Journal of Organometallic Chemistry 696 (2011) 3703–3713

Contents lists available at SciVerse ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Antibacterial 3-(arylamino)-1-ferrocenylpropan-1-ones: Synthesis, spectral, electrochemical and structural characterization

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ARTICLE INFO

Article history:
Received 22 June 2011
Received in revised form
17 August 2011
Accepted 18 August 2011

Keywords:
3-(Arylamino)-1-ferrocenylpropan-1-ones
Mannich bases
Acryloylferrocene
Montmorillonite K-10
Microwave irradiation
Antibacterial activity

ABSTRACT

Syntheses of fourteen new 3-(arylamino)-1-ferrocenylpropan-1-ones have been achieved in good to excellent yields by an aza-Michael addition of different arylamines to acryloylferrocene. The reaction was performed by microwave (MW) irradiation (500 W/5 min) of a mixture of reactants and montmorillonite K-10, without a solvent. The obtained compounds were spectrally and electrochemically (cyclic voltammetry) fully characterized, whereas single-crystal X-ray analysis has been performed for three of them. In a microdilution assay, all of the compounds were shown to have a broad-spectrum effect on Gram-negative and -positive bacteria, although the degree of inhibition varied. A notable activity was observed for all compounds in inhibiting the growth of an important human pathogen *Staphylococcus aureus*.

1. Introduction

Derivatives of ferrocene are of widespread interest in many fields of chemistry, such as organic synthesis [1], coordination chemistry [2], material sciences [1,3,4] and, nowadays, medicinal chemistry [5–10]. This is the consequence of several unique features of these compounds, such as the easiness of derivatization, the outstanding stability in both, aqueous and non-aqueous media, very interesting redox properties, etc. Multifunctional derivatives of this metallocene are particularly valuable, and in this regard Mannich bases (Mannich ketones; β-aminoketones) containing a ferrocene unit might be very interesting.

In general, Mannich bases are versatile synthetic building blocks, which can easily be converted into a range of useful derivatives, such as 1,3-aminoalcohols and products of the substitution of the amino group with some other nucleophile [11,12]. Among many applications of Mannich bases and their derivatives, however, the most important ones are surely those applied in synthesis of pharmaceuticals [11,13–15]. The most famous synthetic approach to Mannich bases is, of course, the Mannich reaction [11,12,16] which, however, has many disadvantages. Drastic reaction conditions and long reaction times (causing many side reactions) are the main ones [11,12,16]. Furthermore, the use of primary amines in this reaction is not suitable, since the obtained products are also good substrates of the same reaction that continues up to the substitution of both hydrogen atoms of the amine group giving, thus, tertiary amines containing two 3-oxo-groups. A very good alternative to the Mannich reaction is the aza-Michael addition – the conjugate addition of amines to the olefinic bond of α,β-unsaturated carbonyls [17]. There are several advantages of this reaction, such as the mild reaction conditions and the possibility to synthesize secondary Mannich bases. A plethora of catalytic systems have been developed for this reaction up to date [18–39]. While the addition of aliphatic amines to Michael acceptors proceeds readily (even without a catalyst [40,41]), aromatic ones did not undergo this reaction easily because of its lower nucleophilicity, particularly when mild conditions and environmental friendly catalysts were used [22,29,31,36,39].

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doi:10.1016/j.jorganchem.2011.08.016

According to the best of our knowledge, there is only one previous report on the addition of amines to some α,β-unsaturated acylferrocenes [31], where the authors described the reaction of several chalcone-type ferrocenes with aliphatic amines under mild conditions (ultrasound irradiation and water as the solvent). The corresponding β-aminoketones were obtained in high yields. However, the reaction failed when aromatic amines were used as the Michael-donors. In continuation of our permanent interest in the synthesis of ferrocene derivatives containing more than one heteroatom in the side chain (interesting from the both the synthetic and medicinal chemistry points of view) [42–48], herewith we wish to report on a suitable synthesis of a series of 3-(arylamino)-1-ferrocenylpropan-1-ones by the addition of various aromatic amines to 1-ferrocenylprop-2-en-1-one (acryloylferrocene).

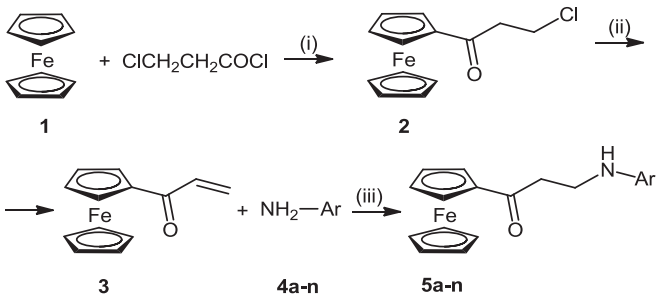
2. Results and discussion

2.1. Synthesis

The primary goal of our study was to find and optimize a procedure for the synthesis of 3-(arylamino)-1-ferrocenylpropan-1-ones with particular attention paid to finding mild enough conditions for the reaction to proceed with an acceptable yield and making use of an environmental friendly catalyst. Knowing that the application of environmentally benign catalysts such as clay [36] and mild reaction conditions [31,39] does not make possible the addition of aromatic amines to Michael acceptors (contrary to aliphatic ones), we expected that the simultaneous use of montmorillonite K-10 (as a solid acidic catalyst) and microwave irradiation would improve the outcome of this approach. It turned out that this idea was quite correct, and that the corresponding Mannich bases were obtained in good to almost quantitative yields.

Our investigations began by the preparation of the intended Michael acceptor – acryloylferrocene (**3**, Scheme 1). This was achieved by Friedel–Crafts acylation of ferrocene (**1**) with 3-chloropropanoic acid chloride in the presence of $AlCl_3$ as the Lewis acid catalyst [49], and the subsequent dehydrohalogenation of the obtained (3-chloropropionyl)ferrocene (**2**) by means of potassium acetate [50].

In order to optimize the synthesis of the title compounds (**5a**–**n**, Scheme 1), aniline (**4a**) was used as the test substrate for the addition to the conjugated enone – acryloylferrocene (**3**). Thus, when ketone **3** (1 mmol) and amine **4a** (1 mmol) were irradiated in a microwave oven (500 W, 5 min) without a catalyst or solvent, and after the usual work-up and flash chromatography (silica gel/toluene, then *n*-hexane–ethyl acetate 9:1), the pure β-aminoketone **5a** (Scheme 1) was obtained in 37% yield. The same result was achieved by a prolongation of the reaction time to 10 min. The next two experiments we performed by irradiating the same mixture of reagents in the presence of 100 mg of montmorillonite K-10 (500 W, 5 and 10 min), and this resulted in an increase of the yield of **5a** up to 55%. Experiments with the increased amount of the catalyst (up to 500 mg) did not affect the yield significantly. Since only small amounts of the starting ketone **3** have been recovered (up to 10%) from the above performed experiments, we concluded that this compound underwent a certain side reaction (most probably some kind of polymerization, because a very polar dark product, which was neither isolated nor identified, formed during the runs). This side product may be the result of multiple Michael additions of the formed β-aminoketones to more molecules of acryloylferrocene, perhaps even leading through tertiary amines to quaternary ammonium salts that would be expected to behave in this way. In order to (statistically) suppress this, the following experiments were performed using a double amount of the amine **4a**. The target compound – β-aminoketone **5a** – was obtained in 85% yield, regardless the reaction time (5 or 10 min).



Scheme 1. Synthesis of 3-(arylamino)-1-ferrocenylpropan-1-ones: (i) $AlCl_3$, CH_2Cl_2, r.t. (ii) CH_3COOK, ethanol, reflux, 2.5 h. (iii) solvent-free, montmorillonite K-10, MW, 500 W, 5 min.

