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Modified *tetra*-oxygenated xanthones analogues as anti-MRSA and *P. aeruginosa* agent and their synergism with vancomycin

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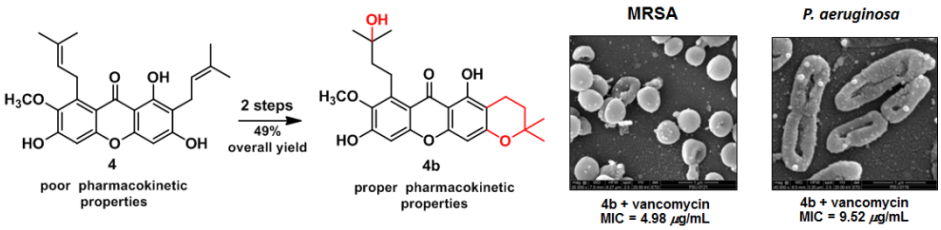
Abstract

Five isolated xanthones from the *C. cochinchinense* and *G. mangostana* were evaluated and tested for antibacterial activities. Isolated **4** and **5** exhibited potent anti-MRSA and *P. aeruginosa* activity, but showed poor pharmacokinetic properties via ADMET prediction. It led us to improve pharmacokinetic properties of **4** and **5** by partially modifying them in acidic condition yielding fourteen analogues. It was found that analogues **4b**, **4d** and **5b** possessed proper pharmacokinetic properties, while only **4b** exhibited the best anti-MRSA and *P. aeruginosa* activity. The SEM results indicated that **4b** may interact with or damage the cell wall of MRSA and *P. aeruginosa*. Moreover, a combination of **4b** and vancomycin exhibits synergistic effect against both MRSA and *P. aeruginosa* at MIC value of 4.98 (MIC = 18.75 μg/mL for **4b**) and 9.52 μg/mL (MIC = 75 μg/mL for **4b**), respectively.

