Bioactive and pharmacologically important pyrano[2,3-c]pyrazoles

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ABSTRACT

Pyranopyrazoles are fused five-six membered rings containing nitrogen and oxygen heterocycles. Interestingly this class of compounds has diverse biological activities such as antimicrobial, anti-inflammatory, anticancer, analgesic, anticonvulsant, vasodilator and molluscidal activity. Recently, this class has been identified as a potential inhibitor of human Chk1 kinase. This review highlights the bioactivity and pharmacological studies of pyrano[2,3-c]pyrazoles.

Keywords: Pyrano[2,3-c]pyrazole, antimicrobial, anti-inflammatory, anticancer, bioactive

INTRODUCTION

The heterocyclic compounds are widely spread in nature and play an important role in life. Due to the characteristic properties, the heterocyclic compounds hold a large area in medicinal chemistry [1]. The chemistry of heterocyclic chemistry has been explored widely in the past two–three decades [2]. The synthesis and the application of heterocyclic compounds of medium size rings became popular [3, 4].

During the recent years, there has been intense research on fused hetercyclic compounds with pharmacological importance. Among the heterocycles the pyranopyrazole class has drawn the attention. Pyranopyrazoles refer to a fused five member pyrazole ring to a six member pyran ring. The pyranopyrazole nucleus is a versatile source of biologically important molecules. There are four possible isomers of pyranopyrazole - pyrano[2,3-c]pyrazole, pyrano[4,3-c]pyrazole, pyrano[3,2-c]pyrazole and pyrano[3,4-c]-pyrazole. The pyrano[2,3-c]pyrazoles (I) are the most popular with pharmacological importance and have been explored most. Pyrano[2,3-c]pyrazoles posses important roles in the field of pharmacological and medicinal chemistry due to the various activities of the heterocycle core.

The pyrano[2,3-c]pyrazole derivatives are known in the literature since early 19th century. After the publication of Junek et al [5] in 1973 and Otto et al [6], tremendous developments have been done on the synthesis of functionalized pyrano[2,3-c]pyrazoles and its application in medicinal chemistry become popular. In this review pharmaceutically important and bioactive pyrano[2,3-c]pyrazoles are highlighted with their activities.
PHARMACOLOGICAL AND BIOLOGICAL EVOLUTION:

Analgesic and Anti-inflammatory activity

Pyrazolopyridines were known as analgesic and anti-inflammatory agents [7]. Ueda et al. [8] thought that the introduction of a pyran ring instead of the pyridine ring might give pharmacologically better active compounds. So, pyrano[2,3-c]pyrazoles were synthesized and tested for analgesic activity in mice.

\[
\begin{align*}
2a: R &= \text{Ph} \\
2b: R &= \text{mCl-Ph} \\
3a: Y &= \text{Br} \\
3b: Y &= \text{CH}_2\text{Cl}
\end{align*}
\]

The analgesic activities \(2a, b\) and \(3a, b\) were examined in comparison with aminopyrine by oral administration to mice. The pharmacological result of compounds \(2a, b\) were almost the equal to that of aminopyrine (70% inhibition) and \(3a, b\) showed 50% inhibition.

Kuo et al [9] synthesized a series of 1- and 2-substituted 3,4-dimethylpyrano[2,3-c]pyrazol-6-one derivatives and 1-substituted 1,6-dihydro-4-methyl-6-oxopyrano[2,3-c]pyrazole-3-acetic acids. These compounds were tested for both analgesic and anti-inflammatory activities. Most of these compounds showed more prominent analgesic activities than anti-inflammatory activities and the result was similar to that of aminopyrine. Among these compounds, 1,3,4-trimethyl-pyrano[2,3-c]pyrazol-6(1H)-one(4) and 2,3,4-trimethylpyrano[2,3-c]pyrazol-6(2H)-one (5) showed more potent analgesic activity.

Vaid et al [10] have prepared some pyrano[2,3-c]pyrazolones and shown analgesic effects.

