Novel Anti-inflammatory Agents Based on Pyrazole Based Dimeric Compounds; Design, Synthesis, Docking and *in Vivo* Activity

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Series of pyrazole ester prodrugs analogues have been synthesized and found to contain highly potent inhibitors of the cyclooxygenase-2 (COX-2) enzyme. The paper describes synthesis of the target pyrazole analogues. The structure of the synthesized mutual ester prodrugs (6—8c) were confirmed by ^{1}H-, ^{13}C-NMR mass spectroscopy (MS) and their purity were ascertained by TLC and elemental analyses. The biological *in vivo* evaluation of these compounds in experimental models (carrageenan-induced oedema) proved the presence of anti-inflammatory activity. Docking studies into the catalytic site of COX-2 were used to identify potential anti-inflammatory lead compounds. One lead derivative was chosen endowed with good binding energies.

Key words prodrug; pyrazole; cyclooxygenase; docking; NMR

Various inflammatory diseases are currently treated with steroidal and non-steroidal anti inflammatory drugs (NSAIDs).[1)] NSAIDs are drug with analgesic, antipyretic and in higher doses anti inflammatory effects. They inhibit synthesis of inflammation mediators.[2,3)] Most NSAIDs act as non-selective inhibitors of the enzyme cyclooxygenase (COX), by inhibiting the metabolism of arachidonic acid.[2)] COX catalyses the formation of prostaglandins (PGs) and thromboxane from arachidonic acid. PGs act as messenger molecules in the process of inflammation. Conventional NSAIDs that non-selectively inhibit major cyclooxygenase isoforms,[4—7)] (COX-1 and COX-2), are widely used to treat the signs and symptoms of inflammation, particularly arthritic pain. COX-1 is the constitutive isoform and is mainly responsible for the synthesis of cytoprotective PGs in gastrointestinal (GI) tract whereas COX-2 is inducible and plays a major role in PG biosynthesis in inflammatory cells.[8—10)] It is believed that the inhibition of COX-1 causes unfavorable GI side effects.[11)] NSAIDs vary in their potency, duration of action and the way in which they are eliminated from the body. Though selective COX-2 inhibitors (coxibs) with better safety profile have been marketed as a new generation of NSAIDs,[12,13)] but careful prospective examination of coxibs has revealed unexpected adverse effects. The two main adverse drug reactions (ADRs) associated with NSAIDs relate to GI effects[14,15)] and renal effects (kidney failure) of the agents. Also, they have shown unexpected cardiovascular adverse effects.[16)] Therefore, development of novel compounds having anti inflammatory activity with an improved safety profile is still a necessity. Literature survey revealed that many pyrazole[17)] derivatives have found their clinical application as NSAIDs. Among the highly marketed COX-2 inhibitors that comprise the pyrazole nucleus, celecoxib is the one which is treated as a safe anti inflammatory and analgesic agent. It is considered as a typical model of the diaryl heterocycle template that is known to selectively inhibit the COX-2 enzyme. Some other examples of pyrazole derivatives as NSAIDs are felcobutazone, mefobutazone, morazone, famprofazone and ramifenazone.[18—21)] But due to serious adverse effects (such as bone marrow depression, water and salt retention and carcinogenesis), the use of pyrazole derivative is limited. This limitation has led to the investigation of new pyrazole derivatives with that more potent activity and less toxicity.

Motivated by the aforementioned findings, it was designed to synthesize novel series of pyrazole derivatives that would act as anti inflammatory agents. The substitution pattern of the pyrazole ring was rationalized so as to be correlated to the diaryl heterocycles template of compounds that are known to act selectively as COX-2 inhibitors, such as celecoxib. Compared with coxibs, new drugs were synthesized by keeping the original pyrazole moiety same in new drugs containing heterocycle (like phthalimide, *N*-pyridone and *O*-pyridone) linked ethylene linker attached to oxygen adjacent to phenyl group containing nitrogen of pyrazole which provides flexibility to the molecule so that it will become easy to fit into the secondary pocket of COX-2 active site. The drugs were synthesized as acid derivatives (containing ester group), initiating the easy uptake of drug (in salt form). Presence of a small hydrophobic group, methyl group in pyrazole nucleus at position 3 can exert important influence on the activity and selectivity of related drug.