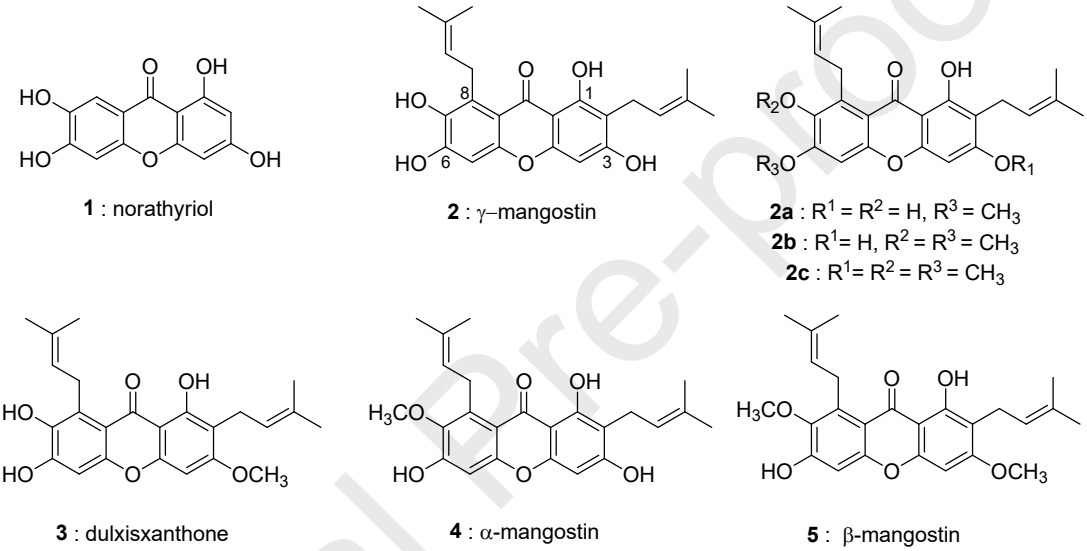
GRAPHICAL ABSTRACT



KEYWORDS: Xanthone, MRSA, *P. aeruginosa*, vancomycin, synergism and ADMET

Bacterial infections are common causes of various diseases such as sepsis, pneumonia, bloodstream infection, gastrointestinal infection, urinary tract infection; however they are successfully cured by treatment with antibiotics. Nowadays, many reports[1] reveal that many microorganisms develop themselves to be the drug-resistance microbes such as *Staphylococcus aureus* shows resistance to the methicillin (MRSA),[1A] *Neisseria gonorrhoeae*[1B] resists to the quinolone, while *Enterobacteriaceae*[1C] resists to the carbapenam. These become a serious public health problem. Moreover, there have been several reports of colistin-resistance which is a last resort treatment for infection caused by drug-resistance bacteria.[2,3] Many antibiotic resistances from various reports around the world showed increment, hence we need a new promising candidate of antibiotics, which should be highly effective but possess low toxicity. Nature has been the important source to provide several medicines for treatment of various types of diseases in humans and animals for many years. Therefore, the natural source should be considered as a good candidate for a new frame of antibiotics. From the previous works on the *Cratoxylum* genus,[4] it revealed that oxygenated xanthones exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria. This result led us to investigate *tetra*–oxygenated xanthone, a major bioactive component from *C. cochinchinense* and *G. mangostana*.

The crude CH_2Cl_2 extracts of the *C. cochichinense* resin *and G. mangostana* hulls were subjected to chemical investigation leading to the isolation of five known 1,3,6,7-*tetra*-oxygenated xanthones identified as norathyriol (**1**),[5A] γ-mangostin (**2**),[5B] dulxisxanthone F (**3**),[5C] α-mangostin (**4**)[5B] and β-mangostin (**5**)[5B] as shown in **Scheme 1**. Their structures were elucidated by NMR analysis and comparison of their spectroscopic data with those reported in the literatures. Compounds **1**–**5** have been evaluated for their antibacterial activity against both Gram-positive and Gram-negative bacteria.



Scheme 1 The structures of compounds **1**–**5** and their derivatives **2a–2c**

From the anti-bacterial activity result as shown in **Table 1**, it showed that compound (**1**) was inactive against both Gram-positive and Gram-negative bacteria except moderately active against MRSA (MIC = 18.75 μg/mL), whereas **2**, an isoprenyl substitute of **1**, exhibited greater anti-bacterial activity against MRSA than **1** (MIC = 4.68 μg/mL). Compound (**4**), a methylated substitute of **2**, showed more potent activity against MRSA, *B. subtilis* and *P. aeruginosa* with the same MIC value of 2.34 μg/mL, as compare to **2** (MIC = 4.68, 18.75, 18.75 μg/mL, respectively), whereas **5**, a dimethylated substitute of **2**, showed moderate activity against MRSA and *P. aeruginosa* with MIC values of 4.68 and 9.37

μg/mL, respectively. We further attempted to partially modify the structure of compounds **2**, **4** and **5** by treatment with MeI/K_2CO_3 in methanol to yield **2a**[6A] (monomethylated of **2**), **2b**[6B] (dimethylated of **2**) and **2c**[6A] (trimethylated of **2**), respectively. Compound **2a** showed potent activity against *P. aeruginosa* (MIC = 4.67 μg/mL) and moderate activity against MRSA (MIC = 9.37 μg/mL). Compound **2b** showed moderate activity against MRSA and *P. aeruginosa* with the same MIC values of 18.75 μg/mL while compound **2c** was inactive. It could be suggested that a methoxyl group at C-7 and on *tetra*-oxygenated xanthone skeleton should be responsible for the effectiveness as anti-bacterial agent as seen in compound **4**.

Table 1 Antibacterial activity (MIC, μg/mL) of **1**–**5**, **2a–2c**, **4a–4f** and **5a–5e**

Compounds	Gram-positive bacteria[a]				Gram-negative bacteria[b]		
	MRSA	*B. subtilis*	*E. faecalis*	VRE	*S. typhi*	*S. sonei*	*P. aeruginosa*
1	18.75	37.5	300	>300	>300	>300	37.5
2	4.68	18.75	9.37	NT	18.75	18.75	18.75
3	9.37	18.75	300	300	150	150	18.75
4	2.34	2.34	150	150	18.75	150	2.34
5	4.68	2.34	300	300	>300	>300	9.37
2a	9.37	75	75	NT	37.5	75	4.67
2b	18.75	75	75	NT	150	150	18.75
2c	>300	75	150	300	300	>300	>300
4a	2.34	2.34	>300	>300	300	300	9.37
4b	18.75	18.75	>300	>300	150	300	75
4c	9.37	9.37	>300	>300	300	300	75
4d	37.5	37.5	>300	>300	300	300	300
4e	>300	>300	300	NT	>300	>300	>300
4f	>300	>300	>300	NT	>300	>300	>300
5a	37.5	150	75	NT	150	150	150
5b	18.75	9.37	150	>300	300	300	18.75
5c	>300	75	75	150	300	300	300
5d	150	150	>300	>300	300	300	300
5e	>300	300	>300	>300	300	300	>300
vancomycin	2.34	2.34	2.34	2.34	2.34	2.34	2.34