Zaki et al [11, 12] synthesized a series of pyrano[2,3-c]pyrazole derivatives. A pharmacological study to evaluate the anti-inflammatory effects of the newly synthesized compounds was performed. The compound \(6a\) showed no anti-inflammatory activity with a lower inhibitory effect compared to diclofenac. Compound \(6c\) reduced edema more effectively than compound \(6a\) with percentage inhibition of 50.40%. Compound \(6b\) reduced oedema more effectively than \(6a\) and \(6c\) with 60.10% percentage inhibition, indicating a high anti-inflammatory effect.

Mandha et al [13] synthesized a series of pyranopyrazoles via multicomponent one-pot approach in aqueous ethanol medium under totally non-catalytic conditions. The synthesized compounds were evaluated for their antibacterial, anti-inflammatory and cytotoxic activities.

Kumar et al [14] synthesized pyrano[4,3-c]pyrazoles. These compounds were found strong analgesic and excellent anti-inflammatory activities.

Antibacterial activity

Hogale et al. [15] synthesized a series of pyrano[2,3-c]pyrazoles that showed antibacterial activity. Ahdi et al [16] synthesized spiro-pyrano-pyrazoles via piperidine catalyze one-pot, four-component reaction of \(\beta\)-ketoesters, hydrazine hydrate, malononitrile, and isatines in aqueous media. The reaction was done at room temperature and the spiro[indoline-3,4'-pyrano[2,3-c]pyrazole]-5'-carbonitriles \(7\) were obtained with high yields and purity via an easy work-up procedure. These compounds were also investigated in vitro for antibacterial activities.
Antimicrobial activity

Bacterial resistance to drug, resulting from the widespread use and misuse of classical anti-microbial agents [17], is a challenging problem in antibacterial development. Efficient syntheses and study of some potent compounds are always required to overcome problem of multi-drug resistant (MDR) bacteria and fungi. Recent research reveals that fact that pyrano[2,3-c]pyrazoles possesses a unique position in medicinal chemistry due to strong microbial activities.

Ei-Tamany et al [18] synthesized pyrano[2,3-c]pyrazole derivatives via the 4-arylidene-3-methyl-1-phenyl-2-pyrazolinones with some active methylene compounds, under Michael reaction conditions. All the newly synthesized compounds were tested against some bacteria and some of the compounds were found to be biologically active.

S. Abdullah El-Assiery et al [19] synthesized pyranopyrazole derivatives and antimicrobial activity of the prepared compounds was tested by the disk diffusion method [20]. Staphylococcus aureus and Bacillus cereus (as Gram positive strains), Serratia marcescens and Proteus mirabilis (as Gram negative strains), Aspergillus fungytus (as fungi) were used for testing the compounds. Moderate to high microbial activity was observed by the compounds. The compound 8 showed moderate activity against Gram negative bacteria strains and low activity against fungi.


Harshad et al [22] synthesized a series of pyrano[2,3-c]pyrazole derivatives of indole by multi-component reactions using the conventional and microwave irradiation approach. Antimicrobial screening of the synthesized derivatives was investigated against eight human pathogens, namely Bacillus subtilis, Clostridium tetani, Streptococcus pneumoniae, Salmonella typhi, Vibrio cholerae, Escherichia coli, Aspergillus fumigatus and Candida albicans. Against fungal pathogen Candida albicans, compounds 9a (R = H), 9b (R = CH3) and 9f (R = F) were found to possess better activity (MIC 250 µg mL−1) as compared to the standard griseofulvin (MIC 500 µg mL−1). Compound 9f showed outstanding activity (MIC 62.5 µg mL−1) against the Gram-negative bacteria Escherichia coli, as compared to the standard ampicillin (MIC 100 µg mL−1) and compound 9e (R = Br) displayed excellent activity (MIC 125 µg mL−1) against the Gram-positive bacteria Bacillus subtilis, as compared to the standard ampicillin (MIC 250 µg mL−1). Compounds 9b, 9d (R = Cl), 9f, and 9h (R = NO2) against B. subtilis (MIC 200 µg...
mL–1) and compounds 9c (R = OCH₃), 9f and 9h (R = NO₂) against Clostridium tetani (MIC 200 µg mL–1) were found significantly active in comparison to ampicillin (MIC 250 µg mL–1).

