2-*N*-methylanilino-3-formylchromones



Scheme 1. Synthesis of 1,2,4-dithiazole derivatives.



Scheme 2. Mechanism of the 1,2,4-dithiazole formation.



Scheme 3. Reaction conditions: (i) xylene reflux 4 h.

with thiobenzamide (**10**)/thioazadinene (**11**) did not occur due to steric reasons.

Therefore, the thionation of substituted 3-formylchromones (**9**) on reaction with thiobenzamide (**11**) or thioazadiene (**10**) may be similarly involving the attack on C2 (Scheme 5).

After thionation, the thioaldehyde further reacts with another molecule of the thiobenzamide (**11**) to yield 3-(5-phenyl-3*H*-[1,2,4]dithiazol-3-yl)chromen-4-ones (**12**) whereas 4-oxo-4*H*-chromene-3-carbothioic acid phenylamides (**17a**–**d**) fail to react with thiobenzamide.

Table 1
Reaction yield (%) of products **17**.

Chromone	Product	% Yield
15a	**17a**	71
15b	**17b**	76
15c	**17c**	78
15d	**17d**	72

In view of the known antimicrobial properties of chromone derivatives and heterocyclic thioamides [23,24], it was decided to synthesize the *N*-phenylthioamides (**17a**–**d**) in addition to chromanyl-1,2,4-dithiazole (**12a**–**e**) and evaluate their antimicrobial activity.

5. Pharmacolology

All the synthesized compounds (**12a**–**e**) and (**17a**–**d**) were evaluated for both antifungal and antibacterial activities.

5.1. Antifungal activity

In vitro antifungal investigations on 3-(5-phenyl-3*H*-[1,2,4]dithiazol-3-yl)chromen-4-ones (**12a**–**e**) and 4-oxo-4*H*-chromene-3-