[a]Methicillin-Resistant *Staphylococcus aureus* ATCC 43300 (MRSA)*; Bacillus subtilis*; *Enterococcus faecalis* TISTR 459; Vancomycin-Resistant *Enterococcus faecalis* ATCC 51299 (VRE); [b]*Salmonella typhi*; *Shigella sonei* and *Pseudomonas aeruginosa*

From the *in vitro* result (**Table 1**), it revealed that among the isolated xanthones (**1**–**5**) and partially modified **2a**–**2c**, compound (**4**) exhibited the most potent activity against

MRSA, *B. subtilis* and *P. aeruginosa* with the same MIC value of 2.34 μg/mL, therefore suitable for further study on the pharmacokinetic properties. In this study, the pharmacokinetic properties were conducted via ADMET PredictorTM software.[7] For instance, a compound with low ADMET problem should be considered to further undergo the process of a new drug development.

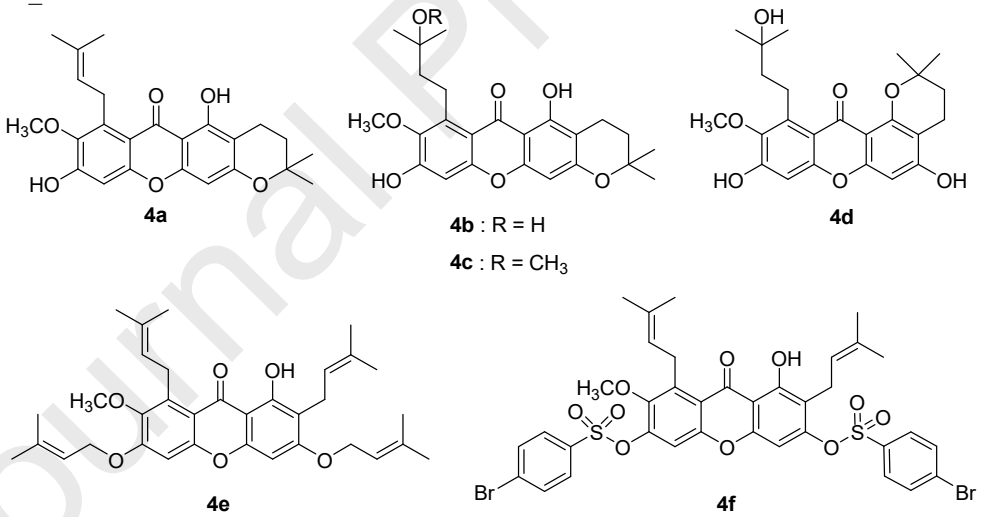
From the ADMET result in (**Tables 2** and **S5**), it revealed that **1** has a low permeability (P_f = 0.0839) in the gut model, whereas compounds **2–5** and **2a–2c** containing isoprenyl side chains on a xanthone nucleus, showing no serious problem about permeability, suggesting that the isoprenyl side chain should be responsible for its proper permeability in the gut tissue and also its anti-bacterial activity. According to the ADMET result, it revealed that compound **4** and the rest of the compounds failed to be used in the *in vivo* model because of the hepatotoxicity (Hp), low fraction unbound of these compounds in the plasma (Fu) and mutagenicity (Mu) problems. Since in **Table 1** compounds **4** and **5** exhibited potent antibacterial activity, thus, further study should be focussed on the structural modification of compounds **4** and **5** in order to improve their pharmacokinetic and pharmacodynamic properties.

