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Original article

Synthesis, antibacterial and anticonvulsant evaluations of some cyclic enaminones

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ABSTRACT

Several cyclic enaminone esters were synthesized, characterized, and evaluated for anticonvulsant and antibacterial activities using standardized tests. A series of enaminones were mainly phenyl analogs of anticonvulsant enaminones, while a second series comprised of compounds bearing the oxazolidinone pharmacophoric moiety found in the synthetic antibacterial linezolid. The enaminone ester bearing an unsubstituted anilino analog showed class 2 anticonvulsant activity. This represents a first report of an unsubstituted anilino enaminone with anticonvulsant activity. The enaminone esters gave interesting UV data, and four analogs displayed potent anticonvulsant activities, while another four compounds showed moderate anticonvulsant activities. Surprisingly, none of the enaminone esters had any significant antibacterial activity.

1. Introduction

Epilepsy is a brain disorder in which clusters of nerve cells, or neurons, in the brain sometimes signal abnormally. In epilepsy, the normal pattern of neuronal activity becomes disturbed causing strange sensations, emotions, and behaviors or sometimes convulsions, muscle spasms, and loss of consciousness. Anticonvulsant drugs are an important part of the treatment program for epilepsy [1]. Once epilepsy is diagnosed, it is important to begin treatment as soon as possible. This is because seizures can be controlled with modern medicines and surgical techniques in about 80% of those diagnosed with epilepsy.

The search for antiepileptic compounds with more selective activity and lower toxicity is an area under intensive investigation; hence one of the objectives of this research was to synthesize enaminones with anticonvulsant activity. Enaminones (Fig. 1) are chemical compounds consisting of an amino group linked through a C=C bond to a keto group [2]. The second objective of this research was to synthesize enaminones containing the oxazolidinone moiety, and evaluate them for anticonvulsant and antibacterial activities.

Oxazolidinone antibacterial agents exemplified by linezolid **1** are totally synthetic, structurally distinct and mechanistically novel class of agents [3,4]. It is effective against Gram-positive pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), *Streptococcus pneumoniae* (*S. pn*), *Streptococcus pyogenes* (*S. py*), and vancomycin-resistant *Enterococcus faecium* (VREF) [5,6]. Early structure–activity relationship (SAR) on oxazolidinones indicated that the C5-acylamino group was essential for good antibacterial activity [7]. However, compounds bearing thio-amides, thioureas, halogen-substituted methyl-amides, ureas, and *N*-carbamates have comparable or superior activity to linezolid [8,9]. Furthermore, radical modifications involving the introduction of O-linked **2a** and N-linked **2b** and **PH-027** (Fig. 1) heterocyles at the C5-position have been shown to give compounds with strong antibacterial activity [9,10]. In this regard, it was hypothesized that the oxazolidinone pharmacophoric moiety bearing enaminones at the C5-position would also exhibit antimicrobial activity.

In addition, it was expected that some of the secondary enaminones bearing the oxazolidinone moiety would display anticonvulsant activity, similar to the anilino and benzylamino enaminone derivatives.

2. Results and discussions

2.1. Chemistry

2.1.1. Synthesis

The cyclohexanone intermediates **5a**,**b** were prepared from the cyclization reactions between the ketones **3a**,**b** and the esters **4a**,**b**

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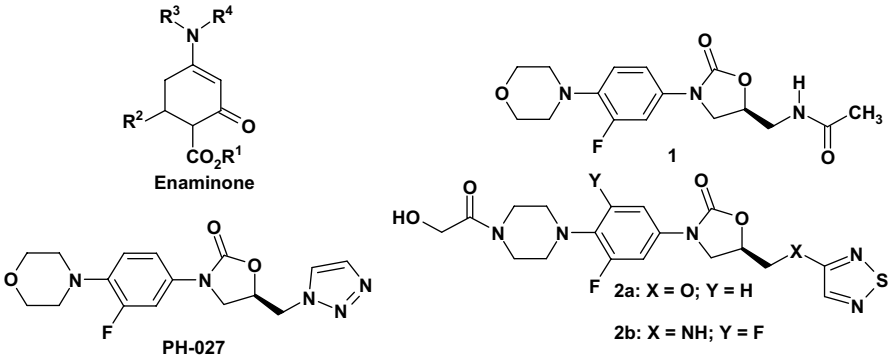


Fig. 1. Chemical structures of enaminones and oxazolidinones.

in alcohol and sodium alkoxide in the respective alcohol (see also Scheme 1). The condensation of the cyclohexanone intermediates **5a**,**b** with a variety of amino compounds yielded the final enaminone esters (Scheme 1 Tables 1 and 2). The 5-aminomethyl oxazolidinone and the morpholine and piperazine related amino derivatives were prepared according to literature methods [10,11]. The enaminone esters **6a**–**z** and **7a**–**m** were completely characterized by UV, IR, NMR, MS, and elemental analyses. The recorded data were as expected for secondary and tertiary enaminones [2], and confirmed the chemical structures of the compounds in Tables 1 and 2.

2.1.2. Ultraviolet absorption of enaminones

In this study the wavelength of maximum absorption (λ_{max}) and absorptivity (ε) values for selected enaminones were determined. The enaminone derivatives produced intense absorption bands under UV spectroscopy and where a compound exhibited more than one band the most intense bands having molar absorptivity (ε) values of >14 000 were selected and reported. The UV data of the enaminones were quite unique for the compounds. While the UV data of enaminones in 95% EtOH and water indicated absorptions in neutral media; those in, 1 M HCl and 1 M NaOH indicated their absorptions in acidic and alkaline media, respectively. Most of the enaminones are basic in nature, and therefore existed in the partially protonated form in the neutral medium. Deprotonation of these enaminones in alkaline solution (1 M NaOH) had small hypsochromic or no significant effect on their λ_{max} values when compared to neutral solutions. The enaminones that normally behave as weak bases exhibited hypsochromic shift on moving from neutral to acidic medium. In acidic medium (1 M HCl), the enaminones were usually protonated. Tertiary enaminones displayed UV absorption at longer wavelengths than secondary enaminones in acidic, alkaline, and neutral solutions. The tertiary enaminones absorbed UV light at the higher end and secondary enaminones towards the lower end of the UV wavelength range 259–336 nm in aqueous media.

From the UV data of the enaminones (Table 3), interesting UV absorption patterns were noted, and generally classified into six sub-groups as follows.