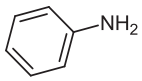
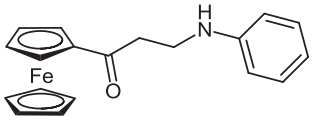
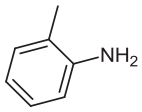
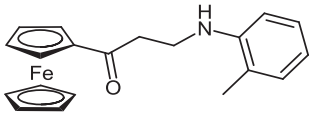
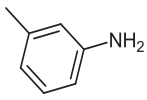
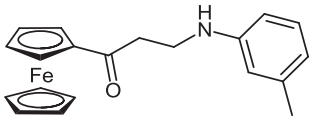
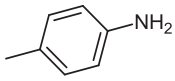
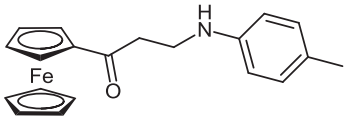
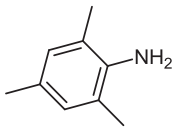
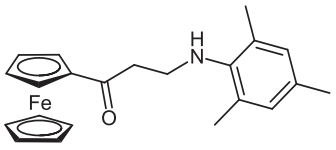
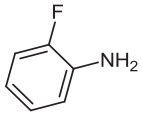
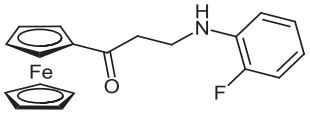
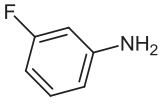
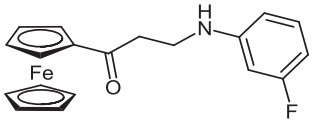
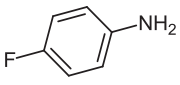
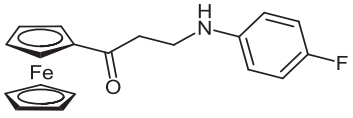
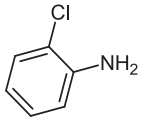
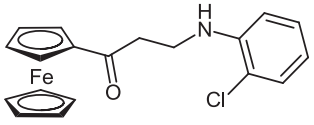
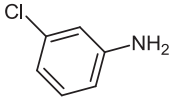
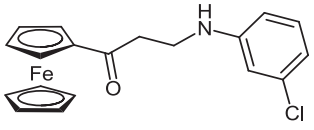
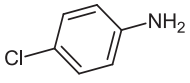
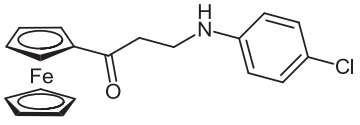
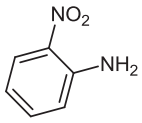
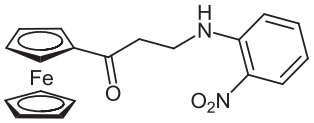
Then the same reaction conditions (1 mmol of acryloylferrocene/2 mmol of arylamine/100 mg of montmorillonite K-10/500 W/5 min) were applied to the reaction of the ketone **3** with the another thirteen substrates **4b**–**n**. The corresponding Mannich bases **5b**–**n** were obtained in good to excellent yields (see Table 1) and were fully spectrally characterized (see below).

As it can be seen from the data listed in Table 1, the lowest yields of the corresponding Mannich bases were achieved when amines **4e**, **4l** and **4n** were used. In the case of **4e** the steric nature (bulkiness) of the substrate is the most likely reason for it, whereas the lowered nucleophilicity of the amino group of the two nitroanilines causes the decrease in the yields of **4l** and **4n**. In order to try to improve the yields in these cases, we performed experiments having a prolonged time of exposure of the reactants and the catalyst to MW irradiation. However, not even doubling the reaction time to 10 min did not cause an increase in obtained yields. Again, the unconsumed amines were recovered almost quantitatively and almost no ketone **3**, further strengthening the notion of multiple Michael additions.

2.2. Spectral characterization

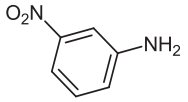
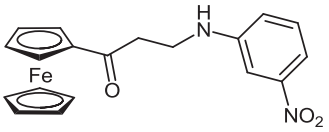
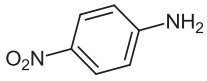
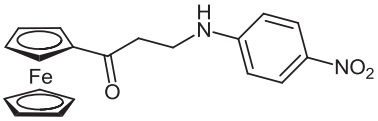
Intense absorption bands were present in the IR spectra of the obtained 3-(*N*-arylamino)propanones **5** for the C=O (at around 1660 cm^{-1}) and secondary NH groups (3340–3390 cm^{-1}, sharp). The ^1H NMR spectra contained typical signals for a monosubstituted ferrocene (two triplets at ~4.76 and 4.50 ppm, and a singlet at ~4.12 ppm) and were also characterized by the presence of two multiplet signals for the protons of the O=C–CH_2–CH_2–N grouping, which were positioned in the region of 3.25–3.83 and 2.97–3.12 ppm (generally in agreement with a previous report [51]). An interesting feature of the ^1H NMR spectra was the occurrence of coupling of the NH protons with those of the adjacent CH_2 group. The N–CH_2 signals appeared as either sharp or somewhat broadened quartets in a number of cases (the *ortho*- and *meta*-substituted anilines with an electron-withdrawing group) from accidental equivalence of the vicinal HN–CH_2 and CH_2–CH_2 couplings (*J ca.* 6 Hz). Such a coupling was not observed for the benzene analogs where the CH_2 group bonded to amino showed little indication of coupling to the NH protons, so NH exchange must have been rapid on the NMR time scale [52] as also seems to be the case with compounds **5a**–**e**,**h**,**n** in the current study. The proton of the secondary amino group was a broadened signal at 3.6–4.8 ppm, typical of NH protons of anilines in $CDCl_3$ solution, with the only exception for compound **5l**, an *o*-nitroaniline (*ca.* δ 8) that had, as expected, an NH signal downfield of this range. The broadening has several sources: partially averaged coupling to neighboring protons, intermolecular exchange with other NH

Table 1
Structures of the newly prepared 3-(arylamino)-1-ferrocenylpropan-1-ones (**5a**–**n**) and corresponding starting amines (**4a**–**n**), as well as the yield of the reactions.

Run	Amine		Product		Yield[a]
1		**4a**		**5a**	85%
2		**4b**		**5b**	89%
3		**4c**		**5c**	80%
4		**4d**		**5d**	95%
5		**4e**		**5e**	59%
6		**4f**		**5f**	98%
7		**4g**		**5g**	89%
8		**4h**		**5h**	83%
9		**4i**		**5i**	93%
10		**4j**		**5j**	87%
11		**4k**		**5k**	93%
12		**4l**		**5l**	64%

(*continued on next page*)

Table 1 (*continued*)

Run	Amine	Product	Yield[a]
13	 **4m**	 **5m**	87%
14	 **4n**	 **5n**	64%

[a] Isolated yields based on the starting enone.

protons, and partially coalesced coupling to the quadrupolar ^{14}N nucleus ($I = 1$), which usually has a short T_1. The labile NH protons were identified by shaking the $CDCl_3$ solutions of the compounds with a drop of D_2O, which resulted in the disappearance of the NH signals and in the transformation of the mentioned quartets (NH–CH_2) to the corresponding triplets, thus corroborating the existence of such NH–CH_2 coupling. It appears that this slow chemical exchange is more pronounced for the more acidic NH protons and those involved in intramolecular hydrogen bonding (the proximity of the electron-withdrawing groups, halogens and the nitro group, in *ortho*- and *meta*-positions).

The aromatic protons in the positions *ortho* to the amino group of the aniline fragment afford diagnostic signals from their high field disposition (at 6.6–6.8 ppm). ^{13}C NMR spectra also corroborate the structure of these ferrocene derivatives. Signals at *ca.* 78.7, 72.4, 69.8 and 69.2 can be attributed to the ferrocene moiety while the other characteristic signals, one at about 200 ppm and two about 38 ppm, are those corresponding to the carbonyl- and methylene carbons, respectively.

2.3. X-ray crystal structure of *5j*, *5l* and *5m*

Most of the synthesized compounds were crystal substances, suitable for X-ray crystal structure analysis. Herein, we present the structures of **5j**, **5l** and **5m** compounds (Fig. 1a–c).

The cyclopentadienyl rings (Cp) of the title compounds **5j** and **5m** are close to an eclipsed geometry. The C1–Cg1–Cg2–C6 torsion angle is 9.5(5)° in **5j** and 11.3(5)° in **5m** (Cg1 and Cg2 are centroids of the corresponding Cp rings). In **5l**, the Cp rings are more eclipsed and the C1–Cg1–Cg2–C6 torsion angle is −4.0(4)°. In all three compounds the Cp rings within the ferrocenyl units are almost parallel with interplanar angles 1.1(4), 2.3(4) and 0.7(5)° for **5j**, **5m** and **5l**, respectively. The Cg1–Cg2 distance (3.295, 3.309 and 3.301 Å) and the Cg1–Fe–Cg2 angle (178.5, 177.4 and 178.5°) are also very similar for all crystal structures.

