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Synthesis, Antibacterial and Antifungal Activities of some new Bipyrazolic Tripodal Derivatives

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Abstract

A series of novel bipyrazolic tripodal derivatives were prepared in one step, in good and excellent yields. The in vitro antibacterial and antifungal activities of these products and their starting materials were screened against two fungal strains (Saccharomyces cerevisiae and Fusarium oxysporum f.sp albedinis) and against bacterial strains (Echerichia coli). Structure-activity relationship (SAR) reflects the effect of substituted drugs. A considerable activity was recorded with respect to the Fusarium oxysporum f.sp albedinis with MIC = 7,05.

Keywords: Bipyrazole; structure–antibacterial activity relationships, antibacterial activity, antifungal activity.

Introduction

The important progression of microbial infections and the recrudescence of resistance towards the antibiotics used nowadays incite the researchers to make more efforts to discover and synthesize new molecules with systemic activity. This activity must be efficient and less toxic for the host cells. In the agricultural environment, the oxysporum Fusarium species is one of the most important fungi organisms in the cultivated soil. It constitutes on its own 40 to 70% of the total Fusaran flora. It is represented by a set of much varying forms regarding morphology and physiology. These forms behave either as saprophytes or parasites of different plants, among these forms there may be various degrees of virulence. The number of pathogenic forms of oxysporum Fusarium is estimated to 80. Some special forms are capable of producing disease in plants belonging to more than one family, such as oxysporum Fusarium f.sp apii which attacks celery and peas, oxysporum Fusarium f.sp vasinfectum which is pathogenic for cotton, tobacco and alfalfa, oxysporum Fusarium f.sp lycopersici which attacks tomatoes, and oxysporum Fusarium f.sp melonis which attacks melon.

The oxysporum Fusarium f.sp albedinis causal agent of vascular fusarium of date palm (Bayoud) constitute with oxysporum Fusarium f.sp cubense causal agent of fusarium of banana trees, the two most serious diseases.

The discovery, development and synthesizing a new efficient, active and less toxic molecule for systemic activities was the aim and subject of many researchers. This is evident from the large number of scientific articles and reviews[1-5].

In this context, pyrazole derivatives are well established in the literature as important biologically active heterocyclic compounds. These derivatives are the subject of many research studies due to their widespread potential biological activities such as anti-inflammatory[6], anti-anxiety[7,8], antipyretic[9], antimicrobial[10], antiviral[11], antitumor[12,13], anticonvulsant[14], antihistaminic[15], antidepressant[16], insecticides[17], and fungicides[17].

On the other hand, it was found that pyrazole-3-carboxylate derivatives have shown potent and selective anti-viral/anti-tumour activity[18]. Also, pyrazole-4-carboxylates act as intermediates for agricultural microbicides and herbicides[19]. Besides it has been observed those pyrazole-4-carboxylates when subjected to in vitro anti-bacterial screening showed activity against some strains of gram-positive bacteria[20].

In our recent work, a series of acyclic and macrocyclic pyrazole compounds containing one, two, three or four pyrazole rings were prepared and demonstrated several applications[21,22].

The present study was carried out to investigate the antibacterial and antifungal inhibitions of several bipyrazole-3-carboxylates which were synthesized in excellent yields. We targeted to study the structure–activity relationship by altering the methyl part and the carboxylate moiety at the 3-position of the pyrazole rings and substitutions at the 2-position of the aniline ring. Herein we report, for the first time, the screening results of the antibacterial and antifungal activities of novel bipyrazolic tripodal derivatives and their starting materials.

Material and Methods

General: All solvents and other chemicals, obtained from usual commercial sources, were of analytical grade and used without further purification. The NMR spectra were obtained with a Bruker AC 300 spectrometer. Elemental analyses were performed by Microanalysis Central Service (CNRS). Molecular weights were determined on a JEOL JMS DX-300 mass spectrometer.

