Asian Journal of Chemistry; Vol. 26, No. 23 (2014), 8025-8028

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16959

Antimicrobial Activities of Monoesters of Succinic Acid

MUHAMMAD IQBAL[1,*], RUBINA IQBAL[1], MUHAMMAD BILAL[2], MUHAMMAD AKRAM[3], IMAM BAKHSH BALOCH[1] and MUSA KALEEM BALOCH[1]

[1]Department of Chemistry, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan
[2]Gomal College of Veterinary Sciences, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan
[3]Medicinal Botanic Centre, PCSIR Laboratories Complex, Peshawar, Khyber Pakhtunkhwa, Pakistan

*Corresponding author: Fax: +92 966 750255; Tel: +92 966 750424-429; E-mail: miqbal_44@yahoo.com

Received: 30 December 2013; *Accepted*: 10 April 2014; *Published online*: 15 November 2014; AJC-16287

Present study describes the antimicrobial activities of the di- and tri-substituted benzyl monoesters (**1-27**) derived from succinic anhydride. The monoesters were screened against two fungi (*C. albicans* and *A. niger*) and two bacteria (*E. coli*, *P. mirabilis*) strains. All the compounds displayed bioactivity against screened microbes. However, the activities were dependent on the nature of the substituents. Zones of inhibition and minimum inhibitory concentrations (MICs) of the monoesters are reported. Chloramphenicol and ketoconazole were used as standard antibacterial and antifungal drugs respectively.

Keywords: Succinic anhydride, Benzyl alcohols, Monoesters of succinic acid.

INTRODUCTION

Fungi and bacteria are a great threat to all living organisms by causing illnesses in them. Rate of spreading diseases by them is higher in contrast to synthesis of drugs that are antifungal and/or antibacterial[1-4]. At present substantial part of the research in chemistry is devoted to the synthesis and characterization of biologically active compounds that would be used against the microbes[5-16]. Monoesters of succinic acid, in addition to their chemical uses, possess medicinal properties like, antiHIV, antitumor, antiseptic, antioxidant, antifungal and antibacterial[10-16] activities. In continuation to our work to establish medicinal use of monoesters[17-20] twenty-seven (**1-27**) new monoesters were prepared from succinic anhydride[7] and explored for antifungal and antibacterial activity. All the reported compounds displayed considerable bioactivities. However, the halogenated esters displayed activity equivalent to standard drugs chloramphenicol and ketoconazole.

EXPERIMENTAL

The microbes used for bioactivities of synthesized compounds (**1-27**) were *Candida albicans* ATCC 10231 (*C. albicans*), *Aspergillus niger* F2723 (*A. niger*), *Escherichia coli* MTCC-739 (*E. coli*) and *Proteus mirabilis* ATCC 14153 (*P. mirabilis*). Ketoconazole purchased from M/s SMS Pharmaceuticals Ltd. Hyderabad and chloramphenicol from Sigma St. Louis, USA was used as standard.

General procedure for the preparation of monoesters 1-27: Twenty-seven (**1-27**) aryl succinic acids were synthesized according to by following a known procedure[7]. Briefly, by adding 20 mmol of corresponding alcohol into a single necked round-bottom flask (100 mL), already containing succinic anhydride (20 mmol), anhydrous *p*-toluene sulfonic acid (0.08 mmol) and toluene (20 mL) under nitrogen atmosphere. The apparatus was equipped with magnetic stirrer, Dean-Stark trap and a reflux condenser. The mixture was refluxed for variable times and allowed to cool up to 25 °C. After cooling, it was poured into saturated aqueous $NaHCO_3$ solution (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 products. The obtained mixture was subjected to separation on column chromatography using mixture of *n*-hexane-ethyl acetate (1:0 → 0:1) to get thirty fractions (1-30). The fractions (17-22) were combined and re-chromatographed on preparative thin layer chromatography using *n*-hexane-ethyl acetate mixture (4:6) as an eluent which yielded colourless amorphous solid pure compounds (**1-27**). Recording UV, IR, ^{1}H NMR and ^{13}C-NMR, analysis and mass measurement, characterized the target substrates[7].

Antifungal activity of monoesters: The disk diffusion method was employed to evaluate antifungal activities of the compounds (**1-27**) against ketoconazole as standard[21]. Briefly, onto the plates with germinating fungal spores were placed filter papers disk soaked with solution (500 ppm) of the

monoesters and incubated at 28 °C for 22 h. and the antifungal activity was determined by measuring the diameter of the inhibition zone in mm.

