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Amberlyst-15 catalyzed synthesis of novel thiophene–pyrazoline derivatives: spectral and crystallographic characterization and anti-inflammatory and antimicrobial evaluation

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Abstract Increasing instances of antimicrobial drug resistance and Inflammation-mediated disorders requires the design and synthesis of new small-molecules with higher affinity and specificity for their potential targets to serve as antibiotics or anti-inflammatory drugs, respectively. The current study presents the synthesis of a series of chalcones, **3**(**a**–**h**) by the reaction of 3-methylthiophene-2-carbaldehyde, **1** and acetophenones, **2**(**a**–**h**) by Claisen–Schmidt approach. The chalcones were efficiently transformed into thienyl-pyrazolines, **5**(**a**–**h**) by their reaction with thiosemicarbazide hydrochloride, **4** in the presence of Amberlyst-15 as a catalyst in acetonitrile at room temperature. Alternatively, the compounds **5**(**a**–**h**) were prepared by conventional method using acetic acid (40%) medium. Structures were characterized by spectral and single crystal X-ray diffraction studies. Preliminary assessment of the anti-inflammatory properties of the compounds showed that, amongst the series, compounds **5b** and **5c** have excellent anti-inflammatory activities. Further, compound **5c** showed excellent activity against *Escherichia coli* (MIC, 15 μg/mL), *Bacillus subtilis* (MIC, 20 μg/mL), *Aspergillus niger* (MIC, 20 μg/mL), and *Aspergillus flavus* (MIC 15 μg/mL), respectively. Compounds **5a** and **5b** were also found to be active against the tested microorganisms.

Keywords Anti-inflammatory · Antimicrobial · Chalcone · Condensation · Carbothioamide

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Abbreviations

TLC	Thin layer chromatography
CCD	Charge couple device
CIF	Crystallographic information file
CCDC	Cambridge Crystallographic Data Centre
NMR	Nuclear magnetic resonance
UPLC	Ultimate performance liquid chromatography
ESI	Electrospray
APCI	Atmospheric pressure chemical ionization
MTCC	Microbial type culture collection
VRV-PL-8a	*Vipera russelli* (Russell's viper) snake venom phospholipase A_2
RFU	Relative Fluorescence Unit

Introduction

Inflammation is a normal response of both the innate and adaptive immune systems whenever the host faces challenge in terms of infection [1]. However, when uncontrolled, inflammation can cause autoimmune/autoinflammatory disorders leading to yet greater conditions such as neurodegenerative disease and cancer. Microbial infections are implicated in inflammation whereby they trigger a persistent and aggressive immune response to antigens/adjuvants secreted by them and against normal human microbiota. In spite of the availability of effective anti-inflammatory agents there is a constant need for newer and safer small-molecules with inflammation countering properties. Despite numerous attempts to search for more effective antimicrobial and anti-inflammatory agents, pyrazoles still remain as the scaffold of choice in bioorganic chemistry. Amongst, the various routes available in the literature, most commonly employed are: highly regioselective synthesis of phosphonyl/sulphonyl pyrazoles by the reaction of chalcones with an Bestmann Ohira Reagent [2], 1.3-dipolar cycloaddition reaction of hydrazones to alkenes/alkynes [3], base catalyzed reaction of *N*-arylbenzamidrazones with 2-(ethoxymethylene)malononitriles [4], and the cyclocondensation reaction of phenylhydrazine hydrochlorides to chalcones [5].

In recent years, heterogeneous catalysis has played a central role in various organic transformations [6, 7]. Amberlyst-15, a macro reticular polystyrene based ion exchange resin with strongly acidic sulfonic group that facilitates its utility as an excellent source of strong acid, has been used in various acid catalyzed reactions in organic synthesis. The growing interest in the use of Amberlyst-15, a heterogeneous catalyst, is mainly due to its role in mild and highly selective transformations/synthesis, and the fact that the catalyst can be regenerated and used several times [8]. For instance, recently it was successfully employed for the synthesis of quinazolinones [9].

Further, it is emphasized here that pyrazoles are ubiquitous scaffolds and are regarded as promising molecules with potential applications in medicinal chemistry.

Pyrazoles were known to exhibit anti-inflammatory [10], antimicrobial and antioxidant [11], anesthetic [12], analgesic [13], antioxidant [14], antipsychotic [15], antidepressant and anticonvulsant [16], activities. In view of wide range of synthetic and biological applications of pyrazoles, we herein report the synthesis of thienyl-pyrazole derivatives and the results of their in vitro evaluation for anti-inflammatory and antimicrobial activities. The demonstrated protocol paves the way for future efforts at synthesizing derivatives of pyrazoles that could find widespread applications in medicinal chemistry.

Experimental

Materials and methods

Melting points were determined by an open capillary tube method and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 and 125 MHz spectrometer respectively. Mass spectra were obtained on ESI/APCI-Hybrid Quadrupole, Synapt G2 HDMS ACQUITY UPLC model spectrometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 elemental analyzer.

Synthesis of chalcones, 3(a–h)

To a solution mixture of 3-methylthiophene-2-carbaldehyde, **1** (5 mmol) and acetophenones, **2**(**a**–**h**) (5 mmol) in methyl alcohol, potassium hydroxide solution (40%, 2 mL) was added. Then the solution mixture was stirred at room temperature for 2–3 h. The progress of the reaction was monitored by TLC. After the completion, the reaction mixture was cooled to room temperature and poured into ice cold water. The solids separated were filtered, washed successively with cold hydrochloric acid (5%) and cold water. The crude solids were recrystallized from methyl alcohol to obtain the compounds **3**(**a**–**h**).

Synthesis of 5-aryl-3-(3-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-carbothioamides, 5(a–h)

Method A Mixture of chalcones **3**(**a**–**h**) (5 mmol), thiosemicarbazide hydrochloride **4** (10 mmol) and Amberlyst-15 (10%, w/w) in acetonitrile (25 mL) was stirred at room temperature for 30–60 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the separated solid was filtered, washed by diethyl ether (2 × 20 mL), dried and treated with ethyl acetate (20 mL). After stirring for 10 min, the mixture was filtered to remove the insoluble catalyst. The filtrate was collected and concentrated under vacuum. The solid isolated was triturated in diethyl ether, filtered and dried to obtain the desired product **5**(**a**–**h**). The recovered catalyst was washed with ethyl acetate, dried and reused effectively for four subsequent reactions.

Method B A solution mixture of chalcones, **3**(**a**–**h**) (5 mmol) and thiosemicarbazide hydrochloride, **4** (10 mmol) in acetic acid (40%) was refluxed on a water bath for 3–4 h. The progress of the reaction was monitored by TLC. After the completion, the mixture was filtered and the filtrate was poured into crushed ice. The separated solids were filtered and washed successively with 5% $NaHCO_3$ and water. The crude solids were recrystallized from methyl alcohol to get target molecules **5**(**a**–**h**) in good yields.

