An Overview on Synthetic Methodologies and Biological Activities of Pyrazoloquinolines

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Abstract: The chemistry of pyrazoloquinolines is well established. This system has proved to be a very attractive scaffold for medicinal chemist in the recent past. Pyrazoloquinolines were extensively studied as bioactive compounds and are known to possess remarkable biological activities such as anti-cancer, anti-anxiety, anti-inflammatory, anti-asthmatic, cerebroprotective and anti-viral among others. For many of the activities the molecular mode of action is known. Recent research efforts have also highlighted the ability of agents based on pyrazoloquinoline skeleton to modulate adenosine A3 receptors and the phosphodiesterase receptors. In this review the developments in the medicinal chemistry of pyrazoloquinolines is discussed.

Keywords: Adenosine receptor, benzodiazepine receptor, Chk1kinase, phosphodiesterase, pyrazoloquinoline, *ras*, topoisomerase II, SAR.

INTRODUCTION

Since the reports of Squires *et al.* and Mohler *et al.* on the high affinity binding sites for benzodiazepines in the rat brain tissues [1-2], a number of synthetic compounds with different structures have been found to possess high affinity for the benzodiazepine receptor either as agonists or as antagonists [3-8]. Non-benzodiazepine compounds with affinity for these sites could be of potential importance as tools for studying the receptor itself and eventually for the introduction into clinical use of new classes of compounds having the same properties as benzodiazepines. Cain and co-workers in a study on β-carbolines have reported some of the requirements that affect the affinity for the receptor [9]. They stated that a planar heteroaromatic system containing at least one nitrogen atom is necessary and that a carbonyl group adjacent to nitrogen atom greatly augments the binding to the receptor. Quinolines and their derivatives satisfy the above structural requirements and have been explored and proved to possess affinity for benzodiazepine receptor. Pyrazoloquinolines represent one such class of derivatives of quinolines. In the year 1982 Yokohama and co-workers recognised pyrazoloquinolines as psychoactive agents useful in the treatment of anxiety and depression by virtue of their benzodiazepine receptor binding affinity and patented the 2-aryl-pyrazolo[4-3-c]quinolin-3-ones as psychoactive agents [7-8]. Since then many groups have devoted their research efforts towards optimisation of CNS activity of these agents [10-14]. In the subsequent years pyrazoloquinolines have emerged as a multivalent scaffold with variety of biological activities. The anticancer potential of the scaffold was identified in the nineties [15-16]. The mechanism of action was investigated and Topoisomerase II was identified as the target enzyme. Recently another mode of anticancer activity of these agents was identified involving checkpoint 1 (CHK1) kinase as the target enzyme [17]. One of the most important aspects of therapeutic potential of this scaffold is its anti-inflammatory activity. The mode of action is known to be inhibition of phosphodiesterase IV (PDE IV) enzyme [18]. The agents of this class have also displayed adenosine A3 receptor antagonist activity [19]. This further substantiates the anti-inflammatory activity of these agents. The PDE IV inhibition is also responsible for the anti-asthmatic activity of these agents [18]. The focus in the recent past has been in the anti-asthmatic potential of the scaffold. Antiviral activity and estrogen receptors modulatory activity are some of the recently identified activities of these agents [20-21].

Despite the numerous activities of pyrazoloquinolines no review dedicated to the scaffold has been published till now. This encouraged us to review this multivalent scaffold. This review highlights the biological activities and synthetic strategies for pyrazoloquinolines reported over the past three decades. An attempt is made to cover the research on the scaffold in various scientific journals as well as patent literatures.

BIOLOGICAL ACTIVITY OF PYRAZOLOQUINOLINES AND RELATED COMPOUNDS

CNS Activity

Perhaps, the benzodiazepine receptor activity of pyrazoloquinolines represents the most interesting pharmacological property for these compounds. Pyrazoloquinolines have been known for their high affinity for central benzodiazepine receptors since 1982 [7-8]. Later SARs and QSARs were reported, predicting the structural requirements which cause change in activity.

Yokohama and co-workers reported 2-aryl pyrazolo[4,3-c]quinolin-3-ones and related derivatives as psychoactive agents useful in the treatment of epilepsy, anxiety or depression. The compounds were evaluated by benzodiazepine receptors binding assay and also by rat metrazol anti-

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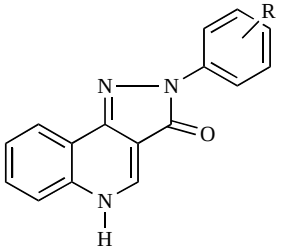
convulsant test. The compounds displayed promising benzodiazepine receptor binding affinity with IC_{50} ranging down to 0.1 nM **(3)** which was lower than the value for diazepam (5 nM) and chlordiazepoxide (400 nM) (Table **1**). The compounds also displayed significant anticonvulsant activity with ED_{50} as low as 0.9 nM [7-8].

Table 1. 2-aryl pyrazolo[4,3-c]quinolin-3-one Derivatives Useful in the Treatment of Epilepsy, Anxiety or Depression



Compd	R	IC_{50}[a] (Benzodiazepine receptor binding)	ED_{50}[b] (Metrazol anticonvulsant test) mg/kg PO (95% CL)
1	H	0.4 nM	Inactive
2	*p*-Cl	0.6 nM	0.9
3	*p*-OCH_3	0.1 nM	2.81
Diazepam	-	5 nM	4.0

[a] C. Braestrup and R. F. Squires, *Proc. Natl. Acad. Sci. U.S.A.* 1977, 74, 3805 & H. Mohler and T. Okada, Life Sci. 1977, 20, 2101.
[b]Rat metrazol anticonvulsant test. G. Zbinden and L. 0. Randall, ***Adu.*** Pharmacol. 1967, 5, 213-291.

Synthesis and structure-activity relationships of a series of 2-(thien-3-yl)- and 2-(thien-2-yl)-2,5-dihydro-*3H*-pyrazolo[4,3-c]quinolin-3-ones were reported by Takada and coworkers (Fig. (**1**). Number of compounds of the series possessed higher affinity for the receptors than diazepam. The data suggested that planarity is one of the structural requirements for binding to benzodiazepine receptors. The activities of agonists and inverse agonists were assessed on the basis of inhibition or facilitation of the pentylenetetrazole-induced convulsions. Thien-3-yl compounds exhibited inverse agonist activity whereas thien-2-yl analogues with a 5'-alkyl group showed agonist activity. Substitution on the quinoline moiety did not enhance *in vivo* activity (**7** and **8**). The most potent compounds were the 5-methylthien-3-yl derivative **(4)** as an inverse agonist and the 5-methylthien-2-yl compound **5** as an agonist [22]. It was further observed that introduction of alkyl groups of different sizes into the unsubstituted inverse agonistic compounds results in a corresponding shift in the activity from an inverse agonist to an antagonist to an agonist [23].

Wang and co-workers used computer-aided conformational analysis, based on molecular dynamics simulation, cluster analysis, and Monte Carlo techniques to design and synthesize compounds in which a benzyloxy substituent was incorporated into a series of pyrazoloquinoline ligands [24]. Earlier studies had shown that the benzyloxy group could act as part of the agonist pharmacophoric determinant in the β-carboline ring system (ZK93423) (Fig. (**2**) [25].

It was determined whether the benzyloxy substituent could be used as an agonist pharmacophoric descriptor for the phenylpyrazolo[4,3-c]quinolin-3-one benzodiazepine receptor ligands. Molecular-modeling techniques indicated that three structurally different classes of heterocyclic ring systems can be constrained to fit (volume and electrostatic potentials) a three-dimensional model for agonist ligands at the benzodiazepine receptor. It was proposed that the benzyloxy substituent on an agonist β-carboline is equivalent to the one on the pyrazoloquinoline series of benzodiazepine receptor ligands. It followed that the benzyloxy group can act as an agonist pharmacophoric descriptor and forces the ligand into an agonist conformation. The results of a determination of

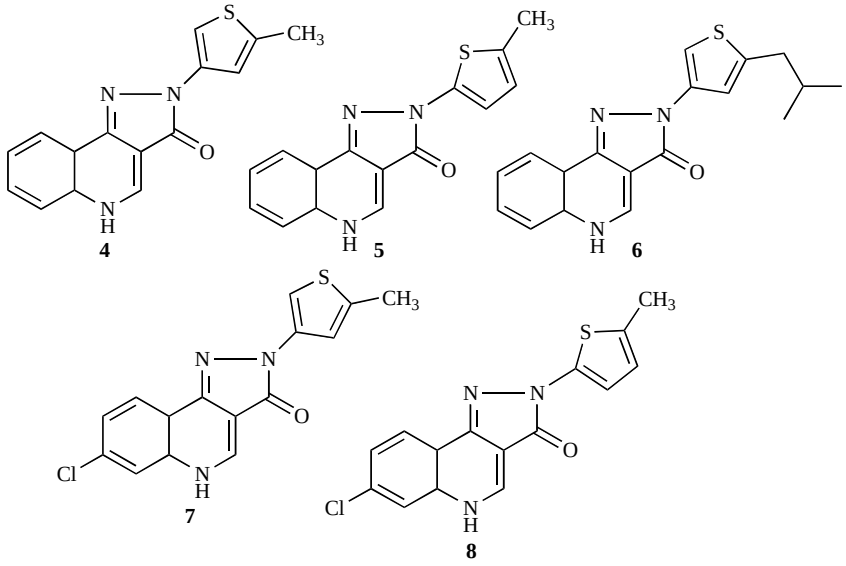
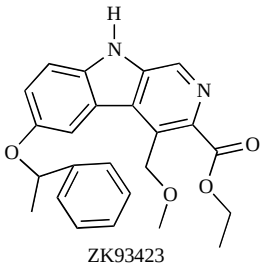


Fig. (1). 2-(thien-3-yl)- and 2-(thien-2-yl)-2,5-dihydro-*3H*-pyrazolo[4,3-c]quinolin-3-ones, agonists and inverse agonists of benzodiazepine receptors.