Antibacterial activity of monoesters: Agar well diffusion method using chloramphenicol as standard was used to establish antibacterial activity of the monoesters[22]. Briefly, wells were dug in the pre-coated agar nutrients media plates with the help a sterile borer. Surface of the agar nutrient was covered with eight-hour bacterial inoculum containing 104-106 colony forming units (CFU/mL). To each well a 1 mL of DMSO solution of monoesters (2-10 mg in DMSO 1 mL) was placed. Two wells were reserved for negative and positive controls. To the well for negative control 1 mL pure DMSO and to the well for positive control 2 µg of chloramphenicol/mL DMSO of were introduced. The plates were incubated immediately at 37 °C for 22 h. The activity was determined by measuring the diameter of the inhibition zone (in mm).

Minimum inhibitory concentrations (MICs) of monoesters (1-27): Minimum inhibitory concentrations (MICs) were determined by agar dilution method[22]. Briefly, 25 mL of the sterilized Mueller-Hinton agar (Oxoid) was added to sterilized test tube containing 1 mL of 2-10 µg/mL of monoesters at 25 °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 °C for 24 h and MIC values were calculated.

RESULTS AND DISCUSSION

Scheme-I listed the structures of antimicrobial monoesters of succinic acid. All of the di- and tri-substituted benzyl esters (**1-27**) were probed for antimicrobial activities using Ketoconazole and Chloramphenicol as standards. *C. albicans* and *A. niger* were employed for antifungal while, *E. coli* and *P. mirabilis* for antibacterial activity. In vitro results of this study are presented in Tables-1 and 2. The obtained results showed that all the compounds exhibited considerable antimicrobial activity against used microbes (Tables 1 and 2). Antimicrobial activities in terms of zones of inhibitions of monoesters **1-27** are provided in Table-1. Zones of inhibition displayed by **25** are *C. albicans* (25.72 mm), *A. niger* (24.17 mm), *E. coli* (26.70 mm) and *P. mirabilis* (26.73 mm) while for **26** the values are *C. albicans* (24.66 mm), *A. niger* (25.14 mm), *E. coli* (26.67 mm) and *P. mirabilis* (26.64 mm). These values are close to values exhibited by standard drugs chloramphenicol and ketoconazole (Table-1). As is evident from values for zones of inhibition for **1-27**, the halogenated compounds **1-10**, **25** and **26** exhibited maximum inhibition zones against microbes. Compounds **11-14** and **27** displayed lower inhibition zones (9-14 mm) as compared with **1-10**, **25** and **26** (Table-1).

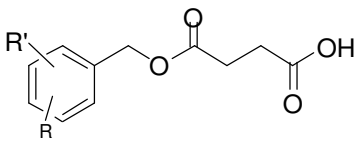
Conclusion

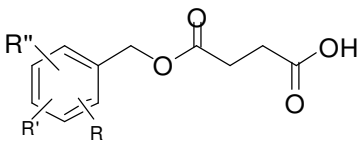
The prepared compounds except **1-10**, **25** and **26** were found to be noticeably bioactive. The highest activity was observed for tri-halogenated derivatives. It can be concluded that the compounds may be used as candidates for antifungal and antibacterial drugs. It is recommended that *in vivo* studies of these compounds may be carried out and their mode of action against these microbes be explored.

in vitro MICs values of monoesters (**1-27**) are presented in Table-2. Dichlorobenzyl derivatives displayed MICs values lower than 3 µg/mL while fluorinated monoesters displayed somewhat higher values (Table-2). Monoesters **9-24** and **27** displayed MICs values between 9 and 11 µg/mL. Compounds **25** and **26** showed as low MICs values as standard drugs (Table-2). As is evident from MICs values in Table-2, monoester **25** exhibited lowest MIC value of 1.67 µg/mL against bacterium *E. coli* while **26** displayed against *P. mirabilis* an MIC value of 1.68 µg/mL. Furthermore, **25** displayed lowest MICs value of 1.71 and **26** 1.73 µg/mL against fungal strains *A. niger* and *C. albicans*, respectively.

ACKNOWLEDGEMENTS

The authors acknowledge Mr. Adnan Amin and Prof. Dr. Muhammad Ayaz Khan, Director Biotechnology and Biochemistry Centre, Gomal University Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan for antifungal and antibacterial studies.





