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Synthesis of Bioactive Succinylanthranilic Acid Ester and its Analogues

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Abstract: A marine natural product, methyl 2-[propanamide-2′-methoxycarbonyl]benzoate (8) and its analogues were synthesized from anthranilic acid (1). Dicarboxylic acids (5-7) were obtained as intermediates which were converted to their corresponding dicarboxylic acid esters (8-10) by their esterification with diazomethane. The comparative chymotrypsin inhibition, anti-bacterial and anti-fungal activities were also carried out for compounds 5-10.

Key words: Synthesis · succinylanthranilic acid ester · analogues and bioassays

INTRODUCTION

Succinylanthranilic acid ester (8) was isolated for the first time by the author from a marine brown alga, *Jolyna laminariodes* Guimaraes collected from the coastline of Karachi, Pakistan [1]. The significant chymotrypsin inhibition of this compound stimulated us to synthesize it for the comparative biological studies. Chymotrypsin, a serine protease enzyme, play a critical role in several biological processes including digestion, blood coagulation, fibrinolysis and reproduction [2]. A number of other diseases such as glomerulonephritis, pancreatitis and inflammations are also associated with the excessive protease activity [3, 4].

A convenient synthetic route has been developed for the synthesis of compound 8 and its analogues. In the first step, anthranilic acid (1) was separately condensed with succinic anhydride (2), maleic anhydride (3) and glutaric anhydride (4) by refluxing the reaction mixture in dry benzene to produce dicarboxylic acids (5-7) which were esterified with diazomethane to produce corresponding dicarboxylic acid esters (8-10, Scheme-1). Compound 8 was characterized as succinylanthranilic acid ester by comparing its TLC and spectroscopic data with the standard sample of 8 isolated from *J. laminariodes*. In addition, structures of compounds 5-10 were determined by using the powerful tool of modern spectroscopic techniques.

Dicarboxylic acids 5-7 showed 36.5%, 27.3% and 23.8% inhibition whereas their corresponding esters 8-10 showed 42.5%, 33.7% and 29.1% inhibition against chymotrypsin, respectively (Table 1). Therefore, it is concluded that the esters possesses more pronounced enzyme inhibition than the corresponding dicarboxylic acids. These compounds also showed anti-bacterial activity against *Escherichia coli*, *Shigella boydii* and *Staphylococcus aureus* along with the antifungal activity against *Curvularia lunata* and *Aspergillus niger*.

Experimental: *General*- Melting points were determined by Buchi-510 melting point apparatus. MS were recorded at 80 ev, IR as liquid film in $CHCl_3$ and UV in MeOH. ^{1}H-NMR were recorded at 400 MHz in CD_3OD. The purity of the samples were checked by TLC on silica gel (G-254) precoated plates.

Synthesis- In the first step, succinic anhydride (0.7 g) was added to 250 ml round bottom flask containing anthranilic acid (1.0 g) in 100 ml dry benzene. The reaction mixture was refluxed for 10 hrs and then immediately filtered followed by crystallization at room temperature. A pure crystalline compound 5 (0.82 g) was obtained and identified as succinylanthranilic acid. In the second step, compound 5 was added in a mixture of diethylether and diazomethane (20 ml each). The reaction flask was kept over night with continous stirring at room temperature. After the evaporation of diethylether compound 8 (0.71 g) was obtained which was identified as succinylanthranilic acid ester. Similarly, compounds 6 and 9 were prepared by using maleic anhydride whereas compounds 7 and 10 were prepared by using glutaric anhydride.