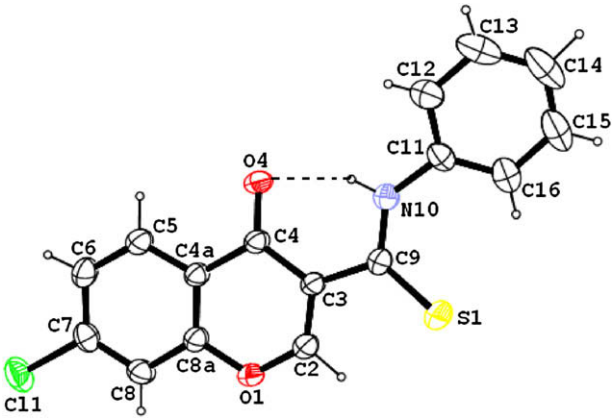
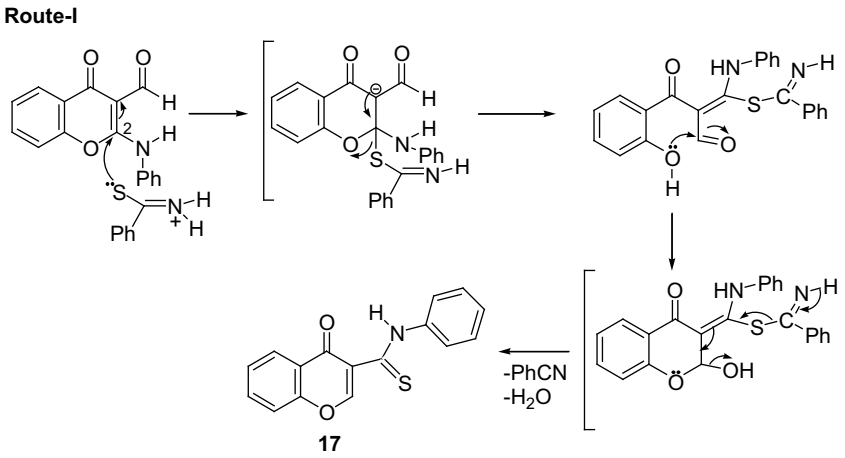
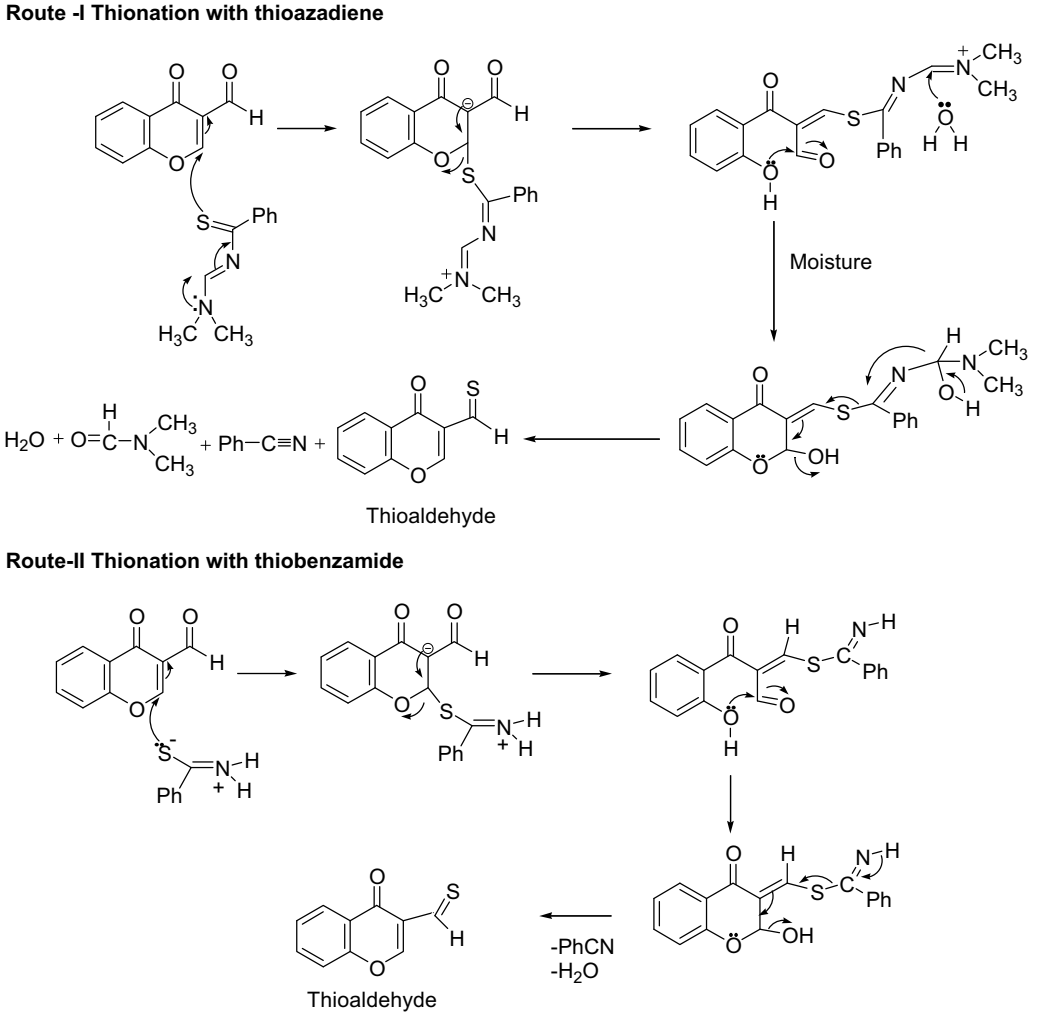


Fig. 3. ORTEP view of **17c**.



Scheme 4. Mechanism of formation of *N*-phenylthioamides.

carbothioic acid *N*-phenylamides (**17a**–**d**) were carried out on fungal strains *Aspergillus niger* (MTCC-281), *Geotrichum candidum* (MTCC-3993), *C. albicans* (MTCC-227) and *Candida tropicalis* (MTCC-230). Fluconazole was used as positive control. Minimum inhibitory concentration (MIC) is the concentration μg/ml required to inhibit bacterial cell proliferation by 50% after exposure of cells to test compounds. Inhibitory potential of the compounds was determined in terms of MIC (μg/ml) using turbidimetry method



Scheme 5. Mechanism of thionation with both thioazadiene and thiobenzamide.

Table 2
In vitro antifungal activities of compounds **12a–e** and **17a–d**.

Compound	MIC (μg/ml)			
	Asperagillus niger	*Geotrichum candidum*	*Candida albicans*	*Candida tropicalis*
	(MTCC-281)	(MTCC-3993)	(MTCC-227)	(MTCC-230)
12a	90	32	30	40
12b	125	30	28	28
12c	50	32	30	42
12d	90	**05**	40	28
12e	150	95	60	52
17a	90	–	–	–
17b	120	–	–	–
17c	80	–	60	60
17d	–	–	75	–
Fluconazole	49	09	24	25

(Table 2). For **12c** maximum inhibition (MIC 50) was observed against *A. niger* followed by **17c** (MIC 80). Compound **12d** with MIC 5 μg/ml was found to be the most active compound against *G. candidum* (Fig. 4). Against *C. albicans* compound **12b** was most active (MIC 28) followed by **12a**,**c** (MIC 30). Compounds **12b**,**d** showed maximum activity (MIC 28) against *C. tropicalis*. It may be mentioned here that compound **12d** showed very high inhibition (MIC 5 μg/ml), which is more than that of fluconazole (MIC 9) used as positive control.

