Synthesis and Biological Activity of Chiral Dihydropyrazole: Potential Lead for Drug Design

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Abstract: Dihydropyrazole, a small bioactive molecule, is a prominent structural motif found in numerous pharmaceutically active compounds. The chiral dihydropyrazole structure has been demonstrated to bear important biological activities such as anticancer, antimicrobial, antimalarial, antinociceptive, antiviral, antitubercular, antiinflammatory, anticonvulsant and steroidal, and can also act as MAO inhibitors, CB1 receptor antagonists and nitric oxide synthase inhibitors. The review describes the latest advances in the synthesis of dihydropyrazole derivatives incorporating physiologically active substances. It is the first attempt at a general and systematic account of the extensive literature data on this subject.

Keywords: Dihydropyrazole, biological activities, SAR, lead.

1. INTRODUCTION

The significance of the dihydropyrazole heterocycle is evidenced by its presence as a structural subunit in a variety of pharmacologically interesting compounds. The naturally occurring biologically active flavonoids and isoflavonoids and suitably substituted α, β-unsaturated ketones may serve as ideal constituents to access dihydropyrazole, a small bioactive molecule prevalent in numerous pharmaceutically active compounds. During the past years, considerable evidence has been accumulated to demonstrate the efficacy of dihydropyrazole derivatives as potential anticancer, antimicrobial, antimalarial, antinociceptive and antiviral agents [1-11]. A great variety of this class of compounds have been synthesized, many of them are currently being tested and/or clinically evaluated for new drug discovery. The purpose of this article is to provide a critical account of the procedures utilized in the literature for the synthesis and biological activity of dihydropyrazole and to review the available information on potential lead in drug design. Furthermore, this article, which covers our recent research in this field, aims to summarize and comment on several data concerning the biological properties of these compounds.

2. BIOLOGICALLY ACTIVITY

2.1. Anticancer Activity

Cancer has been one of the biggest threats to human life and is expected to become the leading cause of death over the next few decades. Development of resistance against the existing anticancer drugs keeps research window open in search of newer anticancer molecules. Unfortunately, the scope in this area looks rather grim as it is extremely difficult to design a molecule which can selectively inhibit the proliferation of abnormal cells without adversely affecting the normal cells. Of late, several scientific reports have appeared on antitumor, antiproliferative or anticancer potential of dihydropyrazole derivatives. Based on these reports, we evaluated their usefulness as antitubercular and antiproliferative agents [12]. Furthermore, Cuberes *et al.* employed some dihydropyrazole derivatives for the treatment of specific cancers such as brain, bone, lip, mouth, esophageal, stomach, liver, bladder, pancreas, ovary, cervical, lung, breast and skin but found them particularly effective in treating colon, bowel and prostate cancers [12].

In 2004, Abdou and his team synthesized 2, 4-dihydropyrazole glucosides **1**-**3** (Scheme **1**) and tested them for their antitumor activities [13]. The effect of these nucleosides on proliferation of HL60 and mouse EL4 cell lines was evaluated. The IC_{50} values for compounds **1**-**3** indicated that HL60 cells were more sensitive than EL4 cells (Table **1**).

Again, in 2005, Manna *et al.* synthesized a series of substituted dihydropyrazoles (Scheme **2**) and tested them for their ability to inhibit *P*-glycoprotein-mediated multi-drug resistance by direct binding to a purified protein domain containing an ATP-binding site and a modulator interacting region [14]. Compounds **4** and **5** were found to bind to *P*-glycoprotein with greater affinity.

In 2009, in the pursuit of preparing dihydropyrazole derivatives with potent activity, Havrylyuk, D. *et al.* obtained some novel thiazolone-based compounds bearing

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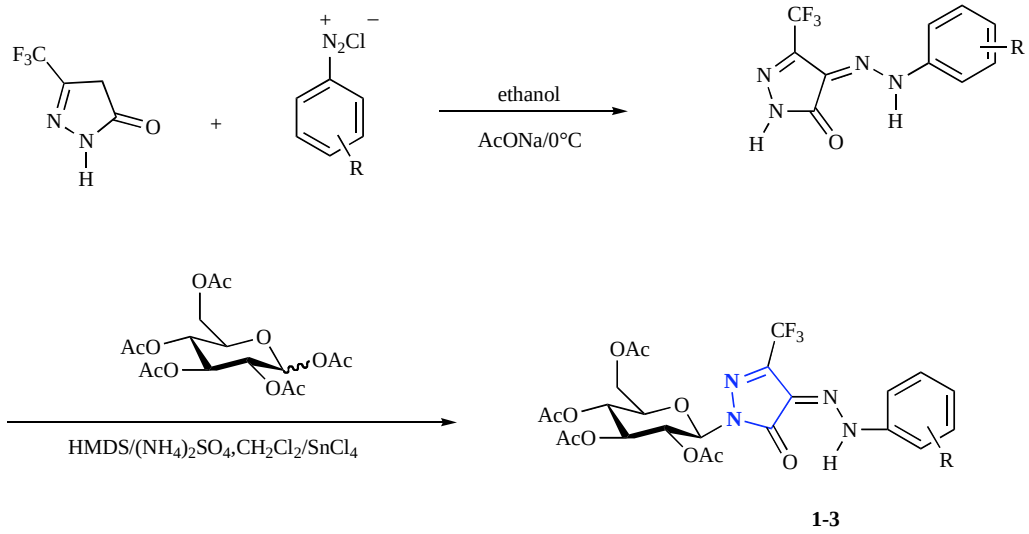
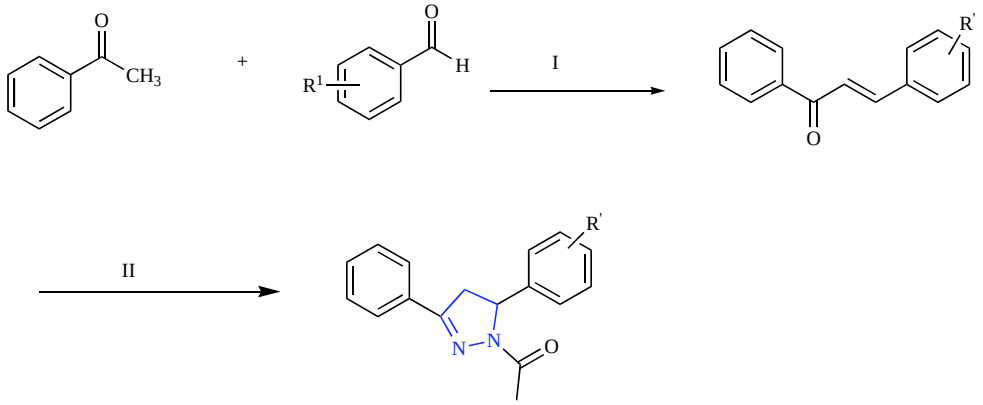


1-3

Scheme 1. Synthetic route to compounds **1-3**.

Table 1. Antitumor Activity of Compounds 1-3

Compd	R	IC_{50} (μM)	
		HL 60 proliferation (human)	EL 4 proliferation (mouse)
1	*m*-CF_3	20.2±1.7	25.3±0.9
2	*p*-F	16.4±1.8	19.4±2.0
3	*m*-F	27.2±1.3	28.7±2.0



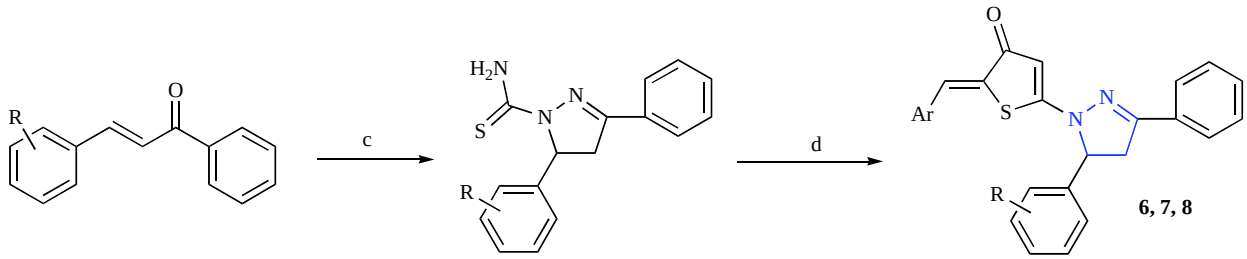
4: R' = H; **5:** R' = 2-OMe

Scheme 2. Synthetic route to compounds **4-5**.

Reagents and conditions: (i) $Ba(OH)_2$, EtOH, 25 °C; (ii) N_2H_4, CH_3COOH, reflux.

5-aryl-3-phenyl-4,5-dihydro-*1H*-pyrazol-1-yl framework [15] and demonstrated their *in vitro* anticancer activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancer cell lines (Tables **2-5**). The SAR study revealed that: (1) anticancer activity of compounds **6, 7** is sensitive to the nature of substituent in position 5 of thiazolonecycle, (2) introduction of *p*-OH group in 5-benzylidene fragment enhanced potency, and (3) linking position of pyrazoline fragment with thiazolone core did not influence antitumor activity.

Subsequently, eleven dihydropyrazole derivatives (Scheme **4**) were synthesized and tested for their antiproliferative activities against PC-3 cell and A431 cell lines *in vitro* [16]. From the IC_{50} values, it is obvious that compounds **9-13** exhibited strong inhibitory activities against PC-3 cell lines comparable to those displayed by 5-fluorouracil as a positive control (Table **4**). Scanning Table **4**, we found that there was clear SAR against PC-3 cell lines. Inspection of the chemical structures of the final compounds suggests that the nature of group $\mathbf{R^2}$ in the title compounds



Scheme 3. Synthetic route to compounds **6-8**.

Reagents and conditions: (c) thiosemicarbazide (1.2 equiv), KOH (2.5 equiv), EtOH, reflux 8 h, 75–80%; (d) Ar-CHO (1.2 equiv), $ClCH_2COOH$ (1.0 equiv), AcONa (2.0 equiv), AcOH, reflux 5 h, 65–69%.

Table 2. Anticancer Activity of Compounds 6, 7 (Screening Data at10^{-5} M Concentration)

Compd	R	Ar	60 Cell lines assay in 1-dose 10^{-5}M conc.				Active
			Mean growth%	Range of growth%	The most sensitive cell line	Growth% of most sensitive cell line	
6	2-OH	4-OH-C_6H_4	1.13	-96.31 to 134.28	SK-MEL-5 (Melanoma)	-96.31	Active
7	2-OH	3,5-$(OMe)_2$-4-OH-C_6H_2	43.59	-57.04 to 123.35	DU-145 (Prostate cancer)	-57.04	Active

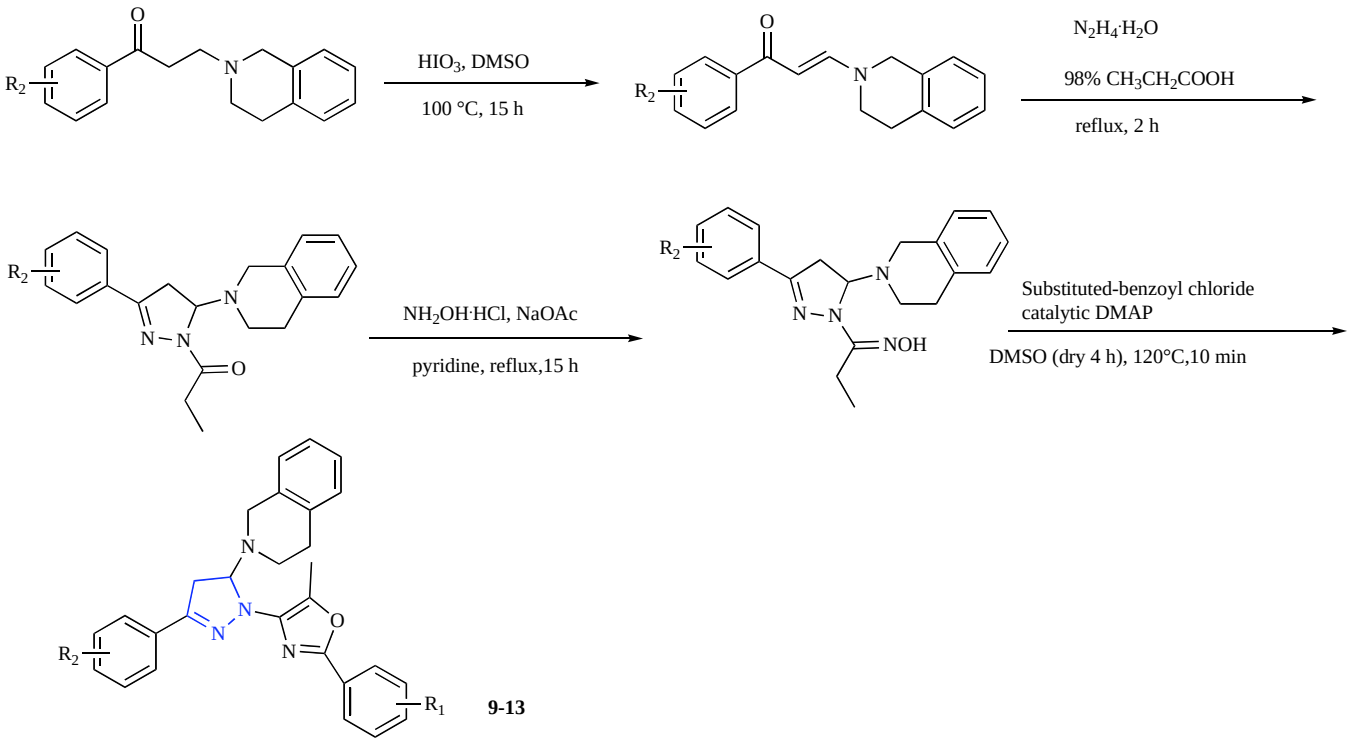
Table 3. Summary of Anticancer Screening Data at Dose-Dependent Assay

Compd	R	Ar	N	Log GI_{50}		
				N1	Range	MG-MID
8	2-OH	Ph	54	54	-5.28 to -4.43	-5.41
6	2-OH	4-OH-C_6H_4	57	57	-5.78 to -4.59	-5.46
7	2-OH	3,5-$(OMe)_2$-4-OH-C_6H_2	57	57	-6.37 to -4.74	-5.61
Compd	**R**	**Ar**	**N**	**log TGI**		
				N2	**Range**	**MG-MID**
8	2-OH	Ph	54	52	-5.54 to -4.00	-4.90
6	2-OH	4-OH-C_6H_4	57	23	-5.48 to -4.00	-4.45
7	2-OH	3,5-$(OMe)_2$-4-OH-C_6H_2	57	55	-5.53 to -4.37	-5.14
Compd	**R**	**Ar**	**N**	**log LC_{50}**		
				N3	**Range**	**MG-MID**
8	2-OH	Ph	54	37	-5.54 to -4.00	-4.38
6	2-OH	4-OH-C_6H_4	57	5	-4.31 to -4.00	-4.02
7	2-OH	3,5-$(OMe)_2$-4-OH-C_6H_2	57	45	-5.27 to -4.00	-4.58

N – number of human tumor cell lines tested at the 2nd stage assay.

N1, N2, N3 – number of sensitive cell lines, against which the compound possessed considerable growth inhibition according to mentioned parameter (parameters log GI50, log TGI and log LC_{50}<-4.00).

significantly influence the antitumor activity. With a fluorinated substituent on the phenyl ring, the compounds exhibited enhanced bioactivity against PC-3 cell lines. Furthermore, the presence of a heterocycle-functional group in the title compounds plays an important role in the antiproliferative activity.



Scheme 4. Synthetic route to compounds **9-13**.

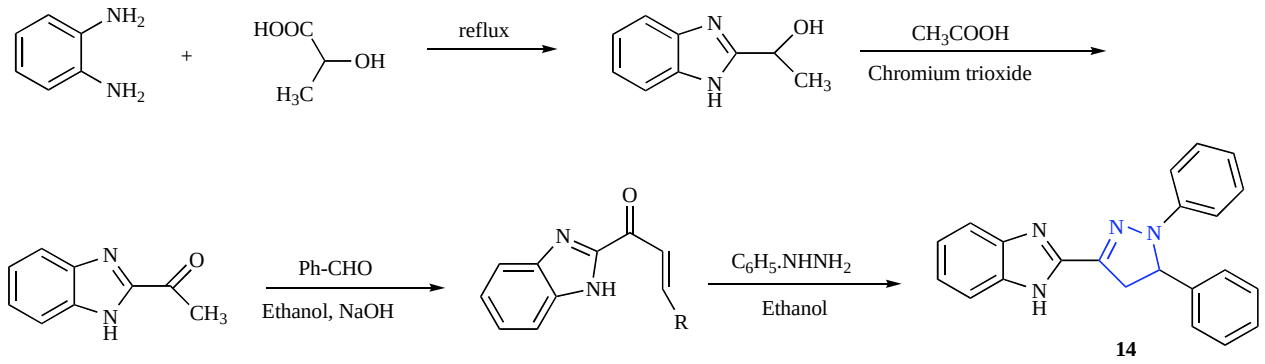
Table 4. Cytotoxic Activity of Compounds 9-13 Against PC-3 and A431 Cell Lines

Compd	R_1	R_2	IC_{50}/(μg·mL)	
			PC-3	A431
9	2-CH=CH_2	H	4.6±0.13	4.6±0.13
10	2-(Furyl-2-yl)benzoyl	H	4.6±0.13	4.6±0.13
11	H	2-F	3.9±0.18	100.3±0.04
12	2-(Furyl-2-yl)benzoyl	2-F	4.6±0.13	4.6±0.13
13	2-CH=CH_2	2-F	4.6±0.13	4.6±0.13
5-Fluorouracil			2.2±0.12	2.1±0.20

Similarly, amongst the various compounds synthesized by Shaharyar *et al*., dihydropyrazole derivative **14** (Scheme **5**) appeared to be the most active anticancer candidate of the series [17]. The results of *in vitro* test of **14** against various lines are depicted in Table **5**; promising activity was observed against Leukemia CCRF-CEM and RPMI-8226 cell lines with GI_{50} values of 2.23 and 2.76 μM respectively. Based on close examination on substitutions, it may be concluded that the role of electron donating groups (–OCH_3) on the phenyl ring at 5^{th} position of pyrazoline has great influence on anticancer activity, also similar role of electron donating group onantipro-liferative activity, although the compound did not exhibit very good activity but remarkable superiority within the series when electron doting substituent was present. This assumption is further supported by the presence of electron donating groups on the phenyl ring particularly (–OCH_3) in the structure of Combretastatin-A4 and its pyrazoline derivatives.

More recently, Liu and his group [18, 19] synthesized a series of novel coumarin derivatives containing 4, 5-dihydropyrazole moiety as potential telomerase inhibitors (Scheme **6**). The bioassay tests showed that compounds **15-18** exhibited potentially high activity against SGC-7901 cell. The modified TRAP assay results revealed that compounds **15** and **17** could strongly inhibit telomerase with IC_{50} values of 2.0±0.07 and 1.8±0.35 μM respectively (Table **6**).

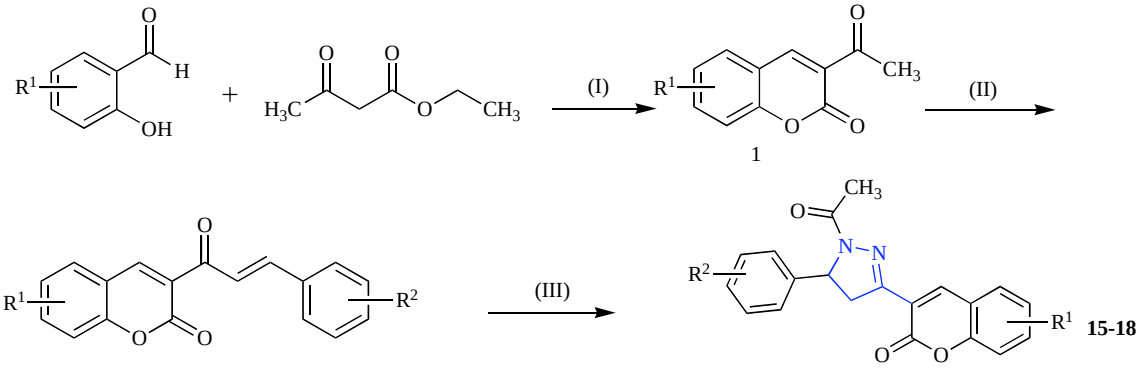
In an effort to elucidate the mechanism by which the title compound can induce anticancer activity in the human gastric cell SGC-7901, molecular docking of the potent inhibitors **15, 18** into ATP binding site of telomerase was performed to simulate a binding model derived from



Scheme 5. Synthetic route to compound **14**.

Table 5. The Results of *In Vitro* Analysis of Compound 14 in μM

Panel	Cell Line	GI_{50}		TGI	LG_{50}
		Concentration per cell line	Subpanel MID		
Leukemia	CCRF-CEM	2.23	53.82	6.82	88.7
	HL-60(BT)	>100		>100	>100
	K-562	>100		>100	>100
	MOLT-4	17.9		>100	>100
	RPMI-8226	2.76		10.3	76.6
	SR	>100		>100	>100
Prostate Cancer	PC-3	6.27	19.69	49	>100
	DU-145	33.1		>100	>100
MID		16.87			



Scheme 6. Synthetic route to compounds **15-18**.

Reagents and conditions: (I) piperrazine, 25~30 °C, 1 h. (II) substituted-benzaldehyde, piperidine, ethanol, reflux, 6 h. (III). 80% NH_2-$NH_2.H_2O$, 98% CH_3COOH, reflux, 2 h.

telomerase structure (3DU6. pdb) (Figs. **1-4**) The compound **15**, **4, 5-**dihydropyrazole ring projects into a hydrophobic region, which is comprised of the side chains of Pro 201, Asp 202, Ser 203, Ala 204, that is important for the potent inhibitory activity of **15**. These residues influenced the accessibility of the hydrophobic pocket that flanks the ATP binding site, and their size can be a key factor in controlling telomerase selectivity. In the other end of the ATP-binding pocket, the O of dihydropyrazole acetyl interacted with the residue Ile 199, which made the 3D structure more stable. Compound **18** resides in a novel location, binds in a distinct manner among the residues (140–343). They can bind well

Table 6. Biological Properties of Compounds 15-18

Compd	R^1	R^2	IC_{50}/(μg·mL)	IC_{50} (μM)
			SGC-7901	telomerase
15	H	H	2.69±0.60	2.0±0.136
16	Br	H	4.6±0.13	4.6±0.13
17	7-F	4-OH	2.98±0.16	
18	7-F	H	8.51±0.70	
Ethidium bromide				2.5 ±0.8
5-Fluorouracil			7.38±0.98	

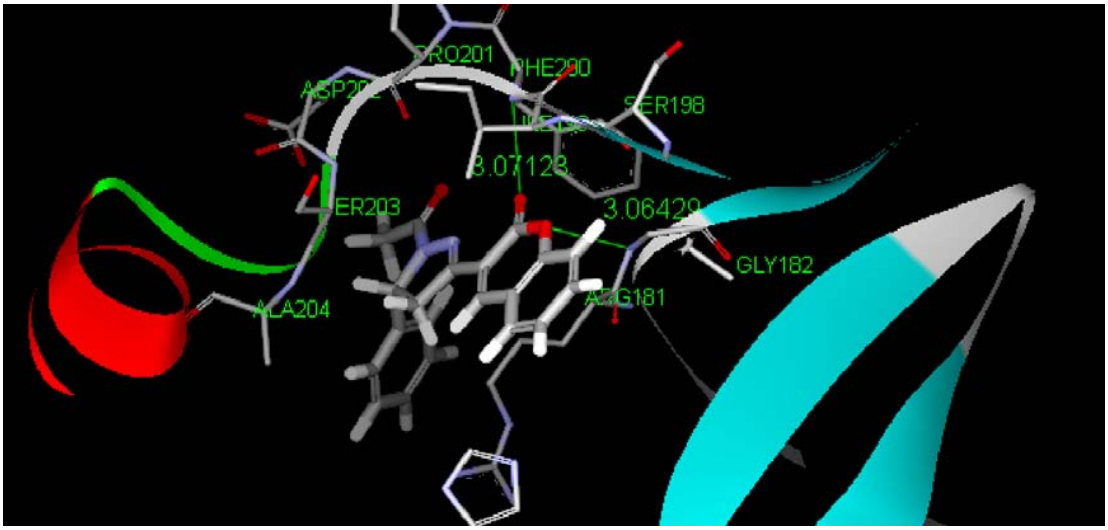


Fig. (1). Molecular docking modeling of compound **15** with telomerase; the small molecule and the critical interaction of 3DU6 are represented by sticks. Panel is a view into the active site cavity.

with the active site. An intramolecular hydrogen bond is observed between the N–H····F: 2.31347 Å, with amino hydrogen group of Ala-255.

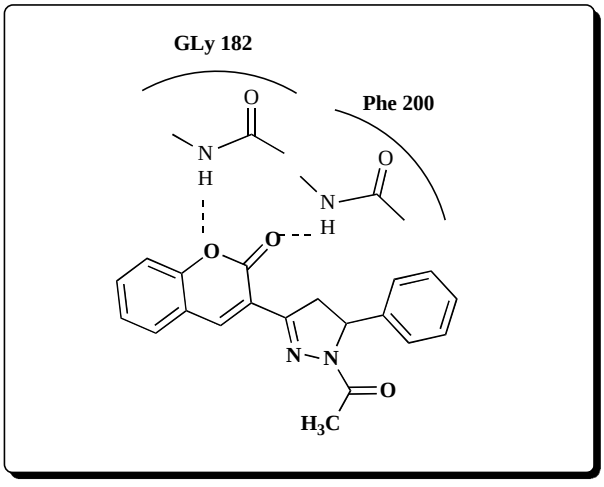


Fig. (2). Schematic representation of the binding mode of **15** in the ATP binding site of 3DU6.

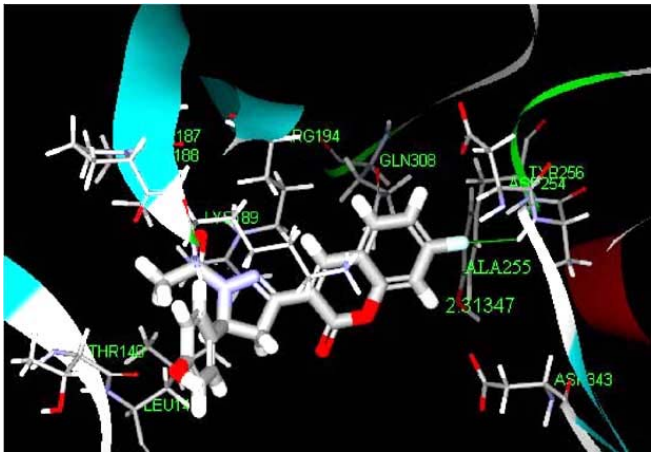


Fig. (3). Molecular docking modeling of compound **18** with telomerase; the small molecule and the critical interaction of 3DU6 are represented by sticks. Panel is a view into the active site cavity.

In another interesting report, Insuasty and co-workers [20] obtained a series pyrazolic chalcones, some of which

were screened by US NCI for their ability to inhibit 60 different human tumor cell lines. The study revealed that compound **19** (Fig. **5**) was associated with remarkable activity against leukemia, renal cancer and non-small cell lung cancer cell lines. According to the *in vitro* bioassays, the most significant GI50 values ranged from 0.04 to 11.4 μM.