The C1=O1 carbonyl group lies approximately in the plane of the substituted Cp ring with the O1–C11–C1–C5 angle −4.5(8), −3.2(8) and −6.9(9)° for the three compounds, respectively. Bond lengths and angles show expected values (Table 2). The C1–C11–C12–C13–N1 fragment, although consisted of single bonds, adopts a similar conformation in all three molecules (Fig. 1a–c). The C1–C11–C12–C13 and the C11–C12–C13–N1 torsion angles are −167.5(4)/74.4(6), −164.4(4)/67.4(7) and −179.2(5)/71.8(7)° for **5j**, **5m** and **5l**, respectively. However, regardless of this similarity, the directionality of N1–C14 bond and the resulting orientation of the C14-phenyl ring are quite different for compound **5l**. The C12–C13–N1–C14 torsion angle describes this difference (74.2(6), 68.7(8) and −175.7(6)° for **5j**, **5m** and **5l**, respectively). The orientation of the phenyl ring with respect to the ferrocenyl unit is well illustrated in Supplementary material (Fig. A1). This conformational particularity of the structure **5l** could be explained by the formation of the N1–H…O2 intramolecular hydrogen bond (Fig. 1c) which does not exist in **5j** and **5m**.

The only significant H-bond donor in all three crystal structures is the N1–H group. In **5j** and **5m**, adjacent molecules form centrosymmetric dimers by hydrogen bonding between N1–H and O1. Geometry of these dimers for both crystal structures is very similar (see Fig. A2 in Supplementary material). In **5l**, the N1–H does not participate in any intermolecular H-bonding. Geometrical parameters for the selected intra- and intermolecular interactions are given in Table 3.

2.4. Electrochemistry

Cyclic voltammetry in acetonitrile containing 0.1 mol/l lithium perchlorate as the supporting electrolyte has been used for the evaluation of electrochemical properties of the compounds **5a**–**n**. The voltammogram of compound **5a** is presented here (Fig. 2) as a representative example, whereas the data of the other compounds are listed in Table 4. As it can be seen from the summarized data, all of the synthesized β-aminoketones exhibited two well defined oxidation waves on the forward potential sweep (O1, at 0.650–0.693 V and O2, at 0.693–1.373 V, respectively) and one reduction wave on the back potential sweep (R1, at 0.592–0.620 V). As depicted in Fig. 2B for **5a**, the reduction peak R1 appeared also when the potential was reversed after O1. Since the difference between the values of these two potentials is close to the theoretical one, O1 and R apparently belong to a reversible redox couple, appearing due to the presence of the ferrocene nucleus. Their position lays more than 200 mV higher than that of the unsubstituted ferrocene (see Fig. A3 in the Supplementary material), as expected for ferrocene derivatives possessing an electron-withdrawing group conjugated to the cyclopentadienyl ring(s). Both the anodic (O1) and cathodic (R1) peak currents are proportional to the square root of the scan rate (as depicted for **5a**, Fig. A4 in Supplementary material), and their ratio is independent of the scan rate, indicating a diffusion-controlled process.

The second oxidation wave (O2) is due to an irreversible oxidation of the aniline unit of these molecules. A study of the

cyclovoltammetry of *N*-alkylanilines [53–55] showed that these compounds undergo irreversible anodic oxidation, producing a single oxidation wave on the first scan. Upon reversal of the scan three cathodic waves are obtained (at the potentials between 0.2 and 0.5 V). In the subsequent anodic scans three anodic waves appeared at the corresponding potentials, making up together with the cathodic ones three reversible couples. It was demonstrated that they belong to the products obtained from the species formed by the anodic oxidation of anilines [53–55]. We assumed that the lack of such type of waves in the cyclovoltammograms of compounds **5a**–**n** is a consequence of their accidental overlapping with the waves belonging to the ferrocene unit. In order to confirm

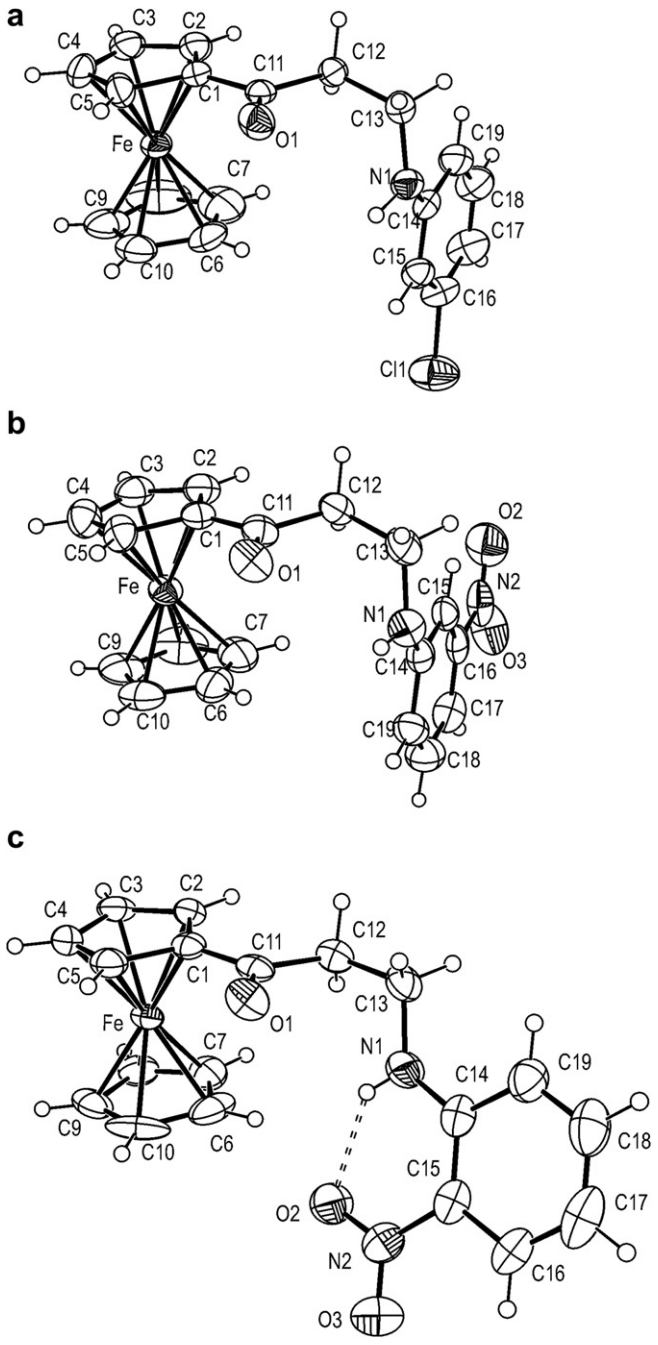


Fig. 1. The molecular structure of **5j** (a), **5m** (b) and **5l** (c; N1–H…O2 is labeled by dashed lines) with the atom numbering scheme. Displacement ellipsoids are drawn at the 50% probability level.

Table 2
Selected bond lengths and angles for **5j**, **5m** and **5l**.

	5j	5m	5l
Bond lengths (Å)			
O1–C11	1.223(6)	1.220(6)	1.218(6)
N1–C14	1.380(8)	1.358(7)	1.351(8)
N1–C13	1.440(7)	1.454(7)	1.452(8)
C1–C11	1.462(7)	1.457(8)	1.459(8)
C11–C12	1.497(7)	1.514(8)	1.507(8)
C12–C13	1.519(7)	1.523(8)	1.519(8)
C16–Cl	1.731(6)		
C–N2		1.470(8)	1.440(9)
N2–O2		1.217(7)	1.237(7)
N2–O3		1.237(7)	1.223(7)
Bond angles (°)			
O1–C11–C1	122.3(5)	122.3(6)	121.6(5)
O1–C11–C12	120.4(5)	121.4(5)	120.4(6)
C1–C11–C12	117.2(5)	116.3(5)	117.9(5)
C11–C12–C13	113.7(4)	112.7(5)	113.2(5)
N1–C13–C12	113.6(5)	113.8(5)	109.6(5)
C14–N1–C13	122.8(5)	123.3(5)	124.6(6)

it, 4-(phenylamino)butan-2-one (**6**) was synthesized and its electrochemical properties investigated by cyclic voltammetry subject to the same conditions. As depicted in Fig. 3, this compound exhibited only one oxidation wave in the first cycle (Fig. 3A, solid curve), but three in the second one (curve b). However, when acetylferrocene was added, these waves overlapped with the electrode response of acetylferrocene (curve c), and we find this to be sufficient evidence to back up the above statements.