General procedure for the preparation of compounds 5-12: The products **5-12** were prepared by the addition of each: aniline, pyridin-2-amine, 2-nitrobenzenamine and 2-methylbenzenamine to **3** or **4**. To a solution of the substituted hydroxymethylpyrazole **3** or **4** (10 mmol) in acetonitrile (25 ml) was added the amine derivatives (10 mmol) and the mixture was continued under stirring at room temperature for 4-5 days. The crude material was evaporated, washed with water and CH_2Cl_2 and purified by silica gel column flash-chromatography to give the target product.

Synthesis of N, N-bis((3,5-dimethyl-*1H*-pyrazol-1-yl)methyl)-2-methylphenylamine 11: White powder. Yield 90%. Mp 104-107°C. ^{1}H RMN (300MHz, CDC_{l3}) δ ppm: 2,10 (s, 3H, phenyl-CH_3), 2,30 (s, 12H, pyrazol-CH_3), 5,40 (s, 4H, N-CH_2-N), 6,65 (s, 2H, pyrazol), 6,60-7,20 (m, 4H, C_6H_4). ^{13}C RMN (75 MHz, CDC_{l3}) δ ppm: 11,50 (pyrazol-CH_3), 14(-O-CH_2-CH_3), 59,65 (N-CH_2-N), 109 (pyrazol, CH), 114-120-129(CH_2, C_6H_5), 140 (pyrazol, CH-CH_3), 143 (C=N), 145 (C_6H_5, C-N), 162,5 (C=O). Anal. Calcd. for $C_{19}H_{25}N_5$: C 70.56, H 7.79, N 21.65, Found: C 70.29, H 7.83, N 21.55; m/z (M+): 323.44

Synthesis of N, N-bis ((3-carboxyethyl-5-methyl-1H-pyrazol-1-yl)methyl)-2-methyl phenylamine 12: White powder. Yield 88%. Mp 142-144 °C. ^{1}H RMN (300MHz, CDC_{l3}) δ ppm: 1,40 (t, 6H, -O-CH_2-CH_3), 2,10 (s, 3H, phenyl-CH_3), 2,30 (s, 6H, pyrazol-CH_3), 4,40 (q, 4H, -O-CH_2-CH_3), 5,50 (s, 4H, N-CH_2-N), 6,65 (s, 2H, pyrazol, CH), 6,60-7,20 (m, 4H, C_6H_4). ^{13}C RMN (75 MHz, CDC_{l3}) δ ppm: 11,50 (pyrazol-CH_3), 14 (-O-CH_2-CH_3), 59,50 (N-CH_2-N), 61(-O-CH_2-CH_3), 109 (pyrazol, CH), 114-120-129(CH_2, C_6H_5), 140 (pyrazol, CH-CH_3), 143 (C=N), 145 (C_6H_5, C-N), 162,5 (C=O). Anal. Calcd. for $C_{23}H_{29}N_5O_4$: C 62.85, H 6.65, N 15.93, Found: C 62.71, H 6.56, N 16.02; m/z (M+): 439.5

Results and Discussion

Chemistry: The synthesis of target drugs was illustrated in scheme-1. Compounds (3, 5 - dimethy l-1 *H* – pyrazol – 1 -yl) methanol 3 and (3-carboxyethyl-5-methyl -1*H*-pyrazol-1-yl) methanol 4 were already reported by several old and recent works.[23-26] The target bipyrazoles 5-12 were prepared, respectively, by condensation of two equivalents of 3 or 4 with one equivalent of amino aryl derivatives (ii-v) (commercially available) under gentle conditions (room temperature, atmospheric pressure, 4-5 days), using anhydrous acetonitrile as solvent. The reaction is very slow but selective at room temperature.

Recently, we have reported[27] the synthesis and antitumor activity of some compounds 5-10 against three human cancer cell lines including breast (MDA-MB231), prostate (PC3) and colorectal (LoVo) cancers.