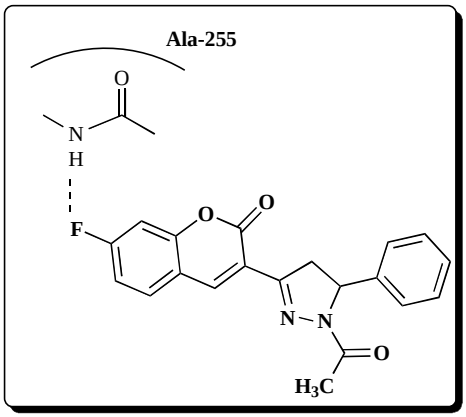


Fig. (4). Schematic representation of the binding mode of **18** in the ATP binding site of 3DU6.

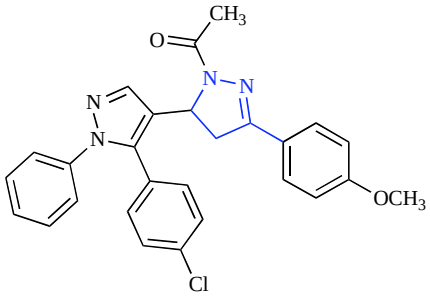


Fig. (5). Structure of compound **19**.

On the other hand, some of the dihydropyrazole derivatives synthesized by Lv, P. C. *et al*. exhibited significant EGFR kinase inhibitory activity [21]. In particular, compound **20** (Fig. **6**) displayed excellent result with IC_{50} of 0.07 μM, which was comparable to the positive control erlotinib. Antiproliferative assay results indicated that selected dihydropyrazole derivatives possessed high antiproliferative activity against MCF-7.

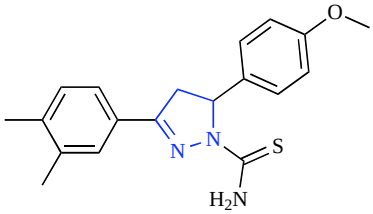


Fig. (6). Structure of compound **20**.

Compound **20** which displayed the most potent EGFR inhibitory activity was selected for further molecular docking study, which was performed on the binding model based on the EGFR complex structure (1M17.pdb). In the binding model, compound **20** is nicely bound to the EGFR kinase with its N–H group project toward the side chain carbonyl group of Asp831, forming a more optimal H-bond interaction. Based on the significant EGFR inhibitory activity of dihydropyrazole derivatives containing thiourea skeleton, it can be concluded that this H-bond plays an important effect in the EGFR inhibitory. Also, the oxygen atom of the methoxy group of compound **20** forms hydrogen bond with Gly697. Furthermore, the intermolecular hydrogen bonds of compound **20**, whose effect has already been counted in the binding energy, were also investigated in order to find useful information for drug design (Figs. **7**-**9**).

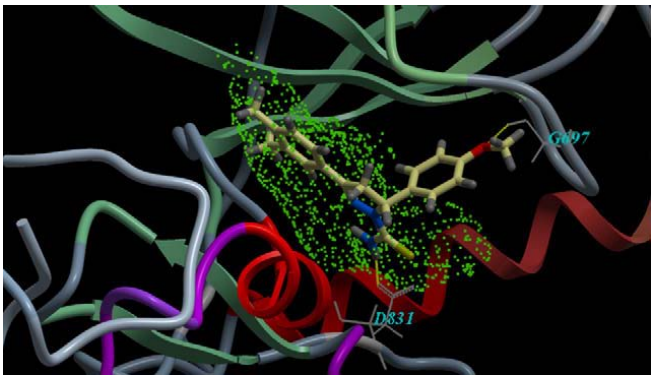


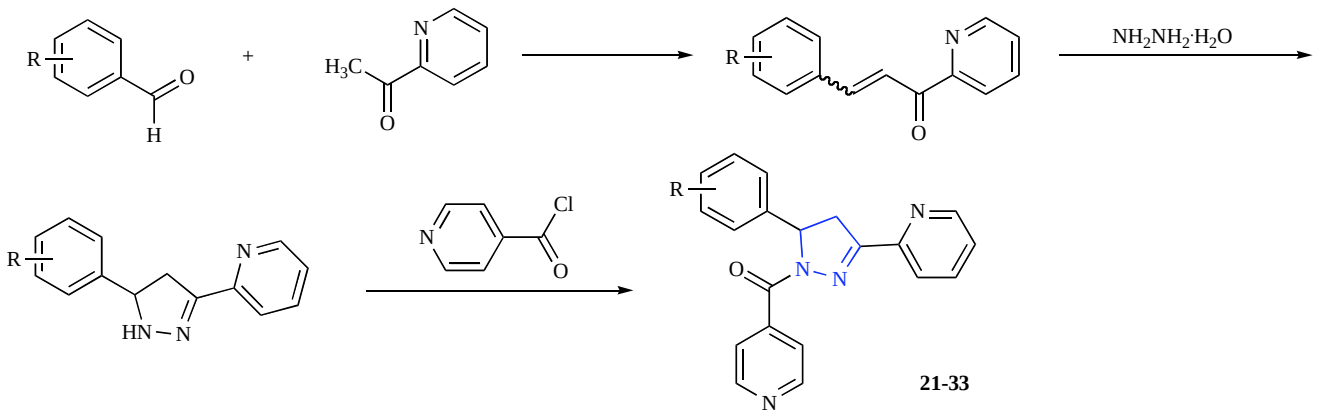
Fig. (7). Molecular docking modeling of compound **20** with EGFR kinase: for clarity, only interacting residues are displayed. The H-bond (yellow line) is displayed as line.

2.2. Antimicrobial Activity

Multidrug resistance is a condition enabling a disease-causing organism to resist distinct drugs or chemicals targeted at eradicating the organism. Recently, multidrug-resistant Gram-positive bacteria, such as methicillin-resistant *S. aureus* and penicillin-resistant *S. pneumoniae* have started to pose serious issues in medical science to tackle with. To overcome the limitations of the known DNA gyrase inhibitors, it has become imperative to identify new class of compounds. Many dihydropyrazole derivatives are well acknowledged to possess a wide range of antibacterial bioactivities. Much attention was paid to pyrazole as a potential antimicrobial agent after the discovery of the natural pyrazole C-glycoside, pyrazofurin which demonstrated a broad spectrum of antimicrobial activity. Selected dihydropyrazole derivatives which were used as potent and selective inhibitors against DNA gyrase could lead to bacterial cell death.

In 2001, Mamolo *et al.* synthesized a series of 5-aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives **21**-**33** (Scheme **7**) and tested their *in vitro* antimycobacterial activities [22]. All the synthesized compounds exhibited interesting activity against the tested strains of *M. tuberculosis* with MIC values ranging from 8 to 16 μg/mL (Table **7**).

Scanning Table **7**, we found that compounds **21**–**33** were inactive against the tested strains of *C. albicans* and *E. coli*, and exhibited a very low activity toward the strain of *S. epidermidis*. Since the substituents on the phenyl residue at



Scheme 7. Synthetic route to compounds **21-23**.

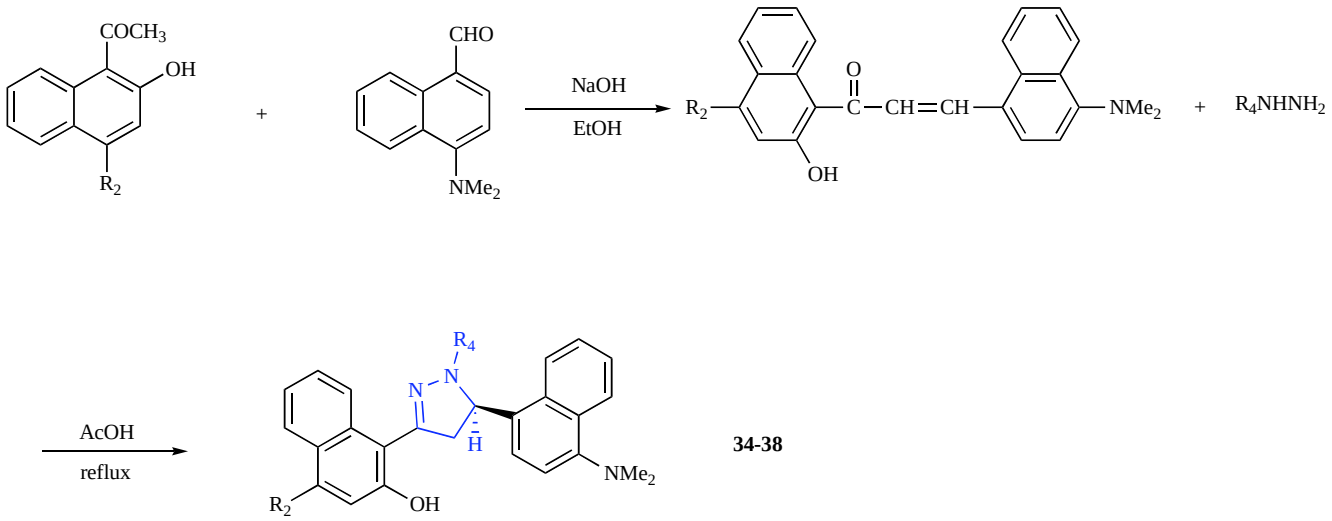
Table 7. Activity of Compounds 21-23 Against *M. tuberculosis* H_{37}Rv and *M. tuberculosis* H4 Clinical Isolate

Compd	R	MIC (μg/mL)	
		M. tuberculosis H_{37}*Rv*	*M. tuberculosis H4 clinical isolate*
21	H	8	8
22	2-Cl	8	8
23	3-Cl	8	8
24	4-Cl	8	8
25	3-Br	8	16
26	3-Br	8	16
27	4-Br	8	16
28	2-F	16	16
29	3-F	16	16
30	4-F	16	16
31	2-CH_3	8	8
32	3-CH_3	8	16
33	4-CH_3	16	16

the 5-position on the cycle do not exert any important modulatory role on the activity, pyrazoline derivatives, modified by the replacement of the substituted phenyl residue with heterocyclic rings, may lead to compounds with higher antimycobacterial activity. The presence of the 2-pyridinyl residue at 3-position on the pyrazoline cycle may exert an important role on the activity of the tested compounds. It will be of interest to verify if analogous 3,5-diaryl-pyrazoline derivatives without the *ortho*-hydroxy substituent on the phenyl ring at the 3-position on the cycle may exhibit antimycobacterial properties. On the other hand, compounds **21**–**33** are characterized by the presence in the 3-position of the 2-pyridinyl substituent, which can contribute to the activity. The replacement of the isonicotinoyl group in compounds **21**–**33** with other acyl derivatives may be important in order to establish the possible significance of the 2-pyridinyl residue with respect to the antimycobacterial activity.

In the following year, Azarifar, D. *et al.* [23] obtained twenty-four 3, 5-dinaphthalene-1-yl substituted 2-pyrazolines bearing certain specific substituents both on the naphthalene and pyrazoline rings (Scheme **8**). The compounds were tested *in vitro* for antimicrobial activity against the test organisms *E. coli, S. aureus, K. pneumoniae, P. mirabillis, S. dysentry and S. typhii* at a temperature of 37 °C (±1°C). The results revealed that compounds **34-38** positively acted against all six organisms, among which **37** was found to be the most effective as it could inhibit the microbial growth at much lower concentrations (Table **8**).

The clear SAR shows that the compounds containing chloro, hydroxo and dimethylamino -$N(CH_3)_2$ group as substituents on the naphthalene rings have been found to be



Scheme 8. Synthetic route to compounds **34-38**.

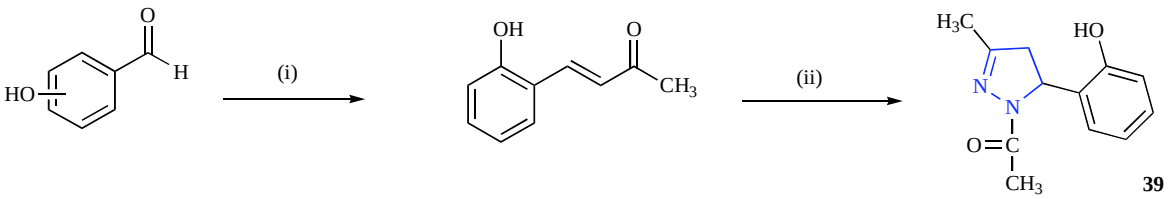
Table 8. Antimicrobial Activity of Compounds 34-38

Compd	R_2	R_4	MIC values (mg/mL) against test organisms					
			E. coli	*S. aureus*	*K. pneumonae*	*S. typhii*	*S. dysentary*	*P. mirabilis*
34	H	H	63	63	31	63	125	63
35	Me	Ph	125	63	125	31	63	63
36	H	$CONH_2$	63	31	16	125	63	31
37	Cl	$CONH_2$	63	16	16	63	31	63
38	Me	$CONH_2$	125	31	63	31	63	63
Chloram-phenicol (standard antibiotic)			-	25	6	12	25	50

very effective antimicrobial agents. In addition, the presence of a carboxamido -$CONH_2$ substituent group at the N-1 position of the 2-pyrazoline rings is shown to contribute substantially to the antimicrobial activity.

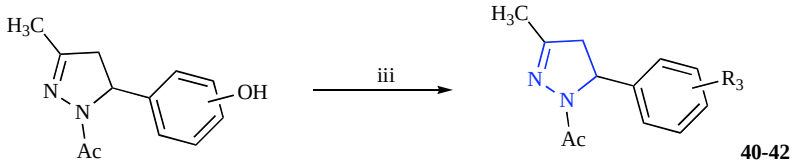
In order to explore antibacterial activity of 5-arylpyrazole derivatives, Liu and co-workers [24] prepared compounds **39** and **40-42** from suitably substituted derivatives as demonstrated in Schemes **9** and **10** respectively. These were subsequently screened for their antibacterial activities against two Gram-positive bacterial strains and two Gram-negative bacterial strains. As could be observed from the data presented in Table **9**, compounds **39** and **40** both displayed potent activity against *B. subtilis* with a common MIC value of 1.562 μg/mL comparable to the figure shown by positive control penicillin, whereas compounds **39** and **41** exhibited significant antibacterial activity against *P. aureus*, being similar to that displayed by Kanamycin (MIC = 3.125 μg/mL). It is also worthwhile to note that compounds **41** and **42** revealed high antibacterial activity against *E. coli*. Based on the experimental data, it can be concluded that *N*-acetyl arylpyrazole derivatives exhibit higher antibacterial activity against selected strains than thiosulfate arylpyrazole and acetamide arylpyrazole derivatives. Furthermore, the fluorine and chlorine substituents play an important role on the antibacterial activity. In addition, the compounds that have phenolic hydroxy groups show a higher antibacterial activity against the tested strains than other compounds.

In continuation to this work, Liu, X. H. *et al.* [25] further synthesized a series of novel dihydropyrazole derivatives from substituted benzaldehydes or alkyl phenyl ketone as shown in Schemes **11-13**. The MICs of the compounds against four bacteria were tested (Table **10**). To elucidate the mechanism by which the pyrazole derivatives induce antibacterial antivity, the inhibitory activity of selected compounds was examined against DNA gyrase isolated from *S. aureus* and *E.coli* (Table **11**). The results indicated that compounds **43** and **47** possessed potent antibacterial antivities to inhibit *S. aureus* and *E. coli.* From the structure–activity relationships, it can be concluded that all5-phenyl-3-methyl-4,5-dihydropyrazole derivatives displayed poor activity against four strains, but only some 3-phenyl-5-phenyl-4,5-dihydropyrazole derivatives showed good activity against bacterial strains, specially against *S. aureus* ATCC 6538 and *P. eruginosa* ATCC 13525. The most active



Scheme 9. Synthetic route to compound **39**.

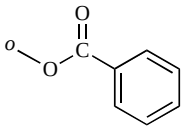
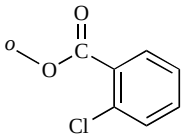
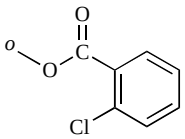
Reagents and conditions: (i): CH_3COCH_3, NaOH, EtOH, 25°C, 15 h; (ii): $N_2H_4 \cdot H_2O$, 98% CH_3COOH, reflux, 2 h.



Scheme 10. Synthetic route to compounds **40-42**.

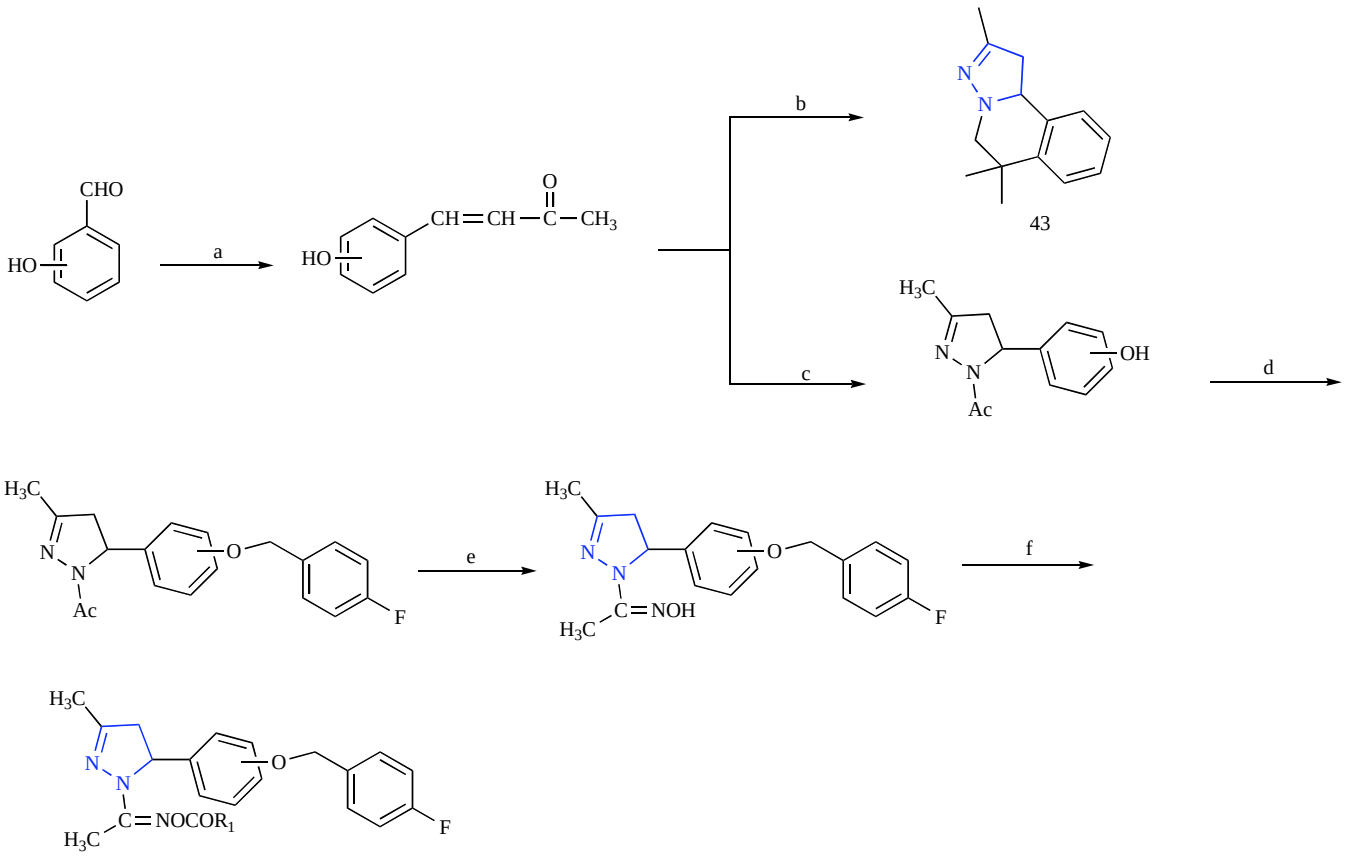
Reagents and conditions: (iii): (a) acid chloride, pyridine, $CHCl_3$, reflux, 6 h. (b) 1- (chloromethyl) benzene, NaOH, CH_3COCH_3, reflux, 10 h.

Table 9. The MICs of Compounds 40-42 Against the Four Strains

Compd	R_3	Microorganisms			
		Gram positive		Gram negative	
		B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*
39		1.562	>50	3.125	>50
42		6.25	>50	6.25	6.25
40		1.562	>50	12.5	>50
41		12.5	>50	3.125	6.25
Penicillin		1.562	1.562	6.25	6.25
Kanamycin		0.39	1.562	3.125	3.125
Novobiocin		0.78	3.125	1.562	3.125

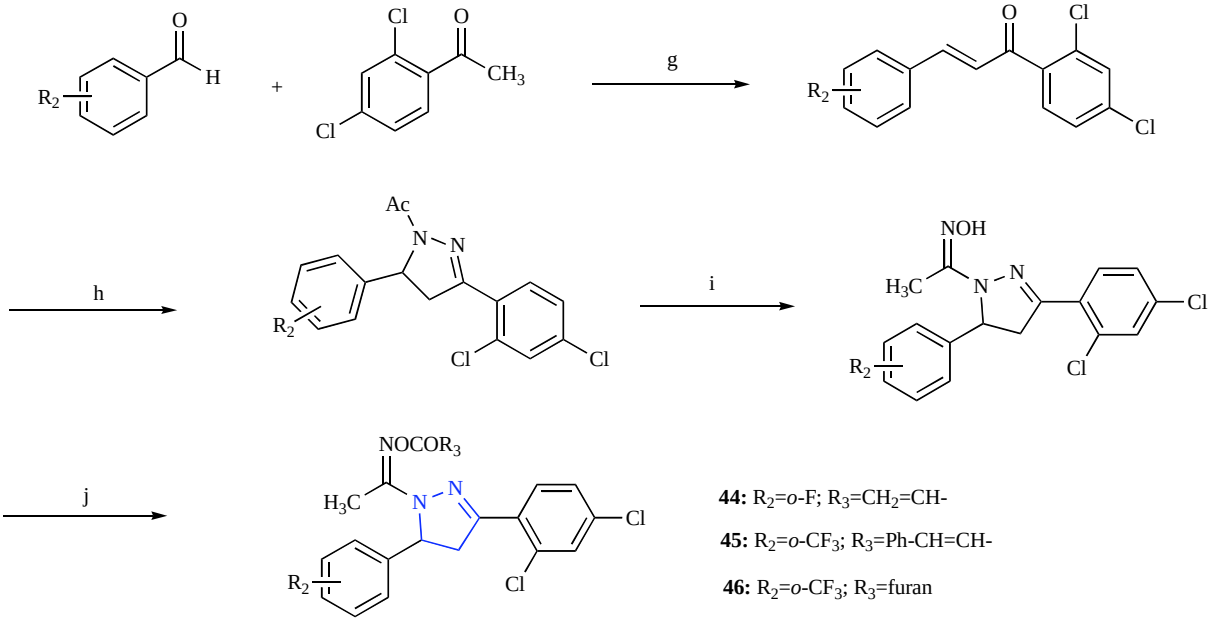
agent against the bacterial strains was 5-methoxy-3-phenyl-4,5-dihydropyrazol. Further, the presence of furan group in the oxime ester part played an important role in the antimicrobial activity, however, introduction of alkyl group in the oxime ester depressed the antimicrobial activities.

In another illustration [26], α,β-unsaturated fluorinated ketones were successfully converted into a series of 3-substituted 5-hydroxy-5-trifluoro [chloro]methyl-1*H*-1-isonicotinoyl-4,5-dihydropyrazoles, as shown in Scheme **14**. The pyrazole derivatives were evaluated against INH-susceptible *M. tuberculosis* and four INH-resistant clinical solates; some of them (**50-55**) showed significant activity against *M. tuberculosis* H37Rv with MIC values being recorded within the range 0.77 μM -18.66 Mm (Tables **12**, **13**). Analysis of the correlation between activity against *M.tuberculosis* H37Rv and the compound's chemical structure allows us to draw some conclusions. It is important to observe that trifluoromethyl-substituted pyrazoles were more active than the respective trichloromethyl-substituted pyrazoles. Antimicrobial activity was dependent on the



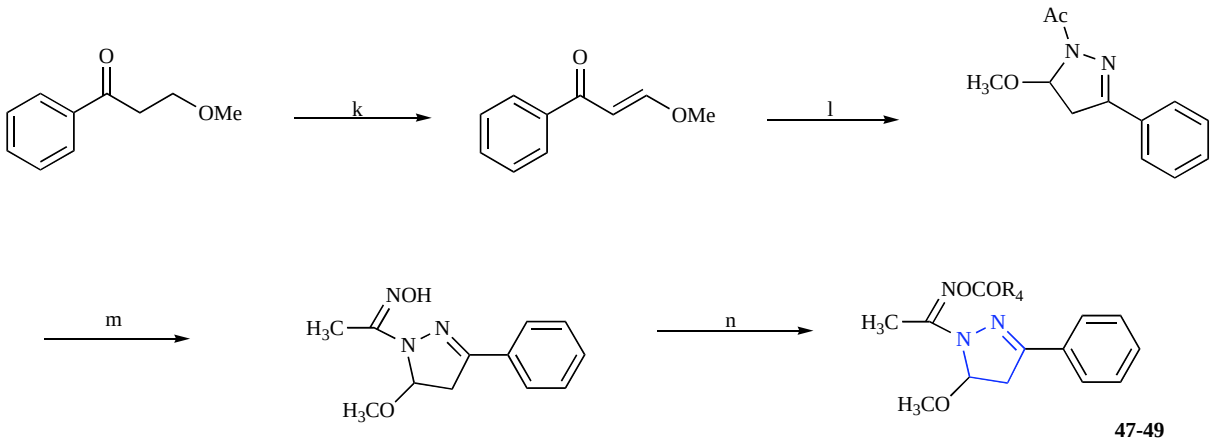
Scheme 11. Synthetic route to the key intermediate.

Reagents and conditions: (a) CH_3COCH_3, NaOH, C_2H_5OH, 25 °C, 15 h; (b) $N_2H_4.H_2O$, 98% CH_3COOH, reflux, 2 h; (c) $N_2H_4.H_2O$, n-butanol, reflux, 10 h; (d) *p*-F-Ph-CH_2Cl, NaOH, $CHCl_3$, reflux, 3 h; (e) $NH_2OH{\cdot}HCl$, $NaCH_3CO_2$, pyridine, reflux, 8 h; (f) RCOCl, NMM, $CHCl_3$.



Scheme 12. Synthetic route to compounds **44-46**.

Reagents and conditions: (g) H_2SO_4, MeOH, reflux, 10 h; (h) $N_2H_4{\cdot}H_2O$, 98% CH_3COOH, reflux, 4 h; (i) $NH_2OH{\cdot}HCl$, $NaCH_3CO_2$, pyridine, reflux, 8 h; (j) RCOCl, NMM, $CHCl_3$.



Scheme 13. Synthetic route to compounds **47-49**.

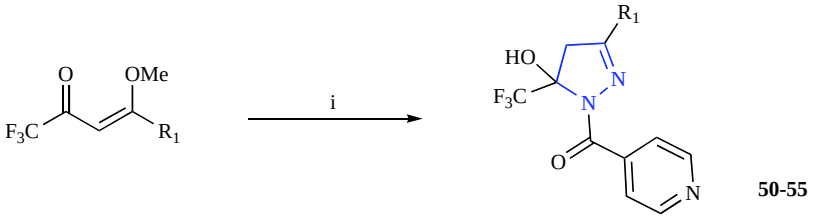
Reagents and conditions: (k) HIO_3.DMSO, 60 °C , 15 h; (l) $N_2H_4.H_2O$, 98% CH_3COOH, reflux, 2 h; (m) $NH_2OH{\cdot}HCl$, $NaCH_3CO_2$, reflux, 10 h; (n) RCOCl, NMM, $CHCl_3$.