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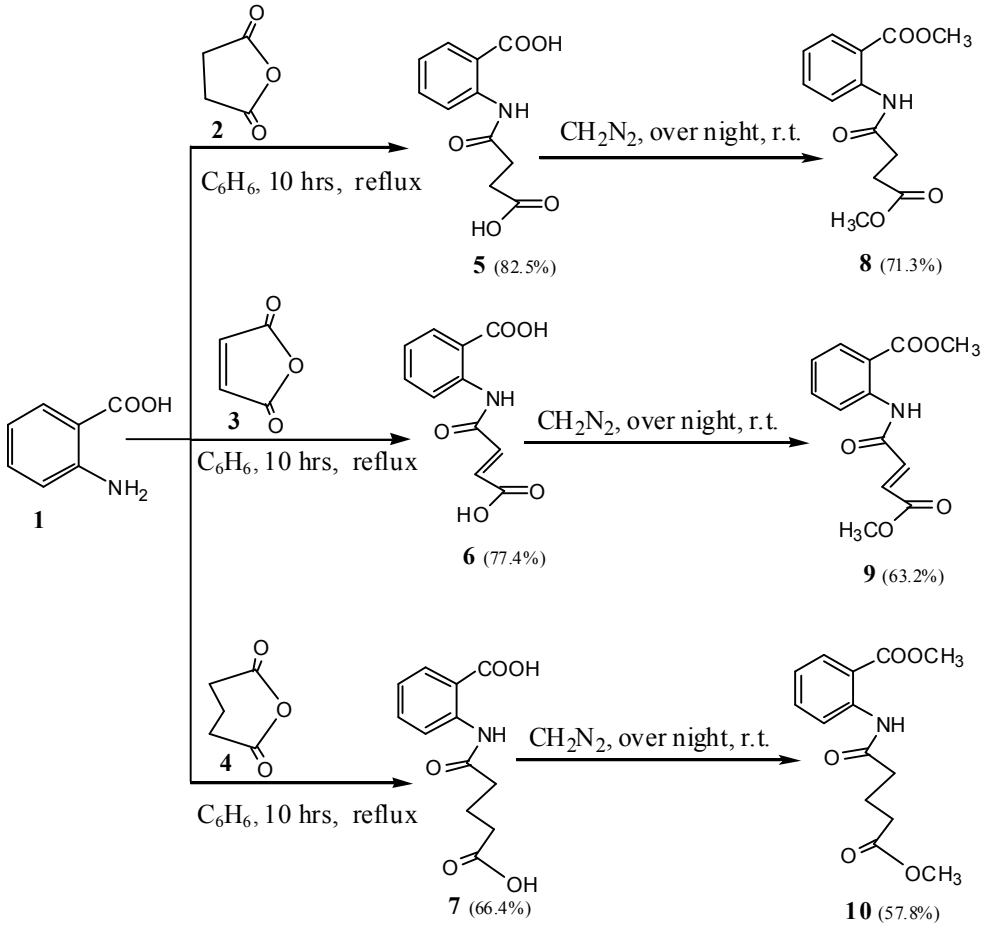

Scheme-1: Synthesis of succinylanthranilic acid ester (**8**) and its analogues.

Table 1: Chymotrypsin inhibition and % yield of compounds 5-10

Dicarboxylic acids	Chymotrypsin Inhibition (%)	% Yield	Dicarboxylic acid esters	Chymotrypsin inhibition (%)	% Yield
5	36.5	82.5	8	42.5	71.3
6	27.3	77.4	9	33.7	63.2
7	23.8	66.4	10	29.1	57.8

***Succinylanthranilic acid (5)*:** (0.825 g, 82.5 % yield, R_f = 0.23 in $CHCl_3$); m.p. 181°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.51 (1H, *d*, $J_{3,4}$ = 7.3 Hz, H-3), 8.02 (1H, *dd*, $J_{6,5}$ = 7.9 Hz, $J_{6,4}$ = 1.6 Hz, H-6), 7.49 (1H, *ddd*, $J_{4,5}$ = 8.5 Hz, $J_{4,3}$ = 7.3 Hz, $J_{4,6}$ = 1.7 Hz, H-4), 7.08 (1H, *dt*, $J_{5,6/5,4}$ = 8.5 Hz, H-5), 2.65 (4H, *m*, 2H-3′/2H-2′); IR (KBr) cm^{-1}: 2800, 1695, 1520, 1440; Uvλ$_{max}$ (MeOH) nm (log ϵ): 293.2 (4.0), 250 (4.6), 211 (4.9); MS *m/z*: 237 (M^+), 175, 146, 137, 119, 92; HREI MS *m/z*: 237.0635 (Calcd. for $C_{11}H_{11}NO_5$: 237.0624); FD MS *m/z*: 237.

***Maleinylanthranilic acid* (6):** (0.774 g, 77.4 % yield, R_f = 0.21 in $CHCl_3$); m.p. 230°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.56 (1H, *d*, $J_{3,4}$ = 8.5 Hz, H-3), 8.10 (1H, *dd*, $J_{6,5}$ = 7.5 Hz, $J_{6,4}$ = 1.5 Hz, H-6), 7.57 (1H, *ddd*, $J_{4,5}$ = 8.5 Hz, $J_{4,3}$ = 7.5 Hz, $J_{4,6}$ = 1.5 Hz, H-4), 7.19 (1H, *dt*, $J_{5,6/5,4}$ = 8.0 Hz, H-5), 6.61 (1H, *d*, $J_{3',2'}$ = 13.0 Hz, H-3′), 6.32 (1H, *d*, $J_{2',3'}$ = 13.0 Hz, H-2′); IR (KBr) cm^{-1}: 2491, 1697, 1613, 1584, 1529, 1483, 1469, 1412, 1302, 1225, 978, 855, 781, 763, 647; UVλ$_{max}$ (MeOH) nm (log ϵ): 311 (3.9), 215 (4.5), 197 (4.3); MS *m/z*: 235 (M^+), 217, 172, 146, 137, 119, 98, 92, 76, 66, 55; HREI MS *m/z*: 235.0472 (Calcd. for $C_{11}H_9NO_5$: 235.0481); FD MS *m/z*: 235.