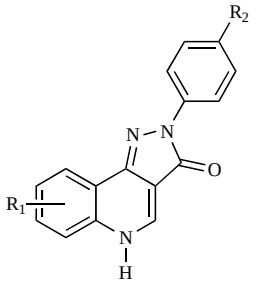


ZK93423

Fig. (2). β-carboline benzodiazepine receptor agonist.

GABA shift ratios for the synthetic ligands indicate that 8-(benzyloxy)-2-phenylpyrazolo[4,3-c]quinolin-3-one **(9)** can be predicted as an agonist at the benzodiazepine receptor (Table **2**).

Table 2. 8-(benzyloxy)-2-phenylpyrazolo[4,3-c]quinolin-3-one, Benzodiazepine Receptor Agonist and 9-(methoxy)-2 Phenylpyrazolo[4,3-c]quinolin-3-one, Benzodiazepine Receptor Antagonist/Partial Agonist



Compd	R_1	R_2	IC_{50}	GABA shift ratio
9	8-$C_6H_5CH_2$-0-	H	3.7 nM	1.3
10	8-$C_6H_5CH_2$-0-	Cl	52.3 nM	0.94
11	9-OCH_3	Ph	0.68 nM	1.09
12	9-OH	Ph	0.74 nM	1.03

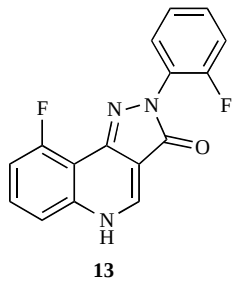


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Fig. (3). 9-fluro-2-(2-fluorophenyl)-2,5-dihydro-3*H*-pyrazolo[4,3-c]quinolin-3-one, a fluorine containing pyrazoloquinoline derivative having affinity for benzodiazepine receptor.

Fluorine containing pyrazolo[4,3-c]quinolin-3-one derivatives were identified as an useful lead for further development of pyrazoloquinolin-3-ones having affinity for benzodiazepine receptor. 9-fluro-2-(2-fluorophenyl)-2,5-dihydro-3*H*-pyrazolo[4,3-c]quinolin-3-one **(13)** represents one such fluorine containing pyrazoloquinolone derivative (Fig. (**3**) [26].

As part of research on pyrazolo[4,3-c]quinolin-3-one skeleton some pyrazolopyrroloquinolin-3-ones were designed and synthesised on the basis of specific literature data and pharmacophore models [27]. Pyrazolo[4,3-c]pyrrolo[3,2-f]quinolin-3-one tetracycle was chosen because it may be considered as pyrazolo[4,3-c]quinolin-3-one modified by a fused pyrrole ring at 8 and 9 position. These positions appeared to be the most suitable as here pyrrole moiety establishes connection with lipophilic region of the model pharmacophore (Fig. (**4**)) [28]. The most potent compounds of the series were those with alkylated pyrrole 8-N, indicating that they fit the lipophilic pocket L_{Di} of the proposed pharmacophore/receptor model establishing hydrophobic interactions and that this pocket is large enough to receive the fourth fused pyrrole ring even when N-ethylated as in compound **(14)** (Fig. (**5**)).

Pyrazoloquinolin-4-ones have also exhibited benzodiazepine receptor binding affinity. Cecchi and co-workers

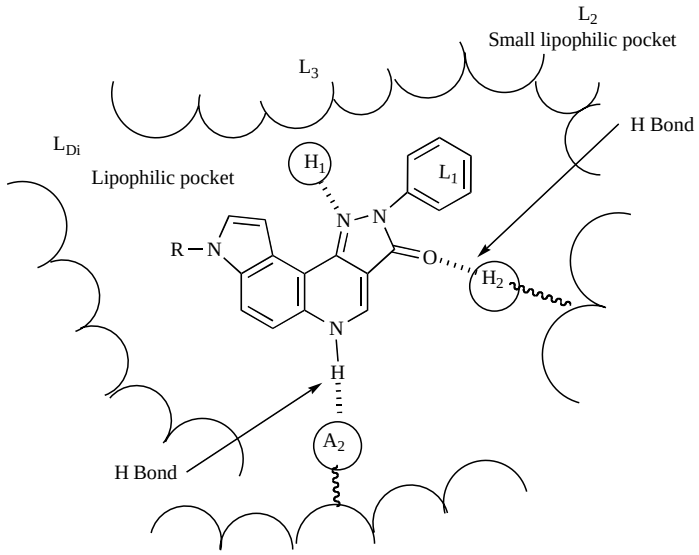

Fig. (4). Proposed pharmacophore model for pyrazolo[4,3-c]quinolin-3-one with a tetracyclic pyrazolopyrroloquinolin-3-ones.

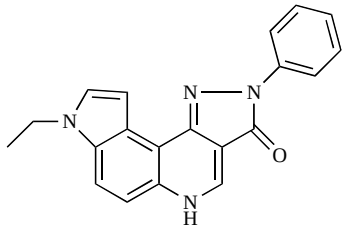

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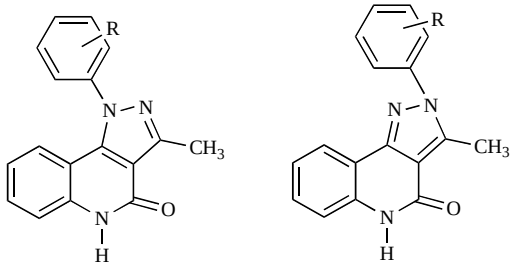
Fig. (5). A tetracyclic pyrazolopyrroloquinolin-3-one.

Table 3. Schematic representation of 1-aryl-3-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-c]quinolin-4-one and 2-aryl-3-methyl-4,5-dihydro-2*H*-pyrazolo-[4,5-c]quinolin-4-one Derivatives



15-16 **17**

Compd	R	Inhibition % (34 μM)[a]
15	3-Cl	94
16	3-CH_3	97
17	3-CH_3	20

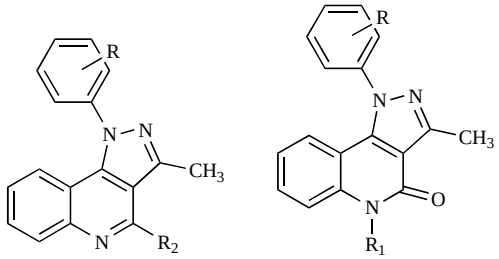
[a] % of inhibition of specific [^{3}H] Flunitrazepam binding at 34 μM compound concentration are means ± SEM of five determinations

prepared some pyrazoloquinolin-4-ones bearing an aryl substitution at position 1 or at position 2 and compared them with their unsubstituted counterparts (Table **3**) [10]. The higher affinity of compounds bearing phenyl substituent indicated the importance of hydrophobic phenyl substituent for the receptor affinity. The structure activity relationships also revealed that the meta aryl derivatives are the compounds with highest affinity towards the receptor [11]. The receptor affinity was found to be in the order: meta > para > ortho. A QSAR study reported by Singh and co-workers on the abovementioned class of compounds further supported the finding, highlighting the importance of meta aryl substitution [14] revealing its favourable hydrophobic interactions with the active site of benzodiazepine receptor. SAR study by Gupta and co-workers highlighted the importance of steric and hydrophobic constants of aryl substituent for the benzodiazepine receptor affinity [13]. The binding study on bovine brain membranes has shown that only the 1-aryl-3-methyl-4,5-dihydro-2*H*-pyrazolo[4,3-c] quinolin-4-ones (**15-16**) possess activity in displacing specific [^{3}H] Flunitrazepam from its receptor site while the 2-aryl derivatives (**17**) are devoid of activity.

In continuation with this work some newer 1-aryl [4,5-c]quinoline derivatives were prepared, some of them bearing 5-N-methyl group **(20 & 21)** while others having 4-methyl piperazinyl group **(18 & 19)** replacing carbonyl group (Table **4**). 5-N-methyl derivatives showed significant increase in the affinity while the 4-methyl piperazinyl derivatives possessed only slight activity, indicating the importance of 5-N-methyl and 4-carbonyl group [12].

Replacement of 3-methyl group with the bulkier phenyl group resulted in enhancement of binding potency, evident by high affinity of the 1,3-diaryl derivative (**23**) as compared to the 1-aryl-3-methyl derivative (**22**) (Table **5**) [29]. In another study the variation of the kind of condensation occurring between pyrazole and the quinoline moiety was investigated for its effect on the inhibitory potency. It was found that isomeric derivatives [4,5-c] showed affinity for the receptor whereas [5,4-c] (**24**) were completely devoid of binding affinity (Table **5**). This confirmed that the position of nitrogen is crucial in the heterocyclic system. Overall the meta substituted 1,3-diaryl[4,5-c]quinolin-4-ones are the most active compounds with activity greater than chlordiazepoxide and close to diazepam.