Table 10. The MICs of Compounds 43-49 Against Four Bacteria

Compd	R	Gram-positive		Gram-negtive	
		B.subtilis	***S. aureus***	***P. fluorescens***	***E. coli***
43		1.25	3.125	3.125	1.562
44	CH_2=CH-	3.125	3.125	12.5	25.0
45	Ph-CH=CH-	1,562	12.5	3.125	6.25
46	2-Furan	1.562	3.125	1.562	12.5
47	C_6H_4-*p*-CF_3	1.562	1.562	1.562	3.125
48	Ph-CH=CH	1.562	6.25	3.125	12.5
49	2-Furan	0.78	6.25	1.562	3.125
Penicillin		1.562	1.562	6.25	6.25
Kanamycin		0.39	1.562	3.125	3.125
Novobiocin		0.78	3.125	1.562	3.125

Table 11. The Inhibitory Activity of Compounds 43-49

Compd		IC_{50} (μg/mL)	
		***S. aureus* DNA gyrase**	***E. coli* DNA gyrase**
43	Isoquinoline	0.25	0.125
46	2-Furan	0.125	1.0
44	CH_2=CH	0. 5	100
45	2-Furan	0. 5	8.0
47	C_6H_4-*p*-CF_3	0.125	0.25
49	2-Furan	4.0	0.25
Novobiocin		0.28	0.31



Scheme 14. Synthetic route to fluorinated pyrazoles **50-55**.

Reagents and conditions: (i) **50:** $NH_2NHC(O)C_5H_4N$, MeOH, r.t. 48 h; **51-53:** $NH_2NHC(O)C_5H_4N$, MeOH, 60-65 ^oC, 16 h; **54-55:** $NH_2NHC(O)C_5H_4N$, MeOH, 20-25 ^oC, 24 h.

Table 12. ***In Vitro*** **Anti-Mycobacterial Activity of Compounds 50-55 Against Five Mycobacterium Strains**

Compd	R_1	MIC(μM)				
		H37Rv	**RGH101**	**RGH102**	**RGH103**	**RGH104**
Isoniazid		1.45	>72.9	>72.9	>72.9	>72.9
50	H	0.77	12	24.13	48	24.13
51	Me	5.71	22.89	366.3	366.3	5.71
52	Ph	18.66	74.63	>298.5	>298.5	18.66
53	4-MePh	2.23	8.94	71.63	286.53	4.47
54	2-thienyl	>293.26	>293.26	>293.26	>293.26	>293.26
55	furyl	9.6	38.46	38.46	19.23	38.46

Table 13. Activity of Compounds 50-53 and Isoniazid Against Non-Tuberculous Mycobacteria

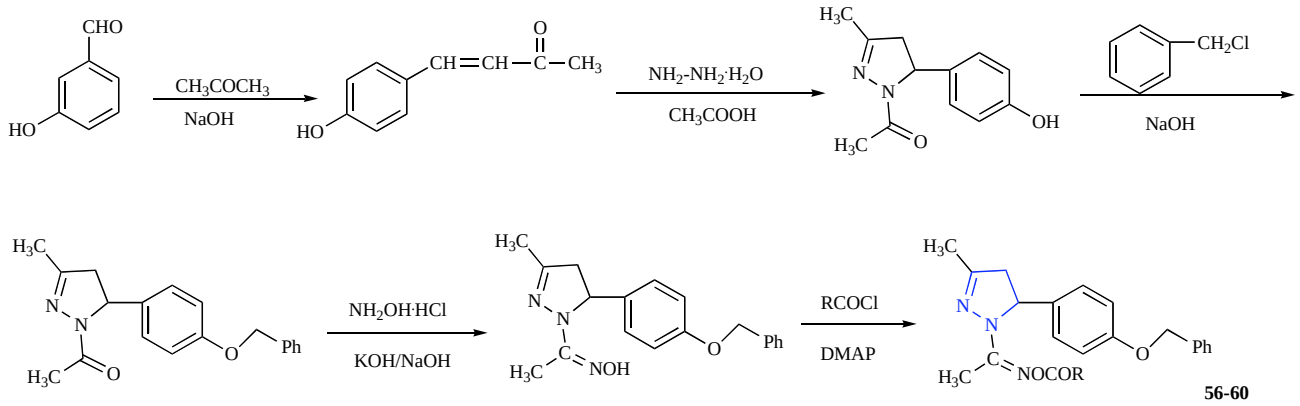
Compd	MIC (μM)				
	50	**51**	**52**	**53**	**INH**
M.avium	6.25	5.90	1.72	38.40	180
M.fortuitum	3.12	183	71.6	>306	180
M.kansasii	6.25	45.75	17.9	38.40	120

R^1 substituent. Furthermore, the substituents of the phenyl ring appear to have a significant effect on antimicrobial activity because the tolyl-substituted compound. Also, the INH moiety is not the only structure responsible for the antimycobacterial activity because pyrazolines with different substituents exhibited very different activities.

Afterwards, starting from 3-hydroxy benzaldehyde, Liu *et al*. [27] synthesized a series of dihydropyrazol derivatives using a five-step synthetic protocol (Scheme **15**). All the compounds were screened for their antibacterial potential *in vitro* against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. The results were indicative of existence of a good correlation between the MIC and the IC_{50} for compounds **56-58** (Tables **14**, **15**), suggesting that inhibition of DNA gyrase by the title compound could lead to the suppression of bacterial cell growth.

On the other hand, when 2-chloro benzaldehyde was employed as the starting material (Scheme **16**) [28] and the synthesized dihyropyrazols were screened for their antibacterial potential *in vitro* against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*, it was observed that **62** and **65** were associated with significant activity with MIC value of 1.562 μg/mL against all four bacteria. The compounds **62**, **63** and **65** displayed moderate inhibition against DNA gyrase (IC_{50}＝1.6-2.5 μg/mL) (Tables **16**, **17**). From the structure-activity relationships, it can be concluded that the dihydropyrazole oxime esters containing triaryl moieties exhibit potentially higher activities against the selected microorganisms than the derivatives having diaryl moities. Further, the presence of functional group and nitro functionalities in the oxime ester part plays an important role in the antimicrobial activities.

Relatively recently, in 2009, Abdel-Wahab and co-workers [29] obtained another series of pyrazoline derivatives (Scheme **17**) and tested them at 100 μg concentration for their *in vitro* antimicrobial activities



Scheme 15. Synthetic route to compounds **56-60**.

Table 14. MIC (μg/mL) of Compounds 56-58

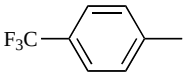
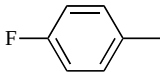
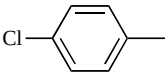
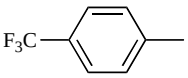
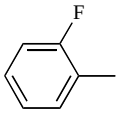
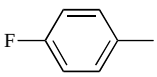
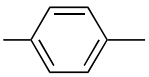
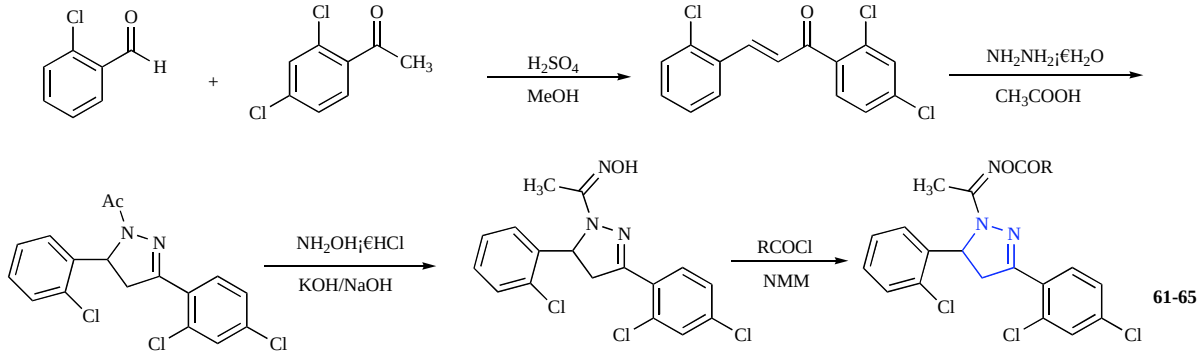
Compd	R	Gram poditive		Gram negative	
		B. subtilis	***S. aureus***	***P. aeruginosa***	***E. coli***
56		1.562	1.562	1.562	1.562
57		1.562	6.25	6.25	1.562
58		3.125	3.125	3.125	3.125
Penicillin		1.562	1.562	6.25	6.25
Kanamycin		0.39	1.562	3.125	3.125

Table 15. Inhibitory Effects of Compounds 56-60 Against DNA Gyrase

Compd	56	59	SPFX	57	58	60
R						
IC_{50} (μg/mL)	1.9	9.5	0.21	2.1	2.5	16.5



Scheme 16. Synthetic route to compounds **61-65**.

Table 16. MIC (μg/mL) of Compounds 61-65

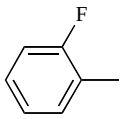
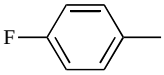
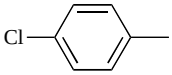
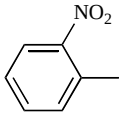
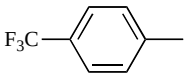
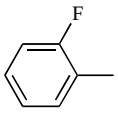
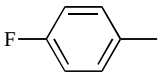
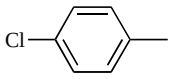
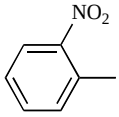
Compd	R	Gram positive		Gram negative	
		B. Subtilis	***S. aureus***	***P. aeruginosa***	***E. coli***
62		1.562	1.562	1.562	1.562
63		3.125	6.25	6.25	3.125
64		6.25	6.25	6.25	12.5
65		1.562	1.562	1.562	1.562
Penicillin		1.562	1.562	6.25	6.25
Kanamycin		0.39	1.562	3.125	3.125

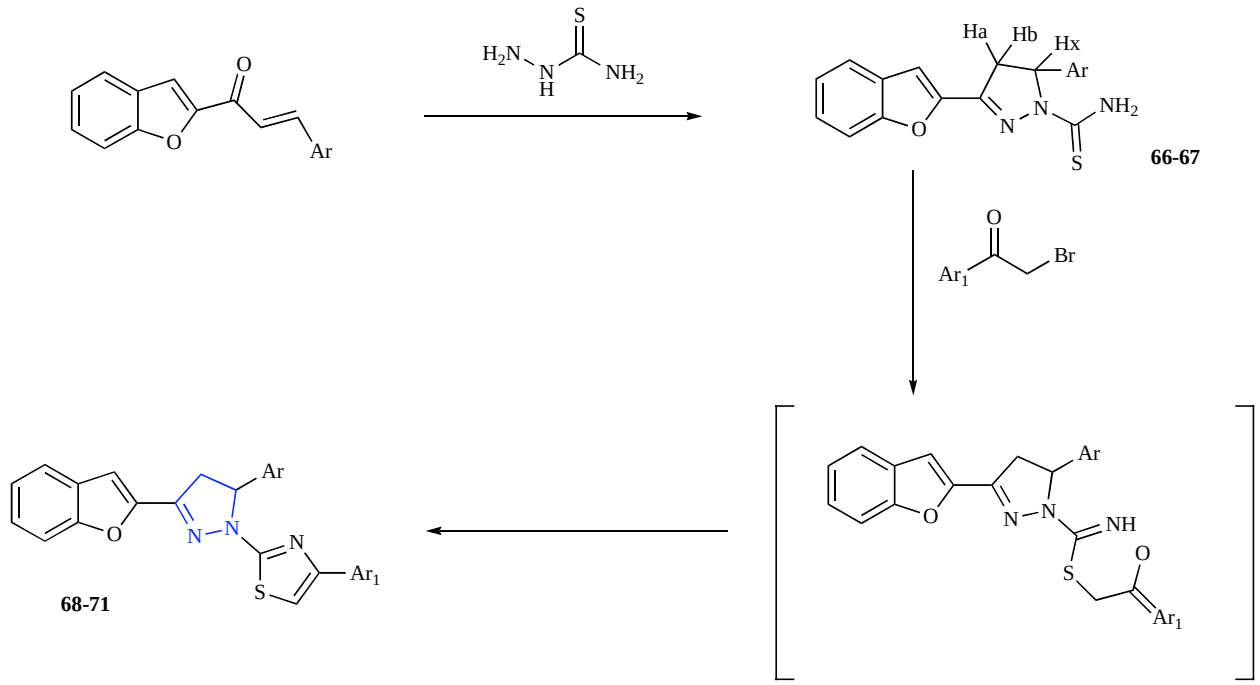
Table 17. Inhibitory Effects of Compounds 61-65 Against DNA Gyrase

Compd	61	62	SP-FX	63	64	65
R						
DNA gyrase IC_{50} (μg/mL)	18.4	2.3	0.29	2.5	4.5	1.6

against the Gram-positive bacteria *S. aureus*, *B. subtilis*, the Gram-negative bacteria *E. coli*, and fungi *C. albicans*, *A. niger*. The results revealed that majority of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms. In general, the inhibitory activity against the Gram-negative bacteria was higher than that of the Gram-positive bacteria. The compound **68** showed excellent activity against Gram-negative bacteria, good activity against Gram-positive bacteria. Most of the tested compounds showed none or weak antifungal activity against A. niger. According to structure–activity relationships (SAR), it can be concluded that benzofuran, pyrazoline, and thiazole moieties are essential for the antimicrobial activity (Table **18**).

Another multi-step synthesis involving the use of microwave irradiation could lead to the generation of a series of 2-[1-(5, 8-dihydro quinoxalino [2, 3-b]indoloacetyl)-3-(1-benzofuran-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl] phenyl derivatives as has been outlined in Scheme **18** [30]. The compounds **72-76** were found to possess good antibacterial activity with each showing promising MIC against *E. coli*, *P. aeruginosa* and *S. aureus*, being comparable to sparfloxacin and norfloxacin. Structure–activity relationships suggested that substituted phenyl ring at 5-position in 4,5-dihydro pyrazole produced various antibacterial activity against gram positive and gram negative bacteria. The *ortho* substitution in phenyl ring with –OH, $–NO_2$ and *para* substitution in phenyl ring by $–OCH_3$ at 5-position pyrazole produced the best antibacterial activity against gram negative bacteria. Unsubstituted phenyl ring at 5-position pyrazole produced moderate antibacterial activity. When phenyl ring was replaced by five member ring at 5-position of pyrazole ring caused reduction in antibacterial activity. The single C–C bond between pyrazole ring and 5-phenyl ring can be replaced by ethenyl bridge resulted in moderate antibacterial activity. *Ortho* substitution in phenyl ring with $–OCH_3$, –Cl and *para* substitution in phenyl ring by –OH, –Cl at 5-position pyrazole produced less or inactive antibacterial activity against gram negative bacteria. The common structure of compounds did not support any antifungal activity against C. albicans (Tables **19**, **20**).

In 2009, Liu *et al.* synthesized a series of new 2-(1-(2-(subsititiuted-phenyl)-5-Methyloxazol-4-yl)-3-(2-substituted-phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-7-substituted-1,2,



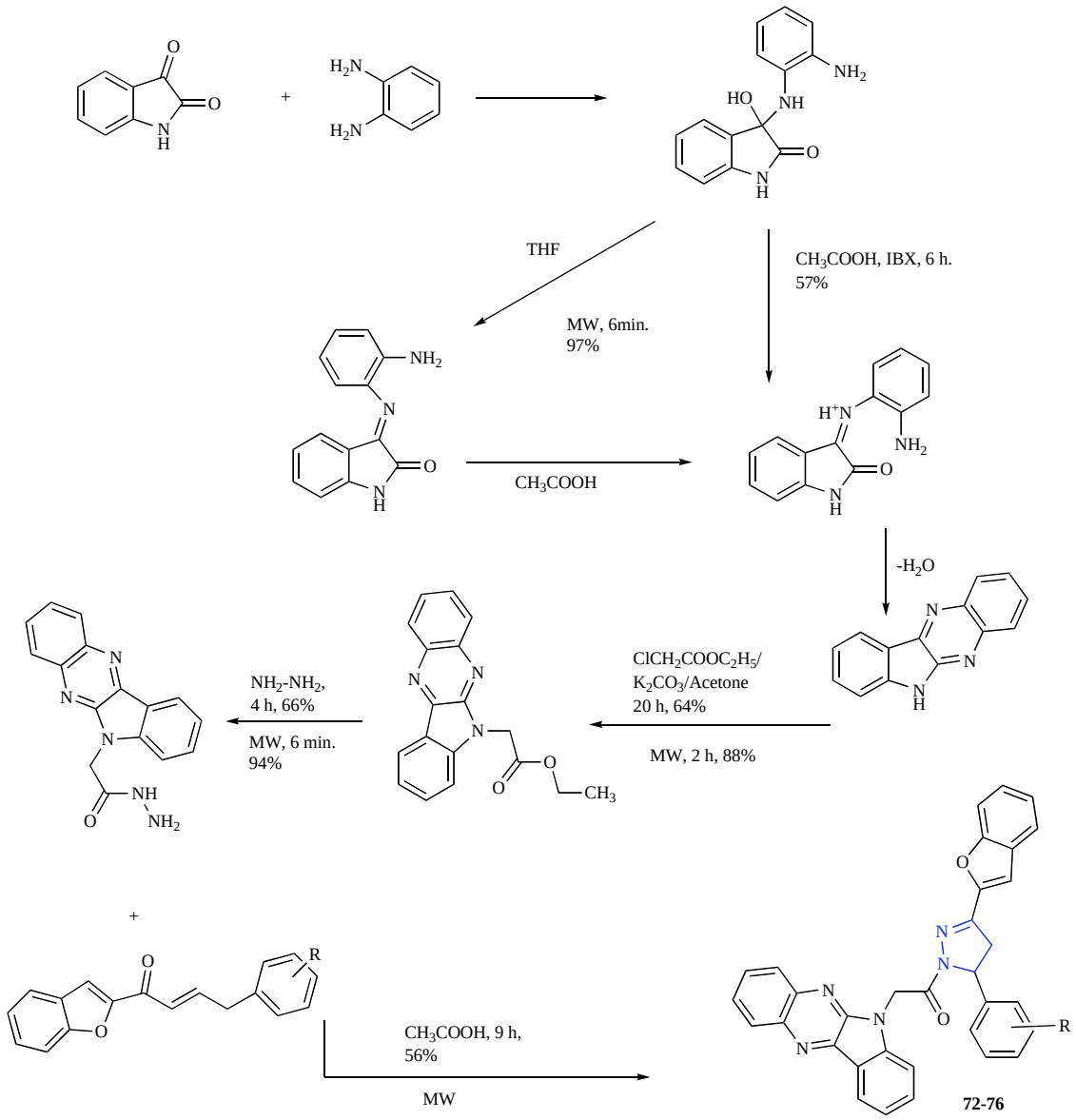
Scheme 17. Synthetic route to compounds **66-71**.

Table 18. The *In Vitro* Antimicrobial Activity of Compounds 66-71

Compd	Ar	Ar_1	Zone of inhibition (mm)				
			S. aureus	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
66	Ph		0	0	0	5	0
67	4-Cl-C_6H_4		0	12	15	0	15
68	Ph	Ph	17	0	25	0	0
69	Ph	4-Br-C_6H_4	0	0	0	0	0
70	4-Cl-C_6H_4	Ph	0	0	12	25	15
71	4-Cl-C_6H_4	4-Br-C_6H_4	0	0	0	25	0
Amoxicillin			20	20	20	-	-
Flucanazol			-	-	-	20	20

3,4-tetrahydroiso-quinoline derivatives [31] (Scheme **19**), some of which showed strong inhibition activity against *S. aureus* DNA gyrase and *B. subtilis* DNA gyrase. From the structure–activity relationships, it can be concluded that some 2-(1-(2-(substituted-phenyl)-5- methyloxazol-4-yl)-3-(2-substitued-phenyl)-4,5-dihydro-1H-pyrazol-5-yl)-7-subs-titued-1,2,3,4-tetrahydroisoquinoline derivatives showed good activity against Gram positive strains, but most of the derivatives displayed poor activity against Gram negative strain. To elucidate the mechanism by which the dihydropyrazole derivatives induce antibacterial activity, the inhibitory activities of selected compounds were examined against DNA gyrase isolated from *B. subtilis* and *S. aureus*. As shown in Table **21**. There was a good correlation between the MICs and the IC_{50s} of compounds **77** and **78**, indicating that inhibition of the DNA gyrase by the pyrazole-oxazole derivatives caused inhibition of bacterial cell growth. But bacterial topoisomerase inhibitors sometimes have poor selectivity against human topoisomerase.

Liu and his group [32] further obtained some novel heterocylcle-substituted dihydropyrazole derivatives through an initial aldol condensation reaction between an aldehyde and a ketone as shown in Scheme **20**. The compounds were screened for their antibacterial potential *in vitro* against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. The results showed that **79-81**possessed significant activity against *B. subtilis* (MIC ≈ 0.39–1.562 mg/mL), whereas **80** and **81** displayed promising results against *S. aureus* (MIC ≈1.562 mg/mL) (Table **22**).



Scheme 18. Synthetic route to compounds **72-76**.

Table 19. Gram-Negative Bacteria Activities of 72-76 Measured by Zone of Inhibition in mm

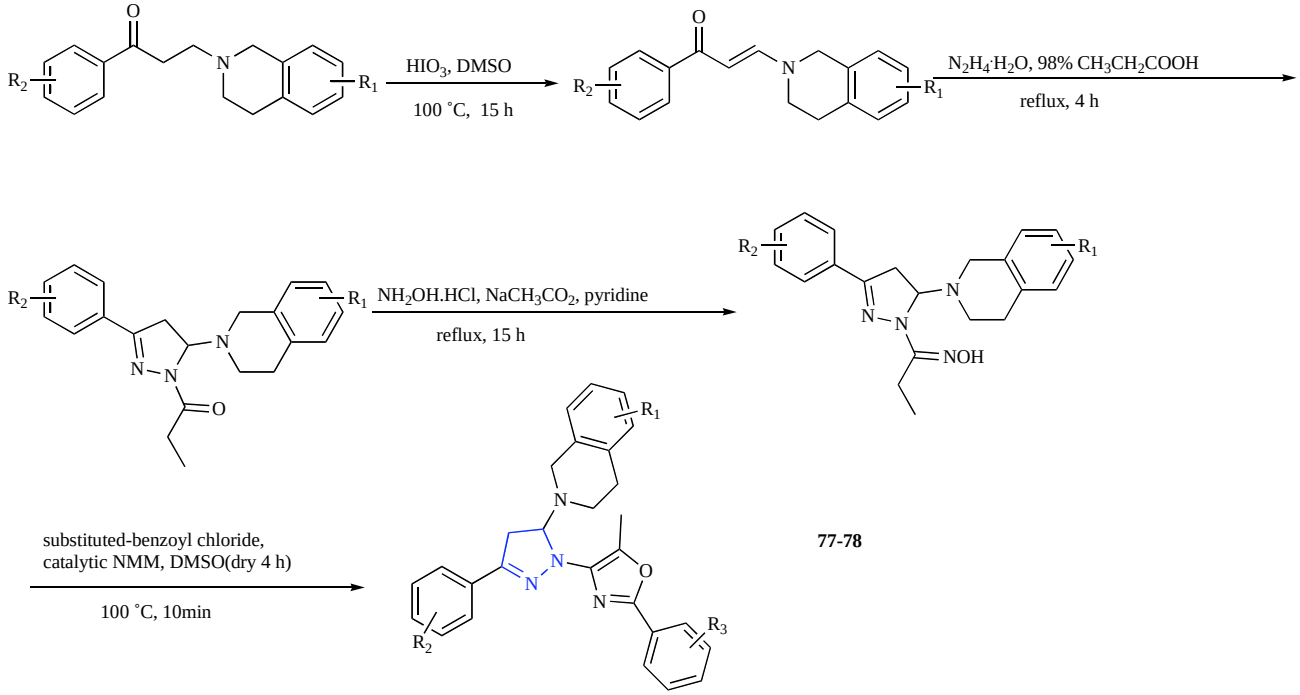
Compd	R	Gram-negative bacteria							
		E. coli		*P. aeruginosa*		*S. typhi*		*S. agalactiae*	
		mm	MIC*	mm	MIC*	mm	MIC	mm	MIC*
72	-OH(*o*)	18	1.00	15	3.5	12	9.5	11	22.0
73	-NO_2(*m*)	13	10.5	16	5.5	14	8	12	26.0
74	-NO_2(*o*)	16	5.5	12	10.5	13	8.5	12	22.0
75	-OCH_3(*p*)	17	5.0	13	12.5	16	4.5	14	19.0
76	-H	14	8.0	11	15.5	20	2	8	28.5
Sparfloxacin		30	0.26	33	4.6	34	0.04	38	12.0
Norfloxacin		20	1.3	28	1.8	22	0.12	32	22.0

*Measured in μg/mL.

Table 20. Gram-Positive Bacteria Activities of 72-76 Measured by Zone of Inhibition in mm

Compd	R	Gram-positive bacteria					
		S. pneumoniae		*S. aureus*		*S. pyogenes*	
		mm	MIC*	mm	MIC*	mm	MIC*
72	-OH(*o*)	13	8.5	13	6.0	10	26.0
73	-NO_2(*m*)	10	13.0	9	12.5	12	28.0
74	-NO_2(*o*)	19	14.5	12	9.5	10	24.0
75	-OCH_3(*p*)	11	12.0	12	8.0	14	23.5
76	-H	8	17.0	16	2.5	12	28.5
Sparfloxacin		34	0.50	36	0.25	38	15.0
Norfloxacin		24	20.2	28	1.5	30	2.6

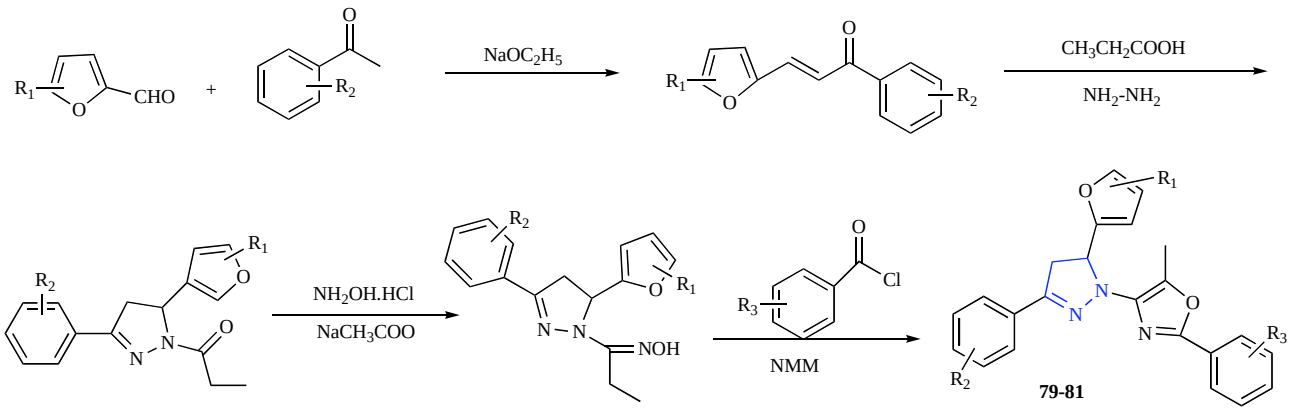
*Measured in μg/mL.



Scheme 19. Synthetic route to compounds **77-78**.

Table 21. Inhibitory Effects of Compounds 77-78 Against DNA Gyrase

Compd	R_1	R_2	R_3	IC_{50}(μg/mL)	
				S.aureus **DNA gyrase**	***B.subtilis*** **DNA gyrase**
77	H	4-Furan	2,4-2F	0.125	0.25
78	7-OMe	4-Furan	2,4-2F	0.25	0.125
Novobiocin				0.25	0.5



Scheme 20. Synthetic route to compounds **79-81**.