Chemistry All compounds were synthesized *via* a general substitution reaction at room temperature. The starting material in the synthesis of substituted pyrazoles had been synthesized from reaction of ethyl acetoacetate **1** with phenyl hydrazine at refluxing temperature[22)] to synthesize 5-methyl-2-phenyl-2,4-dihydro-pyrazol-3-one **2** (Chart 1). Reaction of **2** with carbon disulfide[23,24)] followed by methylation gave 4-(bis-methyl sulfanyl-methylene)-5-methyl-2-phenyl-2,4-dihy-

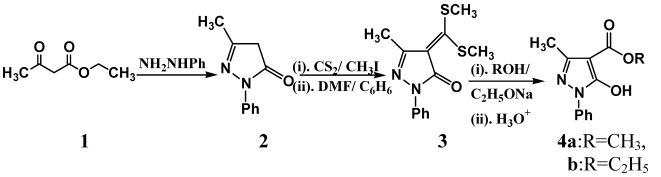

Chart 1

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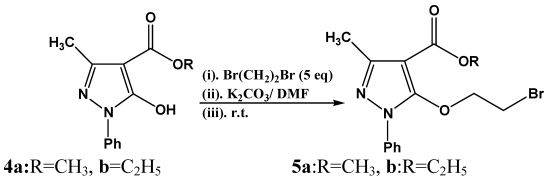

Chart 2

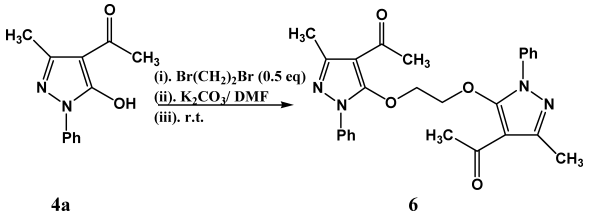

Chart 3

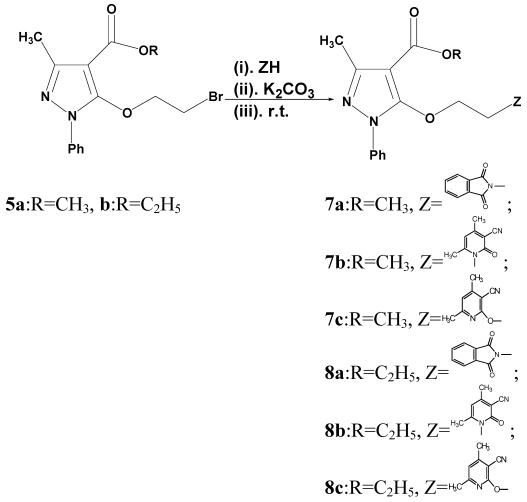

Chart 4

dro-pyrazol-3-one **3** (in 80—90% yield), the key intermediate in the synthesis of 4-substituted pyrazoles. Base catalysed alcoholysis of **3** followed by acidification gave pyrazole-ester **4** in 80% yields. The mass spectrum and elemental analysis are in accordance with the assigned structure. The ^{1}H-NMR spectrum of **4a** had a singlet at δ 2.406 for methyl protons and a singlet at δ 3.920 for methoxy protons and a broad singlet at δ 9.992 for hydroxyl proton. The mass spectrum of **4a** had the base peak at MS (*m*/*z*) M^+ 233. Similarly the ^{1}H-NMR and mass spectra of **4b** were found in accordance to the assigned structures. Treatment of **4a** with excess of 1,2-dibromoethane (5 eq) in presence of a weak base (anhydrous K_2CO_3) and *N*,*N*-dimethylmethanamide (DMF) afforded synthesis of **5a** with quantitative yield and high purity. Similarly, **5b** was obtained by condensation of **4a** with excess of 1,2-dibromoethane in basic medium. All products were obtained in quantitative yield with high purity *via* SiO_2 column chromatography with 5% ethyl acetate in chloroform as eluant mixture. Reaction of **4a** with 1,2-dibromoethane (0.5 eq) in presence of K_2CO_3 and DMF afforded synthesis of **6** with 42.78% yield. The base catalyzed nucleophilic substitution of **5a** with 1 eq of phthalimide in DMF as solvent at room temperature gave **7a** (Chart 4), in good yield. The pure product was obtained by crystallization with 2% ethyl acetate in hexane. Similarly, **5b** reacted with phthalimide to afford synthesis of **8a**. The base catalyzed nucleophilic substitution of **5a** and **5b** separately with 1 eq of pyridone and DMF at r.t. afforded the synthesis of **7b**, **7c**, **8b** and **8c**, respectively.