2.5. Biology

Medicinal chemists are open to the inclusion of ferrocene into their drug design strategies because of the novelty introduced by its presence. Previous workers [56–58] have found that certain Mannich bases possessed *in vitro* antimicrobial activity. Chatten et al. [59] have shown that besides the difference in amine moieties, the substituents in phenyl ketones also exert an influence on

Table 3
Geometrical parameters (Å,°) of hydrogen bonds and selected C–H…O interactions for **5j**, **5m** and **5l**. The C–H…O interactions are given if H…O distance is shorter than 2.7 Å and C–H…O angle is larger than 100°.

D–H…A	D–H	D…A	H…A	D–H…A
5j				
C4–H4…N1^{i}	0.93	3.559(7)	2.69	156
N1–H1N…O1^{ii}	0.91(6)	3.020(7)	2.14(6)	164(4)
C7–H7…Cl1^{iii}	0.93	3.625(13)	2.87	139
Symmetry codes: (i) $x-1, +y+1, +z$; (ii) $-x+1, -y+1, -z+1$; (iii) $-x+1, -y+1, -z+2$				
5m				
C9–H9…O2^{i}	0.93	3.443(10)	2.66	143
C4–H4…O3^{i}	0.93	3.272(10)	2.65	125
N1–H1N…O1^{ii}	0.73(4)	3.142(7)	2.46(5)	156(5)
C19–H19…O1^{ii}	0.93	3.388(7)	2.64	138
Symmetry codes: (i) $-x+1, -y+1, -z+1$; (ii) $-x+2, -y+1, -z$				
5l				
N1–H1N…O1^{i}	0.76(8)	2.984(8)	2.70(8)	105(6)
N1–H1N…O2^{i}	0.76(8)	2.625(8)	2.01(7)	139(7)
C6–H6…O2^{i}	0.93	3.469(10)	2.63	150
C16–H16…O3^{i}	0.93	2.662(11)	2.34	100
C2–H2…O1^{ii}	0.93	3.352(7)	2.57	142
C9–H9…O3^{iii}	0.93	3.373(9)	2.67	133
C12–H12a…O1^{iv}	0.97	3.130(8)	2.44	128
Symmetry codes: (i) x,y,z; (ii) $x+1, +y, +z$; (iii) $-x+1, +y+1/2, -z-1/2$; (iv) $x+1/2, -y+1/2+2, -z$				

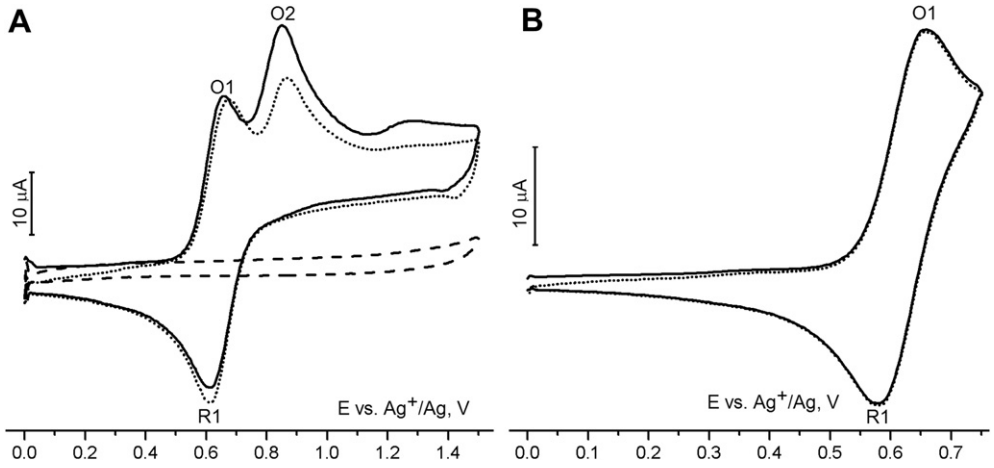


Fig. 2. Cyclic voltammograms of **5a** at the glassy carbon electrode (2 mm diameter) by a 0.1 V s^{-1} scan rate in a 0.1 M acetonitrile solution of $LiClO_4$: A) up to 1.5 V (dashed curve – the electrolyte, solid curve – first scan, dotted curve – second scan) and B) up to 0.75 V (solid curve – first scan, dotted curve – second scan).

activities. These observations together with the fact that ferrocene is widely regarded as a substitute for the aromatic benzene ring prompted the preparation and testing of the antibacterial activity of Mannich products containing a ferrocene system as a part of the ketone moiety. The minimum inhibitory concentration (MIC) of the synthesized compounds **5a**–**n** was measured against growth of six bacteria (three Gram-positive and three Gram-negative) that were chosen to represent the major types of bacteria associated with human disease. The results of these studies and those of minimal bactericidal activity (MBC) are presented in Table 5 as the averages of multiple determinations. The tested bacteria were generally sensitive to these compounds, and as shown in Table 5, the values of MIC for compounds **5a**–**n** varied between 0.02 and 12.50 mg/ml. Growth inhibition of the bacteria was observed for all of the compounds early in the incubation period but the test organism overgrew the inhibition zones within 48 h as reflected in the high differences in the obtained MIC and MBC values. The best results (Table 5) were obtained against a Gram-negative bacterium and an important human pathogen, *Staphylococcus aureus* (MIC values 0.02–0.10 mg/ml), but most of the compounds exhibited activity at least one hundred fold lower than Tetracycline against both Gram-positive and negative bacteria (although the latter seem to be more susceptible to the compounds). Infections caused by *Pseudomonas aeruginosa* are often difficult to treat chemotherapeutically because of the unusually high resistance of the organism to most antimicrobial drugs [60,61] and because resistance to other drugs may evolve rapidly [61]. Therefore, it was not surprising that this organism was the most resistant to nearly all of the compounds studied. When comparing the activity of the herein synthesized Mannich bases, in general, compounds having an electron-acceptor functionality (**5f**–**n**) appeared not to be more or less effective in inhibiting the growth of all bacteria than compounds possessing a electron-donating substituent or no substituent at all (**5a**–**e**). A similar stands for the sets of three regioisomeric compounds (differing only in the position of the substituent on the benzene ring), e.g. **5b**–**d**, since they had a mutually very similar antibacterial effect as well. It was tempting to assume that the steric effects could prevent the *ortho* isomers from interacting with the receptor of the test organisms, however, the differences in potency usually ascribed to substituents at the various positions in the benzene ring have not been found. The other parts of the molecule seem to have a much more important contribution to the activities observed. Some Mannich bases derived from the corresponding acetophenones (analogous to the currently prepared ferrocene derivatives) were found to possess significant antimicrobial activity [62] (e.g. in a disk diffusion assay [62], 1-phenyl-3-(phenylamino)propan-1-one, the analog of compound **5a**, inhibited the growth of *Escherichia coli* with a zone of 15 mm in diameter, while at the same dose per disk the antibiotic ofloxacin had a zone of 22 mm). Since ferrocene is electron donating ($\sigma_{\text{para ferrocene}}$ −0.18 compared to $\sigma_{\text{para phenyl}}$ 0.01) and electron donation to the ketone can occur, one can take this as a possible cause of the decrease in activity in the case of the metallocene containing compounds.

Table 4
Peak potentials obtained by cyclic voltammetry of the Mannich bases **5a**–**n** at the glassy carbon electrode (2 mm diameter) by a 0.1 V s^{-1} scan rate in a 0.1 M acetonitrile solution of $LiClO_4$.

Compound	O1 (mV)	O2 (mV)	R (mV)
5a	665	851	620
5b	653	803	598
5c	653	784	613
5d	693	693	604
5e	662	830	604
5f	647	992	601
5g	644	983	595
5h	638	861	598
5i	647	1031	595
5j	647	1007	595
5k	662	952	610
5l	659	1373	595
5m	650	1166	610
5n	653	1361	592

3. Conclusion

In conclusion, we described herein an easily performable procedure for the synthesis of *N*-aryl-3-amino-1-ferrocenylpropan-1-ones via an aza-Michael addition of the corresponding aromatic amines to acryloylferrocene in good to excellent yields. We unambiguously showed that both, the catalyst (montmorillonite K-10) and the microwave irradiation play an important role in this synthesis. The procedure requires short reaction times, and employs an environmentally friendly, as well as cheap catalyst.

