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Synthesis and antimicrobial activity of 5-substituted-2-phenyl-3-(*o*-carboethoxyphenyl)iminomethyl indoles and their derivatives.

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Abstract : 5-Substituted indole-3-carboxaldehydes (1a-c) on reaction with ethyl anthranilate (2) in ethanol under reflux for 8 h in presence of catalytic amount of concentrated hydrochloric acid gave 5-substituted-2-phenyl-3-(*o*-carboxyphenyl)iminomethyl indoles (3a-c), which on further reaction with hydrazine hydrate in ethanol under reflux for 5 h yielded 5-substituted-2-phenyl-3-(*o*-carboethoxyhydrazidophenyl)iminomethyl indoles (4a-c). Compounds (4a-c) on reaction with acetyl acetone/ethyl acetoacetate and ethylcyanoacetate in ethanol under reflux conditions for 5 h in presence of catalytic amount of glacial acetic acid furnished 5-substituted-2-phenyl-3-[*o*-carboxy-(3′,5′-dimethylpyrazol/3′-methylpyrazol-5′-one/3′-aminopyrazol-5′-one-1′-)ylphenyl]iminomethyl indoles (5a-c), (6a-c) and (7a-c), respectively. All the newly synthesized compounds have been tested for their antimicrobial activity against *E. coli*, *S. aureus*, *P. vulgaris*, *A. niger* and *C. albicans*. Some of the compounds exhibited good activity against all the microorganisms tested.

Keywords : Schiff bases, indoles, pyrazoles, antimicrobial activity.

Introduction

Pyrazoles represent one of the most active classes of organic compounds possessing wide spectrum of biological activities, such as anti-inflammatory, antipyretic and smooth muscle relaxant activities[1,2]. Many pyrazole derivatives have been reported to possess antifungal, antidiuretic and anti-inflammatory[3–6], vasodialotory, respiration stimulating, bronchodialatory and hypotensive activities[7]. Some of the indolyl oxadiazoles, pyrazoles and triazoles have been found to exhibit good antibacterial and antifungal activities[8–12]. We have reported the synthesis and pharmacological evaluation of some substituted indole-2-(N_β-(substituted benzopyran-2′-one-3′-carboxyl)]carboxylhydrazide by making use of 3,5-disubstituted indole-2-(N_β-mono(carbethoxymalonyl)]-carboxyhydrazides[13]. In continuation of our research work on pharmacologically active compounds containing indole nucleus[14], we report here for the first time, the synthesis of 5-substituted-2-phenyl-3-[*o*-carboxy-(3′,5′-dimethylpyrazol/3′-methyl pyrazol-5′-one/3′-amino pyrazol-5′-one-l′-)ylphenyl]iminomethyl indoles (**5a-c**), (**6a-c**) and (**7a-c**), respectively by using 5-substituted-2-phenyl indole-3-carboxyaldehydes as starting materials. The antimicrobial activity of these compounds and their intermediates against *S. aureus*, *E. coli*, *P. vulgaris*, *A. niger* and *C. albicans* have been presented in this paper.

