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Synthesis, Antibacterial Activity and Molecular Docking of Substituted Naphthyridines as Potential *DNA Gyrase* Inhibitors

Farghaly A. Omar,*[a] Mariam Abelrasoul,[b] Mahmoud M. Sheha,[b] Hoda Y. Hassan,[b] and Yasser Musa. Ibrahiem[c]

A series of naphthyridine-3-thiosemicarbazide **7,8(a–e)** and the corresponding cyclized analogs, naphthyridine-3-(1,3,4-oxadiazoles) **9,10(a–e)** were synthesized through modification of the COOH in nalidixic acid (**NA**) and its 6-bromo analogue, as new chemical entities (*NCE*) with enhanced antimicrobial potential. The compounds were screened for antibacterial activity against Gram positive (***G+ve***) strains (*S. aureus*, *B. cereus*); Gram negative (***G-ve***) (*E. coli, K. pneumonia, P. aeruginosa*) and *Mycobac. smegmatis*. Compounds **7 b,c** and **9 b,d** displayed the highest activity against *S. aureus* (minimal inhibitory concentration; *MIC* ~ 6–7 *mM*), whereas *B. cereus* was found to be more susceptible to the brominated oxadiazoles **10 b,d,e** (*MIC* ~ 5.5-5.9 *mM*). Moreover, **10 b,c,d** exhibit similar *MIC* values against *K. pneumonia* and *M. smegmatis*. This demonstrates that bromination of the naphtyridone skeleton results in broader spectrum and enhanced antibacterial profile. In addition, the aryl substituted thioemicarbazides **7 c,d,e** showed inhibitory effect of the growth of *M. smegmatis* at *MIC* ~ 5.4-7.1 *mM*. Molecular docking to *DNA-gyrase* cleavage complex of *S. aureus*, *Mycobac.* (*mTB*) and *Top. IV* of *K. pneumonia* revealed similar binding poses to the co-crystallized quinolone ligands and indicate good correlation of the binding energy (***Δ G***) with the observed *MIC* values of the active compounds. Consequently, *DNA-gyrase* assay was proposed and executed. Most prominent *DNA-gyrase* inhibition showed by the naphthyridinyl-3-thiosemicarbazides, **7 c** and **8 e** (IC_{50}: 1.73 and 4.46 μg/mL respectively); and the oxadiazoles **9 b** and **10 d** (IC_{50}: 3.36 and 3.89 *μg/mL* respectively). Assessment of drug-likeness characteristics illustrates that the synthesized compounds showed agreement to Lipinsiki's and Veper's parameters. The study could offer an exceptional framework that may lead to the discovery of new potent antimicrobial agents.

Introduction

The widespread emergence of multidrug resistant (*MDR*) strains of microbial infections to clinically available drugs puts further momentum to the urgent need for discovery of new and effective antimicrobial agents with novel mechanisms of action. WHO report (*2015*) on global surveillance of antimicrobial resistance stated that the evidence from around the world indicates an overall decline in the total stock of antibiotic effectiveness.[1] Infections caused by *S. aureus, E. coli, P. vulgaris, P. aeruginosa, S.typhi and S. paratyphi* become resistant to the current antibacterials and are the most prevalent of fatal infectious diseases.[2–4] In case of *M. tuberculosis*, the problem is further exacerbated by extensively drug-resistant (XDR) and totally drug-resistant (TDR) forms.[5]

In this context, chemical manipulation of proven leads or drug candidate is a well established strategy affording *new chemical entities (NCE)* with improved therapeutic characteristics. Recent reports revealed approval of a brominated diarylquinoline derivative, *Bedaquiline*, for treatment of drug-sensitive and drug-resistant *Mycobacterium* forms. It has a bactericidal effect against dormant tubercle bacilli through inhibition of mycobacterial ATP synthase subunit C, thus exhibiting a new mechanism of action.[6,7]

Compounds containing quinoline or its isosteric naphthyridine scaffold represent important therapeutic classes that exhibit a wide-spectrum of biological actions, including antibacterial,[8] antiviral,[9] anticancer,[10] and antifungal activities.[11] The antibacterial quinolones has been enormously modified to enhance their antibacterial spectrum and attenuate the developed bacterial resistance.[12] Nalidixic acid (**NA**), the prototype of the quinolone antibacterials, has been considered as outdated drug due to limited activity against Gram negative (***G-ve***) bacteria that infect urinary tract. Nevertheless, nalidixic acid is ineffective against Grampositive (***G+ve***) bacteria including *Staphylococcal, Streptococcal*, and *Pseudomonal* species.[13] Trials involving modifications of the carboxylic functionality of nalidixic acid to the corresponding substituted hydrazides[14,15] or substituted 5-membered heterocycles lead to

[a] *Prof. Dr. F. A. Omar*
Dept. Pharmaceutical Chemistry, Faculty of Pharmacy, October 6 University, www.o6u.edu.eg (Six October City, Giza/Egypt; Postal Code: 12585)
E-mail: farghalyomar@yahoo.com
farghalyomar@o6u.edu.eg
Homepage: https://www.researchgate.net/profile/Farghaly_Omar
[b] *M. Abelrasoul, Prof. Dr. M. M. Sheha, Dr. H. Y. Hassan*
Dept. of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.
[c] *Dr. Y. M. Ibrahiem*
Depart. of Microbiology, National Org. for Drug Control and Research (NODCAR), Giza, Egypt.

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remarkable antimicrobial activity against a wide range of bacterial strains.[16,17] Meanwhile, thiosemicarbazides and 1,3,4-oxadiazoles are known bioisosteric surrogates for carboxylic group in lead optimization procedures.[18] Both pharmacophoric moieties have shown comprehensive antimicrobial and anti-mycobacterial activities.[19–22]

The present investigation reports a new series of substituted naphthyridine skeleton as hybrid entity with thiosemicarbazide and/or 1,3,4-oxadiazole moieties. The modifications involve also introduction of bromosubstituent at C-6 of the naphtyridine nucleus to delineate the effect of bromination on the antibacterial activity. The design concept is intended as to maintain the pharmacophoric features established for inhibition of bacterial DNA-gyrase cleavage complex as a wellknown mechanism for the antibacterial effect of quinolones and naphthyridones. Hybrid molecules have been reported as approach for potentiation and/or attenuation of antibacterial activity towards resistant bacterial strains.[11,14,15] Moreover, halogention of lead compounds has been routinely adopted for improvement of the lipophilic and electronic characteristics resulting in enhanced drug-target interactions. Unlike the known fluoroquinolones, shifting the 6-fluoro substituent by the corresponding 6-bromo might attain a clue to overcome bacterial resistance. This assumption resides on the fact that bromo- derivatives are rarely encountered within the molecular features of antibacterial agents. The synthesized compounds will be investigated for potential antimicrobial activity against *G+ve, G-ve*; and *Mycobacterial* strains. Molecular docking to selected bacterial *DNAgyrase* complexes will be adapted to assist understanding the possible binding modes and interactions between the compounds and their potential target.