(i) Firstly, some enaminones showed slight hypsochromic shift in acid, and bathochromic shift in alkaline solutions when compared to their absorptions in neutral solutions. Enaminones **6a**, **6h**, **6m**, **6n**, **6w**, **7c**, **7d**, and **7m** had UV absorption ranges from 282 to 317 nm in neutral solutions, 259 to 311 nm in acidic solutions, and 283 to 319 nm in alkaline solutions.

(ii) Secondly, some enaminones showed hypsochromic shift in acid, and no change in alkaline solutions when compared to absorptions in neutral solutions. Enaminones **6b**, **6c**, **6e**, **6f**, **6g**, **6i**, **6j**, **6p**, **6s**, **6t**, **6y**, **6z**, **7b**, **7h**, **7i**, and **7j** had UV absorption ranges from 283 to 308 nm in neutral solutions, 259 to 296 nm in acidic solutions, and 283 to 308 nm in alkaline solutions.

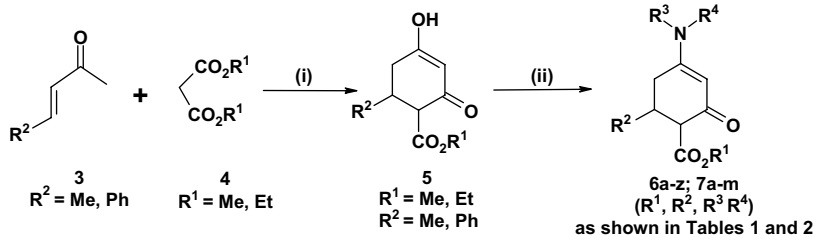
(iii) Lastly, some of the enaminones showed hypsochromic shifts both in acidic and alkaline solutions when compared to their absorptions in neutral solutions. Enaminones **6d**, **6k**, **6l**, **6q**, **6u**, **6v**, and **6x** had UV absorption ranges from 306 to 315 nm in neutral solutions, 271 to 309 nm in acidic solutions, and 281 to 313 nm in alkaline solutions.

(iv) Other enaminones showed bathochromic shifts in acidic solutions, and hypsochromic shifts in alkaline solutions when compared to their absorptions in neutral solutions. Enaminones **6o**, **7a**, **7f**, **7g**, **7k**, and **7l** had UV absorption ranges from 317 to 332 nm in neutral solutions, 319 to 336 nm in acidic solutions, and 293 to 315 nm in alkaline solutions. Similar to a few enaminones previously reported [12,21], these enaminones uniquely display acidic properties.

(v) The enaminone **6r** showed bathochromic shift in both acidic solution (283 nm) and alkaline solution (282 nm), when compared to its absorption in a neutral medium (261 nm).

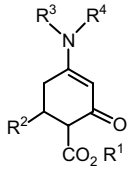
(vi) The enaminone **7e** showed no change in acidic solution (318 nm) when compared to its neutral solution (318 nm). However, enaminone **7e** showed hypsochromic shift in alkaline solution (311 nm) when compared to its absorption in a neutral solution (318 nm).

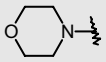
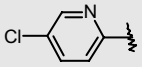
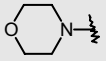
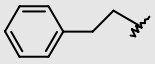
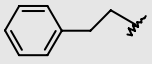
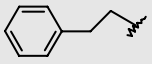
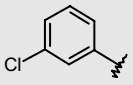
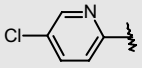
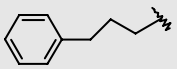
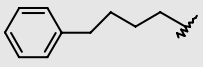
It was observed that the enaminones that showed anticonvulsant activities were within sub-groups (i)–(iii), whereas enaminones in sub-groups (iv)–(vi) including all the heterocyclic analogs



Scheme 1. Synthesis of heterocyclic enaminones. (i) Na/MeOH or Na/EtOH; (ii) R^3R^4NH (1° or 2° amine)/EtOH/heat 4–6 h.

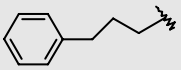
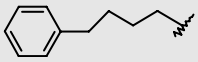
Table 1
Physical constants and anticonvulsant activity of cyclic enaminones



Compound	R^1	R^2	R^3	R^4	*C* log *P*	M^+ ion peaks	Mp (°C)	ADD[a]
6a	$-C_2H_5$	–Ph	–H	4-NO_2Ph	4.570	381.1	132–136	3
6b	$-C_2H_5$	–Ph	–		2.197	330.1	140–142	3
6c	$-C_2H_5$	–Ph	–H		4.498	371.1	144–145	3
6d	$-C_2H_5$	–Ph	–H	4-OHPh	3.512	352.1	197–199	3
6e	$-C_2H_5$	$-CH_3$	–		1.308	268.1	133–135	2
6f	$-C_2H_5$	$-CH_3$	–H		3.606	302.2	131–132	1
6g	$-CH_3$	–Ph	–		2.611	300.1	172–174	3
6h	$-C_2H_5$	–Ph	–		3.140	314.2	156–158	2
6i	$-C_2H_5$	–Ph	–H		4.495	364.1	132–135	3
6j	$-CH_3$	–Ph	–H		3.966	350.1	162–164	3
6k[b]	$-C_2H_5$	$-CH_3$	–H		4.306	321.1	130–131	1
6l[c]	$-C_2H_5$	$-CH_3$	–H	4-BrPh	4.456	354	147–149	1
6m[c]	$-C_2H_5$	$-CH_3$	–H	4-FPh	3.736	292.1	142–144	1
6n	$-CH_3$	–Ph	–H	4-FPh	4.625	340.1	214–215	3
6o	$-CH_3$	–Ph	–H		3.969	357.1	226–229	3
6p	$-CH_3$	–Ph	–H		4.345	364.2	153–154	3
6q	$-CH_3$	–Ph	–H	Ph	3.650	322.1	183–187	2
6r	$-C_2H_5$	–Ph	$-C_2H_5$	$-C_2H_5$	3.573	316.3	114–116	3
6s	$-CH_3$	–Ph	–H		4.874	379.2	158–159	3
6t	$-CH_3$	–Ph	$-C_2H_5$	$-C_2H_5$	3.044	312.3	140–141	3
6u	$-C_2H_5$	–Ph	–H	4-ClPh	5.195	370.1	220–222	2
6v	$-C_2H_5$	–Ph	–H	4-BrPh	5.345	415	211–214	3
6w	$-C_2H_5$	–Ph	–H	4-FPh	4.625	354.2	208–209	3
6x	$-C_2H_5$	–Ph	–H	Ph	4.179	336	171–172	3

(*continued on next page*)

