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Synthesis of *E*- and *Z*-Pyrazolylacrylonitriles and their Evaluation as Novel Antioxidants

Virinder S. Parmar,[a,*] Ajay Kumar,[a] Ashok K. Prasad,[a] Sanjay K. Singh,[a] Naresh Kumar,[a] Shubhasish Mukherjee,[a] Hanumantharao G. Raj,[b] Sanjay Goel,[b] William Errington[c] and Mohindar S. Puar[d]

[a]*Department of Chemistry, University of Delhi, Delhi-110 007, India*
[b]*Department of Biochemistry, VP Chest Institute, University of Delhi, Delhi-110 007, India*
[c]*Department of Chemistry, University of Warwick, Coventry CV4 7AL, England*
[d]*Structural Chemistry Division, Schering Plough Research Institute, Kenilworth, NJ 07033-0539, USA*

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Abstract—A facile synthesis of (*Z*)- and (*E*)-2-(5-arylpyrazol-3-yl)-3-(pyrrol-2-yl)acrylonitriles and (*Z*)-2-(1,3-diarylpyrazol-5-yl)-3-(pyrrol-2-yl)acrylonitriles, and isomerisation of (*Z*)-2-(5-arylpyrazolyl)acrylonitriles to (*E*)-2-(5-arylpyrazolyl)acrylonitriles under basic conditions have been reported. (*Z*)-2-(1,3-Diarylpyrazolyl)acrylonitriles did not undergo isomerisation under the similar conditions. New compounds were identified on the basis of their spectral data (^{1}H-, ^{13}C-, ^{1}H-^{1}H COSY, NOESY, NOE, HMQC NMR, IR, UV and EI mass). The structures of one acrylonitrile and five of their precursor 6-arylpyran-2-ones and cyanomethylpyrazoles were confirmed by X-ray crystallographic studies. Effects of pyrazolylacrylonitriles and their precursors on rat liver-microsomal lipid peroxidation were evaluated in vitro with a view to establish structure–activity relationship and to identify a lead compound.

Introduction

Pyrazoles and their derivatives are widely used as medicines, e.g. as antipyretic,[1,2] antiinflammatory,[3] gastric secretion stimulatory,[4] antidepressant,[5] against rheumatoid arthritis,[6,7] antihypercholesterolemic agents,[8] antibacterial agents,[9] anticonvulsant agents,[10] antifilarial agents,[11] etc. Along with the medicinal applications, this class of compounds are also useful as agrochemicals, e.g. as herbicides,[12,13] fungicides,[14] pesticides[15] and insecticides,[15] and as dyestuffs,[16–18] in sunscreen materials[19] and as analytical reagents.[20] Very few pyrazole derivatives are naturally occurring, may be due to the difficulty of living organisms to construct the $N-N$ bond. Owing to the widespread applications, synthesis and biological activity evaluation of pyrazoles and their derivatives have been a subject of intensive investigations as revealed by enormous literature covering the subject.

Considerable progress has been made in recent years in relating ageing to oxidation in biological cells. The reactive oxygen species (ROS), cause of oxidation in biological cells, are basically involved in detoxification of invading organisms and chemicals, but stray ROS also initiate lipid peroxidation in healthy cells leading to diverse pathologies such as Alzheimer's disease, atherosclerosis, diabetes, Parkinson's disease, etc.[21] Thus, reduction of the rate of these life-limiting metabolic processes by use of chemicals[22–24] has been a subject of current research. In recent years, we have reported the synthesis of several new pyrazoles,[25] pyrazolylcoumarins[26] and isoxazoles,[27] and studied their biological activities.[28] In continuation, we wish to report herein the synthesis of *Z*- and *E*-pyrazolylacrylonitriles and their effects on NADPH-catalysed liver-microsomal lipid peroxidation.

Results and Discussion

(*Z*)-2-(5-Arylpyrazol-3-yl)-3-(pyrrol-2-yl)acrylonitriles **4a–4h** were prepared by coupling of pyrrole-2-carboxaldehyde with the corresponding 5-aryl-3-cyanomethylpyrazoles **3a–3h**[27,29] in quantitative yields according to the procedure outlined in Scheme 1 and described in detail in the Experimental. Arylpyrazoles **3a–3h**, in turn were prepared in four steps starting with the condensation of ethyl cyanoacetate with carbon disulfide in sodium ethoxide, methylation of

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* Corresponding author. Tel.: +91-11-725-6555/7206; fax: +91-11-725 7206; e-mail: vparmar.duchem@axcess.net.in

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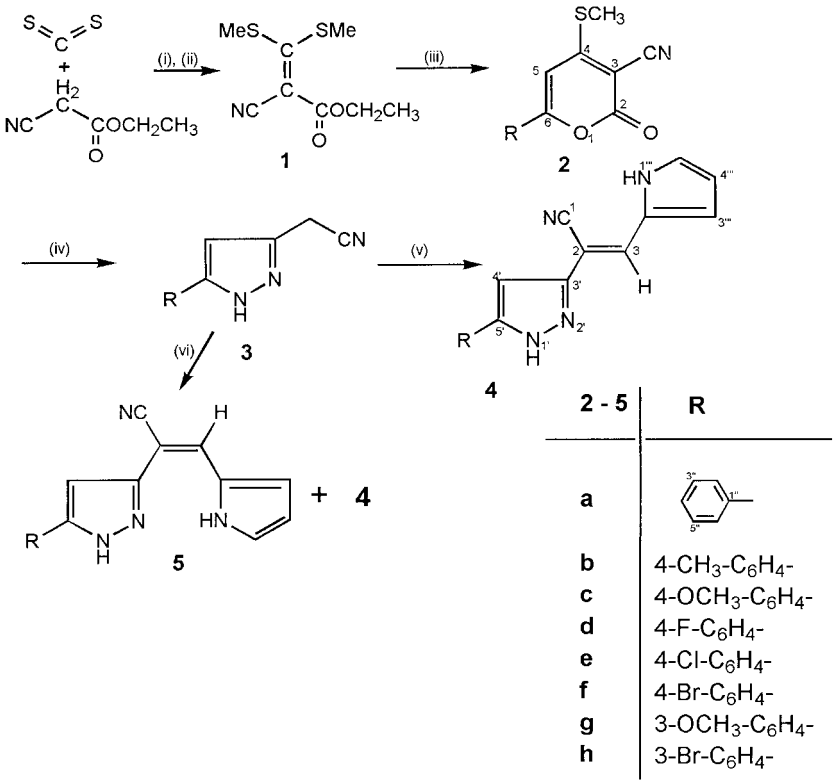
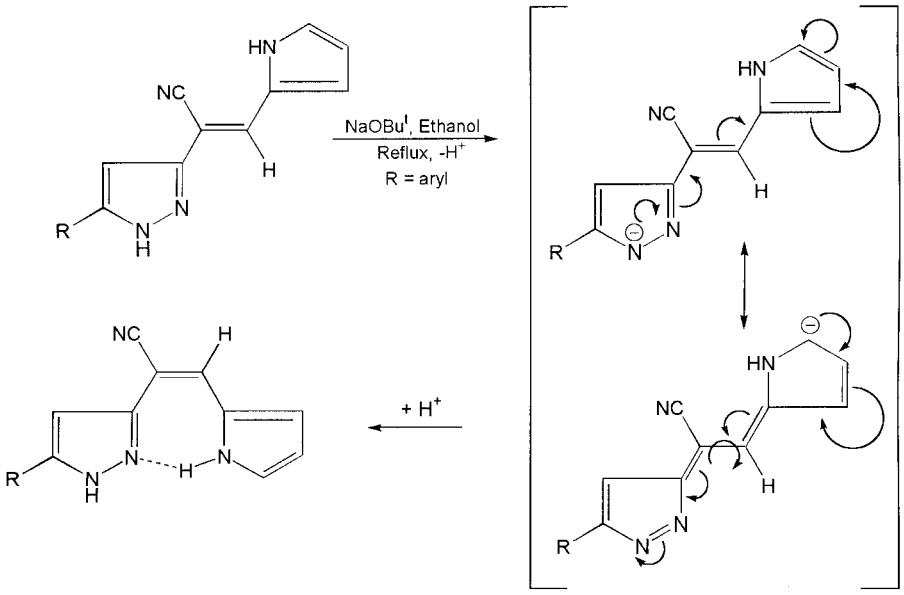


Scheme 1. *Reagents and conditions:* (i) EtOH, Na, 0°C, stirring 5–6 h; (ii) CH_3I, MeOH, 0°C, stirring 2–3 h; (iii) DMF, appropriately substituted acetophenone, KOH, 30°C, stirring 6 h; (iv) MeOH, NH_2NH_2, refluxing 5–6 h; (v) EtOH, $NaOBu^t$, pyrrole-2-carboxaldehyde, stirring 4–5 h; (vi) EtOH, $NaOBu^t$, pyrrole-2-carboxaldehyde, refluxing 20–25 h.

the disodio salt, condensation of resulted ethyl 2-cyano-3,3-*bis*-methylthioacrylate (**1**) with corresponding acetophenones affording pyranones **2a–2h**, followed by reaction with hydrazine in methanol[27,30] (Scheme 1). (*Z*)-Pyrazolylacrylonitriles were well characterised by analysis of their spectral data. The ^{1}H NMR spectra of **4a** and **4b** exhibited resonances for all the protons including the two D_2O-exchangeable broad singlets at around δ 11.50 and 13.40 due to two acidic NH. The proton NMR spectral assignments for compounds **4b** and **4e** were confirmed by ^{1}H-^{1}H COSY NMR. Thus, peaks resonating at δ 6.33 and 7.15 in the ^{1}H NMR spectrum of **4b** assigned to C-4‴H, and C-3‴H and C-5‴H of pyrrole moiety, respectively, showed expected cross peaks in its ^{1}H-^{1}H COSY NMR spectrum. Similarly, peaks at δ 7.29 and 7.70 in the ^{1}H NMR of **4b**, assigned to C-3″H and C-5″H, and C-2″H and 6″H, respectively were confirmed by expected cross peaks in its ^{1}H-^{1}H COSY NMR spectrum. The assignment of ^{13}C NMR spectral values in case of (*Z*)-pyrazolylacrylonitrile **4a** was done on the basis of selective INEPT experiment and carbons of compounds **4b–4h** were assigned accordingly (Table 4).