Results and Discussion

Chemistry

The Synthesis of the intermediates and target compounds was performed according to the reactions outlined in *Scheme 1*, using nalidixic acid (NA) 1 and its 6-bromo analog 2 as starting materials. The key intermediates, nalidixic acid methyl ester 3 and the corresponding 6-bromoester 4, were obtained according to reported mild esterification method developed in our laboratory.[23,24] 6-Bromonalidixic acid 2 has been obtained by treatment of nalidixic acid methyl ester 3 with bromine in methanol followed by hydrolysis and acidification of the resulting 6-bromonalidixic acid methyl ester 4. It is worth mentioning that direct bromination of nalidixic acid results in decarboxylation and subsequent formation of 3-bromo or 3,6-dibromo-naphtyridone derivatives.[25] The structure of 2 has been confirmed on basis of ^{1}H-NMR spectrum (Supporting information S1) characterized by the absence of pair of doublets corresponding to the naphthyridinyl H-5 & H-6 at $\delta=$ 8.5 & 7.5 ppm and the incidence of a singlet signal at $\delta=$ 8.6 ppm due to H-5. The methyl esters **3–4** were converted to the hydrazides **5–6** which were then used for synthesis of the targeted naphthy-ridinyl-3-thiosemicarbazides **7,8(a–e)**. Unlike, the aryl substituted thiosemicarbazide **7,8(b–e)**, the unsubstituted derivatives **7a-8a** were prepared by refluxing the acid hydrazides **5–6** with ammonium thiocyanate in 2 M HCl.[26] Meanwhile, the substituted congeners **7,8(b–e)** were prepared by treating the hydrazides **5–6** with the appropriate arylisothiocyanates in refluxing EtOH.[27] The naphthyridinyl-3-(2-amino-1,3,4-oxadiazoles) **9,10(a–e)** were in turn prepared from the corresponding thiosemicarbazides by cyclodesulfurization using mercuric acetate in acetic acid.[28]

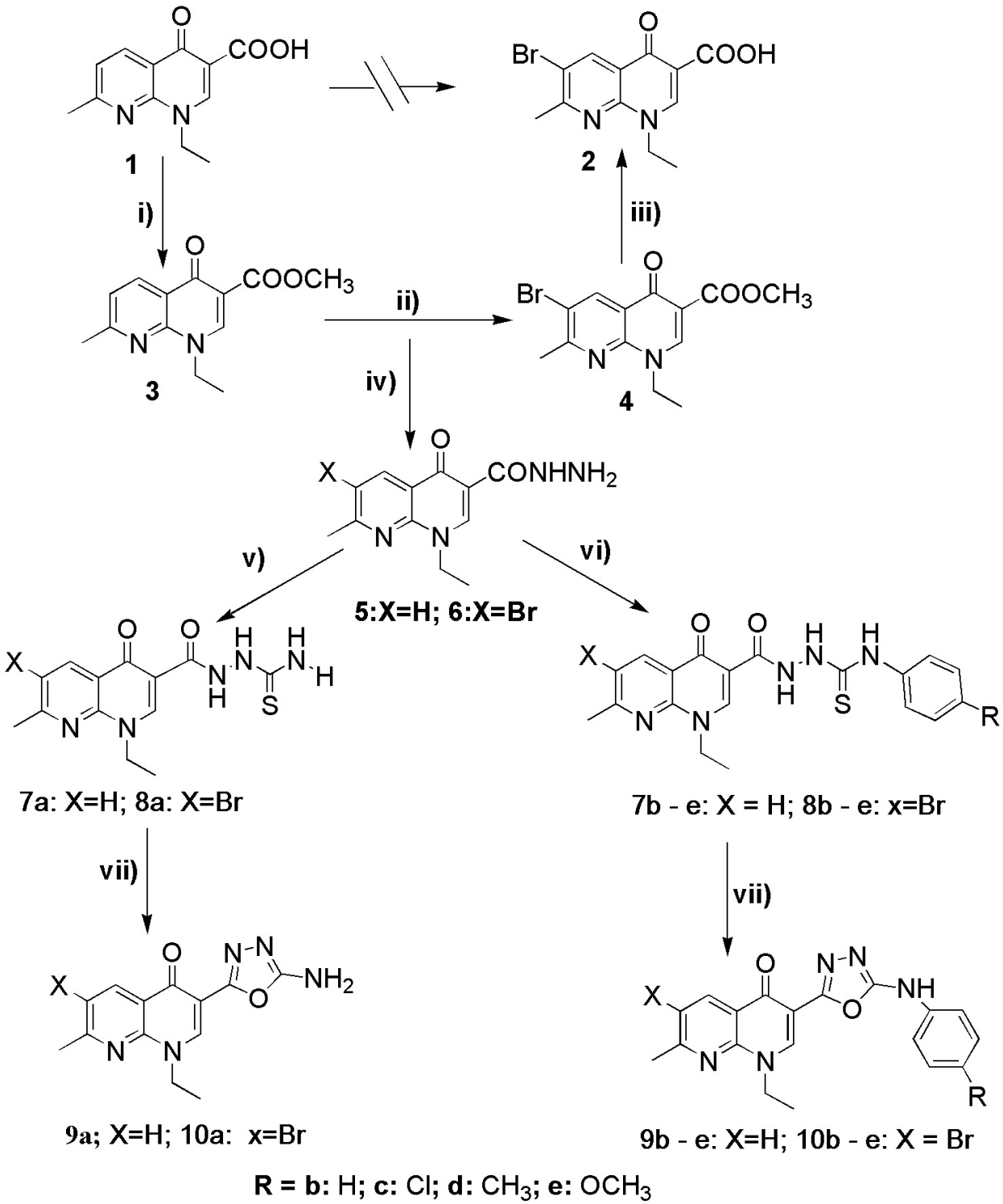
The structures of the synthesized compounds were confirmed by spectral analyses involving IR, ^{1}H-NMR, ^{13}C-NMR (Supporting information S:3 - 9) and MS (S:10 −13) as well as elemental analyses. A special feature in the IR spectra of the thiosemicarbazides, characteristic for thiourea residue (NH-CS-NH), exhibited as strong absorption in the range of 1110–1141 cm^{-1} attributable to C=S. The amidic carbonyl absorption was observed as a broad band in the range of 1635–1680 cm^{-1}.

^{1}H-NMR spectra of the unsubstituted thiosemicarbazides **7a, 8a** are characterized by three broad exchangeable singlets appeared at $\delta=$7.5, 9.4 and 11.2 *ppm*, of the protons of $CSNH_2$, NHCS and CONH respectively. The substituted counterparts **7,8(b–e)** showed the presence of exchangeable singlets for the NH functions at $\delta\sim$ 9.7; 10; and 12.2 *ppm*.