***Glutarnylanthranilic acid* (7):** (0.664 g, 66.4 % yield, R_f = 0.24 in $CHCl_3$); m.p. 81°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.53 (1H, *dd*, $J_{3,4}$ = 8.5, $J_{3,5}$ = 1.5 Hz, H-3), 8.05 (1H, *dd*, $J_{6,5}$ = 8.0 Hz, $J_{6,4}$ = 2.0 Hz, H-6), 7.52 (1H, *ddd*, $J_{4,5}$ = 9.0 Hz, $J_{4,3}$ = 8.5 Hz, $J_{4,6}$ = 2.0 Hz, H-4), 7.08 (1H, *dt*, $J_{5,6/5,4}$ = 8.5 Hz, H-5), 2.49 (2H, *m*, 2H-2′), 1.86 (2H, *m*, H-3′) and 2.32 (2H, *m*, H-4′); IR (KBr) cm^{-1}: 2900, 1695, 1540, 1415, 1305, 1261, 1208, 1163, 1060, 765; UVλ$_{max}$ (MeOH) nm (log ϵ): 388 (2.5), 302 (3.5), 251 (3.9), 221 (4.1), 197 (3.9); MS *m/z*: 251 (M^+), 174, 146, 137, 119, 92, 90, 87, 65, 55; HREI MS *m/z*:

251.0794 (Calcd. for $C_{12}H_{13}NO_5$: 251.0705); FD MS *m/z*: 251.

Succinylanthranilic acid ester **(8):** (0.713 g, 71.3 % yield, R_f = 0.75 in $CHCl_3$); m.p. 51°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.45 (1H, *d*, $J_{3,4}$ = 7.3 Hz, $J_{3,5}$ = 1.9 Hz, H-3), 8.03 (1H, *dd*, $J_{6,5}$ = 7.9 Hz, $J_{6,4}$ = 1.7 Hz, H-6), 7.50 (1H, *ddd*, $J_{4,5}$ = 8.5 Hz, $J_{4,3}$ = 7.3 Hz, $J_{4,6}$ = 1.7 Hz, H-4), 7.14 (1H, *dt*, $J_{5,6/5,4}$ = 8.4 Hz, H-5), 3.95 (3H, *s*), 3.65 (3H, *s*) 2.50 (4H, *m*, 2H-3′/2H-2′); IR (KBr) cm^{-1}: 3275, 1720, 1686; UVλ_{max} (MeOH) nm (log ϵ): 389.6 (2.6), 307.4 (3.8), 251 (4.3), 222.4 (4.6), 197 (4.3); MS *m/z*: 265 (M^+), 234, 202, 174, 151, 115, 55; HREI MS *m/z*: 265.0948 (Calcd. for $C_{13}H_{15}NO_5$: 265.095); FD MS *m/z*: 265.

Maleinylanthranilic acid ester **(9):** *(0.632 g, 63.2 % yield, R_f = 0.71 in $CHCl_3$); m.p. 230°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.36 (1H, d, $J_{3,4}$ = 8.5 Hz, H-3), 8.05 (1H, dd, $J_{6,5}$ = 7.5 Hz, $J_{6,4}$ = 1.0 Hz, H-6), 7.47 (1H, ddd, $J_{4,5}$ = 8.5 Hz, $J_{4,3}$ = 7.5 Hz, $J_{4,6}$ = 1.0 Hz, H-4), 7.08 (1H, dt, $J_{5,6/5,4}$ = 8.0 Hz, H-5), 6.71 (1H, d, $J_{3',2'}$ = 13.0 Hz, H-3′), 6.42 (1H, d, $J_{2',3'}$ = 13.0 Hz, H-2′), 3.94 (3H, s) and 3.65 (3H, s); IR (KBr) cm^{-1}: 3275, 1720, 1697, 1613, 1584, 1529, 1483, 1412, 1302, 1225, 978, 855, 781, 763, 647; UVλ_{max} (MeOH) nm (log ϵ): 389 (3.9), 320 (4.0), 253 (3.8), 235 (4.5), 197 (4.3); MS m/z: 263 (M^+), 232, 172, 151, 146, 137, 119, 92, 76, 55; HREI MS m/z: 263.0781 (Calcd. for $C_{13}H_{13}NO_5$: 263.0794); FD MS m/z: 263.*

