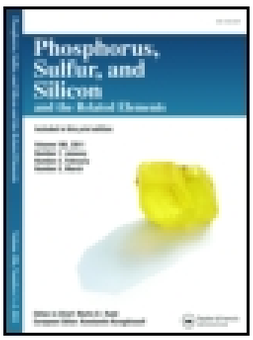

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PHOSPHORUS, SULFUR, AND SILICON AND THE RELATED ELEMENTS
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Ketene dithioacetal mediated synthesis of 1,3,4,5-tetrasubstituted pyrazole derivatives and their biological evaluation

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ABSTRACT

Ketene dithioacetal mediated chemo- and regioselective synthesis of a series of novel 1,3,4,5-tetrasubstituted pyrazole derivatives (**4a-l**) integrated with a bioactive indole nucleus was achieved by reacting substituted 2-(1-methyl-1H-indole-3-carbonyl)-3,3-bis-(methylthio)-acrylonitrile (**2**) and substituted phenyl hydrazine hydrochloride (**3**) in the presence of a catalytic amount of anhydrous K_2CO_3 under reflux conditions. The structures were ascertained by ^{1}H NMR, NOESY, ^{13}C NMR, FT-IR, and HRMS data. *In vitro* cytotoxicity evaluation of the synthesized compounds against MCF 7 (breast carcinoma) and normal Vero (monkey kidney) cell lines revealed that the compound 5-(5-Bromo-1-methyl-1*H*-indol-3-yl)-1-(4-cyano-phenyl)-3-methylsulfanyl-1*H*-pyrazole-4-carbonitrile (**4k**) showed significant cytotoxicity against MCF 7 (GI_{50} = 15.6 μM) with low cytotoxicity against normal Vero cell line. Most of the synthesized compounds were also found to possess excellent anti-inflammatory and antioxidant (DPPH, NO, H_2O_2 and SOR) potential.

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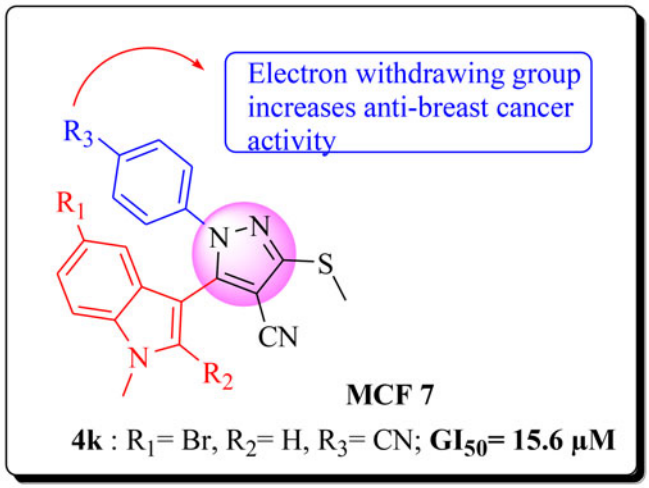
α-Aroylketene dithioacetal; anti-inflammatory; antioxidant; breast cancer; pyrazole

GRAPHICAL ABSTRACT



Introduction

Cancer is one of the most severe clinical problems and among the leading causes of death worldwide. The number of deaths due to cancer alone is more than those caused by AIDS, malaria, and tuberculosis combined,[1] by 2030, there will be 27 million people with cancer and 17 million deaths annually.[2] Numerous compounds, both synthetic and naturally derived, have been screened to evaluate their selective cytotoxic potential. In particular, indoles with 5- or 6-membered heterocyclic systems in the 3-position have gained considerable interest due to their significant anti-cancer activity.[3] Some drugs containing indole and pyrazole heterocycles, such as Ruxolitinib **1**,[4] Crizotinib **2**,[4] indomethacin **3**,[5] celecoxib **4**,[6] have created some hope for the life of cancer patients (Figure 1).

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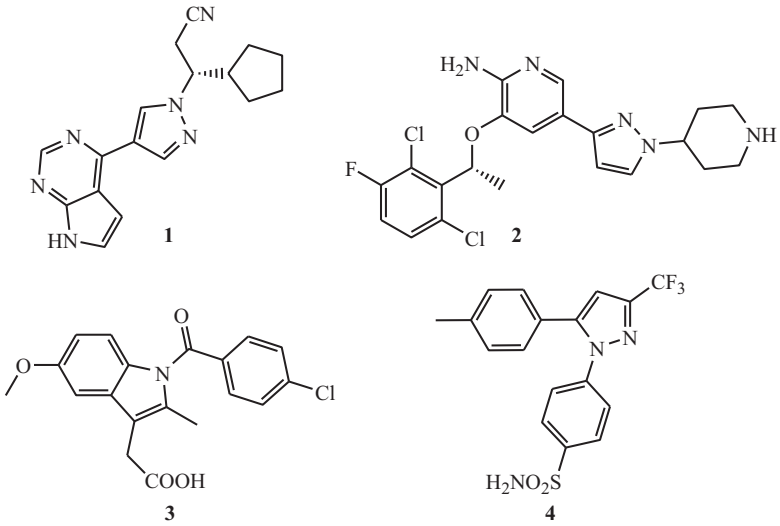