Table 4. Schematic Representation of 1-aryl-3,5-dimethyl-4,5-dihydro-1*H*-pyrazolo-[4,5-c]quinolin-4-one and 1-aryl-3-methyl-4-(4-methylpiperazinyl)-1*H*-pyrazolo-[4,5-c]quinolin-4-one Derivatives

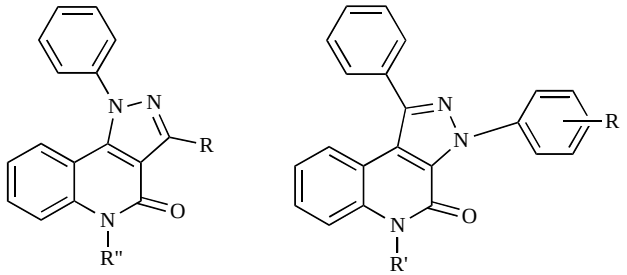


18-19 **20-21**

Compd	R	R_2	Inhibition % (34 μM)[a]	Compd	R	R_1	IC_{50} (μM)[b]
18	3-CH_3	4-methyl piperazinyl	44±4	20	3-CH_3	CH_3	0.26±0.01
19	3-Cl	4-methyl piperazinyl	49±3	21	3-Cl	CH_3	1.0±0.2

[a] % of inhibition of specific [^{3}H] Flunitrazepam binding at 34 μM compound concentration are means ± SEM of five determinations
[b] Concentrations necessary for 50% inhibition (IC_{50}) are means ± SEM of four determinations

Table 5. Schematic Representation of 1-aryl-3-methyl-4,5-dihydro-1*H*-pyrazolo[4,3-c]quinolin-4-one and 1,3-diaryl-4,5-dihydro-1*H*-pyrazolo[4,3-c]quinolin-4-one Derivatives



22-23 **24**

Compd	R	R'	R"	IC_{50} (μM)
22	CH_3	3-CH_3	CH_3	0.26±0.01[c]
23	Ph	3-Cl	CH_3	0.07±0.02[d]
24	3-Cl	H	-	8*

[c]Concentrations necessary for 50% inhibition (IC_{50}) are means ± SEM of four determinations
[d]Concentrations necessary for 50% inhibition (IC_{50}) are means ± SEM of four determinations
*% of inhibition of specific [^{3}H] Flunitrazepam binding at 34 μM compound concentration are means ± SEM of five determinations

Catarzi and co-workers developed pharmacophore model of pyrazolo[3,4-c] quinolin-4-ones (Fig. (**6**)) for their binding at the benzodiazepine receptor. The pharmacophore contained some optional (a_1 and d) and some essential (L_1, L_2 and a_2) pharmacophoric descriptors for receptor recognition. The pharmacophore model highlighted the importance of proton acceptor groups in the molecules [30].

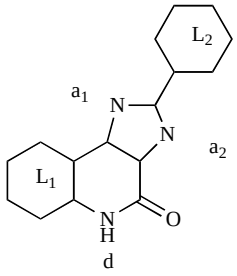


Fig. (6). Schematic two-dimensional representation of essential and optional pharmacophoric descriptors of pyrazolo[3,4-c]quinolin-4-one as benzodiazepine receptor ligand. Essential pharmacophoric descriptors are: L_1 and L_2 lipophilic areas, and a_2 proton acceptor site. Optional binding sites, only affecting the potency of a ligand, are: d proton donor and a_1 proton acceptor sites.

In a study conducted by Allen and co-workers, the effect of functional change from quinoline to isoquinoline over the binding affinity was investigated (Fig. (**7**)) The results showed that pyrazoloisoquinoline have affinity greater than 2nM which was in marked contrast to high pM- low nM affinities of pyrazoloquinolones. The findings indicated the importance of N-H functionality of quinoline nuclei of pyrazoloquinolones [31].

The most important aspect of pyrazoloquinolones as ligands for benzodiazepine receptor is the lack of neurological deficit liability seen with diazepam and other benzodiazepines at antianxiety dose. Thus these agents are likely to produce antianxiety effect without affecting the routine and normal activities of the patient [7-8].

Antagonists of the N-methyl-D-aspartate (NMDA) receptor have attracted considerable attention in recent years as potential therapeutic agents for the treatment of a variety of neurological and neurodegenerative disorders, almost all of the exisiting known antagonists at the glycine site of the N-methyl-D-aspartate (NMDA) receptor have a low propensity for crossing the blood-brain barrier [32-35]. It has been suggested that in many cases this may be due to the presence of a carboxylic acid which is a common feature of most of the potent full antagonists at this receptor. McLeod and co-workers synthesized a series of 2-aryl- 1*H*-pyrazolo[3,4-c]quinoline-l,4(*2H*)-diones which were found to have high-affinity binding at the glycine receptor [36]. In particular, structure-activity studies identified 7-chloro-3,5-dihydro-2-(4-methoxyphenyl)-l*H*-pyrazolo[3,4-c]quinoline-1,4-(2*H*)-dione (**25**) as the most potent of the series of analogues with an IC_{50} of 3.3 nM (Fig. (**8**)). The measured pKa that values in this class of compounds (typically 4.0) indicate that they are of equivalent acidity to carboxylic acids. Inhibition of NMDA-evoked responses in rat cortical slices demonstrated functional antagonism.

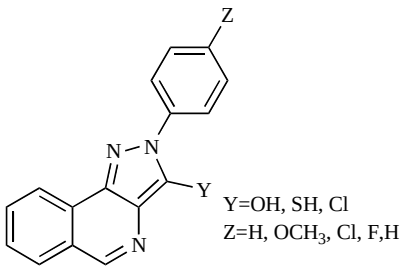


Fig. (7). Pyrazoloisoquinoline derivative having affinity for benzodiazepine receptor.

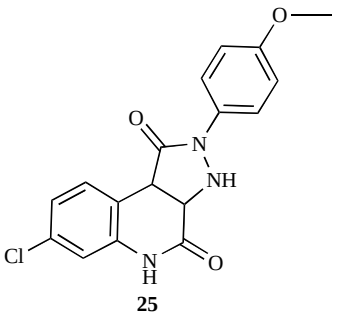


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Fig. (8). 7-chloro-3,5-dihydro-2-(4-methoxyphenyl)-l*H*-pyrazolo [3,4-c]quinoline-1,4-(2*H*)-dione, NMDA antagonist.

Activity at Adenosine Receptors

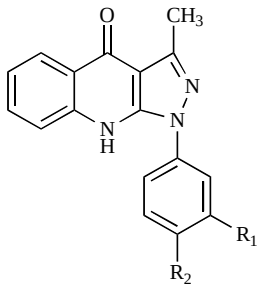
Adenosine, an endogenous modulator of a wide range of biological functions in the nervous, cardiovascular, renal and immune systems, interacts with at least four cell surface receptor subtypes classified as A_1, A_{2a}, A_{2b} and A_3. All four adenosine receptor subtypes are coupled *via* G protein to the enzyme adenylate cyclase in either an inhibitory (A_1 or A_3) or stimulatory manner (A_{2a} and A_{2b}). In addition the A_3 adenosine receptor subtype, that is distributed in different organs (lung, liver, heart, kidney and in low density in the brain) [37-40] also exerts its action through the stimulation of phospholipases C4 and D5. The potential therapeutic applications of activating or antagonising this receptor subtype have been investigated in recent years and in particular, antagonists for the A_3 receptor may prove useful for the treatment of inflammation and in the regulation of cell growth. It has also been suggested that the A_3 receptor plays an important role in brain ischemia, immunosuppresion and bronchospasm in several animal models. From these pharmacological observations highly selective A_3 adenosine receptor antagonists are being sought as potential anti-asthmatic, anti-inflammatory and cerebroprotective agents.

During early nineties Colotta and co-workers focussed their research efforts on the development of subtype-selective agonists and antagonists of adenosine receptor for providing tools which define the structural requirements of each receptor subtype. They synthesised non-xanthine compounds containing tricyclic ring system as antagonists of adenosine receptor [41-44]. In order to understand the structural requirement for anchoring of a ligand to the adenosine receptor recognition site they synthesised and evaluated the A_1 and A_{2a} binding activities of some 1-aryl-1,4-dihydro-3-methyl-1-benzopyrano[2,3-c]pyrazol-4-ones and their isosters 1-aryl-4,9-dihydro-3-methyl-1*H*-pyrazolo [3,4-b]quinoline-4-ones. The only compound to display some A_{2a} binding activity was the 1-[3-methoxyphenyl] derivative **(27)**. However this compound displayed 2.5 fold more activity on A_1 (Table **6**) [45].

In an effort to identify the relationship between structure and binding affinity towards the adenosine receptor subtypes Colotta and co-workers synthesized 2-arylpyrazolo[3,4-c]quinoline derivatives (Table **7**) [46]. Compounds of the series were little active at the A_{2a} receptor and the substituents on the 2-phenyl ring affected differently the A_1 and A_3 affinities. The requirement of 4-amino group for A_1 and A_{2a} receptor-ligand interaction was corroborated by the comparison of A_1 and A_{2a} affinities of the 4-amino derivatives **(36-40)** with those of the 4-ones **(33-35)**. The nanomolar A_3 affinity of compounds having a carbonyl containing group at position 4 proved the importance of the presence at position 4 of either a nuclear (as in 4-ones) or extra nuclear (as in amides and or ureide) carbonyl group for A_3 receptor recognition. It was hypothesized that in these compounds, N-4 region corresponds to the adenosine N-6 one. The A_3 nanomolar affinity of compounds having a carbonyl containing group at position 4 could be due to the presence of a proton donor (which is able to bind to the C=O proton acceptor) in the N-6 region of the adenosine A_3 subtype.