A trait worth noting of the ^{1}H NMR spectra of the synthesized β-aminoketones, possessing electron-withdrawing substituents in the *ortho*- and *meta*-positions of the aniline moiety of the molecules, was the occurrence of coupling of the NH protons with those of the adjacent CH_2 group, indicating a slow NH exchange on the NMR time scale. The N–CH_2 signals appeared as quartets from

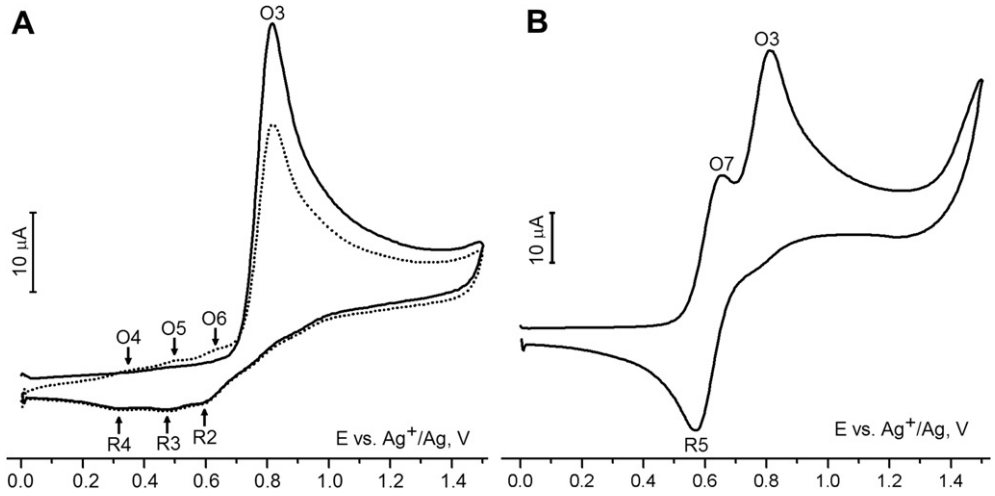


Fig. 3. Cyclic voltammograms of 3 mM solution of 4-(phenylamino)butan-2-one (**6**) at the glassy carbon electrode (2 mm diameter) by a 0.1 V s^{-1} scan rate in a 0.1 M acetonitrile solution of $LiClO_4$: A) without acetylferrocene (solid curve – first scan, dotted curve – second scan) and B) with 3 mM acetylferrocene (first scan).

accidental equivalence of the vicinal HN–CH_2 and CH_2–CH_2 couplings. Such coupling that appears to be in connection with the acidity and/or intramolecular hydrogen bonding was not observed for the benzene analogs or for the compounds having electron-donating or *para*-electron-withdrawing substituents.

The structure of three compounds was unequivocally corroborated by single-crystal X-ray analysis. Besides some conformational similarity in molecular structure of all three compounds, two of them with NO_2 substituent at the phenyl ring show different orientation of the phenyl ring regarding to the rest of molecule. The ferrocene compound with NO_2 in the *ortho* position forms strong N–H…O intramolecular hydrogen bond while other two compounds use the same N–H donor group for formation of geometrically similar centrosymmetric dimers.

All of the compounds appeared to have broad-spectrum effect on Gram-negative and Gram-positive bacteria, although the degree of inhibition varied. A notable exception to the generally medium-low activity is shown by the fact that all compounds inhibited best *S. aureus*. The introduction of either an electron-donating or acceptor group in the *ortho* position to the phenyl resulted in no alteration in activity.

4. Experimental section

4.1. General remarks

All chemicals were commercially available and used as received, except that the solvents were purified by distillation. Chromatographic separations were carried out using silica gel 60 (Merck, 230–400 mesh ASTM), whereas silica gel 60 on Al plates, layer thickness 0.2 mm (Merck) was used for TLC. Melting points (uncorrected) were determined on a Mel-Temp capillary melting

Table 5
Minimal inhibitory (MIC) and minimal bactericidal concentrations (MBC) of the synthesized Mannich bases **5a**–**n**.

	Compound (mg/ml)														
	5a	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**	**5k**	**5l**	**5m**	**5n**	T
Gram (–) bacterial strains															
Escherichia coli ATCC 25922															
MIC	0.78	0.39	0.39	0.39	0.78	0.78	0.78	0.20	0.39	0.78	0.39	0.39	1.56	0.39	1.56
MBC	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	12.50	25.00	25.00	1.56
Salmonella enterica ATCC 13076															
MIC	0.78	6.25	1.56	1.56	0.78	1.56	12.50	0.20	0.78	1.56	0.39	0.39	0.78	0.39	3.12
MBC	12.50	50.00	25.00	12.50	12.50	12.50	50.00	12.50	12.50	12.50	12.50	12.50	12.50	12.50	3.12
Pseudomonas aeruginosa ATCC 27853															
MIC	1.56	0.78	1.56	3.12	3.12	3.12	3.12	3.12	1.56	3.12	3.12	1.56	1.56	0.78	3.12
MBC	25.00	25.00	12.50	25.00	12.50	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	3.12
Gram (+) bacterial strains															
Staphylococcus aureus ATCC 6538															
MIC	0.02	0.02	0.10	0.02	0.02	0.05	0.10	0.02	0.10	0.10	0.05	0.10	0.10	0.02	0.09
MBC	3.12	3.12	6.25	3.12	3.12	3.12	6.25	3.12	6.25	6.25	3.12	6.25	6.25	3.12	0.09
Bacillus cereus ATCC 10876															
MIC	0.39	1.56	3.12	0.10	1.56	0.39	0.39	0.78	0.78	0.78	0.39	0.39	0.39	0.39	1.56
MBC	25.00	50.00	50.00	12.50	25.00	12.50	12.50	25.00	25.00	25.00	12.50	25.00	12.50	12.50	1.56
Clostridium perfringens ATCC 19404															
MIC	0.39	0.20	0.20	0.20	0.20	0.20	0.39	0.39	0.20	0.20	0.20	0.39	0.39	0.20	1.56
MBC	12.50	12.50	12.50	12.50	12.50	25.00	6.25	6.25	12.50	12.50	12.50	12.50	12.50	12.50	1.56

T, Tetracycline (μg/ml).

points apparatus, model 1001. The ^{1}H and ^{13}C NMR spectra of the samples in $CDCl_3$ were recorded on a Varian Gemini (200 MHz) spectrometer. Chemical shifts are expressed in δ (ppm), relative to residual solvent protons as the internal standard ($CDCl_3$: 7.26 ppm for ^{1}H and 77 ppm for ^{13}C). Cyclic voltammetry experiments were performed at room temperature under argon in a three-electrode cell using an Autolab potentiostat (PGSTAT 302N). The working electrode was a glassy carbon disk (2 mm diameter). The counter electrode was a platinum wire, and a silver wire was used as the reference electrode. IR measurements were carried out with a Perkin–Elmer FTIR 31725-X spectrophotometer. Microanalysis of carbon, hydrogen and nitrogen was carried out with a Carlo Erba 1106 microanalyser; their results agreed favorably with the calculated values. The reactions (microwave assisted syntheses) were performed by placing the teflon quivet with the reagents without a solvent in a closed reactor equipped with pressure and temperature control units and irradiating inside the cavity of a MicroSynth (Milestone) according to the following parameters: power 500 W, 5 min.

4.2. Acryloylferrocene (**3**)

Anhydrous $AlCl_3$ (2.0 g, 15 mmol) was suspended in a cooled solution (an ice bath) of 2.8 g (15 mmol) of ferrocene (**1**) and 1.9 g (15 mmol) of 3-chloropropionyl chloride in 100 ml of dry dichloromethane, and the obtained mixture stirred for 5 h. The mixture was quenched with water (100 ml), filtered off (Buchner funnel), and the organic layer was separated. The water layer was extracted with two additional 30 ml portions of dichloromethane, the combined organic layers were washed with saturated solution of $NaHCO_3$ and the solvent distilled off. The crude product was redissolved in toluene, passed through a short column of silica, and the toluene evaporated. The solid residue was placed in a solution of 1.5 g of CH_3COOK in 100 ml of ethanol and refluxed for 2.5 h. After that the ethanol was evaporated, the residue extracted with dichloromethane and the obtained solution dried over anhydrous Na_2SO_4. Flash chromatography (SiO_2/toluene) gave 2.41 g ($\sim$10.5 mmol; $\sim$67% based on ferrocene) of pure acryloylferrocene (**3**). The spectral data of **3** were in agreement with the literature ones [63].