Table 1 (*continued*)

Compound	R^1	R^2	R^3	R^4	*C* log *P*	M^+ ion peaks	Mp (°C)	ADD[a]
6y	$-C_2H_5$	–Ph	–H		4.874	379.1	65–73	3
6z	$-C_2H_5$	–Ph	–H		5.403	392.2	136–140	3

[a] ADD indicates Anticonvulsant Drug Development program classification for potency of evaluated compounds: class 1 > class 2 > class 3. Class 1, active at 100 mg/kg or less; class 2, active at doses >100 mg/kg and up to 300 mg/kg; class 3, inactive at 300 mg/kg dose. Four enaminones exhibited potent activity: **6f** (class 1 in MES), **6k** (class 1 in MES and SCMET), **6l** (class 1 in MES and SCMET), **6m** (class 1 in MES and SCMET); while another four enaminones showed moderate activity: **6e** (class 2 in SCMET), **6h** (class 2 in MES), **6q** (class 2 in SCMET), and **6u** (class 2 in MES).
[b] Ref. [13].
[c] Ref. [14].

in Table 2 were inactive. These results indicated that specific electronic properties of the enaminones contributed to, but were not the only factors responsible for the anticonvulsant activities of the active enaminones. We had suggested in previous reports that electron-withdrawing properties of moieties, partition coefficient (exemplified by *C* log *P* values), hydrogen bonding, hydrophilic–hydrophobic bonding portions, and steric conformations for receptor binding are important determinants for anticonvulsant activity of enaminones [2,12,16].

2.2. Pharmacology

2.2.1. Anticonvulsant activities of cyclic enaminones

The anticonvulsant evaluation of these enaminones in the Anticonvulsant Drug Development (ADD) program [1] indicated that a total of eight of enaminones displayed' anticonvulsant activity, among which four enaminones **6f**, **6k**, **6l**, and **6m**, showed class 1 activity and the other four active enaminones **6e**, **6h**, **6q** and **6u** showed class 2 activity. Class 1 anticonvulsant enaminones are more potent than class 2 anticonvulsant enaminones, whereas class 3 analogs are inactive at 300 mg/kg doses. The enaminones did not display selective anticonvulsant activity. Compound **6e** exhibited class 2 activity against SCMET seizures, compound **6f** exhibited class 1 activity against MES seizures, compound **6h** exhibited class 2 activity against MES seizures, compounds **6k**, **6l**, and **6m** showed class 1 activity against MES and SCMET seizures, compound **6q** exhibited class 2 activity against SCMET seizures, and compound **6u** exhibited class 2 activity against MES seizures. Thus, the anticonvulsant profile of the enaminones was qualitatively different among its analogs. Generally, anticonvulsant enaminones that are active against the MES test, but not the SCMET test, show a pharmacological profile similar to phenytoin, whereas enaminones that are active against SCMET, but not against the MES test, show a pharmacological profile similar to ethosuximide. Enaminones that are active against both MES and SCMET tests show a pharmacological profile similar to valproate. However, we recently reported that anticonvulsant enaminones have several unique mechanisms of action, as explained below in the structure–activity relationship (SAR) of cyclic enaminones. Enaminones **7a**–**m** including those containing the oxazolidinone moiety were inactive in the ADD evaluation.

2.2.2. Attempted correlation of anticonvulsant activity with C *log* P *data*

The *C* log *P* data for the eight anticonvulsant enaminones (**6e**, **6f**, **6h**, **6k**, **6l**, **6m**, **6q**, and **6u**) in Table 1 ranged from 1.308 to 5.195, which also covered the range for the *C* log *P* values for most of the inactive enaminones in Table 1. In support of our previous report [12], no direct correlation could be established between *C* log *P* values and anticonvulsant activity of the enaminones. Moreover, the eight anticonvulsant enaminones have a variety of electron-withdrawing effects that contributed to their anticonvulsant activity.

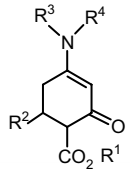
The *C* log *P* values of the heterocyclic enaminones in Table 2 ranged from 2.386 to 5.548, yet none of the 13 enaminones was anticonvulsant. This observation indicated that *C* log *P* data were not very important for the anticonvulsant activity of the enaminones. Instead, the steric hindrance due to the bulky amino groups in these enaminones probably prevented suitable binding with putative receptor sites resulting in compounds that were inactive.

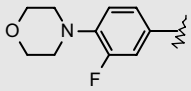
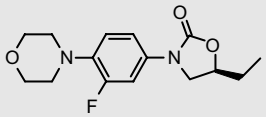
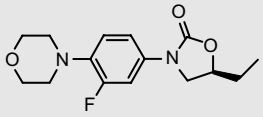
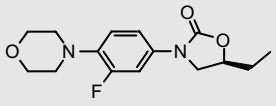
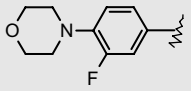
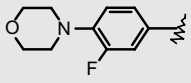
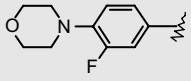
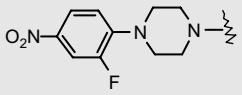
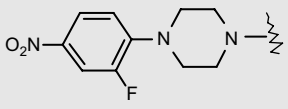
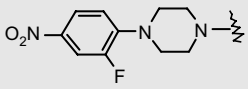
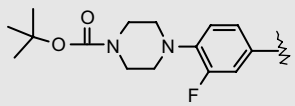
2.2.3. Structure–activity relationship (SAR) of cyclic enaminones

The presence of the enaminone system is essential for anticonvulsant activity, and our current data support our observation that the enaminone pharmacophore existing in a sterically favored conformation is very important for anticonvulsant activity. In this series of enaminones, we observed the rarity of tertiary enaminones (**6e** and **6h**) displaying anticonvulsant activity, although these were only class 2 anticonvulsants and thus were moderately active. However, the NH moiety is still very important for highly potent anticonvulsant activity of secondary enaminones (**6f**, **6k**–**m**). These enaminones were potent class 1 anticonvulsants. The anticonvulsant enaminones in this study were active against SCMET seizures when the substitution at C-6 was phenyl; and protective against MES seizures when the substitution at C-6 was a methyl group. However, the enaminone ester **6q** was an unsubstituted anilino analog which was a class 2 anticonvulsant. The substitution at C-6 in **6q** was a phenyl group. This is the first time we are reporting an unsubstituted anilino enaminone as possessing anticonvulsant activity. The enaminone ester **6u** was a 4-chlorophenylamino analog which was expected to be very highly potent, but it was only a class 2 anticonvulsant. The active compounds were mainly anilino derivatives which have been shown to elevate gamma aminobutyric acid (GABA) levels in the brain, and to reduce the firing frequency of certain neurons in vitro in brain cells or slides [2,13–20].