In continuation of their previous work Colotta and co-workers reported some 2-arylpyrazolo[3,4-*c*]quinolin-4-ones, 4-amines, and 4-amino-substituted derivatives designed as human A_3 adenosine receptor (AR) antagonists [47]. Most of the reported compounds showed a nanomolar affinity towards the hA_3 receptor subtype and different degrees of selectivity that was found to be strictly dependent on the presence and nature of the substituent on the 4-amino group. Bulky and lipophilic acyl groups, as well as the benzylcarbamoyl residue, afforded highly potent and selective hA_3 receptor antagonists (Table **8**). The selected 4-diphenylacetylamino-2-phenylpyrazoloquinoline (**41**) and 4-

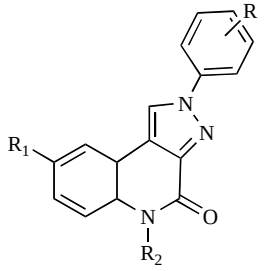
Table 6. A_1 and A_{2a} Adenosine Binding Activity of 1-aryl-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-b]quinoline-4-ones[a]



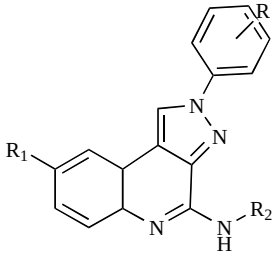
Compd	R_1	R_2	$K_i \pm SEM(\mu M)$[b]	
			A_{1C}	A_{2ad}
26	H	H	3.30 ± 0.28	3.70 ± 0.34
27	OMe	H	0.25 ± 0.021	0.71 ± 0.056
28	NO_2	H	0.47 ± 0.038	>20
29	NO_2	Cl	>20	>20
30	OH	H	2.23 ± 0.17	1.5 ± 0.12
31	NH_2	H	18 ± 1.3	5.16 ± 0.38
32	NH_2	Cl	4.47 ± 0.37	4.46 ± 0.32

[a] The tests were carried out dissolving the tested compounds in DMSO (DMSO buffer, 2%).
[b] The K_i values are means ± SEM of four separate assays, each performed in triplicate.
[c] A_1 binding was measured as displacement of [^{3}H] N^6-cyclohexyladenosine ([^{3}H]CHA) binding.
[d] A_{2a} binding was measured as displacement of [^{3}H]-2-[[4-(2-carboxyethyl) phenethyl]amino]-5'-(*N*-ethylcarbamoyl)adenosine ([^{3}H]CGS 21680) binding.

Table 7. Adenosine Receptor Binding Activity of 2-arylpyrazolo[3,4-c]quinoline Derivatives



33-35



36-40

Compd	R	R_1	R_2	K_i(nM) or % inhibition[a]		
				A_1[b]	A_2[c]	A_3[d]
33	H	H	H	2100 ±170	8600 ± 710	30.8 ± 2.6
34	3-F	H	H	583 ± 49	0%	45.3 ± 3.9
35	4-Cl	H	H	464 ± 39	35%	2.9 ± 0.1
36	H	H	H	69.2 ± 4.8	331 ± 26	551 ± 34
37	3-F	H	H	110 ± 8.9	49.5 ± 3.2	788 ± 69
38	4-Cl	H	H	2290 ± 180	11200 ± 960	150 ± 12
39	H	H	COMe	75.9 ± 6.4	2400 ± 190	48.2 ± 3.7
40	H	H	COPh	42%	3%	2.1 ± 0.1

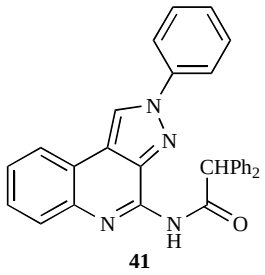
[a] The K_i values are means ± SEM of four separate assays, each performed in triplicate.
[b] Displacement of specific [^{3}H] CHA binding in bovine brain membranes or percentage of inhibition (*I*%) of specific binding at 20 μM concentration.
[c] Displacement of specific [^{3}H]CGS 21680 in bovine striatal membranes or percentage of inhibition (*I*%) of specific binding at 20 μM concentration.
[d] Displacement of specific [^{125}I]N^6-(4-amino-3-iodobenzyl)-5'-*N*-methylcarbamoyl-adenosine([^{125}I]AB-MECA) binding at human A_3 receptors expressed in CHO (Chinese Hamster Ovary) cells or percentage of inhibition (*I*%) of specific binding at 1 μM concentration.

dibenzoylamino-2-(4-methoxyphenyl)pyrazoloquinoline (**42**), tested in an *in vitro* rat model for cerebral ischemia, prevented the irreversible failure of synaptic activity induced by oxygen and glucose deprivation in the hippocampus.

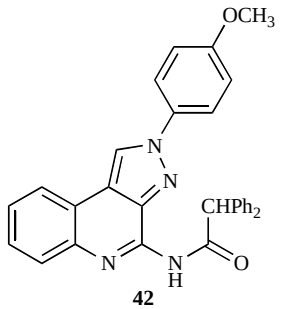
Further research efforts of Colotta and co-workers resulted in synthesis of novel 2-arylpyrazolo[3,4-c]quinolin-4-(hetero)arylamides, designed as human A_3 adenosine receptor antagonists [48]. The new derivatives were endowed with nanomolar hA_3 receptor affinity and high selectivity versus hA_1, hA_{2a} and hA_{2b} receptors. Among the (hetero)aroyl residues introduced on the 4-amino group, the 2-furyl **(43)** and 4-pyridyl **(44)** rings turned out to be the most beneficial for hA_3 affinity (K_i = 3.4 and 5.0 nM, respectively) (Table **9**).

An intensive molecular docking study to a rhodopsin-based homology model of the hA_3 receptor was carried out to obtain a 'structure-based pharmacophore model' that proved

Table 8. 2-Arylpyrazolo[3,4-c]quinolin-4-ones, 4-amines, and 4-Amino-Substituted Derivatives as Human A_3 Adenosine Receptor (AR) Antagonists



41

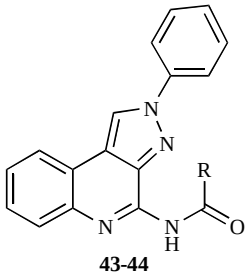


42

Compd	K_i (nM)
41	8.9 ± 0.6
42	17.2 ±1.4

[a] The K_i values are means ± SEM of four separate assays, each performed in triplicate).
[b] Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in CHO cells or percentage of inhibition (*I*%) of specific binding at 1 *n*M concentration.

Table 9. 2-Arylpyrazolo[3,4-c]quinolin-4-(hetero)arylamides as Human A_3 Adenosine Receptor Antagonists



43-44

Compd	R	K_i (nM)
43	2-Furyl	3.4 ± 0.3
44	4-Pyridyl	5.0 ± 0.6

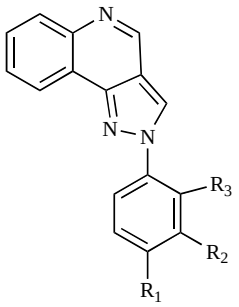
[a] Displacement of specific [^{125}I]AB-MECA binding to hA_3 CHO cells. K_i values are means ± SEM of four separate assays each performed in duplicate.

to be helpful for the interpretation of the observed affinities of the new hA_3 pyrazoloquinoline antagonists.

Some of the pyrazolo[3,4-c]quinoline derivatives, discussed above have shown affinity towards both A_1 and A_3 adenosine receptors while their structural isomers pyrazolo [4,3-c]quinolin-4-one derivatives have shown remarkable selectivity for A_3 receptor over A_1, A_{2a} and A_{2b} receptors. Baraldi and co-workers synthesised 2-arylpyrazolo[4,3-c]quinolin-4-one derivatives as selective A_3 adenosine receptor antagonists having nanomolar affinity for A_3 receptors (Table **10**) [19].

The unsubstituted 2-phenyl derivatives **(45)** showed good affinity and selectivity for A_3 receptor. Introduction of electron donating group at 4-position of 2-phenyl ring such as methyl **(47)** and methoxy **(48)** enhanced the affinity.

Table 10. 2–Aryl pyrazolo[4,3-c]quinolin-4-one Derivative Having Affinity for A_3 Receptors



45-51

Compd	R_1	R_2	R_3	hA_3 K_i (nM)
45	H	H	H	27
46	Cl	H	H	19
47	CH_3	H	H	9
48	OCH_3	H	H	16
49	F	H	H	27
50	H	H	Cl	44
51	Et	H	H	157

[d] Displacement of specific [^{3}H] MRE3008-F20 binding at human A_3 receptors expressed in CHO cells (n=3-6). Data expressed as geometric means with 95% confidence limits.

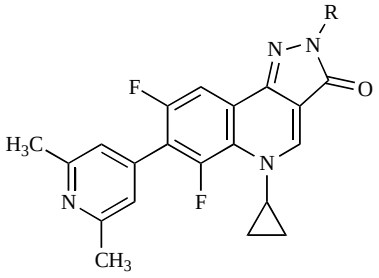
While electron withdrawing group such as chloro **(46)** or fluoro **(49)** at 4-position of 2-phenyl ring produced comparable affinity at the adenosine receptor subtypes. Introduction of groups bulkier than ethyl at 4-position of 2-phenyl ring decreased the affinity. Amide linkage was also identified as crucial structural feature for affinity and selectivity at A_3 receptors.

Anticancer Activity

Pyrazoloquinolines have been investigated thoroughly during the 1990s for anticancer potential. Wentland and co-workers patented pyrazolo[4,3-c]quinolin-3-ones as anti-cancer agent in 1994 (US patent number 5,334,595) [15]. The compounds reported by them were found to be inhibitors of mammalians Topoisomerase II, with EC_{50} values in nano molar range (Table **11**). Compounds were also evaluated in the cell assays using different tumor systems. The tumor systems included: murine tumor: P388.

The two most potent analogues, 52 and 53, contain a cyclohexyl group at the 2-position and had comparable (within 2-fold) potency to the two reference compounds, mAMSA and VP-16(Etoposide). Topoisomerase II inhibition potency did not correlate with several physicochemical properties of the 2-substituent. For example, compounds with relatively small R groups (e.g., H or CH_3) had roughly the same potency as those with much larger groups (e.g., C_6H_5 or 4-acetamidocyclohexyl). Hydrophilic R groups (e.g., CH_2CH_2OH or CH_2CO_2 [assuming extensive ionization at pH 7.4]), in general, contributed to activity to the same extent as hydrophohic groups (e.g., CH_3 or C_6H_5). Also, little difference in potency was seen in compounds having inductively electron donating R groups, (e.g., CH_3) and electron withdrawing groups (e.g., CH_2CO_2Et). In several instances potency was increased by the introduction of a cyclohexyl group at 2-position. However, little correlation between structure (e.g., substitution, stereochemistry, or heteroatom replacement of the 4'-carbon of the cyclohexyl ring) and potency was observed.