Figure 1. Indole and pyrazole heterocycle containing drug molecules.

Recently, some indole-pyrazole hybrid molecules reported by Singh *et al.* were found to be effective inhibitors of tumour promoting activities in an average concentration range of 2.0–3.2 μM, when tested against 60 human cancer cell lines.[7] Zhang synthesized a series of novel 3-(1*H*-indole-3-yl)-1*H*-pyrazole-5-carbohydrazide derivatives and evaluated for their cytotoxic activity against four human cancer cell lines and one of the synthesized compounds was found to arrest the cell cycle at S phase when analyzed by the flow cytometry method.[8] Therefore, it would be of interest to combine both the indole functionality and pyrazole into one molecule.

Inspired by these observations and in continuation of our enduring research programme on the development of novel anticancer and anti-inflammatory agents,[9] herein we have reported library of 1,3,4,5-tetrasubstituted pyrazole derivatives (**4a-l**) integrated with indole nucleus as potent anti-breast cancer, anti-inflammatory and antioxidant agents (Scheme 1).

Results and discussion

Chemistry

In the present study, the synthesis of the desired 1,3,4,5-tetrasubstituted pyrazole derivatives (**4a-l**) has been achieved by chemo and regioselective cyclization of substituted 2-(1-methyl-1H-indole-3-carbonyl)-3,3-bis(methylthio)acrylonitrile (**2**) and substituted phenyl hydrazine hydrochloride (**3**) in the presence of anhydrous K_2CO_3 (3 eq.) under reflux conditions in ethanol (Scheme 1). The starting material for the synthesis of title compounds, namely 2-(1-methyl-1H-indole-3-carbonyl)-3,3-bis(methylthio)acrylonitrile (**2**), was synthesized in good yield from the reaction of substituted 3-(1-methyl-1H-indol-3-yl)-3-oxopropanenitrile (**1**) with carbon disulfide and dimethyl sulfate in presence of sodium *tert*-butoxide.[10] The products were purified by column chromatography using silica gel mesh size, 100–200 and elution with 10% ethyl acetate in hexane. The structures of target molecules were ascertained by IR, ^{1}H NMR, ^{13}C NMR and HRMS data. The CN stretching band at 2219 cm^{-1} in the IR spectrum confirms the chemoselective cyclization and formation of desired product **4** over **5**. To carry out distinction between plausible regioisomers **4** and **6**, the representative compound **4h** was analyzed by 2D NMR (NOESY) spectroscopy in $CDCl_3$. The full NOESY and partial NOESY results are presented in Figure 2.

In 2D NOESY experiment, the weak NOE between -OMe of ring A vs aromatic proton of ring B and strong NOE of CH of ring A with CH of ring B suggested that the aromatic ring B is attached to N1 nitrogen of pyrazole i.e. compound **4h** (Figure 2).

Anticancer activity

All the synthesized 1,3,4,5-tetrasubstituted pyrazole derivatives (**4a-l**) were evaluated for their *in vitro* cytotoxic potencies in human breast cancer cell line MCF-7 by employing the sulforhodamine B (SRB) assay method.[11] Adriamycin is one of the most efficient anticancer drug is employed as reference standard. Three parameters such as GI_{50}, TGI and LC_{50} were determined during the screening process and the results are summarized in Table S1 (Supplemental Materials). Among the compounds screened, compound **4k** (5-(5-Bromo-1-methyl-1H-indol-3-yl)-1-(4-cyano-phenyl)-3-methylsulfanyl-1H-pyrazole-4-carbonitrile) exhibited significant cytotoxicity (GI_{50} = 15.6 μM) against the MCF-7 cell line, whereas compounds **4j**, **4c**, **4h** and **4f** exhibited moderate cytotoxicity (GI_{50} = 47.0–71.6 μM). On the other hand, rest of the compounds showed weak cytotoxic potential against MCF-7 cell line. A comparison of the TGI concentrations of the compounds with adriamycin was also done. Most of the compounds unveiled weak activity against the MCF-7 cell line. The LC_{50} concentrations of the compounds were also appraised to get an indication of the cytotoxic effects of these compounds against the MCF-7 cell line. As like adriamycin (LC_{50} >100 μM), the compounds synthesized showed weak activity (LC_{50} >100 μM).