Glutarnylanthranilic acid ester **(10):** *(0.578 g, 57.8 % yield, R_f = 0.79 in $CHCl_3$); ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.55 (1H, dd, $J_{3,4}$ = 8.5, $J_{3,5}$ = 1.5 Hz, H-3), 8.07 (1H, dd, $J_{6,5}$ = 8.0 Hz, $J_{6,4}$ = 1.8 Hz, H-6), 7.56 (1H, ddd, $J_{4,5}$ = 9.0 Hz, $J_{4,3}$ = 8.5 Hz, $J_{4,6}$ = 1.8 Hz, H-4), 7.08 (1H, dt, $J_{5,6/5,4}$ = 8.0 Hz, H-5), 2.51 (2H, m, 2H-2′), 1.89 (2H, m, H-3′) and 2.30 (2H, m, H-4′), 3.90 (3H, s), 3.55 (3H, s); IR (KBr) cm^{-1}: 2900, 1542, 1413, 1307, 1261, 1209, 1163, 1060, 765; UVλ_{max} (MeOH) nm (log ϵ): 388 (2.5), 302 (3.5), 252 (3.9), 221 (4.1), 197 (3.9); MS m/z: 279 (M^+), 216, 151, 101, 90, 87, 55; HREI MS m/z: 279.1121 (Calcd. for $C_{14}H_{17}NO_5$: 279.1107); FD MS m/z: 279.*

Bioassays: (a) *Chymotrypsin inhibition*: Chymotrypsin inhibition was determined for compounds 5-10 according to the reference [2]. Briefly, increasing conc. of 5-10 (0.05 mM to 1 mM) were incubated with chymotrypsin E.C. 3.4.21.1 (sigma) in 50 mM *Tris*-HCl buffer (P^H 7.6) at 25°C for 30 min. After the addition of the substrate (*N*-succinylphenylalanine-*p*-nitroanilide, 1 mg ml^{-1}), the absorbance of liberated *p*-nitroaniline was measured at 410 nm. Inhibitory activity was calculated as the difference between the enzyme activity in the absence and presence of inhibitor. Results were compared with the phenylmethylsulphonyl fluoride, a standard inhibitor of chymotrypsin.

(b) *Anti-microbial activity*: Anti-bacterial activity was determined by the agar well diffusion method whereas anti-fungal activity was determined by the agar tube dilution method [5, 6]. For anti-bacterial activity one loop of 24 h-old-culture containing ca 10^4-10^6 CFU (Colony Forming Units) was spread on the surface of Mueller-Hinton agar plates. Wells were cut in the medium with the help of a sterile metallic borer and 100 µl of each dilution was added to the respective wells. Zones of inhibition were measured after an incubation period of 24 h.

For anti-fungal activity, test tubes containing sterile Sabouraud dextrose agar were inoculated with different conc. of stock solution of samples and kept in a slanting position at room temperature for solidification. Fungal cultures were inoculated on the slant and growth inhibition was observed after an incubation period of 7 days. Tobramycin and ampicillin were used as a standard anti-bacterial antibiotics whereas nystatin and griseofulvin were used as standard anti-fungal antibiotics for comparative study.

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REFERENCES

1. Atta-ur-Rahman, M.I. Choudhary, A.M. Khan, M. Shabbir, U. Ghani and M. Shameel, 1997. Phytochemistry, 46: 1215.
2. Cannell, R.J.P., S.J. Kellam, A.M. Owsianka and J.M. Walker, 1988. Planta Medica, 54: 10.
3. Hopsu, V.K.H., J.E. Fraki and M. Jarvinen, 1977. Proteinases in Mammalian Cells and Tissues, Barret J.A. ed., North Holand, Amsterdam, pp: 545.
4. Starkey, P.M., 1977. Acta. Biol. Med. Germ., 36: 1549.
5. Paxton, J.D., 1991. Methods in Plant Biochemistry, Vol. 6, Ed. K. Hostettmann, Academic Press, London, pp: 3.
6. Carran, R., A. Maran, J.M. Montero, L. Fernandozlago and A. Dominguez, 1987. Plantes Medicinales et Phytotherapie, 21, 195.