The coupling reaction of pyrrole-2-carboxaldehyde with 3-cyanomethylpyrazoles **3a–3h** in the presence of $NaOBu^t$ is highly temperature dependent. Thus, the reaction carried out at room temperature (25–30°C) afforded exclusively the kinetically controlled (*Z*)-isomers, i.e. the compounds **4a–4h** in 88 to 95% yields in 4–5 h, whereas the same reaction mixture when refluxed at 90–100°C for 20–24 h afforded the thermodynamically stable *E*-isomers, i.e. (*E*)-2-(5-arylpyrazol-3-yl)-3-(pyrrol-2-yl) acrylonitriles **5a–5h** in 52 to 60% yields along with the corresponding (*Z*)-isomers **4a–4h** as minor products (25–30% yields); the composition of equilibrium mixtures of (*Z*)- and (*E*)-pyrazolylacrylonitriles thus formed did not change on further continuation of the reaction. An attempt to isomerise (*Z*)-acrylonitriles by refluxing their solution in methanol failed, whereas addition of $NaOBu^t$ in the reaction mixture and continuation of refluxing allowed the isomerisation of (*Z*)-pyrazolylacrylonitriles to the corresponding (*E*)-isomers. The reaction condition of isomerisation indicates that the base deprotonates the more acidic NH proton of pyrazole ring of acrylonitriles leading to different resonance structures and to single bond character across the double bond; rotation through corresponding single bond results in isomerisation (Scheme 2). The other less plausible explanation of isomerisation of (*Z*)-pyrazolylacrylonitriles to the corresponding *E*-isomers is via deprotonation of the pyrrolic nitrogen or reversible addition of ethoxide ion to acrylonitrile-double bond.

The structures of (*Z*)- and (*E*)-acrylonitriles **4a–4h** and **5a–5h** were unambiguously ascertained on the basis of their TLC behaviour, UV spectra and NMR experiments. The close inspection of the models of (*Z*)- and (*E*)-acrylonitriles indicated that the pyrazole and pyrrole moieties are in *trans* position in *Z*-isomer, which



Scheme 2. Proposed mechanism of isomerisation of (*Z*)-pyrazolylacrylonitriles to (*E*)-pyrazolylacrylonitriles.

reduces steric hindrance between two bulky groups hence it is a favoured product of reaction at room temperature. In basic conditions, the double bond in the acrylonitrile moiety develops a single bond character and at high temperature, when the molecule gets sufficient energy to cross over the energy barrier of steric hindrance, (*E*)-isomers are formed and stabilised by intramolecular interaction between NH of pyrrole moiety and nitrogen of pyrazole moiety as both the moieties come closer to each other in space (Scheme 2). This interaction is revealed by TLC behaviour of isomers as well, e.g. R_f values of (*Z*)- and (*E*)-isomers (ethyl acetate:benzene, 1:9) are between 0.40–0.42 and between 0.60–0.64, respectively, indicating that the *E*-isomer is less polar than *Z*-isomer due to involvement of NH proton in hydrogen bonding intramolecularly. In their UV spectrum, the -CH=C(CN)- chromophore of *E*-isomer absorbs at lower wavelength (353–356 nM) than that of the *Z*-isomer (360–363 nM) which is indicative of the fact that in *E*-isomers, the two bulky groups are *cis* to each other and because of the steric hindrance of the two bulky groups, the planarity necessary for extension of conjugation is disrupted leading to the hypsochromic shift (Table 1). This electronic effect is also observed in the resonance position of C-3H in the ^{1}H NMR spectra and resonance position of C-2 in the ^{13}C NMR spectra of (*E*)- and (*Z*)-acrylonitriles. Smooth extension of conjugation through C-2 and C-3 double bond increases *s*-character and hence electronegativity of these two sp^2-hybridised carbons resulting in the lowering of chemical shift values of C-3 protons in *Z*-isomers, C-3H in *Z*-isomers appears between δ 7.73–7.81, while in *E*-isomers, it is exhibited at between δ 7.31–7.39 (Table 1). Again, in the ^{1}H NMR spectra of acrylonitrites, the pyrrolic NH of *Z*-isomers resonated at lower δ values (δ11.47–11.60) than the *E*-isomers (δ13.90–14.10) indicating that the proton of pyrrolic NH is involved in H-bonding type of interaction with the nitrogen of pyrazole in the case of

Table 1. Comparison of salient features in the NMR and the UV spectral data of (*Z*)- and (*E*)-arylpyrazolylacrylonitriles **4a–4h** and **5a–5h**

Compounds	N-1′ H(δ)	N-1‴ H(δ)	C-3H (δ)	C-2 (δ)	λ_{max} (−CH=C(CN)−)
Z-isomers					
4a	13.50	11.55	7.81	112.0	362
4b	13.30	11.60	7.78	111.8	362
4c	13.20	11.47	7.74	111.9	362
4d	13.40	11.49	7.74	112.0	362
4e	13.51	11.50	7.75	111.9	363
4f	13.50	11.52	7.73	112.1	362
4g	13.43	11.48	7.75	112.2	362
4h	13.50	11.51	7.73	112.2	360
E-isomers					
5a	13.36	14.02	7.39	121.4	353
5b	13.32	13.90	7.31	119.5	356
5c	13.34	13.90	7.32	121.5	356
5d	13.24	13.90	7.34	121.5	353
5e	13.20	14.10	7.34	121.2	356
5f	13.20	13.90	7.35	121.2	356
5g	13.27	13.90	7.34	121.2	356
5h	13.10	13.90	7.35	121.1	356

E-isomers. This interaction is not possible in *Z*-isomers because pyrazole and pyrrole moieties are *trans* to each other. The structures of (*Z*)- and (*E*)-acrylonitriles were also supported by NOESY and NOE experiments. Thus, the NH groups of pyrrole and pyrazole moieties gave a cross peak in the NOESY spectrum of (*E*)-acrylonitriles **5a**, **5b** and **5e** indicating that they are close to each other in space (*cis* geometry). No NH−NH cross peak was observed in the NOESY spectrum of (*Z*)-acrylonitrile **4b** indicating that they are too far away in space (*trans* geometry) to exhibit the dipolar coupling. All other expected cross peaks were observed in the NOESY spectra of the acrylonitriles **4b**, **5a**, **5b** and **5e**. Again, irradiation of C-4′H in compound **4b** gave 4.0% and 9.1% NOE on C-3H and, C-2″H and C-6″H,

respectively indicating that these protons are closer to each other in space. Similarly, irradiation of C-3H gave 5.2% NOE on C-4′H and 1.4% NOE on C-3‴H and C-5‴H (Table 2). These results clearly indicate that C-4′H and C-3H are situated on the same side of the double bond in compound **4b**. Contrary to this, irradiation of C-4′H or C-3H in compound **5e** (*E*-isomer) showed no NOE on each other (Table 2) indicating that they are on the opposite side of the double bond. The ^{1}H NMR spectral peak assignments of (*E*)-acrylonitriles **5b** and **5e** were confirmed by presence of expected cross peaks in their ^{1}H-^{1}H COSY NMR spectra. The ^{13}C NMR spectral signals of (*E*)-acrylonitrile **5a** were carefully assigned by ^{1}H-^{13}C correlation (HMQC) experiment (Table 3) and selective INEPT experiment. The assignments of carbon signals in the ^{13}C NMR spectra of the (*E*)-acrylonitriles **5b–5h** were made accordingly (Table 5).

Table 2. NOE results of compounds **4b**, **5e** and **7d**

Compounds	Proton irradiated	% Enhancement						
		C-2″ H	C-6″ H	C-3H	C-3‴ H	C-4′ H	C-5‴ H	N-1‴ H
4b	C-4′H	9.1	9.1	4.0	—	—	—	—
	C-3H	—	—	—	1.4	5.2	1.4	—
5e	C-4′H	8.5	8.5	—	—	—	—	—
	C-3H	—	—	—	7.8	—	—	2.1
	N-1‴H	25	25	—	—	—	—	—
7d	C-4′H	7.4	7.4	2.9	—	—	—	—

Table 3. ^{1}H and ^{13}C correlation (HMQC) for compound **5a**

Proton irradiated	Carbon observed
C-4‴H (s, 6.38)	C-4‴(110.0)
C-3‴H (6.81)	C-3‴(119.4)
C-4′H (6.96)	C-4′(102.3), C-3′(146.0), C-1″(127.9) and C-2 (121.9)
C-5‴H (7.30)	C-5‴(124.4)
C-3H (7.39)	C-3 (131.2), C-1 (93.8) and C-2‴(128.1)
C-4″H (7.42)	C-4″(128.8)
C-3″H and C-5″H(7.51)	C-3″ and C-5″ (129.1)
C-2″H and C-6″H(7.90)	C-2″ and C-6″(125.6), and C-5′(143.5)