Table 11. Pyrazolo[4,3-c]quinolin-3-ones as Anticancer Agent



Compd	R	Topo II inh.[a] EC_{50} µM	*In vitro* cytox [b] IC_{50} µM
52	cyclohexyl	0.9	0.68
53	$CH(CH_2CH_2)_2CHNH_2$ *(cis)*	0.5	0.44
mAMSA	-	0.72	0.15
VP-16	-	0.81	0.30

[a]Promotion by test agent of covalent complex formation between [^{32}P]-end labeled pBR322 DNA and extensively purified HeLa cell topo II was determined by the SDS/K precipitation method. EC_{50} values were calculated to be the concentration of test compound at which the amount of *DNA* precipitated was equivalent to 50% of the maximum precipitated by mAMSA in a concomitant control experiment, [b]*In Vitro* Cytotoxicity was measured by quantifying clonogenic survival in soft agar following a 1 hour transient exposure of P388 mouse leukemia cells to drug. The IC_{50} value is the concentration of drug which reduced clonogenic survival by 50%.

Overall pyrazoloquinolones were found to possess anti-neoplastic activity as evidenced by their ability to reduce the size of tumor, curing them and increasing the survival of mice.

Recently pyrazoloquinoline derivatives were investigated for their antagonistic activity at checkpoint 1 kinase (Chk1). Chk1 inhibitors are special in their ability to kill cancer cells without causing damage to healthy cells. The cell division cycle comprises of four sequential phases G1, S, G2 and the mitosis M. The cell cycle is driven by ordered activation of kinases named cyclin-dependent kinases. The inhibition of these kinases can block the cell cycle and stop cell proliferation. However, the inhibition of cyclin dependent kinases may induce effects on both cancer and healthy cells.

Three checkpoints in G1, S and G2 are activated in response to DNA damage. The role of the checkpoints is to delay the cell cycle progression when DNA damage occurs in order to provide time for DNA repair. The G1/S checkpoint is p53 (most commonly mutated gene in human cancers) dependent. In the presence of DNA damage, a rapid

induction of p53 activity occurs, inducing cyclin-dependent kinases inhibition and cell cycle arrest to prevent the replication of damaged DNA during the S phase. Thus, in the *p53*-mutated cells, the cascade of signals in response to DNA damage is inactivated and the G1/S checkpoint is abrogated. In the *p53*-mutated cells, in which the G1 checkpoint is lacking, only the G2 checkpoint is able to provide a delay in the cell cycle progression allowing the activation of DNA repair pathways [49-56]. Therefore, the G1 checkpoint defect distinguishes cancer cells from healthy cells and thereby provides a potential target for anticancer therapy. Among the G2 checkpoint effectors are ATM and ATR kinases and downstream Chk1 kinase [10]. Chk1 inhibitors abrogate the G2 checkpoint and sensitize, essentially *p53*-mutated cells, to DNA damaging agents [57]. Pyrazolo[4,3-c]quinolin-4-ones capable of inhibiting CHK1 kinase in nanomolar concentration have been identified by Brnardic and co-workers. Compound **54** (Fig. (**9**)) was identified as the most potent inhibitor of CHK1 Kinase.

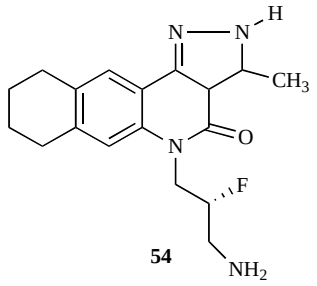


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Fig. (9). A pyrazoloquinoline derivative capable of inhibiting CHK1 kinase in nanomolar concentration.

These agents cause abrogation of cell cycle arrest leading to cell death through mitotic catastrophe and apoptotic pathways [17].

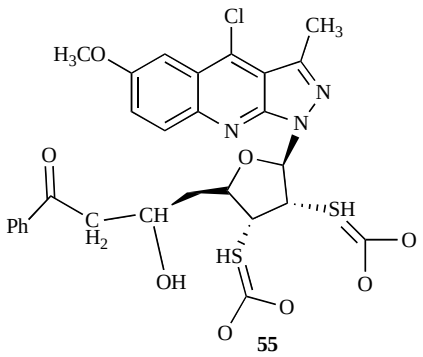
Structure activity relationship revealed that substitution at 4 and 6 position did not provide improvement in activity while substitution at 5-position provide significant increase in activity. A three carbon amine chain was found to be optimal. Introduction of monofluoro substitution in the propyl chain resulted in optimal cell potency as well as CHK1 kinase affinity. These requirements are epitomized by compound **(54)**.

Another mechanism by which pyrazoloquinoline derivatives are known to produce anticancer activity is inhibition of *Ras*. Current evidence suggests that *Ras* oncogenes are responsible for a large percentage of human tumors, some of which include carcinomas of the pancreas, colon, and lung. It is believed that the protein product encoded by the *ras* gene, known as p21, plays an important role in relaying chemical signals within the cell.This 21 kilodalton protein is susceptible to single point mutations which inactivate the intrinsic GTPase activity of the protein resulting in a highly oncogenic form of *Ras.* Activation of the *Ras* pathway is also dependent upon the nature of the ligand/protein complex. When GDP is bound to *Ras* the protein resides in an inactive state; in contrast, the binding of GTP to *Ras* causes the protein to enter an active conformation favoring interaction with downstream effectors and promoting signal transduction. Because the GTP bound form of oncogenic *Ras* is stable, downstream signaling by *Ras* is constitutive resulting in abnormal cell proliferation or carcinogenesis [58-61]. Some pyrazolo[3,4-b]-quinolines were synthesized by Wolin and co-workers in order to accomplish the objective of developing a non-nucleoside compound capable of binding to an allosteric region of *Ras,* ultimately leading to a conformational change so as to prevent GTP ligand binding. Series of ribose and morpholino derivatives of pyrazolo[3,4-b]quinoline ribofuranosides were prepared and evaluated for their ability to inhibit the nucleotide exchange process of oncogenic *Ras* [62]. Compounds **55** from the ribose series and **(56)** from the morpholino series were identified as the most potent inhibitors of *Ras* having IC_{50} of 1.5 and 1 μM respectively (Fig. (**10**)).

One possible strategy, reported to cause moderately positive outcome in various cancer models in cell culture, has been to inhibit the checkpoint signaling to enhance the impact of concomitant treatment with DNA-damaging drugs or radiation. The ability of pyrazoloquinolines to inhibit both topoisomerase II and CHK1 kinase makes them interesting subject of research for time to time. These agents have still not been explored thoroughly for anticancer activity and thus present vast opportunity for practicing medicinal chemist to design anticancer agents based on pyrazoloquinoline scaffold.

Antiviral Activity

Pyrazoloquinoline derivatives have been extensively studied for their antiviral activity in recent past by de Olieviera and co-workers. To explore the antiviral potential of pyrazoloquinolines, several new pyrazolo[4,3-c]quinolin-3-one ribonucleosides and their corresponding heterocycle



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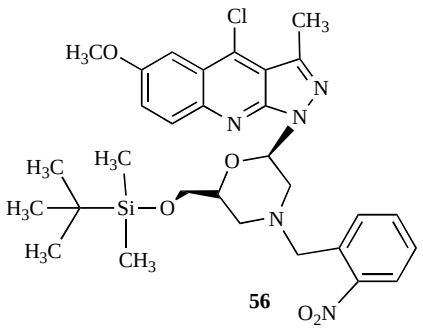


56

Fig. (10). Ribose and morpholino derivatives of pyrazolo[3,4-b] quinoline ribofuranosides.

moieties were synthesized and evaluated against vaccinia virus (VV) and herpes simplex virus type 1 (HSV-1) . The derivatives **(57)** and **(58)** showed modest inhibitory activity against vaccinia virus reaching 70% at a concentration of 100 mM. All heterocyclic compounds showed a modest inhibition against HSV-1, reaching the maximal inhibitory effect around 20–30% (Fig. (**11**)) [20].

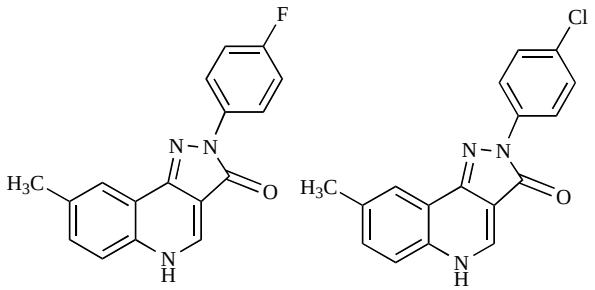
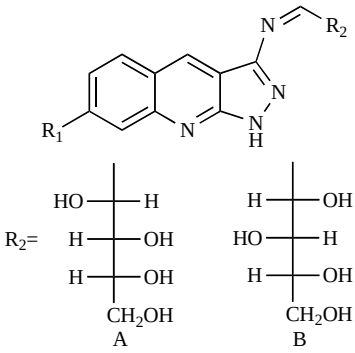


Fig. (11). Anti HSV-1 and Anti vaccinia virus compounds.