Materials and Methods Animals: All experiments have been conducted on adult Wistar strain albino rats of either sex, weighing between 150—200 g. The animals were obtained from the Central Animal House of the Institute of Medical Sciences, B.H.U. They were kept in colony cages under identical housing conditions at an ambient temperature of 25±2 °C and 45—55% relative humidity with a 12 h light–dark cycle in the departmental animal room and fed on standard diet. Animals were acclimatized for a week before use.

Experimental Model of Inflammation: Carrageenin induced paw edema[25] was used throughout the investigation.

Carrageenin Induced Paw Edema in Rats: Target compounds were suspended in aqueous solution of carboxymethyl cellulose (CMC, 1% w/v) and administered orally at a dose level of 10 mg/kg of NSAI drugs nimusilide and their equivalent doses of the target derivatives (**6**—**8C**). After 15 min control animals were similarly treated with aqueous solution of carboxymethyl cellulose (CMC, 1% w/v). One percent carrageenin suspension was prepared as a homogeneous solution in distilled water. A volume of 0.1 ml of carrageenin solution was injected through a 26 gauge needle into the plantar surface of the left hind paw below the plantar aponeurosis. The volume of paw was measured before and at different intervals for 3 h after injection of carrageenin. The difference in paw volume before and after administration of the phlogistic agent was taken as the measure of pedal edema.

Measurement of Paw Volume: The volume of hind paw of the rats up to the ankle joint was measured plathysmographically by the mercury displacement method. The ankle joint of the rats was marked with a skin marking pencil and the paw was dipped in the mercury, so that the mark on the paw coincides with a prefixed line kept constant on the syringe. The level of the mercury was every time brought to the level of this line by adjusting the height of the displaced mercury. The difference in the paw volume before and after injection of the phlogistic agents was taken as a measure of pedal edema. The change in paw volume was expressed in "ml" of mercury displaced.

Statistical Analysis: The difference in the means between each experimental group was first analyzed by Analysis of Variance (ANOVA). When the overall ANOVA was significant ($p<0.05$), the data was further subjected to statistical analysis by the student's *t*-test.

Molecular Docking analysis on COX-2 Inhibitor: A binding study of COX-2 inhibitor was performed using ligand–receptor interaction module of MOE software (Macrovision Europe Ltd.), while docking analysis was conducted using FlexXnet software (Schrodinger). In all the studies, indomethacine was used as a reference inhibitor in COX-2 crystal structure (PDB code: 4COX), considering that size and shape complementarity of indomethacine was similar to the studied compound.

Result and Observations In all experiments, carrageenin was administered into the left hind paw and the paw volume was measured before and at intervals of 30 min, 90 min and 180 min, after carrageenin injection (Table 1).

It was observed that maximum percentage of paw edema

Table 1. Paw Edema at Different Time Interval (ml/Rat) (Mean±S.E.M.)

Group	0 min	30 min	90 min	180 min
Control	0.99±0.067	1.27±0.043	1.36±0.070	1.21±0.072
Nimusilide	1.18±0.064	1.29±0.041	1.25±0.024	1.21±0.021
6	1.16±0.044	1.32±0.029	1.43±0.025	1.33±0.046
7a	1.28±0.064*	1.33±0.061	1.36±0.063	1.28±0.093*
7b	0.84±0.056	0.94±0.044***	1.03±0.057***	0.99±0.043*
7c	0.82±0.038	0.87±0.028***	0.97±0.025***	0.99±0.028*
8a	0.76±0.033	1.02±0.052**	1.11±0.034**	1.01±0.052
8b	1.15±0.050	1.45±0.028	1.44±0.012	1.34±0.045
8c	0.97±0.086	1.25±0.034	1.15±0.050	1.05±0.022

Results are (mean±S.E.M.) of 6 rats in each group. $*p<0.05$; $**p<0.01$; $***p<0.001$.