5.2. Antibacterial activity

In vitro antibacterial studies of various compounds (**12a–e**) and (**17a–d**) were carried out on Gram negative and Gram positive bacterial strains by disk-diffusion assay. The Gram negative strains used were *Escherichia coli* (MTCC-119, facultative anaerobic), *Pseudomonas aeruginosa* (MTCC-741, aerobic) and *Shigella flexneri* (MTCC-1457, facultatively anaerobic). Gram positive strain used is *Staphylococcus aureus* (MTCC-740, facultatively anaerobic). The antibacterial activity was determined at concentrations of 2.5×10^{-5}, 5×10^{-5} and 1×10^{-4} μg/ml (Table 3). Ciprofloxacin and chloramphenicol were used as positive controls. MIC values were determined using turbidimetry method (Table 4). In the case of anaerobic Gram negative bacteria *E. coli* the maximum inhibition of 85.5% (5×10^{-5}, MIC 62) was observed for **17a** followed by 78.28% (5×10^{-5}, MIC 64) for **17d** and 73.7% (5×10^{-5}, MIC 68) for **17b**. Compound **12a** also showed significant activity 56.68% (5×10^{-5}, MIC 88). In the case of *S. flexneri*, maximum inhibition observed was 98.55% (MIC 52) followed by 85.50% (MIC 58) for **12d** and **12a** respectively, at same concentration. It is pertinent to mention here that none of the compounds **12a–e** and **17a–d** were found active against aerobic Gram negative *P. aeruginosa* bacteria. For Gram positive bacteria *S. aureus*, maximum growth inhibition of 92.72%

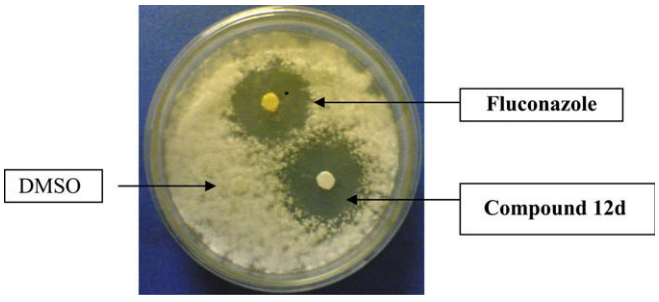


Fig. 4. Zone of inhibition of **12d** against fluconazole on *Geotrichum candidum*.

Table 3
In vitro antibacterial % growth inhibition by compounds **12a–e** and **17a–d**.

Compound	Conc. (μg/ml)	% Growth inhibition			
		E. coli	*S. flexneri*	*P. aeruginosa*	*S. aureus*
		(MTCC-119)	(MTCC-1457)	(MTCC-741)	(MTCC-740)
12a	2.5×10^{-5}	24.83	44.26	–	30.20
	5×10^{-5}	**56.68**	**85.50**	–	**73.88**
	1×10^{-4}	**100.00**	**100.00**	–	**100.00**
12b	2.5×10^{-5}	21.96	27.34	–	26.42
	5×10^{-5}	49.60	55.80	–	**51.50**
	1×10^{-4}	100.00	100.00	–	**100.00**
12c	2.5×10^{-5}	–	28.36	–	–
	5×10^{-5}	12.28	**62.32**	–	23.88
	1×10^{-4}	80.90	**100.00**	–	48.80
12d	2.5×10^{-5}	–	48.30	–	32.86
	5×10^{-5}	14.00	**98.55**	–	**68.65**
	1×10^{-4}	30.50	**100.00**	–	**100.00**
12e	2.5×10^{-5}	–	21.01	–	35.04
	5×10^{-5}	30.00	**60.86**	–	**92.72**
	1×10^{-4}	72.50	**100.00**	–	**100.00**
17a	2.5×10^{-5}	38.46	–	–	35.60
	5×10^{-5}	**85.50**	**55.34**	–	**76.25**
	1×10^{-4}	**100.00**	**100.00**	–	**100.00**
17b	2.5×10^{-5}	–	–	–	–
	5×10^{-5}	**73.75**	–	–	–
	1×10^{-4}	**100.00**	–	–	–
17c	2.5×10^{-5}	–	–	–	–
	5×10^{-5}	42.60	**52.18**	–	43.62
	1×10^{-4}	87.60	**100.00**	–	**81.00**
17d	2.5×10^{-5}	20.36	8.82	–	3.78
	5×10^{-5}	**78.28**	**60.86**	–	**90.90**
	1×10^{-4}	**100.00**	**100.00**	–	**100.00**