X-ray crystallography

A defect free single crystal of approximate dimension 0.25 × 0.27 × 0.30 mm^3 was chosen for X-ray diffraction studies. X-ray intensity data for the title compound were collected at temperature 293 K on a Bruker Proteum2 CCD diffractometer with X-ray generator operating at 45 kV and 10 mA, using CuK_α radiation of wavelength 1.54178 Å. Data were collected for 24 frames per set with different settings of φ (0° and 90°), keeping the scan width of 0.5°, exposure time of 5 s, the sample to detector distance of 45.10 mm. Parameters in CIF format are available as Electronic Supplementary Publication from Cambridge Crystallographic Data Centre.

Anti-inflammatory activity

Inflammation is initiated by manifold cues such as microbial or viral pathogens, autoimmune defects, tissue damage due to oxidative challenge etc. COX-2 has been the main target till date for which anti-inflammatory agents have been designed. However, inhibiting the enzyme soluble phospholipase A2 (sPLA2) which is an upstreame target in the inflammation pathway, will result in substrate depletion for COX-2, thereby bringing down the inflammation, as there will be no pro-inflammatory and inflammatory Prostaglandins (PG) [17]. In this context, we assessed the inhibitory potential of the newly synthesized thiophene–pyrazoline derivatives **5**(**a**–**h**) to block the upstream enzyme sPLA2, rather than LOX and COX-2. This approach will result in multiple enzyme targets within a pathway resulting in combinatorial administration of drugs in order to increase the potency and efficacy of blocking the pathway.

Purification of $sPLA_2$ (VRV-PL-8a) from V. russelli venom

sPLA2 (VRV-PL-8a) from *Vipera russelli* venom was purified to homogeneity by reported procedure [18], and the protein was estimated by Lowry's method [19]. Briefly, *V. russelli* venom (80 mg) was fractionated on pre-equilibrated Sephadex G-75 column (1.5 × 160 cm) using 50 mM phosphate buffer pH 7.0. The protein was resolved into major three peaks. The second peak, constituting about 30% of the total protein, showed major sPLA2 activity. This sPLA2 peak fraction was lyophilized and subjected further to cation exchange chromatographic fractionation employing a pre-equilibrated Carboxymethyl-Sephadex C-25 column (1.5 × 45 cm). The fractions were eluted stepwise using phosphate buffers of

varied ionic strength (50–200 mM) and pH (7.0–8.0). They were resolved into two fractions labeled as V and VIII respectively. The lyophilized fraction VIII was next subjected to Sephadex G-50 column (0.75 × 40 cm) chromatography and eluted using 50 mM phosphate buffer pH 7.0 and obtained peak was checked for sPLA2 activity.

*In vitro inhibition of VRV-PL-8a by thiophene-pyrazole conjugates, **5(a–h)***

In vitro inhibition of sPLA2 (VRV-PL-8a) by the synthesized pyrazole derivatives, **5**(**a**–**h**), was assessed by known procedure [20]. Briefly, a 50 μL activity buffer containing 50 mM Tris–HCl buffer pH 7.5, 10 mM $CaCl_2$ and 100 μM substrate stock (1 mM DMPC in methanol containing 2 mM Triton X-100 in Milli-Q water) were added and incubated for 5 min at 37 °C. Activity was initiated by adding 10 ng of sPLA2 alone or pre incubated with different concentration of thiophene-pyrazole conjugates **5**(**a**–**h**) ranging from 0 to 100 μM for 5 min at 37 °C. Reaction mixtures were incubated for 45 min at 37 °C. 50 μL of quenching solution was added at a final concentration of 2 mM NaN_3, 50 μM ANS and 50 mM EGTA, vortexed for 30 s and incubated for 5 min at RT. 2 μL of this solution was pipetted to measure RFU in a Nanodrop ND3300 Ver 2.8 using an excitation UV-LED (370 ± 10 nm) and emission was recorded at 480 nm in dark condition. Enzyme activity was calculated using the equation;

$$\Delta\text{RFU} = \text{RFU (control)} = \text{RFU (test)};$$

where **Δ**RFU is the change in RFU of test (with sPLA2) with respect to control (without sPLA2) in the presence of inhibitors. The resultant RFU was compared with the standard curve to determine the sPLA2 activity in the presence of inhibitors.

Effect of substrate and calcium concentration on VRV-PL-8a

0.1 mL of the reaction mixture containing 10 mg of $sPLA_2$ alone or the inhibitor (test compounds) concentration at their respective IC_{50} in 50 mM Tris–HCl buffer pH 7.5, 10 mM $CaCl_2$ and 10 μL of varied substrate stock (0–400 μM) was used for sPLA2 assay to check the effect of substrate in presence of test compounds. 50 μL of quenching solution was added at final concentration of 50 μM ANS, 2 mM sodium azide (NaN_3) and 50 mM (ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid) (EGTA) vortexed for 30 s and incubated for 5 min at RT. 2 μL of this solution was pipetted to measure Relative Fluorescence Unit (RFU). Similar set of experiment was conducted, where in reaction mixture containing IC_{50} concentration of inhibitor (test samples) in 50 mM Tris–HCl buffer, pH 7.5, 10 ng of sPLA2, 100 μM substrate and varied concentration of $CaCl_2$ (0–12 mM). Reaction was terminated and RFU was measured as described above.

Antimicrobial activity

The antimicrobial activities of the compounds **5**(**a**–**h**) were determined as minimum inhibitory concentrations (MIC) by serial dilution method [21, 22]. The antibacterial tests were conducted against bacterial pathogens such as *Escherichia coli* (MTCC 1687), *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 737). The antifungal activity was evaluated against fungal strains *Aspergillus niger*, *Aspergillus flavus* and *Candila albicans* (MTCC 227). The antibiotics ciprofloxacin and nystatin were used as positive control against bacterial and fungal species, respectively. Dimethyl sulfoxide was used as solvent control. The experiments were carried out in triplicate; the results were taken as a mean ± standard deviation (SD).