Bekhit and co-workers synthesised a novel series of structurally related pyrazolo[3,4-b]quinoline nucleosides [21]. All the newly synthesized compounds were examined for their *in vitro* antiviral activity against herpes simplex type-1 by two different bioassays, namely; crystal violet staining or the MTS tetrazolium dye measurement. The acute toxicity (LD_{50}) values of the biologically active compounds were also determined. The results obtained were concordant in respect to compounds **(59)** and **(60)** (Table **12**). These two compounds possess inhibitory activity against virus growth in concentrations above 50 mM. The biologically significant compounds **(59)** and **(60)** were further evaluated for their approximate LD_{50} in male mice. Results indicated that the two tested compounds proved to be non-toxic and well tolerated by the experimental animals as evidenced by their LD_{50} values (> 300mg/Kg). The study revealed that the aldopentose derivatives were more promising than the aldohexose derivatives and the compounds represents fruitful means of developing a new class of antiherpetic agent.

Table 12. Pyrazolo[3,4-b]quinoline Nucleosides as Antiherpetic Agents



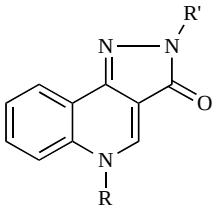
Compd	R_1	R_2
59	CH_3	A
60	OCH_3	B

PDE IV Antagonist Activity

PDE IV inhibitors have potential utility in therapy of asthma and COPD. Theophylline may be considered a prototype of PDE IV inhibitors [63]. However it tends to be associated with a number of unwanted side effects including headache and emesis due to its lack of selectivity for PDE IV receptors [64-67]. Attempts made through rational drug design led to synthesis of compounds that demonstrate improved side effect profile. Such drugs offer an exciting opportunity to selectively downregulate inflammatory cell function as a novel therapeutic approach in treatment of airway disease [68-69].

Pyrazoloquinolones represent one such class of agents. Crespo and co-workers designed and synthesised 2,5-dihydropyrazole[4,3-c]quinolin-3-ones as inhibitors of PDE IV with low emetic potential and anti-asthmatic properties. Compounds of the series exhibited PDE IV inhibition in low micro molar range. Compound **(64)** and **(65)** were the most potent inhibitors with good selectivity for PDE IV (0.4 μm) over PDE3 (>20 μm) (Table **13**).

Table 13. 2,5-Dihydropyrazole[4,3-c]quinolin-3-ones Inhibitors of PDE IV with Low Emetic Potential



Compd	R	R'	PDE III IC_{50} (μM)	PDE IV IC_{50} (μM)
61	Benzyl	H	48	37
62	Benzyl	cyclopentyl	26	0.7
63	Benzyl	Cyclobutyl methyl	>200	0.8
64	cyclohexylmethyl	cyclopentyl	>20	0.4
65	2-thienylmethyl	cyclopentyl	>20	0.4
Rolipram	-	-	242	0.32
Nitraquazone	-	-	>200	0.05

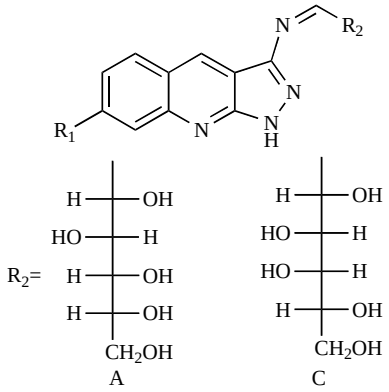
The observed activity of compounds indicated the importance of lipophilic groups for PDE IV inhibition. Best results for N2 position were obtained for compounds with cyclopentyl or cyclobutylmethyl groups. While for N5 position cyclohexylmethyl or 2-thienylmethyl group was identified as optimum.

Antileishmanial Activity

The treatment of leishmaniasis with traditional drugs is associated with numerous limitations, it is highly desirable to

find more effective and safer drugs for the treatment of leishmaniasis [70]. Ahmed and co workers performed screening study to examine the effect of a series of synthesized aldohexose and aldopentose pyrazolo[4,3-b]quinoline derivatives on the growth of *Leishmania donovani* promastigotes [71]. Sixteen compounds were tested, ten of which showed an inhibitory effect on the growth of promastigotes. Compound **(66)** demonstrated potent antileishmanial activity, followed by compounds **(67)** and **(68)** (Table **14**). Some compounds showed less significant activities, while others exhibited little or no activity. Some of these compounds may be potential candidates for future treatment of leishmaniasis.

Table 14. Effect of Pyrazolo[4,3-b]quinoline Derivatives on the Growth of *L. donovani* promastigotes



Compd	R_1	R_2	Viability at 400 µM conc.
66	H	A	0.411 ± 0.010
67	CH_3	A	0.434 ± 0.041
68	OCH_3	C	0.414 ± 0.020

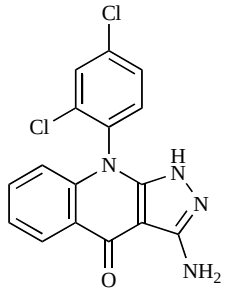
[a] Viability of cells was monitored microscopically and by using the tetrazolium (MTT) colorimetrically. Amphotericin B was used as an antileishmanial control drug.

Antimalarial Activity

Malaria remains one of the most challenging infectious diseases in the world [72]. A significant and increasing problem in malaria control is the resistance of malaria parasites to available chemotherapeutic agents. There is, therefore, a pressing need to identify new antimalarial drugs. Dominguez and co-workers reported the synthesis and spectral characterization of some 3-amino-9-phenylpyrazolo[3,4-b]quinolin-4-ones[73-74]. These compounds proved to be an interesting family of antimalarial agents *in vitro*. They have demonstrated high antimalarial activity against a chloroquine-resistant strain of *Plasmodium falciparum in vitro*. Compound **(69)** was found to be the most potent with IC_{50} of 5.06 µM (Fig. (**12**)). This class of compounds represents an attractive means of developing new antimalarial agents.

Synthetic Procedures for Pyrazoloquinolines and Related Compounds

The synthetic procedures for pyrazoloquinolines are outlined in the following section. The synthetic schemes are classified according to the linkage between the quinoline and pyrazole rings.



69

Fig. (12). 3-amino-9-phenylpyrazolo[3,4-b]-4-quinolones as antimalarial agents.

Pyrazolo[3,4-c]quinoline and pyrazolo[4,3-c]quinoline

The one pot reaction of 3-ethoxalylindoles **(70)** with hydrazine hydrochlorides yields the 2-substituted pyrazolo [3,4-c]quinolin-4-ones **(72)** (Fig. (**13**)). However the reaction fails to occur with 2-fluorophenylhydrazine hydrochloride. The reaction of 2-flurophenylhyrazine hydrochloride with **(70)** yeilds hydrazone (**71**)**,** which rearranges under acidic conditions to yield **(72)**. [30, 47].

Condensation of aniline with ethoxymethylene malonate diethyl ester produce quinolone-3-carboxylate **(73)**, which on treatment with phosphorus oxy chloride and then suitable hydrazine gives pyrazolo[4,3-c]quinolin-3-one **(76)**. **(73)** on treatment with phosphorus pentasulphide yields **(75)** which on further treatment with suitable hydrazine yields **(76)** (Fig. (**14**)) [18].

Suitably substituted phenyl hydrazines on reaction with *o*-nitro acetophenone yields the corresponding phenyl hydrazones **(77)** which can be converted to pyrazole-4-carboxaldehydes by vilsmeir reaction ($POCl_3$ and DMF). The pyrazole-4-caroxyaldehydes on hydrogenation and subsequent boiling of the amine intermediate in ethanol yields the pyrazolo[4,3-c]quinoline compound **(79)** (Fig. (**15**)) [75].

Luca and co-workers in 2004 proposed the use of 2,4,6-trichloro 1,3,5- triazine (TCT, cyanuric chloride) and DMF

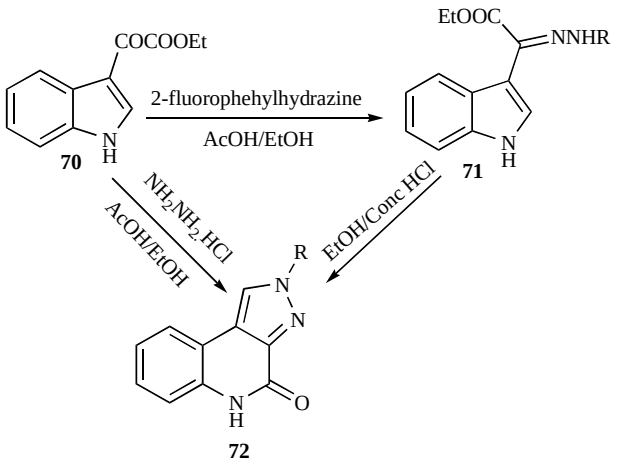


Fig. (13). Synthesis of pyrazolo[3,4-c]quinolin-4-ones from 3-ethoxalylindoles.

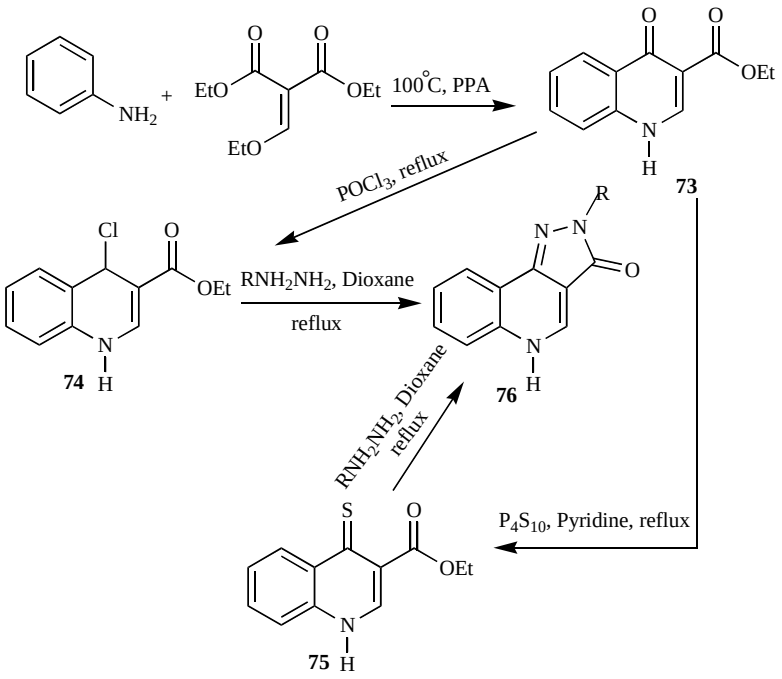


Fig. (14). Synthesis of pyrazolo[3,4-c]quinolin-4-ones from aniline and ethoxymethylene malonate diethyl ester.