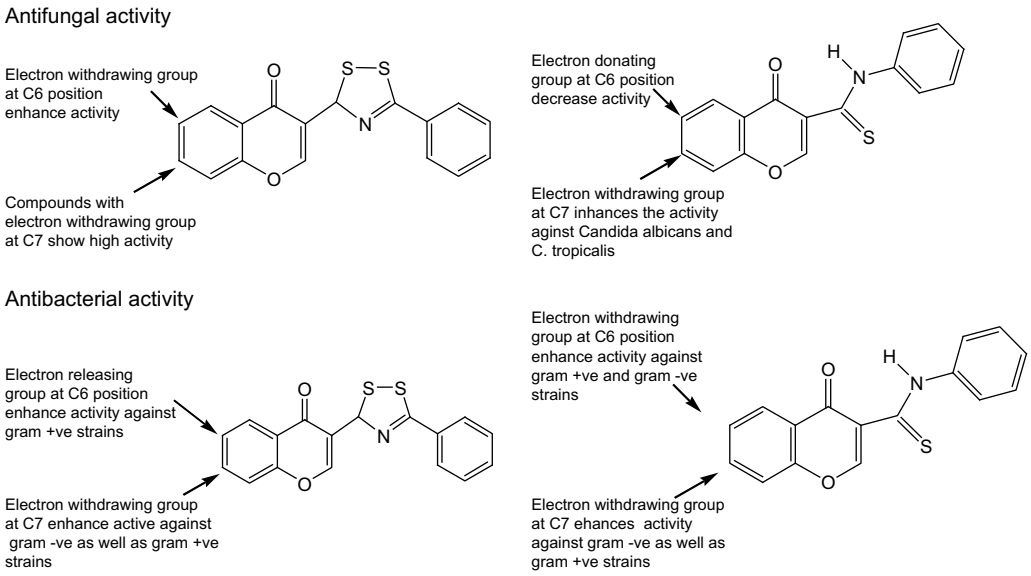
(5×10^{-5} μg/ml, MIC 54) was observed for **12e** followed by 90.90% (MIC 55) for **17d**.

5.3. Structure activity relationships

In vitro antifungal testing revealed compounds bearing electron withdrawing groups at C6 i.e., **12c** and **12b** show maximum activity against *A. niger* and *C. albicans*, respectively, while maximum inhibition was observed in the case of *Candidum geotrichum* (MIC 05) for compound **12d**, which bears electron withdrawing (F, Cl) substituents at C6 and C7. The 4-oxo-4*H*-chromene-3-carbothioic acid *N*-phenylamide derivatives (**17a**,**b**,**d**) are found to be more active against *E. coli*. On the other hand, 1,2,4-dithiazole-chromen-4-one derivative **12d** showed maximum inhibition against *S. flexneri*, when compounds possess electron withdrawing group (Cl) at C6 or C7 positions. Compound **12e** bearing electron releasing group

Table 4
In vitro antibacterial activity MIC (μg/ml) of compounds **12a–e** and **17a–d**.

Compound code	MIC (μg/ml)			
	E. coli	*S. flexneri*	*P. aeruginosa*	*S. aureus*
	(MTCC-119)	(MTCC-119)	(MTCC-119)	(MTCC-119)
12a	**88**	**58**	–	68
12b	100	**90**	–	98
12c	120	80	–	205
12d	350	**52**	–	72
12e	132	**82**	–	**54**
17a	**62**	**92**	–	70
17b	**68**	–	–	–
17c	115	**96**	–	125
17d	**64**	62	–	**55**
Chloramphenicol	2.5	5.6	2.7	13.6
Ciprofloxacin	1.2	1.4	1.8	0.9



($-CH_3$) at C-6 position possesses significant activity against *S. aureus* but lesser than compounds bearing electron withdrawing groups at C_7.