In the oxadiazole series, the pattern of the three exchangeable singlets of the thiosemi-carbazide moiety has been disappeared. Instead there is only one broad exchangeable singlet, corresponds to NH_2 or NH groups in compounds **9a, 10a** appeared at δ 7.1 or 7.4 ppm and at $\delta\sim$ 10.6 - 12.5 *ppm* in case of substituted amino analogues **9,10(b–e)**. In the ^{13}C spectra, the characteristic signals in the aliphatic region e.g. CH_3, one CH_2, as well as CH are directly identified on basis of their characteristic chemical shifts. The C-atoms of the naphthyridine skeleton were assigned with reference to reported data. Furthermore, DEPT experiments at 90° and 135° were used for further verification of the respective C-atoms (Supporting information S: 4,6,8,9). Mass spectrum of compounds **9d** and **10d** as representative for the non-brominated and 6-bromo-naphthyridinyl series were carried out. The molecular ion peaks in each spectrum matched to the calculated values and additionally suggested fragmentation patterns have been proposed (Supporting information S:10-13).

Antimicrobial activity

The primary screening test involved assessment of the antibacterial effect of the synthesized compounds elicited as inhibition zones of the growth of the tested organisms. The target compounds **710(a–e)** were tested *in vitro* in comparison with nalidixic acid for their antibacterial activity against selected Gram positive (*G+ve*) strains (*S. aureus* ATCC 25923, and *B. cereus* ATCC 11778); Gram negative (*G–ve*) (*E. coli* ATCC 25922, *K. pneumonia* ATCC 700603 and *P. aeruginosa* ATCC 27853) and *Mycobac. smegmatis*. Consequently, minimum inhibitory concentrations (*MIC*) were determined for compounds displaying significant growth inhibition zones more than or equivalent to that of nalidixic acid (**NA**) using the Broth micro dilution method.[29] The results summarized in (**Table 1**) indicate that the thiosemicarbazides **7,8(a–e)** display narrow antimicrobial



Scheme 1. Synthesis of the target compounds

Table 1. The antimicrobial *MIC** (*mM*) values of thiosemicarbazide 7,8(a-e) and oxadiazolyl naphthyridones 9,10(a–e):

Cpd. #	*S. aureus*	*B. cereus*	*E. coli*	*K. pneum.*	*P. aerug.*	*M. smeg.*
7a	16.4	-	-	-	-	-
7b	6.6	-	-	-	19.7	-
7c	6	-	-	-	18	6
7d	12.6	-	-	-	18.9	6.3
7e	12.2	-	6.1	-	18.2	6.1
8a	13	6.5	6.5	-	19.5	-
8b	10.8	10.8	-	10.8	16.3	-
8c	10	10	5	10	15	10
8d	10.5	10.5	-	10.5	15.8	10.5
8e	10.2	10.2	-	10.2	10.2	5.1
9a	18.4	-	-	-	-	9.2
9b	7.2	7.2	-	7.2	21.6	-
9c	13	6.5	6.5	6.5	19.5	6.5
9d	6.9	-	-	-	20.7	6.9
9e	13.2	-	-	-	19.8	-
10a	14.2	-	-	7.1	21.4	7.1
10b	11.7	5.9	5.9	5.9	17.6	-
10c	10.8	-	5.4	-	16.3	5.4
10d	11.4	5.7	5.7	5.7	17	5.7
10e	11	5.5	5.5	11	16.5	11
NA	21.5	10.8	4.3	10.8	21.5	10.8

* MIC values (mean ± SEM of n = 3. Values that are significantly higher than nalidixic acid not considered. MIC values of the most active compounds are highlighted.

spectrum (**Figure 1**) as compared to their cyclised counterparts **(figure 2)** of the oxadiazoles **9,10(a – e)**. It is evident that

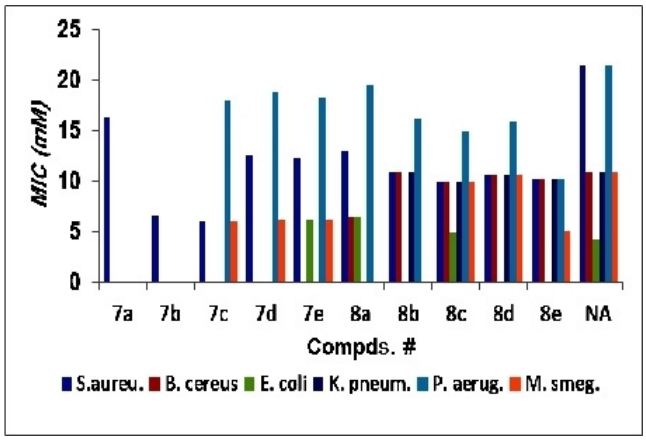


Figure 1. Graphical representation of MIC-values of the naphthyridone-3-thiosemicarbazides 7,8(a-e).

compounds loaded by aryl substituent linked to the thiosemicarbazide **7,8(b – e)** or the oxadiazole moieties **9,10(b – e)** showed selective antibacterial activity against *S. aureus (MIC = 6.0 - 13.0 mM), B. cereus and M. smegmatis (MIC = 5 – 6 mM).* It is worth mentioning that such bacterial strains are resistant to the reference drug. This might allocate the significance of the lipophilic characteristics for the antibacterial activity within this series. Bromination of the naphthyridone nucleus enhances the

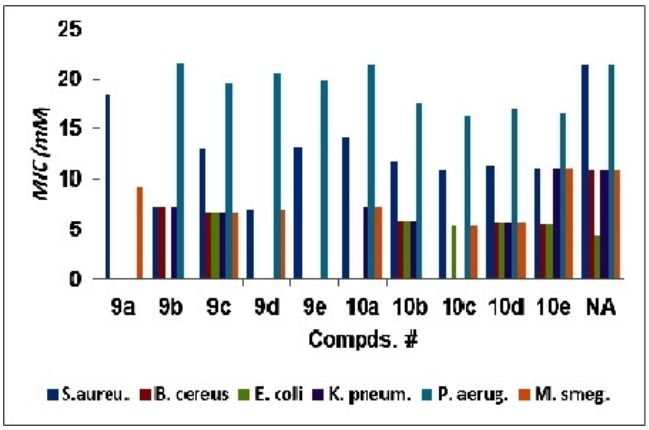


Figure 2. Graphical representation of MIC-values of the naphthyridone-3-oxadiazoles 9,10(a-e).

potency and selectivity towards *B. cereus* as evidenced by the thiosemicarbazide derivative **8a** and oxadiazoles **10(b,d,e);** *and K. pneumonia* by compounds **10(a,b,d).**

The observed *MIC-values (~ 5.5 – 7.2 mM)* are 1.5 – 2 times less than that of the reference drug. In case of *M. smegmatus* the *MIC*-values of the brominated thiosemicarbazide derivatives e.g **8e** or oxadiazoles e.g. **10(a,c,d)** are not significantly different than the non-brominated analogues. The observed enhancement of *MIC-values* due to introduction of 6-bromo substituent is likely due increased lipophilicity of the compounds. This has been established through assessment of molecular characteristics of the synthesized compounds, **(Table 4).** The *logP*-values of the 6-bromo-3-thiosemicarbazides **8a-e** (*miLogP* = 1.13 – 3.25) and 6-bromo-3-oxadiazoles **10a-e** (*miLogP* = 1.84 – 5.03) are approximately double that of the non-brominated analogues **7a-e** (*miLogP* = 0.01 – 2.12) and **9a-e** (*miLogP* = 0.72 −3.90).