1.	R=R'=2,4-Dichloro	**2.**	R=R'=2,5-Dichloro	**3.**	R=R'=2,6-Dichloro	**4.**	R=R'=2,6-Dichloro
		5.	R=R'=3,5-Dichloro	**6.**	R=R'=2,3-Difluoro	**7.**	R=R'=2,4-Difluoro
8.	R=R'=2,5-Difluoro	**9.**	R=R'=2,6-Difluoro	**10.**	R=R'=2,4-Dimethyl	**11.**	R=R'=2,5-Dimethyl
12.	R=R'=3,4-Dimethyl	**13.**	R=R'=3,5-Dimethyl	**14.**	R=R'=3,4-Dinitro	**15.**	R=R'=3,5-Dinitro;R"=H
16.	R=R'=2,6-Diethoxy;R"=H	**17.**	R=R'=3,4-Diethoxy;R"=H	**18.**	R=R'=3,5-Dihydroxy;R"=H	**19.**	R=R'=R"=2,3,4-Trimethoxy
20.	R=R'=R"=2,4,5-Trimethoxy	**21.**	R=R'=R"=3,4,5Ttrimethoxy	**22.**	R=3,Methoxy; R'=4-nitro;R"=H	**23.**	R=4-Methoxy; R'=3-nitro;R"=H
24.	R=2-Methoxy; R'=5-nitro;R"=H	**25.**	R=R'=R"=2,4,6-Trichloro	**26.**	R=R'=R"=2,4,5-Tribromo	**27.**	R=R'=R"=2,4,6-Trimethyl

Scheme-I: Structures of antimicrobial monoesters

TABLE-1
INHIBITION ZONES OF **1-27**

Org (cmpd)	Antifungal activity zone of inhibition (mm)		Antibacterial activity zone of inhibition (mm)	
	C. albicans	*A. niger*	*E. coli*	*P. mirabilis*
1	21.83 ± 0.35	22.54 ± 0.45	22.21 ± 0.55	23.85 ± 0.38
2	22.07 ± 0.28	22.35 ± 0.49	21.82 ± 0.24	21.65 ± 0.22
3	23.35 ± 0.77	23.66 ± 0.52	23.93 ± 0.18	23.22 ± 0.82
4	20.77 ± 0.27	19.75 ± 0.46	21.66 ± 0.33	21.67 ± 0.62
5	21.09 ± 0.41	20.53 ± 0.42	21.28 ± 0.59	21.77 ± 0.31
6	16.62 ± 0.88	16.36 ± 0.13	17.95 ± 0.10	17.89 ± 0.11
7	16.78 ± 0.22	17.02 ± 0.93	17.49 ± 0.23	17.78 ± 0.45
8	17.56 ± 0.16	17.45 ± 0.65	16.83 ± 0.14	17.89 ± 0.24
9	16.88 ± 0.17	17.77 ± 0.48	16.66 ± 0.33	17.22 ± 0.75
10	9.33 ± 0.43	9.34 ± 0.77	9.53 ± 0.98	9.36 ± 0.35
11	9.73 ± 0.39	9.65 ± 0.64	9.45 ± 0.41	9.82 ± 0.91
12	9.87 ± 0.22	10.43 ± 0.62	10.35 ± 0.67	10.63 ± 0.45
13	10.35 ± 0.18	10.18 ± 0.35	10.22 ± 0.46	10.46 ± 0.25
14	8.33 ± 0.36	8.36 ± 0.33	8.38 ± 0.66	8.36 ± 0.38
15	8.27 ± 0.31	8.31 ± 0.27	8.44 ± 0.28	8.28 ± 0.44
16	9.39 ± 0.35	9.35 ± 0.39	9.32 ± 0.33	9.33 ± 0.32
17	9.88 ± 0.85	9.88 ± 0.56	9.31 ± 0.43	9.43 ± 0.38
18	9.15 ± 0.11	9.11 ± 0.15	9.18 ± 0.18	9.23 ± 0.54
19	9.45 ± 0.50	9.50 ± 0.45	9.29 ± 0.25	9.25 ± 0.29
20	10.21 ± 0.13	9.13 ± 0.22	11.13 ± 0.14	10.12 ± 0.15
21	11.25 ± 0.35	11.34 ± 0.26	11.31 ± 0.16	10.45 ± 0.32
22	11.71 ± 0.24	9.23 ± 0.72	9.65 ± 0.34	9.33 ± 0.66
23	11.36 ± 0.25	9.24 ± 0.26	9.32 ± 0.35	9.34 ± 0.33
24	9.68 ± 0.65	9.64 ± 0.69	9.26 ± 0.65	10.76 ± 0.27
25	25.72 ± 0.18	24.17 ± 0.73	26.70 ± 0.71	26.73 ± 0.76
26	24.66 ± 0.15	25.14 ± 0.67	26.67 ± 0.65	26.64 ± 0.68
27	13.48 ± 0.55	13.54 ± 0.49	13.53 ± 0.64	13.59 ± 0.52
Chloramphenicol	28.67 ± 0.50	29.11 ± 0.66	-	-
Ketoconazole	-		27.13 ± 0.43	27.56 ± 0.65