Eight anticonvulsant enaminones in this study offer new compounds with different mechanisms of action and minimal side effects. We had shown previously that enaminones inhibit excitatory postsynaptic currents (EPSCs) in the brain by enhancing extracellular GABA levels [17]; inhibit tetrodotoxin (TTX)-sensitive currents to modulate excessive firing in individual neurons [18]; and elicit anticonvulsant activity on neuronal network responses by suppressing epileptiform activity corresponding to both ictal and interictal events representative of clinical seizures [20]. From our previous findings, part of the anticonvulsant activity is at the synaptic level involving GABA [17], while part is direct on the

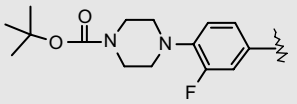
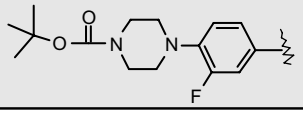
Table 2
Physical constants and anticonvulsant activity of heterocyclic enaminones



Compound	R^1	R^2	R^3	R^4	*C* log *P*	Mp (°C)	ADD[a]
7a	$-CH_3$	$-CH_3$	–H		3.078	189–193	3
7b	$-CH_3$	$-CH_3$	–H		2.386	206–209	3
7c	$-C_2H_5$	$-CH_3$	–H		2.915	202–204	3
7d	$-CH_3$	–Ph	–H		3.275	210–212	3
7e	$-C_2H_5$	$-CH_3$	–H		3.607	164	3
7f	$-CH_3$	–Ph	–H		3.967	220	3
7g	$-C_2H_5$	–Ph	–H		4.496	216	3
7h	$-C_2H_5$	$-CH_3$	–		3.268	162–163	3
7i	$-CH_3$	–Ph	–		3.628	201–202	3
7j	$-C_2H_5$	–Ph	–		4.157	166–167	3
7k	$-CH_3$	–Ph	–H		5.019	222–223	3

(*continued on next page*)

Table 2 (*continued*)

Compound	R^1	R^2	R^3	R^4	*C* log *P*	Mp (°C)	ADD[a]
7l	$-C_2H_5$	–Ph	–H		5.548	196–197	3
7m	$-CH_3$	$-CH_3$	–H		4.130	184–185	3

[a] ADD indicates Anticonvulsant Drug Development program classification for potency of evaluated compounds: class 1 > class 2 > class 3. Class 1, active at 100 mg/kg or less; class 2, active at doses >100 mg/kg and up to 300 mg/kg; class 3, inactive at 300 mg/kg dose.

postsynaptic cell [18]. Enaminones depress population spike (PS) amplitude, and reversibly reduce the number of single PS being transformed into multiple PS in the brain. The multiple spikes following afferent stimulation represent ictal events, while the spontaneous bursts (SB) represent interictal events in seizures. In addition, enaminones suppress the frequency of spontaneous bursts (SB) and suppress afterdischarges (AD) in the brain. The anticonvulsant enaminones in this present study may be acting by one or more of these mechanisms. These properties of enaminones make them good candidates to abort seizures by synaptic and non-synaptic mechanisms, and to prevent additional seizure attacks [20].

2.2.4. Antibacterial activity of selected compounds

The minimum inhibitory concentrations (MIC, μg/ml values) for the selected cyclic enaminones (**7b–e** and **7g**) in comparison to linezolid, **PH-027** and vancomycin are shown in Table 4. Linezolid and **PH-027** are 5-methylacetamido and 5-methyltriazolyl oxazolidines that showed potent antibacterial activity. Unfortunately, none of the new compounds evaluated showed significant

Table 3
UV data of cyclic enaminones in neutral, acidic and alkaline media

Compound	Mol. formula	Mol. wt.	95% EtOH		H_2O		1 M HCl		1 M NaOH	
			λ_{max}	ε	λ_{max}	ε	λ_{max}	ε	λ_{max}	ε
6a	$C_{21}H_{20}O_5N_2$	380.38	284	34 819	282	28 229	259	24 243	283	38 029
6b	$C_{19}H_{23}O_4N$	329.37	303	29 752	308	33 740	296	24 260	308	31 244
6c	$C_{20}H_{19}O_3N_2$	370.84	284	28 889	283	41 208	260	28 662	283	42 731
6d	$C_{21}H_{21}O_4N$	351.96	285	43 895	306	32 096	271	29 217	281	26 104
6e	$C_{14}H_{21}O_4N$	267.20	300	34 469	306	38 288	292	28 515	306	36 058
6f	$C_{18}H_{23}O_3N$	301.18	290	31 209	295	32 600	284	22 661	295	33 026
6g	$C_{18}H_{21}O_3N$	299.37	303	33 413	306	35 204	286	24 270	306	34 700
6h	$C_{19}H_{23}O_3N$	313.40	302	33 590	305	34 914	285	24 300	306	34 377
6i	$C_{23}H_{25}O_3N$	363.46	284	32 042	283	30 932	259	21 574	283	37 679
6j	$C_{22}H_{23}O_3N$	349.43	290	20 197	295	21 679	286	15 579	295	24 162
6k	$C_{16}H_{18}O_3ClN$	319.67	313	30 103	311	31 242	302	26 590	309	29 968
6l	$C_{16}H_{18}O_3BrN$	352.03	314	28 575	314	28 421	306	21 696	311	25 679
6m	$C_{16}H_{18}O_3FN$	291.11	302	22 247	305	24 303	299	20 018	307	23 265
6n	$C_{20}H_{18}O_3NF$	339.34	303	24 403	307	27 010	303	22 672	309	26 159
6o	$C_{19}H_{17}O_3N_2Cl$	356.79	334	30 068	332	29 275	336	20 071	315	19 854
6p	$C_{23}H_{25}O_3N$	363.44	292	26 279	297	29 096	285	22 432	297	30 621
6q	$C_{20}H_{19}O_3N$	321.35	311	26 358	310	28 323	305	22 748	308	26 519
6r	$C_{19}H_{25}ON$	315.4	283	27 448	261	24 787	283	27 276	282	26 946
6s	$C_{24}H_{28}ON$	378.47	291	35 350	296	37 331	284	28 388	296	36 204
6t	$C_{18}H_{23}O_3N$	311.4	284	28 813	283	27 916	259	18 794	283	27 653
6u	$C_{21}H_{20}O_3NCl$	369.84	315	22 533	314	26 563	308	20 922	312	24 956
6v	$C_{21}H_{20}O_3NBr$	414.29	316	28 367	315	30 067	309	23 658	313	29 029
6w	$C_{21}H_{20}O_3NF$	353.37	303	22 668	306	24 714	302	20 657	309	23 811
6x	$C_{21}H_{21}O_3N$	335.38	310	23 873	311	25 450	304	20 260	309	24 333
6y	$C_{24}H_{28}O_3N$	378.47	292	31 473	297	32 385	285	23 996	297	31 935
6z	$C_{25}H_{29}O_3N$	391.49	291	30 604	296	32 000	284	24 546	296	31 281
7a	$C_{19}H_{23}FN_2O_4$	362.40	316	27 739	317	29 696	319	24 375	310	29 007
7b	$C_{23}H_{28}FN_3O_6$	461.49	287	47 541	292	44 495	285	38 432	292	43 232
7c	$C_{24}H_{30}FN_3O_6$	475.52	287	34 805	291	32 638	285	28 295	293	31 533
7d	$C_{28}H_{30}FN_3O_6$	523.57	288	35 274	293	32 216	289	28 968	294	31 153
7e	$C_{20}H_{25}FN_2O_4$	376.43	318	23 602	318	25 318	318	20 993	311	25 078
7f	$C_{24}H_{25}FN_2O_4$	424.48	319	22 296	318	23 921	321	21 355	293	27 633
7g	$C_{25}H_{27}FN_2O_4$	438.50	319	22 670	319	24 794	320	23 403	314	24 926
7h	$C_{20}H_{24}FN_3O_5$	405.43	301	23 549	306	22 738	291	11 759	306	20 446
7i	$C_{24}H_{24}FN_3O_5$	453.46	303	25 150	308	25 336	294	12 382	308	20 568
7j	$C_{25}H_{26}FN_3O_5$	467.48	302	21 050	308	21 300	293	10 773	308	18 582
7k	$C_{29}H_{34}FN_3O_5$	523.58	320	27 350	319	28 766	321	26 052	313	28 966
7l	$C_{30}H_{35}FN_3O_5$	536.60	319	21 881	319	22 914	321	20 393	313	23 043
7m	$C_{24}H_{31}FN_3O_5$	460.51	318	26 282	317	27 968	311	27 068	319	22 359

