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Antioxidant Activities of N-((3,5-dimethyl-1H-pyrazol-1-yl)methyl)pyridin-4-amine derivatives

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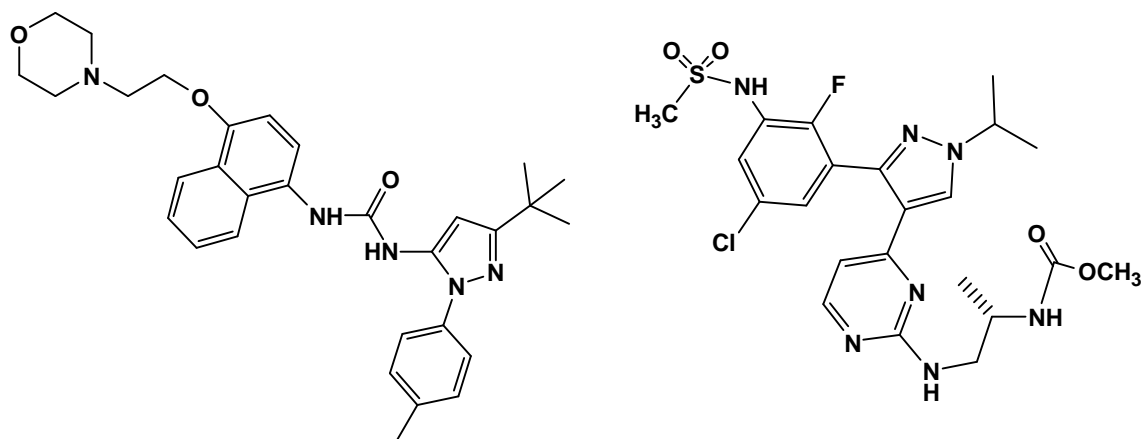
ABSTRACT

A serie of pyrazolic heterocyclic compounds processing -NH function (**1-14**) have been prepared in one step by condensation of one equivalent of (3, 5-dimethyl-1H-pyrazol-1-yl) methanol or (1H-pyrazol-1-yl) methanol with one equivalent of appropriate primary amines. The antioxidant activities of these compounds have been investigated with the rate ranging for the best one such as the compounds **8** (96.64% at 80 mg/mL), **1** (77.31% at 80 mg/mL), **10** (71.71% at 80 mg/mL), **3** (58.79 at 80 mg/mL) and **4** (49.57% at 80 mg/mL).

Keywords: Heterocyclic compounds, tridentate ligand, nitrogen-rich, synthesis, pyrazole, antioxidant activity.

INTRODUCTION

Pyrazoles and their derivatives represent an important class of compounds that find extensive use in the pharmaceutical industry [1-4]. Compounds containing a pyrazole motif are being developed in a wide range of therapeutic areas including metabolic, CNS (Central Nervous System) and oncological diseases [5], Anti-inflammatory agents, such as Celebrex [6-8], and Viagra [9-12]. Substituted pyrazole derivatives have also been employed as drug candidate, for example LGX818, emerged after extensive tweaks to a sulphonamide as inhibitor of the RAF kinase in cancer therapy [13-14]. Another example, BIRB796 which got a new name - doramapimod underwent testing in people with Crohn's disease, rheumatoid arthritis, and psoriasis [15-17] (Figure1).



BIRB 796

LGX818

Figure 1. Structures of BIRB796 and LGX818.

