Bull. Chem. Soc. Ethiop. **2014**, 28(1), 131-136.
Printed in Ethiopia
DOI: http://dx.doi.org/10.4314/bcse.v28i1.15
ISSN 1011-3924

SHORT COMMUNICATION

EVALUATION OF ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF MONOESTERS OF SUCCINIC ANHYDRIDE[a]

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(Received September 20, 2012; revised October 9, 2013)

ABSTRACT. Monoester of succinic acid (**1-29**), synthesised and characterised at our laboratory, were investigated with reference to their antifungal and antibacterial activities. The results concluded that though almost all the compounds were bioactive but the degree of activity was dependent over the substituent attached to benzyl group and order of their bioactivity was iodo > chloro > methoxy > nitro substituted monoesters against the considered microbes.

KEY WORDS: Succinic anhydride, Aryl alcohols, Monoesters of succinic acid, Antifungal and antibacterial activity

INTRODUCTION

Application of synthetic compounds as a substitute for natural medicines for the treatment of ailment has brought a revolution in synthetic chemistry. At present evaluation of synthetic compounds for their antifungal and antibacterial activity is the subject of active chemistry research [1-3]. In spite of the fact diseases caused by microbes are increasing with the passage of time, the microbes have become resistant to the available drugs and hence posing a great threat to human being but no considerable antifungal and antibacterial drugs have been synthesised/discovered from the last two decades. Therefore, it is need of the day to synthesise new antifungal/antibacterial compounds which may ultimately be used as drugs. Literature revealed that natural and synthetic esters of succinic acid had a wide range of applications like industrial, agrochemical and pharmaceutical [4-7]. Some of these are the treatment of HIV, tumours, as antiseptic agents, antioxidants, as enzyme inhibition, resolving the racemic mixtures and in the synthesis of bioactive compounds [8-16].

Therefore, quite a good number of scientists are involved in the synthesis of biologically active compounds and characterise them with reference to their antimicrobial properties [18-20]. In the last report the monoesters of succinic acid (**1-29**) were synthesised and characterised [19] whereas, in the present study the biological activities of these compounds (**1-29**) against various microbes are reported. All the reported compounds were bioactive, however, only the halogenated esters **11-20** displayed activity equivalent to standard drugs chloramphenicol and ketoconazole.

EXPERIMENTAL

General procedure for the preparation of **1-29**. The aryl hydrogen succinates (**1-29**) were synthesized and characterised by following the standard protocol [19]. Briefly, 15 mmol of corresponding alcohol was added to succinic anhydride (15 mmol), anhydrous *p*-toluenesulfonic acid (0.06 mmol) and toluene (15 mL) under the atmosphere of nitrogen in a single-necked round-bottom flask (100 mL). The flask was equipped with magnetic stirrer, Dean–Stark trap and a reflux condenser. The solution was refluxed for 14 h and allowed to cool up to 25 ^{o}C. The

[a]This paper is dedicated to Prof. Dr. G.A. Miana at his 75th birth day.
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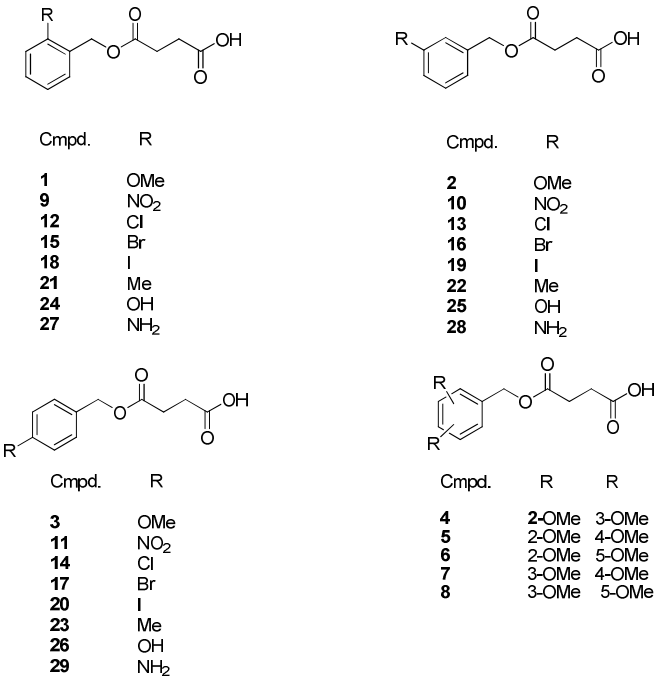
product was then poured into saturated aqueous solution of $NaHCO_3$ (12.5 mL) and the organic layer was extracted with hexane (3 × 25 mL). The organic phase was then washed with brine (10 mL), dried over anhydrous Na_2SO_4 and the excess of the solvent was removed under vacuum to give a resinous product. It was then subjected to column chromatography to get pure monoesters (**1-29**). The target substrates were characterized by UV, IR, ^{1}H-NMR and ^{13}C-NMR and mass measurement. The structure of prepared monoesters (**1-29**) so obtained is presented in Scheme 1.