Table 22. Minimum Inhibitory Concentrations (MIC-μg/mL) of Compounds 79-81

Compd	R_1	R_2	R_3	Gram-positive		Gram-negative	
				B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*
79	H	2-CF_3	$CH=CH_2$	1.562	3.125	50	50
80	2-SO_2NH_2	2-NO_2	2,4-2F	0.39	1.562	50	12.5
81	2-SO_2NH_2	NO_2	2-CF_3	0.78	1.562	50	>50
Novobiocin				0.78	3.125	6.25	6.25
Penicillin				1.562	1.562	6.25	6.25
Kanamycin				0.78	1.562	3.125	3.125

2.3. Antimalarial Activity

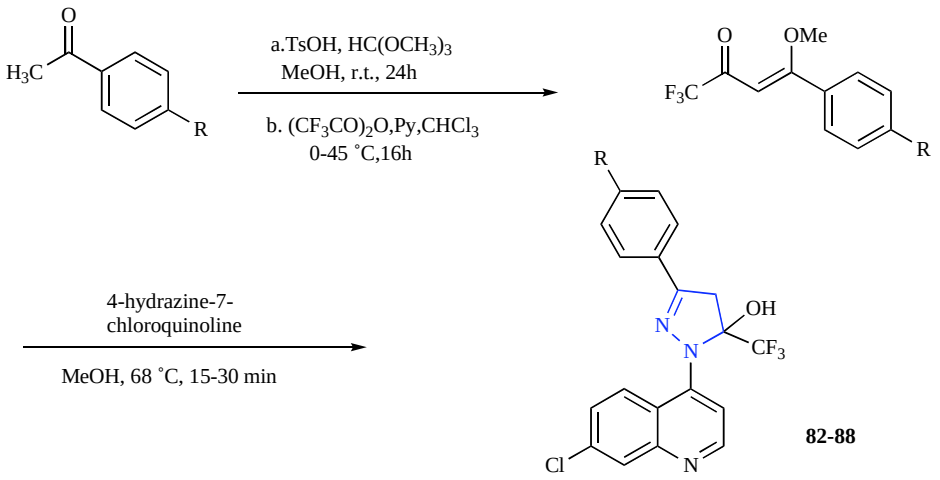
Malaria remains one of the most important diseases of human with over half of the world population at risk of infection. Both the lack of a credible malaria vaccine and the emergence and spread of parasites resistant to most of the clinically used antimalarial drugs and drug combination have aroused an imperative need to develop new drugs against malaria. This suggests that, if a novel chemical class of drug is being discovered against the same target then that could have immense clinical value. During the past few years, some dihydropyrazole analogs have been discovered for clinical trials as potential antimalarial leads. In this context, a series of (4,5-dihydropyrazol-1-yl) chloroquine derivatives **82-88** were accessed from different aryl methyl ketones as depicted in Scheme **21** [33]. The antimalarial activity of these derivatives was evaluated *in vitro* against a chloroquine resistant Plasmodium falciparum clonem. All the compounds displayed appreciable inhibition of growth of *P.* falciparum *in vitro* at concentrations in micromolar level (Table **23**). The halogen substituted compounds have also shown good activity based on the low IC_{50} values. Despite the fact that the more active compounds *in vitro* are at least 10- to 15 times less active than chloroquine, their IC_{50} values are in the micromolar concentration range comparable to recently reported results.

The pharmacophore model contains ring aromatic (RA), positive ionisable (PI), hydrogen bond acceptor (HBA) and aliphatic hydrophobic (HY-ALI) features. Fig. **8** shows the mapping of 1,3,5-trisubstituted pyrazoline for all the pharmacophoric features except PI. In a significant breakthrough to the development of antimalerial drugs, Acharya *et al*. [34] prepared a series of 1, 3, 5-trisubstituted pyrazolines (Scheme **22**, Fig. **9**) from methyl 4-hydroxyphenyl ketone and evaluated them for *in vitro* antimalarial efficacy against chloroquine sensitive (MRC-02) as well as chloroquine resistant strains of *P.* falciparum. Some of the synthesized compounds were shown to possess even better antimalarial activity than chloroquine against resistant strain of *P.* falciparum and were found to be potent in the *in vivo* experiments (Tables **24**, **25**).

Further development in this area was observed recently when Wanare and his group [35] demonstrated antimalerial activity of a series of α-pyranochalcones and pyrazoline analogs by evaluation of the growth of malaria parasite in culture experiments. Compounds **93-95** (Fig. **10**) showed high therapeutic indices suggesting that they were selective in their action against the malaria parasite. This is the first instance wherein chromeno-pyrazolines were found to act as active antimalarial agents. Further exploration and optimization of this new lead could provide novel antimalarial molecules which can ward off issues of cross-resistance to drugs like chloroquine.

2.4. Antinociceptive Activity

Pain is a disagreeable and subjective sensation resulting from a harmful sensorial stimulation that alerts the body



Scheme 21. Synthetic route to compounds **82-88**.

Table 23. Antimalarial Activity of Compounds 82-88 Against *Plasmodium falciparum* W2 Clone *In Vitro*

Compd	R	IC_{50}[a] (μg/mL)
82	H	1.39
83	Me	3.04
84	F	2.13
85	Cl	1.69
86	Br	1.55
87	NO_2	5.71
88	phenyl	2.12
Chloroquin		0.19

[a]The IC_{50} represents concentration inhibitory dose of the parasite growth in relation to control cultures without any drug.

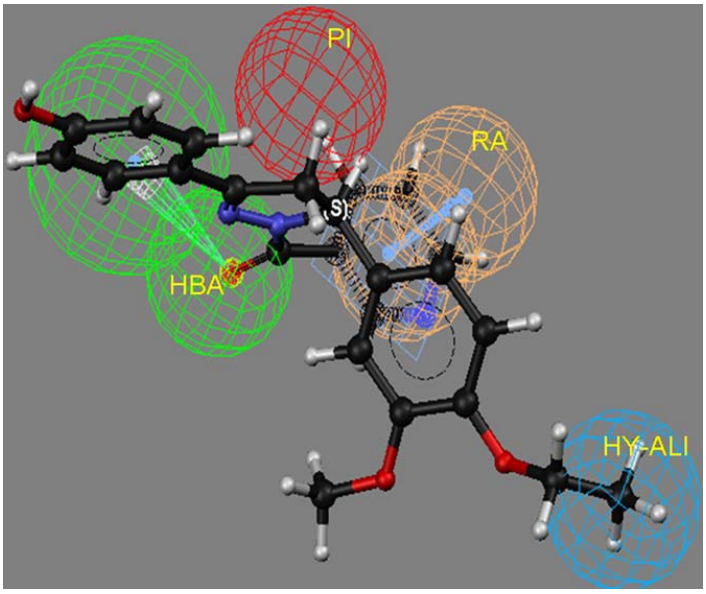
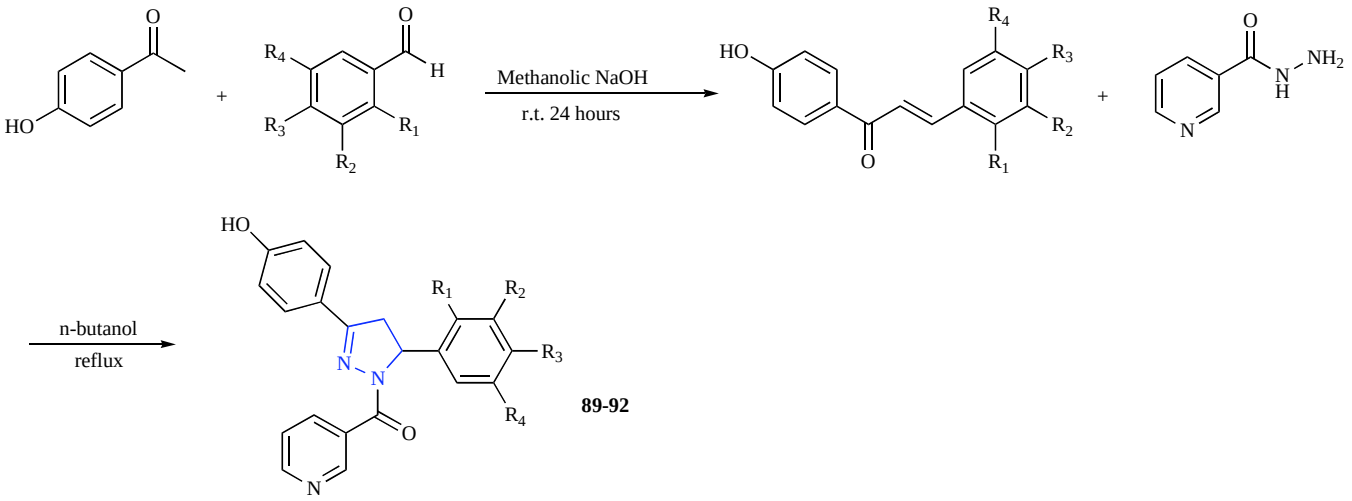


Fig. (8). Pharmacophore mapping of 1,3,5-trisubstituted pyrazoline. The blue contour represents the hydrophobic aliphatic feature (HY-ALI), the orange contour represents ring aromatic feature (AR), the green contour represents hydrogen bond acceptor feature (HBA) and the red contour represents positive ionizable feature (PI)



Scheme 22. Synthetic route to compounds **89-92**.

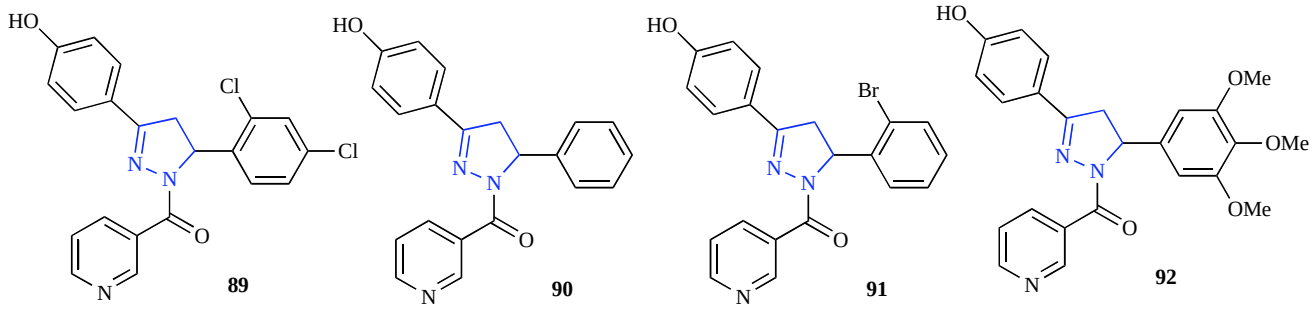


Fig. (9). Structures of compounds **89-92**.

Table 24. Cytotoxicity, *In Vitro* and *In Vivo* Antimalarial Activity of Compounds 89-92

Compd	Cytotox. (μM)	Activity vs MRC-02 strain (μM)	Cytotox./ antimal. ratio	Activity vs RKL9 strain (μM)	Cytotox./ antimal. ratio
90	1.875	0.0304± 0.006	61.67	0.1305± 0.031	14.36
92	0.828	0.0265± 0.005	31.24	0.0425± 0.005	19.48
CQ	66.233	0.0210± 0.003	3153.95	0.177±0.004	374.19

Table 25. *In Vivo* Antimalarial Activity Against P. Berghei ANKA Strain of Compounds 89-92

Compd	% Suppression on day 4[a]	Mean survival time[a] (MSTindays) ±SE	% Suppression on day 4[b]	Mean survival time[b] (MSTindays) ±SE	Rt (min)[e]	Purity(%)[e]
89	68.93	13.22±1.19	60.55	11.55±1.02	12.67	98.72
91	47.52	10.33±1.98	34.98	9.00±1.06	11.64	98.91
92	43.70	11.00±0.78	32.78	9.00±1.55	11.87	98.83
CQ	100[c]	All alive	-	-	-	-
Control	0[d]	6.44±0.97	-	-	-	-

[a]At 50mg/kg/day. [b]At 25mg/kg/day. [c]At 8mg/kg/day. [d]Without drug. [e]HPLC.

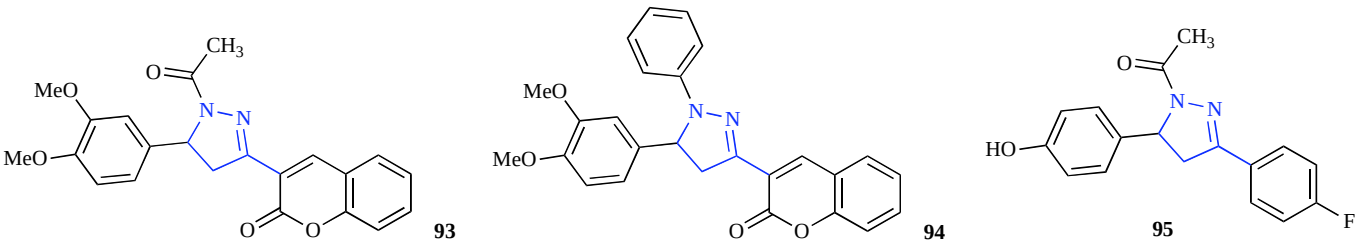
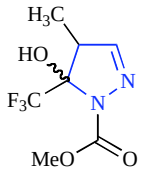


Fig. (10). Structures of compounds **93-95**.

about a current or potential damage to its tissues or organs. Despite the painful sensation, which can be efficiently solved by the removal of the main reason, the pain-causing stimulus cannot always be either easily defined or quickly removed. Contemporary analgesics, like opiates and nonsteroidal anti-inflammatory drugs have some limitations in clinical use, especially for opiates, such as addiction, tolerance and side effects. Some dihydropyrazole compounds were shown to possess analgesic activities mediated by peripheral mechanisms and have been suggested as potential candidates to act as bioactive molecules for the creation of promising new analgesic agents in future. For this purpose, a series of compounds bearing pyrazole scaffold were screened to discover new antinociceptive drugs [36-40]. Fortunately, it was found that 4-methyl-5-trifluoromethyl-5-hydroxy-4,5-dihydro-*1H*-pyrazole methyl ester **96** (Fig. **11**) was associated with significant antinociceptive effects in formalin and hot-plate tests with efficacies similar to dipyrone or morphine**.**



96

Fig. (11). Structure of compound **96**.

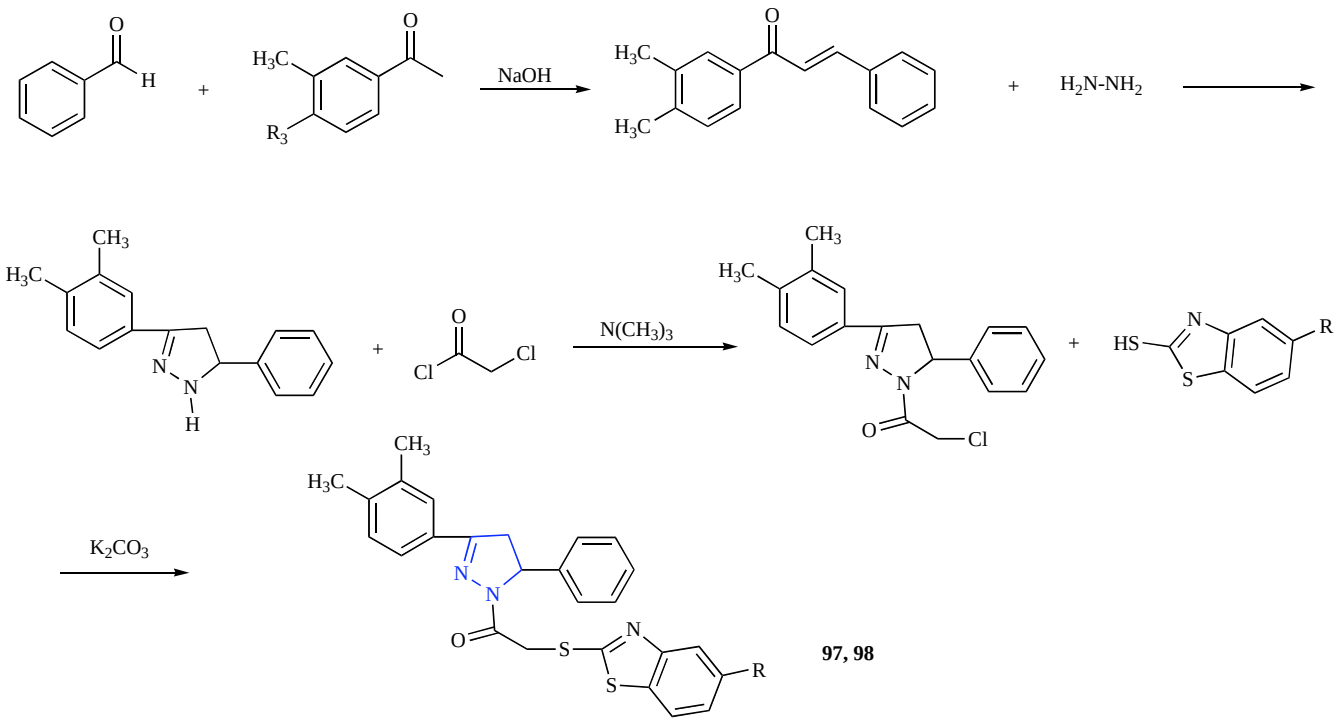
The antinociceptive effect was reversed by the opioid receptor antagonist, naloxone, but not by the alpha2-adrenergic receptor antagonist, yohimbine, nor by pre-treatment with the serotonin synthesis inhibitor, *p*-chlorophenylalanine ethyl ester. Furthermore, compound **96** was a less effective thanmorphine to reduce gastrointestinal transit. In contrast to morphine, this compound did not generate a tolerance to its antinociceptive effect and did not present cross-tolerance with morphine [41]. Thus, its effects were considerably different from the reference analgesic drug dipyrone which may produce significant tolerance and offer cross-tolerance with morphine [42]. It is believed that compound **96** produces antinociceptive action by stimulating the opioid system, but presents fewer side effects compared to morphine or dipyrone.

In 2009, Kaplancikli, Z. A. *et al.* [43] again synthesized some dihydropyrazoline derivatives through an initial crossed aldol condensation reaction (Scheme **23**) and investigated their potential antinociceptive activities. All the compounds (100mg/kg) exhibited significant antinociceptive activities in both hot plate and aceticacid-induced writhing tests, however, **97** and **98** showed much superior results in both the nociception tests (Tables **26** and **27**). The mouse writhing model involves different nociceptive mechanisms, such as sympathetic system, cyclooxygenases and their metabolites and opioid mechanisms. Acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulate the nociceptive neurons sensitive to NSAIDs and/or opioids. When the results of writhing and hot plate tests were considered together, it can be concluded that the antinociceptive activities of the tested compounds may occur by both central and peripheral mechanisms. It was observed that, analgesic activities of all tested compounds were reversed completely by naloxone pre-treatment, which indicates the involvement of the opioid mechanisms in the analgesic action. This effect could be due to the direct opioid receptor agonistic activities of the constituents in the extract and/or induction of endogenous opioid peptide release. These results support the previous studies suggesting opioid mediated analgesic activities of some benzoxazole/ benzimidazole–pyrazoline-derived compounds.

2.5. Antiviral Activity

The incidence of viral infections has been constantly emerging on a global scale. The progress in the development of antiviral drugs has been rather sluggish in comparison to the advancement made in the field of anti-infective chemotherapy. One of the major limitations has been the absence of specific viral 'targets', because host cell pathways are used predominantly for viral replication. Nevertheless, some selected dihydropyrazoe derivatives have revealed interesting antiviral properties e.g. cytotoxic compounds **99** and **100 (**Fig. **12)** were shown to inhibit flavivirus infection in cell culture. They not only inhibited an epidemic strain of WN virus without detectable cytotoxicity (IC_{50} of 28 μM) but also other flaviviruses (dengue, yellow fever, and St. Louis encephalitis viruses), an alphavirus (Western equine encephalitis virus), a coronavirus (mouse hepatitis virus), and a rhabdovirus (vesicular stomatitis virus) [44, 45]. Also, in 2009, Yar, M. S. *et al.* [46] prepared a series of 2- [4-[3-(2,4-dihydroxyphenyl)-1-(2-hydroxybenzoyl-4,5- dihydro-*1H*-5-pyrazolyl]-2-methoxyphenoxy] acetic acid derivatives and demonstrated theirr *in vitro* cytotoxicity and antiviral activity with a minimum cytotoxic concentration of 0.16 μg/mL in human embryonic lung cells.

In addition, the novel dihydropyrazole derivatives obtained in a two-step tricky synthesis by El-Sabbagh *et al.* [47] (Scheme **24**) exhibited significant antiviral activity. In particular, compound **101** was found to be potent against



Scheme 23. Synthetic route to compounds **97-98**.

Table 26. Effects of Compounds 97-98 on Hot Plate Response in Mice

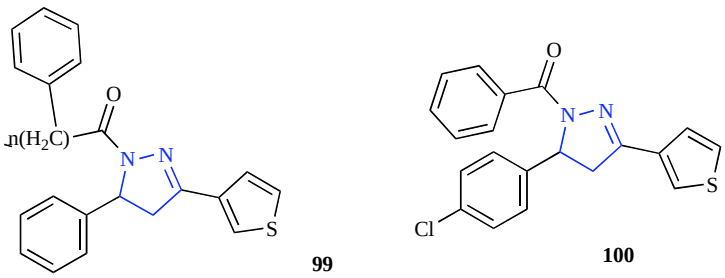
Treatment	R	X	% Analgesia (mean±SEM)
Control			1.74 ±1.55
97 (10mg/kg)	Cl	O	72.6±19.9**
98 (10mg/kg)	H	NH	82.5 ±16.3***
Naloxone(5mg/kg) +97 (10mg/kg)			0.97± 6.78
Naloxone(5mg/kg) +98 (10mg/kg)			0.58± 9.52
Morphine(10mg/kg)			92.9 ±9.03***

Valuesaremean SEM. *P< 0.05, **P <0.01, ***P < 0.001,compared with control.

Table 27. Effects of Compounds 97-98 on Writhing Test in Mice

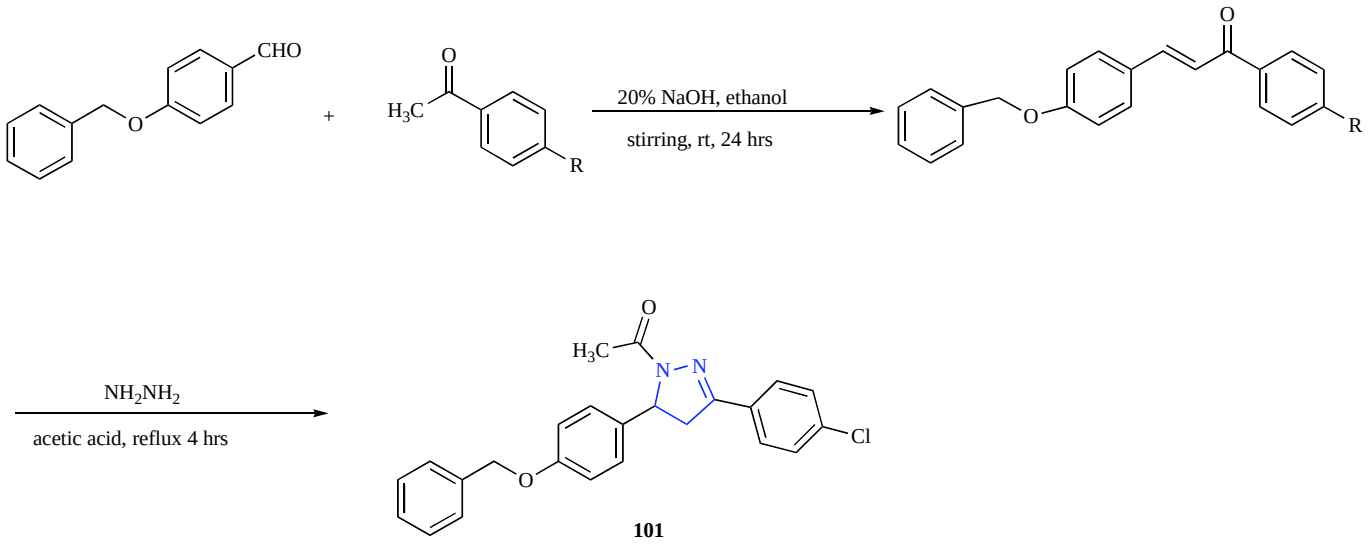
Treatment	R	X	Number of writhing (10min) (Mean±SEM)	% Protection
Control			30.7 ±3.6	—
97 (10mg/kg)	Cl	O	0.57 ±0.42***	98.14
98 (10mg/kg)	H	NH	0.71 ±0.56***	97.67
Naloxone(5mg/kg) +97 (10mg/kg)			24.14 ±1.91	21.39
Naloxone(5mg/kg) +98 (10mg/kg)			24.86 ±2.45	19.07
Morphine(10mg/kg)			3.85 ±0.5***	87.45

Values are mean±SEM. **P < 0.01, ***P <0.001 compared with control.



99 **100**

Fig. (12). Structures of compounds **99, 100**.



101

Scheme 24. Synthetic route to compound **101**.

Table 28. Cytotoxicity and Antiviral Activity of Compound 101 in HEL Cell Cultures

Compd	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50} [b] (μg/ml)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2(G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV
101	100	>20	>20	7±3	>20	>20
Brivudin (μM)	>250	0.08	126	10	>250	>250
Ribavirin (μM)	>250	>250	>250	146	>250	>250
Cidofovir (μM)	>250	3	5	10	>250	5
Ganciclovir (μM)	>100	0.08	0.08	>100	>100	6

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 29. Cytotoxicity and Antiviral Activity of Compound 101 in HEL[a] Cell Cultures

Compd	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50} [b] (μg/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
101	>20	>20	>20	>20
DS-5000	>100	>100	9	0.8
(S)-DHPA (μM)	>250	>250	>250	>250
Ribavirin (μM)	>250	29	146	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 30. Cytotoxicity and Antiviral Activity of Compound 101 in Vero Cell Cultures

Compd	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50} [b] (μg/ml)				
		Parainflu-enza-3 virus	Reovir-us-1	Sindbis virus	Coxsackie virusB4	Punta toro virus
101	20	>4	>4	>4	>4	>4
DS-5000	>100	>100	>100	59	>100	>100
(S)-DHPA (μM)	>250	>250	>250	>250	>250	>250
Ribavirin (μM)	>250	45	>250	>250	>250	146

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

vaccinia virus (Lederle strain) in HEL cell cultures with a 50% effective concentration (EC_{50}) at 7 μg/mL (Tables **28-30**). Based on the observed promising activity of compound **101** against vaccinia virus in HEL cell cultures, we further evaluated this compound against several other poxviruses, including vaccinia virus strains Lister, Western Reserve and Copenhagen. Unfortunately, none of these investigated poxviruses were found to be sensitive to the inhibitory activity of the 4,5-dihydropyrazole **101** at subtoxic concentrations.

2.6. CB1 Receptor Antagonists

Obesity is one of the greatest health threats of this century. The benefits of a controlled dietary intake can be profound for the management of obesity. Hence, significant attention has been directed towards the development of antiobesity drugs that are effective and safe in targeting appetite suppression. It has been found clinically and experimentally that the endocannabinoid system is hyperactive in obese subjects. In addition, CB1 receptor antagonists/inverse agonists represent a promising new approach for reducing body weight and decreasing the co-morbidities associated with excessive adiposity. Cannabinoid CB1 receptor antagonists are currently the subject of intensive research due to their potential in therapeutic applications. Several NCEs with CB1 antagonistic properties have recently been disclosed by many pharmaceutical companies and academic research groups, some of which are close structural analogs of the leading mcompound rimonabant. A considerable numbers of these CB1 antagonists are bioisosteres and are usually derived from rimonabant by the replacement of the pyrazole moiety with an alternative heterocycle like dihydropyrazole.