A major disadvantage in the progress of anticancer drug development is most of the anticancer drugs affect the normal cell growth. Therefore, we have ensured the selectivity by *in vitro* screening against the normal Vero (Monkey, Kidney)

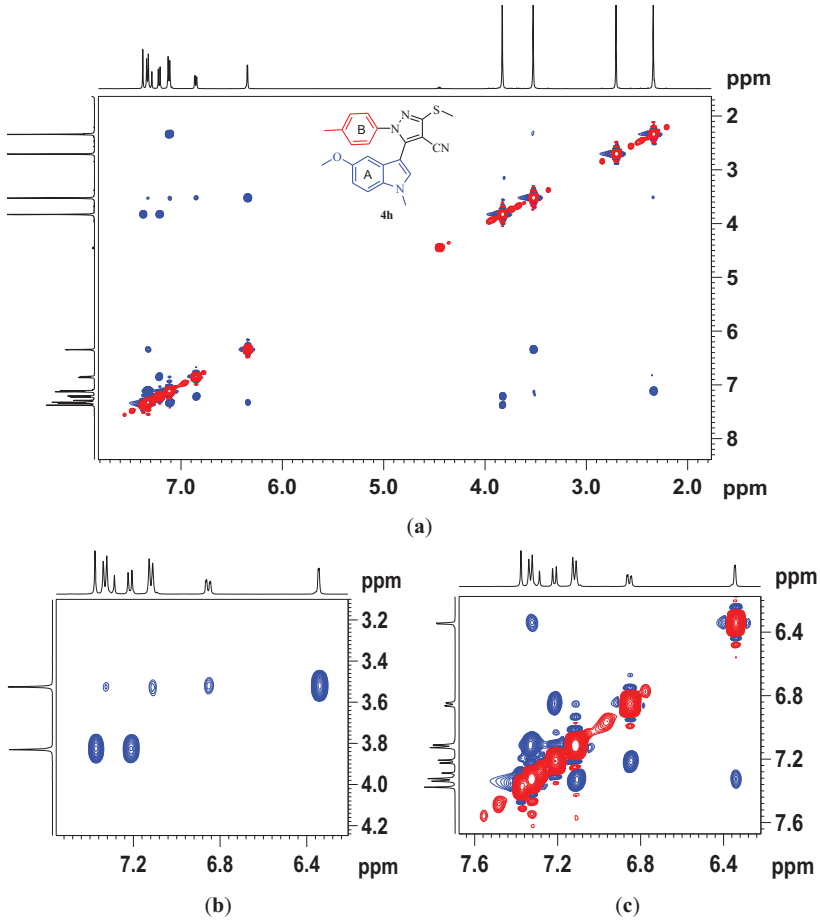


Figure 2. (a) Full NOESY Spectrum; (b) Partial NOESY: aliphatic vs aromatic; (c) Partial NOESY: aromatic vs aromatic region (500 MHz, $CDCl_3$).