To increase the pharmacodynamic properties of **4**, the isoprenyl side chain of **4** was partially modified to 3-hydroxyl-3-methyl butyl group and dimethylchromane ring in the acidic medium to yield compounds **4a**–**4d** (**Scheme 2**). The antibacterial activity of **4a**–**4d** were shown in **Table 1**. Only **4a** showed strong activity against MRSA and *B. subtilis* with MIC value of 2.34 μg/mL, the same as those of **4**, while compound **4b** showed moderate activity against MRSA and *B. subtilis*, whreas inactive against *P. aeruginosa* with MIC values of 18.75, 18.75 and 75 μg/mL, respectively.

Table 2 Prediction of pharmacokinetic properties and toxicity risks of **1**–**5** and **2a–2c** by ADMET prediction TM software

No.	Absorption			Distribution		Metabolism and Excretion		Toxicity risks		ADMET risk[i]	ADMET code
	Pf[a]	Sw[b]	Ow[c]	Fu[d]	Vd[e] (L/kg)	CYP[f] MET_3A4	CYP[f] MET_2C9	Hp[g]	Ra[h] (mg/kg)		
1	0.0839	0.1390	1.79	3.48	0.53	0.182	1.492	2	1205.53	3	Pf, Fu, Hp
2	0.8353	0.0353	4.28	1.42	1.11	2.031	3.415	2	497.26	2	Fu, Hp
3	1.0458	0.0160	4.24	3.36	2.26	2.732	2.487	2	583.39	3	Fu, Ow, Hp
4	0.9307	0.0177	4.58	1.21	1.08	3.433	4.36	2.5	336.11	2	Fu, Mu[j]
5	1.1383	0.0051	4.58	3.17	2.88	4.103	3.156	2	477.29	3	Fu, Ow, Hp
2a	0.9872	0.0190	4.51	1.34	1.05	3.006	2.439	2.5	396.64	2	Fu, Mu
2b	1.2881	0.0031	4.97	3.62	3.39	5.171	1.964	3	452.71	3	Sw, Ow, Mu
2c	1.0863	0.0155	4.92	1.22	1.20	4.404	2.672	2	319.06	2	Fu, Ow

[a]Pf = low permeability if < 0.1; [b]Sw = low solubility if <0.005; [c]Ow = excessive lipophobicity if <-0.891; [d]Fu = low fraction unbound in plasma if <3.5%; [e]Vd = high steady-state volume of distribution if > 5.5; [f]CYP = metabolic risk if and MET_3A4 > 30 and MET_2C9 > 30; [g]Hp = human liver hepatotoxicity if TOX_AlkPhos = Toxic or TOX_GGT = Toxic or TOX_LDH = Toxic and TOX_SGOT = Toxic or TOX_SGPT = Toxic; [h]Ra = acute toxicity in rats if <300; [i]each score related with potential ADMET problem; [j]Mu = mutagenicity if TOX_MUT_risk > 2



Scheme 2 The structures of compounds **4a–4f**

In addition, the pharmacokinetic properties of **4a**–**4d** were conducted via ADMET Predictor (**Tables 3** and **S5**), it revealed that potent compound **4a** possessed low solubility (Sw = 0.0020), excessive lipophobicity (Ow = 4.75) and showed toxicity to the rat (Ra = 299.60 mg/kg). Surprisingly, compounds **4b** and **4d** were moderately active for antibacterial activity against MRSA and *B. subtilis* but they have no ADMET risk (**Table 3**). To increase a permeability of **4** (P_f = 0.9307), a hydroxyl group of **4** was replaced with the isoprenyl and *p*-bromobenzenesulfonyl side chains to yield **4e** and **4f**, respectively. The ADMET result revealed that they have better permeability than **4**, but along with high risk of the other ADMET parameters. From the structure relationship between compounds **4** and **4a**–**4d**, it could be suggested that the replacement of an isoprenyl side chain with a 3-hydroxy-3-methylbutyl group and a chromane ring can obviously improve the pharmacokinetic properties of **4** to be more drug-likeness. It can be summarized that **4b** and **4d** with no ADMET risk have the proper pharmacokinetic properties. However, compound **4b** showed better antibacterial activity than **4d**, see **Table 1**.