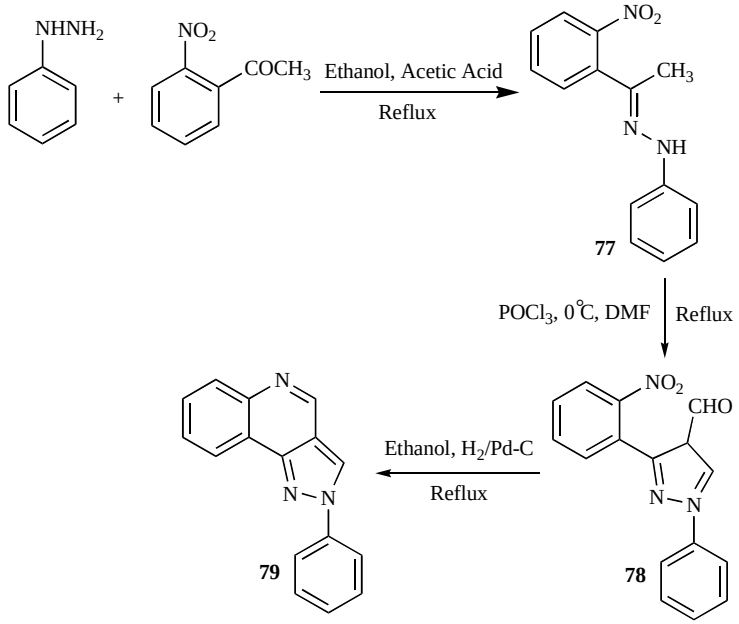


Fig. (15). Synthesis of pyrazolo[3,4-c]quinolin-4-ones using vilsmeir reaction.

instead of the vilsmeir-Haack reaction conditions ($POCl_3$-DMF) to convert the hydrazones **(80)** into pyrazolo carboxaldehydes **(81)** (Fig. (**16**)) [76]. Next the aldehydes were oxidised with $NaOCl_2$ in the presence of sulfamic acid as a scavenger to produce the corresponding carboxylic acids **(82)**. The latter were converted into respective amides **(83)** by reaction with methexylamine hydrochloride in the presence of the uranium coupling reagents TBTU to promote the cylization step. Phenyliodine (III) bis (trifluoro acetate) (PIFA) was selected as the source of hypervalent iodine to induce the generation of expected acylnitrinium intermediates **(84)**. Trifluoro acetic acid (TFA) was used as additive and CH_2Cl_2 as solvent. Finally demethylation of methoxy–protective groups with BBr_3 at -78 ºC yields the desired pyrazolo[4,3-c]quinolin-4-ones **(85)**.

In yet another strategy anthranilic acid was cyclised with ethylchloroacetate to provide benzoxazinones **(86)** [17, 77]. The latter were then reacted with the sodium anion of ethyl acetoacetate to provide acrylates **(87)** which were then cyclised and decarboxylated by treatment upon sodium methoxide to provide 4-hydroxyquinolinones **(88)**. Heating the 4-hydroxyquinolinones with hydrazine affords the pyrazolo[4,3-c]quinolones in over all good yields **(89)** (Fig. (**17**)).

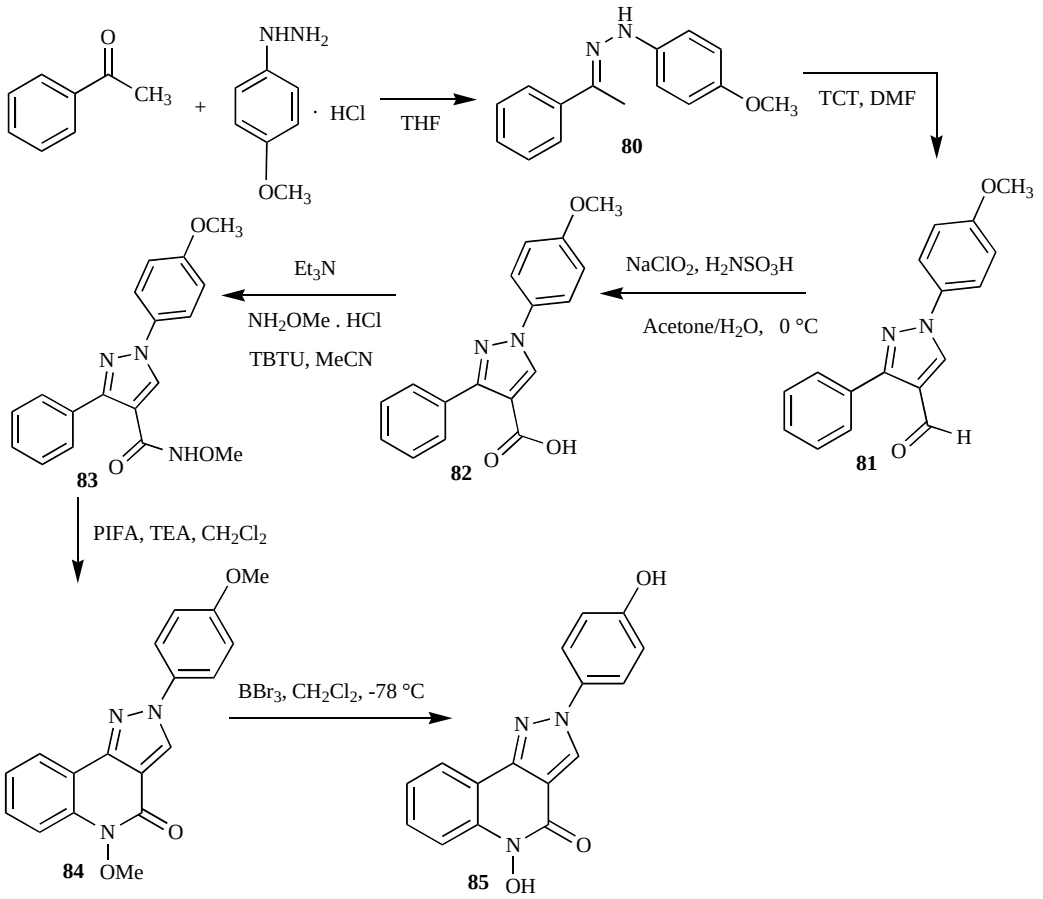


Fig. (16). Synthesis of pyrazolo[3,4-c]quinolin-4-ones using 2,4,6-trichloro 1,3,5- triazine and DMF.

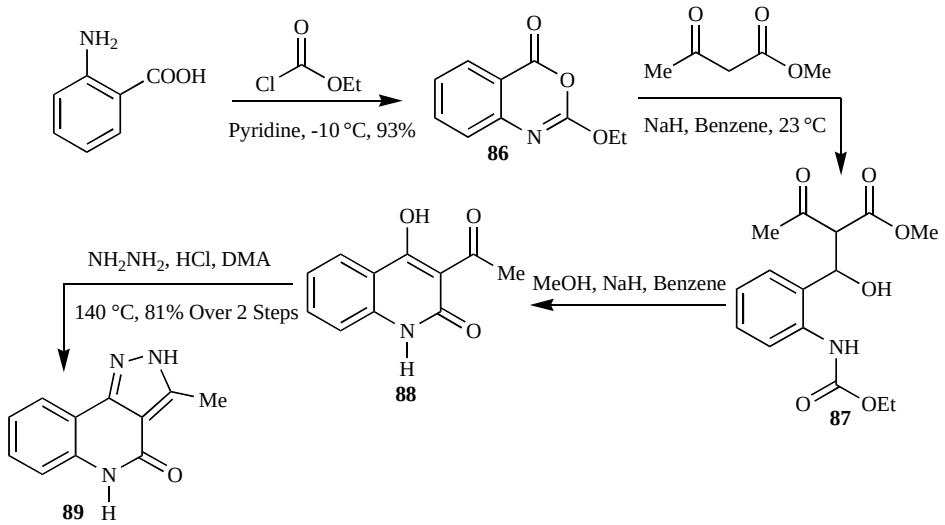


Fig. (17). Synthesis of pyrazolo[4,3-c]quinolin-4-ones using anthranilic acids and ethylchloroacetate.

Diazotisation and subsequent displacement of diazonium salt with CuBr on *o*-nitroaniline led to the intermediate **(91)** that was reduced with H_2 and Raney Ni as catalyst to give the aniline intermediate **(92)**. Condensation of **(92)** with diethyl ethoxymethylenemalonate afforded compound **(93)**, which *via* thermal ring closure in boiling diphenyl ether gave ethyl 8-bromo-4-hydroxyquinoline-3-carboxylate **(94)**. Debromination by hydrogenolysis with Pd/C afforded **(95)**, and treatment of **(95)** with $POCl_3$ led to the **(96).** The derivative thus obtained was refluxed with phenyl hydrazine derivative to afford pyrazolo[4,3-c]quinolin-3-one **(97)** (Fig. (**18**)) [26].