Scheme 1. Structures of monoeasters **1-29**.

Antifungal and antibacterial activities. Antifungal and antibacterial activities of synthesised compounds (**1-29**) were evaluated against *Colletotrichum gloeosporioides*, *Alternaria brassicicola*, *Colletotrichum capsici* supplied from Laboratório de Antibióticos, Universidade Federal de Santa Catarina and *Klebsiella pneumonia NCTC 11228*, *Escherichia coli ATCC 25922*, *Staphylococcus aureus ATCC 25923*, respectively. Ketoconazole purchased from m/s SMS Pharmaceuticals Ltd. Hyderabad (Pakistan) and chloramphenicol from Sigma St. Louis (USA) was used as standard.

Antifungal activity of monoesters. The antifungal activities of the monoesters (**1-29**) were determined by employing hanging drop method considering ketoconazole as standard [21]. Briefly, 500 µg/mL solution of the compounds was employed on the germinating fungal spores. The plates were incubated at 37 ^{o}C for 20 h and the antifungal activity was determined by measuring the diameter of the inhibition zone in mm (Figure 1). The percentage inhibition of spore germination was calculated by observing the germination of the spores under microscope after 8 hours of incubation at 30 ^{o}C using Equation 1.

$$\text{Percent inhibition of spores germination} = \frac{\text{Total No. of germinated spores}}{\text{Total No. of spores}} \times 100 \quad (1)$$

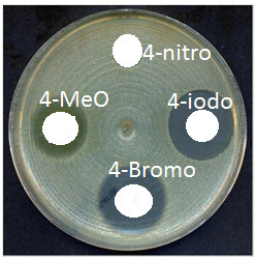


Figure 1. Antifungal activity in terms of zone of inhibition of 4-iodobenzyl hydrogen succinate (**20**), 4-bromobenzyl hydrogen succinate (**17**), 4-methoxybenzyl hydrogen succinate (**3**) and 4-nitrobenzyl hydrogen succinate (**11**) against *A. brassicicola*.

Antibacterial activity of monoesters. The antibacterial activity of the monoesters (**1-29)** was determined by following the agar well diffusion method [22] using chloramphenicol as standard. Briefly, wells were dug in the media using a sterile borer. Using a sterile cotton swab, the surface of the agar nutrient was covered with eight-hour bacterial inoculum containing 10^4-10^6 colony forming units (CFU/mL). Monoesters (6-16 mg in DMSO 1 mL) were placed in the wells. Pure DMSO (1 mL) and chloramphenicol (6 mg/mL DMSO) were introduced into two other wells for negative and positive controls, respectively. The plates were incubated immediately at 37 ^{o}C for 20 h. The activity was determined by measuring the diameter of the inhibition zone (in mm). Growth inhibition zone was calculated with reference to the positive control.

Minimum inhibitory concentration (MIC) of monoesters. The minimum inhibitory concentration (MIC) was determined by agar dilution method [22]. Twenty-five mL of the sterilized Mueller-Hinton agar (Oxoid) was added to sterilized test tube containing 1 mL of 6-16 µg/mL of monoesters at 25 ^{o}C. The mixture was then thoroughly mixed and poured into sterilized petri plates. The microbial suspension with density adjusted to 0.5 McFarland turbidity standard was inoculated (0.05 µL) on to the series of agar plates using micropipette. The plates were then incubated at 37 ^{o}C for 24 h and MIC values were calculated.