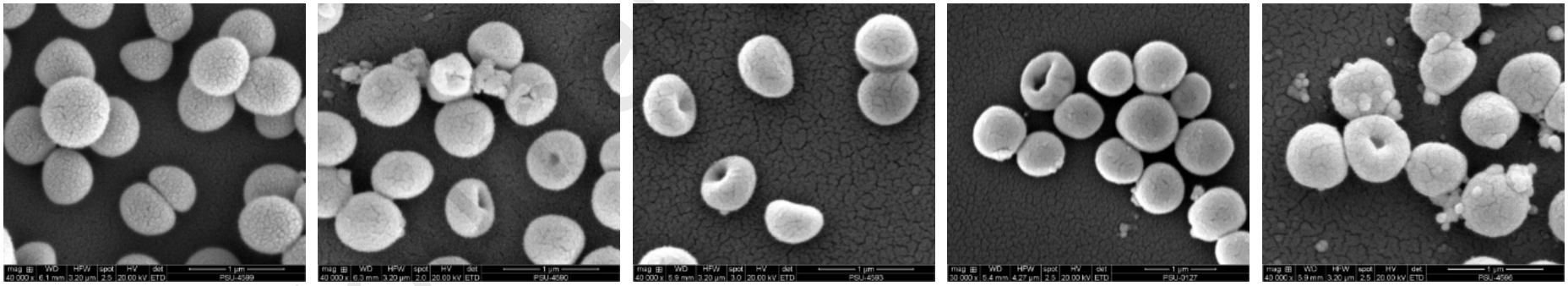
We further studied the possible mode of action of **4**, **4a**, and **4b** against MRSA and *P. aeruginosa* by observing the bacteria cell morphology through scanning electron microscope (SEM) for 24 h after treatment with **4**, **4a**, and **4b**.

From the SEM results (see **Figs. 1** and **2**), it was clearly indicated that the cell morphology of MRSA and *P. aeruginosa* after treatment with **4**, **4a**, and **4b**, most cells were completely deformed whose results were correlated to their good antibacterial activity (**Table 1**). Therefore, it can be suggested that **4**, **4a**, and **4b** may interact with or damage the cell wall of MRSA and *P. aeruginosa* as seen by the formation of pores on the cell wall of MRSA and *P. aeruginosa* (**Figs. 1** and **2**).

Table 3 Prediction of pharmacokinetic properties and toxicity risks **4** and **4a**–**4f** by ADMET prediction TM software

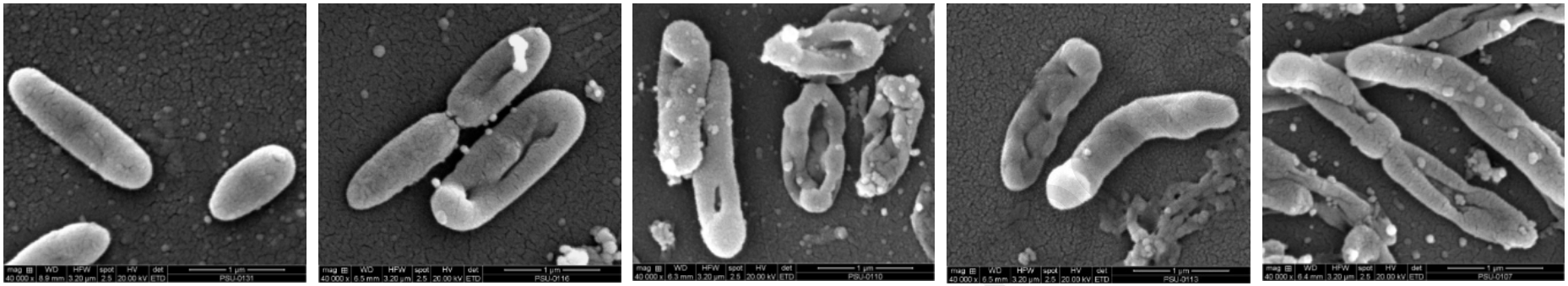
No.	Absorption			Distribution		Metabolism and Excretion		Toxicity risks		ADMET risk	ADMET code
	Pf	Sw	Ow	Fu	Vd (L/kg)	CYP MET_3A4	CYP MET_2C9	Hp	Ra (mg/kg)		
4	0.9307	0.0177	4.58	1.21	1.08	3.433	4.360	2.5	336.11	2	Fu, Mu
4a	0.5622	0.0020	4.75	4.64	3.24	2.823	3.509	2	299.60	3	Sw, Ow, Ra
4b	0.1949	0.0069	3.64	7.98	3.89	3.289	1.833	2	383.85	0	–
4c	0.4029	0.0032	4.45	6.68	3.97	4.167	2.044	2	307.02	2	Sw, Ow
4d	0.1965	0.0178	3.25	6.56	3.34	3.332	1.921	2	368.89	0	–
4e	2.1528	0.0012	7.07	2.02	5.55	60.88	3.903	1.5	394.62	10	Sw, Fu, Ow, Vd, 3A,[a] Hp, ti,[c] Sz,[d] Rb,[e] Ch[f]
4f	2.0880	0.0030	6.72	0.32	0.77	74.348	46.604	0	208.07	11	Sw, Fu, Ow, 3A,[a] C9,[b] ti,[c] Ra, Sz,[d] Rb,[e] Ch,[f] HA[g]