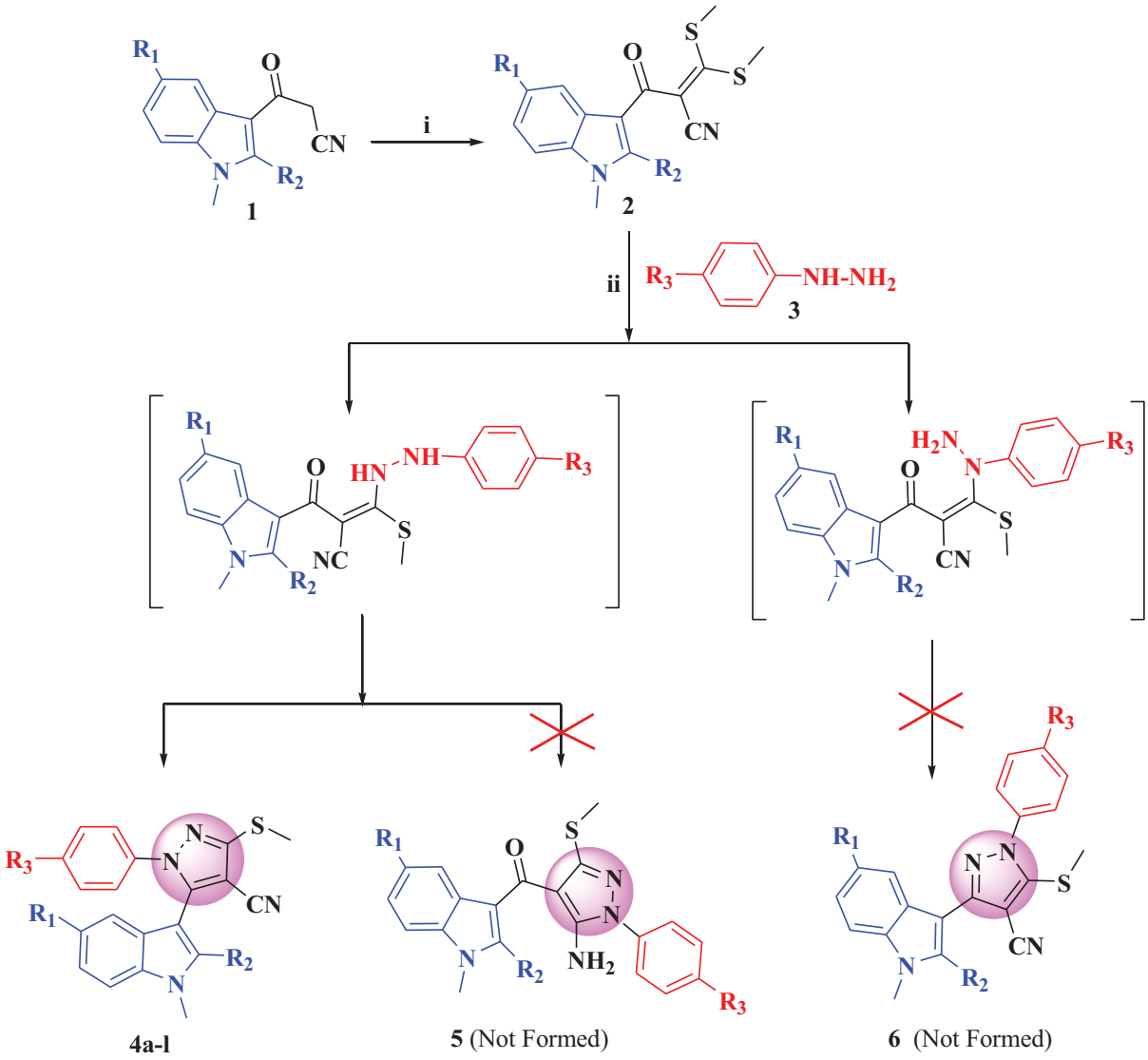
cell line.[12] All the compounds subjected for the cytotoxicity screening against normal Vero cell line surprisingly exhibited very low cytotoxicity (GI_{50} >100μM, TGI >100μM and LC_{50} >100μM) compared to standard drug Adriamycin (GI_{50} = 0.03μM, TGI = 10μM and LC_{50} >100μM). A structure activity relationship (SAR) study revealed that increase in activity was observed with the introduction of electron withdrawing groups at R3 and R1 position whereas, a decrease in activity was observed with electron donating groups.

Anti-inflammatory activity

Protein denaturation is a very well recognised cause of inflammation. In the present study, *in vitro* anti-inflammatory effect of synthesized 1,3,4,5-tetrasubstituted pyrazole derivatives (**4a-l**) was also evaluated against denaturation of egg albumin and results are summarized in Table S2 (Supplemental Materials). Compound **4e** showed excellent inhibition (93.80%) compared to the standard drug diclofenac sodium (90.21%) at 1mM concentration. All other compounds showed good to moderate inhibition of heat induced albumin denaturation (47.23–71.08%).

Antioxidant activity

Reactive oxygen species (ROS) plays key role in the pathophysiological mechanisms and they reacts indiscriminately with almost every type of biomolecules found in living cell and may deviate the cells from its normal physiological functions.[13] Taking into the account of multifactorial character of oxidative stress; involved in many pathological states, al the synthesized 1,3,4,5-tetrasubstituted pyrazole derivatives (**4a-l**) were evaluated for their scavenging activity against a variety of reactive oxygen and nitrogen radicals such as 2,2-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), superoxide (SOR) and peroxide (H_2O_2).[14] Free radical scavenging activity was estimated in terms of percent inhibition and results are depicted in Table S2 (Supplemental Materials). Most of the compounds displayed good to excellent scavenging activity against DPPH, NO, SOR and H_2O_2 radicals compared to the ascorbic acid as a reference standard (Figure S1, Supplemental Materials). Compounds except **4b, 4f** and **4j**, all other compounds exhibited excellent DPPH radical scavenging activity (71.59–44.18%) compared to the ascorbic acid (44.18%). Compounds except **4c** and **4k**, all other compounds displayed excellent NO radical scavenging activity (85.71–48.57%) compared to ascorbic acid (42.63%). Compounds **4j, 4a, 4f** and **4l** were found to be excellent SOR radical scavengers (90.24–74.50%) compared to ascorbic acid (74.07%), however rest of the compounds were moderate SOR scavengers (66.66–15.68%). Compounds **4b, 4f, 4j, 4l, 4c** and **4i** were found to possess significant H_2O_2 scavenging activity (69.72–47.19) as compared to the ascorbic acid (47.17%).