Table 2. Percentage Edema Growth Relative to Control at Different Time Intervals (Mean±S.E.M.)

Group	0 (min)	30 (min)	90 (min)	180 (min)
Control	100±0	130.1±6.54 (30.1)	138.7±4.47 (38.7)	122.3±5.17 (22.3)
Nimusilide	100±0	110.8±2.58 (10.8)	107.90±3.14 (7.90)	102.54±2.52 (2.54)
6	100±0	114.1±2.88 (14.1)	123.4±3.37* (23.4)	114.9±4.25 (14.9)
7a	100±0	103.9±2.59* (3.9)	106.2±3.98* (6.2)	99.6±2.54** (0.4)
7b	100±0	115.1±10.02 (15.1)	123.3±3.83*** (23.3)	120.6±8.34 (20.6)
7c	100±0	106.5±3.67** (6.5)	118.4±3.86 (18.4)	121.4±4.55 (21.4)
8a	100±0	133.9±3.56 (33.9)	146.5±6.54 (46.5)	133.6±7.58 (33.6)
8b	100±0	126.8±5.60 (26.8)	125.6±4.86 (25.6)	117.1±5.86 (17.1)
8c	100±0	129.0±4.53 (29.0)	118.4±3.98* (18.4)	108.7±4.75 (8.7)

$n=6$=number of rats in each group. Results in parentheses indicate percentage change from respective control group. $*p<0.05$; $**p<0.01$; $***p<0.001$.

growth in control group at 90 min was 38.7% which was found to decrease upto 6.2% in the group of rats treated with **7a** and least effect was shown by **8a** drug (Table 2).

Discussion and Conclusion The results of present study shown that compound **7a** had shown to possess maximum inhibitory effect when compared to control group (Fig. 1). It was observed that maximum percentage of paw edema growth in control group at 90 min was 38.7% which was found to decrease upto 6.2% in the group of rats treated with **7a** (Table 2). Also, the values were found statistically very significant. **8c** have also been found to possess very good anti inflammatory property as the percentage paw edema growth was shown to be only 18.4% when compared to that of control group (where it was 38.7% at 90 min). Other drugs *i.e.*, **6**, **7b** and **8b** have shown moderate to intermediate effects on inhibitory properties. But **8a** has shown no effect as anti inflammatory agent. Also, in case of **7c**, the drug was shown to possess good inhibitory property at 90 min when compared to control group as the value dropped from 38.7 to 18.4% but at 180 min, the drug has shown almost negligible effect on inflammation inhibition and the percentage inhibition increased up to 21.4%. Therefore, the results indicated that **7a** is potent inhibitor of inflammation and the anti inflammatory effect of **7a** on inflammagen induced edema may depend on inhibition of the formation of several inflammation mediators. The structures of **7a** (drug with maximum anti inflammatory activity) and **8a** (drug with no anti inflammatory activity) were almost same except that in **7a** ester moiety contained methyl group and in **8a** ester moiety contained ethyl group which being bulkier than methyl group may affect the activity of the particular drug.

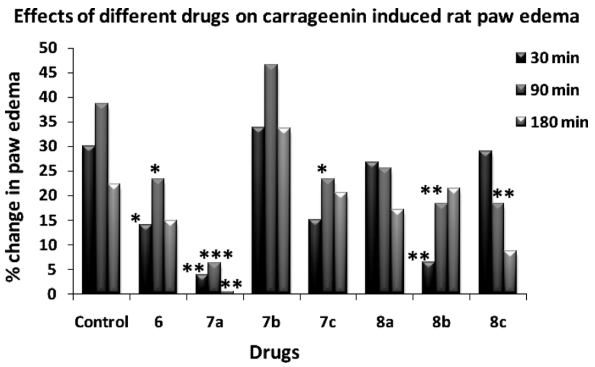

Fig. 1. Effect of Different Drugs on Carrageenin Induced Rat Paw Edema
Vertical lines indicate S.E.; $*p<0.05$; $**p<0.01$; $***p<0.001$.

