Safety Evaluation of Nicotinic Acid Based Pyrazolone Derivatives in Human Cells

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Summary: Pyrazolone is considered to be an active nucleus present in a large number of drugs. Based on their biological profile, we have tested previously synthesized series of pyrazolone derivatives (Ia-Id), (IIa-IId), (IIIa-IIId), and screened those for *in-vitro* antioxidant potential using DPPH assay method. These compounds showed moderate to good antioxidant activity. Furthermore, potential analogs were evaluated for *in-vitro* cytotoxicity via MTT reduction assay in MCF-7 cell line. The compounds exhibited non-toxic effect and did not show 50% inhibition on particular concentration. Based on these observations, we report here that these analogs can be developed into safe drugs.

Keywords: Pyrazolone, Antioxidant, MTT Assay, MCF-7.

Introduction

Heterocyclic rings are the common structural feature of the majority of marketed drugs. Amongst the heterocyclic compounds pyrazolone ring has a unique status in the pharmaceutical industry. It is a five-membered lactam ring containing two nitrogen and one ketonic group [1]. 3-pyrazolone and 5-pyrazolone are the most dominant classes of pyrazolone nucleus as shown in Fig. 1.1. The pyrazolone nucleus exists in three tautomeric forms (Idemudia et al., 2016). Sufficient literature is available on the synthesis, characterization, and biological investigation of pyrazolone derivatives [2].

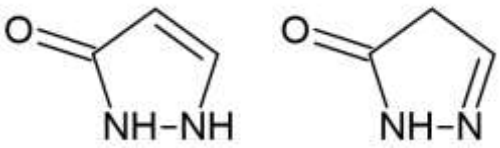

Fig. 1.1: Structure of 3-pyrazolone and 5- pyrazolone.

The biological activities of pyrazolone-5-one depends on the nature of substituents [3]. Recently FDA has approved drugs with pyrazolone nucleus including Edaravone, which has been used for ALS disorder and for brain infarction [4]. Another drug of this class aminophenazone, besides its analgesic and anti-inflammatory activities, is also used in breath test to measure the cytochrome P-450 metabolic activity in liver function evaluation [5]. Dichlorophenazone is a sedative and analgesic drug [6]. Metamizole along with its antipyretic and analgesic effect is also used for preoperative, as anticancer and for acute injury pain [7]. Demethylated antipyrine is a neuroprotective drug, reducing the brain damage by inhibiting endothelial injury. Pyrazolone derivatives have also been reported as antibacterial drugs [8]. These compounds have shown cytostatic effect against many carcinomatic cells including leukemia, melanoma, lung, colon, CNS, ovarian, prostate, and breast cancer cells [9]. New steroidal Pyrazolone containing moieties were synthesized and screened against different cancer cell lines including SW 480, Hep G2, A549, HeLa, and HL-60. These compounds were also screened for antimicrobial assay and MIC by broth dilution method using different bacterial and fungal strains for cancer and antimicrobial activity [10]. Based on the biological profile new pyrazolone derivatives (already synthesized) [11] were screened for in-vitro anticancer activity using MCF-7 cell lines. All compounds showed negligible cytotoxic potential, and were considered as safest candidate for further in-vivo screening on molecular level. Few of the FDA approved marketed drugs with pyrazolone moiety are given in Fig. 1.2.

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Experimental

Chemicals and reagents

All chemicals and reagents used during research were purchased from Aldrich chemical Co., USA. Melting points were recorded by using digital Gallen hamp (SANYO) model MPD.BM 3.5 apparatus and these were uncorrected. The synthesized Nicotinic acid based pyrazolone derivatives are shown in Fig 2.1.