4a = $R_1 = R_2 = R_3 = H$
4b = $R_1 = R_3 = H$, $R_2 = CH_3$
4c = R_1 = Br, $R_2 = R_3 = H$
4d = $R_1 = OCH_3$, $R_2 = R_3 = H$
4e = $R_1 = R_2 = H$, $R_3 = CH_3$
4f = $R_1 = H$, R2 = $R_3 = CH_3$
4g = R_1 = Br, $R_2 = R_3 = H$
4h = $R_1 = OCH_3$, $R_2 = H$, $R_3 = CH_3$
4i = $R_1 = R_2 = H$, R_3 = CN
4j = $R_1 = H$, $R_2 = CH_3$, R_3 = CN
4k = R_1 = Br, $R_2 = H$, R_3 = CN
4l = $R_1 = OCH_3$, $R_2 = H$, R_3 = CN

Scheme 1. Chemo- and regioselective cyclization for the synthesis of 1,3,4,5-tetrasubstituted pyrazole derivatives. **Reagents and conditions:** i) Sodium *tert*-butoxide, CS_2, Dimethyl sulfate, THF; ii) K_2CO_3, Ethanol, Reflux, 3h.

However, all other compounds were moderate H_2O_2 radical scavengers (42.57–17.32%).

Experimental

General methods

All the chemicals and reagents used in experiment were of analytical grade purchased from Sigma Aldrich, Germany and purified if required by standard technique. All the reactions were supervised by thin–layer Chromatography (TLC), which was performed on aluminum sheet pre-coated with silica gel, kiesel gel 60 F_{254} (Merck) and the spots were visualized under UV lamp (254 nm). Melting point of compounds was determined with digital thermometer and was uncorrected. The IR spectra were performed on Infrared FT-IR Spectrometer, Nicolet iS10; Thermo Electron Scientific, USA and values were represented in cm^{-1}. The mass spectra were confirmed on Shimadzu LCMS-2010 EV. ^1H NMR spectra were run on 400 MHz FT-NMR, Bruker AVIII, Switzerland, using $CDCl_3$/DMSO-d_6 solvent and chemical shift values recorded in parts per million on δ scale. Coupling constants (J) are referred in Hertz (Hz). The Supplemental Materials contains sample ^1H NMR and HRMS spectra for products 4 (Figures S2–S21).

General procedure for the preparation of 5-(1-methyl-1H-indol-3-yl)-3-(methylthio)-1-phenyl-1H-pyrazole-4-carbonitrile derivatives (4):

A mixture of substituted 2-(1-methyl-1H-indole-3-carbonyl)-3,3-bis(methylthio)acrylonitrile **2** (302 mg, 1.0 mmol) and substituted phenyl hydrazine hydrochloride **2** (144 mg, 1 mmole) in ethanol (10 ml) in the presence of catalytic amount of anhydrous K_2CO_3 was refluxed for 3 h. The reaction mixture was cooled and poured in ice cold water. The faint brownish solid obtained was filtered, washed with water and recrystallized from ethanol to obtain pure compound **4** (Yield: 86%).

Spectral data of synthesized compounds

5-(1-methyl-1H-indol-3-yl)-3-(methylthio)-1-phenyl-1H-pyrazole-4-carbonitrile (4a): Light brown solid; Yield: 86%; M.P.: 164-166 °C; IR (KBr cm^{-1}): 2930 (CH), 2219 (CN), 1614 (C = C), 1593, 1570; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.44-7.40 (m, 2H), 7.39-7.53 (m, 1H), 7.34-7.32 (m, 1H), 7.31-7.28 (m, 3H), 7.27-7.21 (m, 1H), 6.99-6.96 (m, 2H), 3.86 (s, 3H, NCH_3), 2.72 (s, 3H, SCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 150.10, 142.33, 136.13, 135.10, 130.00, 129.10, 128.12, 127.19, 124.17, 121.76, 120.32, 119.24, 117.01(CN), 110.13, 105.02, 97.66, 32.12(N-CH_3), 13.11(S-CH_3); HRMS m/z calcd for $C_{20}H_{16}N_4S$ 344.1096, found 345.1162 (M + H).