Table 4
Antibacterial activity of heterocyclic enaminones against susceptible and resistant clinical isolates

Compound	*C* log P[a] (measured)[b]	MIC (μg/ml) ranges for							
		MSSA ($n=11$)	MRSA ($n=10$)	MS-CNS ($n=8$)	MR-CNS ($n=3$)	*H. influenzae* ($n=7$)	*S. pn* ($n=6$)	VSE ($n=7$)	VRE ($n=4$)
7b	2.3865	>16	>16	>16	>16	>16	>16	>16	>16
7c	2.9155	>16	>16	>16	>16	>16	>16	>16	>16
7d	3.2755	>16	>16	>16	>16	>16	>16	>16	>16
7e	3.6074	>16	>16	>16	>16	>16	>16	>16	>16
7g	4.4964	>16	>16	>16	>16	>16	>16	>16	>16
PH-027	0.6308	0.5–1	0.5–2	1	0.5–1	32	0.5–1	0.5–2	0.5–2
Lzd	0.76 (0.5321)	2	0.5–2	0.5–1	0.5–1	8	0.5–1	1–2	1–2
Van	n.d.	1–2	0.5–2	1–2	2	>32	0.25–1	1–4	8 to >32

[a] Ref. [24].
[b] Value obtained from the Pharmacia & Upjohn Material Safety Data Sheet January 2000.

antibacterial activity against standard and clinical isolates of Gram-positive and Gram-negative bacteria used in the present study. Surprisingly, compounds **7b**–**d** containing the oxazolidinone pharmacophoric group and the morpholino moiety found in linezolid were also devoid of antibacterial activity. This could be due to the bulkiness and lack of planarity of the enaminone ring structure. In addition, compounds **7e**–**g** having the arylmorpholino moiety found in linezolid were also inactive. The enaminones **7b**–**e** and **7g** showed MIC values >16 μg/ml compared to MIC ranges between 0.5 and 8 for linezolid, **PH-027** and vancomycin, respectively (Table 4).

3. Conclusion

In conclusion, four enaminones showed potent anticonvulsant activity, and another four analogs showed moderate anticonvulsant activity against experimentally induced seizures in mice. These anticonvulsant enaminones included tertiary enaminones **6e** and **6h**, and unsubstituted anilino enaminone **6q** that we are reporting to be active for the first time. Enaminone **6f** was particularly potent and selective against MES test. Most of the enaminones exhibited hypsochromic shifts in acidic solutions and bathochromic shifts in alkaline solutions compared to their neutral solutions. Thus, they generally behaved like basic compounds in solution, although a few enaminones exhibited neutral or acidic properties in solution [12,21]. Our current results support our previous report [12] that the anticonvulsant activity of enaminones is not due to electronic effect alone, but it results from a combination of factors which include electronic and steric effects, lipophilicity, and hydrogen bonding. The anticonvulsant enaminones in this study may be acting by synaptic and non-synaptic mechanisms in similar manners as reported recently [2,20]. However, none of the cyclic enaminones showed significant antibacterial activity, probably due to the presence of the bulky 5-methylenaminone and its lack of fit at the receptor site.

4. Experimental

4.1. Characterization

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The Science Analytical Facilities (SAF), Faculty of Science, Kuwait University, performed all the analyses. Elemental analyses were determined on LECO elemental analyzer CHNS 932 apparatus and were within ±0.4% of the calculated values. ^{1}H NMR spectra were recorded on Bruker DPX 400 NMR (400 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra were measured on a Finnigan MAT INCOS XL mass spectrometer. Ultraviolet (UV) spectroscopy of the enaminones was determined on a UV-3101PC spectrophotometer equipped with computerized programs to plot the spectrum as the sample was being run and to printout the (λ_{max}) value for each enaminone. Molar absorptivity (ε) was then calculated for each compound and ε value rounded to nearest hundred [21–23].