Pyrazolo[4,5-c]quinoline and pyrazolo[5,4-c]quinoline

Benzoylhydrazine **(98)** was obtained by reacting the arylhydrazine hydrochloride with benzoyl chloride which

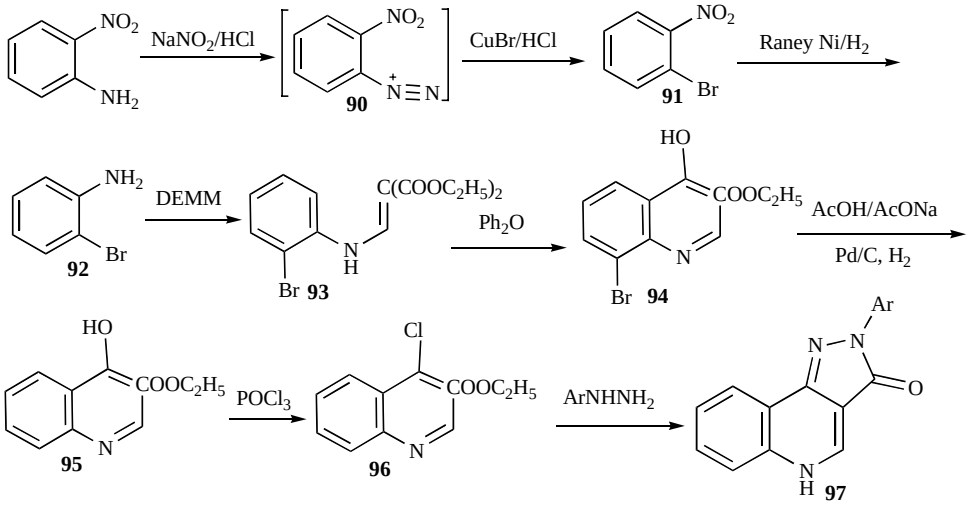


Fig. (18). Synthesis of pyrazolo[4,3-c]quinolin-4-ones using *o*-nitroaniline.

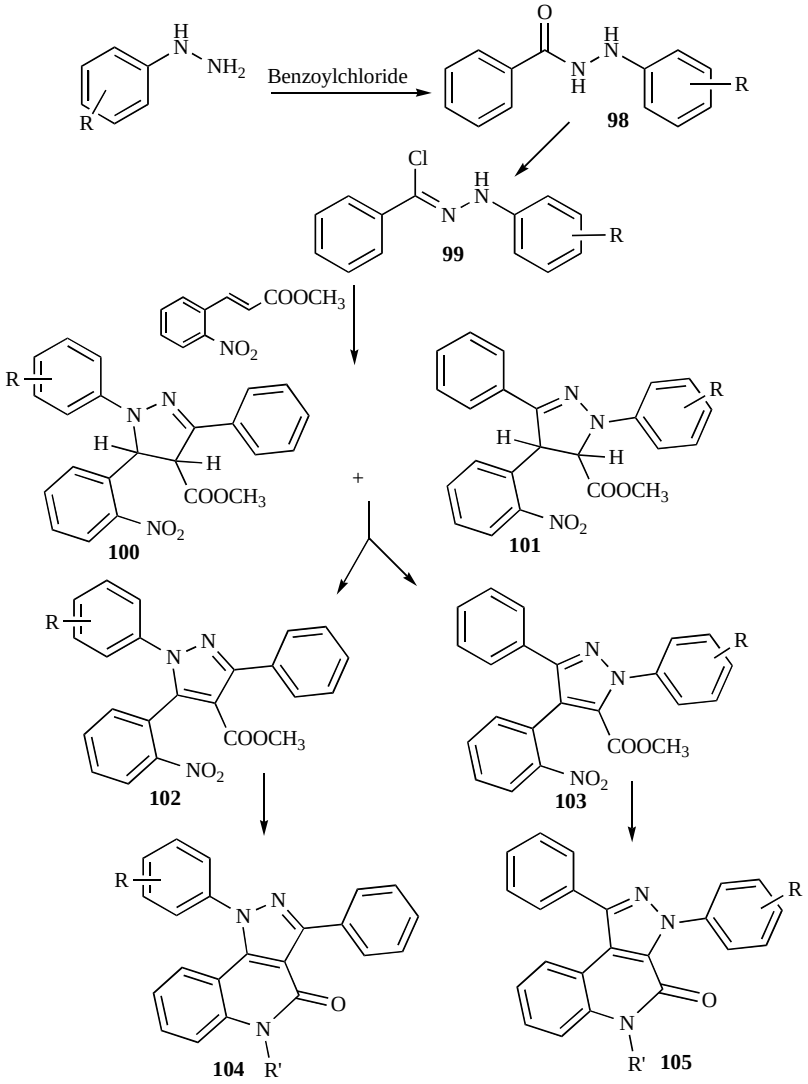


Fig. (19). Synthesis of pyrazolo[5,4-c]quinolin-4-ones using arylhydrazine hydrochloride and benzoyl chloride.

was then converted to (α-chloro-benzy1idene)arylhydrazine **(99)** (Fig. (**19**)). The 1,3-dipolar cycloaddition of (α-chloro-benzy1idene)arylhydrazine to the E isomer of methyl 2-nitrocinnamate gave rise to a mixture of the two isomeric

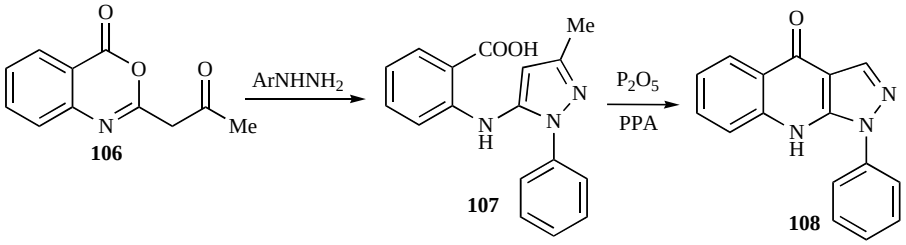


Fig. (20). Synthesis of pyrazolo[3,4-b]quinolin-4-ones using 2-acetonyl-4*H*-3,1-benzoxazin-4-one.

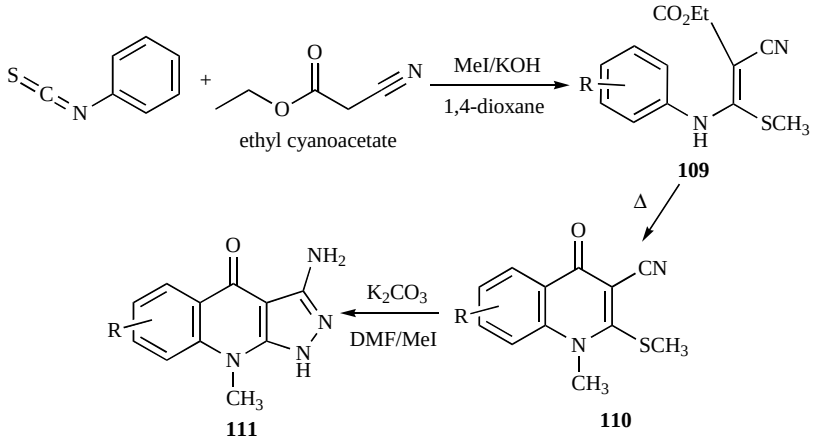


Fig. (21). Synthesis of pyrazolo[3,4-b]quinolin-4-ones using phenyl isothiocyanate and ethyl cyanoacetate.

pyrazolines **(100)** and **(101)**, which were isolated together from the starting materials. Oxidation of the mixture with lead tetraacetate yielded the corresponding triarylpyrazoles **(102)** and **(103)**, which were separated by column chromatography. Reduction and cyclization of **(102)** and **(103)** gave the target compounds 1,3-diarylpyrazolo[4,5-c]-quinolin-4-ones **(104)** and 1,3-diarylpyrazolo[5,4-c]-quinolin-4-ones **(105),** respectively [27].

Pyrazolo[3,4-b]quinoline

The synthesis of the pyrazolo[3,4-b]quinolin-4-one (**108**) is illustrated in Fig. (**20**). Treatment of 2-acetonyl-4*H*-3,1-benzoxazin-4-one (**106**) with arylhydrazines gave the *N-(*l-aryl-3 methylpyrazol-5-yl)anthranilic acids (**107**) Fig. (**20**). By heating the latter with a mixture of P_2O_5 and poly- (phosphoric acid), the l-aryl-4,9-dihydro-3-methyl-lH-pyrazolo [3,4-b]quinolin-4-one (**108**) was obtained [45, 78].

Another approach for the synthesis of pyrazolo[3,4-b]quinolin-4-one compounds is shown in Fig. (**21**). Phenyl isothiocyanate was condensed with ethyl cyanoacetate using potassium hydroxide, and MeI in dry 1,4-dioxane, the resulting N,S-acetal was cyclized thermally and finally the quinolone was N-alkylated regiospecifically by heating with potassium carbonate, DMF and MeI. The final products (**111)** were obtained when (**110)**, reacted with hydrazine hydrate [79].

CONCLUSION

Pyrazoloquinolines possess vast array of pharmacological effects including anticancer, antiviral, antimalarial, antileishmanial, phosphodiesterase IV antagonist and several others, and constitute a lead scaffold for drug development. Accumulating experimental evidence suggests that they interfere with a variety of molecular targets and processes involved. Enormous studies over structure activity relationship have been done and the results are being exploited to design and synthesise new and promising compounds. It is evident form experiments that the type of linkage between the pyrazole and quinoline ring greatly affects the activity. Continued interest in these agents may lay down basis for development of many future clinical drugs. The potential of this class as therapeutic agents is well documented.

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