5-(1,2-dimethyl-1H-indol-3-yl)-3-(methylthio)-1-phenyl-1H-pyrazole-4-carbonitrile (4b): Light brown solid; Yield: 83%; M.P.: 170-172 °C; IR (KBr cm^{-1}): 2972 (CH), 2224 (CN), 1616 (C = C), 1593, 1576; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.73 (d, J = 8.0 Hz, 1H), 7.62-7.58 (m, 4H), 7.50 (d, J= 6.8 Hz, 1H), 7.45-7.42 (m, 2H), 7.27 (d, J= 6.8 Hz, 1H), 3.84 (s, 3H, NCH_3), 2.96 (s, 3H), 2.72 (s, 3H, SCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 151.21, 142.41, 139.41, 138.23, 136.00, 130.11, 128.91, 128.19, 126.65, 125.11, 121.50, 120.24, 115.60(CN), 109.33, 105.08, 97.66, 31.89(N-CH_3), 14.17(S-CH_3), 11.03(Ar-CH_3); HRMS m/z calcd for $C_{21}H_{18}N_4S$ 358.1252, found 359.2001 (M + H).

5-(5-bromo-1-methyl-1H-indol-3-yl)-3-(methylthio)-1-phenyl-1H-pyrazole-4-carbonitrile (4c): Light brown solid; Yield: 83%; M.P.: 176-178 °C; IR (KBr cm^{-1}): 2934 (CH), 2227(CN), 1603 (C = C), 1583; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.70 (s, 1H), 7.64 (m, 3H), 7.55 (d, J= 8.0 Hz, 2H), 7.40-7.32 (m, 3H), 3.87 (s, 3H, NCH_3), 2.68 (s, 3H, SCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 150.33, 139.65, 137.45, 133.23, 130.03, 129.31, 128.19, 126.05, 125.91, 124.00, 123.94, 122.50, 115.21 (CN), 110.33, 105.24, 96.07, 31.99 (N-CH_3), 13.92 (S-CH_3); HRMS m/z calcd for $C_{20}H_{15}{}^{81}BrN_4S$ 424.0180, found 425.0101 (M + H) (^{81}Br).

5-(5-methoxy-1-methyl-1H-indol-3-yl)-3-(methylthio)-1-phenyl-1H-pyrazole-4-carbonitrile (4d): Light brown solid; Yield: 86%; M.P.: 178-180 °C; IR (KBr cm^{-1}): 2994 (CH), 2216 (CN), 1621 (C = C), 1595, 1565; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.47-7.42 (m, 2H), 7.40 (s, 1H), 7.35-7.28 (m, 3H), 7.23 (d, J= 8.8 Hz, 1H), 6.85 (dd, J= 8.8 Hz, 2.4 Hz, 1H), 6.29 (d, J= 2.4 Hz, 1H), 3.84 (s, 3H), 3.50 (s, 3H), 2.72 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 154.21, 142.43, 139.22, 136.23, 130.05, 129.21, 129.00, 128.05, 125.21, 124.90, 115.91(CN), 113.33, 109.23, 105.92, 103.42, 99.07, 55.43(OCH_3), 32.09(N-CH_3), 12.92(S-CH_3); HRMS m/z calcd for $C_{21}H_{18}N_4OS$ 374.1201, found 375.1268 (M + H).

5-(1-methyl-1H-indol-3-yl)-3-(methylthio)-1-p-tolyl-1H-pyrazole-4-carbonitrile (4e): Light brown solid; Yield: 84%; M.P.: 184-186 °C; IR (KBr cm^{-1}): 2932 (CH), 2216 (CN), 1612 (C = C), 1562; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.35 (d, J= 8.0 Hz, 2H), 7.31-7.25 (m, 2H), 7.23 (d, J= 6.8 Hz, 1H), 7.09 (d, J= 8.4 Hz, 2H), 7.05-6.97 (m, 2H), 3.86 (s, 3H), 2.70 (s, 3H), 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 151.67, 144.66, 138.11, 137.07, 136.93, 130.10, 125.04, 124.19, 122.67, 120.76, 120.24, 114.24(CN), 109.71, 101.53, 93.34, 33.32(N-CH_3), 21.10(Ar-CH_3), 14.83(S-CH_3); HRMS m/z calcd for $C_{21}H_{18}N_4S$ 358.1252, found 359.1307 (M + H).