4.3. General procedure for the synthesis of Mannich bases ***5a–n***

Acryloylferrocene (**3**, 1 mmol), the corresponding amine (**4a–n**, 2 mmol) and 100 mg of montmorillonite K-10 were well mixed and irradiated in a microwave oven for 5 min at (500 W). The reaction mixture was extracted with dichloromethane (30 ml), the solvent evaporated and the crude product purified by flash chromatography (SiO_2). Amines were eluted with toluene, whereas ketone **3** and the target Mannich bases **5a–n** were separated by using of a mixed solvent (*n*-hexane/ethyl acetate = 9:1, v/v) as the eluent. In all cases the complete excess of the amines was recovered. The spectral data of compounds **5a–n** follow.

4.3.1. 1-Ferrocenyl-3-(phenylamino)propan-1-one (***5a***)

m.p. 106 °C; IR: ν_{max} (KBr)/cm^{-1} 3358, 3085, 2933, 1655, 1603, 1515, 1498, 1456, 1401, 1274, 1069, 825, 746, 695; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.30–7.10 (m, 2H, Ar), 6.78–6.58 (m, 3H, Ar), 4.76 (t, J = 1.9 Hz, 2H, Fc), 4.49 (t, J = 1.9 Hz, 2H, Fc), 4.21 (brs, 1H, NH), 4.11 (s, 5H, Fc), 3.57 (t, J = 6.1 Hz, 2H, N–CH_2), 3.01 (t, J = 6.1 Hz, 2H, CO–CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.4 (CO), 147.6 (Ar), 129.2 (Ar), 117.4 (Ar), 112.9 (Ar), 78.7 (Fc), 72.3 (Fc), 69.7 (Fc), 69.1 (Fc), 38.5 (N–C), 38.0 (C–C); Anal. Calcd. ($C_{19}H_{19}FeNO$): C, 68.49; H, 5.75; N, 4.20; Found: C, 68.51; H, 5.71; N, 4.23.

4.3.2. 1-Ferrocenyl-3-(o-tolylamino)propan-1-one (***5b***)

m.p. 112 °C; IR: ν_{max} (KBr)/cm^{-1} 3393, 3098, 2918, 1668, 1603, 1503, 1457, 1408, 1260, 1068, 826, 754; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.25–6.98 (m, 2H, Ar), 6.76–6.59 (m, 2H, Ar), 4.76 (t, J = 1.9 Hz, 2H, Fc), 4.49 (t, J = 1.9 Hz, 2H, Fc), 4.14 (brs, 1H, NH), 4.10 (s, 5H, Fc), 3.69–3.54 (m, 2H, N–CH_2), 3.04 (t, J = 6.0 Hz, 2H, CO–CH_2), 2.13 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.5 (CO), 145.6 (Ar), 130.2 (Ar), 127.0 (Ar), 122.4 (Ar), 117.0 (Ar), 109.5 (Ar), 78.7 (Fc), 72.3 (Fc), 69.7 (Fc), 69.1 (Fc), 38.6 (N–C), 38.1 (C–C), 17.4 (CH_3); Anal. Calcd. ($C_{20}H_{21}FeNO$): C, 69.18; H, 6.10; N, 4.03; Found: C, 69.19; H, 6.13; N, 3.99.

4.3.3. 1-Ferrocenyl-3-(m-tolylamino)propan-1-one (***5c***)

m.p. 121 °C; IR: ν_{max} (KBr)/cm^{-1} 3349, 3082, 2934, 1655, 1603, 1457, 1404, 1281, 1265, 1106, 826, 773; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.18–6.92 (m, 1H, Ar), 6.59–6.48 (m, 3H, Ar), 4.75 (t, J = 1.9 Hz, 2H, Fc), 4.48 (t, J = 1.9 Hz, 2H, Fc), 4.13 (brs, 1H, NH), 4.11 (s, 5H, Fc), 3.55 (t, J = 6.1 Hz, 2H, N–CH_2), 2.99 (t, J = 6.1 Hz, 2H, CO–CH_2), 2.27 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 2034 (CO), 147.6 (Ar), 138.9 (Ar), 129.1 (Ar), 118.3 (Ar), 113.8 (Ar), 110.1 (Ar), 78.7 (Fc), 72.2 (Fc), 69.7 (Fc), 69.1 (Fc), 38.6 (N–C), 38.1 (C–C), 21.5 (CH_3); Anal. Calcd. ($C_{20}H_{21}FeNO$): C, 69.18; H, 6.10; N, 4.03; Found: C, 69.17; H, 6.07; N, 4.04.

4.3.4. 1-Ferrocenyl-3-(p-tolylamino)propan-1-one (***5d***)

m.p. 73 °C; IR: ν_{max} (KBr)/cm^{-1} 3351, 3090, 2918, 1656, 1618, 1521, 1456, 1401, 1273, 1070, 824, 807; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.99 (d, J = 8.2 Hz, 2H, Ar), 6.57 (d, J = 8.4 Hz, 2H, Ar), 4.74 (t, J = 1.9 Hz, 2H, Fc), 4.47 (t, J = 1.9 Hz, 2H, Fc), 4.10 (s, 5H, Fc), 4.06 (brs, 1H, NH), 3.53 (t, J = 6.1 Hz, 2H, N–CH_2), 2.98 (t, J = 6.1 Hz, 2H, CO–CH_2), 2.22 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.4 (CO), 145.3 (Ar), 129.7 (Ar), 126.5 (Ar), 113.1 (Ar), 78.7 (Fc), 72.2 (Fc), 69.7 (Fc), 69.0 (Fc), 38.9 (N–C), 38.1 (C–C), 20.2 (CH_3); Anal. Calcd. ($C_{20}H_{21}FeNO$): C, 69.18; H, 6.10; N, 4.03; Found: C, 69.20; H, 6.10; N, 4.05.

4.3.5. 1-Ferrocenyl-3-(mesitylamino)propan-1-one (***5e***)

m.p. 86 °C; IR: ν_{max} (KBr)/cm^{-1} 3378, 3094, 2940, 1655, 1485, 1456, 1376, 1310, 1243, 1021, 821; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.82 (s, 2H, Ar), 4.77 (t, J = 1.8 Hz, 2H, Fc), 4.48 (t, J = 1.8 Hz, 2H, Fc), 4.18 (s, 5H, Fc), 3.62 (brs, 1H, NH), 3.25 (t, J = 5.7 Hz, 2H, N–CH_2), 2.97 (t, J = 5.7 Hz, 2H, CO–CH_2), 2.31 (s, 6H, *o*-CH_3), 2.22 (s, 3H, *p*-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.9 (CO), 143.3 (Ar), 131.2 (Ar), 130.0 (Ar), 129.2 (Ar), 78.7 (Fc), 72.2 (Fc), 69.7 (Fc), 69.1 (Fc), 43.1 (N–C), 39.7 (C–C), 20.5 (*p*-CH_3), 18.1 (*o*-CH_3); Anal. Calcd. ($C_{22}H_{25}FeNO$): C, 70.41; H, 6.71; N, 3.73; Found: C, 70.40; H, 6.70; N, 3.75.

4.3.6. 1-Ferrocenyl-3-(2-fluorophenylamino)propan-1-one (***5f***)

m.p. 89 °C; IR: ν_{max} (KBr)/cm^{-1} 3383, 3096, 2903, 1665, 1619, 1529, 1402, 1261, 1190, 824, 735; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.13–6.50 (m, 4H, Ar), 4.77 (t, J = 1.8 Hz, 2H, Fc), 4.50 (t, J = 1.8 Hz, 2H, Fc), 4.37 (brs, 1H, NH), 4.12 (s, 5H, Fc), 3.71–3.48 (brq, 2H, N–CH_2), 3.02 (t, J = 6.1 Hz, 2H, CO–CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 202.9 (CO), 151.7 (J_{CF} = 238.8 Hz, Ar), 136.2 (J_{CF} = 11.5 Hz, Ar), 124.5 (J_{CF} = 3.4 Hz, Ar), 116.7 (J_{CF} = 7.0 Hz, Ar), 114.6 (J_{CF} = 18.5 Hz, Ar), 111.9 (J_{CF} = 3.3 Hz, Ar), 78.7 (Fc), 72.4 (Fc), 69.7 (Fc), 69.1 (Fc), 38.2 (N–C), 38.1 (C–C); Anal. Calcd. ($C_{19}H_{18}FFeNO$): C, 64.98; H, 5.17; N, 3.99; Found: C, 64.99; H, 5.20; N, 4.01.