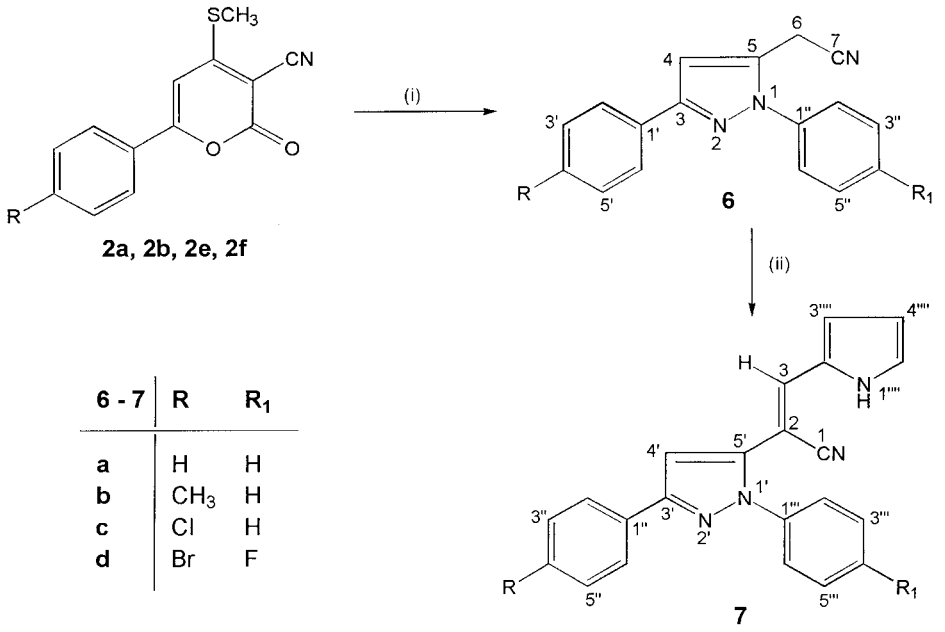
The *Z*- and *E*- structural assignments and proposed driving force for isomerisation of (*Z*)-acrylonitriles to (*E*)-acrylonitriles is supported by the synthesis of (*Z*)-1,3-diarylpyrazolylacrylonitriles **7a–7d** by coupling of pyrrole-2-carboxaldehyde with corresponding 5-cyanomethyl-1,3-diarylpyrazoles **6a–6d** in quantitative yields (Scheme 3). The compounds **6a–6d** were prepared by condensation of appropriate arylhydrazines with the corresponding pyranones **2a**, **2b**, **2e** and **2f** (Scheme 3) in 45 to 52% yields and identified on the basis of their spectral analysis. In the diarylpyrazolylacrylonitriles **7a–7d**, the N-1 of pyrazole ring bears an aryl group and hence pyrrolic NH can not form hydrogen bonding (a driving force for isomerisation). This indicates that the *E*-form will not be an energetically favoured form. The expected result was observed when a solution of the (*Z*)-acrylonitriles **7a–7d** in methanol was refluxed in the presence of NaOBu^t for 48 h without formation of any traces of (*E*)-isomers. This proves that the lowering of energy due to interaction of NH of pyrrole moiety with the nitrogen of pyrazole moiety is a necessary driving force for the isomerisation. The structures of (*Z*)-diarylpyrazolylacrylonitriles **7a–7d** were established on the basis of their spectral analysis. Thus, the ^{1}H NMR spectra of **7a–7d** showed the presence of one D_2O-exchangeable proton as a broad singlet and peaks in the aromatic region due to two aryl groups. The assignments of ^{1}H NMR spectral signals of compound **7d** were confirmed by the presence of expected cross peaks in its ^{1}H-^{1}H COSY NMR spectrum. The *Z*-configuration across the double bond in compound **7d** was proved by NOE experiment. Thus, irradiation of signal at δ 6.80 due to C-4′H gave 2.9% and 7.4% NOE on C-3H and, C-2″H and C-6″H, respectively (Table 2). This proves that the C-4′H and the C-3H are on the same side of the double bond, i.e. geometry across the double bond is *Z(trans)*. The observed NOE between C-3H and C-4′H is also confirmed by the presence of a cross peak in the NOESY spectrum of **7d** due to dipolar coupling of these two protons. The structures of diarylpyrazoles **6a–6d** and (*Z*)-diarylpyrazolylacrylonitriles **7a–7d** were also supported by the presence of all relevant peaks in their ^{13}C NMR spectra (Table 6). All the 5-

Table 4. ^{13}C NMR Chemical-shift values of compounds **4a-4h** (*Z*-isomers)

Carbon No.	4a	4b	4c	4d	4e	4f	4g	4h
C-1	95.5	95.5	93.8	93.6	95.2	95.3	93.8	95.5
C-2	112.0	111.8	111.9	112.0	111.9	112.1	122.2	112.2
C-3	130.9	130.7	131.0	131.3	130.9	131.1	131.4	131.1
C-3′	147.6	147.4	145.8	148.0	147.6	147.6	145.8	147.4
C-4′	99.0	98.5	98.0	99.1	99.4	99.6	99.4	100.0
C-5′	143.6	143.6	143.4	142.6	142.4	143.0	143.5	143.6
C-1″	128.3	127.1	130.7	130.9	127.6	127.1	124.6	127.0
C-2″	125.2	125.1	126.6	127.3	126.9	127.1	117.9	130.9
C-3″	128.9	129.4	114.4	115.8	128.2	131.8	159.8	122.3
C-4″	128.2	137.8	159.3	163.9	132.8	121.3	111.3	124.2
C-5″	128.2	129.4	114.4	115.8	128.2	131.8	130.4	130.9
C-6″	125.2	125.1	126.6	127.3	126.9	127.7	114.9	127.6
C-2‴	127.1	125.1	127.0	127.4	127.0	127.0	127.2	127.6
C-3‴	118.4	118.3	118.3	119.5	118.3	118.2	119.6	118.2
C-4‴	111.1	111.0	111.0	111.1	111.0	111.1	110.6	111.1
C-5‴	123.0	122.9	124.3	124.7	123.1	123.3	124.6	124.2
CH_3/OCH_3	—	20.7	55.4	—	—	—	55.4	—

Table 5. ^{13}C NMR Chemical-shift values of compounds **5a–5h** (*E*-isomers)

Carbon No.	5a	5b	5c	5d	5e	5f	5g	5h
C-1	93.8	93.8	93.9	93.7	93.5	93.5	93.7	93.5
C-2	121.9	119.5	121.5	121.5	121.2	121.2	121.2	121.1
C-3	131.2	131.3	131.3	131.5	131.4	131.9	131.2	131.1
C-3′	146.0	146.0	146.0	146.2	146.1	146.1	145.9	145.9
C-4′	102.3	102.0	101.6	102.5	102.7	102.7	102.5	103.1
C-5′	143.5	143.7	143.6	142.8	142.4	142.4	143.4	142.0
C-1″	127.9	127.9	128.0	128.1	127.7	127.7	124.4	127.7
C-2″	125.6	125.2	127.2	127.9	127.3	127.5	117.7	131.4
C-3″	129.1	129.7	114.6	116.1	129.1	131.9	159.7	122.4
C-4″	128.8	138.6	159.8	163.9	133.3	121.9	110.9	124.5
C-5″	129.1	129.7	114.6	116.4	129.1	131.9	130.1	131.7
C-6″	125.6	125.2	127.2	127.9	127.3	127.5	114.7	130.3
C-2‴	128.1	125.2	127.2	128.1	127.3	127.7	127.8	128.0
C-3‴	119.4	121.4	119.6	119.7	119.4	119.4	119.3	119.4
C-4‴	110.0	111.1	111.1	111.2	111.0	110.9	110.7	110.9
C-5‴	124.4	124.6	124.6	124.8	124.5	124.5	129.3	124.5
CH_3/OCH_3	—	20.8	55.4	—	—	—	55.2	—



6 - 7	R	R_1
a	H	H
b	CH_3	H
c	Cl	H
d	Br	F

Scheme 3. Reagents and conditions: (i) $PhNHNH_2$ HCl/*p*-$FC_6H_4NHNH_2HCl$, MeOH/ pyridine, refluxing 5-6 hrs; (ii) EtOH, $NaOBu^t$, pyrrole-2-carboxaldehyde, refluxing 4-5 hrs.

Table 6. ^{13}C NMR Chemical-shift values of compounds **6a–6d** and **7a–7d**

Carbon No.	6a	6b	6c	6d	Carbon No.	7a	7b	7c	7d
C-3	152.8	152.8	151.5	151.6	C-1	119.0	119.3	119.3	119.0
C-4	105.7	105.6	105.7	105.7	C-2	90.6	91.0	91.0	89.9
C-5	133.2	133.1	133.1	131.6	C-3	136.2	136.0	136.2	136.4
C-6	16.7	16.7	16.7	16.6	C-3′	152.1	152.0	151.5	152.1
C-7	116.1	116.1	116.1	115.8	C-4′	105.9	105.3	105.3	105.2
C-1′	112.8	124.4	124.4	117.4	C-5′	133.1	138.6	139.7	132.3
C-2′	129.2	129.9	129.4	127.7	C-1″	125.6	125.5	125.9	127.8
C-3′	125.7	125.7	127.5	132.3	C-2″	126.3	129.9	129.9	137.3
C-4′	128.8	130.5	134.5	122.9	C-3″	129.2	126.9	127.5	127.7
C-5′	125.7	125.7	127.5	132.3	C-4″	128.8	129.9	134.5	122.8
C-6′	129.2	129.9	129.4	127.7	C-5″	129.2	126.9	127.5	127.7
C-1″	138.1	138.1	134.4	133.6	C-6″	126.3	129.9	129.4	132.3
C-2″	130.1	130.1	130.2	127.9	C-1‴	138.1	138.6	138.9	135.6
C-3″	126.2	126.1	125.7	117.0	C-2‴	129.9	129.9	129.9	128.0
C-4″	129.4	129.3	129.5	165.5	C-3‴	126.0	126.0	126.0	116.7
C-5″	126.2	126.1	125.7	117.0	C-4‴	129.0	129.0	127.5	163.0
C-6″	130.1	130.1	130.2	127.9	C-5‴	126.0	126.0	126.0	116.7
CH_3	—	21.8	—	—	C-6‴	129.9	129.9	129.9	128.0
					C-2⁗	139.4	139.7	139.7	139.1
					C-3⁗	120.6	120.5	120.9	121.1
					C-4⁗	111.6	111.6	111.7	111.8
					C-5⁗	125.8	127.0	127.5	125.9
					CH_3	—	20.5	—	—

arylpyrazolylacrylonitriles **4a–4h** and **5a–5h**, 1,3-diarylpyrazoles **6a–6d** and 1,3-diarylpyrazolylacrylonitriles **7a–7d** have been found to be new compounds.

X-ray crystallography

The structures determined on the basis of spectral analysis of the new pyrazole, 5-cyanomethyl-3-(4-methylphenyl)-1-phenylpyrazole (**6b**), and the new pyrazolylacrylonitrile (*E*)-2-[5-(3-bromophenyl)pyrazol-3-yl]3-(pyrrol-2-yl)acrylonitrile (**5h**) were confirmed by X-ray crystallographic studies. Similarly, the structures of the three lactones, 3-cyano-6-(4-fluorophenyl)-4-methylthio-2*H*-pyran-2-one (**2d**), 3-cyano-6-(3-methoxyphenyl)-4-methylthio-2*H*-pyran-2-one (**2g**) and 6-(3-bromophenyl)-3-cyano-4-methylthio-2*H*-pyran-2-one (**2h**), and a pyrazole, 3-cyanomethyl-5-(4-methylphenyl)pyrazole (**3b**) were finally confirmed by X-ray crystallographic studies. The schematic representation of the molecular structures of the pyran-2-one **2d**, pyrazole **3b** and (*E*)-acrylonitrile **5h** are shown in Figures 1–3, respectively; the structures of **2g**,[31] **2h**,[27] **5h**[32] and **6b**[25] have been reported elsewhere. The crystal structure of (*E*)-acrylonitrile **5h** depicted in Figure 3 clearly exhibits the *E*-geometry across the acrylonitrile double bond and also reveals that in the solid state, an additional tautomer is possible in which the proton at N-1′ has migrated to N-2′ (Fig. 3). Confirmation of the spectroscopically established structures of lactones **2d**, **2g** and **2h**, pyrazoles **3b** and **6b**, and of acrylonitrile **5h** by X-ray crystallography supports the structure deduction of all other acrylonitriles and their precursors.