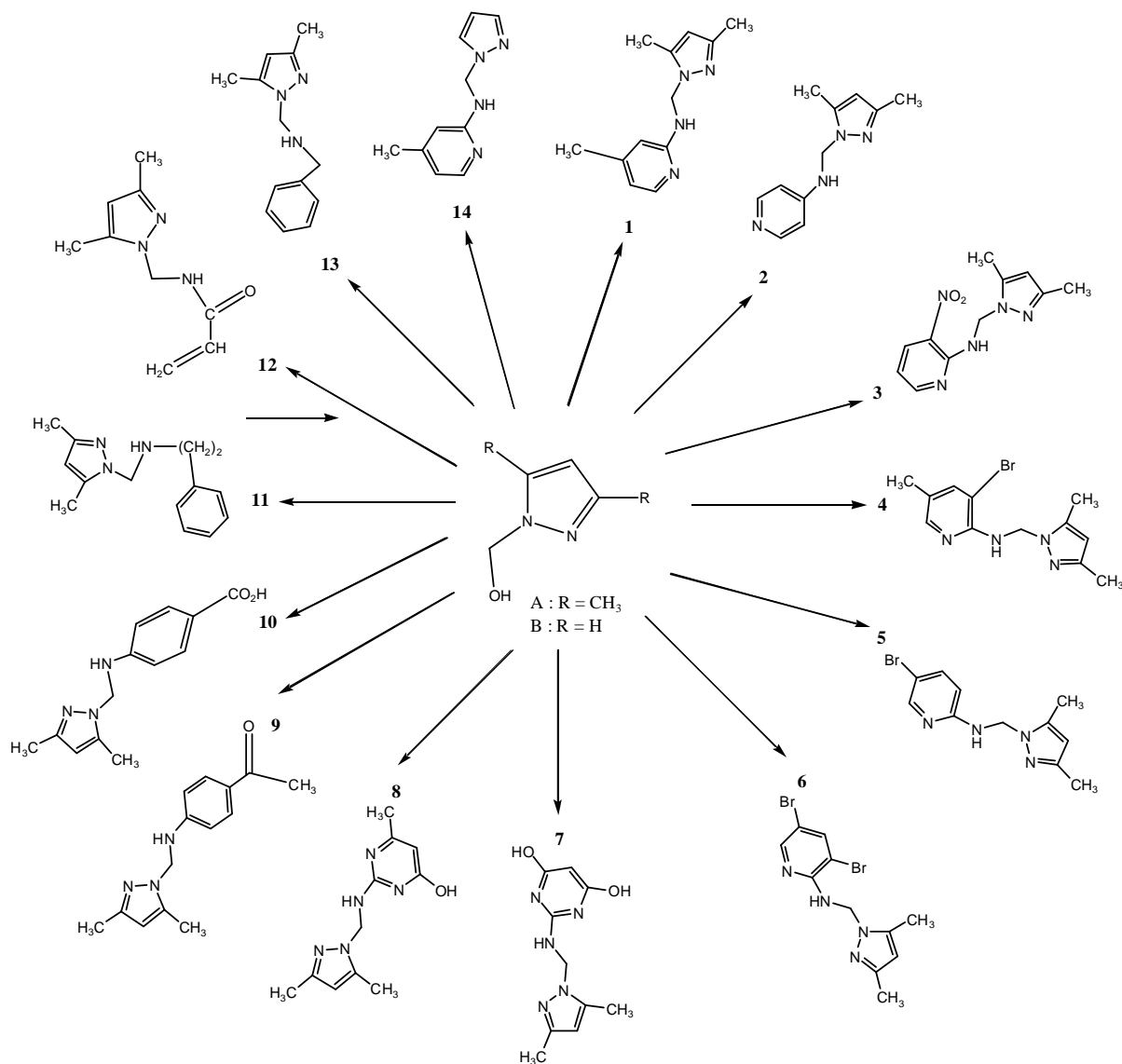
In view of this part and continuation of our research investigations looking for new efficient molecules against diesis [18-24], it was thought worthwhile to synthesize and investigate new library of pyrazolic compounds containing heterocyclic substituents. Present work is concerned with the synthesis of Novel N-((3,5-dimethyl-1H-pyrazol-1-yl)methyl)pyridin-4-amine and the study of their antioxidant activities.

MATERIALS AND METHODS

Melting point are uncorrected, ¹H and ¹³C NMR spectra were recorded at 300 MHz. Chemical shifts are reported in parts per million (ppm) downfield from an internal TMS (trimethylsilane) reference. Coupling constants (J) are reported in hertz (Hz), and spin multiplicities are represented by the symbols s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra (MS) were obtained by using electrospray ionization (ESI) [25].

General method for synthesis

The reaction employed for synthesis of the target pyrazoles **1-14** are illustrated in **scheme1**, where we mixed one equivalent of (3, 5-dimethyl-1H-pyrazol-1-yl) methanol((1H-pyrazol-1-yl) methanol) with one equivalent of an amine in 20ml of acetonitrile (CH₃CN) as solvent. All reactions were carried out at reflux for 4 hours. The liquid residue is dried over MgSO₄, filtered and concentrated in vacuum.