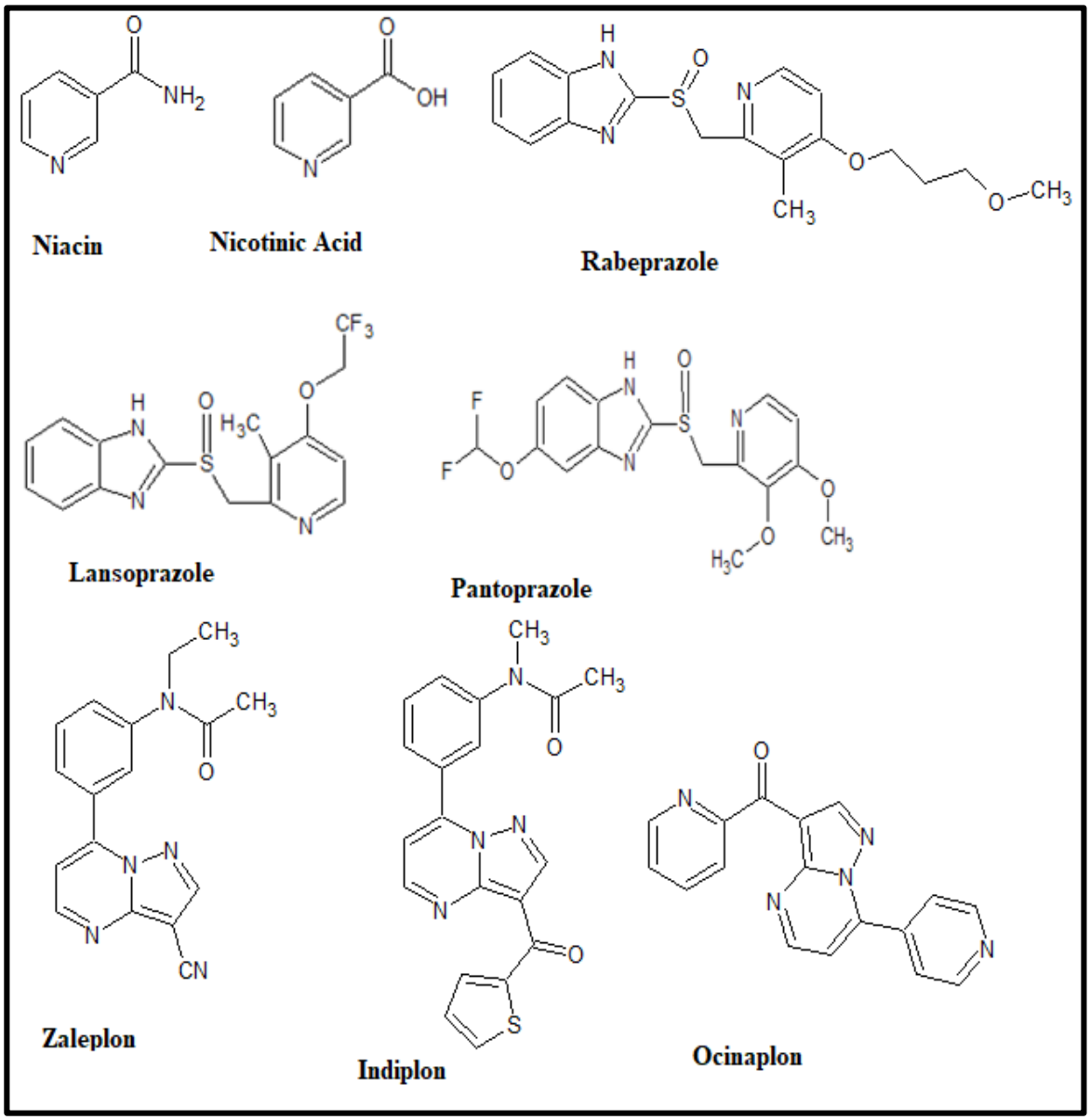

Fig. 1.2: Marketed drugs containing heterocyclic ring system.