Mandour, A. H [23] synthesized and extensively studied three series of pyranopyrazoles- 3-[(N-substituted indol-3-yl)methyleneamino]-6-amino-4-aryl-pyrano(2,3-c)pyrazole-5-carbonitriles (10a-g and 11a-g) and 3,6-diamino-4-(N-substituted indol-3-yl)pyrano(2,3-c)pyrazole-5-carbonitriles (12a-g). The synthesized compounds possess significant anti inflammatory, analgesic and anticonvulsant activities. The anticonvulsant potency of certain tested compounds were more pronounced than both anti-inflammatory and analgesic activities. Moreover, most of the newly synthesized compounds possess potential antimicrobial activity against Escherichia coli and Pseudomonas aeruginosa. The newly synthesized compounds with pyrano(2,3-c)pyrazole nucleus at 3-position of indole moiety show anti-inflammatory, analgesic and anticonvulsant activities which are increased by the presence of halo atoms. The anticonvulsant potency of certain tested compounds was more pronounced than both the anti-inflammatory and analgesic activities.

Mistry et al [24] synthesized a series of pyrano[2,3-d]pyrimidine-5-one derivatives from 6-amino-4-(substitutedphenyl)-5-cyano-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-c]pyrazole derivatives via cyclization using formic acid and acetic acid. All the synthesized compounds have been screened for antibacterial, antifungal and antitubercular activity.

Kassem et al [25] synthesized pyranopyrazoles and pyranoimidazoles. The synthesized new fused pyranopyrazoles, 13a-e incorporated to 8-hydroxyquinoline through a sulfonyl bridge at position 5 and evaluation of their antimicrobial against a variety of pathogenic microorganisms, E. coli, P. aeruginosa (Gram-negative bacteria), S. aureus, B. cereus (Gram-positive bacteria), and one strain of fungi (Candida albicans), at different doses of the tested compounds (100, 50, and 25 mg/disc). Compound 13c was identified as the most active with growth inhibition of 22mm at cocentration 100 mg/disc against E. coli when compared with the reference drug cefatoxime (32 mm) at 30 mg/disc.

Katariya et al [26] synthesized a series of pyrano[2,3-c]pyrazoles via one-pot, multi component reaction (MCRs) using various basic catalyst. All the synthesized compounds 14 were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method with two bacterial strains S. aureus MTCC-96 and B. subtilis MTCC-441 and two fungal strains A. niger MTCC-282 and C. albicans MTCC-227. Very good antibacterial activity was observed for 3,4 dimethoxy derivatives. 2,6 dichloro derivatives shown the activity at minimal inhibitory concentration of 62.5 mg/mL for gram +ve bacteria S.p.- Streptococcus pyogenes MTCC 443, gram negative bacteria E. coli compare to know chloromphenicol and ciprofroxacin 50 mg/mL.

B. N. Amin et al [27] synthesized a series of novel 6-amino-4-(aryl/heteroaryl)phenyl-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carboxylate derivatives (15a-e) and screened for their antibacterial and antifungal activity. The compound 15a showed a moderate inhibition against E.coli and A.niger. However, it showed lower inhibition against S. aureus. The compound 15b and 15c showed a moderate inhibition against S. aureus and E. coli. The compound 15c showed significant antifungal activity against A. niger at 40 µg/mL. The compound 15d showed good inhibition against S. aureus and E.coli with respect to gentamycin. The compound 15e showed no zone inhibition against S. aureus and E.coli. and good inhibition against A. niger.
Anticancer activity
Mohamed et al [28] synthesized pyranopyrazole and pyranopyridines and tested into the cytotoxicity bioassay on Human tumor cell line (HEPG2). The tested compounds were evaluated for their cytotoxicity against the liver Carcinoma cell line (HEPG2) of human. The results indicated pyranopyrazole compounds were moderately active and best results were obtained.

Abidi et al [29] synthesized a series of 6-amino-4-aryl-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-carbonitrile derivatives catalyzed by borax in water via four component coupling reaction. The in vitro cytotoxic activity of the synthesized compounds was investigated against cancer cell lines (SW48, A549, KB, HepG2) in comparison with doxorubicin, a well-known anticancer drug, using MTT colorimetric assay. The synthesized compounds showed good and reasonable cytotoxicity compared with doxorubicin in some studied cell lines Fig.4. The compounds 16a,16b, 16d in KB cell line (IC50 = 8±2.217 µM, 7±2.77 µM, 7.5±1.49 µM respectively), 16f in A549 cell line (IC50 = 31.5±2.02 µM), 16e in HepG2 cell line (IC50 = 22.5±3.09 µM), 16c, and 16f in SW48 cell line (IC50 = 23±0.772 µM, 23±4.97 µM respectively) showed the best results in close to the control drug (IC50 = 6.8±0.78 µM, 6.3±0.65 µM, 5.4±0.5 µM, 4.3±0.12 µM in A549, HepG2, KB, and SW48 cell lines respectively).