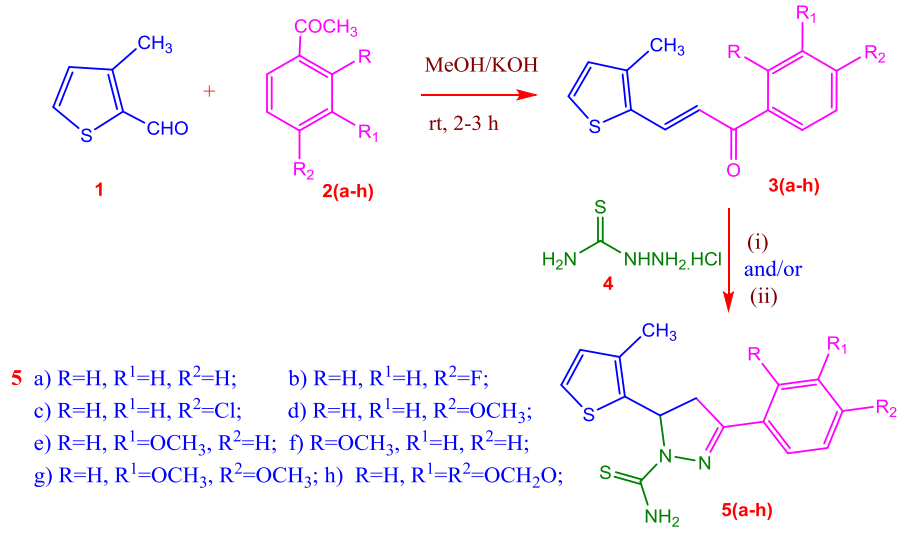
Results and discussion

Synthesis and characterization of compounds, 3(a–h) and 5(a–h)

In search of new anti-inflammatory and antimicrobial agents, our strategic aim was to synthesize thienyl-pyrazole carbothioamides. In this context, initially, the intermediate 1-(aryl)-3-(3-methylthiophen-2-yl)prop-2-en-1-ones, **3**(**a**–**h**) were synthesized by base catalyzed reaction of 3-methylthiophene-2-carbaldehyde **1,** with substituted acetophenones, **2**(**a**–**h**) in methyl alcohol according to our earlier procedure [23]. Then the reaction of chalcones, **3**(**a**–**h**) and thiosemicarbazide hydrochloride **4**, in the presence of catalyst Amberlyst-15 (10%, w/w) in acetonitrile under stirring conditions at room temperature produced thienyl-pyrazole carbothioamides, **5**(**a**–**h**) in good yields. The target compounds, **5**(**a**–**h**) were also synthesized by conventional method in acetic acid (40%) under reflux conditions, which paves way for understanding the catalytic activity of Amberlyst-15 (Scheme 1).

It was observed that, Amberlyst-15 mediated synthesis requires lesser reaction time, and occurs at room temperature in comparison to conventional method which required thermal energy and more reaction time. Interestingly, yields of the products obtained were almost same and/or up to + 4% more in comparison with the conventional heating. More importantly, Amberlyst-15 was recoverable with the solvent ethyl acetate, and is efficiently reused for four consecutive experiments. ^{1}H NMR, ^{13}C NMR, MS studies and elemental analysis provided the structural proof for the compounds **5**(**a**–**h**). Further, amongst the series, the structure of compound **5h** was confirmed by single crystal X-ray diffraction studies.

^{1}H NMR spectra of compounds **5**(**a**–**h**) did not show the signals appearing as doublet for each of the alkenyl protons of chalcones **3**(**a**–**h**), confirming the cycloaddition between chalcones and thiosemicarbazide hydrochloride to form target compounds. Further, the methylene protons of C-4 atom of newly formed pyrazole ring in compounds **5**(**a**–**h**) exhibited typical ABX spin and were of diastereotopic nature. For instance, in ^{1}H NMR spectra, the 4-H_a proton of **5h** appears as doublet of doublet at δ 3.110 (J = 3.2, 17.6 Hz) ppm; whereas, 4-H_b proton appears as doublet of doublet at δ 3.792 (J = 11.2, 17.6 Hz) ppm,



Scheme 1 Schematic diagram for the synthesis of thienyl-pyrazoline carbothioamides, **5**(**a**–**h**)

respectively. Instead of appearing as a triplet, 5-H resonates with both 4-H_a and 4-H_b and appears as doublet of doublet at δ 6.099 (J = 3.6, 11.2 Hz) ppm. The singlets appearing at δ 2.223, 6.069, and 7.980 ppm were due to CH_3, OCH_2O and NH_2 protons, respectively. Array of signals appearing as multiplet in the region δ 7.160–7.246 and doublets at δ 6.732 ppm and δ 7.608 ppm were due to thiophene and aromatic ring protons.

In the ^{13}C NMR spectrum, compound **5h** showed signals at δ 18.63, 106.56 and 160.13 ppm due to CH_3, OCH_2O and C=S carbons. The C-4, C-5 and C-3 carbons of newly formed pyrazole ring showed signals correspondingly at δ 47.30, 59.35 and 155.48 ppm. The appearance of signals for C-4 and C-5 in this region confirms the formation of non-aromatic pyrazoline ring. The signals due to other carbons appear in the aromatic region. Furthermore, in mass spectrum, compound **5h** showed m/z peaks at 345.07 corresponding to its molecular mass. All compounds of the series **5**(**a**–**h**) showed signals with similar patterns. All showed satisfactory elemental analyses compared with theoretical values, which strongly favour the formation of the products.

Further, amongst the series, the structure of the compound 3-(benzo[d][1,3]-dioxol-5-yl)-5-(3-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide **5h** was confirmed by single crystal x-ray diffraction studies. The crystal data and the structure refinement details are given in Table 1. There were two molecules (**A** and **B**) in the asymmetric unit of the crystal. The ORTEP of the molecule **A** and molecule **B** with displacement ellipsoids drawn at 50% probability level is shown in Figs. 1 and 2 respectively. The hydrogen bonding geometry is listed in Table S1, and the packing of molecules when viewed down along c axis is shown in Fig. S1.

Table 1 Crystal data and structure refinement details of the molecule, **5h**

CCDC Number	1557004
Empirical formula	$C_{16}H_{15}N_3O_2S_2$
Formula weight	345.45
Temperature	296 K
Wavelength	1.54178 Å
Crystal system, space group	Triclinic, $P-1$
Unit cell dimensions	a = 9.6550(3) Å; b = 13.2331(4) Å; c = 14.0232(4) Å α = 66.164(2)°; β = 89.317(2)°; γ = 81.675(2)°
Volume/Z	1619.44(9) Å^3/4
Density (calculated)	1.417 Mg/m^3
Absorption coefficient	3.091 mm^{-1}
F_{000}	720
Crystal size	0.25 × 0.30 × 0.27 mm
θ range for data collection	5.64°–64.53°
Index ranges	$-11 \le h \le 10$; $-15 \le k \le 15$; $-16 \le l \le 16$
Reflections collected	22,251
Independent reflections	5372 [R int = 0.0662]
Absorption correction	Multi-scan
Refinement method	Full matrix least-squares on F^2
Data/restraints/parameters	5372/0/417
Goodness-of-fit on F^2	1.027
Final [$I > 2\sigma(I)$]	$R1$ = 0.0602, $wR2$ = 0.1706
R indices (all data)	$R1$ = 0.0777, $wR2$ = 0.1887
Largest diff. peak and hole	0.375 and − 0.346 e/Å^3

The dotted line represents hydrogen bond interactions. The bridging of molecules through N–H···S hydrogen bond interactions between amidogen and sulphur elements results in an R_2^2 (8) ring motif is shown in Fig. S2. The molecular structure is comparable with the molecule reported earlier [24, 25].