6. Conclusion

Conversion of 2-anilino-3-formylchromones **15** to corresponding thioamides (**17**) establishes that the reaction of the former with thiobenzamide **11** proceeds through attack on more electrophilic C2. Therefore, in the light of failure of benzaldehyde **13** and 2-*N*-methylanilino-3-formylchromone **14** to react with thiobenzamide **11** or thiazadiene **12**, it is concluded that thionation of 3-formylchromones also proceeds through initial attack on more electrophilic C2 rather than aldehydic carbon, thereby establishing the mechanism of thionation and hence formation of 3-(5-phenyl-3*H*-[1,2,4]dithiazol-3-yl)chromen-4-ones (**12a**–**e**). All the synthesized compounds (**12a**–**e**) and (**17a**–**d**) were evaluated for antifungal and antibacterial activities. All the compounds (**12a**–**e**) and (**17a**–**d**) display good antifungal activity. However, the dithiazole derivative (**12d**) bearing electron withdrawing (F, Cl) groups at C6 and C7 positions shows high antifungal activity (MIC 05) in comparison to fluconazole (MIC 09) against *G. candidum*. All the compounds (**12a**–**e** and **17a**–**d**) display useful antibacterial activity, but the thioamide derivatives (**17a**–**d**) are relatively more active. Chromone moiety has been shown to be a good replacement for quinolone moiety present in fluroquinolone antibacterials [23] i.e., tertiary nitrogen of quinolones is replaced by isosteric oxygen, therefore, thioamides (**17a**–**d**) may be having the same mode of action as that of fluoroquinolones; the latter are known bacterial DNA gyrase inhibitors [25–31]. These molecules are being developed further.

7. Experimental

7.1. Chemistry

Starting materials, reagents and solvents were purchased from commercial suppliers and purified/distilled/crystallized before use. JEOL AL-300FT (300 MHz) NMR spectrometers were used to record ^1H and ^{13}C NMR (75 MHz) spectra. Chemical shifts (δ) are reported as downfield displacements from TMS used as internal standard and coupling constants (J) are reported in Hz. IR spectra were recorded with Shimadzu FT-IR-8400S spectrophotometer on KBr pellets. Mass spectra, ESI-method, were recorded on Bruker Daltonics Esquire 300 mass spectrometer. Elemental Analyses were carried out on a Thermoelectron EA-112 elemental analyzer and are reported in percent atomic abundance. All melting points are uncorrected and measured in open glass-capillaries on a Veego (make) MP-D digital melting point apparatus. X-ray analysis was recorded at Bruker SMART APEX diffractometer equipped with low-temperature device and the structure was solved by direct methods using SHELXS 97 software (Sheldrick, 1997).

7.2. General procedure – synthesis of 1,2,4-dithiazoles (*12a–d*)

The reactions were carried out by reacting substituted 3-formylchromone (500 mg, 2.8 mmol) with thiobenzamide (767 mg, 5.6 mmol) in RBF using toluene (20 mL) as the solvent at an oil bath temperature of 120 °C. The completion of the reactions was monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure. The crude mixture was purified by column chromatography over silica 60–120 mesh (Loba Cheme 20 g packed in hexane) and eluted with 1% EtOAc in hexane. The purified products (**12a**–**e**) were characterized [16] by various spectroscopic techniques (UV, IR, ^1H and ^{13}C NMR, mass).

7.3. Synthesis of 4-oxo-4H-chromene-3-carbothioic acid N-phenylamides (*17a–d*)

The reactions were carried out by refluxing substituted 2-anilino-3-formylchromones (500 mg, 1.8 mmol) with thiobenzamide (656 mg, 3.7 mmol) in a 100 ml round bottom flask using xylene (20 mL) at the solvent at an oil bath temperature of 145 °C. Progress of reactions was monitored by TLC. After completion of the reaction the solvent was evaporated under reduced pressure. The crude mixture was purified by column chromatography over silica 60–120 mesh (Loba Cheme 20 g packed in hexane) and eluted with 5% EtOAc in hexane. The purified products (**17a**–**d**) were characterized by various spectroscopic techniques (IR, ^1H and ^{13}C NMR, mass) and by elemental analysis.