Scheme 1. General reaction of the synthesis of tested compounds

Antioxidant Activity

DPPH radical scavenging assay

The hydrogen atoms or electron-donating ability of the corresponding samples was determined from the bleaching of purple-colored ethanol solution of DPPH [26]. This spectrophotometric assay uses the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) as a reagent [27, 28]. Radical scavenging activity of samples was measured by slightly modified method of Braca as described in refer [29]. Different concentrations of each sample were prepared in ethanol: 2, 10, 20, 40 and 80 mg/ml. A solution of DPPH in ethanol (54 µg/ml) was prepared and 0.5 ml of this solution was added to 0.5 ml of sample solution in ethanol at different concentrations (2, 10, 20, 40 and 80 mg/ml). The solution of DPPH was prepared daily before measurements. After vortexing, the mixtures were incubated in the dark at ambient temperature and the absorbance was measured at 517 nm after 30 min, using a spectrophotometer (UV/VIS Spectrophotometer OPTIZEN POP) against ethanol. The blank sample was used as 0.5 ml of DPPH solution (54 µg/ml in ethanol) with 0.5 ml of ethanol. Decreasing of the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity (% of inhibition). This activity is given as percent DPPH radical scavenging, which is calculated with the following equation:

$$\% \text{ DPPH radical scavenging} = [(A_0 - A_t)/A_0] \times 100$$

A₀: Absorbance of blank sample;

A_t: Absorbance of tested sample solution at the time t = 30 min.

The experiment was performed in triplicate and the average absorbance was noted for each measure. The same procedure was followed for the positive control: ascorbic acid (0.002, 0.005, 1.8, 3 and 10 mg/ml). The ethanol was used for baseline correction.

RESULTS AND DISCUSSION

Antioxidant Activity

Antioxidant properties of these compounds were evaluated to find a new natural source of antioxidant. The potential antioxidant activity of these samples was determined on the basis of the scavenging activity of the free stable radical DPPH which is considered a good *in vitro* model and is widely used to conveniently assess antioxidant efficacy. The sample activity was compared to the synthetic antioxidant, ascorbic acid which was used as antioxidant reference. The results obtained at different concentrations are given in Table 3.

Table 3. DPPH radical – scavenging activities of different samples.

Conc (mg/mL)	2	10	20	40	80	IC ₅₀ (mg/mL)
1	32.90	34.23	43.61	60.46	77.31	27.51
2	2.16	28.72	31.69	33.71	34.67	-
3	12.97	44.85	53.91	58.02	58.79	15.89
4	0.00	18.06	30.98	46.47	49.57	-
5	0.00	0.00	0.00	0.00	0.00	-
6	0.00	0.00	0.00	0.00	0.00	-
7	1.63	8.16	15.12	29.03	9.52	-
8	0.00	0.00	27.38	82.29	96.64	28.11
9	1.55	7.72	10.41	15.79	0.28	-
10	5.28	26.39	27.55	29.88	71.71	57.12
11	0.00	0.00	0.00	5.63	6.22	-
12	0.00	0.00	0.00	0.00	0.00	-
13	0.00	0.35	1.33	3.30	0.00	-
14	3.23	16.14	22.77	36.02	02.21	
Conc. (mg/ml)	0.002	0.005	1.8	3	10	
Ascorbic Acid	17.40	50.00	97.00	94.00	80.14	0.005

In its radical form, DPPH has an absorbance at 517 nm which disappears when DPPH is reduced by an antioxidant compound or a radical species to become a stable diamagnetic molecule. As a result, the color changes from purple to yellow. This color change is taken as an indication of the hydrogen donating ability of the tested compounds. Antioxidants can react with DPPH and produce 2,2-diphenyl-1-picrylhydrazine [30].