Analytical data of compounds, 3(a–h), 5(a–h)

3-(3-Methylthiophen-2-yl)-1-phenylprop-2-en-1-one, **3a** Obtained from **1** (1.26 g, 10 mol) and **2a** (1.20 g, 10 mmol) in 76% yield (brownish semisolid); ^{1}H NMR ($CDCl_3$, δ ppm): 7.32 (d, 1H, J = 15.5 MHz, CH=), 7.74–7.85 (m, 7H, Ar–H), 7.98 (d, 1H, J = 16.4 MHz, =CH); ^{13}C NMR ($CDCl_3$, δ ppm): 125.6 (1C), 130.1 (1C), 131.6 (1C), 128.6 (2C), 129.8 (2C), 133.5 (1C), 134.3 (1C), 135.0 (1C), 136.7 (1C), 150.2 (1C), 188.5 (1C, C=O). MS m/z: 228.02 (M+, 100); Anal. Calcd. for $C_{14}H_{12}OS$ (%): C, 73.65; H, 5.30. Found: C, 73.52; H, 5.27.

1-(4-Fluorophenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3b** Obtained from **1** (1.26 g, 10 mol) and **2b** (1.38 g, 10 mmol) in 89% yield, (brackish semisolid); ^{1}H NMR ($CDCl_3$, δ ppm): 6.98 (dd, 2H, J = 12.0, 3.1 MHz, Ar–H), 7.31 (d, 1H, J = 15.9 MHz, CH=), 7.46 (dd, 2H, J = 12.3, 2.4 MHz, Ar–H), 7.73–7.92 (m, 2H,

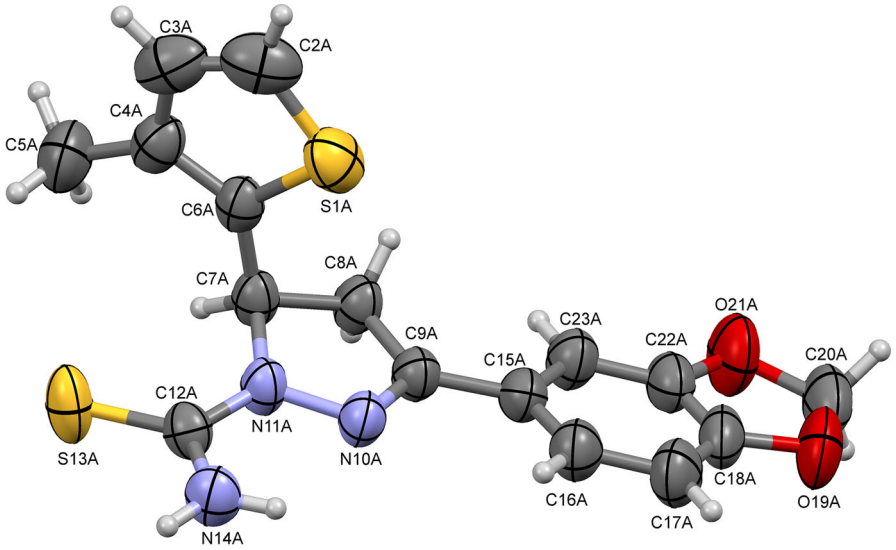


Fig. 1 ORTEP of the molecule **A** with thermal ellipsoids drawn at 50% probability

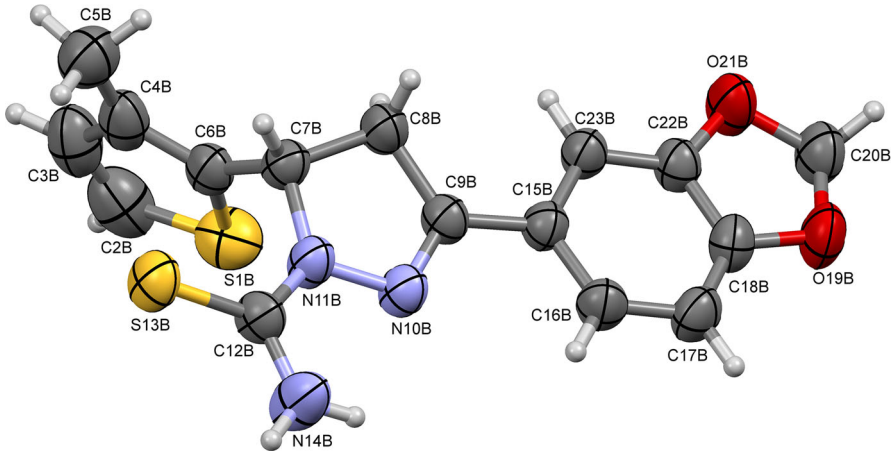


Fig. 2 ORTEP of the molecule **B** with thermal ellipsoids drawn at 50% probability

Ar–H), 8.09 (d, 1H, J = 16.0 MHz, =CH); ^{13}C NMR ($CDCl_3$, δ ppm): 115.6 (1C), 115.6 (1C), 123.8 (1C), 128.9 (1C), 129.8 (1C), 129.9 (1C), 131.4 (1C), 134.9 (1C), 139.8 (1C), 136.6 (1C), 140.2 (1C), 160.6 (1C), 188.4 (1C, C=O). MS *m/z*: 246.05 (M+, 100); Anal. Calcd. for $C_{14}H_{11}FOS$ (%): C, 68.27; H, 4.50. Found: C, 68.20; H, 4.47.