*7.3.1. 4-Oxo-4*H*-chromene-3-carbothioic acid N-phenylamide (**17a**)*

Yield: 71%; orange crystalline solid, mp 121–127 °C (chloroform:hexane, 1:1); IR (KBr) ν_{max} (cm^{-1}): 3360 (NH), 1650(C═O); ^{1}H NMR (300 MHz $CDCl_3$): $\delta = 13.65$ (s, 1H, NH), 9.71 (s, 1H, C_2H), 8.35 (dd, 1H, $J = 7.8$ and 1.8 Hz, ArH), 8.33–7.30 (m, 8H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 187.5$ (C═S), 177.7 (C4), 166.1 (q), 155.5 (C_2), 139.0 (q), 134.9 (CH), 129.3 (CH), 128.8 (CH), 126.8 (CH), 126.6 (CH), 124.2 (CH), 123.3 (q), 120.2 (CH), 118.5 (CH); MS (ESI): *m/z* 282 $(M + H)^+$, 281 (M^+); Anal. calcd. for $C_{16}H_{11}NO_2S$ %: C, 68.31; H, 3.94; N, 4.98. Found %: C, 67.99; H, 3.75; N, 4.31.

*7.3.2. 6-Fluoro-4-oxo-4*H*-chromene-3-carbothioic acid N-phenylamide (**17b**)*

Yield: 76%; light yellow crystalline solid, mp 128–131 °C (chloroform:hexane, 1:1); IR (KBr): ν_{max} 3370(NH), 1645(C═O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 13.55$ (s, 1H, NH), 9.74 (s, 1H, ArH), 8.00–7.30 (m, 8H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 187.7$ (C═S),176.9 (C4), 166.1 (q), 151.3 (C_2), 139.3 (q), 135.3 (CH), 129.4 (CH), 128.8 (CH), 126.9 (CH), 126.3 (CH), 124.2 (CH), 123.0 (q), 120.7 (CH), 118.4 (CH); MS (ESI): Fragment *m/z* 338 ($M + Ca^+$); Anal. calcd. for $C_{16}H_{10}FNO_2S$ %: C, 64.20; H, 3.37; N, 4.68. Found %: C, 64.01; H, 3.06; N, 4.21.

*7.3.3. 7-Chloro-4-oxo-4*H*-chromene-3-carbothioic acid N-phenylamide (**17c**)*

Yield: 78%; light orange crystalline solid, mp 165–168 °C (chloroform:hexane, 1:1); IR (KBr): ν_{max} 3350(NH), 1655 (C═O), 1517, 1220 (S–S) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 13.53$ (s, 1H, NH), 9.68 (s, 1H, C_2H), 8.64 (dd, 1H, $J = 8.4$ and 1.8 Hz, ArH), 8.26–7.32 (m, 7H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 187.7$ (C═S), 176.0 (C_4), 166.1 (q), 151.3 (C_2), 139.8 (q), 131.9 (CH), 129.4 (CH), 128.8 (CH), 126.9 (CH), 126.3 (CH), 124.2 (CH), 123.0 (q), 120.7 (CH), 118.4 (CH); Fragment *m/z* 338 ($M + Na^+$); Anal. calcd. for $C_{16}H_{10}ClNO_2S$ %: C, 61.86; H, 3.19; N, 4.44. Found %: C, 61.10; H, 2.96; N, 4.09.

*7.3.4. 6,7-Dichloro-4-oxo-4*H*-chromene-3-carbothioic acid N-phenylamide (**17d**)*

Yield: 72%; light orange crystalline solid, mp 131–135 °C (chloroform:hexane, 1:1); UV (MeOH): 307, 247 nm; IR (KBr): ν_{max} 3372(NH), 1653(C═O) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 13.1$ (s, 1H, NH), 9.17 (s, 1H, C_2), 9.16 (dd, 1H, $J = 8.4$ and 0.9 Hz, ArH), 8.53–7.63 (m, 6H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 187.2$ (C═S), 176.0 (C_4), 166.4 (q), 153.4 (C_2), 139.4 (q), 131.9 (CH), 128.8 (CH), 127.7 (CH), 127.6 (CH), 127.1 (CH), 124.1 (CH), 123.4 (q), 120.4 (CH), 120.2 (CH); MS (ESI): Fragment *m/z* 348 (M^{+2}); Anal. calcd. For $C_{16}H_9Cl_2NO_2S$ %: C, 54.87; H, 2.59; N, 4.00 Found %: C, 54.61; H, 3.27; N, 3.76.

7.4. Pharmacology

Antibacterial and antifungal activities of synthesized compounds were evaluated using disc-diffusion test for pre-screening of antibacterial and antifungal potential of agents and the broth micro-dilution method to determine the minimum inhibitory concentration (MIC) [32,33].