The structures of the all newly products 11 and 12 were determined on the basis of the corresponding analytical and spectroscopic data.

Biological Assays: The compounds described in this manuscript **1-12** were tested *in vitro* for their activity against: Fungal strains (*Saccharomyces cerevisiae*) isolated from a yeast strain. Fungal strains (*Fusarium oxysporum f.sp albedinis*) isolated from a date palm having a vascular fusariose.Bacterial strains (*Echerichia coli*).

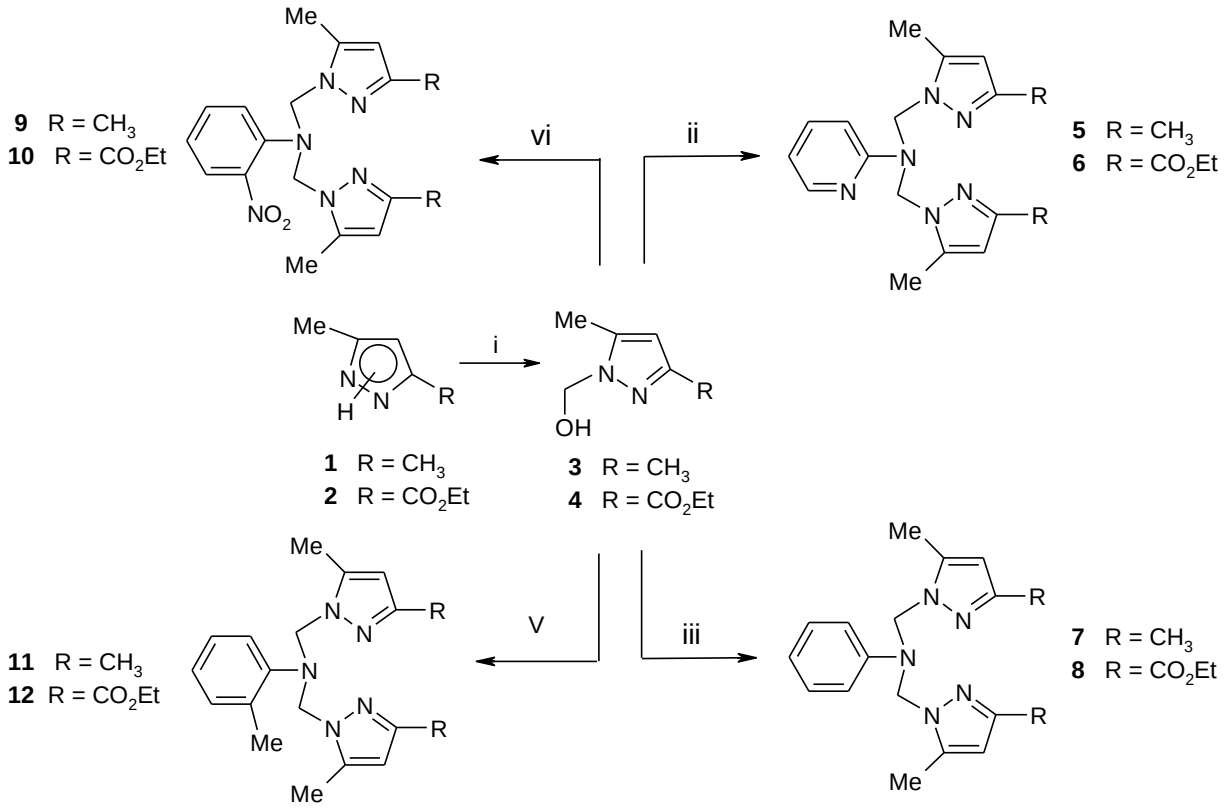
Streptomycin was used as reference compound and better standard in antibacterial assay.

The activities were determined by the agar diffusion technique as previously described[28]. The agar media were inoculated with test organisms and a solution of the tested compound in DMSO/EtOH (50/50) was added to different concentration in the culture media.

