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Original article

Synthesis, antitumor, cytotoxic and antioxidant evaluation of some new pyrazolotriazines attached to antipyrine moiety

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A B S T R A C T

Iminopropanehydrazonoyl cyanide **4** was achieved upon reaction of antipyrine diazonium salt **2** with 3-iminobutanenitrile (**3**) in EtOH/AcONa. 3-Aminopyrazole derivative **5** was obtained upon reaction of **4** with hydrazine hydrate. Diazodization of **5** afforded the diazonium salt **6** which coupled with active methylene compounds **7**–**10**, **19**, **20**, **25**, **29** and **32** in pyridine to give aryl hydrazone derivatives **11**–**14**, **21**, **22**, **26**, **30** and **33**, respectively. Refluxing of compounds **11**–**14**, **21**, **22**, **26** and **33** in acetic acid afforded the pyrazolotriazines **15**–**18**, **23**, **24**, **28** and **35**, respectively. The newly synthesized compounds were screened for their cytotoxic and antioxidant activities. The results showed clearly that compounds **4**, **5**, **13**, **22**, and **24** displayed promising *in vitro* anticancer activity against four different cell lines (HepG2, WI 38, VERO and MCF-7). Compounds **4** and **22** are the more potent antioxidant and anticancer agents. On the other hand, most of the compounds exhibited good cytotoxic activity toward (EAC).

1. Introduction

It is well known that nitriles are widely used as intermediates for a large number of heterocyclic compounds. Aminopyrazole compounds can be readily obtained by the reaction of nitrile derivatives with hydrazine hydrate [1–4].

Also, aminopyrazole is often used for construction of pyrazolotriazine heterocyclic systems *via* diazodization and diazocoupling with active methylene moieties [5–7]. Antipyrine or phenazone derivatives are well known compounds used mainly as analgesic and antipyretic drugs [8]. One of the best known antipyrine derivatives is 4-aminoantipyrine which is used for the protection against oxidative stress as well as prophylactic of some diseases including cancer and these are important directions in medicine and biochemistry [9,10]. Reactive oxygen species (ROS), including free radicals led to a decrease in the antioxidant capacity and may generate other reactive species that damage the living cell. Oxidative stress may arise in a biological system after an increased exposure to oxidants, so the antioxidants play a major role in protecting biological systems against such threats. Different types of antioxidants (vitamins C and E, glutathione, lipoic acid and butylated phenols, etc.) have been widely used in different fields of industry and medicine as compounds that interrupt radical-chain oxidation processes, causing thus a high scientific interest [11,12]. Antipyrine derivatives are strong inhibitors of cycloxygenase isoenzymes, platelet tromboxane and prostanoids synthesis [8,13]. The biological activity of these compounds has also been attributed to its scavenging activity against reactive oxygen and nitrogen species, as well as to the inhibition of neutrophil's oxidative burst. However, besides its well recognized benefits, antipyrine derivatives have been associated with potential adverse effects characterized by leukopenia, most commonly of neutrophils, causing neutropenia in the circulating blood (agranulocytosis). It is worth to mention that there are studies demonstrating that this adverse effect might be exaggerated [14,15].

Moreover, several pyrazole ring systems are associated with antifungal, antitubercular, antibacterial, antiviral anticancer and antioxidant activities [16–18] as well as the biological activities of pyrazolotriazine ring systems are well documented [19–21]. It has been used as adenine analogs, antagonists, antischistosomal, antitumor and antibacterial agents [22–27]. Therefore, it is a real challenge to combine the above mentioned boilable rings together in a molecular framework to see the additive effect of these rings toward the antioxidant and antitumor activities.

2. Result and discussion

The synthetic strategies adopted to obtain the corresponding pyrazolotriazines are depicted in Schemes 1–4. The starting