4.3.7. 1-Ferrocenyl-3-(3-fluorophenylamino)propan-1-one (***5g***)

m.p. 124 °C; IR: ν_{max} (KBr)/cm^{-1} 3362, 3098, 2945, 1654, 1622, 1499, 1457, 1399, 1261, 1154, 1072, 840, 823, 755, 686; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.20–7.01 (m, 1H, Ar), 6.48–6.28 (m, 3H, Ar), 4.77 (t, J = 1.9 Hz, 2H, Fc), 4.51 (t, J = 1.9 Hz, 2H, Fc), 4.39 (brs, 1H, NH), 4.12 (s, 5H, Fc), 3.63–3.46 (brq, 2H, N–CH_2), 3.01 (t, J = 6.0 Hz,

2H, CO–CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.3 (CO), 164.2 (J_{CF} = 242.8 Hz, Ar), 149.5 (J_{CF} = 10.6 Hz, Ar), 130.4 (J_{CF} = 10.2 Hz, Ar), 108.9 (J_{CF} = 2.3 Hz, Ar), 103.8 (J_{CF} = 21.6 Hz, Ar), 99.3 (J_{CF} = 25.3 Hz, Ar), 78.6 (Fc), 72.4 (Fc), 69.8 (Fc), 69.1 (Fc), 38.4 (N–C), 37.8 (C–C); Anal. Calcd. ($C_{19}H_{18}FFeNO$): C, 64.98; H, 5.17; N, 3.99; Found: C, 64.95; H, 5.17; N, 4.00.

*4.3.8. 1-Ferrocenyl-3-(4-fluorophenylamino)propan-1-one (**5h**)*

m.p. 127 °C; IR: ν_{max} (KBr)/cm^{-1} 3399, 3102, 2911, 1664, 1521, 1461, 1400, 1219, 1050, 818, 785; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.97–6.82 (m, 2H, Ar), 6.66–6.54 (m, 2H, Ar), 4.76 (t, J = 1.9 Hz, 2H, Fc), 4.51 (t, J = 1.9 Hz, 2H, Fc), 4.12 (brs, 1H, NH), 4.12 (s, 5H, Fc), 3.52 (t, J = 6.0 Hz, 2H, N–CH_2), 3.00 (t, J = 6.0 Hz, 2H, CO–CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.4 (CO), 155.9 (J_{CF} = 235.1 Hz, Ar), 144.1 (J_{CF} = 1.5 Hz, Ar), 115.7 (J_{CF} = 22.3 Hz, Ar), 113.9 (J_{CF} = 7.4 Hz, Ar), 103.8 (J_{CF} = 21.6 Hz, Ar), 99.3 (J_{CF} = 25.3 Hz, Ar), 78.7 (Fc), 72.4 (Fc), 69.8 (Fc), 69.1 (Fc), 39.3 (N–C), 37.9 (C–C); Anal. Calcd. ($C_{19}H_{18}FFeNO$): C, 64.98; H, 5.17; N, 3.99; Found: C, 65.00; H, 5.21; N, 3.97.

*4.3.9. 3-(2-Chlorophenylamino)-1-ferrocenylpropan-1-one (**5i**)*

m.p. 108 °C; IR: ν_{max} (KBr)/cm^{-1} 3418, 3096, 2921, 1675, 1599, 1504, 1456, 1410, 1325, 1256, 1025, 823, 750; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33–7.10 (m, 2H, Ar), 6.82–6.55 (m, 2H, Ar), 4.77 (t, J = 1.9 Hz, 2H, Fc), 4.77 (brs, 1H, NH), 4.50 (t, J = 1.9 Hz, 2H, Fc), 4.12 (s, 5H, Fc), 3.64 (q, J = 6.1 Hz, 2H, N–CH_2), 3.03 (t, J = 6.2 Hz, 2H, CO–CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 202.7 (CO), 143.5 (Ar), 129.3 (Ar), 127.8 (Ar), 119.4 (Ar), 117.3 (Ar), 110.9 (Ar), 78.7 (Fc), 72.4 (Fc), 69.8 (Fc), 69.2 (Fc), 38.1 (N–C), 38.1 (C–C); Anal. Calcd. ($C_{19}H_{18}ClFeNO$): C, 62.07; H, 4.93; N, 3.81; Found: C, 62.03; H, 4.94; N, 3.78.

*4.3.10. 3-(3-Chlorophenylamino)-1-ferrocenylpropan-1-one (**5j**)*

m.p. 121 °C; IR: ν_{max} (KBr)/cm^{-1} 3353, 3086, 2930, 1654, 1596, 1487, 1456, 1400, 1275, 1248, 1073, 822, 758; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.14–7.00 (m, 1H, Ar), 6.72–6.45 (m, 3H, Ar), 4.76 (t, J = 1.9 Hz, 2H, Fc), 4.50 (t, J = 1.9 Hz, 2H, Fc), 4.36 (brs, 1H, NH), 4.12 (s, 5H, Fc), 3.61–3.46 (brq, 2H, N–CH_2), 3.00 (t, J = 6.0 Hz, 2H, CO–CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.2 (CO), 148.8 (Ar), 135.0 (Ar), 130.2 (Ar), 117.2 (Ar), 112.2 (Ar), 111.4 (Ar), 78.6 (Fc), 72.4 (Fc), 69.7 (Fc), 69.1 (Fc), 38.3 (N–C), 37.8 (C–C); Anal. Calcd. ($C_{19}H_{18}ClFeNO$): C, 62.07; H, 4.93; N, 3.81; Found: C, 62.05; H, 4.96; N, 3.80.

*4.3.11. 3-(4-Chlorophenylamino)-1-ferrocenylpropan-1-one (**5k**)*

m.p. 51 °C; IR: ν_{max} (KBr)/cm^{-1} 3344, 3092, 2933, 1658, 1596, 1509, 1493, 1456, 1396, 1273, 1088, 1066, 824, 799; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.19–7.01 (m, 2H, Ar), 6.65–6.50 (m, 3H, Ar), 4.75 (t, J = 1.9 Hz, 2H, Fc), 4.50 (t, J = 1.9 Hz, 2H, Fc), 4.24 (brs, 1H, NH), 4.11 (s, 5H, Fc), 3.52 (t, J = 6.0 Hz, 2H, N–CH_2), 2.98 (t, J = 6.0 Hz, 2H, CO–CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.3 (CO), 146.2 (Ar), 129.0 (Ar), 121.8 (Ar), 113.9 (Ar), 78.6 (Fc), 72.4 (Fc), 69.7 (Fc), 69.1 (Fc), 38.7 (N–C), 37.8 (C–C); Anal. Calcd. ($C_{19}H_{18}ClFeNO$): C, 62.07; H, 4.93; N, 3.81; Found: C, 62.10; H, 4.94; N, 3.79.

*4.3.12. 1-Ferrocenyl-3-(2-nitrophenylamino)propan-1-one (**5l**)*

m.p. 96 °C; IR: ν_{max} (KBr)/cm^{-1} 3377, 3116, 2935, 1661, 1617, 1568, 1508, 1457, 1399, 1263, 1238, 1149, 823, 743; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.42–8.11 (m, 2H, NH and Ar), 7.60–7.40 (m, 1H, Ar), 6.96 (d, J = 8.5 Hz, 1H, Ar), 6.75–6.59 (m, 1H, Ar), 4.80 (t, J = 1.9 Hz, 2H, Fc), 4.53 (t, J = 1.9 Hz, 2H, Fc), 4.17 (s, 5H, Fc), 3.76 (q, J = 6.6 Hz, 2H, N–CH_2), 3.12 (t, J = 6.6 Hz, 2H, CO–CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 201.5 (CO), 145.0 (Ar), 136.1 (Ar), 126.9 (Ar), 115.3 (Ar), 113.4(Ar), 78.3 (Fc), 72.5 (Fc), 69.7 (Fc), 69.1 (Fc), 38.3 (N–C), 37.6 (C–C); Anal. Calcd. ($C_{19}H_{18}FeN_2O_3$): C, 60.34; H, 4.80; N, 7.41; Found: C, 60.30; H, 4.81; N, 7.41.

