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Antibacterial and antifungal activities of new pyrazolic compounds

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Abstract

Conventional antimicrobials become increasingly ineffective against multidrug resistant pathogens. Nowadays, the search for new bioactive molecules is of paramount importance. Pyrazoles are a new class of bioactive molecules that can enhance the therapeutic arsenal in the coming years. Fifteen compounds based on pyrazole and primary amines were tested in vitro against one fungal strain, namely *Fusarium oxysporum f.sp.albedinis* and three bacterial strains, namely *Bacillus subtilis, Micrococcus luteus* and *Escherichia coli*. The results showed that some compounds containing (-OH) group and phenyl moiety have a considerable effect against tested bacteria and some compounds containing the (-Br) group have higher activity against tested fungus. The obtained results prove that pyrazolic compounds have an antimicrobial potential and inspired us to conduct more researches in order to use them in therapeutics.

Keywords: Pyrazole, Antimicrobial activity, Antibacterial, Antifungal, Bioactive compounds, Synthetic molecules.

Introduction

The emergence of multidrug resistant pathogens is a great challenge for the scientific and medical community (Thumar & Patel, 2011). Thus, the search for new bioactive molecules is of paramount importance to protect human health (Bayrak *et al.,* 2009).

Pyrazole derivatives are heterocyclic bioactive compounds known to exhibit diverse pharmacological activities (Goel *et al.,* 2014).

The aim of this work was to test fifteen new pyrazolic compounds which were synthesized and published previously (Abrigach *et al.,* 2014; Khoutoul *et al.,* 2013; El Kodadi *et al.,* 2004). Their activities in vitro such as antibacterial and antifungal were evaluated against one fungal strain (*Fusarium oxysporum f.sp. albidinis*) and three bacterial strains (*Bacillus subtilis, Micrococcus luteus* and *Escherichia coli*).

Materials and methods

Tested compounds are those described by (Abrigach *et al.,* 2014; Khoutoul *et al.,* 2013; El Kodadi *et al.,* 2004). Their chemical structures are presented in Scheme 1. All culture media used in these tests are obtained from Biokard and the Dimethyl-sulfoxyde (DMSO) from Sigma. From each product, 40 mg was solubilized in 1 ml of DMSO then diluted in 9 ml of distilled water and filtered though a 0.45 µm filter. Optical density of bacterial cultures was measured by JASCO UV/VIS Spectrophotometer 7800. Each test was repeated three times and the mean values were used.

Antibacterial activity test

Culture characteristics of the bacterial strains used for testing antibacterial activity are presented in Table 1.

The used test was that recommended by the NCCLS (National Committee for Clinical Laboratory

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Standards) and described by the AFNOR 2004: NF-U - 47-107. A Petri plate containing Muller-Hinton agar was seeded by 5 ml of bacterial test inoculum at 10^6 CFU.ml^{-1}. After bacterial attachment, the excess of bacterial solution was removed and the WATTMAN paper disks (6 mm) are placed and soaked in the different tested compounds. The disk charges used in this test were 40 µg and 80 µg for each compound. After 24 hours on incubation, diameter of inhibition zones around each disc was measured.