Antioxidant activity studies

Five pyranones, i.e. **2a**, **2b**, **2d**, **2g** and **2h**, seven 5-arylpyrazoles **3a–3f** and **3h**, three 1,3-diarylpyrazoles **6a–6c** and all twenty acrylonitrites **4–4h**, **5a–5h** and **7a–7d** have been tested for their effect on NADPH-catalysed rat liver-microsomal lipid peroxidation. Svingen et al.[33] have demonstrated that NADPH-dependent lipid peroxidation proceeds through the formation of lipid hydroperoxides, called initiation step followed by propagation step which involves the breakdown of hydroperoxides yielding reactive radicals and oxidised products. We have investigated the effect of above pyranones and pyrazole derivatives at both initiation and propagation steps. NADPH-dependent liver microsomal

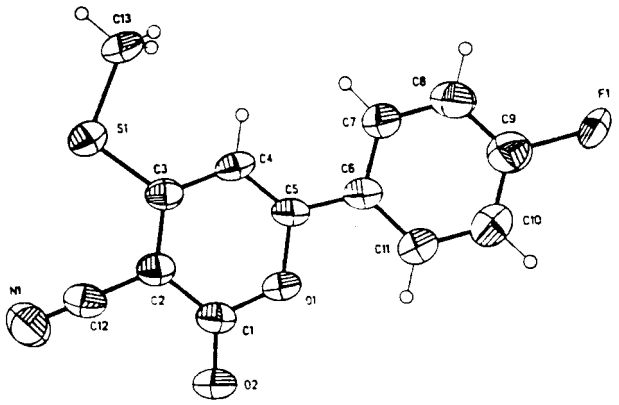


Figure 1. X-ray crystal structure of 3-cyano-6-(4-flourophenyl)-4-methylthio-2*H*-pyran-2-one (**2d**).

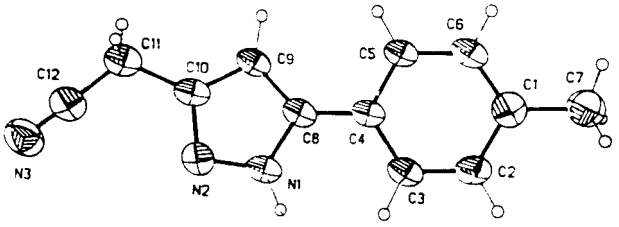


Figure 2. X-ray crystal structure of 3-cyanomethyl-5-(4-methylphenyl)pyrazole (**3b**).

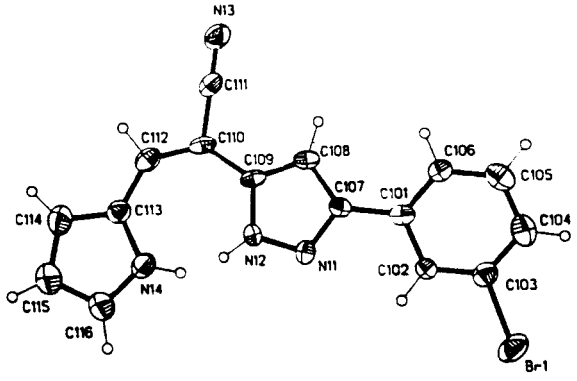


Figure 3. (*E*)-2-[5-(3-Bromophenyl)pyrazol-3-yl]-3-(pyrrol-2-yl)-acrylonitrile (**5h**).

lipid peroxidation was assayed by the method of Ernster and Nordenbrand.[34] All activity testings have been carried out at 100 μM concentrations. The results (Table 7) illustrate that the pyranones **2a**, **2b**, **2d**, **2g** and **2h** do not exhibit significant activity to inhibit the initiation of NADPH-catalysed peroxidation of lipids of rat liver-microsomes. Whereas conversion of pyranones to 3-arylpyrazoles **3a–3f** and **3h** and to 1,3-diarylpyrazoles **6a–6c** increases the activity by 0 to 11 fold in different cases, the maximum increase in activity has been observed in pyrazoles with unsubstituted phenyl ring/rings derived from the corresponding pyranones. The antioxidant activity of 5-arylpyrazoles and 1,3-diarylpyrazoles are comparable. *Z*- and *E*-Pyrazolylacrylonitriles (**4a–4h** and **5a–5h**) showed 3–4 fold increase in their antioxidant activity over the activity of the respective precursor (Table 8). Though diarylpyrazolylacrylonitrile **7c** exhibits a significant increase in the activity over its precursor pyrazole **6c**, activity of compounds **7a** and **7b** remained comparable to their precursor pyrazoles **6a** and **6b**, respectively.

Table 7. Inhibitory effect of pyranones and cyanomethylpyrazoles on lipid peroxidation in rat liver microsomes initiated by NADPH

Compound	% Inhibition of initiation	Compound	% Inhibition of initiation
2a	3	**3d**	27
2b	14	**3e**	33
2d	4	**3f**	10
2g	14	**3h**	18
2h	18	**6a**	33
3a	33	**6b**	33
3b	31	**6c**	26
3c	35		

Table 8. Inhibitory effect of 2-pyrazolyl-3-(pyrrol-2-yl)acrylonitriles on lipid peroxidation in rat liver microsomes initiated by NADPH

Compound	% Inhibition of initiation	Compound	% Inhibition of initiation
4a	53	**4f**	42
5a	73	**5f**	68
4b	69	**4g**	27
5b	95	**5g**	90
4c	52	**4h**	77
5c	67	**5h**	68
4d	20	**7a**	36
5d	62	**7b**	48
4e	42	**7c**	87
5e	69	**7d**	80

Normally the antioxidant property of a compound is attributed to its (a) oxygen radical scavenging ability, (b) ability to inhibit cellular microsomal P-450-linked mixed function oxidation (MFO) reaction and (c) ability to suppress the formation of reactive oxygen species (ROS). Pyrazolylacrylonitriles have neither radical scavenging ability nor can they inhibit MFO (data not shown). But there exists the possibility of pyrazolylacrylonitriles forming a stable mixed ligand complex with ADP and Fe^{2+} (see Experimental) thereby preventing the production of ADP-perferryl radical responsible for ROS formation as we have shown earlier in the case of dioxygenated 4-methylcoumarins.[35–37] *E*-Isomers of acrylonitriles have demonstrated much higher antioxidant activity than *Z*-isomers, possibly because they can form more stable mixed ligand complex with ADP and Fe^{2+} as compared to those formed by the corresponding *Z*-isomers.

Among all lactones and pyrazole derivatives tested for their antioxidant activity, (*E*)-2-[5-(4-methylphenyl) pyrazol-3-yl]-3-(pyrrol-2-yl)acrylonitrile (**5b**) and (*E*)-2-[5-(3-methoxyphenyl)pyrazol-3-yl]-3-(pyrrol-2-yl)acrylonitrile (**5g**) exhibit very high degree of inhibition, i.e. 95 and 90%, respectively of the NADPH-catalysed lipid peroxidation in rat liver microsomes at the initiation stage (Table 8). The IC_{50} values for inhibition of lipid-hydroperoxide formation (Table 9) and activity of inhibition at propagation stage of NADPH-catalysed lipid

Table 9. Antioxidant potential of 2-pyrazolyl-3-(pyrrol-2-yl)acrylonitriles

Compound	[a]IC_{50} (μ moles)	Compound	[a]IC_{50} (μ moles)
4b	9.1	**5e**	12.6
4h	2.1	**5f**	8.7
5a	18.3	**5g**	5.8
5b	8.7	**5h**	12.0
5c	18.1	**7c**	6.6
5d	31.6	**7d**	15.4

[a]IC_{50} values are calculated only for those compounds who have shown more than 60% inhibition at initiation step.

Table 10. Inhibitory effect of 2-pyrazolyl-3-(pyrrol-2- yl)acrylonitriles on linoleic acid hydroperoxide induced lipid peroxidation

Compound	% Inhibition of propagation	Compound	% Inhibition of propagation
4b	72.3	**5e**	10.0
4h	11.2	**5f**	37.1
5a	11.2	**5g**	19.5
5b	30.6	**5h**	26.2
5c	29.8	**7c**	11.1
5d	5.6	**7d**	37.8

peroxidation in rat liver microsomes (Table 10) have been evaluated only for those compounds which have shown more than 60% inhibition of lipid peroxidation at the initiation stage. The minimum IC_{50} value, i.e. 2.1 has been measured for (*Z*)-2-[5-(3-bromophenyl)pyrazol-3-yl] -3-(pyrro-2-yl)acrylonitrile (**4h**) for the inhibition at initiation stage.

Conclusion

A facile synthetic procedure has been developed for the synthesis of a new class of heterocyclic compounds, i.e. (*Z*)- and (*E*)-2-(5-arylpyrazol-3-yl)-3-(pyrrol-2-yl)acrylo nitriles and (*Z*)-2-(1,3-diarylpyrazol-5-yl)-3-(pyrrol-2-yl)acrylonitriles. The mechanism of isomerisation of (*Z*)-2-(5-arylpyrazol-3-yl)-3-(pyrrol-2-yl)acrylonitriles into the corresponding *E*-isomers has been proposed on the basis of investigations carried out. In total twenty four new/novel compounds have been synthesised and subjected to screening of their activity as antioxidants for inhibition of NADPH-catalysed peroxidation of rat liver microsomes which led to the identification of arylpyrazolylacrylonitriles **5b**, **5g** and **4h** as highly potent compounds. Again, this activity evaluation has revealed that methyl- or methoxyphenylpyrazolylacrylonitriles **5b** and **5g** are better antioxidants than unsubstituted, or fluoro- or chloro substituted phenylpyrazolylacrylonitriles. These acrylonitriles may be of significance for the development of a clinical antioxidant agent.