Infrared (IR) spectra were recorded on Perkin Elmer System 2000 FT-IR spectrometer using KBr pellets. Column chromatography was carried out with silica gel [Kieselgel 60, 70–230 mesh (Aldrich)]. TLC was conducted on 0.25 mm precoated silica gel plates ($60F_{254}$, Merck). All extracted solvents were dried over Na_2SO_4, followed by evaporation in vacuum. The calculated partition coefficient (*C* log *P*) values were determined by using the CS ChemDraw Ultra version 6.01, computer software by CambridgeSoft.Com [24].

4.2. Syntheses

4.2.1. General procedure for the preparation of the enaminone derivatives

The cyclohexanedione esters (3.312 mmol) were prepared [12,16] and refluxed with the appropriate amino compounds (2.548 mmol) in absolute ethanol or isopropanol (25 ml) as solvent (Scheme 1). 5-Aminomethyl oxazolidinone was prepared according to literature methods [10,11]. Then, the reaction mixture was evaporated to give a residue, which was recrystallized using suitable organic solvents (isopropanol or ethanol) to give crystalline solids. The following are representative reaction conditions, yields and spectroscopic data of representative compounds.

4.2.2. Methyl 4-(3-fluoro-4-morpholinophenylamino)-6-methyl-2-oxocyclohex-3-ene-1-carboxylate (*7a*)

Recrystallization from isopropanol gave a crystalline solid 734 mg (80%), mp 189–193 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.03 (s, 1H, –NH), 6.95–7.08 (m, 3H, phenyl H), 5.20 (s, 1H, vinyl=CH), 3.74 (t, 4H, $J=5.0$ Hz, morpholine H), 3.63 (s, 3H, OCH_3), 2.99 (t, 4H, $J=5.0$ Hz, morpholine H), 2.33–2.51 (m, 4H, cyclohexyl), 1.02 (d, 3H, $J=6.0$ Hz, CH_3). Anal. CHN (%): 62.9, 6.43, 7.73, found (%): 63.31, 6.38, 7.80.

4.2.3. Methyl 4-(((S)-3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methylamino)-6-methyl-2-oxocyclohex-3-enecarboxylate (*7b*)

Recrystallization from ethanol gave a crystalline yellow solid 538 mg (77%), mp 207–209 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.54 (br s, 1H, –HN, exchangeable with D_2O), 7.52 (dd, 1H, $J=3.0$, 15.0 Hz, phenyl H), 7.17 (dd, 1H, $J=1.0$, 10.0 Hz, phenyl H), 7.08 (t, 1H, $J=10.0$ Hz, phenyl H), 5.03 (s, 1H, vinyl H), 4.82 (br s, 1H, oxazolidinone C_5H), 4.12 (t, 1H, $J=9.0$ Hz, C_4H oxazolidinone), 3.74 (t, 4H, $J=4.5$ Hz, morpholinyl), 3.72 (dd, 1H, C_4H oxazolidinone, overlaps partly with morpholinyl H), 3.62 (s, 3H, OCH_3), 3.40–3.44

(m, 2H, $-CH_2$), 2.97 (t, 4H, $J = 4.5$ Hz, morpholinyl), 2.33–2.51 (m, 4H, cyclohexyl), 0.93 (d, 3H, $J = 5.0$ Hz, CH_3). Anal. CHN (%): 59.86, 6.12, 9.11, found (%): 59.47, 6.13, 9.12.

4.2.4. Ethyl 4-(((S)-3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methylamino)-6-methyl-2-oxocyclohex-3-ene-1-carboxylate (**7c**)

Recrystallization from ethanol gave a crystalline pale yellow solid 406 mg (63%), mp 202–204 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.46 (dd, 1H, $J = 2.0$, 14.0 Hz, phenyl H), 7.10 (dd, 1H, $J = 2.0$, 9.0 Hz, phenyl H), 6.94 (t, 1H, $J = 9.0$ Hz, phenyl H), 5.41 (br s, 1H, NH), 5.21 (s, 1H, vinyl H), 4.89 (m, 1H, oxazolidinone C_5H), 4.26 (q, 2H, $J = 7.1$ Hz, $CO_2CH_2CH_3$), 4.10 (tt, 1H, $J = 2.6$, 9.1 Hz, C_4H oxazolidinone), 3.89 (t, 4H, $J = 4.5$ Hz, morpholinyl H), 3.69–3.74 (m, 1H, oxazolidinone H), 3.55–3.58 (m, 1H), 3.43–3.47 (m, 1H), 3.07 (t, 4H, $J = 4.5$ Hz, morpholinyl H), 3.02 (t, 1H, $J = 11.0$ Hz), 2.57–2.60 (m, 1H), 2.40–2.44 (m, 1H), 2.22–2.32 (m, 1H), 1.31 (t, 3H, $J = 7.1$ Hz, $CO_2CH_2CH_3$), 1.09 (dd, 3H, $J = 2.8$, 6.4 Hz, CH_3). Anal. CHN (%): 60.62, 6.36, 8.84, found (%): 60.79, 6.32, 8.90.

4.2.5. Methyl 4-(((S)-3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methylamino)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (**7d**)

Recrystallized from ethanol gave a yellow crystalline solid 467 mg (66%), mp 210–212 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.90–7.45 (m, 8H, phenyl H), 5.74 (br s, 1H, NH), 5.30 (s, 1H, vinyl H), 4.89 (m, 1H, oxazolidinone C_5H), 4.10 (t, 1H, $J = 9.0$ Hz, C_4H oxazolidinone), 3.89 (t, 4H, $J = 4.5$ Hz, morpholinyl H), 3.42–3.74 (m, 8H), 3.06 (t, 4H, $J = 4.5$ Hz, morpholinyl H), 2.72–2.81 (m, 1H), 2.47–2.59 (m, 1H). Anal. CHN (%): 64.23, 5.78, 8.03, found (%): 64.21, 5.84, 8.04.

4.2.6. Ethyl 4-(3-fluoro-4-morpholinophenylamino)-6-methyl-2-oxocyclohex-3-ene-1-carboxylate (**7e**)

Recrystallized from ethanol to give an off-white crystalline solid 500 mg (43.4% yield), mp 164–166 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.90–6.92 (m, 3H, phenyl H), 6.19 (s, 1H, NH), 5.48 (s, 1H, vinyl H), 4.26 (q, 2H, $J = 7.12$ Hz, $CO_2CH_2CH_3$), 3.89 (t, 4H, $J = 4.5$ Hz, morpholinyl H), 3.05–3.10 (m, 5H, enaminone CH and morpholinyl H), 2.62–2.69 (m, 1H, enaminone H), 2.50 (dd, 1H, $J = 4.6$, 16.5 Hz, enaminone H), 2.37 (dd, 1H, $J = 10.9$, 16.3 Hz, enaminone H), 1.31 (t, 3H, $J = 7.12$ Hz, $CO_2CH_2CH_3$), 1.12 (d, 3H, $J = 6.5$ Hz, CH_3). Anal. CHN (%): 63.82, 6.69, 7.44, found (%): 63.62, 6.47, 7.80.