The ability of compound **7a** to interact with the COX-2 was further assessed by *in silico* studies (Table 3). Figure 2 depicted the compound docked at the active site, using FlexX software for which the total score obtained was −8.72 hydrogen bonding interactions are proposed to occur near the active site of COX-2 (i) carbonyl group of dione **7a** form hydrogen bond with the hydroxyl group of Ser 530 and its

Table 3. Compounds Docking Scores and Compared with indomethacine

Compound	Docking score
Indomethacine	−12.09
6	−6.88
7a	−8.72
7b	−7.54
7c	−8.27
8a	−7.78
8b	−6.82
8c	−6.67

distance is 2.227 Å, this active site is thought to reflect inhibitory binding mode where no viable products can be produced[26] and (ii) nitrogen of the pyrazole ring of **7a** form hydrogen bond with the hydroxyl group of Tyr 355 and its distance is 2.070 Å, bound in the top channel, similar to arachidonic acid.[27] This conformation of product, in which the PGG2/PGH2 species hydrogen-bonds with the constriction site residues and bends in an L-shaped fashion at Tyr-385 and its distance is 2.596 Å, suggests that arachidonic acid was positioned properly for catalysis presented in Fig. 2.

According to these computational studies, **7a** is able to dock into the enzyme active site in the same way that has been reported for indomethacine.[28]

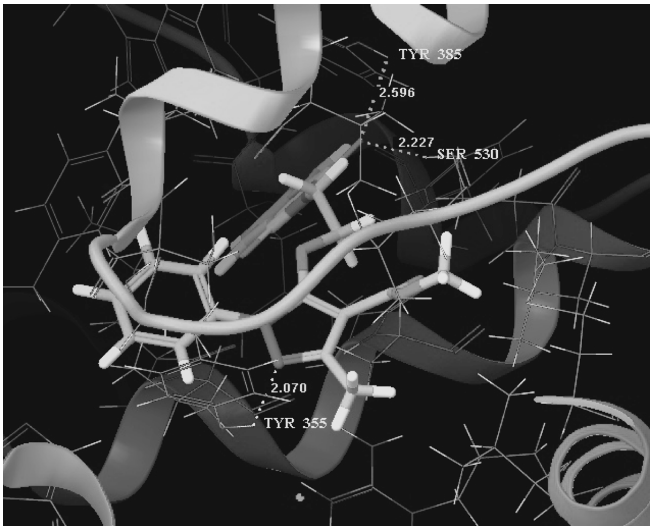

Fig. 2. Docking Analysis of the Compound (**7a**) Shown in Capped Stick Model

Amino acids are in wireframe model, amino acids form hydrogen bonding shown with broken lines and amino acid residues are indicated using three-letters code.

Experimental

5-(2-Bromo-ethoxy)-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic Acid Methyl Ester 5a Compound **4a**[23] (2.60 g, 11.2 mmol), anhydrous K_2CO_3 (2.31 g, 16.8 mmol) and 1,2-dibromoethane (4.8 ml, 56 mmol) were dissolved in 15 ml DMF and stirred at r.t. for 48 h. TLC was checked and reaction mixture was worked up. DMF was removed under pressure through rotary evaporator and crude product was extracted with $CHCl_3/H_2O$ (100/50×2 ml), dried (anhydrous Na_2SO_4), filtered and evaporated. **5a** was obtained *via* column chromatography. Eluant used was 5% ethyl acetate in chloroform. mp=48—50 °C. Yield=3.00 g (78.9 %).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.46 (s, 3H, CH_3), 3.46—3.50 (t, 2H, CH_2), 3.87 (s, 3H, OCH_3), 4.48—4.52 (t, 2H, CH_2), 7.26—7.67 (m, 5H, Ar-H).