RESULTS AND DISCUSSION

All of the monoesters (**1-29**, Scheme 1) were subjected to biological activities using Ketoconazole and Chloramphenicol as controls. Antifungal/antibacterial activity was probed against *Colletotrichum gloeosporioides*, *Alternaria brassicicola*, *Colletotrichum capsici*, *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus. In vitro* results of this study are presented in Tables 1 and 2. The obtained results showed that all the compounds exhibited considerable antifungal/antibacterial activity against used microbes (Table 2). All the microbes were found to be sensitive towards compound **20** displaying MIC values 2.12-2.32 µg/mL. The study also showed that halogenated esters (**12-19**) displayed activity against all the microbes and MICs value ranged 2.14-2.96 µg/mL. While compounds **21-23** were less sensitive towards all the investigated microbes and MICs values ranged from 3.11-4.41 µg/mL and **4-7** compounds were moderately sensitive showing MICs values 4.78-7.78 µg/mL, whereas, compounds **9-11**

displayed no activity (up to 50 μg/mL) against these microbes. *In vitro* results showed that all the microbes showed sensitivity towards compound **20** displaying zone of inhibition in case of *C. gloeosporioides* (22.1 mm), *A. brassicicola* (mm 23.13), *C. capsici* (23.12 mm), *K. pneumonia* (22.12 mm), *E. coli* (22.13 mm) and *S. aureus* (20.12 mm) and were approximately equivalent to standards chloramphenicol (27 mm) and ketoconazole (25 mm).

Zone of inhibition of the halogenated ester **12** against used microbes was in the range of 12.35-17.37 mm. **13** (9.39-14.38 mm), **14** (12.38-16.32 mm), **15** (12.38-16.32 mm), **16** (11.44-16.28 mm), **17** (11.35-13.33 mm), **18** (8.43-21.88 mm) and **19** (14.43-18.50 mm). For rest of the compounds (**1-8**, **21-29**) microbes were resistant and showed very small zone of inhibition (Table 1), whereas compounds **9-11** were almost inactive.

Table 1. Inhibition zones of monoesters **1-29** against fungi and bacteria.

Org /compd	Antifungal activity zone of inhibition (mm)			Antibacterial activity zone of inhibition (mm)		
	C. gloeosporioides	*A. brassicicola*	*C. capsici*	*K. pneumonia*	*E. coli*	*S. Aureus*
1	11.72	12.43	13.47	13.14	12.10	13.74
2	9.96	11.24	10.19	11.11	10.71	10.54
3	13.24	15.55	13.22	13.66	15.82	13.11
4	9.66	8.64	9.45	11.22	10. 55	10.56
5	8.98	9.43	8.45	9.81	10.17	10.66
6	10.50	9.24	6.86	9.29	7.83	8.77
7	8.66	8.89	6.74	7.77	8.37	9.66
8	8.44	9.33	6.72	10.75	9.71	9.77
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	14.33	16.34	17.37	12.35	13.53	14.36
13	12.35	14.38	13.39	11.37	9.39	10.38
14	14.33	15.36	16.36	13.33	12.38	15.36
15	13.27	14.31	16.28	12.27	11.44	13.28
16	12.39	11.35	12.36	11.65	13.32	13.33
17	21.88	21.88	19.28	9.32	11.31	8.43
18	22.15	22.11	23.11	16.19	17.18	16.23
19	16.45	18.50	16.15	14.43	15.29	16.25
20	22.21	23.13	23.12	22.12	22.13	20.12
21	12.25	12.34	11.22	11.34	12.31	11.45
22	12.71	11.23	10.23	9.23	8.65	10.33
23	11.36	12.24	10.35	9.53	11.32	10.34
24	12.68	12.64	12.09	12.66	12.26	12.76
25	12.72	12.17	12.47	12.11	12.70	12.73
26	12.66	12.14	12.36	12.56	12.67	12.64
27	12.48	12.54	12.58	12.33	12.53	12.59
28	12.64	12.63	12.56	12.36	12.61	12.64
29	12.24	12.54	12.48	12.56	12.54	12.49
Chloram-phenicol	26.67	25.11	26.15	-	-	-
Ketocon-azole	-	-	-	25.25	24.13	23.56

Table 2. MICs of monoesters **1-29** against fungi and bacteria.