4.2.7. Methyl 4-(3-fluoro-4-morpholinophenylamino)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (**7f**)

Recrystallization from ethanol gave an off-white crystalline solid 740 mg (57% yield), mp 220–222 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28–7.37 (m, 5H, phenyl H), 6.91–6.96 (m, 3H, phenyl H), 6.40 (s, 1H, NH), 5.55 (s, 1H, vinyl H), 3.89 (t, 4H, $J = 4.5$ Hz, morpholinyl H), 3.75 (dd, 1H, $J = 4.3$, 12 Hz, enaminone CH), 3.68 (d, 1H, $J = 12.0$ Hz, enaminone H), 3.55 (s, 3H, CO_2CH_3), 3.09 (t, 4H, $J = 4.5$ Hz, morpholinyl H), 2.87 (dd, 1H, $J = 12$, 16.5 Hz, enaminone H), 2.61 (dd, 1H, $J = 4.3$, 16.6 Hz, enaminone H). Anal. CHN (%): 67.91, 5.60, 6.60, found (%): 67.23, 5.92, 7.01.

4.2.8. Ethyl 4-(3-fluoro-4-morpholinophenylamino)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (**7g**)

Recrystallization from ethanol gave an off-white crystalline solid 700 mg (52% yield), mp 216–218 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28–7.37 (m, 5H, phenyl H), 6.92–6.95 (m, 3H, phenyl H), 6.12 (s, 1H, NH), 5.56 (s, 1H, vinyl H), 4.01–4.05 (m, 2H, $CO_2CH_2CH_3$), 3.90 (t, 4H, $J = 4.5$ Hz, morpholinyl H), 3.77 (dd, 1H, $J = 4.3$, 12 Hz, enaminone CH), 3.67 (d, 1H, $J = 12.0$ Hz, enaminone H), 3.10 (t, 4H, $J = 4.5$ Hz, morpholinyl H), 2.89 (dd, 1H, $J = 12$, 16.5 Hz, enaminone H), 2.60 (dd, 1H, $J = 4.3$, 16.6 Hz, enaminone H), 1.03 (t, 3H, $CO_2CH_2CH_3$). Anal. CHN (%): 68.48, 6.21, 6.39, found (%): 68.31, 6.13, 6.79.

4.2.9. Ethyl 4-(4-(2-fluoro-4-nitrophenyl)piperazin-1-yl)-6-methyl-2-oxocyclohex-3-ene-1-carboxylate (**7h**)

Recrystallization from isopropanol gave a yellow crystalline solid 1.42 g (79% yield), mp 162–163 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.03 (dd, 1H, $J = 2.5$, 8.6 Hz, phenyl H), 7.96 (dd, 1H, $J = 2.6$, 12.9 Hz, phenyl H), 6.94 (t, 1H, $J = 8.7$ Hz, phenyl H), 5.36 (s, 1H, vinyl H), 4.27 (q, 2H, $J = 7.1$, 14.2 Hz, $CO_2CH_2CH_3$), 3.52–3.63 (m, 4H, piperazinyl H), 3.36–3.43 (m, 4H, piperazinyl H), 3.03 (d, 1H, $J = 11.0$ Hz, enaminone CH), 2.69 (dd, 1H, $J = 4.7$, 16.0 Hz, enaminone H), 2.57–2.64 (m, 1H, enaminone H), 2.17 (dd, 1H, $J = 10.2$, 16.0 Hz, enaminone H), 1.37 (t, 3H, $J = 7.12$ Hz, $CO_2CH_2CH_3$), 1.14 (d, 3H, $J = 6.4$ Hz, CH_3). Anal. CHN (%): 63.14, 7.21, 7.22, found (%): 63.02, 6.97, 7.50.

4.2.10. Methyl 4-(4-(2-fluoro-4-nitrophenyl)piperazin-1-yl)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (**7i**)

Recrystallization from isopropanol gave a yellow crystalline solid 1.08 g (67% yield), mp 201–203 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.03 (dd, 1H, $J = 2.5$, 8.6 Hz, phenyl H), 7.95 (dd, 1H, $J = 2.6$, 12.9 Hz, phenyl H), 7.28–7.39 (m, 5H, phenyl H), 6.93 (t, 1H, $J = 8.7$ Hz, phenyl H), 5.45 (s, 1H, vinyl H), 3.53–3.75 (m, 6H, piperazinyl H and enaminone H, overlaps with CH_3 signal at 3.59 ppm), 3.59 (s, 3H, CH_3, overlaps with piperazinyl and enaminone H at 3.53–3.75 ppm), 3.38–3.41 (m, 4H, piperazinyl H), 2.85 (dd, 1H, $J = 4.3$, 16.0 Hz, enaminone H), 2.64 (dd, 1H, $J = 10.4$, 16.0 Hz, enaminone H). Anal. CHN (%): 63.57, 5.33, 9.27, found (%): 63.27, 5.79, 9.47.

4.2.11. Ethyl 4-(4-(2-fluoro-4-nitrophenyl)piperazin-1-yl)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (**7j**)

Recrystallized from propanol to give a yellow crystalline solid 1.26 g (91% yield), mp 166–167 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.02 (dd, 1H, $J = 2.5$, 8.6 Hz, phenyl H), 7.95 (dd, 1H, $J = 2.6$, 12.9 Hz, phenyl H), 7.26–7.38 (m, 5H, phenyl H), 6.94 (t, 1H, $J = 8.7$ Hz, phenyl H), 5.44 (s, 1H, vinyl H), 4.27 (q, 2H, $J = 7.1$, 14.2 Hz, $CO_2CH_2CH_3$), 3.68–3.76 (m, 2H, enaminone H), 3.52–3.65 (m, 4H, piperazinyl H), 3.22–3.43 (m, 4H, piperazinyl H), 2.86 (dd, 1H, $J = 4.3$, 16.0 Hz enaminone H), 2.64 (dd, 1H, $J = 10.4$, 16.0 Hz, enaminone H), 1.04 (t, 3H, $J = 7.12$ Hz, $CO_2CH_2CH_3$). Anal. CHN (%): 64.23, 5.61, 8.99, found (%): 64.10, 5.79, 9.17.