Experimental

Melting points were determined either in a bath or on a Mettler FP62 instrument and are uncorrected. The UV and IR spectra were recorded on Beckmann DU-2 spectrophotometer and Shimadzu model 435 spectrophotometer, respectively. The ^{1}H NMR spectra were recorded either at Perkin–Elmer 200, Bruker AC-250 or XL-400 instruments at 200, 250 or 400 MHz, respectively. The ^{13}C NMR spectra were recorded on same instruments as for ^{1}H NMR spectral recordings either at 50, 62.8 or 100 MHz. TMS has been used as an internal standard for both ^{1}H and ^{13}C NMR spectral recordings. The chemical shift values are on δ scale and the coupling constants (*J*) are in Hz. The ^{1}H-^{1}H COSY, NOE, NOESY and HMQC NMR studies were performed on a XL-400 or GE-400 spectrometer. The EI mass spectra were recorded either on a varian MAT 311A or Jeol AX 505W mass spectrometer at 70 eV. Analytical TLCs were performed on silica gel coated on 5×20 cm glass plates and/or on precoated Merck silica gel 60 F_{254} plates. Solvent systems used for R_f measurements were **A** (ethyl acetate:benzene, 1:9) and **B** (ethyl acetate:benzene, 1:19). The developing agents were alcoholic $FeCl_3$ solution (3%) or iodine vapours. Reactions were monitored on Shimadzu LC-10AS HPLC instrument with SPD-10A UV–vis detector and Shimpack CLC-ODS (4.6×150 mm) reverse phase column. Solvent system used for HPLC was methanol:water mixture (7:3) at the flow rate of 0.75 mL/min and monitored at 254 nM. 6-Aryl-3-cyano-4-thiomethyl-2*H*-pyran-2-ones **2a–2h** were prepared according to the procedure given in our earlier publication[27] in three steps starting with the condensation of carbon disulfide and ethyl cyanoacetate and dimethylation of disodio salt leading to **1**, followed by condensation with corresponding acetophenones.

General method of preparation of 5-aryl-3-cyanomethylpyrazoles 3a–3h. To a solution of 6-aryl-3-cyano-4-methylthio-2*H*-pyran-2-ones (**2a–2h**, 6 mmol) in methanol (30 ml), hydrazine monohydrate (9 mmol) was added and the reaction mixture was refluxed for 5–6 h. The progress of the reaction was monitored on HPLC and/or on TLC. The reaction mixture was concentrated on completion under reduced pressure, poured over crushed ice (200 g) and stirred vigorously until a brownish-yellow solid precipitated out. It was filtered, washed with water (2×25 mL), dried, and column chromatographed over silica gel using ethyl acetate–petroleum ether as eluent to afford **3a–3h**[27] as white solids, which were crystallised from ethyl acetate to white shining needles in 50 to 60% yields.

General method of preparation of (*Z*)-2-(5-arylpyrazol-3-yl)-3-(pyrrol-2-yl)acrylonitriles 4a–4h. To a solution of 5-aryl-3-cyanomethylpyrazoles[27] **3a–3h** (1.5 mmol) in ethanol (20 mL), $NaOBu^t$ (1.5 mmol) was added, and reaction mixture was stirred for 15 min at 25–30°C followed by addition of pyrrole-2-carboxaldehyde (1.5 mmol) in one lot. Stirring was continued for 4–5 h and progress of the reaction was monitored on HPLC and/or on TLC. The reaction mixture was concentrated on completion under reduced pressure and poured over crushed ice (50 g), when a yellow solid precipitated out. It was filtered, washed with water (2×25 mL), dried over P_2O_5 in a vacuum desiccator and

crystallised to afford pure acrylonitriles **4a–4h** in almost quantitative yields.

(*Z*)-2-(5-Phenylpyrazol-3-yl)-3-(pyrrol-2-yl)acrylonitrile (4a). Crystallised from acetone as a yellow powder (351 mg, 90%), mp 209–10 °C; R_f 0.40 (solvent A); IR (Nujol): 3594, 3490, 3420, 3139, 2208, 1728, 1682, 1608, 1538, 1497, 1325, 1124, 1096, 1029, 972, 880 and 742 cm^{-1}; UV (MeOH): 206, 248 and 362 nM; ^1H NMR (400 MHz, DMSO-d_6): δ 6.38 (1H, brs, C-4‴H), 6.91 (1H, s, C-4′H), 7.15 (1H, brs, C-3‴H), 7.20 (1H, brs, C-5‴H), 7.39 (1H, m, C-4″H), 7.49 (2H, m, C-3″H and C-5″H), 7.81 (3H, m, C-3H, C-2″H and C-6″H), 11.55 (1H, brs, N-1‴H) and 13.50 (1H, brs, N-1′H); ^{13}C NMR (100 MHz, DMSO-d_6): Table 4; EIMS, *m*/*z* (rel. int.): 260 [M^+](100), 259(51), 232(18), 217(44), 204(12), 183(4), 177(5), 156(17), 149(9), 130(7), 129(10), 104(13), 89(9), 77(25), 51(18) and 43(36).

(*Z*)-2-[5-(4-Methylphenyl)pyrazol-3-yl]-3-(pyrrol-2-yl) acrylonitrile (4b). Crystallised from acetone as a yellow powder (369 mg, 90%), mp 215 °C; R_f 0.42 (solvent A); IR (Nujol): 3603, 3176, 2361, 2219, 1726, 1598, 1533, 1342, 1118, 1037, 1020, 908, 789 and 730 cm^{-1}; UV (MeOH): 209, 254 and 362 nM; ^1H NMR (400 MHz, DMSO-d_6: 2.34 (3H, s, CH_3), 6.33 (1H, brs, C-4‴H), 6.82 (1H, s, C-4′H), 7.15 (2H, brs, C-3‴H and C-5‴H), 7.29 (2H, d, J = 7.8 Hz, C-3″H and C-5″H) 7.70 (2H, d, J = 7.8 Hz, C-2″H and C-6″H), 7.78 (1H, s, C-3H), 11.60 (1H, brs, N-1‴H) and 13.30 (1H, brs, N-1′H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 4; EIMS, *m*/*z* (% rel. int.): 274[M^+](100), 273(49), 258(4), 246(8), 231(4), 204(1), 183(1), 156(7), 137(4), 129(3), 118(3), 91(3), 77(1) and 39(2).

(*Z*)-2-[5-(4-Methoxyphenyl)pyrazol-3-yl]-3-(pyrrol-2-yl) acrylonitrile (4c). Crystallised from acetone as a yellow powder (395 mg, 91%), mp 210–11 °C. R_f 0.42 (solvent A); IR (Nujol): 3604, 3321, 3175, 2360, 2219, 1725, 1605, 1270, 1133, 1020, 965, 816, 783 and 728 cm^{-1}. UV (MeOH): 206, 260 and 362 nM, ^1H NMR (250 MHz, DMSO-d_6): δ 3.81 (3H, s, OCH_3), 6.33 (1H, brs, C-4‴H), 6.77 (1H, s, C-4′H), 7.04 (2H, d, J = 8.5 Hz, C-3″H and C-5″H), 7.13 (2H, brs, C-3‴H and C-5‴H), 7.72 (2H, d, J = 8.5 Hz, C-2″H and C-6″H), 7.74 (1H, s, C-3H), 11.47 (1H, brs, N-1‴H) and 13.20 (1H, brs, N-1′H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 4; EIMS, *m*/*z* (% rel. int.): 290 [M^+](100), 289(38), 274(3), 262(6), 247(2), 231(2), 191(1), 183(7), 156(5), 145(5), 134(2), 129(3), 102(1), 77(1) and 39(2).

(*Z*)-2-[5-(4-Fluorophenyl)pyrazol-3-yl]-3-(pyrrol-2-yl) acrylotnitrile (4d). Crystallised from acetone as yellow needles (370 mg, 88%), mp 210 °C; R_f 0.40 (solvent A); IR (Nujol): 3500, 3310, 2342, 2210, 1726, 1608, 1500, 1155, 1120, 1102, 1020, 965, 880, 835, 800 and 735 cm^{-1}; UV (MeOH): 208, 245 and 362 nM; ^1H NMR (250 MHz, DMSO-d_6): δ 6.33 (1H, brs, C-4‴H), 6.87 (1H, s, C-4′H), 7.13 (2H, brs, C-3‴ and C-5‴H), 7.31 (2H, t, J = 8.6 Hz, C-3″H and C-5″H), 7.74 (1H, s, C-3H), 7.85 (2H, dd, J = 5.5 and 8.6 Hz, C-2″H and C-6″H), 11.49 (1H, brs, N-1‴H) and 13.40 (1H, brs, N-1′H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 4; EIMS, *m*/*z* (% rel. int.): 278 [M^+] (100), 277(47), 250(15), 249(8), 222(5), 194(2), 183(2), 156(10), 139(3), 129(5), 122(4), 95(5), 75(3) and 39(2).

(*Z*)-2-[5-(4-Chlorophenyl)pyrazol-3-yl]-3-(pyrrol-2-yl) acrylonitrile (4e). Crystallised from acetone as a yellow powder (409 mg, 94%), mp 243–46 °C; R_f 0.41 (solvent A); IR (Nujol): 3402, 3316, 3191, 2729, 2360, 2228, 1727, 1271, 1253, 1208, 1150, 840, 780 and 729 cm^{-1}; UV (MeOH): 206, 254 and 363 nM; ^1H NMR (250 MHz, DMSO-d_6): δ 6.33 (1H, brs, C-4‴H), 6.92 (1H, s, C-4′H), 7.14 (2H, brs, C-3‴H and C-5‴H), 7.54 (2H, d, J = 8.1 Hz, C-3″H and C-5″H), 7.75 (1H, s, C-3H), 7.82 (2H, d, J = 8.1 Hz, C-2″H and C-6″H), 11.50 (1H, brs, N-1‴H) and 13.51 (1H, brs, N-1′H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 4; EIMS, *m*/*z* (% rel. int.): 296 [$M^+ + 2$](33), 295(31), 294 [M^+] (100), 293(41), 266(4), 258(3), 238(1), 231(15), 204(4), 183(2), 176(2), 156(12), 138(5), 129(7), 111(4), 88(3), 75(6) and 55(4).