7.4.1. Antifungal activity – materials and methods

The following fungal strains were used: *A. niger* (MTCC-281), *C. geotrichum* (MTCC-3993), *C. albicans* (MTCC-227) and *C. tropicalis* (MTCC-230). Fungi were cultivated at 25 °C on Sabouraud Dextrose Agar (SDA) and MIC was determined by using Sabouraud Dextrose Broth (SDB). The compounds (**12a–e**) and (**17a–d**) and standard were dissolved in dimethylsulfoxide (DMSO, 1/10) and applied in different concentrations. Dimethylsulfoxide was used as a negative control and antifungal (Fluconazole) as positive controls.

7.4.2. Antifungal activity

The disk-diffusion assay was applied to determine the growth inhibition of fungi by compounds to be tested. Overnight fungal cultures (100 μL) were spread onto SDA. The compounds were applied to 8 mm disks (Whatman paper No. 1). After 48 h of incubation at 25 °C, the diameter of growth inhibition zones was measured.

7.4.3. MIC determination

The broth dilution test was performed in test tubes. The conidial suspension, which gave the final concentration of 1×10^5 CFU/ml, was prepared. A growth control tube and sterility control tube were used in each test. After 24–72 h incubation at 25 °C, the MIC was determined visually as the lowest concentration that inhibits growth, evidenced by the absence of turbidity.

7.5. Antibacterial activity – materials and methods

The following bacterial strains were used: *E. coli* MTCC-119, *S. aureus* MTCC-740, *P. aeruginosa* MTCC-741, *S. flexneri* MTCC-1457 from Microbial Type Culture Collection, IMTECH, Chandigarh, India.

Bacteria were cultivated at 37 °C in Nutrient Agar. For MIC determination Mueller Hinton Broth (MHB) and Mueller Hinton agar (MHA) was used. The compounds (**12a–e**) and (**17a–d**) and standards were dissolved in dimethylsulfoxide (1/10) and applied in different concentrations. Dimethylsulfoxide was used as a negative control and antibiotics (chloramphenicol and ciprofloxacin) as positive controls.

7.5.1. Antibacterial activity

The disk-diffusion assay was applied to determine the growth inhibition of bacteria by compounds to be tested. 4-h grown bacterial cultures in Nutrient Broth (100 μl) were spread onto MHA and the compounds were applied to 8 mm disks (Whatman paper No.1). After 24 h of incubation at 37 °C, the diameter of growth inhibition zones was measured.

7.5.2. MIC determination

The broth dilution test was performed in test tubes. In two-fold serial dilutions of the compounds, a standardized suspension (McFarland turbidity standard) of test bacteria (100 μl) was added to obtain a final concentration of 5×10^5 CFU/ml. A growth control tube and sterility control tube were used in each test. After overnight incubation at 37 °C, the MIC was determined by measuring optical density at 600 nm as the lowest concentration that inhibits growth, evidenced by the absence of turbidity [34].

References

[1] H.J. Smith, Introduction to the Principles of Drug Design and Action, fourth ed. Taylor Francis, 2006, pp. 567–614.
[2] Ruiz-Herrera, G. San-Blas, Current Drug Targets – Infectious Disorders, vol. 3, Bentham Science Publishers, 2003, 77–91.
[3] A.G. Gilman, The Pharmacological Basis of Therapeutic, tenth ed. McGraw-Hill, 2001, Sec. VIII, pp. 1141–1310.
[4] O.A. Mascaretti, Bacteria versus Antibacterial Agents: An Integrated Approach, ASM Press, 2003, p. 321.
[5] G. Laura, R. Marinella, P. Daniela, Mini Rev. Med. Chem. 7 (2007) 481–489.
[6] M. Bakavoli, M. Nikapour, M. Rahimizadeh, M.R. Saberi, H. Sadeghian, Bioorg. Med. Chem. 15 (2007) 2120–2126.
[7] A.E. Niewiadomy, K. Kulak, C. Lukaszuk, J. Matysiak, M. Kostecka, Rocz. Akad. Med. Bialymst. 50 (2005) 31–35.
[8] G. Cottenceau, T. Besson, V. Gautier, C.W. Rees, A.-M. Pons, Bioorg. Med. Chem. Lett. 6 (1996) 529–532.