Lange *et al*. synthesized a series of novel 3,4-diarylpyrazolines, of which **102** and **103** held considerable promise (Scheme **25**) [48, 49]. In line with the CB_1 receptor affinity results, both the enantiomers (**102** and **103**) showed

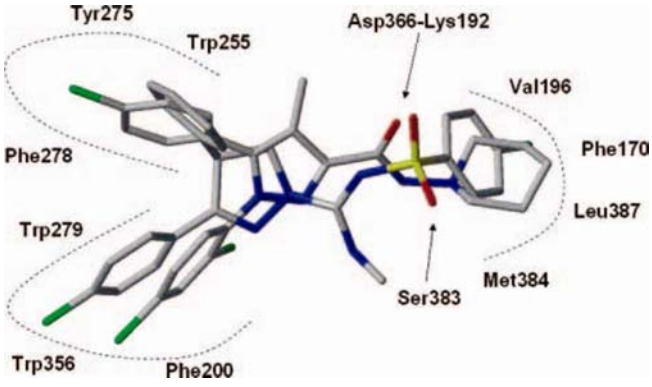
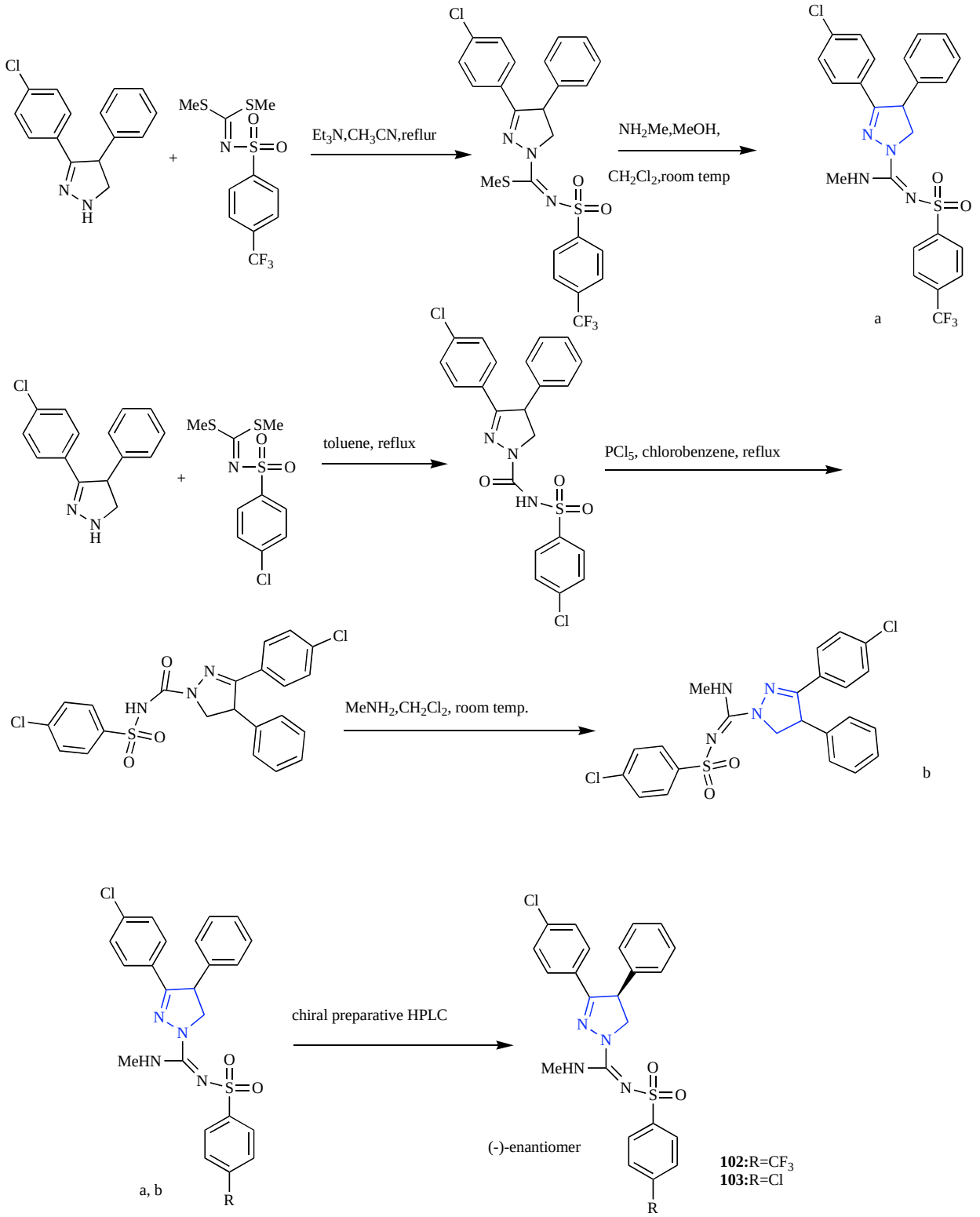


Fig. (13). Receptor-based alignments of **103** and rimonabant.

considerably higher CB1 antagonistic properties with lipophilicity lower than that of SLV319. The key change was the replacement of the arylsulfonyl group in the original series by a dialkylaminosulfonyl moiety. One of the compounds in the series exhibited very high CB1 receptor affinity (*K*i = 24 nM) and very potent CB1 antagonistic activity (p*A*2 = 8.8).

The receptor model was reconstructed.Giving the best fit with rimonabant, the *T*g, conformation **103D** was used as starting conformation for manual docking into the receptor,



Scheme 25. Synthetic route to the compounds **102, 103**.

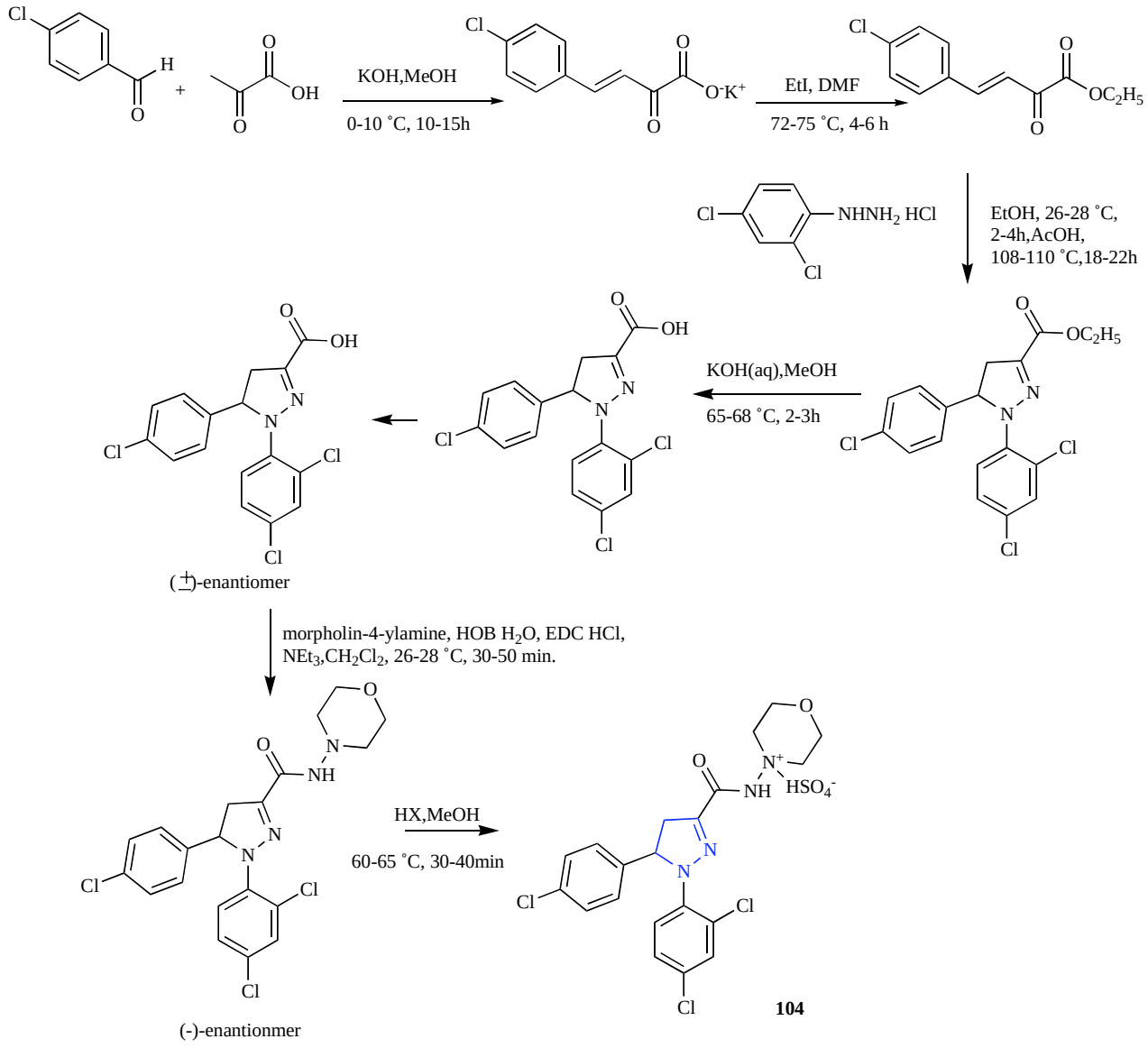
followed by simulated annealing and minimization (Fig. **13**). In the case of **103**, a stacking interaction between the *p*-chlorophenyl ring and Phe170 is possible. The two aromatic rings attached to the pyrazoline core are enclosed by an arrangement of stacked aromatic residues. The *p*-chlorophenyl ring is bound in a pocket formed by Trp279/Phe200/Trp356 while the other ring fits in a cavity created by Tyr275/Trp255/Phe278.

Afterwards, some diaryl dihydropyrazole-3-carboxamide analogues obtained through a condensation reaction between 4-chlorobenxaldehyde and α-keto acid in a basic medium were evaluated for their appetite suppression and body weight reduction capacity in animal models [50]. The optimization of the studies led to the development compound **104** (Scheme **26**) as a potent cannabinoid CB1 receptor antagonist with a significant antiobesity effect in animal models. In the dihydropyrazole motif, the *N*-aminomorpholine is the optimal side chain, and bisulfate salt serves as the more potent bioavailableor component imparting the antiobesity effect. This class of compounds is associated with promising therapeutic potential as a CB1 receptor antagonist to treat obesity. Further explorations are required to be conducted in future. The both enantiomers *R* and *S* (**104a**, **104b**) for the compound **9** were then automatically docked in the binding site defined by the docked posed of ligand**.** The poses were ranked with PLP-1, PLP-1, and Dockscore, and the conformations with the best scores were checked visually (Fig. **14**).

Subsequently, many new dihydropyrazole derivatives were prepared and patented as CB1 receptors, the list of these inventions has been shown in Table **31** [51-58].

2.7. Antitubercular Activity

A recent survey indicates mortality caused by HIV/AIDS is largely and intimately associated with tuberculosis. Current chemotherapy of tuberculosis does not appear to act



Scheme 26. Synthetic route to the compound **104**.

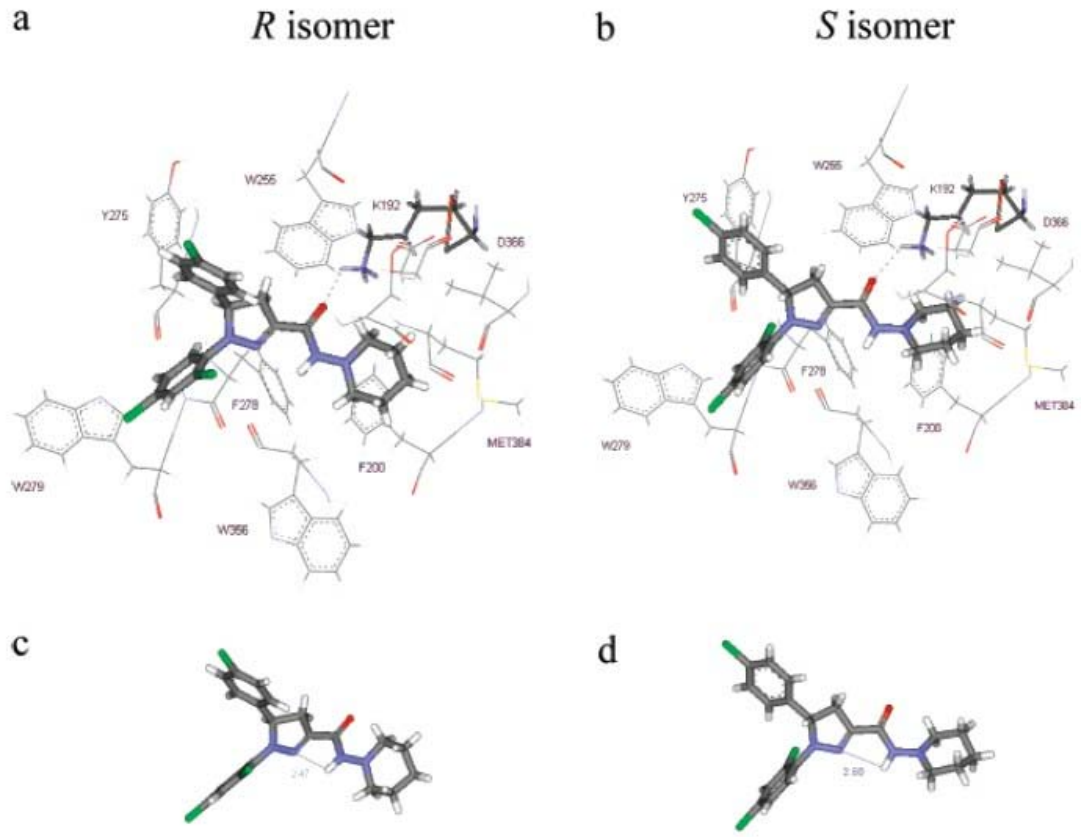
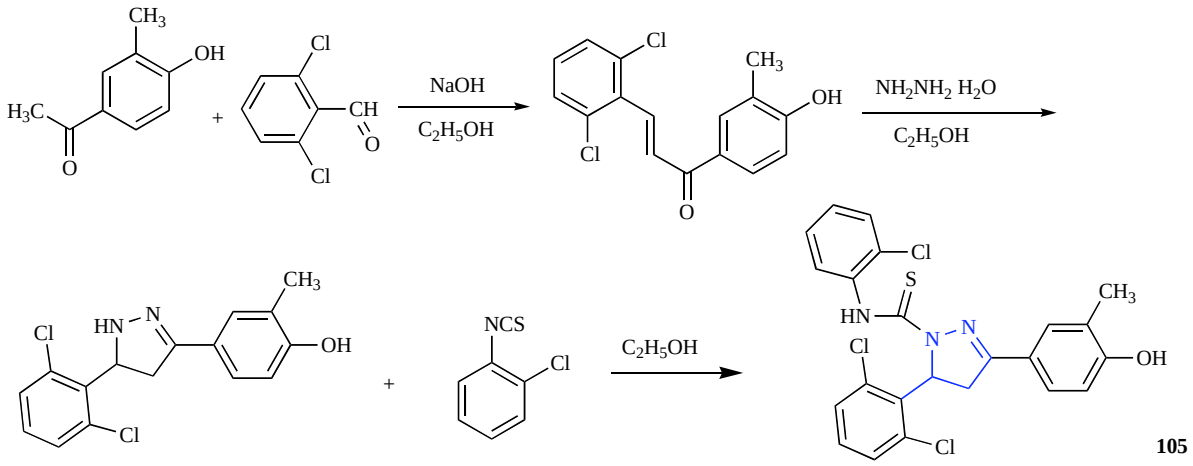


Fig. (14). Docking of the both isomers *R* and *S* (**104a**, **104b**) for compound **104** in the homology model of the CB1 receptor: (a, b) key interactions of *R* and *S* isomers, respectively, such as the H bond with Lys 192 and aromatic stacking of the phenyl rings with Phe 200, Trp 255, Tyr 275, Phe 278, Trp 279, and Trp 356; (c, d) intramolecular H bond in the docked conformer of both *R* and isomers

Table 31. List of Patents on Dihydropyrazoles Used as CB1 Receptors

Patent No.	Patent Date	Invention Disclosed
WO2009037244	26.03.2009	This invention disclosed 5-(hetero)aryl-4,5-dihydro-(*1H*)-pyrazole derivatives as cannabinoid CB1 receptor agonists and also relates to the uses of such compounds particularly their use in administering them to patients to achieve a therapeutic effect in disorders in which CB1 receptors are involved [51]
WO2008152086	18.12.2008	Disclosed invention related to 4, 5-dihydro-(*1H*)-pyrazole derivatives as cannabinoid CB1 receptor modulators [52]
WO2008043544	17.04.2008	Sulphonamide substituted Pyrazoline compounds, their preparation and use as CB1 modulators [53]
WO2007071662	28.06.2007	This invention is directed to 4, 5-dihydro-(*1H*)-pyrazole derivatives as cannabinoid CB1 receptor modulators, pharmaceutical compositions, methods for the preparation and their use [54]
WO2007017125	15.02.2007	CB1 Antagonist or inverse antagonist as therapeutical agents for the treatment of inflammation involving gene expression [55]
US20090082396	26.03.2009	5-(Hetero)aryl-4,5-dihydro-(*1H*)-pyrazole derivatives as cannabinoid CB1 receptor agonists and method for synthesis, pharmaceutical composition etc. Their use in patients to achieve a therapeutic effect in disorders in which CB1 receptors are involved [56]
WO2007009688	25.01.2007	Thiocarbonyl-substituted pyrazoline compounds, their preparation and use as CB1 modulators [57]
WO2007009687	25.01.2007	Carbonyl substituted pyrazoline compounds, their preparation and use as CB1 receptor modulators [58]



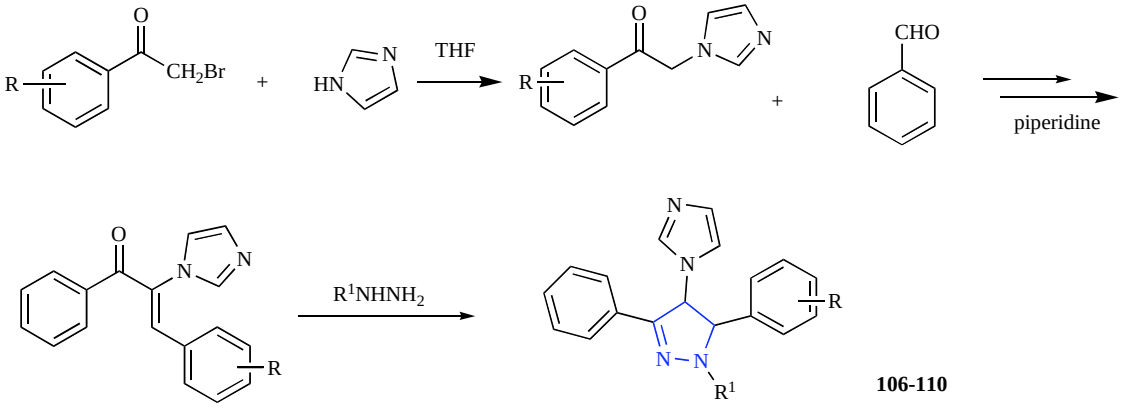
Scheme 27. Synthetic route to compound **105**.

successfully in retroviral infected patients' across to the world. Therefore, development of improved therapy for tuberculosis as novel antitubercular agents has been recognized as a major need for the developing countries as well as developed countries. The mechanism of action needs to be investigated in detail for the treatment of complex tuberculosis cases.

In 2007, Ali *et al.* [59] synthesized compound **105** by the reacting hydrazine hydrate with chalcone (Scheme **27**). This compound was tested for its anti-mycobacterial activity *in vitro* against INH resistant *M. tuberculosis* using the BACTEC 460-radiometric system. Result showed that compound **105** had very high potency and exhibited 90% inhibition at MIC 0.96 mg/mL. Replacement of phenyl substitution at C-5 with a 2-chlorophenyl group in the pyrazoline analogue improves antitubercular activity. These results clearly showed that the presence of *N*-1 2-chlorophenyl substutuent with a dichloro substitution at the C-5 of the pyrazoline derivatives caused a remarkable improvement in anti-mycobacterial activity.

In another attempt, Zampieri [60], synthesized a series of 1-(3, 5-diaryl-4, 5-dihydro-*1H*- pyrazol-4-yl)-*1H*-imidazole derivatives by using a multi-step synthetic protocol as depicted in Scheme **28**. The synthesized compounds **106-110** revealed good antimycobacterial activity with MIC reaching an agreeable figure of 4 μg/mL as shown in Table **32**. In this case the substitution at *N*-1 position of the pyrazoline ring with a phenyl or 4-fluorophenyl moiety, rather than a methyl or hydrogen, improves the antimycobacterial activity. The substitution at the phenyl groups at 3, 5 position of the pyrazole ring, did not appear to be influent for the activity.

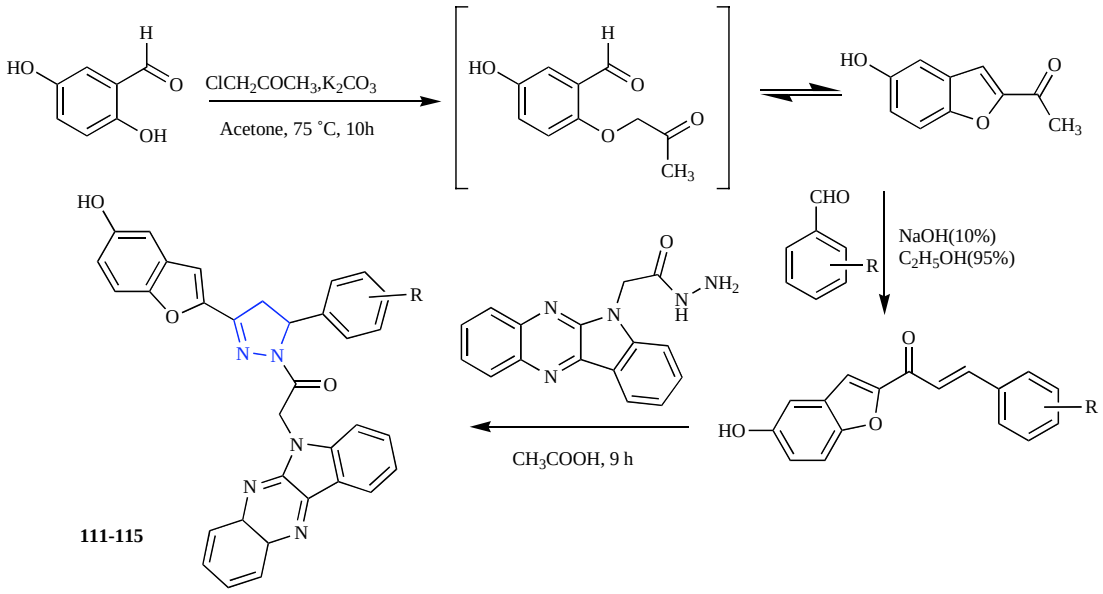
In a recent report, Manna and co-workers [61] prepared some novel 1,3,5-trisubstituted indophenazine pyrazolines **111-115** bearing benzofuran by a cycloaddition of benzofuran chalcone on indophenazine hydrazide (Scheme **29**). These compounds exhibited high antitubercular activity on MTB and MDR-TB with MICs values between 0.16 and 9.78 μg/ml (Table **33**). The potency, selectivity, and low cytotoxicity of the synthesized compounds make them valuable for the design of lead structures with desired activity. Structure activity relationships study recommended that substituted phenyl ring at 5 positions by –OH (*o*) and –OCH_3 (*m*) in 4,5-dihydro pyrazole posses a variable *in vitro* and *in vivo* antitubercular activity against MTB and MDR-TB. The *ortho* and *meta* substituted phenyl ring with –NO_2 produced very good antitubercular activity. Where, unsubstituted phenyl ring found moderate activity. When phenyl ring was replaced by five-membered ring at 5 position of pyrazole ring created good antitubercular activity.



Scheme 28. Synthetic route to compounds **106-110**.

Table 32. Test Results of Compounds 106-110 Against a Strain of *M. tuberculosis* $H_{37}Rv$

Compound	R	R^1	$H_{37}Rv$
			MIC μg/mL
Miconazole	-	-	-
Amphotericin B	-	-	-
Isoniazid	-	-	0.5
106	Cl	Ph	4
107	2,4-$(Cl)_2$	Ph	4
108	Br	4-F-Ph	4
109	Cl	4-F-Ph	4
110	CH_3	4-F-Ph	4



Scheme 29. Synthetic route to compounds **111-115**.

Table 33. *In Vitro* and *In Vivo* Antitubercular Studies of Compounds 111-115 Against *M. tuberculosis* $H_{37}RV$

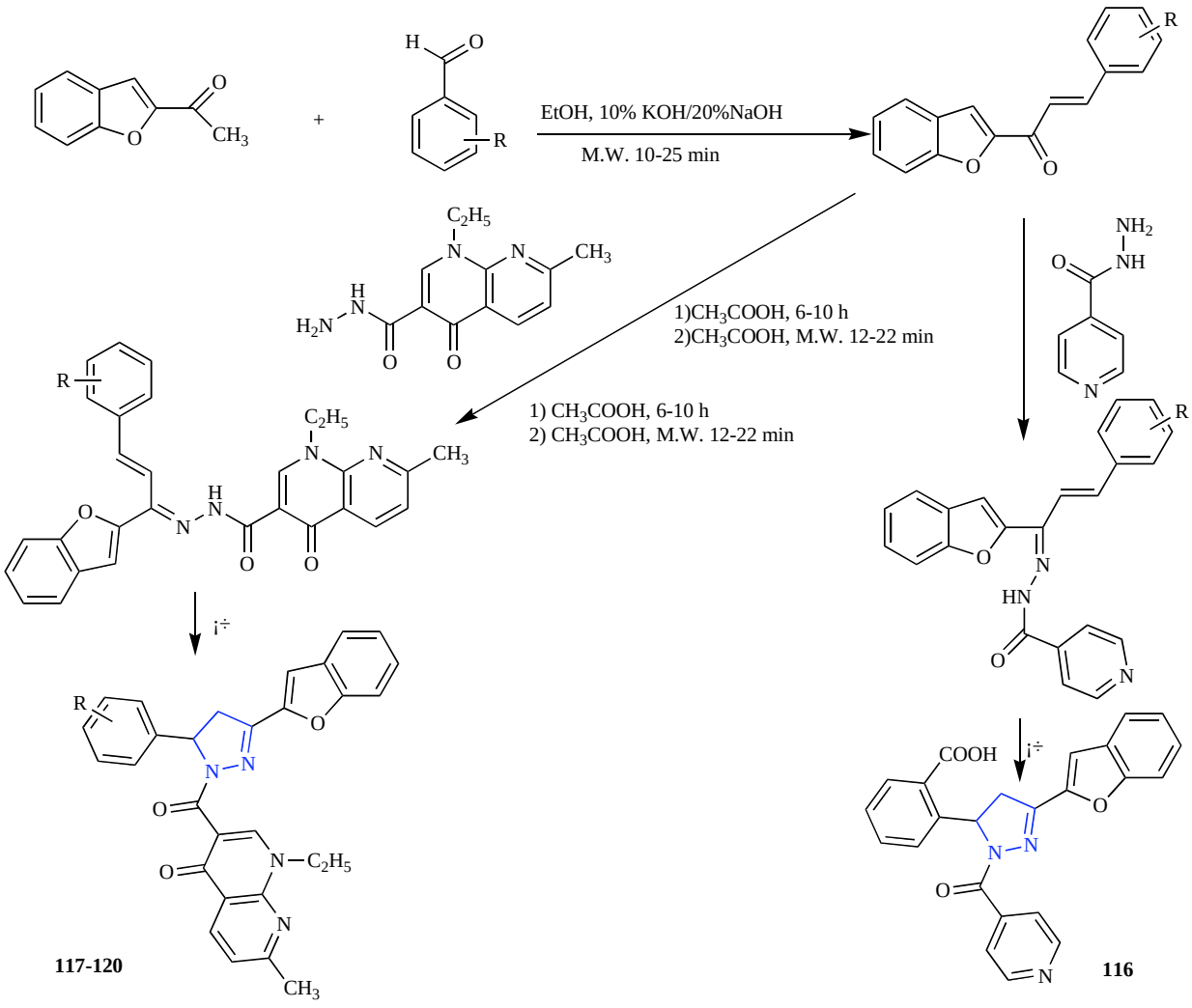
Compound	R	Results against MTB		Results against MDR- TB		IC_{50} (μM)
		MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	
111	–NO_2 (m)	0.62	1.24	1.20	2.00	>180.2
112	–Cl (p)	2.45	4.20	5.23	8.66	>205.4
113	–Cl (o)	1.75	2.56	4.68	8.61	>225.5
114	–NO2 (o)	0.16	0.42	3.24	7.2	>144.3
115	Furan ring	1.1	3.12	6.40	9.78	>198.2
Rifampicin	–	0.5	2.0	4.21	7.37	>77.4
Gatifloxacin	–	0.12	0.5	14.73	28.46	>159.5

The single C–C bond between pyrazole ring and 5-phenyl ring can be replaced by ethylene bridge, results potent inhibitor of *M. tuberculosis*. *Ortho* and *para* positions substituted with –Cl in phenyl ring produced moderate activity. The other substitution in phenyl ring at position 5 in pyrazoline produced less active against *M. tuberculosis.*

Subsequently, new 3-benzofuran-5-aryl-1-pyrazolyl-pyridylmethanone and 3- benzofuran-5-aryl-1-pyrazolylcarbonyl-4-oxo-naphthyridin analogs were prepared by microwave irradiation (Scheme **30**) and then evaluated for *in vitro* and *in vivo* antitubercular activity against multidrug- resistant *M. tuberculosis* stains [62]. Among the synthesized compounds, **116** and **117** displayed maximum *in vitro* antitubercular activities against both MTB and MDR-TB similar to those shown by standard drugs. Rest of the compounds, however, showed only moderate to low antitubercular activity. Furthermore, compounds **116** and **118-120** revealed very satisfactory results in reducing bacterial count in lung and spleen tissues, being in the same range as displayed by standard drug and control (Tables **34**, **35**). The structure-activity relationships study demonstrated that, the naphthyridin ring is more favorable group then pyridinylcarbonyl ring for the potent activity. Also the antitubercular activity may be due to formation of free isonicotinoyl-NAD complex, which may be responsible for the inhibition of mycobacterium cell wall biosynthesis. Carboxylic group contained was found more active against multidrug-resistant *M. Tuberculosis.* Hence, the acidic medium is favorable for the formation of isonicotinoyl-NAD complex, which produced by carboxylic group. The electron-withdrawing group containing naphthyridine ring produced better activity than presence of halogen, furan and other groups in same ring system. Nitro derivatives of pyrazoline containing benzofuran with naphthyridine or pyridines are highly favorable moieties for antitubercular activity.