4.2.12. tert-Butyl 4-(2-fluoro-4-(3-hydroxy-4-(methoxycarbonyl)-5-phenylcyclohex-1-enylamino)phenyl)piperazine-1-carboxylate (**7k**)

Recrystallized from isopropanol to give a greenish crystalline solid, 1.29 g (79% yield), mp 222–223 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.92–7.37 (s, 9H, phenyl H and NH), 5.63 (s, 1H, vinyl H), 3.69–3.79 (q, 2H, enaminone H), 3.63 (br s, 4H, piperazinyl H), 3.57 (s, 3H, CO_2CH_3), 3.05 (br s, 4H, piperazinyl H), 2.86 (dd, 1H, $J = 4.3$, 16.0 Hz, enaminone H), 2.64 (dd, 1H, $J = 10.4$, 16.0 Hz, enaminone H), 1.50 (s, 9H, $C(CH_3)_3$). Anal. CHN (%): 66.52, 6.55, 8.03, found (%): 66.35, 6.44, 8.17.

4.2.13. tert-Butyl 4-(4-(4-(ethoxycarbonyl)-3-oxo-5-phenylcyclohex-1-enylamino)-2-fluorophenyl)piperazine-1-carboxylate (**7l**)

Recrystallization from isopropanol gave a greenish crystalline solid, 1.15 g (73% yield), mp 196–197 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.90–7.35 (m, 9H, phenyl H), 6.71 (s, 1H, NH), 5.56 (s, 1H, vinyl H), 4.27 (q, 2H, $J = 7.1$, 14.2 Hz, $CO_2CH_2CH_3$), 3.69–3.75 (q, 2H, enaminone H), 3.62 (s, 4H, piperazinyl H), 3.01 (s, 4H, piperazinyl H), 2.86 (dd, 1H, $J = 4.3$, 16.0 Hz, enaminone H), 2.62 (dd, 1H, $J = 10.4$,

16.0 Hz, enaminone H), 1.50 (s, 9H, $C(CH_3)_3$), 1.04 (t, 3H, $J = 7.12$ Hz, $CO_2CH_2CH_3$). Anal. CHN (%): 67.02, 6.75, 7.82, found (%): 67.09, 6.53, 8.10.

4.2.14. tert-*Butyl 4-(2-fluoro-4-(4-(methoxycarbonyl)-5-methyl-3-oxocyclohex-1-enylamino)phenyl)piperazine-1-carboxylate (**7m**)*

Recrystallized from isopropanol gave a crystalline solid, 1.28 g (67% yield), mp 184–185 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.91–6.99 (m, 4H, phenyl H and NH), 5.56 (s, 1H, vinyl H), 3.78 (s, 3H, CO_2CH_3), 3.62 (s, 4H, piperazinyl H), 3.05–3.12 (m, 1H, enaminone H, overlaps partly with the piperazinyl singlet at 3.05), 3.05 (s, 4H, piperazinyl H), 2.60–2.68 (m, 1H, enaminone H), 2.57 (dd, 1H, $J = 4.3$, 16.0 Hz, enaminone H), 2.38 (dd, 1H, $J = 10.4$, 16.0 Hz, enaminone H), 1.50 (s, 9H, $C(CH_3)_3$), 1.13 (d, 3H, $J = 6.4$ Hz, CH_3). Anal. CHN (%): 62.46, 6.99, 9.12, found (%): 62.33, 6.71, 9.40.

4.3. In vivo anticonvulsant evaluation of enaminones

The enaminones synthesized were submitted for evaluation by the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological Disorders and Stroke [1]. The antiepileptic drug development (ADD) program initially evaluated anticonvulsant activity for the newly submitted compounds following intraperitoneal (i.p.) administration in mice and oral administration in rats. Two convulsant tests and a toxicity screen are employed for preliminary evaluation. The Maximal Electroshock Seizure (MES) or Maximal Pattern Test is a model for generalized tonic–clonic seizures, while the Subcutaneous Pentylenetetrazol (metrazol) (SCMET) test is a model that identifies anticonvulsant compounds that would raise seizure threshold. Neurotoxicity was evalauated using the standardized rotorod test. Untreated control mice, when placed on a 6 rpm rotorod, can maintain their equilibrium for a prolonged period of time. Neurological impairment can be demonstrated by the inability to maintain equilibrium for a minute in each of three successive trials. Data for the anticonvulsant evaluations are shown in Tables 1 and 2.

4.4. Microbiology

4.4.1. In vitro antibacterial evaluations

The clinical isolates used in the study were obtained from culture collection maintained at the MRSA Reference Laboratory, Faculty of Medicine, Kuwait University. Antibacterial susceptibility testing was performed by disk diffusion and the agar dilution methods as described by the National Committee for Clinical Laboratory Standards (now Clinical Laboratory Standard Institute) [25]. Minimum inhibitory concentrations (MICs, μg/ml) were determined on Mueller–Hinton (MH) agar with medium containing dilutions of antibacterial agents ranging from 0.12 to 16 μg/ml. The test compounds were dissolved in 80% DMSO in water while linezolid and vancomycin were dissolved in 60% ethanol in water and water, respectively. The tests were performed using MH agar plates for all staphylococci and enterococci, and on MH agar plates supplemented with 5% sheep blood to facilitate the growth of *S. pneumoniae*, and *Haemophilus influenzae*. The Gram-positive organisms utilized in this study consisted of methicillin-resistant *S. aureus* (MRSA, $n = 10$), methicillin-susceptible *S. aureus* (MSSA, $n = 11$), methicillin-resistant coagulase-negative staphylococci (MR-CNS, $n = 3$), methicillin-sensitive coagulase-negative staphylococci (MS-CNS, $n = 8$), *S. pneumoniae* ($n = 6$), vancomycin-sensitive (VSE, $n = 7$) and vancomycin-resistant (VRE, $n = 4$) enterococci. The Gram-negative organisms included *H. influenzae* ($n = 7$) clinical isolates and *Escherichia coli* ATCC 25922. The reference strains, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *Enterococcus faecalis* ATCC 29212, *E. coli* (ATCC 25922) and *H. influenzae* ATCC 49247 were used to control the tests. The final bacterial concentration for inocula was 10^7 CFU/ml, and was incubated at 35 °C for 18 h. The MIC was defined as the lowest drug concentration that completely inhibited growth of the bacteria. **PH-027**, prepared according to literature methods [10,11], and linezolid and vancomycin obtained from commercial sources were used as reference antibacterial agents.

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