[a]3A = CYP_3A4_Substr =Yes and MET_3A4_CLint > 30; [b]C9 = CYP_2C9_Substr = Yes and MET_2C9_CLint >30; [c]ti = specific inhibition constant for the CYP 3A4-mediated metabolism of testosterone; [d]Sz = large size; [e]Rb = too flexible; [f]Ch = excessive partial atomic charge; [g]HA = too many H-bond acceptors



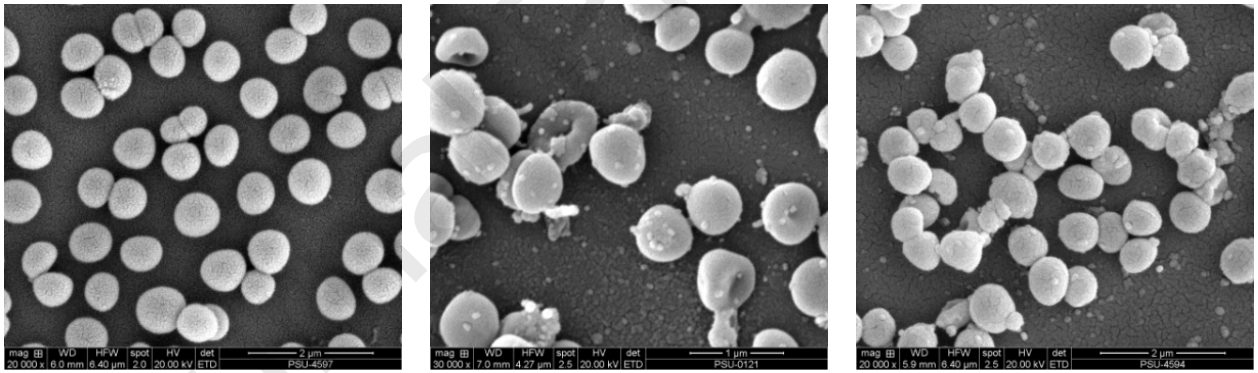
a) control **b) 4** **c) 4a** **d) 4b** **e) vancomycin**

Figure 1 SEM images of MRSA after treatment with **4**, **4a** and **4b**



a) control **b) 4** **c) 4a** **d) 4b** **e) vancomycin**

Figure 2 SEM images of *P. aeruginosa* after treatment with **4**, **4a** and **4b**



control **4b+VCM** **vancomycin**

Figure 3 SEM images of MRSA after co-treatment with **4b** and vancomycin

Further, we decided to extend the study on synergism of potent antibacterial compounds **4** and **4a** with vancomycin (commercially available antibiotic), and also a combination between a moderate antibacterial compound **4b** (no ADMET risk) and vancomycin against MRSA and *P. aeruginosa* strains with the hope of enhancing their activity. FIC index results were shown in **Tables 4** and **5**. From this result in **Table 4**, synergism between **4** and vancomycin (entry 1) against MRSA was observed at MIC value of 0.5850 μg/mL, lower than MIC value of **4** (MIC value of 2.34 μg/mL, **Table 1**). While, a combination of **4b** and vancomycin (entry 3) exhibited synergistic effect against MRSA at MIC value of 4.6875 μg/mL (MIC value of 18.75 μg/mL for **4b**).