2.8. Antiinflammatory Activity

Arachidonic acid is metabolized *via* two pathways in leukocytes: cyclo-oxygenase, leading to the stable prostaglandins, and lipoxygenase, leading to hydroxyacids. Conventional non-steroidal anti-inflammatory drugs that non-selectively inhibit both the major cyclo-oxygenase isoforms (COX-1 and COX-2) are widely used to treat the signs and symptoms of inflammation, particularly arthritic



Scheme 30. Synthetic route to compounds **116-120**.

Table 34. ***In-Vitro*** **Antitubercular Activity and Cytotoxic Results of Compounds 116-120 Against MTB and MDR-TB**

Compound	R	Results against MTB	Results against MDR-TB	IC_{50} (μM)
		MIC (μg/mL)	MIC (μg/mL)	
116	-	2.2	3.2	>170.9
118	-OH (o)	1.2	6.4	>157.6
119	$-NO_2$ (m)	2.3	3.7	>164.5
117	$-NO_2$ (o)	1.9	3.6	>85.4
120	-CH=CH-Ar	5.5	7.4	>168.6
Rifampin	-	0.60	4.2	>74.5
Isoniazid	-	0.32	8.5	>130.5

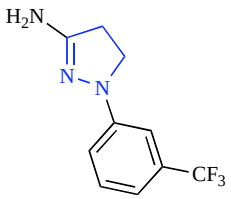
Table 35. ***In Vivo*** **Antitubercular Activities of Compounds 116-120 Against** ***M. tuberculosis*** **ATCC 35801 in Mice**

Compound	R	Lungs (log cfu ± SEM)	Spleen (log cfu ± SEM)
116	-	6.24±0.16	5.42±0.22
118	-OH (o)	4.11±0.62	5.36±0.31
119	$-NO_2$ (m)	2.65±0.43	3.25±0.28
117	$-NO_2$ (o)	5.67±0.22	6.12±0.33
120	-CH=CH-Ar	4.61±0.12	6.32±0.19
Control	-	8.24±0.21	9.42±0.19
Rifampin (25 mg/Kg)	-	3.21±0.11	3.98±0.09
Isoniazid (25 mg/Kg)	-	4.62±0.10	5.45±0.12

pain. COX-1 is the constitutive isoform which is primarily responsible for the synthesis of cyto-protective prostaglandins in gastrointestinal (GI) tract, whereas COX-2 is inducible and plays a major role in prostaglandin biosynthesis in inflammatory cells. Therefore, a high level of selective COX-2 inhibition would help design a therapeutic strategy to alleviate pain and inflammation without any untoward GI toxicity caused by COX-1 inhibition.

Randall *et al.* [63] first noticed that indomethacin is able to inhibit cyclo-oxygenase selectively, whereas inhibition by compound **121** (3-amino-l-(m-(trifluoro-methyl)phenyl)-2-pyrazoline) occurs equally through both the pathways (cyclo-oxygenase and lipoxygenase) (Fig. **15**). The product 5-hydroxy-eicosatetraenoic acid is used as a commercial standard to determine lipoxygenase activity and hydroxyhepta-decatrienoic acid is emoloyed as a standard index for cyclo-oxygenase activity. It appears that indomethacin is far more active in inhibiting cyclo-oxygenase compared to lipoxygenase, while compound **121** is almost equally active against both. These figures were suggestive of their high antiinflammatory activities.

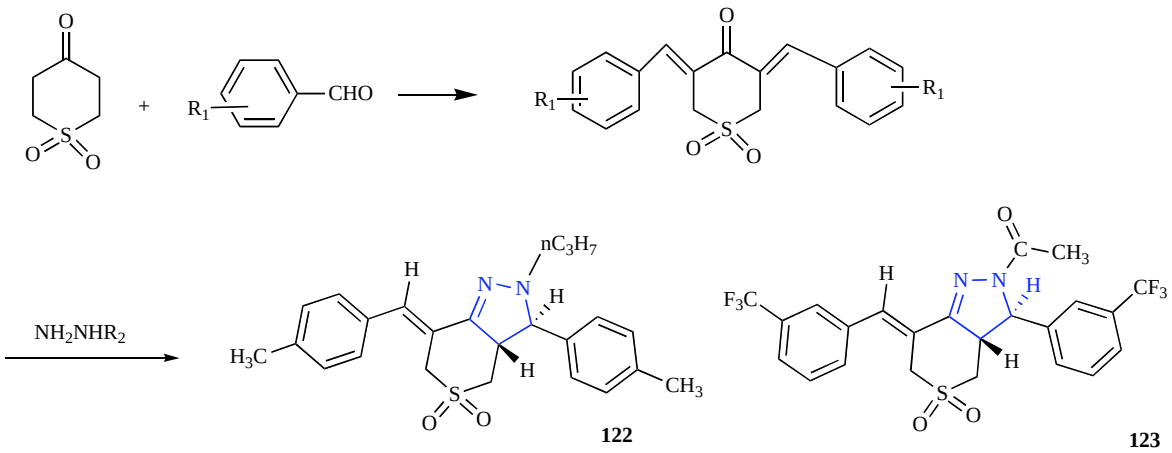
In the pursuit of obtaining more potent compounds, Rovnyak and co-workers [64] prepared a series of novel hexahydrothiopyrano[4,3-c]pyrazoles and related analogues (Scheme **31**) and tested them for their antiinflammatory activities. In general, these compounds displayed appreciable activity in the mouse active arthus and the adjuvant-induced arthritis reactions when administered by the intraperitoneal route. Most importantly, when compound **123** was administered orally, it produced significant inhibition of the systemic lesions in the adjuvant-induced arthritis model, but this activity occurred at or near toxic dose levels (Tables **36**, **37**).



121

Fig. (15). Structure of compound **121**.

In 1993, Nugent and co-workers [65] prepared pyrazoline bisphosphonate tetraethyl esters from vinylidenebispho-ephonic acid tetraethyl ester and diazoketones in ether at 22 ^{o}C as shown in Scheme **32**. Bioactivity results showed that



Scheme 31. Synthetic route to compounds **122-123**.

Table 36. Adjuvant-Induced Arthritis of Compounds 122-123

Adjuvant-induced arthritis			
No.	**Dose, mg/kg**	**Local/systemic,%inhibn**	
		ip	**po**
122	75	28/78	1/0
	150	16/72	17/0
123	75	22/78	0/35
	150	31/97	7/60*

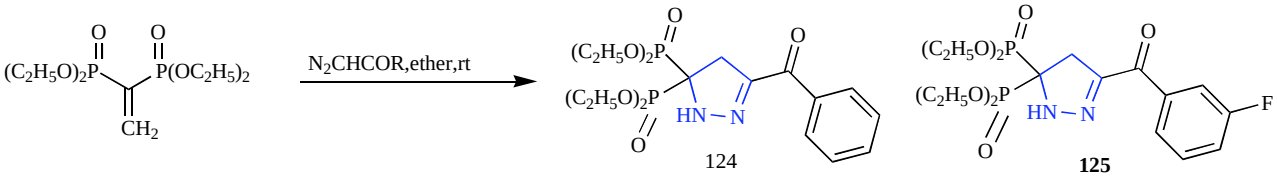
Table 37. Carrageenin-Induced Edema of Compounds 122-123

B. Carrageenin-induced edema		
No.	**ID_{50}, mg/kg**	
	ip	**po**
122	84	250
123	>150(30%)	>200(37%)

compounds **124** and **125** had high antiinflammatory activity and were capable of inhibiting chronic arthritis and inflammation in animals. These compounds might be useful in human being for treating chronic tissue injury associated with arthropathies such as inflammatory joint disease as well as other chronicinflammatory diseases (Tables **38**, **39**).

In the following year Gusar *et al.* [66] studied the effect of several derivatives in comparison with BW755C, the standard inhibitor of cyclooxygenase and lipoxygenase. It was observed that amongst all the thiourea derivatives, the most effective anti-inflammatory drug was the one which had an unsubstituted phenyl nucleus e.g. compound **126** (Fig. **16**). Among thiourea derivatives, the presence or absence of a substituent in the N' position had no significant effect on antiinflammatory activity. At the same time, introduction of a substituent in the phenyl nucleus at position 1 of the pyrazoline ring led to a reduction in activity, as exemplified by the *N*-pyrazolinyl-*N'*-benzoylthioureas. The most effective substance in this series was the one containing an unsubstituted phenyl nucleus, i.e., lab, which reduced edema by 37.5 %, and was slightly more active than the reference compound. Formamidines of the pyrazoline series, containing dimethylamine, piperidine, or morpholine fragments in the side chain and an unsubstituted phenyl nucleus in position 1 of the heterocyclic ring had approximately the same antiinflammatory activities, which were greater than the activity of the compound containing a diethanolamine group.

Similarly, Burguete and co-workers [67] prepared some new ring substituted 3-phenyl-1-(1, 4-di-*N*- oxidequinoxalin-2-yl)-2-propen-1-one derivatives and their corresponding 4, 5- dihydro-(*1H*)- pyrazole analogues as shown in Scheme



Scheme 32. Synthetic route to compounds **124-125**.

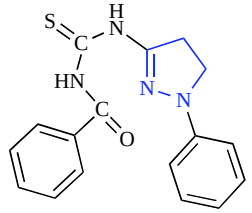
Table 38. Antiarthritic Activity of Compounds 124-125

No.	AIP (%inhibn) (△PV, 28 days)			Antigen-induced arthrities	
	Dose (mg/kg)	Injected paw	Noninjected paw	Dose(mg/kg)	%inhibn
124	100	13	50*	200	48***
	60	23	56*	100	48**
	15	25	31*	50	35*
	5	28	50*	25	16
125	100	22	55*	100	55***
	60	8	46*	50	52***
	15	4	46*	25	42**
	5	0	0		

(***)p<0.001,(**) p<0.01,(*) p<0.05.

Table 39. Results on Delayed Hypersensitivity Granuloma for Compounds 124-125

No.	Dose(mg/kg) sc	% inhibn of granuloma	
		Dry wt	Wet wt
124	100	51***	56***
	50	42***	33*
	25	42***	36*
125	100	65***	58***
	50	51***	45***
	25	49***	42***

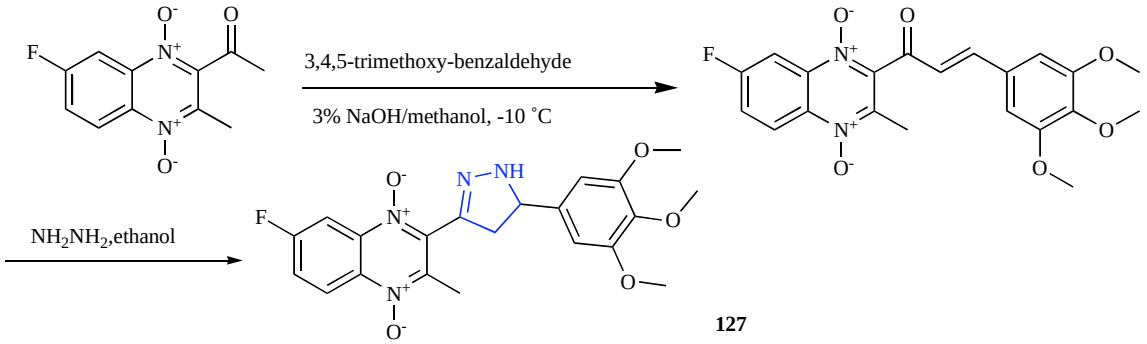


126

Fig. (16). Structure of the compound **126**.

33. Compound **127** showed a modest inhibition of soybean lipoxygenase LOX by the UV absorbance based enzyme assay. However, the experimental results require further validation. Hydrophilicity (lipophilicity with negative sign) is the most significant parameter. Compound **127**, the more lipophilic, is not included in the regression. This fact proceeds in parallel to the observation that low lipophilicity is highly involved to the biological response. Attempts to correlate the *in vivo*/*in vitro* expressions of activity with RM values in a linear or non-linear regression analysis gave statistically non-significant correlations.

Subsequently, a series of 3-(4-biphenyl)-5-substituted phenyl-2-pyrazolines and 1-benzoyl-3-(4-biphenyl)-5-substituted phenyl-2-pyrazolines were prepared by condensation of chalcones with hydrazine hydrate in a mixed solvent consisting of equimolar amount of ethanol and DMF [68]. The newly synthesized compounds (Scheme **34**) were screened for their anti-inflammatory and analgesic activity, and the results were compared with standard drug. The anti-inflammatory activity data showed that compounds with 2, 4,



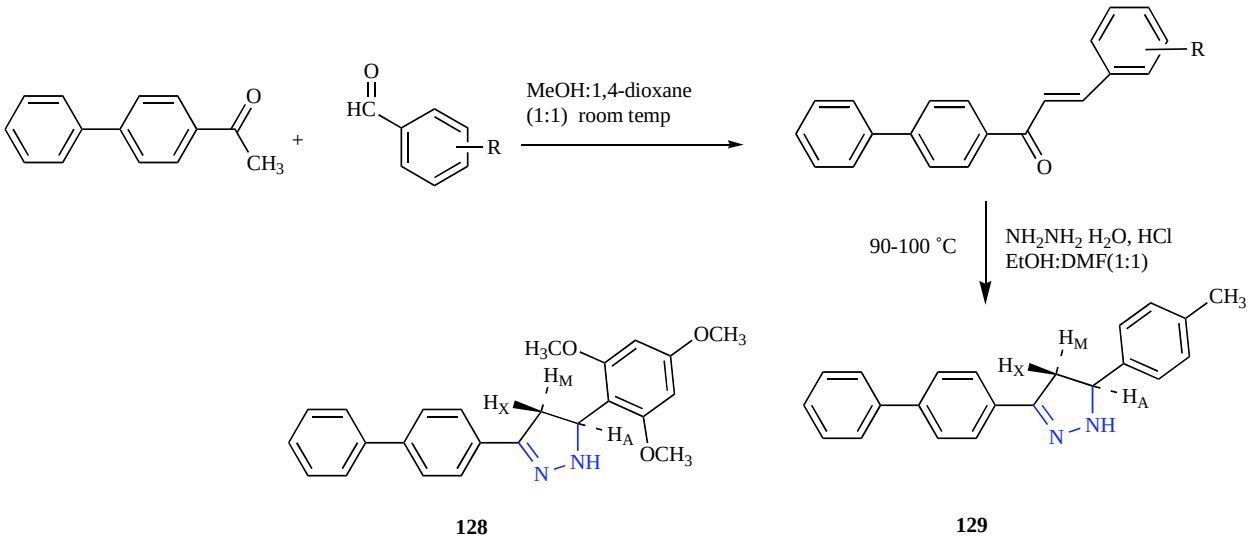
Scheme 33. Synthetic route to compound **127**.

6-trimethoxyphenyl and 4-methylphenyl groups at C-5 of pyrazoline nucleus were associated with most potent activity. Thus, compounds **128** and **129** displaying desired anti-inflammatory activity were further screened for their analgesic activity. It was observed that compound **129** revealed better analgesic activity and has the potential to be employed as a lead drug (Table **40**).

In 2009, Khode and co-workers [69] synthesized a novel series of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines by reacting various substituted 3-aryl-1-(3-coumarinyl) propan-1- ones with phenylhydrazine in the presence of hot pyridine (Scheme **35**). The compounds were screened for *in vivo* anti-inflammatory and analgesic activities at a dose of 200 mg/kg b.w. All compounds exhibited moderate to good anti-inflammatory activity with the percentage inhibition of edema formation (Tables **41**, **42**). The preliminary *in vivo* biological activities of these novel compounds evidenced that the presence of chlorine, fluorine and methoxy groups in the aromatic ring of 5-position of the pyrazoline nucleus gave rise to an increased anti-inflammatory and analgesic activities. Among the prepared compounds, some compounds exhibited significant anti-inflammatory activity in model of acute inflammation such as carrageenan-induced rat edema paw while some compounds showed considerable activity in model of chronic inflammation such as adjuvant-induced arthritis.

In an ingeniously designed synthetic protocol, Shoman and co-workers [70] prepared different series of 3,5-diaryl-2-pyrazoline derivatives by linking either a nitrate ester group or an oxime group to the heterocyclic system through other intervening atoms as shown in Scheme **36**. The synthesized compounds **134a-d**, **135a-c**, **136a, b** were evaluated for their anti-inflammatory activity. The results showed that most of the synthesized compounds had significant anti-inflammatory activities against carrageenan-induced pawedema in rats after 3 h, which was the time required to attain maximum activity for the tested compounds. It is worth mentioning here that hybrid molecules incorporating electron releasing moieties can serve to improve the safety of NSAIDs without altering their effectiveness.

Again, 5-trifluoromethyl-4,5-dihydro-*1H*-pyrazole derivatives **137** and **138** (Fig. **17**) were tested to evaluate their antinociceptive and antiedematogenic effects through acute (1-1000 µmol/kg) and chronic (100 µmol/kg for 15 days) administration in rats which were subjected to a model of adjuvant-induced arthritis [71]. The results indicated that compounds **137** and **138** were associated with antinocic-

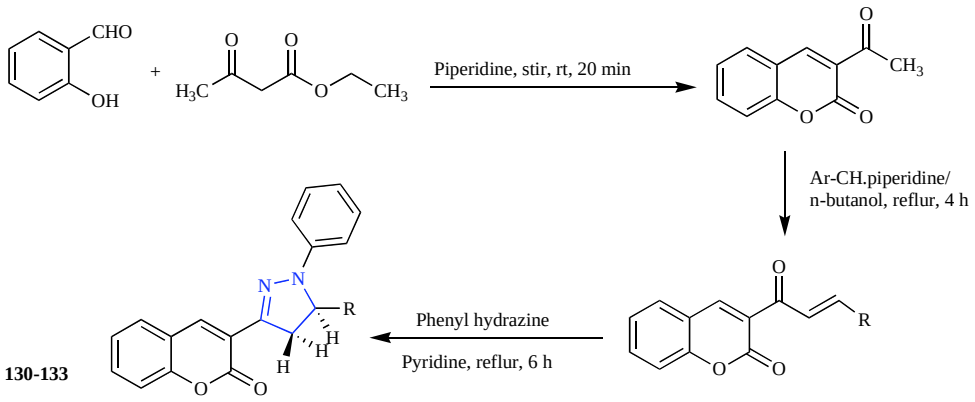


Scheme 34. Synthetic route to compounds **128-129**.

Table 40. Anti-Inflammatory Activity of Compounds 128-129

Compound	Anti-inflammatory activity % inhibition ± SEM	
	After 3 h	After 4 h
128	73.69 ± 3.60	82.45 ± 2.20
129	80.70 ± 3.23	82.45 ± 2.21
Flurbiprophen	73.68 ± 4.51	80.69 ± 3.23

All compounds administered at an oral dose of 41 lmol/kg. *P < 0.01.



Scheme 35. Synthetic route to compounds **130-133**.

Table 41. *In Vivo* Acute Anti-Inflammatory Activity of Compounds 130-133

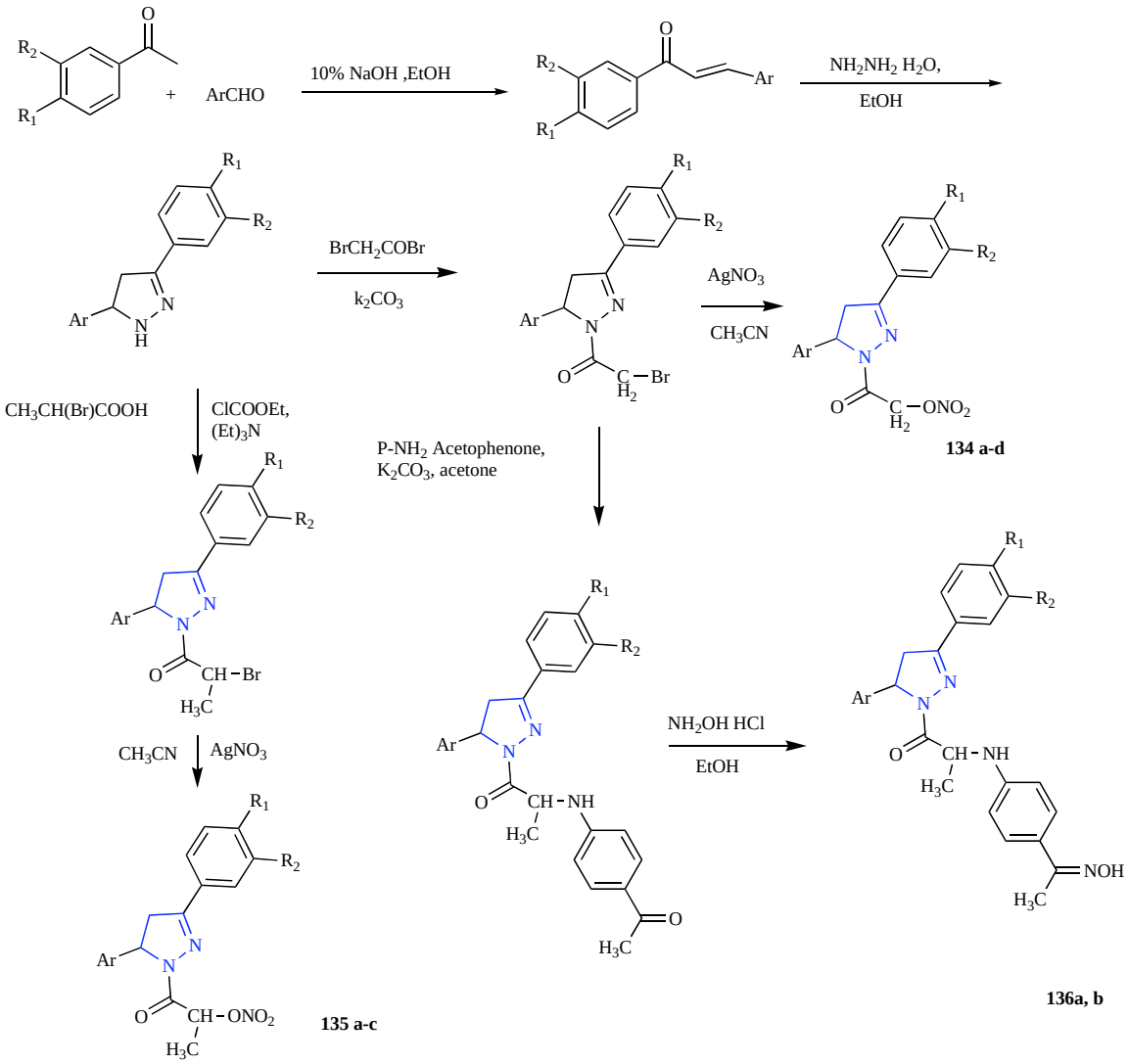
Compound	R	Anti-inflammatory activity	
		% inhibition after 2h (±SEM)	% inhibition after 4h (±SEM)
130	4-Cl- C_6H_4	39.2(±0.026)*	64.7(±0.021)*
131	2,4-$(Cl)_2$-C_6H_3	56.7(±0.030)**	67.5(±0.024)**
132	3-OMe-C_6H_4	38.3(±0.030)*	61.5(±0.013)**
133	4-F-C_6H_4	44.9(±0.023)**	66.7(±0.011)**
Diclofenac		63.7(±0.017)***	78.7(±0.013)***

p<0.05. *p<0.05 vs control at 200 mg/kg b.w; **p<0.01 vscontrol at 200 mg/kg b.w; ***p< 0.001 vs control at 13.5 mg/kg b.w.

Table 42. *In Vivo* Chronic Anti-Inflammatory Activity of Compounds 130-133 in Adjuvant-Induced Arthritis Model

Compound	Anti-inflammatory activity		
	Paw edema volume(mean±SEM)		% inhibition after treatment (on day 19)
	Day 15	Day 19	
control	0.92±0.08	0.87±0.02	05.5±0.24
130	0.87±0.12	0.53±0.07	39.1±0.81*
131	0.86±0.07	0.47±0.06	45.4±1.63*
132	0.81±0.05	0.64±0.09	20.9±1.25
133	0.89±0.04	0.53±0.03	40.5±1.80*
Diclofenac	0.83±0.09	0.39±0.05	53.0±1.92**

p< 0.05. *p<0.05 vs control at 200 mg/kg b.w.; **p <0.01 vs control at 13.5 mg/kg b.w.



No.	R_1	R_2	Ar
a	OCH_3	OCH_3	Furyl
b	OCH_3	OCH_3	2,4-Di-OCH3phenyl
c	OCH_3	OCH_3	2,6-Di-Clphenyl
d	OCH_3	H	2,6-Di-Clphenyl

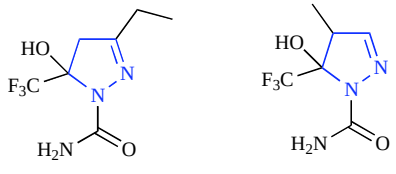
Scheme 36. Synthetic route to compounds **134-136**.

eptive property against chronic inflammatory pain induced by CFA in rats. The antinociceptive effect occurred in the absence of diverse effects, indicating that these compounds may be interesting in the design of new drugs for chronicpain management.