(*Z*)-2-[5-(4-Bromophenyl)pyrazol-3-yl]-3-(pyrrol-2-yl) acrylonitrile (4f). Crystallised from acetone as a yellow powder (467 mg, 91%), mp 233–35 °C; R_f 0.42 (solvent A); IR (Nujol): 3402, 3316, 2228, 1600, 1520, 1320, 1295, 1195, 1120, 1100, 1040, 980, 835, 800 and 740 cm^{-1}; UV (MeOH): 209, 257 and 362 nM; ^1H NMR (250 MHz, DMSO-d_6): δ 6.34 (1H, brs, C-4‴H), 6.92 (1H, s, C-4′H), 7.14 (2H, brs, C-3‴H and C-5‴H), 7.67 (2H, d, J = 8.5 Hz, C-3″H and C-5″H), 7.73 (1H, s, C-3H), 7.77 (2H, d, J = 8.5 Hz, C-2″H and C-6″H), 11.52 (1H, brs, N-1‴H), 13.50 (1H, brs, N-1′H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 4; EIMS, *m*/*z* (% rel. int.): 340 [$M^+ + 2$] (94), 339(47), 338 [M^+](100), 337(34), 310(3), 261(14), 250(7), 231(26), 204(11), 183(3), 176(9), 156(25), 149(19), 129(15), 109(36), 89(11), 77(35) and 43(51).

(*Z*)-2-[5-(3-Methoxyphenyl)pyrazol-3-yl]-3-(pyrrol-2-yl) acrylonitrile (4g). Crystallised from acetone as a yellow powder (391 mg, 90%), mp 158–60 °C; R_f 0.42 (solvent A); IR (Nujol): 3605, 3404, 2360, 2208, 1726, 1607, 1588, 1537, 1328, 1311, 1274, 1169, 1128, 1097, 1038, 970, 940, 864, 832 and 772 cm^{-1}; UV (MeOH): 209, 251 and 362 nM; ^1H NMR (400 MHz, DMSO-d_6): δ 3.83 (3H, s, OCH_3), 6.33 (1H, brs, C-4‴H), 6.91 (1H, s, C-4′H), 6.94 (1H, m, C-4″H), 7.13 (2H, brs, C-3‴H and C-5‴H), 7.37–7.38 (3H, m, C-2″H, C-5″H and (C-6″H), 7.75 (1H, s, C-3H), 11.48 (1H, brs, N-1‴H) and 13.43 (1H, s, N-1′H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 4; EIMS, *m*/*z* (% rel. int.): 290 [M^+](100), 289(91), 274(8), 262(10), 247(5), 217(5), 191(4), 183(2), 162(10), 156(7), 145(5), 129(3), 102(2), 95(1), 77(2) and 39(2).

(*Z*)-2-[5-(3-Bromophenyl)pyrazol-3-yl]-3-(pyrrol-2-yl) acrylonitrile (4h). Crystallised from acetone as yellow plates (484 mg, 95%), mp 239 °C; R_f 0.42 (solvent A); IR (Nujol): 3631, 3429, 2361, 2206, 1726, 1611, 1579, 1170, 1123, 1103, 1036, 1020, 969, 876, 723 and 679 cm^{-1}; UV (MeOH): 208, 254 and 360 nM; ^1H NMR (250 MHz, DMSO-d_6): δ 6.33 (1H, brs, C-4‴H), 6.99 (1H, s, C-4″H), 7.14 (2H, brs, C-3‴ and C-5‴H), 7.42 (1H, t, J = 7.9 Hz, C-

5″H), 7.55 (1H, d, $J=7.9$ Hz, C-4″H), 7.73 (1H, s, C-3H), 7.80 (1H, d, $J=7.9$ Hz, C-6″H), 8.04 (1H, brs, C-2″H), 11.51 (1H, brs, N-1‴H) and 13.50 (1H, brs, N-1′H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 4; EIMS, m/z (% rel. int.): 340 [$M^+ + 2$] (2), 338 [M^+] (3), 256(4), 250(5), 218(57), 205(3), 185(5), 156(1), 154(8), 125(5), 109(100), 77(50) and 39(44).

General method for isomerisation of (*Z*)-acrylonitriles 4a–4h to (*E*)-acrylonitriles 5a–5h. (*Z*)-2-(5-Arylpyrazol-3-yl)-3-(pyrrol-2-yl)acrylonitrile **4a–4h** (0.15 mmol) was added to a solution of NaOBu^t (48 mg, 0.5 mmol) in ethanol (20 mL) and the solution refluxed for 24 h. The TLC showed almost complete conversion of the *Z*-isomer to *E*-isomer. The reaction mixture was concentrated under reduced pressure, poured over crushed ice, when a yellow solid precipitated out. It was filtered, washed with water and dried over P_2O_5 in a vacuum desicator, purification of the crude product by column chromatography afforded **5a–5h** in 80–85% yields.

General method of preparation of (*E*)-2-(5-arylpyrazol-3-yl)-3-(pyrrol-2-yl)acrylonitriles 5a–5h. To a solution of 5-aryl-3-cyanomethylpyrazole **3a–3h** (1.5 mmol) in ethanol (20 mL), NaOBu^t (1.5 mmol) was added followed by addition of pyrrole-2-carboxaldehyde (1.5 mmol) and the reaction mixture was refluxed for 20–25 h. The progress of the reaction was monitored on HPLC and/or on TLC, the reaction was worked up as in the case of preparation of *Z*-isomers; yellow crude product obtained was purified by column chromatography with a solvent system of petroleum ether and ethyl acetate. Elution of the column with 5% and 10% ethyl acetate in petroleum ether afforded compounds **5a–5h** and **4a–4h** as the major and minor products, respectively.

(*E*)-2-(5-Phenylpyrazol-3-yl)-3-(pyrrol-2-yl)acrylonitrile (5a). Crystallised from acetone as a light yellow powder (206 mg, 53%), mp 165–66 °C; R_f 0.62 (solvent A); IR (Nujol): 3316, 3191, 2729, 2360, 2228, 1783, 1727, 1600, 1410, 1339, 1150, 1088, 1039, 986, 959, 871, 840, 799, 770 and 729 cm^{-1}; UV (MeOH): 209, 248 and 353 nM; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.38 (1H, brs, C-4‴H), 6.81 (1H, brs, C-3‴H), 6.96 (1H, s, C-4′H), 7.30 (1H, brs, C-5‴H), 7.39 (1H, s, C-3H), 7.42 (1H, m, C-4″H), 7.51 (2H, m, C-3″H and C-5″H), 7.90 (2H, d, $J=7.0$ Hz, C-2″H and C-6″H), 13.36 (1H, brs, N-1′H), 14.02 (1H, brs, N-1‴H); ^{13}C NMR (100 MHz, DMSO-d_6): Table 5; EIMS, m/z (% rel. int.): 260 [M^+](100), 259(53), 232(18), 204(10), 183(4), 176(5), 156(17), 140(2), 130(7), 129(12), 104(13), 90(4), 77(33), 51(23) and 43(32).

(*E*)-2-[5-(4-Methylphenyl)pyrazol-3-yl]-3-(pyrrol-2-yl) acrylonitrile (5b). Crystallised from acetone as yellow needles (234 mg, 57%), mp 173 °C; R_f 0.60 (solvent A); IR (Nujol): 3272, 2360, 2211, 1726, 1605, 1583, 1533, 1342, 1120, 1093, 1035, 986, 906, 786 and 723 cm^{-1}; UV (MeOH): 206, 254 and 356 nM; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.35 (3H, s, CH_3), 6.34 (1H, brs, C-4‴H), 6.80 (1H, brs, C-3‴H), 6.88 (1H, s, C-4′H), 7.30 (1H, brs, C-5‴H), 7.31 (1H, s, C-3), 7.33 (2H, d, $J=8.1$ Hz, C-3″H and C-5″H), 7.75 (2H, d, $J=8.1$ Hz, C-2″H and C-6″H), 13.32 (1H, brs, N-1′H) and 13.90 (1H, brs, N-1‴H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 5; EIMS, m/z (% rel. int.): 274 [M^+](100), 273(59), 258(5), 246(11), 137(4), 231(5), 203(1),183(1), 182(1), 156(9), 137(4), 129(4), 118(4), 115(3), 91(5), 73(2), 65(41) and 43(7).

(*E*)-2-[5-(4-Methoxyphenyl)pyrazol-3-yl]-3-(pyrrol-2-yl) acrylonitrile (5c). Crystallised from acetone as a yellow powder (239 mg, 55%), mp 159 °C; R_f 0.64 (solvent A); IR (Nujol): 3325, 3174, 2342, 2219, 1726, 1605, 1270, 1163, 1133, 1049, 1024, 964, 901, 883, 815, 774 and 724 cm^{-1}; UV (MeOH): 209, 260 and 356 nM; ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.81 (3H, s, OCH_3), 6.33 (1H, brs, C-4‴H), 6.78 (1H, brs, C-3‴H), 6.81 (1H, s, C-4′H), 7.05 (2H, d, $J=8.8$ Hz, C-3″H and C-5″H), 7.28 (1H, brs, C-5‴H), 7.32 (1H, s, C-3H), 7.79 (2H, d, $J=8.8$ Hz, C-2″H and C-6″H), 13.34 (1H, brs, N-1′H) and 13.90 (1H, brs, N-1‴H); ^{13}C NMR (62.8 MHz, DMSO-d_6). Table 5; EIMS, m/z (% rel. int.): 290 [M^+] (100), 289(39), 274(3), 262(7), 246(2), 231(3), 183(1), 156(6), 145(3), 134(3), 129(3), 73(5), 60(6) and 43(7).

(*E*)-2-[5-(4-Fluorophenyl)pyrazol-3-yl]-3-(pyrrol-2-yl)-acrylonitrile (5d). Crystallised from acetone as a yellow powder (216 mg, 52%), mp 188 °C; R_f 0.60 (solvent A); IR (Nujol): 3200, 3190, 2228, 1600, 1400, 1340, 1320, 1255, 1235, 1140, 1080, 1035, 980, 835, 780 and 720 cm^{-1}; UV (MeOH): 209, 248 and 353 nM; ^{1}H NMR (250 MHz, DMSO-d_6): δ 6.33 (1H, brs, C-4‴H), 6.79 (1H, brs, C-3‴H), 6.91 (1H, s, C-4′H), 7.28 (1H, brs, C-5‴H), 7.34 (1H, s, C-3H), 7.37 (2H, d, $J=8.5$ Hz, C-3″H and C-5″H), 7.90 (2H, d, $J=8.5$ Hz, C-2″H and C-6″H), 13.24 (1H, brs, N-1′H) and 13.90 (1H, brs, N-1‴H); ^{13}C NMR (62.8 MHz, DMSO-d_6: Table 5; EIMS, m/z (% rel. int.): 278 [M^+] (100), 277(47), 250(15), 249(6), 222(4), 208(1), 194(1), 183(1), 156(10), 139(3), 129(5), 122(4), 102(1), 95(4), 75(3) and 41(6).