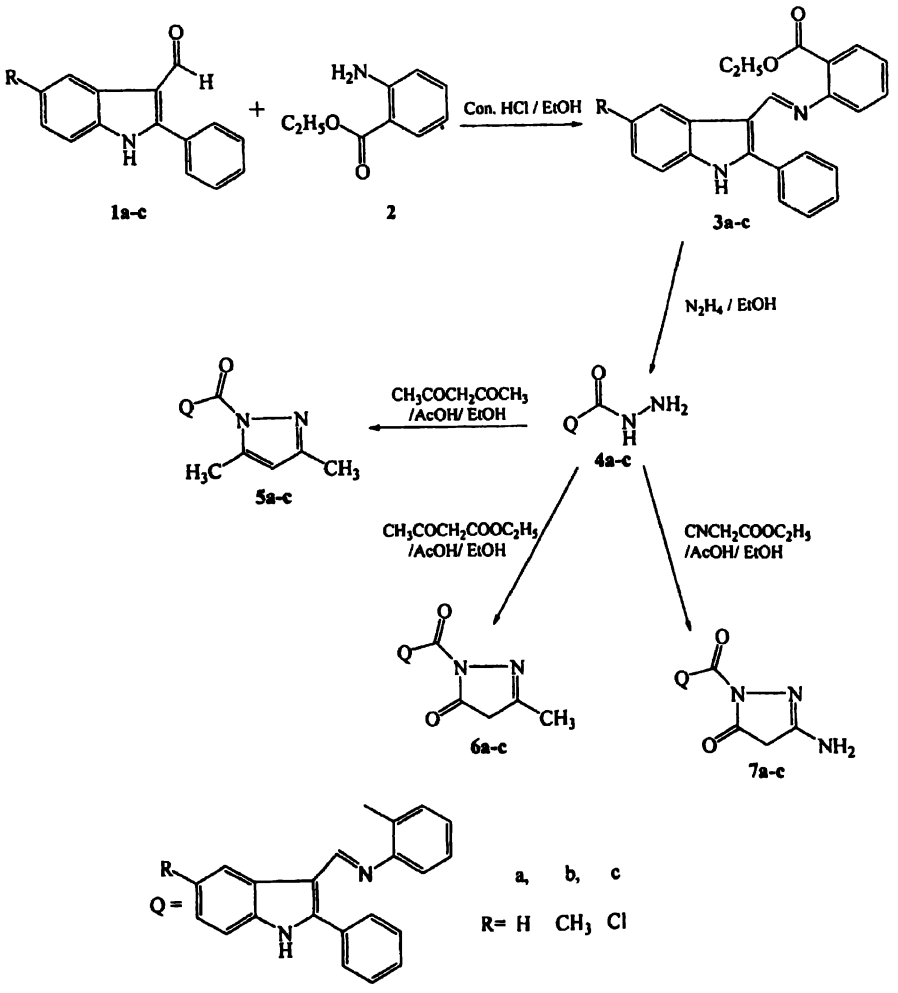
Results and discussion

The target compounds (**5a-c**), (**6a-c**) and (**7a-c**) were prepared by the method outlined in the Scheme 1. The starting compounds 5-substituted-2-phenyl indole-3-carboxyaldehydes (**1a-c**) were prepared according to the literature methods[15,16]. 5-Substituted-2-phenyl indole-3-carboxaldehydes (**1a-c**) on reaction with ethyl anthranilate (**2**) in ethanol under reflux for 8 h in presences on catalytic amount of concentrated hydrochloric acid afforded 5-substituted-2-phenyl-3-(*o*-carboxyphenyl)iminomethyl indoles (**3a-c**) in good yield.

These compounds (**3a-c**) when allowed to react with hydrazine hydrate in ethanol under reflux conditions for 5 h yielded 5-substituted-2-phenyl-3-(*o*-carboethoxyhydrazidophenyl)iminomethyl indoles (**4a-c**) in good yield.

Compounds (**4a-c**) on further reaction with acetyl acetone/ ethyl acetoacetate and ethyl cyanoacetate in ethanol under refluxing condition in presence of catalytic amount of glacial acetic acid afforded 5-substituted-2-phenyl-3-[*o*-carboxy-(3′,5′-dimethylpyrazol/3′-methylpyrazol-5′-one/ 3′-aminopyrazol-5′-one-l′-)ylphenyl)iminomethyl indoles (**5a-c**), (**6a-c**) and (**7a-c**) respectively in good yield (Scheme 1).

trum showed two distinct singlets at 10.10 and 8.90 ppm due to proton on indole nitrogen and an azomethine proton respectively. Twelve aromatic protons have resonated as multiple in the region 6.66–8.45 ppm. Two protons of methylene group and three protons of methyl group of ethyl ester function have appeared as quartet and triplet at 4.32 and 1.41 ppm respectively. These data prove the formation of compound **3c** from compound **1c**.