1-(4-Chlorophenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3c** Obtained from **1** (1.26 g, 10 mmol) and **2c** (1.54 g, 10 mmol) in 86% yield, m.p. 89–91 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.35 (s, 3H, CH_3), 6.87 (d, 1H, J = 2.8 MHz, CH=),

7.11–7.27 (m, 2H, Ar–H), 7.42 (d, 2H, J = 7.2 MHz, Ar–H), 7.92 (d, 2H, J = 7.2 MHz, Ar–H), 8.01 (d, 1H, J = 15.2 MHz, =CH); ^{13}C NMR ($CDCl_3$, δ ppm): 14.2 (1C), 119.0 (1C), 126.4 (1C), 127.6 (1C), 128.3 (1C), 128.8 (1C), 129.7 (1C), 131.4 (1C), 134.3 (1C), 136.0 (1C), 136.5 (1C), 139.0 (1C), 143.1 (1C), 188.3 (1C, C=O). MS *m/z*: 265.07 (MH+, ^{37}Cl, 41), 263.07 (MH+, ^{35}Cl, 100); Anal. Calcd. for $C_{14}H_{11}ClOS$ (%): C, 64.00; H, 4.50. Found: C, 59.83; H, 4.47.

1-(4-Methoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3d** Obtained from **1** (1.26 g, 10 mmol) and **2d** (1.50 g, 10 mmol) in 86% yield, pale yellow oily mass; ^{1}H NMR ($CDCl_3$, δ ppm): 2.33 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 6.84 (d, 1H, J = 5.2 MHz, CH=), 6.92 (d, 2H, J = 8.8 MHz, Ar–H), 7.22–7.27 (m, 2H, Ar–H), 7.96–7.97 (m, 2H, Ar–H), 8.00 (d, 1H, J = 3.2 MHz, =CH); ^{13}C NMR ($CDCl_3$, δ ppm): 14.0 (1C), 55.4 (1C), 112.7 (1C), 119.0 (1C), 119.7 (1C), 120.8 (1C), 127.3 (1C), 129.5 (1C), 131.4 (1C), 135.5 (1C), 139.6 (1C), 142.7 (1C), 159.8 (1C), 189.4 (1C, C=O). MS *m/z*: 259.12 (MH+, 100); Anal. Calcd. for $C_{15}H_{14}O_2S$ (%): C, 69.74; H, 5.46. Found: C, 69.63; H, 5.43.

1-(3-Methoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3e** Obtained from **1** (1.26 g, 10 mmol) and **2e** (1.50 g, 10 mmol) in 87% yield, (light brown semisolid); ^{1}H NMR ($CDCl_3$, δ ppm): 2.36 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 6.89 (d, 1H, J = 4.6 MHz, CH=), 7.10–7.41 (m, 4H, Ar–H), 7.52–7.59 (m, 2H, Ar–H), 8.03 (d, 1H, J = 14.7 MHz, =CH). MS *m/z*: 259.07 (M+, 100); Anal. Calcd. for $C_{15}H_{14}O_2S$ (%): C, 69.74; H, 5.46. Found: C, 69.65; H, 5.42.

1-(2-Methoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3f** Obtained from **1** (1.26 g, 10 mmol) and **2f** (1.50 g, 10 mmol) in 86% yield, m.p. 113–115 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.38 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 6.89 (d, 1H, J = 4.8 MHz, CH=), 7.10–7.42 (m, 4H, Ar–H), 7.53–7.59 (m, 2H, Ar–H), 8.03 (d, 1H, J = 14.8 MHz, =CH); ^{13}C NMR ($CDCl_3$, δ ppm): 14.2 (1C), 55.4 (1C), 113.6 (1C), 113.7 (1C), 119.5 (1C), 126.9 (1C), 130.6 (2C), 131.1 (1C), 131.3 (1C), 134.6 (1C), 134.7 (1C), 142.3 (1C), 163.3 (1C), 187.9 (1C, C=O). MS *m/z*: 259.12 (MH+, 100); Anal. Calcd. for $C_{15}H_{14}O_2S$ (%): C, 69.74; H, 5.46. Found: C, 69.60; H, 5.43.

1-(3,4-Dimethoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3g** Obtained from **1** (1.26 g, 10 mmol) and **2g** (1.80 g, 10 mmol) in 90% yield, m.p. 93–95 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.34 (s, 3H, CH_3), 3.85 (s, 6H, OCH_3), 6.88 (d, 1H, J = 4.8 MHz, CH=), 7.08–7.42 (m, 3H, Ar–H), 7.55–7.59 (m, 2H, Ar–H), 8.04 (d, 1H, J = 14.2 MHz, =CH). MS *m/z*: 288.08 (M+, 100); Anal. Calcd. for $C_{16}H_{16}O_3S$ (%): C, 66.64; H, 5.59. Found: C, 66.49; H, 5.54.

1-(Benzo[d][1,3]dioxol-5-yl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3h** Obtained from **1** (1.26 g, 10 mmol) and **2h** (1.64 g, 10 mmol) in 80% yield, m.p. 111–113 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.38 (s, 3H, CH_3), 6.05 (s, 2H, OCH_2O), 6.87–6.89 (m, 2H, Ar–H), 7.20–7.28 (m, 2H, Ar–H), 7.51 (s, 1H, Ar–H), 7.62 (d, 1H, J = 8.0 MHz, CH=), 8.00 (d, 1H, J = 14.2 MHz, =CH); ^{13}C NMR ($CDCl_3$, δ ppm): 13.6 (1C), 101.2 (1C), 107.4 (1C), 118.7 (1C), 123.7 (1C), 126.4 (1C), 130.7

(1C), 132.4 (1C), 133.3 (1C), 133.9 (1C), 134.4 (1C), 141.9 (1C), 147.6 (1C), 150.9 (1C), 186.9 (1C, C=O). MS *m/z*: 273.11 (MH+, 100); Anal. Calcd. for $C_{15}H_{12}O_3S$ (%): C, 66.16; H, 4.44. Found: C, 66.04; H, 4.40.

5-(3-Methylthiophen-2-yl)-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5a** Obtained from 3-(3-methylthiophen-2-yl)-1-phenylprop-2-en-1-one, **3a** (1.14 g, 5 mmol) and thiosemicarbazide hydrochloride, **4** (1.11 g, 10 mmol) in 72% yield, m.p. 151–153 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.26 (s, 3H, CH_3), 3.37 (dd, 1H, J = 4.9, 6.8 Hz, C_4-H_a), 3.84 (s, 3H, OCH_3), 3.89 (dd, 1H, J = 11.2, 17.7 Hz, C_4-H_b), 5.44 (s, 2H, NH_2), 5.73 (dd, 1H, J = 4.9, 8.6 Hz, C_5-H), 6.78–7.01 (m, 2H, Ar–H), 7.44–7.68 (m, 5H, Ar–H); ^{13}C NMR ($CDCl_3$, δ ppm): 13.2 (1C, CH_3), 44.8 (1C, C-4), 54.4 (1C, C-5), 121.5 (1C), 122.2 (1C), 123.9 (1C), 128.6 (1C), 128.6 (1C), 129.0 (1C), 129.1 (1C), 131.3 (1C), 133.3 (1C), 135.5 (1C), 151.7 (1C, C-3), 164.1 (1C, C=S). MS *m/z*: 301.02 (M+, 100); Anal. Calcd. for $C_{15}H_{15}N_3S_2$ (%): C, 59.77; H, 5.02; N, 13.94; Found: C, 59.62; H, 4.98; N, 13.88.