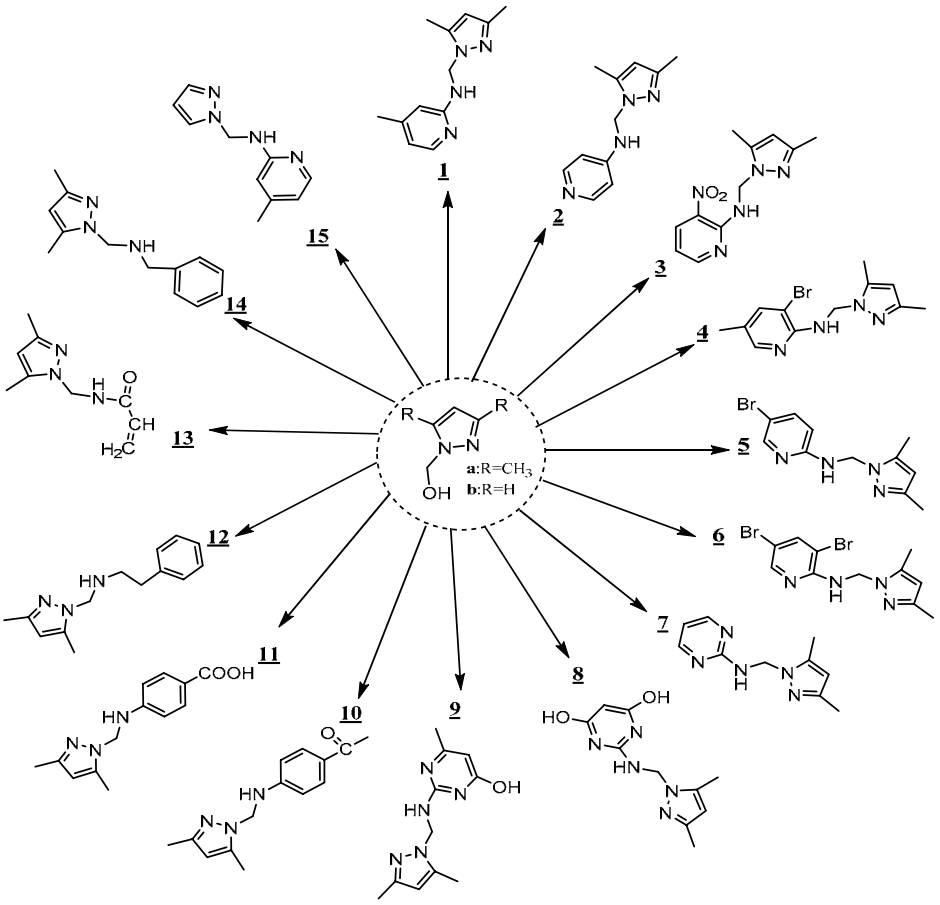
Table 1. Bacterial strains used in antibacterial tests.

Strain	Form	Gram	Culture temperature (°C)
Bacillus subtilis	Bacillus	(+)	30
Micrococcus luteus	Coccus	(+)	37
E. coli	Bacillus	(-)	37

The inhibitory concentration 50 (IC_{50}) was determined using the same bacterial strains in Muller-Hinton broth with decreasing concentrations of the tested products. Optical Density (OD) was measured of each culture at 625 nm after 6 hours of incubation. IC_{50} corresponds to the concentration of tested compound which inhibits 50% of bacterial growth. Gentamicin (1 mg.ml^{-1}) was used as a positive control and distilled water as a negative control.

Antifungal activity test

It was made by agar diffusion technique using the *Fusarium oxysporum f.sp.albedinis.* Each compound was added to the



Scheme 1. Structures of the fifteen tested compounds.

potato dextrose agar (PDA) at different concentrations before culturing the fungus (Boussalah *et al.,* 2013). Fungal cultures were incubated at 28 °C for 7 days. The inhibition percentage of each compound was calculated by the ratio of the mycelium diameter observed when using it over that observed in the negative control. IC_{50} was determined by the linear regression equation between the natural logarithm of the concentrations and the growth inhibition percentages. DMSO was used as a positive control and distilled water as a negative control.

Results and Discussion

Antibacterial activity

For each strain and each tested compound, the diameter of inhibition (DI) and IC_{50} was measured (Table 2). Two different disc charges were used to determine DI: 40 µg and 80 µg. The results reveal that most of the tested compounds showed antibacterial activity with varying levels. However, some are more effective especially: (i) *Bacillus subtilis*: compounds (**12**-**14** and **15**) had an $IC_{50} \leq 1$ mg.ml^{-1} and a $DI_{80} \geq 18$ mm; (ii) *Micrococcus luteus*: compounds (**8**-**9** and **12**) had an $IC_{50} \leq 1$ mg.ml^{-1} and a $DI_{80} \geq 18$ mm; and (iii) *E. coli*: compounds (**2**-**7**-**13**-**14** and **15**) had an $IC_{50} \leq 1$ mg.ml^{-1} and a $DI_{80} \geq 18$ mm.

We specially note that compound **12** had an excellent activity on gram positive tested bacteria (*Bacillus subtilis* and *Micrococcus luteus)* with a $DI_{40} \geq$ 18mm. however, all of the tested compounds have no remarkable effect at 40 µg on *E.coli.*

Table 2. The diameter of inhibition and the IC_{50} of fifteen purazolic compounds tested on three bacterial strains.