(*E*)-2-[5-(4-Chlorophenyl)pyrazol-3-yl]-3-(pyrrol-2-yl)-acrylonitrile (5e). Crystallised from acetone as a yellow powder (266 mg, 60%), mp 226–29 °C. R_f 0.61 (solvent A); IR (Nujol): 3593, 3245, 2725, 2359, 2216, 1724, 1599, 1269, 1153, 829, 787 and 725 cm^{-1}; UV (MeOH): 209, 260 and 356 nM; ^{1}H NMR (250 MHz, DMSO-d_6): δ 6.32 (1H, brs, C-4‴H), 6.79 (1H, brs, C-3‴H), 6.97 (1H, s, C-4′H), 7.28 (1H, brs, C-5‴H), 7.34 (1H, s, C-3H), 7.57 (2H, d, $J=8.5$ Hz, C-3″H and C-5″H), 7.89 (2H, d, $J=8.5$ Hz, C-2″H and C-6″H), 13.20 (1H, brs, N-1′H) and 14.10 (1H, brs, N-1‴H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 5; EIMS, m/z (% rel. int.): 296 [$M^+ + 2$](34), 295(32), 294 [M^+](100), 293(43), 278(4), 266(4), 258(4), 243(10), 231(15), 203(7), 183(2), 156(14), 138(7), 149(20), 129(8), 111(6), 83(6), 75(11) and 43(73).

(*E*)-2-[5-(4-Bromophenyl)pyrazol-3-yl]-3-(pyrrol-2-yl)-acrylonitrile (5f) . Crystallised from acetone as a yellow powder (305 mg, 60%), mp 225 °C; R_f 0.62 (solvent A); IR (Nujol): 3254, 2200, 1600, 1340, 1126, 1060, 1020, 993, 820, 780, 743 and 720 cm^{-1}; UV (MeOH): 209, 260 and 356 nM; ^{1}H NMR (250 MHz, DMSO-d_6): δ 6.33 (1H, t, $J=2.9$ Hz, C-4‴H), 6.77 (1H, brs, C-3‴H), 6.97 (1H, s, C-4′H), 7.28 (1H. brs, C-5‴H), 7.35 (1H, s, C-

3H), 7.68 (2H, d, $J=8.5$ Hz, C-3″H and C-5″H), 7.81 (2H, d, $J=8.6$ Hz, C-2″H and C-6″H), 13.20 (1H, brs, N-1′H) and 13.90 (1H, brs, N-1‴H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 5; EIMS, m/z (% rel. int.): 340 [$M^+ + 2$] (96), 339(50), 338 [M^+](100), 337(35), 310(3), 358(14), 231(25), 203(7), 183(3), 176(5), 156(21), 151(4), 129(13), 102(10), 88(8), 75(11) and 39(8).

(*E*)-2-[5-(3-Methoxyphenyl)pyrazol-3-yl]-3-(pyrrol-2-yl)-acrylonitrile (5g). Crystallised from acetone as a light yellow powder (243 mg, 56%), mp 155 °C; R_f 0.64 (solvent A); IR (Nujol): 3253, 2359, 2212, 1727, 1592, 1573, 1533, 1308, 1271, 1145, 1092, 1048, 980, 901, 857, 842, 785 and 766 cm^{-1}; UV (MeOH): 212, 254 and 356 nM; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (3H, s, OCH_3), 6.33 (1H, brs, C-4‴H), 6.79 (1H, brs, C-3‴H), 6.96 (1H, s, C-4′H), 6.98 (1H, d, $J=8.4$ Hz, C-4′H), 7.28 (1H, brs, C-5‴H), 7.34 (1H, s, C-3H), 7.36–7.44 (3H, m, C-2″H, C-5″H and C-6″H), 13.27 (1H, brs, N-1′H) and 13.90 (1H, brs, N-1‴H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 5; EIMS, m/z (% rel. int.): 290 [M^+](100), 289(53), 274(5), 262(7), 246(3), 217(3), 203(1), 190(2), 183(1), 156(8), 145(5), 134(3), 129(4), 102(2), 73(7) and 44(9).

(*E*)-2-[5-(3-Bromophenyl)pyrazol-3-yl]-3-(pyrrol-2-yl)-acrylonitrile (5h). Crystallised from acetone as yellow needles (274 mg, 54%), mp 210–11 °C; R_f 0.62 (solvent A); IR (Nujol): 3224, 2360, 2342, 2225, 1726, 1596, 1306, 1148, 1118, 1090, 1037, 1020, 967, 898, 884, 730 and 686 cm^{-1}; UV (MeOH): 212, 257 and 356 nM; ^{1}H NMR (250 MHz, DMSO-d_6): δ 6.33 (1H, brs, C-4‴H), 6.79(1H, brs, C-3‴H), 7.05(1H, s, C-4′H), 7.29(1H, brs, C-5‴H), 7.35(1H, brs, C-3H), 7.45(1H, t, $J=7.9$ Hz, C-5″H), 7.60(1H, d, $J=8.2$ Hz, C-4″H), 7.88 (1H, d, $J=7.9$ Hz, C-6″H), 8.14 (1H, brs, C-2″H), 13.10(1H, brs, N-1′H) and 13.90 (1H, brs, N-1‴H); ^{13}C NMR (62.8 MHz, DMSO-d_6): Table 5; EIMS, m/z (% rel. int.): 340 [$M^+ + 2$] (19), 338 [M^+] (20), 337(5), 289(31), 287(31), 250(17), 229(3), 218(75), 185(7), 156(2), 154(8), 125(26), 109(100), 77(59), 65(37) and 39(29).

General method of preparation of 5-cyanomethyl-1,3-diarylpyrazoles 6a-6d. To a solution of 6-aryl-3-cyano-4-methylthio-2H-pyran-2-one **2a**, **2b**, **2e** and **2f** (6.0 mmol) in methanol/pyridine (30 mL), phenylhydrazine/*p*-fluorophenylhydrazine hydrochloride (9.0 mmol) was added and the reaction mixture was refluxed for 5–6 h. The progress of the reaction was monitored on HPLC and/or on TLC. The reaction mixture was concentrated on completion under reduced pressure, poured over crushed ice (200 g) and stirred vigorously until a brownish yellow solid precipitated out. It was filtered, washed with water, dried and column chromatographed over silica gel using ethyl acetate-petroleum ether as eluent to afford **6a–6d** as yellowish white solids which crystallised from ethyl acetate as white shining needles in 45% to 52% yields.

5-Cyanomethyl-1,3-diphenylpyrazole (6a). Crystallised from acetone as white needles (745 mg, 48%), mp 71 °C; R_f 0.50 (solvent B); IR (Nujol): 2361, 2342, 2254, 1766, 1598, 1548, 1504, 759, 723 and 685 cm^{-1}; UV (MeOH): 212 and 260 nM; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.75 (2H, s, C-6H), 6.84 (1H, s, C-4H), 7.36–7.52 (8H, m, C-3″H, C-4″H, C-5″H and Ar′H) and 7.84 (2H, d, $J=6.9$ Hz, C-2″H and C-6″H); ^{13}C NMR (50 MHz, $CDCl_3$): Table 6; EIMS, m/z (% rel. int.): 259[M^+] (100), 231(59), 219(20), 204(5), 180(7), 155(25), 129(10), 116(30), 89(22) and 77(60).

5-Cyanomethyl-3-(4-methylphenyl)-1-phenylpyrazole (6b). Crystallised from acetone as white needles (737 mg, 45%), mp 115–16 °C; R_f 0.54 (solvent B); IR (Nujol): 2724, 2341, 1712, 1595, 1377, 795 and 723 cm^{-1}; UV (MeOH): 213 and 265 nM; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.37 (3H, s, CH_3), 3.77 (2H, s, C-6H), 6.82 (1H, s, C-4H), 7.24 (2H, d, $J=8.0$ Hz, C-3′H and C-5′H), 7.45–7.56 (5H, m, Ar″H) and 7.73 (2H, d, $J=8.0$ Hz, C-2′H and C-6′H), ^{13}C NMR (50 MHz, $CDCl_3$): Table 6; EIMS, m/z (% rel. int.): 273 [M^+] (100), 259(18), 245(45), 233(25), 194(5), 155(20), 130(20), 116(18), 91(20) and 77(37).

5-Cyanomethyl-3-(4-chlorophenyl)-1-phenylpyrazole (6c). Crystallised from acetone as white needles (814 mg, 46%), mp 83–84 °C; R_f 0.50 (solvent B); IR (Nujol): 2359, 1597, 1500, 830, 804, 785, 763 and 699 cm^{-1}; UV (MeOH): 212 and 265 nM; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.77 (2H, s, C-6H), 6.82 (1H, s, C-4H), 7.39 (2H, d, $J=8.4$ Hz, C-3′H and C-5′H), 7.44–7.51 (5H, m, H) and 7.78 (2H, d, $J=8.4$ Hz, C-2′H and C-6′H); ^{13}C NMR (50 MHz, $CDCl_3$): Table 6; EIMS, m/z (% rel. int.): 295 [$M^+ + 2$] (30), 293 [M^+] (100), 277(25), 265(18), 231(23), 198(5), 155 (6), 116(4), 91(3) and 77(18).

3-(4-Bromophenyl)-5-cyanomethyl-1-(4-fluorophenyl)-pyrazole (6d). Crystallised from acetone as white needles (1.107 g, 52%), mp 145–46 °C; R_f 0.50 (solvent B); IR (Nujol): 2359, 2254, 1598, 1511, 1377, 1215, 957, 838, 798, 772, 719 and 610; UV (MeOH): 209 and 265 nM; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.74 (2H, s, C-6H), 6.79 (1H, s, C-4H), 7.24 (2H, d, $J=8.6$ Hz, C-3′H and C-5′H), 7.43 and 7.47 (2H, 2d, $J=4.8$ Hz each, C-3″H and C-5″H), 7.53 (2H, d, $J=8.5$ Hz, C-2″H and C-6″H) and 7.70(2H, d, $J=8.6$ Hz, C-2′H and C-6′H); ^{13}C NMR (50 MHz, $CDCl_3$): Table 6; EIMS, m/z (% rel. int.): 357 [$M^+ + 2$] (96), 355[M^+](100), 329(10), 315(5), 276(6), 249(6), 196(2), 173(9), 134(15), 107(7) and 95(15).