Table 4 MIC (μg/mL) and fractional inhibitory concentration (FIC) index of combination of **4**, **4a**, **4b** and vancomycin against MRSA

Entry	MIC (μg/mL)				Total MIC (μg/mL) of combination	FIC index
	4	**4a**	**4b**	**VCM**[c]		
1	0.5850	–	–	0.2925	0.8775	0.375[a]
2	–	1.1700	–	0.5850	1.7550	0.750[b]
3	–	–	4.6875	0.2925	4.9800	0.375[a]

[a]synergism if FIC = 0.30–0.70,[8A] [b]moderate synergism if FIC = 0.70–0.85[8A], [c]the concentration of VCM start at 1.17 μg/mL.

We further studied the possible mode of action of a combination of **4b** and vancomycin at MIC value of 4.6875 μg/mL against MRSA by observing the cell morphology. From the SEM results (see **Fig. 3**), it was clearly indicated that most cells of MRSA were completely deformed

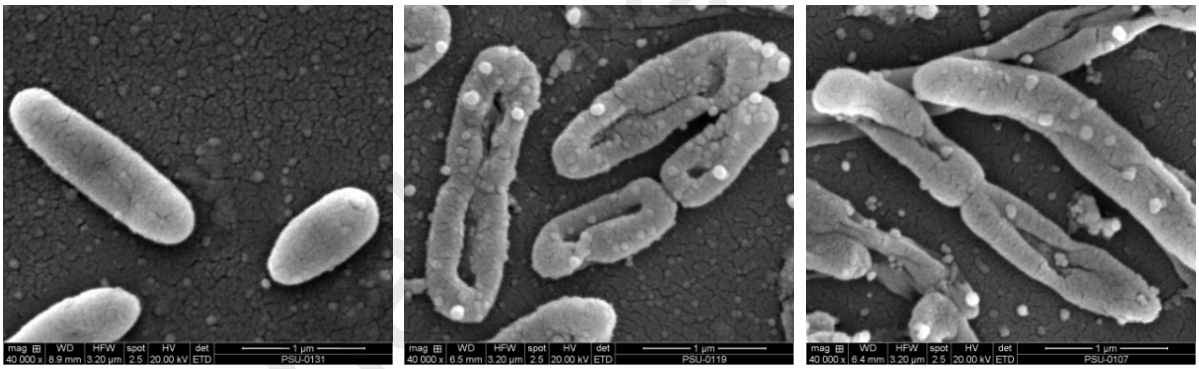
From the result in **Table 5**, all combinations of **4**, **4a**, **4b** with vancomycin exhibit synergistic effect against *P. aeruginosa*, especially for **4** and **4b** exhibit significant synergistic effect against *P. aeruginosa* with FIC index at 0.187. Moreover, a combination of **4b** and vancomycin exhibits strong antibacterial activity against *P. aeruginosa* with MIC value of 9.375 μg/mL compare to the pure form of **4b** (MIC value = 75 μg/mL, **Table 1**).

Table 5 MIC (μg/mL) and FIC index of combination of **4**, **4a**, **4b** and vancomycin against *P. aeruginosa*

Entry	MIC (μg/mL)				Total MIC (μg/mL) of combination	FIC index
	4	4a	4b	VCM[c]		
1	0.2925	–	–	0.1462	0.4387	0.187[a]
2	–	2.3425	–	0.2925	2.6350	0.375[b]
3	–	–	9.3750	0.1462	9.5210	0.187[b]

[a]strong synergism if FIC = 0.10–0.30,[8A] [b]synergism if FIC = 0.30–0.70[8A], [c]the concentration of VCM start at 1.17 μg/mL.

The possible mode of action against *P. aeruginosa* of **4b** and vancomycin was further investigated by observing the cell morphology from SEM (see **Fig. 4**), which was clearly indicated that most cells of MRSA were completely deformed.