5-(2-Bromo-ethoxy)-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic Acid Ethyl Ester 5b Compound **4b**[23] (3 g, 12.1 mmol), anhydrous K_2CO_3 (1.68 g, 12.1 mmol) and 1,2-dibromoethane (5.25 ml, 60.9 mmol) were dissolved in 20 ml DMF and stirred at r.t. for 24 h and TLC checked. Reaction mixture was worked up as above and pure **5b** was obtained as oily liquid *via* column loaded with silica in chloroform. Eluant used was 5% ethyl acetate in chloroform. Yield=3.32 g (77.4 %).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 1.36—1.41 (t, 3H, $OCH_2C\underline{H}_3$), 2.47 (s, 3H, CH_3), 3.45—3.50 (t, 2H, OCH_2), 4.29—4.36 (q, 2H, $OC\underline{H}_2CH_3$), 4.48—4.53 (t, 2H, CH_2), 7.26—7.67 (m, 5H, Ar-H).

Preparation of 1-[5-[2-(4-Acetyl-5-methyl-2-phenyl-2*H*-pyrazol-3-yloxy)-ethoxy]-3-methyl-1-phenyl-1*H*-pyrazol-4-yl]-ethanone 6 Compound **4a** (2 g, 8.6 mmol), anhydrous K_2CO_3 (1.18 g, 8.6 mmol) and 1,2-dibromoethane (0.37 ml, 4.31 mmol) were dissolved in 20 ml DMF and stirred for 24 h at r.t. TLC checked and reaction mixture was worked up. **6** was obtained in pure form through column loaded with silica in chloroform. Eluant used was chloroform. mp=88—90 °C. Yield=1.81 g (42.8%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.45 (s, 6H, CH_3×2), 3.80 (s, 6H, OCH_3×2), 4.50 (s, 4H, CH_2×2), 7.26—7.57 (m, 10H, Ar-H). ^{13}C-NMR ($CDCl_3$, 300 MHz) δ in ppm: 15.36 (CH_3), 51.08 (OCH_3), 74.00 (CH_2), 99.21 (C), 123.07 (CH), 127.43 (CH), 128.91 (CH), 137.38 (C), 150.84 (C), 154.78 (C), 163.33 (C). MS: M^+ at *m/z* 491.

5-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethoxy]-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic Acid Methyl Ester 7a Phthalimide (0.21 g, 1.4 mmol), anhydrous K_2CO_3 (0.19 g, 1.4 mmol) and **5a** (0.5 g, 1.4 mmol) were taken in 20 ml DMF and stirred at r.t. for 24 h. TLC was checked and the reaction mixture was worked up. DMF was removed under pressure and compound was extracted with $CHCl_3/H_2O$ (20/20×2 ml). The $CHCl_3$ layer was dried (anhydrous Na_2SO_4) and filtered and the solvent was removed. Pure **7a** was obtained by crystallization with 2% ethyl acetate in hexane mixture. mp=68—70 °C. Yield=0.33 g (55.2%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.42 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2), 4.50 (s, 2H, CH_2), 7.04—7.77 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$) δ in ppm: 15.18 (CH_3), 37.57 (CH_2), 51.03 (OCH_3), 71.58 (CH_2), 99.46 (C), 123.14 (CH), 127.24 (CH), 128.68 (CH), 131.86 (CH), 133.83 (CH), 137.06 (C), 150.86 (CH), 154.33 (C), 163.19 (C), 167.79 (C). Element Analysis: (i). Calculated: %N=10.37, %C=65.18, %H=4.69. (ii). Found: %N=10.17, %C=65.77, %H=4.82. MS: M^+ at *m/z* 406.