Org /compd	Antifungal activity MIC (µg/mL)			Antibacterial activity MIC (µg/mL)		
	C. gloeosporioides	*A. brassicicola*	*C. capsici*	*K. pneumonia*	*E. coli*	*S. Aureus*
1	4.7 0	4.21	4.71	4.73	4.26	4.75
2	5.2 9	5.31	5.61	5.51	5.76	5.41
3	5.61	4.49	4.51	4.7 1	3.31	3.61
4	4.89	7.58	6.89	6.81	6.41	5.81
5	5.58	6.19	7.68	7.59	7.14	6.68
6	4.81	6.39	6.78	6.89	6.37	7.78
7	4.78	6.18	6.87	7.77	7.38	6.63
8	6.68	6.29	6.71	6.61	7.29	7.72
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	2.31	2.41	2.71	2.51	2.54	2.65
13	2.51	2.81	2.91	2.71	2.9 1	2.86
14	2. 31	2.61	2.61	2. 31	2.80	2.64
15	2.71	2.14	2.86	2.71	2.43	2.80
16	2.91	2.51	2.65	2.81	2.26	2.36
17	2.92	2.88	2.87	2.29	2.12	2.48
18	2.45	2. 50	2.55	2.21	2.91	2.41
19	2.95	2.97	2.94	2.96	2.88	2.78
20	2.12	2.13	2.12	2.22	2.32	2.22
21	3.25	3.40	3.71	3.40	3.11	4.51
22	3.30	3.21	3.41	3.29	3.08	3.31
23	3.63	3.31	3.51	3.56	3.29	3.46
24	6.86	6.41	6.91	6.62	6.21	6.71
25	7.28	7.11	7.41	7.10	7.01	7.32
26	5.64	5.11	6.31	6.53	6.78	6.48
27	4.88	5.41	5.81	5.71	5.32	5.68
28	6.41	6.31	6.51	6.36	6.12	6.51
29	4.91	5.41	4.81	5.61	5.21	4.90
Chloram-phenicol	1.22	1.12	1.12	-	-	-
Ketocon-azole	-	-	-	1.15	1.32	1.22

The prepared compounds showed interesting structure activity relationships while exploring their antifungal and antibacterial activity. Some interesting trends that were noticed included low activities of the compounds having substituents linked through oxygen and having substituent at three position of benzene ring (Tables 1 and 2). Relatively high activity was observed for compounds with substituent at 2 and 4 position of benzene ring. Highest activity was revealed by halogenated monoesters (**12-20**) in general and iodinated monoesters in particular (**18-20**). Monoesters having substituents linked through oxygen to benzene ring (**1-8**) and (**24-29**) displayed relatively less activity as compared to halogenated monoesters. This could be explained on the basis that the presence of halogens could be responsible for enhanced activity. Also substituents like methyl, methoxy and hydroxyl, having +M effect that increased electronic density on the benzene ring, decreased the activity of the compounds (Tables 1 and 2). As is evident from Tables 1 and 2, iodosubstituted monoesters exhibited values close to ketoconazole and chloramphenicol standards. Therefore, the compounds are potential sources as

antibacterial and antifungal agents and can find use in biomedical area in near future. Moreover, *meta*- substituted isomeric monoesters showed lower activities than their *ortho*- and *para*-analogues (Tables 1 and 2).

CONCLUSION

The prepared compounds except **9-11**were found to be noticeably bioactive. The highest activity was observed for iodinated monoesters. It can be concluded that the compounds may be candidates for antifungal and antibacterial drugs. It is recommended that *in vivo* studies of these compounds may be carried out and their mode of action against these microbes be explored.

ACKNOWLEDGEMENTS

We acknowledge Mr Adnan Amin and Professor Dr Muhammad Ayaz Khan, Director Biotechnology and Biochemistry Centre, Gomal University Dera Ismail Khan, KPK, Pakistan for antifungal and antibacterial studies.

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