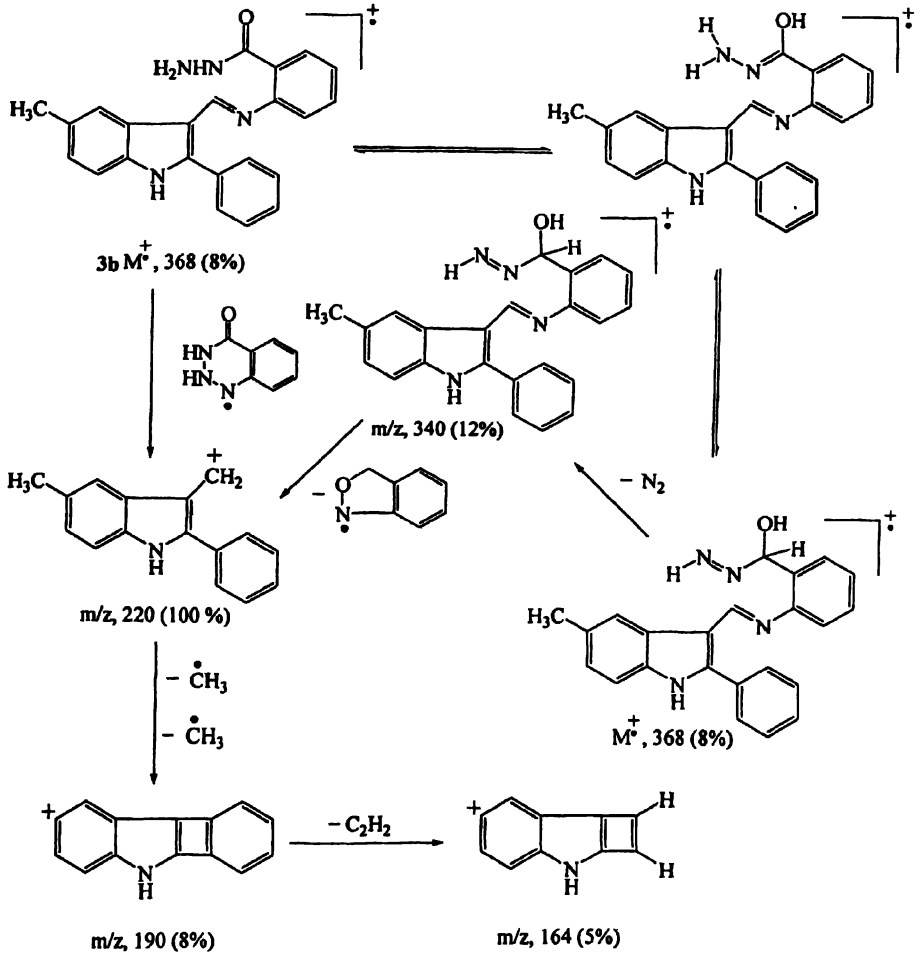
Scheme 1

The IR spectrum of compound **3c** displayed absorption bands at 3325, 1700 and 1660 cm^{-1} due to NH, C=O and C=N functions respectively. It's ^1H NMR spectrum showed two distinct singlets at 10.10 and 8.90 ppm due to proton on indole nitrogen and an azomethine proton respectively. Twelve aromatic protons have resonated as multiple in the region 6.66–8.45 ppm. Two protons of methylene group and three protons of methyl group of ethyl ester function have appeared as quartet and triplet at 4.32 and 1.41 ppm respectively. These data prove the formation of compound **3c** from compound **1c**.

Compound **4c** in its IR spectrum showed absorption bands at 3480, 3450, 3405, 3350, 1680 and 1660 cm^{-1} due to NH/NH_2, C=O and C=N functions respectively.

In its ^1H NMR spectrum (in δ), signals due to protons of CONH, proton on indole nitrogen and azomethine proton have appeared at 12.06, 11.48 and 8.95 ppm as broad singlet, singlet and singlet respectively. Twelve aromatic protons have resonated as multiple in the region 7.0–8.44 ppm. Protons of NH_2 group have appeared as broad singlet at 6.30 ppm. These data clearly prove the conversion of compound **3c** to compound **4c** by its reaction with hydrazine hydrate.

Further the mass spectrum of compound **4b** obtained by the reaction of compound **3b** with hydrazine hydrate under the conditions described for the conversion of **3c** to compound **4c** showed a molecular ion peak $M^{+\cdot}$ at 368 (8%) which is equivalent to its molecular weight. This molecular ion underwent fragmentation by the expulsion of $C_7H_6N_3O$ species gave a fragment ion peak at *m/z* 220 (100%) which is also a base peak. This base peak was also obtained by sequential expulsion of N_2 and C_7H_6NO fragments from the molecular ion via the intermediacy of fragment ion at *m/z* 340 (12%). Further the fragment ion at *m/z* 220 (100%) by the simultaneous loss of CH_3 and CH_3 radicals gave another fragment ion peak at *m/z* 190 (8%). This fragment ion on further loss of C_2H_2 species gave a fragment ion peak at *m/z* 164 (5%) (Scheme 2). The fragment pattern of the molecular ion peak of compound **4b** is in consistency with its structure.