3-(4-Fluorophenyl)-5-(3-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5b** Obtained from 1-(4-fluorophenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3b** (1.23 g, 5 mmol) and thiosemicarbazide hydrochloride, **4** (1.27 g, 10 mmol) in 81% yield, m.p. 146–148 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.25 (s, 3H, CH_3), 3.37 (dd, 1H, J = 4.6, 5.9 Hz, C_4-H_a), 3.87 (dd, 1H, J = 11.0, 17.5 Hz, C_4-H_b), 5.46 (s, 2H, NH_2), 5.73 (dd, 1H, J = 4.7, 9.0 Hz, C_5-H), 6.78 (d, 1H, Ar–H), 7.10 (d, 1H, Ar–H), 7.32 (d, 2H, J = 7.8 Hz, Ar–H), 7.74 (d, 2H, J = 7.1 Hz, Ar–H); ^{13}C NMR ($CDCl_3$, δ ppm): 13.8 (1C, CH_3), 44.7 (1C, C-4), 53.9 (1C, C-5), 114.5 (2C), 120.1 (1C), 120.8 (1C), 123.3 (1C), 129.3 (2C), 131.1 (1C), 132.7 (1C), 151.7 (1C, C-3), 163.3 (1C), 162.9 (1C, C=S). MS *m/z*: 319.06 (M+, 100); Anal. Calcd. for $C_{15}H_{14}FN_3S_2$ (%): C, 56.40; H, 4.42; N, 13.16; Found: C, 56.27; H, 4.40; N, 13.14.

3-(4-Chlorophenyl)-5-(3-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5c** Obtained from 1-(4-chlorophenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3c** (1.31 g, 5 mmol) and thiosemicarbazide hydrochloride, **4** (1.27 g, 10 mmol) in 88% yield, m.p. 56–57 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.25 (s, 3H, CH_3), 3.35 (dd, 1H, J = 4.5, 5.4 Hz, C_4-H_a), 3.87 (dd, 1H, J = 11.1, 18.1 Hz, C_4-H_b), 5.43 (s, 2H, NH_2), 5.73 (dd, 1H, J = 4.7, 8.8 Hz, C_5-H), 6.73 (d, 1H, Ar–H), 7.31 (d, 1H, Ar–H), 7.48 (d, 2H, J = 7.4 Hz, Ar–H), 7.92 (d, 2H, J = 7.1 Hz, Ar–H); ^{13}C NMR ($CDCl_3$, δ ppm): 13.5 (1C, CH_3), 45.3 (1C, C-4), 54.3 (1C, C-5), 119.5 (1C), 120.4 (1C), 121.9 (1C), 128.2 (2C), 128.9 (2C), 132.3 (1C), 134.1 (1C), 135.7 (1C), 151.8 (1C, C-3), 164.1 (1C, C=S). MS *m/z*: 337.05 (M+2, 36.5%), 335.06 (M+, 100); Anal. Calcd. for $C_{15}H_{14}ClN_3S_2$ (%): C, 53.64; H, 4.20; N, 12.51; Found: C, 53.51; H, 4.16; N, 12.49.

3-(4-Methoxyphenyl)-5-(3-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5d** Obtained from 1-(4-methoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3d** (1.27 g, 5 mmol) and thiosemicarbazide hydrochloride, **4** (1.27 g, 10 mmol) in 80% yield, m.p. 162–164 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.26 (s, 3H, CH_3), 3.37 (dd, 1H, J = 4.7, 5.5 Hz, C_4-H_a), 3.83 (s, 3H, OCH_3), 3.92 (dd,

1H, J = 11.3, 17.0 Hz, C_4-H_b), 5.45 (s, 2H, NH_2), 5.72 (dd, 1H, J = 5.0, 8.2 Hz, C_5-H), 6.73 (d, 1H, Ar–H), 7.32–7.78 (m, 5H, Ar–H); ^{13}C NMR ($CDCl_3$, δ ppm): 13.6 (1C, CH_3), 44.6 (1C, C-4), 54.4 (1C, C-5), 55.5 (1C, OCH_3), 113.4 (2C), 120.8 (1C), 121.6 (1C), 123.1 (1C), 128.0 (1C), 128.1 (2C), 133.2 (1C), 151.8 (1C, C-3), 159.3 (1C), 163.1 (1C, C=S). MS *m/z*: 331.08 (M+, 100); Anal. Calcd. for $C_{16}H_{17}N_3OS_2$ (%): C, 57.98; H, 5.17; N, 12.68; Found: C, 57.85; H, 5.14; N, 12.65.

3-(3-Methoxyphenyl)-5-(3-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5e** Obtained from 1-(3-methoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3e** (1.27 g, 5 mmol) and thiosemicarbazide hydrochloride, **4** (1.27 g, 10 mmol) in 77% yield, m.p. 118–120 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.28 (s, 3H, CH_3), 3.35 (dd, 1H, J = 6.9, 17.1 Hz, C_4-H_a), 3.84 (s, 3H, OCH_3), 3.90 (dd, 1H, J = 6.2, 13.2 Hz, C_4-H_b), 5.69 (s, 2H, NH_2), 5.72 (dd, 1H, J = 6.8, 11.9 Hz, C_5-H), 6.73 (d, 1H, J = 4.0 Hz, Ar–H), 6.92–7.36 (m, 4H, Ar–H), 7.85 (d, 1H, J = 7.6 Hz, Ar–H); ^{13}C NMR ($CDCl_3$, δ ppm): 13.9 (1C, CH_3), 45.8 (1C, C-4), 54.4 (1C, C-5), 55.4 (1C, OCH_3), 111.5 (1C), 120.6 (1C), 120.8 (1C), 122.2 (1C), 129.0 (1C), 130.1 (1C), 131.3 (1C), 133.3 (1C), 139.4 (1C), 151.9 (1C, C-3), 155.4 (1C), 158.1 (1C, C=S). MS *m/z*: 331.08 (M+, 100); Anal. Calcd. for $C_{16}H_{17}N_3OS_2$ (%): C, 57.98; H, 5.17; N, 12.68; Found: C, 57.82; H, 5.13; N, 12.66.