[9] P.G. Baraldi, M.G.M.G. Pavani, M.C. Nuñez, P. Brigidi, B. Vitali, R. Gambari, R. Romagnoli, Bioorg. Med. Chem. 10 (2002) 449–456.
[10] T. Besson, C.W. Rees, G. Cottenceau, A.M. Pons, Bioorg. Med. Chem. Lett. 6 (1996) 2343–2348.
[11] V. Thiery, C.W. Rees, T. Besson, G. Cottenceau, A.-M. Pons, Eur J. Med. Chem. 33 (1998) 149–153.
[12] C.W. Pluijgers, J.W. Vonk, G.D. Thorn, Tetrahedron Lett. 12 (1971) 1317–1318.
[13] R.E. Allen, R.S. Shelton, M.G. Van Campen, J. Am. Chem. Soc. 76 (1954) 1158–1159.
[14] W.B. Turner, D.C. Aldridge, Fungal Metabolites – II, Acedamic Press Inc., New York, 1983, p. 152.
[15] S. Alam, J. Chem. Sci. 116 (2004) 325–331.
[16] T. Raj, M.P.S. Ishar, V. Gupta, A.P.S. Pannu, P. Kanwal, G. Singh, Tetrahedron Lett. 49 (2008) 243–246.
[17] K. Shimada, K. Aikawa, T. Fujita, M. Sato, K. Goto, S. Aoyagi, Y. Takikawa, C. Kabuto, Bull. Chem. Soc. Jpn. 74 (2001) 511–525.
[18] I.M. Rafiqul, K. Shimada, S. Aoyagi, Y. Fujisawa, Y. Takikawa, Tetrahedron Lett. 45 (2004) 6187–6190.
[19] J.C. Meslin, H. Quiniou, Tetrahedron 31 (1975) 3055–3058.
[20] G. Sabita, Aldrichim. Acta 29 (1996) 15–25.
[21] G. Singh, R. Singh, N.K. Girdhar, M.P.S. Ishar, Tetrahedron 58 (2002) 2471–2480.
[22] The data for **17c** has been submitted to The Cambridge Crystallographic Data Centre (CCDC No. 702090).
[23] V. Klimesova, M. Svoboda, K. Waisser, M. Machacek, V. Buchta, Z. Odlerova, Arch. Pharm. 329 (1996) 438–442.
[24] F. Wang, R. Langley, G. Gulten, L.G. Dover, G.S. Besra, W.R. Jacobs Jr., J.C. Sacchettini, J. Exp. Med. 204 (2007) 73–78.
[25] M.P.S. Ishar, G. Singh, S. Singh, K.K. Sreenivasan, G. Singh, Bioorg. Med. Chem. Lett. 16 (2006) 1366–1370.
[26] Y. Pommier, A. Tanizawa, in: J. Hickman, T. Tritton (Eds.), Cancer Chemotherapy, Blackwell Scientific Publications Ltd, Oxford, 1993, pp. 214–250.
[27] A.Y. Chen, L.F. Liu, Annu. Rev. Pharmacol. Toxicol. 94 (1994) 194–218.
[28] M. Zewail-Foote, L. Hurley, Anti-Cancer Drug Des. 14 (1999) 1–9.
[29] T.L. MacDonald, E.K. Lehnert, J.T. Loper, K.C. Chow, W.E. Ross, in: M. Potmesil, K.W. Kohn (Eds.), DNA Topoisomerase in Cancer, Oxford University Press, New York, 1991, p. 119 (Chapter 2).
[30] T.D. Gootz, K.E. Birghty, Chemistry and Mechanism of Action of the Quinolone Antibacterials, in: V.T. Andriole (Ed.), The Quinolones, second ed. Academic Press, San Diego, CA, 1998, pp. 27–80.
[31] G. Cianchetta, R. Mannhold, G. Cruciani, M. Baroni, V. Cecchetti, J. Med. Chem. 47 (2004) 3193–3201.
[32] D. Mitic-Culafic, B. Vukovic-Gacic, J. Knezevic-Vukcevic, S. Stankovic, D. Simic, Arch. Biol. Sci. 57 (2005) 173–178.
[33] R.A. Fromtling, J.N. Galgiani, M.A. Pfaller, A. Espinel-Ingroff, K.F. Bartizal, M.S. Bartlett, B.A. Body, C. Frey, G. Hall, G.D. Roberts, F.B. Nolte, F.C. Odds, M.G. Rinaldi, A.M. Sugar, K. Villareal, Antimicrob. Agents Chemother. 37 (1993) 39–45.
[34] J. Hindler, in: C.C. Mahon, G. Manuselis (Eds.), Textbook of Diagnostic Microbiology Special Antimicrobial Susceptibility Tests (1995), pp. 89–96.