TABLE-2
in vitro MICs OF MONOESTERS **1-27**

Org (cmpd)	Antifungal activity MIC (µg/mL)		Antibacterial activity MIC (µg/mL)	
	C. albicans	*A. niger*	*E. coli*	*P. mirabilis*
1	2.55	2.25	2.29	2.78
2	2.23	2.33	2.42	2.31
3	2.45	2.56	2.48	2.75
4	2.47	2.49	2.21	2.49
5	2.85	2.91	2.41	2.86
6	3.18	3.39	3.73	3.66
7	3.78	3.18	3.38	3.63
8	3.68	3.29	4.29	3.72
9	3.65	3.66	4.33	3.79
10	9.54	9.79	9.67	9.44
11	9.18	9.63	9.99	9.87
12	9.33	9.53	9.66	9.77
13	9.64	9.93	9.03	9.98
14	9. 43	9.73	9.92	9.76
15	9.83	9.26	9.55	9.92
16	9.96	9.67	9.38	9.48
17	9.98	9.99	9.43	9.60
18	9.76	9.63	9.01	9.77
19	10.95	10.97	10.88	10.78
20	10.12	10.13	10.32	10.22
21	10.25	10.40	10.11	10.51
22	10.30	10.21	10.08	10.31
23	10.63	10.31	10.29	10.46
24	10.86	10.41	10.21	10.71
25	1.7.2	1.71	1.67	1.86
26	1.73	1.75	1.98	1.68
27	10.86	10.46	10.34	10.73
Chloramphenicol	1.23	1.15	-	-
Ketoconazole	-	-	1.34	1.27

REFERENCES

1. A.R. Katritzky, Z. Wang, A.P. Wells and P.J. Steel, *Org. Prep. Proced. Int*., **27**, 457 (1995).
2. W.G. Kofron and L.G. Wideman, *J. Org. Chem*., **37**, 555 (1972).
3. W. Wang, B. Xu and G.B. Hammond, *J. Org. Chem*., **74**, 1640 (2009).
4. C. Kashima, Y. Shirahata and Y. Tsukamoto, *Heterocycles*, **54**, 309 (2001).
5. E. Rudloff, *Can. J. Chem*., **36**, 486 (1958).
6. N. Vaz, K.N. Vinod, P. Puttaswamy and N.M. Made Gowda, *Am. J. Chem*., **2**, 12 (2012).
7. M. Iqbal, I.B. Baloch and M.K. Baloch, *Chem. J*., **2**, 48 (2012).
8. R.S. Chang, L. Ding, C. Gai-Qing, P. Qi-Choa, Z. Ze-Lin and K.M. Smith, *Soc. Expt. Biol. Med*., **197**, 59 (1991).
9. Y. Maeda, A. Nakayama, N. Kawasaki, K. Hayashi, S. Aiba and N. Yamamoto, *Polymer*, **38**, 4719 (1997).
10. Y. Matsumura, T. Endo, M. Chiba, H. Fukawa and Y. Terao, *Chem. Pharm. Bull. (Tokyo)*, **48**, 304 (2000).
11. P.S. Vraka, C. Drouza, M.P. Rikkou, A.D. Odysseos and A.D. Keramidas, *Bioorg. Med. Chem*., **14**, 2684 (2006).
12. A. Basak, S. Cooper, A.G. Roberge, U.K. Banik, M. Chrétien and N.G. Seidah, *Biochem. J*., **338**, 107 (1999).
13. M.E. Rogers, S.G. Boots and M.R. Boots, *J. Pharm. Sci*., **68**, 903 (1979).
14. D. Todorov, V. Alexieva, E. Karanov, D. Velichkov and V. Velikova, *J. Plant Growth Regul*., **11**, 233 (1992).
15. T. Fujimaki, *Polym. Degrad. Stab*., **59**, 209 (1998).
16. M. Ostermeier, B. Brunner, C. Korff and G. Helmchen, *Eur. J. Org. Chem*., **2003**, 3453 (2003).
17. M. Iqbal, I.B. Baloch and M.K. Baloch, *Chemical Papers*, **67**, 444 (2013).
18. M. Iqbal, I.B. Baloch and M.K. Baloch, *Asian J. Chem*., **25**, 9701 (2013).
19. M. Iqbal, I.B. Baloch and M.K. Baloch. *Bull. Chem. Soc. Ethiop.*, **28**, 131 (2014).
20. M. Amanullah, S.K. Sadozai, W. Rehman, Z. Hassan, A. Rauf and M. Iqbal, *Afr. J. Biotechnol*., **10**, 209 (2011).
21. P.K. Mukherjee, Quality Control of Herbal Drugs and Approach to Evaluation of Botanical, Business Horizons, New Delhi, India, p.256 (2002).
22. D. Gerald and J.O. Lampen, *J. Bacteriol*., **84**, 508 (1962).