Docking study

Several *X-ray* crystal structures of bacterial gyrase illustrate that it is a hetero-tetrameric ($GyrA_2GyrB_2$) enzyme. Only some of them were *DNA-gyrase* cleavage complexes cocrystallised with quinolones.[30–33] The crystal structures of *DNA-gyrase* cleavage complex of *S. aureus* (PDB entry: *5cdq*), *Mycobac. mTB* (PDB entry: *5btd*) and the cleavage complex of topoisomerase *Top. IV* of *K. pneumonia* (PDB entry: *5eix*) co-crystalized with fluoroquinolones, (Supporting information S:14), have been downloaded from protein data bank and used for the present docking study.

Analysis of the cocrystallized *S. aureus* DNA-gyrase cleavage complex with moxifloxacin revealed binding between GyrA: Ser 84 and Glu 88 and the two C=O groups of quinolone nucleus and COOH via water–Mg^{2+} bridge.[32]

In addition there are hydrophobic interactions with adenine and guanine DNA bases and H-bonds with *Arg 122*, *Glu 477* or *Asp 437*. Similarly, levofloxacin binds *ParC*, subunit A of *Topo IV* of *K. pneumonia* through Mg^{2+}; in addition to π–π stacking interaction between *DNA* bases (adenine and guanine) as

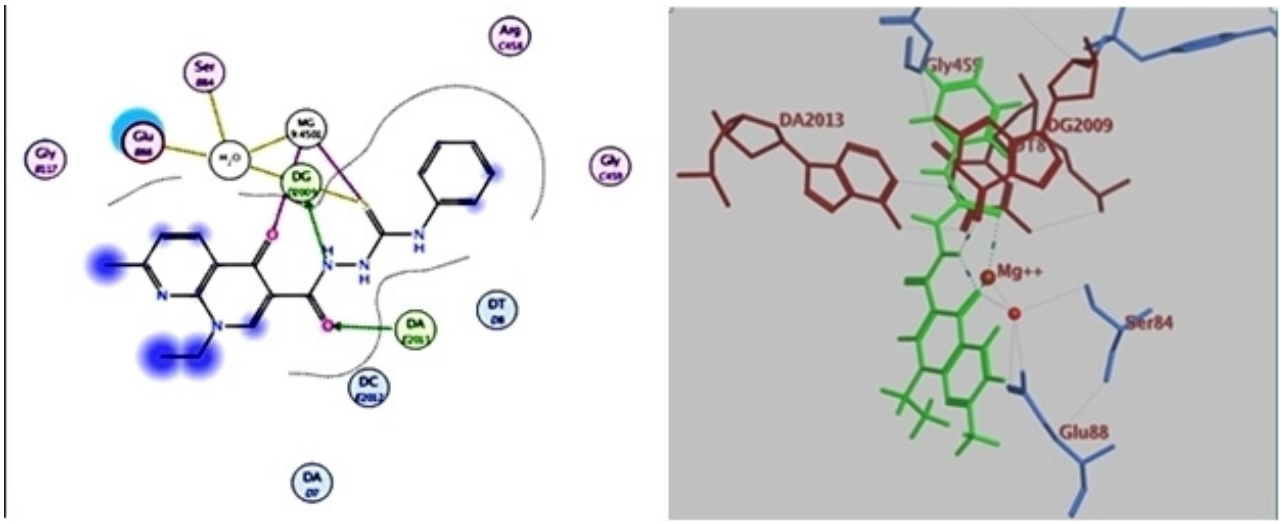


Figure 3. Ligand interaction diagram(A) and 3D representation (B) of (7b) into *S. aureus* DNA-gyrase cleavage complex

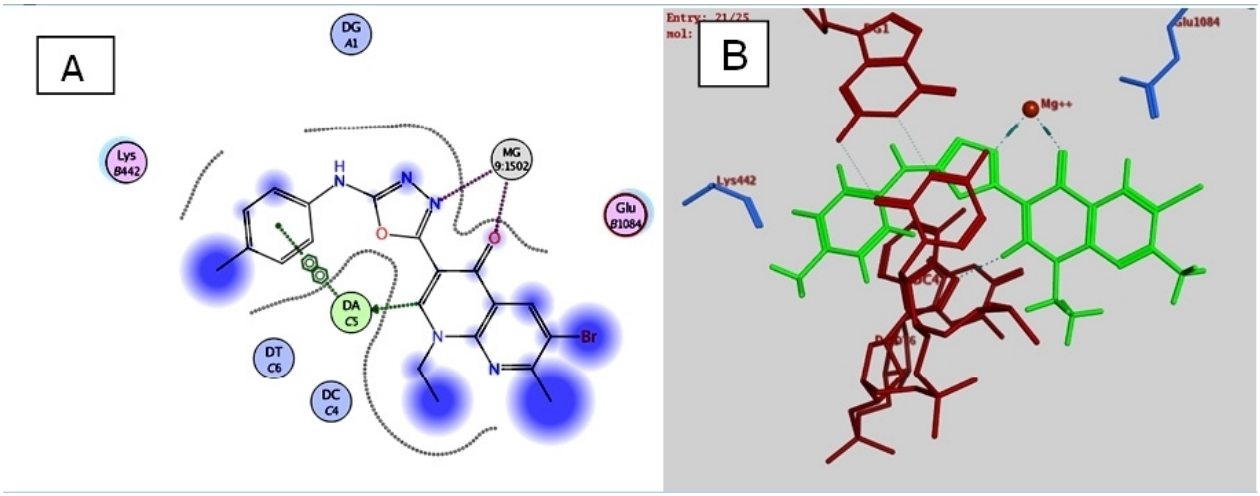


Figure 4. Ligand interaction diagram (A) and 3D-representaion of (10d) into *K. pneumoniae* topoisomerase

second important interaction.[33] Likewise, the crystal structure of *Mycobac. DNA-gyrase* complex co-crystallized with gatifloxacin shows binding of the keto-acid moiety of gatifloxacin molecule to *GyrA: Asp 94* through a hydrated Mg^{2+}. Other significant interactions include *vdWs* interactions of the quinolone ring with two adenine bases of *DNA* and that of the cyclopropyl group with one thymine base.[32]