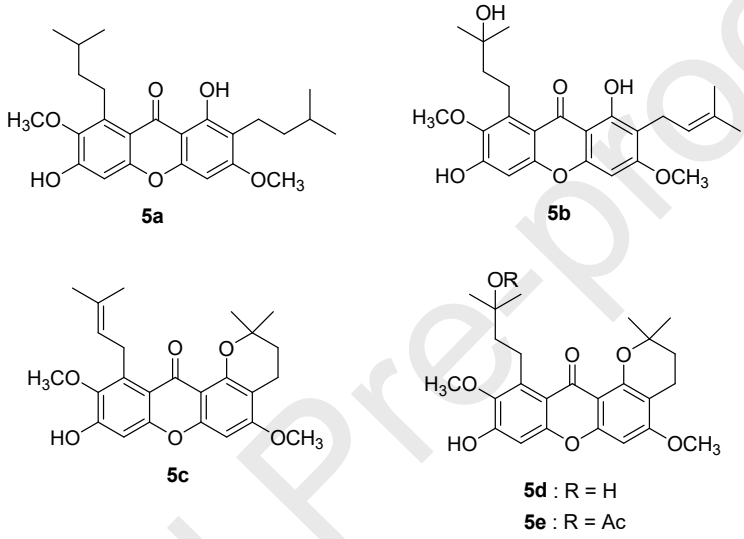


a) control **b) 4b+ vancomycin** **c) vancomycin**

Figure 4 SEM images of *P. aeruginosa* after treatment with **4b** and vancomycin

From the result of antibacterial activity and ADMET, it revealed that **4b** has the proper pharmacokinetic and pharmacodynamics properties that meet criteria for further study on *in vivo* model. For in vivo study, a **4b** would be used in gram scale to complete the project. We also report a partial modifying of **4b** from **4** in two-steps with 49% overall yield (**Scheme S1**), of which compound **4** was mainly found as a major constituent in the hulls of *G.mangostana* a bio-waste after the agricultural product processing in Thailand.

The partial modification of **5** was shown in **Scheme 3**. From the antibacterial activity in **Table 1**, it was shown that only **5b** showed moderate activity against MRSA and *P. aeruginosa* with the same MIC value of 18.75 μg/mL as compare to those of **5** (MIC value of 4.68 and 9.37 μg/mL, respectively), and no ADMET risk (**Tables 6** and **S5**), whereas compounds **5a**, **5c-5f** were inactive for antibacterial activity. In this work, we have no further study on compound **5b** because of its lower polarity than **4b** and **4d**.



Scheme 3 The structures of compounds **5a–5e**

In conclusion, we have isolated xanthones from the *C. cochinchinense* and *G. mangostana* were tested for antibacterial activities. Isolated **4** and **5** exhibited potent anti-MRSA and *P. aeruginosa* activity, but showed poor pharmacokinetic properties. It led us to improve pharmacokinetic properties of **4** and **5** by partially modifying them in acidic condition yielding fourteen analogues. It was found that analogues **4b**, **4d** and **5b** possessed proper pharmacokinetic properties, while only **4b** exhibited the best anti-MRSA and *P. aeruginosa* activity. Moreover, a combination of **4b** and vancomycin exhibits synergistic effect against both MRSA and *P. aeruginosa*.

Table 6 Prediction of pharmacokinetic properties and toxicity risks **5** and **5a**–**5e** by ADMET prediction TM software

No.	Absorption			Distribution		Metabolism and Excretion		Toxicity risks		ADMET risk	ADMET code
	Pf	Sw	Ow	Fu	Vd (L/kg)	CYP MET_3A4	CYP MET_2C9	Hp	Ra (mg/kg)		
5	1.1383	0.0051	4.58	3.17	2.88	4.103	3.156	2	477.29	3	Ow, Fu, Hp
5a	1.1299	0.0023	5.36	5.67	4.23	6.478	1.398	2	517.04	2	Sw, Ow
5b	0.6370	0.0150	3.5	5.34	3.33	2.954	1.760	2	581.27	0	–
5c	0.7958	0.0012	4.72	5.25	4.29	3.327	2.508	2	275.20	3	Sw, Ow, Ra
5d	0.3250	0.0045	3.54	8.51	4.87	3.810	1.346	2	334.10	1	Sw
5e	0.3052	0.0017	3.94	7.84	4.56	3.799	1.649	2	273.52	2	Sw, Ra

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Supplementary data

Experimental section, Synthesis of xanthone analogues, Antibacterial assay, Synergistic study and ^{1}H and ^{13}C NMR spectra (**Figures S1–S19**) of selected compounds. All compounds were predicted under 24 criteria (**Tables S1–S4**) in pH 7.4 condition described in **Table S5**.

References and notes

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