Scheme 2

IR spectrum of compound **5c** displayed bands at 3390, 1700, 1680 and 1660 cm^{-1} due to NH, C=O and C=N/C=N functions respectively. In its ^1H NMR spectrum (in δ), proton on indole nitrogen appeared as a singlet at 8.28 ppm. A multiplet which accounts for twelve aromatic protons and an azomethine proton was observed in the region 7.16–7.76 ppm. Methine proton of pyrazole moiety and protons of two methyl groups attached to pyrazole moiety have resonated as singlets at 6.78, 2.48 and 1.60 ppm respectively. These data are in agreement with the structure of the compound **5c**.

Compound **6c** in its IR spectrum displayed absorptions bands at 3350, 1700, 1680, 1650 and 1630 cm^{-1} due to NH, C=O/C=O and C=N/C=N functions respectively. In its ^1H NMR spectrum (in δ), proton on indole nitrogen and an azomethine proton have resonated as singlets at 10.04 and 9.04 ppm respectively. Twelve aromatic protons have appeared as multiplet in the region 7.10–8.52 ppm. Two methylene protons of pyrazolone moiety and three protons of methyl group attached to 3-position of pyrazolone ring have resonated as singlets at 3.72 and 2.60 ppm respectively. These data confirm the formation of compound **6c** obtained by the reaction of **4c** with ethyl acetoacetate.

IR spectrum of compound **7c** displayed absorption bands at 3425, 3300, 1690, 1680, 1620 and 1590 cm^{-1} due to NH/NH_2, C=O/C=O and C=N/C=N functions respectively. In its ^1H NMR spectrum (in δ), signals due to proton on indole nitrogen and azomethine proton have appeared as two distinct singlets at 10.50 and 8.30 ppm respectively. Twelve aromatic protons have resonated as multiplet in the region 6.60–8.20 ppm. Two protons of amine group attached to 3-position of pyrazolone moiety and methylene protons of pyrazolone ring have appeared as a broad singlet and singlet at 4.10 and 3.54 ppm respectively. These data are in confirmity with structure **7c**.

Compound **7b** synthesized by following the procedure described for compound **7c** starting from compound **4b** in its mass spectrum gave molecular ion peak at M^+ 435 (2%) which is equivalent to its molecular weight. The molecular ion underwent fragmentation by the expulsion of $C_{16}H_{12}N$ species to give a peak at *m/z* 217 (11%) which in turn underwent sequential expulsion of CO molecule and C_2H_2N radical to give a fragment ion peaks at *m/z* 189 (7%) and *m/z* 149 (100%, base peak) respectively. The fragmentation pattern of the molecular ion of the compound **7b** is in confirmity with its structure (Scheme 3).

The physical and spectral data of all the newly synthesized compounds were tabulated in Table 1.

Antimicrobial activity :