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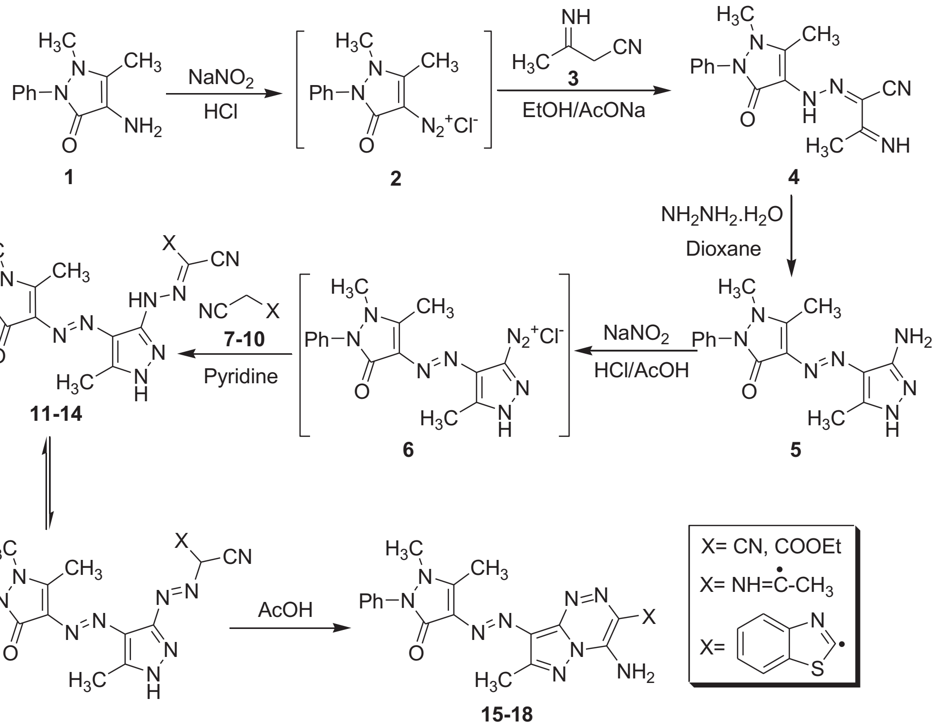
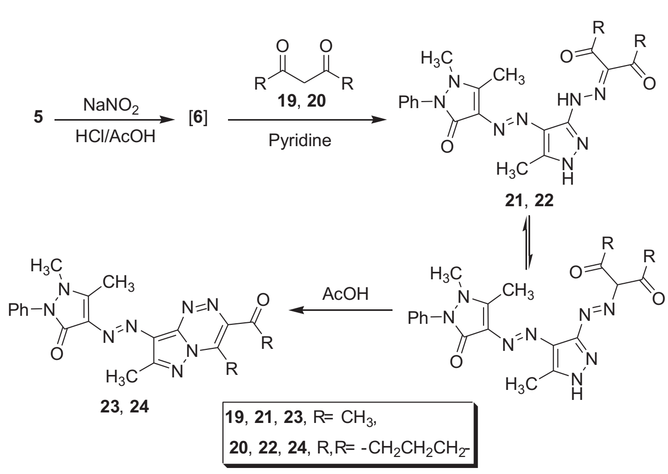


Scheme 1. Synthetic route for 3-substituted-7-methylpyrazolo[5,1-*c*][1,2,4]triazines **15**–**18**.

material, 4-((3-amino-5-methyl-1*H*-pyrazol-4-yl)diazenyl)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**5**) was prepared by coupling of 1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazole-4-diazonium chloride (**2**) with 3-iminobutanenitrile (**3**) [28] in ethanol containing sodium acetate followed by heating the formed *N*′-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-2-iminopropane hydrazonoyl cyanide (**4**) under reflux with hydrazine hydrate in dioxane. Diazotization of aminopyrazole **5** with sodium nitrite in a mixture of acetic acid and hydrochloric acid led to the corresponding diazonium salt [intermediate] **6** which coupled with malononitrile (**7**), ethyl cyanoacetate (**8**), 3-iminobutanenitrile (**9**) or 2-(benzo[*d*]thiazol-2-yl)acetonitrile (**10**) [29] in pyridine to give the corresponding hydrazones **11**–**14**, respectively. Cyclizations of compounds **11**–**14** afforded the desired pyrazolotriazines **15**–**18** under the influence of acetic acid (Scheme 1).



Scheme 2. Synthesis of 3,4-disubstituted-7-methylpyrazolo[5,1-*c*][1,2,4]triazines **23** and **24**.

In similar manner, the diazonium salt **6** reacted with acetylacetone (**19**) or cyclohexane-1,3-dione (**20**) in pyridine to afford products that may be formulated as hydrazone derivatives **21** and **22**, respectively based on both elemental analyses and spectral data. Cyclizations of the resulted hydrazones **21** and **22** under acidic condition gave the desired pyrazolotriazines **23** and **24**, respectively (Scheme 2).

The synthetic potency of **6** was investigated to develop a facile and convenient route to polysubstituted pyrazolotriazine derivatives of expected biological activity [30]. Thus, coupling of compound **6** with 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**25**) [31] in pyridine afforded hydrazone derivative **26** which gave pyrazolopyridotriazine derivative **28** upon refluxing in acetic acid through the intermediate **27**. Furthermore, we have investigated the reactivity of **6** with 3-methyl-1*H*-pyrazol-5(4*H*)-one (**29**) [32] to synthesize dipyrazolo[5,1-*c*:3′,4′-*e*][1,2,4]triazine ring system **31**. Thus, coupling of diazonium salt **6** with compound **29** in pyridine furnished the hydrazone derivative **30** which cyclized in $POCl_3$/DMF to afford the target compound **31** (Scheme 3).

Treatment of **6** with 1-phenyl-2-thiocyanatoethanone (**32**) in pyridine gave hydrazone derivative **33**. The preparation of compound **34** through cyclization of **33** in acetic acid was failed and we obtain the iminothiadiazole **35** (Scheme 4). Assignment of the new synthesized compounds was based on elemental analyses and spectral data (IR, ^{1}H NMR and mass spectra) (*C.f.* Experimental Part).

2.1. Biological studies

2.1.1. Antitumor activity

2.1.1.1. Effect of drugs on the viability of Ehrlich ascites cells (EAC) in vitro. Twenty antipyrine derivatives were tested for cytotoxicity against a well known established model EAC *in vitro* [33]. Results for the ED_{100}, ED_{50}, ED_{25} and IC_{50} values of the active compounds are summarized in Table 1. The data showed clearly that most of compounds have good activities. Thus, it would appear that the