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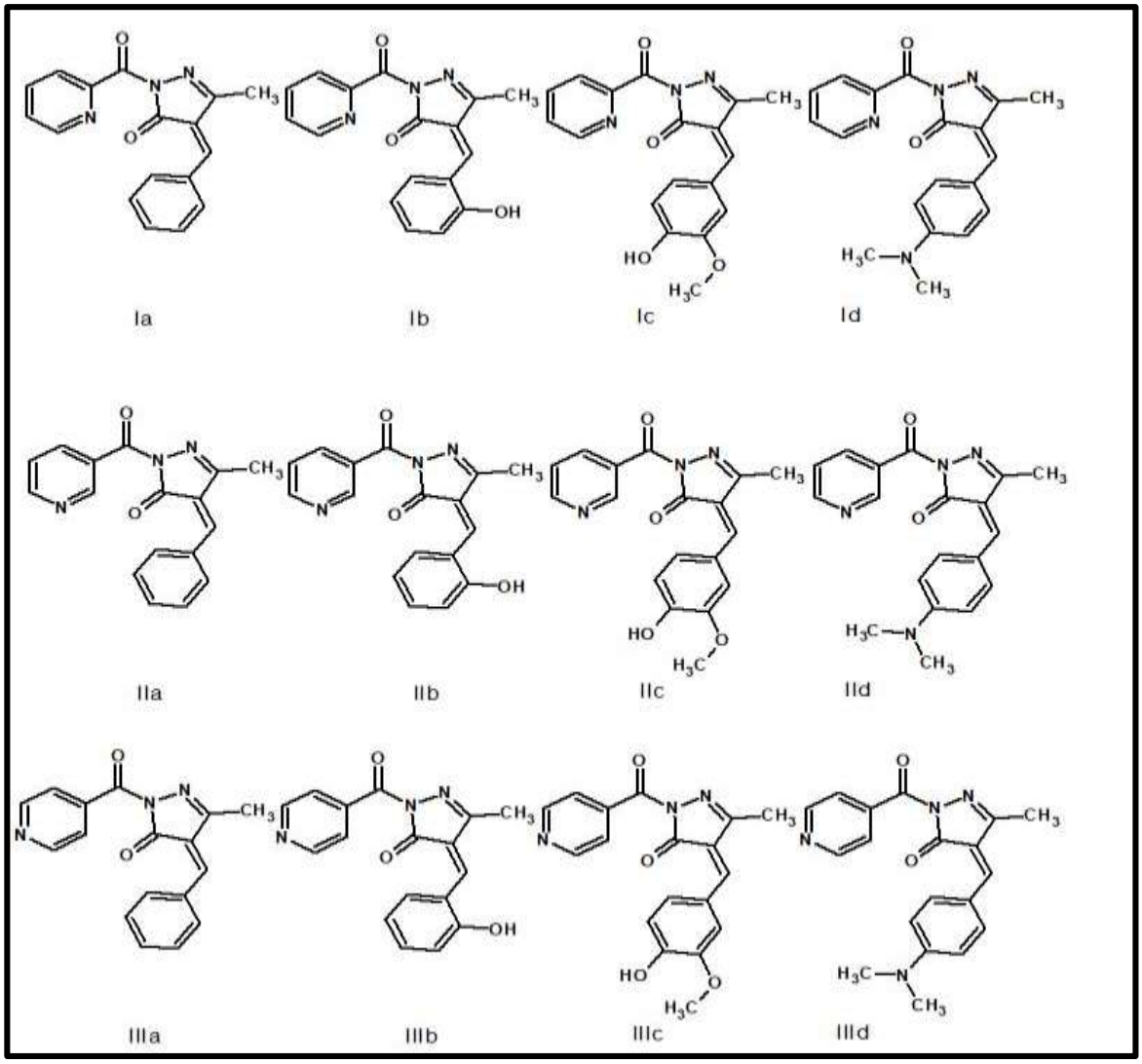
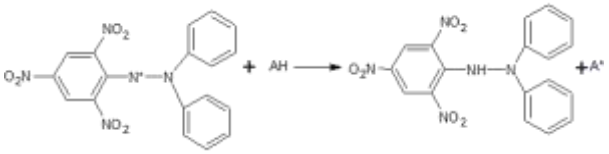


Fig. 2.1: Pyrazolone derivatives synthesized from nicotinic acid, Isonicotinic Acid and Picolinic Acid.

Biological activities

Free Radical scavenging activity by using DPPH method

To check the anti-oxidant activity of synthesized pyrazolone derivatives, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was performed. 1 mg/mL stock solution of test compounds and ascorbic acid (positive control) was Prepared, and subsequent sample concentrations (700, 300, 100, 10, 5, and 1 μg/mL) were prepared using the serial dilution method. Methanol was used to make the 1 mmol DPPH solution. Then, from each dilution, 1 mL of the test sample was obtained and 3 mL of DPPH solution was taken in separate test tubes to make a volume up to 4 ml. All test tubes were stored at room temperature and covered with aluminum foil. The oxidation potential of test compound was demonstrated by change in purple color of DPPH to yellow due to the free radical scavenging activity. Using a UV spectrophotometer, the absorbance was measured at 517nm. Inhibition percentage or free radical scavenging activity was calculated by using formula as under [11, 12].



Diphenylpicrylhydrazide (free radical) Diphenylpicrylhydrazine (non-radical)

Percent inhibition scavenging will be calculated by using the formula given as under.

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

In-vitro Cytotoxicity assay in MCF-7 cell lines

The cytotoxicity of all the synthesized compounds in MCF-7 cell lines was determined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay [13]. Cells ($1x10^5$/well) were placed in 0.2ml of medium in 96-well plates and were kept for 36 hrs. in CO2 incubator. For MTT assay, after incubation, the medium was removed carefully from the wells. MEM (w/o) and FCS was used to wash the each well 2-3 times and 200µl of MTT (5mg/ml) was added. 1ml of DMSO was added to each well and mixed well with the help of micropipette and then left it for 45 sec. Presence of viable cells were visualized by the development of purple colour due to the formation formazan crystals. Concentration required for 50% inhibition of viability (IC_{50}) was determined graphically. Graph was plotted taking concentration on X-axis and relative cell viability at Y-axis.