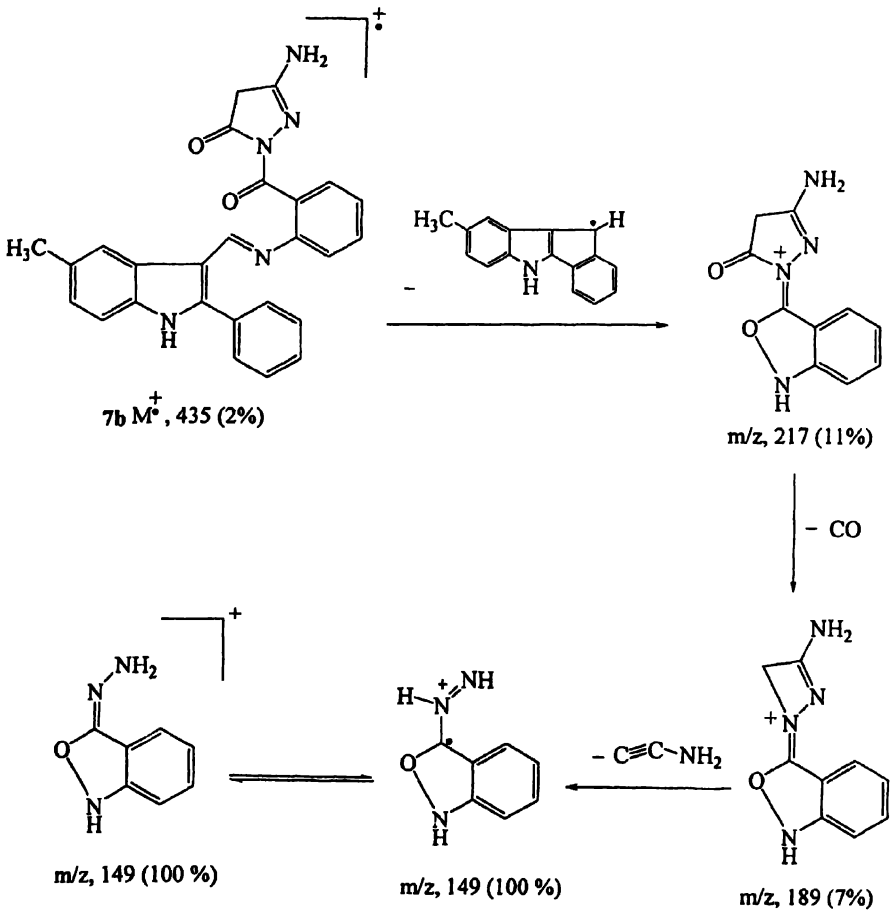
Compounds (**3a-c**), (**4a-c**), (**5a-c**), (**6a-c**) and (**7a-c**) were evaluated *in vitro* for their antimicrobial activity against *E. coli*, *S. aureus*, *P. vulgaris*, and antifungal activity against *A. niger* and *C. albicans* using DMF as solvent at 100 μg/ml concentration by cup-plate method. The activity was compared with the known drugs, Gentamycin and Nystatin for antibacterial and antifungal activity respectively. The zone of inhibition after 24 h of incubation at 37 °C in case of antibacterial activity and 72 h in case of antifungal activity was compared with that of standards. The results showed that the compounds **3c, 4a, 4c, 5a, 5b, 5c, 6a, 6b, 6c, 7a** and **7c** were found to be highly active against *S. aureus*. Compounds **3c, 4a, 4b, 4c, 5b, 6a, 6b, 6c, 7a** and **7c** exhibited high activity against *E. coli*. Compounds **3a, 3c, 4c, 5b, 5c, 6a** and **6c** showed good activity against *P. vulgaris*. Compounds **3a, 5a, 5b, 6a, 6b** and **6c** showed good activity against *A. niger* and compounds **4a, 4c, 5a, 5b, 6a, 6b, 6c, 7a, 7b** and **7c** showed good inhibition against *C. albicans* and rest of the compounds showed very less to moderate activity against all the organisms tested. Out of all the compounds tested, only compounds **5b, 6a** and **6c** showed good activity against all the organisms tested (Table 2).

Experimental

Melting points were determined in open capillary tubes and are uncorrected. IR spectrum (nujol) were recorded on a Hitachi 270-50 infrared spectrophotometer (ν_{max} in cm^{-1}), ^1H NMR spectra (chemical shifts in δ ppm) on EM-390 NMR spectrophotometer using DMSO-d_6/$CDCl_3$ as solvents and TMS as an internal standard and mass spectra on a Jeol D-300 instrument.

Synthesis of 5-substituted-2-phenyl-3-(o-carboethoxyphenyl)iminomethyl indoles (**3a-c**) :

5-Substituted-2-phenylindole-3-carboxaldehydes (**1a-c**)



Scheme 3

Table 1. Physical and spectral data of the synthesized compounds

Compd.	R	M.p. (°C) (Yield in %)	Elemental analysis (%) : Found (Calcd.)			IR (cm^{-1})
			C	H	N	
3a	H	220 (84)	78.51 (78.26)	5.21 (5.43)	7.37 (7.60)	3325 (NH), 1700 (C=O), 1660 (C=N)
3b	CH_3	138 (84)	78.66 (78.53)	5.56 (5.76)	7.60 (7.32)	3340 (NH), 1700 (C=O), 1660 (C=N)
3c	Cl	271 (82)	71.88 (71.64)	4.64 (4.72)	6.68 (6.76)	3325 (NH), 1700 (C=O), 1660 (C=N)
4a	H	290 (85)	74.35 (74.57)	5.26 (5.08)	15.62 (15.82)	3475 (NH), 3425, 3375, 3350 (H/NH_2), 1670 (C=O), 1635 (C=N)
4b	CH_3	268 (86)	75.16 (75.00)	5.18 (5.43)	15.26 (15.21)	3450 (NH), 3400, 3360, 3325 (NH/NH_2), 1660 (C=O), 1630 (C=N)

Table-1 (contd)