3-(2-Methoxyphenyl)-5-(3-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5f** Obtained from 1-(2-methoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3f** (1.27 g, 5 mmol) and thiosemicarbazide hydrochloride, **4** (1.27 g, 10 mmol) in 84% yield, m.p. 132–135 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.28 (s, 3H, CH_3), 3.36 (dd, 1H, J = 4.4, 5.2 Hz, C_4-H_a), 3.81 (s, 3H, OCH_3), 3.88 (dd, 1H, J = 11.6, 17.2 Hz, C_4-H_b), 5.40 (s, 2H, NH_2), 5.71 (dd, 1H, J = 4.8, 8.0 Hz, C_5-H), 6.73–7.02 (m, 4H, Ar–H), 7.34–7.38 (m, 1H, Ar–H), 7.85 (d, 1H, Ar–H); ^{13}C NMR ($CDCl_3$, δ ppm): 13.9 (1C, CH_3), 45.8 (1C, C-4), 54.4 (1C, C-5), 55.4 (1C, OCH_3), 111.5 (1C), 120.6 (1C), 120.8 (1C), 122.2 (1C), 129.0 (1C), 130.1 (1C), 131.3 (1C), 133.3 (1C), 139.4 (1C), 151.9 (1C, C-3), 155.4 (1C), 158.1 (1C, C=S). MS *m/z*: 331.08 (M+, 100); Anal. Calcd. for $C_{16}H_{17}N_3OS_2$ (%): C, 57.98; H, 5.17; N, 12.68; Found: C, 57.80; H, 5.12; N, 12.64.

3-(3,4-Dimethoxyphenyl)-5-(3-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5g** Obtained from 1-(3,4-dimethoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3g** (1.44 g, 5 mmol) and thiosemicarbazide hydrochloride, **4** (1.27 g, 10 mmol) in 89% yield, m.p. 185–187 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.25 (s, 3H, CH_3), 3.35 (dd, 1H, J = 5.4, 6.2 Hz, C_4-H_a), 3.82 (s, 6H, OCH_3), 3.87 (dd, 1H, J = 11.2, 17.1 Hz, C_4-H_b), 5.45 (s, 2H, NH_2), 5.72 (dd, 1H, J = 4.9, 8.4 Hz, C_5-H), 6.75–6.92 (m, 2H, Ar–H), 7.34–7.48 (m, 3H, Ar–H); ^{13}C NMR ($CDCl_3$, δ ppm): 13.3 (1C, CH_3), 43.8 (1C, C-4), 54.5 (1C, C-5), 55.8 (2C, OCH_3), 113.6 (1C), 118.6 (1C), 120.1 (1C), 121.1 (1C), 121.3 (1C), 123.7 (1C), 126.8 (1C), 133.1 (1C), 149.4 (1C), 151.8 (1C, C-3), 152.4 (1C), 159.1 (1C, C=S). MS *m/z*: 361.09 (M+, 100); Anal. Calcd. for $C_{17}H_{19}N_3O_2S_2$ (%): C, 56.48; H, 5.30; N, 11.62; Found: C, 56.41; H, 5.25; N, 11.57.

3-(Benzo[d][1,3]dioxol-5-yl)-5-(3-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5h** Obtained from 1-(benzo[d][1,3]dioxol-5-yl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, **3h** (1.36 g, 5 mmol) and thiosemicarbazide hydrochloride, **4** (1.27 g, 10 mmol) in 75% yield, m.p. 115–117 °C; ^{1}H NMR (C_6D_6, δ ppm): 2.22 (s, 3H, CH_3), 3.11 (dd, 1H, J = 3.2, 17.6 Hz, 4-H_a), 3.79 (dd, 1H, J = 11.2, 17.6 Hz, 4-H_b), 6.06 (s, 2H, OCH_2O), 6.09 (dd, 1H, J = 3.6, 11.2 Hz, 5-H), 6.73 (d, 1H, Ar–H), 7.16–7.24 (m, 2H, Ar–H); 7.60 (d, 1H, Ar–H), 7.98 (s, 2H, NH_2); ^{13}C NMR ($CDCl_3$, δ ppm): 18.6 (1C, CH_3), 47.3 (1C, C-4), 59.3 (1C, C-5), 106.5 (1C, OCH_2O), 111.0 (1C), 113.3 (1C), 126.5 (1C), 127.4 (1C), 130.8 (1C), 135.0 (1C), 137.5 (1C), 145.5 (1C), 152.8 (1C), 153.8 (1C), 155.4 (1C, C-5), 160.1 (1C, C=S). MS *m/z*: 345.07 (M+, 100); Anal. Calcd. for $C_{16}H_{15}N_3O_2S_2$ (%): C, 55.63; H, 4.38; N, 12.16; Found: C, 55.51; H, 4.35; N, 12.10.

Anti-inflammatory activity of compounds, 5(a–h)

The results of sPLA2 inhibition studies of synthesized compounds **5**(**a**–**h**) are tabulated in Table 2. The tested 2-pyrazoline derivatives, **5**(**a**–**h**) inhibited $sPLA_2$ in dose dependent manner with an IC_{50} value ranging from 9.110 to 26.220 μM which are computed and analyzed using sigmoidal Four Parameter Logistic (4PL) curve fit using the equation; $y = \min + (\max - \min)/(1 + (x/EC50)^{(-Hillslope)}$ in which y = % effect, min = minimum effect, max = maximum effect, x = concentration. Two constraints were used for fitting the 4PL function: (a) $0.1 < \min < 1$; (b) $\max < 100$. Amongst the series, **5b**, and **5c** showed significant inhibition against VRV-PL-8a with IC_{50} value of 10.76 and 9.11 μM respectively (Table 2), compared to other structurally related molecules.

To understand the mechanism of action of inhibition, we studied the effect of substrate and calcium concentrations on VRV-PL-8a enzyme inhibition by **5b**, and **5c**. We examined its inhibition as a function of calcium and substrate concentrations. Compounds **5b** at its IC_{50} concentration (10.76 μM) and **5c** (9.11 μM) with varying calcium (0–12 mM) concentrations, showed no change in sPLA2 activity (Table 3).

Preliminary study revealed that compounds **5b** and **5c** were not competing to the calcium binding site or active site on the VRV-PL-8a enzyme. Inhibition of sPLA2

Table 2 In vitro anti-inflammatory activity of thienyl-pyrazoline carbothioamides, **5**(**a**–**h**)

Compound	sPLA2 IC_{50} (μM) ± SEM
5a	14.660 ± 0.132
5b	10.762 ± 0.102
5c	9.110 ± 0.090
5d	18.010 ± 0.165
5e	19.210 ± 0.186
5f	19.488 ± 0.179
5g	26.220 ± 0.224
5h	25.100 ± 0.240

Results are expressed as mean ± standard error of measurement (SEM) (n = 3)

Table 3 Effect of substrate and calcium concentrations on VRV-PL-8a enzyme inhibition by compounds **5b** (IC_{50} − 10.76 μM), **5c** (IC_{50} − 9.11 μM) with varying calcium concentrations (0–12 mM)

$CaCl_2$ (mM)	sPLA2	sPLA2-**5b**	sPLA2-**5c**
0	0	0	0
1	0.112	0.060	0.066
2	0.173	0.085	0.090
4	0.210	0.105	0.122
6	0.250	0.132	0.147
8	0.295	0.165	0.178
10	0.325	0.180	0.185
12	0.335	0.198	0.204

The results are expressed as mean ± SEM (n = 3)

by these compounds is independent of calcium concentration and do not chelate calcium ions required for the enzyme activity.