Compounds	*Bacillus subtilis*			*Micrococcus luteus*			*Escherichia coli*		
	DI_{40}	DI_{80}	IC_{50} (mg.ml^{-1})	DI_{40}	DI_{80}	IC_{50} (mg.ml^{-1})	DI_{40}	DI_{80}	IC_{50} (mg.ml^{-1})
1	11	17	1,12	8	12	3,80	-	8	4,32
2	13	18	1,89	15	19	1,03	11	23	0,90
3	10	16	2,22	8	13	3,42	-	10	4,54
4	12	16	2,65	10	14	-	-	10	-
5	-	12	4,81	9	13	3,41	-	10	-
6	12	17	1,90	-	-	-	-	-	-
7	-	-	-	14	18	1,12	16	28	0,63
8	-	-	-	18	21	0,62	13	17	1,75
9	-	-	-	17	22	0,98	16	19	1,12
10	-	-	-	13	18	2,45	-	13	4,21
11	-	-	-	8	17	4,22	-	11	3,25
12	18	21	0,91	20	33	0,32	-	15	3,45
13	-	-	-	10	13	2,41	12	22	0,82
14	13	18	0,91	12	16	1,91	10	20	0,54
15	12	18	0,83	-	-	-	13	25	0,54
(-) Control	-	-	-	-	-	-	-	-	-
(+)Control	20*	22**	0.04	20*	22**	0.03	20*	22*	0.05

DI_{40} & DI_{80}, Diameter of inhibition in (mm) using 40 µg and 80 µg of compounds in the same order; (-) Control, Distilled water; (+) Control, Gentamycin 1 mg.ml^{-1} used at * 10 µl and ** 20 µl.

Antifungal activity

Most of tested compounds presented an antifungal activity against using fungi. The compounds **4**-**5** and **6** were the most efficient with an $IC_{50} \leq 0,12$ mg.ml^{-1}. The other compounds showed moderate to poor antifungal activities (Table 3).

Antimicrobial activity levels of these compounds may be affected by some minor changes in their molecular substitutes. Thus, the incorporation of -OH group in (**8**-**9**) can explain high activity against *Micrococcus luteus.* The phenyl moiety in (**12**-**14**) can explain their high activity against *Bacillus subtilis*. Finally, high activity of (**4**-**5**-**6**) against *Fusarium*

oxysporum f.sp.albedinis may be due to the presence of the (–Br) group.

Many works proved that certain pyrazole derivatives have a wide spectrum of biological and pharmacological activities such as antifungal (Radi *et al,.* 2012), anti-inflammatory (Tewari & Mishra 2001), anti-anxiety (Wustrow *et al.,* 1998) antipyretic (Wiley & Wiley, 1964), antimicrobial (Pimerova & Voronina, 2001), antiviral (Janus *et al.,* 1999), antitumor (Bouabdallah *et al.,* 2006), anticonvulsant (Michon *et al.,* 1995), antihistaminic (Yildirim *et al.,* 2005) antidepressant (Bailey *et al.,* 1985) and insecticides (Chu & Cutler, 1986).

Table 3. Growth inhibition rates (%) of the *Fusarium oxysporum f.sp.albedinis* at different concentrations of fifteen pyrazolic compounds.

Compounds	Concentration (µg.mL^{-1})					IC_{50} (mg.mL^{-1})
	40	80	160	320	640	
1	0	0	0	0	0	-
2	0	0	54	54	64	0,15
3	0	0	0	0	60	0,62
4	0	4	74	100	100	0,12
5	14	36	84	100	100	0,11
6	0	32	60	100	100	0,12
7	0	0	0	24	80	0,56
8	0	0	0	4	64	0,60
9	0	0	0	28	84	0,58
10	0	0	0	44	52	0,63
11	0	0	30	54	62	0,30
12	20	30	50	60	70	0,16
13	0	0	0	24	72	0,51
14	0	0	0	0	70	0,50
15	0	0	0	24	76	0,49
(-) Control	0	0	0	0	0	-
(+)Control	0	0	0	0	0	-

(-) Control, Distilled water; (+) Control, Dimethyl-sulfoxyde

Conclusion

The in vitro antimicrobial activities of fifteen pyrazolic compounds were evaluated against one fungal strain (*Fusarium oxysporum f.sp.albedinis*) and three bacterial strains (*Bacillus subtilus, Micrococcus luteus* and *Escherichia coli*). The preliminary results showed that some compounds containing -OH group or phenyl moiety (**8**-**9**-**12** and **14**) had a significant antibacterial activity against tested bacteria. Some compounds containing -Br group (**4**-**5** and **6**) had a significant antifungal activity against tested fungus. However, the other compounds gave moderate to poor potency. Further investigations should be conducted to better understand their mechanisms of action and improve their efficiency. Incorporation of phenyl moiety, –OH and -Br groups may be more explored.

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