In the present investigation the proposed docking algorithm was initially validated by self-docking of the co-crystallized ligands to each of the aforementioned targets. Whereby, the ligands were removed from the complex and then docked back into the binding site. Heavy-atom root mean square deviation (*RMSD*) values between top-ranked poses and the experimental crystal structure ranged from ~ 0.52 – 0.9 *Å*. Subsequently, a docking procedures have been achieved for the tested compounds **7,8(a–e) and 9,10(a–e)** using Molecular Operating Environment software (*MOE-dock tool, ver. 2014.0901,* Chemical Computing Group, Canada) to predict their binding modes to bacterial *DNA-gyrase* cleavage complex active site. The studies have successfully identified binding poses for all test molecules, with comparable docking scores, indicating that the proposed compounds could potentially bind to the active site with analogous strengths. The ligand interaction diagrams and the corresponding 3D representations of the binding modes of selected active compounds are illustrated in ***Figures 3–5***

The following binding features are observed regarding the docked compounds:

1. The studied compounds showed similar binding modes as displayed in case of the co-crystallized ligands. As evident in **Figure (3)** the thiosemicarbazide derivative **7 b** coordinate through C=O oxygen of naphthyridone ring and C=S sulfur of the side chain with Mg^{2+}, which in turn linked via a water bridge to ***Ser 84*** and ***Glu 88***. In case of the oxadiazole

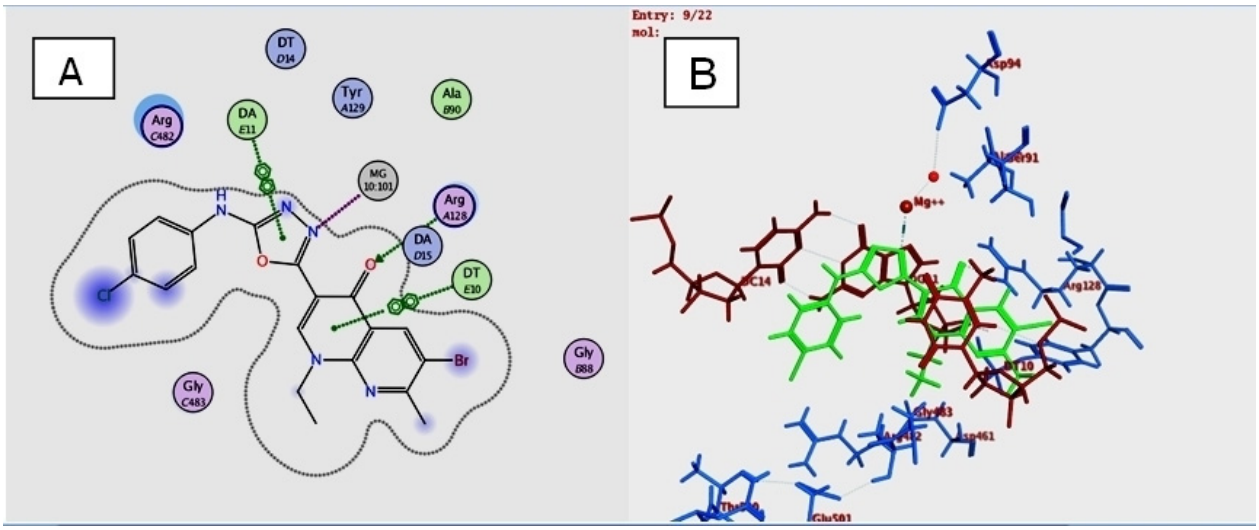


Figure 5. Ligand interaction diagram (A) and 3D-representaion (B) of (10c) into Mycobac. DNA-gyrase cleavage complex

derivatives (**Figures 4** & **5**), the coordination initiated by one of the nitrogen atoms of the oxadiazole ring and/or naphthyridone C=O oxygen.

2. Additionally, π-π interactions of adenine and thymidine DNA bases and naphthyridone, oxadiazole and/or the aromatic moieties of C-3 substituents were observed.
3. H-bonds were perceived between DNA bases and NH or C=O of the thiosemi-carbazide moiety.

Docking simulations based on London ***Δ G***, expressed as E-refine values, as a scoring function were performed to estimate the free energy of interactions of the studied thiosemicarbazides **7,8(a–e)** and oxadiazoles **9,10(a–e)** from a given pose with the respective DNA-gyrase cleavage complexes. This parameter considered as more representative, since it involves energy minimization procedure for both the ligand and target. Consequently, it illustrates the least energy interaction value of the selected pose. (**Table 2)** summarizes the docking scores and the respective ***MIC****-values* of the tested compounds.

The results indicate that the most active compounds against *S. aureus* (**7 b,c; 9 b** and **9 d)** with *MIC* values 6.6, 6.0, 7.2 and 6.9 *mM*, showed the lowest ΔG values (E-refine): −24.68, −26.09, −23.34 and −23.30 *Kcal/mol* respectively. It is obvious that the reference compound, nalidixic acid, which is practically inactive against *S. aureus* exhibits very low binding with the *DNA-gyrase* cleavage complex (E-refine = 7.86). Similar pattern has been logged in case of topoisomerase (*Top. IV*) DNA-gyrase cleavage complex of *K. pneumonia*. The most active compounds are the substituted amino oxadiazoles **9 b,c** and **10 a,b,d** (*MIC values*: 7.2, 6.5 and 7.1, 5.9, and 5.7) displayed the lowest binding energy values corresponding to: −26.57, −26.89, −22.62, −25.63, and −25.53. The results illustrate also that the active site of *Top. IV of K. pneumonia* can embrace bulky substituents of the 6-bromo derivatives **10 a,b,d.** Remarkable correlation is also observed between the *in vitro* antimycobacterial activity and binding free energy (*ΔG)* with *Mycobac. mTB DNA–gyrase* cleavage complex as evident in compounds: **7 c,d,e; 8 e; 9 c,d; and 10 a,c,d.**

Table 2. Interaction energies (*Kcal/mol*) and MIC (*mM*) of the tested compounds with DNA-gyrase/ topoisomerase-IV cleavage complexes