Antimicrobial activity of compounds, 5(a–h)

The results of antimicrobial activities of the synthesized compounds **5**(**a**–**h**) are summarized in Table 4.

All the synthesized series of 2-pyrazoline carbothioamides, **5**(**a**–**h**) exerted a wide range of modest to good in vitro antimicrobial activities having MIC' values ranging from 10.0 to 120 μg/mL against bacteria and 15.0–110 μg/mL against fungal species; in particular, they showed lesser inhibitory effect on *S. aureus* (90.0–120.0 μg/mL) and *C. albicans* (80.0–110.0 μg/mL) organisms. Amongst the series of 2-pyrazolines, **5g** having dimethoxy substitutions, and **5h** having

Table 4 Minimum inhibitory concentrations (expressed as μg/mL)[a] of thienyl-pyrazoline carbothioamides **5**(**a**–**h**) against bacteria and fungi species by serial dilution method

Sample	*S. aureus*	*E. coli*	*B. subtilis*	*A. niger*	*A. flavus*	*C. albicans*
5a	110.0 ± 1.00	25.0 ± 0.50	25.0 ± 0.75	25.0 ± 0.50	25.0 ± 0.60	90.0 ± 0.50
5b	120.0 ± 1.50	20.0 ± 0.50	20.0 ± 0.60	25.0 ± 0.75	25.0 ± 0.75	95.0 ± 0.75
5c	90.0 ± 0.75	15.0 ± 0.50	20.0 ± 0.75	20.0 ± 0.60	15.0 ± 0.70	80.0 ± 0.55
5d	120.0 ± 0.50	60.0 ± 1.00	75.0 ± 1.00	70.0 ± 0.75	75.0 ± 0.60	110.0 ± 1.25
5e	110.0 ± 1.00	50.0 ± 0.70	55.0 ± 1.00	55.0 ± 0.50	50.0 ± 0.40	90.0 ± 1.00
5f	90.0 ± 1.25	55.0 ± 0.50	45.0 ± 0.40	40.0 ± 0.50	45.0 ± 0.60	100.0 ± 1.50
5g	120.0 ± 1.50	90.0 ± 1.00	110.0 ± 1.50	90.0 ± 1.50	100.0 ± 1.25	100.0 ± 2.50
5h	100.0 ± 1.50	90.0 ± 1.50	100.0 ± 1.50	90.0 ± 1.25	90.0 ± 1.00	90.0 ± 1.25
CYP[b]	20.0 ± 0.50	20.0 ± 0.75	30.0 ± 1.00	–	–	–
NYS[c]	–	–	–	25.0 ± 0.75	25.0 ± 0.25	20.0 ± 0.50

[a]Values are mean ± SD of three replicates

[b]Ciprofloxacin—positive control against bacteria species

[c]Nystatin—positive control against fungi species

(benzo[d][1,3]dioxol-5-yl) substitution, exhibited lesser activity against all the organisms tested. Compound **5c** showed excellent activity by inhibiting spore germination of *E. coli* (MIC:15 μg/mL), *B. subtilis* (MIC: 20 μg/mL), *A. niger* (MIC: 20 μg/mL), and *A. flavus* (MIC: 15 μg/mL), respectively.

Promising activities were showed by the compounds **5a** against *E. coli* (25 μg/mL), *B. subtilis* (25 μg/mL). *A. niger* (25 μg/mL) *A. flavus* (25 μg/mL); **5b** against *E. coli* (20 μg/mL), *B. subtilis* (20 μg/mL). *A. niger* (25 μg/mL) *A. flavus* (25 μg/mL), which were incidentally similar to those demonstrated by the respective standard drugs used in the experiment. 2-Pyrazolines **5d**, **5e**, and **5f** having methoxyphenyl substitutions, showed moderate activity against bacterial pathogens *E. coli* (50.0–80.0 μg/mL), *B. subtilis* (45.0–75.0 μg/mL); and against fungal stains *A. niger* (40.0–70.0 μg/mL), *A. flavus* (40.0–75.0 μg/mL).

Though this study has not attempted understanding the mode of action for this family of compounds in antimicrobial and antifungal functions, previous reports with compounds belonging to the same family have speculated that the mode of antimicrobial and antifungal activities of these compounds could be mediated through an elevated production of cytotoxic hydroxyl radicals (due to the molecular properties of the small-molecules) or through the possible inhibition of oxidative stress protection function(s) more critically needed in rich media capable of constant synthesis of free oxide radical [26].

Further, the authors have also raised the possibility whereby the small-molecules bring about their antimicrobial effects by interfering with iron acquisition in a nutrient limiting environment. These compounds can bind to iron more tightly than the corresponding bacterial or fungal siderophores, hence sequestering iron under limiting conditions of this micronutrient. This, in turn, has the potential to reduce iron bioavailability thus showing potent antimicrobial activity under the iron-scarcity condition [26].

Conclusions

To sum up, in the present work, we report a new procedure for the synthesis of thiophene-pyrazole hybrids from chalcones using a recyclable Amberlyst-15 catalyst. The method is an efficient and reliable approach towards the synthesis of pyrazoles. Synthesized compounds were characterized by spectral and crystallographic studies. Preliminary studies on anti-inflammatory activity of the synthesized compounds **5**(**a**–**h**) show that the compounds **5b** and **5c** exhibit excellent anti-inflammatory activities. Further, compound **5c** showed excellent activity by inhibiting *E. coli* (15, 10 μg/mL), *B. subtilis* (20, 15 μg/mL), *A. niger* (20, 20 μg/mL), and *A. flavus* (15, 15 μg/mL), respectively. Compounds **5a** and **5b** were also found to be active against the tested microorganisms. The demonstrated synthesis paves the way for future efforts at synthesizing novel derivatives of pyrazoles that could find widespread applications in medicinal chemistry and helping the efforts aimed at alleviating dreadful diseases caused by microorganism induced inflammation.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest including financial, personal or other relationships with other people or organizations for this article.

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