*4.3.13. 1-Ferrocenyl-3-(3-nitrophenylamino)propan-1-one (**5m**)*

m.p. 91 °C; IR: ν_{max} (KBr)/cm^{-1} 3329, 3098, 2956, 1656, 1620, 1526, 1456, 1347, 1265, 1238, 826, 782; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.60–7.40 (m, 2H, Ar), 7.36–7.21 (m, 1H, Ar), 7.36–7.21 (m, 1H, Ar), 6.98–6.85 (m, 1H, Ar), 4.78 (t, J = 1.9 Hz, 2H, Fc), 4.71 (brs, 1H, NH),

Table 6
Crystallographic data for **5j**, **5m** and **5l**.

Identification code	**5j**	**5m**	**5l**
Empirical formula	$C_{19}H_{18}ClFeNO$	$C_{19}H_{18}FeN_2O_3$	$C_{19}H_{18}FeN_2O_3$
Formula weight	367.64	378.20	378.20
Color, crystal shape	Dark-orange, prism	Dark-orange, plate	Dark-orange, prism
Crystal size (mm^3)	0.32 × 30 × 25	0.36 × 0.31 × 0.10	028 × 0.25 × 0.23
Temperature (K)	293(2)	293(2)	293(2)
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system	Triclinic	Triclinic	Orthorhombic
Space group	$P-1$	$P-1$	$P2_12_12_1$
Unit cell dimensions			
a (Å)	7.5449(7)	9.109(3)	5.8295(7)
b (Å)	9.7317(8)	9.659(2)	13.6390(18)
c (Å)	12.5382(11)	11.242(3)	21.231(4)
α (°)	88.912(7)	65.53(3)	90
β (°)	76.107(8)	72.79(2)	90
γ (°)	69.330(12)	84.94(4)	90
V (Å^3)	833.96(13)	859.3(4)	1688.0(4)
Z	2	2	4
D_{calc} (Mg/m^3)	1.464	1.462	1.488
μ (mm^{-1})	1.067	0.898	0.914
θ range for data collection (°)	3.03–28.97	2.98–28.92	3.14–29.03
Reflections collected	6698	5930	5237
Independent reflections, R_{int}	3803, 0.0458	3877, 0.1109	3564, 0.0470
Completeness (%) to θ = 26.32°	99.6	99.7	99.8
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3803/0/212	3877/0/230	3564/0/230
Goodness-of-fit on F^2	1.090	0.992	1.052
Final R_1/wR_2 indices [$I > 2\sigma(I)$]	0.0889/0.1997	0.0853/0.1856	0.0683/0.1349

4.53 (t, $J = 1.9$ Hz, 2H, Fc), 4.13 (s, 5H, Fc), 3.70–3.54 (brq, 2H, N–CH_2), 3.05 (t, $J = 5.9$ Hz, 2H, CO–CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.3 (CO), 148.6 (Ar), 147.6 (Ar), 129.8 (Ar), 119.2 (Ar), 111.9 (Ar), 105.9 (Ar), 78.4 (Fc), 72.6 (Fc), 69.8 (Fc), 69.2 (Fc), 38.4 (N–C), 37.7 (C–C); Anal. Calcd. ($C_{19}H_{18}FeN_2O_3$): C, 60.34; H, 4.80; N, 7.41; Found: C, 60.32; H, 4.84; N, 7.43.

4.3.14. 1-Ferrocenyl-3-(4-nitrophenylamino)propan-1-one (**5n**)

m.p. 189–190 °C; IR: ν_{max} (KBr)/cm^{-1} 3356, 3107, 2907, 1653, 1604, 1501, 1471, 1319, 1115, 832, 754; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.09 (d, $J = 9.2$ Hz, 2H, Ar), 6.61 (d, $J = 9.2$ Hz, 2H, Ar), 4.79 (t, $J = 1.9$ Hz, 2H, Fc), 4.58 (t, $J = 1.9$ Hz, 2H, Fc), 4.14 (s, 5H, Fc), 3.74 (brs, 1H, NH), 3.66 (t, $J = 6.1$ Hz, 2H, N–CH_2), 3.07 (t, $J = 6.1$ Hz, 2H, CO–CH_2). ^{13}C NMR (50 MHz, $CDCl_3$) δ 204.0 (CO), 153.5 (Ar), 137.5 (Ar), 126.8 (Ar), 111.0 (Ar), 78.3 (Fc), 73.2 (Fc), 70.1 (Fc), 69.5 (Fc), 38.0 (N–C), 38.0 (C–C); Anal. Calcd. ($C_{19}H_{18}FeN_2O_3$): C, 60.34; H, 4.80; N, 7.41; Found: C, 60.33; H, 4.82; N, 7.38.

4.4. X-ray crystallography

Single-crystal diffraction data for **5j**, **5l** and **5m** were collected on a Oxford Diffraction Xcalibur Sapphire3 Gemini diffractometer equipped with Mo Kα radiation ($\lambda = 0.71073$ Å) at room temperature. Data were processed with CrysAlis software [64] with multi-scan absorption corrections applied using SCALE3 ABSPACK [64]. All three crystal structures were solved with SHELXS [65] and refined using SHELXL [65].

The H1n atom attached to N1 was located by difference Fourier synthesis and refined isotropically. All other H atoms were placed at geometrically calculated positions with the C–H distances fixed to 0.93 from C(sp^2); 0.97 and 0.98 Å from methylene and methine C(sp^3), respectively. The corresponding isotropic displacement parameters of the hydrogen atoms were equal to $1.2U_{eq}$ and $1.5U_{eq}$ of the parent C(sp^2) and C(sp^3), respectively.

A summary of crystallographic data is given in Table 6. Figures were produced using ORTEP-3 [66] and MERCURY, Version 2.4 [67]. The software used for the preparation of the materials for publication: WinGX [68], PLATON [69], PARST [70].

4.5. Biology

4.5.1. Test microorganisms

The synthesized Mannich bases **5a**–**n** were tested against a panel of microorganisms (American Type Culture Collection strains), including Gram-positive *S. aureus* ATCC 6538, *Bacillus cereus* ATCC 10876, *Clostridium perfringens* ATCC 19404, Gram-negative *Salmonella enterica* ATCC 13076, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Bacterial strains were maintained on Nutrient agar at optimal temperature of 37 °C at the Microbiology Laboratory (Department of Biology, Faculty of Science and Mathematics, University of Niš).

4.5.2. Screening of antimicrobial activity

Antimicrobial activity was evaluated using a broth microdilution method according to NCCLS (2003) [71]. Minimum inhibitory concentrations (MIC) determination was performed by a serial dilution method in 96 well microtitre plates. Bacterial species were cultured at 37 °C in Mueller Hinton agar. After 18 h of cultivation, bacterial suspensions were made in Mueller Hinton broth and their turbidity was standardized to 0.5 McFarland. Absorbance of every suspension was confirmed on a spectrophotometer (UV–VIS 1650, Shimadzu, Japan). The final density of bacterial inoculi corresponded to 5×10^5 CFU (colony forming units).

Stock solutions of the compounds **5a**–**n** were prepared in 10% (v/v) aqueous dimethyl sulfoxide (DMSO) in the concentration range 0.01–50.00 mg/ml (the diluting factor 2). The bacterial inoculum was added to all wells containing the compounds in appropriate concentrations and the plates were incubated at 37 °C during 24 h. Tetracycline served as a positive control, while the solvent (10% DMSO(aq)) was used as a negative control. The DMSO solvent controls did not produce any measurable inhibition of the test organisms. Replicate tests performed with a specific dilution of a test compound on any given day were in excellent agreement and results obtained with a specific dilution of any given compound on different days were generally in close agreement. One non-inoculated well, free of the antimicrobial agents, was also included to ensure medium sterility.

Bacterial growth was visualized by adding 20 µl of 0.5% (w/w) triphenyltetrazolium chloride (TTC) aqueous solution [72]. Minimal inhibitory concentration (MIC) was defined as the lowest concentration of the compounds **5a**–**n** that inhibited visible growth (red colored pellet on the bottom of the wells after the addition of TTC), while minimal bactericidal concentration (MBC) was defined as the lowest concentration of the compound that killed 99.9% of bacterial cells. To determine MBC, broth was taken from each well without any visible growth and inoculated in Mueller Hinton agar (MHA) for 24 h at 37 °C.

4.5.3. Statistical analysis

All experiments were done in quantiplicate and mean values are presented. In order to evaluate statistically any significant differences among mean values, a one-way ANOVA test was used. p values less than 0.05 ($p < 0.05$) were used as the significance level.

Acknowledgment

This work was supported by the Ministry of Education and Science of the Republic of Serbia (grant 172034).

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2011.08.016.

Appendix A. Supplementary data

CCDC 827956, 827957 and 821837 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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