Cpd. #	*S. aureus*		*K. pneumon.*		*mTB*	
	ΔG	MIC	ΔG	MIC	ΔG	MIC
7a	5.20	16.4	-5.68	16.4	2.54	16.4
7b	-24.7	6.6	-18.7	13.1	-12.3	13
7c	-26.1	6	-14.1	12	-26.3	6
7d	-15.4	12.6	-16.6	12.6	-23.8	6.3
7e	-12.8	12.2	-18.3	12.2	-26.7	6.1
8a	-15.5	13	-16.2	13	-17.0	13.1
8b	-14.5	10.8	-15.9	10.8	8.82	16.3
8c	-18.2	10	-14.5	10	-17.1	10
8d	-13.0	10.5	-16.1	10.5	-17.6	10.5
8e	-14.5	10.2	-17.6	10.2	-24.6	5.1
9a	4.32	18.4	-1.61	18.4	-19.0	9.2
9b	-23.3	7.2	-26.6	7.2	6.57	14.4
9c	-14.2	13	-26.9	6.5	-25.8	6.5
9d	-23.3	6.9	-22.3	13.8	-27.2	6.9
9e	-13.0	13.2	-19.7	13.2	-12.5	13.2
10a	-5.02	14.2	-22.6	7.1	-22.7	7.1
10b	-14.5	11.7	-25.2	5.9	-12.7	11.7
10c	-16.1	10.8	-20.93	10.8	-25.42	5.4
10d	-10.41	11.4	-25.63	5.7	-24.77	5.7
10e	-13.80	11	-23.53	11	-13.61	11
NA	7.86	21.5	-18.12	10.8	-14.11	10.8

Inhibition of DNA-gyrase supercoiling activity

The observed binding characteristics of the synthesized compounds with *DNA Gyrases* in molecular docking inspire the assessment of their potential inhibitory activity against the enzyme. Consequently, *DNA-gyrase* assay has been proposed and executed. Representative compounds of the synthesized series, specifically those having the lowest *MIC*-values and docking scores, were used for assessment of the IC_{50}.

E. Coli DNA-gyrase microplate assay kit (*Inspiralis®*) has been used for the anticipated assay according to the optimized protocol by the manufacturer (Supporting information S:15). This assay is based upon the fact that negatively-supercoiled plasmids form intermolecular triplex ***DNA*** more readily than do relaxed plasmids under some conditions. The assay overcomes some of the problems of gel-based assays, which are time consuming and are therefore inherently low-throughput. In this assay the substrate is relaxed pNO1, a modified form of pBR322 which contains a 'triplex-forming sequence'. The assay can be used to determine the activity of compounds as inhibitors of *DNA*-gyrase either as an initial screen or in the determination of IC_{50} values.

The IC_{50} values of the selected compounds are listed in (Table 3) and represented in (Figure 6). The results indicate that

Table 3. IC_{50} (μg/ml) against E. Coli DNA-gyrase

Cpd. #	IC_{50} (μg/ml)
7a	10.29
8a	51.02
7c	1.73
7e	7.66
8e	4.64
9b	3.36
9c	9.69
10b	40.5
10d	3.89
NA	1.74

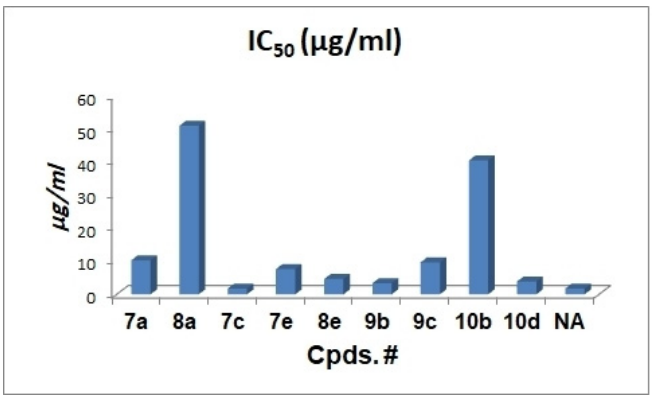


Figure 6. Inhibitory activity (IC_{50} μg/ml) of selected compounds against *E. coli* DNA gyrase

within the naphthyridinyl-3-thiosemicarbazide series the presence of aryl substituent on thiosemicarbazide moiety is crucial as previously evidenced by the *MIC*-value. Compounds 7a and 8a, the non-substituted thiosemicarbazides, showed much higher IC_{50}values (10.29 and 51.02 *μg/ml* respectively). Compound 7c showed the lowest IC_{50} value (1.73 μg/ml), which is similar to that of nalidixic acid. This compound is a 4-chlorophenyl substituted thiosemicarbazide derivative.

Its analogue **7e** with 4-methoxyphenyl substituent showed relatively moderate ***IC₅₀*** value (7.66 *μg/ml*) illustrating the significance of an electron withdrawing group on the aryl substituent for enzyme inhibition.

In case of the cyclized analogues i.e. naphthyridinyl-3-oxadiazoles, **9b** and **10d** are the most active compounds with almost equal ***IC₅₀*** values of 3.36 and 3.89 μg/ml respectively. This advocate that bromination of the naphtyridine nucleus is not a critical modification for inhibition activity of the synthesized compounds against E. coli DNA gyrase. These results matched well with the observed binding scores of the two compounds (E-refine: -26.57 and −25.53 respectively).

Molecular properties and drug-likeness

Molecular properties are a complex balance of various structural features which determines whether a particular molecule is similar to the known drugs or not. It generally means "molecules which contain functional groups and/or have physical properties consistent with most of the known drugs". Hydrophobicity, molecular size, flexibility, and presence of various pharmacophoric features are the main physical properties that influence the behavior of molecules in a living organism. Good bioavailability can be achieved with an appropriate balance between solubility and partitioning properties. Thus, the compliance of the newly synthesized compounds to the Lipinski's rule of five was evaluated.[34] In addition, topological polar surface area (***TPSA***) and number of rotatable bonds (***nrotb***) have been linked to drug bioavailability.[35]

Molecular properties (***TPSA, nrotb, miLogP, OH–NH*** interaction, O–N interaction, molecular weight, and number of violations from Lipinski's rule) of the newly synthesized compounds were calculated using *molinspiration* software and compared to the values of the standard drug, nalidixic acid (**Table 4**). Topological polar surface area (***TPSA***) and lipophilicity (***logP***) values are two important properties for the prediction of oral bioavailability of drug molecules.[36–39] ***TPSA*** is calculated based on the methodology published by *Ertl et al*[39] as the surface areas that are occupied by oxygen and nitrogen atoms and by hydrogen atoms attached to them. Thus, it is closely related to the hydrogen bonding potential of a compound. Moreover, it is considered as a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, and blood–brain barrier penetration. Molecules with ***TPSA*** values around 140 $Å^2$ or more are expected to exhibit poor intestinal absorption.[35] The results shown in **Table (4)** indicate that all the synthesized compounds have ***TPSA*** values $<140\ Å^2$; thus, they are expected to have good intestinal absorption.