5-[2-(3-Cyano-4,6-dimethyl-2-oxo-2*H*-pyridin-1-yl)-ethoxy]-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic Acid Methyl Ester 7b and 5-[2-(3-Cyano-4,6-dimethyl-pyridin-2-yloxy)-ethoxy]-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic Acid Methyl Ester 7c 4,6-Dimethyl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (0.35 g, 2.3 mmol), anhydrous K_2CO_3 (0.65 g, 4.7 mmol) and **5a** (0.8 g, 2.3 mmol) were stirred in 15 ml DMF for 48 h at r.t. Completion of reaction was confirmed *via* TLC. Reaction was worked up. DMF was removed through rota vapour and the product mixture was extracted with $CHCl_3/H_2O$ (50/50×2 ml). The $CHCl_3$ layer was dried (Na_2SO_4), filtered and evaporated and pure **7b** and **7c** were obtained through crystallization with ethyl acetate in hexane mixture. [**7b** was crystallized with ethyl acetate and **7c** was crystallized with 1% ethyl acetate in hexane solution.]

7b: mp=174—176 °C. Yield=0.50 g (52.2%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.38 (s, 3H, CH_3), 2.42—2.43 (d, 6H, CH_3×2), 3.84 (s, 3H, OCH_3), 4.34—4.37 (t, 2H, CH_2), 4.49—4.53 (t, 2H, CH_2), 5.93 (s, 1H, CH), 7.26—7.33 (m, 5H, Ar-H). ^{13}C-NMR ($CDCl_3$) δ in ppm: 15.25 (CH_3), 20.82 (CH_3), 21.46 (CH_3), 44.98 (CH_2), 51.13 (OCH_3), 71.98 (CH_2), 99.63 (C), 101.39 (C), 109.53 (CH), 115.14 (C), 123.42 (CH), 127.83 (CH), 129.00 (CH), 136.99 (C), 150.72 (C), 151.41 (C), 154.34 (C), 158.24 (C), 160.66 (C), 163.25 (C). Element Analysis: (i). Calculated: %N=13.79, %C=65.02, %H=5.41. (ii). Found: %N=13.29, %C=64.98, %H=5.32. MS: M^+ at *m/z* 407.

7c: mp=98—100 °C. Yield=0.32 g (33.4%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.37 (s, 3H, CH_3), 2.42—2.46 (d, 6H, CH_3×2), 3.87 (s, 3H, OCH_3), 4.57—4.64 (double-d, 4H, OCH_2×2), 6.66 (s, 1H, CH), 7.17—7.66 (m, 5H, Ar-H). ^{13}C-NMR ($CDCl_3$) δ in ppm: 15.27 (CH_3), 19.91 (CH_3), 24.31 (CH_3), 51.06 (OCH_3), 65.05 (CH_2), 73.42 (CH_2), 93.89 (C), 99.29 (C), 114.41 (C), 117.68 (CH), 122.77 (CH), 126.96 (CH), 128.79 (CH), 137.36 (C), 150.85 (C), 154.32 (C), 154.85 (C), 160.21 (C), 162.95 (C), 163.38 (C). Element Analysis: (i). Calculated: %N=13.79, %C=65.02, %H=5.41. (ii). Found: %N=13.29, %C=64.63, %H=5.49. MS: M^+ at *m/z* 407.

5-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethoxy]-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic Acid Ethyl Ester 8a Phthalimide (0.20 g, 1.4 mmol), anhydrous K_2CO_3 (0.19 g, 1.4 mmol) and **5b** (0.5 g, 1.4 mmol) were taken in 10 ml DMF and stirred at r.t. for 24 h and the completion of reaction

was confirmed by TLC. The reaction was worked up. DMF was removed under pressure and the product was extracted with $CHCl_3/H_2O$ (30/30×2 ml). The organic layer was dried (anhydrous Na_2SO_4), filtered and evaporated. Pure **8a** was obtained from 2% ethyl acetate in hexane mixture. mp=86—88 °C. Yield=0.44 g (74.1%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 1.31—1.36 (t, 3H, $OCH_2C\underline{H}_3$), 2.42 (s, 3H, CH_3), 3.97—4.00 (t, 2H, CH_2), 4.23—4.31 (q, 2H, $OC\underline{H}_2CH_3$), 4.50—4.54 (t, 2H, CH_2), 7.02—7.79 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$) δ in ppm: 14.31 (CH_3), 15.27 (CH_3), 37.59 (CH_2), 59.93 (CH_2), 71.58 (CH_2), 99.72 (CH), 123.21 (CH), 127.18 (CH), 128.65 (CH), 131.88 (C), 133.81 (CH), 137.09 (C), 150.85 (C), 154.33 (C), 162.81 (C), 167.79 (C). Element Analysis: (i). Calculated: %N=10.02, %C=65.87, %H=5.01. (ii). Found: %N=9.87, %C=65.80, %H=4.97. MS: M^+ at *m/z* 420.