5-(1,2-dimethyl-1H-indol-3-yl)-3-(methylthio)-1-p-tolyl-1H-pyrazole-4-carbonitrile (4f): Light brown solid; Yield: 87%; M.P.: 180-182 °C; IR (KBr cm^{-1}): 2939 (CH), 2222 (CN), 1612 (C = C), 1581, 1560; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.77 (d, J= 8.0 Hz, 1H), 7.64-7.57 (m, 3H), 7.48-7.42 (m, 3H), 7.27 (d, J= 6.6 Hz, 1H), 3.86 (s, 3H, NCH_3), 2.99 (s, 3H), 2.70 (s, 3H, SCH_3), 2.50 (s, 3H, Ar-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 150.33, 140.43, 138.10, 135.07, 136.73, 130.00, 128.04, 126.21, 125.00, 121.46, 121.01, 119.04, 115.01(CN), 109.31, 103.22, 97.22, 31.12(N-CH_3), 21.34(Ar-CH_3), 13.93(S-CH_3), 11.02 (Ar-CH_3); HRMS m/z calcd for $C_{22}H_{20}N_4S$ 372.1409, found 371.2001 (M-H).

5-(5-bromo-1-methyl-1H-indol-3-yl)-3-(methylthio)-1-p-tolyl-1H-pyrazole-4-carbonitrile (4g): Light brown solid; Yield: 88%; M.P.: 176-178 °C; IR (KBr cm^{-1}): 2942 (CH), 2210 (CN), 1605 (C = C), 1585, 1559; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.84 (s, 1H), 7.65 (d, J= 6.8 Hz, 1H), 7.51 (d, J= 8.0 Hz, 2H), 7.40 (d, J= 8.0 Hz, 2H), 7.29 (m, 2H), 3.89 (s, 3H, NCH_3), 2.73 (s, 3H, SCH_3), 2.48 (s, 3H,Ar-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 149.03, 140.11, 136.10, 135.07, 130.73, 130.00, 129.21, 126.31, 126.00, 125.21, 123.51, 121.04, 114.81(CN), 110.31, 109.12, 96.32, 32.03(N-CH_3), 21.23(Ar-CH_3), 12.93(S-CH_3); HRMS m/z calcd for $C_{21}H_{17}{}^{81}BrN_4S$ 438.0337, found 439.0307 (M + H) (^{81}Br).

5-(5-methoxy-1-methyl-1H-indol-3-yl)-3-(methylthio)-1-p-tolyl-1H-pyrazole-4-carbonitrile (4h): Light brown solid; Yield: 86%; M.P.: 178-180 °C; IR (KBr cm^{-1}): 2935 (CH), 2221 (CN), 1620 (C = C), 1560; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.38 (s, 1H), 7.33 (dd, J= 6.4 Hz, 2.0 Hz, 2H), 7.21 (d, J= 8.8 Hz, 1H), 7.12 (d, J= 8.0 Hz, 2H), 6.85 (dd, J= 8.8 Hz, 2.4 Hz, 1H), 6.34 (d, J = 2.4 Hz, 1H), 3.83 (s, 3H), 3.52 (s, 3H), 2.71 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.73, 151.75, 144.93, 138.10, 137.38, 132.08, 130.39, 129.71, 125.21, 124.20, 114.39(CN), 113.68, 110.58, 101.20, 101.08, 92.89, 55.34(OCH_3), 33.50(N-CH_3), 21.07(Ar-CH_3), 14.82(S-CH_3); HRMS m/z calcd for $C_{22}H_{20}N_4OS$ 388.1358, found 389.1410 (M + H).

1-(4-cyanophenyl)-5-(1-methyl-1H-indol-3-yl)-3-(methylthio)-1H-pyrazole-4-carbonitrile (4i): Light brown solid; Yield: 84%; M.P.: 258-260 °C; IR (KBr cm^{-1}): 2927 (CH), 2221 (CN), 1605 (C = C), 1584, 1560; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.60-7.55 (m, 2H), 7.49 (s, 1H), 7.40

(d, J= 8.4 Hz, 2H), 7.39-7.25 (m, 1H), 7.01-6.97 (m, 1H), 6.83 (d, J= 8.0 Hz, 2H), 3.92 (s, 3H), 2.72 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.22, 145.20, 142.76, 137.17, 133.04, 130.22, 124.70, 124.02, 123.17, 121.26, 119.69, 117.92(CN), 113.60(CN), 111.19, 110.17, 100.81, 94.49, 33.49 N-CH_3), 14.41(S-CH_3); HRMS m/z calcd for $C_{21}H_{15}N_5S$ 369.1048, found 370.1104 (M + H).