Additionally, the total number of rotatable bonds in drug molecules represents an important molecular characteristic. Compounds with more than 10 rotatable bonds may have problems with bioavailability.[35] All compounds under investigation (**Table 4**) have two to eight rotatable bonds and they might not have problems with bioavailability. The lipophilic characteristics expressed as (***LogP)*** value have been calculated adopting the methodology developed by Molinspiration (***MiLogP***), which depends on summation of fragment-based

Table 4.
Molecular characteristics* of 7,8(a-e) and 9,10(a-e):

code	Mwt	miLog P	HBA	HBD	TPSA	#RT	Drug score	Solubility (mg/mL)
NA	232.23	0.81	5	1	72.20	2	0.82	68.5
7a	305.36	0.01	7	4	102.05	4	1.30	100.3
7b	381.45	1.45	7	3	88.05	6	1.03	5.27
7c	415.90	2.12	7	3	88.05	6	1.41	0.63
7d	395.48	1.89	7	3	88.05	6	0.99	1.92
7e	411.48	1.50	8	3	97.28	7	0.90	3.12
8a	384.25	1.13	7	4	102.05	4	1.28	8.23
8b	460.35	2.57	7	3	88.05	6	0.84	0.42
8c	494.80	3.25	7	3	88.05	6	1.45	0.05
8d	474.38	3.02	7	3	88.05	6	0.86	0.15
8e	490.38	2.62	8	3	97.28	7	0.90	0.24
9a	271.28	0.72	7	2	99.84	2	0.69	18.67
9b	347.37	3.23	7	1	85.85	4	0.74	0.92
9c	381.82	3.90	7	1	85.85	4	1.08	0.11
9d	361.40	3.67	7	1	85.85	4	0.65	0.33
9e	377.40	3.28	8	1	95.08	5	0.59	0.54
10a	350.17	1.84	7	2	99.84	2	0.75	1.54
10b	426.27	4.35	7	1	85.85	4	0.69	0.07
10c	460.71	5.03	7	1	85.85	4	1.20	0.01
10d	440.30	4.80	7	1	85.85	4	0.63	0.03
10e	456.29	4.41	8	1	95.08	5	0.68	0.04

* ***Mwt:*** molecular weight, ***miLog P:*** lipophilicity parameter, ***HBD:*** # of hydrogen bond donor, ***HBA:*** # of hydrogen bond acceptor, ***TPSA:*** topological polar surface area, # RT: # of rotatable bonds.

contributions and correction factors (*http://www.molinspiration.-com*). It has been shown that for the compound to have a reasonable probability of being well absorbed, ***miLogP*** value must be in the range of −0.4 to 5.[35] On this basis, all the tested compounds were found to have ***miLogP*** values within the acceptable criteria and are expected to have reasonable oral absorption. It is worth mentioning that all compounds have one or zero violation of Lipinski's rule; therefore, they are expected not to have problems with bioavailability.

The drug score combines drug-likeness, ***miLogP***, solubility, molecular weight, and toxicity risks in one handy value that may be used to judge the compound's overall potential to qualify for a drug.[36] A value of 0.5 or more makes the compound a promising lead for future development of safe and efficient drugs.

The overall drug score values for the synthesized compounds were calculated using molsoft software (http://molsoft.-com/ mprop.) and compared to that of the parent drug, nalidixic acid (**Table 4**). Almost all the synthesized compounds possess good drug score values above 0.5.

Conclusions

A synthetic strategy for structural modification of nalidixic acid involving isosteric replacement of the COOH in 3-position and introduction of bromo substituent at C-6 was developed. Results of antimicrobial screening showed that all the synthesized compounds showed selective antibacterial activity against the tested G +ve bacterial strains that are known to be resistant to nalidixic acid. In case of *S. aureus* both the nonbrominated (**7b,c)** and the 6-brominated thiosemicarbazide (**8c-e**) as well as their corresponding oxadiazoles **9b,d** exhibit superior antibacterial activity *(MIC = 6.0 - 10.5 mM)*. However, in case of *B. cereus* the presence of 6-bromo substituent seems to be promising for their antibacterial action as manifested by compounds **8a; 10b,d,e** *(MIC = 5 – 6 mM)*. The antibacterial activity of the tested compounds is not significantly deviating than the reference drug against the G –ve bacterial strains. Only in case of *K. pneumonia* some of the naphthyridinyl-3-oxadiazole derivatives (**9b,c** and **10a,b,d)** are twice as potent as the reference drug. As regards the antimycobacterial activity against *M. smegmatis*, it can be concluded that the achieved structural modifications result in two folds enhancement of the *MIC*-value as illustrated by compounds **7c,d,e**; **8e**; **9c,d**; and **10c,d**. Molecular docking and *DNA gyrase* inhibition assay illustrate that compound **7c** is the most active compound with IC_{50}value = 1.73 $\mu g/ml$ against *E. coli DNA gyrase*. Furthermore, Molecular properties and drug-likeness data illustrate that the studied compounds fulfill lipinski's rule requirements and possess good drug score values. These preliminary encouraging results of the in vitro antibacterial activity of the newly synthesized compounds could offer an exceptional framework that may lead to the discovery of new potent antimicrobial agents.

Supporting Information Summary

Experimental details from the synthetic procedures of the intermediates (**1** −**6**) the targeted naphthyridinyl-3-thiosemicarbazides **7,8(a–e)** and naphthyridinyl-3-(2-Amino-1,3,4-oxadiazoles) **9,10(a–e)** as will as their spectral data (involving ^{1}H-; ^{13}C-NMR and MS) are presented in the supporting Information file. Moreover, this section includes the antibacterial screening method against the selected ***G+ve***, ***G-ve***, ***Mycopbac.*** strains (Comprising: assessment of MIC values, IC_{50} against *E. coli DNA-gyrase*), the 3D representation and docking scores of reference compounds with *DNA- gyrase*.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Antimicrobial · DNA Gyrase · Drug-likeness · Molecular Docking Naphthyridone · Oxadiazole · Thiosemicarbazide

[1] H. Gelband, M. Miller-Petrie, S. Pant, A. White, S. Gandra, J. Levinson, D. Barter, R. Laxminarayan, *The state of world's antibiotics, Center for Disease Dynamics, Economics & Policy (CDDEP):* Washington, D.C, USA, **2015**, p. 8.
[2] D. L. Paterson, *Am. J. Med.* **2006**,*119*, 20–28.
[3] V. Talla, P. Veerareddy, *J. Young Pharm.* **2011**, *3*, 304–309.
[4] C. Kang, S. Kim, D. M. Kim, W. B. Park, K. Lee, H. Kim, M. Oh, E. Kim, *Epidemiol.* **2014**, *25*, 860–867.
[5] T. H. Dellit, R. C. Owens, J. E. Mc Gowan. *J. Clin. Infect. Dis.* **2007**, *44*, 159.
[6] A. H. Diacon, A. Pym, R. Rustomjee, M. Grobusch, R. Patientia, L. Page-Shipp, J. Allen, J. C. Palomino, T. De Marez, R. P. G. Van Heeswijk, *N. Engl. J. Med.* **2009**, *360*, 2397–2405.
[7] N. Veziris, M. Ibrahim, N. Lounis, K. Andries, V. Jarlier, *PLoS One* **2011**, *6*, 2–7.