The reducing abilities of the synthesized compounds were determined by their interaction with the free stable radical for 30 min. As from the table 1, it could be seen that the inhibitory activities of the compounds were in the range of 0 – 96.64 % after 30 min of incubation time at room temperature. It is possible to conclude that some samples were able to give a proton to the stable radical DPPH that is the principle of this method for the determination of antioxidant activity. The best percentages of antioxidant activities were observed for the synthetic antioxidant ascorbic acid (97.00% at 1.8 mg/ml) followed by compounds **8** (96.64% at 80 mg/ml), **1** (77.31% at 80 mg/ml), **10** (71.71% at 80 mg/ml), **3** (58.79 at 80 mg/ml) and **4** (49.57% at 80 mg/ml). However, the compounds **2** (34.67% at 80 mg/ml), **7** (29.03% at 40 mg/ml), **9** (15.79% at 40 mg/ml), **11** (6.22% at 80 mg/ml), **13** (3.30% at 40 mg/ml) and **14** (36.02% at 40 mg/ml), when compared to the standard ascorbic acid, showed weak activities. While the compounds **5**, **6** and **12** did not show any activity in the range of concentration 0 – 80 mg/ml. For these compounds, the antioxidant capacity was dependent on the concentrations tested. As shown in table 1, the antioxidant activity of **1-4**, **8**, **10** increased with the increase of their concentrations from 2 to 80 mg/ml. While, the compounds **9**, **13** and **14** showed, at 40 mg/ml, the highest antioxidant capacity (**9**: 15.79 %; **13**: 3.30% and **14** : 36.02%) after 30 min of incubation time. Above this concentration (40 mg/ml), the antioxidant activity of **9**, **13** and **14** decreased. The same phenomenon was observed for the ascorbic acid, that it produced at 1.8 mg/ml its optimum antioxidant effect.

Structure activity relationship study showed that the antioxidant of the compounds **1-4**; **7-10** and **14** could be attributed to electron donating nature of the substituent's like -OH, -CH₃, and -NO₂ on compounds, reduce free radical DPPH. The more hydrogen donors, the stronger is the antioxidant activity. These antioxidants should display antioxidant activity if one or more the groups like -OH, -CH₃ are free, since they are known to be good hydrogen donors [31, 32]. The free radical scavenging activity is usually expressed as percentage of DPPH inhibition but also

by the antioxidant concentration required for 50 % DPPH reduction (IC_{50}). Basically, a higher DPPH radical scavenging activity is associated with a lower IC_{50} value. IC_{50} value was determined from plotted graph of scavenging activity against the different concentrations of compounds. The scavenging activity was expressed by the percentage of DPPH reduction after 30 min of reaction [33, 34]. The obtained results are summarized in table 1. The results show that the ascorbic acid (0.005 mg/ml) was the most potent of all the compounds, followed by compound **3** (15.89 mg/ml), **1** (27.51 mg/ml) and **8** (28.11 mg/ml), while the compound **10** (57.12 mg/ml) exhibits weak activity. When the obtained results were compared, it observed that the $-CH_3$ substituted in compound **1** (77.31%), showed more DPPH scavenging activity in comparison to the $-NO_2$ substituted in compound **3** (58.79%). While the $-Br$ substituted in compound **5** did not exhibit any activity in the range of concentration 0 – 80 mg/ml. When the compound **4** (49.57%) is substituted with $-Br$ and $-CH_3$, exhibited better activity in comparison to substitution with 2 ($-Br$) for compound **6**. Highest DPPH free radical scavenging activity is shown when hydroxyl ($-OH$) and methyl ($-CH_3$) substitution are done (compound **8** (96.64%)). When the phenyl group is substituted with $-OH$ group, the compound **10** (71.71%) exhibited better activity in comparison to substitution with $-COCH_3$ group in compound **9** (15.79%). The substitution with 2 ($-CH_3$) in **1** (77.31%) increases the antioxidant when compared to the compound **14** (36.02%). The substitution with $COCH=CH-$ in compound **12** did not exhibit any activity. In addition, the substitution with $C_6H_5-CH_2-CH_2-$ (compound **11**: 6.22%), and $C_6H_5 - CH_2-$ (compound **13**: 3.3%) showed weak activities.

CONCLUSION

In conclusion, the 14th synthesized compounds, for the in vitro screening four of them (**8**, **1**, **10** and **3**) have potent antioxidant activity. On the basis of observed results, it may be concluded that the substitution with $-OH$, $-CH_3$ and NO_2 groups increases the DPPH free radical scavenging of the compounds, but the substitution with $-Br$, $COCH=CH-$, $C_6H_5 - CH_2-CH_2-$ and $C_6H_5 - CH_2-$ disfavors the scavenging activity. The evaluation of antioxidant activity described in the manuscript has a certain level of novelty for the first evaluation of the activity of the compounds. Additionally, it is the fact that the good antioxidant is valuable especially for pharmaceutical application in the future.

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