5-[2-(3-Cyano-4,6-dimethyl-2-oxo-2*H*-pyridin-1-yl)-ethoxy]-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic Acid Ethyl Ester 8b and 5-[2-(3-Cyano-4,6-dimethyl-pyridin-2-yloxy)-ethoxy]-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic Acid Ethyl Ester 8c 4,6-Dimethyl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (0.20 g, 1.4 mmol) and anhydrous K_2CO_3 (0.39 g, 2.8 mmol) were dissolved in 15 ml DMF for 30 min and **5b** (0.50 g, 1.4 mmol) was added slowly with stirring. The reaction was stirred at r.t. for 48 h and TLC checked. Reaction was worked up. Solvent was removed *via* rotary evaporator and the product mixture was extracted with $CHCl_3/H_2O$ (50/50×2 ml). The $CHCl_3$ layer was dried (anhydrous Na_2SO_4), filtered and evaporated. The product mixture was crystallized with 5% ethyl acetate in hexane and recrystallized with 2% ethyl acetate in hexane to obtain pure **8b** and **8c**.

8b: mp=154—156 °C. Yield=0.12 g (20.2%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 1.35—1.39 (t, 3H, $OCH_2C\underline{H}_3$), 2.38 (s, 3H, CH_3), 2.41—2.43 (d, 6H, CH_3×2), 4.29—4.37 (double-t, 4H, CH_2, $OC\underline{H}_2CH_3$), 4.50—4.52 (d, 2H, CH_2), 5.92 (s, 1H, CH), 7.25—7.323 (m, 5H, Ar-H). ^{13}C-NMR ($CDCl_3$) δ in ppm: 14.38 (CH_3), 15.36 (CH_3), 20.85 (CH_3), 21.51 (CH_3), 45.02 (CH_2), 60.07 (CH_2), 72.00 (CH_2), 109.52 (CH), 123.46 (CH), 127.83 (CH), 129.02 (CH), 137.06 (C), 150.75 (C), 151.39 (C), 158.23 (C), 160.69 (C), 162.91 (C). Element Analysis: (i). Calculated: %N=13.30, %C=65.71, %H=5.71. (ii). Found: %N=13.03, %C=65.33, %H=5.69. MS: M^+ at *m/z* 421.

8c: mp=86—88 °C. Yield=0.10 g (16.8%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 1.37—1.41 (t, 3H, $OCH_2C\underline{H}_3$), 2.36 (s, 3H, CH_3), 2.42—2.47 (d, 6H, CH_3×2), 4.30—4.37 (q, 2H, $OC\underline{H}_2CH_3$), 4.56—4.59 (t, 2H, OCH_2), 4.64—4.67 (t, 2H, OCH_2), 6.65 (s, 1H, CH), 7.18—7.66 (m, 5H, Ar-H). ^{13}C-NMR ($CDCl_3$) δ in ppm: 14.38 (CH_3), 15.39 (CH_3), 19.96 (CH_3), 24.34 (CH_3), 59.94 (CH_2), 65.09 (CH_2), 73.43 (CH_2), 93.98 (C), 99.54 (C), 114.44 (C), 117.69 (CH), 122.84 (CH), 126.94 (CH), 128.81 (CH), 137.46 (C) 150.92 (C), 154.36 (C), 154.87 (C), 160.24 (C), 163.02 (C). Element Analysis: (i). Calculated: %N=13.30, %C=65.71, %H=5.71. (ii). Found: %N=13.21, %C=65.23, %H=5.32. MS: M^+ at *m/z* 421.

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