4c	Cl	253	67 83	4 56	14 65	3480 (NH), 3450, 3405, 3350 (NH/NH_2),
		(83)	(68 04)	(4 38)	(14 43)	1680 (C=O), 1660 (C=N)
5a	H	275	77 75	5 13	13 19	3350 (NH), 1690 (C=O), 1675, 1650
		(75)	(77 51)	(5 26)	(13 40)	(C=N)
5b	CH_3	271	77 57	5 32	12 77	3375 (NH), 1720 (C=O), 1700, 1680
		(77)	(77 77)	(5 55)	(12 96)	(C=N)
5c	Cl	262	71 52	4 37	12 68	3390 (NH), 1700 (C=O), 1680, 1660
		(73)	(71 68)	(4 64)	(12 04)	(C=N)
6a	H	166	74 15	4 57	13 55	3425 (NH), 1700, 1670 (C=O), 1620,
		(85)	(74 28)	(4 76)	(13 33)	1590 (C=N)
6b	CH_3	275	74 55	4 88	12 65	3375 (NH), 1700, 1670 (C=O), 1650,
		(88)	(74 65)	(5 06)	(12 90)	1630 (C=N)
6c	Cl	264	68 45	4 36	12 55	3350 (NH), 1700, 1680 (C=O), 1650,
		(89)	(68 72)	(4 18)	(12 33)	1630 (C=N)
7a	H	211	71 52	4 75	16 40	3350, 3310 (NH/NH_2), 1700, 1680
		(79)	(71 25)	(4 51)	(16 62)	(C=O), 1630, 1660 (C=N)
7b	CH_3	234	71 49	5 09	16 30	3425, 3375 (NH/NH_2), 1710, 1680
		(83)	(71 72)	(4 82)	(16 09)	(C=O, 1630, 1610 (C=N)
7c	Cl	306	65 68	4 17	15 11	3425, 3300 (NH'NH_2), 1690, 1680
		(86)	(65 93)	(3 95)	(15 38)	(C=O), 1620, 1590 (C=N)

Table 2. Antimicrobial activity of the synthesized compounds

Compd	Conc (μg/ml)	Zone of inhibition (mm)				
		Antibacterial activity			Antifungal activity	
		S aureus	*E coli*	*P vulgaris*	*A niger*	*C albicans*
3a	100	12	15	15	16	14
3b	100	16	17	14	14	14
3c	100	17	15	16		14
4a	100	15	16	14	14	15
4b	100	14	16	13	13	14
4c	100	15	17	16	14	16
5a	100	14	13	13	16	15
5b	100	15	15	17	17	16
5c	100	14	13	15	12	13
6a	100	16	16	15	17	16
6b	100	14	15	14	17	17
6c	100	18	20	21	21	19
7a	100	15	16	14	14	15
7b	100	12	12	14	13	15
7c	100	15	17	14	14	15
Gentamycin	100	18	20	20		
Nystatin	100				21	19
DMF	-					

(0 001 mol) and ethyl anthranilate (2) (0 001 mol) were refluxed in ethanol (10 ml) with catalytic amount of concentrated hydrochloric acid (1–2 drops) for 8 h on water bath Excess of ethanol was removed by distillation Resi-

due obtained was further purified by crystallisation in methanol to afford (**3a-c**) in good yield.

Synthesis of 5-substituted-2-phenyl-3-(o-carboethoxy-hydrazidophenyl)iminomethyl indoles (**4a-c**) :

An aqueous solution of hydrazine hydrate (15 ml, 80%) was added to a suspension of appropriate 5-substituted-2-phenyl-3-(*o*-carboethoxyphenyl)iminomethyl indoles (**3a-c**) (0.046 mol) in ethanol (30 ml). The resultant mixture was refluxed for 5 h, cooled to room temperature and separated solid filtered, washed with ethanol, dried and crystallized in ethanol to furnish (**4a-c**) in good yield.

Synthesis of 5-substituted-2-phenyl-3-[o-carboxy-(3',5'-dimethylpyrazol/3'-methylpyrazol-5'-one/3'-aminopyrazol-5'-one-1'-)ylphenyl]iminomethyl indoles (**5a-c**), (**6a-c**) and (**7a-c**) :

To a solution of (**4a-c**) (0.001 mol) in ethanol (5 ml), appropriate diketones like acetyl acetone/ethyl acetoacetate/ethyl cyanoacetate (0.001 mol) were added and the mixture was refluxed for 5 h in presence of catalytic amount of glacial acetic acid. Excess of ethanol was removed by distillation and the crystalline residue obtained was filtered, washed with little ethanol, dried and crystallized from ethanol to afford (**5a-c**), (**6a-c**) and (**7a-c**), respectively.

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