Eruçu et al [30] synthesized 6-amino-5-cyano-3-trifluoromethylpyranopyrazole-4-spiro-oxindole derivatives and evaluated for in vitro cytotoxicity against cell lines U937 (human histiocytic lymphoma) and B16F10 (mouse melanocarcinoma) by using MTT assay [31]. Total twelve compounds were tested. The potency of the compound 17 was best when nitro group was present in spirooxindole moiety and a chlorine atom in pyrazole moiety. The potency of the compounds decreased marginally when nitro group was absent or replaced by other halogen groups in oxindole but chlorine atom still retained in pyrazole moiety.

Antiplatelet activity
An antplatelet drug [32] is a generic term, describing agents which decrease platelet aggregation and inhibit thrombus formation.

Huang et al. [33] synthesized a series of 1- and 2-aryl methyl-3,4-dimethylpyrano[2,3-c]pyrazol-6-one derivatives and examined for their antplatelet activities. Some of these compounds showed significant inhibitory activities. Among them, 1-phenylmethyl-3,4-dimethylpyrano[2,3-c]pyrazol-6(1H)-one (18) and 2-(2'-methoxyphenyl)methyl-3,4-dimethylpyrano[2,3-c]pyrazol-6(2H)-one (19) were the most effective. These inhibitors acted in a concentration-dependent manner.
Chk1 kinase inhibitory activity

The checkpoint kinase 1 (Chk1) [34] is a key component of the DNA damage response, a molecular network deputed to maintain genome integrity. Nevertheless, cancer cells aberrantly exploit these circuits to overcome chemotherapy-induced cytotoxicity. Chk1 inhibitors have been developed as a chemopotentiating strategy and different molecular mechanisms underlying the synergism with chemotherapeutics have been uncovered. Checkpoint-1 kinase plays an important role in the G2M cell cycle control, therefore its inhibition by small molecules is of great therapeutic interest in oncology [35]. The compound 4-(6-amino-3-methyl-2,4-dihydropyran-2,3-c]pyrazol-4-yl)benzene-1,2-diol (20) has been identified as a potential inhibitor of human Chk1 kinase by Foloppe et al [36].

Ramtekkar et al [37] reported the virtual screening of an in-house library of 2499 pyranopyrazole derivatives against the ATP-binding site of Chk1 kinase using Glide 5.0 program, which resulted in six hits. All these ligands were docked into the site forming most crucial interactions with Cys87, Glu91 and Leu15 residues.

Vasodilatory activity

Sato et al. [38] prepared the 5-aminoethylpyrano[2,3-c]pyrazole derivatives by amination of corresponding chloroethyl compounds. 5-(2-(diethylamino)ethyl)-1,3,4-trimethylpyrano[2,3-c]pyrazol-6(1H)-one (21) was found to possess hypotensive, vasodilating and hypoglycemic activities. Several pyrano[2,3-d]pyrazol-4-ones have demonstrated an affinity toward A1 and A2a adenosine receptors.

Yu et al [39] demonstrated the pharmacological effects of (2-(4′methoxyphenylmethyl)-3,4-dimethylpyrano[2,3-c]pyrazol-6(2H)-one) (21) and (2-(2′-thienylmethyl)-3,4-dimethylpyrano[2,3-c]pyrazol-6(2H)-one) (22) on rat isolated thoracic aorta have been examined. In high potassium medium (60 M M), Ca^{2+}(0·03–3 M)-induced vasoconstriction was inhibited by 21 and 22 (10–100 µg mL^{-1}). It is concluded that 21 and 22 relaxed the rat aorta by suppressing the Ca^{2+} influx through both voltage-dependent and receptor-operated Ca^{2+} channels.