General method of synthesis of (*Z*)-2-(1,3-diarylpyrazol-5-yl)-3-(pyrrol-2-yl)acrylonitriles 7a–7d. To a solution of 5-cyanomethyl-1,3-diarylpyrazoles **6a–6d** (1.5 mmol) in ethanol (20 mL), NaOBu^t (1.5 mmol) was added and the reaction mixture was stirred for 15 min at 25–30 °C followed by addition of pyrrole-2-carboxaldehyde (1.5 mmol) in one lot. Stirring was continued for 4–5 h and progress of the reaction was monitored on HPLC and/or on TLC. The reaction mixture was concentrated on completion under reduced pressure and poured over crushed ice (50 g). The yellow solid that precipitated out was filtered, dried over P_2O_5 in a vacuum dessicator and the crude product was purified by column-chromatography using ethyl acetate:petroleum ether (1:49) as eluent and finally crystallised to afford **7a–7d** in almost quantitative yields.

(*Z*)-2-(1,3-Diphenylpyrazol-5-yl)-3-(pyrrol-2-yl)acrylonitrile (7a). Crystallised from acetone as yellow needles (441 mg, 87%), mp 120–23 °C; R_f 0.60 (solvent B); IR (Nujol): 3403, 2727, 2360, 2207, 1592, 1126, 1039, 976, 767, 749 and 732 cm^{-1}; UV (MeOH): 218, 252 and

359 nM; ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.33 (1H, brs, C-4‴″H), 6.63 (1H, brs, C-3‴″H), 6.84 (1H, s, C-4′H), 7.05 (2H, s, C-3H and C-5‴″H), 7.35–7.55 (8H, m, C-3‴H and C-5‴H and Ar″H), 7.88 (2H, d, J = 6.9 Hz, C-2‴H and C-6‴H), 9.75 (1H, brs, NH); ^{13}C NMR (50 MHz, $CDCl_3$): Table 6; EIMS, m/z (% rel, int): 336 [M^+] (100), 335(95), 308(6), 269(58), 233(50), 205(15), 168(18), 154(3), 129(10), 104(15) and 91(40).

(*Z*)-2-[3-(4-Methylphenyl)-1-phenylpyrazol-5-yl]-3-(pyrrol-2-yl)acrylonitrile (7b). Crystallised from acetone as yellow needles (472 mg, 90%), mp 176–78 °C; R_f 0.64 (solvent B); IR (Nujol). 3403, 2726, 2360, 2208, 1642, 1590, 1323, 1127, 1041, 776, 752, 723 and 700 cm^{-1}; UV (MeOH): 209, 253 and 363 nM; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.39 (3H, s, CH_3), 6.33 (1H, brs, C-4‴″H), 6.62 (1H, brs, C-3‴″H), 6.81 (1H, s, C-4′H), 7.04 (2H, s, C-3H and C-5‴″H), 7.24 (2H, d, J = 8.0 Hz, C-3″H and C-5″H), 7.46–7.58 (5H, m, Ar‴H), 7.77 (2H, d, J = 8.0 Hz, C-2″H and C-6″H) and 9.70 (1H, brs, NH); ^{13}C NMR (50 MHz, $CDCl_3$): Table 6; EIMS, m/z (% rel. int.): 350 [M^+] (100), 349(95), 335(25), 322(5), 283(45), 258(15), 233(47), 194(14), 154(5), 119(99), 91(60) and 77(25).

(*Z*)-2-[3-(4-Chlorophenyl)-1-phenylpyrazol-5-yl]-3-(pyrrol-2-yl)acriylonitrile (7c). Crystallised from acetone as yellow needles (526 mg, 95%), mp 175–77 °C; R_f 0.62 (solvent B); IR (Nujol): 3403, 2726, 2360, 2208, 1642, 1589, 1323, 1126, 1041, 781, 752 and 723 cm^{-1}; UV (MeOH): 216, 252 and 365 nM; ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.34 (1H, brs, C-4‴″H), 6.62 (1H, brs, C-3‴″M), 6.81 (1H, s, C-4′H), 7.04 (2H, s, C-3H and C-5‴″H), 7.40 (2H, d, J = 8.6 Hz, C-3″H and C-5″H), 7.44–7.57 (5H, m, Ar‴H), 7.81 (2H, d, J = 8.6 Hz, C-2″H and C-6″H) and 9.69 (1H, brs, NH); ^{13}C NMR (50 MHz, $CDCl_3$): Table 6; EIMS, m/z (% rel. int.): 373 [$M^+ + 2$] (33), 371 [M^+] (100), 370(80), 333(7), 303(40), 268(15), 233(45), 205(20), 129(10), 91(51), 77(43) and 67(22).

(*Z*)-2-[3-(4-Bromophenyl)-1-(4-fluorophenyl)pyrazol-5-yl]-3-(pyrrol-2-yl)acrylonitrile (7d). Crystallised from acetone as yellow needles (605 mg, 93%), mp 197–201 °C, R_f 0.60 (solvent B); IR (Nujol): 3413, 2725, 2360, 2203, 1659, 1643, 1589, 1325, 1223, 1169, 1154, 1124, 1041, 980, 847, 783, 750, 723 and 692 cm^{-1}; UV (MeOH): 211, 259 and 364 nM; ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.36 (1H, brs, C-4‴″H), 6.65 (1H, brs, C-3‴″H), 6.80 (1H, s, C-4′H), 7.07 (1H, brs, C-5‴″H), 7.09 (1H, s, C-3H), 7.21 (2H, d, J = 9.0 Hz, C-3″H and C-5″H), 7.45–7.60 (4H, m, Ar‴H), 7.75 (2H, d, J = 9.0 Hz, C-2″H and C-6″H) and 9.70 (1H, brs, NH); ^{13}C NMR (50 MHz, $CDCl_3$): Table 6; EIMS, m/z (% rel. int.): 434 [$M^+ + 2$] (98), 432 [M^+] (100), 431(45), 406(3), 367(15), 351(40), 324(20), 276(20), 251(80), 229(25), 176(23), 156(18), 129(23), 109(90), 95(46), 81(25), 69(44) and 57(23).

X-ray crystallography. The crystallographic measurements on compounds **2d** and **3b** were made using a Siemens SMART area-detector diffractometer. Graphite monochromated Mo-K_α radiation was used in all cases. The structures were solved using SHELXTL-PLUS[38] and refined with SHELXL-96.[39] The crystal data of compounds **2d** and **3b** are given below.

3-Cyano-6-(4-fluorophenyl)-4-methylthio-2*H*-pyran-2-one (2d). $C_{13}H_8FNO_2S$, M = 265.26, T = 180(2)K, λ = 0.71073 Å. Monoclinic a = 38.0091(10), b = 4.90430(10), c = 14.46 090(10) Å, β = 108.478(2)°, V = 2556.66(9) Å^3, space group C2/c, Z = 8, D_x = 1.378 Mg/m^3, μ = 0.260 mm^{-1}, F(000) = 1088. Crystal size 0.40×0.40×0.06 mm; θ range for data collection 2.26–24.99°, limiting indices $-49 < h < 43$, $-6 < k < 6$, $-19 < l < 16$; reflections collected 5993; independent reflections 2232 [R(int) = 0.0394; refinement method full-matrix least-squares on F^2; data/restraints/parameters 2232/0/173; goodness-of-fit on F^2 1.046; R(F)[I > 2σ(I)] = 0.0624; wR(F^2) = 0.1664; largest diff. peak and hole 0.596 and −0.238 e Å^{-3}.

3-Cyanomethyl-5-(4-methylphenyl)pyrazole (3b). $C_{12}H_{11}N_3$, M = 197.24, T = 180(2)K, λ = 0.71073Å. Monoclinic a = 12.5575(7), b = 14.4585(3), c = 5.8342 (3)Å, β = 103.097(2)°, V = 1031.72(8)Å^3, space group $P2_1/c$, Z = 4, D_x = 1.270 Mg/m^3, μ = 0.079 mm^{-1}, F(000) = 416. Crystal size 0.42×0.34×0.10 mm; θ range for data collection 1.66 to 23.00°; index range $-16 < h < 16$, $-18 < k < 18$, $-7 < l < 6$; reflections collected 3433; independent reflections 1418 [R(int) = 0.1336]; refinement method full-matrix least-squares on F^2; data/restraints/parameters 1418/0/138; goodness-of-fit on F^2 0.997; R(F)[I > 2σ[I] = 0.0940; wR(F^2) = 0.2285; largest diff. peak and hole 0.323 and −0.344 e Å^{-3}.

Effect of pyrazolylacrylonitriles and their precursors on NADPH-catalysed liver microsomal lipid peroxidation. a) Assay for inhibition of lipid peroxidation

Detailed assay procedure has been described in our earlier commnunication.[35] In short, rat liver microsomes (1 mg protein) were preincubated with Tris–HCl (0.025M, pH 7.5) and test compound (100 μM in DMSO) was added at 37 °C for 10 min, followed by the addition of ADP (3 mM) and $FeCl_3$ (0.15 mM). The reaction for inhibition of enzymatic lipid peroxidation was started by the addition of NADPH (0.5 mM) and incubation of the reaction mixture continued for 10 min. The products of lipid peroxidation were quantified by the estimation of thiobarbituric acid reactive substances (TBARS) thus formed as described earlier.[35] The inhibitory potential of various pyrazolylacrylonitriles was assessed by determining the inhibitor concentration for 50% inhibition of lipid peroxidation (IC_{50}).

b) Assay for inhibition of propagation of lipid peroxidation

For this purpose, linoleic acid hydroperoxide was incubated with EDTA and $FeSO_4$ in order to propagate the lipid peroxidation as described by Sato et al.[40] The test compound at a concentration of 100 mM was added to the reaction mixture to determine the effect of linoleic acid-induced lipid peroxidation.

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