Cell viability (%) = Mean OD/ control OD x 100

Result and Discussion

New pyrazolone derivatives synthesized (already reported) were initially screened for *in-vitro* antioxidant potential. Based on their activity, all were screened for in-vitro cytotoxicity activity by MTT assay using MCF-7 cell lines. None on them exhibited cytotoxicity against MCF-cell lines. The detailed results and their discussion is given as under.

Free Radical scavenging potential

All derivatives were initially screened for in-vitro antioxidant activity (Previously Published). Results showed that all compounds exhibited good antioxidant potential as compared to standard drug ascorbic acid (Vitamin C).

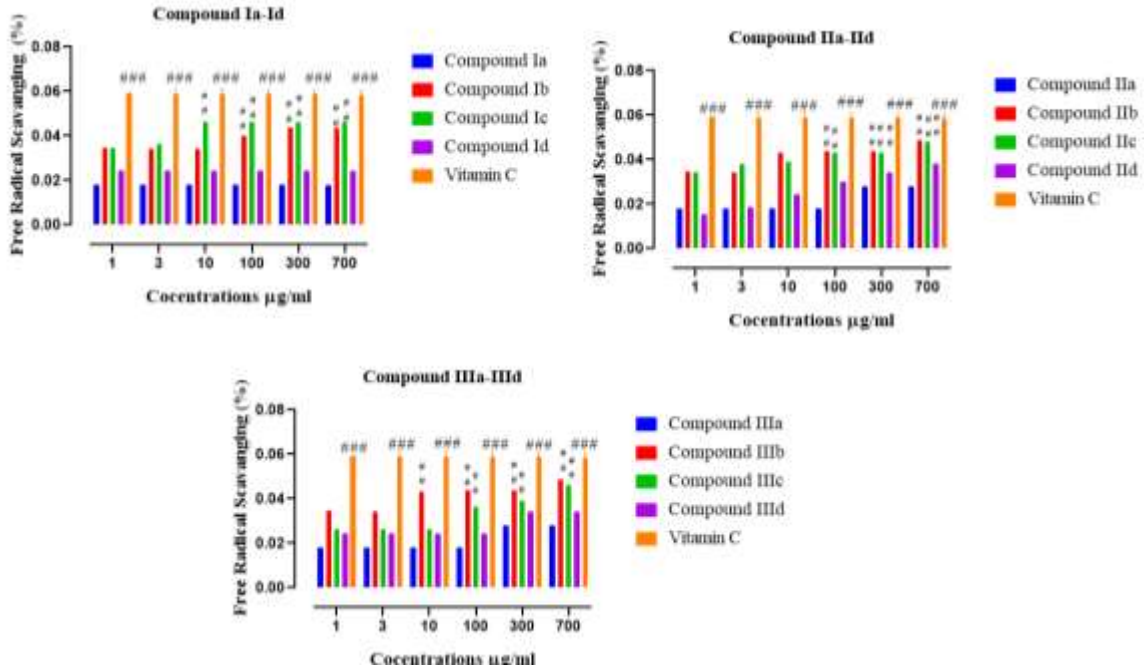


Fig. 3.1: The percentage Free Radical Scavenging potential of all newly synthesized Pyrazolone derivatives (Ia-Id), (IIa-IId), (IIIa-IIId) and standard drug ascorbic acid. All velues are presented in the form of standard error of mean (± SEM) . Symbol *** or ### show significant difference at p<0.001, while * and ** show significant difference at p< 0.05 and p< 0.01, respectively. Symbol # represented the significant difference relative to disease group and symbol * showed the significant difference compared to the control group.

In-vitro Cytotoxicity

All analogs were screened for *in-vitro* cytotoxicity assay on MCF-7 cell lines to investigate their toxic profile. The results revealed nontoxic effect and did not show 50% inhibition of cells at particular concentrations. Results showed the cell survival rate at particular concentration. Thus it is concluded that these derivatives can be further used for *in-vivo* analysis on molecular level. All results are shown in the given Figure (3.2) below.