The growth is followed by a count of bacteria and yeast colonies and measurement of mycelium diameter. The inhibition percentage of a molecule is equal to the ratio of the colonies number or the mycelium diameter of the culture in presence of a dose of the tested compound over the colonies number or the mycelium diameter of the reference culture multiplied by 100. The minimum inhibition concentration (MIC) is the least dose of the compound which caused inhibition of the micro organism growth.

The most tested compounds show growth inhibitory action for fungus *oxysporum Fusarium* f.sp. *albedinis*. However, they have no effects on bacteria *E. coli* and yeast *Saccharomyces cerevisiae.* The level of inhibition depends on the compound nature and its concentration. We can thus conclude that introduction of different substituent in 3-position of the pyrazole rings and in 2-position of the aniline ring has an impact on the antibacterial activity.

Result in table-1 shows that monopyrazolic derivatives (**1-4**) did not exhibit any interestingly effect against two fungal and bacterial strains examined. However, products containing two pyrazole rings showed differential activity against *oxysporum Fusarium* f.sp. *albedinis*, as evident by the MIC of 7,05 values.



i = Formol 35%, EtOH; ii = Pyridin-2-amine, room temperature for 4-5 days; iii = Aniline, room temperature for 4-5 days; vi = 2-Nitrobenzenamine, room temperature for 4-5 days; v = 2-Methylbenzenamine, room temperature for 4-5 days.

Scheme-1
Structure of synthesized compounds

Table- 1
Rate of inhibition of the growth of *Fusarium Oxysporum* Fsp. *Albedinis* According to the concentration of the compounds tested

Compounds	Concentration (µg/ml)								MIC (µg/ml)	MIC (µM)
	1,25	2,5	5	10	20	40	80	160		
1	0	0	8,67	29,30	48,70	50,34	66,53	73,88	5	52,04
2	0	0	4,33	12,11	20,13	40	69,81	95,66	5	32,45
3	0	0	0	0	0	0	0	0	-	-
4	0	0	0	0	0	0	0	0	-	-
5	0	7,35	14,44	47,89	68,22	76,66	89,12	100	2,5	8,05
6	0	0	8,88	23,56	43,78	61,53	65,02	74,51	5	11,73
7	0	10,66	23,76	43,33	47,55	51,56	86,60	100	2,5	8,08
8	0	0	0	0	0	13,33	16,66	23,5	40	94,07
9	0	4,75	10,25	31,21	52,76	69,41	77,64	93,65	2,5	7,05
10	0	0	9,87	22,90	45,55	52,22	73,44	100	5	10,63
11	0	0	0	0	0	5,57	11,1	12,90	40	123,84
12	0	0	0	0	0	0	5,55	20,51	80	182,14

The bipyrazolic derivatives present two regions for SAR evaluation, the increased potency of compounds **9** and **10** could be attributed most probably to the electron-attracting nitro group in the phenyl region. Replacement of nitro group with an electron-donating methyl group resulted in complete loss of activity (compounds **11-12**). The increased potency of compounds **5** and **6** could be attributed equally to a +π effect of nitrogen in the phenyl region.[29]

In another variation, we alternated the methyl part and the carboxylate moiety at the 3-position of the pyrazole region. As shown in Table 1, compounds: **5** (MIC = 8,05), **7** (MIC = 8,08) and **9** (MIC = 7,05) had considerably better activity than the other compounds with carboxylate moiety; indicating methyl might be a good substitution for modification.

Conclusion

In conclusion, new bipyrazole derivatives drugs were prepared from easily accessible starting materials in few steps. The preliminary *in vitro* test results of these compounds against the three studied micro-organisms such as *Saccharomyces cerevisiae*, *Echerichia coli* and *Fusarium oxysporum f.sp albedinis* show a significant activity toward a causal agent of vascular fusarium of date palm (Bayoud).

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