[8] A. Naeem, S. Badshah, M. Muska, N. Ahmad, K. Khan, *Molecules* **2016**, *21*, 268–287.
[9] S. Huang, J. Qing, S. Wang, H. Wang, *Org. Biomol. Chem.* **2014**, *1*, 2344–2348.
[10] C. Manera, A. M. Malfitano, T. Parkkari, V. Lucchesi, F. Stefano, S. Bertini, C. Laezza, A. Ligresti, J. R. Savinainen, E. Ciaglia, S. Pisanti, P. Gazzerro, V. Di Marzo, P. Nieri, M. Macchia, M. Bifulco, *Eur. J. Med. Chem.* **2015**, *97*, 10–18.
[11] N. Aggarwal, R. Kumar, P. Dureja, J. M. Khurana, *Eur. J. Med. Chem.* **2011**, *46*, 4089–4099.
[12] C. M. Oliphant, W. Casper, G. M. Green, K. Permanente, *Clin. Pharmacol.* **2002**, *65*, 455–464.
[13] R. R. Somani, A. G. Agrawal, P. P. Kalantri, P. S. Gavarkar, E. D. Clercq, *Int. J. Drug Des. Discov.* **2011**, *2*, 353–360.
[14] G. Grover, S. G. Kini, *Eur. J. Med. Chem.* **2006**, *41*, 256–262.
[15] T. Aboul-Fadl, A. A. Radwan, H. A. Abdel-Aziz, M. Baseeruddin, M. I. Attia, A. Kadi, *Dig. J. Nanomater. Biostructures*. **2012**, *7*, 329–338.
[16] N. Aggarwal, R. Kumar, P. Dureja, J. M. Khurana, *Chem. Biol. Drug Des.* **2012**, *79*, 384–397.
[17] Ł. Popiołek, M. Gawrońska-grzywacz, *Int. Res. J. Pure Appl. Chem.* **2015**, *7*, 191–202.
[18] A. Kadi, N. El-Brollosy, O. Al-Deeb, E. Habib, T. Ibrahim, A. El-emam, *Eur. J. Med. Chem.* **2007**, *42*, 235–343.
[19] P. Shetty, M. Raghavendra, B. M. Praveen, S. Cheruku, K. Manjunath, *Mol. Divers.* **2016**, *20*, 391–398.
[20] A. Mazumder, M. Shaharyar, *Med. Chem. Res.* **2015**, *24*, 2514–2528.
[21] A. P. Liesen, T. M. De Aquino, C. S. Carvalho, V. T. Lima, J. M. De Araújo, J. G. De Lima, A. R. De Faria, E. De Melo, A. J. Alves, E. W. Alves, A. Q. Alves, A. Góes, *Eur. J. Med. Chem.* **2010**, *45*, 3685–3691.
[22] O. U. Tan, K. Ozadali, P. Yogeeswari, D. Sriram, A. Balkan, *Med. Chem. Res.* **2012**, *21*, 2388–2394.
[23] F. A. Omar, *Alex. J. Pharm. Sci.* **1994**, *8*, 65–69.
[24] A. F. Youssef, F. A. Omar, H. A. El-sherief, G. A. Abuo-Rahma, Design, *Bull. Pharm. Sci. Assiut University* **1998**, *21*, 15–26.
[25] A. F. Youssef, F. A. Omar, H. A. El-Sherief, G. A. Abou-Rahma, *1st Pharm. Sci. Conf. (faculty of pharmacy-Assiut University- Assiut/Egypt)* **1998**, 20–34.
[26] M. A. Metwally, S. Bondock, H. El-azap, E. M. Kandeel, *J. Sulfur Chem.* **2011**, *32*, 489–519.
[27] G. L. Almajan, I. Saramet, C. Draghici, C. Enache, *Rev. Chim.* **2008**, *59*, 305–308.
[28] N. R. Rivera, J. Balsells, K. B. Hansen, *Tetrahedron Lett.* **2006**, *47*, 4889–4891.
[29] J. M. Andrews, *J. Antimicrob. Chemother.* **2001**, *48(1)*, 5–16. doi:10.1093/jac/48.suppl_1.5
[30] P. F. Chan, V. Srikannathasan, J. Huang, H. Cui, A. P. Fosberry, M. Gu, M. M. Hann, M. Hibbs, P. Homes, K. Ingraham, J. Pizzollo, C. Shen, A. J. Shillings, C. E. Spitzfaden, R. Tanner, A. J. Theobald, R. A. Stavenger, B. D. Bax, M. N. Gwynn, *Nat. Commun.* **2015**, *6*, 10048.
[31] B. D. Bax, P. F. Chan, D. S. Eggleston, A. Fosberry, D. R. Gentry, F. Gorrec, I. Giordano, M. Hann, A. Hennessy, M. Hibbs, J. Huang, E. Jones, J. Jones, K. K. Brown, C. J. Lewis, E. W. May, M. R. Saunders, O. Singh, C. E. Spitzfaden, C. Shen, A. Shillings, A. J. Theobald, A. Wohlkonig, N. D. Pearson, M. N. Gwynn, *Natur.* **2010**, *466*, 935–940.
[32] T. R. Blower, B. H. Williamson, R. J. Kerns, J. M. Berger, *Proc. Natl. Acad. Sci.* **2016**, *113*, 1706–1713.
[33] D. A. Veselkov, I. Laponogov, X. S. Pan, J. Selvarajah, G. B. Skamrova, A. Branstrom, J. Narasimhan, J. V. N. V. Prasad, L. M. Fisher, M. R. Sanderson, *Acta Crystallogr. Sect. D, Struct. Biol.* **2016**, *72*, 488–96.
[34] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Deliv. Rev.* **2001**, *46*, 3–26.
[35] D. F. Veber, S. R. Johnson, H. Y. Cheng, B. R. Smith, K. W. Ward, K. D. Kopple, *J. Med. Chem.* **2002**, *45*, 2615–2623.
[36] A. Jarrahpour, J. Fathi, M. Mimouni, T. Ben Hadda, J. Sheikh, Z. H. Chohan, A. Parvez, *Med. Chem. Res.* **2012**, *21*, 1984–1990.
[37] A. Parvez, M. Jyotsna, M. H. Youssoufi, T. Ben Hadda, *Phosphorus Sulfur Silicon Relat. Elem.* **2010**, *7*, 1500–1510.
[38] A. Parvez, J. Meshram, V. Tiwari, J. Sheikh, R. Dongre, M. H. Youssoufi, T. Ben Hadda, *Eur. J. Med. Chem.* **2010**, *45*, 4370–4378.
[39] P. Ertl, B. Rohde, P. Selzer, *J. Med. Chem.* **2000**, *43*, 3714–3717.

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