In another related study, Rathish*et al.* [72] synthesized new 1, 3, 5-trisubstituted pyrazolines bearing benzene sulfonamides and tested them for their anti-inflammatory activity at the dose of 20 mg/kg in carrageenan-induced rat paw edema model; the volumes of paw edema were measured at 0, 3 and 5 h. Compounds **139** and **140** (Fig. **18**) displayed higher activity than celecoxib throughout the study (at 3 and 5 h).

2.9. MAOs Inhibitor

Monoamine oxidases (MAOs) are a family of enzymes that are responsible for the metabolism of monoamine



137 **138**

Fig. (17). Structures of compounds **137, 138**.

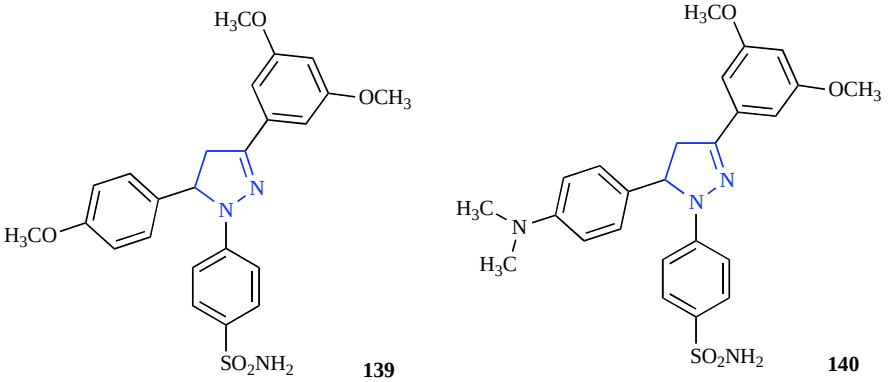


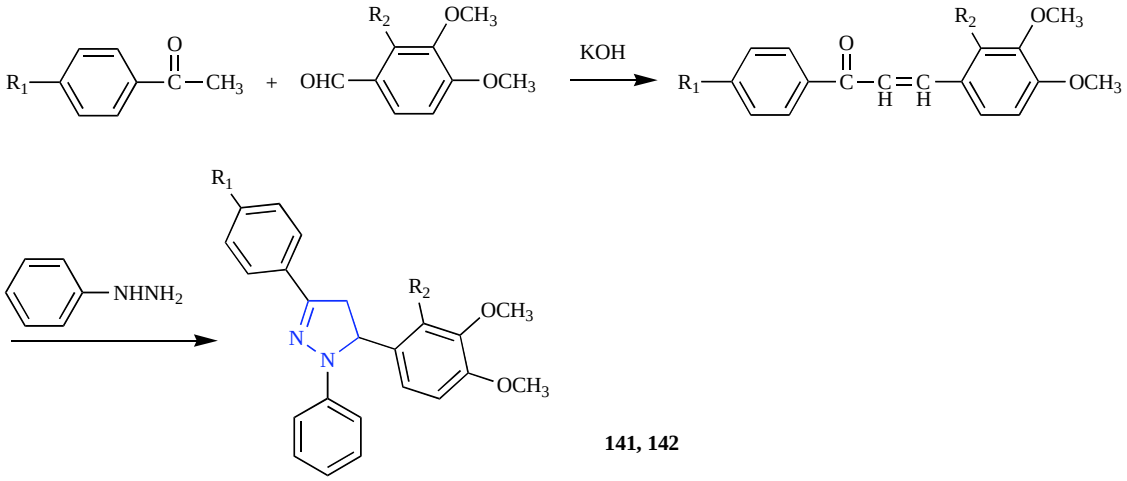
Fig. (18). Structures of compounds **139, 140**.

neurotransmitters. They are widely distributed among mammals, plants, and prokaryotic and eukaryotic microrganisms which can catalyze oxidatively amines to aldehydes. As a result, MAO inhibitors (MAOI) have been studied extensively for the treatment of several psy-chiatric and neurological diseases. MAO-A inhibitors are often employed as antianxiety drugs, whereas MAO-B inhibitors can act as coadjuvant in the treatment of Parkinson's and Alzheimer's diseases.

In 1996, Palaska, E. *et al.* [73] synthesized new 1, 3, 5-triphenyl-2-pyrazoline derivatives (Scheme **37**) by reacting 1, 3-diphenyl-2-propen-l-one with phenylhydrazine. The antidepressant activities of these compounds were screened by the porsolt behavioral despair test which identified compounds **141** and **142** with significant antidepressant activity when compared with clomipramine and tranylcypromine (Table **43**). A methyl substituent at the position 3 of the pyrazoline ring enhances the antidepressant activity; on the other hand, replacement of this methyl group by chloro or bromo substituents decreases the activity. In addition, introduction of a chloro substituent at position 5 of the phenyl ring lowers the antidepressant activity.

Likewise, the novel series of 1-acetyl-3, 5-diphenyl-4, 5-dihydro-(*1H*)-pyrazole derivatives prepared by Manna and coworkers [74] exhibited reversible and non-competitive inhibition to all types of investigated amine oxidases. In particular, the compounds showed potent inhibition activity against monoamine oxidases with significantly lower C_{50} values e.g. 1-acetyl-3-(2, 4-dihydroxy-phenyl)-5-(3-methylphenyl)-4, 5-dihydro-(*1H*)-pyrazole **143** (Scheme **38**) showed great potential to inhibit monoamine oxidase with a Ki of about 10^{-8} M. The docking studies rationalized the relevant inhibitory activity of compound **143** towards MAO-B, as due to the formation of several favourable interactions with the catalytic site of the enzyme. Notably, residues Tyr398 and Tyr435, both interacting with the disubstituted phenyl ring of **143**, have been already underlined. The importance of the 4-OH group in properly positioning the disubstituted phenyl ring of **143** by H-bond formation was pointed out. The introduction of one Cl in position 4 of the phenyl ring at C5 increased the activity. Notably, the in-silico introduction of such a substituent in the docking geometry of **143** revealed that this atom might be properly accommodated between the side chains of residues Leu171, Leu345, Tyr326 and Phe343, in a favourable hydrophobic environment (Fig. **19**).

In 2004, France and coworkers [75] synthesized a novel series of 1-acetyl-3-(4-hydroxy-and2, 4- dihydroxyphenyl) -5-phenyl-4, 5-dihydro-(*1H*)-pyrazole derivatives in a single step as shown in Scheme **39**. The compounds were evaluated

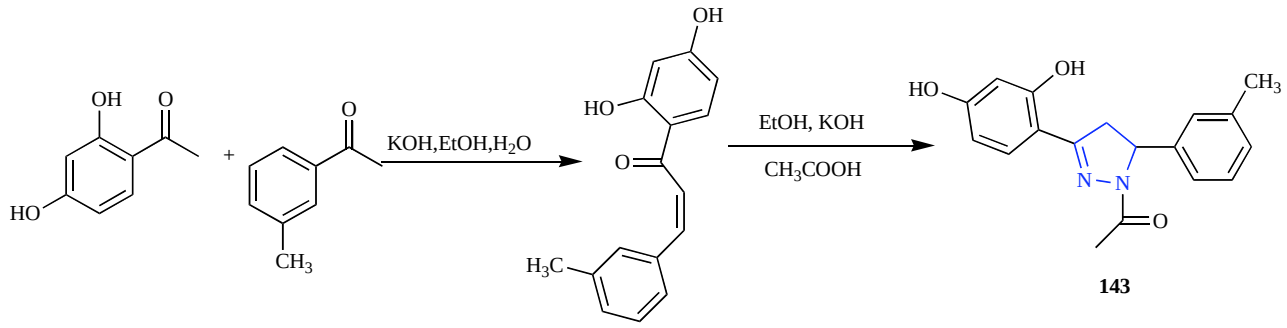


141, 142

Scheme 37. Synthetic route to compounds **141-142**.

Table 43. Antidepressant Activities of Compounds 141-142

Compound	R_1	R_2	Duration of immobility (s)	Change from control (%)
141	CH_3	CH_3	20.8±3.6	−49.51
142	CH_3	Cl	24.5±4.3	−40.53
Clomipramine 10 mg/kg	-	-	27.3±5.1	−33.74
Tranylcypromine 10 mg/kg	-	-	22.8±2.6	−44.66



143

Scheme 38. Synthetic route to compound **143**.

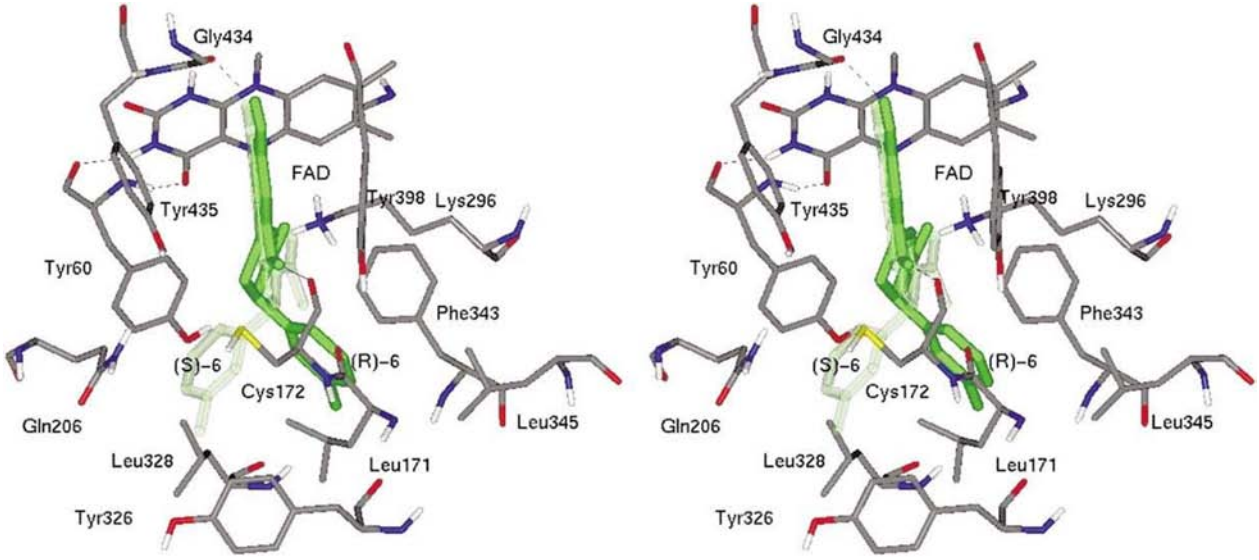
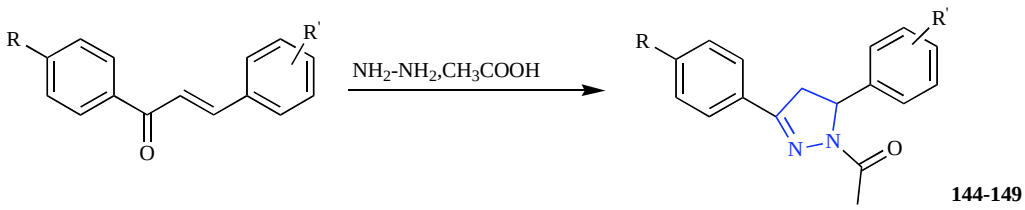


Fig. (19). Superimposition (relaxed stereoview) of the calculated recognition geometries (thick) of (R)-143 (green) and (S)-143 (light green) located at their proper respective position inside the catalytic site of MAO-B (thin, coloured by atom types). For the sake of simplicity only the MAO residues useful for the discussion are shown. Hydrogen atoms bound to heteroatoms are displayed. Hydrogen-bonding interactions are depicted as dashed lines.

for their potential to selectively inhibit the activity of the A and B isoforms of monoamine oxidase (MAO). The results demonstrated that compounds 144-149 were more reversible, potent, and selective inhibitors of MAO-A compared to MAO-B. The inhibitory activities on MAO-A ranged from 8.0×10^{-9} to 9.0×10^{-9} M (Table **44**). The biological results also indicate not only the influence of the para-substituted hydroxyl group on the aromatic ring bonded to C3 of the pyrazoline ring, but also that of the *ortho*-substituted methoxyl group on the aromatic ring bonded to C5 of the pyrazoline nucleus.

Furthermore, Franco and coworkers [76] synthesized 1-thiocarbamoyl-3, 5-diaryl-4, 5-dihydro-(*1H*)-pyrazole derivatives (Scheme **40**) and investigated their selective inhibition activity on the A and B isoforms of monoamine oxidase (MAO). It is evident from the data presented in Table **45** that all the synthesized compounds possessed high activity against both the isoforms of MAO-A and MAO-B. The presence of a 4-chlorophenyl substituent in the 5 position helps to enhance the activity against both MAO-A and MAO-B. The stereochemistry may be an important modulator of biological activity. The docking study was

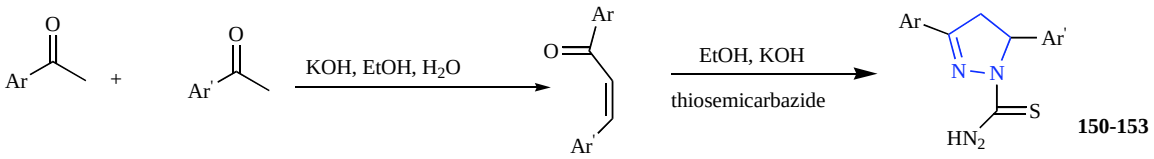


Scheme 39. Synthetic route to compounds **144-149**.

Table 44. Monoamine Oxidase Inhibitory Activity of the Compounds 144-149

Compound	R	R'	MAO IC_{50}	MAO-A IC_{50}	MAO-B IC_{50}	SI selectivity
144	4-OH	3-CH_3	$9.5\times10^{-6}\pm1.00$	$8.0\times10^{-9}\pm0.05$	$1.9\times10^{-5}\pm0.03$	2375
145	4-OH	2-OCH_3	$3.0\times10^{-5}\pm0.05$	$8.8\times10^{-9}\pm0.01$	$1.0\times10^{-4}\pm0.06$	11363
146	4-OH	4-OCH_3	$3.6\times10^{-6}\pm0.03$	$9.0\times10^{-9}\pm0.08$	$7.2\times10^{-6}\pm0.28$	800
147	2,4-OH	4-CH_3	$9.5\times10^{-6}\pm0.08$	$9.0\times10^{-9}\pm0.02$	$4.2\times10^{-5}\pm0.07$	4666
148	2,4-OH	2-OCH_3	$4.0\times10^{-5}\pm0.02$	$8.0\times10^{-9}\pm0.01$	$1.3\times10^{-4}\pm0.03$	16250
149	2,4-OH	4-OCH_3	$1.0\times10^{-5}\pm0.04$	$9.0\times10^{-9}\pm0.04$	$8.3\times10^{-5}\pm0.05$	9222

SI: selectivity index = IC_{50} (MAO-B)/IC_{50} (MAO-A).



Scheme 40. Synthetic route to the compounds **150-153**.

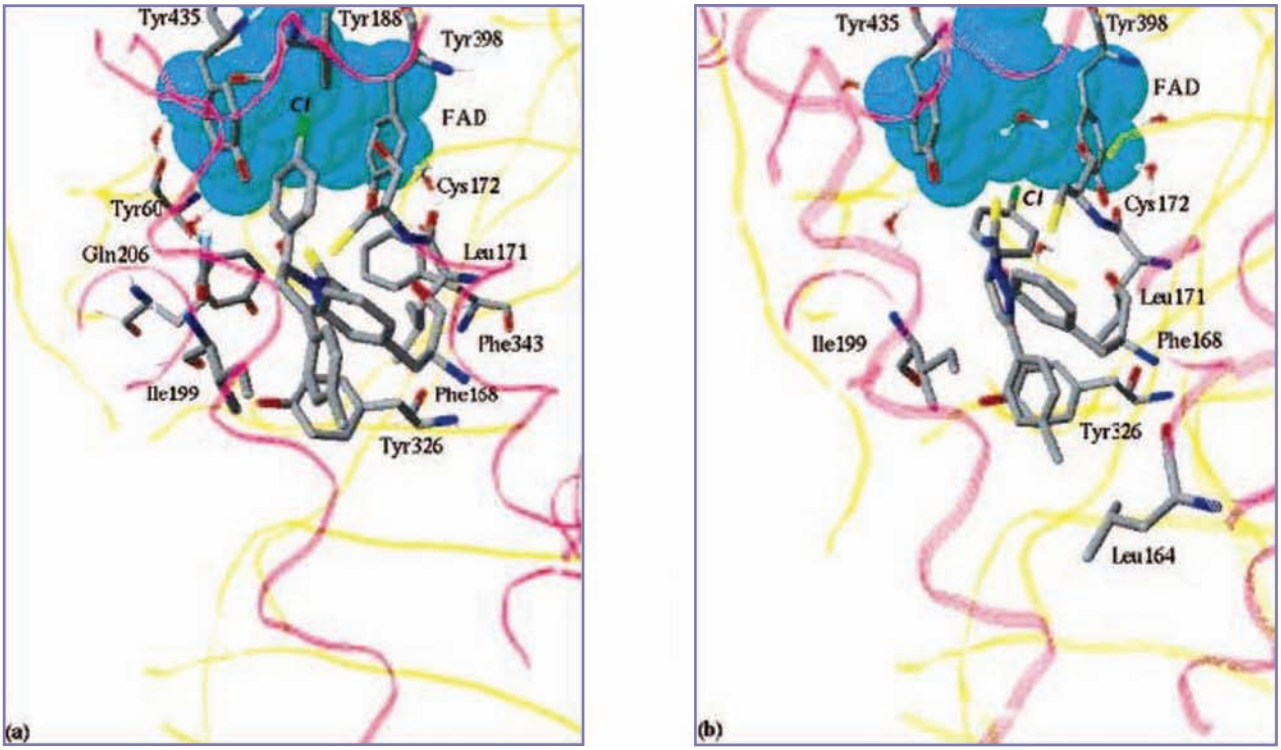
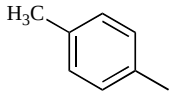
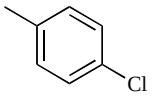
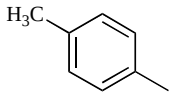
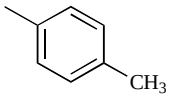
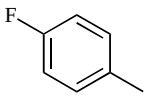
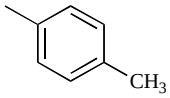
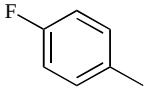


Fig. (20). GLUE-GROMACS binding configurations of complexes [MAO-B/(*S*)-**1**] (a), [MAO-B/(*R*)-**1**] (b).

Table 45. Physicochemical Properties of Compounds 150-153

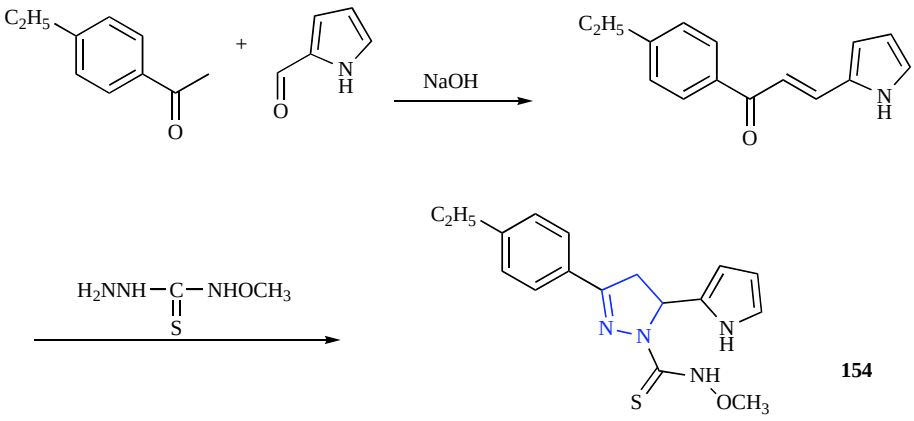
Compound	Ar	Ar'	K_iM MAO-A	K_iM MAO-B	SI B/A	SI A/B
150			3.1×10^{-8} (±0.05)	1.5×10^{-9} (±0.02)	0.05	20.70
151			6.0×10^{-9} (±0.03)	1.0×10^{-8} (±0.07)	1.60	0.60
152			7.0×10^{-9} (±0.07)	5.0×10^{-8} (±0.06)	7.10	0.14
153			8.0×10^{-9} (±0.03)	3.7×10^{-7} (±0.01)	46.25	0.021

carried out with the aim of proposing possible binding modes of the MAO enantioselective compound **150.** In the same enzymatic cleft, its enantiomer (*R*)-**150** was similarly oriented as far as regards the aromatic ring positions. No Ar' stacking was observed, but the van der Waals contacts were evident with the isoalloxazine FAD ring, Tyr398, and Tyr435. Moreover, with this residue the thiourea *S* atom established a first hydrogen bond and a second one with a water molecule close to Cys172. Similar to its enantiomer, the Ar moiety (*R*)-**150** interacted with the same cluster of hydrophobic residues (Fig. **20**).

In 2007, Nesrin *et al.* synthesized a novel series of 1-thiocarbamoyl-3-substituted phenyl-5-(2-pyrrolyl)-4, 5-dihydro-(*1H*)-pyrazole derivatives (Scheme **41**) [77] and investigated them for their ability to inhibit selectively the activity of the A and B isoforms of monoamine oxidase. The compound **154** where the phenyl residue was substituted by methoxy moiety and allyl group by a thiocarbamoyl moiety, exhibited the best antiinflammatory activity. It is suggested that these *N*-substituted pyrazole derivatives can be evaluated as both MAO-B inhibitors and antiinflammatory analgesics which may have promising features in the treatment of AD. Non-competitive and irreversible inhibition of rat liver MAO by these derivatives suggested that these compounds cannot enter the small active site cavity of the enzyme and may interact tightly with another binding site or with some other reactive groups present in the molecule.

Yabanoglu and co-workers [78] prepared new 1-*N*-substituted thiocarbamoyl-3-substituted phenyl-5-pyrolyl-2-pyrazoline derivatives **155-157** carrying a *p*-methoxy group on the phenyl ring. They exhibited inhibition of rat lung SSAO irreversibly in a time-dependent manner and could be used (Fig. **21**) to discriminate between Cu- and FAD-containing amine oxidases and to determine the possible roles of SSAO in physiological events and also in some SSAO-related disorders (Table **46**). These compounds may have promising features as anti-parkinson agents if their SSAO-inhibitory effects can be supported by *in vivo* studies.

Again, series of *N*-1-propanoyl-3, 5-diphenyl-4, 5-dihydro- (*1H*)-pyrazole derivatives (Scheme **42**) [79] were prepared and subsequently assayed as inhibitors of MAO-A and MAO-B isoforms. Most of the tested compounds showed inhibitory activity with micromolar values and MAO-A selectivity. Among them**,** the best MAO-A inhibitory activity was displayed by compounds **158** and **159**



Scheme 41. Synthetic route to compound **154**.

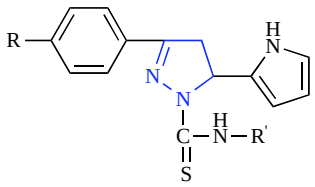


Fig. (21). Structures of compounds **155-157**.

(pIC_{50} = 6.70) (Table **47**). The best MAO-A selectivity was measured for compound **158** (pSI = 2.70), unsubstituted on the A ring, while the best MAO-B selectivity was observed for compound **162** (pSI=1.82), substituted with fluorine and methyl group on the A and B rings, respectively. When methyl and/or methoxy groups are present on the aromatic rings the compounds show poor activity against both the isoforms. Since compound **158** combines the best MAO-A inhibitory activity with the best A-selectivity, it was selected in the following docking study for a better understanding of such biological properties (Fig. **22**).

In 2008, Jun and coworkers synthesized a series of pyrazoline derivatives with β-amino acyl group [80] (Scheme **43**) and evaluated them for their ability to inhibit dipeptidyl peptidase IV. Amongst them, carboxylic acid substituted pyrazoline derivative **163** displayed best results in terms of reducing the inhibitory activity toward CYP3A4 enzyme. X-ray co-crystal structure of initial hit compound was determined Fig. **23**).

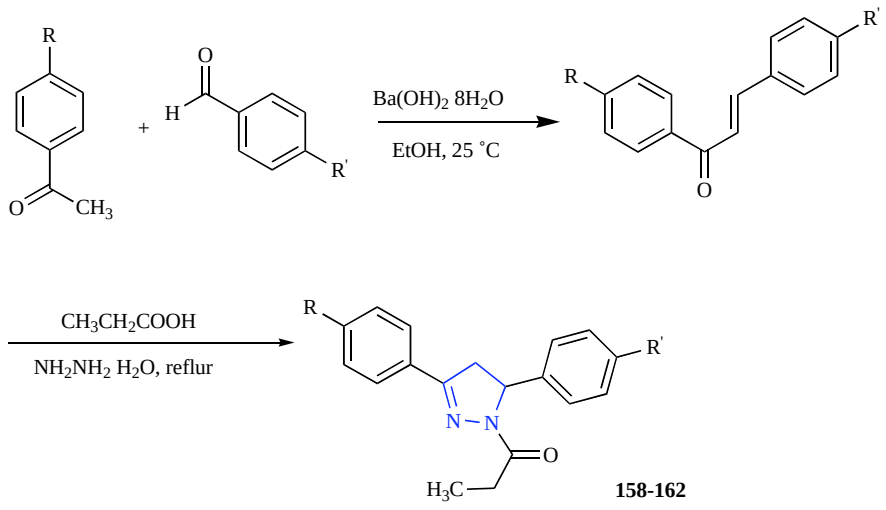
The two hydropyrazole derivatives **164-165** (Scheme **44**) prepared by Amar *et al.* [81] displayed moderate antiparkinsonian activities (Table **48**) (relative potencies to Benzatropine).

In 2009, Can *et al.* examined effects of some 1,3,5-trisubstituted-2-pyrazoline derivatives [82] on depression, anxiety and spontaneous locomotor activity parameters of mice. Effects of the test compounds at 50, 100 and 200 mg/kg doses on exploratory behaviors of mice in hole-board tests were observed. Compounds **166** and **167** (Fig. **24**) showed antidepressant-like activities. These two pyrazoline derivatives significantly shortened the immobility and prolonged the swimming times without any change in the climbing times of mice at 100 mg/kg doses (Table **49**).

In 2010, Sule and coworkers [83] synthesized eight new 1-[(*N*, *N*-disubstituted thiocarbamoylthio) acetyl]-3-(2-thienyl) -5-aryl-2-pyrazolines and investigated their antidepressant-like effect. The results showed that compounds **168-170** (Fig. **25**) could enhance the total distance travelled and horizontal activity. Although compound **169** and clomipramine do not share common chemical structure, they show some behavioral similarities in forced swimming and motor activity tests (Tables **50**, **51**).

Table 46. IC_{50} Values for the Inhibition of Rat Lung SSAO by Compounds 155-157

Compound	R	R'	IC_{50} for SSAO(μM)	
			Preincubation 0 min	Preincubation 60 min
155	OCH_3	CH_3	70.11 ± 6.34	42.10 ± 4.26
156	OCH_3	CH_2CH_3	230.57 ± 19.50	170.16 ±13.90
157	OCH_3	$-CH_2CH=CH_2$	280.30 ± 20.31	225.30 ±16.44
Semicarbazide	-	-	12.82 ± 1.20	5.40 ± 0.46



Scheme 42. Synthetic path to compounds **158-162**.