The A_{2A} receptor is responsible for regulating myocardial blood flow by vasodilating the coronary arteries, which increases blood flow to the myocardium, but may lead to hypotension. Just as in A_{1} receptors, this normally serves as a protective mechanism, but may be destructive in altered cardiac function.

Colotta et al [41] synthesized a series of pyrano[2,3-c]pyrazol-4-ones and evaluated for bovine brain adenosine A_{1} and A_{2A} receptor binding affinity. Substituent at positions 5 and/or 6 were varied in order to define the structure-activity relationships in these new kinds of adenosine receptor ligands. The most selective and potent among the reported compounds was the 1,4-dihydro-1-phenyl-3-methyl-6-(3-aminophenyl)-pyrano[2,3-c]pyrazol-4-one 23 which showed a 27-fold selectivity for A_{1} receptor and a K_{i} value of 84 nM.

Molluscicidal activity


Myrboh et al [45] extensively compiled the revived on the synthesis of pyrano[2,3-c]pyrazoles. Both classical and modern methodologies were studied for a wide variety of pyrano-[2,3-c]pyrazoles, ranging from two-component traditional reactions to four-component protocols. After first synthesis of 6-amino- pyrano[2,3-c]pyrazole by Junek et al [5], several studies on multi coupling reactions are done. Vasuki et al [46] reported four component green synthesis of pyranooyrazole in water. Gogoi et al [46] reported the first enantioselective synthesis of biologically active 6-amino-5-cyanodihydropyran[2,3-c]pyrazoles synthesis through a cinchona alkaloid-catalyzed tandem
Michael addition and Thorpe-Ziegler type reaction between 2-pyrazolin-5-ones and benzylidene malononitriles. The desired products were obtained in excellent yields with mediocre to excellent enantioselectivities (up to >99% ee) [47, 48]. Pawar et al [49] synthesized pyranopyrazoles by multi component reactions. Jaybal et al [50] recently synthesized a number of 6-amino -5-nitro pyrano[2,3-c]pyrazoles (24) by combinatorial synthesis of a the compounds library. Application of nanoparticles in the synthesis was also recently explored for making bioactive pyrano[2,3-c]pyrazoles [51, 52].

![Image](image.png)

D.M. Pore et al [53] reported a catalyst-free multicomponent reaction (MCR) capable of affording a wide range of novel spiro pyranopyrazole derivatives from pyrazolone.

Yang et al [54] synthesized a series of novel 1H,4H-dihydro-pyrano-[2,3-c]pyrazoles (25) and 2H,4H-dihydro-pyrano[2,3-c]pyrazoles (26) with aromatic aldehydes obtained from lignin. In vitro antioxidant using microwave-assisted technology and cytotoxic activities of these compounds were evaluated. The structure activity relationship (SAR) studies showed that the introduction of methoxy group in aromatic groups of dihydro-pyrano[2,3-c]pyrazoles could significantly increase their radical scavenging activities. The substituted moieties at N or C-3 position of dihydro-pyrano[2,3-c]pyrazoles could potentially influence on their antioxidant activities. These compounds might have potential as promising agents for curing some free radical-related diseases or food additives.

Khoobi et al [55] synthesized a new series of tacrine-based acetylcholinesterase (AChE) inhibitors, poly-functionalized hybrid molecules. The benzene ring of tacrine was replaced with an aryl-dihydropyrano[2,3-c]pyrazole. Most of target compounds showed potent and selective anti-AChE activity at sub-micromolar range. The docking study of compound 27 with AChE enzyme revealed that the (R)-enantiomer binds preferably to catalytic anionic site (CAS) while the (S)-enantiomer prone to be a polyamides (PAS) binder.

**CONCLUSION**

It has been established that the pyrano[2,3-c]pyrazole is one of the most promising pharmacophore. Many pyrano[2,3-c]pyrazoles are biologically and pharmacology importance compounds and have been tested for a number of therapeutic areas and found to show good activity such as anticancer, antiinflammatory, antimicrobial and ChK1 kinase inhibitor. This class compounds has already drawn the attention in the medicinal chemistry. In future research and studies on pyrano[2,3-c]pyrazole derivatives may lead to enantiopure candidates with potential clinical application.

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