1-(4-cyanophenyl)-5-(1,2-dimethyl-1H-indol-3-yl)-3-(methylthio)-1H-pyrazole-4-carbonitrile (4j): Light brown solid; Yield: 82%; M.P.: 158-160 °C; IR (KBr cm^{-1}): 2937 (CH), 2225 (CN), 1609 (C = C), 1580, 1567; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.87 (d, J= 8.0 Hz, 2H), 7.70 (d, J= 8.4 Hz, 1H), 7.62 (d, J= 8.0 Hz, 2H), 7.50-743 (m, 2H), 7.21 (d, J= 6.4 Hz, 1H), 3.85 (s, 3H, NCH_3), 3.01 (s, 3H), 2.71 (s, 3H, SCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 150.12, 143.44, 142.21, 137.23, 135.04, 129.22, 126.10, 121.80, 121.17, 119.69, 118.43(CN), 114.53(CN), 112.09, 110.07, 105.12, 99.99, 31.12(N-CH_3), 13.31(S-CH_3), 11.09(Ar-CH_3); HRMS m/z calcd for $C_{22}H_{17}N_5S$ 383.1205, found 384.1704 (M + H).

5-(5-bromo-1-methyl-1H-indol-3-yl)-1-(4-cyanophenyl)-3-(methylthio)-1H-pyrazole-4-carbonitrile (4k): Light brown solid; Yield: 87%; M.P.: 240-242 °C; IR (KBr cm^{-1}):

2941 (CH), 2220 (CN), 1602 (C = C), 1583, 1560; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.96 (s, 1H), 7.89 (d, J= 8.0 Hz, 2H), 7.64-7.60 (m, 3H), 7.27-7.20 (m, 2H), 3.84 (s, 3H, NCH_3), 2.72 (s, 3H, SCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 149.43, 142.32, 140.21, 134.23, 131.13, 126.22, 126.01, 125.80, 122.17, 121.13, 117.63(CN), 115.13(CN), 113.01, 111.07, 105.92, 103.92, 95.89, 32.22(N-CH_3), 12.51(S-CH_3); HRMS m/z calcd for $C_{21}H_{14}{}^{81}BrN_5S$ 449.0133, found 450.0204 (M + H) (^{81}Br).

1-(4-cyanophenyl)-5-(5-methoxy-1-methyl-1H-indol-3-yl)-3-(methylthio)-1H-pyrazole-4-carbonitrile (4l): Light brown solid; Yield: 88%; M.P.: 256-258 °C; IR (KBr cm^{-1}): 2929 (CH), 2226 (CN), 2217 (CN), 1621 (C = C), 1605, 1583; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.61-7.57 (m, 4H), 7.44 (s, 1H), 7.25 (s, 1H), 6.90 (d, J= 6.8 Hz, 1H), 6.24 (d, J= 6.8 Hz, 1H), 3.88 (s, 3H), 3.52 (s, 3H), 2.72 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.10, 144.11, 143.11, 138.05, 129.92, 128.10, 126.90, 117.93(CN), 114.13(CN), 112.59, 111.67, 111.02, 109.34, 107.23, 105.01, 101.34, 96.89, 55.78(OCH_3), 32.02(N-CH_3), 12.71(S-CH_3); HRMS m/z calcd for $C_{22}H_{17}N_5OS$ 399.1154, found 398.1599 (M-H).

Conclusion

In conclusion, ketene dithioacetal mediated chemo- and regioselective synthesis of novel 1,3,4,5-tetrasubstituted pyrazole derivatives (**4a-l**) was achieved and structural investigations of regioisomers was accomplished by using 2D NMR (NOESY). All the synthesized compounds were *in vitro* evaluated for their anti-inflammatory, antioxidant and cytotoxic potential against breast carcinoma (MCF-7). Among the compounds under investigation, 5-(5-Bromo-1-methyl-1*H*-indol-3-yl)-1-(4-cyano-phenyl)-3-methylsulfanyl-1*H*-pyrazole-4-carbonitrile (**4k**) exhibited significant antitumor activities. The introduction of electron withdrawing group at *p*-position of phenyl ring of aryl pyrazole leads to increase in activity. As the structures of synthesized compounds are analogues to coxib family, most of the compounds found to possess excellent anti-inflammatory and antioxidant (DPPH, NO, SOR and H_2O_2) potential. The compound 5-(1,2-Dimethyl-1H-indol-3-yl)-3-methylsulfanyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**4e**) exhibited excellent anti-inflammatory activity compared to diclofenac sodium. These results suggest the cogent use of these compounds for the design and development of novel anti-cancer and anti-inflammatory agents.

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