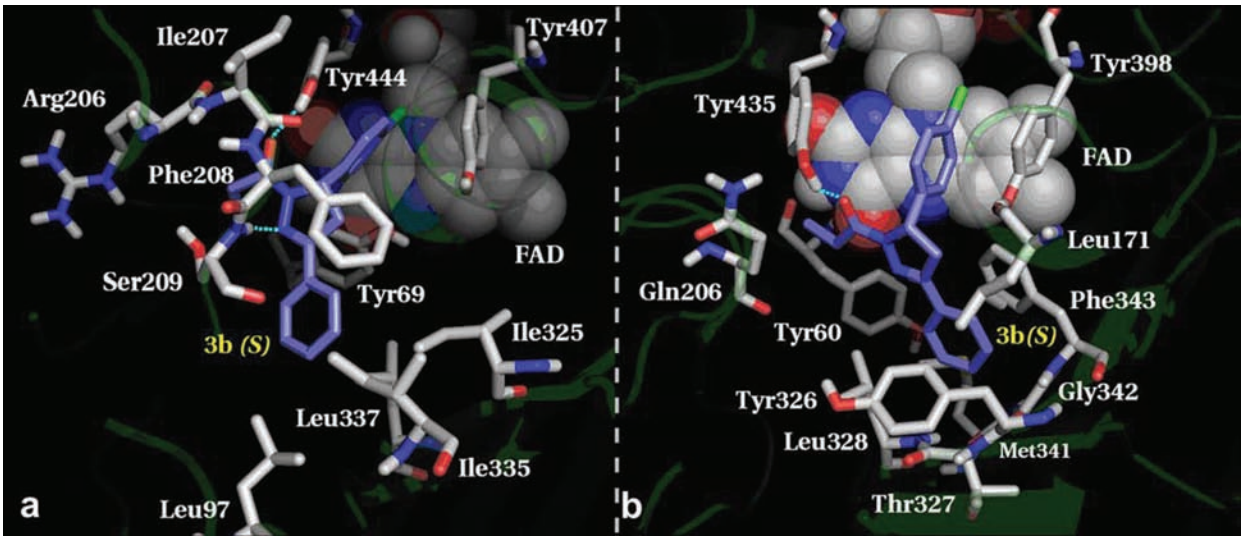
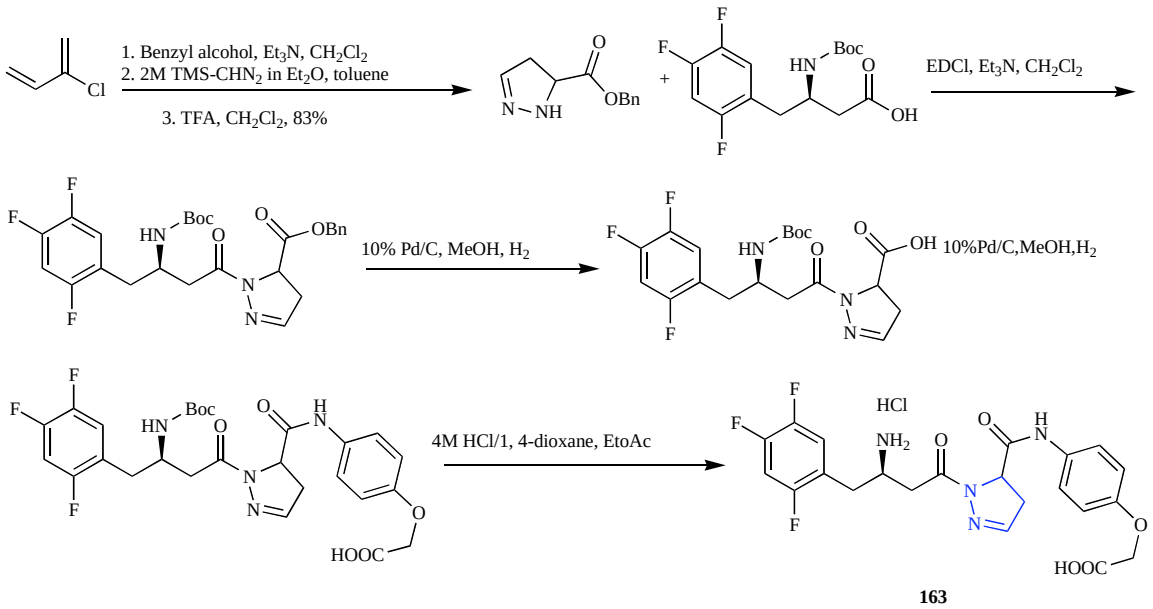


Fig. (22). MAO-A (*S*) 158 (a) and MAO-B (*S*)158 (b) OMD global minimum energy configurations. Interacting residues are represented in stick and FAD cofactors are displayed as spacefill CPK rendering. (*S*) 158 is depicted in stick with violet colored carbon atoms.

Table 47. MAO Inhibitory Activity Data of Compounds 158-162

Compound	R	R'	$pIC_{50(MAO-A)}$	$pIC_{50(MAO-B)}$	$pIC_{50(MAO-A)}$-$pIC_{50\ (MAO-B)}$
158	H	Cl	6.70	4.00	2.70
159	Cl	Cl	6.70	5.00	1.70
160	F	H	6.09	6.70	-0.61
161	F	F	6.00	6.82	0.82
162	F	CH_3	5.00	6.82	-1.82
Moclobemide	-	-	4.94	2.00	2.94
Toloxatone	-	-	6.42	4.82	1.60
Selegiline	-	-	4.42	6.00	-1.58



Scheme 43. Synthetic route to compound **163**.

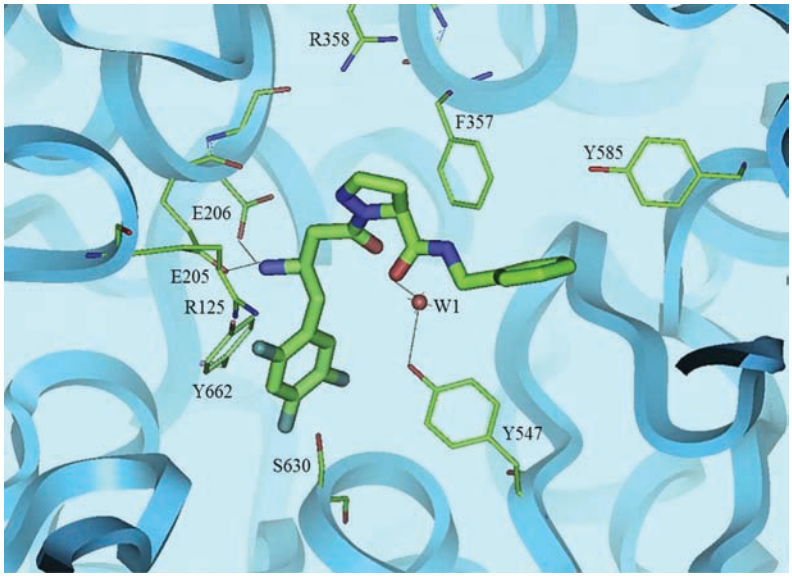
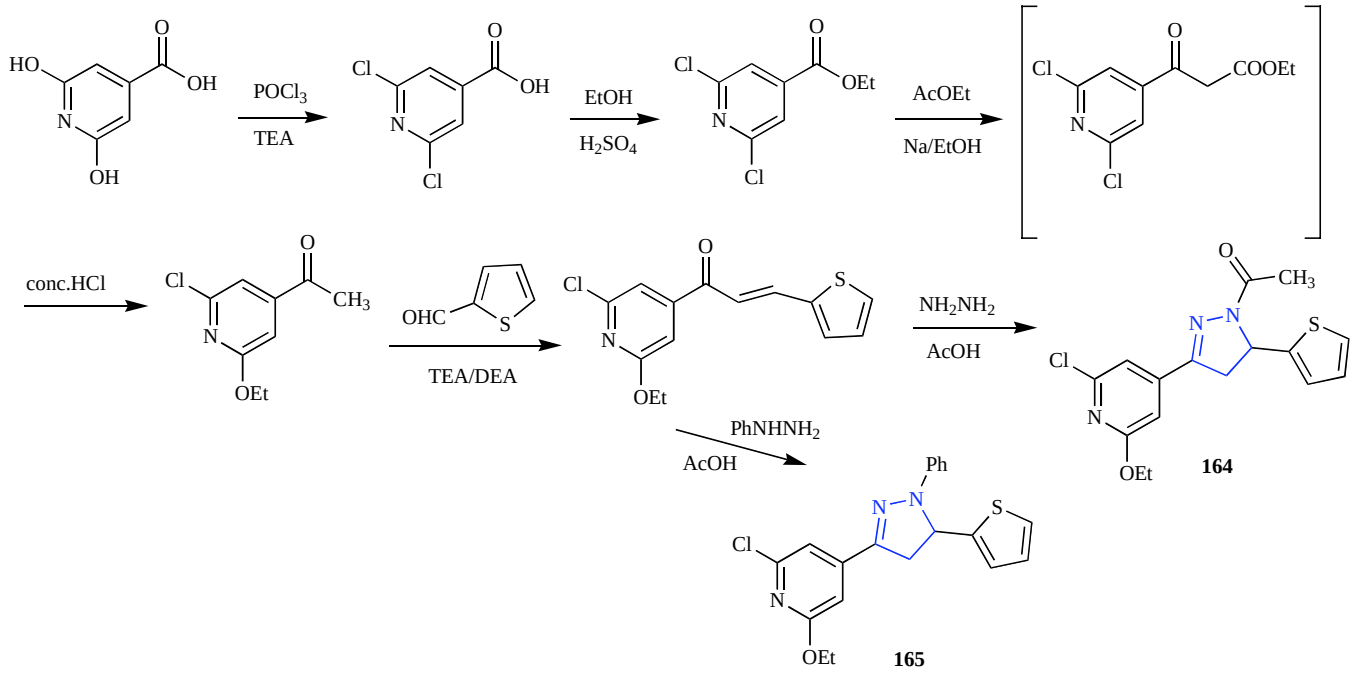


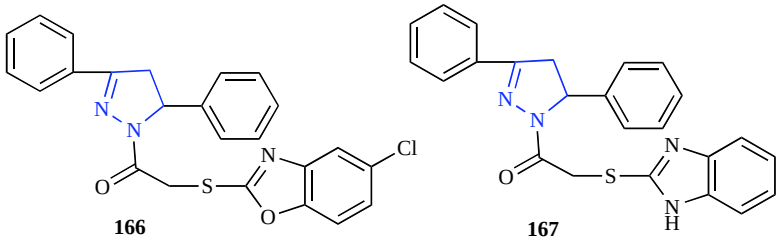
Fig. (23). X-ray co-crystal structure of pyrazoline derivative.



Scheme 44. Synthetic route to compounds **164-165**.

Table 48. Antiparkinsonian Activities of Compounds 164-165 in Comparison with Benzatropine

Compound	Salivation and lacrimation score	Tremors score	Decrease from oxotremorine rectal temperature /% ± SE	Relative potency compared to benzatropine mesilate ± SE
Control	0	0	0	0
Benzatropine	1	1	25.0 ± 0.400	1.00 ± 0.09
164	2	2	11.0 ± 0.100	0.41 ± 0.031
165	1	1	17.0 ± 0.300	0.65 ± 0.07



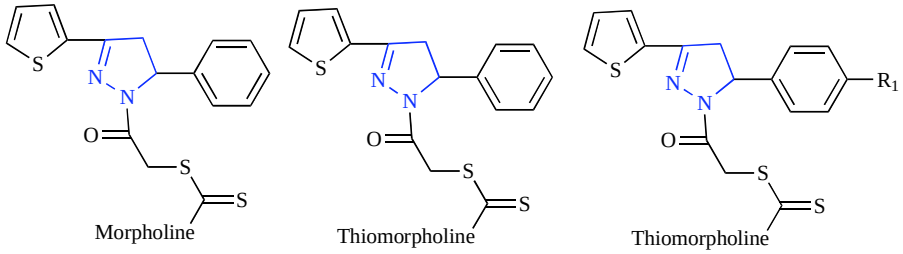
166 **167**

Fig. (24). Structures of compounds **166-167**.

Table 49. Effects of Compounds 166-167 on Climbing, Swimming and Immobility Times of Mice in MFST

Group	Dose	Climbing time (s)	Swimming time (s)	Immobility time (s)
Control	-	41.6 ± 2.8	121.2 ± 7.2	91.9 ± 6.5
Fluoxetine	10 mg/kg	33.3 ± 3.8	180.8 ± 6.9[c]	53.1 ± 5.2[c]
166	100 mg/kg	35.6 ± 3.6	173.1 ± 10.2[b]	58.5 ± 4.8[b]
167	100 mg/kg	34.3 ± 5.5	165.1 ± 12.1[a]	64.9 ± 5.8[a]

Values are given as mean ± S.E.M. Significance compared with control values, [a]p < 0,05, [b]p < 0,01, [c]p < 0,001, One-way ANOVA, post-hoc Tukey's test, n = 7.



168 **169** **170**

Fig. (25). Structures of compounds **168-170**.

Table 50. Effect of Compounds 168-170 on Motor Activity. Data are Given as Mean ± Standard Error of the Mean (SEM)

Group	N	Dose(mg/kg)	Horizontal activity	Distance	Resting(%)
TW80	8	100	1246±117	1460±135	78±2
168	5	100	1501±130	1740±155	77±3
169	6	100	1899±48*	2104 ± 87	73 ± 3
170	5	100	1757 ± 243	1705 ± 195	72 ± 5
Clomipramine	5	10	1870 ± 754	2006 ± 780	73 ± 10
Tranylcypromine	6	10	564 ± 95**	663 ± 141**	89 ± 2
Tranylcypromine	7	20	283 ± 75***	328 ± 89***	93 ± 2*

* P<0.05; ** P<0.01; *** P<0.001 difference from control (TW80).

These results suggest that the *N*, *N*-disubstituted dithiocarbamate moiety of pyrazoline derivatives is associated with antidepressant therapeutic potential.

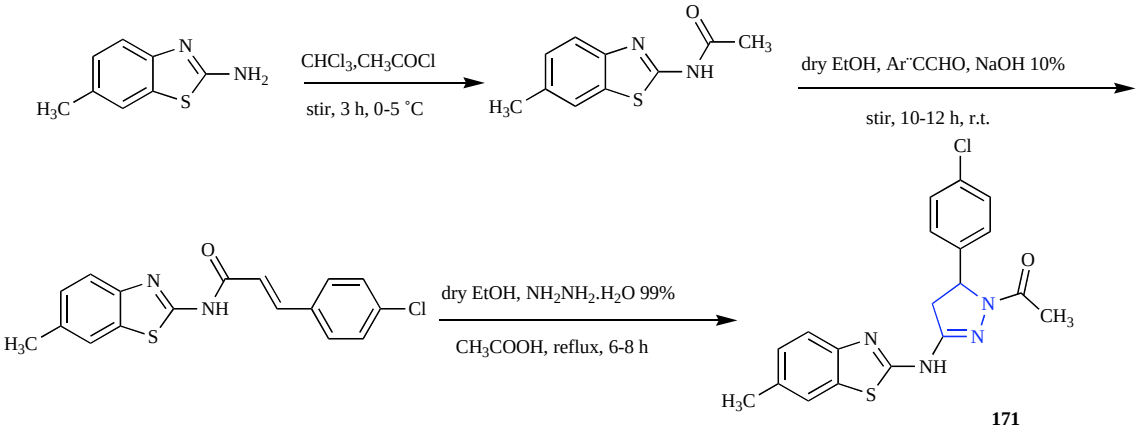
2.10. Anticonvulsant Activity

A significant amount of research has been conducted in recent years for the development of novel therapeutics with potential to be employed as newer anticonvulsant drugs. These drugs have proven to be effective in reducing seizure, whilst their therapeutic efficacy is associated with adverse side effects such as headache, nausea, hepatotoxicity, anorexia, ataxia, drowsiness, gastrointestinal disturbances and hirsutism. These observations stress the need for preparing newer agents which prompted Amnerkar *et al*. [84]

Table 51. Latency and Immobility Time of Mice on Forced Swim Test for Compounds 168-170. Data are Given as Mean ± SEM

Group	n	Dose(mg/kg)	Latency	Immobility(s)
Saline	8		60 ± 12	154 ± 20
Vehicle (TW80)	8		68 ± 13	167 ± 17
168	5	100	73 ± 8	148 ± 23
169	5	100	72 ± 10	84 ± 15**
170	5	100	80 ± 17	134 ± 15
Clomipramine	5	10	101 ± 8	108 ± 14*
Tranylcypromine	6	10	89 ± 14	108 ± 13*
Tranylcypromine	7	20	31 ± 5	65 ± 14***

* P\0.05; ** P\0.01; *** P\0.001, difference from control (saline and TW 80).



Scheme 45. Synthetic route to compound **171**.

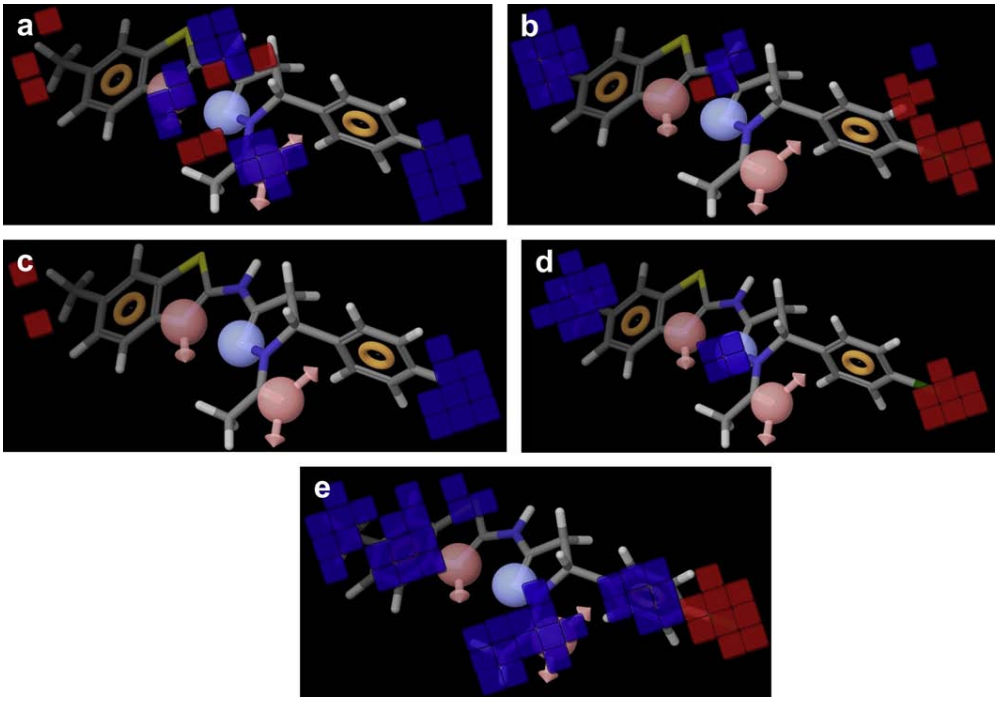
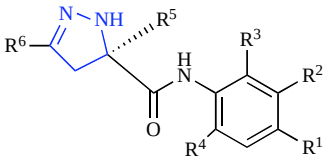


Fig. (26). Phase 3D plots of crucial pharmacophore region based on model displayed with compound **171**. Positive coefficient favored areas are represented by blue cubes. Negative coefficient favored areas are represented by red cubes. (a) Hydrogen-bond acceptor; (b) Hydrogen bond donor; (c) Negative ionic groups; (d) Positive ionic groups; (e) Hydrophobic region.

to design 6-substituted-2-[(1-acetyl-5-substituted)-2- pyrazolin-3-yl] aminobenzothiazole (Scheme **45**). The results showed that **171** exhibited an ED_{50} of 25.49 µmol/kg, TD_{50} of 123.87 µmol/kg and high protective index (PI) of 4.86 compared to standard drug phenytoin showing its promise as an anticonvulsant agent. The anticonvulsant activity (ED50) generated in the present studies for the series of 6-substituted-[3-substituted-prop-2-eneamido] benzo-thiazoles and 6-substituted-2-[(1-Acetyl-5-substituted)-2-pyrazolin-3-yl]aminobenzothiazoles was employed for the generation of 3D-QSAR models with the aim that these models could provide useful pharmacophoric information for the future efforts in the development of more potent molecules in these series of chemical classes (Fig. **26**).

2.11. Steroidal Activity

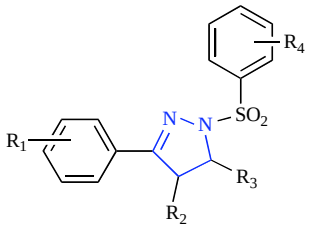
Zhang *et al.* designed a novel series of dihydropyrazole **172** derivatives (Fig. **27**) and evaluated them by *in vivo* screening as tissueselective and rogen receptor modulators. Structure activity relationships were investigated at the $\mathbf{R^1}$ to $\mathbf{R^6}$ positions as well as the core dihydropyrazole ring and the anilide linker. In general, strong electron-withdrawing groups at the $\mathbf{R^1}$ and $\mathbf{R^2}$ positions and a small group at the $\mathbf{R^5}$ and $\mathbf{R^6}$ position are optimal for bringing about AR agonist activity [85].



172

Fig. (27). Structure of compound **172** derivatives.

Jones *et al.* identified 4-substituted pyrazoline derivatives **173** (Fig. **28**) by docking of compounds into a PR homology model. The synthesized derivatives were tested and exhibited functional antagonism of PR [86].



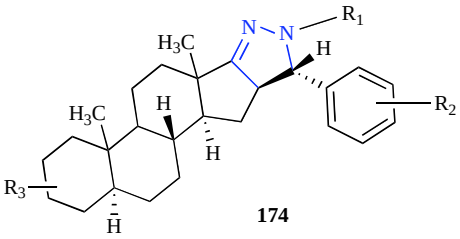
173

Fig. (28). Structure of compound **173** derivatives.

A series of androstano [17, 16-c] dihydropyrazoles and their oxidized derivatives **174** (Fig. **29**) were synthesized from 3β- hydroxyandrostan-17-one as the starting material and evaluated them for their anti-androgenic activity compared to that of cyproterone as a positive control. Some of the compounds exhibited better antiandrogenic activity than the reference drug [87].

2.12. Nitric Oxide Synthase Inhibitor

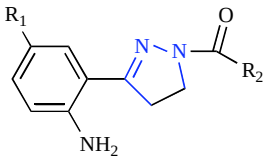
Nitric oxide synthase is an enzyme found in human body that contributes to synaptic transmission from one neuron to another. It also helps transmission to the immune system and dilating of blood vessels. This is done by the synthesis of nitric oxide (NO) from the terminal nitrogen atom of L-arginine in the presence of NADPH. There are three known isoforms of NOS, two are constitutive (cNOS) i.e. neural (nNOS) & endothelial (eNOS) and the third is inducible (iNOS) which is associated with biological functions in brain and other parts of the body.



174

Fig. (29). Structure of compound **174** derivatives.

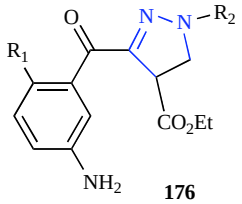
Camacho *et al.* designed and synthesized 19 nNOS inhibitors bearing a 4,5-dihydro-*1H*-pyrazole unit **175** (Fig. **30**) for developing novel compounds with neuroprotective activity. In particular, the compounds 1-cyclopropanecarbonyl-3-(2-amino-5- chlorophenyl)-4,5-dihydro-*1H*-pyrazole and 1-cyclopropanecarbonyl-3-(2-amino-5-methoxyphenyl)-4,5-dihydro-*1H*-pyrazole revealed better activities with inhibition percentages of 70% and 62%, respectively [88].



175

Fig. (30). Structure of nNOS inhibitors.

Similarly, Carrión *et al.* reported preparation and the preliminary evaluation of a series of 1-alkyl-3-benzoyl-4,5-dihydro-1*H*-pyrazoles **176** (Fig. **31**) as potential inhibitors of both neuronal and inducible nitric oxide synthases (nNOS and iNOS) [89].



176

Fig. (31). Structure of compound **176** derivatives.

3. CONCLUSIONS

The chemistry of dihydropyrazole moiety has been widely investigated from the early 1990s, but the most considerable advances in both the synthetic methodologies and the biological evaluation of these derivatives have been made in the recent. Although several strategies and methodologies have been applied to achieve conveniently the synthesis of these compounds, further research must, however, be undertaken in order to design and develop

efficient, practical, and scalable synthetic routes to some of these compounds and their analogues for biological and preclinical studies. A classical synthesis of dihydropyrazole derivatives involves the base (acid)-catalyzed aldol condensation reaction of aromatic ketones and aldehydes to give α, β-unsaturated ketones (chalcones) which subsequently undergo a cyclization reaction with hydrazines affording 2-pyrazolines. In this method, hydrazones are formed as intermediates, which can be subsequently cyclized to 2-pyrazolines in the presence of a suitable catalytic reagent like acetic acid. Vinylidenebisphoephonic acid tetraethyl ester and diazo ketones are also frequently employed in synthetic methodologies.

It has been found that many dihydropyrazole derivatives have considerable biological activities, which stimulated the research activity in this area. Subtle changes and modifications in the parent unit of their structures can lead to the development of potent therapeutic agents in future.

4. OUTLOOK

Of late, many novel dihydropyrazole derivatives have been prepared and patented, but a great challenge still lies ahead in the pursuit of developing more active molecules by making minor structural modifications on dihydropyrazole moiety.

ACKNOWLEDGEMENTS

The authors wish to thank the National Natural Science Foundation of China (No. 20902003) and the opening foundation of the Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, grant No. 2009GDGP0106.

ABBREVIATIONS

CB1	=	Cannabinoid Receptor 1
MAOs	=	Monoamine Oxidase
SAR	=	Structure Activity Relationship
HL60	=	Human Promyelocytic Leukemia
EL4	=	Mouse Lymphoma
PC-3	=	Human Prostate Cancer
A431	=	Human Epidermoid Carcinoma Cancer
EL4	=	T Lymphocytic Leukemia
SGC-7901	=	Human Gastric Cancer
CCRF-CEM	=	Hman Lukemic Lmphoblasts
RPMI-8226	=	Human Myeloma
NCI	=	National Cancer Institute
EGFR	=	Epidermal Growth Factor Receptor
MICs	=	Minimum Inhibitory Concentrations
MCF-7	=	Human Breast Adenocarcinoma
MTT	=	3-(4,5-Dimethyl-2-Thiazyl)-2,5-Diphenyl-2H-Tetrazolium Bromide
DMSO	=	Dimethyl Sulfoxide
MH	=	Mueller-Hinton
PBS	=	Phosphate Buffered Saline
ELISA	=	Enzymelinked Immunosorbentassy
TRAP	=	Tlomere Rpeat Aplification Potocol
B. subtilis	=	*Bacillus subtilis*
E. coli	=	*Escherichia coli*
P. fluorescens	=	*Pseudomonas fluorescens*
S. aureus	=	*Staphylococcus aureus*
S. pneumoniae	=	*Streptococcus pneumoniae*
K. pneumoniae	=	*Klebsiella pneumoniae*
P. mirabillis	=	*Proteus mirabillis*
S. dysentry	=	*Shigella dysentry*
S. typhii	=	*Salmonella typhii*
M. tuberculosis	=	*Mycobacterium tuberculosis*
C. albicans	=	*Candida albicans*
A. niger	=	*Aspergillus niger*
SLV319	=	3-(4-chlorophenyl)-*N*-[(4-chlorophenyl)sulfonyl]-4,5-dihydro-*N'*-methyl-4R-phenyl-*1H*-pyrazole-1-carboximidamide
SSAO	=	Semicarbazide-Sensitive Amine Oxidase
CYP3A4	=	Cytochrome P_{450} 3A4

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Received: December 06, 2010 Revised: April 11, 2011 Accepted: May 26, 2011