Pyrazolon is an important moiety amongst heterocyclic compounds with diverse biological effects including, anti-bacterial, anti-inflammatoy, anti-tuberculosis, anti-viral, anti-convulsant and anti-cancer potential. From the diverse biological potential of Pyrazolone nucleus, new series of pyrazolone derivatives were synthesized and investigated for their cytotoxicity profile. All the synthesized compounds were initially screened for their antioxidant potential *in vitro* through DPPH assay in terms of free radical scavenging activity. The results of compounds belonging to three series are shown in Fig 3.1. The compound Ic and Ib were seem to be most potent antioxidant agents in series I; IIb and IIc in series II while IIIb and IIIc in series III (Fig 3.1) (published data in Iranian Journal of Basic Medical Sciences).

Multiple established reports revealed that Pyrazolone derivatives exhibited not only anti-oxidant and free radical scavenging effect but also activate many anti-oxidant systems yielding species with unpaired electrons delocalized in the heterocyclic ring. The ability of these derivatives as anti-oxidant agents credited to their proton donor ability to neutralize the free radicals.

Selected compounds were screened for their *in-vitro* cytotoxicity in MCF-7 cell line (breast cancer). The anti-cancer activity of all synthesized compounds on MCF-7 cell lines was determined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay (Fig 3.2). Selected compounds did not show 50% inhibition against cell lines. All derivatives exhibited cell survival potential (>80% to 100%) in MCF-7 cell line. These results show that the pyrazolone derivatives are good and safe candidates for further biological activities.

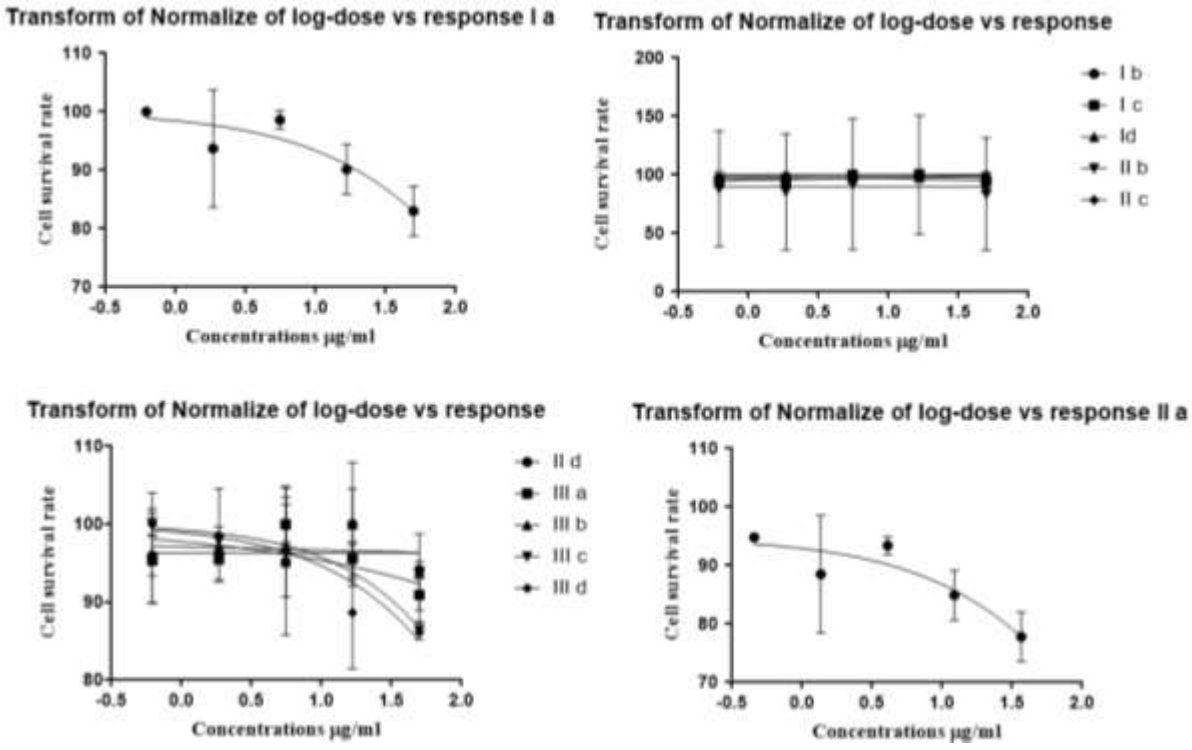


Fig. 3.2: Represented the cell survival rate of all synthesized pyrazolone derivatives. (Ia-Id), (IIb-IId), (IIIa-IIId) on MCF-7 cell lines. The analogues showed cell survival rate at particular concentration.

Conclusion

It is concluded that the synthesized Pyrazolones derivatives are safer candidate for development into safer drugs.

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